

Gas Propulsion of Microprojectiles for the Transformation of Biological Cells

by David Francis Sarphie

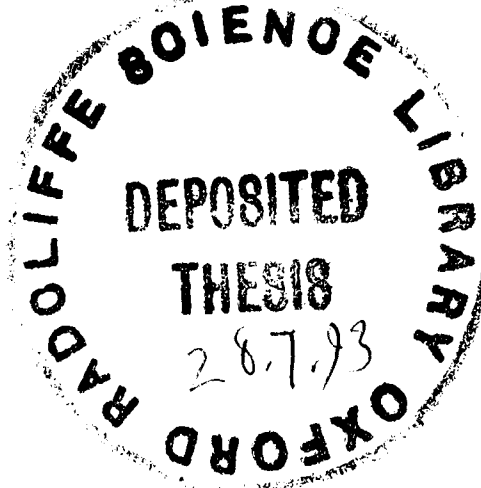
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ABSTRACT

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Bombardment of intact cells and tissue with DNA-coated microprojectiles represents a novel approach to the genetic transformation of biological material. In this thesis, a gas propulsion particle gun is developed for such a purpose. The particle gun utilises gas dynamics to control particle velocity and spread, thereby enabling optimisation of transformation efficiencies for varying types of target material. The dynamics associated with particle acceleration are shown to relate to transient, shock tube flow.

Measurements from schlieren high-speed video photographs of the shock structure of an underexpanded jet demonstrate good agreement with empirical correlations of previous researchers; pitot tube measurements of the nozzle exit Mach number are made and shown to be in good agreement with theoretical contact surface velocities.

A novel optical particle velocimeter is used to measure particle time-of-flight between axial locations in the system's target chamber, providing a consistent, distance-averaged measurement of particle velocities. Particle velocities are measured for a range of system pressure ratios and driver gases. Variation in particle velocities is seen to be similar to theoretical variation in contact surface velocities. Analytical theory is used to predict small gas-particle velocity lag. Particle velocities with helium as a driver gas are shown to be considerably higher than those with air.

High speed video recording of chalk particles exiting the nozzle is used to visualise the spatial and temporal variation in particle spread as a function of nozzle pressure ratio. This technique demonstrates that particle spread increases with increasing nozzle pressure ratio, as gas dynamics theory indicates.

Conditions for bombardment of maize suspension cells are experimentally optimised. Significant rates of transient genetic expression are achieved with both air and helium as driver gases. High levels of transient genetic expression are also found with bombardment of maize coleoptiles and the leaves of various dicot species.

Transformation efficiencies for bombardment of HL60 human leukaemia cells show dramatic increases over efficiencies seen with conventional techniques not involving cell bombardment. For other cell types the gas propulsion device described here appears to give rates of transient genetic expression similar to those reported for commercial systems using microprojectiles. Other data for the performance of such systems are too limited at present to allow comparisons of controllability and reproducibility of bombardment efficiency.

He who bends to himself a Joy
Doth the winged life destroy;
But he who kisses the Joy as it flies
Lives in Eternity's sunrise.

- William Blake -
Gnomic Verses xvii.1

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NOTATION

Symbols used throughout this thesis:

Roman

a	velocity of sound (m/s)
d	diameter (mm) (m in Re)
L	nozzle length (mm)
M	Mach number
p	absolute pressure (bar)
r	radial distance (mm)
Re	Reynolds number
t	time
u	gas velocity (m/s)
v	particle velocity (m/s)
p_o/p_∞	nozzle pressure ratio ($= p_4/p_1$)

Greek

γ	ratio of specific heats, c_p/c_v
ρ	density (kg/m^3)
μ	dynamic viscosity (kg/ms)

Subscripts

o	total (upstream) conditions
cl	centreline
e	exit conditions
g	gas
j	jet
p	particle
∞	ambient conditions

Other symbols by chapter:

Chapter 3

p_4/p_1	diaphragm pressure ratio ($= p_o/p_\infty$)
p_2/p_1	shock strength
x_m	axial distance to first Mach disc (mm)

Greek

β_c	$(M_c^2 - 1)^{1/2}$
δ	nozzle divergence angle (degrees)
Δ	cell length
λ	jet wavelength (mm)
Φ	velocity potential
ϕ	angle between intercepting shock and upstream streamline
ψ	angle between jet axis and tangent to jet boundary

Subscripts

1	conditions in low pressure region
2	conditions of gas traversed by shock wave
3	conditions of gas traversed by expansion wave front
4	conditions in high pressure region
c	curvature
cs	contact surface
s	shock wave

Superscripts

*	critical condition
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Chapter 4

e	pitot misalignment error
p_{o1}	stagnation pressure ahead of shock wave (bar)
p_{o2}	measured pitot pressure (bar)

Chapter 5

Roman

K	constant for Kleinstein model
m	mass (kg)
R	resistance (Ω)
Re_p	Reynolds number ($= (u - v)d_p\rho/\mu$)
S_p	Stokes particle time constant ($= m_p/(6\pi r_p\mu)$)
u_m	axial jet velocity (m/s)
u_o	initial gas velocity (m/s)
V	voltage (V)
$v_{p,o}$	initial particle velocity (m/s)
x_c	dimensionless core length (Kleinstein model)
$x_{m,p}$	distance to first Mach disc with particle addition

Greek

α	reciprocal Stokes time constant ($= 6\pi\mu r/m_p$)
Δ	velocity lag ($= (u_g - v_p)/u_g$)
λ	$2a/\alpha$
ξ	dimensionless distance ($= ax/u_o$)
ϕ	initial particle/gas velocity ratio ($= v_{p,o}/u_o$)
τ	dimensionless time ($= \alpha t/2$)

Chapter 6

Roman

A	light absorption
c_1, c_2, c_3	constants in impact analysis
c_t	velocity of sound in target
d	thickness of optical medium
d_p	particle diameter (μm)
e	transition impact distance
I	light intensity
I_o	initial light intensity
K	nose shape constant
P	penetration distance (μm)
V_o	target chamber vacuum (bar, gauge)

Greek

α	material-dependent constant of absorption
σ_y	target yield strength (N/mm^2)
τ_o	target shear strength

All notation utilised in Appendices are defined therein.

Abbreviations

<i>CFU</i>	colour-forming units
<i>LED/PD</i>	light-emitting diode/photodiode
<i>LDV</i>	laser Doppler velocimeter
<i>MOC</i>	method of characteristics
<i>SEM</i>	scanning electron microscopy
<i>TD</i>	target distance
<i>TGE</i>	transient genetic expression
<i>TOF</i>	time of flight

PREFACE

This thesis is intended to be of use both to geneticists interested in the physical mechanisms of the "biolistics" technique and to engineering researchers interested in the development of applications in the growing field of biotechnology.

The units of pressure utilised herein are *absolute bar*, unless denoted with a *g* for gauge pressure.

CHAPTER 1. INTRODUCTION

1.1 Motivation

The ability to efficiently alter the genetic make-up of a cell or tissue is fundamental to the fields of genetics and genetic engineering. Genetically transformed cells, tissues or species are of significant commercial value in research, medicine and agriculture. Human insulin (hI), for example, is currently being produced by bacteria which have been genetically engineered. hI was the first therapeutic agent produced by recombinant DNA technology to achieve regulatory approval and is now available in quantities large enough to be commercially significant, as shown by one study which found that the estimated 1985 U.S. market for hI was \$345 million (Elander [1991]).

In addition, various plant species have been genetically transformed to produce plants having higher yields, more disease-resistance, or abilities to grow in less-hospitable climates and soils. Because they comprise 52% of the food consumed by man (Vasil [1990]), cereals (wheat, rice, maize, barley, millets, sorghum) have become not surprisingly a major target for such genetic manipulation by researchers. Indeed, as shown in Table 1.1, the global financial value of these cereal crops have made them ripe for the attentions of geneticists attempting to improve strains to optimise certain characteristics.

Table 1.1 Global Value of Cereal Crops (1986) - from Christou *et al.* [1992]

Crops	Value (US\$, billions)
Sugarcane	143
Rice	108
Wheat	60
Sugar beet	44
Potato	36
Maize	35
Cotton	27
Soybean	21
Tobacco	20

Genetic manipulation is not limited to the field of agriculture but is also making inroads into the pharmaceutical industry. Here, researchers have sought to genetically convert microorganisms into minifactories capable of manufacturing desired gene products. This is not a new goal. For thousands of years, man has used naturally-occurring bacteria and yeasts for his own purposes. As early as 6000 B.C. yeast were utilised by people who populated what is now Israel, Syria, and Palestine to ferment alcoholic beverages. The penicillin first identified by Fleming in 1929 as an antibacterial agent (Fleming [1929]) was only later clinically useful once microbial, and later genetic, methods of cultivating improved penicillin-producing strains were developed (Tabor [1989]). Strategies have been implemented to combine genetic engineering and improved fermentation technologies to produce commercially-important proteins in large quantities at low cost. Efforts have initially focused on the low-volume, high-value proteins with applications in the medical field. Some of these have included Interleukins 1,2, and 3, which stimulate T-cell growth for the benefit of the immune system; Factors VIII and IX, which are blood clotting factors for the treatment of haemophilia; Tissue-specific plasminogen activator (tPA), which dissolves fibrinogen clots in the treatment of myocardial blockage; and Human growth hormone (hGH), which is useful in regulation of growth and development (Tabor [1989]). These represent but a few of the more important applications of genetic engineering solutions to problems arising in the pharmaceutical industry. The macromolecule of greatest importance for genetic transformations is deoxyribonucleic acid (DNA). A DNA molecule consists of a pair of nucleotide chains twisted together in a double-helix or, in the words of Richard Dawkins (1976), an "immortal coil". Its self-replicating ability causes this biological polymer to be the essential carrier of genetic information in most organisms¹. The primary aim of any gene transfer process, therefore, is stable insertion of a particular DNA (recombinant DNA: rDNA) into the nuclear genome of cells capable of giving rise to a whole transformed plant (Draper and Scott [1991]).

¹RNA viruses (*e.g.*, retroviruses) represent the primary exception.

It has been the development of novel gene transfer methods over recent years which has permitted the introduction of alternate genetic information into living cells and intact tissue in the laboratory, enabling researchers to transform the genetic structure of certain bacterial, fungal, plant and animal cells. At other times biologists have used similar techniques to introduce various substances such as proteins or biological stains into living cells. The cell membrane and, in the case of plant cells, the cell wall have posed significant obstacles which have excluded many macromolecules. Conventional technologies for introducing biological material into living cells have included electroporation, direct DNA uptake mechanisms, microinjection mechanisms, fusion mechanisms, and the use of infectious agents. Each of these techniques, however, suffers from certain practical disadvantages, and gene delivery is presently a limiting step in numerous research fields. A need exists, therefore, for a gene-delivery system which can facilitate a rapid transformation of various types of cells and tissues with a single apparatus and a single basic protocol.

1.2 Current Gene Transfer Techniques

The importance of introducing alternate genetic information into biological cells is demonstrated by the large amount of work which has been done in this area and the specialized technologies which have been developed to achieve this end. The gene transfer techniques discussed below concentrate on their application to the transformation of plant cells. A large percentage of previous work with plant cells has dealt with cultured, totipotent suspension cells. Ideally, gene transfer into *pollen* (or egg cells) would theoretically give rise to genetically transformed gametes which could be used to generate directly wholly transformed plants via normal fertilisation procedures. However, gene transfer into pollen has yet to be developed as a routine technology. Nevertheless, it has been recognised for several decades that totipotent plant cells can be stimulated to regenerate into whole plants *in vitro*, via organogenesis (shoot formation) or embryogenesis, as long as the cells have been provided with the correct hormonal and nutritional conditions (Skoog and Miller [1957]). Adventitious shoots or embryos are thought to arise from single activated cells. Therefore, as long as totipotent cells can be

identified which are both competent and accessible for gene transfer, genetic transformation is a possibility and will give rise directly to non-chimeric² transformed plants (see, *e.g.*, Draper *et al.* [1988]). The main aim in any gene transfer procedure is to insert DNA stably into the nuclear genome of cells which are capable of giving rise to a whole transformed plant (*e.g.*, totipotent cells) (Draper and Scott [1991]). Some of the gene transfer techniques in current use will be discussed below.

1.2.1 Electroporation

Electroporation is a technique for introducing a variety of molecules into cells. The technique involves the exposure of a suspension of protoplasts³ to brief, rapidly-pulsed high-voltage electric discharges. Protoplasts have been successfully transformed using either a high-voltage rectangular wave pulse of short duration (*i.e.*, 1.5kV per cm² for 10µs; Shillito *et al.* [1985]) or a lower voltage pulse of much longer duration (*i.e.*, 350V over 54ms; Fromm *et al.* [1985]). These rapid electrical pulses form holes or pores in the plasma membrane of the protoplasts of approximately 30nm in diameter (Okada, *et al.* [1986]) through which DNA fragments from the surrounding medium may diffuse. Upon completion of the electrical discharge, the pores in the cell membrane close, capturing any DNA fragments which have diffused into the cell. For a general discussion of electroporation, see Shigekawa *et al.* [1988], or Miller *et al.* [1988].

A major drawback in using electroporation to introduce DNA into living cells is the need to determine empirically optimum values for a large number of parameters before a transformation of any cell line can be attempted. These parameters include voltage, pulse time, capacitance, composition and resistance of electroporation medium, state of cell growth prior to electroporation, concentration of DNA, temperature and cell density. In addition, although the technique has been used successfully to transform both dicotyledons (plants with two cotyledons, such as tobacco or tomato) as well as monocotyledons (most

²A chimeric organism has tissues of varying genetic structure within the same specimen.

³Cells from which the cell walls have been enzymatically removed.

cereals and grains of greatest commercial importance), including maize (Fromm *et al.* (1986)), wheat (Lorz *et al.* [1985], and rice (Uchimiya *et al.* [1986]), electroporation requires the relatively laborious and time-consuming task of removal of the plant cell wall to form protoplasts before the voltage can be applied. These protoplasts must then regenerate their walls before they are able to divide and regenerate into intact plants. Although progress has been made in regenerating plants from transformed protoplasts (Rhodes *et al.* [1988]), it remains currently quite difficult to regenerate intact plants from protoplasts (Peng *et al.* [1990]); this requirement is a severe limitation on the applicability of electroporation.

Other disadvantages to electroporation include: (i) the reduction in overall cell viability caused by the necessary high applied voltages, (ii) the inability to specifically target particular cells, especially in a complex, multicellular organism. Additionally, this method is limited to *in vitro* suspension systems.

1.2.2 DNA Uptake Mechanisms

Uptake mechanisms generally involve suspensions of single cells. As with electroporation, the use of uptake mechanisms to transform plant cells, requires the enzymatic removal of the cell wall. Uptake mechanisms, therefore, are time-consuming and relatively labour-intensive, although a large number of cells can be treated *en masse*. Uptake mechanisms generally involve one of three techniques: i.) enhancement of cell membrane permeability through use of calcium and temperature shock, ii.) use of surface-binding agents such as polyethylene glycol (PEG) or calcium phosphate, iii.) phagocytosis of particles such as liposomes, organelles or bacteria into the cells.

Some of the earliest uptake mechanisms used by researchers involved the use of calcium (Ca) (Mandel *et al.* [1972]) and temperature shock (Dityatkin *et al.* [1972]) to enhance cell membrane permeability.

A second more-successful uptake mechanism has involved the use of the long-chain hydrophobic polymer polyethylene glycol (PEG) (see, *e.g.*, Draper *et al.* [1982], Krens *et al.* [1982], Hain *et al.* [1985]), often in combination with calcium phosphate-precipitated DNA (Wigler *et al.* [1979]). Polyethylene glycol

at high concentrations (15 - 25% PEG 6000) precipitates ionic molecules, such as DNA, and stimulates their uptake by the protoplasts without causing gross damage to the protoplasts.

Phagocytosis of particles into a cell represents a third uptake mechanism. Suitable particles have included liposomes of DNA (Uchimiya *et al.* [1982], Deshayes *et al.* [1985]), organelles (Potrykus [1973]), and bacteria (Cocking [1972]).

Following DNA uptake through application of any of the above three techniques, the protoplast populations are allowed to recover at high cell densities in normal protoplast growth medium. Upon completion of cell wall reformation, normal cell division occurs, and the cells may be plated out for further study.

Thus far, researchers have only been able to achieve relatively low transformation efficiencies using these uptake techniques (*i.e.*, the proportion of cells that take up and express foreign DNA has generally been less than 1 in 10,000; see, *e.g.*, Krens *et al.* [1982]). Also, special selection procedures are required to recover the rare cells which have been genetically transformed.

1.2.3 Cell Fusion Mechanisms

Cell fusion techniques integrate the genetic information of two different cells into one. In plant cell cultures, cell walls must again be dissolved enzymatically and the naked protoplasts fused together, forming a new cell. The descendants of the new fused cell express certain characteristics of both the parent cells; however, it is currently not possible to predict what characteristics will be expressed in the progeny. The results obtained using cell fusion methods, therefore, are unpredictable and generally not reproducible.

Fusion can be induced with electric currents, PEG and virus particles. For general discussions of cell fusion mechanisms, see Uchidaz *et al.* [1980], and Harris [1970].

In animal cell cultures, the cell fusion products of antibody-secreting cells and "immortal" cells (myelomas) are known as hybridomas. Hybridomas are used in medicine and research to produce monoclonal antibodies. Hybridomas, like

plant cell fusion products, react unpredictably, and are expensive to produce and maintain.

1.2.4 Microinjection

Microinjection involves the stable introduction of DNA or other exogenous materials through a micropipette directly into the cytoplasm or nuclei of target cells (Crossway *et al.* [1986], Neuhaus *et al.* [1988]). Typically, an extremely-fine glass micropipette having a diameter in the range of 0.1 to 0.5 microns is inserted directly into the cytoplasm or nucleus of the cell. Exogenous material (such as DNA) is then injected through the inner bore of the micropipette. It is possible for a skilled technician to inject between 500 and 1000 cells per hour (Reich *et al.* [1986]). In addition, high frequencies of transformation have been reported (15 - 20%; Celis [1984]); microinjection is, therefore, useful when few cells/protoplasts are available.

Micropipette injection, however, requires a substantial initial capital expenditure for the elaborate equipment, which includes a micropipette puller for making the needles and micromanipulator to position the needles correctly for injection. In addition, extensive training is necessary before a technician can master this tedious technique. Finally, extremely high pressures are required to induce bulk flow through capillary apertures smaller than one micron, and regulation of such bulk flow can be difficult. Moreover, microinjection techniques suffer from limitations in cell recovery. Direct microinjection of plant cells is complicated by the presence of a rigid cell wall. While protoplasts can be formed and treated, microinjection of plant cell protoplasts is made difficult by their extreme fragility. Overall, micropipette microinjection can be an effective gene transfer method; however, because of the relatively small number of cells that can be transformed in any given experiment, the high cost of the sophisticated equipment that is required and the level of skill required for the labour-intensive procedure, it may not be a gene transfer method available to a vast majority of researchers in the field.

1.2.5 Biological Vectors or Infectious Agents

The most common method for transferring genes into plant cells involves conjugal transfer from living bacteria, primarily *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* (the bacteria responsible, respectively, for crown gall formation and "hairy root" disease). The DNA to be transferred is first engineered into plasmids derived from the bacterial tumour-inducing (Ti) plasmid, and the bacteria are then incubated in culture with the recipient plant cells (Klee *et al.* [1987]). *Agrobacterium tumefaciens* has the innate ability to transfer a portion of DNA from a Ti plasmid harboured therein into an infected plant cell. The cultured, transformed cells must then be regenerated into genetically-homogenous plants. This method is limited to plants susceptible to *Agrobacterium* infection - principally, dicots (Fraley *et al.* [1986]) - which can be regenerated from cultures. Therefore, the range of monocotyledonous cereal crops important as sources of staple foodstuffs are not presently transformable using *Agrobacterium* infection.

Viral infection represents yet another means of transferring foreign DNA into some cells. Retroviruses (RNA viruses) can be used to deliver genes into animal cells (Anderson [1984]). When the virus enters the cell its RNA acts as a template for reverse transcription of complementary DNA which integrates into the genome of the host cell. This DNA can be isolated and inserted into a plasmid, which, with additional genes added, can be used to transform cells. However, viruses are difficult to work with, and since a particular virus will only infect certain cells, the technique is not applicable to the broad spectrum of living cells, tissue or species. Moreover, some viral agents may actually be hazardous to the technicians working with them or to the environment by creating the potential for the evolution of new pathogens.

1.2.6 "Biolistic" Bombardment Techniques

Major cereal species such as wheat and maize are not readily attacked by *Agrobacterium* nor will their protoplasts easily regenerate into whole plants. This

has created the need for the development for an alternate approach to gene transfer. Particle bombardment (commonly referred to as "biological-ballistics" or "biolistics") has been developed to meet just such a need. Biolistics is a physical method for gene delivery which involves the acceleration of DNA-coated particles (or microprojectiles) through the cell walls directly into the cells. Klein *et al.* [1987] and Sanford *et al.* [1988] have shown that nucleic acids can successfully be delivered into plant cells on the surface of small (1 - 3 μm), high-density (tungsten) microprojectiles which have been accelerated using an explosive charge. Alternatively, McCabe *et al.* [1988] have accelerated micron-sized gold particles using shock waves initiated by a high voltage electric discharge vaporising a water droplet. Schematic diagrams of the two primary microprojectile acceleration devices are provided in Figure 1.1.

The technology described in Klein *et al.* [1987] involves coating tiny tungsten particles with plasmid DNA and then loading these particles on the surface of plastic "bullet" (known as the "macroprojectile"). Under a partial vacuum, the macroprojectile is fired by cordite explosion (usually a .22 cartridge). The bullet strikes a plastic stopping plate, with which it fuses, stopping its motion; however, the momentum and small size of the dense microprojectiles cause them to be thrown from the surface of the macroprojectile, through a small hole in the stopping plate. The momentum thereby achieved by the microprojectiles has been shown to be adequate for the penetration of target plant tissue.

The device of McCabe *et al.* [1988] differs in that a sonic shock wave created by the vaporisation of a water droplet through high-voltage electric discharge is utilised to accelerate a mylar carrier sheet, on the surface of which has been placed the DNA-coated microprojectiles. The carrier sheet is stopped by a retaining mesh, but the microprojectiles have sufficient momentum to continue and penetrate the target plant tissue.

Application of the biolistics direct delivery technology has been shown to be successful in achieving high rates of transient gene expression (TGE) for a variety of plant species, among them the important cereal crops. TGE implies that DNA from the microprojectiles has entered a number of the target cells and that a marker gene from the DNA plasmid has diffused into the cells' nuclei to an extent sufficient to cause expression of a detectable enzyme (β -glucuronidase

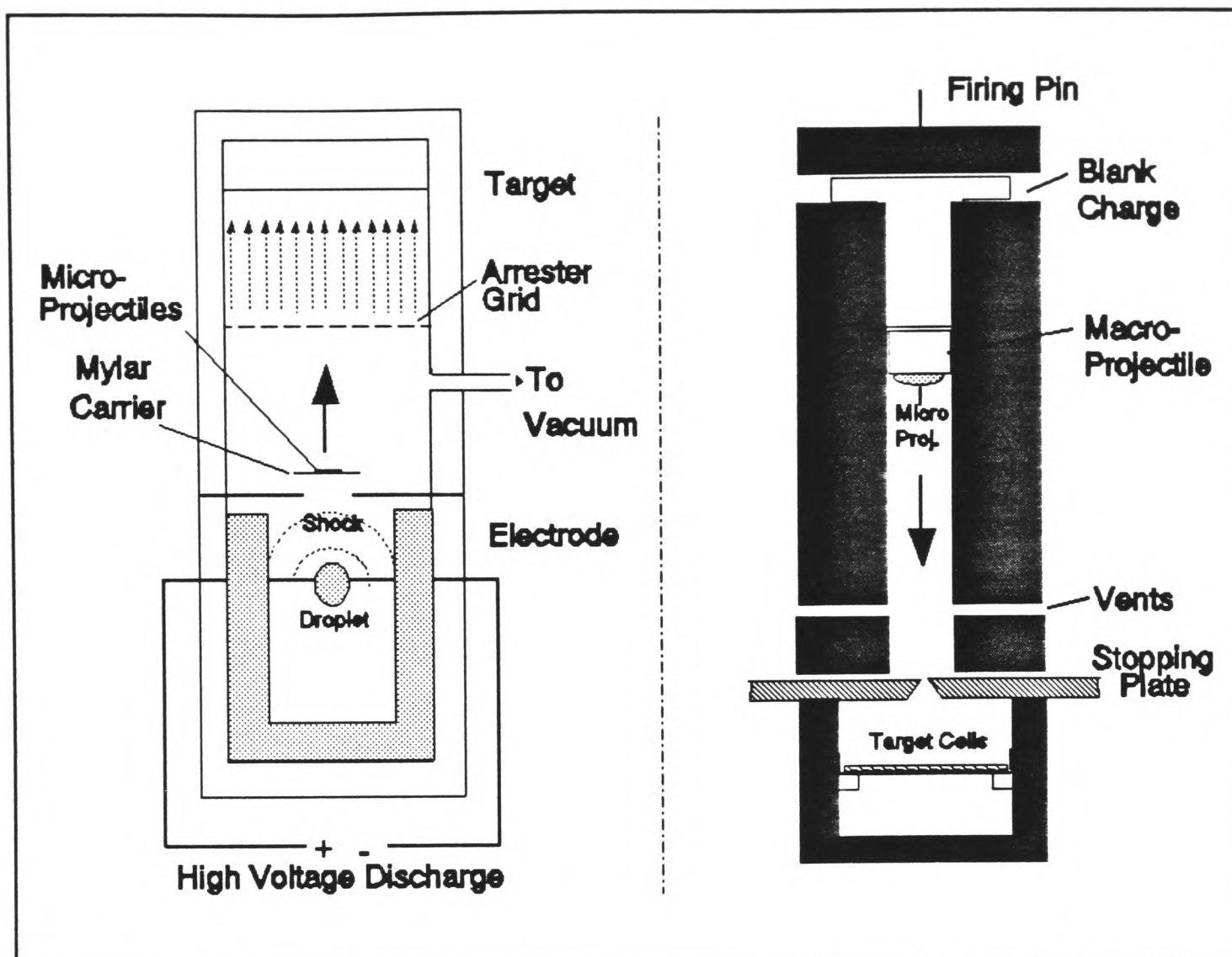


Figure 1.1 Microprojectile acceleration devices. i) Electrostatic, ii) Ballistic.

or GUS). A histochemical assay (Jefferson [1987]) comprising the appropriate amino acid substrate (applied 48-hours post-bombardment) is used to visually distinguish the expressing cells (the cells turn a vivid blue colour and are easily distinguished under a light microscope). TGE represents the first step along the road to the primary goal: achieving stable genetic transformation. Stable genetic transformation is achieved when the exogenous DNA becomes adequately incorporated into the plant germ line and is transmitted to subsequent generations.

Sufficiently high levels of TGE have been achieved using the biolistics device of Klein *et al.* [1987] and Sanford *et al.* [1988] for many cereal crops, including maize (*Zea maize*) (Klein *et al.* [1988]) and rice, wheat and soybean (Wang *et al.* [1988]). In addition, stable transformation has been shown for maize through introduction of a selectable marker gene which expresses herbicide-(kanamycin-) resistance (Klein *et al.* [1989], Fromm *et al.* [1990]). Meanwhile, stable transformation of wheat has also been achieved using particle

bombardment technology (Vasil *et al.* [1991]). Field trials of transgenic soybean (Christou *et al.* [1990]) and rice (Christou *et al.* [1991]), stably transformed using the electric discharge procedure of McCabe *et al.* [1988], have also been reported.

It is, therefore, apparent that the direct-delivery technology associated with these particle bombardment techniques has been quite successful in achieving high rates of gene transfer and genetic transformation. This does not mean, however, that certain deficiencies and disadvantages to the current technology do not exist. First, in both mechanisms described above, achieving consistent, reproducible trajectories of the tungsten (or gold) suspension loads remains an elusive goal. Run-to run differences in the placement of microprojectile suspensions onto the macroprojectiles impair targeting, because the microprojectile suspensions penetrate target tissue at random locations in the target dish. This is thought to be related to the mechanism through which the particle suspensions are flung off the macroprojectile. Because the eventual target location for particle penetration of target tissue is not known *a priori*, this problem increases the number of bombardments that must be carried out for any particular experiment. Secondly, the velocity of the microprojectile suspension is controlled only by characteristics related to the actual acceleration mechanism for the two devices described above (gunpowder load or electric discharge voltage) and these characteristics have shown considerable run-to-run variation (Miller *et al.* [1990]). Indeed, to date no reliable measurement of the actual microprojectile velocities for various run conditions have been carried out. Finally, adequate control of particle velocity for achieving alternate goals (*i.e.*, greater depth of penetration for the transformation of three dimensional tissue explants) has yet to be demonstrated with the above devices.

Although gas-driven devices for the genetic transformation of plant cells have been designed and tested recently (see, *e.g.*, Morikawa *et al.* [1989], Oard *et al.* [1990], Sautter *et al.* [1991], Russell *et al.* [1991]), none of these devices has adequately addressed the above deficiencies associated with the bombardment devices of Klein *et al.* [1987] and McCabe *et al.* [1988]. Therefore, a need for resolution of these issues exists.

1.3 Thesis Objectives

This thesis will describe the development of a novel bombardment device which takes advantage of classical gas dynamics techniques for propulsion of microprojectiles. A large percentage of the work will centre on development of experimental techniques for measurement of physical parameters associated with operation of the gas propulsion particle gun. Among these physical parameters are shock wave and contact surface velocities, particle velocities, and underexpanded jet expansion. Control of these physical parameters will be exploited to control particle distribution across a target and particle penetration into a target. Control of these physical parameters will be extended to biological experiments as a means of optimising transient genetic expression, primarily in experiments involving the bombardment of maize suspension cells. *A primary purpose of this thesis will be to show conclusively that acceleration of microprojectiles in the manner utilised here is related to transient, shock tube flow as opposed to fully-expanded nozzle flow.*

The design of a novel gas propulsion particle gun will be described in **Chapter 2**. A schematic diagram and description of the operation of the apparatus will be presented. Of prime importance will be the description of a convergent-divergent supersonic nozzle, and its design using a two-dimensional method of characteristics technique will be described. Finally, classical methods of evaluating boundary layer growth (both in transient, shock tube flow and in steady, fully-expanded nozzle flow) will be utilised.

A theoretical description of operation of the apparatus based on classical gas dynamics techniques will be presented in **Chapter 3**. Shock tube theory will be used to understand the transient nature of the flow and to provide flow field information at the nozzle exit. Previous research in underexpanded free jets will be reviewed, with particular emphasis on jet structure. In addition, results of approximate techniques for computing the boundary of underexpanded, supersonic jets will be compared with results of a more rigorous method of characteristics.

Experimental gas dynamics studies will be described in **Chapter 4**. Schlieren visualisation of the jet structure will be compared with measurements of the pitot pressure distribution along the free jet centre-line. Resulting parameters for jet structure will, in turn, be compared with classical empirical relations. The variation in measured Mach number at the exit with upstream stagnation pressure will be shown to be in excellent agreement with the corresponding relationship from shock tube theory. This will form the first piece of evidence for the conclusion that the microprojectile trajectories of concern here should be analysed using transient shock tube theory as opposed to fully-expanded nozzle flow.

Experimental evidence from measurement of particle velocities presented in **Chapter 5** will reinforce the conclusion that the microprojectiles are governed by transient shock tube flow. A novel, purpose-built particle velocimeter will be utilised in conjunction with a thin-film temperature gauge in the nozzle to show that the particulate microprojectiles arrive at the nozzle exit just behind the primary shock wave, before steady nozzle flow has been established. Another experiment, involving the replacement of the convergent-divergent nozzle with a cylindrical shock tube, will show that particle velocity is governed by transient effects. Finally, particle velocities throughout the target chamber will be presented for a range of pressure conditions and for two gases: air and helium.

Visualisation of particle distribution and target penetration will be described in **Chapter 6**, and control of these parameters will be demonstrated. A novel optical scanning technique for determining particle distribution on parafilm targets at various target distances will be utilised. In addition, high-speed video analysis of particle expansion from the nozzle and scanning electron microscopy (SEM) of bombarded targets will be used to provide further evidence of the extent of particle spread across targets. Particle penetration of agar for various conditions will be compared with empirical formulations from the literature.

Chapter 7 will present results of application of the gas propulsion particle gun to actual biological problems. Physical parameters of the system will be optimised for bombardment of maize suspension cells. Results from previous chapters will be shown to be useful in providing guidelines for this optimisation.

Application of the technique to mammalian cells as a technique for gene therapy will be documented. In addition, the novel application of the technique for the transfection of human leukaemia cells will be described.

Finally, conclusions from this work and ideas for the future extension of this work to other fields for alternate applications will be presented in **Chapter 8**. Of particular note will be the extension of the technique to other areas within the medical field, including determination of the potential of the device as a novel method of drug delivery.

CHAPTER 2. MATERIALS AND METHODS

2.1 Overview

Previous workers in the field (Oard *et al.* [1990]) have estimated that the particle velocity necessary to cause dense micro-sized particles to penetrate the surface of target cells and become incorporated into the interior of the cells is approximately 500m/s. Although this estimate was based on a rather dubious measurement of the macroprojectile rather than microprojectile velocity, the value did serve as a guideline for the initial design. In order to achieve the necessary acceleration of the DNA-coated microprojectiles, several physical mechanisms were initially considered. Among these were centrifugal and electromagnetic methods of particle acceleration. While each acceleration method theoretically had potential advantages, it was believed that lack of adequate particle control would be a major disadvantage for each. It was decided that controlled shock propagation and gas expansion through a convergent-divergent nozzle held the greatest promise for achieving controlled particle acceleration; therefore, this was the particle acceleration method chosen.

The Mark I prototype (not shown) was designed and built. A feasibility study, supported by ICI Seeds, Jealott's Hill, Berkshire, showed that sufficient particle penetration in a cured agar substrate could be achieved. In addition, particle distribution across a target surface was evaluated and was shown to be a controllable function of target distance and nozzle pressure ratio (Sarphie and Bellhouse [1990]). The Mark II design retained the basic operational features of the original prototype but was repackaged to fit a laminar flow hood and to improve "user-friendliness." This Mark II design will be the one described here.

Figure 2.1 shows a schematic diagram of the gas propulsion particle gun. Primary components of the system include a supply cylinder which acts as a reservoir for compressed gas, a diaphragm cassette, a convergent-divergent supersonic nozzle, and a target chamber. To operate the system, the biological material being genetically transformed (*e.g.*, suspension cells, leaf or root tissue)

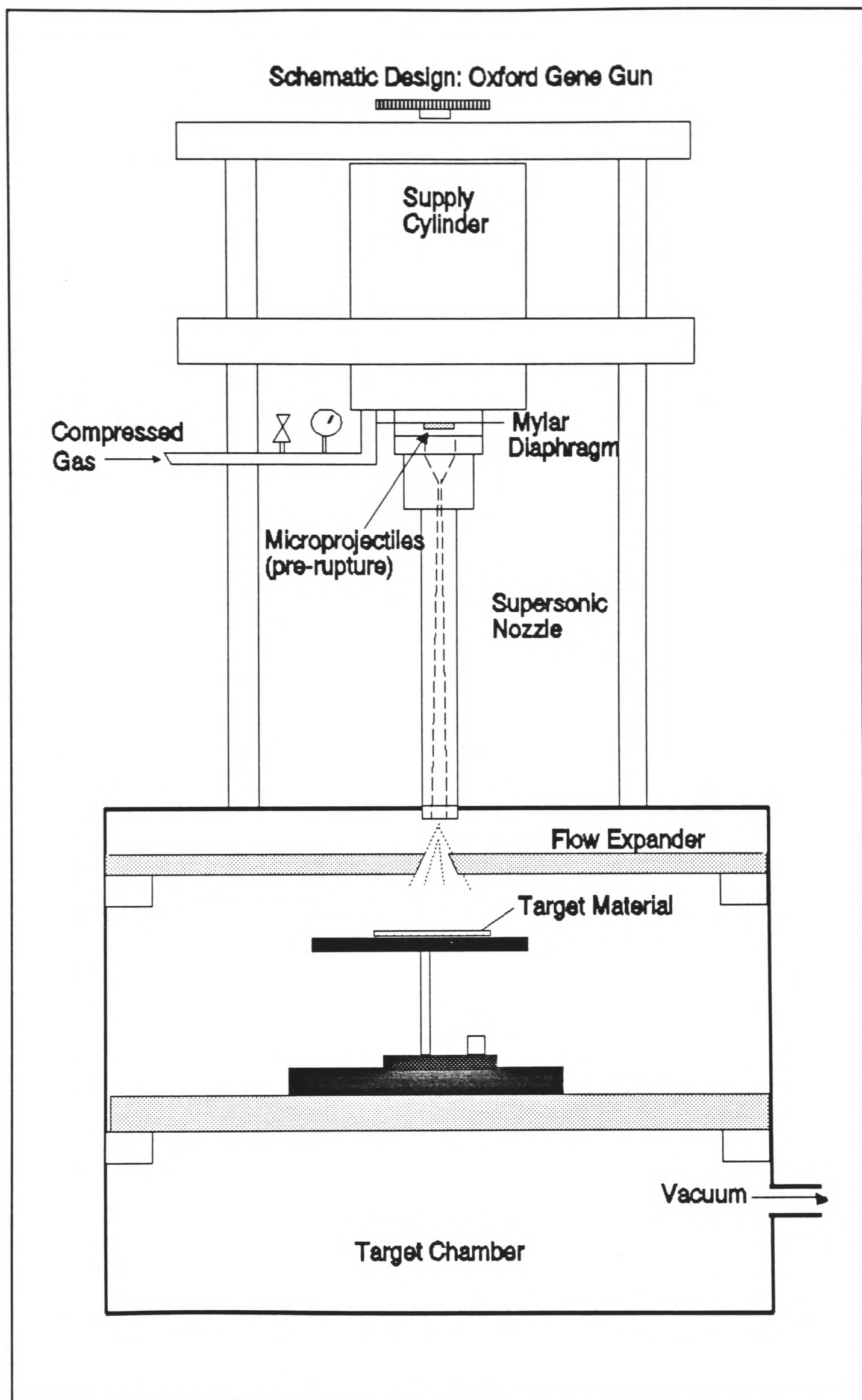


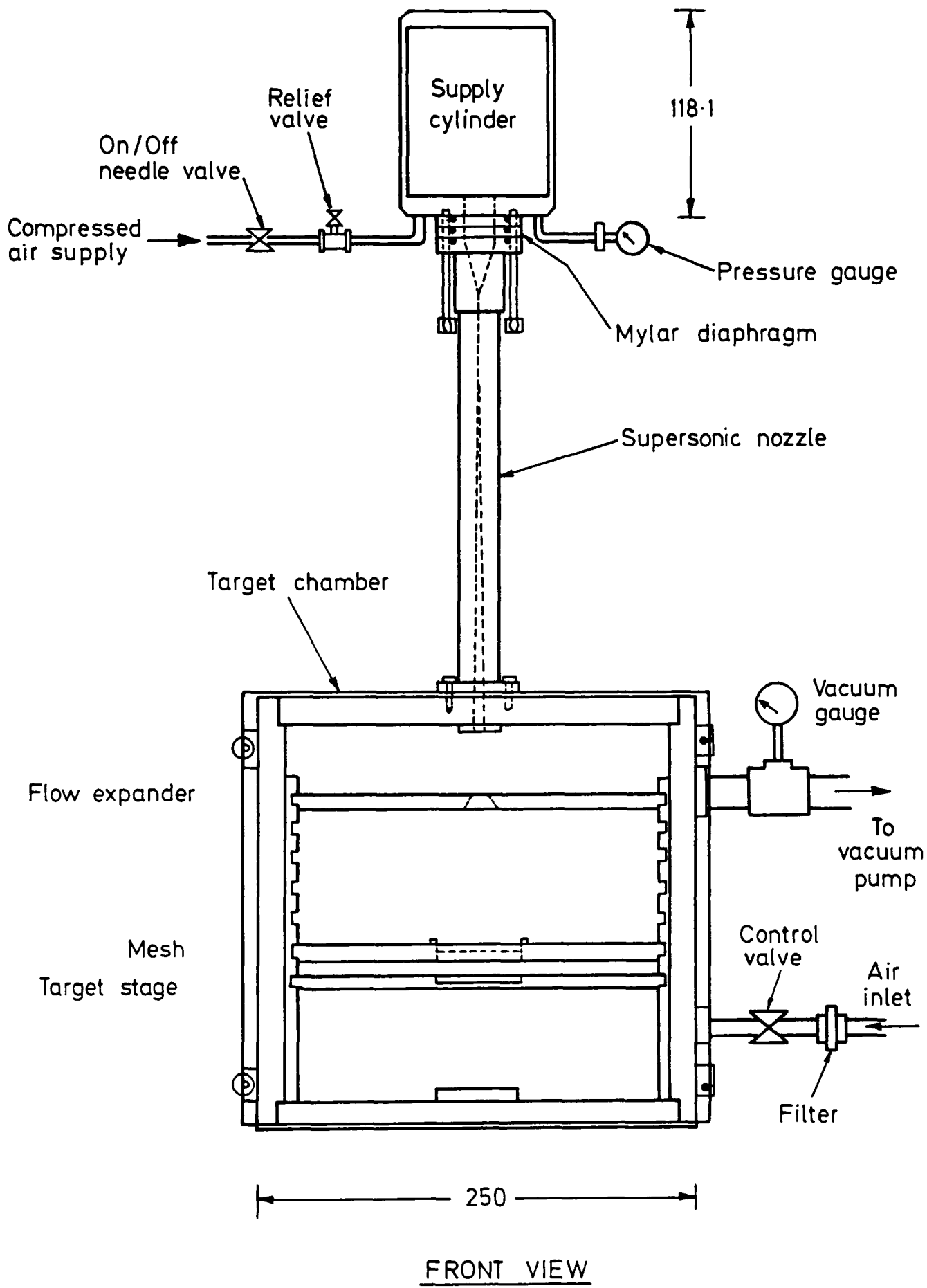
Figure 2.1 Schematic diagram of gas propulsion particle gun.

is placed on one of several shelves in the target chamber. A small volume (usually less than 10 μ l) of an ethanol-based suspension containing micron-sized tungsten particles coated with DNA (for description of coating technique, see **Section 7.2.1**) is dried onto a sterile mylar diaphragm within the diaphragm cassette, and the cassette is fitted into the apparatus. Tungsten particles have routinely been used because of the high density of tungsten, the ease with which plasmid DNA can be precipitated onto a particle's surface, and the easy availability from industrial suppliers. Other groups working in this field have achieved success with gold particles. After the mylar cassette has been slotted into the apparatus, a high-pressure source slowly pressurises the upstream supply cylinder with either air or helium through a constrictive needle valve. Simultaneously, a vacuum pump evacuates the target chamber (if a vacuum is desired). The thickness of the diaphragm determines the pressure difference (upstream pressure minus target chamber pressure, $p_o - p_\infty$) at which the mylar ruptures. Rupture of the diaphragm causes the propagation of a shock wave downstream through the nozzle and immediate expansion of compressed gas flow behind it. Transient gas flow associated with this shock wave propagation accelerates the tungsten particles through the convergent-divergent nozzle and into the target chamber toward the target tissue. For some applications a flow expander is used to diffuse the gas flow which follows the particles once steady flow is established. This prevents, to a large degree, blow away of fragile, lightly-attached target cells by causing further expansion of the jet without disrupting the trajectory of the much-denser microprojectiles. The entire operation has been designed to have a relatively quick turnover rate for biological experiments which can require up to 30 bombardments per day. An average bombardment requires approximately five minutes.

2.2 Component Design

An engineering drawing of the gas propulsion particle gun is shown in Figure 2.2. A photograph of the apparatus installed in a laminar flow hood at ICI Seeds is presented in Figure 2.3. The following sections will highlight design

THIRD ANGLE PROJECTION



Note: All dimensions in mm

Figure 2.2 Engineering drawing of Mark II rig.

DEPARTMENT OF ENGINEERING SCIENCE OXFORD UNIVERSITY		DRAWING No.	
TITLE		DESIGN LAYOUT : Mark II	
TOLERANCES		CHK'D.	
MATERIALS		TRD.	
FINISH		DRN.	
MODIFICATION		SCALE	
ISSUE	DATE	A II - 1	

features of various components of the gas propulsion particle gun, including the supply cylinder, supersonic nozzle, and target chamber.

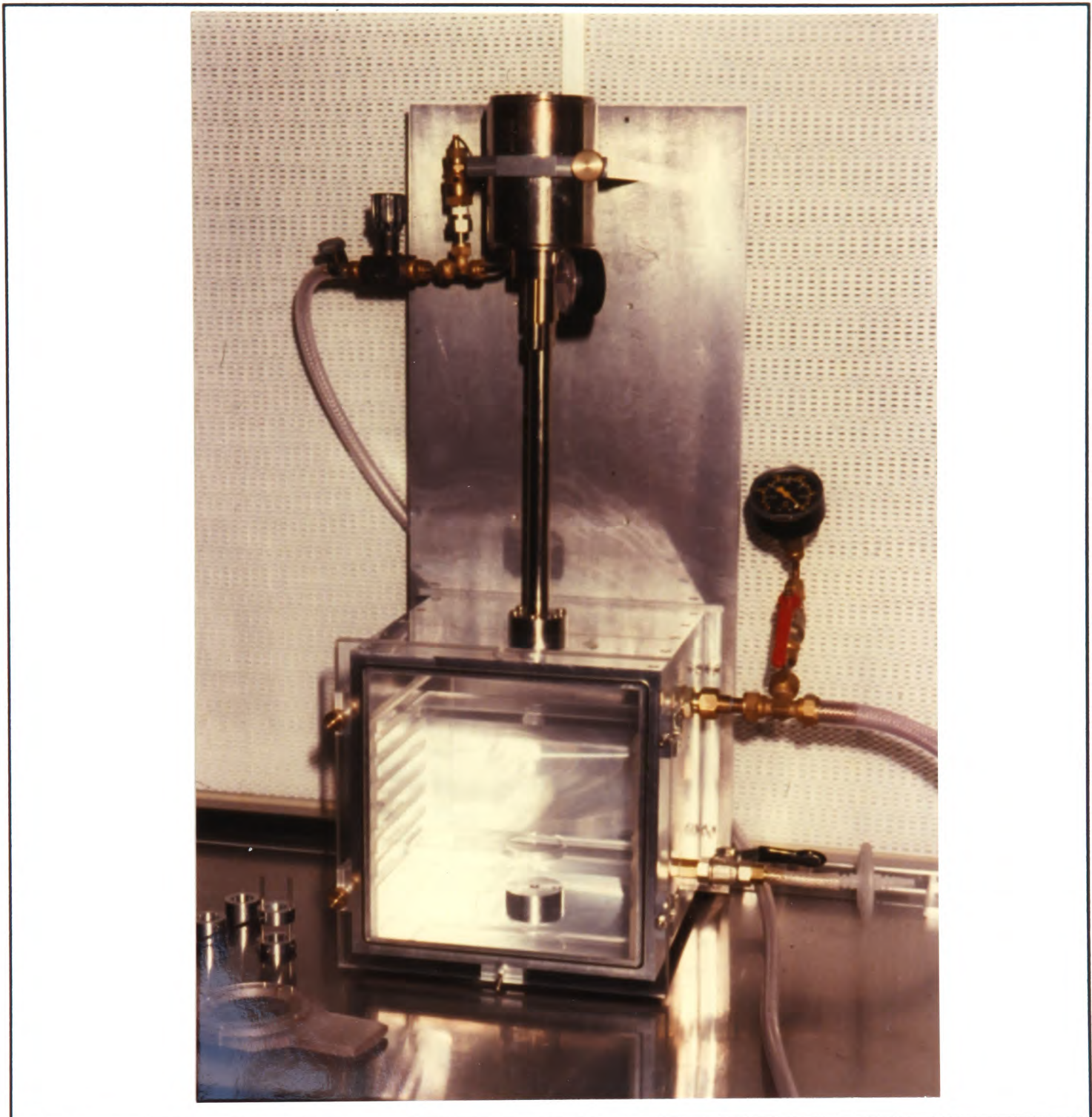


Figure 2.3 Photograph of gas propulsion particle gun.

