

Novel Methods in Imaging Mass Spectrometry and Ion Time-of-Flight Detection

A thesis submitted for the degree of Doctor of Philosophy

by

Benjamin Winter



Jesus College, University of Oxford

Trinity Term, 2014

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Abstract

Imaging mass spectrometry (IMS) in microscope mode allows the spatially resolved molecular constitution of a large sample section to be analysed in a single experiment. If performed in a linear mass spectrometer, the applicability of microscope IMS is limited by a number of factors: the low mass resolving power of the employed ion optics; the time resolution afforded by the scintillator screen based particle detector and the multi-hit capability, per pixel, of the employed imaging sensor. To overcome these limitations, this thesis concerns the construction of an advanced ion optic employing a pulsed extraction method to gain a higher ToF resolution, the development of a bright scintillator screen with short emission lifetime, and the application of the Pixel Imaging Mass Spectrometry (PImMS) sensor with multi-mass imaging and time stamping capabilities.

Initial experimental results employing a three electrode ion optic to spatially map ions emitted from a sample surface are presented. By applying a static electric potential a time-of-flight resolution of $t/2\Delta t = 54$ and a spatial resolution of $20\text{ }\mu\text{m}$ are determined across a field-of-view of 4 mm diameter. While the moderate time-of-flight resolution only allows particles separated by a few Dalton to be distinguished, the instrument is used to demonstrate the multi-mass imaging capabilities of the PImMS sensor when being applied to image grid structures or tissue samples.

An improved time-of-flight resolution is achieved by post extraction differential acceleration of a selected range of ions (up to 100 Da) using a newly developed five electrode ion optic. This modification is shown to correct the initial velocity spread of the ions coming off the sample surface, which yields an enhanced time-of-flight resolution of $t/2\Delta t = 2000$. The spatial resolution of the instrument is found to be $20\text{ }\mu\text{m}$ across a field-of-view of 4 mm. Adjusting the extraction field strength applied to the ion optic of the constructed mass spectrometer allows the optimised mass range to be tuned to any mass of interest. Ion images are recorded for various samples with comparable spatial and ToF resolution. Hence, studies on tissue sections and multi sample arrays become accessible with the improved design and operational principle of the microscope mode IMS instrument.

A fast and efficient conversion of impinging ions into detectable flashes of light, which can consequently be recorded by a fast imaging sensor, is essential to maintain the achievable time-of-flight and spatial resolution of the IMS instrument constructed. In order to find a suitable fast and bright scintillator to be applied in a microchannel based particle detector, various inorganic and organic substances are characterised in terms of their emission properties following electron excitation. Poly-*para*-phenylene laser dye screens are found to show an outstanding performance among all substances analysed. An emission life time of below 4 ns and a brightness exceeding that of a P47 screen (industry standard) by a factor $2\times$ is determined. No signal degradation is observed over an extended period, and the spatial resolution is found to be comparable to commercial imaging detectors. Hence,

these scintillator screens are fully compatible with any ion imaging application requiring a high time resolution.

In a further series of mass spectrometric experiments, ions are accelerated onto a scintillator mounted in front of a multi pixel photon counter. The charged particle impact stimulated the emission of a few photons, which are collected by the fast photon counter. Poly-*para*-phenylene laser dyes again show an outstanding efficiency for the conversion of ions into photons, resulting in a signal enhancement of up to $5\times$ in comparison to previous experiments, which employed an inorganic LYSO scintillator.

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Chapter 1

Introduction and Motivation

The identification and localisation of molecular distributions are two key parameters in the understanding of how diseases affect a tissue section, where administered drugs are localised in an organism, and where a specific compound can be found within a sample array. Microscope mode imaging mass spectrometry (IMS) is an effective tool which has the capability to answer these questions in a single experiment. Originally developed in the 1960s, it has received considerable interest over the last 20 years, as novel, soft sample ionisation methods such as MALDI (Matrix assisted laser desorption and ionisation) and SIMS (Secondary ion mass spectrometry) have been introduced.

Microscope mode IMS experiments are performed by simultaneous ablation and ionisation of a large sample section. The emitted ions are then extracted using electrostatic fields, which are chosen such that the ions are separated according to their mass-to-charge ratio and spatially mapped onto a time and position sensitive detector.

Although the technology and the concept of microscope mode IMS have been around for more than 50 years, only a few mass spectrometers, employing mainly electrostatic analysers, have been developed so far. The reason for this arises from several experimental limitations. Firstly, the design of the ion optics and the mass analyser is challenging, as the spatial information of the sample needs to be maintained while the ions are separated according to their mass-to-charge ratio. Secondly, after extraction, the ions are mapped

onto a detector, which needs to be capable of detecting the position and the arrival time of multiple ions simultaneously. In most cases, these systems consist of a microchannel plate and a scintillator which translate the position of an impinging ion into a distinct light flash. The spatial position of this flash can be observed by a camera. However, the relatively long fluorescence lifetime of commercially available scintillators, and the use of standard imaging cameras, which allow only one ion image to be collected per time-of-flight (ToF) cycle, limit the application of microscope mode IMS. Only recently have newly developed CMOS based cameras been capable of detecting multiple ion events with a sufficient time precision per pixel such that multi-mass imaging becomes accessible.

Considering all these experimental challenges, in conjunction with the fact that the same spatial and molecular information can be collected with many conventional mass spectrometers by rastering the ionisation source across a sample to collect one-dimensional mass spectra, it is not surprising that most research groups focus on the use of this microprobe mode. In this approach the spatial resolution obtained depends on the ionisation area per raster step. Therefore, the higher the required spatial resolution is and the larger the sample area becomes, the longer the acquisition time extends. This issue may be overcome by employing IMS in microscope mode, since the simultaneous analysis of large sample sections reduces the acquisition time significantly.

The work in this thesis describes the construction of a linear microscope mode IMS instrument, the development of a new organic scintillation detector, which allows ion imaging measurements to be performed with an enhanced timing resolution, and the application of scintillator screens to detect the arrival time of an impinging ion directly. In this chapter, a general introduction to the basics of mass spectrometry, velocity map imaging, and imaging mass spectrometry can be found. Furthermore, a brief technical description of the state-of-the-art fast ion imaging systems will be given, as they are used throughout all chapters of this thesis. A complete outline of the thesis is presented at the end of this chapter.

1.1 The Concept of Mass Spectrometry

The history of mass spectrometry has its origin in the early 20th century, when Sir Joseph John Thomson investigated the electrostatic and magnetic deflection of positively charged rays [1], which were detected on a photographic plate such as that shown in the assembly in Figure 1.1. Experiments were first performed with hydrogen and later with oxygen, carbon, and nitrogen atoms, each of which showed its own parabolic pathway resulting in a shifted detection position on the photographic plate. Thomson concluded that the deflection d of the particles can be described by:

$$d = \frac{z}{mv} A, \quad (1.1)$$

where z is the charge, m the mass, v the velocity of the particle, and A a scaling factor depending on the applied fields. Hence, each particle with the same initial velocity, but a different m/z value, is deflected to a different detection position [1].

One year later, Thomson demonstrated the real potential of the new technique using a spectrograph modified by his student Francis William Aston. Thomson investigated the deflection of a beam of neon ions through a magnetic and electric field, and observed two separated signal stripes, corresponding to ^{20}Ne and ^{22}Ne , on the phosphographic screen. Thereby, Thomson provided the first experimental proof of the existence of isotopes. Aston followed up the work of his supervisor and presented the first fully functional mass

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Figure 1.1: Illustration of the cathode tube employed as first mass spectrometer by J. J. Thomson in 1906. The drawing was taken from Reference [1].

spectrometer [2], which allowed him to identify 212 of the 287 isotopes known today [3]. Following his work, Aston was awarded the Nobel Prize in Chemistry for the “*discovery of his spectrograph, of the isotopes for a large number of non-radioactive elements and for the suggestion of the whole-number rule in 1922*”.

An alternative concept to the position sensitive m/z detection of ion beams was presented by William Stephens in April 1946 at the American Physical Society meeting in Cambridge, Massachusetts. He suggested that “*microsecond pulses of ions are selected every millisecond from a low voltage ion source. In travelling down the vacuum tube, ions of different m/z have different velocities and consequently separate into groups spread out in space*.”[4]. By employing a time resolved detector, the ion flux can be measured as a function of the flight time of the particles and a direct mass spectrum can be obtained. One of the first spectrometers adapting the described concept was presented by Cameron and Eggers in 1948 [5], who accelerated an ion beam using a three electrode ion optic. To preselect the start of the ToF measurement, the continuous ion beam was interrupted by deflecting the particles using two pulsed electric plates. In the experiment, all the ions received a potential energy kick which accelerated them to their final velocity, v_f , of

$$v_f = \left(\frac{2zE}{m} \right)^{\frac{1}{2}}, \quad (1.2)$$

where z is the charge of the particle, E the strength of the electric field and m the mass of the particle. In Equation 1.2 it is obvious that the final velocity is dependent on the m/z ratio; heavier ions reach a lower final velocity while lighter ones travel faster through the flight tube. The different velocities of the particles convolute into a different ToF for a given length (D) of the field free vacuum tube. The ToF for a specific particle can be calculated according to:

$$t_{\text{ToF}} = \left(\frac{m}{2zE} \right)^{\frac{1}{2}} D. \quad (1.3)$$

Assuming that the investigated ions have no initial velocity before accelerating them towards the detector, and that the acceleration of the particles occurred instantly at $t = 0$,

the ToF spectrum can be directly converted into m/z values:

$$\frac{m}{z} = \left(\frac{2E}{D^2} \right) t_{\text{ToF}}^2. \quad (1.4)$$

The ToF technique described is commonly named as time-of-flight mass spectrometry (ToF-MS) and is one of the most frequently used chemical analysis methods today. The performance of the spectrometer is defined by a number of parameters, such as the mass resolution and the mass accuracy. The former is a key selling point for the manufacturers of these systems, as the higher the resolution of the instrument becomes, the better they are suited to assign chemical species to specific signals or to analyse isotopic distributions. Basically, the value given for the mass resolution determines how close two peaks can be before they overlap and distinction becomes impossible.

To define the mass resolution for a singly charged molecule ($z = 1$) Equation 1.4 needs to be differentiated to

$$dm = \left(\frac{2E}{D^2} \right) 2t_{\text{ToF}} dt_{\text{ToF}}, \quad (1.5)$$

where dm and dt_{ToF} correspond to the uncertainty in the mass and time domain. By dividing Equation 1.4 by Equation 1.5, and replacing dm with the full-width-at-half-maximum (FWHM) value of the mass peak and dt_{ToF} with the FWHM of the ToF peak, the mass and ToF resolution are defined as:

$$\frac{m}{\Delta m_{\text{FWHM}}} = \frac{t_{\text{ToF}}}{2\Delta t_{\text{FWHM}}}. \quad (1.6)$$

The second specification parameter, the mass accuracy (MA), can be simply described as the closeness of a measured mass (m_{Exp}) to the actual mass of a molecule (m_{Lit}). Mathematically it can be defined as

$$\text{MA} = \left| \frac{m_{\text{Lit}}}{m_{\text{Exp}}} - 1 \right| \quad (1.7)$$

and is usually given on the order of parts per million (ppm).

It should be noted that these parameters may be defined differently in the literature [6, 7]. The specification parameters used in this work are consistent with IUPAC definitions [8].

Nowadays, ToF mass spectrometry applications are the basis of a number of analytical

techniques, including molecular dynamics studies using velocity map imaging, or surface analysis in imaging mass spectrometry. Both concepts will be introduced, and the relation between each of them discussed later in this chapter.

1.2 Ionisation Techniques for Surface Analysis

A key challenge for mass spectrometry in surface analysis applications is to transfer the sample molecules from the solid sample surface into the gas phase, whilst simultaneously ionising the ablating particles. Ideally, this process should avoid fragmentation or clustering in a way that the parent ion peaks can be identified in the collected spectrum. Furthermore, the process should be compatible with a large mass range and analyte types, so that phospholipids in the low mass range (≤ 1000 Da) may be analysed at the same time as proteins in the kDa mass domain. In the following, two ionisation techniques will be introduced, which may be applied to imaging mass spectrometry.

1.2.1 Secondary Ion Mass Spectrometry

In secondary ion mass spectrometry (SIMS), the impact of a primary ion beam of several kiloelectronvolts of kinetic energy is used to eject secondary particles, such as neutrals, charged analytes, electrons or photons from a solid surface [9, 10]. The ablation and particle emission is usually described as a sputtering process (see Figure 1.2): The primary ions impact on the surface and penetrate into the sample, where their kinetic energy is absorbed due to collisions with atoms and molecules in the material. Thereby, a collision cascade within the sample is triggered. If this induced motion reaches an analyte located near the sample surface, sufficient momentum may be transferred to cause cleavage and the particle is emitted from the surface [10].

For mass spectrometric applications the ion beam is usually operated with an ion flux of about $<10^{13}$ impacts per square centimetre. Thereby, erosion of the sample is avoided, whilst larger mass fragments are observed. However, the applicable mass range for imaging

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Figure 1.2: Schematic illustration of the SIMS process. The ion impact induces a collision cascade in the sample (1-3) which triggers the emission of a secondary particle from the surface of the sample (4). The figure was taken from Reference [13].

mass spectrometry using static SIMS is limited to molecules with masses not exceeding 1000 Da [11, 12]. Heavier molecules are usually fragmented upon ion impact.

The sample area affected by the impact of a single primary ion depends not only on the kinetic energy, but also on the kind of the particle in the primary beam. The damage caused by the impact of a 3 keV Ar^+ particle onto a silica surface was found to be 25 nm in diameter, using a scanning tunnelling microscope analysis [14, 15]. Other primary ions employed for SIMS include either small ions, for instance Ga^+ , In^+ , Au^+ , Ar^+ and Cs^+ or more recently ion clusters such as Au_n^+ , SF_5^+ , Bi_n^{m+} and C_{60}^+ [16]. A comparison of a typical particle impact of a small ion (Ga^+) and an ion cluster (C_{60}^+) on a silver surface ($10 \times 10 \times 10 \text{ nm}^3$ sample, containing 612,000 atoms) is presented in Figure 1.3 [17]. For the simulation, both particle energies were set to 15 keV. The time sequence obtained for the small ion beam shows that the particle penetrates quite far into the silver surface, causing limited damage to the sample. Only a few secondary particles are released from the surface. In contrast to this, the impact of the buckminsterfullerene causes significantly more damage to the upper layers of the sample. The number of emitted secondary particles is 15 times greater than that from the Ga^+ beam. The reason for this is that the kinetic energy in the buckminsterfullerene is distributed over the 60 carbon atoms, resulting in an energy of 250 eV for each carbon atom. This energy exceeds the C-C bond strength

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Figure 1.3: Comparison of the temporal evolution of a collision of a Ga^+ ion and a C_{60} fullerene ion cluster at 15 keV kinetic energy with a $\text{Ag}\{111\}$ surface at normal incidence. The layers in the silver material are coloured so that the displacement becomes more obvious. The figure was adapted from simulation presented by Postawa and coworkers [17].

(~ 5 eV) of the C_{60} so that the fullerene collapses upon impact, releasing 60 independent 250 eV carbon atoms, which cause a collision cascade in the surface layers. As a result, more ions are ejected than with the small ion beam. Furthermore, in a number of studies it has also been observed that the ion yield for masses exceeding 1000 Da was somewhat increased using polyatomic primary ion beams [18–21]. Again, this was associated with the fact that the collision cascade is triggered by a number of impinging atoms, and motion is mainly induced in the surface region of the sample [22].

SIMS ionisation methods have been a crucial element in the evolution of imaging mass spectrometry over the last 20 years. It has demonstrated that very high spatial resolutions can be achieved in microprobe mode imaging, as ion beams can be focused down to sub micrometre diameters, allowing the imaging of single cells [23]. However, as discussed in the section above, the applicable mass range for most experimental procedures is limited to below 1000 Da [12]. One typical example can be found in the work from Ewing and coworkers, who demonstrating the capabilities of SIMS by imaging of cholesterol in

macrophage cells, employing a C₆₀ primary ion gun [24]. The primary ion beam diameter was focused down to 200 nm on the sample, which can be seen as the limiting factor in terms of the achievable spatial resolution. Among others, further studies applied the method to investigate biomolecule distributions in rat brains [25], to locate fatty acids in a zebra finch section [26], and to image distributions of di/triacylglycerols in human adipose tissue [27]. All these experiments demonstrate that SIMS as an ionisation method can be an effective tool for a wide range of bioimaging applications.

1.2.2 Laser Desorption and Ionisation Techniques

Alongside the success of the application of primary particle beams, laser induced desorption and ionisation processes have evolved as another major method of choice in mass spectrometry. The range of available methods starts with simple laser ablation techniques, using a short pulse at high photon flux to heat the electrons in the sample to a high temperature. Depending on the nature of the sample, this may lead to either a direct bond cleavage within the sample molecules or a Coulomb explosion, resulting in enhanced sample fragmentation, and a low parent ion yield. The method is mainly applied to elemental and isotope analysis of surface samples [28, 29].

The yield of non fragmented analyte molecules was increased using a laser desorption mass spectrometer (LD-MS), where a significantly lower laser fluence is applied to the sample [30]. The method was initially applied to organic sodium and potassium salts so that a mass spectrometric analysis resulted mainly in the molecular species plus an alkali cation [31]. By performing LD-MS on non-volatile biomolecules, Meuzelaar and coworkers were also able to show that it is possible to observe small biomolecules such as digitonin (1228 Da) [32]. However, despite these promising results, it is quite difficult to compare LD-MS experiments performed from different research groups, given that in order to ionise various organic compounds, experimental parameters including the laser wavelength, the pulse length, and the focusing properties, needed to be adjusted [33–36]. Hence, it was impossible to fully characterise the desorption and ionisation process [37–39].

In the mid 80s, a new concept addressing the limited applicability of the LD-MS was invented. Hillenkamp, Karas and Bachmann used LD-MS to investigate the influence of the laser wavelength on the ion formation of differently absorbing amino acids [40]. At an ionisation wavelength of 266 nm it was found that alanine has a desorption and ionisation threshold 10 times higher than that of the amino acid tryptophane. However, by mixing both compounds into one sample, both analyte peaks were observed at the desorption threshold of tryptophane. Here, tryptophane acted as a UV light absorbing matrix, assisting the molecular ion formation of the non-absorbing compound. The concept of matrix assisted laser desorption and ionisation (MALDI) was later demonstrated by the same group by collecting mass spectra containing the molecular ion peak of proteins such as bovine albumin (67000 Da) [41, 42]. Similar results to the work of Hillenkamp were found in the same year by Tanaka and coworkers, who showed the first intact desorption of protein and polymer molecules for mass signals of up to 10^5 Da using a suspension of metal particles (cobalt powder, 30 nm in diameter, in glycerol) as the absorbing matrix [43].

Today, for most applications, MALDI is the method of choice for the desorption and ionisation of intact macromolecules, such as carbohydrates, proteins, peptides and oligonucleotides. To perform conventional MALDI-MS, the sample molecules are mixed in a ratio of $1:10^3 - 10^5$ into a suitable, absorbing matrix, and the mixture is co-crystallised onto the sample plate of the applied MS instrument. The most common UV matrices applied today are either sinapinic acid (SA) [44], 2,5-dihydroxybenzoic acid (DHB) [45], or α -cyano-hydroxycinnamic acid (CHCA) [46]. Their corresponding molecular structures are shown in Figure 1.4. These aromatic components show a strong absorption around 300 nm to 360 nm, which can be accessed by common N₂ (337 nm) or Nd:Yag (355 nm) laser systems.

In principle, the role of the matrix used in the MALDI process is threefold: firstly, it allows the analyte molecules to be isolated from impurities in the dilution and recrystallisation process of the sample preparation stage; secondly, as the matrix shows

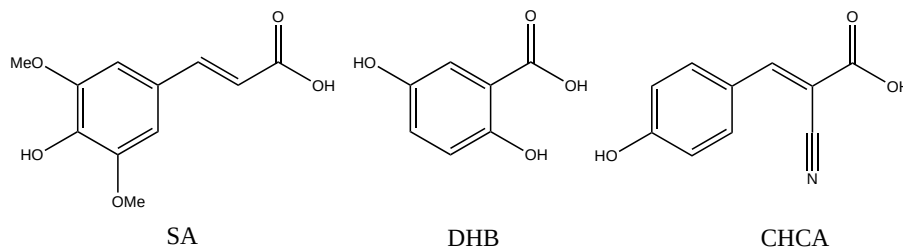


Figure 1.4: Chemical structures of the MALDI matrices sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), and α -cyano-hydroxycinnamic acid (CHCA).

a strong absorption band at the emission wavelength of the ionisation laser, the majority of the laser fluence is used for the electronic excitation of the matrix molecules; and finally, it allows for a non-fragmenting transition of the analyte into a gas phase, molecular ion. However, despite the fact that MALDI is one of the most popular ionisation methods used today, and that it has been around for more than 20 years, the details of the desorption and ionisation mechanism are still not fully understood, and investigations are ongoing. Extensive reviews on the processes involved can be found in References [47] and [48].

The desorption of a sample in MALDI MS is triggered by a short UV laser pulse (0.5 -10 ns) which leads to a quick thermal excitation of the sample molecules, followed by a disintegration process. It was found that this process is significantly dependent on the applied laser fluence. If the laser power is increased slowly, no ion signal is observed until the fluence exceeds a certain ablation threshold [47, 49].

The ionisation mechanism of the sample molecules can be described following two different approaches: either by the lucky survivor model or by the gas phase protonation theory.

The lucky survivor model was initially established and later modified by Karas, Schafer, and coworkers [48, 50]. Here, it is assumed that the analyte molecules are ionised in the sample preparation stage, when the analyte and the matrix molecules are mixed in solution. The charge gained is then retained in the crystallisation process, with the result that the matrix/analyte crystal mixture obtained consists of a variety of charged particles, such as protonated analyte molecules, protonated matrix molecules, and corresponding counter ions. In the desorption process, clusters of matrix and analyte ions are formed, which may

have various charge distributions, depending on the particles contained. The charge in the clusters, may be redistributed and neutralised in the expanding ion plume. However, a number of particles will remain charged after complete vaporisation. These particles (lucky survivors), which are mainly protonated or deprotonated matrix and analyte molecules, may then be analysed in the mass spectrometer.

The lucky survivor model is backed up by the work of Krüger and coworkers, who added a pH-indicating dye to the analyte/matrix mixture to measure the degree of protonation prior to, and after, crystallisation [51]. For both phases the same pH value was determined, indicating that analyte and matrix ions are present in the crystalline sample before irradiation of the laser.

The second model, the gas phase protonation theory, is based on the idea of transferring energy between the matrix molecules in a way that the ionisation threshold energy may be exceeded. Thereby, the energy of the incident UV laser is insufficient to ionise the matrix molecules directly, and multi-photon ionisation processes are unlikely to occur [52]. Therefore, the required energy may be gained by a pooling mechanism. This process was initially considered by Ehring and coworkers [53] and later described by Knochenmuss *et al.* [54].

Equations 1.8 to 1.11 below show a number of possible energy transfer reactions occurring after ablation of the sample. In the first step, a matrix molecule in a ground state, S_0 , absorbs the energy of the incident radiation, and is excited into the S_1 state (1.8). The energy gained may then be transferred to a neighbouring matrix molecule, which is either in the ground state (1.9), or already in an excited state (1.10). In the latter case, higher excited states S_n may be accessed. However, the internal energy of the excited states S_n may still be insufficient to allow a direct ionisation of the matrix molecules, so that a further energy *pooling* collision with an excited matrix molecule is required (1.11), before an ionised matrix molecule, m^+ , and a proton are obtained. This proton may then be attached to an analyte ion while the ablated cluster expands.





This theory is backed up by studies undertaken by Knochenmuss and Selz [55], who noticed that excitons in solid DHB molecules can move across the sample, and annihilation or pooling reactions can occur. A detailed description of the dynamics of the process can be found in Reference [54].

In order to address which of the two postulated ionisation mechanisms is actually occurring in a MALDI process, Jaskolla and Karas recently applied deuterated esters as MALDI matrices [56]. In the case that deuterated analyte ions are observed in the mass spectra, the gas phase protonation model would be applicable, but in the case that protonated analyte molecules are detected, the prediction according to the lucky survivor model would be more appropriate. In the experiment, both compounds were found. However, the ratio of the protonated and deuterated analytes depended on the experimental parameters. For example, an increase in laser fluence led to more deuterated analyte molecules (gas phase protonation model), while a protonation of the matrix solution prior to crystallisation led to more protonated ions (lucky survivor model). These results indicated that both models can be used to describe the ionisation process occurring in a MALDI analysis depending on the experimental conditions used.

1.3 Ion Imaging

The idea of ion imaging was initiated by the work of Chandler and Houston in late 1980s [57], who used a three electrode linear ToF spectrometer in order to investigate the photodissociation of methyl iodide. They passed a skimmed molecular beam through a central hole in the first electrode (repeller), which was then intersected by a 266 nm laser beam to dissociate the parent molecule, and a 330 nm laser pulse, which ionised the CH₃ photofrag-

ments state selectively using a (2+1) REMPI (resonance enhanced multi photon ionisation) process. The resulting ions were accelerated in an electric field, expanding between the repeller and the following two grounded grid electrodes, to be detected on a position sensitive detector, which consisted of a pair of microchannel plates (MCP) and a phosphor screen. The ion image observed for the CH_3 fragment ions showed the shape of the expanding ion cloud, which reflected the nature of the photodissociation process.

Even though the experiments from Chandler and Houston successfully demonstrated the ability to map a photodissociation processes, a number of problems limited the obtainable velocity resolution [58]. For example, ions were dissociated in a rather large section of the expanding molecular beam, and due to the different origin of the fragments, a blurring was introduced in the ion images. Another limiting factor were the properties of the employed ion optics: the electric field lines within the optics were, due to the grid electrodes, completely parallel to each other. Hence, all the ions formed were accelerated at the same rate towards the detector, but they were not focused as a function of their kinetic energy.

This issue was addressed almost a decade later by Eppink and Parker, who replaced the two grids with open electrodes [59]. Based on the work from Davisson and Calbick in the 1930s [60, 61], it is known that the interaction of charged particles with electrodes is similar to that of light particles with optical elements. For example, a round aperture electrode behaves similarly to a spherical lens and, depending on the applied potential, it is either divergent or convergent. Eppink and Parker made use of this principle and applied an electrostatic field to the first two electrodes, while the last one remained grounded to ensure a field free flight tube. The extraction field ratio between the repeller and the extractor plates (second electrode) was adjusted so that the final detection coordinate for the fragment ions was dependent on their transverse velocity. This corresponded to a projection of the back focal plane, in this case the velocity mapping plane, onto the detector. An illustration of the ion trajectories for velocity map imaging (VMI) is presented in Figure 1.5. Here, it is also obvious that ions of different origin in the finite interaction region were mapped onto the same position, so that the velocity mapping resolution was

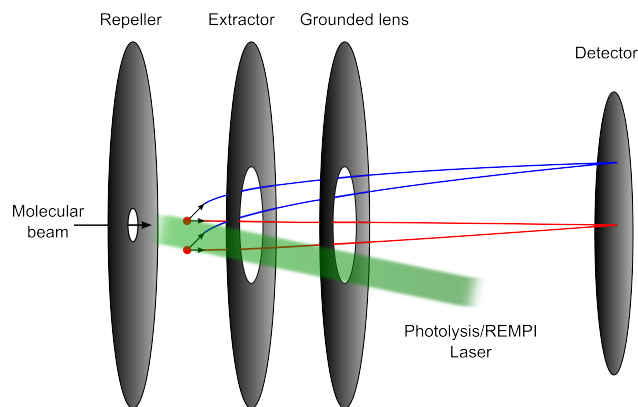


Figure 1.5: Illustration of a typical velocity map imaging experiment. After photodissociation and ionisation the emitted ions are mapped as a function of their initial velocity onto the two dimensional detector.

improved in comparison to the experiment of Chandler and Houston. At the same time, due to the ion optic assembly and the field free flight tube, it was possible to separate the photodissociation products as a function of their m/z values. Thus, due to the optimisation for velocity mapping conditions, the mass resolution was found to be reduced in comparison to a conventional ToF mass spectrometer.

The idea of studying photodissociation processes with velocity map imaging is based on the conservation of energy, i.e. the total energy of the process must be identical before and after the photodissociation process. However, the distribution of energy changes within the process. For the complete energetic description of a photodissociation process, a number of factors need to be considered. These are: the energy of the photons, $h\nu$, the internal (E_{Int}) and kinetic energy (E_{Kin}) of the particles involved, and the dissociation energy of the parent molecule D_0 . For a typical two fragment dissociation process ($\text{AB} + h\nu \rightarrow \text{A} + \text{B}$) the energy balance can be written as follows:

$$E_{\text{Int}}(\text{AB}) + E_{\text{Kin}}(\text{AB}) + h\nu - D_0 = E_{\text{Int}}(\text{A}) + E_{\text{Int}}(\text{B}) + E_{\text{Kin}}(\text{A}) + E_{\text{Kin}}(\text{B}). \quad (1.12)$$

The internal energy of the particles involved is distributed over electronic, vibrational and rotational states. However, if the photodissociation occurs in an adiabatic molecular beam expansion, such as in a typical velocity map imaging experiment, the parent molecules are cooled to populate only a few low-lying levels of their internal energy components.

Therefore, the internal energy of the parents can often be neglected in the above energy balance. Furthermore, if the molecular beam is skimmed before entering the interaction region, the transverse velocity component of the parent molecule can be neglected as well. The origin of the fragment ions can then be treated as point source. Hence, the kinetic energy of a fragment ion can be measured by velocity mapping the expanding ion cloud (Newton sphere). Thereby, the investigated particle is usually ionised using a state selective REMPI process. As a result, the internal energy of the co-fragment can thus be calculated.

By scanning the dissociation wavelength, velocity map imaging allows the energy barriers for the dissociation channels to be measured directly. Once the excitation energy of the laser is high enough to populate a higher lying excited state, a new dissociation channel leading to the probed fragment may be opened, and its threshold energy may be calculated.

In conventional velocity map imaging the Newton sphere is collected by integrating the ion image over the entire ToF peak of the photofragment. In this way, a two dimensional projection of the three dimensional ion distribution is obtained. However, to determine the kinetic energy release, it is necessary to measure only the velocity distribution of the ions which dissociate perpendicular to the ToF axis. Therefore, the fragment velocity either needs to be extracted from the ion image *via* an Abel transformation [62], or the entire Newton sphere needs to be reconstructed using programs such as the pBASEX software or the onion peeling algorithm [63, 64].

As discussed above, the size of the expanding Newton sphere contains information about the height of energy barriers for the dissociation channels and the resulting energetic levels populated in the photofragments. At the same time, details on the photoexcitation step leading to bond cleavage can be extracted from the angular distribution of the photofragments [65, 66]. For an electric dipole transition the transition dipole moment of the parent molecule lies preferentially parallel to the electric vector of the dissociation laser [67]. As a result of this effect, an anisotropic Newton sphere may be observed. For a diatomic molecule, the transition dipole moment can either be oriented parallel or per-

pendicular to the molecular axis. The angle between the electric vector of the light and the dissociation direction is defined as θ . In the case that the dissociation occurs *via* a parallel orientation of transition dipole moment and molecular axis, the obtained velocity mapped image shows a distribution which can be described by a $\cos^2 \theta$ function. For a perpendicular orientation, a $\sin^2 \theta$ distribution is obtained.

Velocity map imaging has evolved to an experimental technique which allows the studying of simple photodissociation processes [68], chemical reactions [69, 70], and inelastic scattering processes [71, 72]. In Chapter 2 of this thesis, the ion optic design of a velocity map imaging system is adapted to imaging mass spectrometry. The technique of velocity map imaging is also applied in Chapter 5, in which the imaging performance of a new scintillator screen is explored.

1.4 Imaging Mass Spectrometry

1.4.1 Advantages of Imaging Mass Spectrometry

As already discussed, imaging mass spectrometry is an effective tool which allows the experimentalist to create a molecular map of a sample surface with high chemical specificity. In contrast to other surface analysis methods, IMS is able to access complex molecular mixtures over a wide mass range in a single experiment. Hence, it can be seen as an alternative approach to staining or fluorescence microscopy [73]. While immunological analysis methods are limited to a certain set of available antibodies, IMS has the capability to analyse the complete chemical composition of a sample directly - it can thus be seen as a label free analysis [74]. No prior knowledge about the target analyte is required, as it is in fluorescence spectroscopy. Due to these advantages, IMS plays a key role in drug development [75], in the analysis of lipid biomarkers in cancer research [76], as well as in fields such as neuroscience [77] and pathology [78].

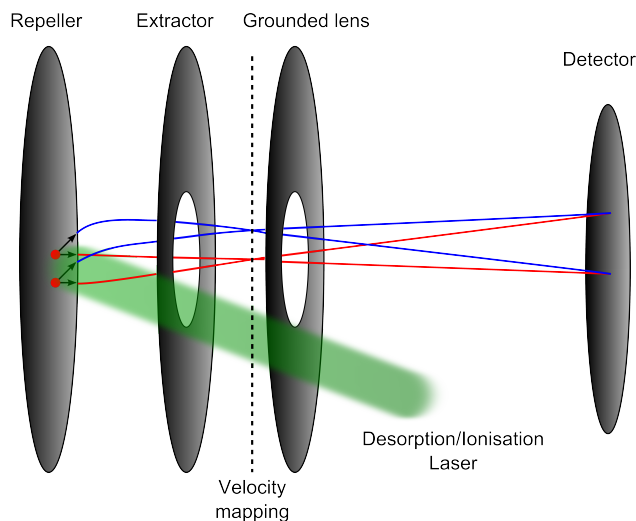


Figure 1.6: Illustration of the ion trajectories of two ions coming off a surface in a linear microscope mode imaging mass spectrometry experiment. The ions pass through the velocity mapping plane and are spatially mapped onto a two dimensional detector. A magnified, inverted image of the particle distribution on the sample surface is obtained.

1.4.2 Microscope Mode IMS

The purpose of an optical microscope is to magnify a small section of a sample, allowing the user to observe microscopic structures in more detail. Following this idea, microscope mode imaging mass spectrometry is based on the principles of a linear light microscope, with the difference being that charged particles travel through an electrostatic lens assembly, which is an analogue to the magnifying optics of a conventional light microscope.

As illustrated in Figure 1.6, in a microscope mode IMS experiment sample molecules are desorbed and ionised across the entire illuminated area, following one of the ionisation mechanisms discussed in Section 1.2. By adjusting the applied voltages of the electrostatic lens to those for spatial mapping conditions, the ejected ions are extracted, separated by their m/z ratio, and projected as a magnified image onto a two dimensional particle detector. In Figure 1.6, it can be seen that the ions pass through the velocity mapping plane, which shows the close relationship between VMI and IMS ion imaging experiments.

The spatial resolution obtained in microscope mode depends on three parameters: the quality of the defocused light or particle beam; the spatial focusing capabilities of

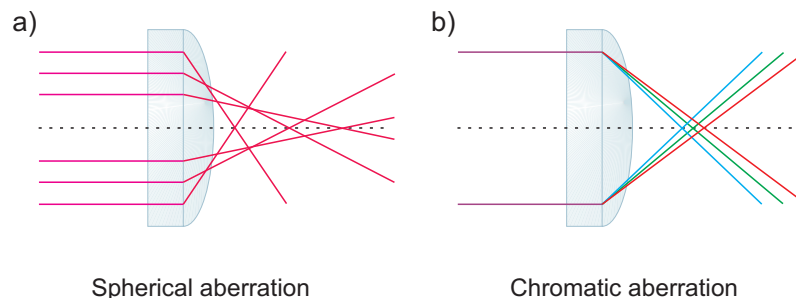


Figure 1.7: *a)* Illustration of spherical aberration for identical particles, travelling parallel to each other, at different distances to the optical axis of the system. *b)* Illustration of chromatic aberrations for particles of various energies travelling on identical trajectories parallel to the molecular axis of the system.

the ion optics; and the intrinsic spatial resolution of the detector assembly. The former parameter is essential for IMS experiments, as it needs to be ensured that the ionisation and desorption beam has a top-hat intensity profile across the entire field-of-view. An uneven profile would result in hot spots, from which ions with higher kinetic energy are ejected. These cause blurring in the image and make a chemical analysis of the sample difficult, as the sensitivity is inconsistent across the sample. A way to minimise this problem for laser desorption experiments is to use optical fibres to even out the beam profile [79]. However, the larger the field-of-view becomes, the greater the laser power that needs to be deposited onto the sample. Therefore, fibres with a large core are required.

Once the ions are desorbed and accelerated in the electric field, spherical and chromatic aberrations affect the spatial mapping properties of the spectrometer. Both effects are illustrated in Figure 1.7. In the case of spherical aberrations, the “refractive index” of the electrostatic lens is dependent on the distance to the optical axis. Hence, an electrostatic deflection of two identical particles travelling at different distances parallel to the optical axis of the system results in two different focal points - the further outside a particle passes through the lens, the shorter is the focal length. For IMS applications, this means that the larger the field-of-view of the spectrometer becomes, the more significant is the effect of spherical aberrations, which reduce the spatial resolution of the system. Chromatic aberrations can be described by the fact that the refraction of the particles is dependent on their kinetic energy. A high energy particle has a longer focal length than a low energy

one, which may cause a blurring effect in the observed ion image.

Furthermore, for high resolution IMS, it is essential that the employed detection system has a sufficiently high spatial resolution. These systems usually consist of a double chevron MCP assembly coupled either *via* a scintillator to a pixel based imaging sensor or directly to an electron sensitive imaging device. While the achievable spatial resolution is most likely not limited by the pore pitch of the MCPs, it may be somewhat reduced by the pixel size of the imaging sensors. Further properties of commonly used imaging detectors will be discussed in Section 1.5.

A second key parameter defining the performance of a microscope IMS instrument is the achievable ToF resolution. In comparison to conventional mass spectrometry systems, it is more challenging to optimise this parameter, as the spatial information needs to be maintained in the mass analyser of the instrument. Therefore, systems such as quadrupole [80], quadrupole ion traps [81], orbit traps [82, 83] and ion cyclotron resonance mass analysers [84–86] are not compatible with microscope mode IMS. Furthermore, to improve the resolution, conventional ToF mass spectrometry may operate with a delayed ion extraction method [87, 88], which is not suitable for microscope IMS, as the spatial resolution is reduced while the ion cloud expands. The effect will be further discussed in Section 3.1. Another approach in ToF-MS involves a reflectron to correct the kinetic energy spread of ions coming off a surface. However, until now no-one has introduced a reflectron into a microscope IMS instrument, as it is complicated to maintain the spatial mapping condition over a wider mass range [89, 90]. This only leaves ToF analysis in combination with electrostatic and magnetic sector analysers as compatible techniques [91].

The idea of constructing a microscope mode imaging mass spectrometer goes back to the early 1960s. Castaing and Slodzian sent a charged particle beam onto an Al-Mg-Si crude foundry leading to a secondary ion emission process [92]. Emitted metallic ions were extracted using a conventional ion optic and spatially mapped into a magnetic sector. Due to the properties of the magnetic sector field, the ion path was turned by 90° and ions of different masses were spatially separated focused onto a movable aperture. By adjusting

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Figure 1.8: Illustration of the first microscope mode imaging mass spectrometer (left) and an obtained ion image of a crude foundry (right). Both figures were adapted from Reference [92].

the position of the aperture, each of the three different metal ions could be selected to be spatial mapped onto a phosphorescent screen. A diagram of the spectrometer and a resultant ion image taken from the original publication are presented in Figure 1.8.

Due to the magnetic sector, the mass analyser instrument of Castaing and Slodzian was limited to the detection of one ion species at a time. Furthermore, the system had the disadvantage that if the aperture width was not small enough, the image obtained would be blurred, as multiple ion images belonging to different particle masses overlapped on the detector. This problem was circumvented by Schueler who proposed a microscope mode imaging mass spectrometer (TRIFT analyser) consisting of an extraction optic and three electrostatic analysers, which were optimised to reduce chromatic aberrations while optimising the mass resolution of the instrument [93]. The system was designed for operation with the SIMS ionisation method and had a field-of-view of 300 μm . A spatial resolution of 1-5 μm , and a mass resolution $m/\Delta m$ of ≥ 7500 were reported.

In 2004, Luxembourg and Heeren introduced the name microscope mode imaging mass spectrometry by modifying Schueler's triple focusing mass spectrometer so that it could

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Figure 1.9: MULTUM imaging mass spectrometer. The ions are cycled through the four electrostatic analysers in order to gain a higher mass resolution before being ejected onto the imaging detector. The illustration was taken from Reference [99].

operate with the soft ionisation method MALDI [94]. To prove the performance and the concept of the experiment, Luxembourg *et al.* imaged grid structures of the applied DHB matrix, the peptide hormone insulin and the neuropeptide *Substance P*. A spatial resolution of 4 μm for a 200 μm diameter wide field-of-view was reported. Following this work, this instrument has demonstrated its outstanding performance in a number of experimental studies: for example, a whole rat cross section was imaged to localise a pharmaceutical metabolite [95]. Furthermore, fast imaging detectors such as a delay line detector [96] or CMOS based imaging sensors [97, 98] were successfully combined with the mass spectrometer to allow multi-mass imaging.

Another mass spectrometer concept based on electrostatic analysers is the MULTUM II instrument, illustrated in Figure 1.9 [99, 100]. In this instrument, four electrostatic analysers are arranged so that ions can be cycled through the assembly in order to gain a higher ToF resolution. According to the *perfect focusing condition* the spatial image is maintained and, once the ions are released, they are projected onto the imaging detector [101]. Experiments have shown that, if the instrument was operated in linear mode, meaning without the use of the electrostatic analysers, a spatial resolution of 3 μm for a field-of-view 400 μm diameter was found. Once the ions were cycled, the spatial resolution suffered from distortions with increasing number of turns. However, the ToF resolution

was improved to $m/\Delta m = 130000$ after 500 turns [102].

The experiments performed with the triple focusing and with the MULTUM mass spectrometer have demonstrated that it is possible to achieve a high spatial resolution and a good ToF resolution ($t/2\Delta t \geq 1000$) at the same time. However, a key challenge in microscope mode IMS is to combine the spectrometer with a fast imaging detector to access the gained information. An overview of the applicable fast detection systems will be provided in Section 1.5.

The key argument for performing surface analysis experiments in microscope mode IMS is the high throughput, as large sections of a sample can be imaged within a single experiment. In this way the total acquisition time is significantly lower than in microprobe mode. Disadvantages are that fast imaging detectors are required, which may be more expensive than standard mass spectrometry detectors, and that the employed mass analyser are limited to ToF resolutions of $t/2\Delta t \leq 10^3$. Hence, studies involving the analysis of isotope distributions may not be possible.

1.4.3 Microprobe Mode IMS

The principle of microprobe mode imaging mass spectrometry is similar to that of any other profiling technique: an ion or laser beam is focused onto a small subsection of a sample surface and the ejected molecules are analysed according to their mass to charge ratio. The resulting mass spectrum is stored and the sample plate is moved to the next position to be analysed. By repeating this procedure in a raster pattern across the region of interest, a complete data set of the molecular constitution of the sample is accumulated. The corresponding ion image can be reconstructed by plotting the mass spectral intensity as a function of the sample position.

Microprobe mode IMS is a technically relatively straightforward approach which can be performed, to a certain extent, in most MALDI mass spectrometers available today. It has the advantage that it can be combined with any available mass analyser without limiting the obtained spatial resolution. However, this resolution does depend on the size

of the ionised region, as each experimental step corresponds to a single pixel in the final ion image: while the spatial information from within the ionised region is lost, the spatial resolution of the total image is increased by decreasing the ionisation volume and reducing the distance of the raster steps. Most MALDI instruments employ preset laser spot sizes ranging between 25 to 200 μm in diameter [103, 104]. These spot diameters are usually well suited for the analysis of simple protein distribution in tissue samples, for instance in a cross section of a mouse brain [105].

In order to compensate for the ionisation area limited resolution, methods such as oversampling have been introduced [106]. With this technique, the spatial resolution of the experiments operated with an ionisation laser of several hundred micron diameter could be improved down to 25 μm resolution [107]. In another approach, a MALDI laser was focused down to sub-micrometre spot sizes using a back side irradiation setup, to enable the direct imaging of single cells [108]. The drawback of the method is that the time taken to collect an image of a given size is increased.

While microprobe mode SIMS experiments have already been in use for a number of years, IMS has experienced a swift development, after Caprioli introduced MALDI imaging to the community [109]. While in 1998 only 69 publication dealt with the concept of imaging mass spectrometry, this number increased to 828 publications in 2013 [110]. The milestones in the development of IMS were recently reviewed by Chughtai and Heeren [111].

One of the most impressive applications using microprobe IMS was performed by Caprioli and coworkers, who studied drug, protein and metabolites distributions in a whole-body mouse section [112]. One of the ion images obtained is reprinted in Figure 1.10. By being capable of screening tissue sections of the size shown, IMS should become a promising technology to accelerate the development of drugs by providing a overall picture of where, in a tissue section, individual drugs and metabolites are distributed. Furthermore, protein and metabolite changes as a function of the drug absorption can be monitored to provide a better understanding of the involved molecular processes.

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Figure 1.10: Whole-body IMS protein analysis of rat tissue section: *a*) Shows an optical image of a rat sagittal tissue section placed on four gold MALDI target plates. *b*) Ion image overlay of organ signals: brain, green m/z 21952; thymus, light blue m/z 6893; thoracic cavity, purple m/z 7921; liver, blue m/z 14321; kidney cortex, yellow m/z 4643; cecum wall, cyan m/z 5155; testis, orange m/z 14400; and muscle, red m/z 11836 (B). The white scaling bar corresponds to a distance of 1 cm on the sample. The figure was taken from Reference [112].

1.5 Detection Systems for Ion Imaging

The fast and efficient detection of charged particles is a key element of a number of applications in science, including particle physics experiments, electron microscopy, velocity map imaging and imaging mass spectrometry. For the latter two, it is essential to convert (with a high precision) the ToF and the position information of arriving ions into a detectable ion image. For this purpose, typically one or more microchannel plates are applied to convert the signal of incoming ions into a burst of electrons. A detailed description about the properties and the operation of MCP detectors can be found in Section 6.1.2.

Once the electrons are released from the back MCP, they can be accelerated onto various detection systems. These need to fulfil a number of requirements to be ideally suited for ion imaging application. In the first instance, the detection efficiency of electrons needs to be sufficiently high such that all ion events are recorded and the obtained signal level is not reduced further. At the same time, the position of the impinging electrons needs to be detected accurately, in order to avoid a loss in spatial resolution caused by distortions or blurring effects. Another key role, especially for imaging mass spectrometry applications,

can be associated with the time precision of the system. Ideally, this should not be lower than the ion arrival time separation achieved using the applied ion optic system and the mass analyser. Furthermore, if multiple ion peaks need to be recorded consecutively, it is essential that the detection device is capable of detecting and registering multiple events per detection position within a single ToF cycle. In the case that the experiment performed operates only with a few events per experimental cycle, it is also beneficial if the detection system can be operated with a high repetition rate, which allows the overall collection time to be reduced.

In the following subsections, three detection systems will be introduced using either a delay line or an imaging sensor based approach to detect the spatial and ToF information. The advantages and disadvantage of these systems will be discussed and a brief overview of their application to ion imaging will be provided. A more detailed description about the various detection systems can be found in recent reviews from Vallance, Brouard and coworkers [113], and Heeren and coworkers [114].

1.5.1 Analog Readout - Delay Line Detectors

Delay line anodes can be described as simple, position and time sensitive detection systems, which are mounted directly behind an MCP detector. Based on the theory of delay-line techniques presented by Lampton in 1987 [115], Eland and Pearson developed the first spatially resolved delay line detector in 1990 [116, 117]. It consisted of twenty concentric conductive rings, each fed into an integrated circuit delay line chip. With a specific delay, depending on the ring number, two output signals could be observed and correlated, allowing the position and the arrival time of a detected event to be determined.

Today, delay line anodes are mainly built in two different ways; either as a two dimensional, orthogonal cross wire detector, such as that presented in Figure 1.11, or as hexanode delay line system, in which three groups of two conductive wires are mounted at a 120° angle with respect to each other [118, 119]. The principle of operation of both systems differs only slightly from the one presented by Eland and Pearson. In the case that an

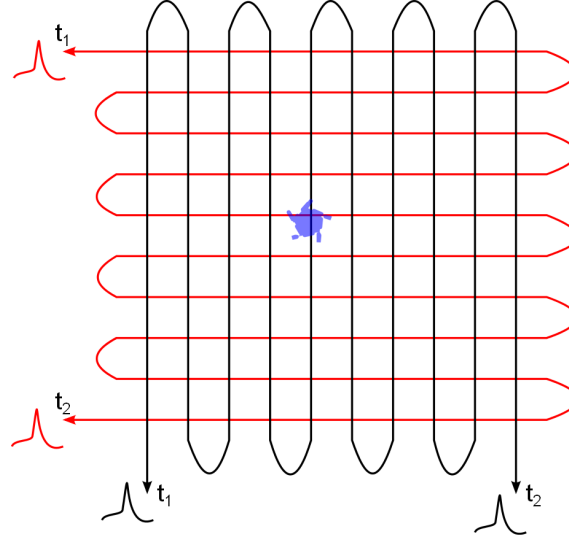


Figure 1.11: Schematic illustration of the operation principle of a crossed wire delay line detector. One ion event triggers the detection of four time dependent signals. By correlating the obtained signals, the arrival time and the impact position of the event can be reconstructed.

impinging electron cloud is detected on one position on the delay line, a signal is induced in the conductive wire travelling with the same speed towards both ends of the detector. By monitoring the arrival time of the two signals at both ends of the line, it is possible to reconstruct the spatial coordinate of the event, as the delay between the two signals correlates directly to the distance travelled. Additionally, the actual arrival time can be determined by calculating the time it takes for a signal to propagate from the position of the impact to one end of the delay line, and subtracting this value from the time the signal was measured at the output. The operation of delay line detectors requires fast electronics in order to read out the pulses at the end of the delay line accurately. Typically, these pulses are preamplified, passed through a constant fraction discriminator, and then time stamped with a time-to-digital converter (TDC). The achievable time resolution is on the order of 25 ps, and a typical dead time of 100 ns can be assumed to reset the electronics [113]. However, by replacing the TDC with an ADC (analog-to-digital converter) the dead time can be reduced to below 1.5 ns [120].

The use of delay line anodes as imaging detectors becomes difficult for applications in which multiple events are observed simultaneously, since the correlation between the

signals arriving at both ends of the delay line becomes impossible. Therefore, by using the crossed delay line system, employing two individual anodes, up to two events can be detected simultaneously, as for each event four correlated signals can be observed. By using the hexanode approach, this number can be increased to up to three or four simultaneous events. Thus, the total number of recorded events depends on the impact positions on the lines [118].

One big advantage of delay line anodes is that they can be operated in the kHz range or higher, which makes them ideally suited to imaging applications which operate at low count, but high repetition rates. Typical examples are ion-ion and ion-electron coincidence experiments [121, 122]. The application of delay line detectors to microscope mode imaging mass spectrometry was so far only reported in a few publications. For example, Toyoda and Awazu coupled a delay line detector in combination with an ADC readout to a linear imaging mass spectrometer, allowing spatial imaging with a $230\text{ }\mu\text{m}$ resolution on the detector screen [123]. The peaks observed in the ToF spectrum were limited by the ion optic resolution to a FWHM value of 24 ns. For these measurements, a frame rate of 42 Mfps was achieved. Similar results were obtained in initial test experiments by Froesch and coworkers, who coupled a hexanode delay line detector to the triple focusing imaging mass spectrometer of Schueler and Heeren [93, 96]. In later work, Heeren and coworkers used a SIMS IMS approach to collect ion images including micrometre scale features from a tissue sample [124]. The spatial resolution was determined to be $4\text{ }\mu\text{m}$ with a pixel resolution of 250 nm. Following these studies it can be concluded that IMS with delay line detectors is possible. No loss in spatial resolution was observed in comparison to conventional detection systems. However, ion count rates and saturation effects were not discussed in these publications, and this still need to be explored.

1.5.2 CCD Cameras

Another approach to collect ion images is to convert the electrons emitted from the MCP into light which can be detected by an imaging sensor. For this purpose, a scintillator screen

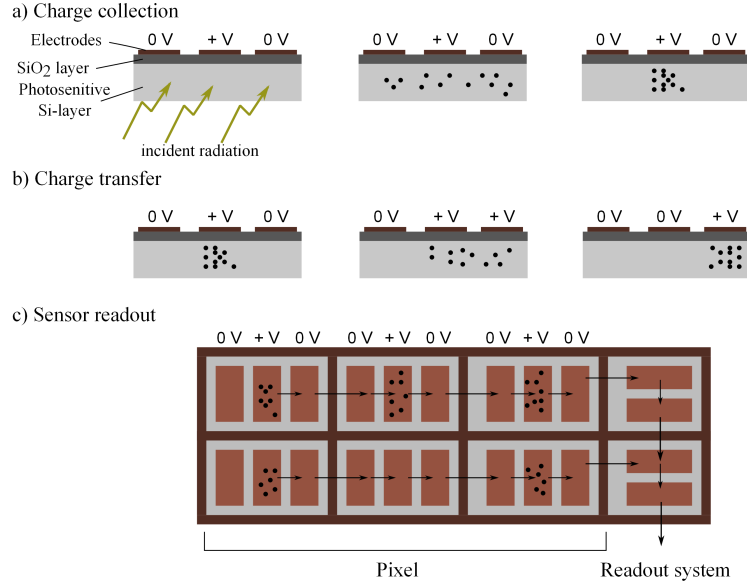


Figure 1.12: Schematic illustration of the operation principle of a simple CCD camera: *a)* charge collection within a pixel; *b)* charge transfer within a single pixel; *c)* sensor readout into a pixel array, located on the outside of the CCD chip.

is mounted behind the MCP, onto which the amplified electrons are accelerated. The screen absorbs the kinetic energy of the impinging particles, which triggers an emission of photons at the impact position. As the scintillator screen is deposited on a transparent substrate, the light flash can be observed from the back of the screen. Typical scintillator screens employed in imaging detectors are either P47 ($\text{Y}_2\text{SiO}_5\text{:Ce}$) or P43 ($\text{Gd}_2\text{O}_2\text{S:Tb}$). Both of these show a high electron-to-photon conversion efficiency and a suitable spatial resolution. However, experiments requiring a high time resolution may suffer from the long emission lifetime which extends over 70 ns for the P47 screen and over a few milliseconds for the P43 screen [125]. A detailed description of the processes involved can be found in Chapter 5.

The emitted light flashes may then be collected using a CCD (charge coupled device) image sensor. These are relatively simple devices, originally developed as memory for data storage [126], but also converted to operate as imaging sensors [127]. A simplified illustration of the operation of a CCD imaging sensor is presented in Figure 1.12. In *a)* the layout of a single pixel is illustrated. If the pixel is exposed to light, electron-hole pairs are created in the photosensitive p-doped silica layer. The resulting electrons are then stored by a capacitor within the pixel until the entire chip is read out. Panels *b)* and *c)* in

Figure 1.12 illustrate the read out process of a single pixel and a section of a CCD chip. Here, the charge, which has been collected in *a*), is moved by gating the electrodes within each pixel. In this way, it is possible to transfer the collected charge in each row, pixel-by-pixel, along the entire sensor. In each step, the charge entering the readout pixel at the end of the row is amplified and converted into a voltage, which then can be monitored by the readout electronics. After all the pixels in the CCD columns are read out, the CCD chip can record the next image and the procedure is repeated.

Following the description of the CCD charge collection and transfer process, it is obvious that these kinds of chips have a number of limitations for applications such as imaging mass spectrometry. For example, with conventional CCD cameras it is not possible to collect multiple ToF peaks independently within the ToF. The detector needs to be gated over one preselected time interval within the entire spectrum. Hence, multi-mass imaging becomes impossible. Furthermore, the readout of the entire CCD chip is inherently slow, so that frame rates of no higher than a few tens per second may be achieved.

In order to overcome the limitations a set of memory registers, in which the collected charge is transferred at a set time, may be integrated into the CCD pixels [128]. The actual frame rate of such a camera depends only on the time it takes for the charge to be moved from one register to another, which is usually on the order of a few nanoseconds. Once all registers are filled up, the camera needs to be read out, following the same principle as described above. Simulations predicting the specification of these kinds of detectors have indicated that by using the *in situ* storage system, a maximum frame rate of a few 100 Mfps may be achieved [129]. A drawback of this approach is that, by increasing the number of registers in a CCD pixel, the light sensitive area is reduced. This may be compensated for by increasing the actual pixel size. An example of a sensor with 100 registers per pixel operating at a frame rate of 1 Mfps can be found in the work of Etoh and coworkers [130]. Furthermore, by taking an excessive amount of frames, the size of the image files obtained increases enormously, so that data handling can be challenging. Another disadvantage in the application of these systems to ion imaging is that the time

when the collected charge is transferred into the registers needs to be preselected. This means that prior knowledge of the peak distribution in the ToF spectrum is required in order to synchronise the camera with the arrival time of the fragments of interest. One commercially available fast framing camera is the DALSA Zenith (Teledyne DALSA), which was recently applied by Brouard, Vallance and coworkers to both velocity map imaging and microscope mode imaging mass spectrometry studies [131, 132]. The camera system reported had a pixel resolution of 64×64 and 16 available registers. The highest frame rate achievable using this system was reported to be 100 Mfps, corresponding to a timing precision of 10 ns.

1.5.3 CMOS Cameras

More recently, a new class of time resolved CMOS (Complementary Metal Oxide Semiconductor) pixelated sensors was introduced to the field of ion imaging. The CMOS technology, which is similarly employed in microprocessors, static RAM, and other digital circuits, allows a more advanced sensor design with higher pixel-functionality in comparison to conventional CCD cameras [114]. Efficient signal treatment after radiation induced charge collection enables every single pixel to convert the collected charge to a voltage, amplify the signal, and digitise the obtained data. Further signal processing steps such as thresholding, noise reduction, and discrimination may be included. The drawback of functionalising the CMOS pixel results is an increased number of transistors, which need to be included in the pixel design. Hence, the photosensitive area per pixel may be reduced or the size of the pixel needs to be increased.

With the advantage that all essential camera functionalities can be placed on a single chip, CMOS based detectors are usually more flexible than standard CCD device. Two detectors specifically designed for particle imaging applications are the Timepix/Medipix sensor [133], originally developed at CERN, and the PImMS (Pixel Imaging Mass Spectrometry) sensor [134], developed by a collaboration of the STFC-Rutherford Appleton Laboratory and the University of Oxford. Although both sensors were fabricated in differ-

ent processes and their specifications differ, both detectors use a time stamping approach to record individual events. If the incident light on a single pixel exceeds a predetermined threshold value, the time of arrival or the number of events occurring in a preselected time window may be stored in the inbuilt memory. The pixel is read out together with the entire sensor at the end of each experimental cycle. As only actual events are recorded by the CMOS sensor, the size of the entire data set is reduced in comparison to images collected with a fast framing CCD camera. Furthermore, no prior knowledge about the ToF spectrum is required. The time resolution obtained depends on the speed of the internal clock of the sensor, while the number of “frames” depends on the size of the inbuilt counter; for instance a 12-bit counter corresponds to 4096 individual frames.

The Timepix sensor currently has an internal clock cycle speed of 10 ns, and a spatial resolution of 256×256 pixels, which may be increased by mounting multiple chips next to each other [114]. Each pixel can be operated in one of the three modes: (1) event counting mode, where each pixel counts the number of events; (2) ToF mode, where the occurring time of an single event is stored and read out at the end of the experimental cycle; (3) time over threshold mode, where the number of frames during which a detected event exceeds a preselected threshold value is measured. For ion imaging applications, the measurement of the arrival time of a particle is the most important mode. However, it should be noted that each pixel has only one register allowing only one event to be stored within an experimental cycle. This might be sufficient for experiments employing a low ion count rate, as the probability that one pixel gets hit multiple times is low. If the sensor is exposed to high ion count rates, it is less likely to detect all the ions arriving later in the ToF spectrum. To be applied to ion imaging studies, the Timepix sensor was configured to detect electrons directly from the MCP using a metal pad to transfer the charge. In this way time resolution limitations, which may be imposed by a long scintillation decay, were avoided.

Since its first presentation in 2009 the Timepix sensor has demonstrated its capabilities in a number of velocity map imaging and imaging mass spectrometry studies [135]. One of

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Figure 1.13: *a)* Shows a photograph of the PImMS sensor bonded to the sensor board. The picture was adapted from Reference [139]. In *b)* a photograph of the assembled PImMS camera is presented.

the first applications using this new generation imaging sensor was performed by Jungmann and coworkers, who employed Timepix to image the entire three dimensional Newton sphere distribution of NO^+ [136]. Later, the sensor was applied to imaging mass spectrometry experiments, comprising of studies imaging the spatial distribution of biomolecule arrays [98], analysing the molecular constitution of mouse testis tissue [137] and using SIMS ionisation methods to obtain high spatial resolution images [138].

In contrast to the Timepix sensor, the PImMS sensor can only be operated in ToF mode, and is designed as a light sensitive device. Hence, the camera needs to be coupled to a standard imaging detector, consisting of a stack of MCPs and a scintillator screen. The prototype camera comprises of an array of 72×72 pixels which can be operated at a clock speed of 12.5 ns over 4096 frames. Its pixel architecture consists of four memory registers per pixel, so that up to four events may be detected within an experimental cycle [139]. Initial experiments including velocity map imaging studies of various molecules indicate that the register depth of four is sufficient to detect the majority of the occurring events [140]. Currently, the second generation of the of PImMS is under development, which will extend the number of pixels to 324×324 . Furthermore, the possibility of operating PImMS at a clock speed of 6.25 ns is to be explored. Two photographs, one of the PImMS sensor bond to the camera board and one of the assembled camera, are presented in Figure 1.13.

1.6 Outline of the Thesis

The work presented in this thesis describes the construction and characterisation of a novel, linear microscope mode imaging mass spectrometer, as well as the development of a new organic scintillation detector. Initial tests investigating the abilities and limitations of a standard velocity map imaging spectrometer operated as IMS instrument are presented in Chapter 2. By employing the new CMOS based PImMS sensor to acquire microscope mode IMS images of tissue samples and grid structures, the potential of multi-mass imaging will be explored. In Chapter 3, ion trajectory simulations will be undertaken to design a new five electrode ion optic which allows to improve the ToF resolution of the spectrometer using a pulsed extraction method. Chapter 4 details the experimental implementation of the designed ion optic. The newly developed imaging mass spectrometer will be fully characterised in terms of its ToF and spatial resolution, and its mass tunability. Chapter 5 describes the development of a new kind of scintillator screen with a fluorescence lifetime of sub-10 ns, allowing ion imaging experiments to be performed with an enhanced ToF resolution. The developed scintillator screens are not only applicable to IMS, they also demonstrate an outstanding ion-to-photon conversion efficiency when being applied with a single photon avalanche diode as a direct ion detector for ToF mass spectrometry (see Chapter 6). Further applications to fields such as medical imaging will also be discussed. In Chapter 7, a new concept of an ultra-fast ToF imaging detector will be presented.

Chapter 2

A Three Lens Ion Optic Applied to Imaging Mass Spectrometry

Velocity map imaging is one of the most common techniques applied to study photodissociation dynamics in physical chemistry today. Electrostatic fields are applied to an ion optic to velocity map the resulting photofragments onto a two dimensional imaging detector. By adjusting the applied electric field ratios, the experiment can be converted to map ions as a function of their origin position instead of their initial velocity. Hence, in this chapter, the potential and limitations of the use of a standard VMI instrument as a surface analysis microscope mode imaging mass spectrometer will be explored. For this purpose, ion trajectory simulations using the SIMION 8.0 software will be compared to experimental results. Furthermore, initial tests employing the newly developed CMOS based PImMS sensor will be presented. These experiments, despite not being performed under optimum conditions, demonstrate the capabilities of the combination of a fast imaging sensor and a microscope mode imaging mass spectrometer.

2.1 Ion Trajectory Simulations

2.1.1 Simulation Principles of SIMION 8.0

When designing and developing an ion optic it is essential to predict the ion trajectories in the mass spectrometer. One program which allows simulating the properties of such an instrument is SIMION 8.0 [141]. Here, a two or three dimensional potential grid array is created in which an ion optics assembly can be modelled. The electrodes are represented by defined potential arrays (PAs) to which a user defined voltage can be applied. The potential of the remaining array points can be calculated by solving the Laplace equation, in which the potentials of the electrodes act as boundary conditions. In this way, a three-dimensional electrostatic model of an entire mass spectrometer can be created.

Before the trajectories of particles flying through the electrostatic array can be simulated, it is necessary to define the initial particle conditions. These comprise of the following parameters: the time of birth at the position (x,y,z) , the initial flight direction defined by the azimuth (xz plane) and the elevation (xy plane) angle, the initial velocity, which is defined by the particle's kinetic energy ($v = \sqrt{2E_{\text{Kin}}/m}$), and the mass-to-charge ratio. Once the particles are created, their trajectories can be calculated following an iterative ToF sequence. In each of these steps, the electrostatic force applied to a single particle is calculated, and its velocity and flight direction is adjusted for the next iteration. Repulsion forces such as Coulomb interactions between the charged particles may be taken into account in the simulation software.

Among various other parameters, SIMION 8.0 can record the time and spatial position of the ion impact on a solid surface. These two parameters are the key factors, which need to be considered to design any kind of mass spectrometer. The input parameters for the ion trajectory simulation and the associated analysis procedures employed in this work will be explained in the next sections.

2.1.2 Ion Parameters and Analysis Procedure

One key element for the accuracy of the SIMION 8.0 simulations is the understanding and prediction of the ion plume expansion from a sample surface. This plume depends on the applied ionisation technique. As the ion optics designed in this work will be applied to MALDI-MS, the initial ion parameters were set according to previous studies, which determined the initial velocity and angle distribution for this process.

The initial velocity distribution was studied by Beavis and Chait, who measured the ToF of polypeptide molecules (molecular masses 1 kDa to 15.6 kDa) as a function of the extraction fields applied [142]. Figure 2.1 shows the velocity distributions obtained for three particles following a MALDI process. The measurements indicated that the ions travel at the same mean velocity regardless of the molecular mass, peaking around 750 m/s, with an overall Gaussian shaped velocity distribution. Following these experiments, the initial ion velocity distribution for the SIMION 8.0 simulations were set using the equation

$$g(v) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(v - \bar{v})^2}{2\sigma^2}\right), \quad (2.1)$$

with a mean velocity of $\bar{v} = 750$ m/s, and a root-mean-square deviation of $\sigma = 150$ m/s.

The second factor which needs to be taken into account is the angular distribution of the ions leaving the surface. This effect was investigated in a number of publications [143–145]. In the simulations presented in this thesis, the initial ion distribution was set according to the work of Zhang and Chait, who imaged the spatial distributions of ions arriving

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Figure 2.1: Experimentally determined initial velocity distribution for ions of the mass 1030 Da, 5730 Da and 15590 Da formed in a MALDI process. The results were taken from Reference [142].

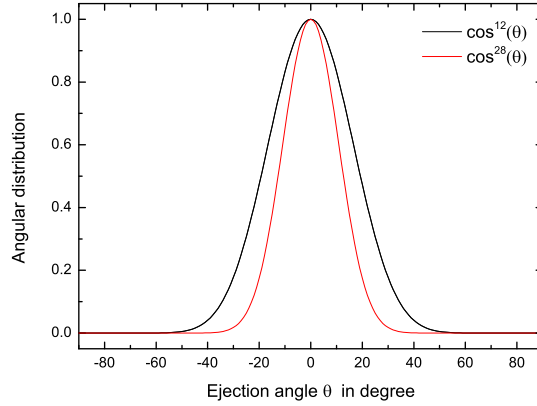


Figure 2.2: Ejection angle distributions for a $\cos^{12}(\theta)$ and $\cos^{28}(\theta)$ function.

at a detector located at the end of the ToF tube [146]. By observing the distributions of several ion species at acceleration voltages between 5 and 20 kV, it was possible to extrapolate the most likely axial velocity distribution at an extraction potential of 0 kV. The angular distribution obtained for the matrix molecules (<300 Da) fits well with a $\cos^{12}\theta$ distribution, while the peaks detected for a protonated dynorphin ion (~ 1600 Da) fit to a $\cos^{28}(\theta)$ function (see Figure 2.2). The angle θ describes the deviation from the ToF axis. Consequently, the angular distribution observed for lighter ions is broader than the more forward peaking distribution obtained for heavier ions. Hence, the initial angular ion distribution for the ion trajectory simulations undertaken was set according to $\cos^{12}(\theta)$, as this wider ion spread should result in an upper limit for the spatial and ToF resolution. The distribution of ions around the ToF axis was assumed to be symmetric to form a cone distribution. A further description of the ion parameters applied can be found in Reference [147].

To determine the spatial and ToF resolution of the mass spectrometer simulated, a one dimensional array of four spots with 200 ions each was created on the repeller plate. One of these spots was located in the centre of the mass spectrometer with the others extending into one direction. The initial ion parameters of each spot were set according to the description above, resulting in a random distribution within the given approximations.

One ion per spot was created without any transverse velocity and with the mean velocity, so that it could be used as a reference ion. The ratio of the separation of these four ions on the repeller and on the detector allowed the magnification of the instrument to be determined. To define the spatial resolution, it was assumed that all four ion spots on the repeller were point sources. Hence, the size of each ion hit on the detector correlated directly, *via* the determined magnification factor, to the spatial resolution of the system. However, the intensity distributions obtained as a function of the ion hit size was incompatible with a Gaussian distribution. Therefore, the average displacements of all impacting ions from the relevant reference ion were calculated. The spatial resolution for each of the four spots was determined by taking two times the standard deviation ($2\sigma_{\text{dis}}$) of the displacement from the reference ions. This value was found to be a good approximation for the FWHM of the ion intensity distribution obtained. The absolute spatial resolution of the instrument was calculated by taking the average value of the spatial resolutions determined for each of the four spots.

In order to obtain an image with a constant spatial resolution across the entire field-of-view it is not sufficient to determine the spatial resolution as an average value of the four spots, as the most central spot might have a higher spatial resolution while the outer ones are distorted due to aberrations. To address this issue, the standard deviation of the spatial resolution of the four spots was determined. The lower the value for the standard deviation, the smaller were the distortions expected in the later image.

The ToF resolution of the mass spectrometer was determined by measuring the arrival times of ions of the same mass and calculating the resulting standard deviation, σ . The FWHM value for the arrival time distribution was then determined according to

$$\text{FWHM} = 2\sqrt{2\ln(2)}\sigma, \quad (2.2)$$

which corresponded directly to the uncertainty in the arrival time (Δt). Finally, according to the definition $t_{\text{ToF}}/2\Delta t$, the ToF resolution was determined.

2.1.3 Simulations for the VMI Instrument

Following the initial description of velocity map imaging in Chapter 1.3, a standard VMI ion optic was modelled in SIMION 8.0. The distances between the three electrodes were 14 mm between the repeller and the extractor, and 21 mm between the extractor and the grounded lens. The two latter lenses had an aperture of 20 mm each, and the total flight length was 613 mm. Each electrode was 1 mm thick and had a diameter of 92 mm.

In order to find the optimum operation conditions the potentials applied to the repeller and extractor electrodes were optimised for the mass of 372 Da, which corresponds to the molecular ion mass of crystal violet. This molecule was chosen for two reasons: Firstly, the laser induced desorption and ionisation process at 355 nm results in a high ion flux in the spectrometer; and secondly, due to its blue colour, the quality of spatial structures created on a sample surface can be monitored using a light microscope. This allows a later comparison between the ion and the optical images. The distances between the ion point sources in the spot array were set to 0.66 mm, so that the simulated field-of-view covered an area of 4 mm in diameter on the repeller plate.

For the optimisation of the ion optic potentials, the repeller voltage was set to 10 kV and the extractor voltage scanned until the optimum spatial mapping condition was found. A resulting plot of the ToF resolution, the spatial resolution, the standard deviation of the spatial resolution of the individual spots, and the magnification of the instruments as a function of the extractor voltage is presented in Figure 2.3. In the simulations, the best spatial resolution of 10 μm was determined for an extractor potential of 9665 V. However, it was noticed that the spatial standard deviation between each spot of the array showed the lowest value at 9659 V. Hence, to reduce distortions in the obtained spatial image, it was decided to proceed for the next simulations with an intermediate extractor potential of 9662 V. The ToF resolution detected across the whole voltage scan decreased slightly towards higher extractor potentials. At the spatially optimised potential, a ToF resolution of $t/2\Delta t = 159$ and an image magnification of $10.3\times$ were predicted for ions of the mass 372 Da.

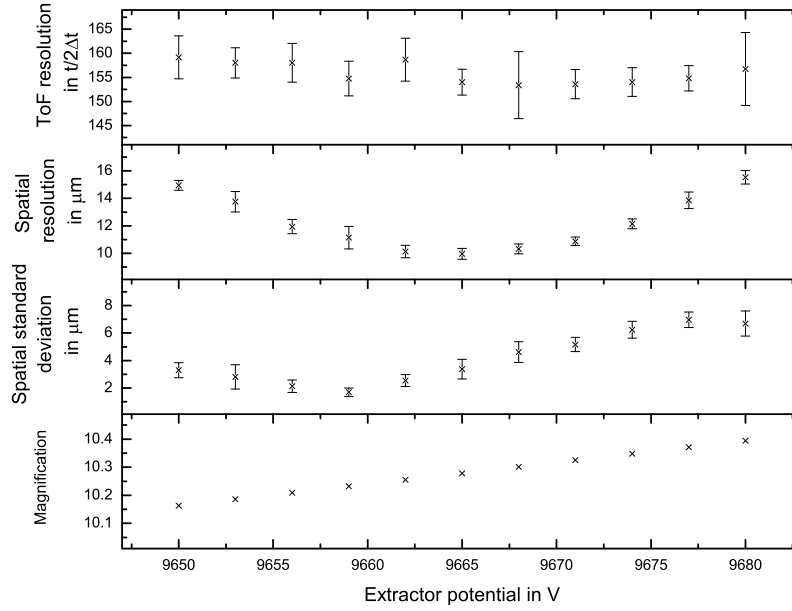


Figure 2.3: Ion trajectory simulation results for a three electrode ion optic applied to IMS. The mass spectrometer specifications determined are presented as a function of the applied extractor potential. The repeller was held constant at 10 kV.

The simulations presented in Figure 2.3 indicate that a reasonably high spatial resolution for the given field-of-view can be achieved. However, the ToF resolution does not exceed $t/2\Delta t = 160$ using a three electrode ion optic under spatial mapping conditions. A further point which needs to be addressed is how the optimised focusing properties change as a function of the ion mass. Figure 2.4 shows the mass dependent specifications of the employed ion optic, operated with the optimised voltage settings from above. Here, the ToF resolution decreases as the particle mass increases. In the same way, the spatial resolution is reduced from sub-10 μm at 200 Da to more than 40 μm at 1000 Da. Furthermore, the optic aberrations indicated by the spatial standard deviation show the smallest influence on the spatial mapping at the optimised ion mass (372 Da). The distortions are increased at the lower and upper end of the mass spectrum. The magnification factor of the mass spectrometer is almost constant across the entire investigated mass range.

All the changes observed in the mass spectrometer specifications can be explained: for example, the ToF resolution determined is reduced with increasing particle mass, as the

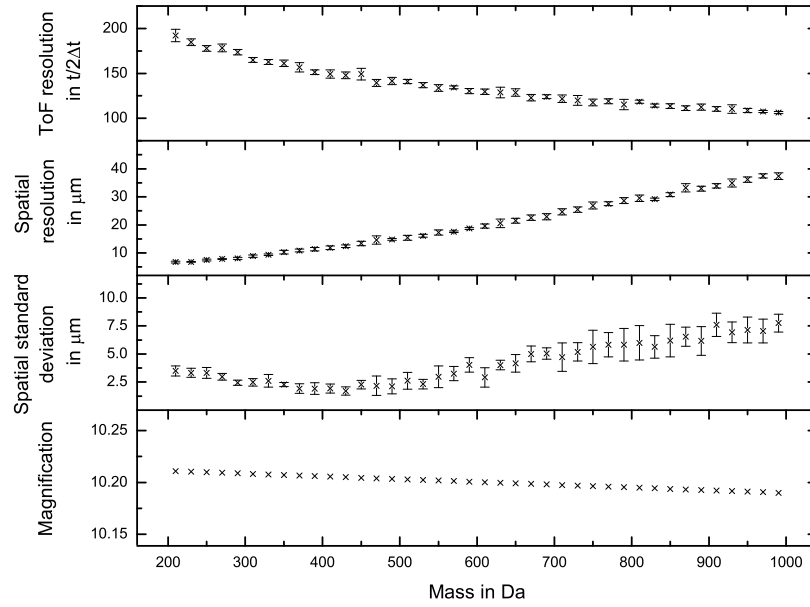


Figure 2.4: Illustration of the mass depended spectrometer specifications of a three electrode ion optic, operated with ion optic settings which were optimised for ions of the mass of 372 Da.

time separation between two adjacent fragments decreases according to

$$\Delta t_{m,m+1} = \sqrt{\frac{D^2}{2zeEs}} \left(\sqrt{(m+1)} - \sqrt{m} \right), \quad (2.3)$$

where $\Delta t_{m,m+1}$ is the time separation between two fragments, m the particle mass, z the charge number, e the elementary charge, E the field strength, s the acceleration distance, and D the flight tube length. As a result, it can be assumed that with increasing particle mass, m , two peaks may start to overlap. Furthermore, peaks of heavier ions are broadened as the initial velocity distribution of the ions cannot be fully corrected using a static extraction field.

To obtain the optimum spatial resolution the electric fields applied to the ion optics need to be adjusted so that the spatial mapping plane is located on the detector. In Figure 2.4, the experiment was initially optimised to spatial map ions of the mass 372 Da. However, the best resolution was observed at 200 Da. The reason for this is that in the simulations it was assumed that all the ions come off the surface with an average speed of 750 m/s in a $\cos^{12}\theta$ distribution, resulting in a kinetic energy spread which differs

significantly for heavier and lighter ions. This kinetic energy spread defines the effect of chromatic aberrations on the spatial resolution obtained, which results in an improvement in the average spatial resolution in the low mass range.

The shape of the spatial standard deviation plot can be explained by the fact that the spatial mapping plane was matched to the detector position for ions of mass 372 Da. For lighter or heavier ions, this plane is located either in front of, or behind, the detector. As a result, the lighter ions show a reduced spatial resolution for the most outer ion spot, while the resolution for the central spot is reduced if heavier ions are imaged with the extraction settings applied.

The magnification, which is determined by comparing the terminal position of the four reference ions, was found to be almost constant over the mass range. However, the slight decrease towards higher ion masses can be associated with the kinetic energy dependent focusing properties of the electrostatic lenses.

The SIMION 8.0 simulations presented in the paragraphs above were all performed using a repeller potential of 10 kV. To address the effect of the strength of the extraction field on the spatial and ToF mapping resolutions, the repeller voltage was scanned from 500 V to 20 kV, while the extractor voltage was set according to the best spatial mapping conditions for ions of the mass 372 Da. The results for the spatial and ToF analysis are presented in Figure 2.5. Both parameters show their optimum values at the strongest extraction field applied. Spatial resolutions of sub-10 μm can be achieved in combination with a ToF resolution of $t/2\Delta t = 200$ at 20 kV extraction. However, due to experimental limitations in the apparatus employed in this thesis, it was not possible to apply more than 10 kV on the electrodes. Furthermore, the improvement in spatial resolution from 10 kV to 20 kV is almost negligible, while the ToF resolution is increased from $t/2\Delta t = 160$ to $t/2\Delta t = 200$ in a non-linear fashion.

Following the ion trajectory simulations, it can be stated that a standard three lens VMI spectrometer, optimised for an experimental field-of-view of 4 mm, allows IMS to be performed with a spatial resolution of down to $\sim 10 \mu\text{m}$ using analyte ions of the mass

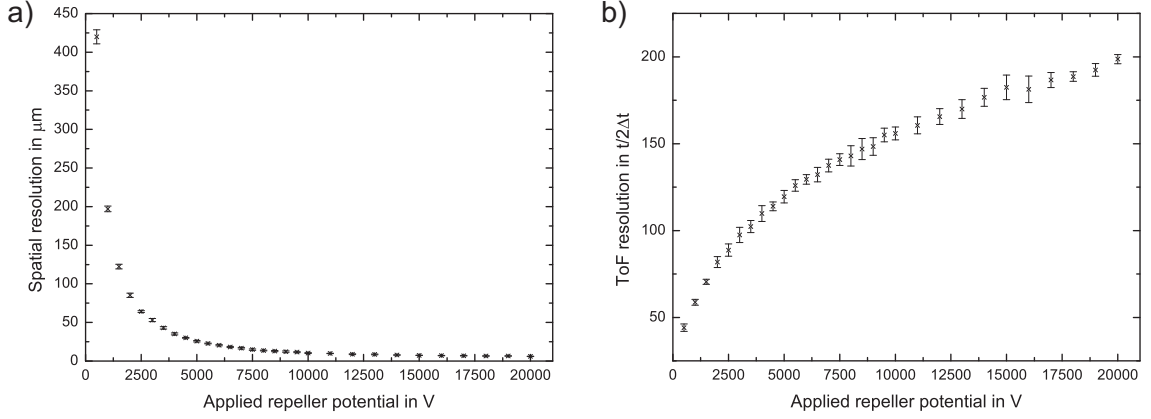


Figure 2.5: Ion trajectory simulation results of the spatial and ToF resolutions obtained by adjusting the strength of the extraction field.

of ~ 370 Da. The magnification factor of the instrument was determined to be $10.3\times$, while the ToF resolution was limited to $t/2\Delta t = 160$. The experimental verification of the simulated resolutions will be presented in the next sections.

2.2 Experimental Techniques and Methods

The experiments presented in this chapter were performed on a modified velocity map imaging mass spectrometer. The instrument comprises of three main parts: the laser system, the ion optics, and the ion detector. The properties of each of these will be described in the next paragraphs. Furthermore, the sample preparation methods will be introduced.

2.2.1 Optical Systems

For the direct laser ablation and ionisation processes undertaken throughout this thesis a 10 Hz, 355 nm, 3rd harmonic Nd:YAG laser (Continuum Powerlite 8010) was employed. Before entering the mass spectrometer the beam was attenuated and split using a 20:80 beam splitter. Whilst the less intense part of the beam was detected using a photodiode to determine the start of the ToF measurement, the more intense part was defocused in the vertical direction using a 30 cm plano-concave cylindrical lens, mounted at a distance

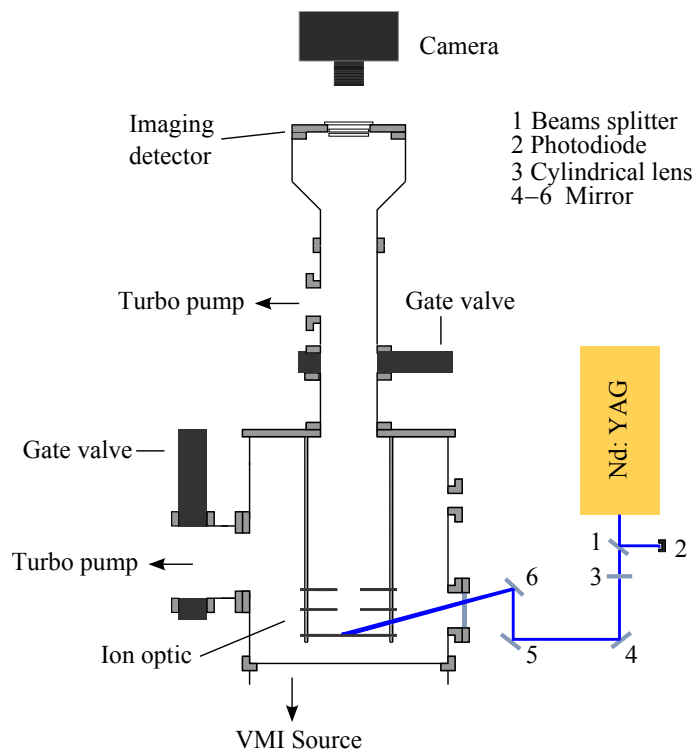


Figure 2.6: Schematic drawing of the laser optic assembly and experimental setup of the modified velocity map imaging spectrometer.

of 80 cm from the mass spectrometer. The resulting defocused laser beam was directed onto the sample at a 5° angle, passing the ion optics between the repeller and the extractor electrode. The incidence angle ensured a defocusing in the horizontal direction, so that an almost uniform laser profile was obtained across the sample. Laser hot spots needed to be avoided as these would prevent a uniform ionisation. Depending on the samples employed, the laser power in the collimated beam was kept between 20 and 100 mJ per pulse. A schematic drawing of the experimental setup is presented in Figure 2.6.

2.2.2 Ion Optic and Vacuum Chamber Specifications

In order to perform IMS experiments with a conventional VMI spectrometer (see Figure 2.6), the existing connection between the molecular beam source and VMI chamber was replaced with a blank flange to reduce the volume of the mass spectrometer. The remaining parts comprised of the ion optic and the detection chamber, separated by a gate



Figure 2.7: Photograph taken of the repeller plate. To load a sample, an ITO coated quartz plate was mounted in the central groove.

valve. Both chamber sections were individually evacuated by two turbo molecular pumps. The model employed in the ion optic chamber was a 510 l/s Pfeiffer Vacuum TMH-521P, while the detection chamber was held at vacuum using a smaller 60 l/s Pfeiffer Vacuum TMU-071P pump. Both were backed by a Leybold Trivac D40V rotary pump. The pressure typically found in both chambers was around 2×10^{-7} mbar. A second gate valve was employed to isolate the attached turbo pump from the ion optic chamber, which could be separately evacuated using a second Leybold Trivac D40V rotary pump.

The ion optic of the instrument was manufactured from stainless steel according to the dimensions used in the SIMION 8.0 simulations above. The electrodes were held in position by four ceramic (Macor) isolated stainless steel rods mounted on the flange of the latter ToF tube. A picture of the repeller plate employed is presented in Figure 2.7. In contrast to the ion trajectory simulations, the repeller plate was about two millimetre thick with a square groove cut into the centre of the lens. Here, an indium tin oxide (ITO) coated glass sample plate ($25 \times 25 \times 1.1$ mm) could be fit in. To ensure an electrical connection between the ITO coating and the metal plate a conductive wire was taped between both. Experiments were performed at pressures no higher than 5×10^{-6} mbar.

2.2.3 Detector and Data Acquisition

The detection assembly for the IMS experiments consisted of a set of microchannel plates, a phosphor screen, a photomultiplier (PMT), and either a CCD or the PImMS camera. The microchannel plates (Burle APD 3075) employed had a size of 75 mm, and were arranged in chevron geometry. Each channel had a pore diameter of 25 μm , with a pitch of 32 μm . In normal operation a total gain of 1.5 kV was applied across the MCP assembly. In the case that it was necessary to pulse the MCPs to avoid large amounts of signal at early ToFs, the back face of the front MCP was initially grounded and then turned on by pulsing it up to 0.75 kV using a fast voltage switch (DEI PVM-4150).

The electrons ejected from the back MCP were further accelerated onto a P47 phosphor screen, which emitted discrete flashes of light with a fluorescence lifetime of 70 ns. For the experiments employing a conventional CCD camera the phosphor screen potential was set at 3.5 kV, while for the PImMS camera, the applied potential, and thereby the brightness of the fluorescent signal, needed to be increased to 4.5 kV. Conventional ToF spectra were recorded using a liquid light guide coupled PMT, with the guide pointed at the phosphor screen.

The intensified CCD camera (Photonic Science) employed in the IMS experiments had a pixel number of 576×768 , and was mounted at a distance of 30 cm from the phosphor screen. In order to collect ion images for specific ToF peaks, the signal was integrated over a minimum window of 100 ns by triggering the intensifier. If a shorter integration window was required, the intensifier gate needed to be overlapped with the gating of the MCP.

In the first step of the acquisition, a threshold level was set for the charge collected at each of the pixels of the CCD camera, with every pixel below the set threshold being set to zero. The detected events were then centroided using a pattern matching algorithm (built into LabVIEW 2012) that searches for circles and reduces them to an (x, y, r) coordinate, where r is the radius of the circle. The value of r was then used as a further discriminator for noise, leaving just an (x, y) coordinate for the real event. This routine presented a marked improvement in the observed spatial resolution.

When the PImMS camera was employed, it was mounted at a distance of 20 cm behind the phosphor screen. Due to the principle of operation of PImMS, the complete ToF spectrum as well as all the spatial positions of the events were collected simultaneously. MCP pulsing was only necessary if large peaks at the beginning of the ToF needed to be removed to avoid damaging the detector. For the experiments reported in this section, the internal clock of PImMS was set to 25 ns, which was the optimum time precision at the time the experiments were performed. The data recorded with the PImMS was not further processed. Ion images were created by selecting the relevant ToF peak, and plotting the spatially resolved intensity distribution using either the PImMS processing suite or a C++ based program [148, 149].

2.2.4 Sample Preparation

To test the spatial mapping specifications of the VMI ion optics it is necessary to create a well defined structure on a surface. Following earlier work, it turned out that grid structures of known dimensions allow an easy determination of the spatial mapping properties of an IMS apparatus. A number of methods may be employed to create structures on a surface. For example, an airbrush can be used to spray an analyte through a grid stencil, which is removed after the process [150]. However, the disadvantage of this method is that, depending on the user, different and partly uneven coatings are obtained. Furthermore, the airflow from the airbrush might lift up the grid stencil, resulting in an uneven grid size on the target plate.

Another method to create a defined pattern on a surface is to coat a grid in an analyte and stick it onto the sample plate after the preparation [94]. Thereby, special care needs to be taken that the grid sticks to the surface over the whole field-of-view. Otherwise, distortions and a loss in ToF and spatial resolution can be expected. Another disadvantage of this method is that the particles are desorbed from the surface of the grid, which stands out from the actual sample plate surface. In this way the actual sample plate is not probed. This can be done by coating the sample plate with an analyte before the grid is

mounted on top. Although this method showed promising results, it is not feasible for IMS instruments in which the laser hits the sample plate at a shallow angle, as shadow effects may be observed [151].

A more professional sample preparation can be achieved using a commercial, computer controlled sample sprayer or spotter systems. These allow a completely even coating of surfaces with MALDI matrices in a short preparation time. However, it needs to be noted that these systems are extremely expensive ($>£20000$).

A compromise between the commercial systems and a reasonably cheap option that allows uniform sample coatings or pattern creation are home-built spray systems such as an oscillation capillary nebulizer (OCN) sprayer [152, 153]. A schematic drawing of an OCN sprayer is presented in Figure 2.8. The systems mainly consist of two capillaries. The inner, smaller one is used to transport the analyte solution, while the outer one is used to create an annular gas stream, which causes oscillations at the tip of the inner capillary. Then high frequency oscillations trigger a nebulisation of the analyte solution, resulting in a fine even coating on the sample plate below [12].

Besides the OCN sprayer, electrosprayers are a comparable system for creating uniform coatings on a surface. Here, the analyte solution is pushed through a positively charged

Figure has been removed due to Copyright restrictions

Figure 2.8: Illustration of a typical OCN sprayer apparatus. The figure was taken from Reference [12].

needle, mounted right on top of a sample plate. By grounding the surface of the sample plate an almost uniform electrospray spot can be obtained. For applications in MALDI mass spectrometry, Hensel and Axelsson reported a high reproducibility in conjunction with low spot-to-spot variations of the resulting samples [154, 155]. The matrix crystals were analysed to be smaller than 1 μm , resulting in a fine and homogeneous layer. In this way, the matrix crystals have almost no effect on the topology of the samples. A disadvantage for tissue analysis using this method is that, due to the small crystal size, only a small amount of analyte is incorporated into the matrix layer. Therefore, a reduced sensitivity can be observed [156].

Both home-built spraying devices described above are reliable in producing homogeneous and reproducible layers. Furthermore, both have the advantage that independent of the user, similar samples are preparable, as the operation is fully automated. However, the OCN sprayer has one key disadvantage in the preparation process of a grid pattern. If a grid is placed as stencil on top of the sample plate, it may be elevated by the continuous gas flow from the OCN sprayer. Therefore, it was decided to construct an electrospraying device, which works without any air flow. After the preparation process the grid can be removed and a negative pattern with a high spatial resolution can be obtained.

A picture of the constructed electrosprayer can be found in Figure 2.9. It was operated with a syringe pump (KDS Legato 100) to control the solvent flow. Inside the electrosprayer, a standard injection needle (G21), with the tip filed off to form a flat-hat shape, was used to release the spray. The needle could be charged up to 7 kV. Disposable ITO coated quartz slides were used as sample plate, which were mounted in the electrosprayer on an xy -adjustable stage, allowing each position on the plate to be reached with the spray. To obtain an optimum electrospray, the applied voltage, the flow rate, and the needle to sample plate distance needed to be adjusted, so that a small droplet was formed on the needle. These settings needed to be adjusted in the case where the solvent was changed or a different spot size was required. To create a grid pattern, an electroformed nickel mesh was placed on the ITO plate by sticking it down with a droplet of acetone. This method

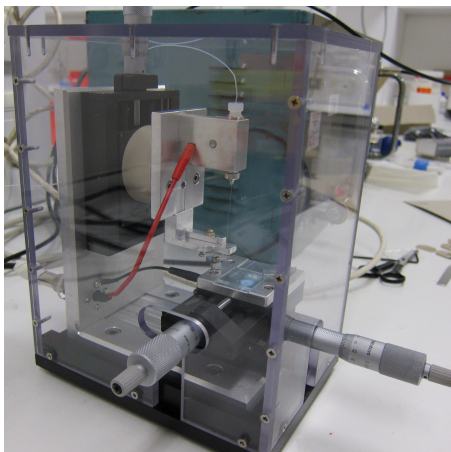


Figure 2.9: Photograph taken of the home-built electrosprayer. The spray was created on the tip of a charged injection needle. A grounded ITO coated quartz plate was mounted on a translational stage to allow the coating of sample.

ensured that the mesh did not lift up from the surface while coating.

For the samples investigated employing the high resolution CCD camera, crystal violet was dissolved at a concentration of 0.3 mg/ml in 50% ethanol. The prepared solution was electrosprayed through a nickel mesh (Goodfellows NI008720) with a wire thickness of 67 μm , and a pitch of 500 μm , using a flow rate of 50 $\mu\text{l/h}$ and an applied voltage of 4.6 kV. Typical spray times for high resolution samples were 60 seconds. After the deposition process, the grid was removed, and the sample was placed in the imaging chamber. Microscope photographs taken of a prepared crystal violet grid are presented in Figure 2.10. By creating a contour plot across the square sections of the sample, and by fitting the signal rise and decay distance from 20% to 80% an average spatial resolution of 11.0 μm was determined [157].

Grid experiments using PImMS were also performed using crystal violet as a sample. Thereby, the spray time was increased to up to 5 minutes, as a thicker layer, and therefore higher signal levels, were required. Furthermore, to demonstrate the capabilities of PImMS for biological imaging, a 10 μm thick section of a mouse brain was covered with a thin layer of the MALDI matrix α -cyano-4-hydroxycinnamic acid (CHCA) using the electrosprayer. The CHCA concentration was 1.0 mg/ml in 50% ethanol and it was sprayed at a flow rate of 100 $\mu\text{l/h}$, at an applied voltage of 5.0 kV, onto the tissue section. Afterwards the sample

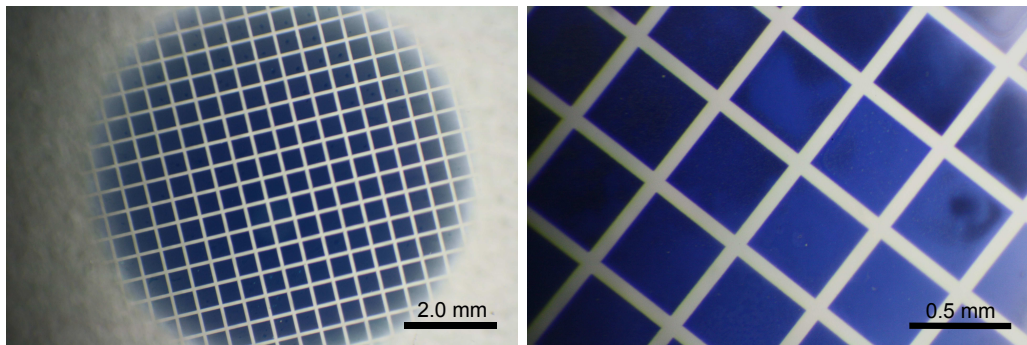


Figure 2.10: Optical microscope images of a crystal violet sample. The grid pattern was created using an electro-sprayer with a nickel mesh as a stencil.

was attached to the repeller plate and loaded into the mass spectrometer.

2.3 Experimental Results and Discussion

2.3.1 IMS Using a CCD Camera

A typical ion image obtained of the crystal violet grid sample using the three lens VMI spectrometer is presented in Figure 2.11a. For the measurements, the repeller and extractor potential were set to 10.0 kV and 9.7 kV respectively. These settings were chosen according to the ion trajectory simulations above. The camera gate was set to collect the parent ion peak at 372 Da over 2000 laser shots. In the image, the pitch of the squares corresponds to a distance of 0.5 mm on the sample. As up to 8 square sections can be spotted in the horizontal and up to 9 squares in the vertical direction, the field-of-view can be assumed to be roughly $4 \times 4.5 \text{ mm}^2$. Larger sample areas may be imaged by increasing the laser spot size further. However, the defocusing of the laser causes problems for the chosen field-of-view. Due to inhomogeneities in the laser profile, an uneven signal distribution across the whole sample is observed in the presented image. In the future, this issue needs to be addressed by the use of fibre optical cables (see Chapter 1), or by the use of a diffuser to mix the photon trajectories.

To determine the spatial resolution and the magnification, an intensity profile plot along the yellow line shown in the image was created. By measuring the distance between

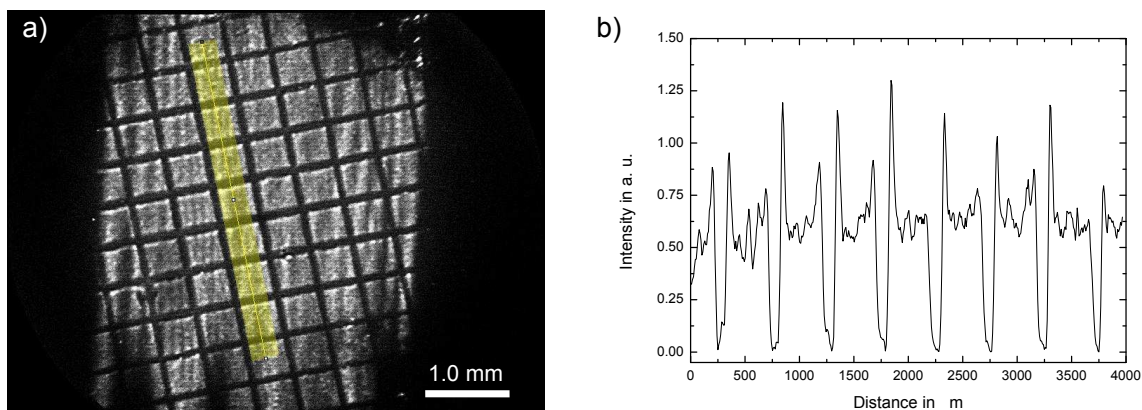


Figure 2.11: *a)* Presents an ion image recorded of the 372 Da crystal violet parent ion peak. Using the cross section plot (see panel *b*) along the yellow bar the spatial resolution of the imaged was determined to be $20 \pm 9 \mu\text{m}$.

the square sections, the size of the squares in pixel was correlated to the sample size on the repeller, so that each pixel in the image corresponds to a distance of $9 \mu\text{m}$ on the sample. From the contour plot (see Figure 2.11*b*) an average spatial resolution of $20 \pm 9 \mu\text{m}$ was determined by fitting the rise range from 20% to 80% of the signals [157]. To define the magnification of the system, the size of the phosphor screen of 75 mm needed to be taken into account. By increasing the brightness of the image, the actual dimensions of the screen became visible, so that by virtually expanding the observed grid structure a maximum sample size of 6.4 mm could be imaged. By comparing both values, the full observable sample size and the actual detector size, a magnification factor of $11.7\times$ was determined.

The cross section plot presented in Figure 2.11*b*, is not only useful to determine the spatial resolution. It also shows an interesting feature which is not immediately obvious in the ion image. On the rising edge side of each square an intense spot can be identified across the ion image. This feature can be assigned to the shallow incident angle of the laser onto the surface. Due to this, the edge of a sample is exposed to higher laser fluence than the opposite side of the square. If the layer thickness of the sample is further increased, this feature will become more dominant, until only a negative image of the grid remains and the squares are bleached out. Recognising this fact suggests that the use of an electrosprayer

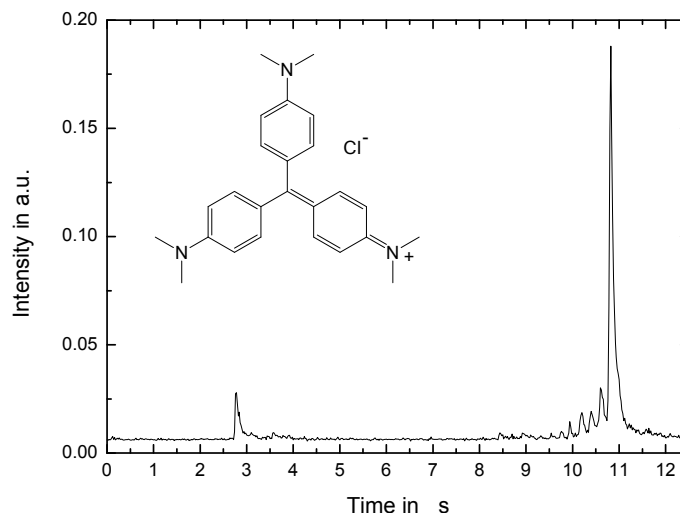


Figure 2.12: ToF spectrum of crystal violet at 10 kV extraction potential. The ToF resolution for the parent ion peak at 11 μ s was determined to be $t/2\Delta t = 54$.

for the sample preparation is sensible, as relatively thin layers can be obtained (~ 200 nm).

Corresponding to the ion image presented above, the ToF spectrum and the chemical structure of crystal violet are illustrated in Figure 2.12. The main peak in the spectrum at 11 μ s ToF can be assigned to the 372 Da parent ion of crystal violet. The smaller peaks at the early ToF side of the parent ion are a result of the consecutive loss of methyl groups. By fitting Gaussian profiles to the ToF peaks exceeding 9 μ s flight time, their FWHM values were extracted. These allowed a resolution of $t/2\Delta t \approx 54$ to be determined.

The Figures 2.11 and 2.12 present an ion image and a ToF spectrum recorded using an extraction potential of 10.0 kV applied to the repeller electrode. In the case that the extraction field strength is reduced, it can be expected that both mass spectrometer specifications will become worse. Experimentally, at 4.0 kV extraction potential the spatial resolution was found to be 33 ± 9 μ m and the ToF resolution was determined to be $t/2\Delta t = 49$. Both values are added into the ion trajectory simulation graphs presented in Figure 2.13. The spatial resolution determined in the experiments at 4.0 kV matches the simulation graph quite well, while at 10.0 kV the experimental specifications are somewhat lower than the simulated ones. This deviation at higher extraction voltages can be associated with several experimental limitations. For example, to obtain the optimum spatial

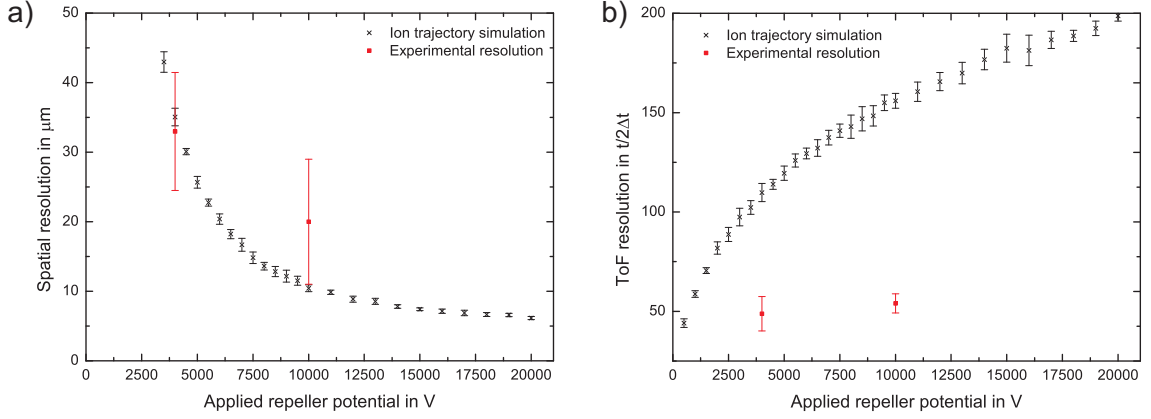


Figure 2.13: Comparisons of the experimentally determined spatial and ToF resolutions to the previously simulated mass spectrometer specification. Both resolutions are presented as a function of the applied extraction field.

resolution, the simulations undertaken were optimised down to an accuracy of 3 V. This is almost impossible in the experiments, as especially at higher voltages the range in which the ion images seems to be in focus extends up to 50 V. In order to choose the optimum value, a voltage scan needs to be performed using the identical sample. However, a sample lasts only for a couple of thousand laser shots, and if the sample is replaced, the spatial resolutions might not be comparable as the quality of the samples might differ. Hence, it is quite challenging to determine the optimum experimental settings. Furthermore, as a single pixel on the CCD camera corresponds to the distance of 9 μm on the sample, it is not reasonable that a spatial resolution of 10 μm can be achieved.

While the spatial resolutions determined almost match the specifications simulated, a significant deviation between the theoretical and experimental ToF resolutions are observed in Figure 2.13b. Independent of the applied extraction field, the experimental resolutions are found to be worse than expected. The reason for this is that in the previous simulations an ideal detector response was assumed. However, the detector employed a slow P47 phosphor screen, which has a decay component that broadens the peaks in the ToF domain, resulting in a decreased ToF resolution.

2.3.2 IMS Using the PImMS Camera

To take full advantage of microscope mode IMS experiments it is essential to detect multiple ions of different masses spatially resolved within a single experimental cycle. This is not possible using a conventional CCD camera, where only a summed image over a preselected time window can be acquired. Thereby, the timing information of each individual event gets lost. This shortfall may be overcome by employing the time stamping, CMOS based PImMS camera. To demonstrate the performance of PImMS in combination with IMS, two example applications will be presented in this section.

Figure 2.14 shows a 2000 shot ToF spectra taken of a crystal violet sample with the PImMS camera. For this measurement the time precision of PImMS was set to 25 ns and the created ions were extracted using a potential of 4.0 kV on the repeller and 3.9 kV on the extractor electrode. Following the labelling in the spectra, nine ToF peaks can be identified. The two large peaks appearing at early ToFs can be allocated to sodium and potassium,

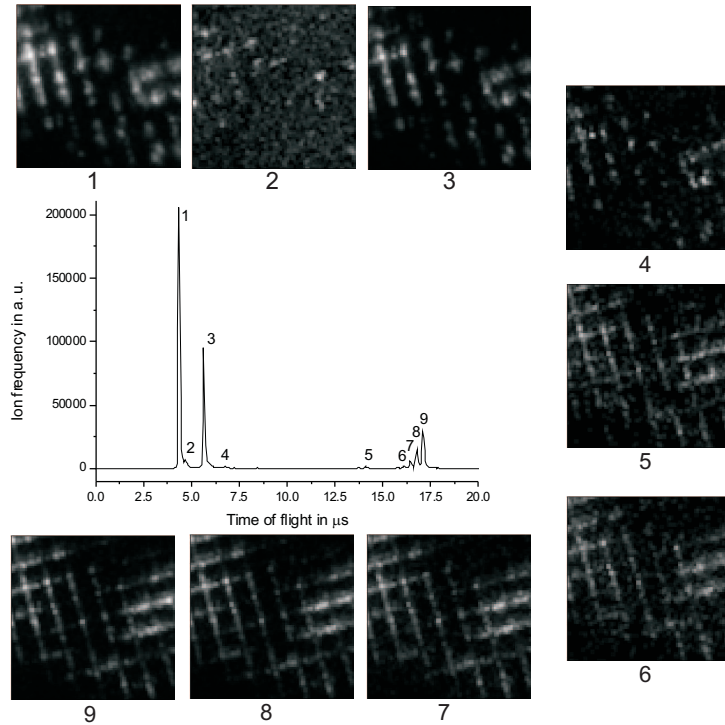


Figure 2.14: ToF spectrum with allocated ion images for a crystal violet grid. The data set was collected using the new PImMS camera. .

while the later ones arise from fragment and the parent ion peaks of crystal violet. For the latter, a ToF resolution of $t/2\Delta t \approx 48$ was determined. This value is quite similar to the one obtained with the PMT in the previous section. Following this agreement, it seems that the time resolution for the presented measurement is not limited by the PImMS camera, but most likely by the convolution of the mass spectrometer resolution and the phosphor screen properties.

By restoring the spatial information for a set ToF window, ion images for each of the labelled peaks can be created. Obviously, for these experiments the spatial resolution is limited by the 72×72 pixel array of PImMS, so that with the given pixel size and magnification each pixel corresponds to an area of $48 \times 48 \mu\text{m}^2$ on the sample. As a result it would be very difficult to resolve the $66 \mu\text{m}$ wide gap between the square section in the samples prepared. But, as described above, if the sample thickness ($>1 \mu\text{m}$) is increased, only the edge of each square section lights up, forming a negative grid structure. In this way, it is possible to create an easily observable pattern on a surface.

In Figure 2.14, the nine ion images obtained for each mass peak are presented. All of these show the expected grid structure. The uneven intensity distribution across each image can again be assigned to the poor laser profile and an inhomogeneous response of the CMOS pixels of the prototype camera. To determine an upper value for the spatial resolution obtained, it can be assumed that each line has its origin from a line structure with an infinitely small width. In this way, the FWHM of each line observed yields an estimate value of the spatial resolution. Corresponding to the intensity profile, a Lorentz function was chosen, which resulted in a spatial resolution of $\sim 99 \mu\text{m}$ for the crystal violet parent ion peak.

The images presented prove that it is possible to record multiple ion images simultaneously using PImMS. However, one of the key features of PImMS is that it can detect up to four events per pixel within a single experimental cycle. In order to prove the multi-hit detection abilities of PImMS, an analysis of the registers across the whole sensor was performed, resulting in a histogram of how many events were stored in each register.

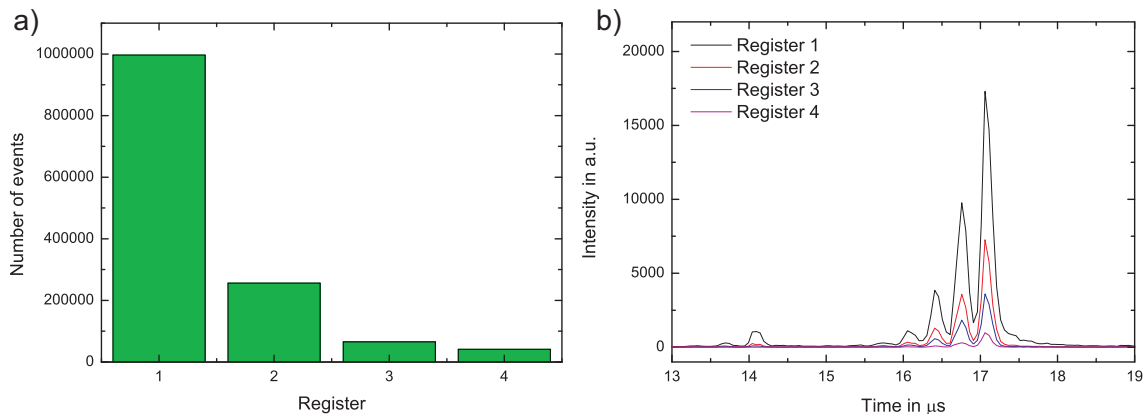


Figure 2.15: *a)* Histogram showing the total number of ions detected in each register of PImMS for the crystal violet data set. *b)* Mass spectra of the crystal violet sample, extracted from each individual PImMS register.

The histogram is plotted in Figure 2.15*a*. About 75% of the events detected are found in the first register, but all the remaining registers are also populated. For the spectrum presented the fourth register contains about 4% of the events, which means that some of the pixels detected up to four signals within a single ToF cycle. The contribution of each register to the ToF spectrum can be identified by plotting the spectrum for every single register separately. The plot is presented for the crystal violet peaks in Figure 2.15*b*. As expected, it can be recognised that the later the peak appears in the ToF spectrum, the more populated the second, third and fourth register become. However, as most events of the latest ToF peak are still found in the first register, it can be assumed that PImMS would be suitable to handle more complicated spectra than the one presented here.

One of the major applications of imaging mass spectrometry is the analysis of biological samples, such as tissue sections. To give an example for the application of PImMS to this field, a mouse brain section, kindly supplied by the Pharmacology Department, University of Oxford, was imaged over 5000 shots using the three lens ion optic spectrometer. The ToF spectrum obtained, displayed in PImMS time bins of 25 ns each, and the corresponding ion images are presented in Figure 2.16. Furthermore, a photograph of the tissue section investigated is shown in the diagram, to allow a correlation with the actual tissue sample. The ToF spectrum shows an unexpected structure for a tissue sample. This is due to the

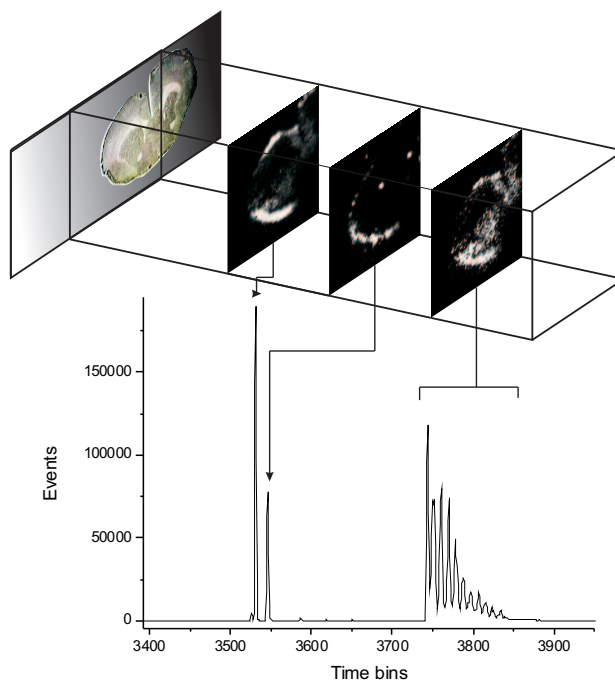


Figure 2.16: IMS data set recorded of a mouse brain tissue section using the PImMS camera.

fact that the MCPs were operated at a lower gain in the time bin region between 3400 and 3750. From time bin 3750 the MCPs were operated with the standard voltages applied. This operation scheme was necessary, as the strong sodium and potassium peaks, observed around time bin 3550, are so dominant in the ToF spectrum that no further signals were detected initially. By reducing the applied potential the signal strength of the two peaks is reduced so that the laser power could be increased and, once the MCPs were back in normal operation mode, later ToF peaks were observed. These later peaks cannot be assigned to a certain group of biomolecules, as they most likely do not reflect distinct ion masses. It is more likely that a saturation effect of the detector is observed.

The ion images obtained for the two cations and the later ion signals show all the structure of the mouse brain but are quite different from each other. While the early sodium peak shows a particle distribution across the entire tissue section, with an enhanced concentration at the lower and upper edge of the section, the potassium signals can mainly be observed in the lower part of the tissue. The biomolecules detected at later ToFs show,

in contrast to the other ion images, no enhanced particle concentration in any part of the image. The distribution is almost uniform. Due to this fact, PImMS demonstrates again its potential to be applied to IMS, as multiple mass peaks were imaged simultaneously and different spatial information are determined from different peaks in the ToF domain.

2.4 Conclusion

The experiments and simulations presented in this section, employing a three lens VMI ion optic, demonstrate that microscope mode IMS can be performed with a spatial resolution of sub-20 μm for a field-of-view of 4 mm in diameter using a linear instrument. However, the limitations of the simple ion optic design became obvious, since the ToF resolution obtained is not sufficient to resolve isotopic mass peaks in the spectra. A second limiting factor in terms of the ToF resolution measured is the fluorescence lifetime of the P47 phosphor screen. As the ToF spectra presented were recorded with a PMT it was impossible to determine shorter ToF signals than the emission signal from the phosphor.

The PImMS camera demonstrated in a proof-of-concept experiment its applicability to imaging mass spectrometry. Multi-mass imaging was performed in two example studies, wherein one employed a tissue section to show the application of the camera to bioimaging. However, in the experiments presented, the ToF resolution achieved was limited due to the spectrometer properties. The spatial resolution was mainly restricted by the low number of pixels of the PImMS sensor, which will be resolved once PImMS 2 is available.

In order to access a higher ToF resolution, the ion optic design needs to be improved to allow a better separation of the individual mass peaks. Furthermore, a faster and brighter scintillator would allow to access higher ToF resolutions, so that single isotope peaks may be identified. These points will be addressed in the following chapters.

Chapter 3

The Development of a Pulsed Ion Extraction Method

This chapter details the development of a novel pulsed extraction method, allowing imaging mass spectrometry to be performed with an enhanced mass resolution in a linear ToF instrument. A mathematical description based on the first order lens equations is presented to explain the concept of a post extraction differential acceleration process. Following this, ion trajectory simulations are employed to design two ion optic assemblies, both of which have the capability of improving the specifications of the imaging spectrometer presented in Chapter 2.

3.1 The Post Extraction Differential Acceleration Method

High ToF resolution is an essential requirement for the accurate determination and identification of molecules in mass spectrometry. Using a direct ion extraction technique, the ToF resolution of particles of the same mass is generally limited by four factors, illustrated in Figure 3.1(*a-d*). In case *a*, ions are not formed simultaneously. Hence, a direct acceleration

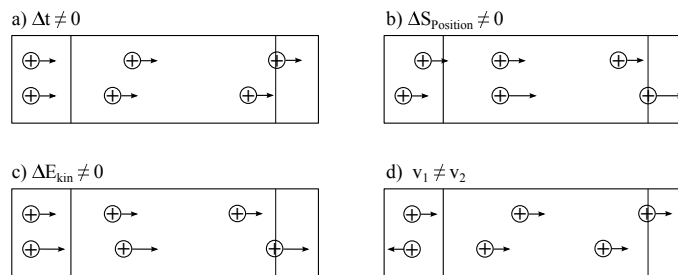


Figure 3.1: Four factors reducing the ToF resolution in direct ion extraction mass spectrometry: *a*) both particles are not ionised at the same time; *b*) both ions are created at different distances from the repeller plate; *c*) both particles have a different initial kinetic energy; *d*) both particles have a different initial flight direction.

results in a corresponding arrival time spread in the detection plane. In case *b*, sample molecules are ionised at different distances from the repeller plate. The ions closer to the repeller plate gain a higher final velocity due to a longer acceleration distance. Depending on the applied extraction potential this may cause a further broadening of the arrival time. The same effect is observed in the case that the ions are formed with different initial kinetic energies (case *c*). This results again in a wider velocity distribution, causing an arrival time spread. If the initial velocity vectors of the ions do not point parallel along the ToF axis (case *d*), the width of the recorded ToF peak is also increased, as the initial flight direction of some ions needs to be corrected in the extraction field. Both the particle's velocity (case *c*) and the initial flight direction (case *d*) contribute to the total axial velocity of the particle. Hence, the ion's axial velocity distribution can be used to describe the reduction in ToF resolution using a direct extraction method.

In the design and the operation mode of a ToF mass spectrometer all the discussed effects need to be addressed and minimised to achieve a high ToF resolution. In a MALDI experiment, samples are ionised using a laser pulse of less than 10 ns, so that all ions are ejected within a narrow time frame. A reduction in the ToF resolution due to non-simultaneous ion formation is therefore negligible. Furthermore, the arrival time dispersion caused by the different initial formation positions of the ions is insignificant, as the sample molecules are located on the repeller plate.

In Figure 3.1*b*, a point in the middle section of the ToF is identified, where the ions are

focused in one plane. By placing this time focus on the detector position, the resolution can be improved significantly. This principle is the basis of the commonly applied delayed extraction method [88]: by leaving a delay of a few microseconds between laser desorption and application of the extraction potentials, the ion plume has time to expand into the field free region. Afterwards, ions with a higher axial velocity are located further away from the repeller plate than ions with lower initial axial velocity. Once the extraction voltage is applied, the ions are accelerated at the same rate, but over different distances, achieving a time focusing in the detection plane according to the description of case *b*. The two remaining effects (*c* and *d*) limiting the ToF resolution in terms of the ion extraction can thus be compensated for using a delayed extraction method.

It has previously been shown that the delayed extraction method is a very useful extension to linear ToF mass spectrometry [88]. However, this extraction technique is not ideally suited to applications in microscope mode imaging mass spectrometry, as a field free expansion of the ion plume leads to a diffusion of the ion trajectories coming off the sample surface. The spatial information is therefore reduced before the ion extraction occurs, and may not be reconstructed to obtain an image of the surface. Hence, imaging mass spectrometry in microscope mode has mainly been performed using a direct ion extraction. By using this method, a reasonably high spatial, but a low ToF resolution is typically achieved (see Chapter 2).

Recently, Aoki and coworkers have introduced a novel ion extraction technique, named post extraction differential acceleration (PEDA), to gain higher ToF resolution for a small portion of the mass spectrum [158]. The advantage of the postulated technique for applications in microscope mode imaging mass spectrometry is that the spatial resolution is maintained whilst the ions are extracted from the sample plate directly. The ion optic employed was based on five electrodes, consisting of a repeller and an extractor electrode, followed by an einzel lens. In operation, the extractor electrode was pulsed to a higher potential once the ions of interest were situated between the extractor and the einzel lens. Thereby, the differential acceleration allowed the initial velocity spread of the ions coming

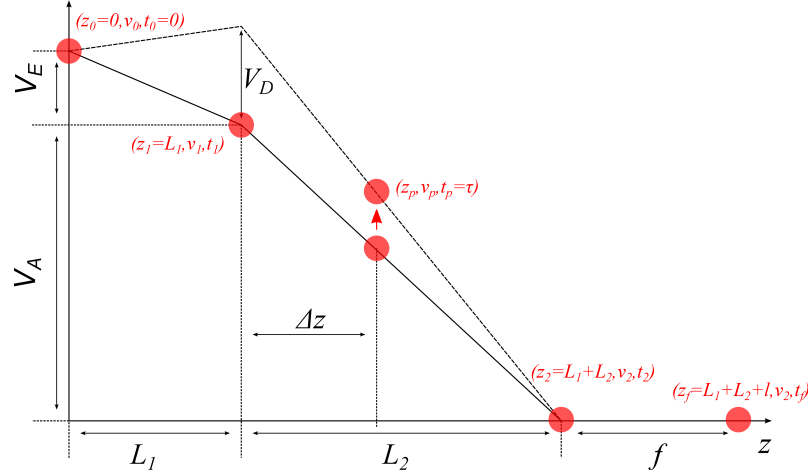


Figure 3.2: Graphical description of the PEDDA extraction method. See text for details. [158]

off the surface to be corrected, which resulted in a time-focusing effect in the detection plane.

The process of the PEDDA method is illustrated in Figure 3.2. For the mathematical description of the technique, it can be assumed that all the singly charged ions of the mass m are generated directly on the repeller (z_0) at the time t_0 . The initial velocity of the particles is labelled as v_0 . The further extraction and acceleration of the ions occurs through the electric field gradient caused by the voltage difference of V_E between the repeller and the extractor electrodes. When the ions pass the extractor electrode, after flying a distance L_1 in the time t_1 , their velocity v_1 can be calculated by vector addition, taking the initial velocity v_0 and the velocity v_a , which the particle gained in the electric field, into account. Explicitly,

$$v_1 = \sqrt{v_0^2 + v_a^2} = \sqrt{\frac{2}{m} \left(\frac{m}{2} v_0^2 + eV_E \right)}, \quad (3.1)$$

where e is the elementary charge. The velocity v_1 can also be described in the time domain:

$$v_1 = v_0 + at_1 = v_0 + \frac{eE}{m} t_1 = v_0 + \frac{eV_E}{mL_1} t_1, \quad (3.2)$$

where E is the electric field strength ($E = V_E/L_1$) and L_1 the distance between the repeller

and the extractor electrode. By combining the Equations 3.1 and 3.2 it follows:

$$L_1 = v_0 t_1 + \frac{eV_E}{2mL_1} t_1^2. \quad (3.3)$$

Solving the equation for the time t_1 results in the expression:

$$t_1 = -\frac{v_0 m L_1}{eV_E} \left(1 - \sqrt{1 + \frac{2eV_E}{mv_0^2}} \right). \quad (3.4)$$

The equation above describes the time at which the ions leave the extraction region between the repeller and extractor, and enter the post extraction region of the ion optics, located between the extractor and the third, grounded electrode.

The electric field in the post acceleration region can be described by V_A/L_2 , where V_A is the extractor potential and L_2 the distance between extractor and third lens. To enable time focusing in the detection plane, the applied voltage on the extractor electrode is increased at the switch time ($t = \tau$), while the ions are located at the position $z = z_P = z_1 + \Delta z$. The velocity of the ions at z_P can be defined as v_P . Δz describes the distance the ions have travelled between the extractor electrode and the einzel lens prior to pulsing. This distance can be calculated according to Equation 3.3:

$$\Delta z = v_1 (\tau - t_1) + \frac{eV_A}{2mL_2} (\tau - t_1)^2. \quad (3.5)$$

The ion velocity at the point z_P depends on the initial ion velocity, and on the kinetic energy the particle gains in both acceleration fields. For the calculation, Equation 3.1 is used, taking into account the energy conservation law:

$$v_P = \sqrt{\frac{2}{m} \left(\frac{m}{2} v_0^2 + e \left(V_E + V_A \frac{\Delta z}{L_2} \right) \right)}. \quad (3.6)$$

To calculate the final ion velocity when the ions leave the ion optics, the potential after increasing the extractor voltage needs to be considered. At the switch position z_P , the electric field can be determined as follows:

$$\Delta V = V_D \left(1 - \frac{\Delta z}{L_2} \right). \quad (3.7)$$

Once the ions leave the extraction and differential acceleration region ($z_2 = L_1 + L_2$) at the time t_2 , the final velocity v_2 can be determined by:

$$v_2 = \sqrt{\frac{2}{m} \left(\frac{m}{2} v_0^2 + e (V_E + V_A + \Delta V) \right)}. \quad (3.8)$$

According to Equation 3.3 it can be written:

$$L_2 - \Delta x = v_P (t_2 - \tau) + \frac{e}{2m} \frac{(V_A + V_D)}{L_2} (t_2 - \tau)^2 . \quad (3.9)$$

Solving Equation 3.9 for t_2 results in:

$$t_2 = \tau + \frac{mv_P L_2}{e(V_A + V_D)} \left(-1 + \sqrt{1 + \frac{2e(V_A + V_D)(L_2 - \Delta x)}{v_P^2 m L_2}} \right) . \quad (3.10)$$

The equations above allow the velocity and the time an ion passes a certain position within the ion optic to be calculated as a function of the switch time and the applied potentials.

To understand the time focusing of the PEDDA method it is necessary to compare ions with high and low initial kinetic energies. The ions get extracted directly in the region L_1 , leading to a velocity spread observed for v_1 . According to Equation 3.1, the velocity spread depends on the extraction potential V_E : if a weak extraction field (V_E small) is applied, the initial axial velocity spread of v_0 has a much larger effect on the calculated ion velocities at the end of the extraction region, while, if a strong extraction field (V_E large) is applied, the overall ion velocity spread is less dependent, on the initial kinetic energy of the particles (i.e., the second term Equation 3.1 becomes more dominant).

At the switch time ($t = \tau$), the potential applied to the extractor is increased. At this moment, the ions with a higher initial axial velocity have travelled further through the post extraction region than the ones with a lower initial axial velocity. Hence, the travelled distance Δz varies slightly for the observed ions. As a result, the final velocity v_2 of the ions located closer to the extractor electrode will be higher than the one observed for ions which have travelled further through the PEDDA region prior to switching (see definition of the final velocity in Equation 3.8). After the ions have left the ion optics they travel through a field free region of length l and terminate at the detector. The time it takes the ions to pass through this field free region is defined as l/v_2 . Hence, the total ion ToF, t_{ToF} , can be determined by:

$$t_{\text{ToF}} = t_2 + \frac{l}{v_2} . \quad (3.11)$$

As the position of the electrodes and the total length of the flight tube are fixed in an experiment, the switch time τ and the applied voltages must be chosen in a way that

ions with a lower initial axial velocity are closing up in the detection plane with the ones, which left the sample surface with a higher initial velocity. As a result, an enhanced ToF resolution can be observed.

Aoki *et al.* demonstrated in their proof of concept experiment using a crystal violet sample that the achievable ToF resolution was improved from $t/2\Delta t = 60$ for a direct ion extraction to $t/2\Delta t = 1450$ employing the PEDDA method. By operating the same spectrometer with the delayed extraction technique a ToF resolution of $t/2\Delta t = 1572$ was determined. However, in the latter experiment no spatially resolved ion image was observed. Comparing these results, it can be concluded that the PEDDA method corrects the initial kinetic energy spread nearly as well as the established delayed extraction technique.

One of the major shortfalls of PEDDA is that the initial kinetic energy spread of the ions is corrected while the ions fly through the mass spectrometer. Therefore, only the ions which are situated at the switch time between the extractor and the first grounded lens are affected. Furthermore, this correction is not similar for each axial point within the post extraction region. The best correction is achieved for ions located exactly in the middle between both plates, when the extractor potential is raised. The applicable mass range was estimated to be $\pm 20\%$ from the optimum. Unfortunately, it was not detailed how much the ToF resolution varied within in this range. The spectrometer used in the publication from Aoki *et al.* was designed to image samples of the size of $300 \times 300 \mu\text{m}$. The spatial resolution for this field-of-view was determined to be between $4.0 \mu\text{m}$ and $5.5 \mu\text{m}$, using a 20% to 80% intensity rise fitting method [157].

Employing the PEDDA technique provides an enhanced ToF resolution while conserving the spatial distribution of the ions ejected from a sample surface. However, a number of questions still need to be addressed. For instance, is it possible to tune the optimised ToF mass range to allow imaging of other ions of interest in a separate experiment? What are the limits in terms of ToF resolution achievable? What is the time separation between individual particles of different masses, and is it possible to employ the method for larger fields of view? These questions will be addressed in the following sections of this chapter.

3.2 Designing a PEDA Optic

The implementation of the PEDA method into the linear IMS instrument employed in Chapter 2 requires a major redesign of the ion optics. For this purpose, ion trajectory simulations were performed to optimise the five electrode assembly from Aoki and coworkers for a field-of-view of 4 mm in diameter. About 70 different ion optic designs were modelled, adjusting the position, the sizes, and the aperture diameters of the electrostatic lenses. The field-of-view was increased stepwise from an initial value of 300 μm to the final 4 mm. Within each iteration, all geometry parameters were optimised to achieve the best possible spatial and ToF resolutions. In this subsection, the pulsing mechanism for the SIMION 8.0 simulations, the final design of the ion optic, and a discussion of geometric parameter are presented.

3.2.1 Pulsing Scheme for the PEDA Simulations

To simulate the PEDA process accurately, it is essential to provide a sensible function of the rise time for the switching process of the extractor electrode. Based on previous experiments undertaken in this laboratory, a rise time of about 40 ns can be assumed for the electrode potentials to be adjusted using a fast, high voltage switch [159]. Furthermore, the shape of the voltage pulse was assumed to follow an arctan function, so that the total applied potential can be calculated according to

$$V_{\text{Applied}} = V_{\text{Ex}} + \left(\frac{V_{\text{High}} - V_{\text{Ex}}}{\pi} \right) \times \left[\arctan \left(\left(\frac{4\pi}{\Delta t_{\text{rise}}} \right) \left((t_{\text{ToF}} - t_{\text{switch}}) - \left(\frac{\Delta t_{\text{rise}}}{2} \right) \right) \right) + \frac{\pi}{2} \right], \quad (3.12)$$

where V_{Ex} stands for the initial extractor potential, V_{High} the final extractor potential, Δt_{rise} for the rise time, t_{ToF} for the total ToF, and t_{switch} for the switch time. A typical voltage time plot for the switching process is presented in Figure 3.3. Here, the extractor was pulsed at the switch time of 2.86 μs from $V_{\text{Ex}} = 4730 \text{ V}$ to $V_{\text{High}} = 5400 \text{ V}$. The rise time was set to be 40 ns. It can be noted that, at the switch time, the applied function has already risen to 5% of the entire voltage step (potential difference). In this way, it

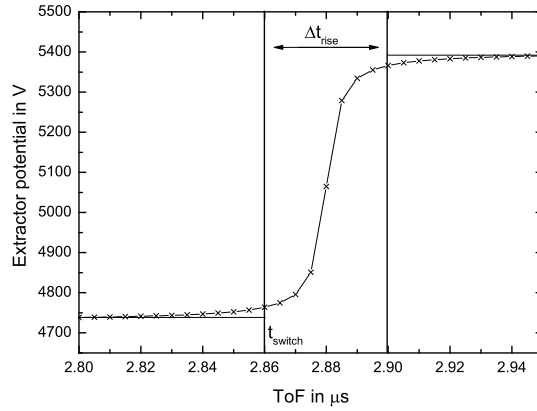


Figure 3.3: Typical rise time function applied in the ion trajectory simulations. The extractor potential was raised from 4730 V to 5400 V within a time of 40 ns. The switch time was set to 2.86 μ s.

was ensured that only the steeper section of the rise function was applied to change the extractor potentials. Once the rise time expired, the function had reached 95% of the entire voltage step, and the remaining difference was changed instantly. The function was included in all PEDDA ion trajectory simulations.

3.2.2 Ion Optic Design and Operation

In order to redesign a similar ion optic for the IMS instrument in Oxford, which is optimised for an enhanced field-of-view of 4 mm in diameter, a number of modified electrode assemblies needed to be analysed in terms of their ToF and spatial imaging properties. For the ion trajectory simulations the initial ion parameters were set according to the description in Chapter 2. The determination of the mass spectrometer parameters followed the procedures introduced in Section 2.1.3. Each spectrometer was optimised for an ion mass of 390 Da. For each presented data point, six independent simulations employing different ion sets were performed to determine the average resolutions and the corresponding standard deviation.

In operation, the achievable ToF and spatial resolution of each individual ion optic design depended on five parameters: the repeller potential, the initial extractor potential,

the extractor potential after pulsing, the pulse time and the applied voltage to the einzel lens (first electrode grounded, second charged, last grounded). To find the optimum operation settings, the simulations were performed iteratively. In the first step, the repeller potential was set to 5000 V, the extractor to 4700 V and the einzel lens to 1800 V. Without pulsing the extractor plate, both the extractor and the einzel lens potentials were adjusted to give the best spatial resolution on the detector. Once the ions were spatially mapped as four separated spots, the switch time for the extractor pulse was set in a way that the 390 Da ions were situated halfway between the extractor and the first electrode of the einzel lens. The applied extractor potential after pulsing was set to 5400 V. To optimise the mapping conditions, each ion optic potential was adjusted for spatial mapping. Once the best spatial settings were found, the switch time was readjusted, so that the highest ToF resolution was observed. This step was recapped with another spatial mapping optimisation procedure, so that after several iterations both the optimum spatial and ToF resolution for the designed ion optic were determined.

By observing the ion trajectories, the applied electric field lines, and the resulting ToF and spatial resolution as functions of the electrode design, some general trends became obvious. For example, it was found that the ToF resolution of the initial instrument of Aoki and coworkers could be improved significantly by reducing the aperture size of the einzel lens. Additionally, it was noted that the further apart the extractor and the einzel lens were, the higher the observed ToF resolution became. The reason for this is that the initial velocity spread could be corrected more accurately. However, this coincided with a reduction in the observed spatial resolution. Furthermore, simulations have shown that the aperture size of the extractor needs to be rather large, to create parallel field lines in the post acceleration region. This ensured that all ions located between the extractor and the einzel lens experience the same electrical force to accelerate them towards the detector in the PEDAs stage. In this way, aberrations may be avoided.

The final design of the ion optic is illustrated in Figure 3.4. Each electrode has a diameter of 100 mm and a thickness of 1.10 mm. The distance between the repeller and

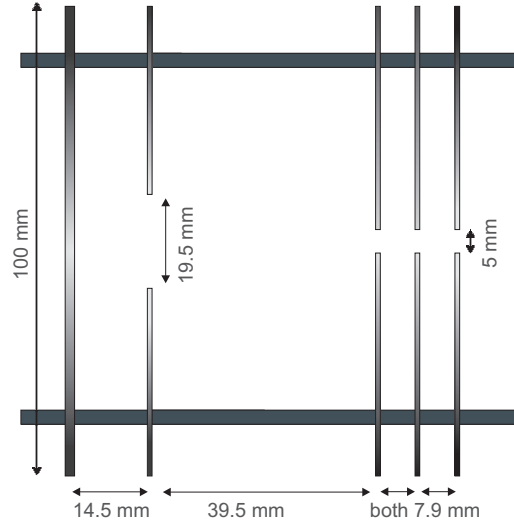


Figure 3.4: Illustration of the final ion optic design, optimised to operate with the PEDDA extraction technique. The total flight tube length, measured from the repeller electrode to the detector, was 980 mm.

the extractor is 14.5 mm. The extractor had an aperture of 19.5 mm in diameter while the einzel lens, consisting of three identical disks with 5.00 mm apertures and a spacing of 7.90 mm, was mounted at a distance of 39.5 mm from the extractor. The total flight length, from the repeller to the detector was 980 mm. For ions of the mass 390 Da the ion optic was operated at its optimum specifications by applying 5000 V to the repeller, 4730 V to the extractor and 1800 V to the einzel lens. The extractor potential was raised at the switch time of 2.80 μ s to 5400 V. Employing these settings, a spatial resolution of 20 μ m, a ToF resolution of $t/2\Delta t = 2500$ and a magnification factor of 10.5 $\times$ were determined.

In order to explore the mass-dependent focusing properties of the ion optic, the optimised settings were applied and a mass scan performed. Figure 3.5 shows illustrative plots, including the mass-dependent ToF and spatial resolutions, as well as the standard deviation of the spatial resolution, and the magnification factor. The first plot indicates a ToF resolution exceeding a value of $t/2\Delta t = 1000$ in a mass range from 340 Da to 430 Da, peaking at the optimised mass of 390 Da. The observed peak structure of the plot can be explained by the fact that only the initial velocity spread of the ions situated between the extractor and the einzel lens is corrected. Ions located at the switch time in other parts of the ion optic are mapped with a similar ToF resolution as the one obtained with the VMI

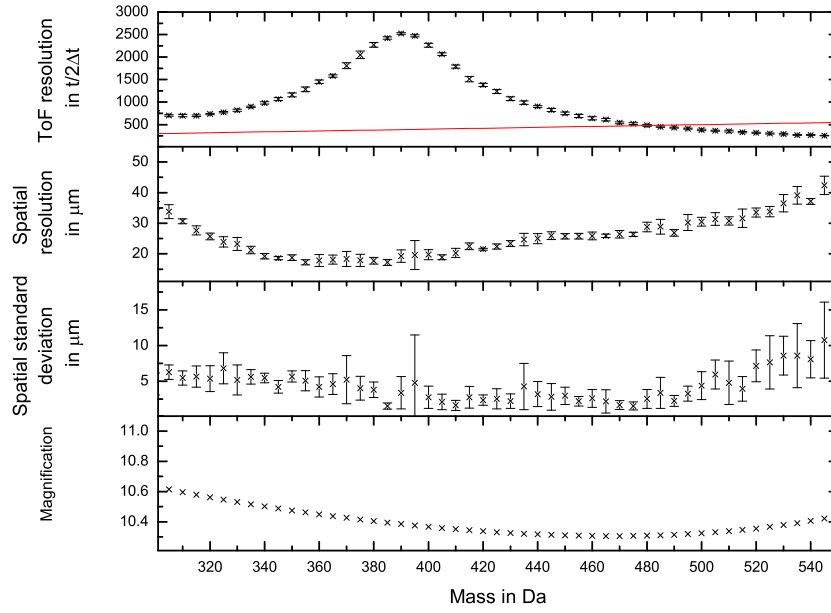


Figure 3.5: Mass dependent spectrometer specifications determined using the PEDAs extraction technique. The simulated instrument was optimised to show the highest ToF and spatial resolution at 390 Da.

optic described in the previous chapter. The red line in the time resolution plot indicates the ToF resolution which is necessary to allow the distinction of peaks separated by 1 Da (unitary mass resolution). This value is exceeded for the entire spectrum, up to 480 Da.