2.2.1 Supply Cylinder

The supply cylinder was designed to contain a maximum safe operating pressure of 15bar, although constraints due to the pressure regulator ensured that the working pressure did not exceed 12bar. The basic design of the supply

cylinder consists of a 78.6mm internal diameter stainless steel tube with 5.7mm wall thickness. A stainless steel plug with a thickness of 15mm is welded at one end of the supply cylinder, per British Standard Design Handbook. A 16mm bore section connects the other end of the supply cylinder to the diaphragm cassette. Two stainless steel screws secure the supply cylinder to the diaphragm cassette. A mechanical pressure gauge and relief valve (pre-set to open at pressures one-bar above working pressure) ensure the safety of the cylinder during operation. In addition, the supply cylinder has been pre-tested and certified using a standard water pressure test at the Oxford Engineering Science facility. This test involves pressurising the cylinder with water up to a pressure equal to twice the safe working pressure (*i.e.*, 30bar) and holding that pressure for one-half hour to check for leakage.

The capacity of the various supply cylinders utilised varied from approximately 50ml (I.D. = 25.2mm) to 500ml. The 500ml cylinder comprised the preferred embodiment for most of the biological experimentation with maize cells, because most of the developmental and particle velocity work had been associated with the large cylinder. The importance of varying the capacity of the supply cylinder will be seen in **Chapter 5**, where it will be seen that reducing cylinder capacity reduces cell blow away effects *without* affecting measured particle velocities.

2.2.2 Diaphragm Cassette

Essential to the operation of the system is the production of a sharply defined pressure/shock front which must be allowed to propagate freely downstream. Suitable release mechanisms for generating this gas shock front must open *very* rapidly. Possible mechanisms that were considered included a special, quick-opening solenoid valve and a rupturable membrane. Because an appropriate valve was not readily available, the approach involving a rupturable membrane was chosen. Mylar, a common diaphragm material in the Osney shock tubes, was used as the diaphragm material for the gas propulsion particle gun. Mylar or its equivalent brand name *Melinex* (HiFi Industrial Film, Stevenage) is a polyester film used frequently as a dielectric material in the electronics industry.

The pressure difference at which the diaphragm ruptures, calculated using the tensile stress on a hemispherical surface, is dependent on the thickness of the mylar. Preliminary experiments have shown that mylar rupture pressure varies approximately linearly with film thickness. This is seen in Figure 2.4. Mylar diaphragms with thicknesses varying from 1.5 μm to 36 μm were used.

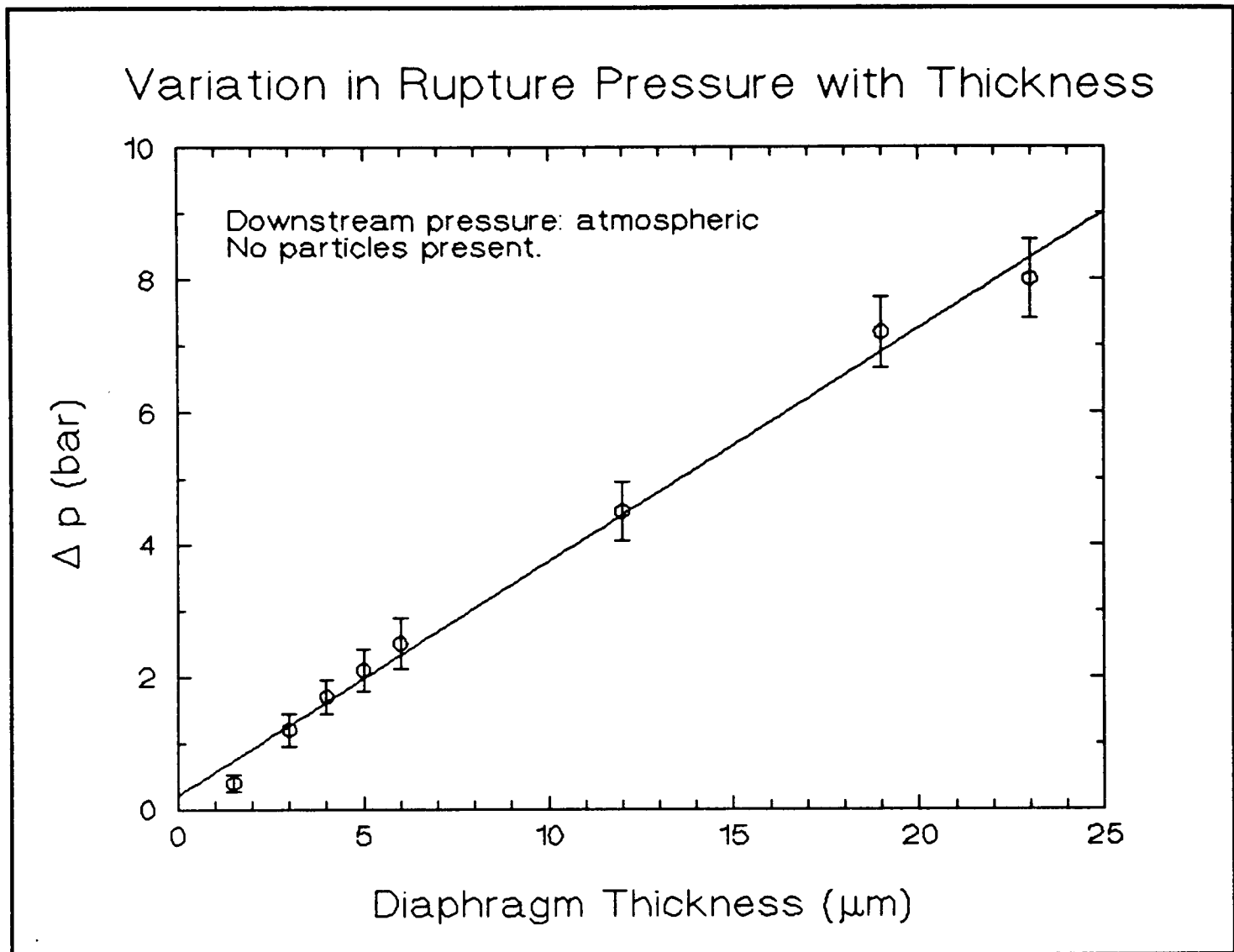


Figure 2.4 Variation in diaphragm rupture pressure with film thickness.

The rupture of the diaphragm causes the propagation of a shock wave and the expansion of the pressurised gas downstream through the supersonic nozzle. This mylar membrane is sealed between two stainless steel flanges with an encircling O-ring which acts as a sealing gasket, leaving a 16mm diameter circular area exposed to the pressure difference. In the preferred operational protocol, the ethanol-based suspension of microprojectiles is "painted" onto the upper surface of the diaphragm and the ethanol allowed to dry prior to bombardment.

Upon diaphragm rupture the particles are immediately entrained in the accelerating flow.

2.2.3 Supersonic Nozzle

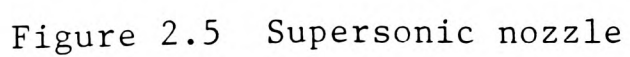
When the bombardment system was originally conceived, two different particle acceleration mechanisms were postulated: i.) particle acceleration due to quasi-steady, supersonic nozzle flow, ii.) particle acceleration due to transient effects associated with shock wave propagation downstream. It was decided that an axisymmetric nozzle achieving a nominal exit Mach number 2.33 would ensure that either acceleration mechanism would achieve the nozzle exit design velocity.

Nozzle Design

A convergent-divergent, supersonic nozzle of large aspect ratio (length to exit diameter = 50) was designed using a two-dimensional method of characteristics technique, after Cheers [1963]. **Appendix A** provides a brief description of the technique utilised in the nozzle design. The large aspect ratio was thought to be desirable in order to provide sufficient particle time-of-flight to allow the particles to achieve the design velocity at the nozzle exit. Figure 2.5 shows a sketch of the design of the convergent-divergent nozzle.

The internal contour of the nozzle was designed for the proper area ratio (Exit Area/Throat Area) to achieve the necessary supersonic conditions (given sufficient pressure ratio), while taking into account the development of a compressible boundary layer along the nozzle wall. Two compressible boundary layer codes developed at Stanford University in the mid-Eighties were employed to aid prediction of boundary layer growth in the nozzle for *quasi-steady flow*: STANLAM for laminar flow and STANTURB for turbulent flow. The results of these computed predictions for boundary layer development are also presented in **Appendix A**, along with a brief explanation of the technique. Because it was uncertain whether the *transient* development of a boundary layer in the shock tube-like flow would affect particle trajectories, a calculation of this transient

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boundary layer was also carried out, using an approximate technique after Mirels [1962]. A summary of this method and the resulting approximation for bombardment conditions can be found in **Appendix A**.

Nozzle Fabrication

The nozzle was fabricated using a method designed specifically for this particular application by John Greenford. A brass plug, the external dimensions of which were shaped to represent the internal contour of the nozzle (the design of which was discussed in the above section), was temporarily fixed inside a 22mm outer diameter stainless steel tube. The volume between the plug and tube was then filled with a liquid resin which hardened over heat after 48 hours. The contour of the contracting part of the nozzle was milled out and the two pieces joined together near the nozzle throat. Care was taken to ensure that the joint was as smooth as possible to prevent the formation of an unwanted boundary layer trip.

2.2.4 Target Chamber

The target chamber comprises a 220mm x 220mm x 220mm dural housing with a transparent perspex door hinged along one side of the chamber. A rubber gasket is secured about the entire periphery of the opening for engagement by the door in the closed position to provide a vacuum-tight seal. Grooves spaced approximately 20mm apart have been milled into opposite sides of the chamber for adjustably supporting a flow expander, target mesh, and petri dish containing target cells/tissue during biological transformation experiments. Alternatively, the grooves were also used to support holders for light-emitting diode/photodiode pairs when particle velocimetry measurements were undertaken. A vacuum hose attached to the side of the chamber connects with a vacuum pump (SpeediVac 2, Severn Sciences, Bristol) to permit evacuation of the chamber to a moderate vacuum (0.1bar).

Flow Expander

Preliminary bombardment experiments with loosely-attached maize suspension cells showed that a potential problem with the use of the gas propulsion system was cell dispersal from the target petri dish ("cell blow away"). Careful examination of the cells which had been dispersed showed the presence of tungsten particles, implying that cell blow away was occurring after the delivery of the tungsten particles¹. Several approaches to the solution of this problem were evaluated: i.) use of a sticky alginate to attach the target cells to the target petri dish, ii.) use of smaller upstream supply cylinder to limit the amount of gas in the system, iii.) use of a driver gas of decreased density (*i.e.*, helium), and iv.) use of a flow expander to aerodynamically cause further expansion of the free jet as it exits the nozzle. Prof. Terry Jones of the Osney Laboratory, Oxford University, provided the original idea for the use of a flow expander. Discussion of the application of the first three of these approaches can be found later in the text. The fourth approach, use of a flow expander, was found particularly useful during early bombardment experiments with maize suspension cells, with air as the driver gas. The flow expander was designed such that the diameter of the bore on the upper surface (10mm) would very nearly match the approximate jet diameter at a axial location of 40mm for slightly underexpanded jet conditions. The internal surface of the flow expander diverges at a half-angle of 24.2°, so that the flow theoretically exits the flow expander at $M = 3.54$ for the Prandtl-Meyer expansion. It is unclear whether the full expansion to this higher Mach number actually occurs; nonetheless, the flow expander proved quite successful in reducing effects of cell blow away.

¹This has important implications for modelling of the system dynamics, as seen in Chapter 5.

CHAPTER 3. GAS PROPULSION: THEORY

3.1 Introduction

In order to develop a theoretical model for the operation of the gas-propulsion system described in **Chapter 2**, it will be helpful to first consider the operation of a shock tube. This will provide insight into pressure and velocity conditions at the nozzle exit. A theoretical model and numerical calculations will be used. In the second part of the chapter, the theory of underexpanded free jets will be developed, with particular emphasis on approximating the jet structure. The shock structure will be described. Finally, jet boundary location will be evaluated using two numerical techniques: an axisymmetric method of characteristics as well as an approximate "pseudo-nozzle" procedure.

3.2 Shock Tube Model

It will be shown experimentally in **Chapter 5** that the particle dynamics of the system are highly transient in nature. Evidence will be presented which shows particle velocities to vary with diaphragm pressure ratios in a manner similar to what would be expected in a shock tube. Further experimental data will reveal that the particles are carried along by the contact surface propagating just behind the primary shock wave. Therefore, a brief theoretical description of the operation of a shock tube will prove important in describing the operation of the system.

3.2.1 Theory

A shock tube is a cylindrical channel initially divided by a diaphragm into regions of higher and lower pressure, as illustrated in Figure 3.1 (a). Utilising the nomenclature of Liepmann and Roshko [1957], conditions in the high pressure (compression) chamber are subscripted with a 4, whilst those in the low pressure

(expansion) chamber are denoted by a 1. At time $t = 0$ the diaphragm bursts, creating a wave motion in both chambers, as shown in Figure 3.1 (b). The initial "step" pressure distribution splits: a shock wave propagates into the expansion chamber, while an expansion wave front traverses the compression chamber.

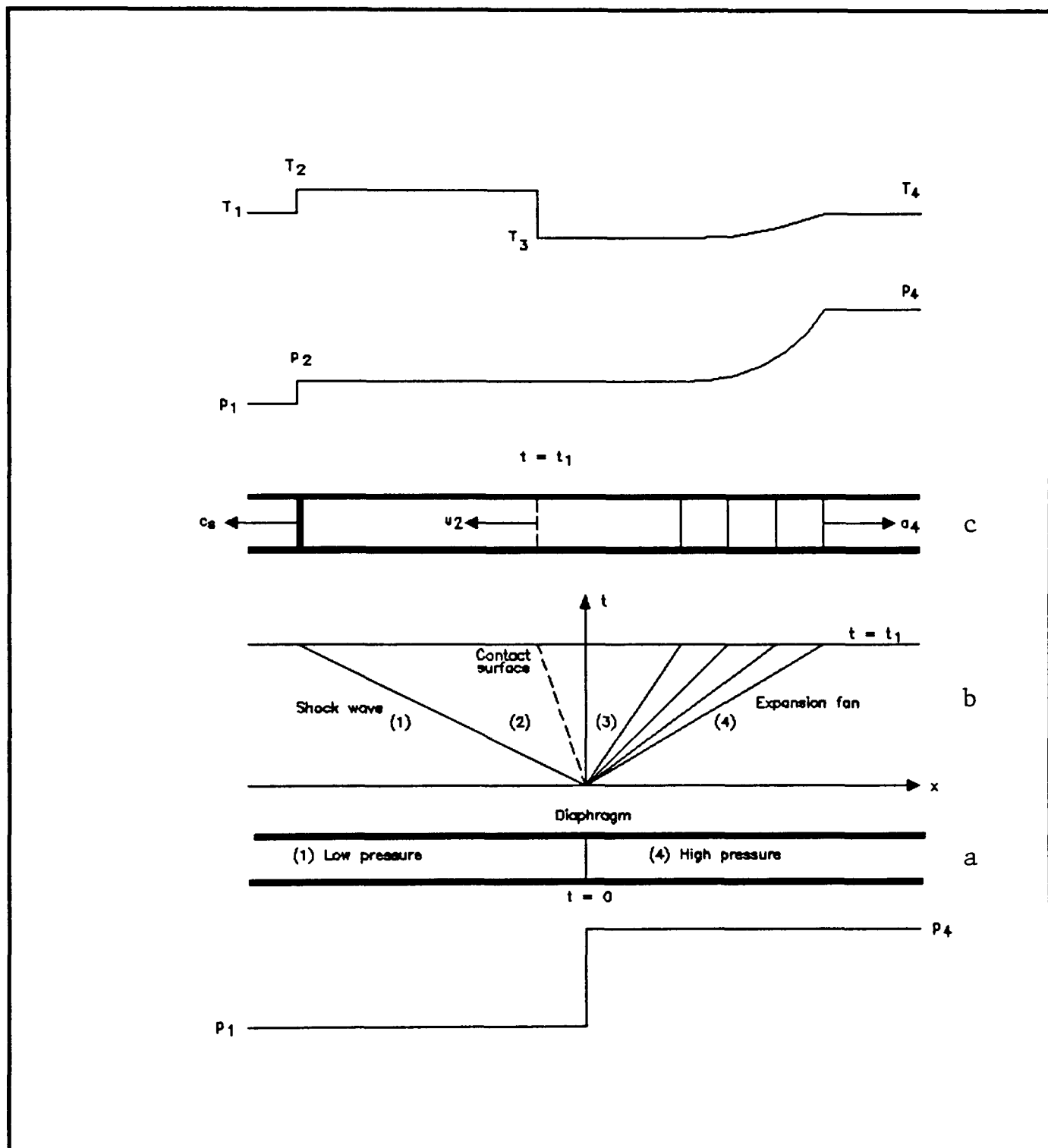


Figure 3.1 Schematic diagram of shock tube wave motion.

Figure 3.1 (b) also shows the position of the *contact surface*, which marks the boundary between the gases which were on either side of the diaphragm initially. Neglecting diffusion, gases 1 and 4 will remain separated by the contact surface throughout the expansion-compression process. The physical conditions of the gas through which the *shock wave* has traversed are denoted by a 2, whilst

those of the gas traversed by the *expansion wave front* are denoted by a 3. The theoretical pressure and temperature distributions at a later time ($t = t_1$) are shown in Figure 3.1 (c).

Because the contact surface is a free boundary, the following conditions are necessary:

$$p_2 = p_3 \quad (3.1)$$

$$u_2 = u_3 \quad (3.2)$$

The fluid velocities for shock and expansion waves are then used to derive the basic shock tube equation:

$$\frac{p_4}{p_1} = \frac{p_2}{p_1} \left[1 - \frac{(\gamma_4 - 1)(a_1/a_4)(p_2/p_1 - 1)}{\sqrt{2\gamma_1}\sqrt{2\gamma_1 + (\gamma_1 + 1)(p_2/p_1 - 1)}} \right]^{\frac{-2\gamma_4}{(\gamma_4 - 1)}} \quad (3.3)$$

This shows diaphragm pressure ratio, p_4/p_1 as a function of shock strength, p_2/p_1 . The pressure jump across a shock is a common definition of shock strength; it is related to the shock Mach number, M_s , as :

$$\frac{p_2}{p_1} = 1 + \frac{2\gamma}{\gamma + 1}(M_s^2 - 1) \quad (3.4)$$

or, rearranging:

$$M_s = \left[\frac{\gamma - 1}{2\gamma} + \frac{\gamma + 1}{2\gamma} \frac{p_2}{p_1} \right]^{1/2} \quad (3.5)$$

Equation (3.5) demonstrates that shock speed in a shock tube is related to pressure jump across the shock, p_2/p_1 . Equations (3.3) and (3.5), which result from the Rankine-Hugoniot relations, are shown graphically in Figure 3.2 for diaphragm pressure ratios of interest in the current study.

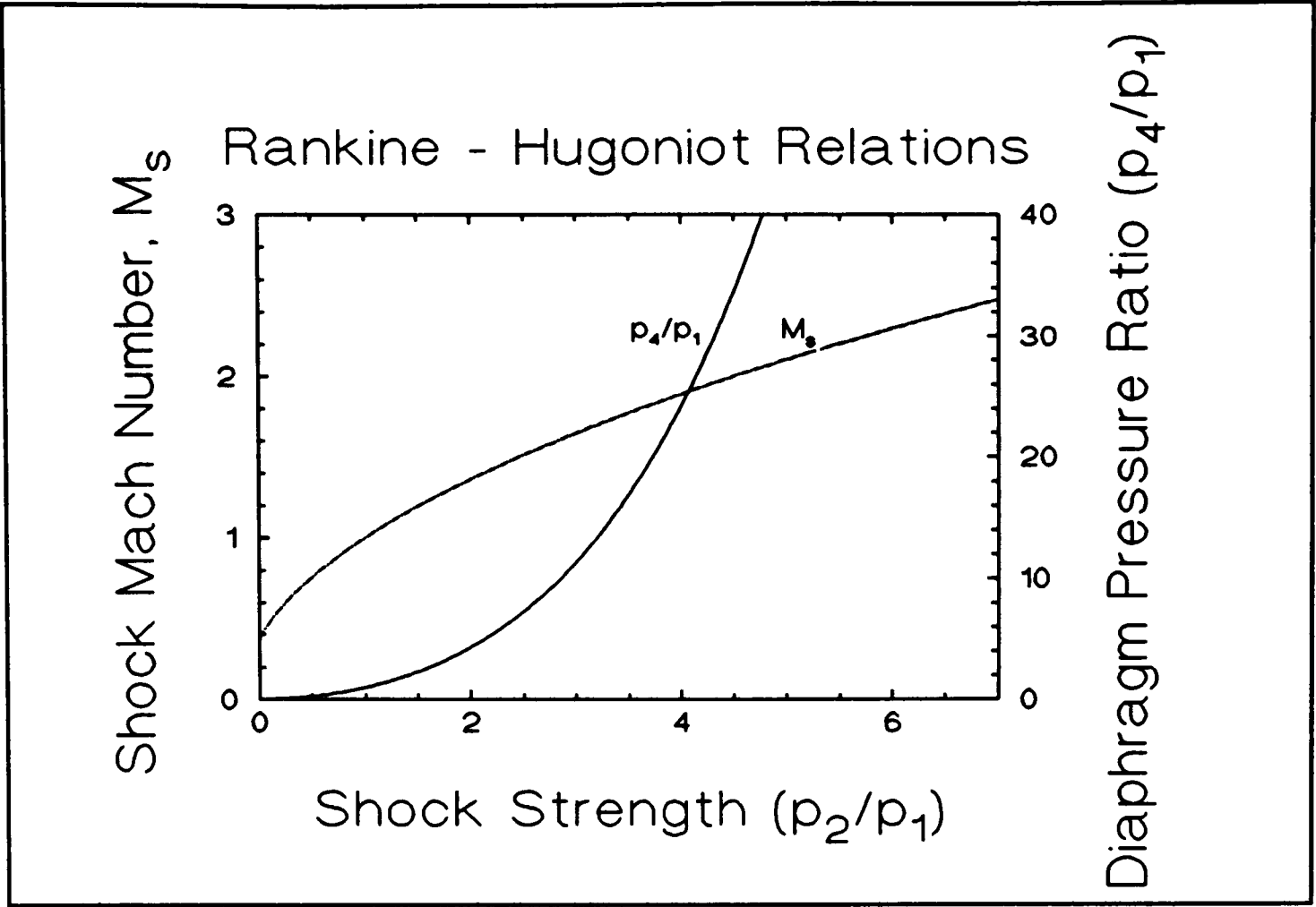


Figure 3.2 Theoretical relationship between diaphragm pressure ratio, shock Mach number, and shock strength for air ($\gamma = 1.4$).

The variation in contact surface velocity, v_{cs} , as a function of shock strength may be derived by considering the following system:

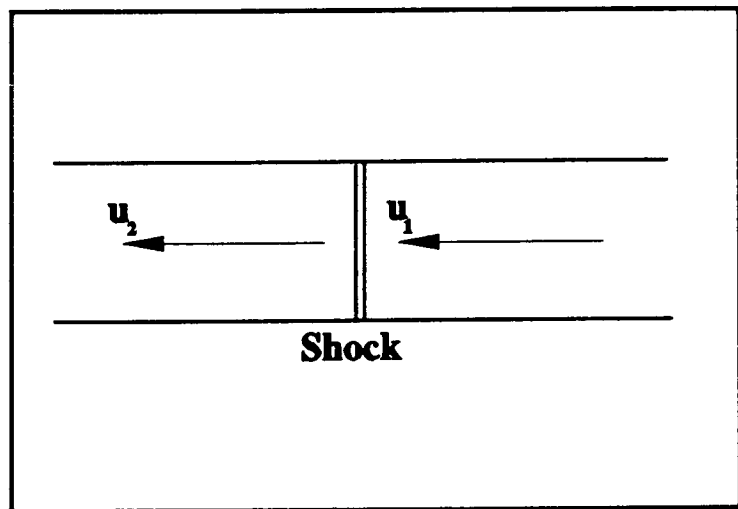


Figure 3.3 Shock stationary.

Here the shock is assumed for the moment to be stationary; the fluid in front of and behind the shock can be seen to travel at the velocities shown. The density ratio across the shock is related to the velocity variation through the continuity equation and the following Rankine-Hugoniot relation:

$$\frac{\rho_2}{\rho_1} = \frac{1 + \frac{\gamma+1}{\gamma-1} \frac{p_2}{p_1}}{\frac{\gamma+1}{\gamma-1} + \frac{p_2}{p_1}} = \frac{u_1}{u_2} \quad (3.6)$$

In the above system $v_s = M_s a_1$ and $v_{cs} = u_1 - u_2 = v_s(1 - u_2/u_1)$. Substituting with (3.5) and (3.6), contact surface velocity can be shown to be:

$$v_{cs} = a_1 \left(\frac{p_2}{p_1} - 1 \right) \sqrt{\frac{2/\gamma}{(\gamma+1)p_2/p_1 + (\gamma-1)}} \quad (3.7)$$

The variation in contact surface velocity with diaphragm pressure ratio for air ($\gamma = 1.4$) and helium ($\gamma = 1.67$) is shown in Figure 3.4.

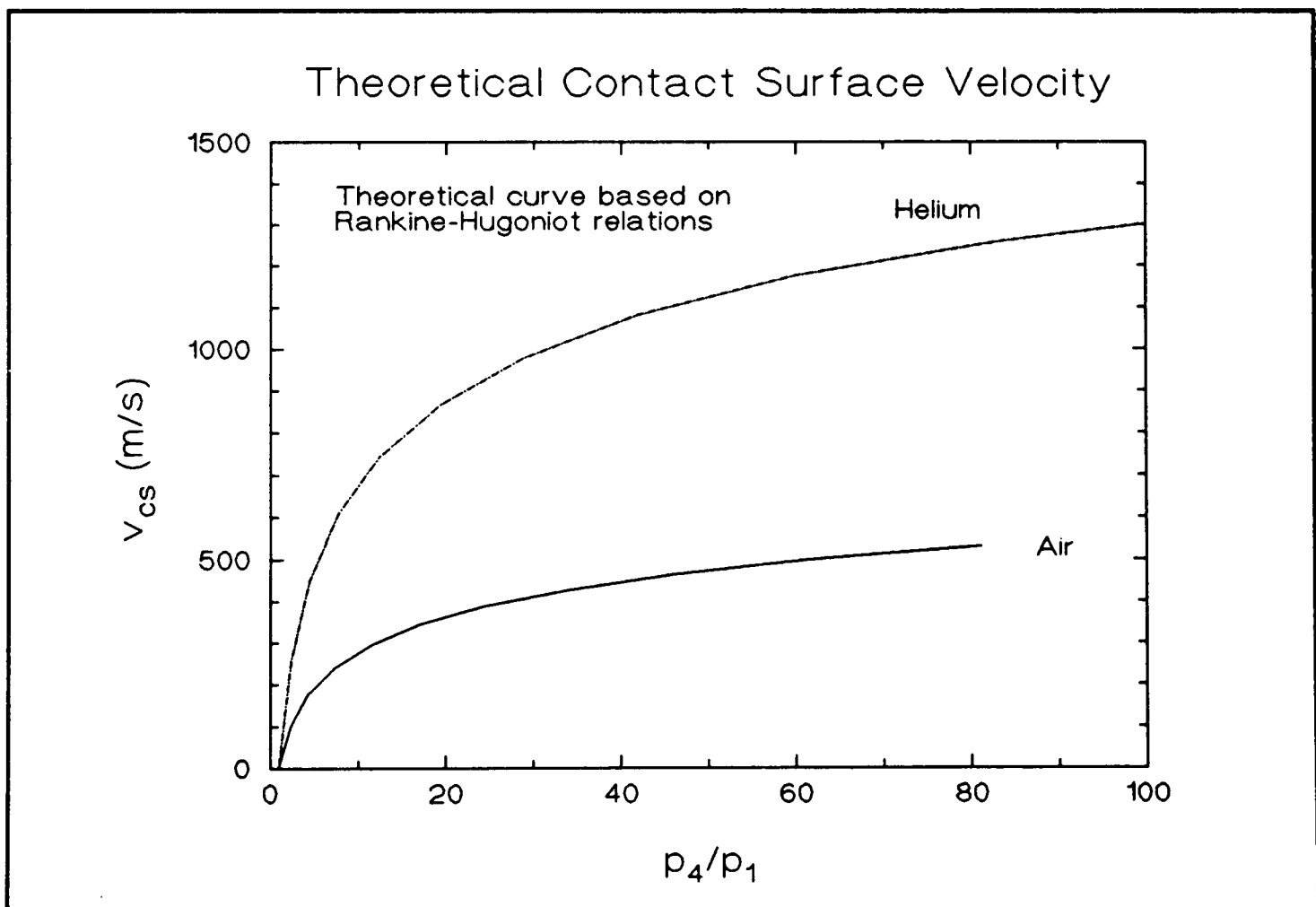


Figure 3.4 Contact surface velocity as a function of diaphragm pressure ratio.

3.2.2 Numerical Shock Tube Calculations

A series of numerical calculations of the transient operation in the nozzle section of a shock tube were made. The author is indebted to Ken Badcock of the Oxford University Computing Laboratory who made the calculations. The objectives of this work were: (1) to examine numerically the transient nature of flow within the system, (2) to provide a full description of the flow field, and (3) to provide a basis for comparison with experimental data.

The numerical method used is based on the thin layer Navier-Stokes equations and uses a discrete finite difference scheme. The method is implicit across the tube and explicit along the tube, permitting resolution of the boundary layer. A brief outline of the computational method can be found in Badcock [1991].

The actual nozzle geometry was input into the program. It was found to be necessary, however, to start the calculations at the throat section of the nozzle, thereby eliminating numerical difficulties in calculating the flow field in both a convergent section and divergent section simultaneously. The overall effects of neglecting the convergent section are thought to be minimal, especially in the later stages of the divergent section, the region of greatest importance in the current study. The running conditions that were modelled are shown below:

	Run #1	Run #2	Run #3
Gas:	Air	Air	Air
p_o (bar abs.):	2.7	6.8	77.0
p_∞ (bar abs.):	0.1	0.1	1.0
p_o/p_∞ :	27.0	68.0	77.0

This represents a range of pressure ratios relevant to the operation of the system in biological bombardment experiments.

The results of the runs show that numerically the effect of boundary layer growth is negligible. This can be seen in Figure 3.5, which shows the velocity field for Run #1. Although some separation is apparent near the throat, the boundary

layer displacement thickness is seen to be minimal at the nozzle exit. This confirms the calculated results for transient boundary layer growth of Mirels [1966] (see Appendix A). Although the numerical mesh size appears somewhat coarse (especially near the nozzle surface), these numerical findings tend to show that boundary layer growth is not significant for the biological conditions under study here. Whether these computational results for boundary layer growth are physically-significant or are artifacts of poor mesh sizing is unclear. Also, it should be noted that shorter arrows at the final downstream x-location were caused by the preliminary abortion of the numerical routine and are of no physical significance.

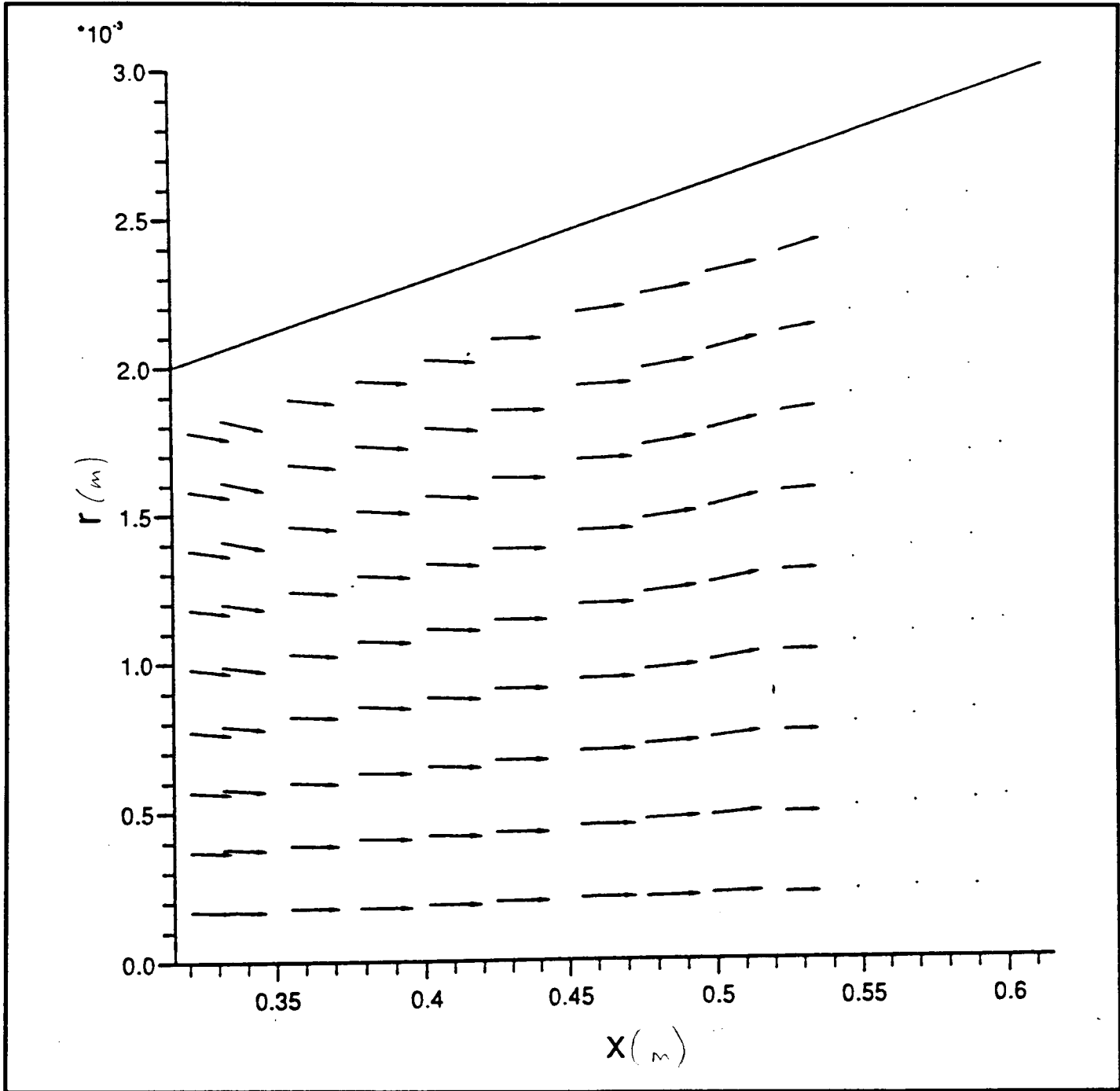


Figure 3.5 Calculation of velocity field for Run #1.

The axial pressure, temperature and velocity distributions at a time 350 μ secs after membrane rupture are shown in Figure 3.6 a,b,c, respectively. From the axial pressure distribution one sees the corresponding positions of the shock waves and expansion fans for the three run conditions. These shock and expansion positions are labelled for Run #3 in Figure 3.6 (a). From examination of Figure 3.6, several points should be emphasised. The notes which follow refer to Run #3, because its variation is most pronounced in the figure. The "snapshot" of the shock in Figure 3.6 (a) shows *not* the abrupt change in pressure that is expected theoretically but shows instead a more gradual increase. The temperature jump across the shock in Figure 3.6 (b) is also shown to be gradual rather than abrupt. These discrepancies are most likely numerical artifacts. As expected theoretically, $p_2 = p_3$. The position of the contact surface is shown, located where the temperature rises to its maximum value behind the shock. The decrease from T_2 to T_3 in Figure 3.6 (b) is again more gradual than theory would predict (see Figure 3.1), and again program numerics are hypothesised as the reason. Figure 3.6 (c) shows a "snapshot" comparison of the axial velocity distributions and displays, among other things, the velocities of the contact surface for each of the runs. The results show that $u_2 = u_3$, as theory predicts.

Figure 3.7 a,b,c show the transient variation in pressure at three points along the nozzle for the three run conditions, respectively. These points correspond to locations of static pressure taps on the experimental nozzle. The distances of each tap from the nozzle throat are: Port 1, 150mm; Port 2, 200mm; and Port 3, 250mm. Note that as the shock tube pressure ratio, p_o/p_∞ , (or, using the terminology of Liepmann and Roshko [1957], p_4/p_1) increases, the pressure jump across the shock (p_2/p_1) also increases. Similarly, the shock Mach number increases.¹ The values of p_2/p_1 and M_s so calculated from the numerical results are replotted with those resulting from the Rankine-Hugoniot relations (Figure 3.2) to show theoretical consistency. This is shown in Figure 3.8.

¹ M_s can be calculated from Figure 3.7 by measuring time difference between successive pressure rises and dividing by appropriate distance.

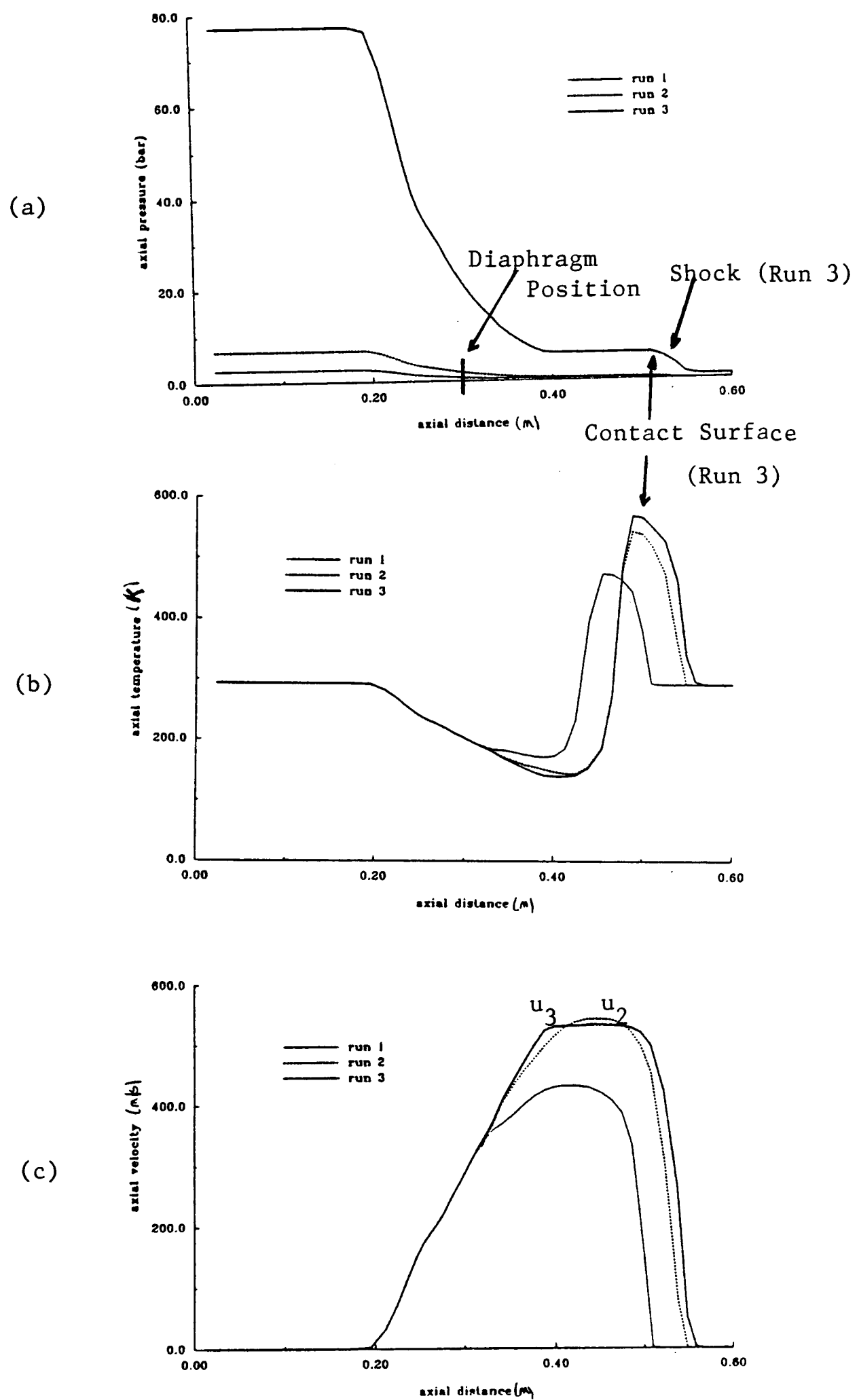


Figure 3.6 Computed axial distributions of (a) pressure, (b) temperature, and (c) velocity for 3 conditions at 350 μ s after membrane rupture.

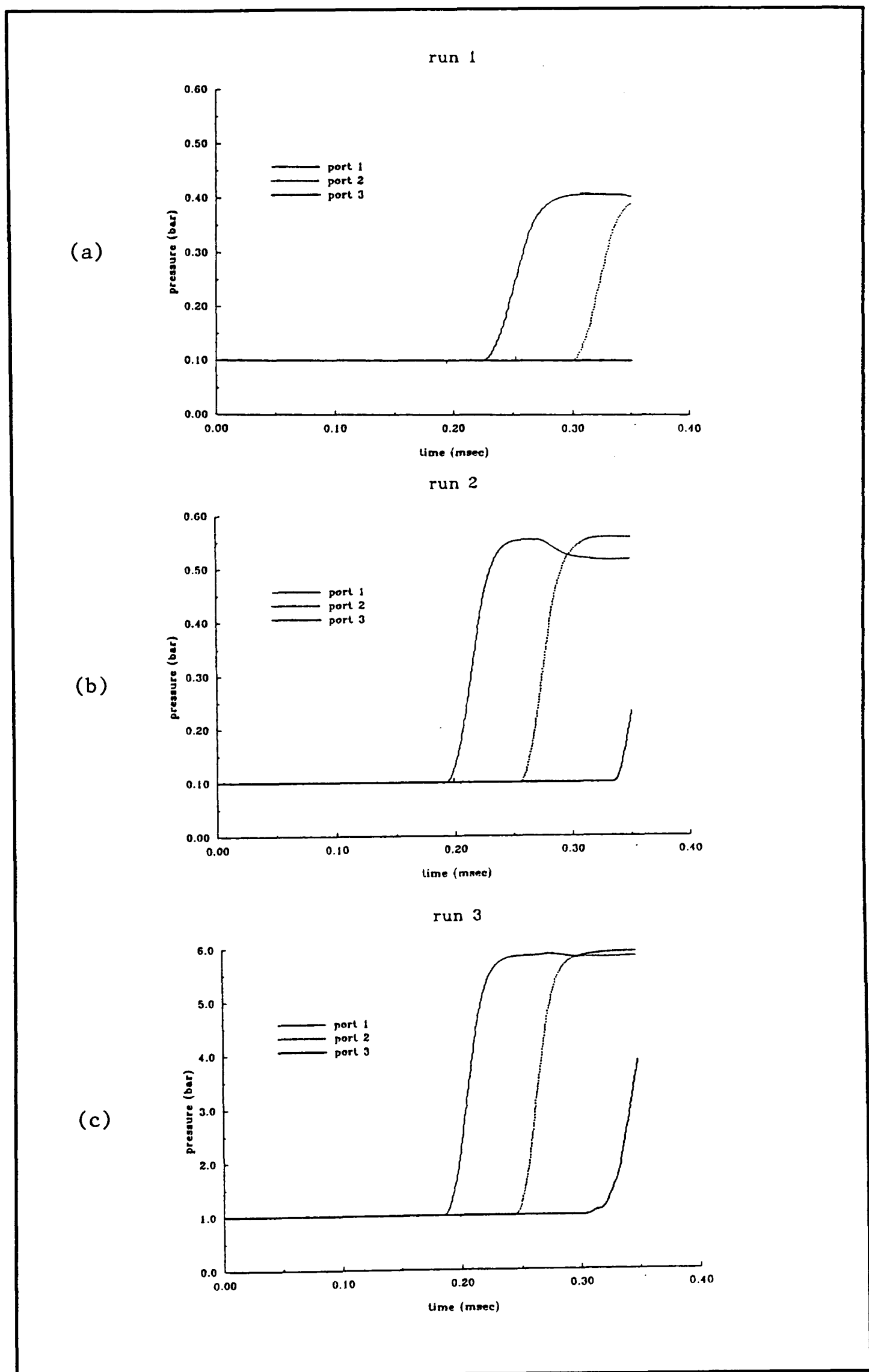


Figure 3.7 Computed transient variation in pressure at three axial locations for three run conditions (from Badcock [1992]).

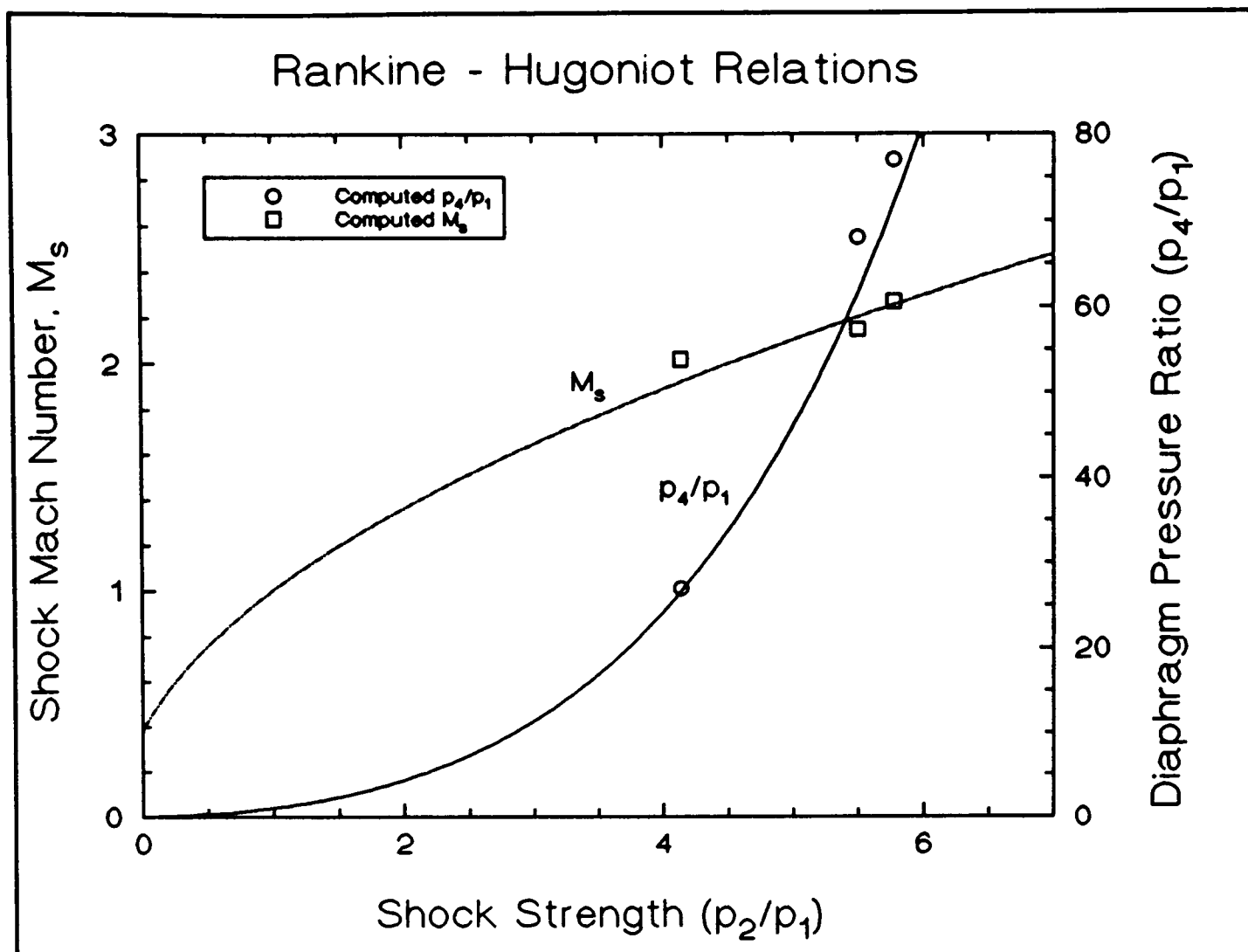


Figure 3.8 Comparison of computed values of p_2/p_1 and M_s with Rankine-Hugoniot relations, $\gamma = 1.4$ (computed values from Badcock [1992]).

3.3 Theory of Underexpanded Free Jets

Because of its importance in the understanding of jet and rocket performance, the flow behind underexpanded nozzles has been carefully studied over the past thirty years. A combination of theoretical, numerical, and experimental investigations has led to a better understanding of the structure of the flow field.

3.3.1 General Flow Field Structure

A diagram showing the essential features of the flow field structure generally observed behind an underexpanded axisymmetric nozzle is presented in Figure 3.9. The gas emerging from the nozzle exit goes through an expansion fan

which expands it to the ambient pressure (back pressure) of the jet boundary. The condition of constant pressure along the jet boundary forces the boundary to be bent back toward the axis of symmetry. This change in the flow direction along the boundary (which narrows the flow field) causes compression waves, developed at the intersection of the initial expansion waves with the jet boundary, to form in the flow. These compression waves coalesce to form the intercepting shock pictured in the diagram. The relative configuration of the intercepting shocks is dependent on the degree to which the nozzle is underexpanded. For slightly underexpanded nozzles, the intercepting shocks converge at the axis to form a diamond or *X* configuration. As the nozzle becomes more underexpanded, the intercepting shocks become too strong and no longer meet at the axis of symmetry but are connected through a normal shock (Mach disc), also pictured in Figure 3.9. In either case (Mach disc or no Mach disc) reflected shocks form which intersect with the jet boundary, reflecting as expansion waves, thereby repeating the whole process. Viscous effects gradually damp this process until it is no longer observed.

Previous investigators have observed that the degree to which an inviscid, supersonic nozzle is underexpanded depends on the nozzle pressure ratio, p_o/p_∞ . Because the nozzle exit pressure, p_e , is a function only of the nozzle area ratio (constant for a given geometry) and the stagnation pressure upstream of the throat, p_o , the nozzle pressure ratio can be related to the static pressure ratio at the nozzle exit, which is p_e/p_∞ . In general, a mismatch occurs between p_e and p_∞ . The situation in which p_e is greater than p_∞ is termed *underexpanded*.

The study of the underexpanded jet in the past has focused on calculation of the positions of the jet boundary and intercepting shock, the location of the first Mach disc, and the wavelength of the periodic structure. Ground-breaking experimental work conducted by Love and Grigsby at the NACA Langley Aeronautical lab in the Fifties found that for a jet exhausting into a static atmosphere, the important parameters influencing jet structure are the static pressure ratio at the nozzle exit (p_e/p_∞), the Mach number at the nozzle exit (M_e), and, to a small extent, the divergence angle of the nozzle (δ_e) (Love and Grigsby [1955], Love *et al.* [1959]). The influence of each of these on jet structure (periodic structure, first Mach disc location, and jet boundary location) will be

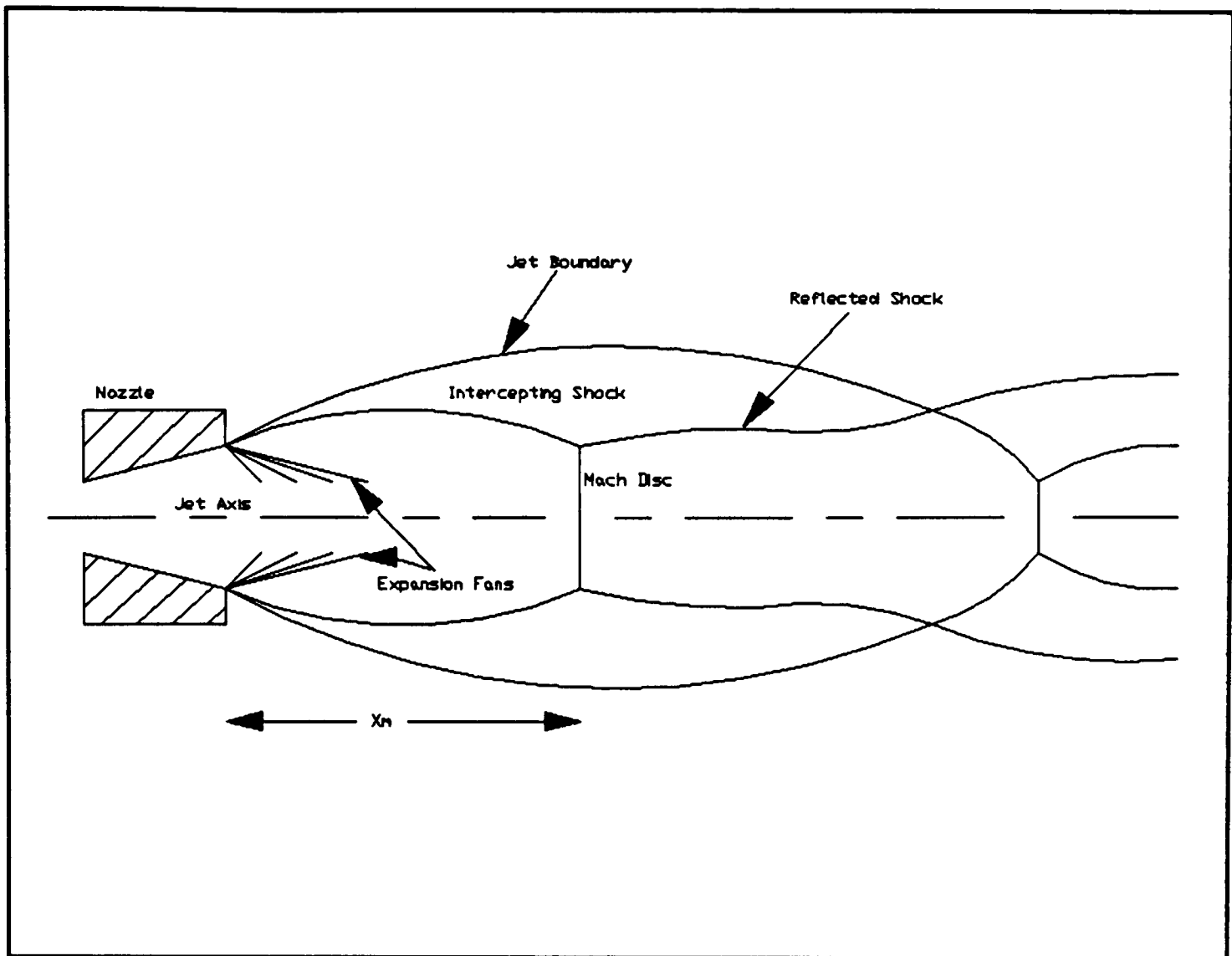


Figure 3.9 Diagram of jet structure behind an underexpanded nozzle.

reviewed in succeeding sections.

3.3.2 Jet Periodic Structure

A gaseous jet exhausting supersonically from an orifice or nozzle into still air or vacuum was known to display a periodic or "diamond" structure at least as early as Rayleigh [1880]. Osborne Reynolds [1886] made a significant advance with his discovery of a critical pressure ratio:

$$\frac{p_{\infty}^*}{p_o} = \left[\frac{2}{\gamma + 1} \right]^{\frac{\gamma}{\gamma + 1}} \quad (3.8)$$

Pressure ratios below this critical value cause the velocity of the gas at the narrowest part of the constriction to attain the local speed of sound. L. Mach [1897] was one of the first to study the initial section or period of a supersonic jet

in detail. Using schlieren and interferometric techniques, he showed that at low total pressures, p_o , the conical waves intersect to form an X configuration, but as p_o increases the shock waves become stronger, and a shock wave normal to the stream appears. This normal shock wave has since become known as a *Mach disc*.² Mach also found from quantitative evaluation of interferometric photographs that the air density as the jet emerges is, in general, higher than the ambient density. At large distances from the orifice, however, the jet density drops significantly below the atmospheric density.

Prandtl [1904] was probably the first to derive a theoretical relationship for the wavelength (or period) in an underexpanded free jet. His derivation³ modelled the wavelength of the periodic structure by perturbing the trivial case of matched pressure ($p_e = p_\infty$). Using linearised supersonic flow theory, Prandtl showed that:

$$U = vx + \Phi \quad (3.12)$$

where Φ is the perturbed velocity potential. Φ must satisfy the wave equation:

$$(M^2 - 1) \frac{\partial^2 \Phi}{\partial x^2} = \frac{\partial^2 \Phi}{\partial y^2} + \frac{\partial^2 \Phi}{\partial z^2} \quad (3.13)$$

where x is the coordinate parallel to the jet axis. This equation may be transformed into polar coordinates and the solution found from separation of variables, $\Phi = X(x) R(r)$. The wave equation can be solved for axially-symmetric flow using the Bessel function of the first kind:

$$\Phi = A \cos(\omega x) J_0(\omega r \sqrt{M^2 - 1}) \quad (3.14)$$

For constant pressure along the jet boundary ($r = \pm D/2$), the first zero of the Bessel function can be found to be:

²Mach used the term *gerader Verdichtungsstoss*.

³Prandtl's derivation was later translated by Birkhoff and Zarantonello [1957].

$$(\omega D/2)\sqrt{M^2-1} = \beta_1 \quad (3.15)$$

This gives:

$$\frac{\lambda}{D} = \frac{2\pi}{\omega D} = 1.3\sqrt{M^2-1} \quad (3.16)$$

since $\pi/\beta_1 = 1.3$ and $\lambda = 2\pi/\omega$.

Other researchers have utilised experimental techniques to study the periodic structure of underexpanded free jets. Emden[1899] used schlieren and shadowgraph visualisation techniques to develop an empirical relationship between "excess pressure" ($p_o - p_\infty$) and "cell" length, Δ , for a sonic jet exhausting into atmosphere.⁴ This relationship shows good agreement with an empirical correlation also developed by Prandtl [1904]. Hartmann and Lazarus [1941] used pitot measurements to determine axial pressure distributions for p_o up to 7 kg/cm². Axial pressure curves were obtained in jets exhibiting both the simple *X* configuration and the more complex normal shock interaction observed by Mach. The structure of corresponding jets, visualised using schlieren photography, showed good agreement with the pitot pressure distributions. Love *et al.* [1959] derived a semi-empirical relationship based on an extensive series of experiments using schlieren visualisation to evaluate effects of jet Mach number, nozzle divergence angle, and jet static-pressure ratio. The correlation of Love *et al.* [1959] is split into two parts at $p_e/p_\infty = 2$. This corresponds approximately to the pressure ratio at which the jet structure changes from an *X* configuration to that involving a normal shock. Correlations for initial dimensionless free jet wavelength are summarised in Table 3.1 and shown graphically in Figure 3.10.

The correlations for jet wavelength show substantial variation, although much of this variation arises from differences in experimental set-up. The three earliest correlations resulted from experimental observation of gas exhausting from *sonic orifices* ($M_e = 1$). The correlations of Love *et al.* [1959] and the data

⁴Nomenclature used by previous researchers will be modified to the standard adopted in this thesis.

of Wilcox et al. [1957], however, resulted from conditions of supersonic flow at the nozzle exit ($M_e > 1$). Supersonic exit conditions are most appropriate for comparison with results of the present study (see Chapter 4).

Table 3.1 Correlations for Free Jet Wavelength

Investigation	Dimensionless Wavelength, λ/d_e	Note
Emden [1899]	$0.89\sqrt{p-0.9}$	
Prandtl [1904]	$1.2\sqrt{p-0.9}$	
Hartmann, Lazarus [1941]	$0.30 + 0.98\sqrt{\frac{p_o - 1.96}{1.033}}$	
Love <i>et al.</i> [1959]	$1.55\sqrt{M_e^2\left(\frac{p_e}{p_\infty}\right) - 1 - 0.55\beta_e}$	$p_e/p_\infty < 2$
Love <i>et al.</i> [1959]	$1.52\left(\frac{p_e}{p_\infty}\right)^{0.437} + 1.55\left(\sqrt{2M_e^2 - 1} - 1\right) - .55\beta_e + 0.5\left[\frac{1}{1.55\sqrt{\left(\frac{p_e}{p_\infty} - 2\right)\beta_e - 1}}\right]$	$p_e/p_\infty \geq 2$

Note: $p = (p_o - p_\infty)/p_\infty$

$$\beta_e = (M_e^2 - 1)^{1/2}$$

3.3.3 Location of First Mach Disc

Because of the relative ease with which it could be experimentally visualised, the downstream location of the first Mach disc was also one of the first free jet properties to be characterised. Ashkenas and Sherman [1966] looked at

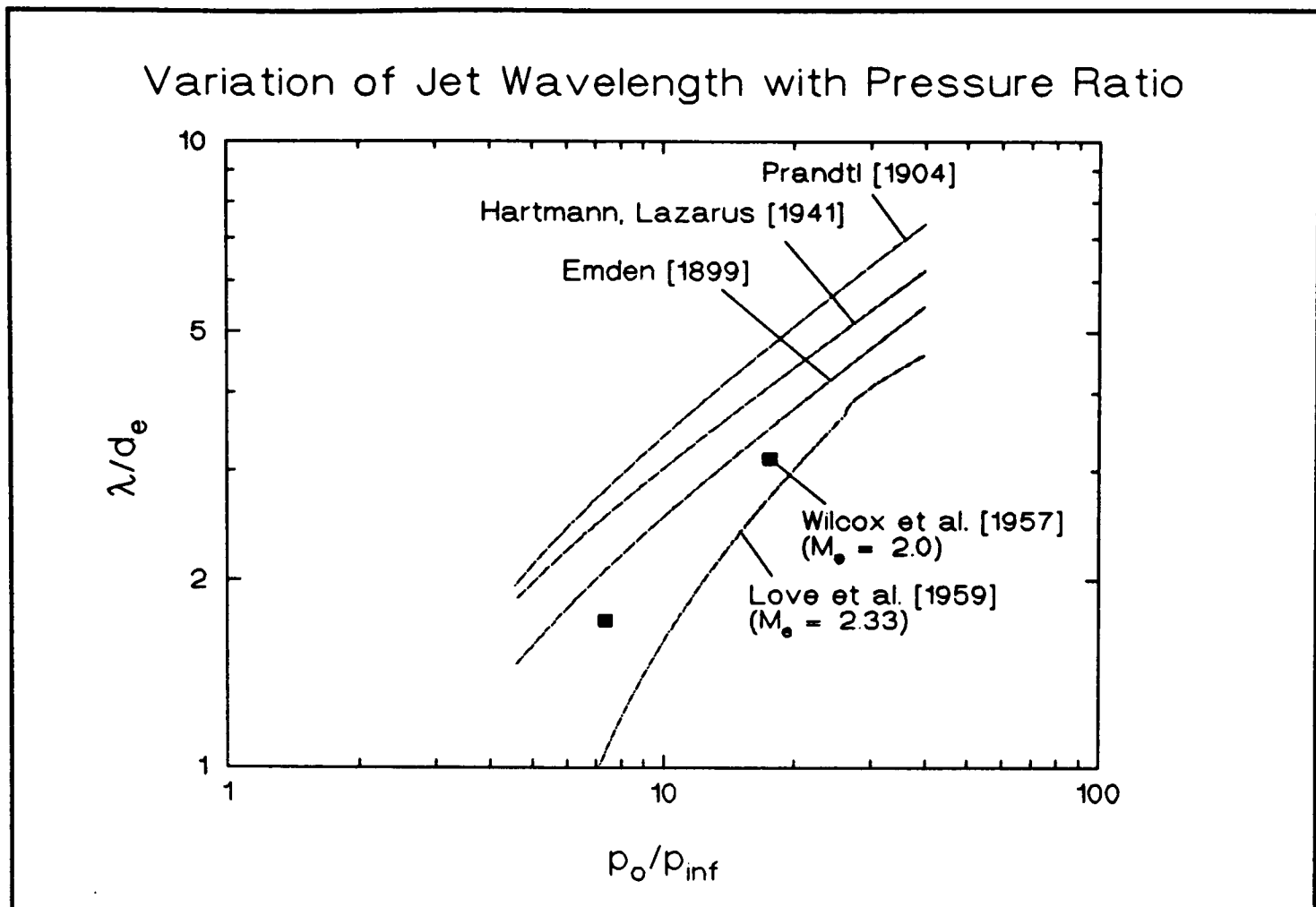


Figure 3.10 Correlations due to previous researchers for free jet wavelength.

axial impact pressure distributions for a wide range of pressure ratios and several gases exhausting from sonic orifices. They used the axial location of initial pitot pressure increase as the primary criterion for determination of Mach disc location. Crist *et al.* [1966] evaluated Mach disc location for underexpanded sonic jets with p_o up to 1000bar and T_o up to 4200°K for a variety of non-reacting gases. Initial Mach disc location was found to be insensitive to ratio of specific heats (γ), nozzle lip geometry, or absolute pressure level and was found to vary as the square root of the overall pressure ratio, p_o/p_∞ . The correlation shown for Love *et al.* [1959] was reduced by the author based on data presented in that report for $M_e = 2.0$. The correlations for first Mach disc location due to these researchers are summarised in Table 3.2 and shown graphically in Figure 3.11. The graph demonstrates good agreement among researchers for the nozzle pressure ratios of concern in the present study.

Table 3.2 Location of First Mach Disc

Investigation	Empirical Correlation, x_m/d_e	Note
Love <i>et al.</i> [1959]	$3.54 \log \left(0.2708 \frac{p_o}{p_\infty} \right)$	<i>Data</i>
Ashkenas, Sherman [1966]	$0.67 \sqrt{p_o/p_\infty}$	p_o/p_∞ 15-17,000
Crist [1966]	$0.645 \sqrt{p_o/p_\infty}$	

Note: $\beta_e = (M_e^2 - 1)^{1/2}$

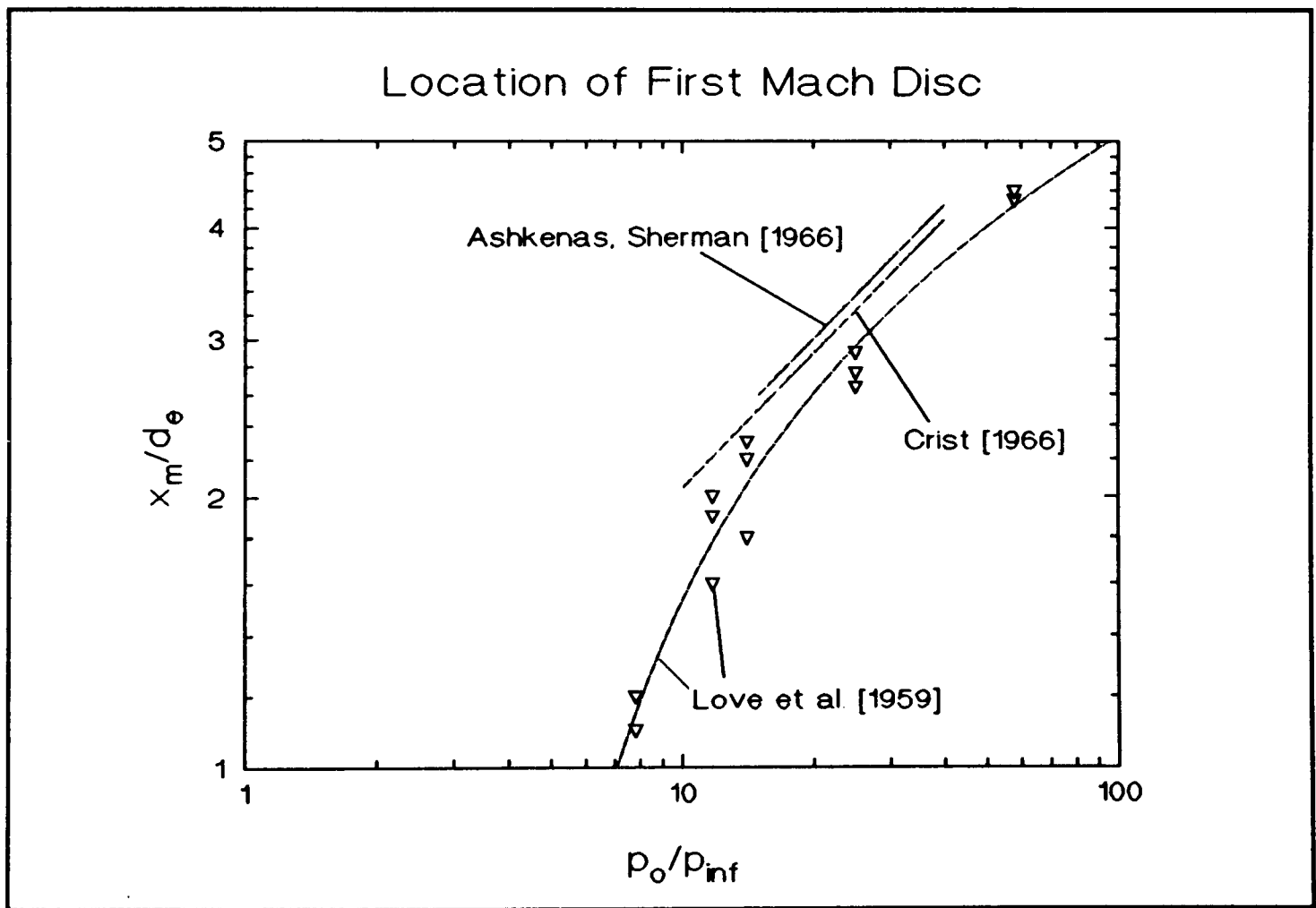


Figure 3.11 Correlations due to previous researchers for location of first Mach disc ($M_e = 2.33$).

Other researchers have used the numerical method of characteristics⁵ (MOC) or similar techniques to compute the flow fields of underexpanded free jets. These computed flow fields have then been used to predict initial Mach disc location. Adamson and Nicholls [1959] calculated the axial pressure distribution on the centreline of the flow using an approximate technique based on the assumption of a fictitious nozzle extension. They assumed that the Mach disc occurs at the point where ambient pressure, p_∞ , would be attained behind the shock (*i.e.*, assuming the shock exists at the end of the fictitious nozzle extension) along the jet axis. Eastman and Radtke [1963] made the assumption that the location of the Mach disc must coincide with a point of *minimum* pressure, using the MOC to generate flow field information. More recently, Chang and Chow [1974] assumed the location of the Mach disc to be governed by the appearance of a triple point shock configuration and the condition that the central core flow reaches "choking at the throat". They iterated upon Mach disc location until the slipline (between subsonic and supersonic flows behind the Mach disc) was such that sonic conditions were reached at a point of minimum area. Using this technique and the MOC, they obtained theoretical results which agreed well with the earlier experimental data of Love *et al.* [1959].

3.4 Calculation of Jet Boundary Location

Calculation of the location of the real jet boundary as the jet expands from the nozzle is important in understanding the performance of the apparatus described in **Chapter 2**. It will be shown later that control of this expansion leads to control of particle trajectory.

The inviscid supersonic flow expanding from a diverging axisymmetric nozzle remains an unsolved problem in potential flow theory. It has been shown, however, that the details of flow in the nozzle exit region have little influence on the flow field in the region more than one nozzle diameter downstream (Ladenburg *et al.* [1949], Ashkenas and Sherman [1966]). Most successful theoretical treatments of the supersonic region have been based on the method

⁵See Appendix C for a general description of the method of characteristics.

of characteristics, which yields surprisingly accurate flow information at a number of discrete mesh points. Many investigators have calculated the ideal expansion from underexpanded nozzles using various method of characteristics techniques (Kelber and Jarvis [1952], Love and Grigsby [1955] Love *et al.* [1959]). However, approximate methods for calculating the expansion of the jet boundary might provide initial insight. One such method, after Adamson and Nicholls [1959], will be used to calculate jet boundary expansion for various nozzle conditions. These results will then be compared with results of a method of characteristics calculation for similar nozzle conditions. It should be emphasised that in both cases, only the *ideal* expansion will be evaluated. This should, however, give a good approximation to the *real* expansion, despite ignoring the effects of a viscous mixing region along the jet boundary. Only experimental evidence for the particular nozzle under study will determine the attributes of the actual expansion.

3.4.1 Approximate Methods

Several researchers have developed approximate techniques for predicting jet boundary location of underexpanded jets. Cain [1991] has provided a useful review of the more important of these approximate methods. Inviscid techniques can generally be divided into two main categories: 1.) those applying a Newtonian pressure-plus-centrifugal correction to a source-flow model and 2.) those assuming a fictitious "nozzle-extension". While the first of these categories is generally thought to provide a better physical model of jet expansion, particularly at high jet Mach numbers and high levels of nozzle exit pressure ratio, p_e/p_∞ , the second category of inviscid techniques will prove useful for conditions of interest in the current study.

In the first of the above categories, Albini [1965] and Hubbard [1966] have proposed an approximate method of determining jet boundary location for a jet exhausting axially into a moving stream of air.⁶ Because their technique assumes inviscid flow, calculation is required of a dividing streamline between the mass flow exhausting from the nozzle and that originating in the free stream. The

⁶The analysis described herein remains valid for $M_\infty = 0$ (*i.e.*, no free stream flow).

location of this dividing streamline is found by equating the ambient pressure on the boundary to the sum of the Newtonian core flow pressure and that pressure necessary to balance the centrifugal acceleration of the shock layer gas as it curves along the intercepting shock. Cain [1991] has used a source flow model and approximation due to Boynton [1967] for the density variation along a radial streamline to show that the Albini/Hubbard model leads to:

$$p_{\infty} = \rho_e u_{\infty}^2 \sin^2 \phi + \int_0^{\delta} \frac{\rho u^2}{R_c} dy \quad (3.19)$$

where ϕ is the angle between the intercepting shock and upstream streamline, R_c is the radius of curvature of the dividing streamline, and δ is the inner shock layer thickness. Albini's method varies from Hubbard's in that Albini's method assumes u to be constant across the shock layer and equal to the local tangential component of jet velocity at the intercepting shock. In Hubbard's method, u is assumed to be constant along any streamline in the shock layer and equal to the tangential component of the jet velocity at the point where it crossed the intercepting shock. Both methods have shown good agreement with experimental data, especially for high jet Mach numbers ($M_e > 3$).

Adamson and Nicholls [1959] have developed a straight-forward approximate technique which provides insight into the physics of the expansion at the nozzle lip. The method assumes constant pressure along the jet boundary. A stream tube along the jet boundary is assumed to form a "nozzle extension". Infinitesimal increases in flow area lead, through isentropic relations, to infinitesimal decreases in pressure. Because pressure along the jet boundary must remain constant, this decrease in pressure is balanced by an equivalent pressure increase, occurring through an infinitesimal change in flow angle. A more complete description of the technique can be found in **Appendix B**. It should be emphasised that this method is only valid for small area increases and small changes in flow direction. Therefore, the method should only be applied for relatively low exit pressure ratios ($p_e/p_{\infty} < 10$).

This approximate technique was used to calculate jet boundary location for a range of pressure ratios, nozzle divergence angles and exit Mach numbers. The

technique was implemented using a LOTUS 1-2-3 spreadsheet program. A Newton-Raphson algorithm was utilised to find Mach number as a function of area ratio. Results were verified with calculations presented by Adamson and Nicholls [1959]. The results for air at $M_e = 2.33$ and $\delta_e = 0^\circ$ are shown in Figure 3.12. This shows the increase in jet boundary radius as jet pressure ratio increases. This is to be expected as the level of pressure mismatch at the nozzle exit largely determines jet expansion. Other factors which affect jet expansion include nozzle divergence angle, δ_e , and nozzle exit Mach number, M_e . The effects of changes in these parameters on jet expansion are shown in Figure 3.13 and Figure 3.14, respectively. Figure 3.13 shows that jet boundary radius increases as nozzle divergence angle increases. Figure 3.14 shows that increasing nozzle exit Mach number causes decreased jet expansion. In both cases, jet pressure ratio is held constant. Although the increase in boundary radius with nozzle divergence angle is somewhat intuitive, the variation with Mach number may not be so. The decrease in jet boundary radius with increasing Mach number is due to increasing momentum flux in the direction parallel to the jet axis. Increased momentum flux in this direction exceeds any increases in momentum flux in directions perpendicular to the jet axis, thereby causing the jet to lengthen. It should be remembered that with this approximate method of calculating the inviscid jet boundary, only the diverging part of the flow is considered. As $\psi \rightarrow 0$, i.e., as the boundary tends to become parallel to the jet axis, $1/\tan \rightarrow \infty$ and $x \rightarrow \infty$.⁷

⁷Indeed, spreadsheet memory restrictions limit r/r_e to values less than 2.15.

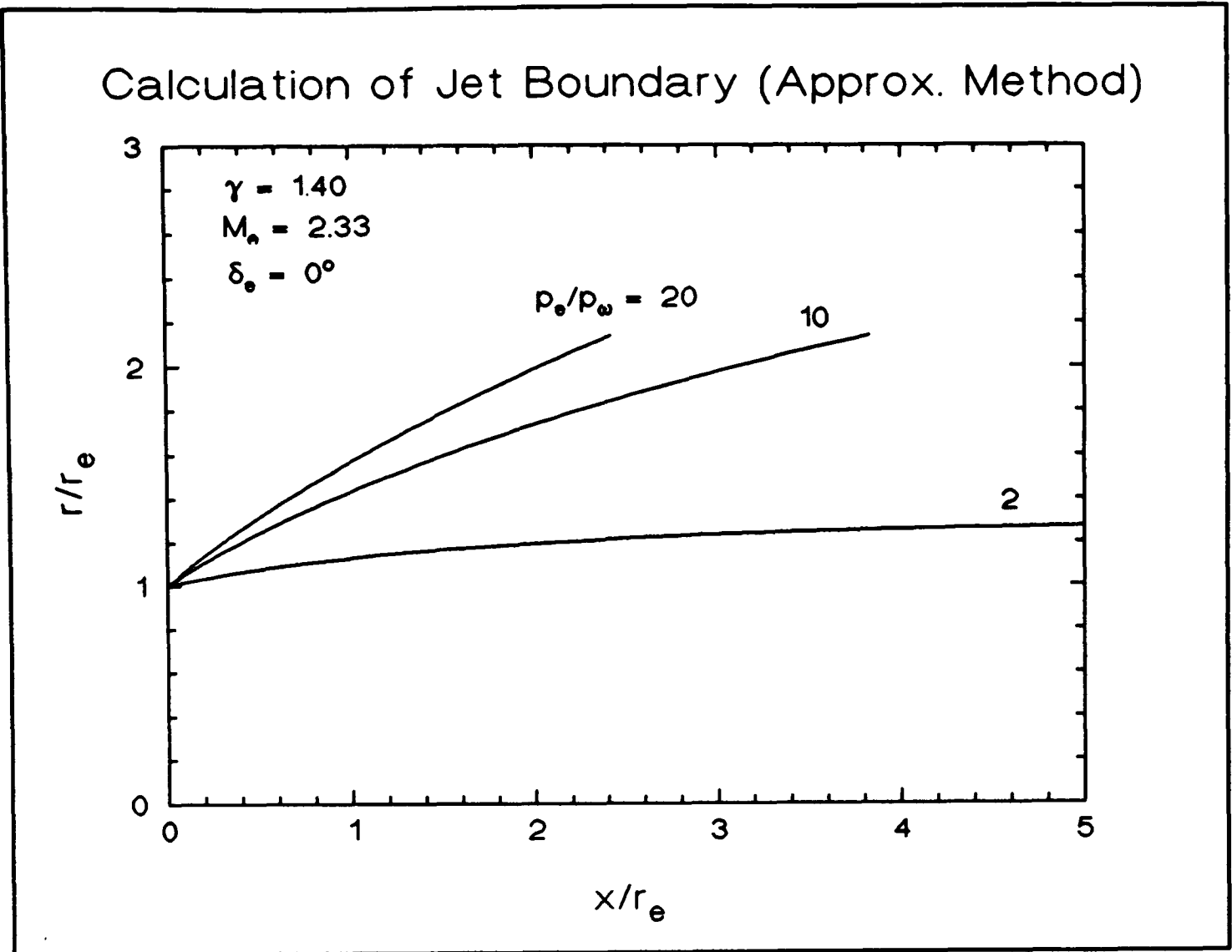


Figure 3.12 Comparison of jet boundaries calculated using an approximate method after Adamson and Nicholls [1959] for a range of jet pressure ratios.

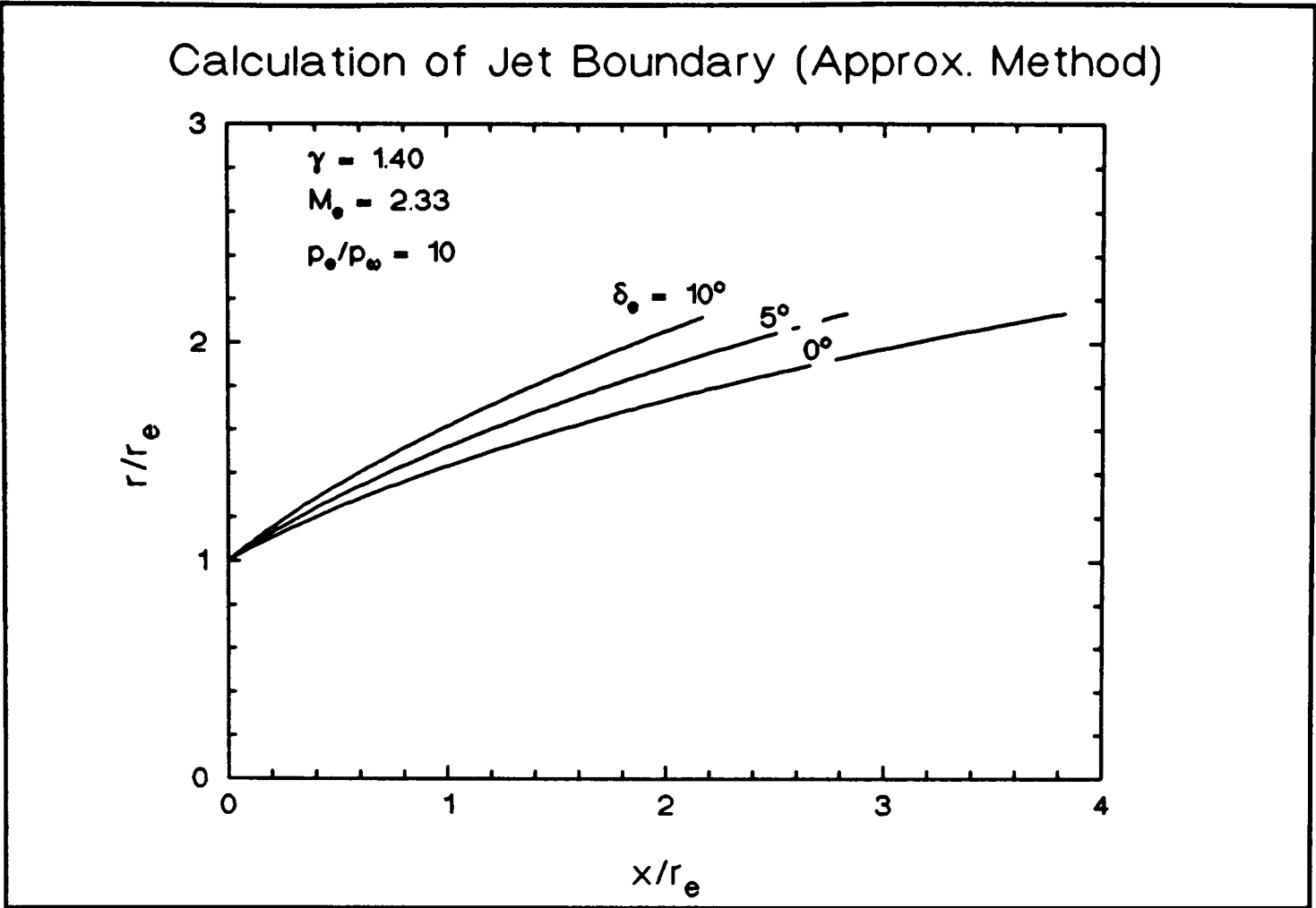


Figure 3.13 Comparison of jet boundaries calculated using an approximate method after Adamson and Nicholls [1959], for a range of nozzle divergence angles.

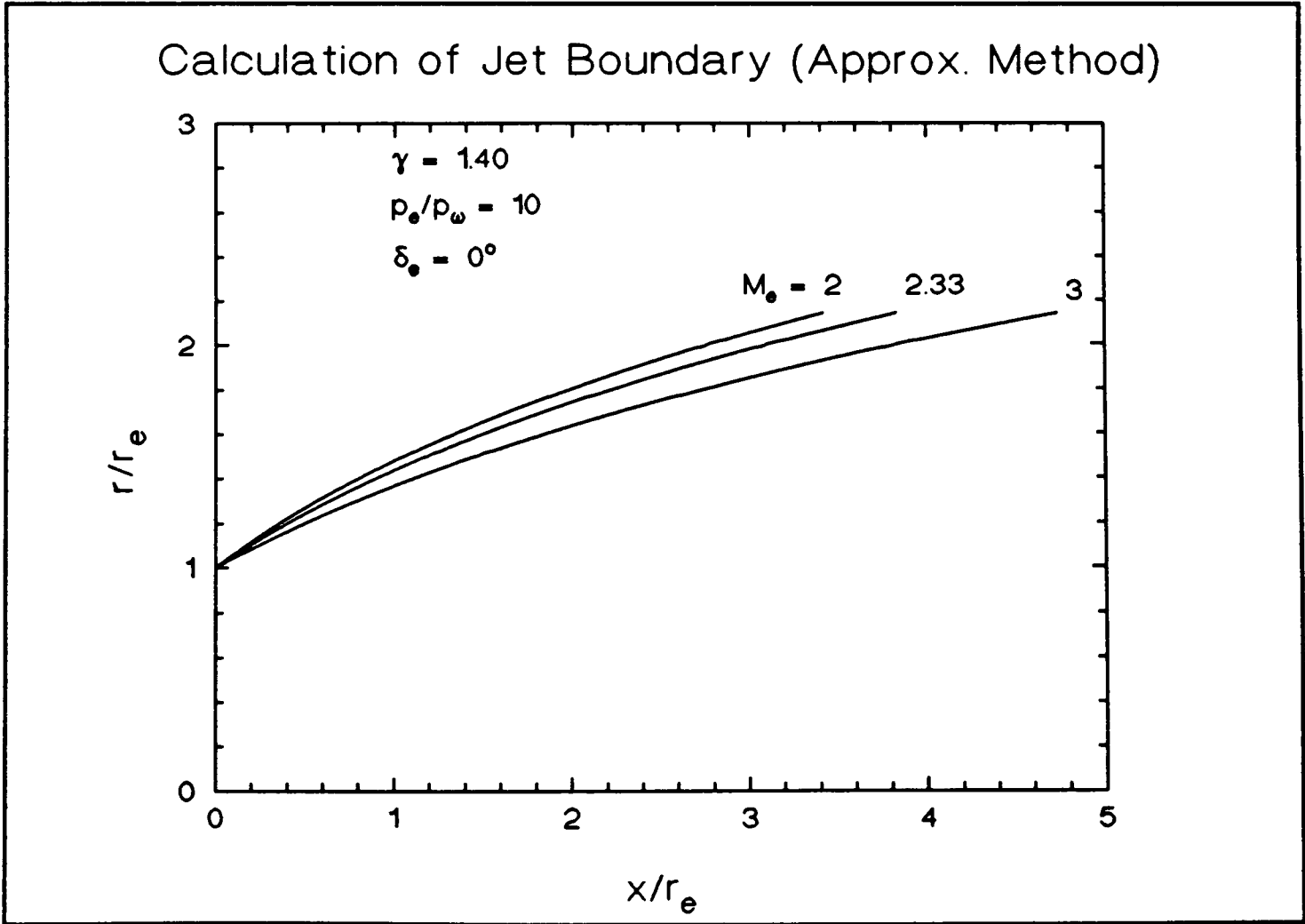


Figure 3.14 Comparison of jet boundaries calculated using an approximate method after Adamson and Nicholls [1959], for a range of exit Mach numbers.

3.4.2 Axisymmetric Method of Characteristics Calculation

A second more exact method of computing the ideal boundary location of an underexpanded free jet involves the method of characteristics (MOC). Owen and Thornhill [1948] were among the first to carry out an entirely numerical MOC computation. They calculated the flow field for a steady supersonic air jet exiting from a slightly supersonic circular orifice into a vacuum. Their calculated axial pressure distribution showed good agreement with pitot pressure measurements of Hartmann and Lazarus [1941] and interferometric measurements of Ladenburg *et al.* [1948].

Love *et al.* [1959] carried out an exhaustive series of jet boundary calculations using the MOC to evaluate effects of p_e/p_∞ , M_e , γ , and δ_e on jet expansion from axisymmetric convergent-divergent nozzles. Some of the conclusions they reached were:

- a.) Jet expansion increases monotonically with increasing jet pressure ratio, p_e/p_∞ .
- b.) Initial boundary inclination, α , decreases with increased M_e . However, maximum jet diameter increases and axial location of maximum diameter moves downstream from jet exit plane.
- c.) Initial boundary inclination decreases with increasing γ . Furthermore, maximum free jet diameter decreases and axial location of maximum diameter moves closer to the exit plane.
- d.) Initial boundary inclination increases with increasing nozzle divergence angle, δ_e . In addition, maximum jet diameter increases and axial location of maximum diameter moves closer to jet exit plane.

Since the underlying theory of the method of characteristics is well-known (see Ferri [1949] or Liepmann and Roshko [1957]), only a brief outline of the main features is necessary. This is presented in **Appendix C**. The method assumes inviscid, isentropic flow from an axisymmetric convergent-divergent

nozzle. For a given geometry, the exit Mach number is easily determined from supersonic gas tables (see, for example, NACA TN 1428). Similarly, if the upstream total pressure, p_o , is known, the supersonic gas tables provide the nozzle exit pressure, so that p_e/p_∞ is known *a priori*.

The axisymmetric method of characteristics was implemented for a set of conditions relevant to the system under study ($M_e = 2.33$, $p_e/p_\infty = 2$, $\delta_e = 0^\circ$). Due to symmetry only one-half of the flow-field was considered. Figure 3.15 shows the resulting characteristics net. The positions of the lattice points shown were not known *a priori* but were determined step-by-step using a LOTUS 1-2-3 spreadsheet program.

The results of the characteristics calculation were compared with results of an MOC calculation presented by Love *et al.* [1959] for similar flow conditions. As shown in Figure 3.16, the current MOC calculation ($M_e = 2.33$) falls between the two relevant curves in the results of Love *et al.*: $M_e = 2.0$ (top curve) and $M_e = 2.5$ (bottom curve). This gives confidence that results from the MOC technique described above are genuine.

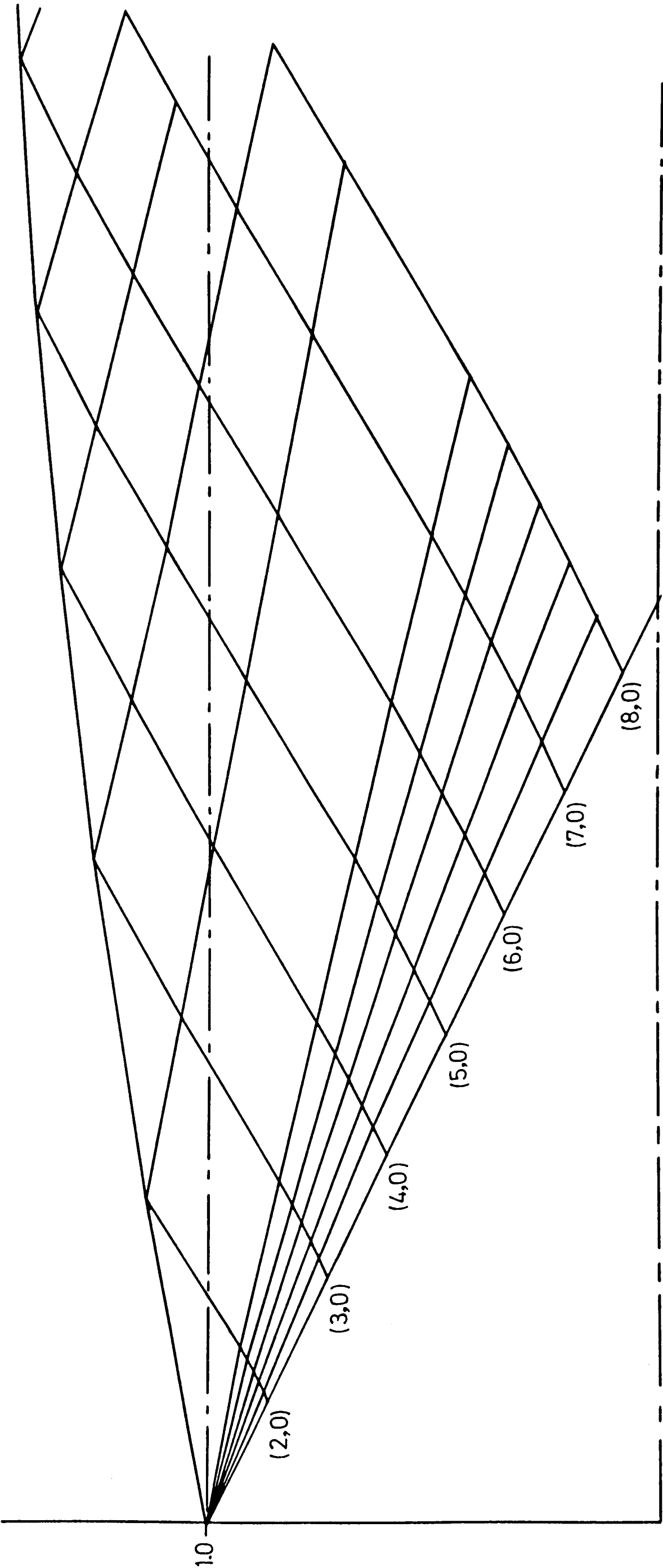


Figure 3.15
 Characteristics net for jet boundary calculation ($M_e=2.33$, $p_e/p_{inf}=2$, $\delta_e=0^\circ$).