The optimum spatial mapping conditions are found in a narrow range between 320 Da and 460 Da. In the lower and higher mass regions, the spatial resolution decreases to values above $25\ \mu\text{m}$ as the spatial focus plane shifts along the ToF axis as a function of mass. Furthermore, heavy ions are still located in the extraction area between the repeller and the extractor plate when the extractor potential is increased. Due to the pulsing, the field gradient in this region is inverted so that for the relevant ions the spatial mapping plane is no longer located in the detection region. All the applied potentials are chosen in such a way that the additional acceleration caused by pulsing of the extractor plate results in a spatial mapping of the ions of interest. Therefore, it is also no longer possible to spatially map the ions which have already passed the post extraction region. The standard deviation plot of the spatial resolution of the four spots shows a reasonable low deviation of $\sim 5\ \mu\text{m}$ in the overlapping optimised ToF and spatial mass range. Hence, it can be assumed

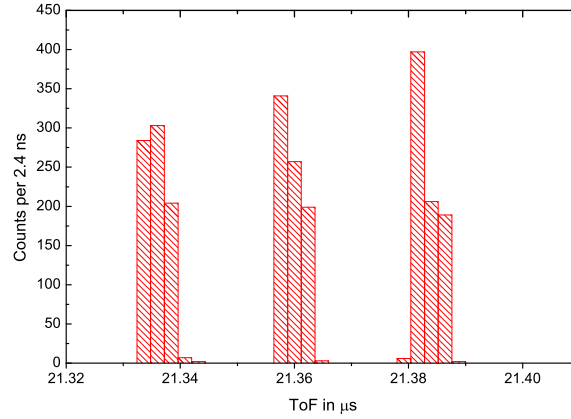


Figure 3.6: Simulated ToF spectrum for ions of the mass 389 Da, 390 Da and 391 Da. The time separation between these signals was found to be on the order of 22 ns. No detector convolution was applied to the spectrum.

that little distortions of the image are expected. The magnification factor shows a slightly decreasing trend over the range of interest, between 340 Da to 430 Da. However, as the change in image magnification is rather small, no major effect on the image properties are expected.

The timing specifications of a ToF-MS instrument are not only defined by its ToF resolution. It is also essential that the mass separation allows the detection of adjacent peaks with the detection system employed. A typical ToF spectrum simulated for the developed ion optic is presented in Figure 3.6. Three ToF peaks corresponding to the ion masses of 389 Da, 390 Da and 391 Da are shown. The time separation between two adjacent peaks was approximately 22 ns. Furthermore, the peak width was determined to be 8 ns. It should be pointed out that the presented ToF spectra do not include a detector convolution. However, it is immediately obvious that by employing a P47 scintillator screen in an imaging detector the achievable ToF resolution will be reduced significantly.

Following the simulation results presented, it can be concluded that the PEDAs technique can be applied to enhance the ToF resolution for a 4 mm diameter field-of-view. However, by increasing the observable area of the spectrometer, the spatial resolution was decreased to 20 μm , which is significantly worse than the 4.0 μm to 5.5 μm resolution

reported by Aoki and coworkers [158]. The expected ToF resolution was simulated to be $t/2\Delta t = 2500$, which would correspond to the best achieved resolution reported for a linear microscope mode IMS instrument so far. Nevertheless, PEDA, in its current design, is not a technique which could be used for scientific mass spectrometric analysis as the mass range in which the ToF resolution exceeds $t/2\Delta t = 1000$ is limited to 100 Da around the optimised mass peak. Therefore, the technique needs to be modified to be applicable to a broader mass range.

3.3 Mass Tunability

The specifications of a microscope mode imaging mass spectrometer are defined by its magnification, spatial resolution, ToF resolution, and accessible mass range. The value of each of these parameters is a result of the mass dependent focusing properties of the ion optic. An ideal spectrometer would operate under optimum and constant specifications across a broad mass range. As suggested from the simulations undertaken above, it was noted that the applicable mass range for the PEDA method is rather narrow, so that the technique is limited to only a few experimental studies. To address this limitation, in this section the mass tunability of the spectrometer specification is explored. The geometry of the ion optic is maintained, while the spectrometer is operated under slightly different conditions to access multiple mass ranges. Even though multiple experimental runs would be necessary to record multiple ion images for various masses over a wide mass range, the advantage of the rather large field-of-view and the enhanced mass spectrometer specifications would still be realised.

3.3.1 Delayed Pulse Optimisation

The first approach to adjust the optimised mass range of the PEDA IMS instrument was based on delaying the pulse time of the extractor electrode. The extended delay time thus ensures that heavier ions are located between the extractor plate and the einzel lens when

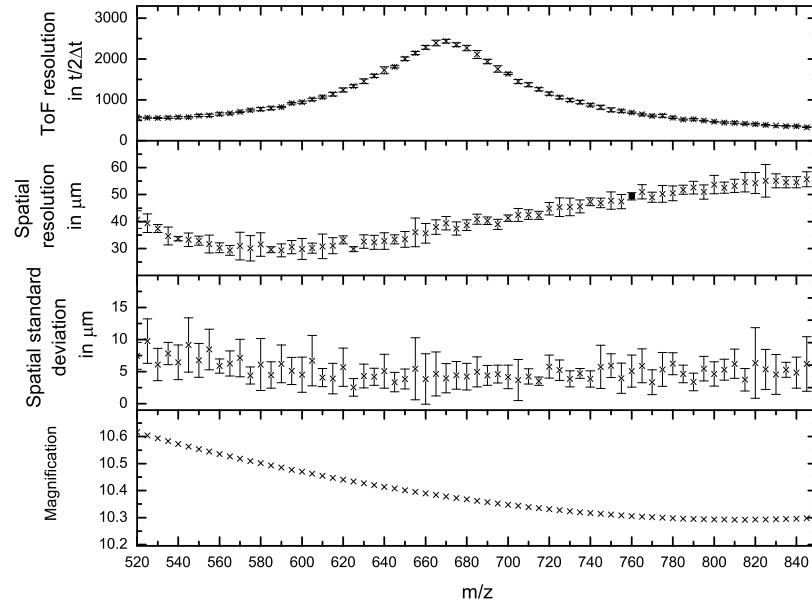


Figure 3.7: Mass spectrometer specifications obtained using PEDa at a delay time of $3.64 \mu\text{s}$. The overlap between the spatial and ToF optimised mass range has been lost, leading to a reduction in the spectrometer specifications.

the post acceleration potential is applied. In Figure 3.7, the mass spectrometer specifications are presented for a mass range between 520 Da and 850 Da. The delay time was adjusted to $3.64 \mu\text{s}$, which resulted in a ToF focusing of the 680 Da ion peak. An optimum ToF resolution $t/2\Delta t = 2430$ was achieved, which is slightly lower than the resolution obtained for 390 Da. Furthermore, in Figure 3.7 can be noted that the best spatial resolution is never better than $35 \mu\text{m}$ at the optimised ToF resolution. Adjustments to the applied extractor, pulsed and einzel lens potentials allowed, in further simulations, to improved this value to $33 \mu\text{m}$. However, a completely restoration of the mass spectrometer specifications achieved for the 390 Da ions was impossible. Therefore, a more advanced method needs to be established to shift the optimised mapping conditions to ions of different masses.

3.3.2 Velocity Corrected Ion Extraction in Imaging Mass Spectrometry

The operation principle of a ToF mass spectrometer is based on the fact that a range of ions is accelerated in a constant electric field so that lighter ions gain a higher final velocity than heavier ones. Due to the kinetic energy distribution, the trajectories that ions follow along the spectrometer will also vary, thus leading to different spatial focusing properties on the detector. Therefore, it is impossible to build an ion optic which gives a constant spatial resolution over a wide mass range. An approach to extend the applicable mass range of PEDDA is to turn this principle around: by accelerating ions of different mass in separate experiments to the same final axial ion velocity, it should be possible to spatially-map both sets of ions onto the same detection position. As a result of this, the spatial mapping and ToF resolution would be maintained. It should be noted that throughout this section the spatial and ToF mapping parameters are treated as functions of the particle's velocity in the detector direction (axial velocity). This term may be replaced by the particle's kinetic energy.

The principle described above provides the advantage that for spatial mapping of multiple different mass ranges no modifications of the ion optics are required. A theoretical and simulated approach defining the mass spectrometer parameters for four different mass ranges will be presented in the following sections.

3.3.2.1 Charged Particles in a Uniform Electric Field

For the design and for the understanding of an ion optic it is essential to predict the trajectories of ions or electrons. These can be determined for an electrostatic element using linear equations. In this section, an example calculation for a charged particle passing through a uniform electric field will be presented. On the basis of the determined equations the principle of the mass-tuning capabilities of a mass spectrometer will be discussed. All the equations employed were based on the first chapter of the book "*Charged particle optics*" by Liebl [160].

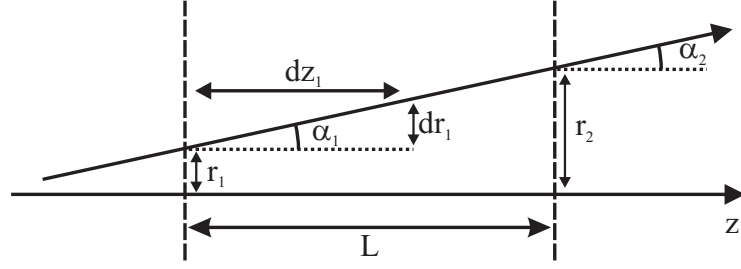


Figure 3.8: Diagram describing the trajectories of a particle crossing a field free drift space along the ToF axis, z .

The trajectory of a particle crossing a cylindrically symmetric system such as that shown in Figure 3.8 can be described by two parameters: the distance from the optical axis z is given by r_i ; and the slope in flight direction, which can either be defined by $r'_i = dr/dz$, or as a function of the angle, α_i , between the optical axis z and the flight direction ($r'_i = dr/dz = \tan \alpha_i$). Figure 3.8 shows the trajectory of a particle travelling through a field free drift space of the length L . The position and the flight direction of the particle at the end of the flight tube can be determined using the linear algebra definitions

$$r_2 = r_1 + Lr'_1 \quad \text{and} \quad r'_2 = r'_1 .$$

These equations are rather simple in comparison to the case, in which a particle travels through a charged electric field. This is illustrated in Figure 3.9. Here, a charged particle enters in the z direction a uniform electric field, which expands between the planes P_n and P_{n+1} . The corresponding applied potentials are labeled V_n and V_{n+1} . Both spaces to the left of P_n and to the right of P_{n+1} are field free. The initial velocity for a particle entering the electric field at P_n can be defined as a function of the initial kinetic energy, $E_{\text{Kin},n}$, and the particle's mass, m :

$$v_n = \sqrt{\frac{2E_{\text{Kin},n}}{m}} . \quad (3.13)$$

If the kinetic energy is defined as a function of an initial potential ($E_{\text{Kin},n} = qeV_n$) then the particle velocity can also be defined as

$$v_n = \sqrt{\frac{2E_{\text{Kin},n}}{m}} = \sqrt{\frac{2qeV_n}{m}} , \quad (3.14)$$

where q is the charge of the particle and e is the elementary charge.

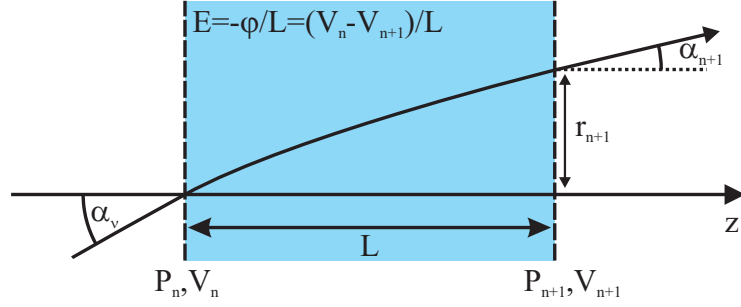


Figure 3.9: Illustration of the trajectories of a charged particle travelling through a uniform electric field between P_n and P_{n+1} .

If a positively charged particle passes through the electric field, as shown in Figure 3.9, its kinetic energy is either increased, for $V_n > V_{n+1}$, or decreased, for $V_n < V_{n+1}$, depending on the applied electric field. Therefore, the final kinetic energy of the ion depends on both the applied electric field and the initial particle potential defined above:

$$E_{\text{Kin},n+1} = qe (V_n + (V_n - V_{n+1})) . \quad (3.15)$$

According to Equation 3.15, the final particle velocity can be defined as:

$$v_{n+1} = \sqrt{\frac{2qe (V_n + (V_n - V_{n+1}))}{m}} . \quad (3.16)$$

The Equations 3.15 and 3.16 allow the general calculation of the kinetic energy and the ion velocity for any particle motion in an electric field.

The trajectory in Figure 3.9 shows the case in which an ion does not fly parallel along the ToF axis, z . Therefore, the definition of the particle velocity from above can be used in combination with the differential equation of motion to determine the particle's axial velocity in the z direction. Before the particle enters the electric field, its velocity, dz_n/dt , can be calculated according to:

$$\frac{dz_n}{dt} = v_{z,n} = v_n \cos \alpha_n . \quad (3.17)$$

The particle's velocity in the orthogonal direction, r , is given by:

$$\frac{dr_n}{dt} = v_n \sin \alpha_n . \quad (3.18)$$

Once the particle has entered the electric field between P_n and P_{n+1} the velocity dz/dt along z does not only depend on the initial particle velocity at P_n . Moreover, the acceleration

within the electric field needs to be taken into account. The time dependent velocity along z may be:

$$\frac{dz}{dt} = v_n \cos \alpha_n + \frac{eqE}{m}t. \quad (3.19)$$

Here, the electric field strength is given by $E = (V_n - V_{n+1})/L$, where L is the distance between P_n and P_{n+1} along z . According to Equation 3.19 it can be written:

$$z = v_n t \cdot \cos \alpha_n + \frac{eqE}{2m}t^2. \quad (3.20)$$

As the applied electric field is completely uniform in the plane orthogonal to z , the speed in r direction depends only on the initial velocity at P_n , and can be written as:

$$\frac{dr}{dt} = \frac{dr_n}{dt} = v_n \sin \alpha_n. \quad (3.21)$$

For the case that the position $r_n \neq 0$, the ion starting position is shifted and needs to be taken into account by:

$$r = r_n + v_n t \cdot \sin \alpha_n. \quad (3.22)$$

These differential equations of motion allow the calculation of the position and the velocity of a given particle as a function of the time it has spent in the electric field. To define the final parameters r_{n+1} and r'_{n+1} at P_{n+1} , r needs to be defined as a function of z . For this purpose, the time t can be determined from Equation 3.20:

$$t = \frac{mv_n}{eqE} \left(\sqrt{\cos^2 \alpha_n + \frac{2eqE}{mv_n^2}z} - \cos \alpha_n \right). \quad (3.23)$$

By replacing t in Equation 3.22, r can be determined according to:

$$r = r_n + \sin \alpha_n \cdot \frac{mv_n^2}{eqE} \left(\sqrt{\cos^2 \alpha_n + \frac{2eqE}{mv_n^2}z} - \cos \alpha_n \right). \quad (3.24)$$

The Equation 3.24 describes the trajectory of the particle as a function of z . The value of r_{n+1} can be determined by setting $z = L$:

$$\begin{aligned} r_{n+1} &= r_n + \sin \alpha_n \cdot \frac{mv_n^2}{eqE} \left(\sqrt{\cos^2 \alpha_n + \frac{2eqE}{mv_n^2}L} - \cos \alpha_n \right) \\ &= r_n + r'_n \cdot \frac{mv_n L}{eq(V_n - V_{n+1})} \left(\sqrt{\cos^2 \alpha_n + \frac{2eq(V_n - V_{n+1})}{mv_n^2}} - \cos \alpha_n \right). \end{aligned} \quad (3.25)$$

The slope at P_2 can be found by differentiating Equation 3.25 with respect to z , at the

distance L :

$$\begin{aligned} r'_{n+1} &= \frac{dr_{n+1}}{dz} = \frac{dr_{n+1}}{dL} \\ &= r'_n \cdot \frac{mv_n}{eq(V_n - V_{n+1})} \left(\sqrt{\cos^2 \alpha_n + \frac{2qe(V_n - V_{n+1})}{mv_n^2}} - \cos \alpha_n \right). \end{aligned} \quad (3.26)$$

The final ion velocity in the z direction is dependent on the initial velocity and the velocity gained within the electric field region. It can be readily shown to be given by the expression

$$v_{z,n+1}^2 = v_{z,n}^2 + \frac{2eq(V_n - V_{n+1})}{m}, \quad (3.27)$$

which can be written as:

$$v_{z,n+1} = \frac{dz_{n+1}}{dt} = \sqrt{v_{z,n}^2 + 2\frac{eq(V_n - V_{n+1})}{m}}. \quad (3.28)$$

By combining Equation 3.17 with the assumption $v_{z,n} = dz_n/dt$, the speed in the z direction at point P_2 can be calculated to first order.

In the case that the initial particle velocity is independent of the particle mass (see the description of the MALDI ion plume in Chapter 2), it can be seen that, after passing through a uniform and constant extraction field, the ToF, the final position, the flight direction, and the velocity in z direction are all only dependent on the mass m of the particle (see Equations 3.23, 3.25, 3.26 and 3.28). As a result, it is clear that in a mass spectrometric experiment two particles of different masses are firstly detected after a different flight time, and secondly mapped onto a different position on the detector. Hence, the observed spatial and ToF resolution, as well as the magnification factor of a spectrometer, vary for different particles.

However, it is also important to note that in the Equations 3.23, 3.25, 3.26 and 3.28, the ToF, the final position, the flight direction, and the velocity in z direction, can be maintained by keeping the ratio $(eq(V_n - V_{n+1}))/m$ constant. Hence, for two particles of different mass-to-charge ratios, it is possible to adjust the acceleration potential according to the ratios

$$\frac{(V_n - V_{n+1})_1}{(V_n - V_{n+1})_2} = \frac{m_1/q_1}{m_2/q_2} = S, \quad (3.29)$$

where S is a linear scaling parameter. Within the simple model presented above, all the

specifications describing the particle trajectories are preserved.

In the case that this principle is applied to an entire imaging mass spectrometer, it should be possible to re-tune the ion optics by adjusting the strength of the applied electric field so that any particle of interest can be detected with the same spatial and ToF resolution. This means for the PEDDA instrument that multiple ion optic settings need be defined, in which a range of ion masses can be imaged. Hence, the spectrometer can be tuned to collect ion images over multiple ion mass ranges with the same resulting spectrometer specification.

3.3.2.2 Velocity Corrected Ion Extraction

The simple model discussed above indicates that the applied electric field strength should be adjusted according to the particle's mass-to-charge ratio. However, to simulate a real ion optic arrangement, higher order effects such as curved electric fields need to be considered. Therefore, instead of adjusting the applied electric fields only as a function of mass-to-charge ratio, the mapping conditions were matched by comparing the axial velocity distributions of the particles using the ion trajectory simulation program SIMION 8.0. The individual velocity of each ion was measured at the exit of the extractor electrode. A typical comparison of the resulting distributions obtained for two different ion masses is presented in Figure 3.10. An average velocity of $15.7 \text{ mm}/\mu\text{s}$ was found for a 390 Da ion extraction, while 480 Da ions travelled with a velocity $14.2 \text{ mm}/\mu\text{s}$ towards the detector. By adjusting the acceleration field, while preserving the voltage ratio between the repeller and extractor, the velocity of the 480 Da ions was matched to the one obtained in the initial 390 Da experiment. The determined scaling factor was then used to adjust all further potentials applied to the ion optic. Due to the fact that the optimised particles travel with the same velocity, it is reasonable to assume that the delay time for the extractor pulse can be retained at $2.8 \mu\text{s}$.

Following the velocity correction performed for the 480 Da ions, the applied electrode potentials were set to 6130 V on the repeller, 5799 V on the extractor, 6620 V as the pulse

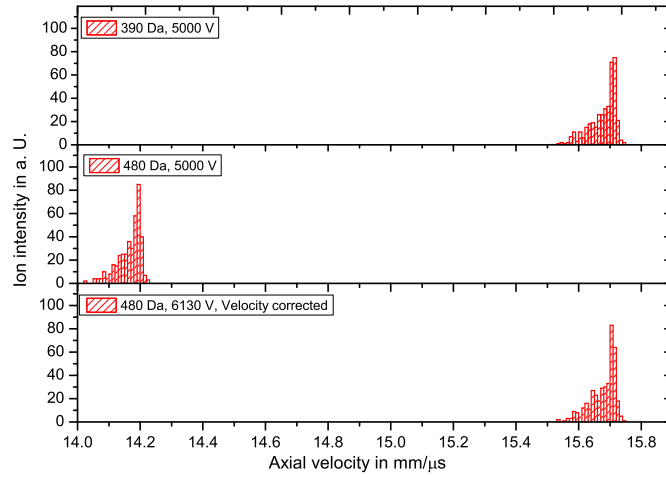


Figure 3.10: The ion velocity distribution (axial component) measured at the exit of the extractor electrode. By adjusting the acceleration field, while keeping the applied ratios the same, the velocity distribution of the 480 Da ions can be matched to the distribution obtained for the 390 Da ions.

voltage, and 2207 V on the einzel lens. These values corresponded to a potential increase by a factor of 1.226. Figure 3.11 shows the resulting, mass dependent spectrometer specification determined after velocity correction. Here, the ToF resolution, spatial resolution, standard deviation of the spatial resolution of the four spots and magnification are plotted as a function of the m/z values.

A comparison of the mass scans obtained for the 390 Da and the 480 Da settings (Figures 3.5 and 3.11) shows that the ToF resolution achieved for both optimised mass ranges peaks at $t/2\Delta t = 2500$. Furthermore, both scans show a ToF resolution exceeding $t/2\Delta t = 1000$ for a range spanning 100 Da around the optimised mass. The red line marking the unitary ToF resolution is not undercut for almost the entire mass range plotted. For both data sets, the spatial resolution ranges from 20 μm to 30 μm in the ToF optimised section of the mass spectrum. Thus, it can be concluded that, by employing an identical ion optic, it is not only possible to tune the optimised ToF resolution, but also the achievable spatial resolution. The two remaining parameters, the standard deviation of the spatial resolution and the magnification, confirm the tunability of the instrument,

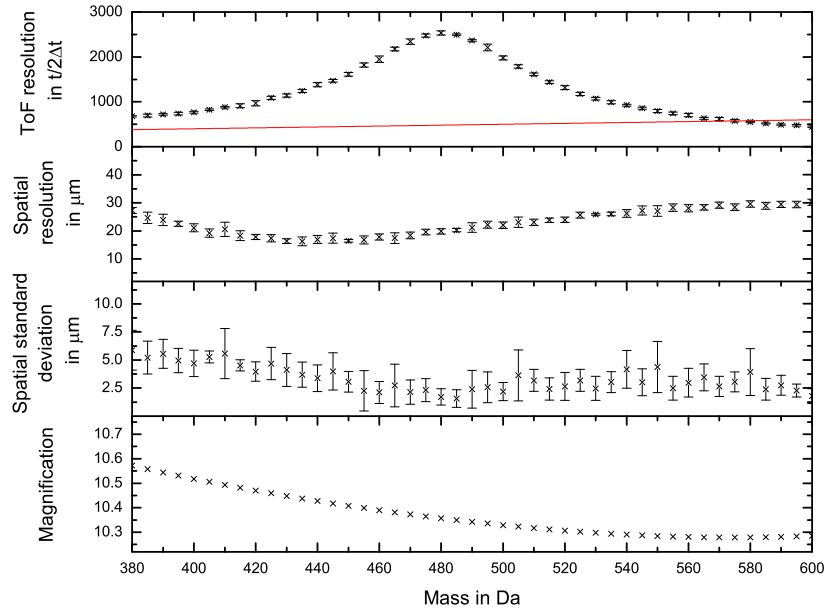


Figure 3.11: Mass spectrometer specification obtained after application of the velocity correction to the 480 Da ion set. An increase in the extraction field strength allowed to maintain the specifications which were previously determined for the 390 Da settings.

as again the same specifications were determined employing the 390 Da and 480 Da ion optic settings.

Following the concept of velocity correction, four adjacent mass ranges peaking at 390 Da, 480 Da, 580 Da and 680 Da were investigated using the ion trajectory simulations. All of these can be accessed by applying an extraction potential up to 10 kV. The electrode potentials determined are listed in Table 3.1 and the resulting mass spectrometer specifications for all four combined mass ranges are presented in Figure 3.12. The vertical lines

Peak in Da	Repeller in V	Extractor in V	Pulse in V	Einzel in V
390	5000	4730	5400	1800
480	6130	5799	6620	2207
580	7355	6957	7943	2648
680	8575	8111	9261	3088

Table 3.1: Applied ion optic potentials for the four mass ranges investigated. All settings were determined using the velocity correction in SIMION 8.0 (see text for details).

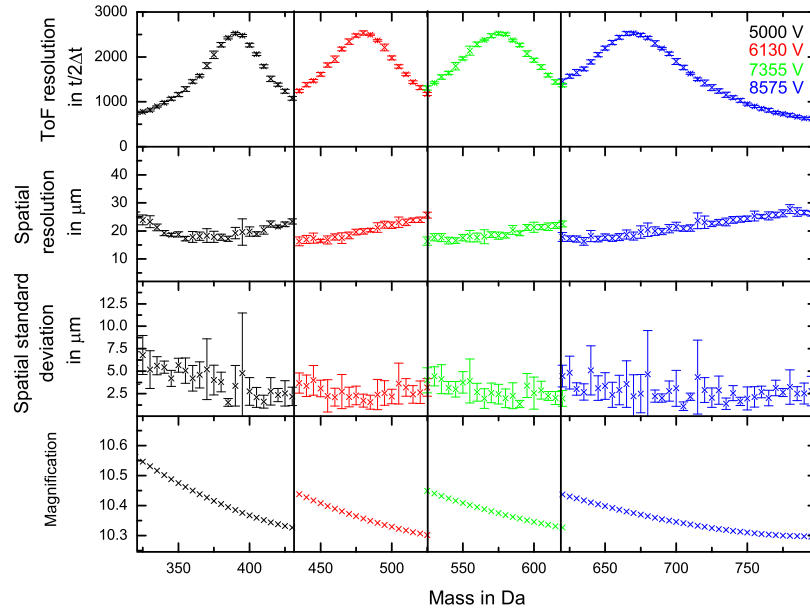


Figure 3.12: Mass dependent IMS specifications obtained using velocity corrected ion extraction over four independent mass ranges. The data sets demonstrate that the spectrometer specifications can be reproduced in each investigated mass range.

through the plot indicate the cutoff of one applicable mass range to another. As indicated by the ToF resolution plot, it can be noted that for the chosen optimised ion masses, the mass resolution across the entire range does not fall below a resolution of $t/2\Delta t = 1000$. In the case that a higher ToF resolution is required, the number of applied mass ranges may be increased. Figure 3.12 shows also that the spatial resolution is found to be no worse than $30 \mu\text{m}$ across the entire simulated mass range. Hence, together with the restored spatial standard deviation and magnification, it can be concluded that all the spectrometer specifications are preserved for throughout the velocity correction based simulations.

As the ion optic is optimised stepwise for an increasing ion mass, the strength of the employed extraction field is successively increased, which has the effect that the total flight time for the optimised ions is almost identical. As the constant ToF resolution indicates, these peaks are still sufficiently narrow, but their time separation is reduced due to the strength of the extraction field. This is obvious in Figure 3.13, where three consecutive peaks separated by one mass unit (389-391 Da and 679-681 Da) are presented as a function of their ToF using the 390 Da and the 680 Da settings. Both data sets are plotted in 2.4 ns

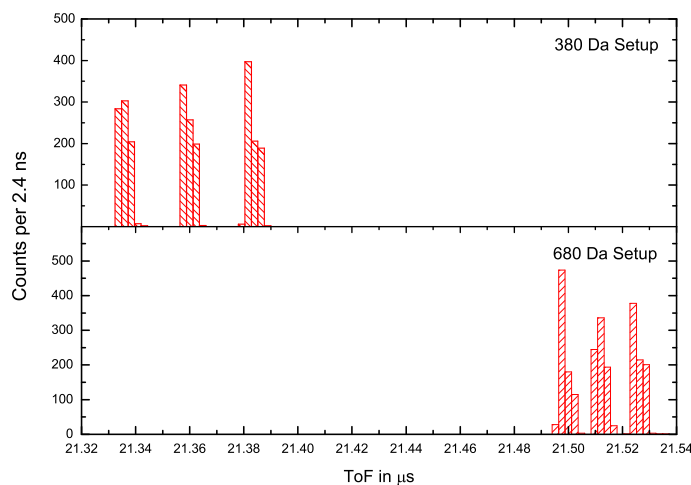


Figure 3.13: ToF spectra simulated for two ion optic settings: in the top panel the 390 Da ion optic settings were applied to simulate the flight times of ions of the mass 389 Da to 391 Da; in the bottom panel ToF spectra of the masses 679 Da to 681 Da are presented. These were simulated using the 680 Da ion optic settings.

wide time bins. The observed peak to peak time separation for the 680 Da settings is on the order of 12 ns, which is only half of the time measured for the low mass settings. As a result, state of the art fast imaging systems, such as the PImMS camera, may not be suitable to image the isotopic distribution of a sample in the higher optimised mass ranges.

3.4 Enhancing the Spatial Resolution - Introduction of an Aperture

The ion trajectory simulations undertaken to define the specifications of the PEDDA ion optic above were performed using the ion sets defined in Section 2.1.3. It was assumed that all the ions come off the surface with an average speed of 750 m/s in a $\cos^{12} \theta$ distribution. As a result of this distribution a significant number of ions are ejected with a high transverse velocity leading to an increased effect of aberrations in the observed ion image. However, as noted in Section 1.4.2, in microscope mode imaging mass spectrometry the ion trajectories pass through the velocity map imaging plane (back focal plane) before being spatially

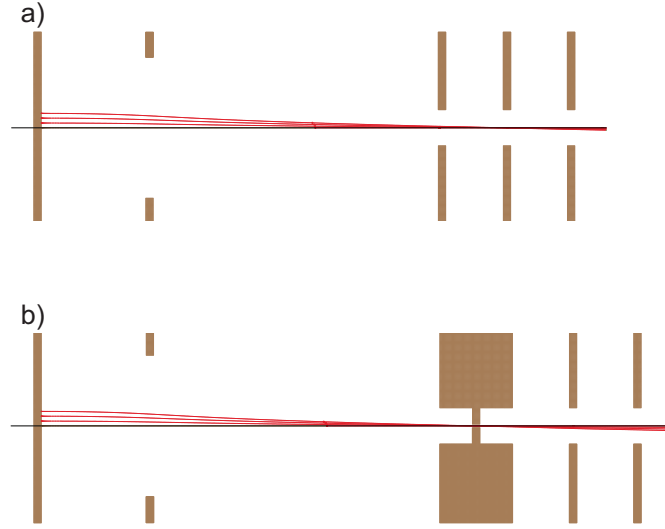


Figure 3.14: *a)* Ion trajectories of four particles coming off the surface with zero transverse velocity. The velocity map focus point is located directly in the middle of the einzel lens. *b)* Ion trajectories for a spatial map ion optic which employs an aperture to cut out ions with higher transverse velocity.

mapped onto the detector. In this plane, only the ions with zero transverse velocity are focused into the centre of the ion optic. The remaining particles are mapped depending on their transverse kinetic energy to cross the VMI plane further from the central axis. Hence, by placing a central aperture in the velocity map imaging plane of the mass spectrometer, it is possible to cut out those particles with higher transverse velocity. Thus, the aberration caused by the energy dependent focusing properties of the ion optics can be removed from the ion image, and the spatial resolution is improved.

For the PEDAs ion optic designed above, the velocity mapping plane was located in the middle of the einzel lens (See Figure 3.14*a*). Therefore, to introduce a grounded aperture into the ion optic, its basic design needed to be adjusted. This was initially done by moving the einzel lens several millimetres towards the detector and introducing two grounded electrodes. The first one of these was located at the same position as the einzel lens in the original assembly. In this way it was ensured that the post extraction acceleration field extended as far as in the first PEDAs spectrometer designed. The second electrode introduced was the aperture, located just in front of the einzel lens. In later configurations, the two newly introduced lenses were combined with the entrance electrode

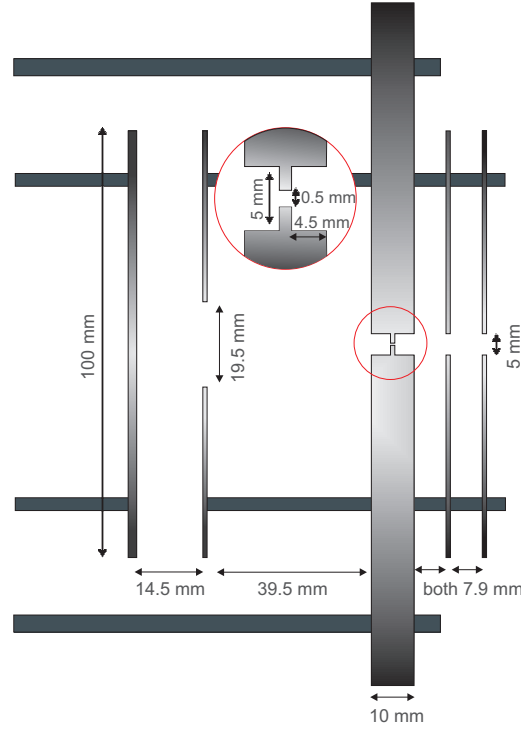


Figure 3.15: Schematic illustration of the developed ion optic including an aperture in the third lens. The total flight length was 980 mm.

of the einzel lens. A typical example is presented in Figure 3.14*b*. In this design, the middle section was employed to cut out ions with a high transverse velocity.

The dimensions of the ion optic employed for the simulations are illustrated in Figure 3.15. An aperture of 0.5 mm diameter was applied, for which only 25% of the ions generated were transmitted onto the detector. Optimum specifications were accomplished following an optimisation for ions of the mass 390 Da, by employing 5000 V on the repeller, 4718 V on the extractor, 5400 V as the pulse potential, 1800 V on the einzel lens and a pulse time of 2.76 μs .

Figure 3.16 presents a comparison of the ToF and spatial resolutions determined as a function of the particle mass for both the initially designed PEDDA spectrometer and the aperture instrument. By removing ions with a high transverse velocity, the mass resolution and the applicable mass range were found to be almost unaffected. The slight increase in ToF resolution at the optimised mass of 390 Da can be associated with the fact that aberrations causing an uncertainty in the ToF domain are removed by the aperture.

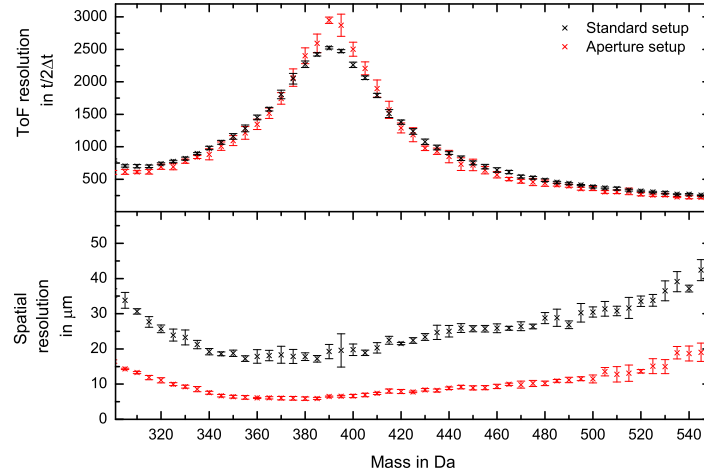


Figure 3.16: Comparison of achievable ToF and spatial resolution for both the standard and the aperture ion optic assemblies.

However, it cannot be ruled out that modifying the ion optic design does not change the ToF focusing properties. A more significant effect can be seen in the spatial resolution plot of the spectrometer (see Figure 3.16). A $10\text{ }\mu\text{m}$ resolution for a mass range extending from 320 Da to 480 Da was determined. At the same time, the aperture ensured that the applicable mass range (in which the spatial resolution is no worse than $30\text{ }\mu\text{m}$) extended over the entire simulated range from 300 Da to 550 Da. Thus, it may be concluded that the introduction of the aperture improves the mass spectrometer specification by removing aberrations.

In order to tune the optimised mass range using the aperture based ion optic, a velocity correction for the ions of the mass 480 Da, 580 Da and 680 Da was performed. Table 3.2 presents the determined electrode potentials, while the resulting mass spectrometer speci-

Peak in Da	Repeller in V	Extractor in V	Pulse in V	Einzel in V
390	5000	4718	5400	1800
480	6097	5753	6585	2195
580	7392	6975	7983	2661
680	8712	8221	9409	3136

Table 3.2: Applied ion optic potentials for the aperture based mass spectrometer.

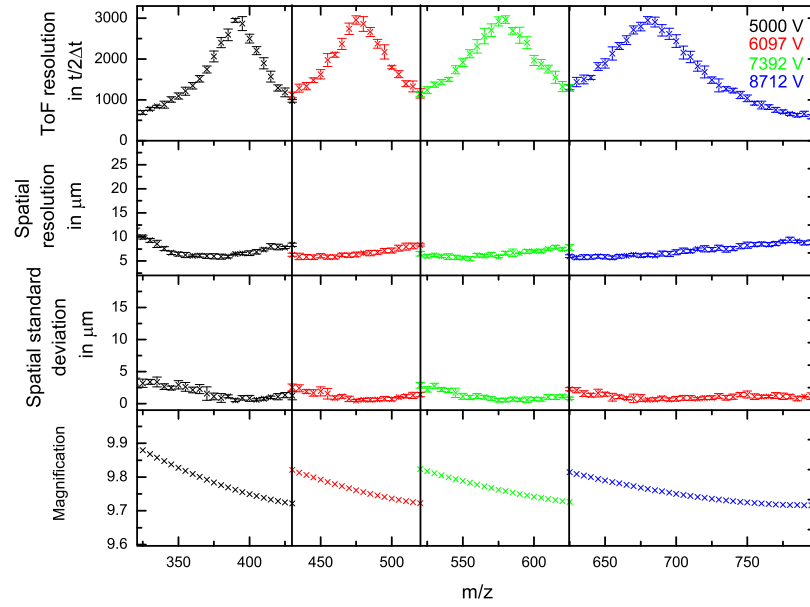


Figure 3.17: Application of the ion velocity correction to the aperture based mass spectrometer. The specifications were maintained over the four mass range settings.

fication for all the optimised mass ranges can be found in Figure 3.17. In agreement to the simple electrode PEDAs instrument used in the sections above, the ToF resolution determined across the four mass ranges was found to be better than $t/2\Delta t = 1000$. Furthermore, the spatial resolution of the system was found to be better than $10 \mu\text{m}$ for all four ion optic settings applied. The last two parameters, the standard deviation of the spatial resolution of the four spots and the magnification factor, were determined to be consistent across the entire investigated mass range. Hence, it can be concluded that comparable images can be collected for any ion mass between 340 Da to 730 Da.

3.5 Conclusion

In this chapter the applicability of the PEDAs method to an ion optic optimised for a field-of-view of 4 mm diameter was investigated. ToF resolutions of up to $t/2\Delta t = 2500$ in combination with a spatial resolution of sub- $25 \mu\text{m}$ were found for the optimised ion mass using ion trajectory simulations. By employing a newly introduced velocity corrected ion

extraction, it was demonstrated that, by resetting the ion optic potentials, the spectrometer can be tuned to focus any particle of interest within a mass range of hundreds of Daltons. In this way, it is possible to scan stepwise through an extended mass range, so that every mass of interest may be imaged and identified, with the same spectrometer specifications being achieved. Furthermore, by introducing an aperture to cut out ions which are emitted with high transverse velocity from the sample surface it is possible to reduce aberration in the ion image. Thereby, the number of detected ions is reduced. In order to test the capabilities of the designed ion optics, and to validate the velocity corrected ion extraction, both designs were constructed and mounted in the existing VMI spectrometer. The experimental results are presented and discussed in the next chapter.

Chapter 4

Post Extraction Differential Acceleration Mass Spectrometry

The ion trajectory simulations undertaken for a five electrode ion optic in Chapter 3 show that the ToF resolution of a linear microscope mode imaging mass spectrometer can be significantly improved using a PEDA extraction technique. To prove the predicted specifications experimentally, the two designed ion optic assemblies were fitted into the ToF imaging spectrometer employed. However, the aim of the study was not only to validate the imaging properties of the instrument, but also to test the ability of the system to be tuned to different ion masses. To assess the mass spectrometer specifications, an ion imaging study was performed to validate the achievable spatial resolution, while a fast readout system was employed to determine the ToF resolution. The experimental procedures and the results obtained will be presented in this chapter.

4.1 Experimental Techniques and Procedures

4.1.1 Ion Optic Assembly and Pulsing Procedure

The ion optic systems were manufactured from stainless steel according to the dimensions presented in Chapter 3. Both repeller plates were again machined with a central groove to match the size of the ITO coated quartz plates ($25 \times 25 \times 1.1$ mm). The remaining experimental setup was equivalent to the one described previously in Section 2.2.1.

A key feature of the PEDDA based ion optic is the pulsing of the extractor electrode, allowing the differential acceleration of a selection of ions. To ensure that the applied voltage is adjusted rapidly, a fast, high voltage switch (Behlke, HTS121) was employed. In order to minimise the capacitance of the charged metal, and thereby reduce the rise time, the switch was mounted directly on the electric feedthrough to the vacuum chamber. For the experiments undertaken, an estimated rise time of ~ 40 ns was assumed [159]. The timing of the pulse was selected relative to the trigger of the ionisation laser. The chosen electrode potentials will be justified in the following sections.

4.1.2 Readout System – Signal Detection and Processing

For the initial experiments presented in Chapter 2, a combination of a conventional chevron MCP assembly, a P47 phosphor screen and a liquid light guide connected to a photomultiplier tube was used to measure the ToF spectra. However, the timing resolution of the system was limited by the fluorescence lifetime of the P47 phosphor screen employed. Hence, the detection system was not suitable for determining the ToF resolution of ions arriving within only a few nanoseconds of each other. Therefore, in order to monitor the ToF performance of the spectrometer, a detection system with a higher timing precision needed to be employed.

Conventionally, high resolution ToF spectra are collected using either an MCP coupled to a cone anode, an electron multiplier tube, or a Faraday cup (see Chapter 6). These systems usually operate at a sub-nanosecond time precision. However, none of these detectors

are designed to collect ions over such a large detection area as the MCP detectors commonly used in ion imaging applications (40 to 80 mm diameter). Thus, by employing these conventional systems an improved ToF resolution would be measured, as the resolution reduces, due to lens aberrations, towards the outer part of the ion image. To determine the actual ToF resolution for the spatially mapped area it is therefore essential to employ a ToF detection system which offers the same size detection area as the imaging detector.

The most convenient way of measuring the ToF spectra would be to extract the ion arrival time while recording the spatial image on the same detector. Thereby, it needs to be ensured that a sensitive and precise ion arrival time can be determined. However, in this work it was not possible to construct an MCP readout system which was sufficiently fast to monitor the current flow sensitively while the detector was operated in “imaging mode”. Therefore, it was decided to read out the conductive surface of the phosphor screen. For this purpose, the detector needed to be operated such that the phosphor screen remained grounded. Thereby, the input face of the front MCP was charged to -2.1 kV, while the back face of the second MCP was set to -0.3 kV. This corresponded to a total gain of 1.8 kV across the MCP assembly. However, in comparison to the “imaging mode” the potential difference between the output face of the electron multiplier system and the phosphor screen was reduced to 0.3 kV, which resulted in a dimmer fluorescence. Hence, a simultaneous detection of the ToF signals and the spatial image became impossible. Furthermore, the potential applied to the input face of the MCPs (-2.1 kV) resulted in an electric field expanding into the ToF tube, affecting the ion trajectories. Therefore, to avoid a change in the mapping conditions of the ToF spectrometer, a fine mesh (Precision Eforming, MN4, 20 lines per inch, 95% transmission) was mounted about 2 mm from the front face of the MCP. By grounding this grid, a field free flight tube could be ensured. The 2 mm flight distance between the grid and the MCP did neither affect the ToF resolution nor the spatial resolution.

The circuit diagram for the phosphor screen readout is presented in Figure 4.1. A $50\ \Omega$ resistor was placed at the output of phosphor screen resulting in a $50\ \Omega$ coaxial connection

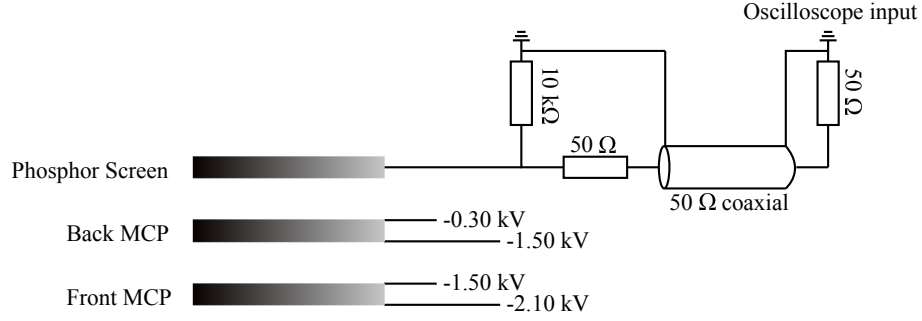


Figure 4.1: Illustration of the circuit diagram of the employed phosphor screen readout system.

to an oscilloscope. Due to the identical termination at both ends of the connection, charge reflections, resulting in a broadening of the observed signal, were avoided. To test the performance of the readout system, a sample was irradiated by the UV laser beam, and ejected electrons were accelerated towards the detector by applying a potential of -1.9 kV to the repeller electrode. The resulting ToF signals were recorded using a LeCroy DDA 960 oscilloscope with a sampling precision of 250 ps. Each ToF spectrum was averaged over 200 shots. Two typical electron ToF spectra are presented in Figure 4.2.

Due to the small electron kinetic energy spread relative to the accelerating potential, it can be assumed that the electron arrival time distribution is the shortest ToF signal which can be measured in the mass spectrometer. Hence, the rise and decay times of the ToF peak can be assigned as a measure of the limitations of the assembled readout system. The measurements indicate that the full signal rise time from 0% to 100% was ~ 3.8 ns, while the decay time from 100% to 10% was determined to be ~ 43 ns (see Figure 4.2).

In mass spectrometry, the ToF resolution is defined as $t_{\text{Peak}}/2\Delta t_{\text{FWHM}}$. However, a complete fit to determined the FWHM of the peaks shown in Figure 4.2 would be significantly influenced by the long decay time caused by the readout system. Therefore, fitting the complete structure of the peak is not a suitable procedure to determine the ToF resolution obtained with the ion optics. In a first approximation, it can be assumed that a Gaussian shaped ToF peak distribution is obtained for particles investigated with the constructed spectrometer. Hence, to determine the detector deconvoluted ToF resolution using the phosphor screen ToF readout, the FWHM value of the ion signal was determined

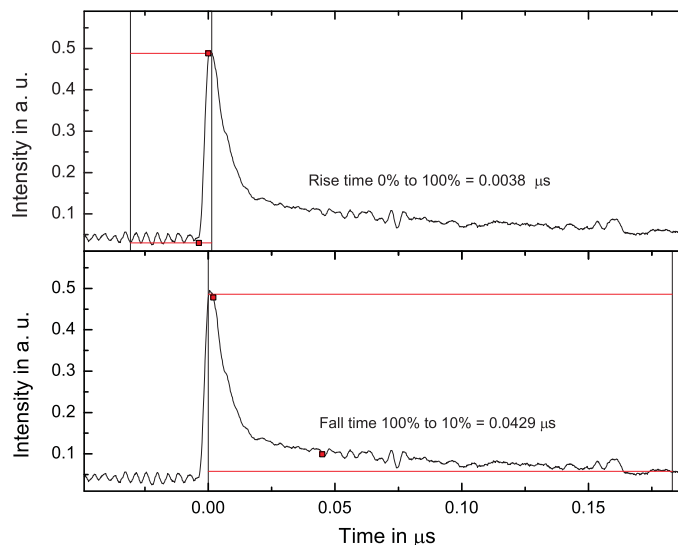


Figure 4.2: Typical electron ToF peaks recorded with the home-built phosphor screen readout system. In the upper spectrum the rise time from 0% to 100%, and in the lower panel the fall time from 100% to 10% were determined.

by measuring the rise time of the peak from 50% to 100% of the signal. By doubling the determined rise time the FWHM was calculated. Further improvement in the signal processing may be achieved by employing a time-to-digital converter, which is triggered by the rising edge of a peak. For these systems the peak decay is neglected as well. According to the described fitting method the FWHM value for the observed electron signal was determined to be 3.24 ns.

4.1.3 Sample Selection and Preparation

In order to determine the specifications of the ion optics employed, both the spatial and the ToF resolution needed to be defined within each of the four mass ranges simulated in Chapter 3. To characterise the former parameter, grid structures of a selection of dyes were created using the sample preparation procedures described in Chapter 2.

To measure the ToF resolution across each of the simulated mass ranges, two polyethylene glycol (PEG) polymers were chosen as samples. The first had an average mass of 400 Da and the other a mass of 550 Da. These will be referred to as PEG 400 and PEG 550 in the following sections. The PEG polymers have been chosen in preference to dyes

No	Molecule	Chemical Formula	CAS
1	Crystal violet	$C_{25}H_{30}N_3Cl$	548-62-9
2	Rhodamine 6G	$C_{27}H_{29}N_2O_3Cl$	989-38-8
3	Rhodamine 101	$C_{32}H_{31}N_2O_7Cl$	116450-56-7
4	Exalite 384	$C_{38}H_{42}$	N/A
5	Exalite 389	$C_{33}H_{34}O_2$	N/A
6	Exalite 404	$C_{50}H_{58}$	N/A
7	PEG 400	$H(C_2H_4O)_nOH$	25322-68-3
8	PEG 550	$CH_3(C_2H_4O)_nOH$	9004-74-4

Table 4.1: Detailed list of the sample molecules which were employed to define either the spatial or the ToF resolution of the designed imaging mass spectrometers.

for the ToF measurements, as they ionise in a MALDI process, and allow a number of mass peaks to be analysed simultaneously. The peaks, corresponding to different chain lengths, are usually separated by 44.03 Da per C_2H_4O group.

Samples were prepared by adding 2 μl of a PEG polymer to a standard matrix solution (5 mg/ml α -cyano-4-hydroxycinnamic acid (CHCA) in 99.9% methanol and 0.1% trifluoroacetic acid) and electrospraying the solution onto a sample plate. The solvent flow rate was set to 100 $\mu l/hr$ and a potential of 5 kV was applied to the needle. The distance between the needle and the sample plate was about 12 mm, resulting in a spot diameter of 5 to 10 mm. The spray time varied from 1 to 5 minutes per sample.

A complete list of the sample molecules employed in this chapter is presented in Table 4.1.

4.2 Experimental Results

In this section the experimental specifications of the first PEDA based IMS instrument will be determined. Initial spatial mapping experiments will be presented which are used to optimise the applied ion optic potentials for the first mass range. Furthermore, to allow accurate mass spectrometry the mass calibration function for the spectrometer will be determined. The tunability of the spectrometer in terms of its spatial and ToF resolution will be explored and discussed later in the section.

4.2.1 Optimising the Ion Optic Settings

In the ion optic simulations undertaken in Chapter 3, the applied electrode potentials were optimised for ions with a mass of 390 Da. However, due to a number of experimental factors the potentials needed to spatially map ions of the selected mass might differ from those given by the simulations. Hence, it is necessary to determine and optimise the experimental settings for the best spatial resolution before calibrating the spectrometer.

In an initial test, the spatial mapping performance of the spectrometer was characterised by observing the parent ion peak of an electrosprayed crystal violet grid structure (372.28 Da). Each square section of the produced grid had a length of 433 μm , while the adjacent gap was about 67 μm . In the ion trajectory simulations, the optimum spatial mapping potentials were found to be 5000 V on the repeller, 4730 V on the extractor, 5400 V as the pulse voltage, and 1800 V on the einzel lens. The pulse time was determined to be 2.8 μs for the ToF optimisation of the 390 Da peak.

Initial ion images taken with the predicted ion optic potentials indicated that the experimentally achieved spatial resolution was somewhat lower than expected. Therefore, the applied extractor potential was adjusted to refocus the ions to map a more clearly resolvable grid structure. A selection of cut-outs of ion images representing six applied extractor potentials between 4750 V and 4786 V is presented in Figure 4.3. The spatial resolutions obtained, following the same fitting method as described Chapter 2, are presented in Figure 4.4. The plot indicates that the optimum spatial resolution is likely to be located in the section between 4760 V and 4770 V.

The ion trajectory simulations performed above indicated that the optimum spatial resolution achievable using the PEDDA ion optic should be on the order of 20 μm . Hence, to narrow down the range of the applied extractor potentials, a crystal violet grid structure containing a gap size of 19.8 μm and a square size of 234.2 μm was created using the electrosprayer. An optical microscope photograph taken of the sample is presented in Figure 4.5. Thus, if the ion optics are capable of imaging features of just below 20 μm size, the gaps between the square sections should only be obvious within a rather narrow

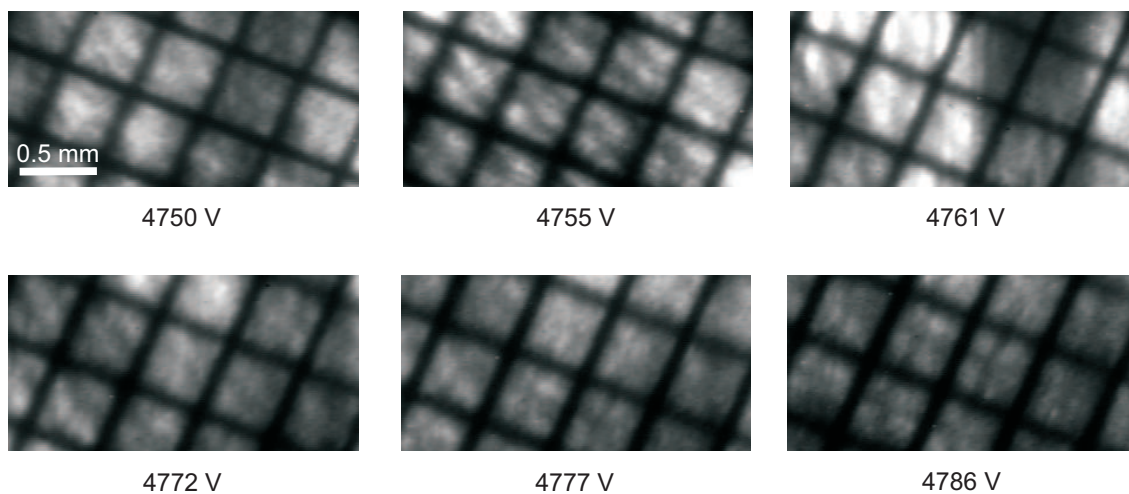


Figure 4.3: Ion images collected for the 372.28 Da crystal violet peak. The images were taken using the indicated extractor potentials. Both the repeller and the einzel lens were operated by applying the previously simulated potentials.

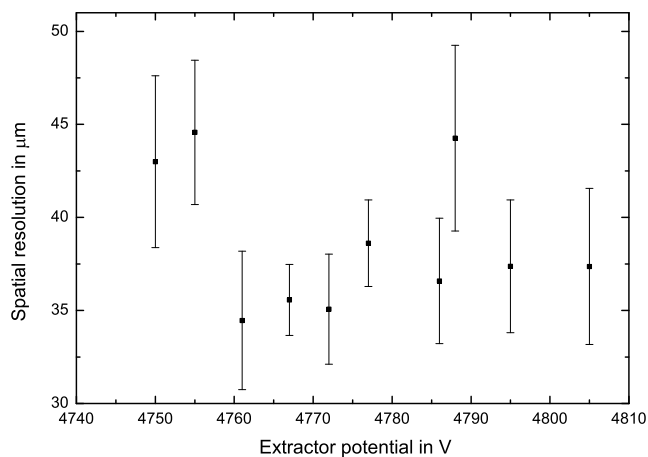


Figure 4.4: Determined spatial resolution for the crystal violet ion images presented in Figure 4.3. Optimum specifications were achieved between 4760 V and 4770 V applied to the extractor electrode.

range in the extractor potential scan.

A series of ion images representing multiple extractor potentials is presented in Figure 4.6. As the observed structures are not clear and uniform enough, the spatial resolution could not be determined using the standard fitting procedure. A crude grid structure can be recognised in the ion images taken at an extractor potential of 4762 V and 4763 V. Hence, for the following experiments the applied potentials were set to 5000 V on the repeller, 4763 V on the extractor, 5400 V as pulsed voltage and 1800 V on the einzel lens. The extractor was pulsed at a delay of 2.80 μ s relative to the laser trigger.

The discrepancy between the simulated and experimental extractor potentials is probably due to the fact that the applied voltage in the experiment is fed through a high voltage switch, which may reduce the output voltage to the electrode. A second possible explanation for the observed deviation is that the ions investigated in the experiment had a mass of 372 Da, while the simulations were optimised for particles of the mass of 390 Da. As a result, slightly different ion optic focussing conditions may be required.

4.2.2 Time-of-Flight Calibration

The correct identification and assignment of ToF peaks to certain molecules or fragments is the key challenge in mass spectrometry. Therefore, the calibration function, which transforms the ToF information into m/z values, needs to be chosen carefully to avoid later misinterpretations of the recorded spectra. A calibration function for a mass spectrometer is determined by observing multiple ToF peaks with a known m/z value. Typical calibration substances can be supplied by the spectrometer manufacturer.

An ideal way of calibrating a mass spectrometer accurately is to employ polymers such as polyethylene glycols, which show multiple peaks corresponding to different chain lengths across a wide mass range. The basic problem of applying these polymers directly as a calibrant in a novel mass spectrometer is that it is possible to misassign the number of polymer units in the mass spectrum. Such an error would result in a wrong calibration function, so that any later identification of sample molecules becomes impossible. Therefore, in this

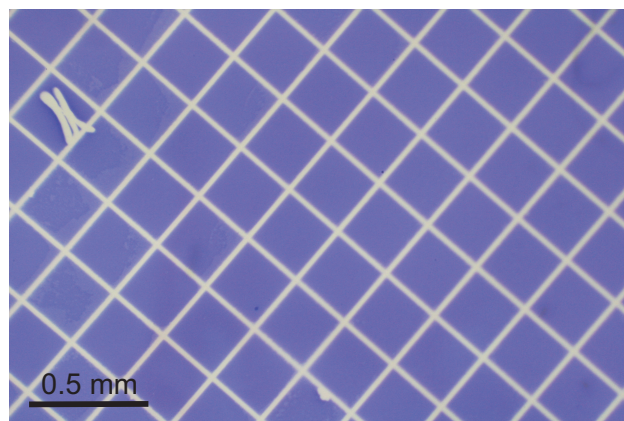


Figure 4.5: Optical microscope picture taken of a crystal violet sample. The grid employed as stencil in the electrospraying process had a wire diameter of $19.8\ \mu\text{m}$ and a square size of $254\ \mu\text{m}$.

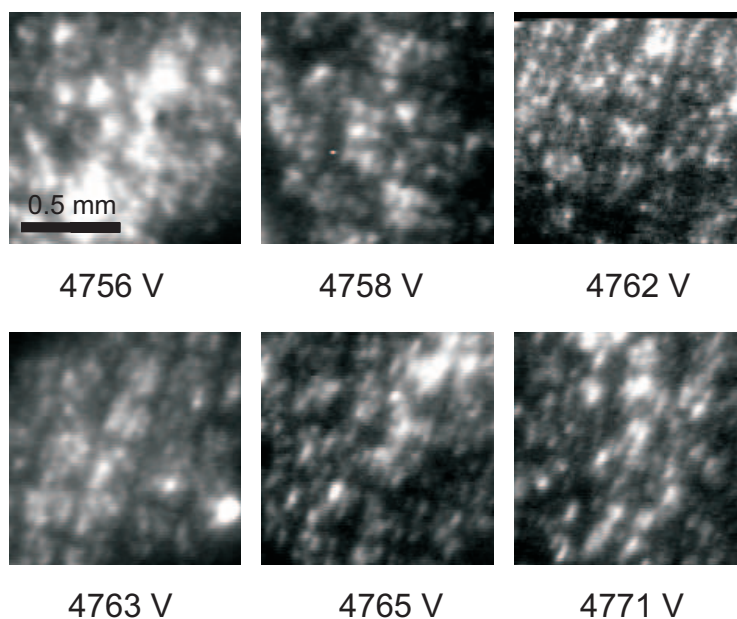


Figure 4.6: Ion images taken of the crystal violet grid sample presented in Figure 4.5. The best resolved grid structure was observed using an extractor potential of 4763 V.

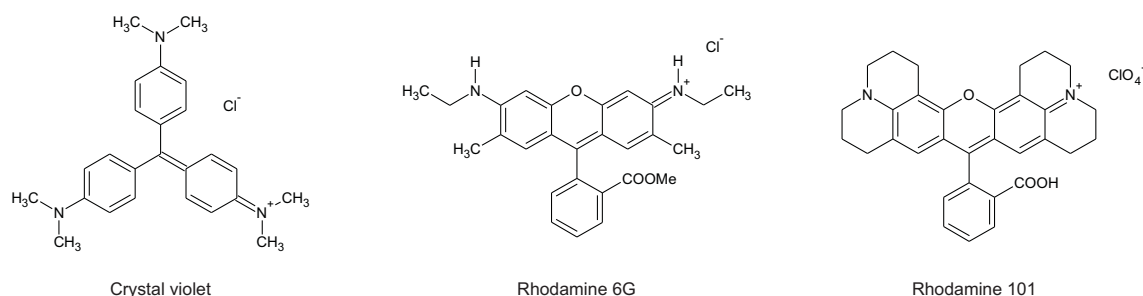


Figure 4.7: Molecular structures of the dyes employed for the preliminary mass spectrometer calibration.

work, a preliminary mass calibration function was determined by measuring ToF spectra of a number of previously characterised dye molecules. The pre-assignment using these samples allowed the absolute chain order of the polymer peaks to be identified. These were later employed to define a more accurate calibration function for the spectrometer.

For the preliminary dye calibration, mass spectra of the dyes crystal violet, rhodamine 6G, and rhodamine 101 were acquired using a commercial MALDI ToF spectrometer (SAI LT2 PLUS). The resulting spectra provided an accurate identification of the expected parent ion mass and/or the resulting fragmentation pattern. By analysing the same compounds in the new microscope mode imaging mass spectrometer, similar peak distributions were recorded using the fast readout system, described in Section 4.1.2. The chemical structures of the three dyes are illustrated in Figure 4.7.

Excerpts of the ToF spectra of the three dye samples, recorded with the new PEDABased imaging mass spectrometer, are shown in Figure 4.8. In the spectrum presented for the crystal violet dye, five clearly separated peaks can be observed. Each of these shows a rudimentary isotope distribution. The ToF peak at $21\ \mu\text{s}$ can be assigned to the parent ion at 372.28 Da, while the four consecutive peaks recorded at earlier ToFs correspond to the loss of CH_2 fragments from the three terminal nitrogen methyl groups of the crystal violet molecule. In the rhodamine 6G spectrum only the parent ion peak at 429.53 Da can be identified, while the third calibration spectrum taken of the rhodamine 101 dye reveals two mass peaks: one for the parent ion at 491.60 Da, and one which can be assigned to the loss of a CO_2 group, resulting in an ion mass of 447.60 Da. The accurate mono-isotopic

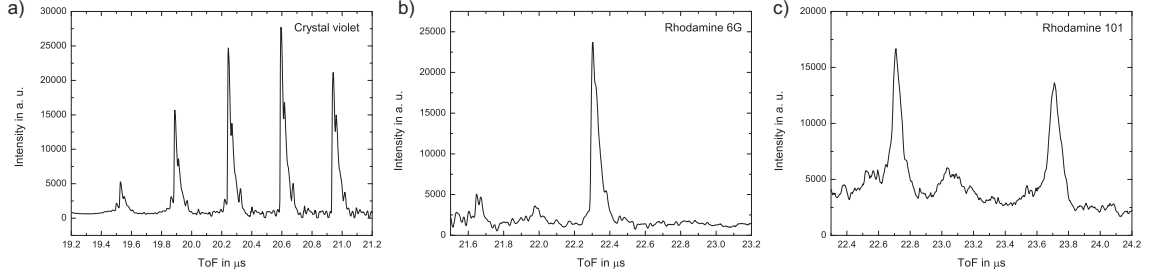


Figure 4.8: The ToF spectra of crystal violet (a), rhodamine 6G (b) and rhodamine 101 (c) recorded using the newly developed PEDA based mass spectrometer.

masses of all the peaks considered in the following initial calibration are presented in Table 4.2.

A first-order ToF calibration function can be created by fitting the experimentally determined ToF peaks as a function of their m/z values according to:

$$t_{\text{ToF}} = a + b\sqrt{\frac{m}{z}}. \quad (4.1)$$

The resulting fit to the collected data points for the three dyes is presented in Figure 4.9a.

The function obtained can be applied to convert the ToF peaks collected for a PEG 400 sample into m/z values. As indicated by the resulting mass spectrum in Figure 4.9b, the observed peaks cover the area of interest between 340 Da to 540 Da. It can be seen that the recorded PEG peaks are separated either by ~ 16 Da or ~ 28 Da, which can be assigned to the polymer chains with either a sodium ($[\text{H}(\text{C}_2\text{H}_4\text{O})_n\text{OH}+\text{Na}]^+$) or potassium ($[\text{H}(\text{C}_2\text{H}_4\text{O})_n\text{OH}+\text{K}]^+$) cation attached. The identification of the peaks and the overall structure of the mass spectrum was found to be in agreement with the previous work of

Peak	Molecule	Ion	m/z
1	Crystal violet	$[\text{M}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{Cl}]^+$	330.21
2	Crystal violet	$[\text{M}-\text{CH}_2-\text{CH}_2-\text{Cl}]^+$	344.26
3	Crystal violet	$[\text{M}-\text{CH}_2-\text{Cl}]^+$	325.26
4	Crystal violet	$[\text{M}-\text{Cl}]^+$	372.28
5	Rhodamine 6G	$[\text{M}-\text{Cl}]^+$	429.22
6	Rhodamine 101	$[\text{M}-\text{CO}_2-\text{ClO}_4]^+$	447.59
7	Rhodamine 101	$[\text{M}-\text{ClO}_4]^+$	491.60

Table 4.2: List of the allocated dye mass peaks, which were further applied to determine the mass spectrometer calibration function presented in Figure 4.9.

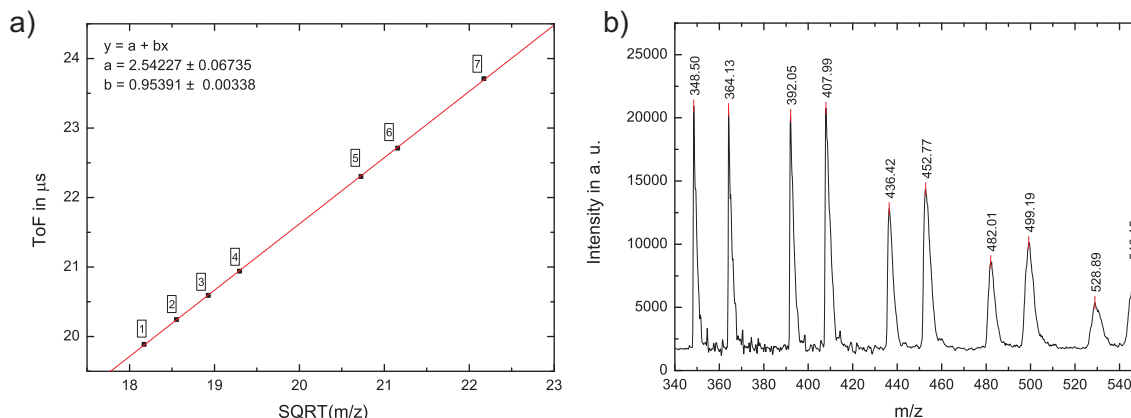


Figure 4.9: a) First order calibration plot of the dye mass peaks identified in the ToF spectra presented in Figure 4.8. The labeled peaks are assigned to the relevant molecule and fragment ions in Table 4.2. An intercept value of 2.54 and a slope of 0.95 were determined for the calibration fit. b) Presents a PEG 400 mass spectrum acquired using the new IMS instrument. The ToF spectrum recorded was converted into m/z values using the dye calibration function. The assignment of the PEG 400 peaks to their molecular formula can be found in Table 4.3.

Griffiths and coworkers [161]. A comprehensive list of the observed ions, the corresponding literature mass, and the determined mass using the preliminary dye calibration function is presented in Table 4.3.

In the range between 350 Da and 500 Da, the calibration function resulted in an uncertainty of ± 2 Da. This inaccuracy can be associated with the following factors: firstly, the dye-based calibration was determined over three separate experiments in which the potentials applied to the electrodes were set manually for each ToF measurement, which may have caused inconsistencies in the applied voltages. In later experiments this problem was circumvented, as the applied potentials were set and controlled by a computer program. In this way, it was ensured that the same calibration function can be applied to every further experiment. A second reason for the incorrect identification of the PEG peaks might arise from the calibration function. In the case that the relationship between the ToF and the square root of the m/z values is not purely linear, a first-order calibration function is insufficient to predict the absolute m/z values correctly.

The quality of a calibration function can be assessed by determining its accuracy for the case in which it is applied to the original ToF peaks used in the calibration. If the

Fragment	Literature m/z	Experimental m/z
$[\text{H}(\text{C}_2\text{H}_4\text{O})_7\text{OH}+\text{Na}]^+$	349.18	348.50
$[\text{H}(\text{C}_2\text{H}_4\text{O})_7\text{OH}+\text{K}]^+$	365.16	364.13
$[\text{H}(\text{C}_2\text{H}_4\text{O})_8\text{OH}+\text{Na}]^+$	393.21	392.05
$[\text{H}(\text{C}_2\text{H}_4\text{O})_8\text{OH}+\text{K}]^+$	409.18	407.99
$[\text{H}(\text{C}_2\text{H}_4\text{O})_9\text{OH}+\text{Na}]^+$	437.24	436.42
$[\text{H}(\text{C}_2\text{H}_4\text{O})_9\text{OH}+\text{K}]^+$	453.21	452.77
$[\text{H}(\text{C}_2\text{H}_4\text{O})_{10}\text{OH}+\text{Na}]^+$	481.26	482.01
$[\text{H}(\text{C}_2\text{H}_4\text{O})_{10}\text{OH}+\text{K}]^+$	497.24	499.19
$[\text{H}(\text{C}_2\text{H}_4\text{O})_{11}\text{OH}+\text{Na}]^+$	525.29	528.89
$[\text{H}(\text{C}_2\text{H}_4\text{O})_{11}\text{OH}+\text{K}]^+$	541.26	546.15

Table 4.3: Identified mass peaks in the PEG 400 spectrum. Both the literature and the experimentally determined m/z values for the observed peaks are listed. The preliminary dye calibration allowed for determination of the ion mass with an upper limit of ± 5 Da.