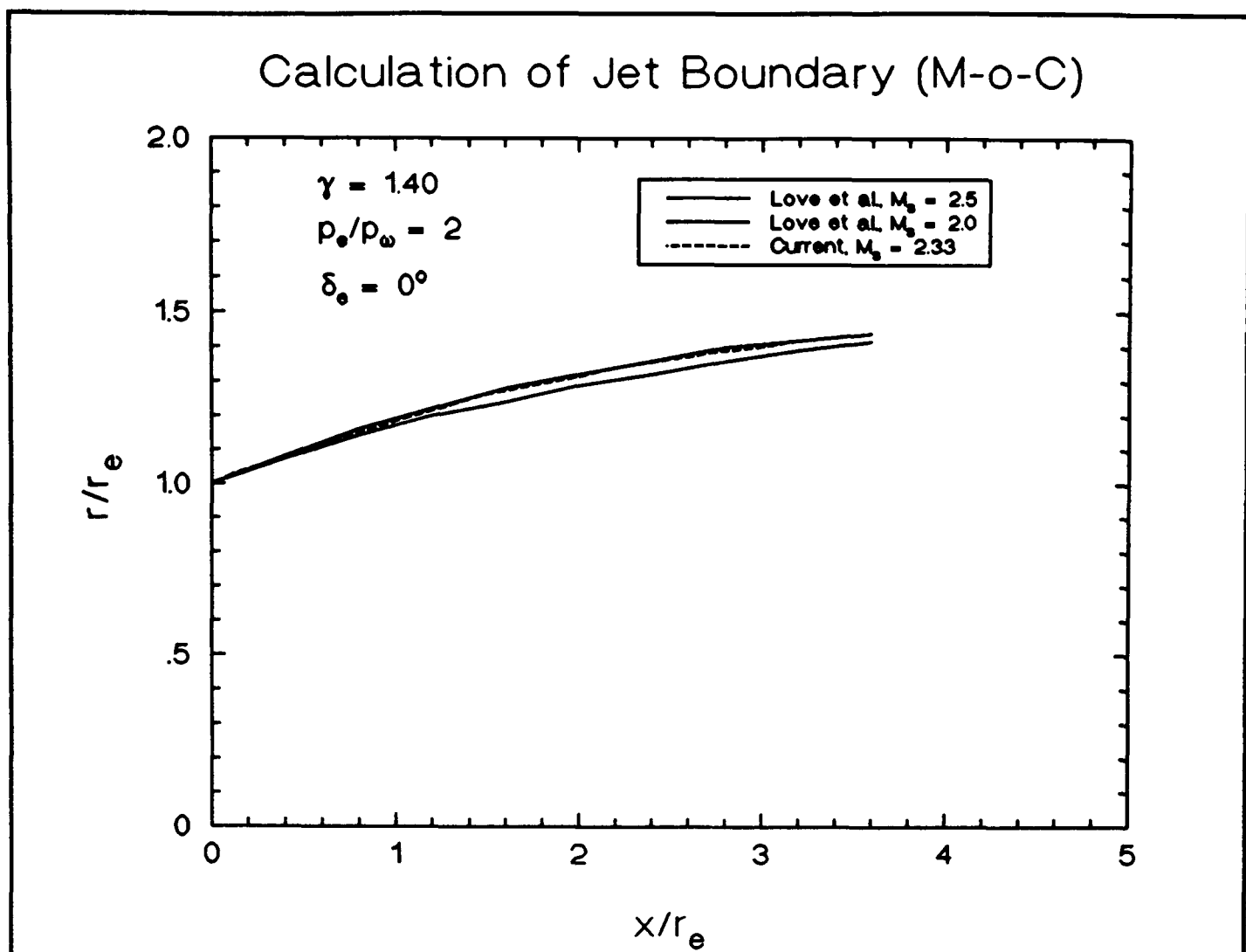


Figure 3.16 Comparison of MOC results: Love *et al.* [1959] ($M_e = 2.0$ (top curve) and $M_e = 2.5$ (bottom curve)) vs. Current ($M_e = 2.33$).

It is also important to determine how closely the approximate method of calculating jet boundaries compares with the more exact method of characteristics for similar flow conditions. Just such a comparison is shown in Figure 3.17 for $M_e = 2.33$, $p_e/p_\infty = 2$, and $\delta_e = 0^\circ$. Although the two curves vary by as much as 12% for the larger values of x/r_e , the approximate method still represents a viable method for fast calculation of jet boundaries. The MOC technique is useful when more exact jet boundary predictions are required and calculation time (and cost) are relatively unimportant. It should be noted that MOC computer packages now exist at most major aeronautical research institutes. These pre-packaged programs are, generally speaking, flexible enough to handle most requirements.

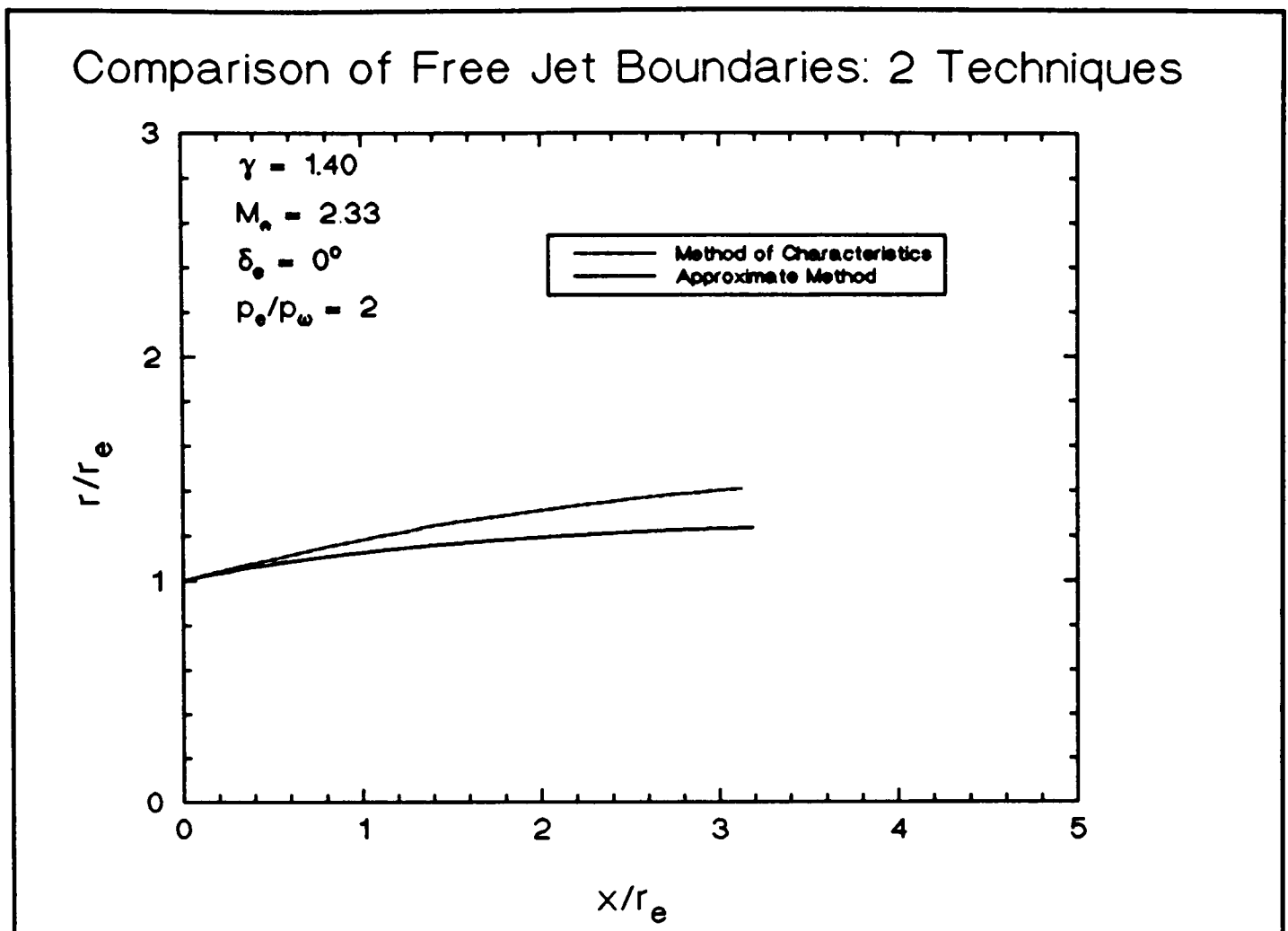


Figure 3.17 Comparison of jet boundary calculations from MOC and approximate techniques.

3.5 Conclusions

A theoretical description of the apparatus under study has been presented. Reference has been made to shock tube theory in order to understand the transient nature of the operation and to provide flow field information at the nozzle exit. This will be found to be useful later in understanding the dynamics of particle acceleration in the present system. Previous research into underexpanded free jets has been described, with particular emphasis on the understanding of jet shock structure. This will provide a baseline with which the current nozzle may be compared. Finally, an approximate technique for computing jet boundaries has been developed and compared with a more exact method of characteristics technique. Adequate agreement between the two techniques has been demonstrated. Application of these techniques for the prediction of jet boundary location for varying nozzle exit pressure ratios has demonstrated the increase in jet expansion as exit pressure ratio increases.

CHAPTER 4. EXPERIMENTAL STUDIES IN GAS DYNAMICS

4.1 Introduction

Before evaluating the particle dynamics associated with the gas propulsion system under consideration, it will first be helpful to look at the gas dynamics. Of particular importance here is the structure of the underexpanded (or, in some cases, matched pressure) free jet. An understanding of how this free jet structure can be controlled may lead us to an understanding of how the system might be optimised for the biological experiments. In this chapter, the free jet structure will be evaluated first using a flow visualisation technique. Results from this study will be compared with theoretical predictions of free jet structure from **Chapter 3**. Next, corresponding pitot pressure measurements will be discussed and compared with results from the flow visualisation study. Pitot pressure measurements made at the nozzle exit will be used to determine exit Mach numbers for a range of nozzle pressure ratios. Also, measurements of impact pressure will be described. Finally, general conclusions of importance to the biological experiments will be considered.

The author wishes to thank members of staff at the Southwell (Osney) Laboratory¹ for making their schlieren system so readily available. Also, plaudits are in order for John Mooney of the Engineering Science Photographic Department, who devoted considerable time and effort to obtain the highest quality high-speed cine images. The SERC's loan of a high-speed video camera and the efforts of Mr. Peter Goodyear in setting up this equipment are recognised. Finally, the assistance of Part II student Graham Glendinning with many of the experiments discussed herein is gratefully acknowledged.

¹Department of Engineering Science, University of Oxford

4.2 Materials and Methods

4.2.1 Flow Visualisation: Schlieren System

The schlieren technique was chosen for flow visualisation of the free jet, primarily due to availability and the relative ease with which quality photographs could be obtained. The basic principles employed are well-known, dating back to work done by Foucault [1859], with Töpler [1866] being the first to apply the technique to the study of rapidly moving shock waves in air. In gas dynamics laboratories, the schlieren technique has been a common tool for at least 45 years (Barnes and Bellinger [1945], Liepmann [1946]). Because the basic principles are well-known, only a brief discussion of the schlieren method will be included here.

Schlieren Experimental Method

The schlieren technique depends on the fact that the speed of light varies indirectly with the density (and, therefore, index of refraction) of the medium through which it passes. Since light passing through one part of the field is retarded differently from that passing through another part, the light rays are refracted. In plane flow, the deflection of a light ray can be shown to be proportional to the density gradient through which it is transmitted. Because of this, previous researchers (Weyl [1945], Schafer [1949]) have been able to show that to a rough approximation the schlieren method measures the first derivative of the refractive index (and, therefore, the density variation). In any flow field (such as the free jet under consideration here), variations in density occur both spatially and temporally; therefore, the refraction of transmitted light can be expected to vary both spatially and temporally.

The basic principle of the schlieren system is that part of the light deflected by density gradients is intercepted before it reaches the viewing screen. This causes that part of the field through which it has traversed to appear darker.²

Figure 4.1 shows a plan view of the schlieren system in use at the Osney Laboratory. The source is shown end-on and is formed by placing a tungsten bulb behind a slit in a metal plate. A large concave ^{MIRROR} on the source side of the working section reflects a parallel beam of light through the working section to a corresponding concave mirror on the viewing side of the working section. This second concave mirror focuses the light to a point. The light expands back out before being focused on a ground glass screen for visualisation. Crucial to the set-up is the placement of a knife-edge, as shown. This intercepts light deflected in the plane *normal* to the knife edge. Therefore, horizontal density gradients are visualised with a vertical knife edge, and *vice versa*.

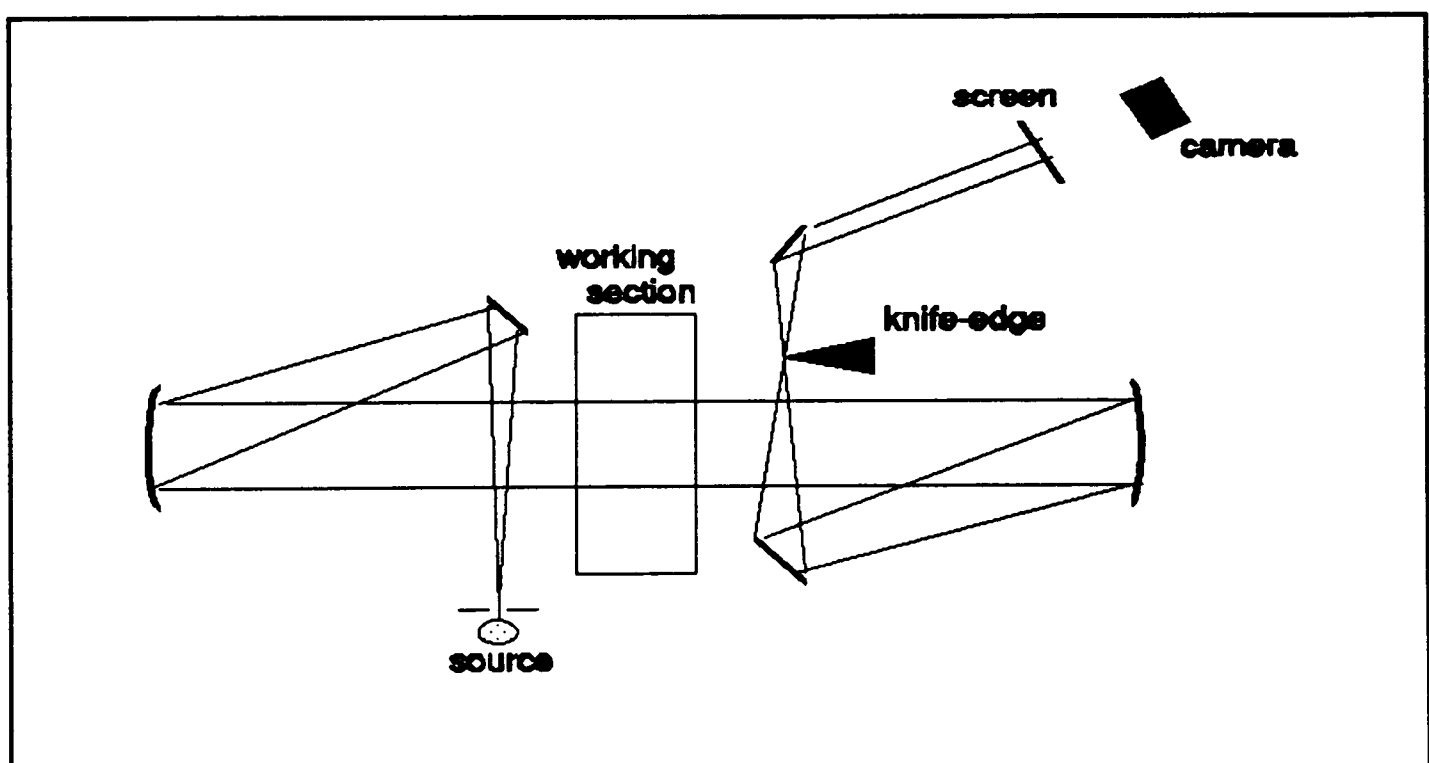


Figure 4.1 Set-up of schlieren system at Osney Laboratory.

The schlieren system at Osney is normally operational with the group's transonic wind tunnel. In order to minimise the probability of breakage, it was decided to work around the wind tunnel instead of attempting to move the optical

²The arrangement can be reversed if *undeflected* light is intercepted and deflected light is allowed to pass.

system. This had the advantage that the optical system was already somewhat aligned in one consistent horizontal plane. Despite this, the many degrees of freedom inherent in the system made it a time-consuming operation to obtain a resolved source image on the knife edge and test image on the screen.

The nozzle was bolted vertically to the edge of the wind tunnel in front of the schlieren glass of the tunnel's working section. Although this proved a convenient and workable position for the nozzle, it meant that the large target chamber could not be included in the set-up. Therefore, operating with a downstream vacuum was not possible. Since the free jet emerged in a vertical direction, a horizontal knife edge was used to visualise density gradients in the flow direction, whilst a vertical knife edge was used to visualise density gradients normal to the flow direction.³

In obtaining a quality schlieren image, two factors conflict: *increasing* sensitivity leads to *decreasing* image brightness. This is true because sensitivity to density gradients increases as the amount of light allowed to pass the knife edge decreases. This, however, leads to a dimmer image which makes high-speed photography difficult. A compromise obtained through trial-and-error is required. Two high-speed photographic methods were employed for capturing the flow structure from the ground glass screen:

1) **High-speed video camera** The high-speed video camera (Kodak Ektapro) had a digital picture and was capable of achieving framing rates between 50 and 1000 frames per second. 1000 frames of fluid memory ensured that the entire expansion process was captured with only manual triggering necessary. (The camera was triggered by hand upon hearing the diaphragm rupture). Photographic stills could be obtained by printing onto thermal paper or by photographing a freeze frame on a high-definition television screen.

2) **High-speed cine-camera** This could run at any speed up to 1000 frames per second, although approximately two seconds were required to allow the film to reach the required speed. To account for this, the film was manually started

³In the discussion that follows, a horizontal knife edge will be denoted as "perpendicular" (to the flow direction), whilst a vertical knife edge will be denoted as "aligned".

approximately four seconds before diaphragm rupture to ensure that the film was running at the proper speed during the event.

Schlieren Run Conditions

As mentioned above, the schlieren set-up precluded the possibility of obtaining a downstream vacuum. Therefore, p_∞ was limited to atmospheric pressure. In addition, safety considerations limited the upstream pressure, p_o , to 12bar absolute. Assuming the exit Mach number, M_e , attains its design value of 2.33 (*i.e.*, boundary layer displacement thicknesses are negligible), supersonic flow tables (NACA TN 1428, for example) can be used to show that exit pressure, p_e , will not quite achieve matched condition:

$$p_o \left(\frac{p}{p_o} \right)_{M_e=2.33} = p_e \quad (4.1)$$

or,

$$p_e \leq 0.916 \text{ bar} < p_\infty \quad (4.2)$$

Because the level of mismatch is so slight, however (implying slightly *overexpanded* conditions), it is probably fair to assume that the resulting flow structure visualised does not vary substantially from that which would result from *matched pressure* conditions.

4.2.2 Pitot Pressure Measurements

As with the schlieren technique, researchers have used pitot pressure measurements to study supersonic flow fields for many years (Kane and Maslach [1950], Ladenburg [1955]). Therefore, only a brief summary of the important principles is necessary here.

The total pressure, p_o , is defined as that pressure the fluid would obtain if brought to rest isentropically. Therefore, it can be considered as a measure of entropy. In the flow field of concern in the present study, many intersecting

shocks occur, causing the entropy to change with downstream position. The pitot tube permits the measurement of this variation.

A pitot tube consists of a small-bore (in the present case, I.D. = 0.8mm) tube with one end open and facing upstream into the flow. The other end may be connected to a manometer, although to obtain transient fluctuations, it is usually more beneficial to use a high frequency response pressure transducer. In the tube, the flow is brought isentropically to rest, as required, giving a reading of the total or stagnation pressure at the mouth of the tube.

In supersonic flow, a detached shock exists just ahead of the tube opening, as shown in Figure 4.2. Therefore, the measured pressure does not indicate the true total (stagnation) pressure, but shows, instead, the total pressure *behind* the shock. Using energy relations and entropy change at a normal shock, a relationship between p_{o1} , p_{o2} , and M_1 can be described:

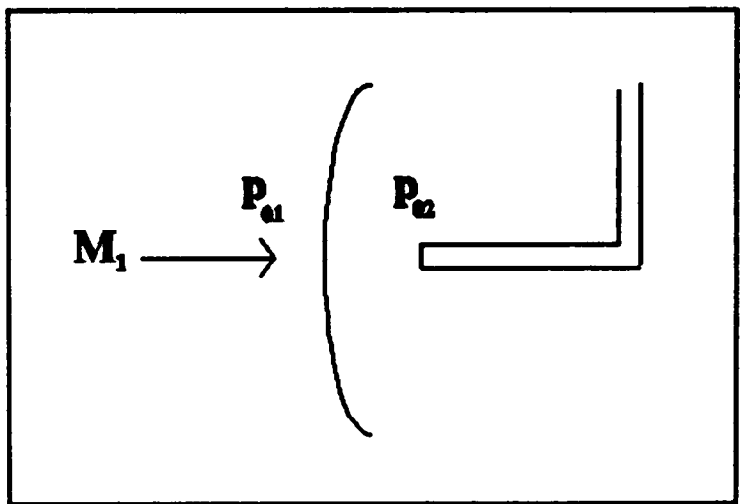


Figure 4.2 Pitot tube with stand-off shock.

$$\frac{p_{o1}}{p_{o2}} = \left(\frac{2\gamma}{\gamma+1} M_1^2 - \frac{\gamma-1}{\gamma+1} \right)^{\frac{1}{\gamma-1}} \left(\frac{1 + \frac{\gamma-1}{2} M_1^2}{\frac{\gamma+1}{2} M_1^2} \right)^{\frac{\gamma}{\gamma-1}} \quad (4.3)$$

where γ represents the ratio of specific heats. This equation may be used to determine the Mach number in isentropic, supersonic flow, where p_{o1} is equal to the upstream (reservoir) pressure.

In situations where the flow is not isentropic (*i.e.*, supersonic flows containing shock waves), the Mach number can be determined from a ratio of static to pitot pressures. This is known as the Rayleigh supersonic pitot formula:

$$\frac{P}{P_{o2}} = \frac{\left(\frac{2\gamma}{\gamma+1} M_1^2 - \frac{\gamma-1}{\gamma+1} \right)^{1/(\gamma-1)}}{\left(\frac{\gamma+1}{2} M_1^2 \right)^{\gamma/(\gamma-1)}} \quad (4.4)$$

Pitot Pressure Experimental Method

In order to characterise the total pressure in the free jet of concern here a large number of pitot traversals were required for various stagnation pressures. Due to the transient nature of the flow field, however, no attempt was made to move the position of the pitot tube during the expansion process. Therefore, in any given "event" only the pressure at one downstream location (as a function of time) could be obtained.

A fast-response piezoelectric differential pressure transducer (Kistler, type 7251) was utilised for the highly-transient measurements. Initially, the transducer was located outside the target chamber, and a 30cm length of stainless-steel tubing (I.D.: 0.8mm) led to the probe tip. It soon became obvious, however, that this was an unsuitable arrangement for making a quickly-varying transient measurement, as the length of tubing reduced the "fast-response" characteristics of the transducer. This was apparent from the output traces, which showed longer rise times than would have been expected.

A calculation of the frequency response of the tubing is useful in demonstrating the response characteristics of a particular pitot measurement set-up. Consider the frequency response of the original set-up. The cavity connecting the probe tip and transducer can be shown to resonate at the Helmholtz frequency (Schaaf and Cyr [1949]), given by $l = \lambda/4$ or $f = c/4l$, where c is the sound speed in the appropriate medium. For air ($c = 340\text{m/s}$) and $l = 30\text{cm}$, the Helmholtz frequency is only of the order of 300Hz. As mentioned above, this was shown to be insufficient for the transient measurements required.

The set-up was altered, therefore, to include the transducer inside the target chamber, thereby substantially reducing the probe-to-transducer length.

The final set-up for the pitot measurements is shown in Figure 4.3. A purpose-built probe tip ($l = 3\text{cm}$, I.D. = 1mm) was screwed into one of the two ports of the differential transducer. A frequency response of 3kHz resulted, which was considered adequate for the required measurements.

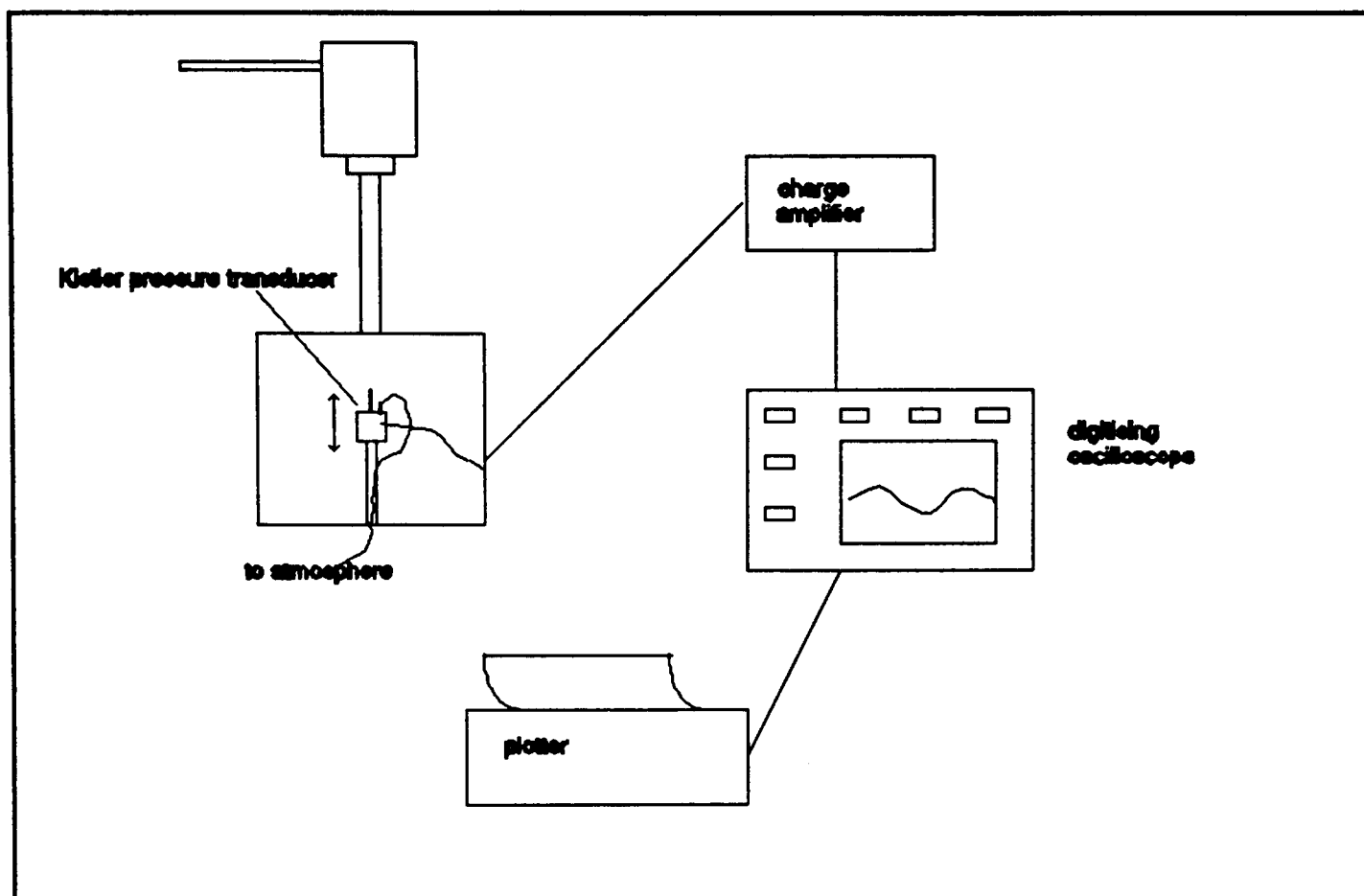


Figure 4.3 Set-up for pitot pressure measurements.

Axial displacement of the transducer was enabled by a purpose-built sliding clamp. This consisted of a perspex securing plate for the transducer, with a stainless steel, 4mm O.D. rod screwed into the back. The telescoping rod was then threaded into a bolt hole at the bottom of the target chamber. Before each run, the axial position of the transducer was verified and secured using a grub screw through the cylinder. The second port of the differential pressure transducer remained at atmosphere⁴. The transducer output was boosted with a charge amplifier (Kistler, type 5007). The amplified output signal was triggered on a digitising oscilloscope (Hewlett Packard 54200A). A hard copy of the

⁴This implies that all measured pressures were actually gauge pressures. Unless noted with a (g), all pressures shown in this thesis are in *absolute atmospheres*.

captured data could be saved on a high quality graphics plotter (Hewlett Packard 7090A).

Each run was recorded as pressure *versus* time for the appropriate axial displacement. Several stagnation pressure conditions were evaluated at axial locations throughout the target chamber, although priority was given to measurements near the nozzle exit plane. At least one pitot pressure traversal was made at a nozzle pressure ratio matching the ratio at which schlieren visualisation was conducted. The oscilloscope time scale was chosen to display the temporal degeneration of the plume. In analysing the data, the temporal variation in upstream and target chamber pressures were considered. Calibration of the transducer was carried out using a mercury manometer.

Sources of potential error for the pitot pressure measurements include: accuracy in reading stagnation pressure, p_o , ($\pm 0.1\text{bar}$, or up to 5% of full scale value), and insufficient pressure transducer frequency of response (independent measurements have shown that the maximum error introduced by the transducer is $< 5\%$). Other potential sources of error including yaw-angled probe positioning probably have only minor impacts on error (see Ladenburg [1955] for a general discussion of this problem). Therefore, the maximum potential error for the pitot pressure measurements is $< 10\%$, although the actual error is probably somewhat smaller than this. Nonetheless, this maximum error value is within the limits necessary for the purposes of this thesis.

4.2.3 Impact Pressure Measurements: Method

Axial measurements of impact pressure were undertaken using a flat-plate impact transducer (Kistler, type 6121A1; plate diameter: 5mm), kindly loaned by the Engineering Department. The primary purpose of this measurement was to simulate the actual initial force felt by target plant cells in a biological experiment. It was thought that these impact measurements could provide useful data in solving the early problem of plant cell blow away (see **Chapter 7**). The jet impingement characteristics were considered over a much shorter time scale than for the pitot measurements, to enable analysis of the initial transient effects due

to the passage of the shock and contact surface. The transducer was calibrated using an evacuated target chamber and a mercury manometer.

The experimental set-up (see Figure 4.4) was similar to that for the pitot measurements; however, the addition of a computer (IBM Portable PC), with the accompanying IEEE data bus (Brain Boxes, Liverpool) permitted the digitised data to be saved for further analysis.

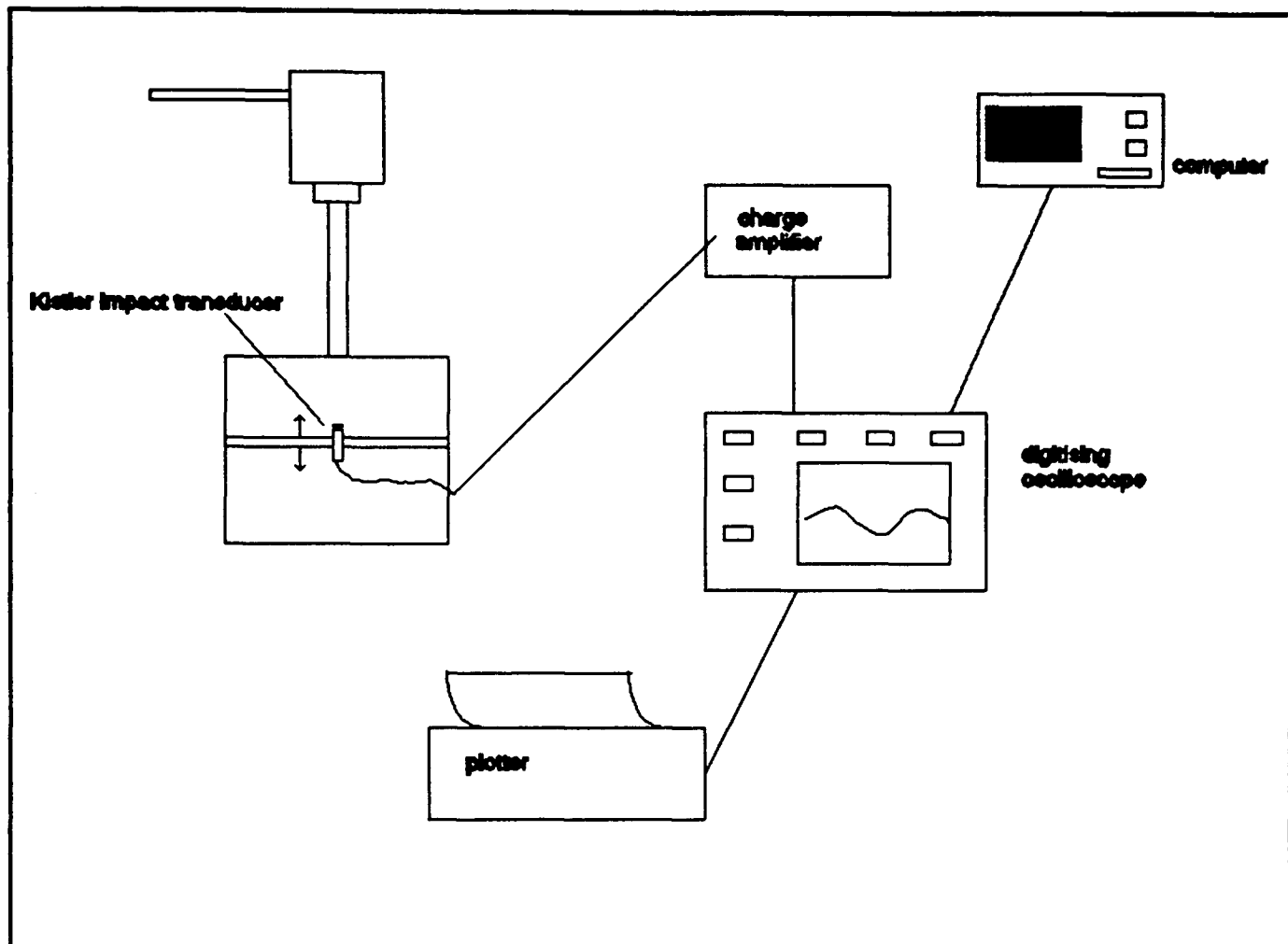


Figure 4.4 Experimental set-up for jet impact measurements.

4.3 Results and Discussion

4.3.1 Schlieren Results

Typical photographic stills from the high-speed video which display the structure of the free jet as it emerges from the nozzle are shown in Figure 4.5 and Figure 4.6. Run conditions for Figure 4.5 were: $p_o = 10.6\text{bar}$; $p_\infty = 1\text{bar}$, while the video was framing at 50 frames/second. Although this is a relatively-slow framing rate for a transient event of this nature, the jet structure changes very little over the course of the first milliseconds. Presumably, this is due to the flow being choked at the nozzle throat. The knife edge is perpendicular to the flow direction in Figure 4.5, and, therefore, density gradients *along* the flow direction are displayed. This is very useful in portraying the cellular (diamond) pattern predicted theoretically by Prandtl [1904], and seen by many early researchers in the field (see Section 3.3.2). Also shown in Figure 4.5 is a typical measurement of the wavelength, λ .

In the case of Figure 4.5, higher density areas appear brighter, as light is refracted towards those areas and past the knife edge. This is the case with the intercepting shocks of the slightly-overexpanded jet. Since density increases across a shock, the oblique shocks from the nozzle lip can be seen as the bright fringe which bounds the dark, still-expanding exit flow. These shocks can be seen to be increasing in strength, as the brightness increases toward the intersection with the axis (as theory would suggest). At the axial location where the shocks intersect (the position of greatest pressure and density change), the image is brightest. Similarly, the strong reflected shocks are very bright. An expansion fan forms where the reflected shock meets the jet boundary; this is not obvious in the photograph, because an expansion fan represents a *gradual* change (decrease) in density. The formation of a second intercepting shock from the reflected expansion fan, the shock itself reflecting and coalescing is, however, seen as a bright fringe. The structure repeats in a periodic manner as described in the theory, until viscous effects cause it to fade downstream.

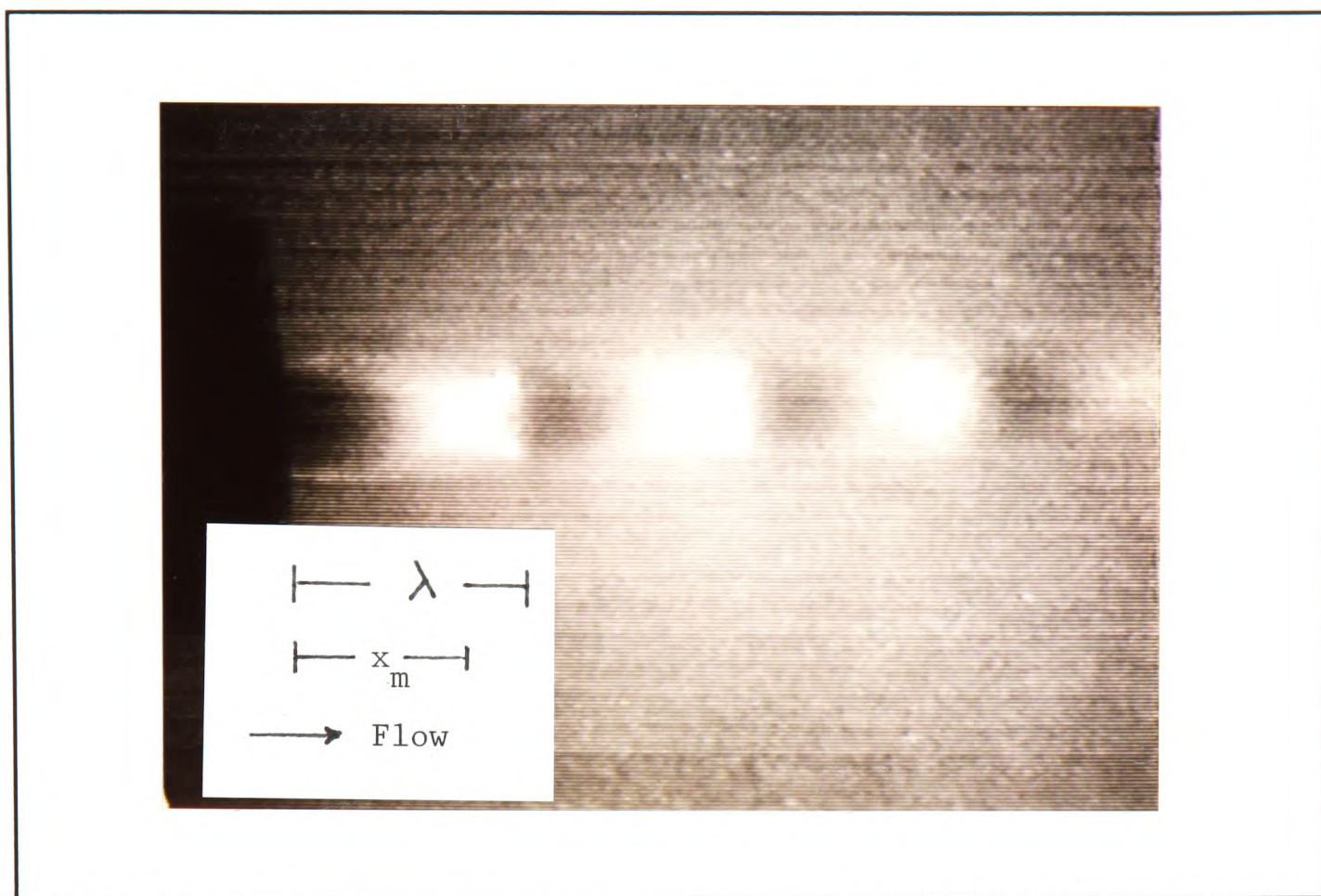


Figure 4.5 Schlieren photograph of free jet (taken from high-speed video). Run conditions: $p_o = 10.6\text{bar}$, $p_\infty = 1\text{bar}$; perpendicular knife edge.

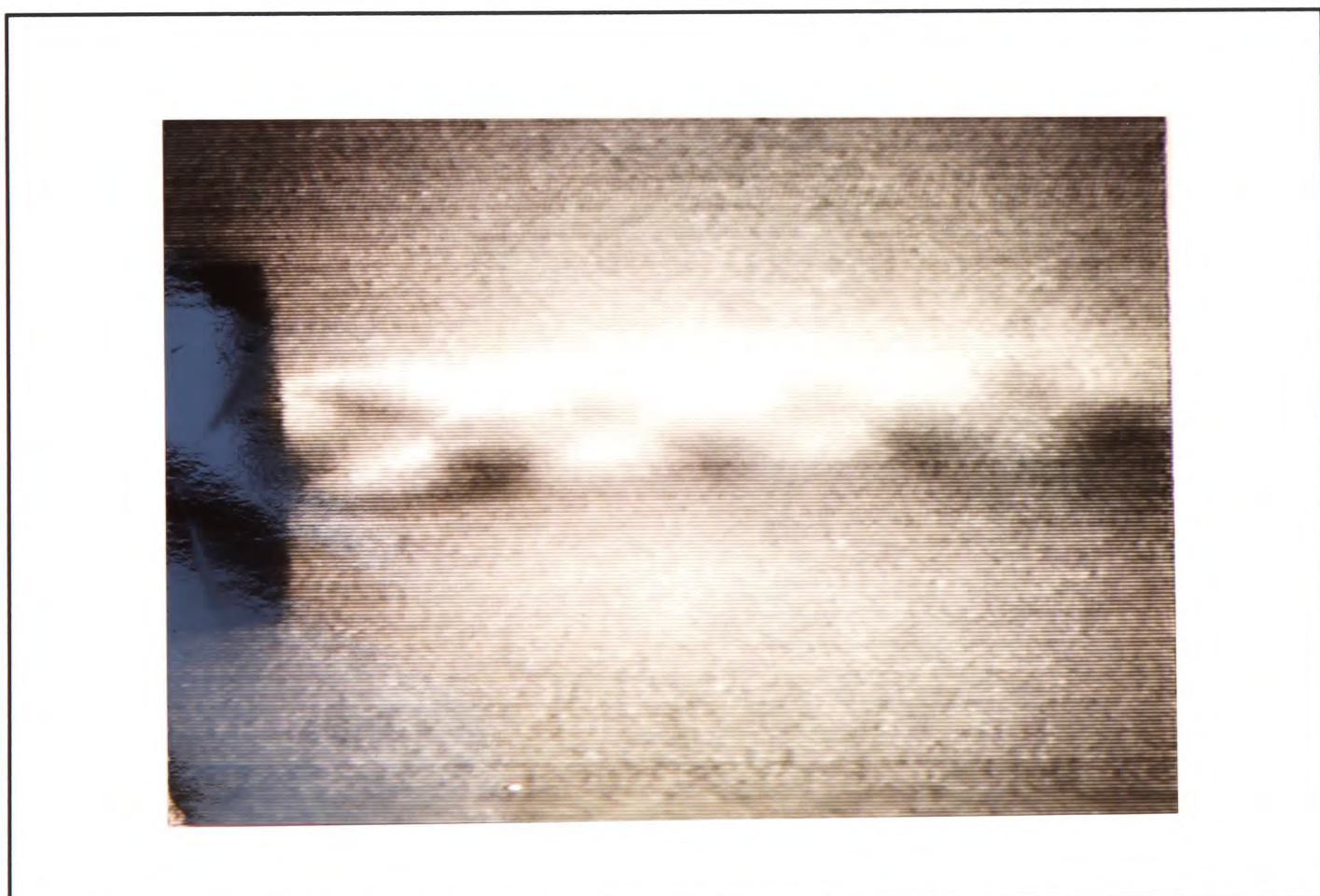


Figure 4.6 Schlieren photograph of free jet. Run conditions: $p_o = 10.0\text{bar}$, $p_\infty = 1\text{bar}$; aligned knife edge.

The knife edge is aligned with the flow direction for Figure 4.6 ($p_o = 10.0$ bar; video speed = 125 frames/sec). Therefore, density gradients normal to the flow are displayed. This emphasises such flow characteristics as jet boundary particularly well, but boundary layer effects are still indistinct. This is not unexpected, as the boundary layer is thin in relation to the jet (see Section 3.2.2) and does not support a large density gradient.

The change in jet structure for various conditions of upstream pressure, p_o , are shown in Figure 4.7 to Figure 4.9. Figure 4.7 shows minimal shock cellular structure for $p_o = 4.0$ bar, while Figure 4.8 displays only slightly more cellular structure for $p_o = 6.0$ bar. The periodicity of the cellular structure is more apparent in Figure 4.9, which shows the jet structure for $p_o = 8.4$ bar. The structure at the lower pressures, being less evident initially, decays rapidly; the time averaging of the high-speed photography (125 frames/sec) may blur the little periodicity that may be present.

Jet Periodic Structure

The wavelengths of the jet periodic structures for all schlieren runs were measured manually from the still photographs. These were normalised with the nozzle exit diameter (6mm) and plotted as a function of upstream gauge pressure in the first instance, as shown in Figure 4.10. Much of the scatter seen is due to errors in making manual measurements off photographic stills. This error is limited to approximately 5%. In each case either the jet exit diameter or nozzle O.D. were used as references for the measurements.