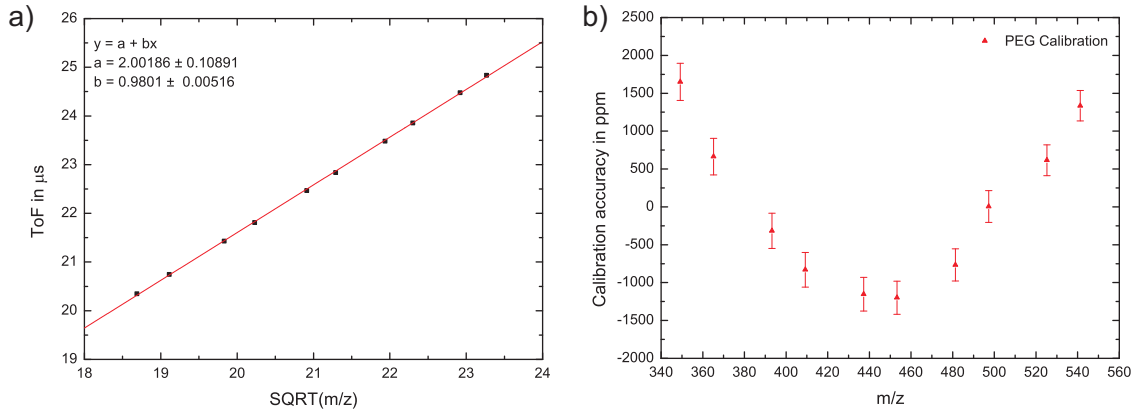


Figure 4.10: a) Calibration plot obtained by fitting a linear function to the determined PEG 400 ToF peaks. b) Presents the resulting calibration accuracy plot.

function is ideally suited, each ToF of the observed peaks would be predicted precisely.

The accuracy of the calibration ($A_{\text{Calib.}}$) can be calculated as follows:

$$A_{\text{Calib.}} = \frac{t_{\text{Peak}} - t_{\text{Calib. plot}}}{t_{\text{Calib. plot}}} 10^6. \quad (4.2)$$

For a perfect calibration function an accuracy of 0 ppm should be determined across the recorded spectrum. However, if the function is used to identify mass peaks in an unknown ToF spectrum, it can be assumed that the uncertainty in the mass accuracy is increased, as further experimental factors may need to be considered.

Figure 4.10 presents both a first-order calibration function fitted to a PEG 400 spectrum and the associated calibration accuracy plot. The latter shows a dependence on the particle mass, which indicates that the applied calibration function is not ideally suited, and higher order calibration functions need to be considered to enhance the mass accuracy of the spectrometer. Extending the linear function with a Taylor expansion up to the second term allows a fit of the data set according to [162]:

$$t_{\text{ToF}} = a\sqrt{\frac{m}{z}} + b\frac{m}{z} + c. \quad (4.3)$$

The resulting calibration function fitted to the PEG 400 ToF peak positions and the associated calibration accuracy plot are presented in Figure 4.11. In the latter graph a scatter plot is observed across the entire calibration range, indicating that the function employed is suitable to predict the actual m/z values for the investigation of unknown samples. An average accuracy of 300 ppm was determined for the self-calibration function applied. This value is on the same order as that determined for a commercial, linear MALDI mass spectrometer (SAI LT2 PLUS, 100 ppm) [163].

To determine the mass accuracy of the spectrometer it was necessary to apply the second-order calibration function to the dye spectra presented above. The function to

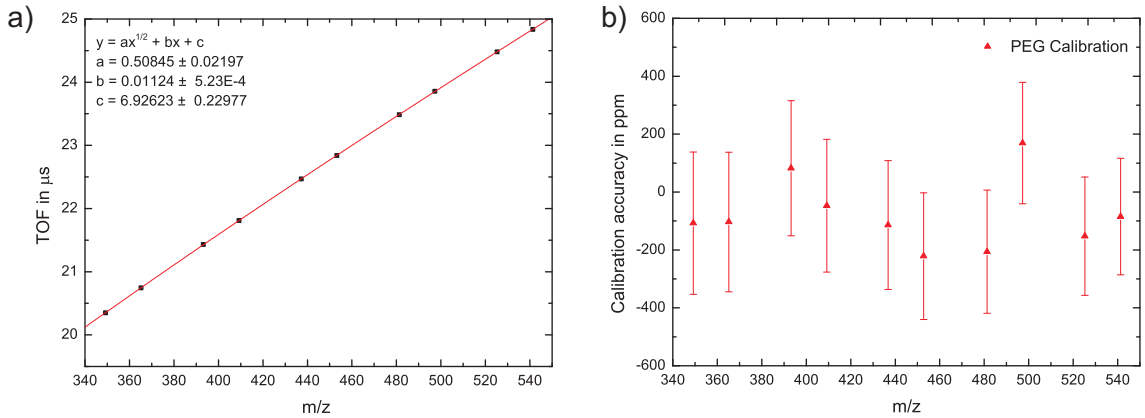


Figure 4.11: a) Shows the 2nd order calibration using the identified PEG 400 peaks as calibration points. The function parameters were determined as ~ 0.51 for a , ~ 0.01 for b and ~ 6.93 for c . b) Illustrates a calibration accuracy plot for the determined PEG 400 calibration function.

Molecule	Literature m/z	PEG Calib. m/z	Deviation m/z	Mass Accuracy in ppm
Crystal violet	330.21	330.64	0.43	1299
Crystal violet	344.26	344.84	0.60	1748
Crystal violet	358.26	358.95	0.69	1936
Crystal violet	372.28	373.06	0.77	2093
Rhodamine 6G	429.53	430.05	0.52	1206
Rhodamine 101	447.59	447.41	-0.18	391
Rhodamine 101	491.60	490.93	-0.67	1359

Table 4.4: Determined mass peaks for the dye ToF spectra using the second order calibration function of the PEG 400 sample. The mass accuracy was found to be no worse than 2100 ppm.

convert the ToF spectrum into m/z values is given by:

$$\frac{m}{z} = \frac{-a^2 \left[\sqrt{\left(\frac{4b \cdot t_{\text{ToF}} - 4bc}{a^2} \right) + 1} \right] - 2b(c - t_{\text{ToF}})a^2}{2b^2}. \quad (4.4)$$

A comparison of the accurate and the determined mass peaks for the dye samples is listed in Table 4.4. The mass accuracy values presented were determined using the previously introduced definition in Equation 1.7. An upper limit of ~ 2100 ppm was found for the application of the calibration function to the dye peaks.

4.2.3 Optimising the Switch Time for PEDA

The time at which the extractor electrode is pulsed to a higher potential defines the ToF focusing properties of the mass spectrometer. By delaying the switch time, the mass range over which the optimum ToF focusing occurs can be shifted to heavier ions, while an earlier switch time results in the ToF focusing of light ions. However, it was shown before that by shifting the ToF optimised mass range, the spatial resolution of the system may be reduced for the relevant ions. Hence, it is essential for the PEDA technique that the switch time is chosen such that both the optimum spatial and ToF resolution overlap for a narrow selection of ion masses. According to the ion trajectory simulations for the applied ion optic settings (switch time $2.80 \mu\text{s}$), the spatially optimised mass range extends from 340 Da to 430 Da. This was found to be in agreement with the experimental spatial

resolutions determined in Section 4.2.1.

To investigate the behaviour of the ToF focusing properties of the assembled spectrometer, a switch time scan was performed using the PEG 400 polymer as sample. During the scan, a potential of 5000 V was applied to the repeller, 4763 V to the extractor, 1800 V to the einzel lens, and the pulse voltage was set at 5400 V. The switch time was adjusted in 50 to 100 ns steps, and six ToF spectra were acquired over 200 shots for each set of experimental parameters applied. To ensure that the signal levels obtained were comparable in each spectrum, the signals acquired with the readout system were thresholded with an upper and lower limit. Before the measurements were performed, the detector was switched back to the settings used for spatial imaging and the laser was aligned to illuminate the entire field-of-view on the sample. This was necessary as the measured ToF resolution is dependent on the origin of the observed ions.

Figures 4.12 and 4.13 show the experimental and simulated ToF resolutions determined as a function of the switch time. At a switch time of 2.60 μs , the best experimental ToF resolution can be found for an ion mass around 350 Da. However, in comparison to the ion trajectory simulations, the optimum resolution could not be determined in the experiments, as the polymer spectrum did not show peaks below 350 Da. By delaying the pulse time, the optimum ToF resolution shifts towards higher masses. At a switch time of 2.70 μs , a clear peak structure can be observed in the experimental data set. The optimum ToF resolution is found to be $t/2\Delta t = 2000$, which is in good agreement with the simulated data set. At 2.80 μs the experimental peak maximum is shifted to 390 Da, for which the ion optic design was initially optimised.

The scans performed at switch times between 2.85 μs to 3.30 μs indicate that it is possible to shift the ToF resolution peak further towards higher ion masses, while maintaining the $t/2\Delta t$ specifications. In the resolution plots of switch times between 3.00 μs and 3.30 μs , a double peak structure is observable in both the experimental and the simulated mass scans. This structure results from a second ToF focusing effect within the ion optic. The resolution determined for this second peak was found to be dependent on the time

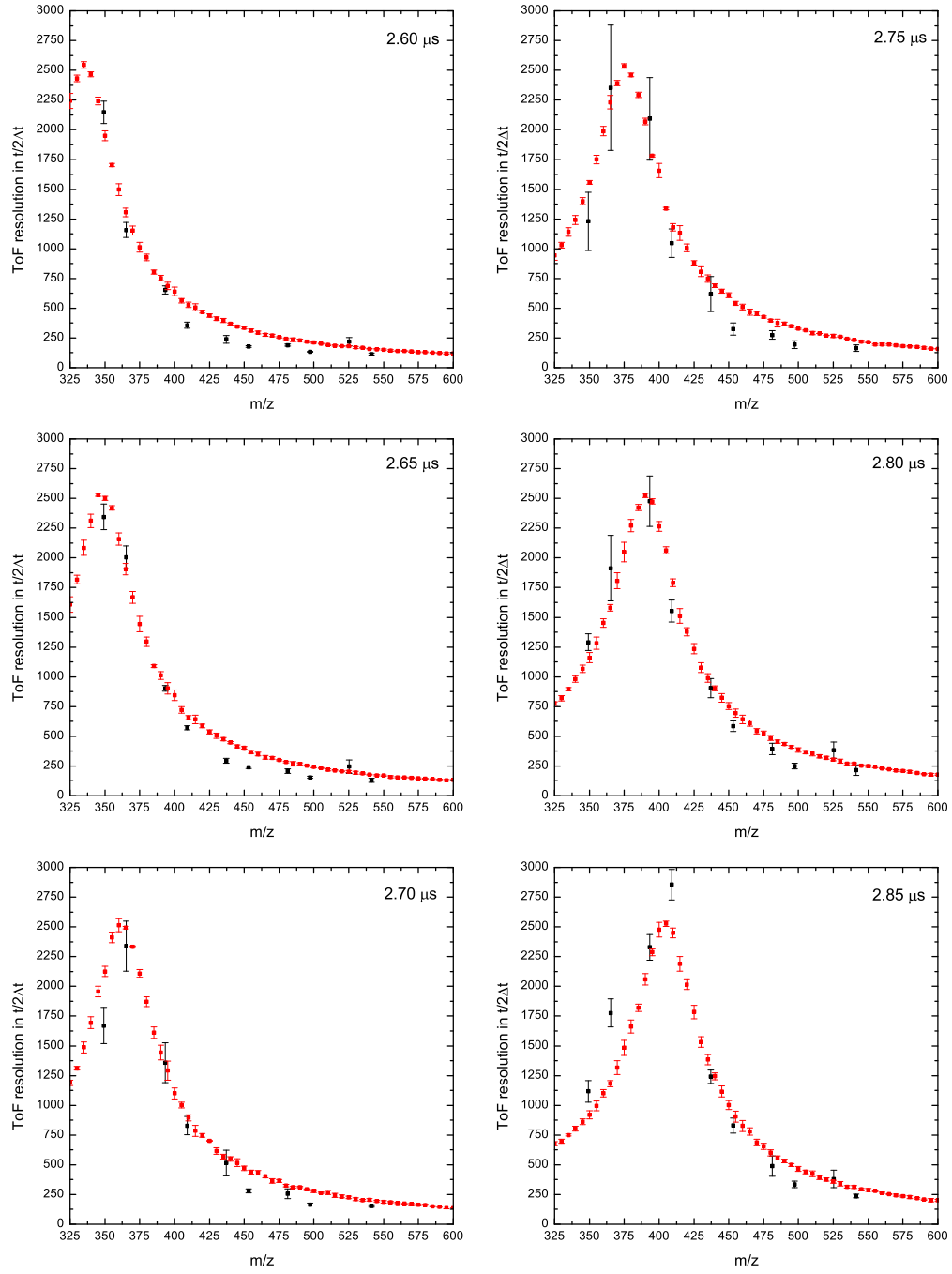


Figure 4.12: Switch time scan from 2.60 μs to 2.85 μs for the post extraction acceleration process. The ion trajectory simulated data is shown in red and the experimental values are illustrated in black. Per sample point six ToF spectra were collected and analysed.

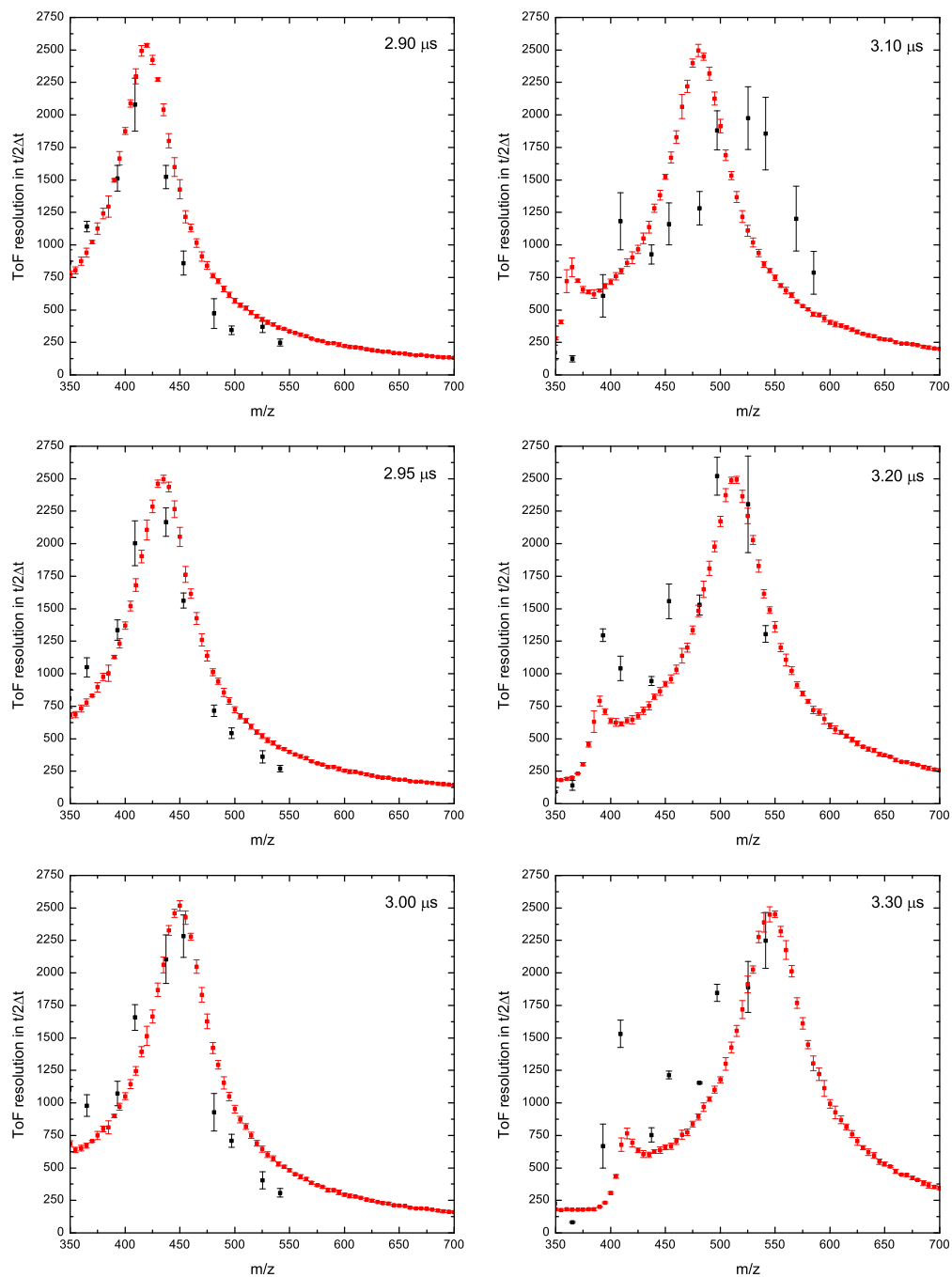


Figure 4.13: Switch time scan from 2.90 μs to 3.30 μs for the post extraction acceleration process. The simulated data is shown in red and the experimental values are illustrated in black. Per sample point six ToF spectra were collected and analysed.

it takes to raise the potential applied to the extractor electrode: the longer the rise-time became, the higher the observed ToF resolution. However, despite extensive investigations, the appearance of this second ToF focused peak cannot be fully explained.

It is also worth noting that the overall mass range in which the ions are ToF focused decreases towards shorter switch times, as fewer ion masses are situated within the post extraction acceleration region when the extractor is pulsed to a higher voltage. If the pulse is applied later, the resolvable mass range becomes broader as the mass separation decreases, and more ion masses are located in the post extraction region.

The maximum ToF resolution achieved at the peak of each of the switch time experiments was found to be between $t/2\Delta t = 2000$ and $t/2\Delta t = 2800$. Hence, it can be stated that by adjusting the delay of the extractor pulse, various ion masses may be detected with an enhanced ToF resolution. However, this method of tuning the optimum mass range is not ideally suited to imaging mass spectrometry, as the spatial resolution and the magnification change depending on the pulse time (See Chapter 3). In further experiments a switch time of $2.80 \mu\text{s}$ was chosen, as an overlap of the optimised ToF and spatial resolution was ensured.

4.2.4 Time-of-Flight Measurements for Tunable Mass Spectrometry

In this section the concept of velocity corrected imaging mass spectrometry will be investigated in terms of the ToF focusing properties for different m/z ranges. For this purpose, the PEG polymers were used to measure a large number of mass peaks covering a range from 350 Da to 760 Da. The initial potentials applied to the ion optic were set according to the previous measurements: 5000 V on the repeller; 4763 V on the extractor; 1800 V on the einzel lens; 5400 V as a pulse voltage. The delay time for the extractor pulse was set to $2.80 \mu\text{s}$.

The velocity correction in the ion trajectory simulations indicated that it is possible to re-optimize the mass spectrometer for ions of the mass 480 Da by increasing the strength

Settings	Type	Repeller	Extractor	Pulse vol.	Einzel
		in V	in V	in V	in V
390 Da	Simulations	5000	4730	5400	1800
	Experimental	5000	4763	5400	1800
480 Da	Simulations	6130	5797	6620	2207
	Experimental	6130	5840	6620	2207
580 Da	Simulations	7355	6952	7943	2648
	Experimental	7355	7003	7943	2647
680 Da	Simulations	8575	8111	9261	3088
	Experimental	8575	8168	9261	3088

Table 4.5: Applied ion optic settings for each of the four mass ranges optimised. The potential applied to the extractor was chosen slightly differently in the simulations and the experiment. The extractor electrode was pulsed at a delay of $2.8 \mu\text{s}$ to the trigger of the ionisation laser.

of the extraction field. For this purpose, the repeller potential was raised to 6130 V while the potentials of the remaining electrodes were calculated by multiplying the applied 390 Da ion optic settings with the ratio of $6130/5000 = 1.226$. As shown in Table 4.5, the only parameter which was changed from in the ion trajectory simulations was the extractor potential. This one was set according to on the optimum spatial mapping settings determined above.

The calculation of the potentials applied to higher mass ranges was based on the repeller settings determined in the ion trajectory simulations in Chapter 3. All resulting voltages are listed in Table 4.5. The ToF spectra, the associated calibration functions and the resulting ToF resolutions are presented in the following sections.

4.2.4.1 First Mass Range - 390 Da

A representative ToF spectrum taken of the PEG 400 polymer with the 390 Da ion optic settings applied is shown in Figure 4.14a. All of the observed PEG peaks arrived within a time window between $19 \mu\text{s}$ and $27 \mu\text{s}$. Within this ToF region, the time focusing effect of the PEDa process can be recognised in the peak structure of the observed molecules; early ToF peaks show a slightly longer rise time, whilst a significantly shorter rise time is

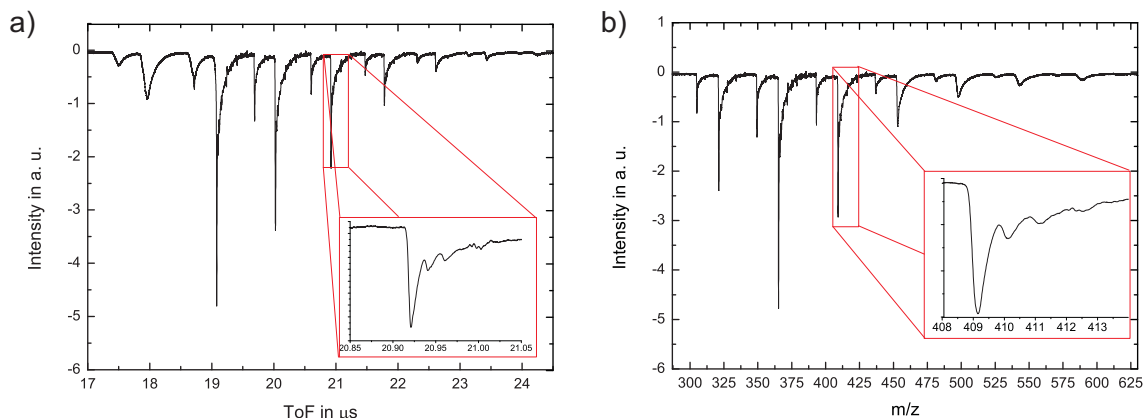


Figure 4.14: ToF spectrum (a) and the converted mass spectrum (b) of the PEG 400 sample, measured using the 390 Da ion optic settings applied.

observed for the peaks detected between 20 μs and 22 μs . Ions with flight times exceeding more than 23 μs show a much broader structure, and cannot be resolved as well as the earlier peaks. In the insert in Figure 4.14a an enlarged section of the ToF spectrum showing the 20.92 μs peak is presented. The observed signal comprises of one main and two smaller peaks. The first can be allocated to the major isotopic ion peak, while the latter peaks are identified as further isotopologues of the PEG molecule. Heavier isotope peaks are not observed as they are obscured by the electrical noise of the readout system.

The spectrum, with its well-known peaks, was translated into m/z values using the second-order calibration function determined in Section 4.2.2. In the resulting mass spectrum, presented in Figure 4.14b, PEG peaks covering the range between 300 Da to 600 Da can be identified. Again, the 20.92 μs equivalent peak is shown enlarged in the mass domain. Here, the isotopologues can be clearly identified.

For each ToF peak observed, the ToF resolution was determined according to the fitting method described in Section 4.1.2. The experimental and previously simulated ToF resolutions are plotted as a function of the particle mass in Figure 4.15. Both data sets show a similar peak structure. However, while in the simulations a ToF resolution of $t/2\Delta t = 2500$ is determined for ions of the mass 390 Da, a somewhat reduced ToF resolution of $t/2\Delta t = 2200$ is found for the 393.21 Da peak in the experimental data. In the low mass range, between 300 Da and 350 Da, a rather large deviation between the

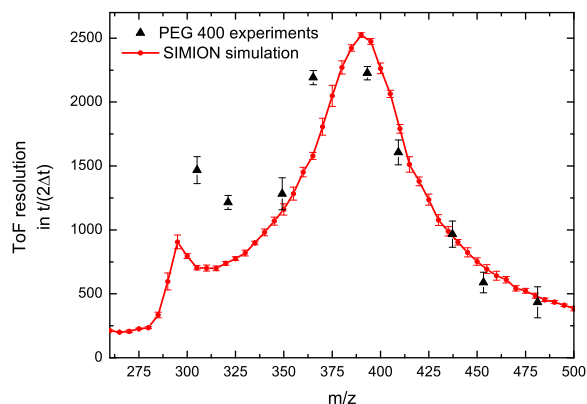


Figure 4.15: Obtained mass dependent ToF resolution plot for the 390 Da ion optic settings. The red curve indicates the simulated data set, while the black data points were determined by fitting the ToF peaks of the acquired PEG 400 spectrum.

simulations and the experiment can be observed: the second ToF focusing peak shows a significantly higher ToF resolution in the spectrum collected with the newly constructed mass spectrometer.

4.2.4.2 Second Mass Range - 480 Da

Due to the change in the ion optic settings applied for the following mass ranges investigated, it is essential to calibrate each range to allow for an accurate identification of the PEG mass peaks. However, an individual calibration on the basis of dye peaks is rather time consuming. Hence, it was ensured that the determined ToF resolution was assigned to the associated molecular mass correctly, by observing the ToF shift of the prior identified PEG peaks using the four different ion optic settings. An overview of the resulting ToF spectra is presented in Figure 4.16. Fourteen peaks of the PEG 400 sample can be observed and assigned to their specific ions. Peak No 1 corresponds to the 305.16 Da ion representing the chemical formula $[\text{H}(\text{C}_2\text{H}_4\text{O})_6\text{OH}+\text{Na}]^+$, while the second peak arises from the replacement of the sodium ion with a potassium ion ($[\text{H}(\text{C}_2\text{H}_4\text{O})_6\text{OH}+\text{K}]^+$). The following ToF signals relate to the same peaks in successive increasing chain orders. The direct comparison of the four spectra indicates that the region of the optimum ToF resolution is

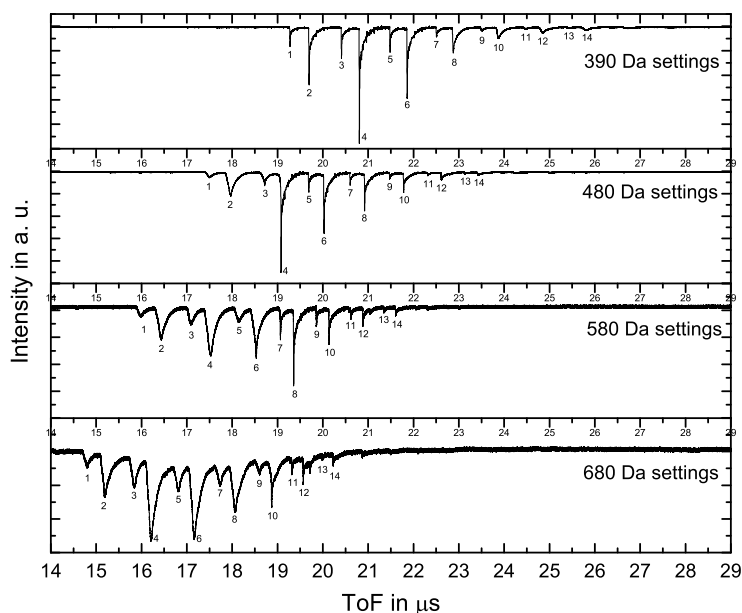


Figure 4.16: ToF spectra of the PEG 400 sample recorded using each of the four mass range settings applied. The peak No 1 can be assigned to the $[\text{H}(\text{C}_2\text{H}_4\text{O})_6\text{OH}+\text{Na}]^+$ ion peak.

always located around $20 \mu\text{s}$.

Figure 4.17 shows the second-order calibration plot and the mass dependent ToF resolution plot determined for the second velocity corrected mass range. The experimentally determined ToF resolution is again found to be in good agreement with the ion trajectory

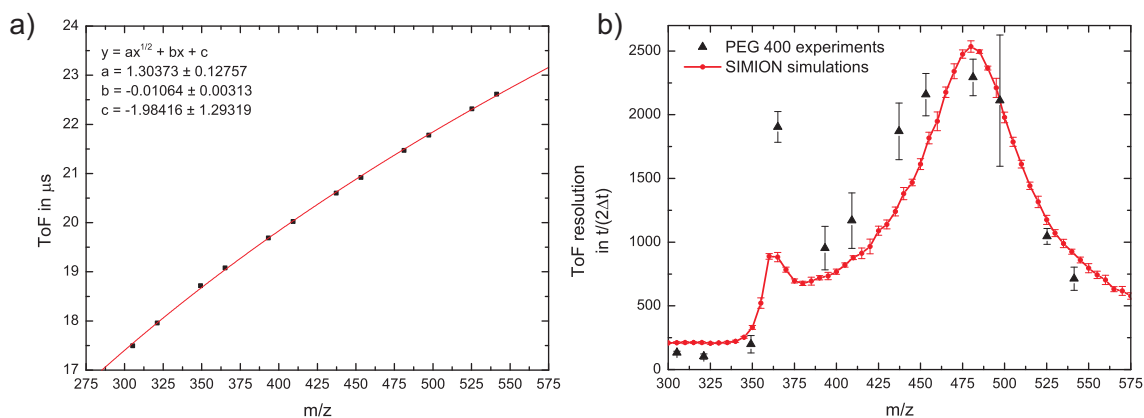


Figure 4.17: a) Second order calibration fit of the PEG 400 sample using the 480 Da ion optic settings. The fitting parameters determined for the calibration are indicated in the graph. b) Comparison of the experimental and simulated mass dependent ToF resolutions determined for the second mass range.

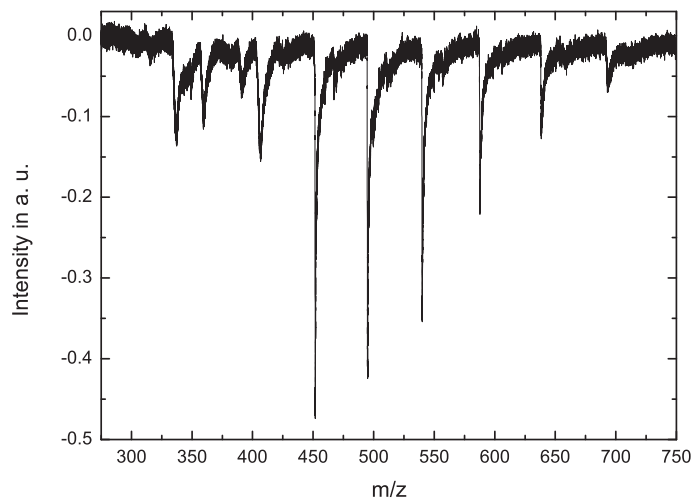


Figure 4.18: Mass spectrum recorded of the PEG 550 sample using the 580 Da ion optic settings. The spectrum was converted into m/z values by applying a second order calibration function (PEG 400).

simulations. The highest resolution of $t/2\Delta t = 2200$ is observed for particles with the mass of 481.26 Da. Hence, according to the determined ToF resolutions for the second mass range, it can be concluded that at least the ToF resolution of the spectrometer can be tuned using a velocity corrected ion extraction.

4.2.4.3 Third Mass Range - 580 Da

Operating the spectrometer with the third mass range settings applied allows the ToF resolution in the mass range from 530 Da to 630 Da to be optimised. For the previously used PEG 400 sample it was noted that the signal levels degrade for peaks exceeding 500 Da. Therefore, it was necessary to add a second polymer (PEG 550) with a higher average mass to define the ToF resolution across the entire optimised mass range. A typical PEG 550 mass spectrum, preliminarily calibrated using a function based on the PEG 400 ToF peaks, is presented in Figure 4.18. The observed peaks allocated to their molecular formulas are listed in Table 4.6.

It should be pointed out that using the PEG 400 calibration function it is possible to determine the m/z values of the PEG 550 ions in the range from 430 Da to 570 Da with a mass accuracy of up to 1500 ppm. However, for heavier particles the applied calibration is

Ion	Literature m/z	Calib. PEG 400 m/z	Difference m/z	Accuracy in PPM
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_8\text{OH}+\text{Na}]^+$	407.23	406.66	0.57	1402
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_9\text{OH}+\text{Na}]^+$	451.25	451.79	-0.54	1195
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_9\text{OH}+\text{K}]^+$	467.23	467.29	-0.06	128
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_{10}\text{OH}+\text{Na}]^+$	495.28	495.04	0.24	484
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_{11}\text{OH}+\text{Na}]^+$	539.30	540.10	-0.80	1426
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_{12}\text{OH}+\text{Na}]^+$	583.33	587.71	-4.38	7453
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_{13}\text{OH}+\text{Na}]^+$	627.36	638.47	-11.11	17401
$[\text{CH}_3(\text{C}_2\text{H}_4\text{O})_{14}\text{OH}+\text{Na}]^+$	671.38	693.79	-22.41	32301

Table 4.6: Identified mass peaks of the 550 Da PEG sample. The calibration was based on the PEG 400 ToF measurement. Both the actual mass difference and the associated mass accuracy are presented for each mass peak observed.

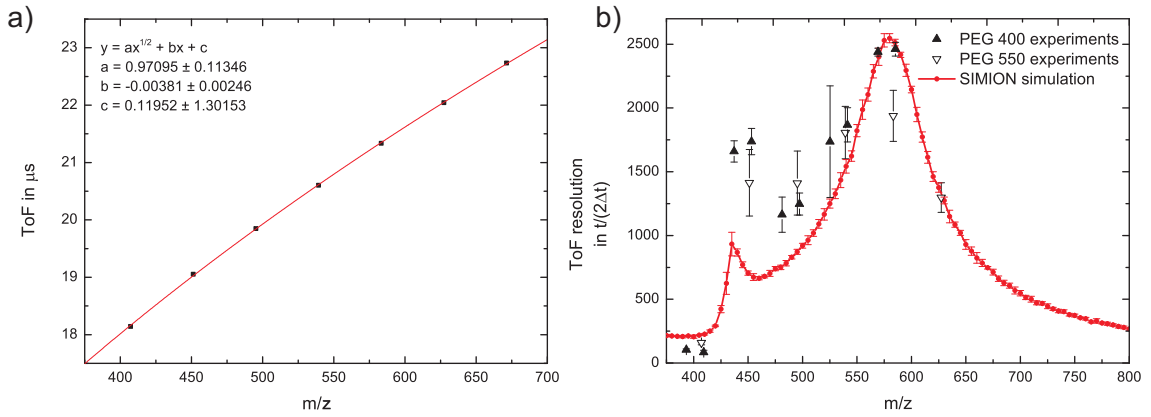


Figure 4.19: *a)* Calibration fit determined of the PEG 550 mass peaks recorded using the 580 Da ion optic settings. *b)* Experimental and simulated mass dependent ToF resolutions determined for the third mass range ion optic settings.

found to be insufficient. This is mainly due to the fact that the applied PEG 400 calibration does not allow the determination of peak positions in the higher mass range. When the extraction field is inverted, ions exceeding masses of 630 Da are still located between the repeller and extractor electrodes. As a result, these are repelled by the increased extractor potential and their ToF is extended accordingly. This effect is not taken into account in the original PEG 400 calibration function. Hence, for the later analysis of unknown samples, it is essential to re-calibrate the spectrometer for ion peaks covering the whole mass range. A second-order calibration plot based on the identified PEG 550 peaks is presented in Figure 4.19 *a*.

The mass dependent ToF resolution plot determined for both PEG samples is presented in Figure 4.19*b*. The peak structure predicted by the ion trajectory simulations is maintained in the experimental data sets. Hence, it can be concluded that the optimised mass range can be tuned by adjusting the particle's axial velocity. The highest ToF resolution exceeding $t/2\Delta t = 2000$ is found to be between 570 Da and 590 Da, which is in agreement with the ion trajectory simulations. However, for both PEG samples a slightly different ToF resolution is observed in the optimised mass region. This is most likely due to the fact that both spectra were recorded with a different laser fluence applied to the sample. Laser hot-spots may have produced ions with a broadened velocity distribution, which causes aberrations affecting the ToF resolution.

4.2.4.4 Fourth Mass Range - 680 Da

In the fourth investigated mass range, both PEG samples were applied to determine the mass dependent ToF resolution. To cover the whole range of interest between 500 Da and 800 Da, a calibration function was determined as a function of the PEG 550 peaks observed (see Figure 4.20*a*).

The mass dependent ToF resolution plot for both applied PEG samples is presented in comparison to the simulation results in Figure 4.20*b*. The optimum ToF resolutions for both the simulation and the experimental results are found at around 670 Da. However, the experimentally determined ToF resolution is somewhat lower than the simulated one, and the observed peak structure is shifted towards lower masses. Again, this might be due to hot-spots in the laser profile causing aberrations. Another possibility might be that the ion trajectory simulation assumed an inaccurate initial velocity distribution for the ion masses exceeding 600 Da. As the principle of the velocity corrected ion extraction is based on the assumption that all ions come off the surface with a mass independent velocity distribution, it might be possible that a slight change in the actual distribution results in an insufficient adjustment to ToF focus higher ion masses. As a result, the ToF resolution curve may be shifted towards lower or higher m/z values.

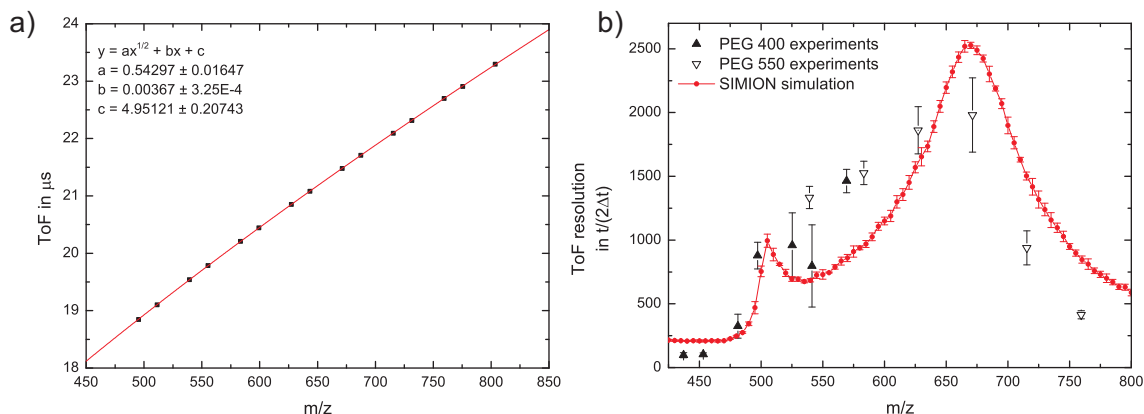


Figure 4.20: a) Calibration plot created for the fourth PEDA optimised mass range. b) Comparison of the experimental and simulated mass dependent ToF resolutions determined.

4.2.4.5 Summary

Following the results presented in this section, it can be concluded that it is possible to scan the ion optic properties successively to match various mass ranges using the applied velocity corrected ion extraction method. For each of the optimised ion optic settings a calibration function was determined so that the newly developed instrument can be applied to identify any analyte with an accuracy of $\pm 1\text{-}2$ Da (≤ 2000 ppm) over a mass range from 340 Da to 730 Da. A list of the determined calibration functions is presented in Table 4.7. The ToF resolution was determined to be $t/2\Delta t \approx 2000$ at the optimised particle masses, and, for each of the applied ion optic settings, an isotopic resolution covering a range of more than 120 Da was observed. Hence, the spectrometer has proven its capabilities in terms of ToF resolution tunability.

However, it should be pointed out that the determination of the ToF resolution followed a rising edge fitting method, which might lead to an inaccurate results as the falling edge of the peak is not fully considered. A more precise ToF resolution would be determined by employing a detector which is capable of recording the full ToF peak with a high timing precision, so that the entire peak may be fit in the ToF analysis.

VC Settings	Calib. range	a	Fitting parameters b	c
390 Da	340-530 Da	0.50845 ± 0.02197	$0.01124 \pm 5.2 \times 10^{-4}$	6.92623 ± 0.22977
480 Da	350-550 Da	1.30373 ± 0.12757	-0.01064 ± 0.00313	-1.98416 ± 1.29318
580 Da	450-750 Da	0.97095 ± 0.11346	-0.00381 ± 0.00246	0.11952 ± 1.30153
680 Da	500-800 Da	0.54297 ± 0.01647	$0.00367 \pm 3.3 \times 10^{-4}$	4.95121 ± 0.20743

Table 4.7: Determined calibration function parameters for each of the mass ranges optimised using velocity corrected ion extraction for IMS. A ToF spectrum can be converted into m/z values by applying the fitting parameters to Equation 4.4.

4.2.5 Imaging Verification of the Velocity Correction

To completely validate the principle of the ion velocity correction, it is necessary to define the spatial resolution achieved in each of the four simulated mass ranges. Ion imaging experiments were performed by switching the detector back into imaging mode, such as described in Chapter 2. All images presented in this section were acquired over 2000 ToF cycles using a conventional CCD camera. By gating the detector such as previously described (see Chapter 2), it was ensured that only a single ToF peak was recorded. To improve the image quality, the events detected by the camera were thresholded, event counted, and centroided. The difference between a software optimised image and the raw image is illustrated in Figure 4.21.

In this section, grid structures (wire diameter $67\text{ }\mu\text{m}$, pitch $500\text{ }\mu\text{m}$) of the dyes exalite 384, exalite 398, exalite 404, rhodamine 101 and rhodamine 6G are imaged and analysed to determine the spatial resolution of the system as a function of the ion optic settings for multiple ion masses. For each of the investigated dye peaks one ion image per ion optic setting was recorded and analysed.

In the following, two series of ion images recorded for the exalite 384 and exalite 404 dye will be presented and discussed. All remaining images considered in the analysis can be found in the Appendix A of this thesis. In both of the following illustrations a microscope

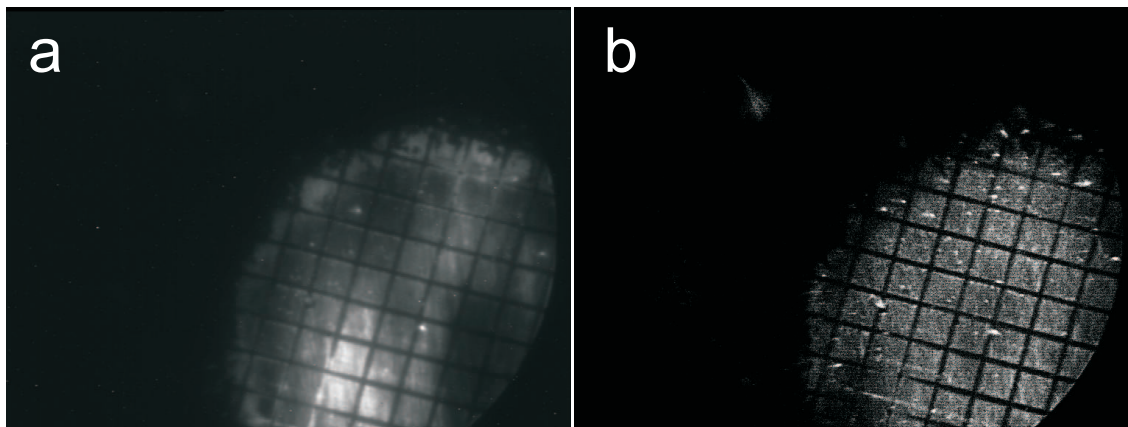


Figure 4.21: Comparison between a raw, unoptimised ion image (*a*), and a thresholded, centroided, and event counted image (*b*) taken of an exalite 384 grid sample.

photo of the imaged grid structure will be presented, to allow a direct optical comparison of the microscopic properties of the samples to the obtained ion image. Furthermore, the determined resolution and magnification values, as well as the corresponding cross section analysis plots are presented.

Exalite 384

Figure 4.22 shows four ion images of the 498.33 Da exalite 384 peak, each taken with one of the defined ion optic settings applied. The mass peak was identified using the calibration functions determined above, and confirmed by recording a mass spectrum with a conventional MALDI mass spectrometer. In all ion images presented, a clear grid structure can be observed. Following the ion trajectory simulations it was expected that the optimum spatial resolution could be achieved by employing the ion optic settings optimised for the second mass range. A 20% to 80% rising edge analysis of the grid patterns indicated that experimentally an optimum spatial resolution of $19 \pm 2 \mu\text{m}$ for the first, and $19 \pm 3 \mu\text{m}$ for the second ion optic settings were observed. In the following third and fourth mass range the spatial resolution dropped to $22 \pm 5 \mu\text{m}$ and $29 \pm 7 \mu\text{m}$. An almost constant magnification factor of $\sim 9.8\times$ was found for all applied ion optic settings.

Exalite 404

The exalite 404 mass spectrum consisted of one peak corresponding to a mass of 658.45 Da. Based on the ion trajectory simulations, this ion mass should show its optimum spatial resolution using the fourth mass range settings. The prediction can be confirmed in Figure 4.23. By operating the spectrometer with the 680 Da ion optic settings, a resolution of $17 \pm 3 \mu\text{m}$ was determined in the ion image. In the case that the ion optic potentials optimised for lower mass ranges were applied, a decrease in spatial resolution was noted. For example, the spatial resolution obtained using the 390 Da ion optic settings was found to be $54 \pm 6 \mu\text{m}$. The magnification factor of the mass spectrometer was determined for the first mass range settings as $9.0\times$. This factor increased to $9.7\times$ when employing the

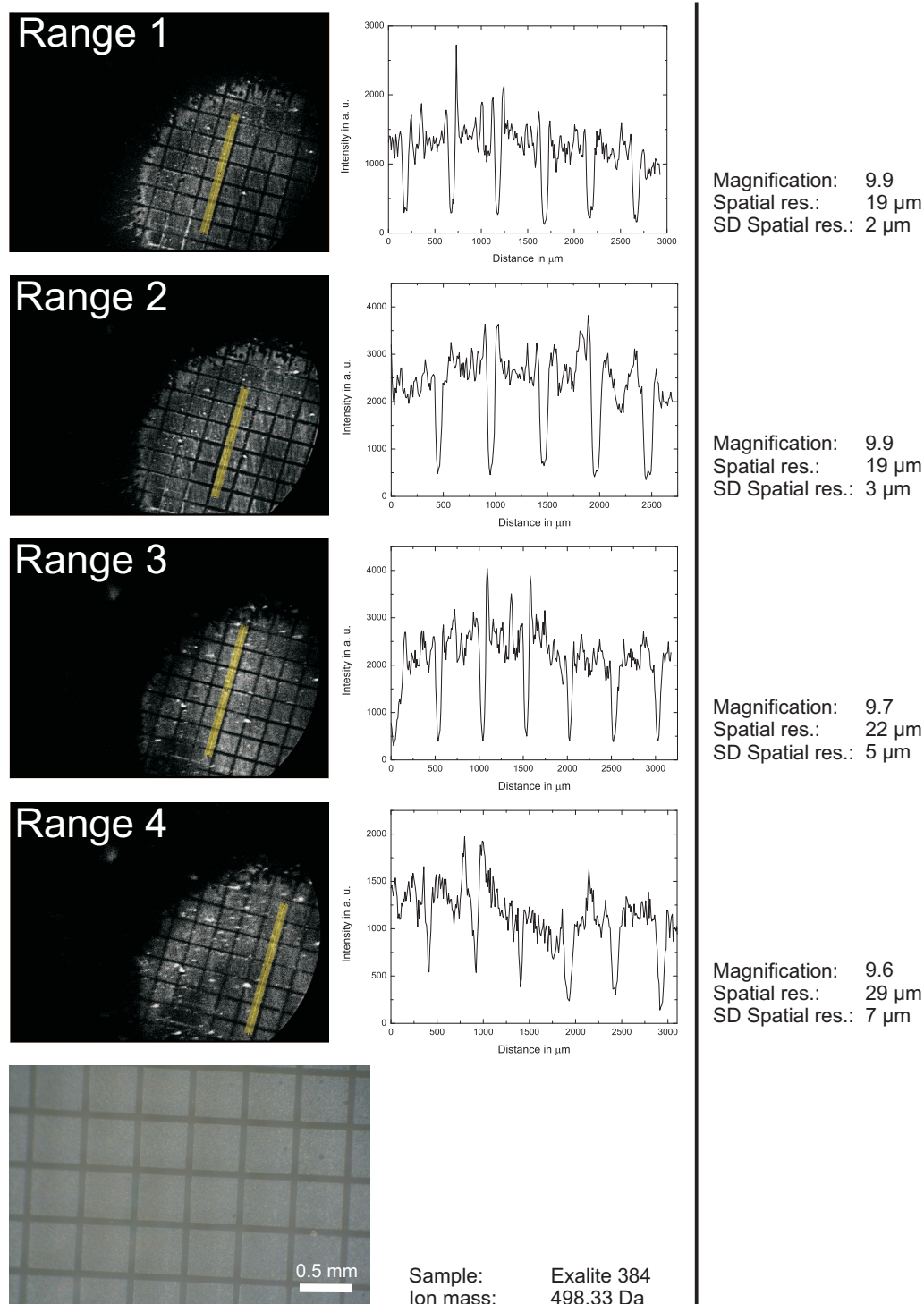


Figure 4.22: In the left section of the figure, four ion images collected of an exalite 384 sample (498.33 Da) with each of the four ion optic settings applied are presented. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

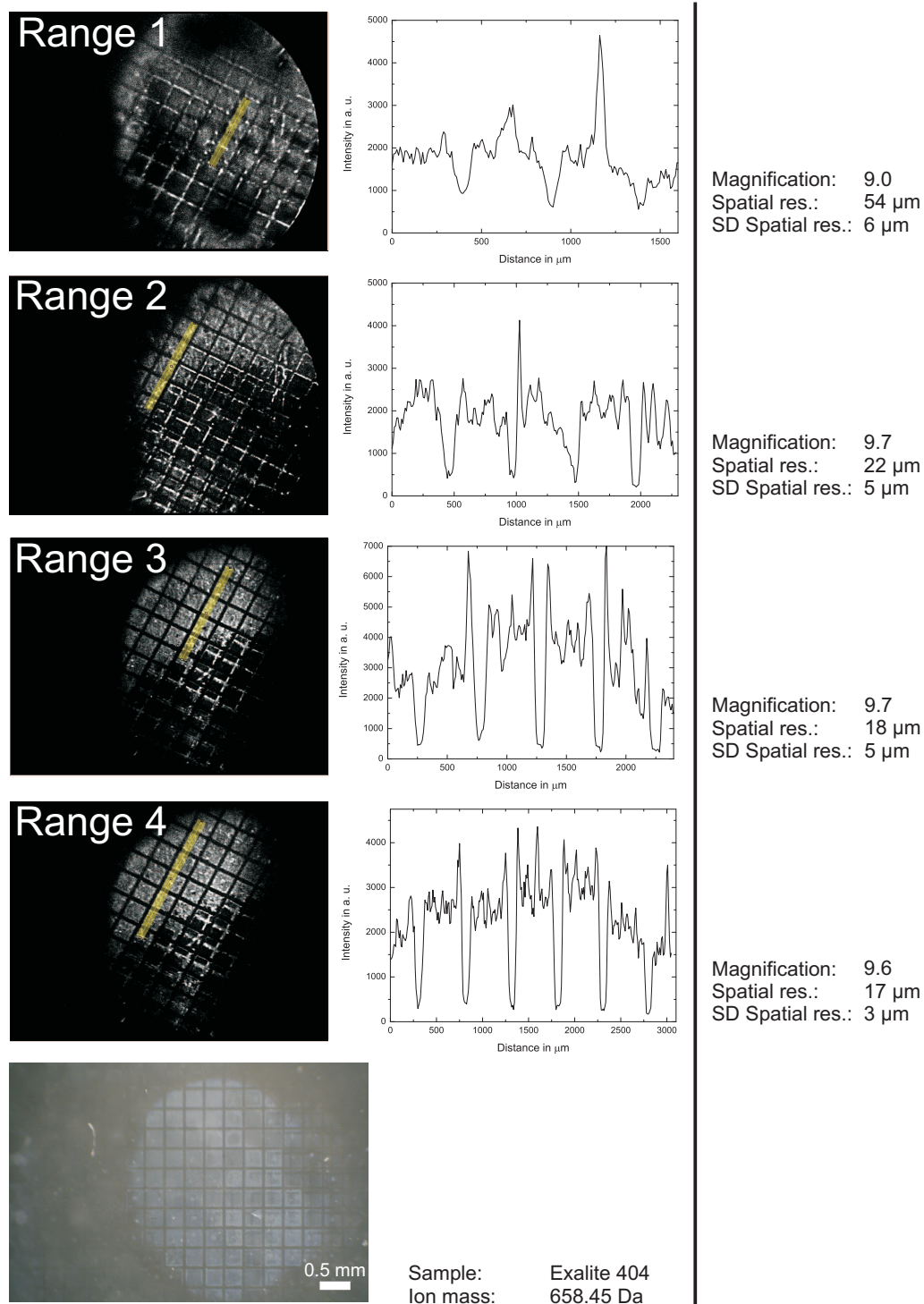


Figure 4.23: In the left section of the figure, four ion images collected of an exalite 404 sample (658.45 Da) with each of the four ion optic settings applied are presented. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

ion optic potentials optimised for 680 Da.

All the ion images recorded show a clear, positive grid structure at the upper side of the ion images. However, this structure converts into a negative grid pattern in the lower image section. By comparing the ion images obtained with the microscope photography of the sample, it can be proven that the line structure is an actual feature of the sample. Hence, an image distortion due to ion optic effects can be excluded. This negative grid structure can be observed in a number of images and is most likely caused by a microlensing effect of the grounded grid stencil in the electrospraying process.

Spatial analysis of the four mass ranges

The graphs in Figure 4.24 and 4.25 show the experimental and simulated spatial resolutions as a function of the ion mass for each of the four ion optic settings applied. In Figure 4.24a, the results obtained for the 390 Da optimised settings are presented. The ion trajectory simulations, illustrated by the black squares, indicate that a spatial resolution of sub-20 μm should be found around 375 Da. Furthermore, it can be noted that within the range from 325 Da to 450 Da a resolution of 25 μm should not be exceeded. The experimental data set, illustrated by the red squares, shows a spatial resolution of 20 μm to 30 μm in the range between 400 Da and 500 Da, which is in agreement with the ion trajectory simulations. For ions exceeding 600 Da a significant difference between the simulated and the experimental

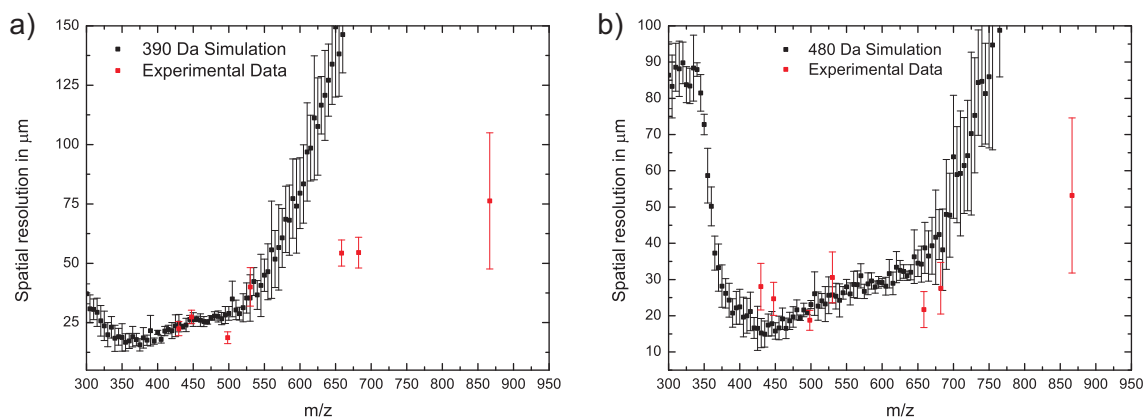


Figure 4.24: Comparison of the experimental and simulated spatial resolutions obtained using the ion optic settings optimised for 390 Da (a) and 480 Da (b).

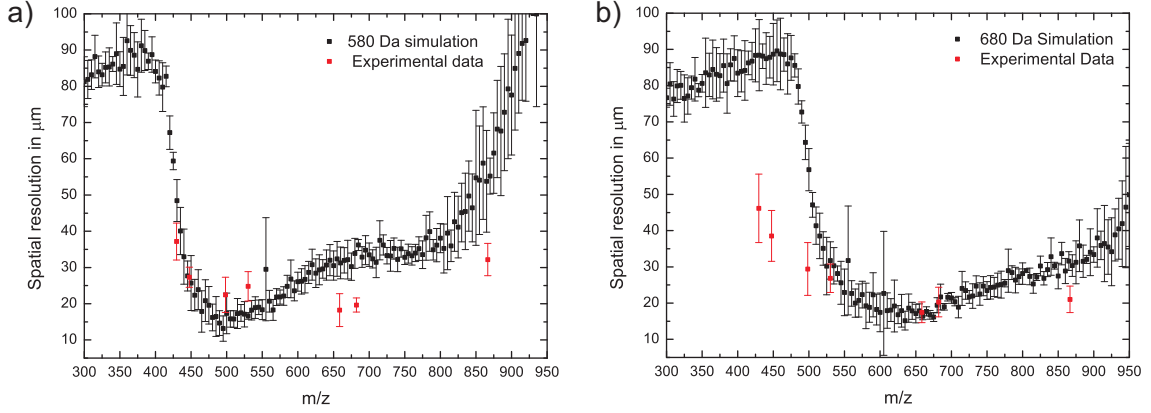


Figure 4.25: Comparison of the experimental and simulated spatial resolutions obtained using the ion optic settings optimised for 580 Da (a) and 680 Da (b).

spatial resolution is observed. Here, the simulations suggest a much steeper resolution decrease than the one determined in the experiments. Nevertheless, the fact that the best spatial resolution was located in the optimised range confirms the value of the simulations and the spatial optimisation procedure undertaken in Section 4.2.1.

In Figure 4.24b, the results obtained for the 480 Da optimised ion optic settings are presented. The experimental data set confirms the prediction, as a resolution of sub-20 μm can be found at 500 Da. Furthermore, it can be seen that experimentally obtained resolutions between 650 Da and 700 Da did not exceed 30 μm , resulting in a wider optimised mass range than that predicted by the ion trajectory simulations. However, more importantly, the result obtained indicates that it is possible to shift the spatially optimised region by changing the extraction field strength towards higher ion masses.

The graph for the third mass range settings illustrates again an agreement between the simulation and the experimental results (see Figure 4.25a). The optimum spatial resolution was expected to be located around 580 Da. An interpolation of the analysed ion masses shows that a resolution of around 20 μm can be assumed at this point. Moreover, in the adjacent mass region, between 490 Da to 550 Da and 650 Da to 700 Da, the spatial resolution was determined for both data sets as $\sim 25 \mu\text{m}$.

The last velocity corrected range, presented in Figure 4.25b, shows the best experimentally observed spatial resolution between 670 Da and 690 Da, which matches predictions

from the simulations. In this graph it becomes even more obvious that the velocity correction allows the tuning of the best focused range, as all the fragments with masses below 550 Da show a decreasing spatial resolution.

The four graphs together indicate that the velocity correction allows a tuning of the mass spectrometer to achieve spatial resolutions of around 25 μm at any mass of interest between 390 Da and 680 Da. The experimental data sets show a reasonably good agreement with the simulations undertaken. The major difference detected in this work was observed in the high mass region of each optimised setting, where the ions were spatially better focused in the experiment than expected in the simulations. This fact may be explained by the fitting method applied. For low resolution images it is rather difficult to set the threshold level for the 20% to 80% rise fit correctly, as the signal differences between square and non-square sections become less obvious. Another possible explanation for the observed differences might be that the simulation parameters employed for the initial ion velocity spread and the angular distribution were not set correctly. The initial kinetic energies and angular distribution for the simulations were based on the data measured for an expanding matrix plume. However, in this study the dyes were ionised using a direct laser ablation process, which may have resulted in a slightly different velocity and angular ion distribution.

Following the presented results, it can be concluded that not only the ToF optimised, but also the spatially optimised section of the mass spectrum can be tuned using a velocity corrected ion extraction method. The magnification factor was determined for the spatially optimised sections of the mass spectrum with $\sim 9.8\times$. Hence, all the spectrometer specifications including a ToF resolution exceeding $t/2\Delta t = 2000$, a spatial resolution of sub-25 μm , and a constant magnification factor, were maintained while tuning the spectrometer.

4.3 Initial Results Employing an Aperture

Ion trajectory simulations undertaken in Section 3.4 indicated that the spatial resolution of the PEDAs-based ion optic may be improved by adding a contrast aperture in the velocity mapping plane of the instrument. In this section, initial results will be presented using an aperture based ion optic assembly which was manufactured according to the illustration in Figure 3.15.

For characterisation experiments undertaken employing the modified PEDAs instrument an exalite 389 grid sample was investigated, and the spatial resolution of the 682.31 Da ion peak analysed. Three ion optic settings, initially optimised for 390 Da, 480 Da and 680 Da, were employed to image the sample. The chosen potentials, which were based on the ion trajectory simulations undertaken above, were previously listed in Table 3.2. The extractor electrode was pulsed at a delay time of 2.76 μs .

Figure 4.26 presents a series of the three ion images of the exalite 398 sample acquired over 2000 experimental cycles each. The first ion image was taken with the settings optimised for 390 Da. A clear grid structure (pitch 500 μm , wire size 67 μm) can be observed across the entire field-of-view, which can be estimated to have a diameter of 5 mm. In contrast to earlier experiments, it can be seen that the ion images is far more homogeneous, and no laser hot spots introducing a blurring effect are observed across the sample. This is most likely due to the fact that the aperture cuts out all ions which are ablated from the sample surface with a high initial transverse velocity. As a result, only the ions

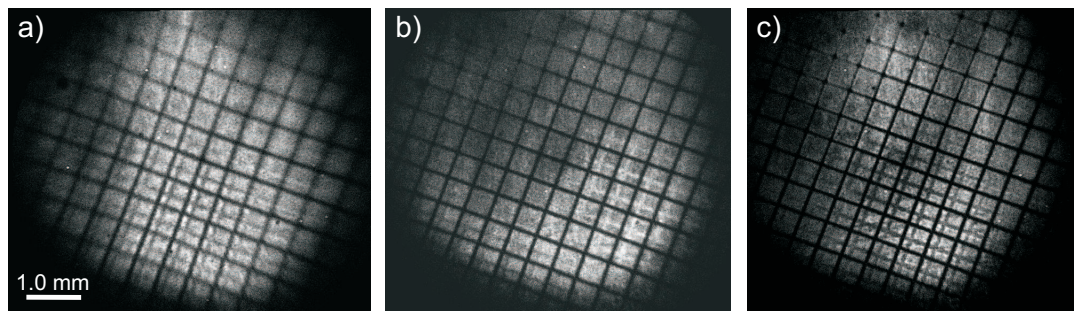


Figure 4.26: Ion images recorded of an exalite 398 (682.31 Da) grid structure using different ion optic settings: *a)* Optimised for 390 Da ions; *b)* Optimised for 480 Da; *c)* Optimised for 680 Da.

which leave the hot spot with no transverse velocity are mapped onto the detector, and a more evenly distributed ion image is observed. A second interesting feature can be spotted in the first image presented: while in the middle section the grid structures are clearly resolved, the squares at the outer part of the image seem to be distorted. This effect can be explained due to the positioning of the velocity focus point of the imaged ion mass in the spectrometer. For the 390 Da settings, only ions of mass 390 Da have their velocity mapping plane located at the position of the aperture, while for ions with a higher mass the focal point can be found further down the ToF tube. Hence, ions coming off the surface at the edges of the field-of-view with a velocity vector pointing straight along the ToF axis, crash into the aperture electrode. Only those ions which come off the surface at a certain angle, pointing towards the centre of the spectrometer, can pass the aperture. As a result, the position information determined by mapping the ions onto the detector is distorted, as the initial velocity vector of the ions causes a spatial shift in their detection position. The effect should be reduced if the ion optic settings optimised for ions closer to the mass of 682.31 Da are applied. For the image presented, an average spatial resolution of $35 \pm 3 \mu\text{m}$, and a magnification factor of $9.48\times$ were determined by analysing a cross section plot. Due to the aperture, the spatial resolution observed for the applied ion optic settings was improved significantly in comparison to the experiments performed in Section 4.2.5, where a resolution of $54 \pm 7 \mu\text{m}$ was reported.

The second image in the series was taken using the ion optic settings optimised for 480 Da. Here, the distortion of the outer part of the ion image is reduced significantly in comparison to the 390 Da settings, as the velocity mapping plane of the ions is located closer to the aperture. The grid structures are resolved across the entire field-of-view, and a spatial resolution of $27 \pm 2 \mu\text{m}$ was determined from a cross section plot. In the experiment using the simple PEDDA instrument, a similar spatial resolution of $28 \pm 7 \mu\text{m}$ was found. Hence, it is surprising that the aperture did not improve the spatial resolution obtained.

The third image presented in Figure 4.26 was taken using the ion optic settings optimised for 680 Da. According to the ion trajectory simulations, a resolution of sub- $10 \mu\text{m}$

can be expected for the 682.31 Da peak. However, the actual resolution achievable in the imaging experiment is limited by two factors. Firstly, for the observed magnification each pixel of the camera corresponded to a distance of 10 μm on the sample. Secondly, the accuracy with which a sample can be prepared using the electrosprayer is on the order of 11 μm (see microscope images in Section 2.10). Hence, it is almost impossible to measure resolutions below 10 μm in the later experiment.

An analysis of a cross section plot of the collected ion image resulted in a spatial resolution of 23 ± 4 μm . This value is surprisingly worse than the spatial resolution determined for the PEDa ion optic employed in the previous section (20 ± 4 μm). The magnification of the mass spectrometer was determined to be $10.0\times$.

The fact that the introduction of an aperture did not improve the spatial resolution of the mass spectrometer may be associated, among other parameters, with the properties of the sample employed. In order to assess this point, a second exalite 398 sample with a grid structure of 254 μm pitch and a wire size of 19.8 μm was prepared. The ion image obtained of the 682.31 Da peak applying the 680 Da ion optic settings is presented in Figure 4.27a. As a grid structure is observable over the most parts of the ion image, a resolution of 19 μm or better can be assumed. In Figure 4.27b, a cross section plot for the investigated sample is presented. According to the standard spatial analysis procedure, a resolution of 15 ± 3 μm was determined for the marked section. Hence, it can be stated that the aperture improves the spatial resolution of the mass spectrometer. However, the fact that this spatial resolution is dependent on the applied sample means that the sample preparation method employed may not be suitable to create comparable grid structures with sub-20 μm resolution.

A second positive effect of the aperture ion optic is that the applicable mass range in which a high spatial resolution is observed is extended significantly. Ion images with sub-35 μm resolution were recorded for mass ranges of more than 300 Da width. Hence, the applicable PEDa mass range only depends on the ToF resolution achieved.

In future work, novel methods to extend the ToF focused mass range of the PEDa

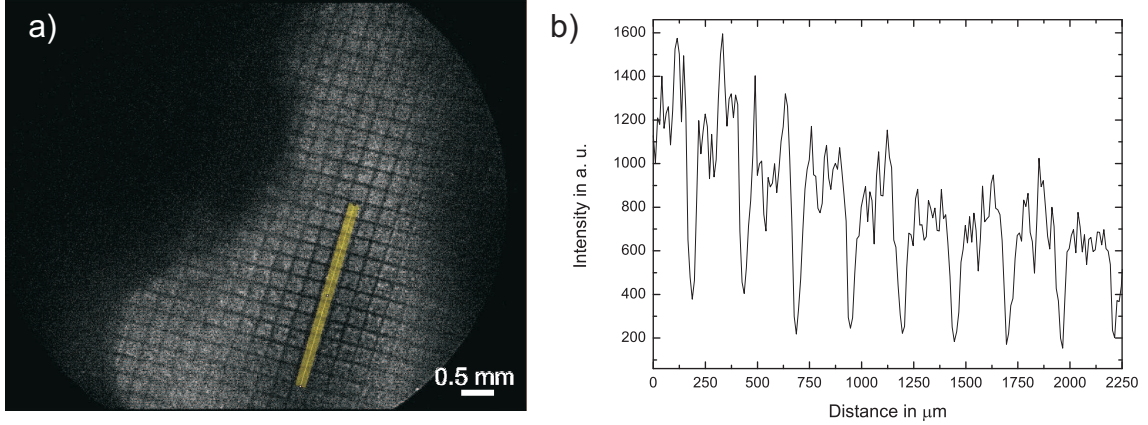


Figure 4.27: a) Ion image of the 682.31 Da exalite 398 peak. The gap size of the imaged grid structure was $19.8 \mu\text{m}$. b) Presents the cross section plot of the ion image. The spatial resolution was determined to be $15 \pm 3 \mu\text{m}$.

method need to be developed. These might involve multiple pulses or using a slow rise function over a longer period of time to allow a wider range of particle masses to be investigated simultaneously. Furthermore, the actual ToF resolution obtained with the aperture configuration needs to be explored experimentally. An optimum resolution of $t/2\Delta t = 2900$ was predicted by the ion trajectory simulations (see Section 4.2.4).

4.4 Conclusion

In this chapter, the specifications of two PEDAs-based microscope mode imaging mass spectrometers have been determined. The first instrument, consisting of simple plane electrodes, allowed spatial distributions across field-of-view of 4.0 mm diameter with spatial resolution of $\sim 20 \mu\text{m}$ to be imaged. The ToF resolution achieved was on the order $t/2\Delta t = 2000$ if the optimum settings for the investigated mass peak were applied. By introducing a velocity corrected ion extraction, it was demonstrated that the spectrometer can be optimised for any mass of interest, whilst the imaging and ToF specifications were maintained. Comparing the obtained results to the work of Aoki and coworkers [158], it should be noted that the ToF resolution achieved using the PEDA technique was improved significantly. Furthermore, the applicability of the technique was improved, as it

was shown that it can be used to image any mass of interest with comparable spectrometer specifications.

The aperture based PEDAS spectrometer was assembled in order to probe how the spatial resolution of the system may be improved by cutting out ions leaving the sample surface with high transverse velocity. In this case, a spatial resolution of $\sim 15 \mu\text{m}$ was determined. Furthermore, a mass range of up to 300 Da width was imaged with spatial resolutions no worse than $35 \mu\text{m}$. However, further work is needed to explore all the specifications of this system.

As a result of the simulations and experiments that have been undertaken, it can be concluded that the achievable overall performance of the spectrometer is no longer limited by the ion optic design, but by the time resolution of the imaging detector. Hence, more advanced particle detectors are required to allow the performance of the spectrometer to be exploited.

Chapter 5

Development of a Fast Scintillator for Ion Imaging Applications

A “complete” microscope mode imaging mass spectrometer requires an ion extraction optic, which allows simultaneously a high ToF and high spatial resolution to be achieved, and an imaging detector, which can detect and translate the impacting ions into light flashes without limiting the instrument specifications. The construction and characterisation of the spectrometer was demonstrated in Chapter 4. However, the ToF and spatial imaging measurements needed to be performed in two separate experiments, as the emission lifetime of the P47 phosphor screen severely limited the temporal resolution of the mass spectrometer. Therefore, to match the timing precision of the imaging detector to the specifications of the spectrometer, it is essential to employ a faster scintillator screen. In this section the development of new, dye-based scintillators will be presented. Key performance characteristics of such scintillators, including the time response, the emission range, the sensitivity, and the spatial resolution, will be investigated separately in the following.

5.1 Introduction

In 1903 Sir William Crooks made an astonishing discovery. A couple of minutes prior, he had spilled radium particles over his zinc sulphide screen. But, rather than collecting and recovering all of the particles, he inspected the screen under his microscope. Instead of the expected continuous glowing of the zinc sulphide, he observed discrete flashes of light, each stimulated by the emission of a single alpha particle. The first scintillator was developed [164].

More than 100 years later, scintillators are employed in detectors and as emitters in a number of devices and experiments, ranging from television and computer monitors, to particle physics experiments [165]. Depending on the application, the required specifications of the employed scintillators vary considerable. For example, the emission wavelength range is crucial for the application of scintillators in night vision devices. If the emission is shifted outside the visible emission range, human eyes cannot recognise the flashes any more. However, one requirement is the same for all scintillators: they should convert the kinetic energy of an impacting particle, or incident ionising radiation, into a detectable, discrete flash of light with a high efficiency. If the scintillation process is stimulated by absorption of a particle's kinetic energy, it is also beneficial if the brightness of the scintillation response shows a linear relationship to the energy of the impacting particle.

For ion imaging applications, such as velocity map imaging or imaging mass spectrometry, it is essential that the detector provides a high spatial resolution. This is achieved when light is emitted from small distinct spots on the scintillator screen that correlates with the position of initial particle impact at the detector. One crucial aspect determining the spot size is the overlap of the absorption and emission spectra of the employed scintillator. Such overlap should be avoided; otherwise emitted photons can be reabsorbed from adjacent scintillator particles, which might cause a secondary fluorescence process. This phenomenon is often referred to as crosstalk. For position sensitive detectors, it is also crucial that the sensitivity of the scintillator is uniform across the whole detector. If this is not the case, the recorded fluorescence features can lead to the misinterpretation of

observed ion image intensity distributions. Besides the spatial resolution aspects, for some applications, such as ToF determination, fast scintillation is required. This time response (fluorescence lifetime) is defined by the specific rise and fall time of the scintillator fluorescence. Furthermore, in terms of the screen preparation it is advantageous for commercial applications if scintillators can be produced in an automated process.

Unfortunately, there are no scintillators which fulfil all of the requirements mentioned above. Therefore, the type of the scintillator needs to be chosen according to its specific application. Generally, scintillator materials can be subdivided into two categories: inorganic and organic ones. The former usually provides the highest light output and a linear energy response, but a slow decay time. Organic scintillators show short fluorescence lifetimes, but they are in the most cases dimmer than the inorganic counterparts. The scintillation mechanism for both kinds of scintillators will be discussed in the following subsections.

5.1.1 Inorganic Scintillation

Inorganic scintillators are metal based compounds, which usually offer a high emission output with reasonably fast decay times [166]. The scintillation mechanism depends on the electronic band structure of the material. This comprises of the low lying valence band, the forbidden band gap, and the conduction band. The electrons located in the valence band are bound, whereas the ones in the conduction band have sufficient energy to migrate through the solid material.

In the case that a charged particle impinges onto a pure crystal, its kinetic energy is absorbed and an electron located in the valence band may be excited into the conduction band (see Figure 5.1). This results either in a direct ionisation of the crystal, or the electron can return into the remaining hole in the valence band. The latter process coincides with the simultaneous emission of a photon, whose energy correlates directly to the separation between the conduction and the valence band. However, the process described is both far too inefficient to allow the material to be used in a scintillation device and the energy of the emitted photon is too high to be located in visible range. Thus, its practical applications

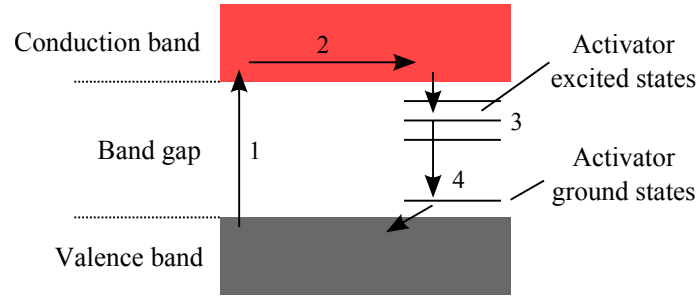


Figure 5.1: Illustration of the scintillation mechanism of an inorganic scintillator. An electron from the valence band is excited into the conduction band (1). There it can migrate through the crystal until it hits an ionised activator (2). An excited neutral is formed within the forbidden band gap from where an emission process to the activator ground state may occur (3-4).

are limited.

To improve the scintillation performance, the inorganic crystals are doped by the introduction of selected metallic compounds. These activators introduce energy states within the forbidden band of the crystal, leading to a modification of the emission wavelength and the efficiency of the emission process. In the case that an electron in the main material of the crystal is excited by the absorption of the kinetic energy of an impacting particle, the created electron-hole pair can migrate independently through the crystal. Once a positive hole hits the activator, this one is immediately ionised, as its ionisation barrier is lower than the one of the main crystal. The ionised activator can then recombine with a free electron from the conduction band so that an excited neutral within the forbidden band of the crystal lattice is formed. The states of the excited neutral are identified in Figure 5.1 by the horizontal lines within the forbidden band gap (see label 3). From here, the activator can get de-excited by emitting a photon, whose energy is defined by the energy gap, to the activator's ground state. For these transitions a typical emission lifetime of 30-500 ns is observed.

In the above explanation of the scintillation mechanism, it was assumed that the translation of the electron in the conduction band and that of its corresponding hole are independent from each other. However, both may stay associated and form an exciton. Here, the electron and the hole are attracted to each other by Coulomb interactions and they

migrate together through the crystal. Once they hit an activator atom, this is excited and a direct scintillation process can occur.

Generally, the scintillation processes are in direct competition with a number of quenching mechanisms. For example, excited activator atoms can relax to the ground state through a radiationless conversion mechanism, or the direct transition to the activator ground state is formally forbidden. In the latter case, a second excitation by either thermal processes, or the approach of a second electron, is required to transfer the activator to a higher state in which the selection rule for the de-excitation can be bypassed. Such emitted light may cause a long afterglow of the scintillator.

5.1.2 Organic Scintillation

Organic scintillators are usually based on aromatic hydrocarbon compounds which show a fluorescence lifetime of a few nanoseconds. A scintillation process occurs most likely within the aromatic system of the molecule: the kinetic energy of an impacting particle can be absorbed by exciting free π -valence electrons into higher states ($\pi \rightarrow \pi^*$ -transition). In contrast to a photon excitation process occurring in the gas phase, no transition rules apply for an electron or ion impact excitation on a solid scintillator screen. Hence, a series of singlet (spin 0) or triplet states (spin 1) may be populated. However, as shown later in this Chapter, both a photon and an electron excitation process result most likely in the population of the same final excited states, leading to fluorescence in the same wavelength range. A simplified energy diagram is illustrated in Figure 5.2 (Jablonski diagram). The respective singlet (S) and triplet (T) electronic states are annotated with the first subscript label, while the second label indicates the vibrational state. For organic scintillators the energy difference between the electronic ground and the first excited singlet state ranges from 3 to 4 eV, while the vibrational levels are separated by roughly 0.15 eV.

If the scintillator is operated at room temperature (thermal energy of 0.026 eV), almost all the molecules are in the electronic ground state. An impact of a charged particle, and the absorption of its kinetic energy, excites the scintillator molecules to a vibronic level

within the manifold of higher electric states as symbolised by the blue absorption arrows in Figure 5.2. The excited molecules usually relax within a few picoseconds *via* a radiationless internal conversion to the ground vibrational level of the S_1 state, denoted as S_{10} .

The actual scintillation process occurs due to fluorescence from the S_{10} level to a range of vibrational excited states in the manifold of the electronic ground state. Here, the number and the population of the final vibrational levels should, to a certain extent, define the width of the emission spectrum observed for the applied organic scintillator. The fluorescence lifetime is defined *via* the constant τ , which can be determined by fitting the fluorescence decay using the equation

$$I = I_0 e^{-t/\tau}, \quad (5.1)$$

where I is the absolute emission intensity, I_0 the initial intensity and t the time component.

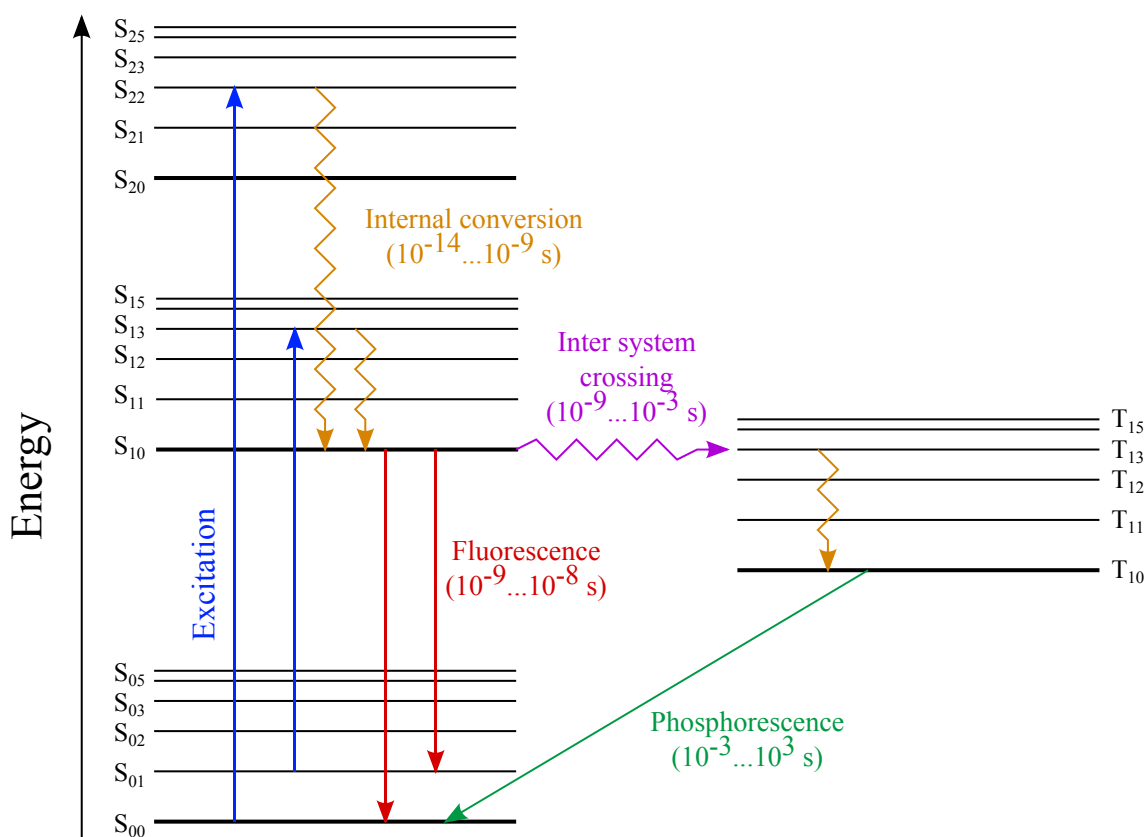


Figure 5.2: Jablonski diagram illustrating the excitation and relaxation mechanism, leading to fluorescence or phosphorescence processes.

Figure 5.2 shows also a second possible relaxation mechanism *via* an intersystem crossing to the lowest triplet state, T_1 . As the lifetime of this triplet state is typically 10^{-3} s to 10^3 s, the de-exitating phosphorescence to the singlet ground state occurs over a relatively long period of time. The emission spectrum associated with phosphorescence is usually red shifted and does not overlap with the fluorescence spectrum, since the triplet state commonly lies energetically below the first excited singlet state. However, this process as well as other radiationless relaxation mechanisms should be avoided, as they may quench the fluorescence of the scintillator.

Another photon emission process observable is the delayed fluorescence. Here, molecules which were transferred into the triplet state can be thermally excited back into the S_1 state. This results in a delayed photon emission from the S_1 state.

One big advantage of organic scintillators is that, in the most cases, their absorption and emission spectra do not overlap. Therefore, the emitted fluorescence photons have insufficient energy to re-populate the S_1 level by re-excitation of ground state molecules. The effect of re-absorption and re-emission can be avoided and the scintillator is transparent to its emitted photons.

Organic scintillators can be subdivided into three groups according to their morphology: liquid, plastic and crystalline scintillators. Due to their vacuum compatibility only the latter two can be employed in an MCP detector. The properties of liquid scintillators will not be discussed further in the thesis.

Plastic scintillators are prepared by dissolving the organic luminescent material in a precursor, which can easily be polymerised. Common plastic matrices are polyvinyltoluene and polymethylmethacrylate. The big advantage of these scintillators is that they can be produced and cut in any form and shape to match specific applications. Furthermore, the plastic polymer protects the scintillator against degradation damage through prolonged exposure to the impact of particles with a high kinetic energy. Additionally, the plastic matrix prevents the luminescent molecules from evaporating under vacuum conditions, which permits their application in MCP ToF detectors.

A typical example of a crystal scintillator is anthracene. It is one of the oldest and most efficient scintillators known today. Due to its high scintillation efficiency, it is used as a standard reference for all other organic scintillators. However, organic crystal scintillators are quite fragile in comparison to plastic scintillators, and it is difficult to produce them in larger sizes. Another point is their uniformity, which might vary across the scintillator screen, resulting in an uneven sensitivity. Further disadvantages of crystalline scintillators are their thermodynamic properties: for example, anthracene is not vacuum stable and sublimates readily within seconds. Thus, it is not possible to employ this scintillator in mass spectrometry, ion imaging experiments or other applications operated under vacuum conditions.

5.1.3 Properties and Applications of Inorganic and Organic Scintillators

The choice of a scintillator for a certain application depends on the required emission properties. In general, if a fast light emission is required then an organic scintillator is the material of choice, while if a high light output is preferential, an inorganic scintillator is preferred.

For most inorganic scintillators the photon emission efficiency follows a simple empirical rule: in order to create an electron-hole pair it is necessary to excite the material with three times the energy of the band gap between the conduction and the valence band. The resulting light output is, in general, proportional to the absorbed kinetic energy. However, nonlinear effects can appear due to the quenching processes discussed previously. In comparison with the inorganic scintillators, organic ones convert only a smaller portion of the kinetic energy into light. Non-radiative quenching processes, such as vibrational relaxation, are a dominant feature of these scintillators. For the standard organic scintillator, such as stilbene and anthracene, the emission efficiency is linearly proportional to the kinetic energy of the impacting particle. However, the light output depends strongly on the kind of particle. For example, protons show a weaker fluorescence signal than electrons. Craun

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Figure 5.3: Scintillation efficiency plot for electrons and protons impinging with different kinetic energies onto an anthracene scintillator. The figure was adapted from Reference [167].

and Smith demonstrated that the higher the impact energy of the particle, the lower is the light output discrepancy between a proton and an electron excitation [167]. A typical comparison graph for anthracene is presented in Figure 5.3.

Generally, it is hard to compare scintillators directly as they emit light at different wavelengths, and respond differently to the nature of the impacting particles. However, it is instructive to list the properties of a number of commercial organic and inorganic scintillators as shown in Table 5.1. Here, it should be mentioned that the listed relative efficiencies of each scintillator indicate a general trend. Due to differences in the kinetic energy of the impacting particles, in each case, the absolute value of the relative intensity may change slightly [165].

The scintillators listed in Table 5.1 are employed for various purposes. The one with the highest light output is P22, which is commonly used in image intensifier tubes for night vision devices. It provides a high spatial resolution, and its emission spectrum peaks at 530 nm, which is located in the green region of the visible spectrum. However, the scintillator cannot be employed in an ion imaging detector as its decay constant for light emission is rather long. Hence, most ion imaging detectors consist of a P43 or P47

Scintillator	Chemical information	Max. emission wavelength (nm)	Emission efficiency as % of anthracene	Decay constant (ns)	Scintillator type
Anthracene	$C_{14}H_{10}$	447	100	30	Organic crystal
Stilbene	$C_{14}H_{12}$	410	50	4.5	Organic crystal
BC-400/EJ-212	Polyvinyltoluene	423	65	2.4	Plastic
BC-404/EJ-204	Polyvinyltoluene	408	68	1.8	Plastic
BC-408/EJ-200	Polyvinyltoluene	425	64	2.1	Plastic
P22	ZnS:Cu, Al	530	1631-1755	> 1 ms	Inorganic
P43	$Gd_2O_3S:Tb$	545	483-725	> 1 ms	Inorganic
P46 / YAG	$Y_3Al_5O_{12}:Ce$	550	102-110	88	Inorganic
P47	$Y_2SiO_5:Ce$	420	145-156	70	Inorganic
YAP	$YAlO_3:Ce$	370	109-117	27	Inorganic

Table 5.1: Properties of a number of organic and inorganic scintillators. Most of the data was adapted from the book “*Radiation Detection and Measurement*” by Glenn F. Knoll [165].

scintillator coupled to a set of MCPs. The emitted photons are detected by either a PMT or a CCD/CMOS based camera device.

Another typical application of the P47 scintillator screens is found in scanning electron microscopy (SEM, see Figure 5.4a). In the surface analysis an electron beam is rastered over a conductive sample, mounted on the stage in the bottom of the machine. The impacting electrons cause an emission of secondary electrons of up to 50 eV. These electrons can be collected by an P47 based Everhart-Thornley secondary electron detector (see Figure 5.4b) mounted sidewise to the sample plate. A second P47 scintillator detector mounted just above the sample plate has a hole in its centre through which the electron beam is guided onto the target. This scintillator is used to detect the back scattered electrons.

Scintillators employed in SEMs are required to show a high sensitivity for electron detection, have a high brightness, and a fast decay time. The scintillation efficiency is mainly important to improve the signal-to-noise ratio, resulting in an increase in the contrast of the back scattered electron signal. This allows a more reliable determination of the chemical composition of the sample. The decay time of the applied scintillator is important for the repetition rate and therefore for the scanning speed of the experiment. For that reason, it is not sensible to employ a P43 screen, as the collection time is increased. P47 is, due

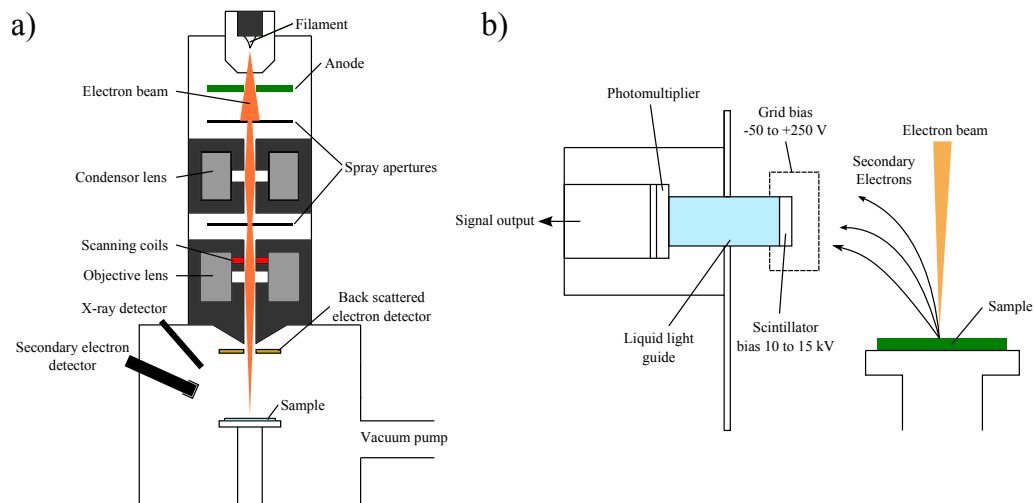


Figure 5.4: Illustration of an scanning electron microscope (a) and an Everhart-Thornley secondary electron detector (b).

to its relative fast decay time and high brightness, the most common applied material in SEMs. Organic crystal scintillators are not employed as they suffer from a low brightness and may out-gas under ultra high vacuum conditions.

Organic plastic scintillators are often used as a conversion unit in electron multiplier tubes and bipolar MCP detectors to allow high timing resolution in mass spectrometry. In both cases the emitted light is detected by a PMT. Such a system represents one of the most common detector assemblies employed in ToF mass spectrometry. A typical rise time of such a detector is on the order of <1.2 ns, and the FWHM is shorter than 2.4 ns [168]. However, MCP-scintillator detectors need to be operated with a high gain, up to 30 kV, to ensure the sensitive detection of impacting particles.

As Table 5.1 indicates, organic and inorganic scintillators are available to cover the whole visible range. However, no known scintillator has the characteristics of a high brightness, a fast decay constant (<10 ns), and vacuum stability, which are required to perform the imaging mass spectrometry experiment described in Chapter 4. Hence, throughout the following sections of this chapter, the development of a new scintillator screen that meets these performance criteria will be described.

5.2 Scintillating Material

In order to investigate the application of various scintillators in terms of their ToF and imaging performance in MCP-based particle detectors, a number of inorganic and organic materials need to be tested and characterised. One initial criteria was that the most intense emission of the screen must lie in the range from 380 nm to 600 nm, which matches the sensitivity of the most CCD and CMOS based imaging sensors. At the same time, the emission lifetime of the scintillator should be as short as possible.

5.2.1 Inorganic Scintillators

Typical metal based scintillators showing a fast fluorescence process are P47($\text{Y}_2\text{SiO}_5\text{:Ce}$), QM58 ($\text{YAlO}_3\text{:Ce}$) and GK31 (ZnO:Ga). The P47 screen emits in the range from 380 nm to 480 nm, has a peak wavelength around 400 nm, and exhibits an emission lifetime of 70 ns [169]. For QM58, a far shorter emission lifetime of ~ 23 ns is observed [169]. However, the peak of the emission wavelength is shifted into the UV to 370 nm. The GK31 scintillator screen decays to a 50% signal level in 0.3 ns, and has a peak emission wavelength of 390 nm [169].

The emission characteristics of metal based phosphor screens are usually very much dependent on the characteristics of the scintillator layer: typical factors are the grain size, the layer thickness, and the density of the phosphor particles distributed in a silica matrix layer. It is generally found that a reduction in the particle size of the phosphor correlates with a loss of efficiency [170]. An increasing layer thickness also gives rise to a decrease in the efficiency (light output), and is associated with a loss in spatial resolution due to light scattering within the layer [171]. However, it is possible to maintain the conversion efficiency for thicker phosphor layers by increasing the kinetic energy of impinging particles. In this thesis, in order to find the most suitable screen for application in imaging detectors, the three metal scintillators mentioned above were prepared using a sedimentation process and compared with various organic based scintillators. The sedimentation process will be described in detail in Section 5.3.

5.2.2 Organic Scintillators

5.2.2.1 Introduction

The application of organic compounds as scintillator has been investigated for a long period of time, and extensive studies were performed as early as in the 1950s, e.g. [172]. In this work the organic scintillation properties following excitation by ultraviolet light and ^{60}Co γ -rays were investigated. 55 pure crystalline compounds were studied and empirical trends

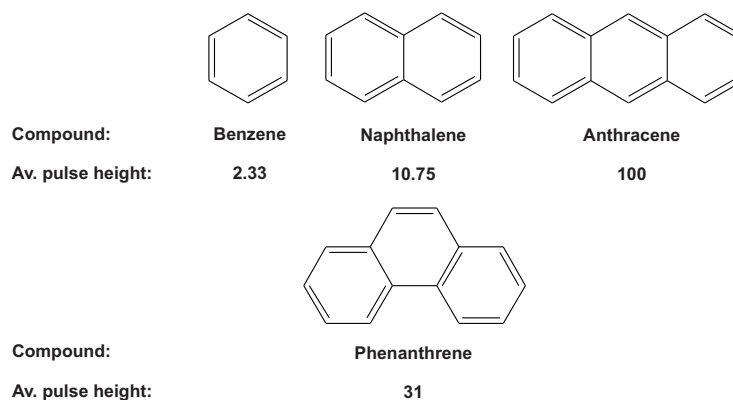


Figure 5.5: Illustration of the chemical structures of a selection of organic scintillators studied by Sangster and Irvine [172]. The scintillation efficiencies relative to anthracene are symbolised as a function of the average pulse height determined for the fluorescence process.

between molecular structure and emission efficiency were established. The chemical structure and the relative scintillation efficiency (average pulse height) of benzene, naphthalene, anthracene and phenanthrene are illustrated in Figure 5.5. The efficiencies determined for these organic compounds were found to be correlated with the extent of the delocalised π -electron system: while benzene emits light with a low efficiency, anthracene and its derivatives are the most efficient organic scintillators known today. However, a further increase of the aromatic system beyond the 3-fused ring system of anthracene does not necessarily result in an improved light output, as factors such as steric hindrance or loss of symmetry may reduce the scintillation efficiency. A typical example is phenanthrene, which exhibits a lower scintillation efficiency despite having the same sized π -electron system as anthracene - the scintillation process is quenched due to hydrogen repulsions, which leads to more stable “kinked polycyclic benzenoids” [173].

5.2.2.2 Applications of Laser Dyes

A typical class of organic scintillators applied to absorb incident radiation in the UV, and re-emit at a longer wavelength *via* a fluorescence process are *laser dyes*. Beside the fact that they show an intense light output after photoexcitation, they also need to have a short emission lifetime, as lasing of more than 10 ns results in a weak peak energy.

Dye	Absorption Peak in nm	Emission Peak in nm	Fluorescence lifetime in ns
α -NPO	326	398	1.9
Stilbene 3	349	425	0.9
Coumarin 460	370	445	3.1
Coumarin 519	412	490	3.5
Rhodamine 6G	530	560	4.4
Rhodamine 101	564	594	5.3
DCM	464	644	2.0

Table 5.2: Photochemical specification of a selection of laser dyes in ethanol solution. The table was adapted from the PhD thesis of Liu [175].

For operation in a dye laser system the dye needs to be dissolved in methanol, ethanol or dioxane. Thus, the solvent will affect the dye's emission properties: for example, the absorption and emission spectra may be shifted, and the fluorescence lifetime may be changed. Such an effect is caused by the viscosity, the polarity, and the structure of the solvent, and is commonly termed the solvent effect [174]. A comprehensive time resolved fluorescence study of a number of laser dyes in ethanol solution was undertaken by Liu in 1997 [175]. The specifications determined are presented in Table 5.2. All dyes investigated decay within 6 ns after photoexcitation, while the emission wavelength range could be selected by choosing a specific dye.

In a further study, Liang and coworkers absorbed various laser dyes as monolayers onto tin and indium oxide surfaces, and measured the fluorescence lifetime of these components after photoexcitation (355 nm) using a streak camera, which operated with a picosecond timing resolution [176]. Emission lifetimes on the order of 50 to 60 ps were reported for rhodamine B and eosin, indicating that the fluorescence lifetime of dyes absorbed onto semiconductors may be up to two orders of magnitude shorter than observed in solution phase.

More recently, Dubois *et al.* used fluorescent organic scintillator materials to build an ion-to-photon conversion detector (IPD) for applications in ToF mass spectrometry [177]. The detector consisted of a layer of scintillator sample, spray coated onto a glass plate, and mounted in front of a fast photo multiplier tube. The principle of operation utilised

the fact that ions impinging on the organic substrate effect the emission of photons, which can be detected by the PMT. The concept of this detector is not entirely new. Already in 1950 Richards and Hays observed light flashes caused by the impact of small ions onto a scintillator, consisting of either silver activated zinc sulphide, thallium activated sodium iodide and/or anthracene [178].

The organic scintillators applied by Dubois and coworkers were 2-(4-*t*-Butylphenyl)-5-(4-biphenyl)-1,3,4-oxiadiazole (But-PBD), 2,5-bis(5'-*tert*-butyl-2-benz-oxazolyl)thiophene (BBOT), 2-(4'-biphenyl,1-6-phenyl-benoxazole) (PBBO) and rhodamine B [177]. All of these scintillators can be classified as laser dyes, although none of these is in frequent use, with the exception of rhodamine B [179]. According to Dubois *et al.*, But-PBD was found to be the most efficient scintillator tested, allowing analyte signal of up to 70000 Da to be detected. In later work Dubois, Knochenmuss and coworkers compared the single ion detection efficiencies of a BBOT based IPD with a capacitor coupled MCP detector [180]. The more efficient Bu-PBD scintillator used in previous studies was not applied as it degraded under vacuum conditions. It was shown that the relative detection efficiency of the IPD, similar to the MCP-based detector, decreased with increasing mass of the detected fragment.

The IPD project was taken a step further by introducing a post acceleration step in front of the scintillator screen [181]. This proved to be an efficient way to enhance the signal levels detected by up to 70%. Generally, a higher ion-to-photon yield was achieved with smaller charged particles. The time response of the BBOT based IPD was determined as ~ 6 ns. By placing a conversion dynode in front of the scintillator, ions were converted into electrons before impinging onto the scintillator. The electron-to-photon conversion of BBOT was determined to be slightly better than the one found for a BC-404 (see efficiencies in Table 5.1).

Most studies of the scintillation processes of laser dyes to date are based on either light, γ -particle, or X-ray excitation. To the knowledge of the author, no study apart from the investigations undertaken by Dubois *et al.* considered the detection of low energy electrons

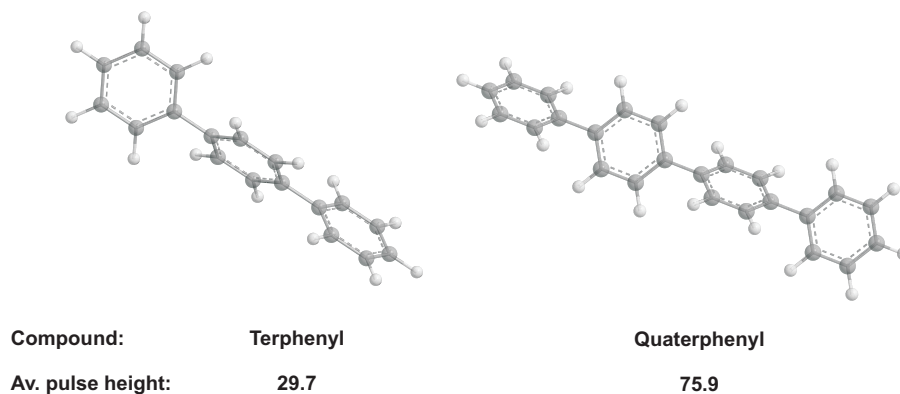


Figure 5.6: Illustration of the chemical structures of *para*-terphenyl and *para*-quaterphenyl. The structures illustrated above were optimised using the Avogadro 1.1.0 software (force field method *Ghemical*). The scintillation efficiencies relative to anthracene were determined by Sangster and Irvine [172].

(≤ 10 keV) using a laser dye based (particle-to-photon) detector. However, following the BBOT study it seemed that this class of molecules did not show a significantly higher electron-to-photon conversion efficiency than a conventional plastic scintillator (e.g. BC-404). For the purpose of developing a fast imaging MCP scintillation detector the electron-to-photon conversion efficiency needs to be increased significantly.

5.2.2.3 Poly-*para*-phenylene Scintillators

Another class of efficient organic scintillators are poly-phenyl derivatives such as illustrated in Figure 5.6. In the chain, all adjacent phenyl units can freely rotate about the main molecular symmetry axis, which avoids steric conflicts of the *ortho* and *meta* located hydrogen atoms. Despite the free rotation of the phenyl units in the gas and liquid phase, all carbon atoms are sp^2 hybrids, and π -electron delocalisation may extend throughout the whole system. Irvine and Sangster noted that the scintillation efficiency of these components increases with the number of integrated phenyl units (see Figure 5.6) [172]. The reason for the improved scintillation properties may be associated with the extension of the delocalised π -system, which increases the probability that an electron is elevated into an excited state.

Derivatives of the presented *para*-terphenyl molecule are nowadays a component of a

number of products. Typical examples are their application in sunscreen lotions [182], or as laser dyes [183–186]. In both cases, the molecules are chosen as they have a large absorption cross section in the UV range. Further characteristics of these compounds are that they show after photoexcitation an exceptional high stability, a high photon-to-photon conversion efficiency, and a short emission lifetime.

Over the last decade several theoretical models have been developed to predict the optical properties of poly-*para*-phenylene derivatives as a function of their molecular structure. Such models take into account the number of linked phenyl units, the terminal substitution groups, and bridging between the phenyl units. The most recent prediction model was introduced by Merz and coworkers [187]. Therein, the researchers described a shift in the peak wavelength of the emission as a function of the electron donor or acceptor strength of terminal substitution groups, as defined by the Hammett parameter (σ_{Hammett}). By substituting *para*-terphenylene with a selection of donor and acceptor groups in the terminal position, and measuring the wavelength shift, $\Delta\lambda_{\text{Shift}}$, with reference to the unsubstituted molecule, a linear relationship to the Hammett parameter was determined,

$$\Delta\lambda_{\text{Shift}} = -104 \pm 7 \times \left(\frac{\sigma_{\text{Hammett}}}{n} \right), \quad (5.2)$$

where n is the number of phenyl units. It can be concluded that a more negative value of the Hammett parameter corresponds to a higher degree of electron density donated towards the phenyl units. This allows more electrons to afford optical transitions, which leads to a reduced energy gap between the ground singlet and first excited electronic state. As a consequence, a fluorescence process involving these electronic states results in the emission of a photon shifted towards a longer wavelength. These results were found to be in good agreement with earlier experimental studies from Kauffmann, Fleitz and coworkers [188–190], who also reported a reduction in the photo stability and an extended fluorescence lifetime for poly-*para*-phenylene derivatives substituted with terminal electron donating groups.

The emission wavelength of poly-*para*-phenylene derivatives is not only dependent on the terminal substituents, but also on the relative torsion angle of the phenyl rings. This

parameter can be controlled by introducing integrated bridges between the *ortho* and *meta* position of two adjacent phenyl units. Experimental studies indicated that the photostability of bridged *para*-quaterphenyls increases following the order $\text{CH}_2\text{CH}_2\text{<O<CH}_2$ [191, 192]. If two units are linked by a methylene bridge they are constrained to lie in the same plane, while non-bridged adjacent units usually have a torsion angle between 30 and 40 degrees. This fact is important as the retention of planarity between adjacent phenyl rings preserves the π -delocalisation throughout the fluorophore leading to more efficient fluorescence.

To predict the emission wavelength as a function of the number of phenyl units, n , and their relative torsion angle, ϕ , which is defined as a function of the reference plane of the molecule, Merz and coworkers normalised the peak emission wavelength recorded for various poly-*para*-phenylene derivatives according to their Hammett parameters [187]. The resulting peak emission wavelength was only dependent on the properties of the phenyl units, and could be predicted by the empirically derived equation

$$\lambda_{\text{Peak}}(n_{\text{eff}}) = \lambda_{\infty} + (\lambda_1 - \lambda_{\infty}) e^{-a(n_{\text{eff}}-1)}, \quad (5.3)$$

where n_{eff} is given by

$$n_{\text{eff}}(n, \tau) = n - (n - 1) \left(\frac{\tau}{90^\circ} \right)^2, \quad (5.4)$$

and

$$\tau(n, \phi_i) = \sum_{i=1}^{n-1} \frac{|\phi_i - \phi_{i-1}|}{n - 1}. \quad (5.5)$$

In the Equations 5.3 to 5.5 λ_{∞} corresponds to the emission wavelength of a molecule with $n \rightarrow \infty$ phenyl units, λ_1 is defined as the emission wavelength of just one phenyl unit (benzene), a is an empiric correlation parameter, and ϕ_i stands for the torsion angle of each phenyl unit. Equation 5.4 covers both extreme orientation cases: If all the phenyl rings are bridged, and are therefore located in one plane, then τ should be zero and the emission wavelength is only dependent on the number of involved phenyl units. But if no bridges hold the phenyl units in plane, then the effective ring number transforms to unity. However, the simple approach of summing over all torsion angles of the molecule and calculating the

average value gave qualitative good results (see Equation 5.5). By fitting Equation 5.3 as a function of n_{eff} to the Hammett parameter corrected emission wavelength of a number of poly-*para*-phenylene derivatives, λ_{∞} could be determined as 420 ± 25 nm. With this result it is possible to predict according to Equations 5.3 and 5.2 the emission wavelength of any poly-*para*-phenylene dye as a function of its structural properties. The prediction model was again found to be in good agreement with experimental studies from Fleitz, Schneider and Seliskar, who characterised the photo-physical properties of 37 poly-*para*-phenylene dyes as a function of their chain length and substitution groups [190, 193, 194].

Models like the one from Merz *et al.* aid the design and optimisation of specific poly-*para*-phenylene derivatives employed as laser dyes. Considering the previous experimental and theoretical work, described above, substituted poly-*para*-phenylene molecules are identified as possible candidates for scintillator molecules, as they are more stable and more efficient than other known organic species in the chosen emission range.

5.2.2.4 Applied Organic Scintillators

The four poly-*para*-phenylene dyes chosen for the purpose of building an electron detector are illustrated in Figure 5.7. 9,9,9',9'-tetrapropyl-9*H*,9'*H*-2,2'-bifluorene (exalite 384), 2,7-bis(4-methoxyphenyl)-9,9-dipropyl-9*H*-fluorene (exalite 389), 9,9,9'',9''-tetraethyl-7,7''-di-*tert*-pentyl-9',9'-dipropyl-9*H*,9'*H*,9''*H*,9''*H*-2,2':7',2''-terfluorene (exalite 398), and 1,4-bis(9,9-diethyl-7-(*tert*-pentyl)-9*H*-fluoren-2-yl)benzene (exalite 404) are all commercially available poly-*para*-phenylene laser dye molecules, which have, due to their molecular structure, the potential to serve as a fast and efficient scintillator following charged particle excitation.

The idea of using exalite dyes as a scintillator in a particle detector is not completely new. In 1997 Tymianski and Walker patented a scintillator system for the purpose of detecting high energy particles and ionising radiation, such as X-rays, γ -rays, α -particles, β -particles and thermal neutrons [195]. The scintillator comprised of three components. An organic solvent or a solid polymer matrix; a polycyclic aromatic compound, such as a substituted naphthalene derivative, usually present to an abundance of 5% or more; a

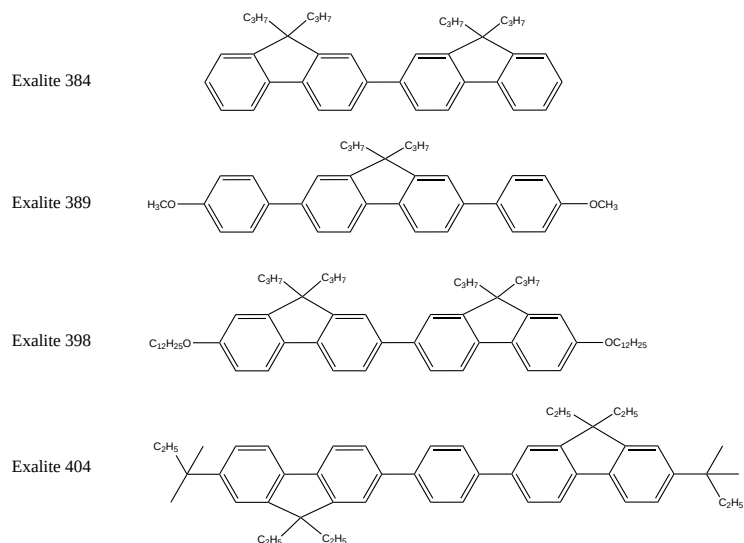


Figure 5.7: Molecular structures of the applied exalite dyes.

fluorophore added in up to 3% ratio of the relative weight of the scintillator. Exalite 389 was one of a number of components employed as a fluorophore or emission wavelength shifter. Experimentally, the incident penetrating radiation loses energy to the solvent matrix, which is transferred to the polycyclic aromatic compound. When the emission spectrum of this compound overlaps with the absorption spectrum of the fluorophore, light excitation of the fluorophore and subsequent re-emission in the range of 400 to 500 nm is recorded using a photon detection device. In the work of Tymianski and Walker, the scintillator device was meant to detect high energy particles. For example, electrons from β -decay processes usually have a kinetic energy up to 1 MeV. This value is far higher than the kinetic energies of electrons detected from an output of an MCP or an electron multiplier tube (1-10 keV). The mixture of solvent and matrix employed is used to protect the organic molecules from structure damage *via* interactions with high energy radiation. For ion imaging or mass spectrometry applications this requirement is of less importance. Therefore, a fluorophore is chosen by the required emission wavelength and employed directly as scintillation screen without the need of a polymer matrix and subsidiary scintillation compounds. As a result, the electron-to-photon conversion efficiencies are expected to be far higher as reported in the patent.

A further indicator that exalite dyes may have the potential to operate in a particle detector with a short fluorescence lifetime, can be found in a study undertaken by Inamdar and coworkers, who measured the decay constant for the fluorescence of solvated exalite 398 molecules [196]. It was shown that the decay constant does not exceed 1.15 ns for a variety of solvents. For application in a dye laser, exalite molecules are readily dissolved in dioxane, and pumped with a Nd:YAG laser beam (355 nm). Thereby, all of the chosen poly-*para*-phenylene derivatives show a maximum light output on the order of 20% of the pump energy [197, 198]. This value represents an outstanding conversion efficiency achieved with commercially available laser dyes.

Apart from the exalite dyes, further organic compounds were identified as potential electron sensitive scintillators. These are mainly molecules commonly applied as laser dyes, or fluorescent tags for microscopy. Typical examples of laser dyes are rhodamine molecules (rhodamine 6G, rhodamine 101), coumarin dyes (coumarin 440) and DCM. These compounds were selected as their emission spectra are shifted towards higher wavelengths than the exalite dyes, and whose fluorescence may be captured sensitively with available PMT or camera technology. Two typical fluorophores applied to microscopy are fluorescein and curcumin. These molecules are usually excited using a blue light source, resulting in the re-emission of photons between 520 nm and 530 nm. A comprehensive list of all scintillators investigated in this thesis is presented in Table 5.3, and the remaining corresponding molecular structures are illustrated in Figure 5.8.

5.3 Scintillator Screen Preparation

The quality of a prepared scintillator screens has a significant impact on the specifications determined in the later characterisation measurements. For example, it needs to be ensured that the luminescent particles form a homogeneous layer across the screen surface, as an uneven distribution may result in an inconsistent fluorescence response. A second key parameter is the reproducibility of the screens. Here, it needs to be ensured that the same preparation procedure results in screens with comparable performance.

Compound	Chemical Formula	Category
P47	$\text{Y}_2\text{SiO}_5:\text{Ce}$	Inorganic / Scintillator
QM58	$\text{YAlO}_3:\text{Ce}$	Inorganic / Scintillator
GK31	$\text{ZnO}:\text{Ga}$	Inorganic / Scintillator
Exalite 384	$\text{C}_{38}\text{H}_{42}$	Organic / Laser dye
Exalite 389	$\text{C}_{33}\text{H}_{34}\text{O}_2$	Organic / Laser dye
Exalite 398	$\text{C}_{62}\text{H}_{90}\text{O}_2$	Organic / Laser dye
Exalite 404	$\text{C}_{50}\text{H}_{58}$	Organic / Laser dye
BBOT	$\text{C}_{26}\text{H}_{26}\text{N}_2\text{O}_2\text{S}$	Organic
Rhodamine 6G	$\text{C}_{27}\text{H}_{29}\text{ClN}_2\text{O}_3$	Organic / Laser dye
Rhodamine 101	$\text{C}_{32}\text{H}_{31}\text{N}_2\text{O}_3 \text{ ClO}_4$	Organic / Laser dye
Coumarin 440	$\text{C}_{10}\text{H}_9\text{NO}_2$	Organic / Laser dye
DCM	$\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}$	Organic / Laser dye
Fluorescein	$\text{C}_{20}\text{H}_{12}\text{O}_5$	Organic / Fluorescence tag
Curcumin	$\text{C}_{21}\text{H}_{20}\text{O}_6$	Organic / Fluorescence tag

Table 5.3: Overview of all compounds whose electron impact stimulated scintillation properties are investigated in this thesis.

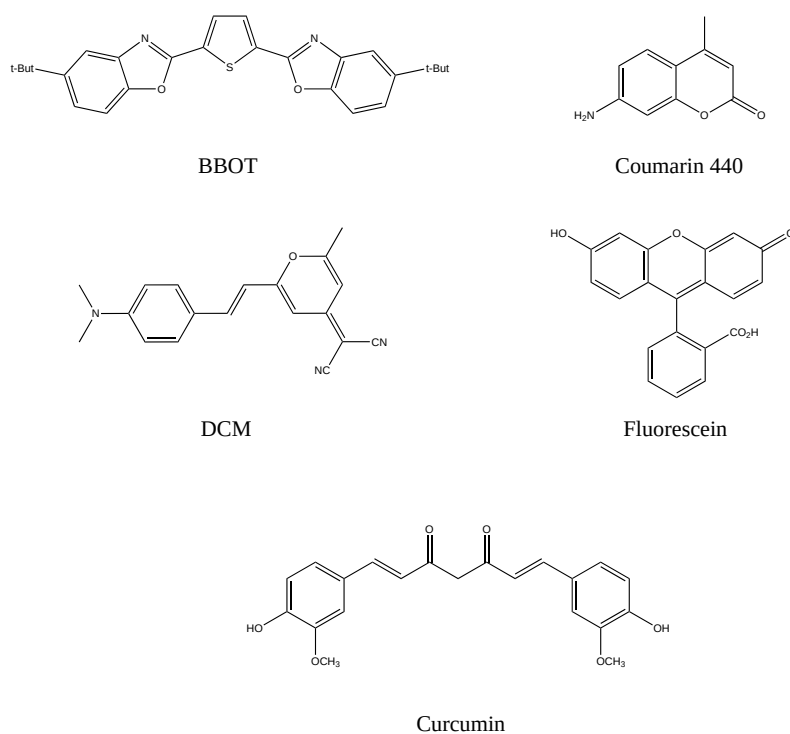


Figure 5.8: Molecular structures of organic compounds investigated for their scintillation properties following electron impact excitation.

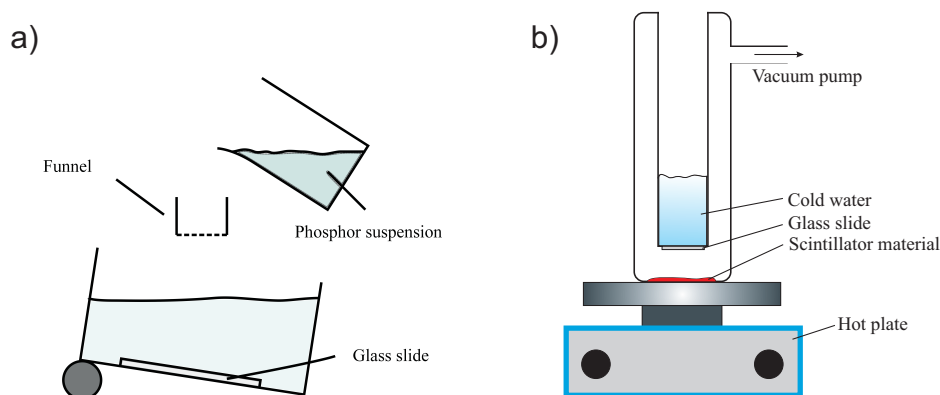


Figure 5.9: Illustration of the sedimentation process (a), and the vacuum sublimation apparatus (b).

Commercially available phosphor screens, such as P43 or P47, are usually prepared by a brushing process [166]. Here, the phosphor particles are distributed through a sieve onto a binder coated glass plate. Then, the particles are brushed into the (heated) binder until a homogeneous coating is achieved. Other preparation methods involve a sedimentation process in which the phosphor particles are incorporated into a silica layer. In a similar way, organic plastic scintillators are often integrated into a polymer matrix.

In this thesis, in order to compare and test the specifications of a number of scintillators, two different sample preparation methods were applied. Inorganic phosphors were deposited using a sedimentation process, while organic dye screens were prepared *via* vacuum sublimation. The substrates for all of the scintillator screens described in this chapter were quartz slides of 1.1 mm thickness, coated with a layer of the transparent conducting compound indium tin oxide (ITO). Square (25 mm) and circular (50 mm diameter) substrates were used. In the following subsections both preparation methods are described in more detail, and the overall detector assembly is presented.

5.3.1 Sedimentation

The sedimentation process, illustrated in Figure 5.9a, is similar to that used to produce phosphor screens in small-scale commercial operations. Clean ITO-coated substrates were placed in a tilted beaker ($\sim 8^\circ$) with the conductive side facing upwards. Barium chloride

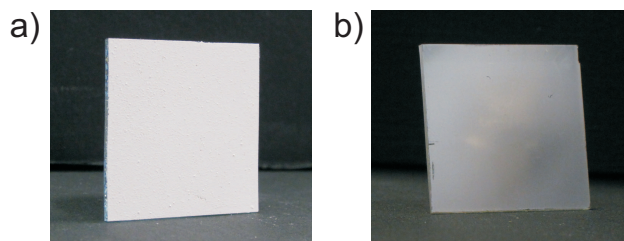


Figure 5.10: Photographs taken of a P47 scintillator screen prepared using the sedimentation process (*a*) and an exalite 404 sample which was prepared by vacuum sublimation (*b*). Both screens had a size of 25×25 mm.

solution (1.16 mM in deionised water) was added to the beaker until the plates were submerged to a depth of 5 cm. A mixture of 0.15 g of phosphor powder (Phosphor Technology, UK, 95% of particles $< 7.2 \mu\text{m}$) in 20 mL of 0.37 M potassium silicate solution was prepared, stirred thoroughly, and poured slowly through a sieve to form a suspension mixture within the beaker. After a sedimentation period of 20 minutes, the remaining solution was siphoned from the beaker, taking care to avoid generating turbulence which might damage the screens. The newly prepared screens were dried in an oven for 20 minutes at 60°C prior to installation within an imaging detector. Typical phosphor thicknesses on screens prepared using this method were around $350 \mu\text{m}$ (micrometre measurement).

A photograph of a P47 phosphor screen prepared according to the sedimentation protocol is shown in Figure 5.10*a*. Inhomogeneities in the screen surface may be caused either by turbulence during solvent removal or air bubbles trapped during the sedimentation step.

5.3.2 Sublimation

Organic scintillators were prepared using a vacuum sublimation apparatus, illustrated in Figure 5.9*b*. A quantity of dye (0.05 g to 0.15 g) was placed in the base of the sublimation apparatus (Ace vacuum sublimation apparatus, 60 mm internal diameter). The clean ITO sample plate was mounted, coated-side-down, on the cold finger of the apparatus, which was then sealed and evacuated down to a pressure of 14 mbar. The cold finger was filled with a water/ice mixture, and heat was applied to the bottom of the sublimation chamber using a hot plate. Depending on the material, the dye crystals either melted

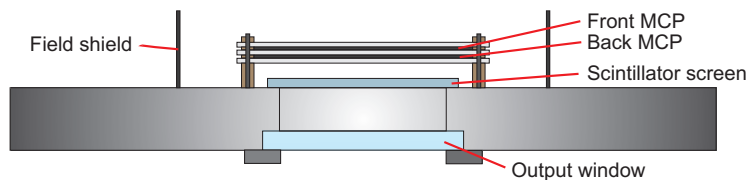


Figure 5.11: Cross section schematic diagram of the ion imaging detector assembly. The gap between the back of the MCPs and the scintillator screen was about 1.5 mm. A grounded shield divided the MCPs and various electrical feedthroughs to avoid the distortion of electron trajectories in the vicinity of the detector due to electrical fields.

and evaporated, or sublimed, and subsequently crystallised onto the ITO plate. Care was taken to ensure complete evaporation/sublimation of the dye sample in order to achieve reproducible scintillator screen thicknesses of around 200 μm (micrometre measurement). A typical scintillator screen prepared using exalite 404 dye is presented in Figure 5.10*b*.

This method has been proven to produce uniform, and reproducible crystalline sample layers. It can be applied to most of the organic materials listed in Table 5.3. Metal based scintillators were not prepared in this way, as the required sublimation temperature is too high.

5.3.3 Detector Assembly

The scintillator screens prepared according to one or the other procedure were mounted on a vacuum flange between a chevron-pair of 40 mm microchannel plates and an 65 mm anti-reflective coated fused silica window (see Figure 5.11). Electrical feedthroughs mounted in the flange allowed to control of the potentials applied to the MCPs and scintillator screen. For some of the calibration and comparison measurements described below, composite screens were fabricated using smaller sections of several different scintillators.

5.4 Time Resolution Measurements

5.4.1 Electron Excitation

Following the preparation procedure given in Section 5.3.3, each of the assembled detectors was mounted in a standard velocity map imaging spectrometer. A schematic drawing including the relevant dimensions of the three lens ion optic is presented in Figure 5.12. The total drift length was approximately 730 mm. In order to collect ToF spectra a PMT (Hamamatsu, H6780-20) with a rise time of 0.78 ns was mounted behind the output window of the detector assembly. The PMT signal was recorded using a fast oscilloscope with a 0.125 ps sampling precision. Ion images were collected using either an intensified CCD or the PImMS camera.

To characterise the fluorescence lifetime of the various screens, electron ToF spectra were recorded, as these provide a suitable fast signal for the measurement. For this purpose, methyl iodide was seeded in helium (5% mixture), and supersonically expanded into the chamber *via* a pulsed valve (General Valve 10 series) to form a molecular beam. This

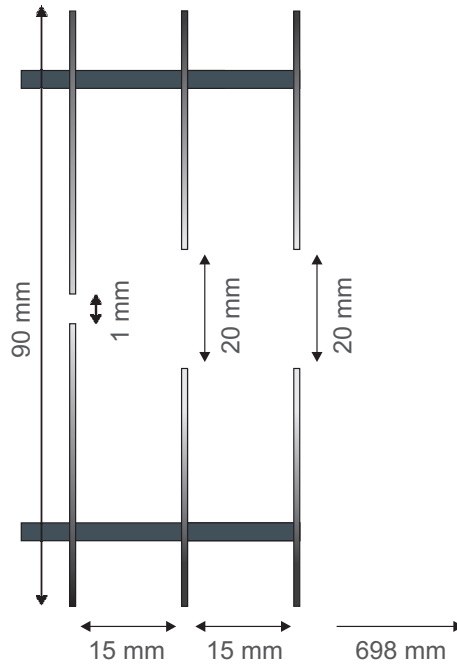


Figure 5.12: Schematic diagram of the ion optic employed in the velocity map imaging spectrometer.

beam was skimmed with a 0.5 mm skimmer, before entering the ion optic through a central aperture in the repeller plate. At a central point between the repeller and the extractor plate, the molecular beam was intersected by a focused beam of 118 nm radiation, generated by focusing the third harmonic of a Nd:YAG (Continuum Surelite I) into a gas cell filled with a phase-matched mixture of 24 mbar of Xe and 325 mbar of Ar [199]. Following the ionisation of the sample, liberated electrons were immediately accelerated towards the detector by applying a potential of -2.0 kV to the repeller, while grounding the two remaining electrodes. The gain applied to the detector was adjusted for every scintillator individually, to ensure that a sufficient signal level for the determination of the rise and the decay time was obtained. Furthermore, for some scintillators ion images of the parent ion peak of the methyl iodide sample were collected under VMI conditions. These measurements served as test of whether the fluorescence signal is detectable with a conventional CCD camera and the CMOS based PImMS camera. Furthermore, the images collected provided preliminary information on the brightness of some scintillators.

ITO coated glass slide

In the first part of the scintillator testing procedure, it was necessary to check if any photon signal arises due to scintillation of the metallic coating of the quartz slides. For this purpose an electron ToF spectrum was acquired over 1000 shots, with a potential of 1.5 kV applied across the MCPs and 3.5 kV applied to the ITO coated glass plate. The resulting electron spectrum (Figure 5.13a) reveals two peaks. The first can be attributed to scattered laser light, while the second peak appearing at a later time corresponds to electrons impinging onto the screen. According to Equation 5.1 the fluorescence lifetime of the electron signal could be fitted with a decay constant of $\tau = 7.3 \pm 1.1$ ns.

Contributions to the photon yield due to scintillation of the ITO coating will add to the electron ToF spectrum recorded for each of the screens fabricated, and will influence the timing specifications extracted. However, the signal levels observed in the presented measurement were at least several orders of magnitude weaker than the light emission

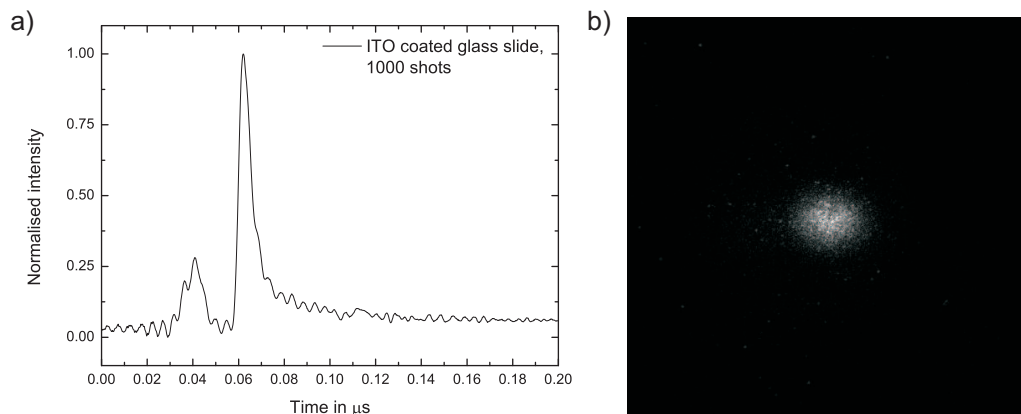


Figure 5.13: a) Electron ToF spectrum recorded using a clean ITO coated glass plate. b) Parent ion VMI image of methyl iodide taken with an ITO coated quartz plate as scintillator.

recorded in subsequent measurements for substrates coated with a layer of inorganic or organic scintillator. Hence, the ITO scintillation contributed a negligible amount to the recorded electron signals, and was ignored in the subsequent analysis.

In Figure 5.13b an ion image of the ionised methyl iodide molecule is presented. The image was acquired over 2000 laser shots using a CCD camera in combination with the clean ITO plate. Despite the low scintillation efficiency it was still possible to record an ion image. However, no signal was observed employing the non-intensified PImMS camera.

Scintillator tests

Figure 5.14 shows the electron ToF signals recorded using the exalite 404, the exalite 389 and the P47 scintillator screens. All spectra were acquired over 100 experimental cycles. When the detector was operated using the inorganic scintillator the potential applied across the MCPs was set to 1.3 kV, while the P47 screen was charged to 3.5 kV. For the two exalite screens a stronger fluorescence signal was observed such that the detector could be operated with 1.1 kV across the MCPs and 2.5 kV applied to the scintillator screen. An exponential fit to the falling edge of the P47 electron signal resulted in a decay constant of $\tau = 69.3 \pm 0.8$ ns. This value is in agreement with the data sheets of commercial P47 scintillator screens [169]. In Figure 5.14 it can also be seen that both exalite screens show

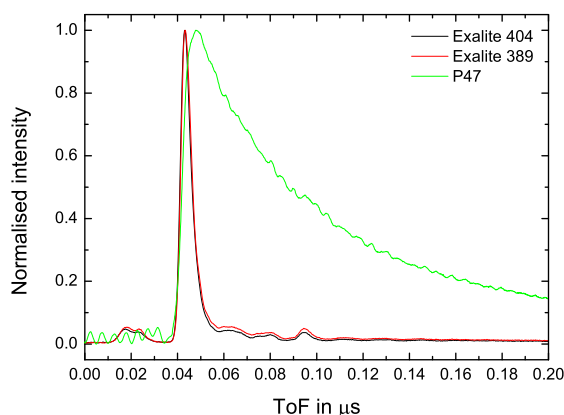


Figure 5.14: Normalised electron ToF signals recorded for the exalite 404, exalite 389 and P47 scintillator screens.

very short fluorescence lifetimes. The decay constants were determined as 3.7 ± 0.5 ns for the exalite 389 screen and 3.7 ± 0.2 ns for the exalite 404 scintillator.

It was shown previously that an MCP-based position sensitive detector containing a P47 scintillator screen can, due to the effective conversion of impinging electrons into photons, successfully be coupled to a PImMS or a CCD camera to acquire images of ion distributions (see Chapter 2). The preliminary electron ToF data suggest that the exalite scintillator screens are at least of a comparable efficiency to the P47 screen, and therefore may also be coupled to a suitable camera to acquire ion images. To test the hypothesis a velocity map image of the parent ion peak of methyl iodide was recorded using an intensified CCD camera (see Figure 5.15a). For the application of the PImMS camera, the applied MCP potential was raised to 1.5 kV and the scintillator potential was set to 3.2 kV. The PImMS image presented in Figure 5.15b shows a far greater contribution of background signal to the molecular beam spot. This background may be due to a higher gas pressure in the chamber, such that the 118 nm radiation ionises sample molecules outside of the intersection with the molecular beam at the centre of the ion optic. Another possibility is that, due to the higher detector settings, a more intense signal was observed. Hence, molecules which were ionised along the laser propagation direction were detected more sensitively. However, independent of this observation, the emission characteristics of the

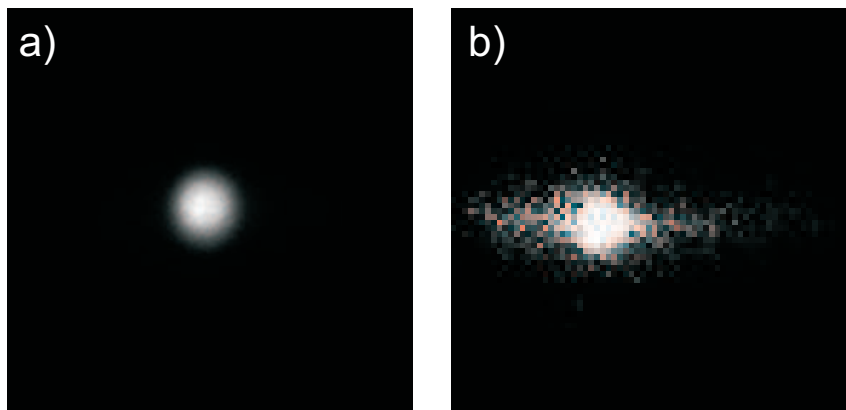


Figure 5.15: Ion images taken of the methyl iodide parent ion peak under velocity map imaging conditions using the exalite 404 scintillator screen. The image in *a)* was acquired over 2000 frames using an intensified CCD Camera. *b)* Shows a 2000 shot image taken with the PImMS camera. Both ion images were recorded with a different magnification.

exalite 404 were found to be suitable for performing imaging studies. Ion images recorded using the exalite 389 screen showed a comparable signal to the ones presented for the exalite 404 scintillator.

Summary

The fluorescence lifetimes determined for all applied scintillator screens are compiled in Table 5.4. In order to give a more comprehensive description of the determined emission properties, the rise time (from 10% to 100% of peak intensity), and the fall time (from 100% to 10% of peak intensity) of the signal were measured additionally. A short rise time is particular important for photon detection devices which trigger off the rising edge of a signal. Employing an exalite 404 or exalite 389 scintillator instead of a P47 screen allows the absolute timing information determined with such a detection device to be improved.

A comparison of the results of these electron ToF measurements reveal that four scintillator screens show the same short decay constant for the observed signal. These are the organic exalite 389, exalite 404 and curcumin screens, as well as the inorganic GK31 scintillator. The identical temporal emission characteristics determined for these scintillators suggest that the absolute fluorescence lifetime may be limited by various experimental

Compound	τ in ns	t_{rise} in ns	t_{fall} in ns	Signal strength
ITO coating	7.3 ± 1.1	3.3 ± 0.4	13.0 ± 1.3	very weak
P47	69.3 ± 0.8	6.7 ± 0.2	168 ± 2.3	strong
QM58	39.6 ± 0.1	5.8 ± 0.4	97.0 ± 1.7	medium
GK31	3.7 ± 0.1	3.5 ± 0.3	7.5 ± 1.3	weak
Exalite 384	4.7 ± 0.5	4.2 ± 0.8	9.3 ± 1.5	strong
Exalite 389	3.7 ± 0.5	3.5 ± 0.6	7.7 ± 1.0	strong
Exalite 398	9.8 ± 0.4	5.1 ± 1.1	26.1 ± 1.9	strong
Exalite 404	3.7 ± 0.2	3.5 ± 0.2	7.7 ± 0.3	strong
BBOT	14.5 ± 0.5	7.0 ± 0.3	30.1 ± 1.8	strong
Rhodamine 6G	-	-	-	no signal
Rhodamine 101	-	-	-	no signal
Coumarin 440	-	-	-	not stable
DCM	37.9 ± 1.3	5.0 ± 1.1	38.2 ± 2.4	weak
Fluorescein	5.4 ± 3.2	-	-	very weak
Curcumin	3.7 ± 0.6	4.1 ± 0.2	7.2 ± 0.3	medium

Table 5.4: Timing parameters determined for the various scintillator screens following the detection of a pulse of electrons. The decay constants (τ) representing the emission lifetime of the screens were determined using a single exponential fit to the data. t_{rise} is defined as the time taken for the signals to rise from 10% to 100% of the peak maximum, and t_{fall} describes the signal decay times from 100% to 10%.

factors. These are the pulse width of the ionisation laser, the time resolution of the ion optics under the chosen focusing conditions, and the PMT time precision. All factors serve to broaden the electron ToF signal. Indeed, ion trajectory simulations indicated that the temporal spread of electrons arriving at the detector, under the operating conditions of the spectrometer, is ~ 3 ns. Thus, for these scintillators 3.7 ns represents an upper limit to the true fluorescence decay time.

As Table 5.4 indicates, it was not possible to determine the emission characteristics of the rhodamine scintillator screens as no electron ToF signal was observed. Furthermore, the coumarin dye was not stable under vacuum conditions (10^{-6} mbar). The ToF spectra recorded for all remaining scintillator screens can be found in Appendix B.

Comparing the molecular structures of the organic scintillators (see Figures 4.7, 5.7 and 5.8) which show a medium or strong fluorescence signal following electron impact excitation, reveals that these compounds are chain based molecules. The delocalised π -electron

system expands for these scintillators, due to their planar geometry, over almost the entire molecule. The organic scintillators showing either no or only a weak fluorescence signal are more compact, which may affect steric effects that result in a weaker fluorescence signal (e.g., rhodamine and fluorescein dyes). This observation is in close agreement with the conclusions of the photoexcitation studies discussed in Section 5.2. The absolute efficiency of the screens will be further investigated in Section 5.6.

The four scintillators showing the shortest fluorescence lifetime are the GK31, exalite 389, exalite 404 and curcumin samples. However, as the fluorescence signals observed for curcumin and GK31 were relatively weak, further characterisation measurements were performed only for the exalite 389 and exalite 404 screens. In order to compare the results of this study with the previously presented work by Zenobi and coworkers [177, 180, 181], the BBOT sample will also be further characterised. All three screens will be compared to the P47 screen, which currently serves as an industry standard.

5.4.2 Light Excitation

A second decay time characterisation measurement was performed using a Fluoromax 4 fluorescence spectrometer (Horiba Scientific) at the Rutherford Appleton Laboratory, United Kingdom. The spectrometer allows the fluorescence lifetime of solid scintillator samples, after photo excitation, to be determined. A schematic drawing of the instrument is presented in Figure 5.16. The spectrometer comprised of two light sources, one broadband emission source and a 337 nm excitation LED. Emission of the former was wavelength selected using a monochromator before irradiating the scintillator screens. The incident radiation of either of the two light sources stimulated fluorescence from the scintillator, which was recorded in a 90 degree angle to the excitation. Emitted light passed through a wavelength filter and then through a second monochromator which both served to filter out the excitation wavelength and to select a narrow wavelength window of the fluorescence emission spectrum. The remaining photons were recorded using a single photon counting PMT located behind the emission monochromator.

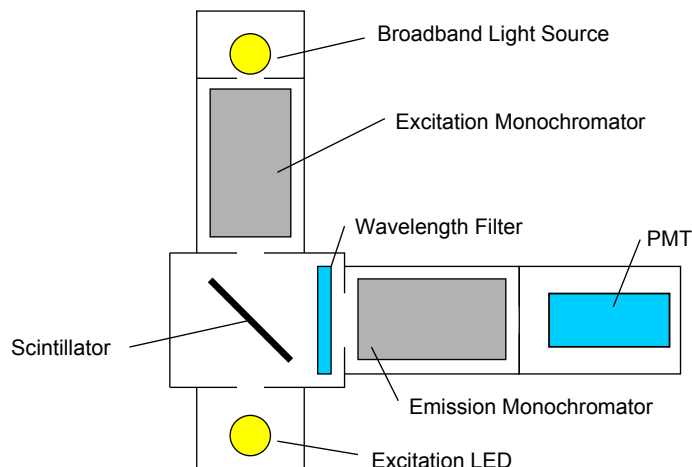


Figure 5.16: Schematic drawing of the Fluoromax 4 fluorescence spectrometer.

Fluorescence lifetime measurements using the Fluoromax 4 spectrometer were performed only for the exalite 404 dye sublimated onto an ITO plate. The luminescent sample was excited using a 337 nm LED flash. A typical decay spectrum acquired over 2000 individual measurements at an emission wavelength of 450 nm (± 2 nm) is presented in Figure 5.17. By fitting a mono-exponential function to the observed fluorescence signal a decay constant of $\tau = 1.58 \pm 0.03$ ns was determined. A measurement closer to the emission maximum, which should be located around 404 nm, was not possible, as the strong scintillator emission saturated the PMT of the spectrometer. Due to the fact that in the undertaken measurements only a prompt fluorescence process was observed, phosphorescence or delayed fluorescence involving triplet states populations can be excluded for the investigated excitation process.

The fluorescence lifetime measured for the exalite 404 screen was found to be < 2 ns. This time constant is on the order of the decay time of the LED (1.5-2.0 ns). Therefore, it is possible that the determined fluorescence decay constant may be limited by the lifetime of the LED flash. In the case that the light and electron impact excitation of the exalite dye leads to the fast population of the same energy levels, a similar fluorescence process can be assumed following both excitation methods. Hence, the light excitation measurements indicate further that the fluorescence lifetimes determined in the ion imaging experiments were limited rather by experimental parameters than by the properties of the scintillator

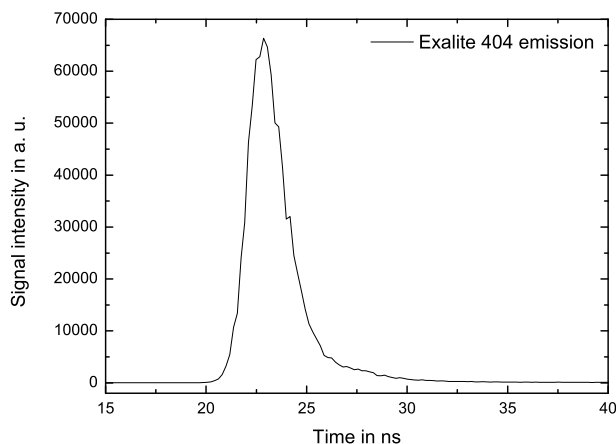


Figure 5.17: Fluorescence signal recorded for a crystalline exalite 404 scintillator after 337 nm flash lamp excitation.

screen.