Figure 4.11 shows a comparison of jet wavelength measurements from the present study with those of previous researchers (see Section 3.3.2). This graph shows good agreement in the general trend of the data with the results of all previous researchers. However, the values of λ/d_e at a given value of p_o/p_∞ found in the present study are somewhat less than those of the classical correlations (Prandtl [1904], Hartmann and Lazarus [1941], and Emden [1899]). The primary reason for this deviation relates to differences in experimental set-up. Whereas the classical studies mentioned above involved investigation of underexpanded,

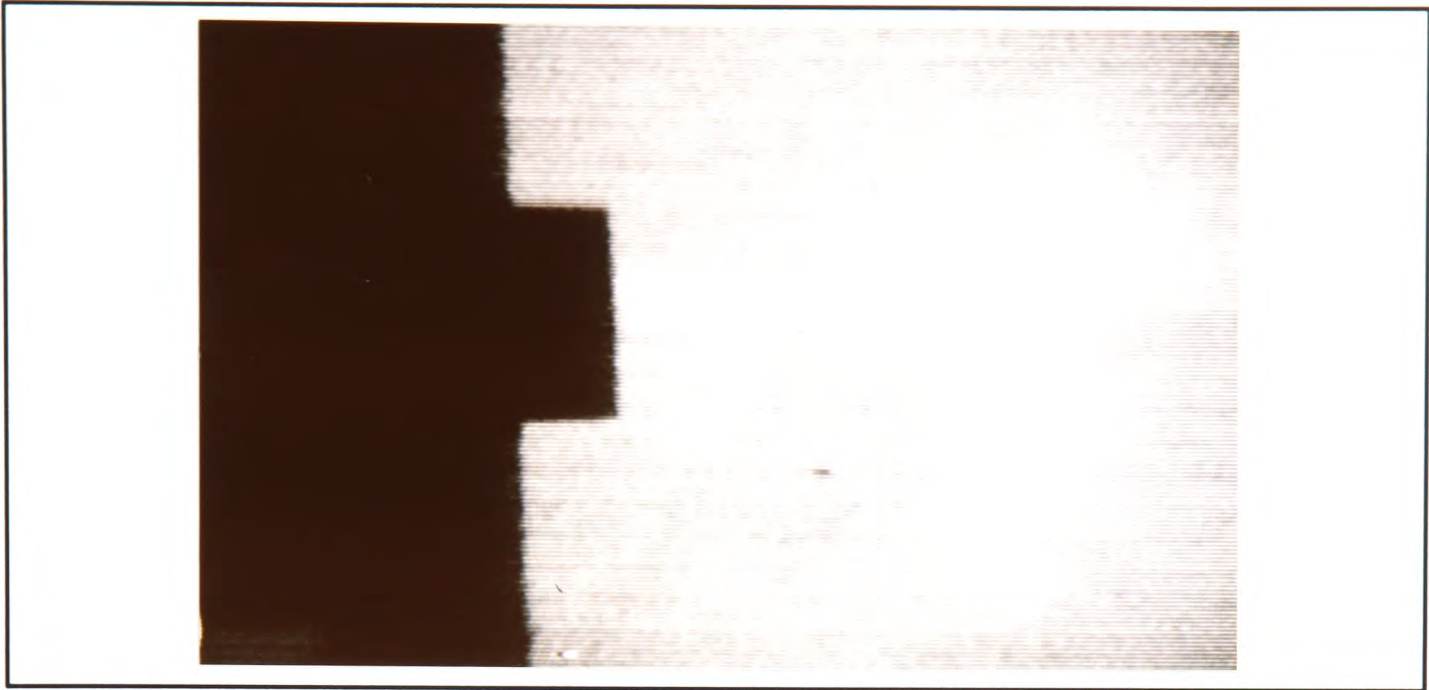


Figure 4.7 $p_o = 4.0\text{bar}$, $p_\infty = 1\text{bar}$; knife edge aligned with flow.

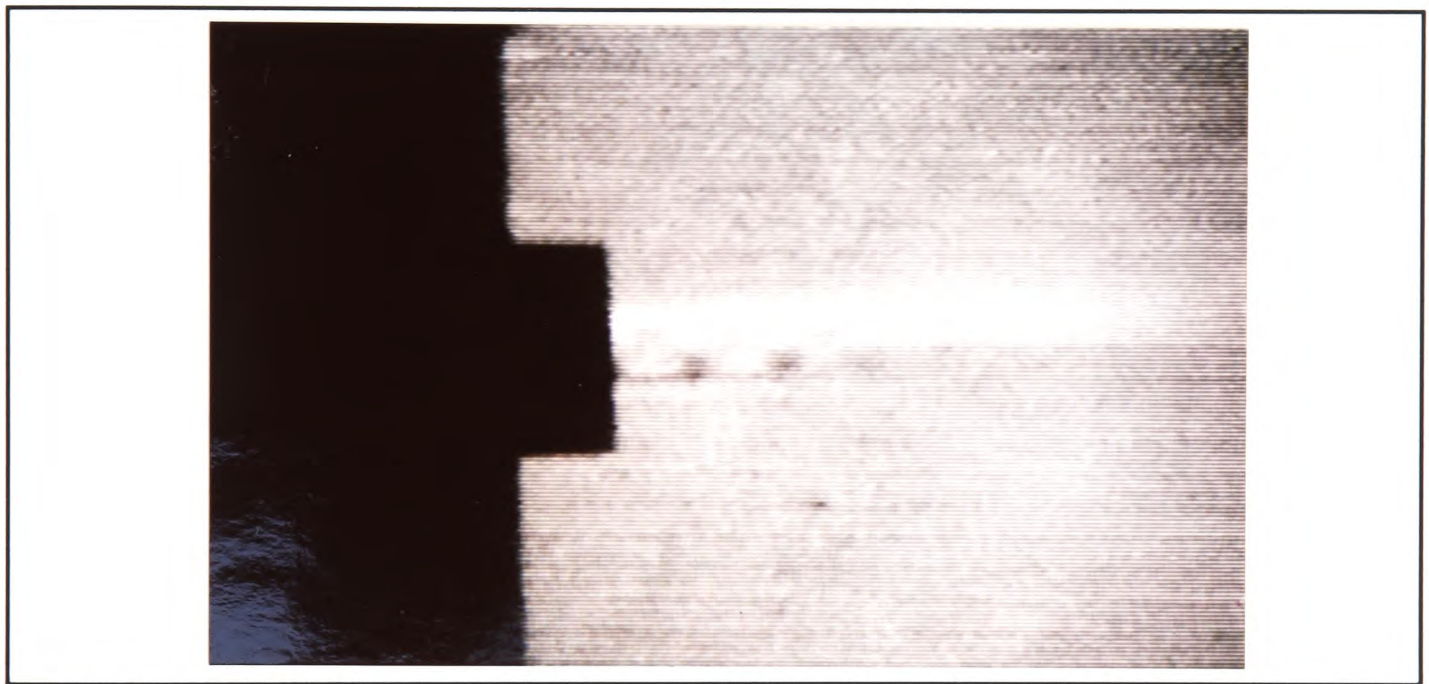


Figure 4.8 $p_o = 6.0\text{bar}$, $p_\infty = 1\text{bar}$; knife edge aligned with flow.

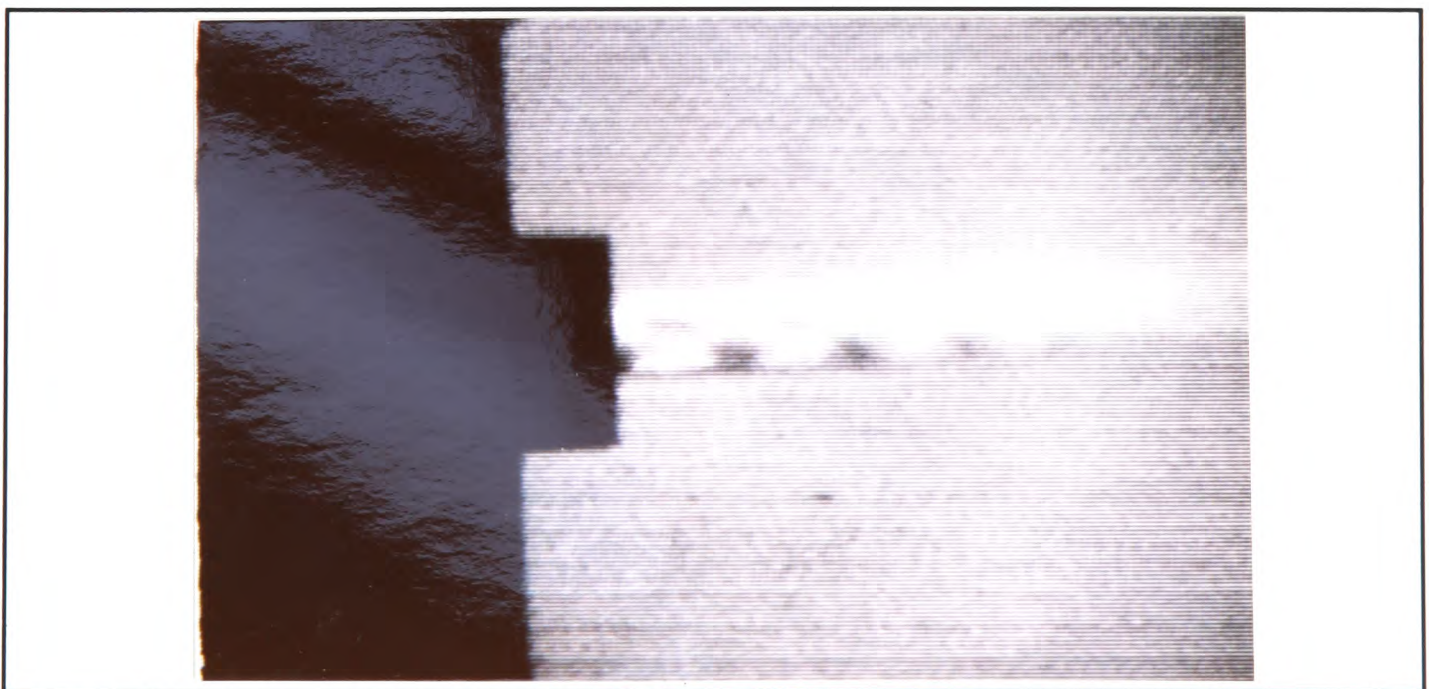


Figure 4.9 $p_o = 8.4\text{bar}$, $p_\infty = 1\text{bar}$; knife edge aligned with flow.

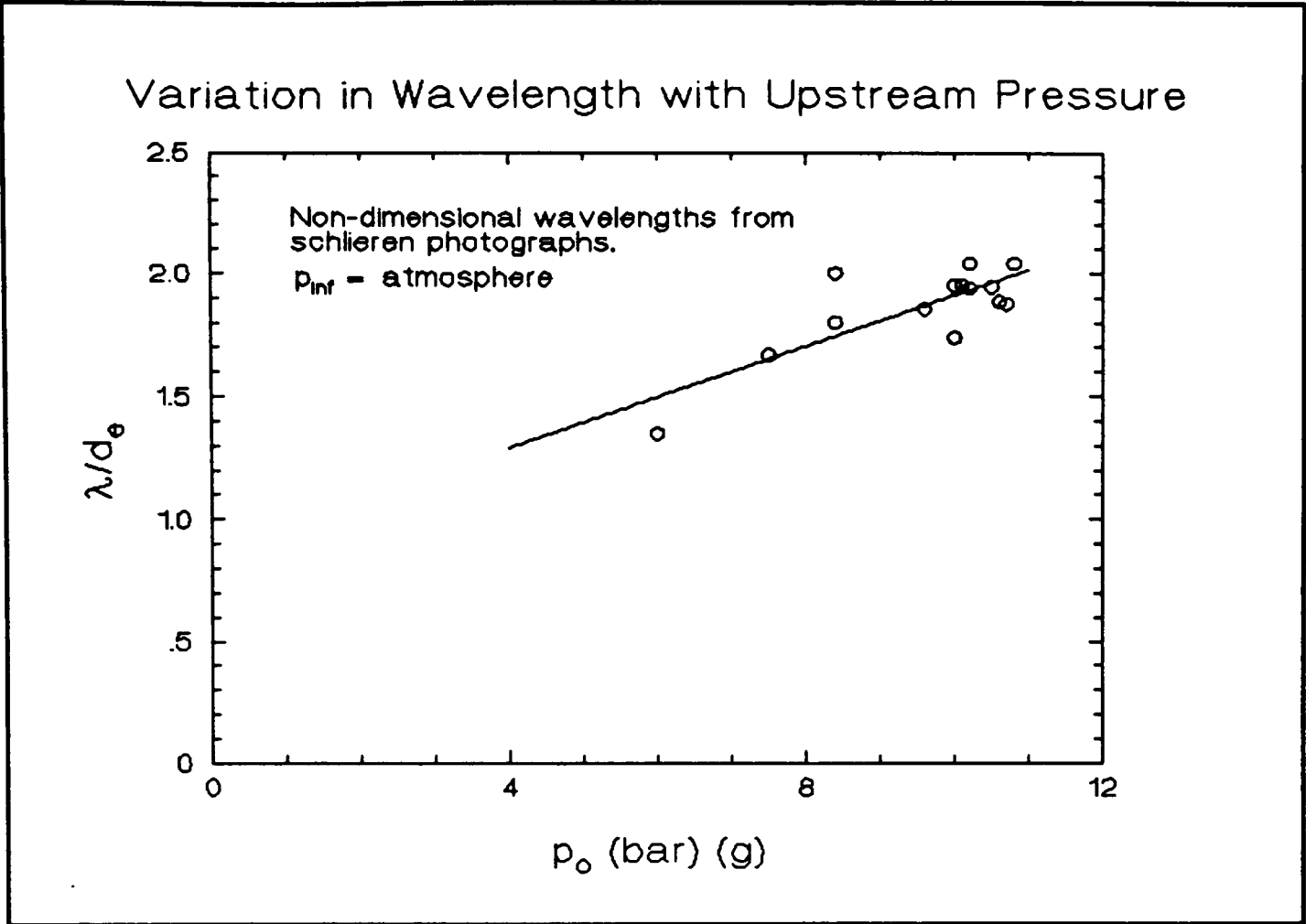


Figure 4.10 Variation in non-dimensional wavelength with upstream pressure.

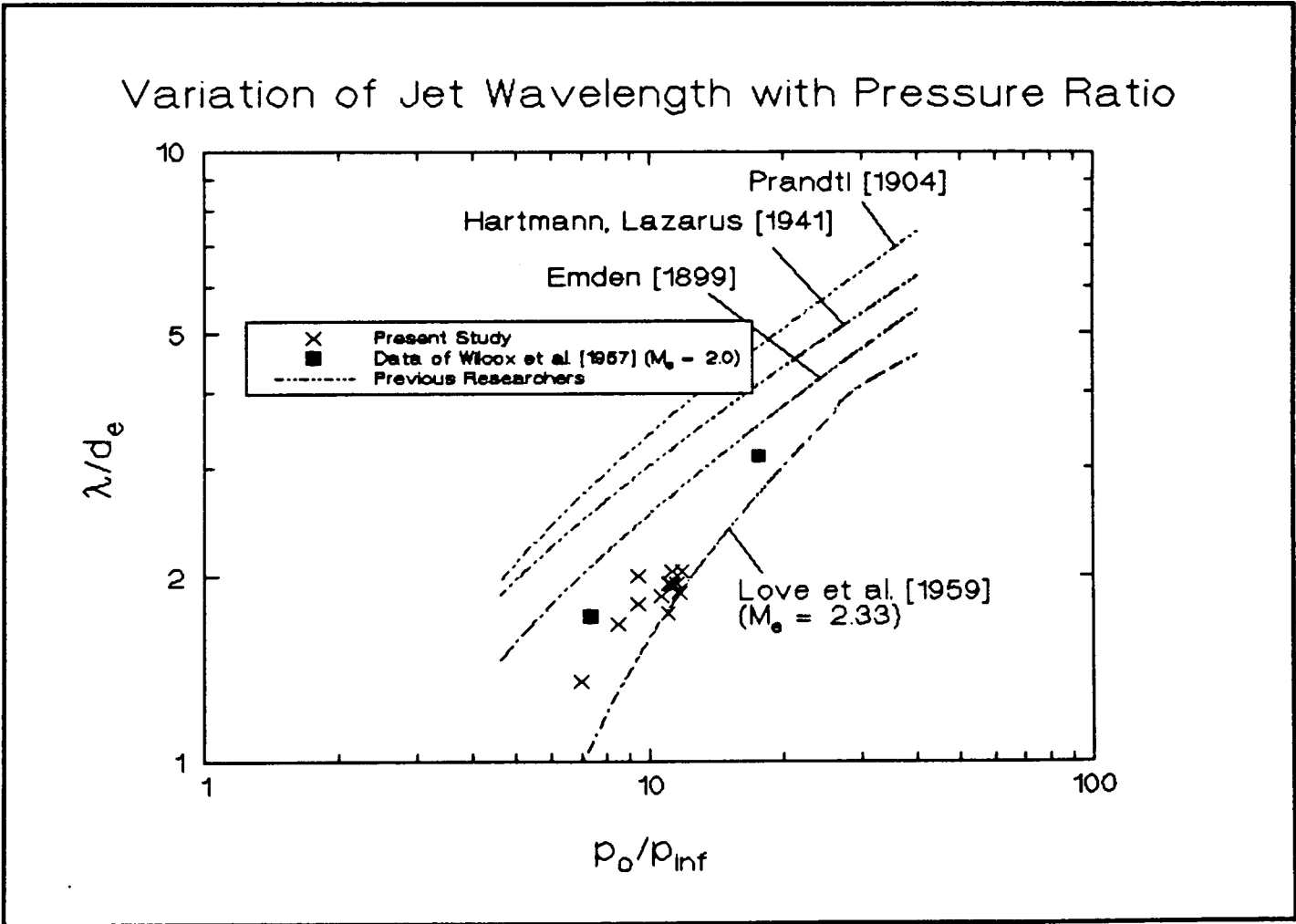


Figure 4.11 Comparison of wavelength measurements of current study with those of previous researchers.

orifice flow ($M_e = 1$), the present study examined matched pressure conditions from a convergent-divergent nozzle of high aspect ratio ($L/d_e = 50$) with *supersonic* exit conditions ($M_e > 1$). When the present data is compared with data from studies involving *supersonic* exit conditions (*i.e.*, Wilcox *et al.* [1957], Love *et al.* [1959]), studies which evaluated jet periodicity from supersonic convergent-divergent nozzles, much better agreement is found. This can also be seen in Figure 4.11.

The variation in jet periodic structure with time for the two knife edge configurations is shown in Figure 4.12 and Figure 4.13. Figure 4.12 ($p_o = 10.8\text{bar (g)}$) displays the frame-by-frame change in the jet structure for a knife edge perpendicular to the flow. The framing rate of 125 frames/second corresponds to 8ms between each frame. The jet quickly obtains a periodic structure; this structure can then be seen to degrade both temporally and spatially, as the upstream stagnation pressure decreases (as in a blowdown wind-tunnel). As the pressure ratio p_o/p_∞ continues to decrease, the diamonds are forced back upstream. Since the wavelength is a function of pressure ratio, it is also, in this case, a function of time. Figure 4.13 ($p_o = 10.2\text{bar (g)}$) displays the temporal change in jet structure for a knife edge aligned with the flow. Here the effect on jet boundary location is particularly emphasised. The initial frame ($t = 0\text{ms}$) shows a slight expansion of the jet from the nozzle, signifying that the nozzle is slightly *underexpanded* for this initial condition. As the upstream reservoir continues to blow down, the jet becomes more *overexpanded*. This is particularly apparent toward the end of the run ($t = 56\text{ms}$) where the jet boundary appears to begin contracting immediately upon exiting the nozzle.

Analyses of the temporal and spatial degradation in jet wavelength based on these two series of photographs are presented in Figure 4.14 and Figure 4.15. Figure 4.14 shows that non-dimensional wavelength appears to diminish almost linearly as a function of time for both runs. This is somewhat unexpected, as stagnation pressure (and, therefore, nozzle pressure ratio, p_o/p_∞) varies approximately exponentially. Figure 4.15 shows a linear degradation in wavelength for each downstream shock cell. This follows from viscous damping.

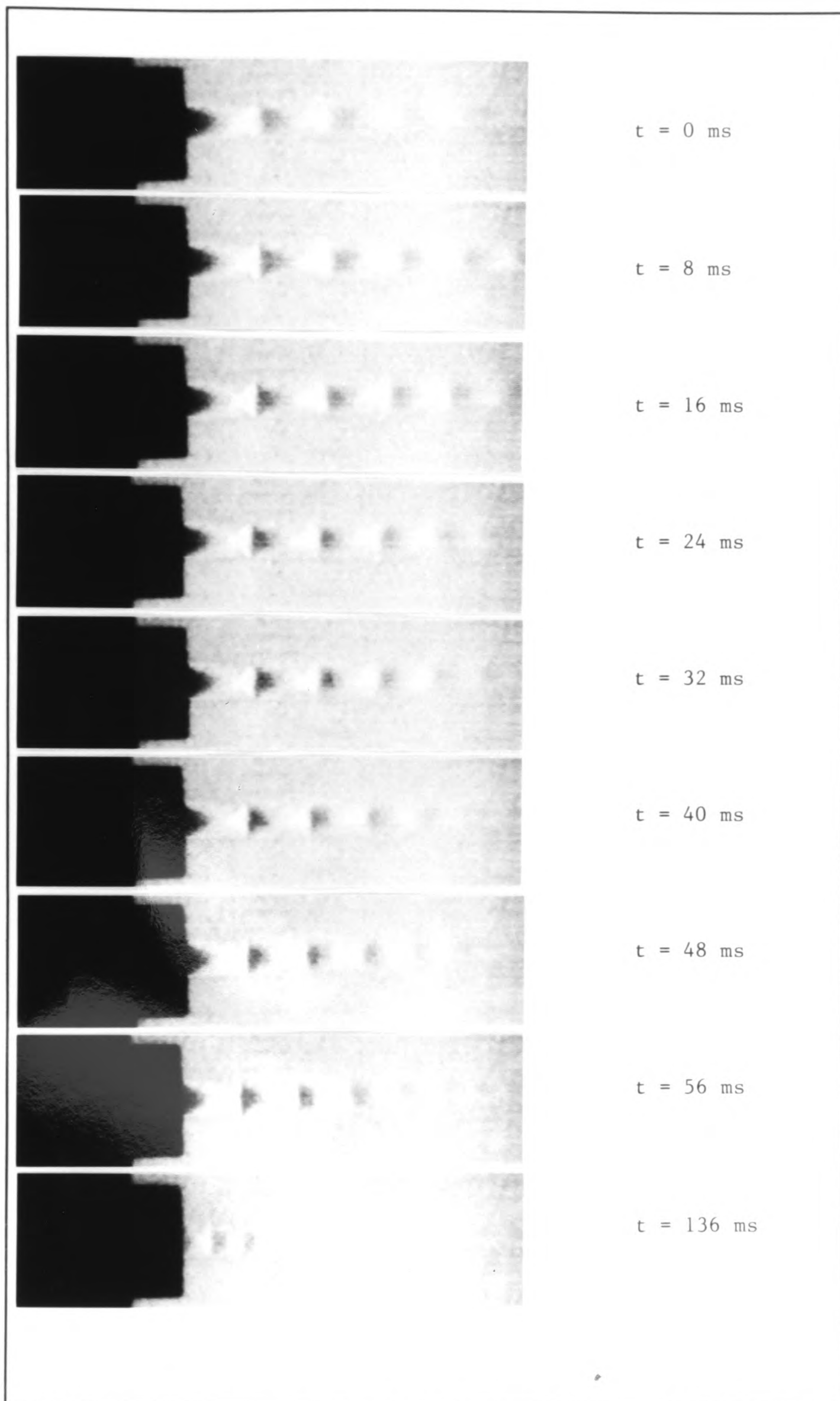


Figure 4.12 Frame-by-frame variation in jet structure at 125 fps ($p_o = 10.8\text{bar}$ (g); perpendicular knife edge).

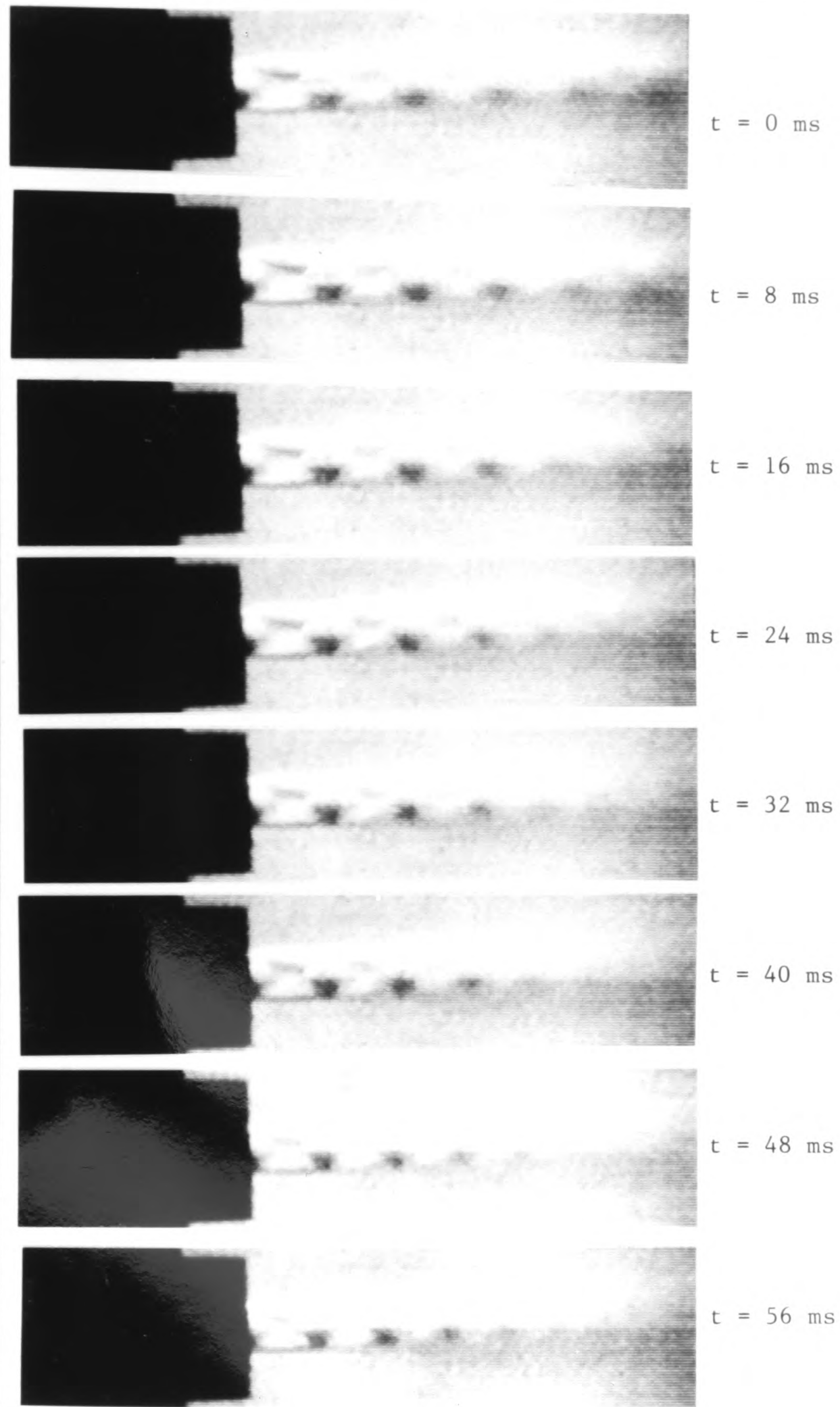


Figure 4.13 Frame-by frame variation in jet structure at 125 fps ($p_o = 10.2\text{bar}$ (g), aligned knife edge).

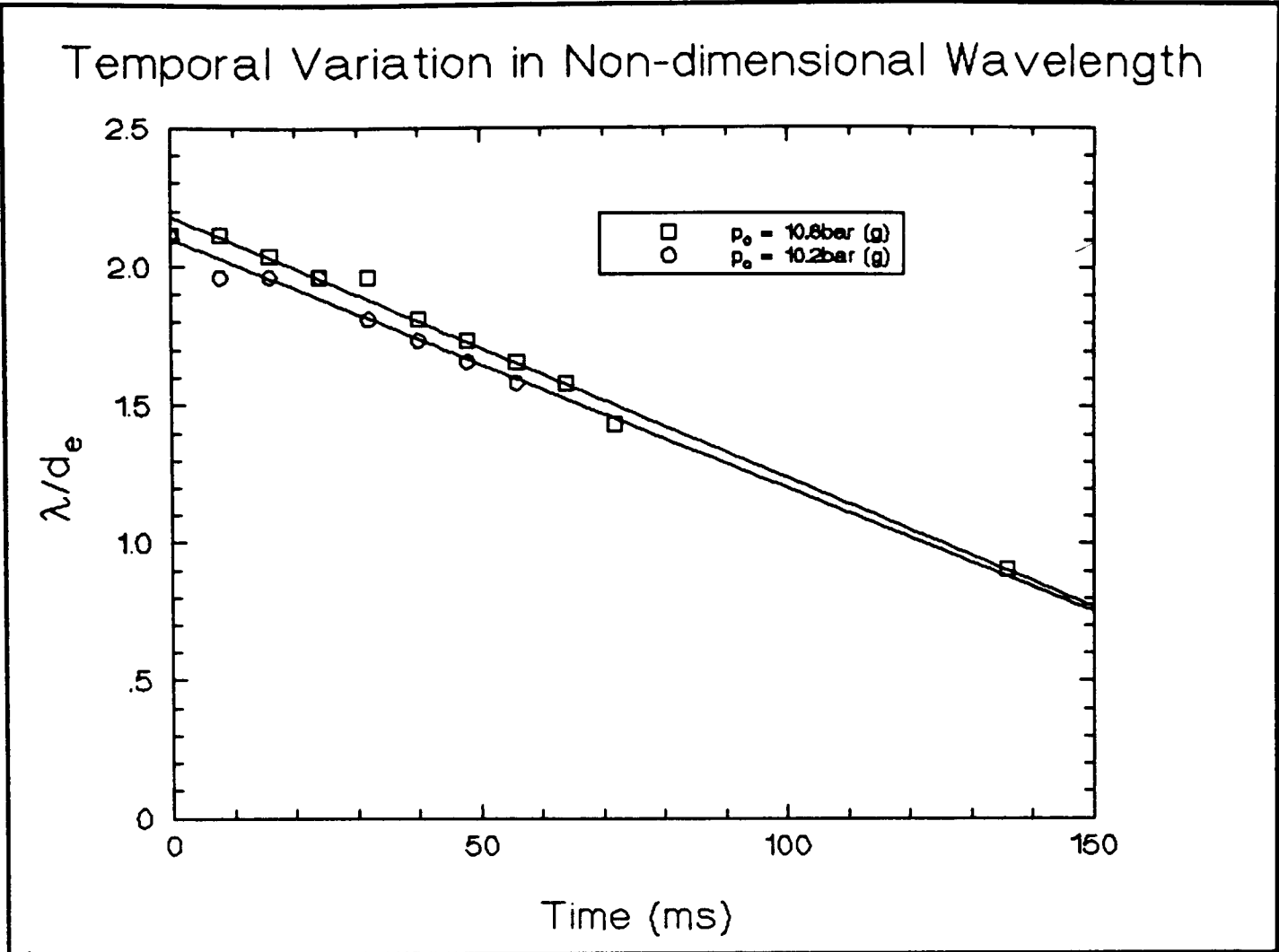


Figure 4.14 Temporal variation in non-dimensional wavelength.

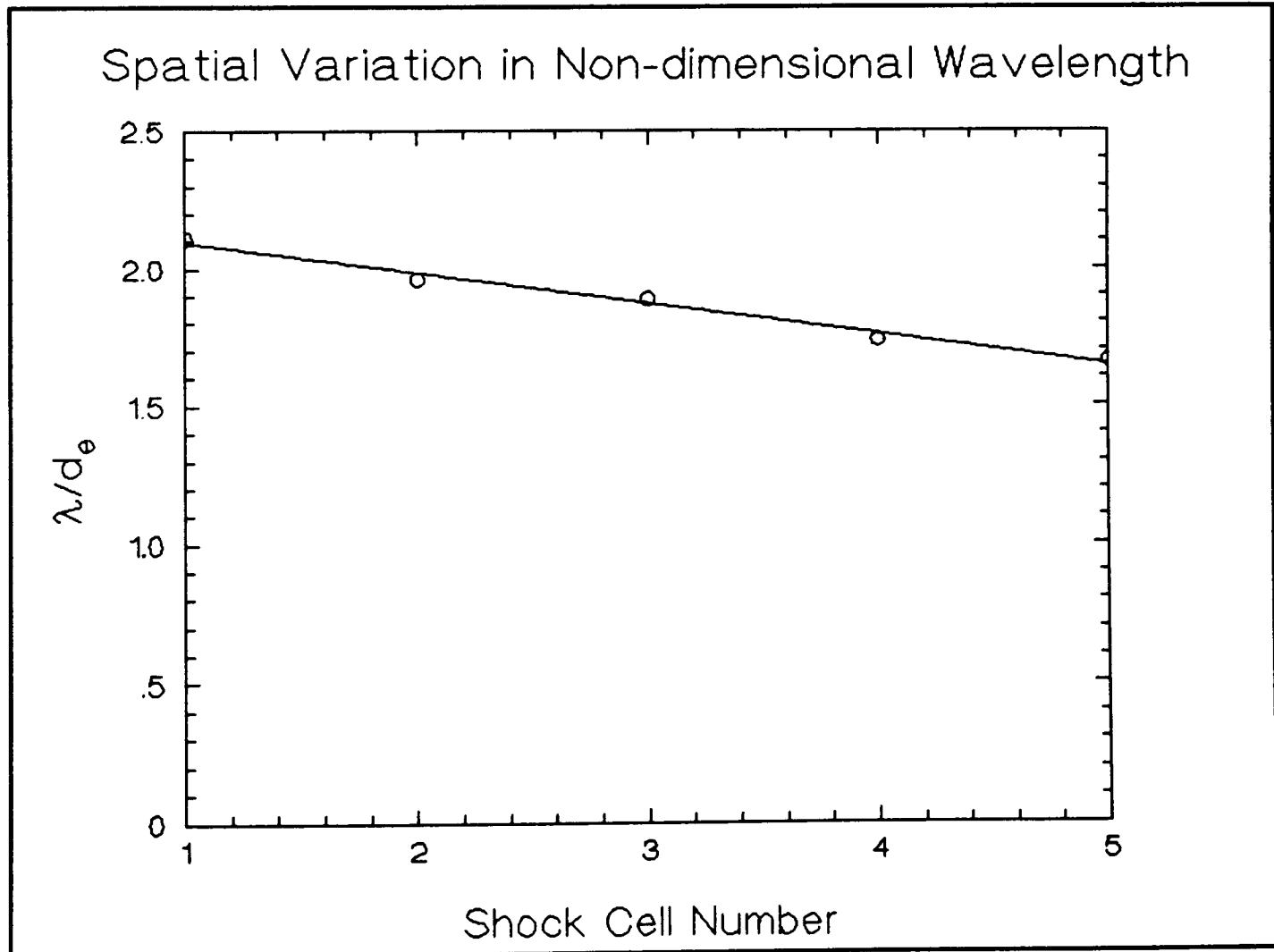


Figure 4.15 Spatial variation in non-dimensional jet wavelength ($p_o = 10.8 \text{ bar (g)}$).

Location of First Mach Disc

The location of the first Mach disc (or, for lower nozzle pressure ratios (p_o/p_∞), the location where intercepting shocks cross the jet axis) was found experimentally in the present study for a range of nozzle pressure ratios by evaluating schlieren photographs. The criterion used was the point of maximum brightness within the first cell where the intercepting shocks intersect. The result of this study is shown in Figure 4.16, which compares results from the present study with results of previous researchers. Correlations from Crist [1966] and Ashkenas and Sherman [1966] were plotted together with experimental data from Love *et al.* [1959]. Results from the present study show excellent agreement, particularly with the data of Love *et al.* [1959], which is to be expected, as the Love data involved measurements from a supersonic convergent-divergent nozzle ($M_e = 2.0$).

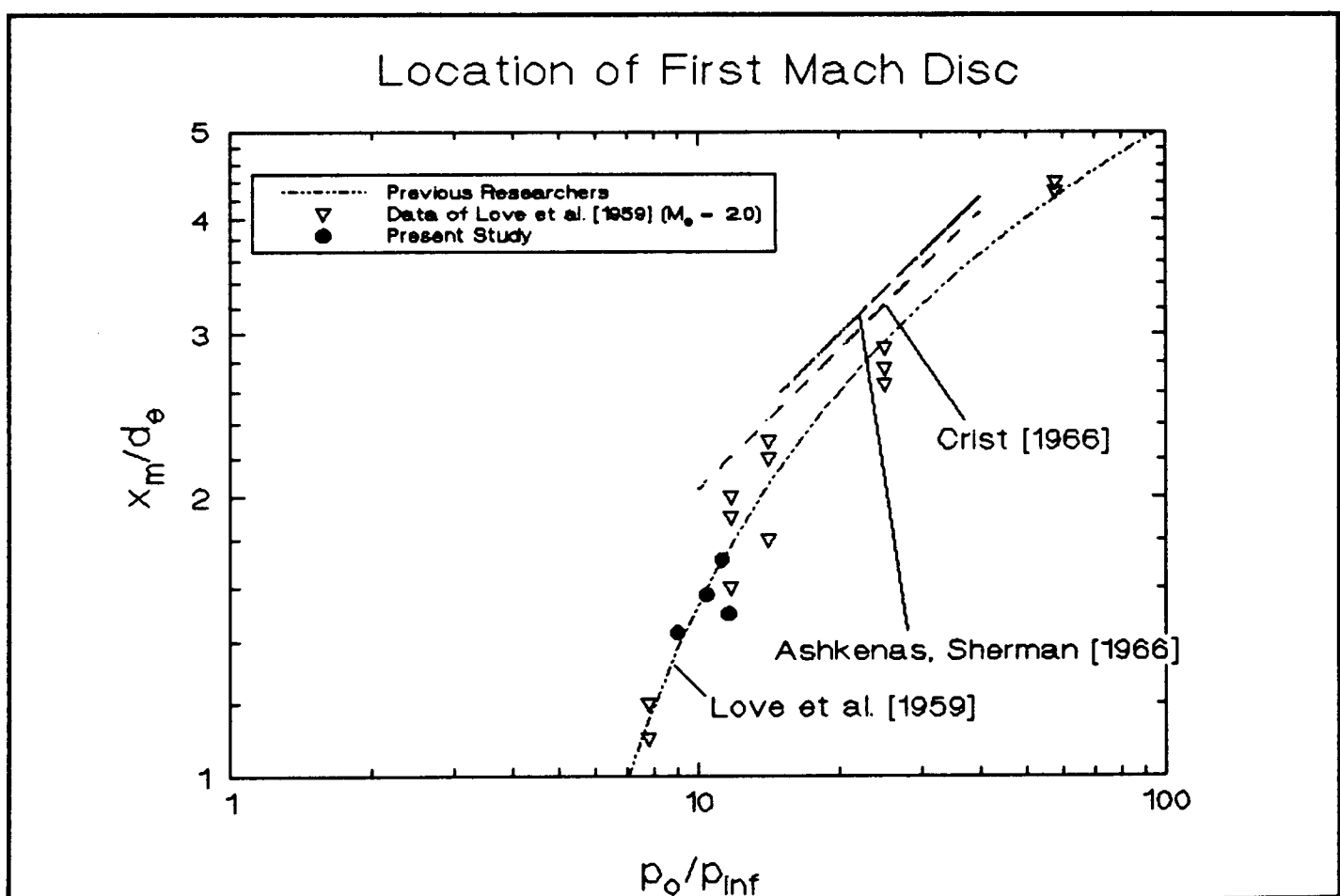


Figure 4.16 Comparison of Mach disc location for present study with results of previous studies.

Jet Boundary Location

Schlieren photographs made with the knife edge aligned with the flow (thereby emphasising density gradients in the radial direction) were useful in displaying the resulting jet boundary location. It may be recalled from Section 3.4.1 that an approximate method after Adamson and Nicholls [1959] is useful in predicting the location of underexpanded free jets for a given nozzle exit pressure ratio, p_o/p_∞ . This approximate method was utilised to predict jet boundary location for a nozzle pressure ratio similar to that used for one of the schlieren photographs (see photograph in Figure 4.6, $p_o/p_\infty = 11.0$). A comparison of the prediction and the actual boundary location measured from the photograph is given in Figure 4.17.

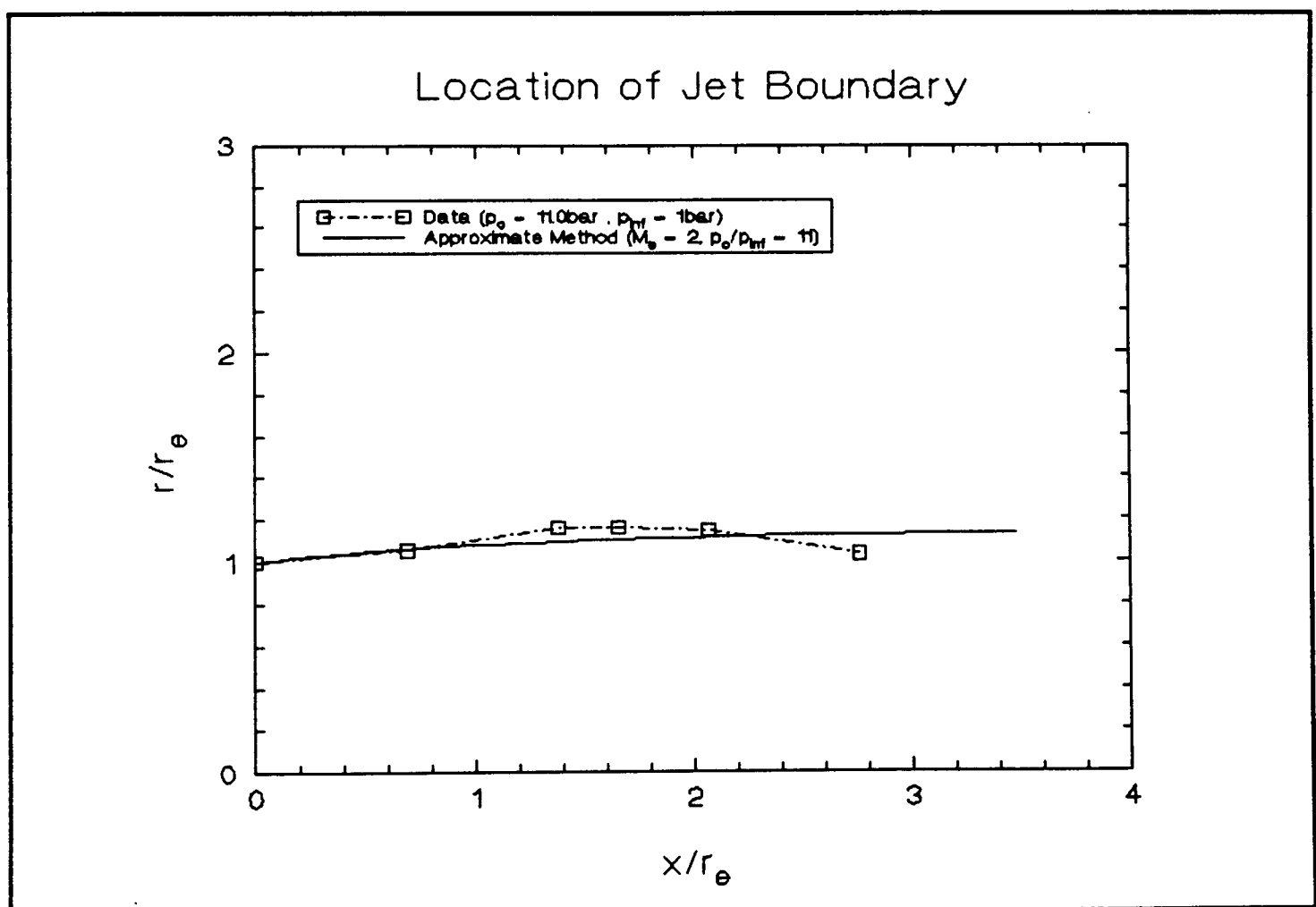


Figure 4.17 Comparison of actual and predicted jet boundary location ($p_o/p_\infty = 11.0$).

The graph shows good agreement between the predicted and actual locations, especially for $x/r_e < 1$ (i.e., near the nozzle exit), although beyond this the measured data tends to show slightly larger expansion. In general, this shows that the approximate technique is a valid method for predicting jet boundary location. Due to limitations discussed above, only a limited range of nozzle pressure ratios were evaluated using the schlieren technique. In Chapter 6, however, the approximate technique will be compared with high-speed visualisation of particle-laden jet expansion for a much wider range of nozzle pressure ratios.

4.3.2 Pitot Pressure Results

A complete investigation of pitot pressures over the first 40mm downstream was undertaken for several stagnation pressure/target chamber combinations. Before embarking on these series of runs, the differential pressure transducer was calibrated using a mercury manometer. This calibration curve, which is presented in Figure 4.18, shows a linear response.

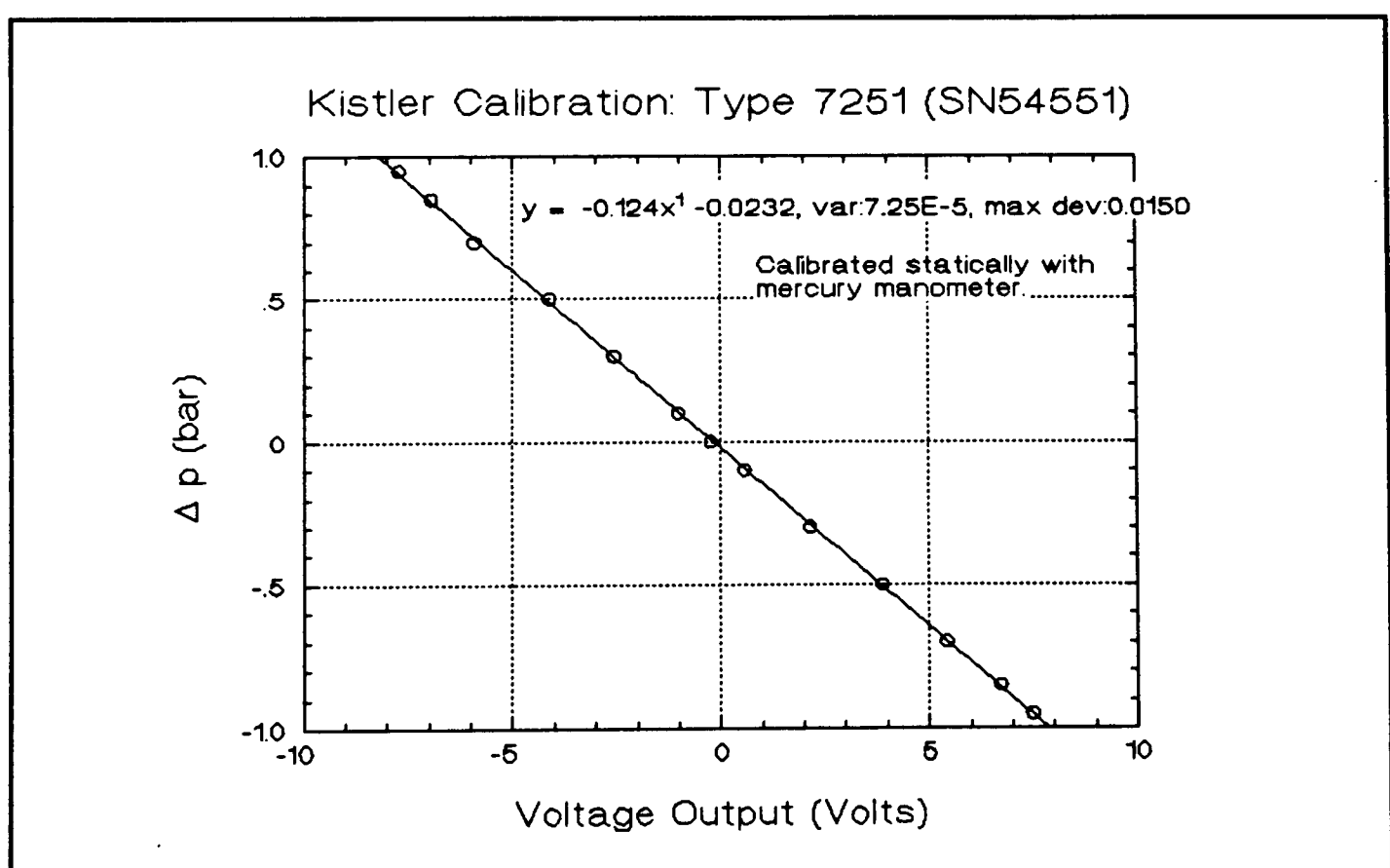


Figure 4.18 Calibration curve for Kistler differential pressure transducer.