5.5 Photoemission Spectra

A second key parameter which needs to be characterised for the selected scintillator screens is the emission wavelength range after an electron impact excitation process. It is essential that the screens mainly emit in the near UV and visible regions of the spectrum, as most imaging sensors and PMTs show their highest photon sensitivity in this wavelength range. In order to investigate the photon emission range after electron excitation, a monochromator (Applied Photophysics, f/3.4 Monochromator) coupled to a PMT was mounted behind the output window of the scintillation detector. Following the description of the ion imaging spectrometer given in the Section 5.4, the detector was again illuminated uniformly by ionising methyl iodide and extracting the resulting electrons with a repeller potential of -6.0 kV. In all measurements, the detector was biased with 1.3 kV across the MCPs, and a potential of 3.5 kV was applied to the scintillator screen. This values resulted in an electron impaction energy of 2.2 keV on the scintillator sample. The signal levels were optimised for each scintillator by adjusting the widths of the monochromator input and

output slits. The maximum slit width applied resulted in a wavelength resolution better than 5 nm. Emission spectra were derived by taking the integrated signal within the electron ToF peak, which was acquired over 100 shots at small wavelength increments in the range from 300 to 600 nm. The resulting spectrum was further adjusted by considering the wavelength dependent sensitivity curve of the PMT and the light absorption of the ITO coated glass plate.

As the exalite and BBOT samples are usually applied as laser dyes in the solution phase it is also interesting to investigate how the photoemission spectrum in solution compares with the electron excitation spectrum of the solid scintillator. Hence, the emission spectra of all organic compounds dissolved in dichloromethane (1 mg/ml) were recorded following photoexcitation at 337 nm. Furthermore, an emission spectrum of the solid exalite 404 scintillator, following 337 nm LED excitation, was recorded using the Fluoromax 4 fluorescence spectrometer (Horiba Scientific). The resulting emission spectra are presented in Figure 5.18.

The emission spectra recorded for the exalite 389 dye are presented in Figure 5.18*a*. Following optical excitation in the solution phase at 337 nm, an emission peak extending from 360 nm to 480 nm was measured, with a peak maximum at 389 nm. A steep rise in the photon emission is observed at the shorter wavelengths, while the emission “tails off” slowly towards longer wavelengths. Furthermore, in the photon excitation solution phase spectrum indications of a double peak structure can be observed close to the peak emission of the exalite 389 scintillator. This may suggest that the fluorescence may occur from or to manifolds of different vibrational modes in either the upper or lower electronic level in the organic molecule. The emission spectrum recorded following the electronic excitation in the imaging detector is shifted towards longer wavelength. Apart from this, a similar peak shape, with a slightly shorter emission tail at the high wavelength end of the spectrum is observed.

The electron and photon excitation emission spectra recorded for the exalite 404 screen show an overlapping peak structure (see Figure 5.18*b*). All recorded spectra peak at

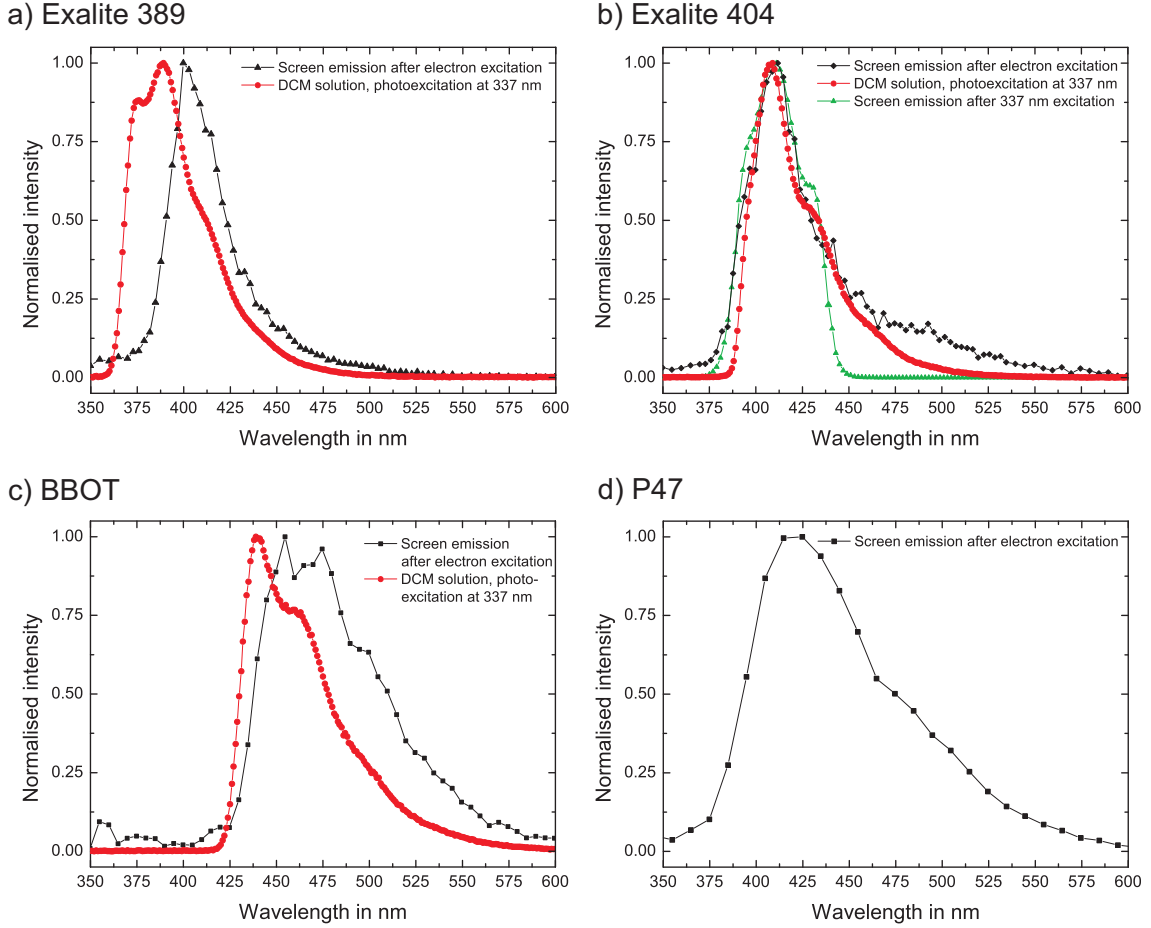


Figure 5.18: Fluorescence emission spectra of selected scintillator samples: *a)* Shows the emission of exalite 389 following 337 nm photo excitation in dichloromethane solution and after electron impact excitation in an MCP detector. *b)* Shows both spectra recorded using exalite 404 as scintillator. Furthermore, an emission spectrum recorded following a photo excitation a solid exalite 404 screen is presented. *c)* Presents the emission spectra recorded for the BBOT sample following a photo excitation process in solution and an electron impact excitation of a solid screen. *d)* Shows the electron excitation emission spectrum of a P47 scintillator screen.

an emission wavelength of 412 nm. However, a difference can be spotted in the longer wavelength tails: while the solid photoexcitation spectrum shows a relatively sharp cut-off at 450 nm, a long extending “tail” is observed for the photoexcitation of the dissolved dye and the electron excitation spectrum.

The third scintillator investigated was BBOT (see Figure 5.18*c*). In contrast to the emission spectra of the exalite dyes, the BBOT emission spectra following both electron and photon excitation are shifted to longer wavelengths. Hence, the optical emission of

BBOT is more compatible with the sensitivity range of most standard imaging sensors. Comparing the photon and electron excitation emission spectra, a clear shift towards higher wavelengths can be observed for the electron impact stimulated fluorescence. In the electron emission spectrum a double peak structure can be observed, which may again indicate the involvement of multiple vibrational state manifolds in the fluorescence process.

The similarities between the photon and electron-impact excitation emission spectra seem to indicate that regardless of which electronic state(s) is initially populated, emission occurs primarily from the same electronic state, presumably following a rapid relaxation from higher states *via* rapid internal conversion processes (see discussion in Section 5.1.2). When employed in solution in commercial dye lasers, the investigated dyes emit from their first excited singlet state [200], and to first order this appears to be the case for organic scintillation in the solid phase investigated in this work. Furthermore, it was initially thought that the long wavelength tail in the solid state emission spectra of all investigated dyes indicates that the emission occurs from the first triplet state. This might be possible, since excitation *via* charged particle collisions does not obey optical selection rules, and an ensemble of molecules in both excited singlet and triplet states may be formed. A phosphorescence process from a triplet state is associated with a longer emission lifetime. As none of the electron ToF spectra indicated a significant contribution to the photon yield from longer-lived emission processes, this de-excitation process can be excluded for the investigated sample molecules.

Figure 5.18*d* shows a representative emission spectrum recorded for a sedimented P47 phosphor screen following a charged particle excitation process. By comparing the organic spectra with that of the P47 screen, it can be seen that all three organic scintillator screens emit within the P47 emission range. Thus, each new scintillator has the potential to replace the comparatively slow P47 screen equivalently.

5.6 Scintillator Sensitivity

The suitability of a scintillator screen is determined by its electron-to-photon conversion efficiency. A brighter and more sensitive scintillator screen reflects an improved signal-to-noise ratio, and allows a more efficient detection of impinging particles. Hence, especially in ion imaging applications where cameras are used to record events, it is beneficial if more photons are distributed over the pixels of the sensor. This allows centroiding and cluster analysis to be performed to gain a higher spatial resolution.

In Section 5.4, a relative statement for the observed scintillation efficiency was presented. The exalite dyes were identified as the most efficient scintillator screens among the applied materials. However, this value is not yet quantified. The absolute value of the photon-to-electron conversion efficiency is a difficult parameter to access with the ion imaging spectrometer available in the research lab, since the absolute numbers of impinging electrons, as well as the absolute photon output, need to be determined. Furthermore, even in the case that the light output of a single scintillator is measured quantitatively in a VMI experiment, such measurements of the scintillation efficiency are not reproducible as experimental conditions such as the source pressure, the composition of the sample gas, the laser power and the background pressure in the chamber may fluctuate over time leading to large variations of ion flux.

To circumvent this problem, a segmented screen was fabricated whereby two quadrants consisted of P47 phosphor screens and the remaining two quadrants are composed of the scintillator of interest. By operating multiple scintillator screens in a segmented experiment it is possible to determine their relative conversion efficiencies. Furthermore, as two scintillators of each kind were mounted simultaneously it is possible to compare the photon output at different electron excitation energies, as the scintillation process might start at a threshold and saturate at a certain electron energy for each scintillator. The assembled screen is presented in Figure 5.19a.

In the experiments a potential of 1.1 kV was applied across the MCPs which was held constant for all the measurements undertaken. Furthermore, one of each pair of

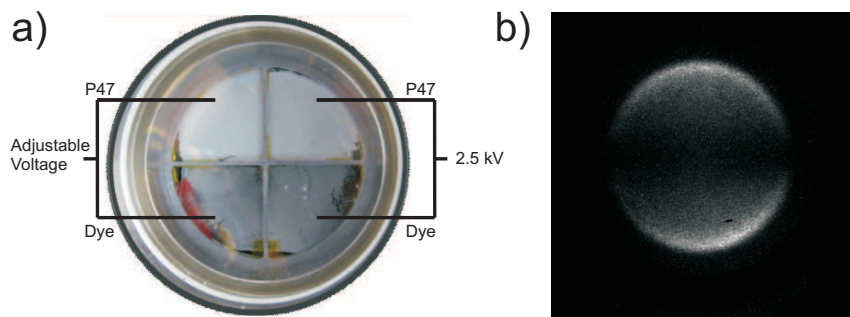


Figure 5.19: a) A typical quadrant detector employed for the relative scintillation efficiency measurements. By operating one of each scintillators at a different acceleration potential a relative value for the brightness of the screens can be determined. The total diameter of the composite screen was 40 mm. b) Ion image of the I^+ fragment formed in the photolysis of ethyl iodide at 245.2 nm. The example image was collected using a conventional P47 detector. A ring diameter of ~ 25 mm was determined.

scintillators was held at 2.5 kV, while the potential applied to the second of each pair was incremented in steps of 100 V up to a maximum of 4.0 kV relative to ground. In this way the relative energy of the impinging electrons could be adjusted. The resulting fluorescence was observed by acquiring an ion image accumulated over 2000 laser shots using a standard intensified CCD camera.

An imaging detector is used to detect ions and record their spatial distribution. Therefore, in this set of experiments, I^+ ions formed *via* the photolysis of ethyl iodide at 245.2 nm were velocity mapped onto the detector. The sample was seeded at 4% in Helium to a backing pressure of ~ 1000 mbar behind the general valve. For all the measurements, the repeller potential was set to 2.00 kV and the extractor was operated with 1.47 kV applied.

A typical ion image detected with a single scintillator screen is presented in Figure 5.19b. The iodine atoms are formed initially with a fairly narrow velocity distribution resulting in a single “ring”. Additionally, a strong anisotropy can be seen in the image, which suggests that the transition dipole moment is mainly aligned parallel to the C-I-bond axis ($\cos^2 \theta$ distribution). A more detailed description of the photodissociation mechanism of ethyl iodide can be found in the work of Tang and coworkers [201].

In the experiments undertaken in this work the position sensitive detector was mounted on a translational stage and positioned such that the centre of the ion image is aligned

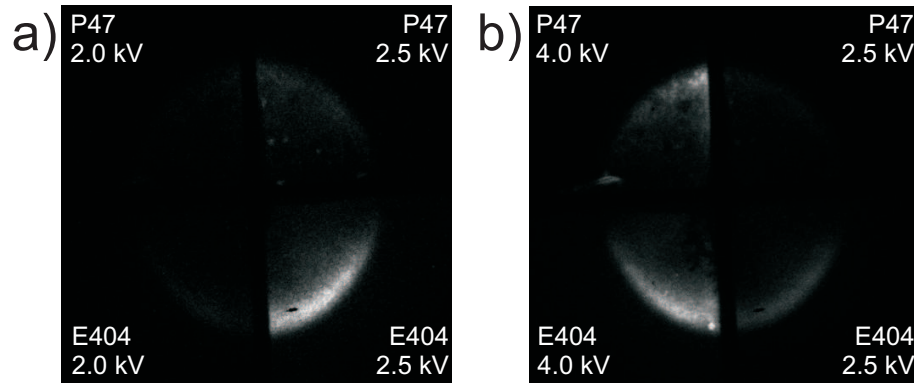


Figure 5.20: Ion images recorded with the segmented scintillator detector. The scintillator in each section and the corresponding applied potentials are denoted in both images.

with the centre of the segmented scintillator screen. Thus, the main advantage of the horizontally symmetric image is that, with careful positioning of the detector, four identical intensity distributions are observed. By integrating the signal intensity for identical regions in each quadrant of the image, a relative value of the electron-to-photon conversion efficiency for each screen can be determined.

A comparison of partial ion images collected using a segmented exalite 404 and P47 screen is presented in Figure 5.20. Even a cursory examination reveals that the exalite samples, located on the bottom side of the segmented screen show a more efficient electron-to-photon conversion than the P47 samples held at the same potential. By dividing the value of the integrated signal intensity for each scintillator by the corresponding value for the P47 screen held at a constant potential of 2.5 kV, the relative electron-to-photon conversion rate is calculated. Values of the relative conversion efficiency for each scintillator, plotted as a function of the acceleration potential between the back face of the MCPs and the scintillator screen, are presented in Figure 5.21.

Such a plot reveals that the exalite 389 sample shows less efficient electron-to-photon conversion over the voltage range studied. In reference to the P47 efficiency curve, the photon emission was found to be reduced by a factor of two. The BBOT sample shows a comparable brightness to the inorganic P47 screen. A far higher relative electron-to-photon conversion was observed for the exalite 404 scintillator screen. Here, the signal levels

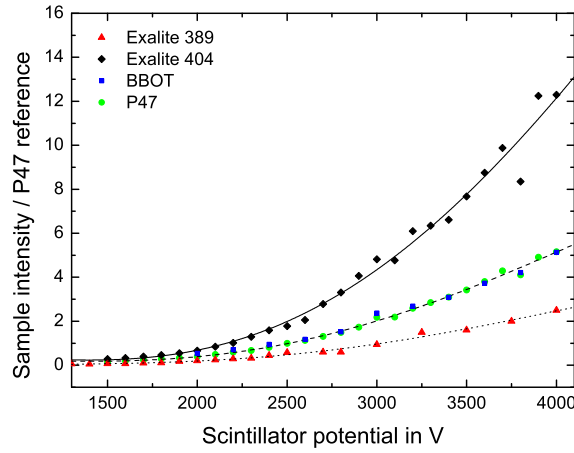


Figure 5.21: Relative scintillation efficiencies determined for the exalite 389, exalite 404, BBOT and P47 scintillator as a function of the incident electron energy. All efficiencies are plotted relative to a reference P47 screen, held at 2.5 kV (1.4 keV electron energy). The data sets were fitted with 4th order polynomial functions.

obtained exceeded the P47 output over the entire voltage range. By applying a scintillator gain of 2.0-3.0 kV (0.9-1.9 keV impact energy) an up to 2.5 times higher conversion rate was measured for the exalite 404 sample relative to P47. Fluorescence saturation effects were not observed for any of the applied samples. However, these may appear at higher electron energies. For the measurements undertaken it was not possible to increase the MCP to scintillator bias further, as damaging of the detector due to electrical arcing needed to be avoided.

For the experimental results presented, it should be pointed out that the determined relative conversion efficiencies are only valid in comparison to the sedimented P47 phosphor screens prepared in this study. The electron-to-photon conversion efficiency of commercial phosphor screens may differ significantly from the samples prepared in this work (Section 5.3). For the presented experiments it is almost impossible that an optimised P47 screen composition was prepared. However, the superior performance of the exalite 404 screen over a commercial P47 screen was demonstrated further in a separate experiment presented in Section 5.9.

Due to the outstanding performance of the exalite 404 screen, the remaining charac-

terisation measurements were only performed using this scintillator.

5.7 Stability

The continuous electron bombardment of organic crystals deposited on a quartz plate is a harsh process for the molecules involved to withstand. Therefore, it might be expected that the observed signal levels degrade over time due to irreversible structural damage of the organic scintillator molecules.

In order to investigate the signal loss due to cumulative damage, a two-segment scintillator screen was formed by mounting an exalite 404 screen beside a P47 inorganic screen. The assembled detector was placed in the VMI spectrometer and set to a constant potential of 1.1 kV across the MCPs and 2.5 kV (~ 1.4 keV electron energy) on both scintillator screens. In the measurements the MCPs were bombarded uniformly with electrons (-2.0 keV), which were created following the same experimental procedures as described in Section 5.4. The integrated signal of both scintillators was monitored over an exposure time of 47 hours using a CCD camera. On average 600 events were recorded across both scintillator screens per ToF cycle, at a repetition rate of 10 Hz. This corresponds to an incident electron current of ~ 6000 electrons per second arriving at the front MCP of the detector. As a result an electron current of around 3×10^9 electrons per second on the scintillator is calculated by assuming a typical MCP gain of 5×10^5 . Hence, over the entire experimental period a total of 5×10^{14} electrons impinged onto the segmented scintillator screen.

Two electron images, one collected at the start and one at the end of the 47 hours stability test, are presented in Figure 5.22. By comparing the ratio of the detected signal levels in both screens no sign of signal degradation due to damage of the organic scintillator is observed. A plot of the relative signal intensity as a function of the exposure time is illustrated in Figure 5.23. For the exalite 404 screen a constant emission with an intensity around twice that of the P47 reference is determined. Again, no sign of cumulative damage resulting in a reduced photon emission from the exalite screen can be noted. This

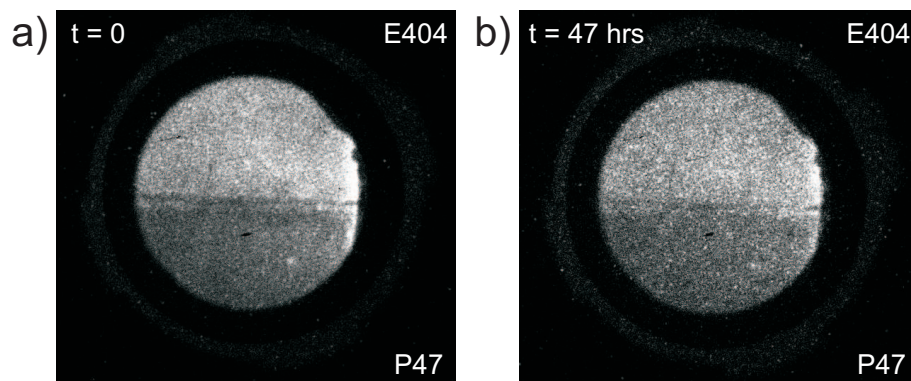


Figure 5.22: Electron images recorded at the start and at the end of the stability test using a segmented scintillator screen. Both presented images were acquired over 2000 shots each.

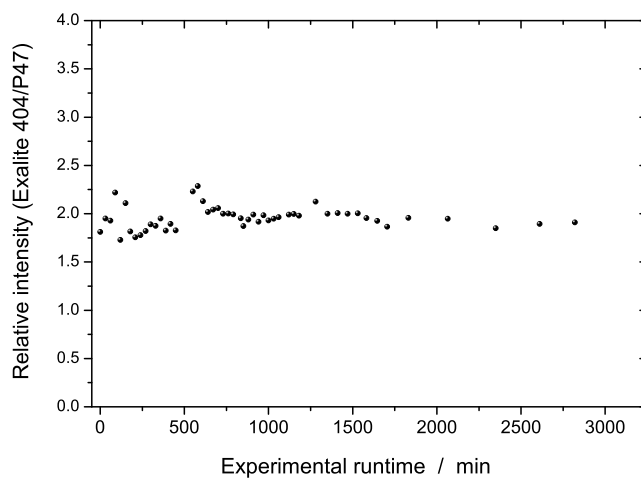


Figure 5.23: Plot of the time dependent signal intensity ratio determined for the segmented exalite 404 and P47 screen. The relative electron-to-photon conversion efficiency was found to be constant over the period of 47 hours.

observation is consistent with the fact that exalite 404 scintillator screens used for several weeks in our laboratory have not shown a signal degradation so far. However, it is almost certain that the employed exalite screens will be damaged if the energy of the impinging electrons is further increased. This issue may be addressed by encapsulating the scintillator molecules in a polymer matrix which can be used to partially absorb the impacting particle energy.

5.8 Layer Thickness

Another factor that might affect the emission efficiency of the exalite 404 scintillator is the sample thickness, which can be varied in the sublimation process. In order to investigate the effects of the layer thickness, two samples were prepared - one consisting of 150 mg of dye and another consisting of 50 mg. A photograph of the two scintillators mounted beside each other in the imaging detector is presented in Figure 5.24*a*. The top scintillator corresponds to the thinner sample, while the bottom one is the thicker sample. To compare the scintillation output, the detector was again mounted in the VMI spectrometer and a spatially uniform electron signal was monitored. The image obtained is illustrated in Figure 5.24*b*. Across both scintillators an evenly distributed electron signal is observed,

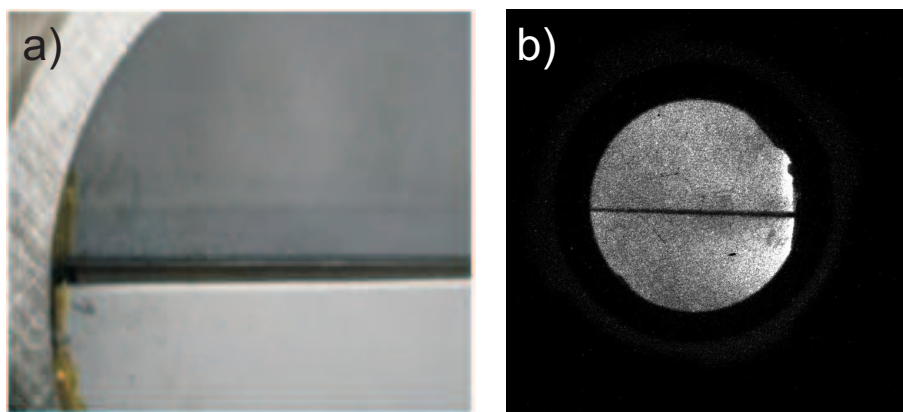


Figure 5.24: *a)* Photograph taken of two exalite scintillator screens with different layer thickness mounted in the imaging detector. *b)* Electron image recorded with the segmented detector. Despite the difference of exalite 404 layer thickness, no differences in the scintillation efficiency were observed.

and no intensity variations due to a thinner or thicker sample can be seen. Despite this result, it must be assumed that both an infinitely thin or thick layer will result in a total loss of signal. However, it can be concluded that small differences in layer thicknesses do not have a significant effect on the emission response. It suggests that small inhomogeneities in the sample thickness across a larger screen will not affect the light output. This has important consequences for the accuracy of measured intensity distributions.

5.9 Spatial Resolution Characterisation

From the previous tests exalite 404 emerged as the most promising scintillator for use in ion imaging applications. The scintillator shows a fast fluorescence decay coupled with a strong emission output in the required wavelength range. Nevertheless, one further factor has so far been ignored. For ion imaging applications it is essential that the developed detector must allow ion imaging with a high spatial resolution to be performed.

To test the imaging capabilities of the new detector, a 50 mm diameter scintillator screen was produced using the standard sublimation process, and was placed in the detector housing. The 40 mm open area MCPs were placed on top of it. This assembly allowed testing of the home-built detector in comparison to a commercial P47 imaging detector (Model: Burle APD 3075FM, 75 mm open area).

To compare the spatial resolution of the two detector assemblies, it was decided to apply both in a velocity map imaging photodissociation study. Investigating the photodissociation dynamics allows, depending on the chosen molecular species, sharp and narrow velocity distributions to be imaged.

One example molecule providing a sharp velocity distribution upon photodissociation is carbonyl sulphide (OCS). The molecule is excited *via* a strong absorption band between 200 and 250 nm, which corresponds to a transition from the $1^1A'(X^1\Sigma^+)$ ground electronic state to the $2^1A'(1^1\Delta)$ excited electronic state (see Figure 5.25). This electronic forbidden transition can only occur due to a vibrationally induced change in the bond angle, transforming the linear $1^1\Sigma^+$ state into a $1^1A'$ state. The following dissociation produces a

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Figure 5.25: Illustration of the singlet potential energy surfaces of OCS. *a)* Shows the energy surface along the C-S bond axis and a fixed length of the C-O bond for a linear OCS molecule. *b)* Illustrates the energy surfaces as function of the bond angle, γ . Both diagrams were taken from Reference [203].

sulphur (1D_2) and a carbon monoxide ($X^1\Sigma^+$) fragment. If the photo dissociation is performed at roughly 230 nm, the CO fragment can be simultaneously probed using a (2+1) REMPI process from the $X^1\Sigma^+(v=0)$ ground state *via* the $B^1\Sigma^+(v'=0)$ state. As the absorption cross section of OCS at 230 nm is quite strong ($11 \times 10^{-20} \text{ cm}^2\text{molecule}^{-1}$) and the Frank-Condon factor of the probe transition is rather large (0.98778), the resulting ion images should show a reasonably high signal level [202].

The detected CO fragment is rotational excited and its bimodal REMPI spectrum of the *Q*-branch following the photo dissociation of OCS can be found in the work of Sato *et al.* [204]. Ion images obtained for the rotational levels $J = 40$ to $J = 53$ show a sharp velocity distribution for the CO fragments obtained. The OCS molecule relaxes after photo excitation to the $2^1A'(1^1\Delta)$ state *via* a conical intersection into a $3^1A'(1^1\Pi)$ state, which is repulsive along the C-S bond axis. As a result, CO fragments in the $X^1\Sigma^+$ low rotational state are obtained. CO fragments in higher rotational excited states may be accessed by crossing the energy barrier on the $2^1A'(1^1\Delta)$ surface prior to dissociation [204].

Experimentally, OCS was seeded in helium in a 10% ratio. For the one-colour photodissociation and ionisation process, a dye laser was pumped by the 3rd harmonic of a Nd:YAG laser, and the frequency doubled laser output was focused into the interaction region of the

VMI spectrometer. Experiments were performed using three different extraction settings. The repeller was charged to either 2.0 kV, 4.0 kV or 6.0 kV, and the extractor was set to 0.745 times the voltage applied to the repeller. The last electrode remained grounded. The commercial detector was operated with a potential of 1.5 kV applied across the MCPs and 3.5 kV to the phosphor screen. Ion images collected using the exalite detector were recorded with 1.3 kV across the MCPs and 3.0 kV on the scintillator screen.

The differences in the applied detector potentials were used for practical reasons. On the one hand, it was impossible to detect any ion signal with the commercial detector operating at the settings of the exalite 404 detection assembly. On the other hand, if the home-built detector was operated using the same potentials as applied to the commercial detector, a very strong fluorescence signal was observed, which caused blurring effects due to a saturation of the camera. Hence, for the purpose of comparing the imaging capabilities of both assemblies, the detectors were operated using settings which allowed ion images to be recorded with a high spatial resolution. All ion images were acquired over 7000 shots each, using a conventional intensified CCD camera.

The ion images obtained with the P47 detector system are compared directly to those collected with the exalite 404 detector in Figure 5.26. As both image series were recorded with a different detector, the employed CCD camera was removed and reattached to the instrument upon changing detector. This resulted in a change of magnification, as the camera was somewhat closer or further away from the scintillator screen. Therefore, for the data analysis, the images were rescaled to compare the recorded velocity distributions. A first visual inspection of Figure 5.26 suggests that the new scintillator reproduces the Newton spheres with the same sharpness and quality as the P47 detector. In fact, the exalite 404 images seem to show an improved signal-to-noise ratio, as the background noise is a less dominant feature in the images. Furthermore, a signal from the vibrationally excited parent molecule is apparent as a second ring around the outer part of the main Newton sphere in the images recorded with the home-built detector.

Fragment velocity distributions were extracted from the collected ion images using the

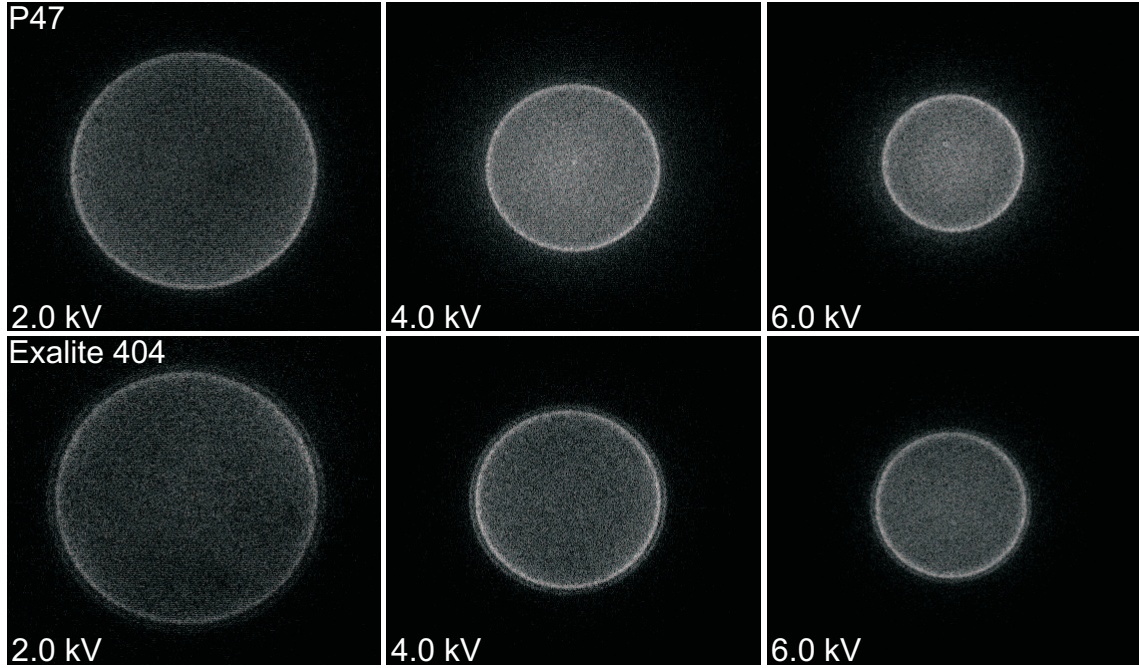


Figure 5.26: CO^+ fragment ion images of the photodissociation of OCS. The images were recorded of CO fragments in the $J = 45$ state following a (2+1) REMPI process along the $\text{X}^1\Sigma^+(v = 0)$ to $\text{B}^1\Sigma^+(v' = 0)$ transition. The top row shows the images recorded with a commercial P47 detector, while the bottom ones were collected with the home-built exalite 404 detector. Images in the first column were collected with 2 kV, the one in the middle with 4 kV and the one in the third row with 6 kV acceleration voltage applied to the ion optics.

velocity map image analysis software pBASEX (Polar Basis Function Expansion) [63]. The pBASEX algorithm describes the angular distribution of photofragments with an expansion of Legendre polynomials as a function of the light polarisation used in the dissociation process. Furthermore, a discrete number of Gaussian functions with a well defined width, σ , are used to describe of the velocity distribution of the Newton sphere. A more detailed description of the fitting algorithm can be found in Reference [63].

The velocity distributions extracted from the recorded ion images are presented in Figure 5.27. The main peak in both the P47 and exalite 404 distributions are in excellent agreement for all the different acceleration voltages applied. There are no major difference between the widths of the peaks. Therefore, it can be concluded that signal blurring effects induced by the application of the exalite 404 scintillator are on the same order as with the P47 screen. Furthermore, by observing the second smaller peak (higher velocity), which

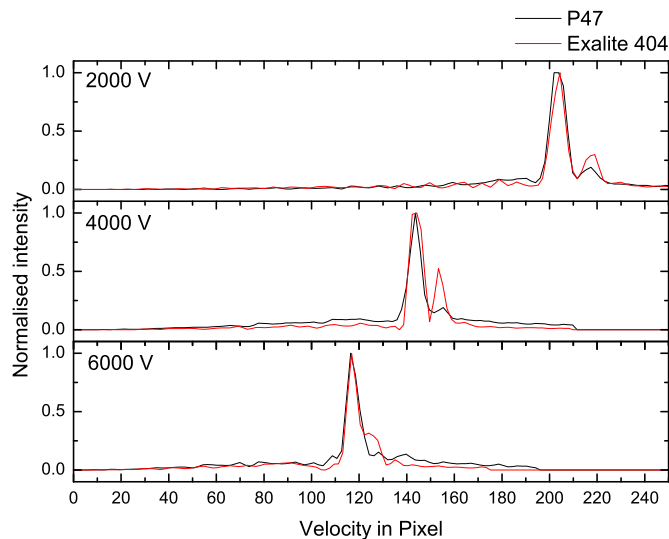


Figure 5.27: Velocity distribution of CO-fragment ion images collected at different acceleration voltages with the P47 detector (black) and the exalite 404 detector (red).

arises from the dissociation of vibrationally excited OCS molecules, it can be established that these fragments were detected more sensitively with the exalite 404 detector. However, since the images were recorded in two separate experiments, this observation might have various origins. For instance, slightly different experimental molecular beam conditions, or a small degree of signal saturation of the inner ring might result in an enhanced detection of vibrationally excited parent ion dissociation products. The β -parameters determined for the images series recorded with both detectors were found with an equivalent value of 0.1 ± 0.05 . Hence, a non-uniform response due to the exalite 404 layer properties can be excluded.

All in all, it has been established that the new imaging detector fulfils all the requirements to be employed in VMI studies. Furthermore, the different detector settings applied in the imaging study back the results from the sensitivity test (see Section 5.6), in which a superior electron-to-photon conversion efficiency was determined for the exalite 404 screen in comparison with the P47 sample. Hence, the use of the novel scintillator screen allows experiments to be performed using a lower gain applied to the imaging detector, which may potentially extend the lifetime of the employed MCPs.

5.10 Application Using PImMS

A remaining point which should be briefly addressed is whether the fluorescence emitted from the exalite 404 scintillator screens is recorded more sensitively than that of a P47 screen using the PImMS camera. Due to the fact that the emission spectra of the exalite 404 screen is shifted further towards the blue end of the UV/Vis spectrum than that of the P47 screen, it might be the case that the emitted photons are not recorded as efficiently by the PImMS pixels. The sensitivity range those pixels has not been fully characterised yet.

To address this point, both a fabricated exalite 404 and a P47 screen were simultaneously mounted in a segmented imaging detector. A uniform distribution of electrons was created and accelerated towards the detector using the experimental procedures described previously. For the measurement a potential of 1.6 kV was applied across the MCPs, while the scintillator screens were operated at 3.7 kV.

Figure 5.28 shows a representative electron image taken at the described settings. It can be seen that in the lower section of the image, in which the exalite 404 screen is located, an enhanced electron detection is observed. Hence, from this simple measurement it may

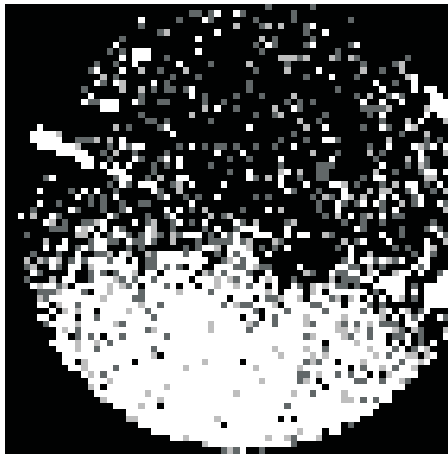


Figure 5.28: Electron image recorded with the PImMS camera using a segmented imaging detector. On the top side a P47 screen was mounted while on the bottom an exalite 404 screen was located. It can be clearly seen that a higher detection efficiency is observed using the newly developed screen.

be concluded that even though the exalite 404 scintillator emits somewhat further in the blue spectral region, a higher detection probability is observed using the PImMS camera. A more detailed analysis of the application of the new screens coupled to the PImMS camera will be addressed in future work [149].

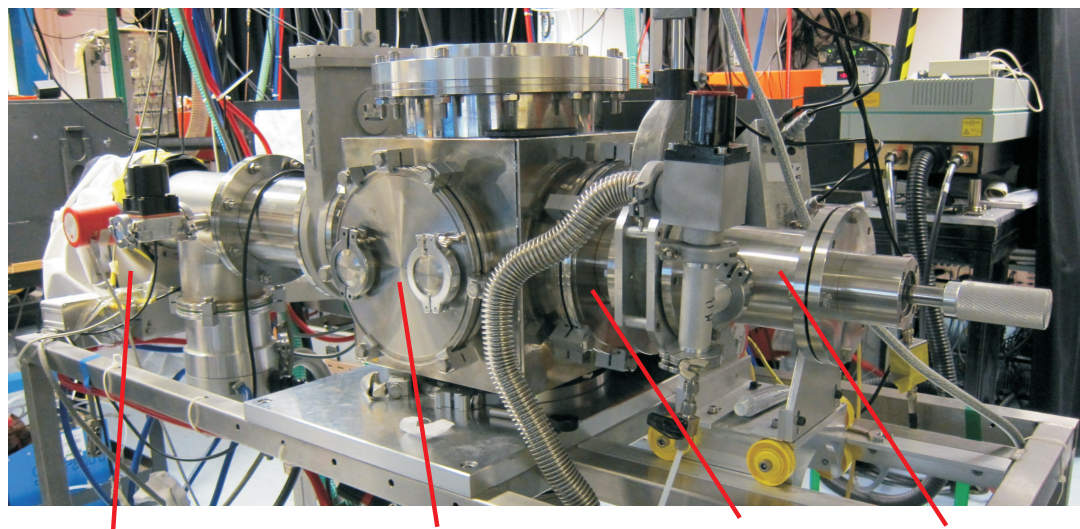
5.11 Application to Imaging Mass Spectrometry

One motivation for developing a new, fast scintillator screen was its possible application to microscope mode imaging mass spectrometry. Following the successful instrumental development process described in Chapters 2 to 4, a new dedicated imaging mass spectrometer was constructed. The details of the instrument and its structural changes in comparison to its predecessor will be described in this section. Furthermore, first results obtained with the new exalite 404 imaging detector applied to this mass spectrometer will be presented.

5.11.1 Instrumental Design

A photograph taken of the assembled mass spectrometer is presented in Figure 5.29. In the construction and design of the spectrometer a number of modifications were introduced, which allow the instrument to be better tailored to imaging mass spectrometry than the instrument which was employed in the previous chapters. The key modifications concern a sample loading system, a more flexible ionisation laser angle, a more versatile ion optic with an exchangeable contrast aperture, the option to mount laser optics inside the spectrometer, and the introduction of a number of vacuum ports, which allow various ionisation methods to be applied.

The practicability of the VMI spectrometer employed for the previous IMS studies was limited by the fact that the entire chamber needed to be vented and opened, when the sample was exchanged. The procedure, until the vacuum was restored and the introduced sample could be investigated, took up to 30 minutes. Hence, only a few samples could be analysed within a working day. To address this issue a sample loading system was designed



Detector and Camera Flange for modifications Ion optic chamber Sample loading system

Figure 5.29: Photograph taken of the newly constructed imaging mass spectrometer.

which allows the sample exchange to be done within less than 5 minutes. The system is presented in Figure 5.30 and 5.31. Two samples are mounted side by side in two grooves located in a metallic sample plate holder. In operation, this plate is pushed into the repeller plate of the ion optics whereby one of the two samples is located in the centre of the ion optic. By rotating the sample holder, the second sample can be analysed. To exchange the samples, the holder is removed from the repeller plate and pulled back behind a gate valve. By closing this valve the sample holder is isolated from the spectrometer. Then the samples can be exchanged by venting the sample holder chamber. Afterwards the samples are transferred back into the ion optic located in the main chamber by evacuating the sample holder chamber down to 10^{-2} mbar and then opening the gate valve.

Another major difference to the previously used instrument is that the incident angle of the ionisation laser (Figure 5.30, Laser I) onto the sample is much steeper. This is possible because the beam passes through a hole in the extractor lens before irradiating the sample on the repeller plate. Thereby, the laser fluence should be more evenly distributed over the entire sample and edge effects (see Chapter 2 and 4) should be reduced. As indicated in Figure 5.30 it is also possible to introduce the ionisation laser through a port located at the back of the ion optic. Thereby, the laser irradiates the sample from the back through

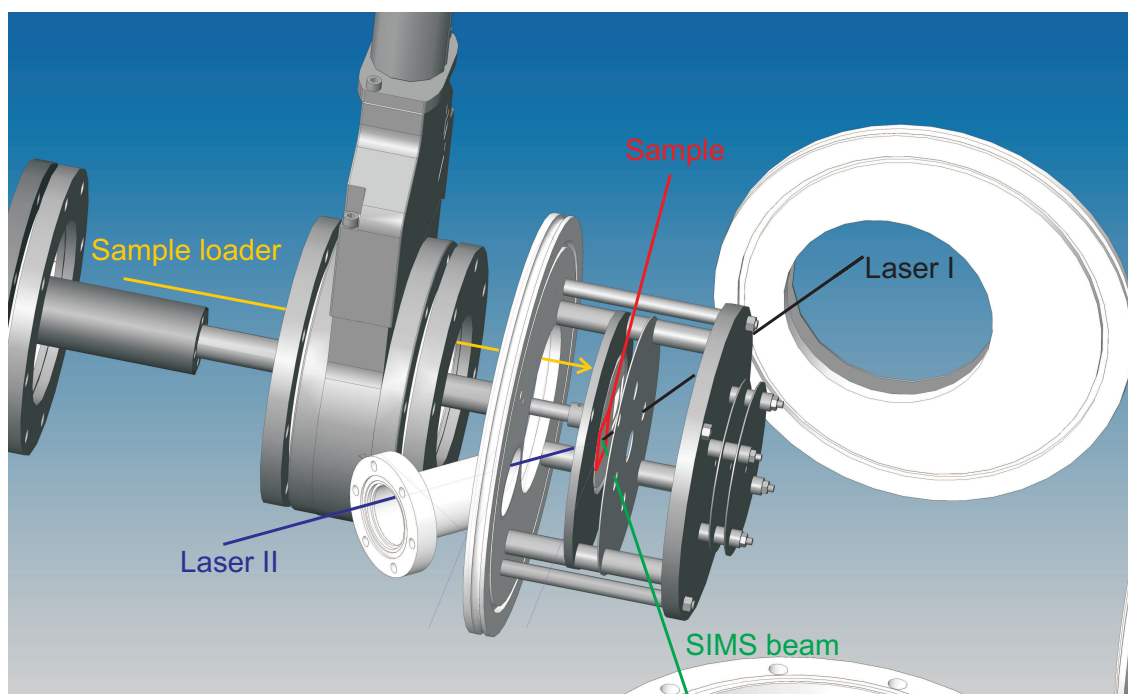


Figure 5.30: CAD cross section of the newly constructed imaging mass spectrometer. In contrast to earlier instruments a sample loading system is attached to the left hand side. The sample may be ionised using three different techniques (see text for details).

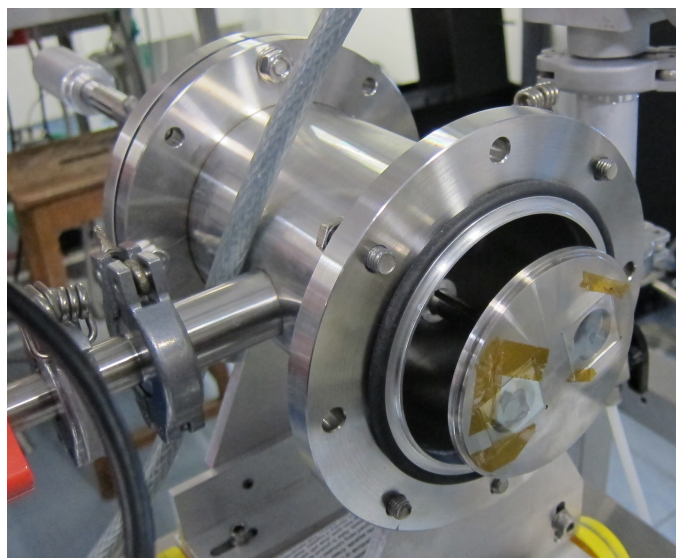


Figure 5.31: Photograph of the sample holder after removing it from the mass spectrometer. Two samples applied to ITO coated glass plates were placed in the grooves and electrically connected to the metallic holder.



Figure 5.32: Aperture electrode of the ion optic of the new IMS instrument. The central aperture could be removed and replaced with a differently sized one.

a transparent sample plate, which may result in a more sensitive ablation process [108].

A further modification, which may be introduced in future work, is the attachment of an ion gun on the empty flange on the side of the spectrometer (see Figures 5.29 and 5.30). The gun needs to be mounted such that the emitted particle beam passes the extractor through one of holes before terminating on the sample. Employing an ion gun may allow an even ionisation across the entire sample and thereby improve the image quality obtained.

The ion optic employed in the new spectrometer was almost the same as the aperture instrument constructed in Chapter 4. The only modification introduced was that the size of the employed aperture could be changed by removing and replacing the central part of the electrode (see Figure 5.32). This allows the effect of the size of the aperture, in future work, to be studied more extensively. The total length of the flight tube was 100 cm, allowing IMS to be performed with the same specifications as achieved with the previously employed instruments.

For the work presented in this section the ion optic was operated with an aperture of 2 mm diameter. SIMION 8.0 simulations indicated that about 98% of the ions emitted from the sample surface should pass the aperture electrode without being terminated on its surface. The ionisation laser was introduced from the side of the ion optic, as shown in

Figure 5.30 (Laser I).

5.11.2 Spatial Imaging

In order to demonstrate the use of the newly developed scintillator in combination with the imaging mass spectrometer, the assembled detector was attached to the experiment and a grid sample of exalite 404 ($67\text{ }\mu\text{m}$ wire and $433\text{ }\mu\text{m}$ pitch) was imaged. The experiment was performed using the same procedures and settings as described in Chapter 4. Ion images were collected using a conventional intensified CCD camera.

Figure 5.33 shows an ion image obtained using the combination of the new spectrometer and the exalite 404 detector. It is worth mentioning that this image is the first one ever recorded with the new spectrometer. Hence, the relatively poor spatial resolution can be associated with a number of experimental parameters, such as not perfectly optimised ion optic potentials or a uneven laser profile. In the image presented two bright lines can be noted on the right hand side. These arise from two hair or dust particles which were located on the sample plate and coated with the dye investigated. Hence, they show an

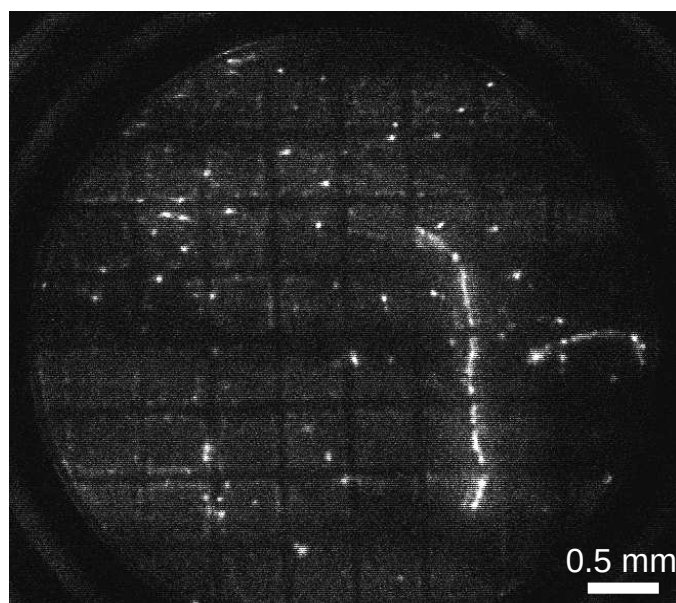


Figure 5.33: First ion image recorded with the new microscope mode imaging spectrometer equipped with the newly developed exalite 404 detector and an intensified CCD camera.

actual feature of the sample. Nevertheless, the image collected serves as proof that the newly developed imaging detector can be applied to IMS and that grid structures can be imaged. The spatial resolution of the grid image was on the order of $\sim 30 \mu\text{m}$, while a magnification factor of $9.4\times$ was determined.

5.11.3 Time-of-Flight Measurements

Figure 5.34 presents a ToF spectrum recorded using a fast PMT pointed directly at the scintillator screen of the imaging detector. Three isotope peaks of the exalite 404 sample are observed in the spectrum (658.45 Da, 659.45 Da, 660.45 Da). The time separation between each of ToF peaks was measured to be 20 ns. Hence, the application of a P47 phosphor screen would not allow the mass of these ions to be assigned accurately. The ToF resolution was determined to be $t/2\Delta t = 1030$. This value is in good agreement with an ion trajectory simulation undertaken for the ion optic settings applied, which predicted a ToF resolution of $t/2\Delta t = 1191$. By adjusting the applied potentials and the PEDAs switch time further, this resolution may be improved in future experiments. Ion trajectory simulation undertaken for the investigated particle mass indicated that a ToF resolution

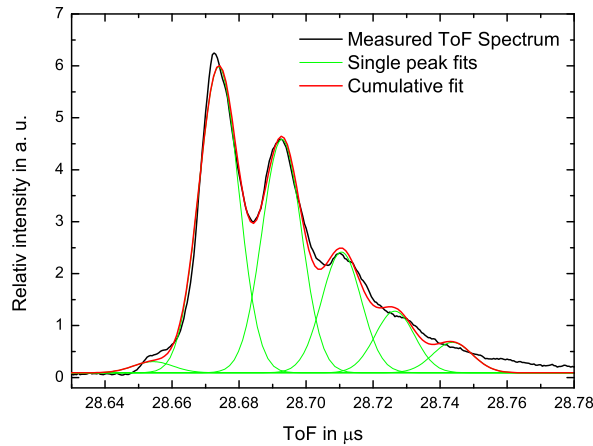


Figure 5.34: ToF spectrum recorded of the parent ion peak of the exalite 404 sample. A rudimentary isotopic resolution is observed. A Gaussian fit allowed the ToF resolution to be determined with $t/2\Delta t = 1030$.

of $t/2\Delta t = 2500$ should be achievable.

It can be concluded that by combining the new exalite 404 detector with a microscope mode imaging mass spectrometer, a higher ToF resolution can be directly accessed. Further studies combining a microscope mode imaging mass spectrometer with the exalite 404 detector and the PImMS camera are ongoing, but these will not be discussed further in this thesis.

5.12 Conclusion

In this chapter, the development of a new scintillator, which sets new benchmarks in terms of temporal specification and brightness, has been described. Careful considerations of the class of the applied material allowed the selection of a specific category of dyes, which, due to their molecular structure, are ideally suited to be applied in a scintillation detector. The new observation brings into question the generally held belief that a fast scintillator shows (in the most cases) a weak fluorescence signal.

The exalite 404 scintillator was tested for its time response, brightness, emission spectrum, and spatial resolution. In each category, comparable or superior results were determined in comparison with an inorganic P47 scintillator screen, which is the current industry standard.

For a complete characterisation of the exalite 404 scintillator screen, future work is required to determine the absolute decay constant after electron excitation. So far only an upper limit of ~ 4 ns was determined in the experiments undertaken. Furthermore, the absolute detection efficiency needs to be determined by counting the number of emitted photons as a function of the number of impacting electrons and their kinetic energy. Even brighter and faster scintillator screens may also be developed by investigating the electron-to-photon conversion efficiency of a number poly-*para*-phenylenes as a function of their substituents and chain length.

Chapter 6

Development of a Direct Ion Detection System

This chapter details the application of a number of scintillator screens to a direct ion detection system using a single-photon avalanche diode (SPAD) as a light sensor. The system will be tested for its suitability in mass spectrometry, and will be compared to commercial ToF detectors. Furthermore, possible medical applications of the assembled detection system will be discussed briefly.

6.1 Time-of-Flight Detection Systems

The importance of the detection system for mass spectrometry is highlighted by Koppenaal and coworker's description of the detector as the “eye” of a mass spectrometer [205]. The required specifications can be subdivided into two categories; analytical parameters and operational parameters. In the former group, attributes such as a fast response, a good signal-to-noise ratio, a high stability, a wide mass range response, a mass independent response, and a high saturation level are important, while the latter category includes robustness to damage, and low maintenance/replacement costs. A number of different detector systems are available today, which are based on either direct charge detection, or

the absorption and conversion of the kinetic energy of an impacting ion. The properties and characteristics of the most common ToF detector systems will be described in the following.

6.1.1 Faraday Cup

The simplest detector employed in ToF mass spectrometry is a Faraday cup [80, 206]. It consists of a metallic cylinder, in which the incoming ion beam is caught. Once these ions hit the collector base plate they are immediately neutralised by either accepting or donating electrons. By amplifying and monitoring the discharged current, a ToF spectrum can be acquired.

Faraday cups have a relatively simple design, and are therefore a cheap and reliable detection system for ToF mass spectrometry. However, the main disadvantage is their low sensitivity. For example, a typical ion beam experiment results in the detection of currents in the subfemto- to nanoampere range, where 1 fA corresponds to a rate of 6242 ions/s [205]. To record the signal obtained, it is necessary to amplify it by factors of 10^9 to 10^{13} . Despite this amplification, Faraday cups are still less sensitive than electron multiplying detectors.

One source of timing inaccuracy of a Faraday cup detector arises from the detection of secondary electrons (see Figure 6.1). These are emitted from ions impacting on the collector base plate, and are detected on the walls of the Faraday cup. To suppress the detection of secondary electrons the shape of the cup can be modified, or magnetic fields can be applied to prevent secondary electron ejection [207, 208].

Today, these detectors are mainly employed in applications such as isotope ratio mass

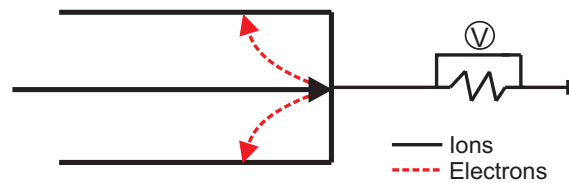


Figure 6.1: Detection scheme of a Faraday cup detector. The red arrows indicate secondary electron emission upon impact of the primary ion.

spectrometry, where a sector field analyser is used to map multiple isotopes onto a series of Faraday cups. This idea was extended by Knight and Lorincik, who combined a micro array of Faraday cups with a fast CMOS readout chip [209, 210]. Due to their small physical size, in comparison to conventional Faraday cup arrays these chips allow the mass resolution of a sector field instrument to be improved.

6.1.2 Electron Multiplier

A second category of ToF detectors are the electron multiplier (EM) systems, which are the most common ion detection system employed in mass spectrometry today. Figure 6.2 illustrates the design of three kinds of EMs: the discrete dynode, the continuous dynode and the microchannel plate.

The discrete dynode EM is a typical ion or photon counting detector [80, 205, 206]. It consists of a stack of separately mounted dynodes. The first of these is used to absorb the kinetic energy of an impinging charged particle, which stimulates a secondary electron emission. By applying an individual, increasing bias voltage to each further dynode, electrons are guided and accelerated through the detector. Once they hit the next dynode, more electrons with a lower initial kinetic energy are emitted, which are then further accelerated in the electrostatic field expanding towards the next dynode. In this way, an electron cascade is released. In the case that the EM is employed to detect incom-

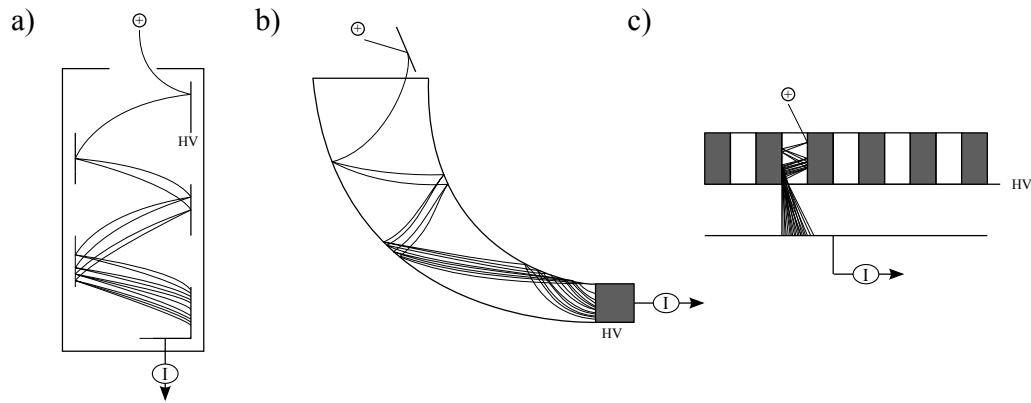


Figure 6.2: Typical electron multiplier systems: *a)* Dynode EM; *b)* Continuous dynode EM; *c)* Microchannel plate EM.

ing photons, a photocathode is mounted just in front of the detector, which releases and accelerates electrons towards the first dynode.

In normal operation the signal measured for a single ion hit is amplified by a factor of 10^6 or more. However, the obtained multiplication depends on the number of dynodes, the bias voltage employed, and the work-function of the dynode coating. The ion signal can be read out by monitoring the current on the final anode. This current can then either be digitised after current-to-voltage conversion, or thresholded and counted to determine the arrival time of the detected particles. The latter mode allows single ion counting with a limit of $< 10^6$ counts per second. Above this threshold, the ion count versus obtained signal level loses its linear relationship. Discrete dynode electron multipliers are used mainly in photomultiplier tubes, where high signal amplification and low noise levels are required.

Another electron multiplier system is the continuous dynode EM, better known by the name channeltron [205]. It is a single dynode EM consisting of a curved, funnel shaped tube. The channel walls are coated with a resistive film on an isolation surface. By applying a bias voltage across the whole channel, the impact of charged particles or high energy photons creates an electron cascade, which is amplified along the tube. By collecting the charge with an anode located at the output of the EM, an arrival time for the detected ions can be determined. Continuous dynodes are efficient and robust detectors, which are often employed in SEMs, mass spectrometry applications, and as an electron counting detector in nuclear physics experiments.

The operation of microchannel plate EMs is based on the same concept as the continuous dynode system [205, 211]. Each plate consists of an array of 10^4 to 10^7 individual parallel channels, which allow the position and the arrival time of an impacting particle to be resolved. The spatial resolution is limited by the size and the separation of the microchannels. Typical tube diameters are 5 to 100 μm with a depth of up to 1 mm. All channels are oriented with a bias angle about 5° to 15° towards the plate surface to improve the signal amplification due to collisions with the channel walls. Both the front and the rear surfaces of the electrodes are usually coated either with a NiChrome or an Inconel

layer (both alloys of nickel and chromium), which allow a bias voltage to be applied across the plate. The gain factor of a single microchannel plate is usually on the order of 10^2 to 10^4 . If a higher electron amplification is required, two (Chevron assembly) or three (Z assembly) MCPs can be stacked. In these assemblies, all the adjacent plates are mounted such that the bias angles of the microchannels alternate. If a chevron MCP assembly is used the gain increases to the factor 10^6 while with the Z stage a gain of 10^7 can be achieved. On the one hand, the use of multiple microchannel plates improves the obtained signal levels; however, on the other hand, the spatial resolution is reduced as electrons can be injected into multiple channels at each transition point.

The advantage of microchannel plates in comparison to other EMs is their fast time response, which is a function of the channel length and the applied voltage. Hence, the time precision of the detector is reduced from 0.5 ns for a single MCP to 1.5 ns using a chevron assembly. Channeltrons usually operate with a typical resolution of 18 ns to 20 ns and have a dead time of 10^{-2} s [211]. Due to the high number of single channel EMs in a microchannel plate, the effective dead time is reduced to 10^{-7} s to 10^{-8} s.

MCPs are sensitive to a number of particles, ranging from UV and X-ray photons to charged particles and electrons. One major field of application is mass spectrometry, where they are used to detect ions up to the macromolecular range (100 kDa). The number of detected events N_{Events} per incident ions N_{Ions} is defined *via* the detection efficiency Q_E [212]:

$$N_{\text{Events}} = N_{\text{Ions}} \times Q_E(\theta, \theta_B, E, Z_i, z, V_O, V_{\text{coll}}, L, h, d, A_{\text{open}}), \quad (6.1)$$

where θ is the incident angle of the impacting ions on the MCP, θ_B the bias angle of the microchannels, E the kinetic energy of the particles, Z_i the atomic number, z the electronic charge of the particle, V_O is the electron acceleration potential difference along the microchannel walls, V_{Coll} is the potential difference between an ion acceleration field and a charge on the MCP surface, L is the length of the microchannels, h is a factor describing the penetration from the NiChrome or Inconel coating into the microchannels, d is the microchannel diameter and A_{open} is the open area ratio.

The ion detection efficiency of MCPs was investigated in a number of studies [212–222]. However, due to the different experimental conditions, such as the employed MCP, the ion beam incidence angle, the ion kinetic energies, and the particle masses, it is almost impossible to describe quantitatively the relationship between Q_E and the various experimental and operational parameters in Equation 6.1. One of the most comprehensive studies investigating the absolute detection efficiencies of microchannel plates was performed by Krems and coworkers in 2005 [213]. Single or multiply charged ions with masses of 14 Da to 132 Da were accelerated to energies ranging from 0.25 keV to 25 keV, and the corresponding signal levels observed upon impact onto an MCP detector were monitored. The resulting absolute sensitivity graph is presented in Figure 6.3. Here, the absolute detection efficiency of the particles is illustrated as a function of the impact energy, divided by the square root of the particle mass. For low $E/\sqrt{m}$ values, the detection efficiency was determined to be relatively low, while at higher values an upper limit of around 57.8 ± 8.4 % can be observed. This limiting value is associated with the open area ratio of the microchannel plates (60%). From Figure 6.3 it can also be concluded that for a constant kinetic energy the detection efficiency decreases with increasing ion mass. The detection efficiencies measured by Krems *et al.* are in good agreement with a number of other studies, e.g. [221].

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Figure 6.3: Absolute detection efficiency graph for microchannel plates. The graph was taken from the work from Krems and coworkers [213].

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Figure 6.4: Both panels show the secondary electron yield as a function of the ion velocity for the labelled molecules [224]. In the high velocity range (*a*), the electron emission yield has a linear relationship to the ion velocity, while in the low velocity range (*b*) the emission rate is exponentially dependent on the ion velocity.

The observation that MCPs suffer from a decrease in detection efficiency at larger masses can be associated with the secondary electron emission process, which becomes less efficient as the molecular mass is increased [223]. In other words, the MCP electron emission rate observed using a constant acceleration field is mainly dependent on the velocity of the impacting particles. This effect was studied in a number of publications in the past [224–226]. For example, Brunelle and coworkers investigated the secondary electron emission yield following the impact of large molecules (700 Da to 66000 Da) accelerated to velocities ranging from 5×10^3 to 7×10^4 ms⁻¹ onto a CsI surface. Typical plots for the secondary electron emission yield as a function of the particle's velocity can be found in Figure 6.4. For ion velocities in the range from 1.5×10^4 to 7×10^4 ms⁻¹ the electron yields show a linear relationship with the ion velocity, while in the lower velocity range an exponential relationship is observed.

MCP detectors applied in mass spectrometry are usually operated either in combination with a fast scintillator and PMT, or with a metal anode [211]. The properties of the first system were discussed in Chapter 5. However, it should be mentioned that the main advantage of the scintillator based detector is that the MCP is electronically decoupled

from the PMT. Hence, a direct readout from the PMT provides the ToF spectrum. If a higher timing resolution is required, a cone anode structure may be used to detect the charge of electrons emitted by the MCP detector. The cone structure was found to be advantageous relative to a plane design, as it allows a constant impedance of $50\ \Omega$ along the entire detector matched to the coaxial readout connection [227]. Time resolutions down to 62 ps were reported by Girard and Bolore for the detection of α -particles emitted from a ^{252}Cf source [228].

Despite the fact that MCP detectors are the most common systems employed in mass spectrometry, they still suffer from a number of further limitations. They are both expensive and fragile. Reliable operation is only possible under vacuum conditions, which do not exceed 10^{-5} mbar, as arcing might damage the detector. Hence, alternative systems allowing the direct detection of ions are of considerable interest to the community. One of these systems is the IPD detector which was already discussed in Chapter 5 [177]. However, in recent years the properties of such detectors have not been further investigated.

6.2 Single-Photon Avalanche Diodes

Single-photon avalanche diodes (SPADS) can be described as a photomultiplier on the basis of semiconductor technology [165]. SPADs consist of a number of p- or n-doped silica layers, formed by the introduction of impurities whose individual units contribute either three (p-doping) or five (n-doping) valence electrons into the crystal lattice. These introduced electrons and electron holes allow a semiconductor to be conductive. A second effect of the doping is that allowed energy states in the band-gap between the conduction and the lower lying valence band are created. p-dopants create states just above the valence band, while donor dopants create states near the conduction band. Depending on the concentration of the dopant, the semiconductor layers can be classified as listed in Table 6.1.

If a p-doped layer is situated next to an n-doped layer the connection is described as a p-n junction (See Figure 6.5). These junctions are an elementary part of semiconductor

Symbol	Impurity concentration
n^-	1 Donor / 10^{10} Atoms
n	1 Donor / 10^7 Atoms
n^+	1 Donor / 10^4 Atoms
p^-	1 Acceptor / 10^{10} Atoms
p	1 Acceptor / 10^7 Atoms
p^+	1 Acceptor / 10^4 Atoms

Table 6.1: Classification of semiconductors as a function of the dopant concentration. The values listed in the table are given for a silica based semiconductor.

devices, as they control their electronic properties. At the interface of both layers, a concentration gradient between the free movable electrons in the n-doped layer and the free electron holes in the p-doped layer is formed. Triggered by a diffusion force, electrons from the n-doped layer are immediately pushed towards the p-doped layer to fill empty electron holes at the interface. At the same time, the free electron holes start moving in the opposite direction. Both of these motions form a space charge region at the interface of the two layers: a positive charge at the n-doped side, and a negative charge at the p-doped one. By increasing the charge in the interaction region, the increasing electric field force counteracts the diffusion force to reach an equilibrium state. The potential difference between both sides of the interaction region is called the built-in potential. The

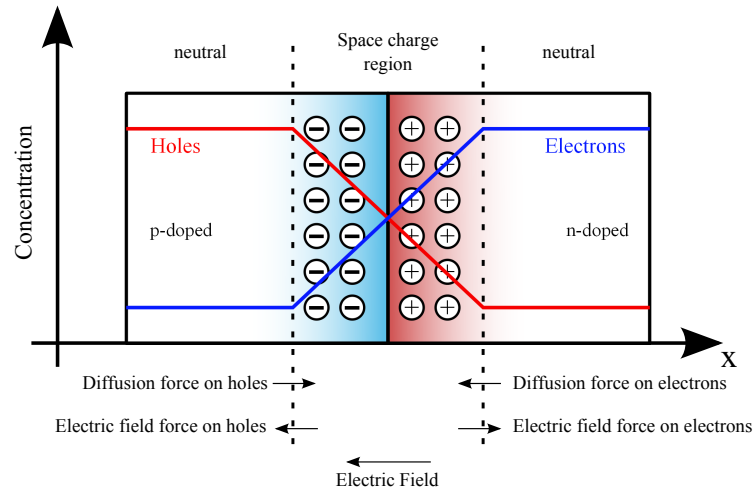


Figure 6.5: Typical charge distribution in a p-n junction. The arrows indicate the forces applied to the electrons and holes. In the interaction region between the p-doped and the n-doped layer a space charge region is formed.

size of the space charge region depends on the dopant concentration, the semiconductor base material, and the temperature.

In the case that a negative charge is applied to the p-layer and a positive charge to the n-layer, the built-in potential and the space charge region are increased as electrons and holes are pulled away from the p-n junction. Due to the increase in the space charge region, no current can flow through the semiconductor. However, if the applied electrical field is increased further to a critical level (breakdown voltage), the space charge region can break down and a current can begin to flow. This avalanche breakdown process is triggered either by thermal energy or photon absorption, which leads to a separation of an electron hole pair. Due to the high potential differences (breakdown voltage) on the p- and n-doped sides of the diode, both the electron and the empty hole move immediately towards the favourable side of the semiconductor. If the electron moves fast enough it collides with atoms located closer to the n-doped end of the diode without being self absorbed. In this process more electrons are separated from their corresponding holes, and both the hole- and electron-current is increased. In avalanche diodes, this process is used to determine the arrival time of a single photon.

A typical schematic of an avalanche photodiode (APD) is illustrated in Figure 6.6. During the operation of an APD a reversed bias, which is just below the breakdown threshold, is applied to the diode. The resulting field strengths are illustrated in the lower section of Figure 6.6. The p^- -layer can be identified as a drift region, in which the field gradient is relatively shallow. A sharp increase in the field strength can be observed in the p-doped layer, before the strength drops again in final n^+ -doped semiconductor. Due to the strong electric field, the electron-hole pair multiplication as a result of the avalanche process is the most effective in this region. Therefore, the last two layers are commonly described as a multiplying region.

If a photon enters the avalanche diode, an electron hole pair in the p^- region can be formed. Due to the applied strong electric field, the electron is accelerated towards the n-doped layer, and an avalanche is launched. The current output varies linearly with the

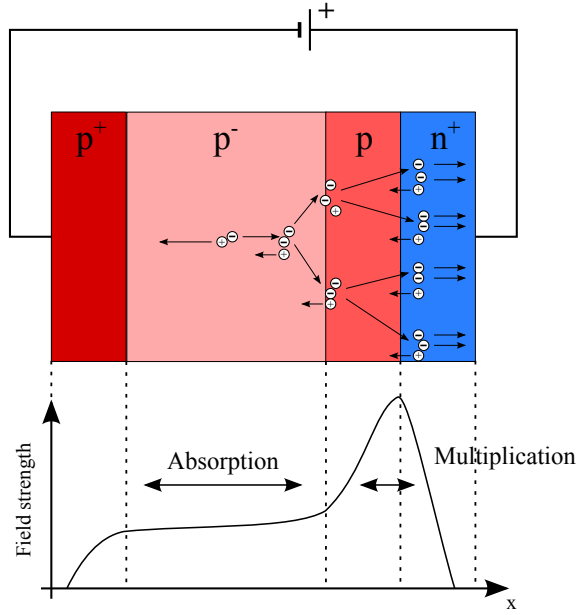


Figure 6.6: Schematic diagram of a typical layer based avalanche diode. The break-down process is illustrated. In the p^- -doped region a photon is absorbed and the released electron initiates an electron cascade towards the cathode.

optical signal level. Typical amplifications factors of 100 to 1000 are achieved using this method [229].

If an APD is operated above the breakdown voltage of the system (usually 50 to 100 V), single photon counting becomes possible [230–233]. These kinds of devices are usually described as Geigermode-APD or single-photon avalanche diodes [165]. Here, the incidence of a single photon causes an avalanche of electrons and electron holes in the system, resulting in an amplification factor of 10^8 . The output current rises, on a sub-nanosecond timescale, to a steady level in the milli-ampere range, and thereby the rising edge of the signal corresponds to the arrival time of the photon. However, as the current keeps flowing at the same rate once the avalanche is triggered, it is necessary to quench the process by lowering the bias voltage below the break down level with a quenching circuit. For this process, either a passive or active quenching circuit is employed. The former uses the fact that a current is quenched by lowering the applied voltages *via* a large resistor (typically several hundred kilo-Ohms), while the latter employs a circuit in which a comparator recognises an increase in the output current, and sends out an pulse

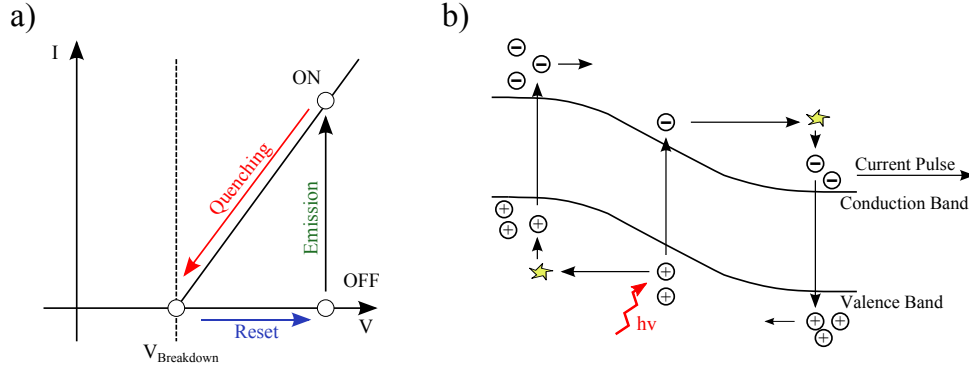


Figure 6.7: *a)* The electron avalanche process illustrated as a function of the applied bias and the output current for a SPAD sensor; *b)* Band model illustration of the electron avalanche process in the multiplication zone.

corresponding to the arrival time before the applied voltage is reduced. Once the current flow has stopped, the applied bias is reset to above the break down voltage so that the next photon can be detected. The dead-time of the sensor is, typically, on the order of 20 ns.

Figure 6.7*a* illustrates the operational principle of a SPAD sensor as a function of the applied voltage and the output current of the diode. The avalanche starts with a quick rise of the current, which is quenched by reducing the applied bias on the diode. Raising the bias above the breakdown voltage resets the diode. Figure 6.7*b* shows the electron avalanche in the band model in the multiplication zone between the p- and n^+ -doped layers. The energetic decrease of the valence and conduction band allows the creation and acceleration of electrons, which are released in an avalanche process.

The application of a single SPAD pixel sensor for the detection of simultaneously impinging photons is always connected to the loss of the intensity information, as only one photon can be detected at a time. Therefore, there has been considerable interest in assembling arrays of small SPAD sensors, named multi-pixel photon counters (MPPC) [232]. In such an array, each diode has an open area of only a few tens of microns. Hence, if the MPPC is combined with a scintillator, the photons emitted for an event can be captured in multiple individual diodes. The number of employed SPAD pixels needs to be sufficiently large so that the detection probability allows no more than one photon

to be detected per diode. However, conventional scintillators may produce up to many thousands of photons per pulse, which means that 10^4 or more SPAD cells are required. By connecting all the SPAD cells in parallel, the analog output current/voltage can be summed. As each diode gives the same output pulse once the avalanche is started, the pulse height of the summed signal corresponds directly to the number of detected photons. So far, MPPCs of up to 5×5 mm diameter have been employed to observe scintillation processes. If larger area detectors are required, several of these array detectors may be combined [165].

MPPC sensors in combination with scintillators have recently been tested for a number of applications. For example, the scintillators LYSO ($\text{Lu}_{1.8}\text{Y}_{0.2}\text{SiO}_5(\text{Ce})$) and LuYAP ($\text{Lu}_{0.8}\text{Y}_{0.2}\text{AlO}_3:\text{Ce}$) were applied to detect X-ray photons for mammography (18-27 keV) and for general X-ray imaging (40-74 keV) [234]. Further studies applied these detection devices to Positron Emission Tomography (PET) [235].

Aside from medical imaging applications, the combination of an MPPC sensor attached to a scintillator screen was recently applied by Vallance, Brouard and coworkers as a direct ion detector for mass spectrometry [236]. The goal of the project was to develop a ToF detector that can operate without an electron multiplier. It was assumed that ions impinging onto a scintillator cause a weak fluorescence signal, which can subsequently be recorded by an MPPC sensor, allowing single photons to be counted. Using an MPPC array in combination with a scintillator as a ToF detector has many advantages in comparison to the standard MCP-scintillator-PMT assembly. Firstly, due to the fact that no high voltages are required to operate the detector, damage due to arcing can be avoided, and secondly, the cost of the detector parts are reduced significantly, as MCPs, a PMT, and external power supplies are not required. Furthermore, such sensors can be operated under non-vacuum conditions, which may open new possibilities in charged particle detection.

Vallance *et al.* applied an LYSO crystal to convert the kinetic energy of the impinging ions into photons. However, despite observing discrete ToF peaks, the major limitation of these experiments was the low ion-to-photon conversion of the applied scintillator. The

ion extraction potential needed to be set no lower than 6.0 kV to stimulate fluorescence to be detected by the MPPC sensor. Furthermore, the achievable ToF resolution was limited by the long fluorescence lifetime (40 ns) of the LYSO crystal. It can be concluded that for direct ion detection, it is essential to combine the MPPC sensor with a faster, brighter scintillator screen.

In the work presented in this thesis, both limitations described above are addressed by employing a number of the newly developed scintillators, introduced in Chapter 5. To explore the sensitivity and the threshold impact energy for fluorescent photon detection for the employed detection systems, ToF spectra were recorded at different acceleration potentials to create a detection efficiency plot, in analogy to the MCP sensitivity curves presented above. The experimental details and results will be presented in the next sections.

6.3 Experimental Specifications and Techniques

The direct ion detection assembly characterised in this work comprised of a scintillator and an MPPC sensor. The employed Hamamatsu MPPC module (S10362-11-025C) has an active area of $1 \times 1 \text{ mm}^2$, a gain of 10^5 to 10^6 , and a typical pixel size of $25 \text{ }\mu\text{m}$. Overall, 1600 pixels are connected to one output channel so that a summed photon signal is recorded. The absorption spectrum of the SPAD array extends from 390 nm to 470 nm, peaking at roughly 420 nm. Time resolutions on the order of 200 ps (FWHM) are achievable with the MPPC module. However, the reset time for each single diode extends up to 20 ns. A microscope photograph of the sensor is presented in Figure 6.8a.

For its application in a mass spectrometer, the sensor was mounted on a DN40 CF flange and connected *via* an electrical feedthrough to the Hamamatsu C10507-11-025U MPPC controlling module, the circuit diagram for which is presented in Figure 6.8b. To record a signal from the MPPC sensor, the current was read out and converted into a voltage, which was subsequently recorded by an oscilloscope. The waveform obtained from the analog output was automatically adjusted such that a signal of 0.1 V corresponded to

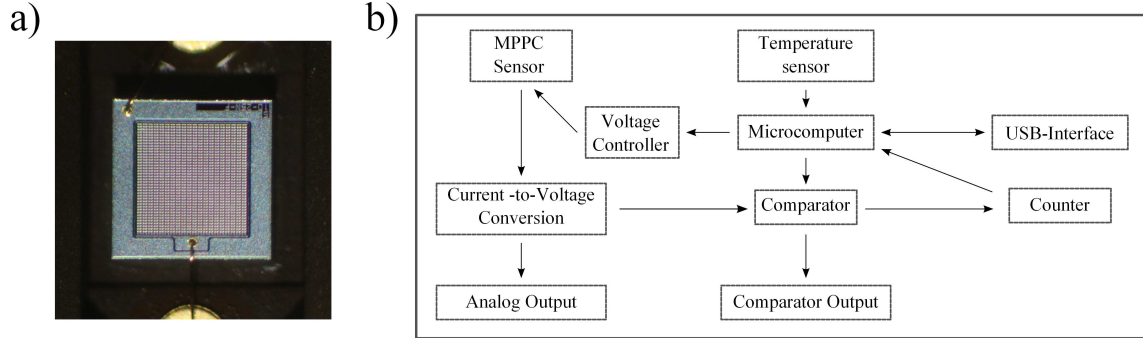


Figure 6.8: *a)* Photograph taken of the SPAD array (MPPC) sensor. The size of the open area is about $1 \times 1 \text{ mm}^2$. *b)* Illustration of the readout electronics of the MPPC module. The time spectrum can be collected using either the analog or the comparator output.

the detection of one photon. If more photons were collected simultaneously (within one frame), the signal increased linearly up to an upper readout saturation of 1.0 V.

By operating the readout module in comparator mode an acquisition gate of 1 to 100 ms could be chosen in which the signals recorded were thresholded at levels equivalent to either 0.5, 1.5, 2.5 or 3.5 photons. Signal levels which did not exceed this preset threshold were removed from the data set. Once a signal passed the threshold criteria, the rising edge of the peak was identified as the photon arrival time, which was indicated by a TTL output pulse from the comparator. Counting all the observed TTL pulses as a function of the arrival time allowed a ToF spectrum to be generated. The spectra presented in this section were recorded using the analog readout, as the intensity information of the ToF peaks was of primary interest.

The second component of the detector was the scintillator. In this work, the scintillators exalite 389, exalite 404, BBOT, coumarin 503 and P47 were coupled to the MPPC sensor described above, and their direct ion detection efficiencies investigated and compared to a LYSO crystal. Exalite and BBOT based scintillators were prepared using the vacuum sublimation technique (see Section 5.3), while for the P47 and coumarin 503 screens a sedimentation process was employed. Microscope cover slips with a thickness of 0.5 mm were used as substrate for all scintillator samples. To match the size of the scintillator to the size of the MPPC chip, the cover slips were cut to roughly $2 \times 2 \text{ mm}^2$ while the

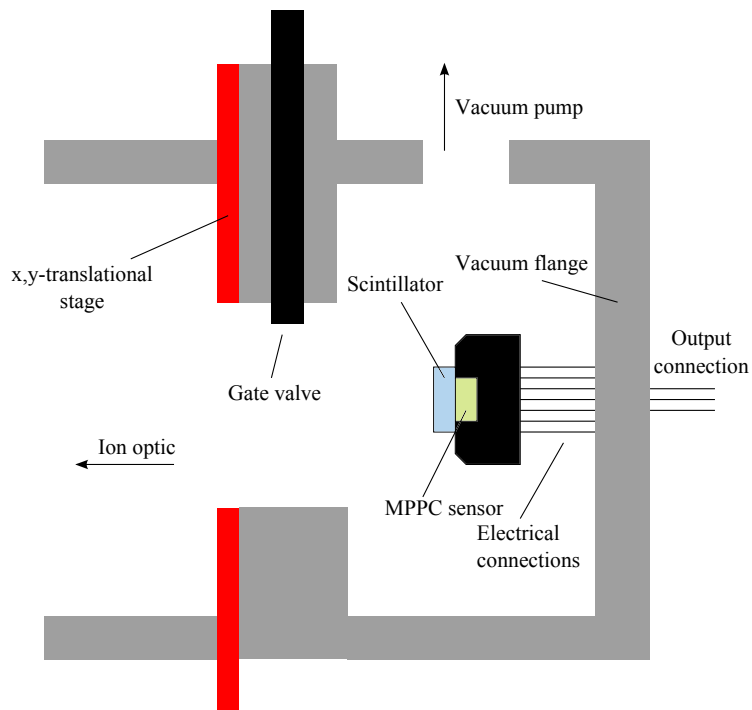


Figure 6.9: Illustration the assembled direct ion detector. The scintillator was mounted in front of the MPPC sensor using optical grease.

inorganic LYSO crystal had a size of $1.5 \times 1.5 \times 0.2 \text{ mm}^3$. The scintillators were attached to the front side of the MPPC sensor by applying a small amount of optical grease (see Figure 6.9).

The performance of the assembled detector was investigated by mounting it in the VMI mass spectrometer, which was previously described in Section 5.4. By closing a gate valve, located directly in front of the MPPC sensor, the detection unit could be separated from the main spectrometer. This setup allowed for a quick scintillator exchange by avoiding the necessity to vent the entire VMI spectrometer.

To create ions, a pulsed molecular beam was intersected either by a focused 193 nm laser beam from a Neweks PSX100 excimer laser (10 Hz, 2.5 mJ per pulse) or by 118 nm radiation (see Chapter 5). In the measurements, the extraction potentials applied to the ion optic were adjusted in small increments from 2 kV to 10 kV (repeller potential), while maintaining the VMI condition. This procedure allowed ion signals of different kinetic energy to be studied. ToF spectra were acquired over 400 laser shots by recording the

analog output of the MPPC control module using an oscilloscope (LeCroy DDA 260).

The target gas molecules used to provide an ion signal in these direct ion detection measurements were butanone and acetone. Butanone was seeded at 5% in helium and injected into the spectrometer at a backing pressure of 1000 mbar using a pulsed general valve. Acetone was admitted into the source region of the VMI spectrometer *via* passage of helium, at a constant backing pressure of 1050 mbar, through a bubbler filled with acetone, positioned prior to the pulsed general valve. This method demonstrably affords a near-constant flux of target gas molecules over prolonged periods of data acquisition (see Section 6.4.3.2).

6.4 Experimental Results

6.4.1 Background Signal

The applied MPPC sensor is capable of counting single photons, and is therefore highly sensitive to background signals such as photons scattered from the ionisation laser. A typical ToF spectrum recorded with the molecular beam off, but with the pulsed laser beam passing through the source region of the VMI mass spectrometer, is presented in Figure 6.10. Two large peaks can be observed at early ToFs, both with a peak height of about 400 V, indicating that in every acquired spectrum the signals recorded exceeded the 10 photon equivalent saturation threshold of the MPPC sensor. Both signals disappeared when the gate valve was closed to separate the detection unit from the remaining spectrometer. Hence, the peaks observed can be assigned to the detection of scattered photons from the ionisation laser pulse. However, while the entire laser pulse has a duration of sub-10 ns, the peaks observed extend up to 1.5 μ s in the ToF spectrum.

A possible explanation for this observation is that scattered light and stimulated fluorescence from the scintillator screen saturate all SPAD sensors at $t = 0$. The second peak appears at a time delay equivalent to the dead time when when all of the SPAD cells are reset. As the MPPC sensor is highly sensitive to any photons emitted by the scintillator,

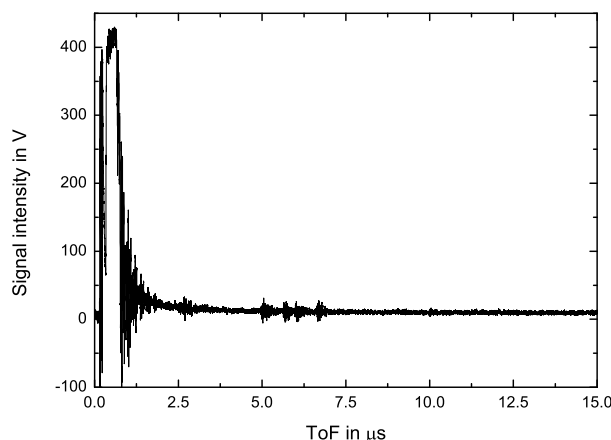


Figure 6.10: ToF background spectrum recorded with an exalite 404 scintillator and the MPPC sensor. The molecular beam was turned off while the dissociation and ionisation laser intersected the spectrometer. All signals observed can either be associated with electrical noise or scattered light.

it may be assumed that a weak scintillator fluorescence signal replenished the SPAD cells, which led to a second saturation effect. The exponential tail observed on the falling edge of the second peak may also be associated with the emission from the scintillator itself.

A further feature noted in the background spectrum in Figure 6.10 are small electrical noise peaks at later ToFs. These voltage spikes were present in all measurements undertaken, independent of whether the gate valve was closed or not. Hence, they can only be assigned to electrical noise from the sensor or the readout electronics. However, these noise spikes were reproducible and were removed from the recorded analyte ToF spectra by background subtraction.

6.4.2 Time-of-Flight Measurements

The ability to detect the impact of charged particles with a high sensitivity and a high time resolution are key characteristics of a ToF detector applied to mass spectrometry. By employing the combination of a fast and efficient scintillator with an MPPC sensor as a direct ion detector both requirements may be fulfilled. Prior to a thorough investigation of the detection efficiencies, initial proof-of-concept measurements were performed

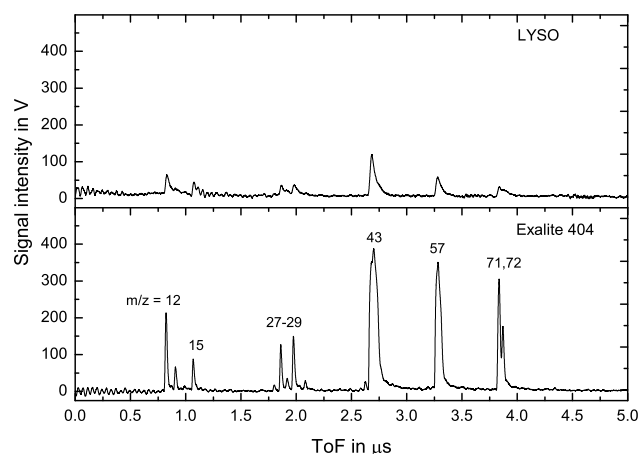


Figure 6.11: ToF spectra of butanone collected using a LYSO and an exalite 404 scintillator as ion-to-photon conversion unit for the direct ion detector. The presented spectra were background subtracted to remove the early noise peaks.

demonstrating the application of an organic and inorganic scintillator to direct ion detection in mass spectrometry. Such measurements allow an initial comparison of the timing properties and relative sensitivities afforded by each scintillator applied.

Figure 6.11 shows two representative butanone ToF spectra recorded using an exalite 404 and a LYSO scintillator attached to an MPPC sensor. Ions were formed using a multi-photon ionisation process at 193 nm and extracted directly by applying an acceleration potential of 10 kV to the repeller electrode. The extractor was set such that the VMI conditions were maintained. A crude inspection of both spectra reveals that the signal levels observed for the exalite 404 scintillator screen are significantly higher than the ones obtained for the LYSO crystal. Furthermore, ToF peak distributions recorded using the exalite 404 screen allowed ion signals separated by a signal mass unit to be resolved. As no isotopic distribution were obtained for the LYSO spectrum, it can be assumed that the emission lifetime of this scintillators defines the time precision of the assembled direct ion detector.

By enlarging the 12 Da carbon ion peak in both spectra and normalising the intensities, the timing properties of the scintillator screens can be compared directly (see Figure 6.12). Following an analysis a FWHM of 16 ns was found for the peak recorded using the exalite

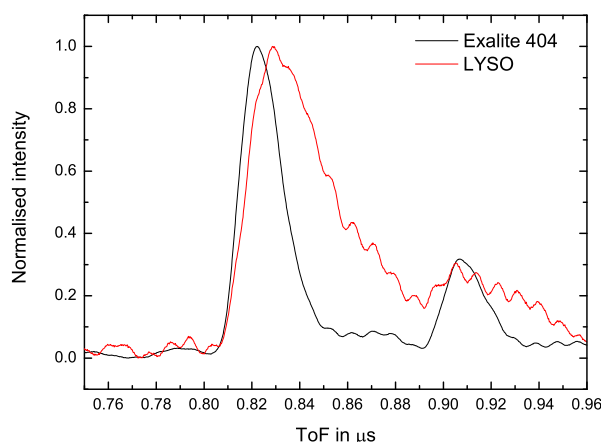


Figure 6.12: Enlarged section of the normalised 10 kV acceleration spectrum recorded for butanone. The main peak observed at $0.822 \mu\text{s}$ ToF can be assigned to the 12 Da carbon cation. The spectra were acquired using the exalite 404 and the LYSO scintillators as ion-to-photon conversion unit.

404 scintillator. The peak width determined for the LYSO spectrum was found to be 36 ns (FWHM). Hence, it can be concluded that the application of the organic scintillator screen allows for a better timing resolution to be achieved. However, comparing the determined time resolution of the exalite 404 scintillator screen with ion trajectory simulations undertaken for the spectrometer, it was found that the observed specifications were mainly defined by the intrinsic resolution of the VMI instrument. Hence, it was not possible to probe the absolute timing capabilities of the detector. This resolution is, following the description of the timing properties of MPPC sensors in the sections above, most likely not limited by the sensor, but by the fluorescence lifetime of the exalite 404 scintillator. However, to perform a study defining the absolute timing properties of the assembled direct ion detector, it needs to be combined with a mass spectrometer which offers an enhanced timing resolution. One example of such a system is the IMS instrument constructed in Chapter 4 and 5.

6.4.3 Direct Ion Detection Efficiency

6.4.3.1 Initial Consideration

To quantify the absolute detection efficiency of the direct ion detector it is essential to measure the absolute number of recorded photons as a function of the number of impinging particles and their kinetic energy. The former parameter may be easy to access with the MPPC array, as it allows single photons to be counted. Thereby, the open area ratio of the chip allows the detection of up to 10% of all the photons emitted from the applied scintillator. In contrast to this, it was not possible to quantify the absolute number of impinging ions in the employed VMI experiment. This number depends in general on experimental conditions such as the concentration of analyte molecules in the molecular beam, the power of the dissociation and ionisation laser and the absorption cross section of the particles.

To circumvent the described quantification problems, the detection efficiencies of the various scintillator MPPC combinations needed to be determined relative to one another. Hence, for a quantification of the detection efficiency it was essential that the experimental conditions, and thereby the number of detected particles, were maintained throughout the entire series of measurements. This was especially challenging as the detector needed to be removed from the VMI spectrometer to exchange the scintillator, which might affect the experimental conditions.

While performing the proof-of-concept experiments presented in Section 6.4.2 it was noted that the signal levels for the butanone sample decreased over time, as the backing pressure of the sample gas reduced quickly under the chosen experimental conditions. This effect excluded the use of this system for the quantification of the detection efficiencies of the different direct ion detection assemblies. To avoid this problem of the limited sample reservoir, the analyte molecule was changed to acetone which was seeded in helium using the bubbler injection system. As a result, a high sample density in the molecular beam was maintained over an extended period of time. Furthermore, the ionisation radiation was changed to 118 nm, which allowed the direct ionisation of acetone molecules with a

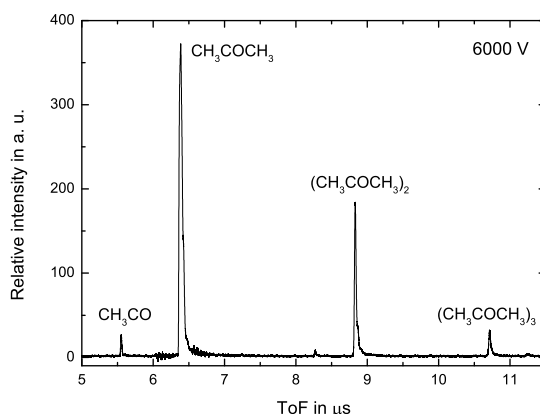


Figure 6.13: Enlarged section of the 6 kV acetone ToF spectrum recorded with the exalite 389 scintillator.

single photon (9.7 eV).

An enlarged section of a 6 kV acceleration spectrum of acetone recorded with a exalite 389 scintillator mounted in front of the MPPC sensor is presented in Figure 6.13. The peak observed at 5.5 μs was assigned to the 43 Da CH_3CO^+ fragment, the peak at 6.4 μs to the parent ion $\text{CH}_3\text{COCH}_3^+$ (58 Da), the peak at 8.8 μs to the $(\text{CH}_3\text{COCH}_3)_2^+$ cluster at 116 Da and the peak at 10.7 μs to the $(\text{CH}_3\text{COCH}_3)_3^+$ cluster at 174 Da.

The results presented were recorded using VMI potentials applied to the ion optic. This had the effect that the observed parent and cluster ion peaks, which were formed without any transverse kinetic energy, were mapped onto a single spot. As a result, the absolute number of detected particles was dependent on the position of the direct ion detector. Therefore, the detector was aligned onto this zero kinetic energy spot using a translational stage, such that the signal of the acetone parent ion peak was maximised. Hence, a relatively high ion flux was observed for the relevant ToF signals.

6.4.3.2 Signal stability

To determine the signal stability observed for the acetone signal, a number of comparison measurements were performed. Figure 6.14a presents the integrated signal level of the 58 Da acetone peak measured over the period of 1.5 hours with a BBOT scintillator ap-

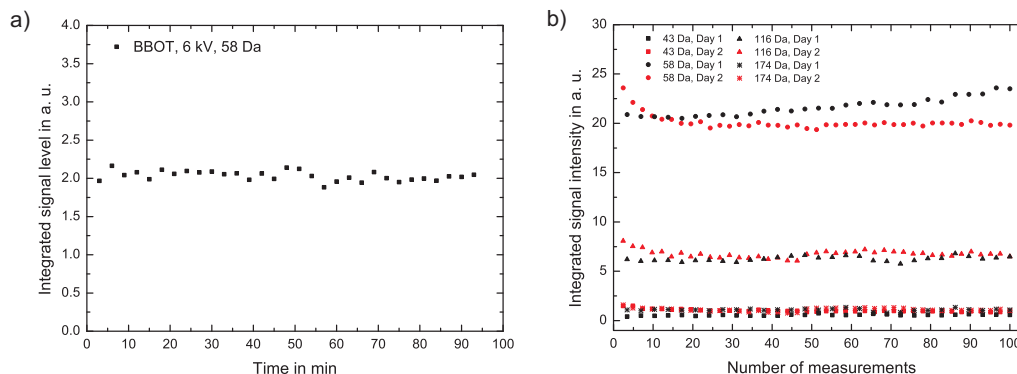


Figure 6.14: Signal stability analysis. *a)* Shows the signal levels observed for the parent ion peak of acetone as a function of the experimental time. The observed signal is stable for a period of at least 90 minutes. *b)* Comparison of a number of measurements taken at two different days using the exalite 389 scintillator. Comparable signal levels were observed for all mass speaks.

plied. Typical signal fluctuations observed are on the order of 10%. As a characterisation measurement employing an entire acceleration voltage scan from 1 kV to 10 kV was usually performed within an hour, these fluctuations can be assumed as typical error for one scan. In order to compare the relative signal levels of the scintillators to one another, the ion flux on the screens did not only need be stable over one measurement, but also consistent over all measurements undertaken. To ensure that the recorded signal intensities were comparable, a number of ToF spectra employing an exalite 389 screen as a detector were recorded before and after all the characterisation measurements were performed (two days separation, see result in Section 6.4.3.3). The integrated signal levels determined for the investigated mass peaks are presented in Figure 6.14*b*. It can be seen that the two sets of measurements result in comparable ion signal levels within a maximum error of 20%. The consistency of these results validates the choice of acetone as a target molecule for the purpose of comparing the various scintillator based direct ion detectors.

It should also be mentioned that none of the investigated scintillator samples suffered from noticeable physical damage due to the direct impact of ions. Furthermore, no signal loss was observed for samples which were exposed to ion impacts for several hours.

6.4.3.3 Sensitivity

The response of the MPPC sensor depends only on the number of employed pixels, the open area ratio and its wavelength dependent sensitivity, while the scintillator's ion-to-photon conversion efficiency depends on its photo-physical properties. To investigate which scintillator affords the highest ion-to-photon conversion efficiency, ToF spectra of all the scintillator screens were recorded using acceleration voltages in the range from 1 to 10 kV.

Figure 6.15 shows a series of ToF spectra recorded at different extraction potentials with the exalite 389 scintillator mounted in front of the MPPC sensor. As the kinetic energy of the impacting ions is increased, the parent ion peak starts to saturate the detector above 5 kV, while further peaks begin to appear in the spectrum. This observation indicates that the MPPC sensor only allows an operation within a narrow dynamic range. As a result the intensity information of the ion signals exceeding the dynamic range of the sensor is lost.

A more detailed analysis of the signal increase as a function of the acceleration potential can be achieved by integrating the parent ion and cluster ion peaks, and plotting the resulting integrals as a function of the applied extraction potential. To ensure that independent of the scintillation lifetime the complete ToF peak was considered in the analysis, the integration windows were chosen to be $0.4 \mu\text{s}$ per peak. The results determined for the acetone parent ion and the two acetone cluster peaks using the exalite 389, exalite 404, LYSO, BBOT, P47 and coumarin 503 scintillators are presented in Figures 6.16 and 6.17.

In all three plots, the highest signal levels are observed using the exalite 389 scintillator, followed by the exalite 404. These results are in agreement with the observation of the individual conversion efficiencies of the butanone measurements presented above. Depending on the relative peak heights of the acetone signals, the first measurable parent ion signal is detected at 1 kV acceleration potential using the exalite 389 screen. The increase of the observed signal level for this screen follows an almost linear trend, which is surprising as in the ToF spectra presented in Figure 6.15 a saturation effect for the 58 Da peak can be observed. However, only the peak height is saturated, while the width of the

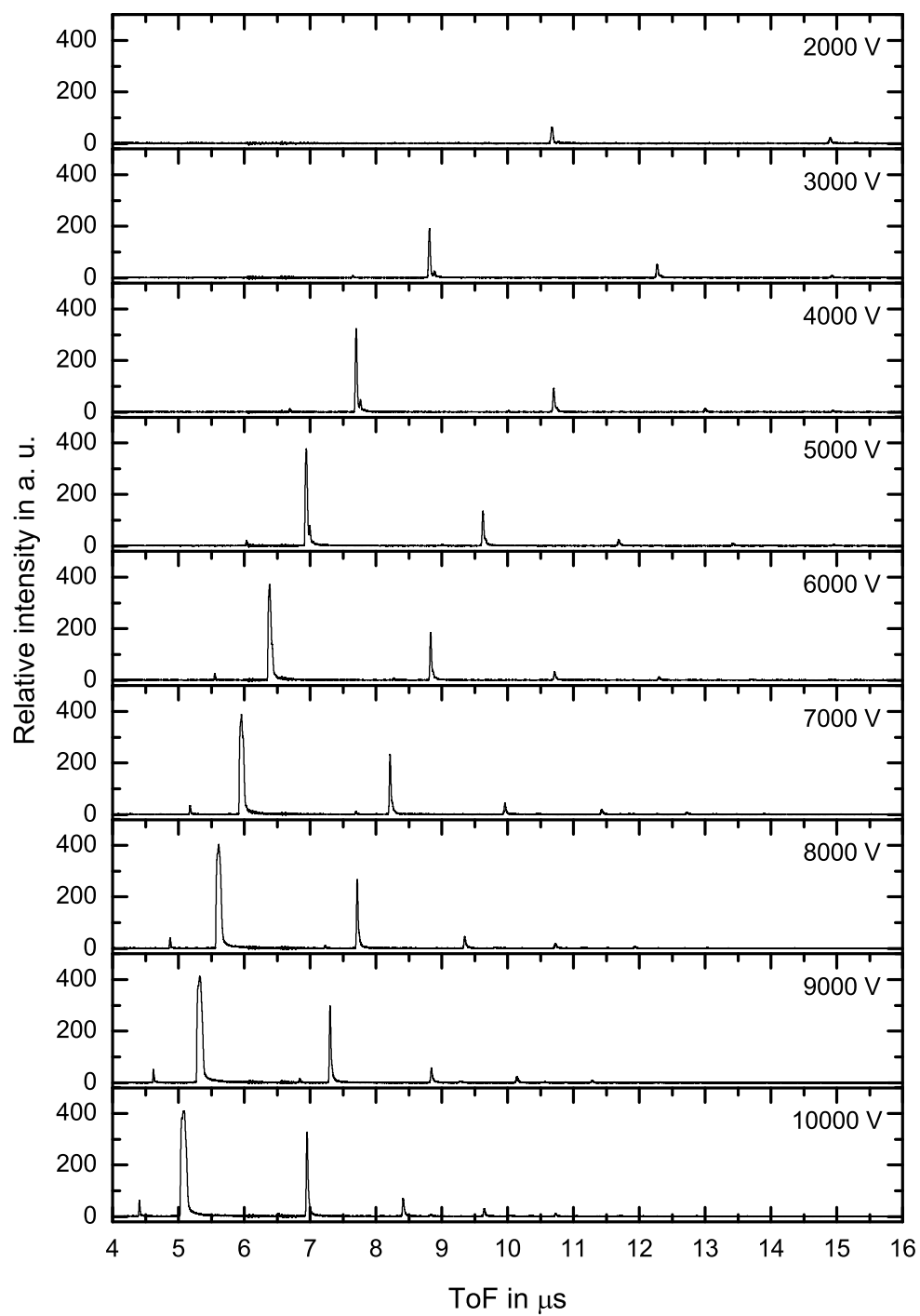


Figure 6.15: ToF spectra of acetone taken with the exalite 389 MPPC direct ion detector using different acceleration potentials.

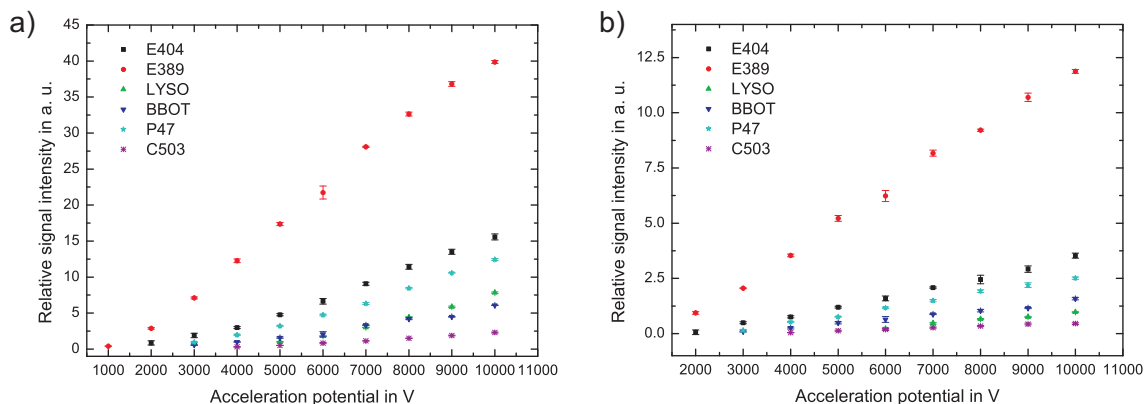


Figure 6.16: Determined relative detection efficiencies for the various scintillator screens employed as a function of the acceleration potential applied. The error bars illustrated represent the standard deviation of the repeated measurements. *a)* Shows the results obtained for the 58 Da acetone parent ion peak and *b)* presents the signal intensities determined for the 116 Da acetone cluster ion peak.

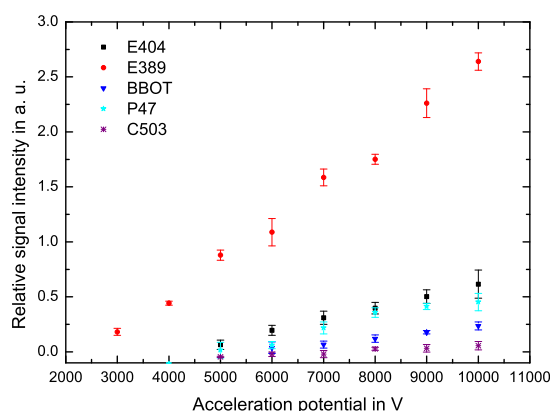


Figure 6.17: Signal intensities, observed for different scintillator screens, plotted as a function of the applied acceleration voltage for the second cluster ToF peak of acetone (174 Da).

peak increases by applying higher acceleration potentials. As a result, the integral of the signal keeps increasing using higher acceleration potentials. Thereby, the integration value is most likely determined with a smaller value, than if the entire peak height would be measured with a non saturated sensor.

The peak integrals determined for the exalite 404 peaks are on the order of 25% to 40% of those observed with the exalite 389. This result is somewhat surprising, as after electron detection the exalite 404 screen showed a better performance, when compared to

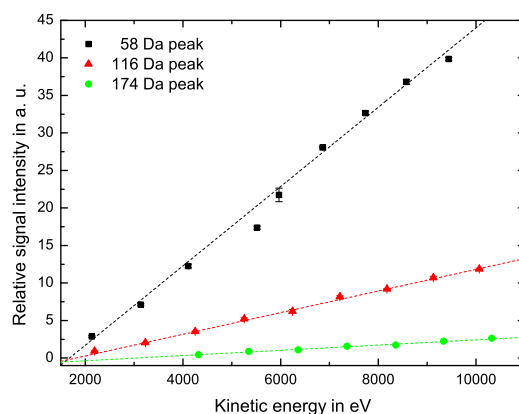


Figure 6.18: Ion signal levels observed using the exalite 389 scintillator. By plotting the signal levels as a function of the kinetic energy of the ions, a linear relationship can be observed.

the exalite 389 scintillator (see Chapter 5). There are a number of reasons that might explain the change in the scintillation efficiency. For example, the scintillation process might mainly occur on the top layer of the screen, which, due to a slightly different layer thickness, might lead to a higher self-absorption or scattering of the emitted light in the exalite 404 screen. Furthermore, differences in the crystal composition or the molecular structure might play a role. However, it was not possible to address the exact reason for this behaviour in this work.

The remaining scintillators show worse signal-to-noise ratios in comparison to the exalite screens. Peak intensities detected for the ToF signals using the BBOT, LYSO, P47 or coumarin 503 scintillators were on the order of 5% to 15% of those observed for the exalite 389 screen. This is a surprising result as the most SPAD studies mentioned above, employed inorganic scintillators such as the LYSO crystal. However, these undertaken investigations were focused on the detection of high energy particles to stimulate the fluorescence of the scintillators, which may have led to a different scintillation response than observed in this study.

Figure 6.18 shows a plot of the integrated signal levels as a function of the kinetic energy of the detected particles, recorded using the exalite 389 scintillator. The three mass

peaks investigated show a linear trend for their signal-to-kinetic energy ratio. Hence, in contrast to the detection of charged particles using an MCP detector (see Section 6.1.2), the release of photons following an ion impact on a scintillator surface is found to be directly proportional to the kinetic energy of the impinging particles for the investigated energy range. As a result, the loss in detection efficiency at higher masses noted using MCP based detectors might not occur to such an extent using the scintillator based direct ion detector. However, this effect needs to be investigated in future work.

The measurements presented in this section were mainly recorded using the molecular beam spot of the acetone signal. Hence, a high particle density is likely such that multiple ion hits might be required to stimulate the emission of a single photon. In future experiment it would be interesting to detect the impact of a single ion onto a scintillator surface and to measure the photon emission rate as a function of the impact energy. These measurements would allow the absolute ion-to-photon conversion efficiency to be determined.

6.4.4 Layer Properties

The organic scintillators used in the experimental series above were prepared with comparable thicknesses in the vacuum sublimation process (150 – 200 nm). As ions are significantly larger than electrons, the layer thickness may play a key role for the efficiency of the ion-to-photon conversion. For example, fluorescence may occur only from the layers closest to the surface, as it is likely that most ions do not penetrate far into the organic layer. The resulting fluorescence photons must then be transmitted through the remaining scintillator layers to reach the SPAD sensor. As such, there may exist a scintillator thickness above which the detected fluorescence signal falls dramatically, corresponding to the inability of emitted photons to penetrate through the scintillator layer.

In order to briefly address this issue, a number of exalite 404 screens were prepared with different layer thicknesses, using the previously introduced electrospray deposition method (see Chapter 2). During the preparation process, a potential of 5.0 kV was applied to the needle, and the flow rate of a saturated exalite 404 solution in acetone was set to

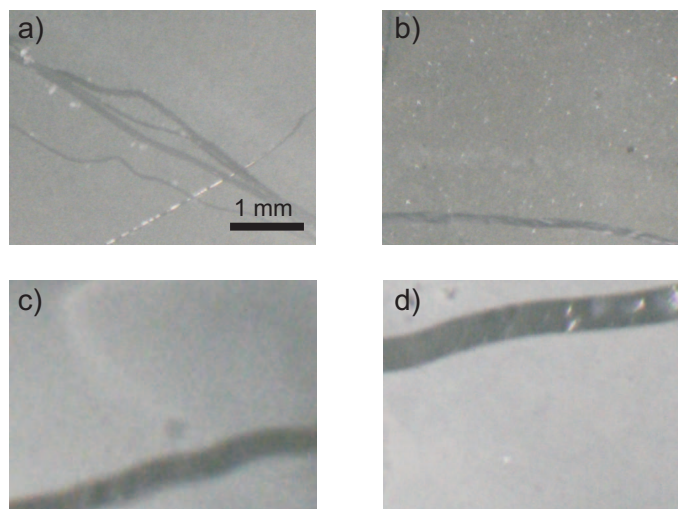


Figure 6.19: Microscope photographs of the employed exalite 404 scintillator screens. Following the order *a* to *d* the spray time was increased by one minute each (1-4 min). The scratches noted in each sample were deliberately added to allow an analytical measurement of the sample thickness.

350 μl per hour. Samples were sprayed for either one, two, three or four minutes. Typical spot sizes of 10 mm diameter were achieved. Microscope photographs of all the samples investigated are presented in Figure 6.19. Here, it can be clearly noted that the layer thickness is increased with a longer spray time. A surface analysis (Profilometer) of the samples indicated that the sample *b* had a layer thickness of 200 nm, which is comparable to the sublimated scintillator screens. The layer thickness of sample *d* was determined to be 860 nm.

The created screens were mounted onto the photosensitive area of the MPPC chip, and the acetone signal levels were recorded using acceleration potentials of 6 kV and 10 kV. The resulting integrated signal levels for the 58 Da peak are illustrated in Figure 6.20. As expected, the scintillator thickness seems to affect the direct ion detection and therefore the ion-to-photon conversion efficiency of the applied screens. The results obtained indicate that the sample which was sprayed for 2 minutes (200 nm thickness) shows the best results. The signal decrease observed for very thin sample layers may be attributed to the fact that not enough scintillator molecules are deposited to convert the kinetic energy of the impinging particles into a fluorescence signal. Thicker samples might reduce the efficiency

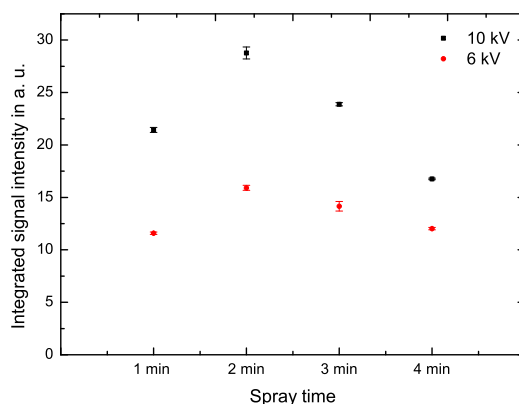


Figure 6.20: Exalite 404 layer thickness test: the highest signal level (acetone, 58 Da peak) was achieved using electrosprayed sample layers of about 200 nm thickness (2 min. spray).

of the screens, due to the discussed scattering and re-absorption effects in the organic layers.

Comparing the obtained signal levels of the electrosprayed samples to those previously recorded using sublimated samples (see Figure 6.16a), it can be noted that depending on the deposition method a significant change in scintillation efficiency is observed. Electrosprayed samples showed an enhanced ion-to-photon conversion efficiency. This effect may be due to the molecule density and the crystal size of the organic layers. Nevertheless, significantly more measurements are required to fully understand the scintillation process as a function of the layer properties. In order to do this, an accurate determination of the individual layer thicknesses and a consistent crystal size across the samples are required.

Following the measurements undertaken in this section it was noted that the measured exalite 404 signal levels never exceeded those determined for the exalite 389 screen. As a result, it can still be stated that the exalite 389 scintillator shows the most promising results for operation in combination with an MPPC sensor as a direct ion detector. Furthermore, the exalite 389 emission efficiency can probably be increased by optimising the layer thickness of the prepared screens.

6.5 Conclusion and Outlook

6.5.1 Application in Mass Spectrometry

The preliminary experiments described in the sections above indicate that organic scintillators in combination with an MPPC sensor may be of particular use as a direct ion detector in mass spectrometric applications. The introduction of the newly developed exalite 389 and exalite 404 scintillator screens resulted in an improvement of the absolute detection efficiency in comparison to the previously employed LYSO crystal (see Reference [236]). The best performance among the investigated scintillator screens was achieved using the exalite 389. This is a somewhat surprising result, as for the detection of electrons the exalite 404 screen outperformed the other organic scintillators (see Chapter 5).

One major limitation of the experiments undertaken can be attributed to the unknown ion flux on the detector. The knowledge of this parameter is essential to define the absolute ion-to-photon conversion efficiency for the investigated scintillators. Therefore, future experiments addressing the quantum efficiency of direct ion detectors will need to allow a quantification of the ion flux. This can be achieved either by mounting a Faraday collector in front of the detector (see Reference [181]), or by ensuring that only a single ion is accelerated onto the detector at a time.

The fluorescence signals observed with the MPPC sensor in the measurements above were likely triggered by the simultaneous impact of multiple ions at the same time. In the case that this assumption is confirmed, it will be essential to trigger the scintillation process from the impact of a single ion in future experiments. This may be achieved by introducing a further ion acceleration stage in front of the detector, such as illustrated in Figure 6.21. Here, a grounded grid is mounted in front of the detector and a conductive coated scintillator screen is attached to the MPPC sensor. By charging the surface of the scintillator screen with a negative potential it is possible to attract and further accelerate ions towards the detector. Thereby, the absolute ion-to-photon conversion efficiency may be improved.

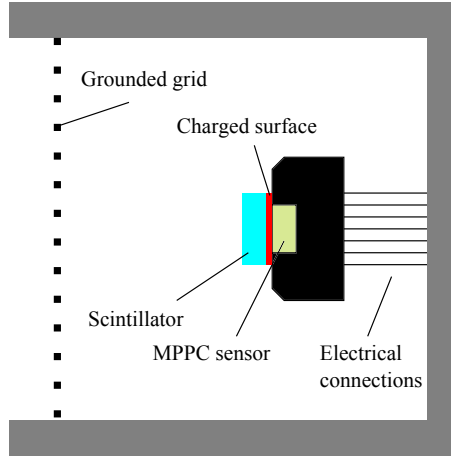


Figure 6.21: Schematic drawing of a direct ion detector employing a post extraction ion acceleration stage between a grounded grid and the MPPC sensor.

A further issue observed in the experiments was the limited dynamic range of the applied MPPC array, which allowed only up to 10 photons to be detected simultaneously, using a sensor with 1600 individual SPAD cells. That means, per “frame” analysed, only 0.6% of the entire sensor can effectively be used to capture impinging photons before the chip is saturated. Therefore, in future experiments the MPPC sensor needs to be read out differently or an improved version needs to be applied to allow more photons to be detected simultaneously.

One advantage of the MPPC chips besides their single-photon counting capabilities is their sub-nanosecond timing resolution. Unfortunately the experiments performed in this work could not verify the high time precision of the detector as this was limited by the resolving power of the VMI ion optics. Additionally, it was not possible to detect fast electron signals as such early ToF peaks are overlapped by the background signal due to scattered laser light. Nevertheless, it can be assumed that the time resolution of the ToF detector is limited by the emission properties of the scintillators employed. These specifications were determined in Chapter 5. However, further experiments are required to demonstrate the timing specifications of the direct ion detection system.

Two dimensional arrays of individual SPAD sensors are currently under development. If these devices are coated with a thin layer of an efficient scintillator, they might provide

a competitive alternative to conventional electron multiplier based ToF detection systems, where both the arrival time and the spatial distribution of impacting ions are captured simultaneously. Hence, these devices would be an asset to all experiments which require a spatial distribution to be recorded in conjunction with a high timing precision.

6.5.2 Medical Imaging Applications

The presented mass spectrometry measurements revealed that the exalite scintillators outperform the LYSO crystals in terms of particle detection efficiency. Furthermore, it was shown that the organic dyes show an improved timing resolution. Hence, the application of an MPPC array in combination with an exalite scintillator may be of considerable interest to other applications besides mass spectrometry, which currently rely on the use of inorganic LYSO crystals [237].

One typical example would be nuclear medical imaging techniques such as Time-of-Flight Positron Emission Tomography (ToF-PET, see Figure 6.22) [238, 239]. In this method, a radioactive tracer isotope, incorporated into a specific biomolecule, is injected into the blood of a patient. The biocompound (usually sugar based molecules) is used to direct and concentrate the radioactive material in the tissue section of interest. Within the tissue the radioactive compound undergoes a β -decay process emitting a positron. This positron migrates through the neighbouring tissue sections, but usually no further than

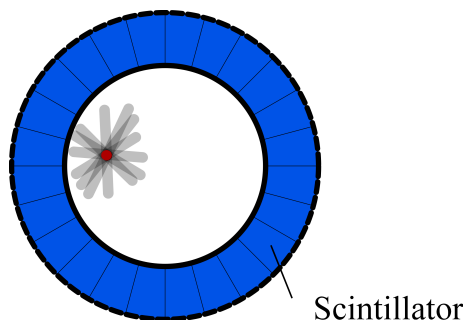


Figure 6.22: Illustration of the operation principle of ToF-PET. Two gamma rays are emitted from a point source, which is surrounded by a ring of scintillator based MPPC detectors. By determining the delay between the arrival time of the two photons, the position of the source can be located.

1 mm. In the process, the kinetic energy of the particle is reduced until it can interact with an electron from the surrounding tissue. The interaction of the encounter produces two gamma photons (511 keV), which are ejected in two opposite directions (electron-positron annihilation). By mounting a detector ring consisting of an array of inorganic scintillator crystals with an attached photomultiplier or silicon avalanche photodiodes around the patient, the two correlated photons can be collected. An event is categorised as a photon pair if both detection events occur within a short time window. By determining the delay between the two events observed, the origin position of the particles can be determined. The precision of this measurement depends on how accurately the arrival time of the gamma particles can be measured. Therefore, fast particle detectors are required to allow an accurate localisation.

Currently, LSO (Lu_2SiO_5), LYSO, BGO ($\text{Bi}_4\text{Ge}_3\text{O}_{12}$), BaF_2 , and LaBr_3 are the most commonly chosen scintillator for PET scanning [240–243]. In most cases, the scintillators are combined with an EM or MPPC sensor.

By applying the fast exalite screens in combination with an MPPC sensor to this technique the time resolution and thereby the spatial resolution of the ToF PET instruments may be improved. The higher scintillator brightness may simultaneously lead to an increase in sensitivity, while reducing the scan time of the instrument. In this way, the radioactive dose for the patient may be lowered, while an improved image is obtained. However, such an application relies on the assumption that the exalite screens are sensitive to gamma radiation, which will need to be addressed in future work.

Chapter 7

Outlook - Imaging with Ultra High Timing Precision

The work described in this thesis has focused on the development of both a linear microscope mode mass spectrometer and a compatible high resolution ToF detector. The milestones achieved in the development of the spectrometer are the design of a new ion optic, capable of imaging simultaneously a large sample section, and obtaining a ToF resolution that is able to distinguish isotope peaks up to an ion mass of 700 Da. The spatial resolution achieved in microscope mode has been shown to allow the identification of sample features down to sub-20 μm , within a field-of-view of up to 4 mm. By combining the spectrometer with the newly developed imaging detector, comprising of MCPs and the exalite 404 screen, both the spatial and the ToF information can be translated into a fluorescence signal, which can be recorded by either a CMOS based sensor or a CCD camera in combination with a fast PMT.

Both camera detection systems have their advantages and disadvantages for applications to imaging mass spectrometry. One suitable CMOS system is the PImMS camera, which allows multi-mass imaging with an upper time precision limit of 6.25 ns (currently 12.5 ns). This is a considerable advantage over the CCD camera, where only one selected mass fragment can be imaged within an experimental cycle. However, if the CCD camera

system is employed, a spatial image with a higher resolution can be recorded as the total number of pixels is higher. Furthermore, if cameras are employed in conjunction with a PMT, the ToF information may be recorded with a sub-nanosecond timing precision while a sharp image is collected. The limitations of both camera systems may be improved by constructing a hybrid delay line scintillator imaging detector. The concept of its operation, and the design of the proposed system will be outlined in the following.

7.1 The Hybrid Delay Line Scintillation Detector

As noted in Chapter 1, delay line anodes offer both a precise measurement of the arrival time of an impinging particle and a fast repetition rate. However, they are limited in their ability to detect multiple particles arriving at the detector simultaneously. Experiments employing delay line anodes therefore need to be operated with low count rates to allow a compatible detection. In contrast to delay line devices, imaging detectors comprising of a scintillator and camera allow the position of multiple, simultaneous events to be determined accurately. However, these systems do not allow the time of the individual events to be determined with a precision such as with conventional delay line detectors.

By constructing a hybrid detector comprising of both systems, the high time resolution of a fast delay line anode can be accessed together with the positional information and high count rate of an imaging sensor. However, a major challenge is how to correlate the accurate timing information from the delay line anode with the accurate spatial coordinate determination from the ion image for each particle. In this chapter the new detector device in combination with suitable data acquisition and analysis algorithms is described. As a result the detector offers time and position resolved particle detection with sub-nanosecond time resolution, high throughput and high repetition rates.

The new detector comprises of a transparent quartz substrate, on which a conductive, transparent wire (for example ITO) is applied, operating in a similar way as a conventional, single delay line anode. On top of the delay line, a scintillator coating is deposited, and the screen is mounted behind an MCP assembly.

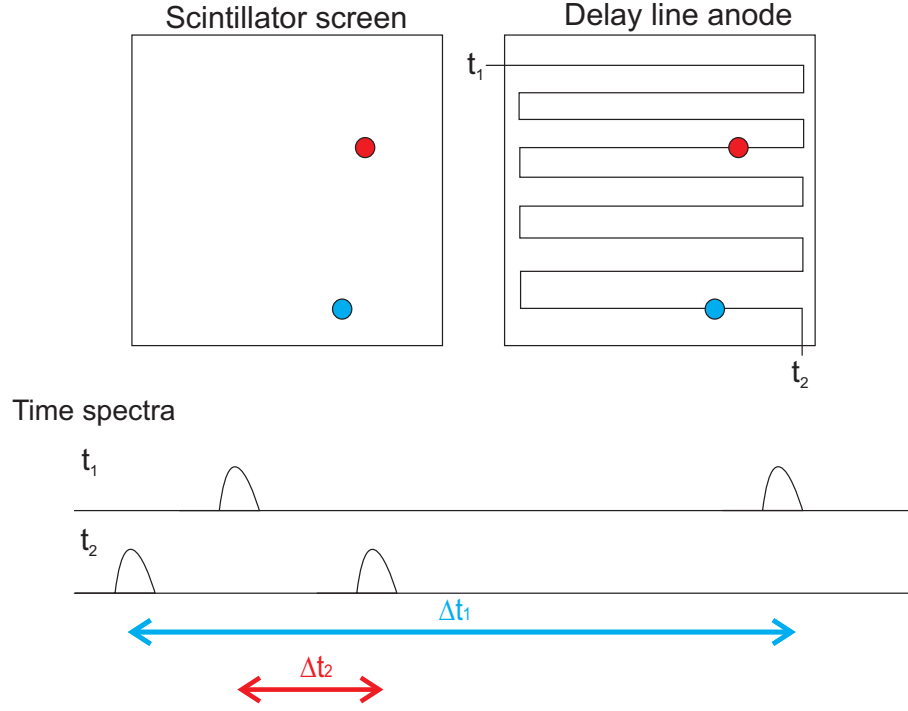


Figure 7.1: Readout concept of the hybrid delay line scintillator imaging detector. See text for details.

During operation, charged particles impinging on the front face of an MCP stack are converted into a burst of electrons. These electrons are accelerated onto the prepared scintillator layer, and are converted into discrete flashes of light. The emitted photons are then transmitted through the transparent delay line to be detected by an imaging sensor. In this way, the camera records an image of all particles detected within a ToF cycle. Simultaneously, the electron impact of each event induces an electrical pulse that is transmitted to both ends of the delay line. The arrival times of all pulses throughout each ToF cycle are measured with high time precision, and stored for further processing. A typical scintillator screen image, and the corresponding impact positions on the delay line, are illustrated in Figure 7.1.

The camera image shows the accurate impact position of all particles, but provides no timing information. The assignment of an arrival time to each particle is achieved by correlating its impact position with the pulse sequences recorded by the delay line. If the propagation speed for the delay line anode is known, it is possible to calculate from

the observed event in the scintillator image an expected time delay, Δt . This value is equivalent with the delay time at which the signals arrive at each end of the delay line anode ($\Delta t = t_1 - t_2$). Hence, it is possible to search for a pair of pulses in the recorded time spectra of t_1 and t_2 , which matches the predicted Δt value (see example in blue and red in Figure 7.1). After identification of the correlated pulses, the exact arrival time of the particle can be determined with a precision that is determined by the properties of the delay line detector. By repeating this process for every event recorded in the image, a position and arrival time for every detected ion can be determined. Hence, the total number of events identified is increased significantly in comparison to a commercial delay line detector.

7.2 Experimental Limitations

The ability of the described system to correctly assign spatial events to their delay line pulses depends on how accurately the position and the pulse arrival time can be measured using the hybrid detector. The error in the localisation of an event using a commercially available high resolution camera is almost negligible. A larger uncertainty in the event allocation process is introduced due to the timing properties of the delay line. For a commercial delay line detector a signal propagation speed on the order of 0.5 mm/ns can be assumed [244]. Hence, if analog-to-digital converters with a time resolution of 2 ns are employed to determine the pulse arrival time on both ends of the delay line, the uncertainty in the position of the event can be assumed to be on the order of 2 mm. As a result, two ion hits, which are observed within a time window of 4 ns and in a distance of 2 mm, can no longer be identified as individual events.

A second limitation arises from pulse broadening effects in the transmission line. In the case that too many pulses are overlapped, a direct assignment to the expected Δt value becomes difficult. However, as a measurement of the pulses travelling through the ITO delay line has not been performed yet, it is hard to assess this value. Hence, the actual limitations expected for the device cannot be fully quantified in the discussion given in this

work.

7.3 Readout Modes

The detection system proposed may be operated in three different modes, depending on if it is combined with a CCD camera or with the PImMS sensor. The modes will be described in the following.

CCD Readout Mode

In the case that a CCD camera is used to capture the fluorescence signal of the scintillator screen, a summed image is acquired over one entire ToF cycle. By analysing the observed ion pattern, an arrival time for each event can be determined following the description above. In the case that two events are detected at the same spatial position, only one can be identified in the CCD image. However, two matching Δt values will be found in the t_1 and t_2 spectra for the specific position, so that two events at different arrival times can be identified using the hybrid detector. In this way, it is possible to perform multi-mass imaging using a conventional CCD camera.

Enhanced Throughput CMOS Readout Mode

If the CCD readout mode is applied to experiments with an extremely high ion flux, it might be the case that the detector is no longer capable of accurately identifying the individual events. To overcome this limitation, either a fast framing CCD or a CMOS camera may be employed to collect the spatial information within a certain time window of the total experimental ToF cycle. PImMS provides time and position information of events recorded within a time window of 12.5 ns. By analysing each recorded time bin individually, it is possible to reduce the search window for the Δt allocation in the data analysis significantly. As a result, the multi-mass imaging capabilities of the hybrid detector are further improved.

High Repetition CMOS Readout Mode

One limiting factor of state-of-the-art CMOS and CCD based detectors is that they often cannot be read out with a high repetition rate as the amount of data is inherently large. In contrast to this, the repetition rates achieved with a delay line are only limited by its detection system (TDC, ADC).

Due to its CMOS architecture, the PImMS camera records single events over 4096 individual time bins. In the case that the width of each individual time bin is increased to be as large as an individual ToF cycle, it is possible to measure an entire ToF spectrum with correlated ToF information within each of these individual bins using the proposed hybrid detector. Thereby, the frame rate of the detector is only defined by the length of the collection time of the individual time bins. In this way, it is possible to collect ion images for 2048 individual ToF cycles without reading out the imaging sensor (after signal collection one time bin should be empty to allow the different ToF cycles to be distinguished).

In the case that the collection window of each time bin of PImMS is set to $100\ \mu\text{s}$, an experiment could be performed every $200\ \mu\text{s}$, which results in a repetition rate of 5 kHz. However, the experiments will be limited by the number of registers employed in each pixel of the camera, as saturation effects of individual pixels with increasing number of experimental cycles may reduce the sensitivity of the hybrid detector. Later versions of PImMS or other CMOS devices may allow a continuous readout of the filled registers. For these devices the high repetition CMOS readout mode will become available.

7.4 Conclusion

The detector proposed may improve the time resolution and multi-mass imaging capabilities of the current state-of-the-art systems. However, the actual limitations of the system need to be investigated after the practical implementation of the system. This will be explored in future work.

Bibliography

- [1] Thomson, J. J. *Rays of Positive Electricity and their Application to Chemical Analysis*; Longmans, Green and Co, London, 1913.
- [2] Aston, F. W. *Philosophical Magazine* **1919**, *38*, 707–715.
- [3] Downard, K. M. *European Journal of Mass Spectrometry* **2007**, *13*, 177–190.
- [4] Stephens, W. *Bull. Am. Phys. Soc.* **1946**, *21*, 22.
- [5] Cameron, A. E.; Eggers, D. F. *Review of Scientific Instruments* **1948**, *19*, 6075–6077.
- [6] McLafferty, F. W.; Turecek, F. *Interpretation of Mass Spectra*; University Science Books, Mill Vally, USA, 1993.
- [7] Sparkman, D. O. *Mass Spec Desk Reference*; Global View Publishing, Pittsburg, USA, 2006; Vol. 2nd.
- [8] *Database*; <http://goldbook.iupac.org>.
- [9] Evans, C. A.; Blattner, R. J. *Ann. Rev. Mat. Sci.* **1978**, *8*, 181–214.
- [10] Williams, P. *Applications of Surface Science (1977-1985)* **1982**, *13*, 241–259.
- [11] Belu, A. M.; Graham, D. J.; Castner, D. G. *Biomaterials* **2003**, *24*, 3635–3653.
- [12] Rubakhin, S. S.; Sweedler, J. V. *Mass Spectrometry Imaging - Principles and Protocols*; Springer New York Dordrecht Heidelber London, 2010.

- [13] Sigmund, P. *Physical Review* **1969**, *184*, 383–416.
- [14] Pacholski, M. L.; Winograd, N. *Chemical Reviews* **1999**, *99*, 2977–3005.
- [15] Zandvliet, H. J. W.; Elswijk, H. B.; Van, E. J., Loenen; Tsong, I. S. T. *Physical Review B: Condensed Matter and Materials Physics* **1992**, *46*, 7581–7587.
- [16] Winograd, N. *Analytical Chemistry* **2005**, *77*, 142A–149A.
- [17] Postawa, Z.; Czerwinski, B.; Szewczyk, M.; Smiley, E. J.; Winograd, N.; Garrison, B. J. *Journal of Physical Chemistry B* **2004**, *108*, 7831–7838.
- [18] Fuoco, E. R.; Gillen, G.; Wijesundara, M. B. J.; Wallace, W. E.; Hanley, L. *Journal of Physical Chemistry B* **2001**, *105*, 3950–3956.
- [19] Wong, S. C. C.; Hill, R.; Blenkinsopp, P.; Lockyer, N. P.; Weibel, D. E.; Vickerman, J. C. *Applied Surface Science* **2003**, *203-204*, 219–222.
- [20] Weibel, D.; Wong, S.; Lockyer, N.; Blenkinsopp, P.; Hill, R.; Vickerman, J. C. *Analytical Chemistry* **2003**, *75*, 1754–1764.
- [21] Weibel, D. E.; Lockyer, N.; Vickerman, J. C. *Applied Surface Science* **2004**, *231-232*, 146–152.
- [22] Zaric, R.; Person, B.; Krantzman, K. D.; Garrison, B. J. *International Journal of Mass Spectrometry and Ion Processes* **1998**, *174*, 155–166.
- [23] Passarelli, M. K.; Ewing, A. G. *Current Opinion in Chemical Biology* **2013**, *17*, 854–859.
- [24] Piehowski, P. D.; Carado, A. J.; Kurczy, M. E.; Ostrowski, S. G.; Heien, M. L.; Winograd, N.; Ewing, A. G. *Analytical Chemistry* **2008**, *80*, 8662–8667.
- [25] Touboul, D.; Kollmer, F.; Niehuis, E.; Brunelle, A.; Laprevote, O. *Journal of the American Society for Mass Spectrometry* **2005**, *16*, 1608–1618.

- [26] Amaya, K. R.; Monroe, E. B.; Sweedler, J. V.; Clayton, D. F. *International Journal of Mass Spectrometry* **2007**, *260*, 121–127.
- [27] Malmberg, P.; Boerner, K.; Chen, Y.; Friberg, P.; Hagenhoff, B.; Mansson, J.-E.; Nygren, H. *Biochimica et Biophysica Acta, Molecular and Cell Biology of Lipids* **2007**, *1771*, 185–195.
- [28] Humayun, M.; Davis, F. A.; Hirschmann, M. M. *Journal of Analytical Atomic Spectrometry* **2010**, *25*, 998–1005.
- [29] Rege, S.; Jackson, S.; Griffin, W. L.; Davies, R. M.; Pearson, N. J.; O'Reilly, S. Y. *Journal of Analytical Atomic Spectrometry* **2005**, *20*, 601–611.
- [30] Vastola, F. J.; Mumma, R. O.; Pirone, A. J. *Organic Mass Spectrometry* **1970**, *3*, 101–104.
- [31] Mumma, R. O.; Vastola, F. J. *Organic Mass Spectrometry* **1972**, *6*, 1373–1376.
- [32] Posthumus, M. A.; Kistemaker, P. G.; Meuzelaar, H. L. C.; Ten Noever de Brauw, M. C. *Analytical Chemistry* **1978**, *50*, 985–991.
- [33] Tabet, J. C.; Cotter, R. J. *Analytical Chemistry* **1984**, *56*, 1662–1667.
- [34] Van Breemen, R. B.; Tabet, J. C.; Cotter, R. J. *Biomedical Mass Spectrometry* **1984**, *11*, 278–283.
- [35] Hardin, E. D.; Fan, T. P.; Blakley, C. R.; Vestal, M. L. *Analytical Chemistry* **1984**, *56*, 2–7.
- [36] Lindner, B.; Seydel, U. *Analytical Chemistry* **1985**, *57*, 895–899.
- [37] Van der Peyl, G. J. Q.; Isa, K.; Haverkamp, J.; Kistemaker, P. G. *Organic Mass Spectrometry* **1981**, *16*, 416–420.
- [38] Van der Peyl, G. J. Q.; Haverkamp, J.; Kistemaker, P. G. *International Journal of Mass Spectrometry and Ion Physics* **1982**, *42*, 125–141.

- [39] Joest, B.; Schueler, B.; Krueger, F. R. *Zeitschrift fuer Naturforschung, Teil A: Astrophysik, Physik und Physikalische Chemie* **1982**, *37A*, 18–27.
- [40] Karas, M.; Bachmann, D.; Hillenkamp, F. *Analytical Chemistry* **1985**, *57*, 2935–2939.
- [41] Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F. *International Journal of Mass Spectrometry and Ion Processes* **1987**, *78*, 53–68.
- [42] Karas, M.; Hillenkamp, F. *Analytical Chemistry* **1988**, *60*, 2299–2301.
- [43] Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, Y.; Yohida, T. *Rapid Communications in Mass Spectrometry* **1988**, *2*, 151–153.
- [44] Beavis, R. C.; Chait, B. T.; Fales, H. M. *Rapid Communications in Mass Spectrometry* **1989**, *3*, 432–435.
- [45] Strupat, K.; Karas, M.; Hillenkamp, F. *International Journal of Mass Spectrometry and Ion Processes* **1991**, *111*, 89–102.
- [46] Beavis, R. C.; Chaudhary, T.; Chait, B. T. *Organic Mass Spectrometry* **1992**, *27*, 156–158.
- [47] Dreisewerd, K. *Chemical Reviews* **2003**, *103*, 395–425.
- [48] Karas, M.; Krueger, R. *Chemical Reviews* **2003**, *103*, 427–439.
- [49] Ingendoh, A.; Karas, M.; Hillenkamp, F.; Giessmann, U. *International Journal of Mass Spectrometry and Ion Processes* **1994**, *131*, 345–354.
- [50] Karas, M.; Gluckmann, M.; Schafer, J. *Journal of Mass Spectrometry* **2000**, *35*, 1–12.
- [51] Krueger, R.; Pfenninger, A.; Fournier, I.; Glueckmann, M.; Karas, M. *Analytical Chemistry* **2001**, *73*, 5812–5821.