A typical transducer plot of voltage *versus* time is given in Figure 4.19. Due to the transducer set-up, *decreases* in voltage output are associated with *increases* in differential pressure. The initial voltage drop corresponds to the pressure increase as the jet exits the nozzle. This is followed by a decay in pressure; passage of shocks toward the nozzle account for the temporary halts in pressure decay with time. This is true because a pitot tube will register higher pressures behind a shock. Also of interest here is the total duration of the "blast" event. This trace shows that for these conditions ($p_o = 2.7\text{bar}$, $p_\infty = 0.25\text{bar}$), flow occurs over approximately nine time divisions or 450ms. This information will be useful in demonstrating that a large proportion of the "blast" is actually sub-sonic.

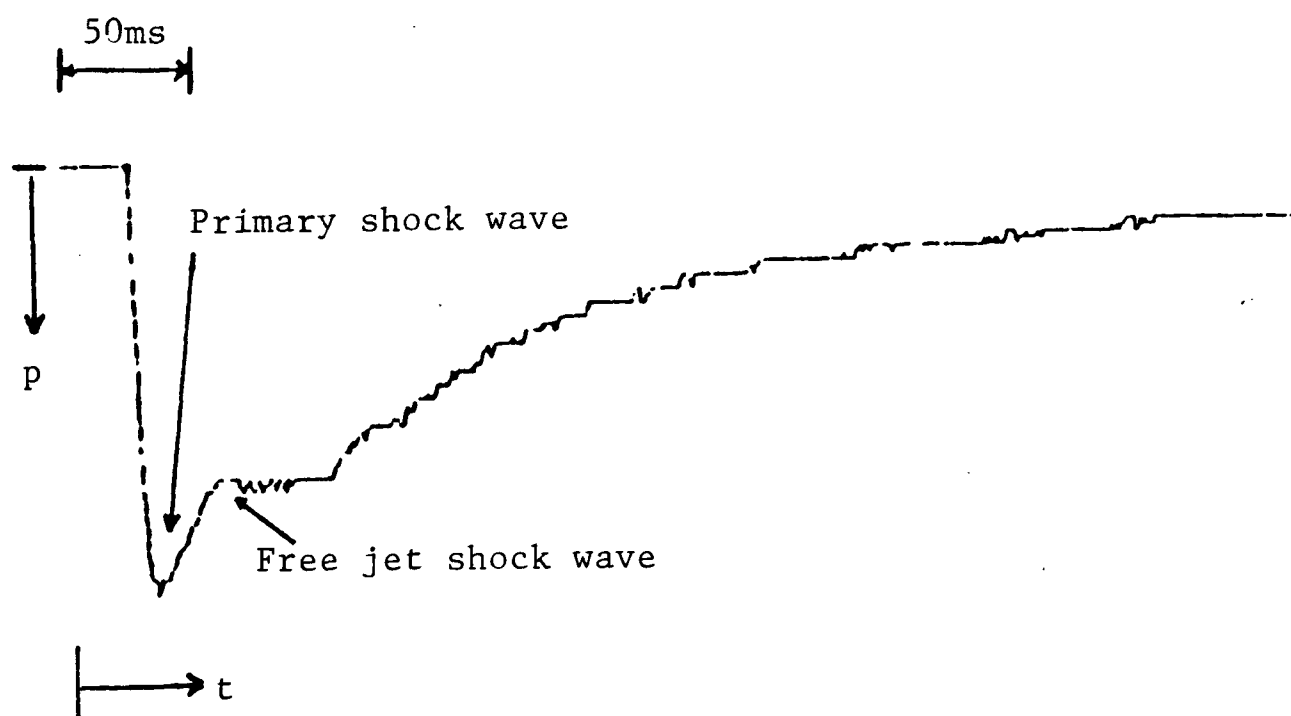


Figure 4.19 Typical plot of voltage *vs.* time.

Jet Structure

In analysing the data, pitot pressure, p_{o2} , was normalised against back pressure, p_{∞} , as well as stagnation pressure, p_o , both of which vary with time. The variation of p_{o2}/p_{∞} as a function of non-dimensional downstream distance, x/d_e , and as a function of time is of particular interest, as it displays the temporal degradation in the diamond structure.

Figure 4.20 through Figure 4.23 compare results from schlieren high speed videos with pitot pressure distributions at comparable *pressure ratios* and at comparable times in the flow. It should be noted, however, that it was not possible to keep the absolute pressure conditions similar between the schlieren runs and pitot pressure runs. This was true primarily for two reasons: a.) with no target chamber for the schlieren runs, it was not possible to obtain a downstream vacuum, and b.) pressure limit on differential transducer ($\Delta P < 1\text{bar}$) prevented runs with $p_o > 8\text{bar}$. Nonetheless, previous researchers (Prandtl [1904], Ashkenas and Sherman [1966], for example) have reported that it is p_o/p_{∞} which affects jet structure to the greatest extent. Therefore, every effort was made to keep the pressure ratios similar between the optical runs and pressure runs.

Figure 4.20 displays the pressure variation at 0ms and compares it with the schlieren photograph at a comparable time and pressure ratio. As the flow expands from the nozzle, the pressure decreases, until the oblique shocks from the nozzle are reached. The pressure rises across the shock, expands, and repeats. Therefore, the pressure exhibits a periodic structure directly comparable with theory and with the schlieren runs. The relationship between the pitot pressure measurements and the schlieren photographs is apparent, with the pressure minima corresponding to the bright fringes of intercepting and reflecting shocks.

Figure 4.21, Figure 4.22, and Figure 4.23 display the temporal correlation between pressure and optical measurements at $t = 25, 50$ and 100ms , respectively. The periodic structure of the jet can be seen to be diminishing faster in the pitot

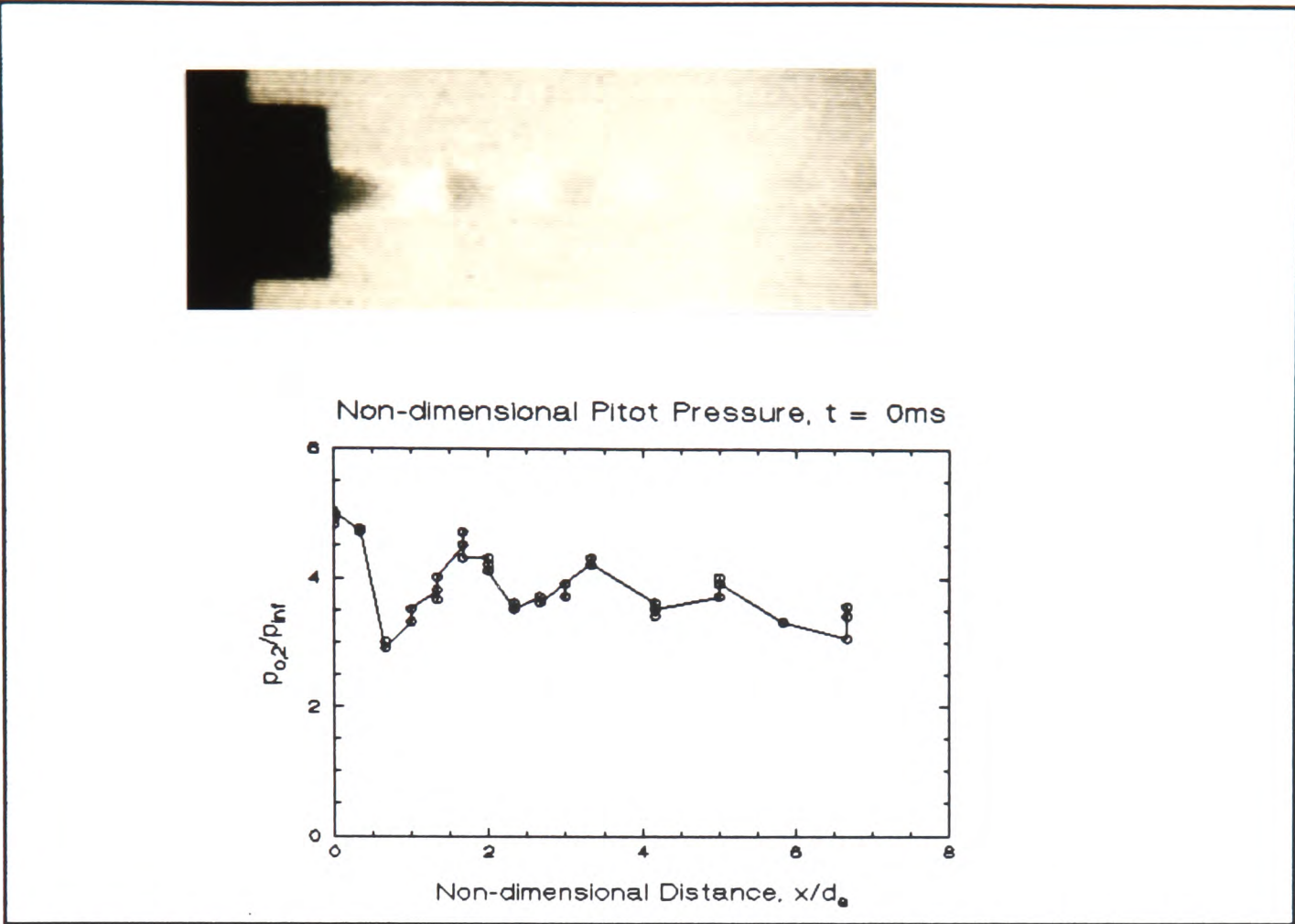


Figure 4.20 Correlation of schlieren and pitot measurements, $t = 0\text{ms}$.

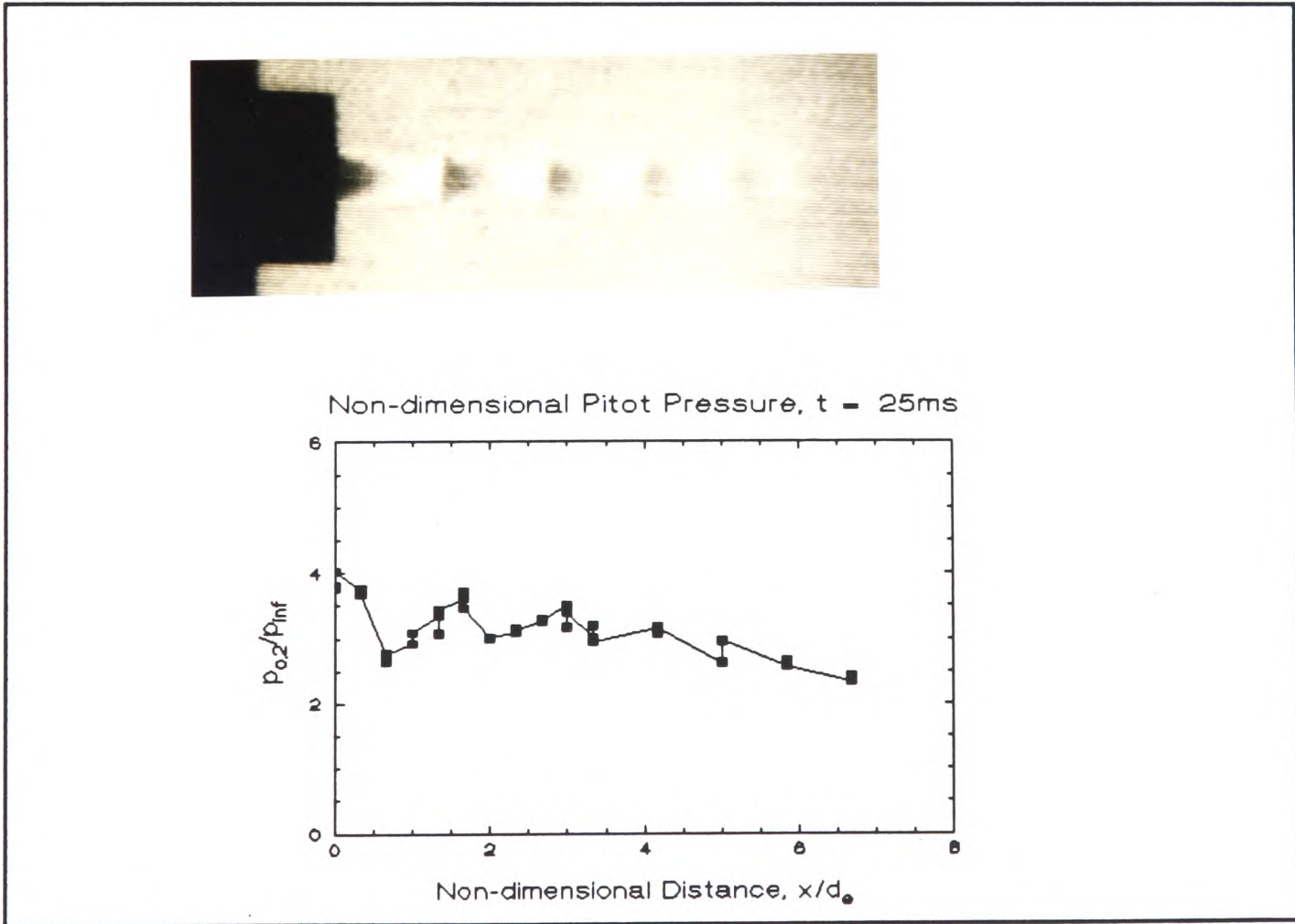


Figure 4.21 Correlation of schlieren and pitot measurements, $t = 25\text{ms}$.

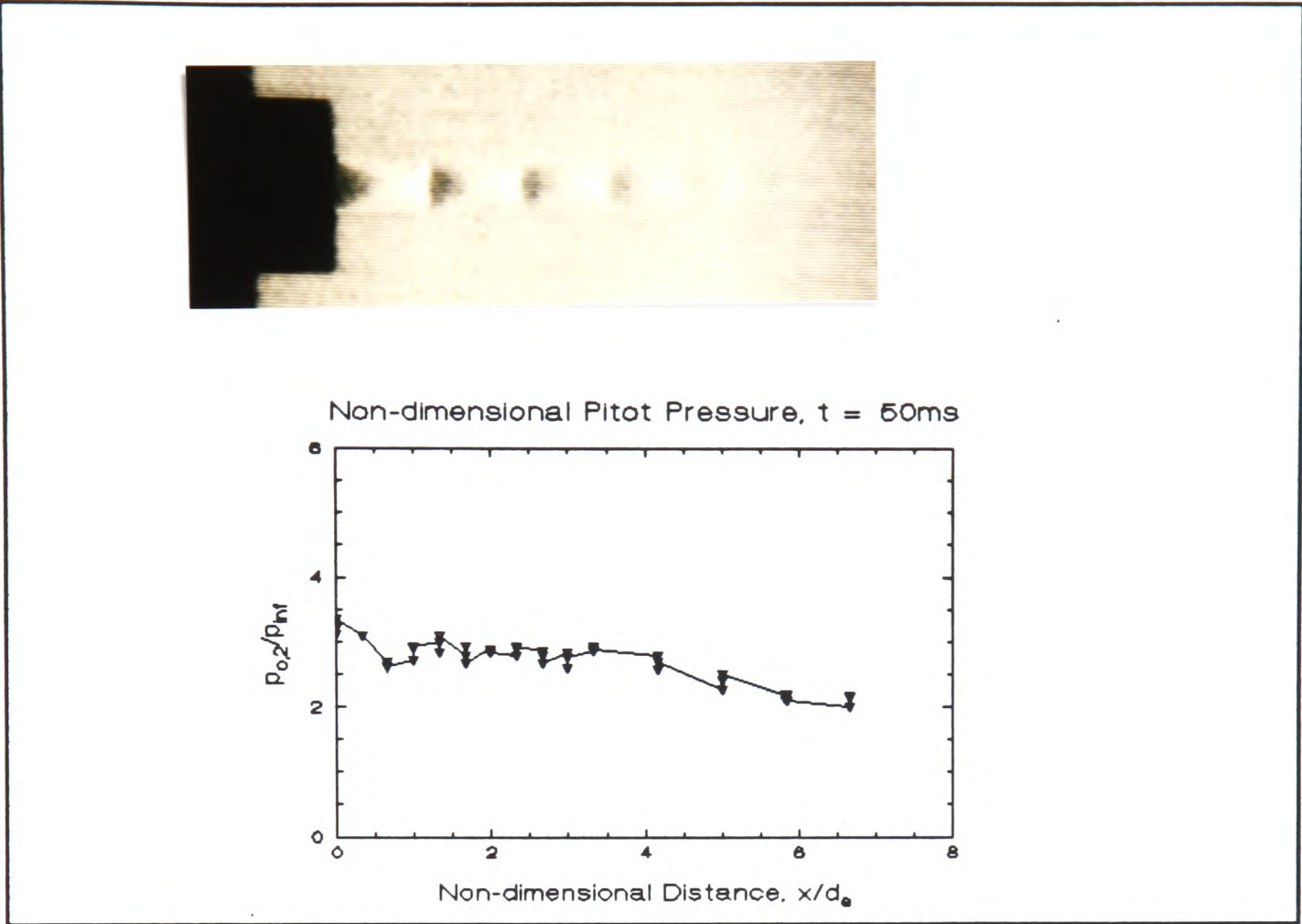


Figure 4.22 Correlation of schlieren and pitot measurements at $t = 50\text{ms}$.

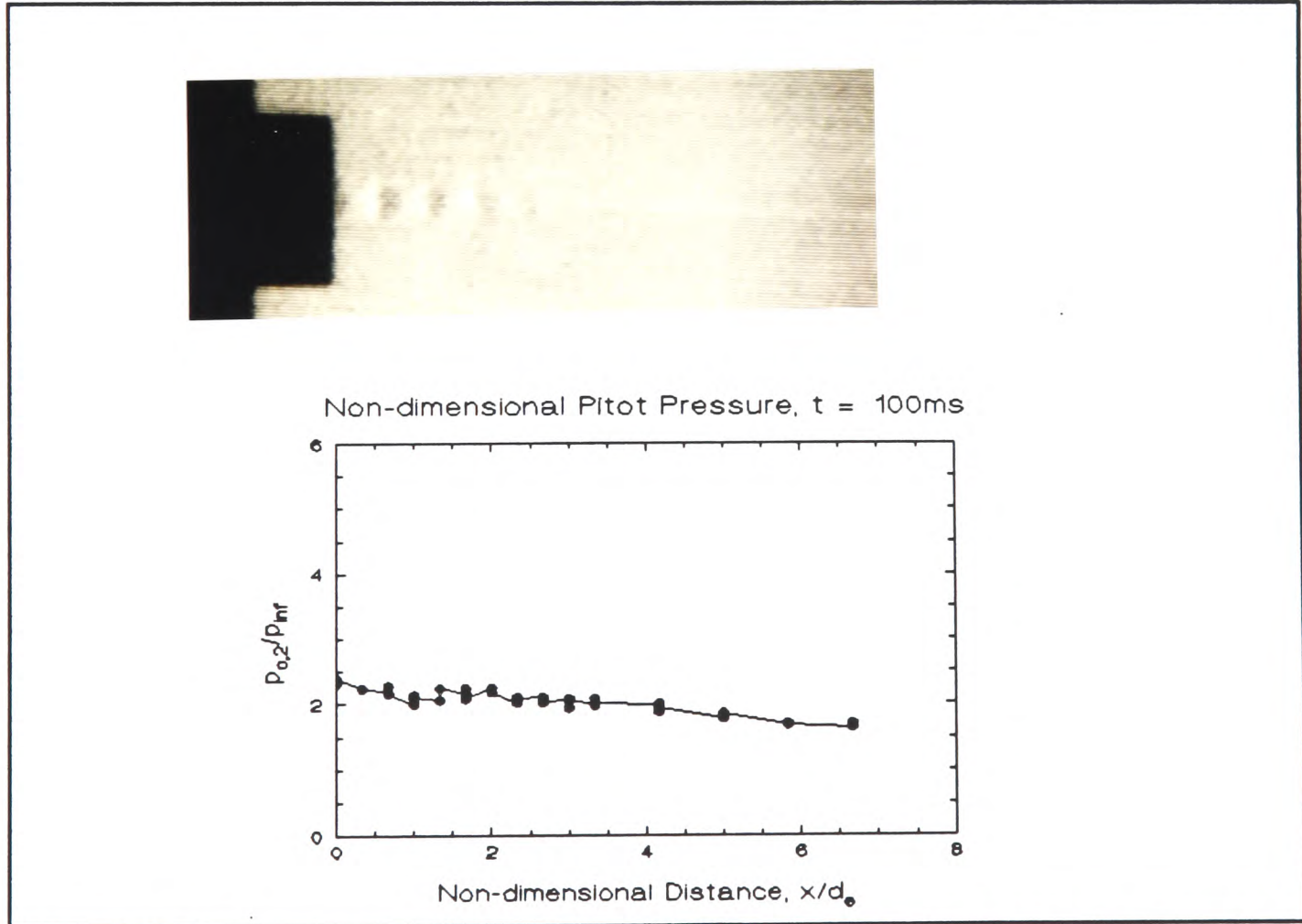


Figure 4.23 Correlation of schlieren/pitot pressure measurements at $t = 100\text{ms}$.

pressure measurements than in the schlieren measurements. By 100ms, in fact, minimal periodicity is apparent in the pressure measurements, while schlieren photographs show jet structure evident up until at least 150ms. This may be explained by the differing run conditions for the two types of tests. The schlieren runs were conducted, as explained above, with expansion into atmosphere ($p_{\infty} = 1\text{bar}$) which may be assumed to remain constant during the "blast". The pitot pressure measurements, on the other hand, were made with the jet expanded into an evacuated target chamber. Thus, the back pressure continually increased during any pitot pressure run. This, presumably, increased the rate at which the jet became over-expanded, increasing the rate at which the diamonds were forced back into the nozzle. Pressure limitations on the differential transducer and resin strength prevented pitot measurements from being made at conditions similar to schlieren conditions. In all cases, however, initial pressure ratios were constant between the two types of runs.

An error in radial positioning of the pitot tube will also cause a shift in pressure readings, as seen in Figure 4.24. If the tube is eccentric from the jet axis by an amount e , and the angles of oblique shocks are 30° , say, the axial shift will be $e/\tan 30^\circ$. In the case of incident and reflected waves, this offset effect will cause a blurring, with the single point of interception being seen as a region between the two sets of shocks.

The four pitot pressure curves from time points $t = 0, 25, 50$ and 100ms are brought together on one graph in Figure 4.25. Again, the periodicity of the jet structure is emphasised, and the reduction in λ/d_e with time is particularly apparent. This shows good agreement with Figure 4.14 above.

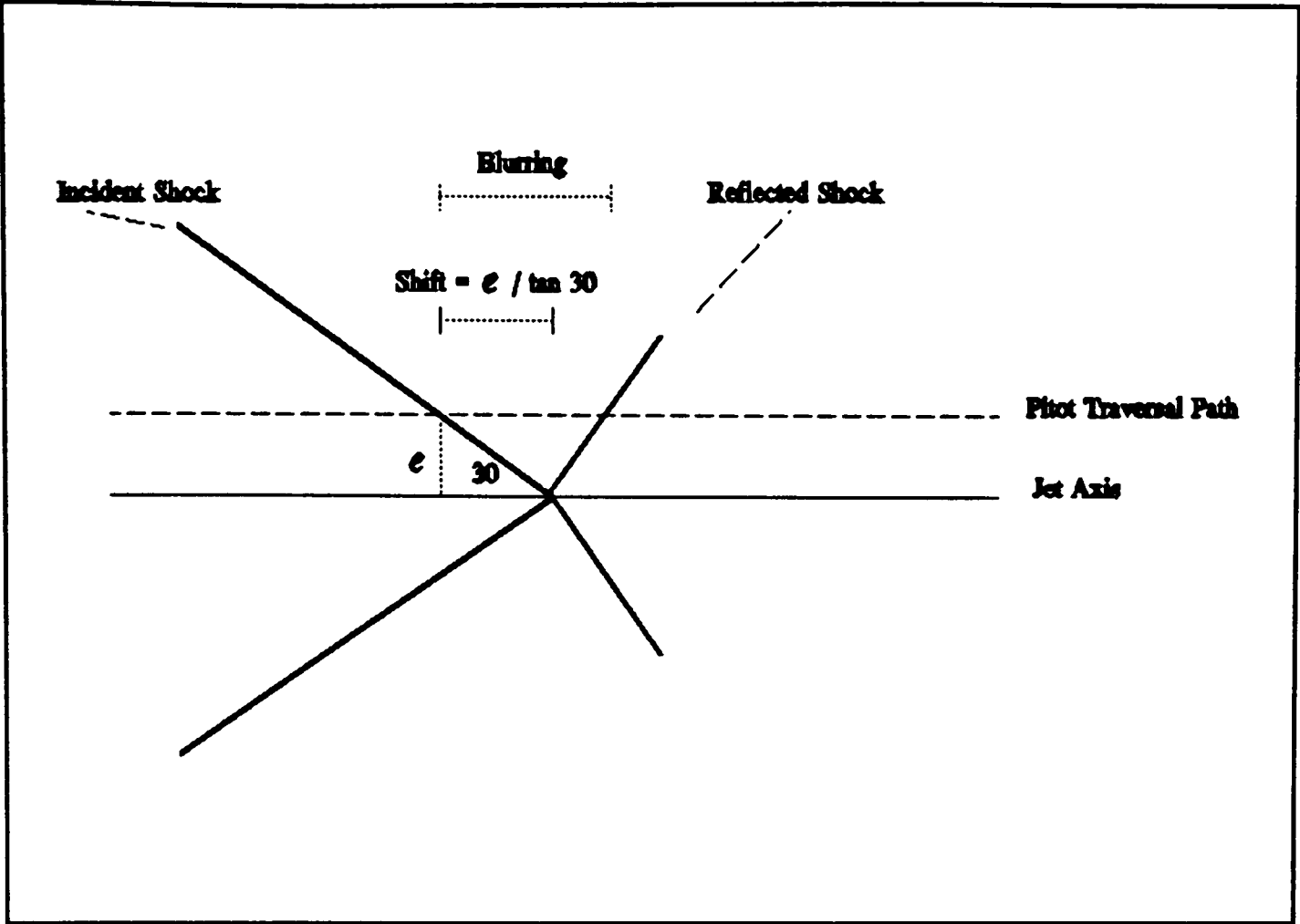


Figure 4.24 Effect of mis-alignment of pitot probe.

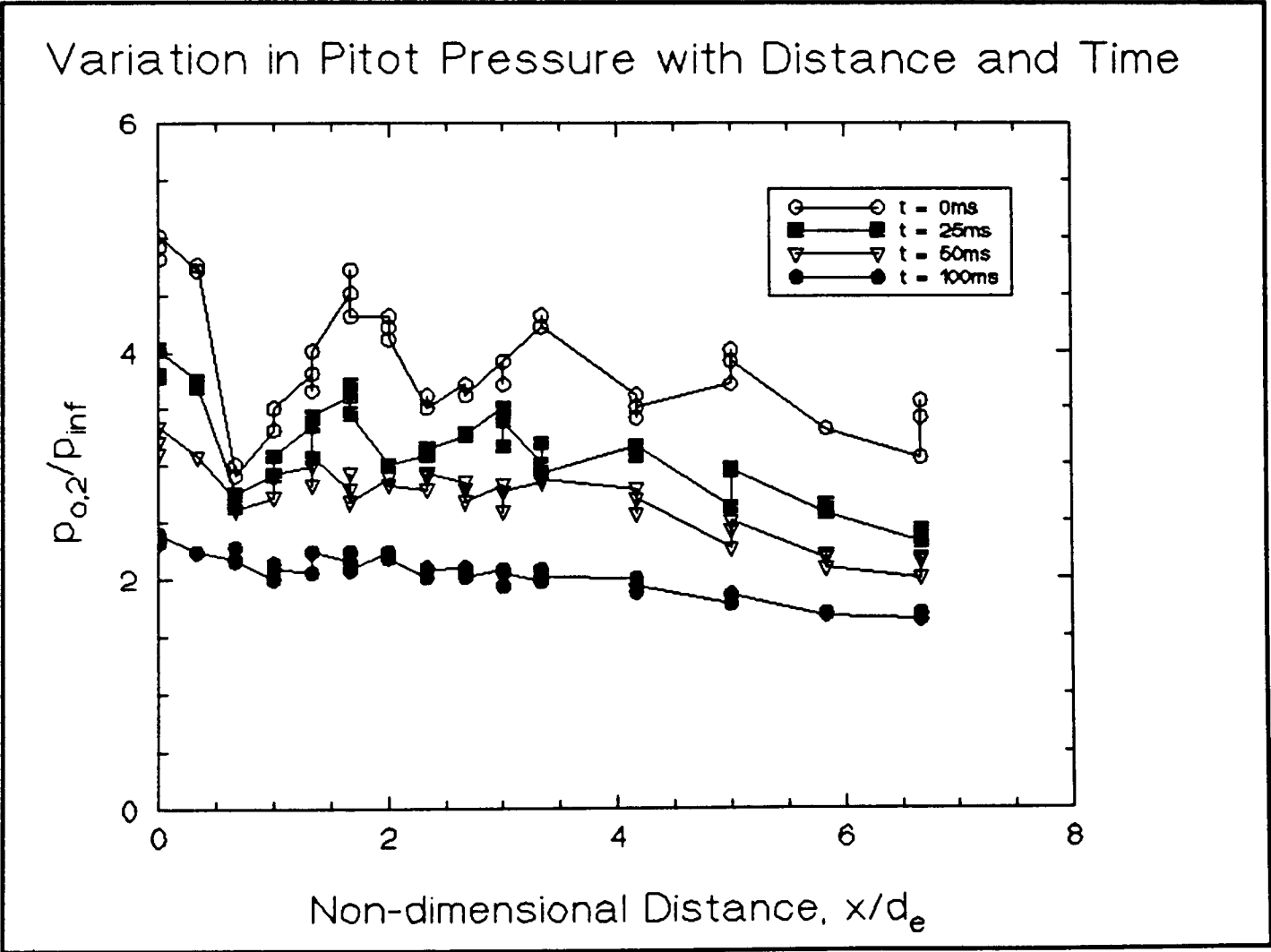


Figure 4.25 Variation in non-dimensional pitot pressure with distance and time. (Conditions: $p_o = 2.7\text{bar}$ (abs), $p_\infty = 0.25\text{bar}$)

A comparison of normalised pitot pressure axial surveys for two nozzle pressure ratios, ($p_o/p_\infty = 26.0$ and 10.8), is presented in Figure 4.26. This figure shows pitot pressure (behind the normal shock) normalised with initial target chamber back pressure, p_∞ . It is more useful to normalise pitot pressure measurements made *downstream* of the first shock cell with p_∞ as opposed to normalising with stagnation pressure, p_o . This is illustrated in Figure 4.26, which shows the periodic curves from the two conditions falling close together, especially downstream of the first shock cell.

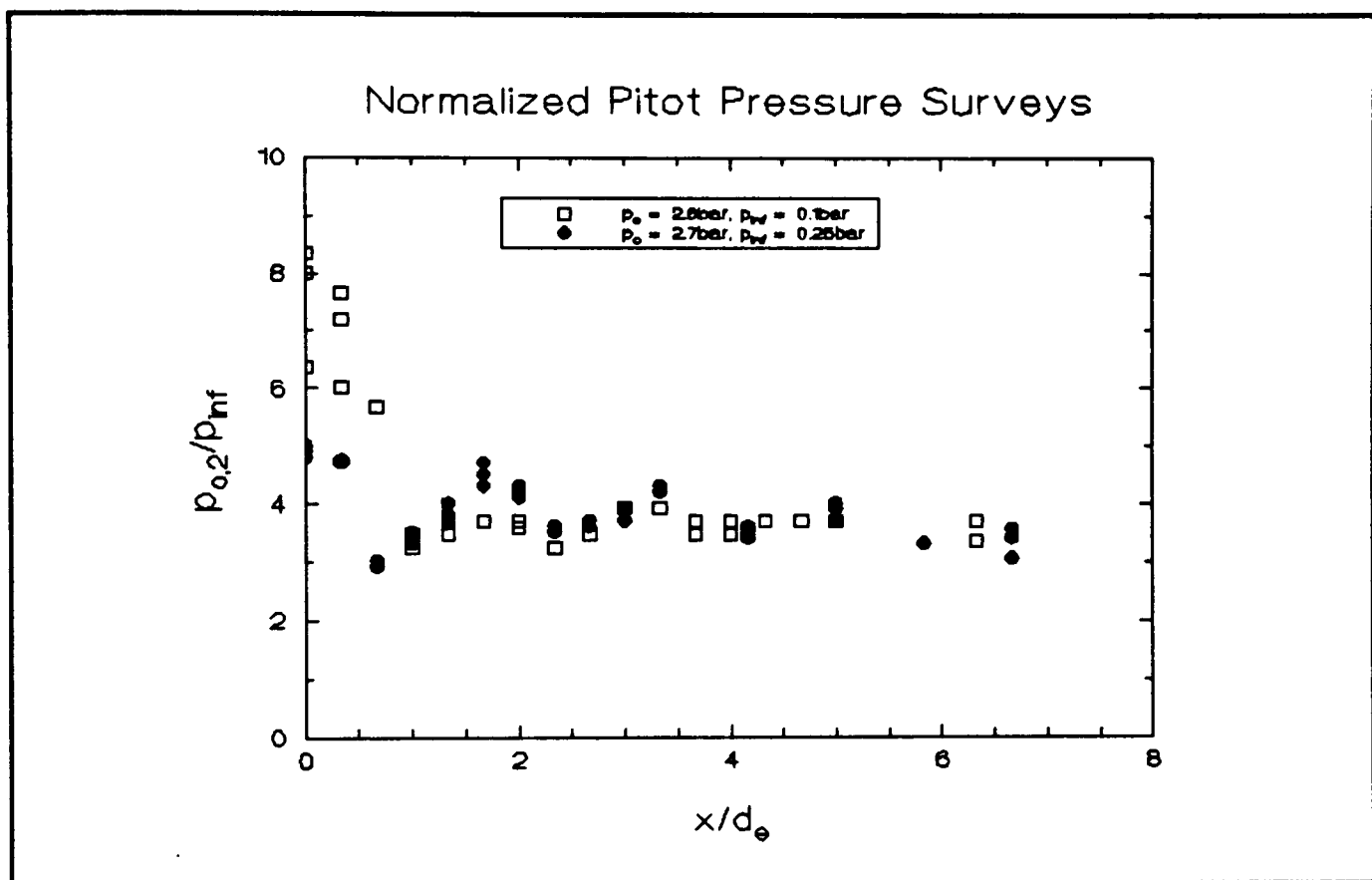


Figure 4.26 Normalised pitot pressure surveys for two nozzle pressure ratios.

4.3.3 Mach Number Determination

The preferred method of determining Mach number in a supersonic flow involves measurement of pitot pressure (behind the normal shock), $p_{o,2}$, and local static pressure, p . Measurement of local static pressure can be difficult, however, as any static probe will potentially disrupt the flow and cause formation of an oblique shock wave, thus altering the value of the static pressure measured. Due

to the transient nature of the current set-up, it was decided that accurate measurement of local static pressure was not feasible. Therefore, an alternative method of determining local static pressure was necessary.

The basic shock tube equation (3.3) gives shock strength, p_2/p_1 , implicitly as a function of diaphragm pressure ratio, p_4/p_1 . This equation was used to derive the theoretical static pressure behind the shock, p_2 , at the nozzle exit plane for various experimental values of p_4/p_1 (or p_o/p_∞). The Rayleigh supersonic pitot formula, Equation (4.4), was then be applied with the measured pitot pressure (behind a normal shock) to determine local Mach number and local flow velocity. Figure 4.27 shows the resulting flow velocities which were determined in this manner. Also shown is the theoretical variation in contact surface velocity (see Section 3.2.1). From the graph, it can be seen that the measured flow velocities show excellent agreement with the theoretical curve.

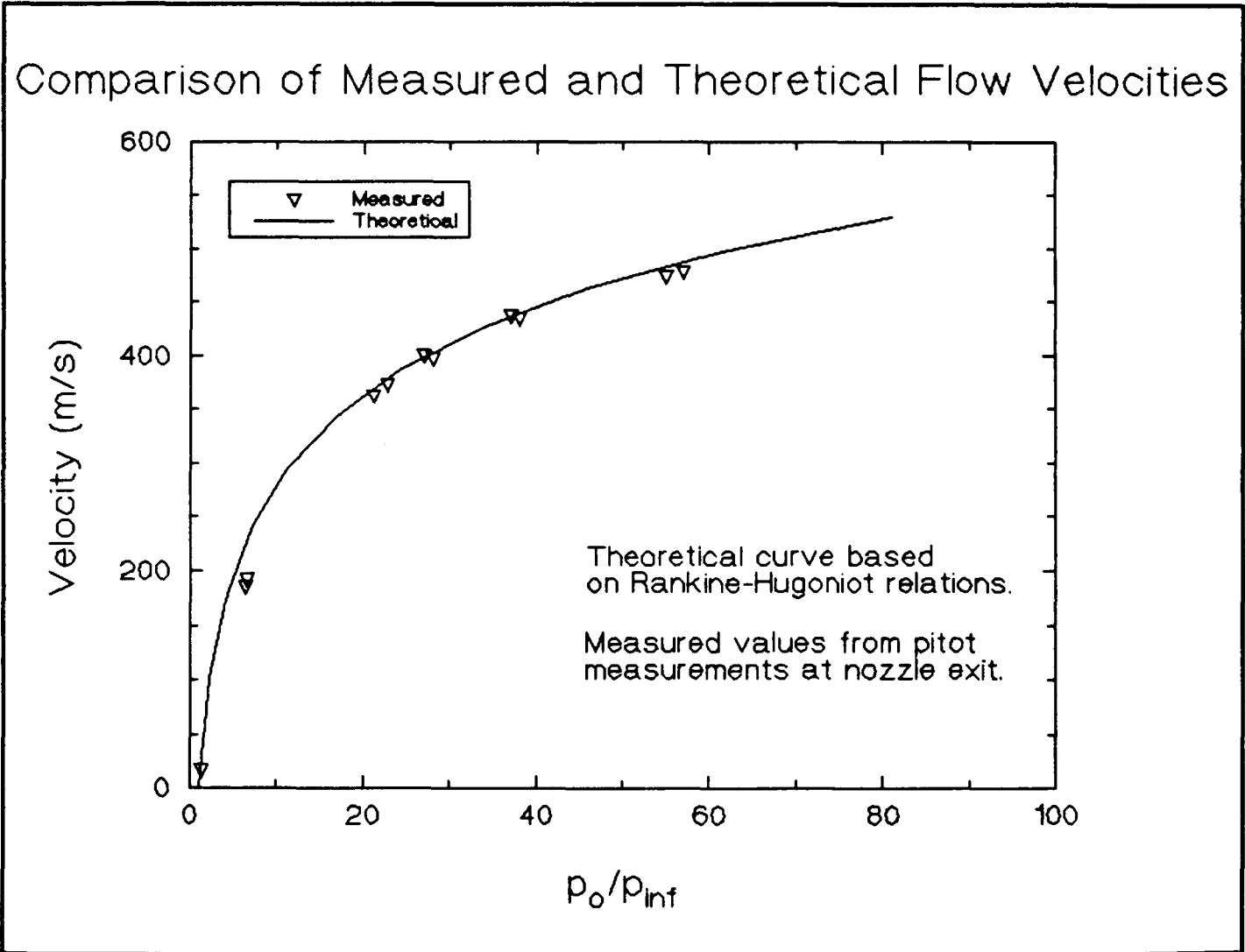


Figure 4.27 Comparison of measured flow velocities at the nozzle exit with theory.

4.3.4 Jet Impact Measurements

Impact pressure measurements were undertaken at a variety of test conditions to evaluate the initial force of the free jet throughout the target chamber. This has particular ramifications for the biological experiments; the measurement technique represents an ideal method of determining the pressure which tends to "blow away" the plant cells. Figure 4.28 shows a sample oscilloscope trace from the impact pressure measurements. Important here is the fast dynamic response of the sensor to the rise in pressure due to the jet. Impact pressure was taken to be the average of the pressures over the first 6ms after the initial pressure rise.

The results of an impact pressure survey along the jet axis with increasing non-dimensional distance, x/d_e , are shown in Figure 4.29 for $p_o/p_\infty = 11.0$. Although the technique is not sensitive enough to detect the detail of the free jet structure (because the force of the free jet is integrated over the area of the 5mm

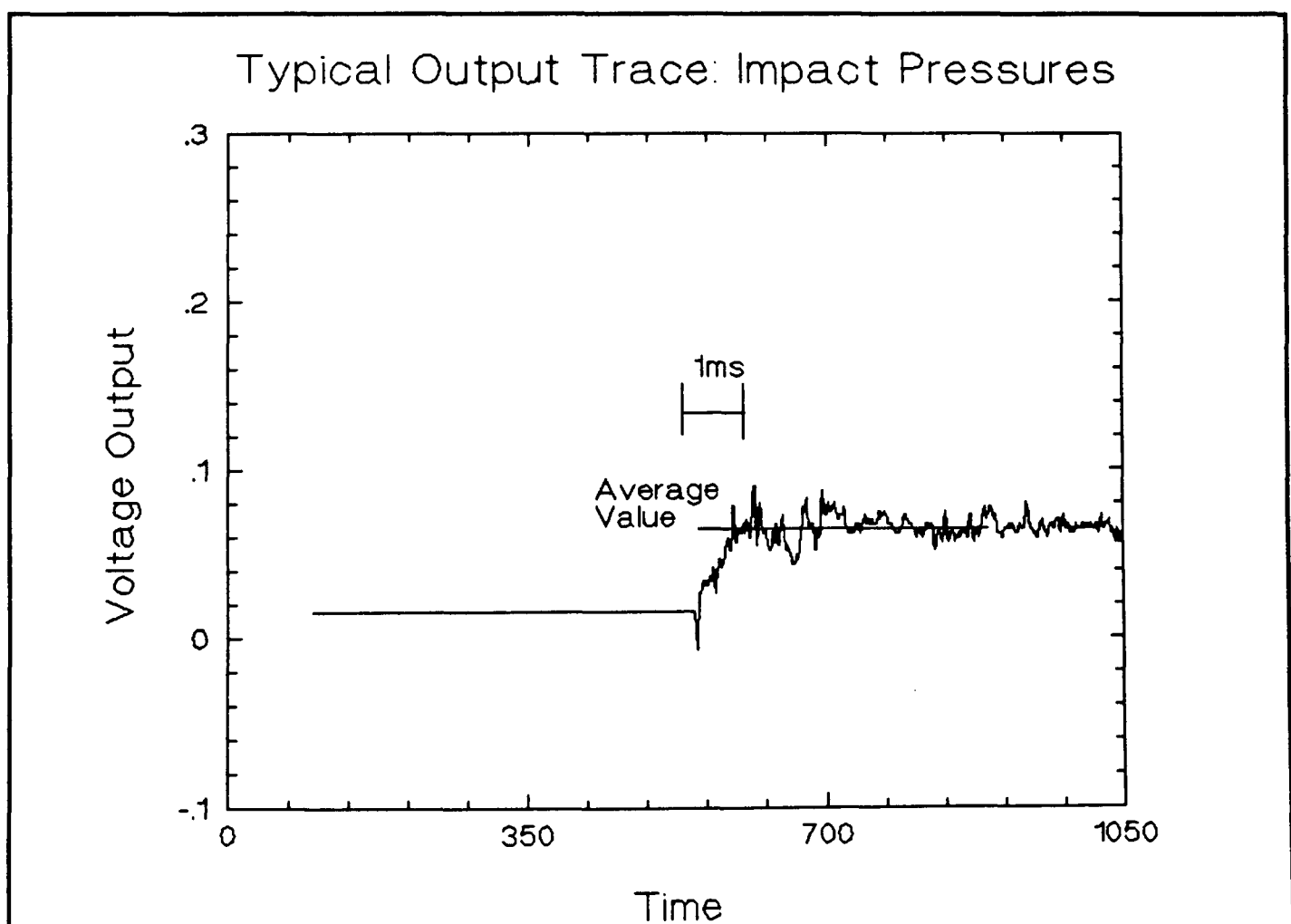


Figure 4.28 Sample oscilloscope trace from impact pressure measurements.

dia. sensor), the position of the first Mach disc may be noted from the local minimum at approximately $x/d_e = 1.2$. The results display the apparent decline in impact pressure along the jet axis. This has important ramifications in determining placement of target plant cells in the target chamber for biological experiments.

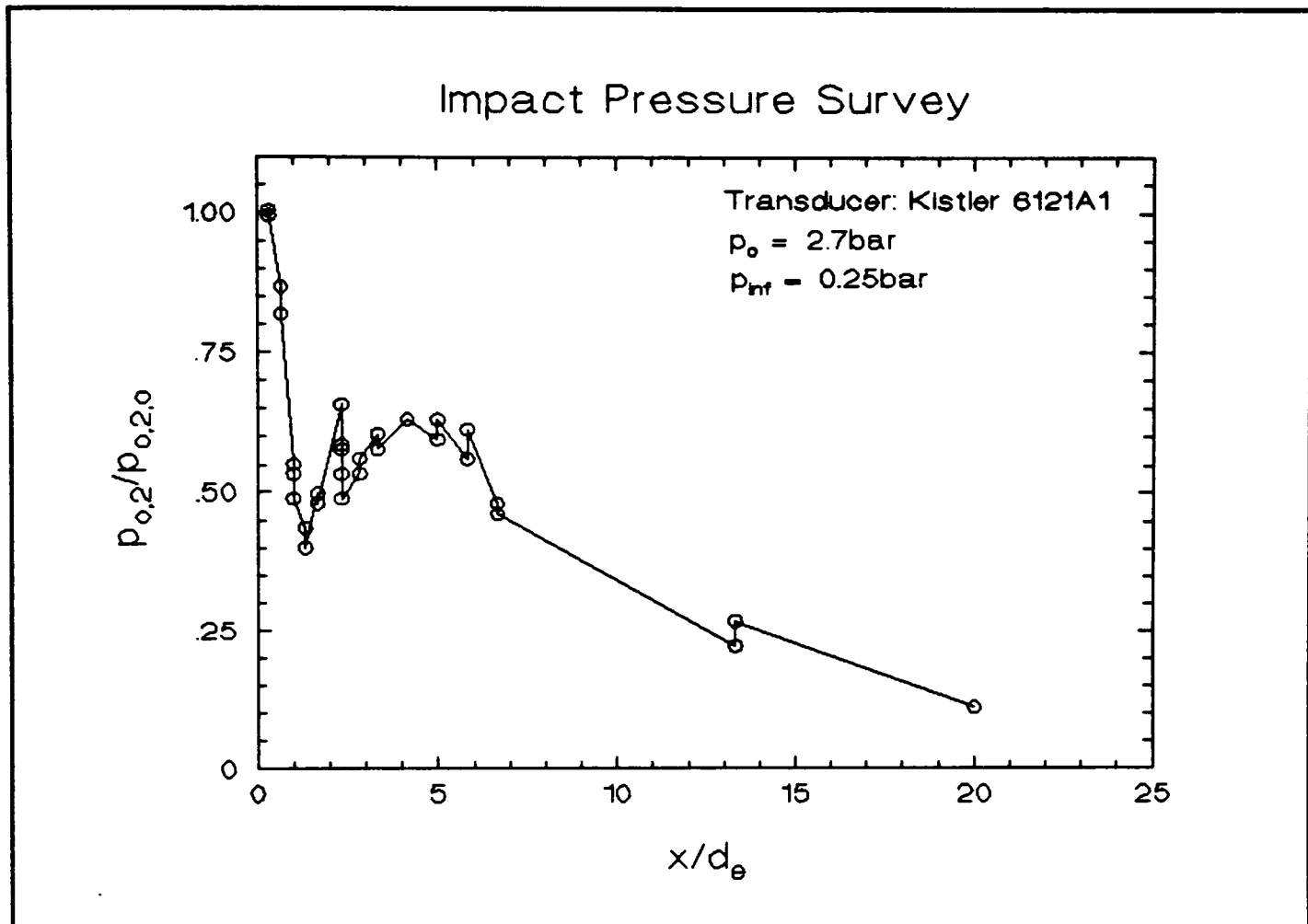


Figure 4.29 Impact pressure survey through target chamber ($p_o = 2.7\text{bar}$, $p_\infty = 0.25\text{bar}$).

A second series of impact pressure measurements was undertaken to evaluate the effect of placement of a *flow expander* in the target chamber. As described in **Chapter 2**, the purpose of the flow expander is to further expand the core flow of the jet in order to reduce impact pressures, thereby reducing the cell blow away effect. Figure 4.30 shows the reduction in impact pressure due to the introduction of a flow expander (FEx #1: $\text{Dia}_1 = 10\text{mm}$, $\text{Dia}_2 = 19\text{mm}$) at $x/d_e = 6.67$. This will later (**Chapter 7**) be shown to be quite effective in biological experimentation.

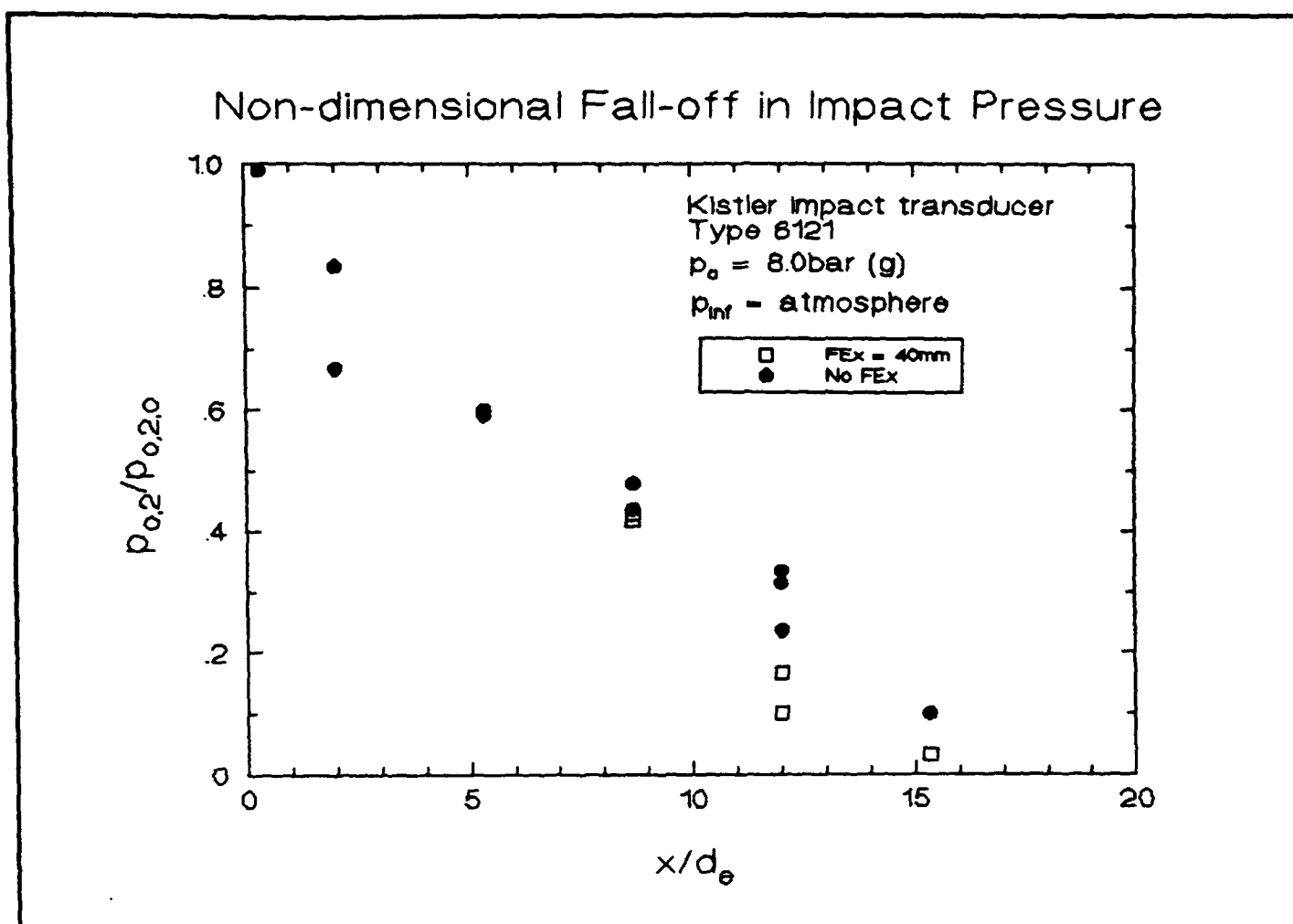


Figure 4.30 Effect of flow expander on jet impact pressures.

4.4 Relevance to Biological Experiments

One of the primary purposes of the work described in this chapter has been to provide information of use to the biological experiments. To this end several points should be emphasised:

a.) a combination of schlieren visualisation and pitot pressure measurements has demonstrated the temporal variation in jet structure, displaying the fall-off in supersonic flow and the onset of subsonic flow. This is useful in helping to determine the point at which cell blow away occurs and the minimum amount of gas necessary in the upstream supply cylinder.

b.) schlieren photographs have enabled the measurement of jet boundary location; this location has showed good agreement with an approximate solution after Adamson and Nicholls [1959]. This shows the viability of using the approximate solution to predict jet boundary location at different nozzle pressures. This will be useful in biological experiments to predict jet spread at various target distances and, potentially, to predict distribution of transformed cells.

c.) pitot pressure measurements have been used to determine the flow Mach number and flow velocity for a variety of nozzle pressure ratios. This information, in conjunction with particle velocities (see Chapter 5) is essential in calibrating the apparatus, especially for differing types of biological materials.

d.) impact measurements have shown the decline in impact pressures with increasing distance from the nozzle exit. In addition, the introduction of a flow expander in the target chamber has been shown to further decrease impact pressures. This provides information on target positioning for plant cell experiments.

4.5 Conclusions

The purpose of this chapter has been to use classical gas dynamics measurement techniques to evaluate the free jet as it emerges from the supersonic convergent-divergent nozzle. The jet structure has been studied, using both schlieren visualisation techniques and pitot pressure measurements. Measurements of the wavelength of the periodic structure and the location of the initial Mach disc have shown good agreement with the results of previous researchers, particularly with those of researchers evaluating supersonic ($M_e > 1$) nozzles. The temporal variation in this structure has likewise been evaluated, providing useful information concerning the shift from supersonic to subsonic flow. Measurements from schlieren photographs have been used to find jet boundary location. Good agreement has been shown between these measurements and an approximate technique after Adamson and Nicholls [1959].

Pitot pressure axial surveys have been compared with schlieren photographs made at similar nozzle pressure ratios, and good agreement has been found, providing confidence in the accuracy of the individual measurements. Pitot pressure measurements have been used to determine Mach number variation at the nozzle exit as a function of nozzle pressure ratio, p_0/p_∞ . Excellent agreement has been found between measured values of flow velocity and theoretically-derived values.

Impact pressure surveys through the target chamber have provided valuable information on potential location of target cells in the biological experiments. Impact pressure has been shown to decline with increasing distance from the nozzle exit, particularly at values of $x/d_e > 7$. Introduction of a flow expander in the target chamber has been shown to further decrease impact pressures by expanding the jet's core flow.

The information gained in this chapter will be combined with particle velocity measurements in **Chapter 5** and particle distribution/penetration measurements in **Chapter 6** to aid in designing the biological experiments and interpreting their results.

CHAPTER 5. TRANSIENT PARTICLE GUN OPERATION

5.1 Introduction

5.1.1 Chapter Objectives

During the early stages of development of the gas propulsion particle gun of interest in the current study, the physical mechanism underlying the motion of the micron-sized particles was unclear. The purpose of this chapter will be the determination of this mechanism of particle acceleration and the presentation of experimental results confirming this conclusion. To this end two hypotheses have been considered: i.) particle dynamics are associated with quasi-steady, fully developed nozzle flow; ii.) particle dynamics are related to the transient dynamics associated with shock tube flow.

Before the experiments described in this chapter were undertaken, arguments in favour of either hypothesis could be developed. Studies of underexpanded free jets without particles in **Chapter 4** had shown that the free jet produced by the particle gun's nozzle behaves in a manner analogous to that seen by previous researchers in the field, displaying the classic diamond configuration, for example. Therefore, quasi-steady, fully-developed supersonic nozzle flow certainly occurs at one stage of particle gun operation. On the other hand, pitot measurements at the nozzle exit had provided evidence in favour of transient effects. Initial gas velocities were seen to correlate well with theoretical contact surface velocities related to shock tube flow. In addition, the method of particle entrainment (*i.e.*, direct placement of the microprojectiles on the bursting diaphragm) and the fact that particle Reynolds numbers are of the order of one provided confidence that little slippage occurs between gas and particle velocities. This would imply that particles are accelerated by the initial slug of expanding gas - the contact surface - which follows the primary shock wave downstream in shock tube flow.

5.1.2 Chapter Thesis

Based on the evidence that will be provided in this chapter, it will be shown that hypothesis ii.) is more descriptive of the actual physical mechanism associated with particle acceleration - *i.e.*, transient, shock tube dynamics are of primary importance. One experiment will show that the time scale for particle delivery to the nozzle exit is approximately $550\mu\text{s}$ after diaphragm rupture (varying with nozzle pressure ratio p_o/p_∞) - closer to the time scale of shock/contact surface propagation ($500\mu\text{s}$) and significantly less than the approximately 1.8ms required for the commencement of steady flow (Smith [1966]). A schematic diagram of the time scales for steady-state nozzle flow, shock flow and particle detection is seen in Figure 5.1.

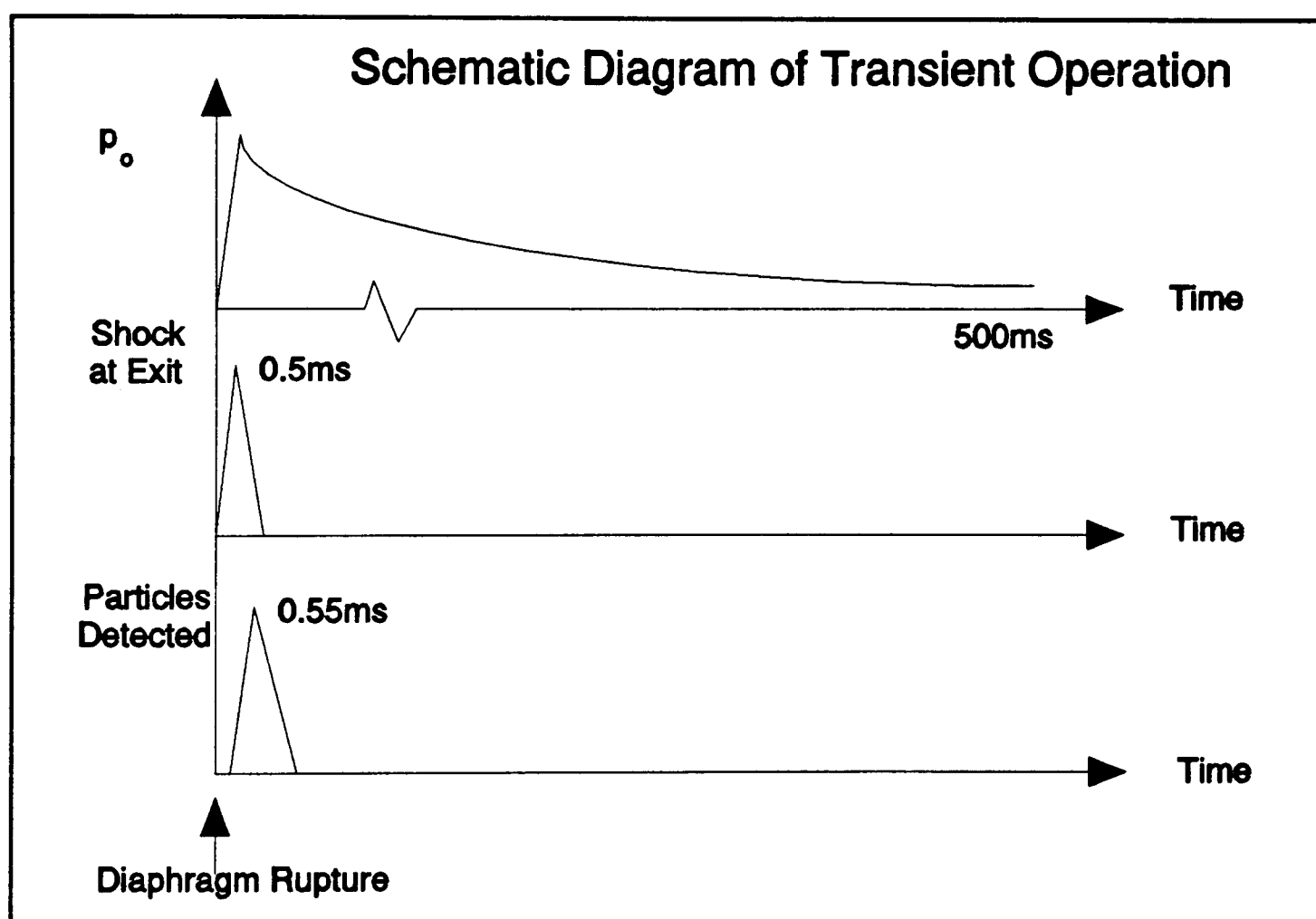


Figure 5.1 Schematic diagram displaying time scales of particle delivery, shock flow, and nozzle flow.

The results of several other experiments will provide additional evidence for the conclusion that transient effects predominate. An experiment evaluating particle time-of-flight between two locations in the target chamber for varying

volumes of upstream cylinder size will show that little variation in particle time-of-flight occurs - even for cylinders only 1/10th the size of the original supply cylinder. In addition, particle velocity measurements for a range of nozzle pressure ratios and at a range of locations in the target chamber will show particle velocities to vary in a manner analogous to the variation in contact surface velocity. Finally, an experiment involving the replacement of the convergent-divergent supersonic nozzle with a cylindrical tube to form a classical shock tube will be shown to result in particle velocities higher than the sonic velocity - theoretically the maximum particle velocity if quasi-steady flow is the principal underlying physical mechanism.

In other sections of this chapter the theoretical background of gas-particle free jets will be discussed. An analytical technique will be presented which shows that the velocities of micron-sized particles of interest in the current study closely follow the local gas velocities. A novel particle velocimeter for the measurement of particle velocities in the target chamber will be described. Results of the experiments described above will be presented. These results will clearly show that particle velocities at the nozzle exit are directly related to transient propagation of the primary shock wave and the contact surface which follows this shock wave. Also, particle velocity measurements in the target chamber will be presented which will enable calibration of the gas propulsion particle gun for conditions relevant to biological bombardment conditions. Finally, particle velocities within the target chamber will be related to velocity fall-off of an underexpanded jet.

5.2 Particle-laden Jets: Historical Background

The general behaviour of solid particles in nozzle flows received a good deal of attention in the 1950's and 60's, primarily in the aerospace industry. Researchers and rocket scientists were keen to understand the negative effects of metal oxide combustion products, which comprised upwards of 40% by weight of the total combustion products of the early solid rockets (Hoglund [1962]). Unable

to do any expansion work, solid particles only decrease the effectiveness of the nozzle expansion process, reducing the efficiency of the rocket motor. Gilbert *et al.* [1955] showed that slippage of these particles in the gas flow stream (particle lag) is a significant contributor to losses in specific impulse of solid rocket motors. Using Stokes' law, which assumes inertial effects to be negligible (so that particle drag is proportional to gas-particle velocity difference), Gilbert *et al.* found that for their conditions (linearly-accelerating gas velocities), the velocity of a 1 μ m diameter particle typically follows the gas velocity closely, whereas a 10 μ m dia. particle shows significant velocity lag. An analytical technique similar to that of Gilbert *et al.* will be applied later for evaluation of the particle dynamics of the current system under study.

Other workers have concentrated on evaluating particle slippage in supersonic flows. Bailey *et al.* [1961] used a one-dimensional analysis similar to that of Gilbert *et al.* [1955] to numerically compute particle velocity lag within an axisymmetric rocket nozzle (propellant: Arcite 372). Bailey *et al.* compared the velocity lag for two different sizes of particles: average particle radius of 2 μ m versus average particle radius of 5 μ m. Their results show that particles of radius 2 μ m achieve 90% of gas velocity within 2.6 throat diameters of the throat and asymptotically approach the gas velocity within 11 throat diameters. Significantly greater lag is seen with the larger (5 μ m) particles. Marble [1963] computed the flow of a gas-particle mixture through an axisymmetric rocket nozzle with the aim of re-designing the conventional nozzle contour to minimise particle lag losses. He found that particle velocity lag can be reduced by approximately 40% through optimum nozzle contouring, although this has only a marginal effect in reducing specific impulse losses.

The change in free jet shock-structure with particle addition has been of particular interest to workers, especially in the aerospace/defense industry. Changes in plume structure of hypersonic vehicles affect plume signature, thereby altering mechanisms by which such vehicles are identified. As described earlier (Section 3.3.3), an important parameter pertinent to supersonic free jets issuing from underexpanded nozzles is the distance from the nozzle exit plane to the first normal shock wave (Mach disc). Early researchers sought a better understanding of the change in this distance with particle addition as an aid in determining

plume radiation effects from solid rockets. Lewis and Carlson [1964] used schlieren photography to measure the distance to the first Mach disc, $x_{m,p}$, for two conical nozzle designs and three gases. They found that addition of alumina particles (mean diameter: $2.8\mu\text{m}$) to the continuous jet caused a significant reduction in $x_{m,p}$. Jarvinen and Draper [1967] modelled a particle-laden jet as a pseudo-nozzle, where the particles are assumed to take no part in the expansion of the plume from the nozzle exit. The assumption of no particle velocity lag was also made. This allowed the calculation of a gas-only exit plane Mach number which decreased with increased particle loading. Theoretical predictions of Mach disc location as a function of particle mass fraction were then developed. These theoretical predictions showed good agreement with the experimental data of Lewis and Carlson [1964]. Bauer [1965] developed a self-similar solution based on the assumption of complete gas-particle thermal equilibrium. The theoretical model Bauer obtained also corresponds well with the data of Lewis and Carlson. More recently, Sommerfeld [1989] used a shadowgraph technique to determine experimentally the change in Mach disc location with increased particle loading. His finding that the Mach disc location moves upstream toward the orifice as particle loading increases agrees with results of previous researchers. Sommerfeld, however, also found significant particle velocity lag through measurements with a laser-Doppler velocimeter. This is not surprising, however, as his particles (spherical glass beads; mean dia.: $45\mu\text{m}$) were significantly larger than those used by previous researchers. His conditions were such that the assumption of Stokes' flow was not valid.

The dynamics of particle-laden jets have also been investigated in the combustion industry with the aim of optimising spray injection mechanisms. In addition, workers seeking a better understanding of the phenomena surrounding explosions have studied the passage through a dusty gas of a shock wave (Carrier [1958], Rudinger [1964]). An analytic approach was used by Igra *et al.* [1987] to model the motion of $10\mu\text{m}$ dust particles retained within a spherical diaphragm prior to rupture. Their analysis found that the dust becomes confined within a spherical layer whose radius increases with time. The peak in the dust spatial density was found to be positioned very near the contact surface and tended to

follow that surface quite closely as a function of time. Soo [1990] has presented an excellent review of various aspects of particle-laden gas dynamics.

5.3 Analytical Approaches

5.3.1 Analytical Solution of Particle Motion

Gilbert *et al.* [1955] used a Stokes' approximation and integrated the equation of motion for linearly-accelerated one dimensional gas flow to calculate analytically the particle velocity lag. A similar analysis will be used as a starting point to calculate particle velocities in the current system under study. The analytical solution assumes inertial effects to be negligible compared to viscous forces, such that $Re_p < 1$, where Re_p is particle Reynolds number. For flow around bodies at very small particle Reynolds numbers separation along the body does not occur, and no disturbed wake develops behind the body. Streamlines show fore-and-aft symmetry. In such flows the assumption of Stokes' drag can be applied. Many textbooks (e.g., White [1974]) present good derivations of Stokes' flow. The analysis that follows assumes a linearly-accelerating fluid flow field. Strictly-speaking, Stokes' flow is not applicable in the early stages of particle acceleration, where $u_{gas} - v_p$ is maximum (and, presumably, $Re_p > 1$). However, Stokes' drag represents a lower bound on the actual drag force for such conditions and consequently provides a conservative estimate of the real drag force. A final caveat concerning the analysis should be noted: the analysis is for a single particle and assumes no particle-particle interaction. It will be shown later (**Chapter 6**) that in the system under consideration particle mass fractions are small enough such that particle interactions are negligible. In addition, scanning electron micrographs in **Chapter 6** will reveal that particle clumping is not a significant problem; therefore, the mean particle radius utilised in the analysis is probably not far off from the real mean particle radius.

Consider, then, the motion of particles in such a flow. The equation of motion for spherical particles can be written:

$$m \frac{d^2x}{dt^2} = 6\pi\mu r(u_g - v_p) \quad (5.1)$$

With a general flow field $u_g(x)$, is not usually integrable. However, an exact solution may be obtained if $u_g(x)$ is assumed to be linear with x . Therefore,

$$u_g(x) = u_o + ax \quad (5.2)$$

The resulting second order linear ordinary differential equation has as initial conditions:

$$\frac{dx}{dt} = v_{p,o} \big|_{x=0, t=0} \quad (5.3)$$

Let α be a reciprocal Stokes time constant, where $\alpha = 6\pi\mu r/m_p = 9\mu/2\rho_p r^2$. Also, let $\lambda = 2a/\alpha$, where a represents the velocity gradient in the gas. The second order differential equation initial value problem can be solved using the Laplacian transform method. By making the physical parameters dimensionless, particle motion can be shown to be:

$$\xi = e^{-\tau} \left(\cosh \tau \sqrt{1+2\lambda} + \frac{\phi\lambda+1}{\sqrt{1+2\lambda}} \sinh \tau \sqrt{1+2\lambda} \right) - 1 \quad (5.4)$$

where $\xi = ax/u_o$, $\tau = \alpha t/2$, and $\phi = v_{p,o}/u_o$. The relative particle velocity lag, $\Delta = (u_g - v_p)/u_g$ can be written:

$$\Delta = \frac{\phi(1+\lambda) - 1 + (1-\phi)\sqrt{1+2\lambda} \coth \tau \sqrt{1+2\lambda}}{1 + \lambda\phi + \sqrt{1+2\lambda} \coth \tau \sqrt{1+2\lambda}} \quad (5.5)$$

This analytical solution was used to predict trajectories of 1 μ m-diameter tungsten particles in the system under consideration. Solutions are plotted in dimensionless form in Figure 5.2 for two values of λ : a.) $\lambda = 0.08288$, where the gas flow accelerates linearly along the entire nozzle length (0.300m) to an assumed exit velocity (600m/s); b.) $\lambda = 0.24865$, where velocity gradient of the gas flow field is three times greater, so that the gas accelerates linearly to the same maximum velocity over a 0.100m nozzle length. The exit velocity assumed in this analysis is representative of particle velocities actually measured, as described

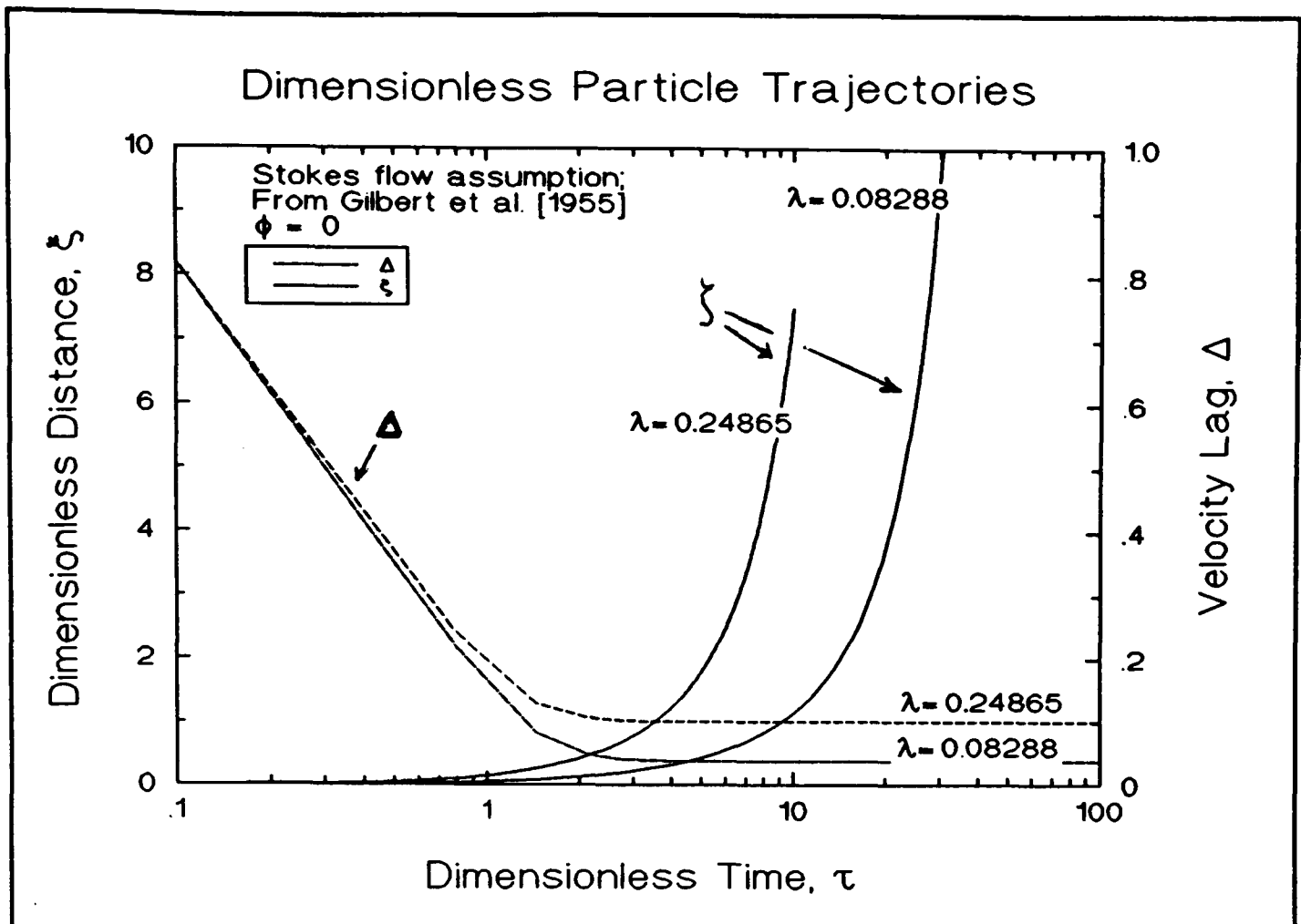


Figure 5.2 Analytical solution of particle motion in linearly-accelerating flow field, using Stokes' flow approximation.

below. Evaluation of particle motion for two values of flow field velocity gradient permits the comparison of the final values of velocity lag attained. As can be seen in the figure, even for the condition of large flow field velocity gradient (Case b.), particles quickly acquire velocities within 10% of the gas velocities. Note that as flow field velocity gradient approaches 0 (for a given Stokes' reciprocal time constant, α), particle velocity lag approaches 0. Similarly, increases in α lead to decreases in particle lag. Clearly, since α is inversely related to the square of particle radius, r_p is of major significance.

These theoretical results show that the small radius of the particles used in the current study (approx. $0.5\mu\text{m}$) lead to small values of velocity lag (*i.e.*, particle velocities closely follow gas velocities). Figure 5.2 shows the velocity lag to be less than 10% even for the highest value of gas velocity gradient that would be expected in the present set-up.

5.3.2 Stokes Number

Marble [1963] has characterised particle dynamics associated with shock passage through dusty gases by describing a Stokes particle time constant, S_p ($= 1/\alpha$):

$$S_p = \frac{m_p}{6\pi r_p \mu} \quad (5.6)$$

This quantity relates inertial force potential associated with particle mass to drag force potential and represents the time required for a particle to increase its velocity relative to the gas by e^{-1} of its original value. Note that in fact $S_p \propto r_p^2$, so that, as intuition would hold, smaller particle radii lead to smaller relaxation times. For situations where S_p is on the order of the local characteristic gas relaxation time, little particle slippage will occur.

For the micron-sized tungsten particles of concern here, S_p can be shown to be approximately 95 μ s. Because this is considerably less than the approximate time required for shock/contact surface propagation through the nozzle (500 μ s), particle slippage is not expected to be a significant factor in transient flow through the shock tube/nozzle.

5.4 Particle Velocimetry Measurements: Technique

Yanta *et al.* [1976] have demonstrated the feasibility of velocity measurement within a supersonic flow field using laser-Doppler velocimetry (LDV). The principles upon which LDV is based are well known and have been documented in several texts and review articles (see, for example, Durst *et al.* [1976]). This was originally the method intended to be used for making particle velocity measurements in the current system under study. As no LDV system was readily available, however, a new measurement technique was required. It was decided that a non-invasive, optical technique for making time-of-flight measurements between two axial locations in the target chamber would be suitable. This method suffers the disadvantage of being unable to discern velocity at a single point. Indeed, with this method particle velocities must be averaged over a finite axial distance. On the positive side, however, the system was easily designed and relatively inexpensive to build.

5.4.1 Design of Optical Particle Velocimeter

The general set-up of the optical particle velocimeter (OPV) is shown in Figure 5.3. The system comprises two light emitting diode / photodiode detector (LED/PD) pairs placed in parallel in the target chamber. Purpose-built holders ensure that the LED/PD pairs are centred in the target chamber. The LEDs (Farnell, TLMP7005; peak λ : 660nm, intensity: 300mcd at 20mA) emit light which is collimated to an included angle less than 6 degrees by lenses on the tips of the LEDs. Light from each LED passes through the jet centreline to a PIN photodiode detector (RS, BPX65; working area: 1mm²). The signal output from each optical detector is then amplified by a bi-polar operational amplifier (RS, NE531N), using a standard amplifier circuit, designed with the aid of a colleague of the author, Joost de Kock. A circuit diagram is shown in Figure 5.4.

Crucial to the design is the slew rate (response time) of this op-amp, which is quoted by the manufacturer as 35 V/ μ s. This gives a response time of the order

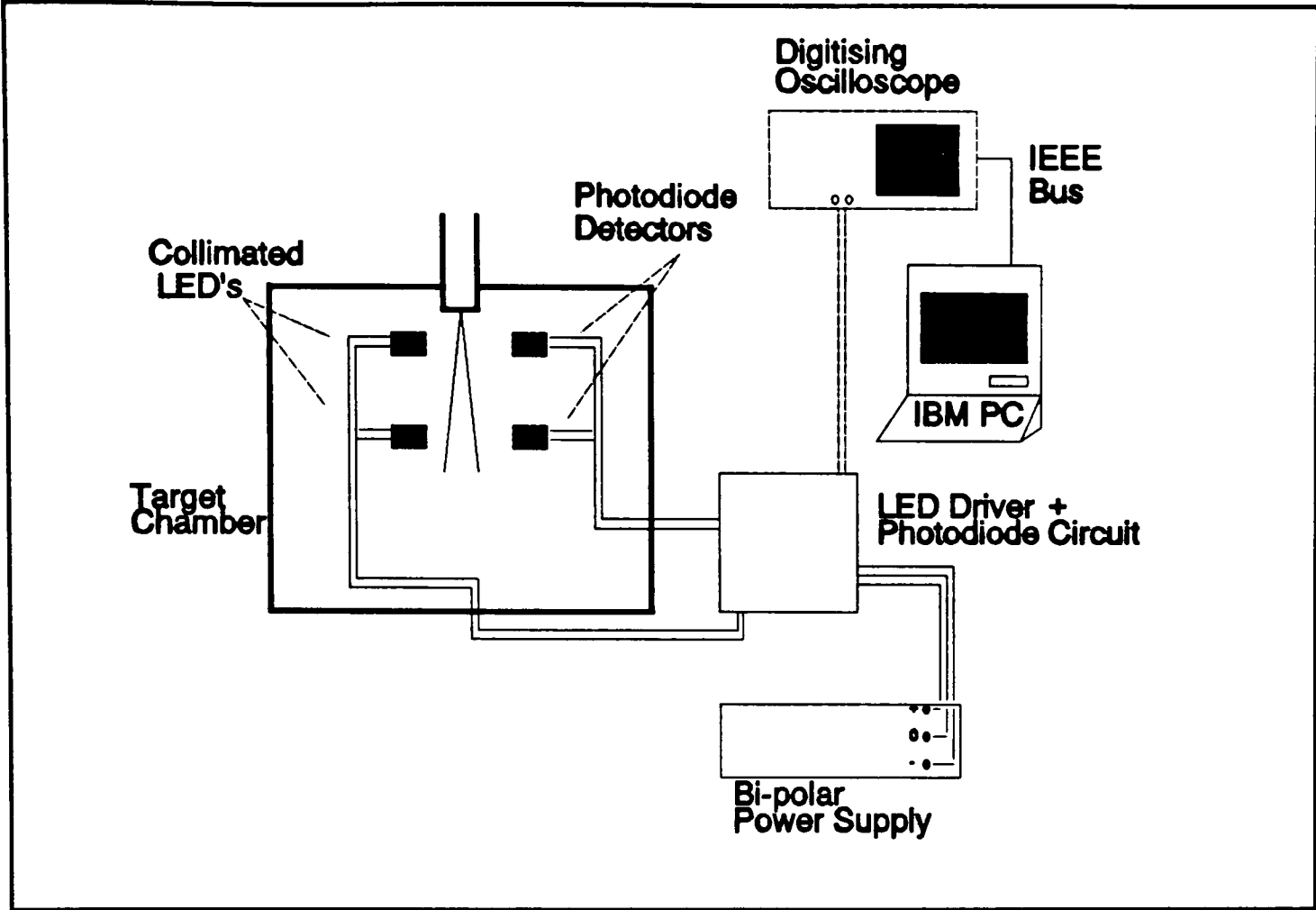


Figure 5.3 General set-up of optical particle velocimeter.

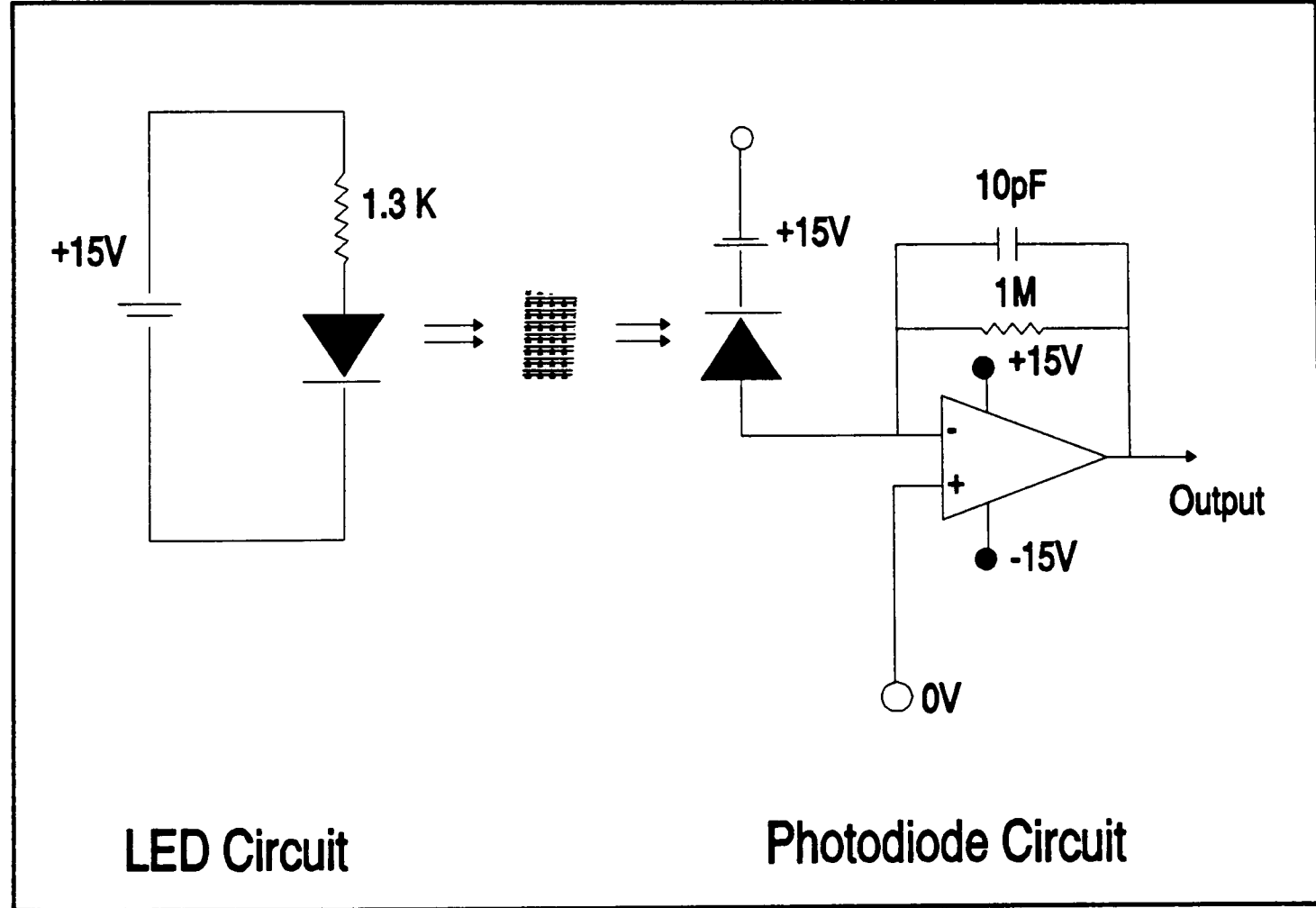


Figure 5.4 Circuit diagrams of LED and photodiode/op-amp circuits.

of $0.1 \mu\text{s}$ for the voltages of concern here, or less than 1% of the magnitude of the measurements. A bi-polar power supply powers both the op-amp circuit and LED circuit. The amplified signals are triggered on a Hewlett Packard digitising oscilloscope (Model 54200A). Typical waveform traces from the photodiodes (via the op-amps) are shown in Figure 5.5. This voltage data is then bussed to an IBM Portable PC via an IEEE interface card (Brain Boxes, Liverpool). At the PC the voltage waveforms are downloaded in binary form onto floppy disk to facilitate data analysis on a IBM-386 PC.

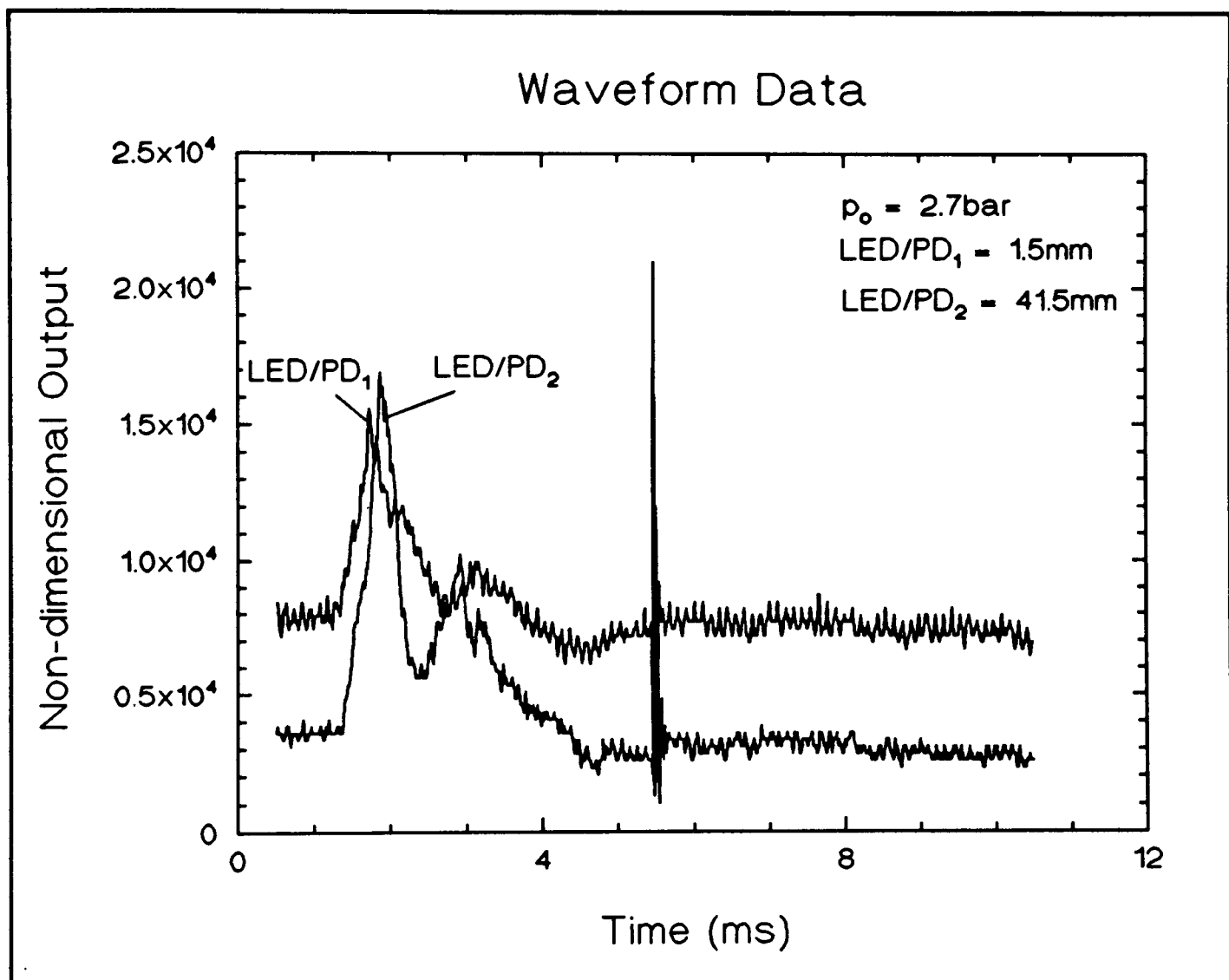


Figure 5.5 Typical waveform signals output from photodiodes.

The system works in the following manner: as groups of particles in the jet pass between an LED and a photodiode, light reaching the working area of the photodiode is decreased through reflection, causing a rise in the output voltage. The output voltage decreases as the light reaching the detector returns to its original level. It should be emphasised that the technique suffers from several

disadvantages: a.) it is only capable of determining average particle velocity between locations LED/PD₁ and LED/PD₂ of the LED/photodiode pairs - *i.e.*, instantaneous local velocities are integrated across that distance and cannot themselves be distinguished; b.) the optical technique integrates across its entire line-of-sight, usually through the jet axis. In doing so, however, it also "sees" particles in the slower-moving mixing region along the jet boundary. This may distort the measurements somewhat, although, presumably, in a consistent manner.

It was demonstrated that the waveforms are related only to particle motion by making ten test runs with *no* particles present in the flow. In *no* case did the oscilloscope trigger or register any waveforms. This discounts the possibility that these waveforms might be artifacts of system vibration or shock wave impingement.

5.4.2 Data Analysis

Amplified waveform signals from the two photodiodes were analysed to determine the time delay. Since the axial distance between the photodiodes was known, an average particle velocity (averaged over distance) could be determined. In order to calculate this time delay, a standard cross-correlation technique was implemented, using a FORTRAN program written by the author. (The mathematical theory underlying the technique is briefly outlined in **Appendix D**. A listing of the FORTRAN program XCORR can also be found in this Appendix.)

5.4.3 Verification of Technique

A standard method of verifying a cross-correlation algorithm is to transform a waveform signal by a given number of time points along the time axis and then to cross-correlate this transformed signal with the original. The output should show a peak consistent with the artificially-introduced time delay. This was done for the cross-correlation algorithm and program used by the author. A typical

waveform signal was transformed 50 time points along the time axis. This transformed signal was then cross-correlated with the original signal. As can be seen in Figure 5.6, the result of the XCORR program is a peak at 50, which is consistent with expectations.

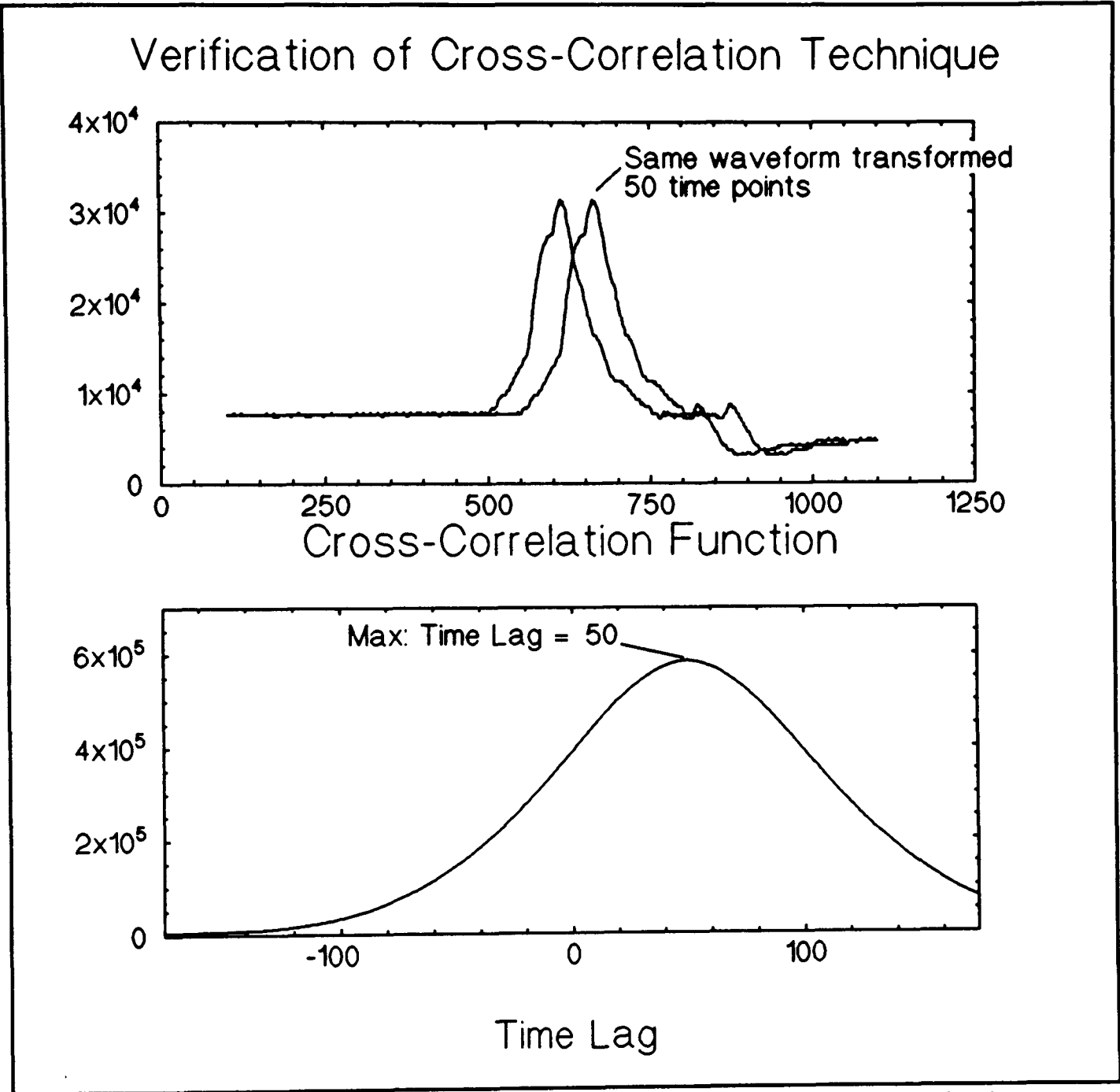


Figure 5.6 Cross-correlation of transformed signal with original; a. two waveforms, b. cross-correlation function.