- [52] Karbach, V.; Knochenmuss, R. *Rapid Communications in Mass Spectrometry* **1998**, *12*, 968–974.
- [53] Ehring, H.; Karas, M.; Hillenkamp, F. *Organic Mass Spectrometry* **1992**, *27*, 472–480.
- [54] Knochenmuss, R. *Analyst* **2006**, *131*, 966–986.
- [55] Setz, P. D.; Knochenmuss, R. *Journal of Physical Chemistry A* **2005**, *109*, 4030–4037.
- [56] Jaskolla, T. W.; Karas, M. *Journal of the American Society for Mass Spectrometry* **2011**, *22*, 976–988.
- [57] Chandler, D. W.; Houston, P. L. *Journal of Chemical Physics* **1987**, *87*, 1445–1447.
- [58] Whitaker, B. J. *Imaging in Molecular Dynamics: Technology and Applications*; Cambridge University Press, UK, 2003.
- [59] Eppink, A. T. J. B.; Parker, D. H. *Review of Scientific Instruments* **1997**, *68*, 3477–3484.
- [60] Davisson, C. J.; Calbick, C. J. *Physical Review* **1931**, *38*, 585.
- [61] Davisson, C. J.; Calbick, C. J. *Physical Review* **1932**, *42*, 580.
- [62] Dasch, C. *Applied optics* **1992**, *31*, 1146–1152.
- [63] Garcia, G. A.; Nahon, L.; Powis, I. *Review of Scientific Instruments* **2004**, *75*, 4989–4996.
- [64] Manzhos, S.; Loock, H.-P. *Computer Physics Communications* **2003**, *154*, 76–87.
- [65] Rakitzis, T. P.; Zare, R. N. *Journal of Chemical Physics* **1999**, *110*, 3341–3350.
- [66] Rakitzis, T. P.; Kandel, S. A.; Alexander, A. J.; Kim, Z. H.; Zare, R. N. *Journal of Chemical Physics* **1999**, *110*, 3351–3359.

- [67] Solomon, J. *Journal of Chemical Physics* **1967**, *47*, 889–894.
- [68] Houston, P. L. *Accounts of Chemical Research* **1995**, *28*, 453–460.
- [69] Kitsopoulos, T. N.; Buntine, M. A.; Baldwin, D. P.; Zare, R. N.; Chandler, D. W. *Science* **1993**, *260*, 1605–1610.
- [70] Ahmed, M.; Peterka, D. S.; Suits, A. G. *Chemical Physics Letters* **1999**, *301*, 372–378.
- [71] Kohguchi, H.; Suzuki, T.; Alexander, M. H. *Science* **2001**, *294*, 832–834.
- [72] Suits, A. G.; Bontuyan, L. S.; Houston, P. L.; Whitaker, B. J. *Journal of Chemical Physics* **1992**, *96*, 8618–8620.
- [73] Allan, V. J. *Protein Localisation by Fluorescence Microscopy: A Practical Approach*; Oxford University Press, UK, 2000.
- [74] Nimesh, S.; Mohottalage, S.; Vincent, R.; Kumarathasan, P. *International Journal of Molecular Sciences* **2013**, *14*, 11277–11301.
- [75] Todd, P. J.; Schaaff, T. G.; Chaurand, P.; Caprioli, R. M. *Journal of Mass Spectrometry* **2001**, *36*, 355–369.
- [76] El Ayed, M.; Bonnel, D.; Longuespee, R.; Castellier, C.; Franck, J.; Vergara, D.; Desmons, A.; Tasiemski, A.; Kenani, A.; Vinatier, D.; Day, R.; Fournier, I.; Salzet, M. *Medical Science Monitor* **2010**, *16*, 233–245.
- [77] Wisztorski, M.; Croix, D.; Macagno, E.; Fournier, I.; Salzet, M. *Developmental Neurobiology* **2008**, *68*, 845–858.
- [78] Norris, J. L.; Caprioli, R. M. *Proteomics: Clinical Applications* **2013**, *7*, 733–738.
- [79] Avila, G. *FRD and scrambling properties of recent non-circular fibres*; [http: www.eso.org](http://www.eso.org).

- [80] de Hoffmann, E.; Stroobant, V. *Mass Spectrometry - Principles and Applications*; John Wiley & Sons Ltd, 2007.
- [81] Paul, W.; Steinwedel, H. *Zeitschrift für Naturforschung A* **1953**, *8*, 448–450.
- [82] Makarov, A. *Analytical Chemistry* **2000**, *72*, 1156–1162.
- [83] Hu, Q.; Noll, R. J.; Li, H.; Makarov, A.; Hardman, M.; Cooks, R. G. *Journal of Mass Spectrometry* **2005**, *40*, 430–443.
- [84] Comisarow, M. B.; Marshall, A. G. *Chemical Physics Letters* **1974**, *25*, 282–283.
- [85] Comisarow, M. B.; Marshall, A. G. *Chemical Physics Letters* **1974**, *26*, 489–490.
- [86] Marshall, A. G.; Hendrickson, C. L.; Jackson, G. S. *Mass Spectrometry Reviews* **1998**, *17*, 1–35.
- [87] Wiley, W. C.; McLaren, I. H. *Review of Scientific Instruments* **1955**, *26*, 1150–1157.
- [88] Vestal, M. L.; Juhasz, P.; Martin, S. A. *Rapid Communications in mass spectrometry* **1995**, *9*, 1044–1050.
- [89] Alikhanov, S. *Sov. Phys. JETP* **1957**, *4*, 452–455.
- [90] Mamyrin, B. A.; Karataev, V. I.; Shmikk, D. V.; Zagulin, V. A. *Sov. Phys. JETP* **1973**, *37*, 45–48.
- [91] Pol, J.; Strohm, M.; Havlicek, V.; Volny, M. *Histochemistry and Cell Biology* **2010**, *134*, 423–443.
- [92] Castaing, R.; Slodzian, G. *Compt. Rend.* **1962**, *255*, 1893–1895.
- [93] Schueler, B. W. *Microsc. Microanal. Microstruct.* **1992**, *3*, 119–139.
- [94] Luxembourg, S. L.; Mize, T. H.; McDonnell, L. A.; Heeren, R. M. A. *Anal. Chem.* **2004**, *76*, 5339–5344.

- [95] Klerk, L. A.; Altelaar, A. F. M.; Froesch, M.; McDonnell, L. A.; Heeren, R. M. A. *Int. J. Mass Spectrom.* **2009**, *285*, 19–25.
- [96] Froesch, M.; Luxembourg, S. L.; Verheijde, D.; Heeren, R. M. A. *European Journal of Mass Spectrometry* **2010**, *16*, 35–45.
- [97] Jungmann, J. H.; MacAleese, L.; Buijs, R.; Giskes, F.; de Snaijer, A.; Visser, J.; J. Visschers; Vrakking, M. J. J.; Heeren, R. M. A. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 2023–2030.
- [98] Jungmann, J. H.; MacAleese, L.; Visser, J.; Vrakking, M. J. J.; Heeren, R. M. A. *Analytical Chemistry* **2011**, *83*, 7888–7894.
- [99] Hazama, H.; Yoshimura, H.; Aoki, J.; Nagao, H.; Toyoda, M.; Masuda, K.; Fujii, K.; Tashima, T.; Naito, Y.; Awazu, K. *J. Biomed. Opt.* **2011**, *16*, 046007/1–046007/7.
- [100] Aoki, J.; Toyoda, M. *J. Mass Spectrom. Soc. Jpn* **2013**, *61*, 23–33.
- [101] Ishihara, M.; Toyoda, M.; Matsuo, T. *Int. J. Mass Spectrom* **2000**, *197*, 179–189.
- [102] Hazama, H.; Aoki, J.; Nagao, H.; Suzuki, R.; Tashima, T.; Fujii, K.-i.; Masuda, K.; Awazu, K.; Toyoda, M.; Naito, Y. *Appl. Surf. Sci.* **2008**, *255*, 1257–1263.
- [103] Stoeckli, M.; Farmer, T. B.; Caprioli, R. M. *Journal of the American Society for Mass Spectrometry* **1999**, *10*, 67–71.
- [104] Stoeckli, M.; Chaurand, P.; Hallahan, D. E.; Caprioli, R. M. *Nature Medicine* **2001**, *7*, 493–496.
- [105] Seeley, E. H.; Caprioli, R. M. *Proceedings of the National Academy of Sciences of the United States of America, Early Edition* **2008**, 1–6.
- [106] Li, Y.; Shrestha, B.; Vertes, A. *Analytical Chemistry* **2007**, *79*, 523–532.
- [107] Jurchen, J. C.; Rubakhin, S. S.; Sweedler, J. V. *Journal of the American Society for Mass Spectrometry* **2005**, *16*, 1654–1659.

- [108] Zavalin, A.; Todd, E. M.; Rawhouser, P. D.; Yang, J.; Norris, J. L.; Caprioli, R. M. *Journal of Mass Spectrometry* **2012**, *47*, 1473–1481.
- [109] Caprioli, R. M.; Farmer, T. B.; Gile, J. *Anal. Chem.* **1997**, *69*, 4751–4760.
- [110] *Database*; <https://scifinder.cas.org/>.
- [111] Chughtai, K.; Heeren, R. M. A. *Chemical Reviews* **2010**, *110*, 3237–3277.
- [112] Khatib-Shahidi, S.; Andersson, M.; Herman, J. L.; Gillespie, T. A.; Caprioli, R. M. *Analytical Chemistry* **2006**, *78*, 6448–6456.
- [113] Vallance, C.; Brouard, M.; Lauer, A.; Slater, C. S.; Halford, E.; Winter, B.; King, S. J.; Lee, J. W. L.; Pooley, D. E.; Sedgwick, I.; Turchetta, R.; Nomerotski, A.; John, J. J.; Hill, L. *Physical Chemistry Chemical Physics* **2014**, *16*, 383–395.
- [114] Jungmann, J. H.; Heeren, R. M. A. *Rapid Communications in Mass Spectrometry* **2013**, *27*, 1–23.
- [115] Lampton, M.; Siegmund, O.; Raffanti, R. *Review of Scientific Instruments* **1987**, *58*, 2298–305.
- [116] Eland, J. H. D.; Pearson, A. H. *Measurement Science and Technology* **1990**, *1*, 36–40.
- [117] Eland, J. H. D. *Measurement Science & Technology* **1994**, *5*, 1501–1504.
- [118] Jagutzki, O.; Cerezo, A.; Czasch, A.; Dorner, R.; Hattas, M.; Huang, M.; Mergel, V.; Spillmann, U.; Ullmann-Pfleger, K.; Weber, T.; Schmidt-Bocking, H.; Smith, G. *Nuclear Science, IEEE Transactions on* **2002**, *49*, 2477–2483.
- [119] Kirchmann, P. S.; Rettig, L.; Nandi, D.; Lipowski, U.; Wolf, M.; Bovensiepen, U. *Applied Physics A: Materials Science & Processing* **2008**, *91*, 211–217.
- [120] Da Costa, G.; Vurpillot, F.; Bostel, A.; Bouet, M.; Deconihout, B. *Review of Scientific Instruments* **2005**, *76*, 013304/1–013304/8.

- [121] Wang, C.; Wang, B.; Okunishi, M.; Roeterdink, W.; Ding, D.; Zhu, R.; PrÃEmper, G.; Shimada, K.; Ueda, K. *Chemical Physics* **2014**, *430*, 40–46.
- [122] Lehmann, C. S.; Ram, N. B.; Janssen, M. H. M. *Review of Scientific Instruments* **2012**, *83*, 093103/1–093103/4.
- [123] Yoshimura, H.; Hazama, H.; Aoki, J.; Toyoda, M.; Naito, Y.; Awazu, K. *Japanese Journal of Applied Physics* **2011**, *50*, 056701/1–056701/7.
- [124] Klerk, L. A.; Lockyer, N. P.; Kharchenko, A.; MacAleese, L.; Dankers, P. Y. W.; Vickerman, J. C.; Heeren, R. M. A. *Analytical Chemistry* **2010**, *82*, 801–807.
- [125] Leo, W. R. *Techniques for Nuclear and Particle Physics Experiments*; Springer-Verlag, 1994; Vol. 2nd Edition.
- [126] Boyle, W. S.; Smith, G. E. *Bell Syst. Tech. J.* **1970**, *49*, 587–593.
- [127] Amelio, G. F.; Tompsett, M. F.; Smith, G. E. *Bell Syst. Tech. J.* **1970**, *49*, 593–600.
- [128] Kosonocky, W. F.; Yang, G.; Kabra, R.; Ye, C.; Pektas, Z.; Lowrance, J.; Mastrocola, V.; Shallcross, F.; Patel, V. *Electron Devices, IEEE Transactions on* **1997**, *44*, 1617–1624.
- [129] Dao, V. T. S.; Etoh, T. G.; Tanaka, M.; Nguyen, H. D.; Cuong, V. L.; Takehara, K.; Akino, T.; Nishi, K.; Aoki, H.; Nakai, J. *Sensors* **2010**, *10*, 16–35.
- [130] Etoh, G. T.; Poggemann, D.; Kreider, G.; Mutoh, H.; Theuwissen, A. J. P.; Ruckelshausen, A.; Kondo, Y.; Maruno, H.; Takubo, K.; Soya, H.; Takehara, K.; Okinaka, T.; Takano, Y. *Electron Devices, IEEE Transactions on* **2003**, *50*, 144–151.
- [131] Brouard, M.; Campbell, E. K.; Johnsen, A. J.; Vallance, C.; Yuen, W. H.; Nomerotski, A. *Review of Scientific Instruments* **2008**, *79*, 123115/1–123115/8.
- [132] Brouard, M.; Johnsen, A. J.; Nomerotski, A.; Slater, C. S.; Vallance, C.; Yuen, W. H. *Journal of Instrumentation* **2011**, *6*, C01044.

- [133] Llopart, X.; Ballabriga, R.; Campbell, M.; Tlustos, L.; Wong, W. *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment* **2007**, *581*, 485–494.
- [134] Nomerotski, A.; Adigun-Boaye, S.; Brouard, M.; Campbell, E.; Clark, A.; Crooks, J.; John, J. J.; Johnsen, A. J.; Slater, C.; Turchetta, R.; Vallance, C.; Wilman, E.; Yuen, W. H. *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment* **2011**, *633*, 243–246.
- [135] Gademann, G.; Huismans, Y.; Gijsbertsen, A.; Jungmann, J.; Visschers, J.; Vrakking, M. J. J. *Review of Scientific Instruments* **2009**, *80*, 103105/1–103105/7.
- [136] Jungmann, J. H.; Gijsbertsen, A.; Visser, J.; Visschers, J.; Heeren, R. M. A.; Vrakking, M. J. J. *Review of Scientific Instruments* **2010**, *81*, 103112/1–103112/7.
- [137] Jungmann, J. H.; Smith, D. F.; MacAleese, L.; Klinkert, I.; Visser, J.; Heeren, R. M. A. *Journal of the American Society for Mass Spectrometry* **2012**, *23*, 1679–1688.
- [138] Kiss, A.; Jungmann, J. H.; Smith, D. F.; Heeren, R. M. A. *Review of Scientific Instruments* **2013**, *84*, 013704/1–013704/7.
- [139] John, J. J. et al. *JINST* **2012**, *7*, C08001.
- [140] Clark, A. T. et al. *Journal of Physical Chemistry A* **2012**, *116*, 10897–10903.
- [141] *SIMION 8.0*; <http://simion.com/>.
- [142] Beavis, R. C.; Chait, B. T. *Chemical Physics Letters* **1991**, *181*, 479–484.
- [143] Boekelmann, V.; Spengler, B.; Kaufmann, R. *European Mass Spectrometry* **1995**, *1*, 81–93.
- [144] Ens, W.; Mao, Y.; Mayer, F.; Standing, K. G. *Rapid Communications in Mass Spectrometry* **1991**, *5*, 117–123.

- [145] Kaufmann, R.; Spengler, B.; Luetzenkirchen, F. *Rapid Communications in Mass Spectrometry* **1993**, *7*, 902–910.
- [146] Zhang, W.; Chait, B. T. *International Journal of Mass Spectrometry and Ion Processes* **1997**, *160*, 259–267.
- [147] Halford, E. Fast Detectors and Spatial Imaging Mass Spectrometry. M.Sc. thesis, 2011.
- [148] Lee, J. W. *PhD Thesis* **2014**,
- [149] Halford, E. *PhD Thesis* **2014**,
- [150] Brouard, M.; Johnsen, A. J.; Nomerotski, A.; Slater, C. S.; Yuen, W. H.; Vallance, C. *JINST* **2011**, *6*, C01044.
- [151] Yuen, W. H. Ion Imaging Mass Spectrometry, PhD Thesis. 2012.
- [152] Wang, L.; May, S. W.; Browner, R. F. *Journal of Analytical Atomic Spectrometry* **1996**, *11*, 1137–1146.
- [153] Perez, J.; Petzold, C. J.; Watkins, M. A.; Vaughn, W. E.; Kenttamaa, H. I. *Journal of the American Society for Mass Spectrometry* **1999**, *10*, 1105–1110.
- [154] Hensel, R. R.; King, R. C.; Owens, K. G. *Rapid Communications in Mass Spectrometry* **1997**, *11*, 1785–1793.
- [155] Axelsson, J.; Hoberg, A.-M.; Waterson, C.; Myatt, P.; Shield, G. L.; Varney, J.; Haddleton, D. M.; Derrick, P. J. *Rapid Communications in Mass Spectrometry* **1997**, *11*, 209–213.
- [156] Altelaar, A. F. M.; Van, J.; Minnen; Jimenez, C. R.; Heeren, R. M. A.; Piersma, S. R. *Analytical Chemistry* **2005**, *77*, 735–741.
- [157] Colliver, T. L.; Brummel, C. L.; Pacholski, M. L.; Swanek, F. D.; Ewing, A. G.; Winograd, N. *Anal. Chem.* **1997**, *69*, 2225–2231.

- [158] Aoki, J.; Hazama, H.; Toyoda, M. *J. Mass Spectrom. Soc. Jpn.* **2011**, *59*, 57–61.
- [159] Slater, C. S. Studies of Photoinduced Molecular Dynamics Using a Fast Imaging Sensor, PhD Thesis. 2014.
- [160] Liebl, H. *Applied Charged Particle Optics*; Springer, 2008.
- [161] Griffiths, W. J.; Jonsson, A. P.; Liu, S.; Rai, D. K.; Wang, Y. *Biochemical Journal* **2001**, *355*, 545–561.
- [162] Christian, N. P.; Arnold, R. J.; Reilly, J. P. *Analytical Chemistry* **2000**, *72*, 3327–3337.
- [163] *Data sheet, SAI LT2 PLUS, Scientific Analysis Instruments Ltd*;
<http://www.saiman.co.uk>.
- [164] Crookes, W. *Chemical News* **1903**, *87*, 241.
- [165] Knoll, G. F. *Radiation Detection and Measurement*; John Wiley & Sons, Inc, 2010; Vol. 4th Edition.
- [166] Yen, W. M.; Weber, M. J. *Inorganic Phosphors - Composition, preparation and optical properties*; CRC Press, 2004; Vol. 1.
- [167] Craun, R. L.; Smith, D. L. *Nuclear Instruments & Methods* **1970**, *80*, 239–244.
- [168] *Photonis BiPolar TOF datasheet*; [http: www.photonis.com](http://www.photonis.com), 2011.
- [169] *Data sheet, Phosphor Technology LTD, UK*; <http://www.phosphor-technology.com>.
- [170] Kingslake, R.; Thompson, B. J. *Applied optics and optical engineering*; Academic Press, London, 1980; Vol. 6nd Edition.
- [171] McGee, J. D. *Photo-electronic Image devices*; Academic Press, London, 1966; Vol. 22A.

- [172] Sangster, R. C.; Irvine, J. W. *Journal of Chemical Physics* **1956**, *24*, 670–715.
- [173] Poater, J.; Visser, R.; Sola, M.; Bickelhaupt, F. M. *Journal of Organic Chemistry* **2007**, *72*, 1134–1142.
- [174] Birks, J. B.; Georghiou, S. *Journal of Physics, B., Proceedings of the Physical Society, Atomic and Molecular Physics* **1968**, [2] *1*, 958–965.
- [175] Liu, L.-I. Time-resolved fluorescence studies of dye polymers as excited by laser and beta radiation, PhD Thesis. 1997.
- [176] Liang, Y.; Goncalves, A. M. P.; Negus, D. K. *Journal of Physical Chemistry* **1983**, *87*, 1–4.
- [177] Dubois, F.; Knochenmuss, R.; Zenobi, R. *International Journal of Mass Spectrometry and Ion Processes* **1997**, *169/170*, 89–98.
- [178] Richards, P. I.; Hays, E. E. *Review of Scientific Instruments* **1950**, *21*, 99–100.
- [179] Brackmann, U. *Lambdachrome Laser Dyes*, 3rd ed.; Lambda Physik AG, 2000.
- [180] Dubois, F.; Knochenmuss, R.; Zenobi, R.; Brunelle, A.; Deprun, C.; Le, Y., Beyec *Rapid Communications in Mass Spectrometry* **1999**, *13*, 786–791.
- [181] Dubois, F.; Knochenmuss, R.; Zenobi, R. *Rapid Communications in Mass Spectrometry* **1999**, *13*, 1958–1967.
- [182] Skubsch, K.; Mundt, C.; Eitrich, A. *Cosmetic foams containing organic micropigments.*; DE102004047287A1, 2006.
- [183] Ziegler, L. D.; Hudson, B. S. *Optics Communications* **1980**, *32*, 119–120.
- [184] Tomin, V. I.; Alcock, A. J.; Sarjeant, W. J.; Leopold, K. E. *Optics Communications* **1978**, *26*, 396–400.
- [185] Godard, B.; de Witte, O. *Optics Communications* **1976**, *19*, 325–328.

- [186] McKee, T.; Stoicheff, B. P.; Wallace, S. C. *Optics letters* **1978**, *3*, 207–208.
- [187] Merz, T. A.; Waddell, P. G.; Cole, J. M. *Journal of Physical Chemistry C* **2013**, *117*, 8429–8436.
- [188] Kauffman, J. M.; Kelley, C. J.; Ghiorghis, A.; Neister, E.; Armstrong, L.; Prause, P. R. *Laser Chemistry* **1987**, *7*, 343–351.
- [189] Kauffman, J. M.; Kelley, C. J.; Ghiorghis, A.; Neister, E.; Armstrong, L. *Laser Chemistry* **1988**, *8*, 335–348.
- [190] Fleitz, P. A.; Seliskar, C. J.; Steppel, R. N.; Kauffman, J. M.; Kelly, C. J.; Ghiorghis, A. *Laser Chemistry* **1991**, *11*, 99–107.
- [191] Barnett, M. D.; Daub, G. H.; Hayes, F. N.; Ott, D. G. *Journal of the American Chemical Society* **1959**, *81*, 4583–4586.
- [192] Rinke, M.; Guesten, H.; Ache, H. J. *Journal of Physical Chemistry* **1986**, *90*, 2666–2670.
- [193] Schneider, D. J.; Landis, D. A.; Fleitz, P. A.; Seliskar, C. J.; Kauffman, J. M.; Steppel, R. N. *Laser Chemistry* **1991**, *11*, 49–62.
- [194] Seliskar, C. J.; Landis, D. A.; Kauffman, J. M.; Aziz, M. A.; Steppel, R. N.; Kelley, C. J.; Qin, Y.; Ghiorghis, A. *Laser Chemistry* **1993**, *13*, 19–28.
- [195] Tymianski, J. R.; Walker, J. K. *Organic scintillator systems and optical fibers containing polycyclic aromatic compounds*; US5606638A, 1997.
- [196] Inamdar, S. R.; Mannekutla, J. R.; Mulimani, B. G.; Savadatti, M. I. *Chemical Physics Letters* **2006**, *429*, 141–146.
- [197] *Laser dye data sheet, Radiant Dyes Laser & Accessories GmbH*; <http://www.radiant-dyes.com>.
- [198] *Laser dye data sheet, Sirah Laser- und Plasmatechnik GmbH*; <http://www.sirah.com>.

- [199] Kung, A. H.; Young, J. F.; Harris, S. E. *Applied Physics Letters* **1973**, *22*, 301–302.
- [200] Duarte, F. J.; Hillmann, L. *Dye laser principles*; Academic Press Limited, London, 1990.
- [201] Tang, Y.; Lee, W.-B.; Hu, Z.; Zhang, B.; Lin, K.-C. *Journal of Chemical Physics* **2007**, *126*, 064302/1–064302/8.
- [202] Moore, J., John H.; Robinson, D. W. *Journal of Chemical Physics* **1968**, *48*, 4870–4874.
- [203] Brouard, M.; Green, A. V.; Quadrini, F.; Vallance, C. *Journal of Chemical Physics* **2007**, *127*, 084304/1–084304/15.
- [204] Sato, Y.; Matsumi, Y.; Kawasaki, M.; Tsukiyama, K.; Bersohn, R. *Journal of Physical Chemistry* **1995**, *99*, 16307–16314.
- [205] Koppenaal, D. W.; Barinaga, C. J.; Denton, M. B.; Sperline, R. P.; Hieftje, G. M.; Schilling, G. D.; Andrade, F. J.; Barnes, I., James H. *Analytical Chemistry* **2005**, *77*, 418A–427A.
- [206] Downard, K. *Mass Spectrometry - A Foundation Course*; The Royal Society of Chemistry, 2004.
- [207] Harasimowicz, J.; Welsch, C. P. In *Faraday Cup for Low-energy, Low-intensity Beam Measurements at the USR.*; JACOW,, Ed.; Proceedings of Beam Instrumentation Workshop, 2010; pp 5–8.
- [208] Pruitt, J. *Nuclear Instruments and Methods* **1966**, *39*, 329–334.
- [209] Knight, A. K.; Sperline, R. P.; Hieftje, G. M.; Young, E.; Barinaga, C. J.; Koppenaal, D. W.; Denton, M. B. *International Journal of Mass Spectrometry* **2002**, *215*, 131–139.

- [210] Lorincik, J.; Denton, M. B.; Sperline, R. P.; Young, E. T.; Williams, P. *Analytical Letters* **2011**, *44*, 1050–1057.
- [211] Wiza, J. L. *Nuclear Instruments & Methods* **1979**, *162*, 587–601.
- [212] Fraser, G. W. *International Journal of Mass Spectrometry* **2002**, *215*, 13–30.
- [213] Krems, M.; Zirbel, J.; Thomason, M.; DuBois, R. D. *Review of Scientific Instruments* **2005**, *76*, 093305/1–093305/7.
- [214] Hosokawa, S.; Takahashi, N.; Saito, M.; Haruyama, Y. *Review of Scientific Instruments* **2010**, *81*, 063301/1–063301/4.
- [215] Takahashi, N.; Hosokawa, S.; Saito, M.; Haruyama, Y. *Physica Scripta, T* **2011**, *T144*, 014057/1–014057/3.
- [216] Gilmore, I. S.; Seah, M. P. *International Journal of Mass Spectrometry* **2000**, *202*, 217–229.
- [217] Yagi, S.; Nagata, T.; Koide, M.; Itoh, Y.; Koizumi, T.; Azuma, Y. *Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* **2001**, *183*, 476–486.
- [218] Gao, R. S.; Gibner, P. S.; Newman, J. H.; Smith, K. A.; Stebbings, R. F. *Review of Scientific Instruments* **1984**, *55*, 1756–1759.
- [219] Muller, A.; Djuric, N.; Dunn, G. H.; Belic, D. S. *Review of Scientific Instruments* **1986**, *57*, 349–353.
- [220] Sakurai, T.; Hashizume, T. *Review of Scientific Instruments* **1986**, *57*, 236–239.
- [221] Oberheide, J.; Wilhelms, P.; Zimmer, M. *Measurement Science & Technology* **1997**, *8*, 351–354.
- [222] Peko, B. L.; Stephen, T. M. *Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* **2000**, *171*, 597–604.

- [223] Frank, M. *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment* **2000**, *444*, 375–384.
- [224] Brunelle, A.; Chaurand, P.; Della-Negra, S.; Le, Y.; Beyec; Parilis, E. *Rapid Communications in Mass Spectrometry* **1997**, *11*, 353–362.
- [225] Westmacott, G.; Ens, W.; Standing, K. G. *Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* **1996**, *108*, 282–289.
- [226] Beuhler, R. J.; Friedman, L. *Nuclear Instruments & Methods* **1980**, *170*, 309–315.
- [227] Wurz, P.; Gubler, L. *Review of Scientific Instruments* **1994**, *65*, 871–876.
- [228] Girard, J.; Bolore, M. *Nuclear Instruments & Methods* **1977**, *140*, 279–282.
- [229] Hering, E.; Bressler, K.; Gutekunst, J. *Elektronik für Ingenieure und Naturwissenschaftler*; Springer Berlin Heidelberg, 2005; Vol. 5.
- [230] Stipcevic, M.; Skenderovic, H.; Gracin, D. *Optics Express* **2010**, *18*, 17448–17459.
- [231] Cova, S.; Lacaita, A.; Ghioni, M.; Ripamonti, G.; Louis, T. A. *Review of Scientific Instruments* **1989**, *60*, 1104–1110.
- [232] Cova, S.; Ghioni, M.; Lacaita, A.; Samori, C.; Zappa, F. *Applied Optics* **1996**, *35*, 1956–1976.
- [233] Dautet, H.; Deschamps, P.; Dion, B.; MacGregor, A. D.; MacSween, D.; McIntyre, R. J.; Trottier, C.; Webb, P. P. *Proceedings of SPIE-The International Society for Optical Engineering* **1993**, *1885*, 240–250.
- [234] Valais, I. G.; Michail, C. M.; Stratos, L. D.; Konstantinidis, A.; Cavouras, D. A.; Kandarakis, I. S.; Panayiotakis, G. S. **2008**, *55*, 785–789.

- [235] Britvitch, I.; Johnson, I.; Renker, D.; Stoykov, A.; Lorenz, E. *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment* **2007**, *571*, 308–311.
- [236] Wilman, E. S.; Gardiner, S. H.; Nomerotski, A.; Turchetta, R.; Brouard, M.; Vallance, C. *Review of Scientific Instruments* **2012**, *83*, 013304/1–013304/4.
- [237] Szawlowski, M.; Meier, D.; Mahlum, G.; Wagenaar, D. J.; Patt, B. E. Spectroscopy and Timing with Multi-Pixel Photon Counter (MPPC) and LYSO Scintillators. 2007.
- [238] Spanoudaki, V. C.; Levin, C. S. *Sensors* **2010**, *10*, 10484–10505.
- [239] Casey, M. E. *Improving PET With HD-PET + Time of Flight*; 2008.
- [240] Moszynski, M.; Kapusta, M.; Nassalski, A.; Szeziesniak, T.; Wolski, D.; Eriksson, L.; Melcher, C. L. **2006**, *53*, 2484–2488.
- [241] Moses, W. W. *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment* **2007**, *580*, 919–924.
- [242] Grodzicka, M.; Moszynski, M.; Szczesniak, T.; Szawlowski, M.; Wolski, D.; Baszak, J. **2012**, *59*, 3294–3303.
- [243] Szczesniak, T.; Kapusta, M.; Moszynski, M.; Grodzick, M.; Szawlowski, M.; Wolski, D.; Baszak, J.; Zhang, N. **2013**, *60*, 1533–1540.
- [244] Harper, S. M.; Hu, W.-P.; Price, S. D. *Journal of Physics B: Atomic, Molecular and Optical Physics* **2002**, *35*, 4409–4423.

Appendix A

Ion Image Analysis for Velocity Corrected Ion Extraction

Exalite 398 In the mass spectrum of exalite 398 sample a number of ToF peaks were observed and identified using the previously determined calibration functions, as well as a comparison mass spectrum taken with a commercial MALDI spectrometer. For the spatial analysis three peaks representing the ion masses 530.07 Da, 682.31 Da and 866.69 Da were chosen to be imaged.

The first mass peak at 530.07 Da (see Figure 7.2) is expected to show the best spatial resolution using the settings optimised for 480 Da and 580 Da. Experimentally, the qualitatively best ion image was found using the third mass range settings. A spatial resolution of $25 \pm 4 \mu\text{m}$ was determined. The worst spatial resolution was found using the 390 Da ion optic settings with $40 \pm 8 \mu\text{m}$. For the second and fourth mass range settings, spatial resolutions of $31 \pm 7 \mu\text{m}$ and $27 \pm 4 \mu\text{m}$ were determined. The magnification factor was found to decrease towards the higher mass optimised settings from $10.0\times$ to $9.5\times$.

The ion images collected of the 682.31 Da ion peak were expected to show the best spatial resolution using the fourth mass range settings. The spatial images presented in Figure 7.3 show the sharpest grid structures with a resolution of $\sim 20 \mu\text{m}$ using the ion optic settings optimised for 580 Da and 680 Da. The first mass range showed a quite blurry grid structure for which the spatial resolution was estimated to be $54 \pm 7 \mu\text{m}$. In the second

mass range, a clearer grid structure was observed resulting in a resolution of $28 \pm 7 \mu\text{m}$. From the first to the third mass range the magnification increased from $8.7\times$ *via* $9.6\times$ to $9.9\times$. The 680 Da settings did not show a further increase in the magnification.

The first mass range image of the 866.69 Da peak barely shows a grid structure (Figure 7.4). Thus, a low spatial resolution with $76 \pm 29 \text{ Da}$ was determined. However, the applied rising edge fitting method is not ideally suited for this image, as no clearly resolved gap between the square sections was observed. In agreement to the ion trajectory simulations, the spatial resolution increased by employing the higher mass optimised ion optic settings to reach its optimum value at the 680 Da settings with $21 \pm 4 \mu\text{m}$. Even better resolutions may be determined if the sample peak is imaged using a higher velocity corrected mass range setting. The magnification factor increased continuously from $7.8\times$ the first mass range to $9.9\times$ the last one.

Rhodamine 6G Rhodamine dyes were chosen to probe the lower mass range ion optic settings. The first dye to be imaged was rhodamine 6G with a parent ion mass of 429.59 Da. This mass is located between the optimised settings of the first and the second mass ranges. By comparing the spatial resolutions obtained in Figure 7.5, it can be noted that by employing the 390 Da ion optic settings a spatial resolution of $22 \pm 3 \mu\text{m}$ was found, while for the 480 Da settings a reduced resolution of $28 \pm 6 \mu\text{m}$ was determined. By employing the 580 Da and 680 Da optimised ion optic settings, the obtained resolution was further reduced. The magnification of the ion images showed a slightly decreasing trend from $9.8\times$ for the lower, to $9.4\times$ for the higher ion mass settings.

Rhodamine 101 The rhodamine 101 sample was used to image the 447.59 Da mass peak. Here, the optimum spatial resolution was expected to be obtained by employing the second mass range ion optic settings. Figure 7.6 confirms this assumption: a spatial resolution of $25 \pm 5 \mu\text{m}$ was determined for the 480 Da ion optic settings. By employing the other ion optic settings, the spatial resolution was found to deteriorate. The observed magnification factor was of the same order as the one determined for the rhodamine 6G

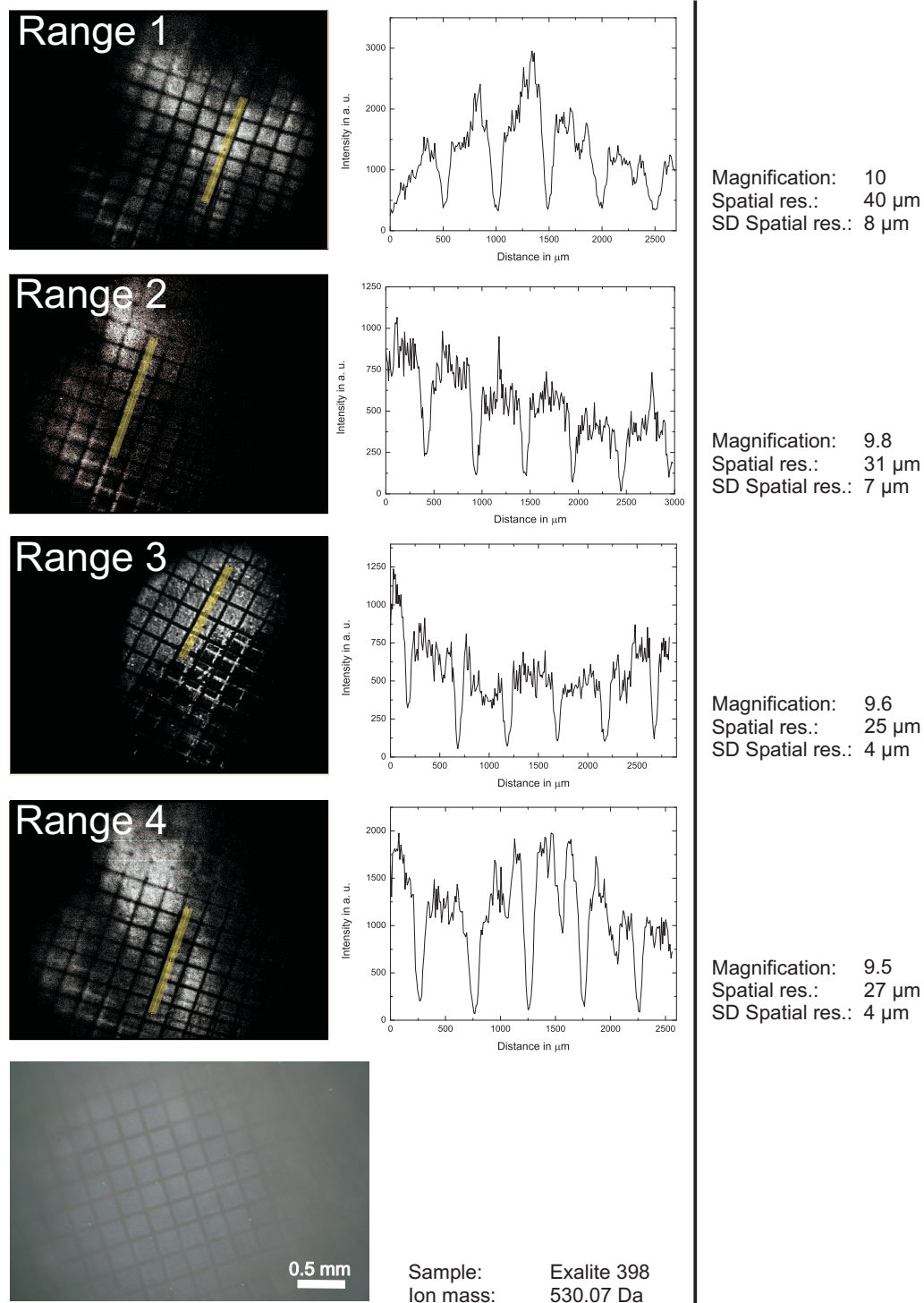


Figure 7.2: Ion images of the exalite 398, 530.07 Da mass peak collected with each of the four ion optic settings applied are shown in the left section of the figure. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

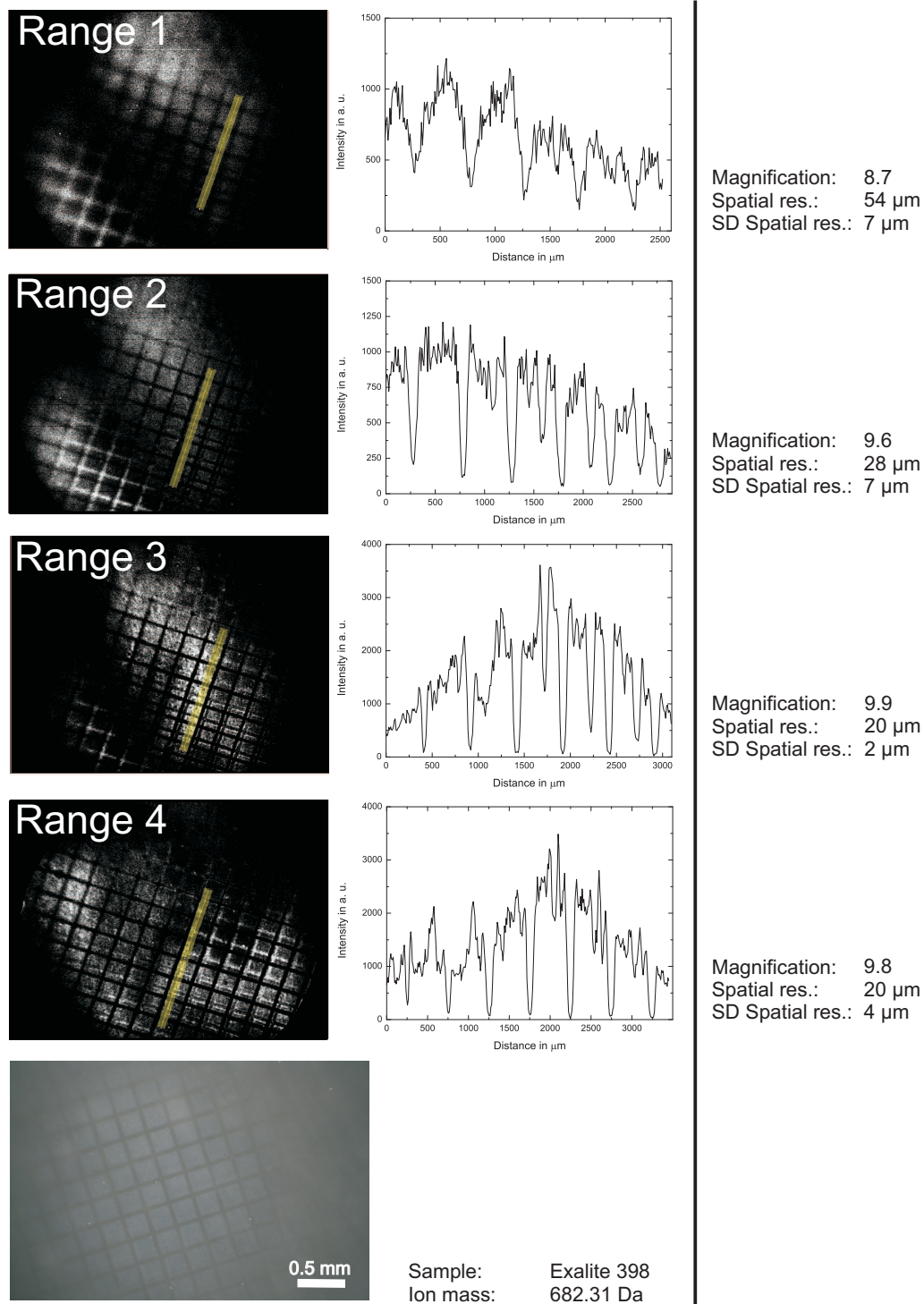


Figure 7.3: Ion images of the exalite 398, 682.31 Da mass peak collected with each of the four ion optic settings applied are shown in the left section of the figure. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

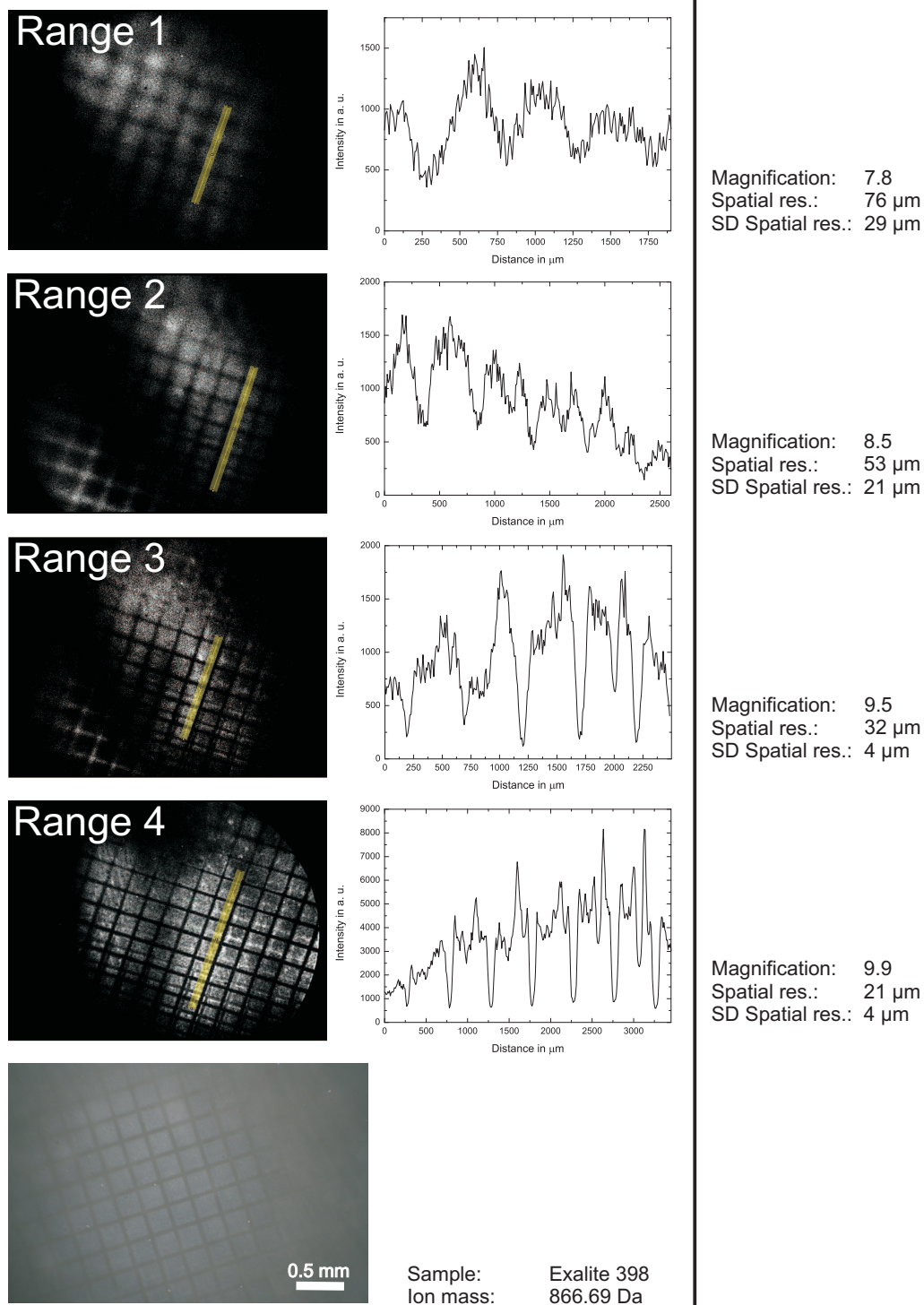


Figure 7.4: Ion images of the exalite 398, 866.69 Da mass peak collected with each of the four ion optic settings applied are shown in the left section of the figure. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

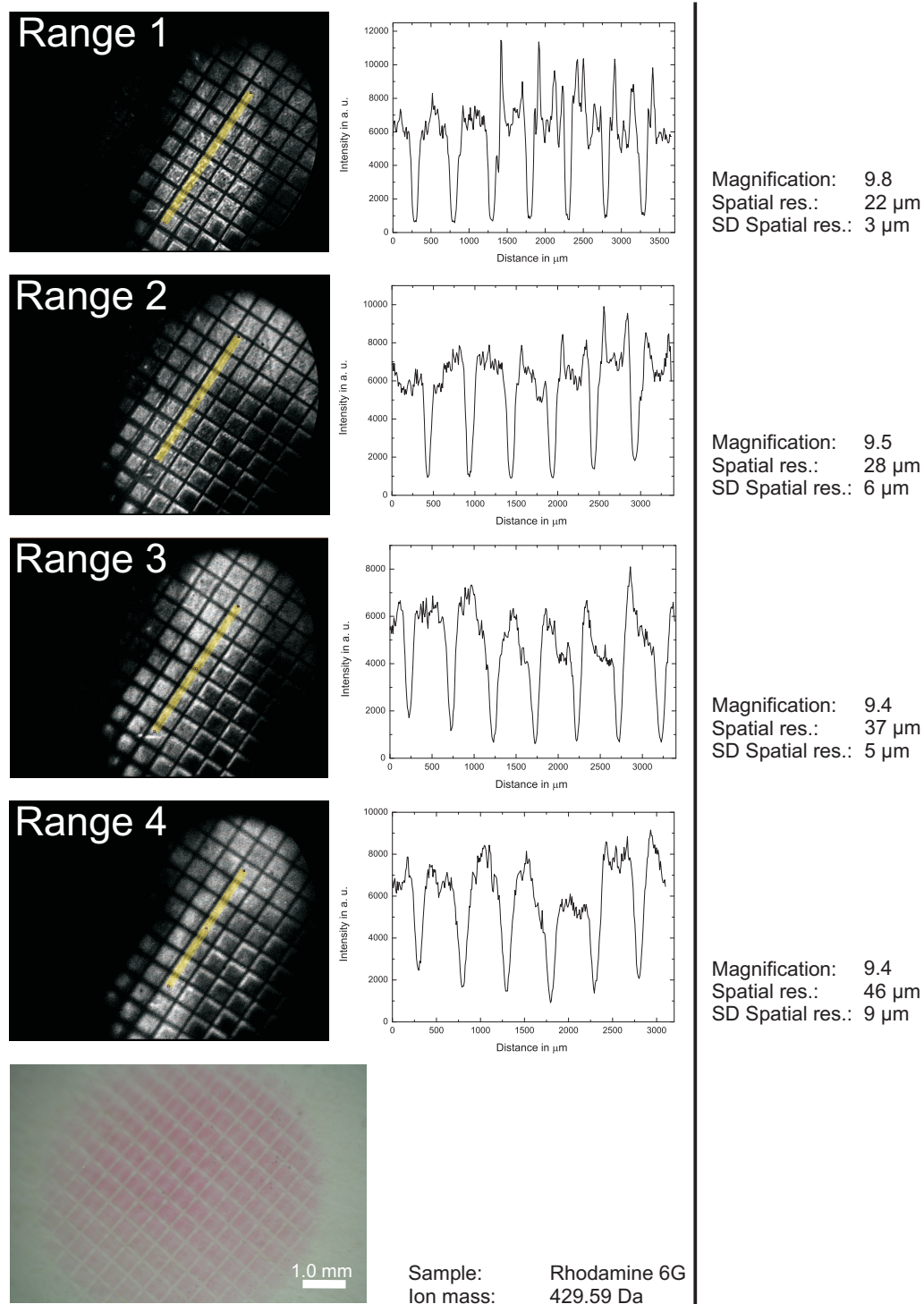


Figure 7.5: Ion images of the rhodamine 6G, 429.59 Da mass peak collected with each of the four ion optic settings applied are shown in the left section of the figure. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

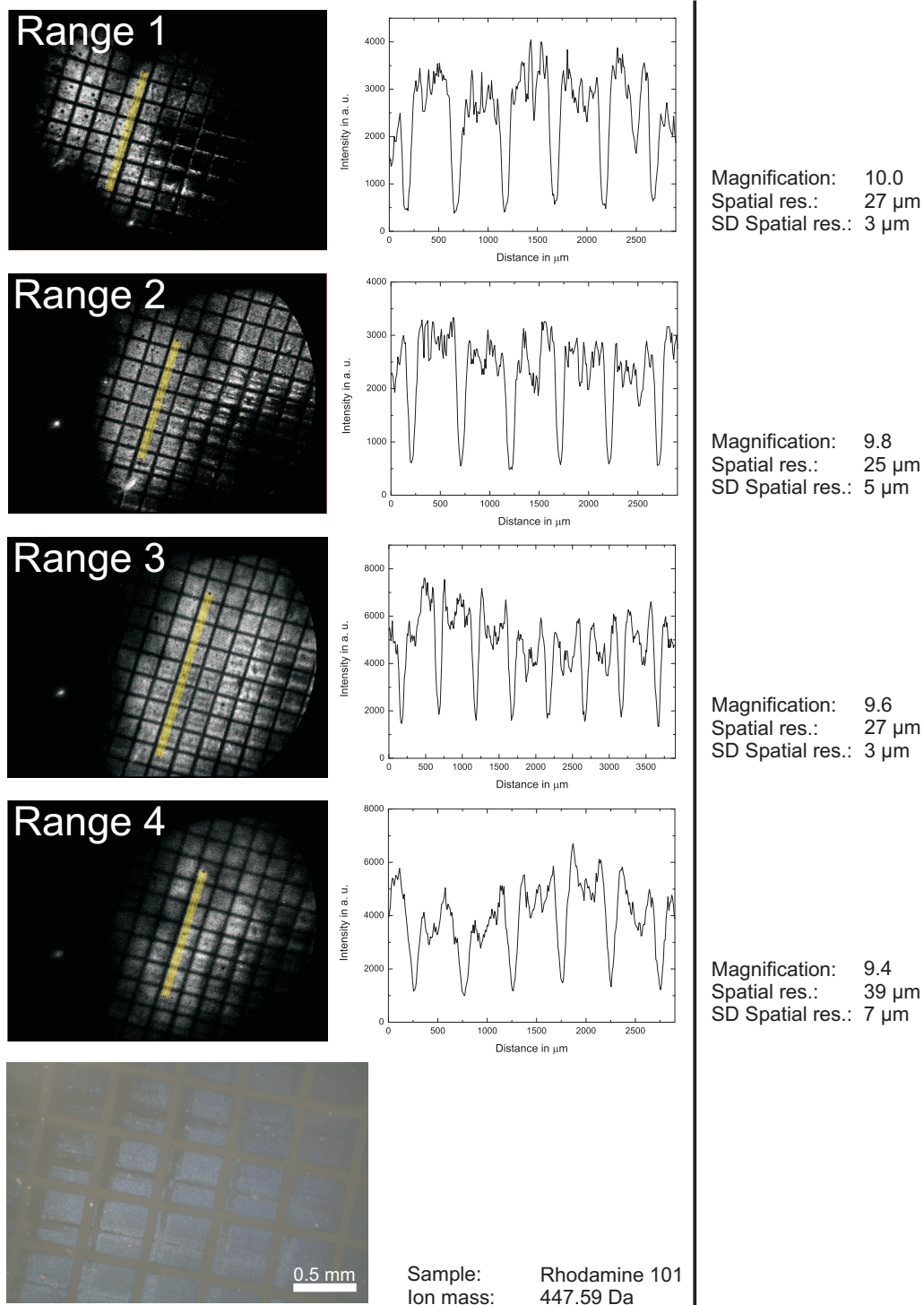


Figure 7.6: Ion images of the rhodamine 101, 447.59 Da mass peak collected with each of the four ion optic settings applied are shown in the left section of the figure. The corresponding intensity plots (width 20 pixels) are presented in the middle section, and the final results are listed on the right for each of the mass ranges applied. Furthermore, a microscope photo of the employed grid sample is illustrated in the bottom left part of the figure.

sample.

Appendix B

Scintillator Emission Analysis

QM58

The further scintillator tested was a sedimented QM58 sample. For the collection of the ToF spectra, the MCPs were set to operate at a bias of 1.3 kV and the scintillator was charged to 3.5 kV. The corresponding electron signal, acquired over 100 shots, is presented in Figure 7.7*b*. Again, two peaks can be identified in the spectrum, of which the first one can be allocated to the incident laser light and the second one corresponds to the electron signal. The exponential decay fit converged with decay constant of 39.6 ± 0.1 ns. Using the QM58 scintillator, it was possible to collect a CCD ion image of the parent ion peak of the methyl iodide sample (Figure 7.8). No image was taken using the PImMS camera.

GK31

The GK31 scintillator screen was expected to show the shortest fluorescence lifetime of the inorganic screens applied. Indeed, the decay constant was determined to be 3.7 ± 0.1 ns using an exponential fit (See spectrum in Figure 7.7*c*). The spectrum was taken with the same detector settings applied as for the measurements above. Additionally, the CCD camera was used to collect an ion image of the methyl iodide parent molecules. However, the signal levels obtained were weaker than the ones of the QM58 screen. Hence, it was again impossible to acquire an image with the PImMS camera.

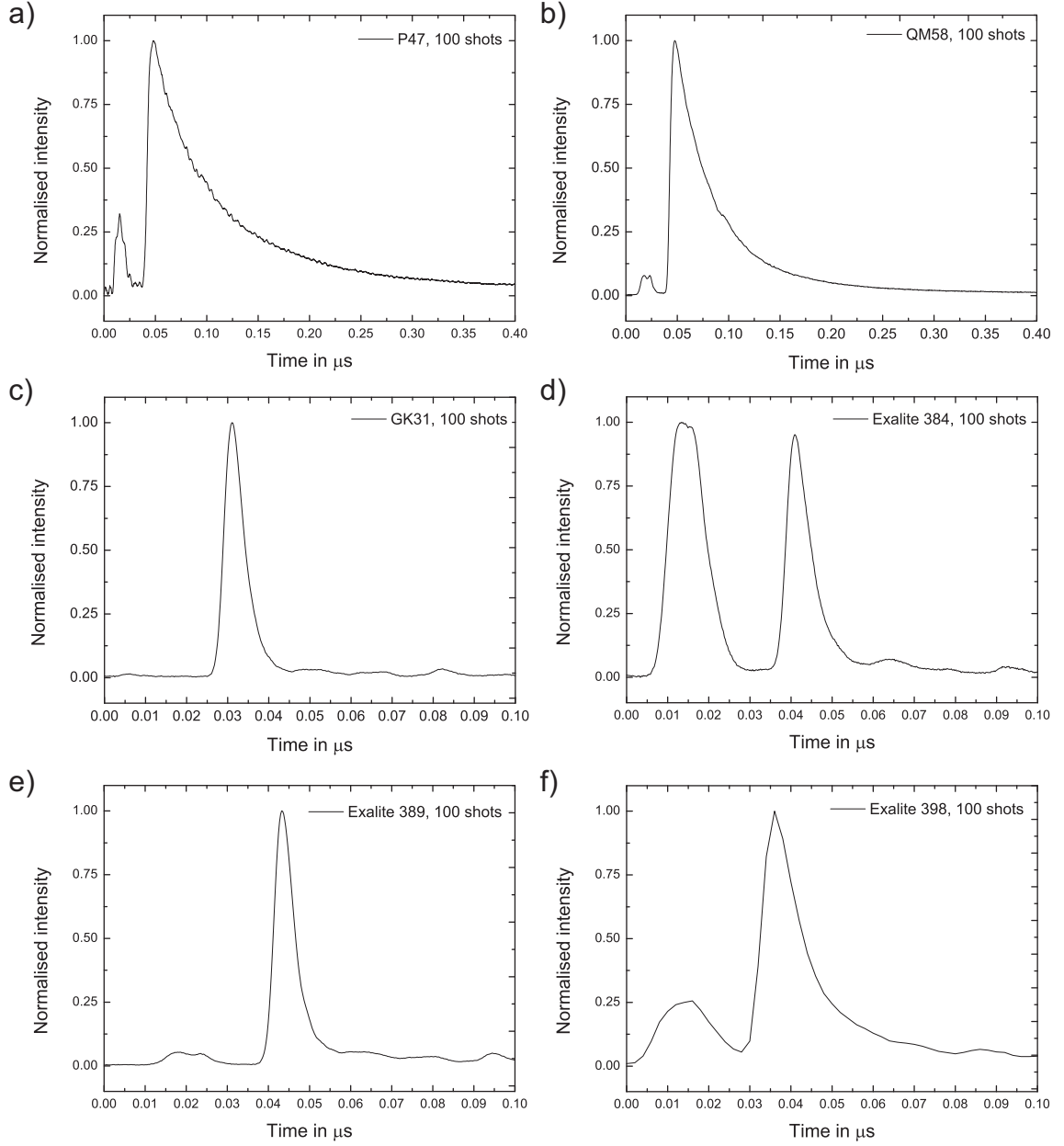


Figure 7.7: Electron ToF spectra collected employing the following scintillator screens: P47 (a), QM58 (b), GK31 (c), exalite 384 (d), exalite 389 (e), exalite 398 (f).

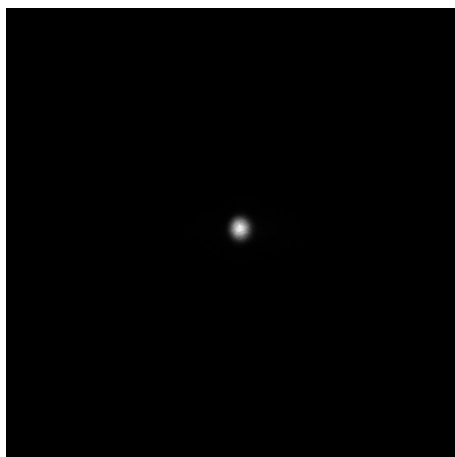


Figure 7.8: Ion image taken of the methyl iodide parent ion peak under velocity map imaging conditions employing the QM58 scintillator screen. The image was acquired over 2000 frames using a conventional, intensified CCD camera.

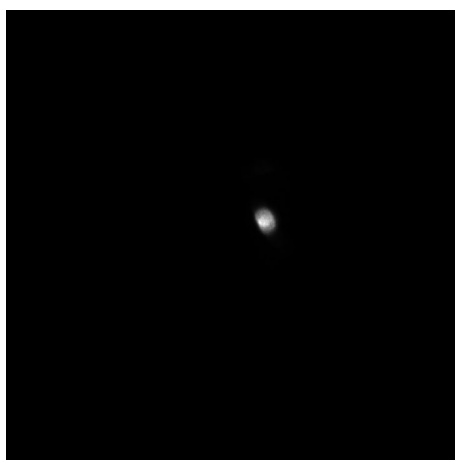


Figure 7.9: Ion image taken of the methyl iodide parent ion peak under velocity map imaging conditions using the GK31 scintillator screen. The image was acquired over 2000 frames using a conventional, intensified CCD camera.

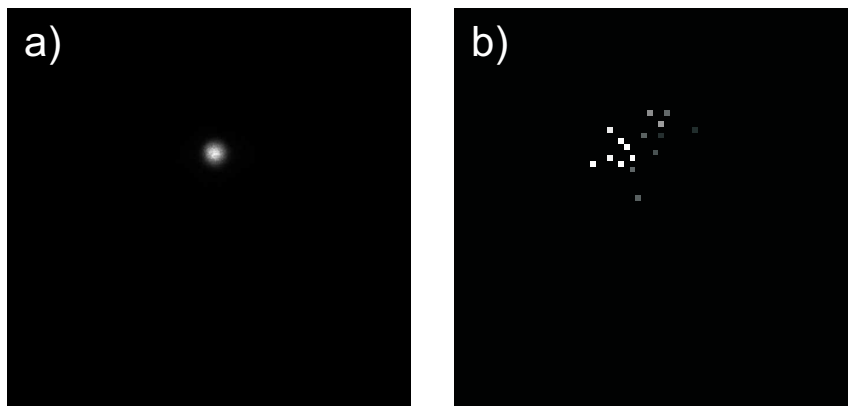


Figure 7.10: Ion images taken of the methyl iodide parent ion peak under velocity map imaging conditions using the exalite 389 scintillator screen. The image in Panel a) was acquired over 2000 frames using an intensified CCD Camera. Panel b) shows a 2000 frame image taken with the PImMS camera.

Exalite 384

A further scintillator investigated was the exalite 384 screen (Figure 7.7d). The observed fluorescence signal was surprisingly strong so that the detector could be operated with 1.1 kV on the MCPs and 2.5 kV on the scintillator screen. A decay constant of $\tau = 4.7 \pm 0.5$ ns was determined.

Exalite 389

Another really fast scintillator was the exalite 389 detector. For all the ToF and CCD camera measurements the MCPs were set to 1.0 kV and the scintillator was set to 2.5 kV. The exponential fit of the electron signal resulted in a decay constant of 3.7 ± 0.5 ns (See Figure 7.7e). The shape of the decay curve indicates that the organic scintillator emits light in a mono exponential decay.

Exalite 398

Another scintillator of the same quater-*para*-phenylene “family” is exalite 398. In contrast to exalite 389 it showed a longer fluorescence signal after electron excitation (See electrons ToF spectrum in Figure 7.7f). A decay constant of 9.8 ± 0.4 ns was determined. The presented ToF spectrum was recorded with the detector potentials set to 1.0 kV across

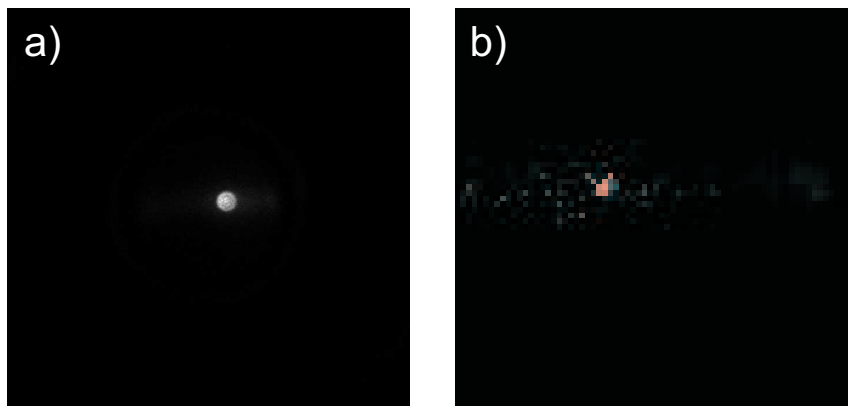


Figure 7.11: Ion images taken of the methyl iodide parent ion peak under velocity map imaging conditions using the exalite 398 scintillator screen. The image in Panel a) was acquired over 2000 frames using an intensified CCD Camera. Panel b) shows a 2000 frame image taken with the PImMS camera.

the MCPs and 2.0 kV applied to the scintillator screen. The associated ion image of methyl iodide showed a sharp molecular beam spot in the middle, with a bit of blurred background along the laser propagation direction (Figure 7.11a). This effect is also obvious in the PImMS image, in which a hot spot for the parent ions in the molecular beam is collected in the middle, while a blurred background signal is observed along the laser direction (Figure 7.11b). The PImMS image was collected with 1.6 kV across the MCP and 4.0 kV applied to the scintillator.

BBOT

For the developed BBOT based IPD detector Zenobi and Coworker determined a response time of ~ 6 ns [177, 180, 181]. However, the term “response” was not further defined in the publication discussed. Hence, it remains unclear whether the authors used the phrase to describe the rise or fall time. The decay constant determined from the electron ToF spectrum presented in Figure 7.12b was found to be $\tau = 14.5 \pm 0.5$ ns. Furthermore, the rise time from 10% to 100% of the signal was measured with ~ 7.0 ns. For the presented measurement, the detector was operated with a potential of 1.3 kV across the MCPs and 3.5 kV applied to the scintillator screen. For the BBOT and all other remaining scintillator screens no further ion imaging studies were undertaken.

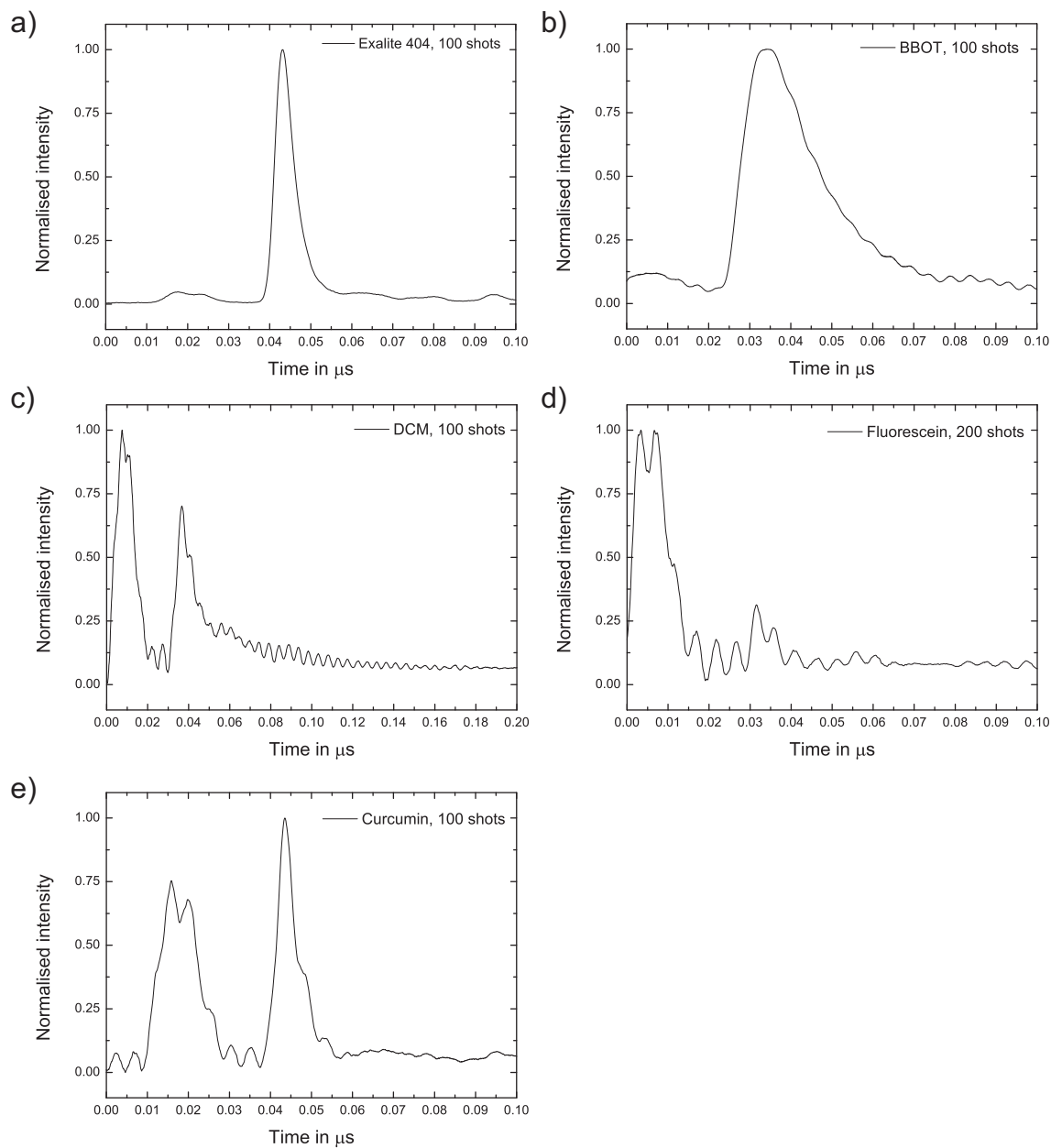


Figure 7.12: Electron ToF spectra collected employing the following scintillator screens: exalite 404 (a), BBOT (b), DCM (c), fluorescein (d), curcumin (e).

DCM

The DCM scintillator screen showed in comparison to the exalite scintillators a very dim fluorescence. Hence, to collect an electron PMT signal the detector potentials needed to be raised to 1.5 kV across the MCPs and 3.5 kV on the scintillator. The spectrum presented in Figure 7.12*c* was acquired over 100 shots and the signal was fitted by a line superposition of two exponential decays following the function

$$I = Ae^{(-t/\tau_1)} + Be^{(-t/\tau_2)}, \quad (7.1)$$

where A and B are relative amplitudes of both decay components. For the DCM scintillator the decay constants τ_1 and τ_2 were determined with 5.0 ± 0.5 ns and 37.9 ± 1.3 ns.

Fluorescein

The fluorescein sample investigated showed a very dim fluorescence signal using detector settings of 1.5 kV across the MCPs and 3.5 kV on the scintillator screen. An exponential decay fit to the obtained electron ToF spectra (200 shots) presented in Figure 7.12*d* resulted in a decay constant of ~ 5 ns. Due to the low signal levels obtained no further investigations were undertaken.

Curcumin

The last organic compound which was applied as scintillator was curcumin. For the electron ToF measurements the detector was operated at a potential of 1.35 kV across the MCPs and 3.5 kV on the scintillator screen. The electron ToF signal presented in Figure 7.12*e* indicates a very short fluorescence lifetime. Fitting an exponential decay function to the falling edge of the peak resulted in a decay constant of $\tau = 3.7 \pm 0.6$ ns. However, in comparison to the exalite screens, a far dimmer fluorescence signal was observed.