Consistency in the measurement system is necessary to demonstrate that response times in the photodiodes and operational amplifiers are negligible. Consistency was evaluated by making a series of three runs at three combinations of LED/photodiode positions. The positions were chosen such that time-of-flight measurements from the first two runs could be used to calculate a result which

could then be checked with the third run. This was done for two different total pressures, p_o , where p_∞ was equal to atmospheric pressure. A matrix of test conditions and results is shown in Table 5.1.

Table 5.1 Demonstration of Velocimeter Consistency

Run #	p_o (bar)	LED/PD ₁ (mm)	LED/PD ₂ (mm)	T-o-F(m) (μ s)	T-o-F(c) (μ s)
A	4.8	1.5	41.5	83.33	161.67
B	4.8	1.5	81.5	245.0	
C	4.8	41.5	81.5	158.14	
D	7.8	1.5	41.5	71.67	186.05
E	7.8	1.5	81.5	247.50	
F	7.8	41.5	81.5	175.83	

Notes: (m) - Measured directly.
(c) - Calculated from two previous runs.

Table 5.1 demonstrates that the optical particle velocimeter described above is self-consistent. Particle time-of-flight between axial locations 41.5mm and 81.5mm from the nozzle exit plane is *calculated* from runs A and B and *measured directly* in C. As shown in the table for $p_o = 4.8\text{bar}$, there is good agreement between the calculated value and the measured value (within 2.2%). The same holds true for $p_o = 7.8\text{bar}$, although here the discrepancy rises to 5.8%.

The final system check utilised a flow visualisation technique to verify particle velocities independently. This involved use of a high speed video camera (Kodak EktaPro 1000), kindly loaned by the SERC. This could be set at 1000 full frames per sec or up to 6000 partial frames per sec. A strobe light accompanying the camera was capable of 6000 Hz. The camera was focused on the exit plane of the nozzle during the experiment. Due to its relatively easy visibility, finely ground French chalk (0.1g; avg. particle dia. = $10\mu\text{m}$) was used to simulate tungsten particle trajectories. A still photograph taken from the video output is shown in Figure 5.7. System conditions were: $p_o = 8.0\text{bar}$; $p_\infty = \text{atmospheric pressure}$. Video conditions were: 6000 partial frames/sec. The still photograph shows that visible chalk particles move approximately 33mm from the nozzle exit

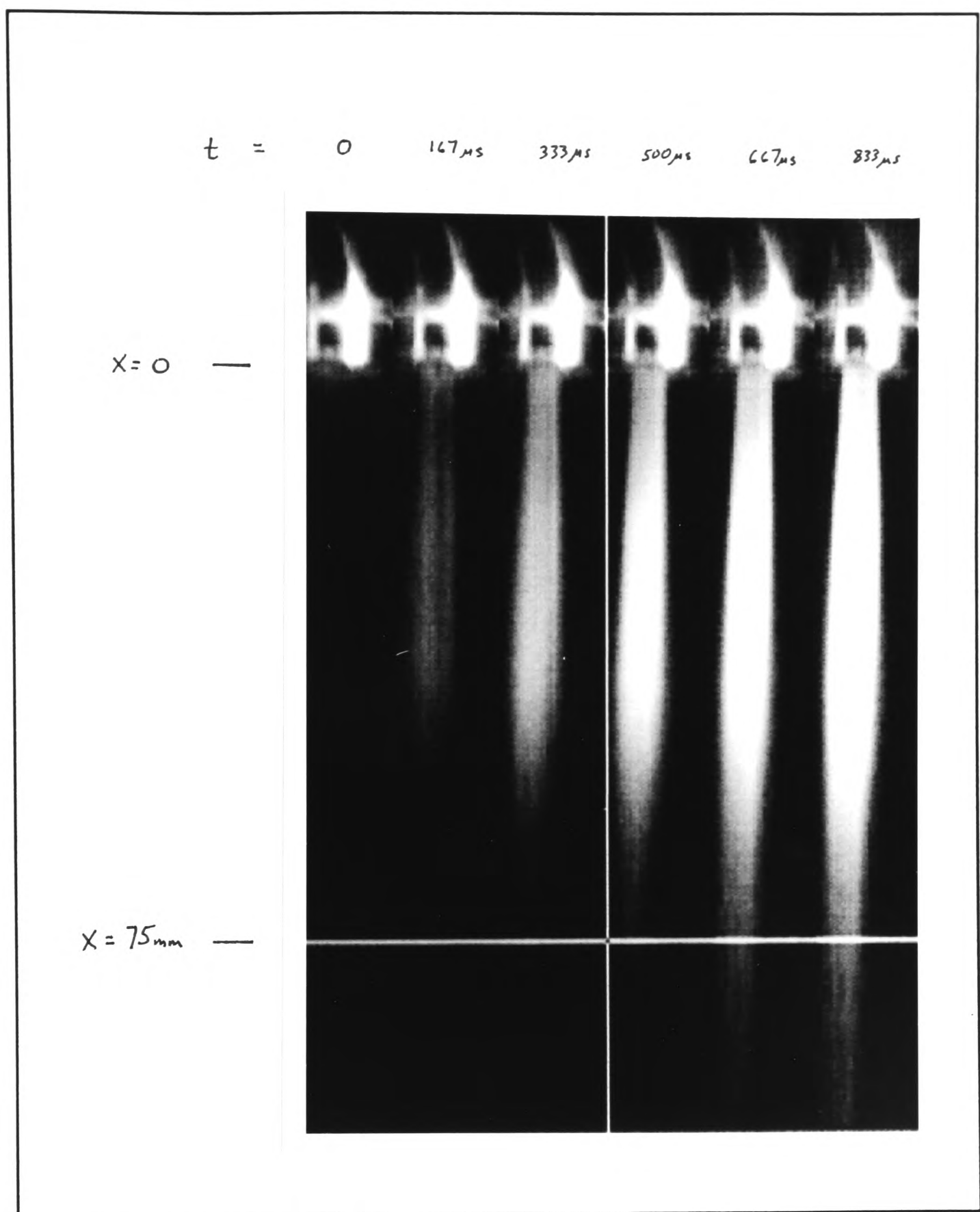


Figure 5.7 Photographic still of particle-laden jet expansion (particle: chalk; 6000 fps).

nozzle exit plane within the first 6000th of a second. As the jet expands in the following three time points, the leading edge of the particles reaches a constant velocity. This constant velocity is estimated from the photograph to be approximately **105 m/s**; the obvious difficulties and experimental errors in making such an approximation from a photograph must be noted. This does, however, provide a ballpark figure which can be compared with results from the more

sensitive optical particle velocimeter. In order to acquire the optical particle velocimeter result for the same conditions, two runs were necessary: measuring particle time-of-flight between 1.5mm and 41.5mm and then between 1.5mm and 81.5mm. The results are shown in Table 5.2 below. This table shows that in the axial location of interest (roughly between 40mm and 80mm), the distance-averaged particle velocity is: $0.040\text{m}/(500-180)\mu\text{s}$ or **125 m/s**. This is within 20% of the value obtained using the high-speed video camera, excellent agreement considering all the areas for potential experimental error (*i.e.*, measuring distances on the still photograph and experimental variation from run to run.)

Table 5.2 Time-of-Flight Measurements for Chalk Dust

Run #	p _o (bar)	LED/PD ₁ (mm)	LED/PD ₂ (mm)	T-o-F(m) (μs)
A	8.0	1.5	41.5	180
B	8.0	1.5	81.5	500

It should be noted that chalk particles used in the particle gun are expected to show velocities significantly less than those for micron-sized tungsten particles due to the fact the Stokes particle time constant for the chalk particles is 10 times greater than that for the tungsten particles. The higher Stokes number for chalk implies greater gas-particle velocity lag and, correspondingly reduced particle velocities for the accelerating flow conditions.

5.4.4 Shock Trigger: Use of Thin Film Gauge

It was found to be useful to measure the time delay between passage of the primary shock past a given point on the nozzle and exit of the particles from the nozzle. This would provide useful information, it was felt, concerning the transient nature of the event. The experiment would determine whether particles are carried in the steady, fully-expanded nozzle flow, as originally thought, or are carried closely behind the primary shock wave as it propagates downstream after diaphragm rupture. There are several methods of determining shock passage over a particular location in shock tube flow. One involves measurement of static pressure rise, using a fast-response pressure transducer such as Kulite (see, for example, Ainsworth [1990]). Another technique for detecting shock passage involves measurement of transient temperature rise using a thin-film gauge. This was the technique chosen for the measurements described in **Section 5.5.1**.

The thin film gauge may be used to measure the unsteady surface temperature associated with passage of a shock wave and associated expansion waves. If a large ballast resistor, R_o , is utilised in the circuit, as shown in Figure 5.8, current I is steady, and the output voltage signal can be shown to be:

$$V = \frac{V_o R}{R + R_o} \quad (5.7)$$

As the shock wave passes, the temperature of the gauge is suddenly increased, and the gauge resistance, R , is consequently increased. The output signal may be read directly on a digital oscilloscope.

Purpose-built thin film gauges were fabricated with the help of Trevor Godfrey of Osney Laboratory. Columnar alumina probes (3mm dia., 15mm long) with 0.5mm filleted edges were each painted with platinum thin film, using a single hair of a paint brush. This is shown in Figure 5.9. These were fitted into perspex shieldings which were threaded to screw into specific locations on the nozzle. Leads were soldered a short distance away from the thin film gauges and encased in perspex sleeving to reduce the possibility of extraneous thermal effects.

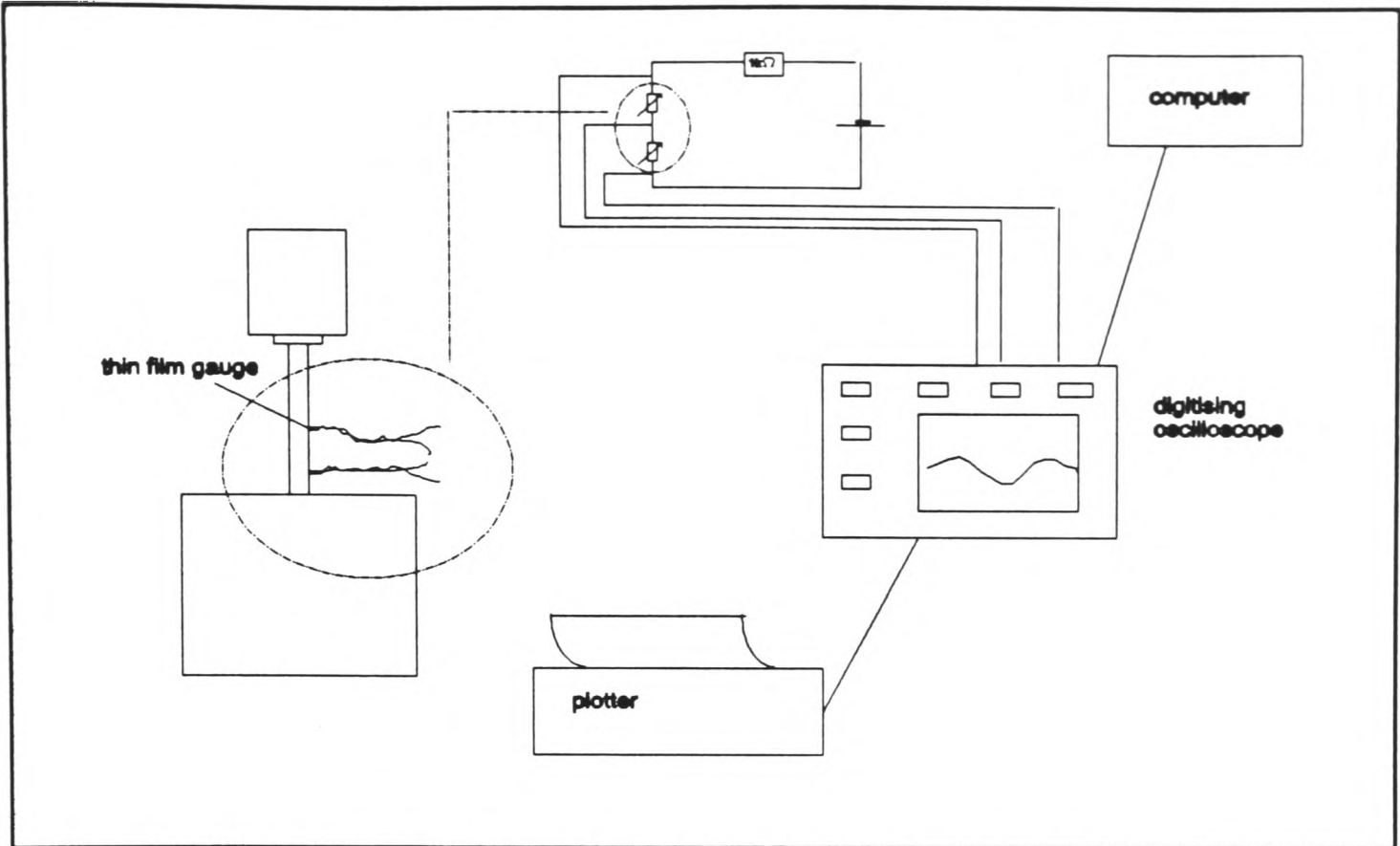


Figure 5.8 Set-up for transient thin-film gauge measurements.

The alumina was slotted into the perspex sleeving, so that it was flush with the inner nozzle surface and was oriented such that the thin film was perpendicular to the flow direction to ensure a sharper gauge response.

Preliminary tests showed that a ballast resistance of 500Ω supplied by a dc-voltage source of 12V was adequate to ensure steady current and achieve a trigger signal for the digital oscilloscope.

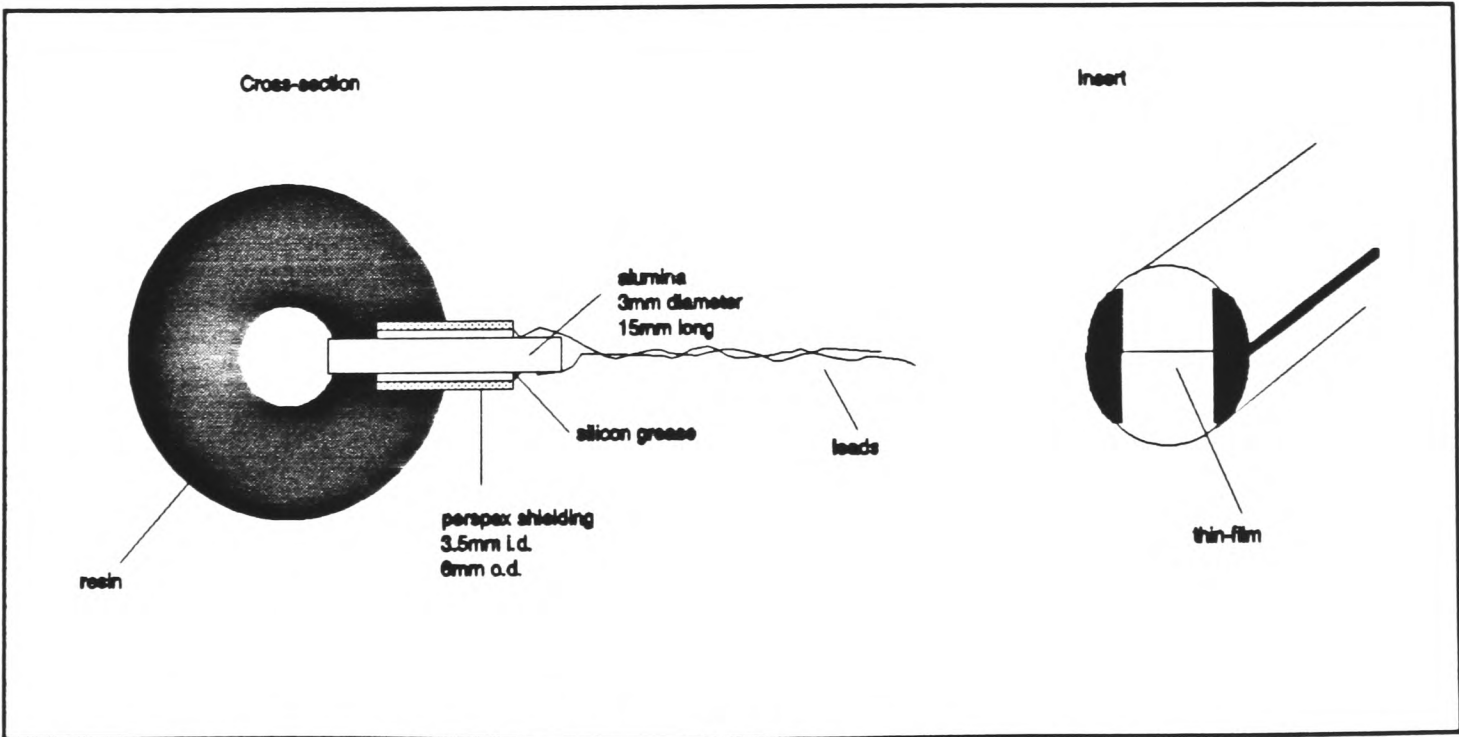


Figure 5.9 Cross-section of thin film gauge.

5.5 Experimental Confirmation of Acceleration Mechanism

The experiments described in this section were conducted to determine the physical mechanism of particle acceleration. Results of the experiments described below confirm that transient effects associated with shock tube flow are predominant in accelerating the microprojectiles in the gas propulsion particle gun.

5.5.1 Time Between Shock and Particles

The purpose of this experiment was the determination of the time delay between shock passage and particles exiting the nozzle. Knowledge of this time delay is important in ascertaining whether particle arrival time is closer to shock arrival time or to the approach of steady flow. Voltage variation of a thin film gauge (TFG) placed 50mm upstream from the nozzle exit on the nozzle was used to trigger the digital oscilloscope, as described in Section 5.4.4 . An LED/PD pair placed close to the nozzle exit (54mm from the position of the TFG) was used for particle detection. Allowance was made for actual shock propagation time between the positions of the TFG and LED/PD. This was done using the theoretical shock propagation velocity (see Equations 3.3 and 3.5). Subtracting this shock propagation time from the actual measured time delay between TFG trigger and particle detection at the LED/PD allowed determination of shock-to-particle time at the nozzle exit. The resulting time delay as a function of nozzle pressure ratio ^{is} are shown in Figure 5.10.

Smith [1966], working at the Osney laboratory in the mid-60s, developed a correlation for the time delay between diaphragm rupture and the onset of *steady flow* in a diverging, blow down nozzle. He showed that this time delay to steady flow for a nozzle of conical shape can be represented by:

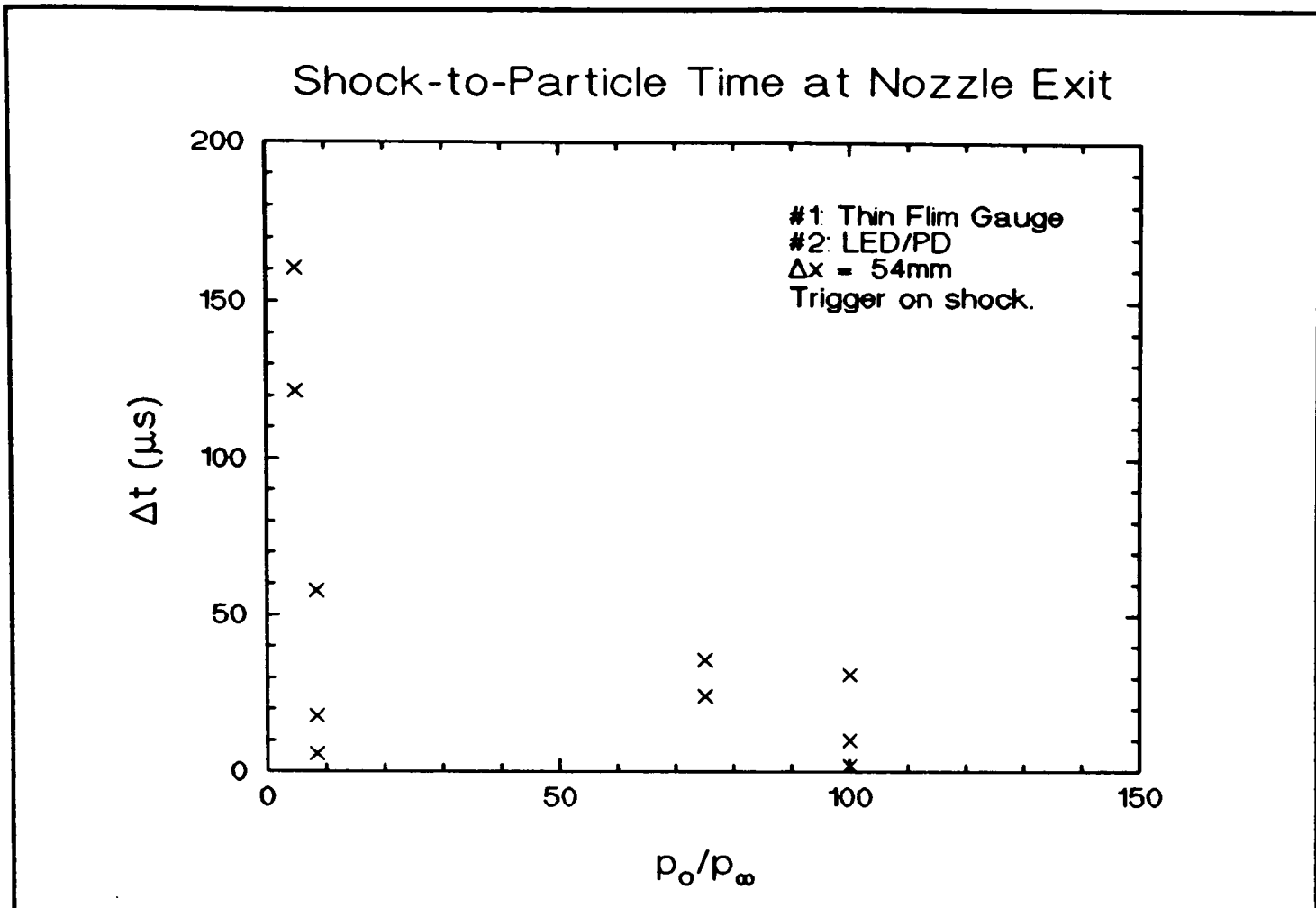


Figure 5.10 Time delay between shock and particle passage at nozzle exit, reduced from TFG - LED/PD time delay.

$$t_{sf} = \frac{0.76r_t}{\beta a_4} \left[M^{0.5} \left(1 - \frac{1}{M} \right) + \frac{1}{15} (M^{1.5} - 1) + \frac{1}{25} (M^{2.5} - 1) \right] \quad (5.8)$$

where r_t is nozzle throat radius, β is tangent of nozzle half angle, and a_4 is sound speed in the stagnation region. Using this correlation for the current geometry and conditions of interest, one may show the time delay to steady flow to be:

$$t_{sf} = 1789.8 \mu s \quad (5.9)$$

Therefore, steady flow does not occur at the nozzle exit until approximately 1.8ms after diaphragm rupture. The results shown in Figure 5.10, however, can be used to show that particle arrival time is significantly less than the time necessary to achieve steady flow. This is shown in Figure 5.11, which shows the time delay between diaphragm rupture and particle detection at the nozzle exit. Figure 5.11 uses the data of Figure 5.10 and again accounts for the theoretical time of shock propagation along the nozzle. These results show that particle detection occurs significantly before the theoretical onset of steady flow at the nozzle exit.

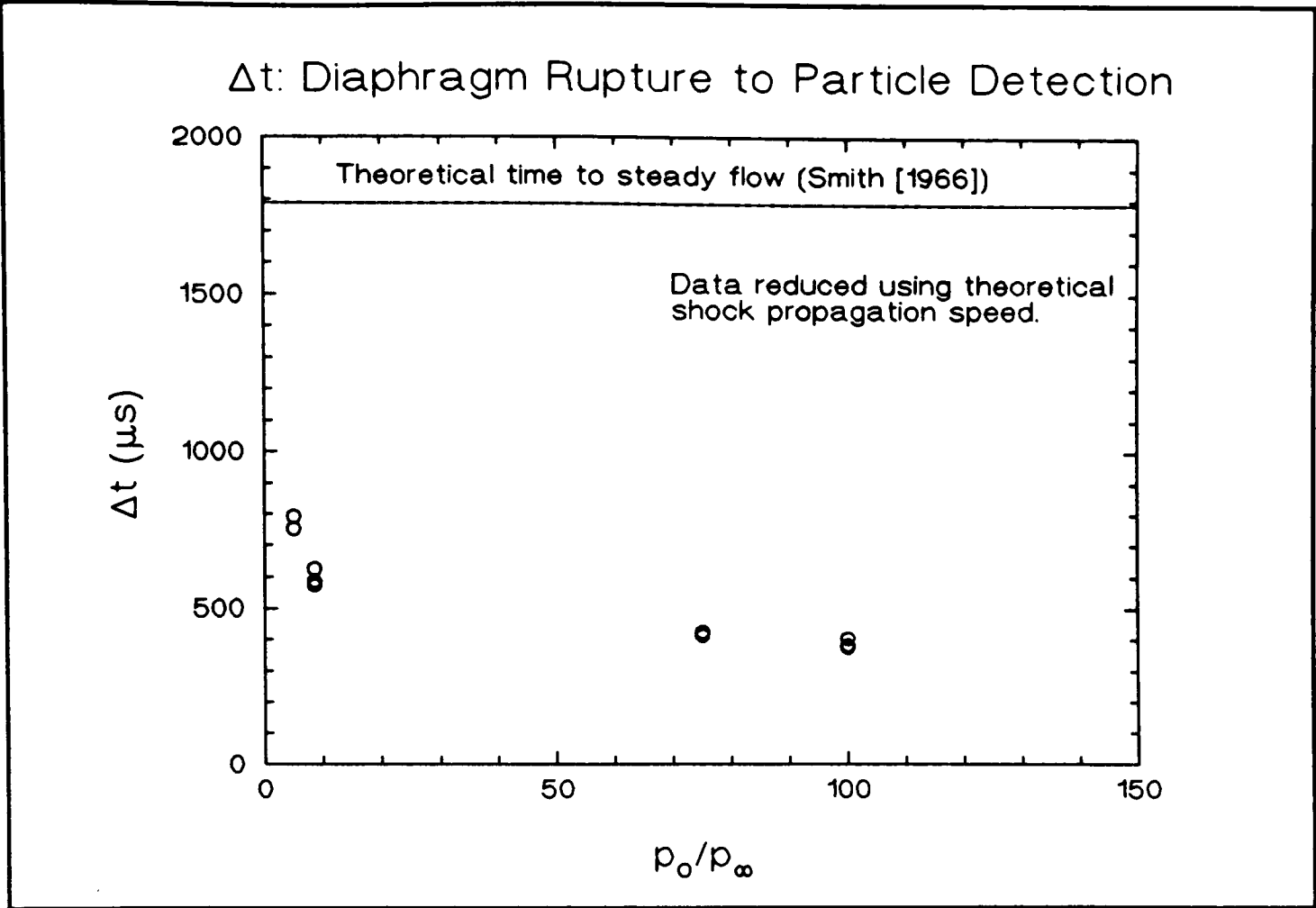


Figure 5.11 Time delay between diaphragm rupture and particle detection at the nozzle exit.

5.5.2 Particle Time-of-Flight Vs. Upstream Cylinder Volume

The objective of this experiment was the determination of variation in particle time-of-flight between two points in the target chamber for differing volumes of the supply cylinder. It was believed that significant variation in particle time-of-flight (and, therefore, particle velocity) would be observed if quasi-steady mechanisms were responsible for particle acceleration. One advantage of smaller upstream cylinders in the biological bombardment experiments is the reduced target cell blow away that has been observed. Two LED/PD pairs (LED/PD₁¹ and LED/PD₂) were placed in the target chamber at positions 1.5mm and 41.5mm from the nozzle exit, respectively. Supply cylinders of 50, 90 and 500ml capacity were tested for air at $p_o = 9.0\text{bar}$ and $p_\infty = \text{atmospheric pressure}$. The resulting time delay between the two LED/PD pairs is shown in Figure 5.12.

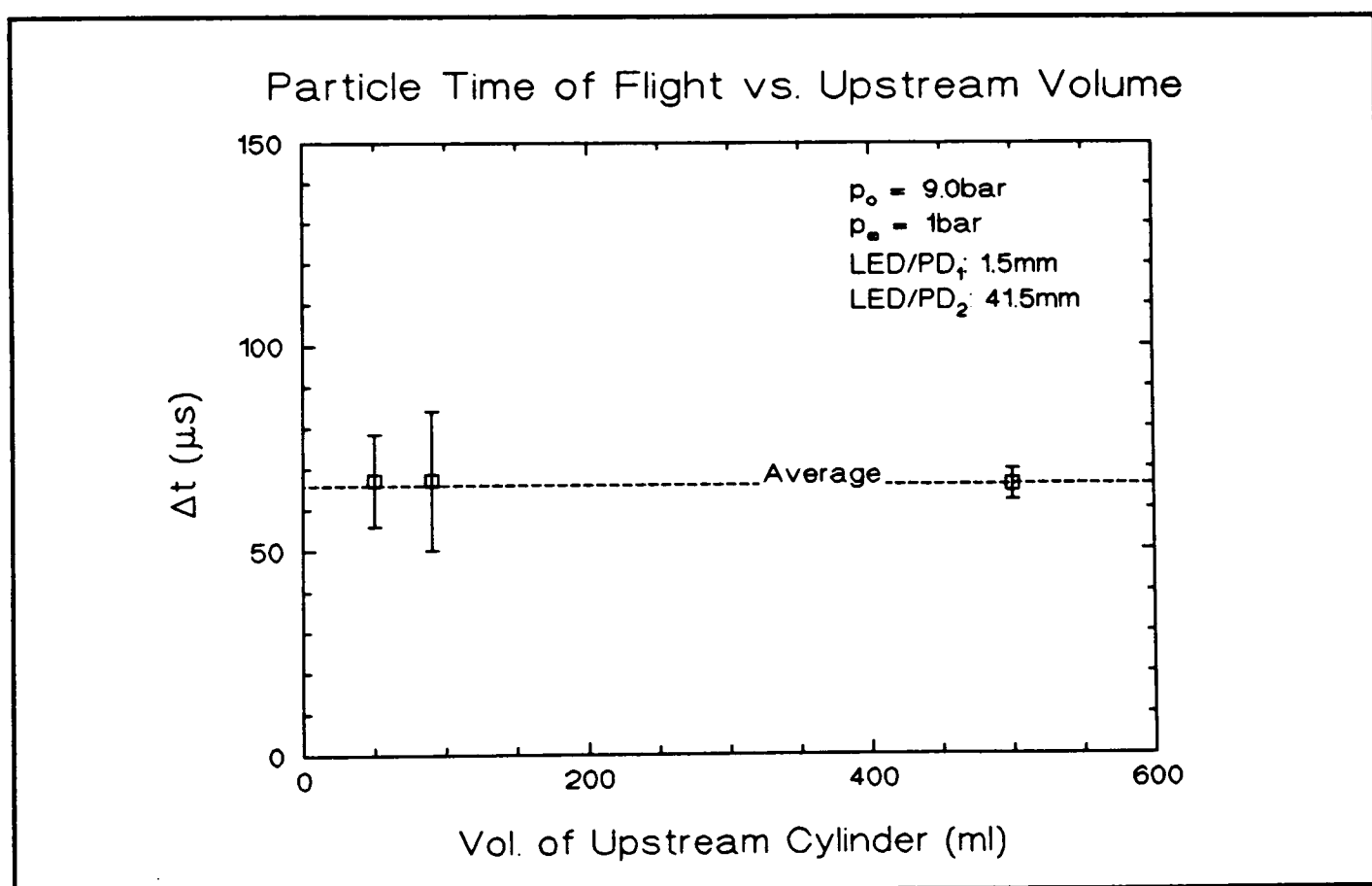


Figure 5.12 Variation in particle time-of-flight for differing supply cylinder volume ($p_o = 9.0\text{bar}$, $p_\infty = 1\text{bar}$).

¹Throughout this chapter, LED/PD₁ will refer to the LED/PD pair nearest the nozzle exit plane; LED/PD₂ will correspondingly refer to the second LED/PD pair.

Figure 5.12 shows that little variation occurs in particle time-of-flight for the differing volumes of supply cylinder. This implies that quasi-steady, fully-expanded nozzle flow is probably not the primary mechanism for particle acceleration.

5.5.3 Particle Velocities for Cylindrical Shock Tube

A final experiment to determine the mechanism of particle acceleration involved the replacement of the supersonic, convergent-divergent nozzle with a cylindrical tube. For quasi-steady "nozzle" flow, the maximum theoretical particle velocity that can be obtained with this set-up is sonic velocity, while particle velocities exceeding sonic velocity are obtainable if transient effects are predominant. A 6mm I.D. cylindrical tube (matching the exit I.D. of usual nozzle) was utilised in place of the normal nozzle. Two LED/PD pairs were used to detect particle passage at two positions near the nozzle exit in the target chamber. Upstream gauge pressure was varied between 0 and 10bar; target chamber pressure was held constant at 1bar (atmospheric pressure). The results, shown in Figure 5.13, show that particle velocities clearly exceed sonic velocity at $p_o > 2.0\text{bar}$ (gauge). This confirms the results of the two previous experiments, showing transient shock tube effects to be predominant for particle acceleration.

Measurements of particle velocity at discrete locations in the target chamber will be presented in the next section. These results, when plotted as a function of nozzle pressure ratio, will show particle velocities to vary in a manner analogous to velocities associated with shock tube flow.

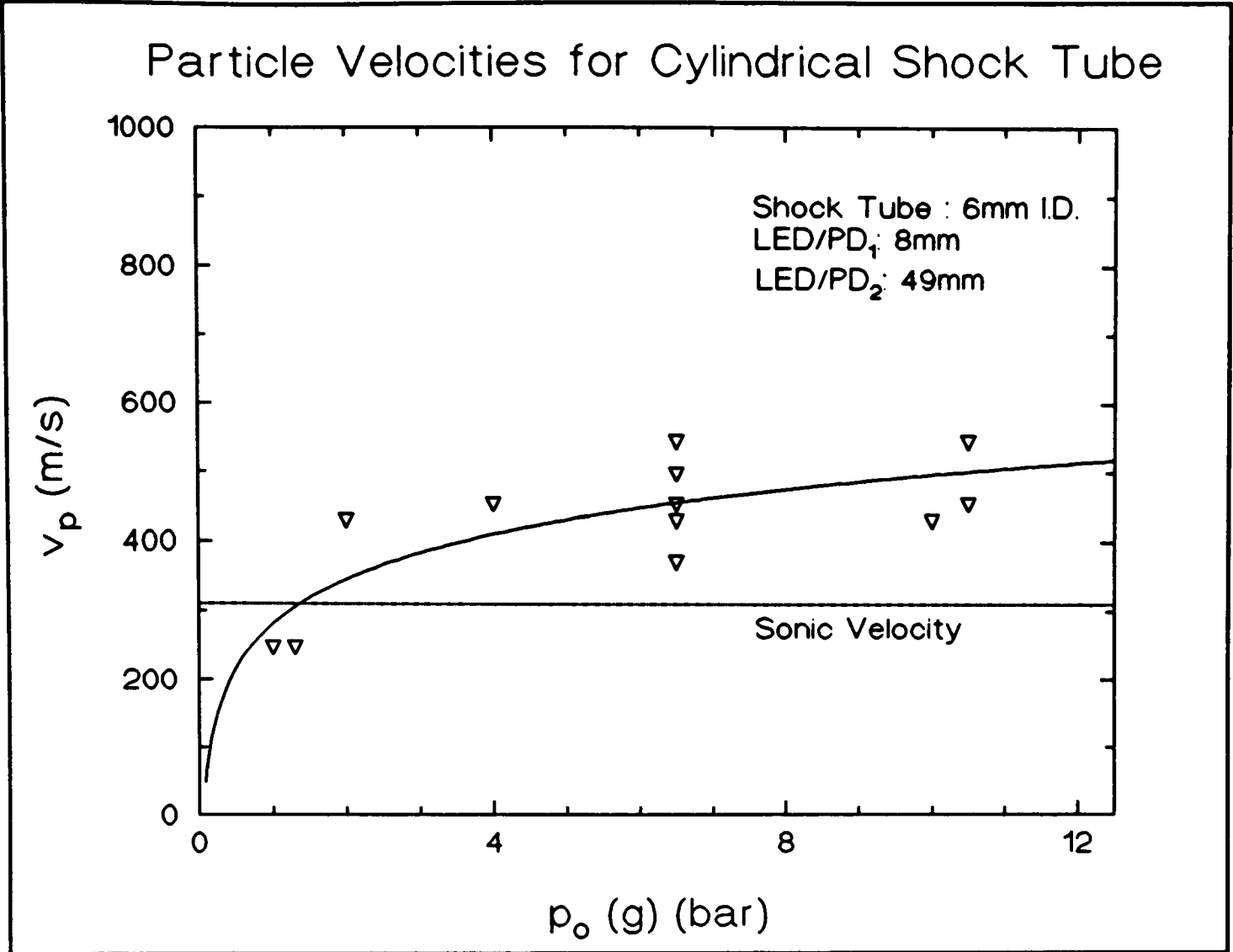


Figure 5.13 Particle velocities for cylindrical shock tube.

5.6 Particle Velocimetry Measurements: Results

5.6.1 Variation in Particle Velocity with Upstream Pressure

The objective of this particular set of experiments was the determination of the effect of upstream stagnation pressure, p_o , on particle velocities at various distances, x , from the nozzle exit plane. p_o was varied from approximately 1bar to 9.5bar - *i.e.*, the range of interest in the biological experiments. The LED/photodiode pairs were positioned as follows: since the oscilloscope was triggered off the output signal of the first photodiode, LED/PD₁, the greatest consistency in triggering was found with the first LED/PD pair as near as possible to the nozzle exit plane. Therefore, in each case the first LED/PD pair was located 1.5mm from the exit plane of the nozzle. The second LED/PD pair was then positioned at discrete intervals in the target chamber: 41.5mm, 81.5mm, or 121.5mm from the nozzle exit plane, depending on the particular conditions of the experiment being conducted.

Typical waveform data output from the two photodiodes together with the corresponding cross-correlation functions are presented in Figure 5.14 and Figure 5.15 for upstream stagnation pressures of 3.6bar (gauge) and 9.0bar (gauge), respectively. The figures demonstrate a *decreasing* time lag between waveform signals as p_o *increases*. Figure 5.14, for example, shows a time delay between the two signals of 390 μ s for $p_o = 3.6$ bar (gauge), while Figure 5.15 shows a time delay of 260 μ s. $\wedge$ for $p_o = 9.0$ bar(g).

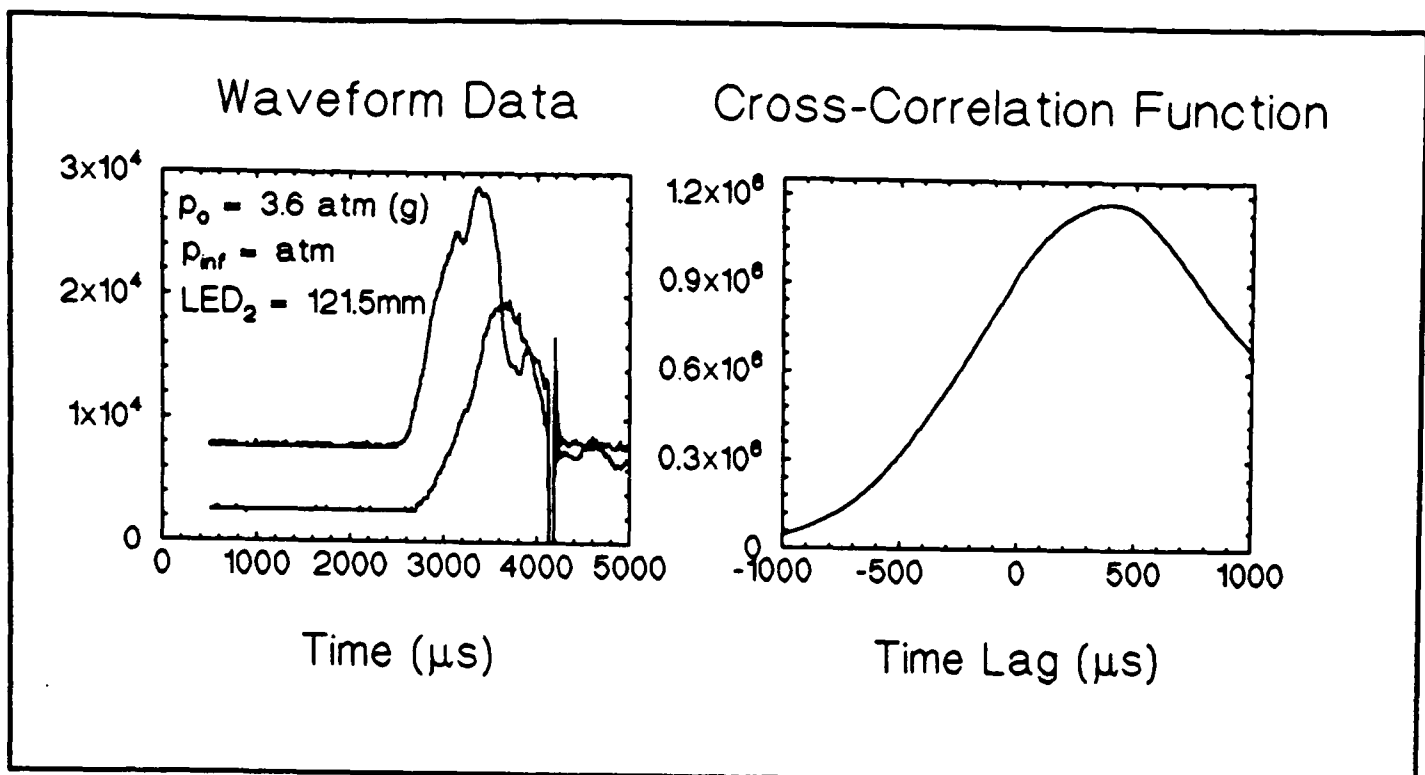


Figure 5.14 Waveform data and cross-correlation function for $p_o = 3.6 \text{ bar (g)}$.

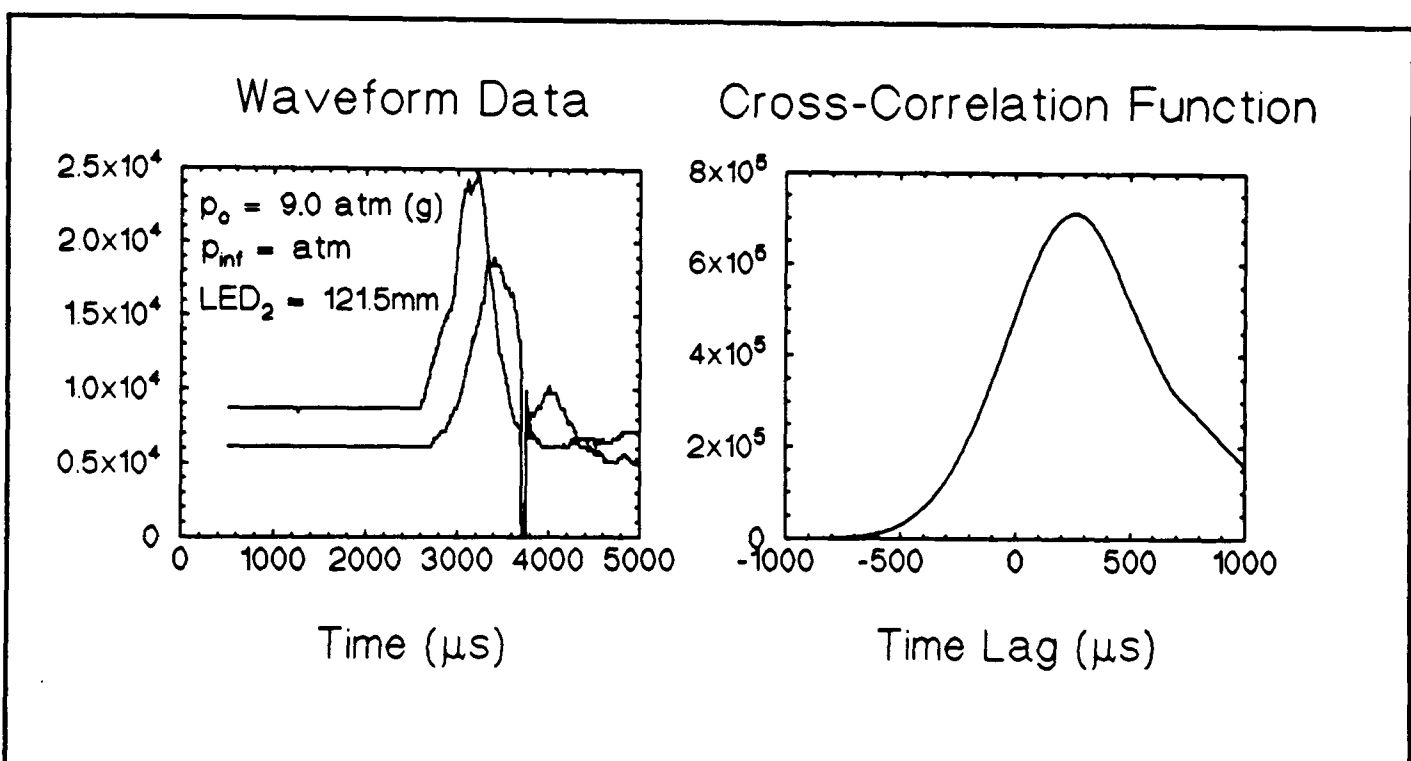


Figure 5.15 Waveform data and cross-correlation function for $p_o = 9.0 \text{ bar (g)}$.

Further evidence for this trend is provided in Figure 5.16, which displays the variation in particle time-of-flight (t-o-f) with p_o , parametric with the three locations for LED/PD₂ in the target chamber. Three comments can be made about the general trends apparent:

- 1.) Particle t-o-f appears to increase significantly as $p_o \rightarrow 0$. This seems intuitive, since the driving force is diminished as $p_o \rightarrow 0$.

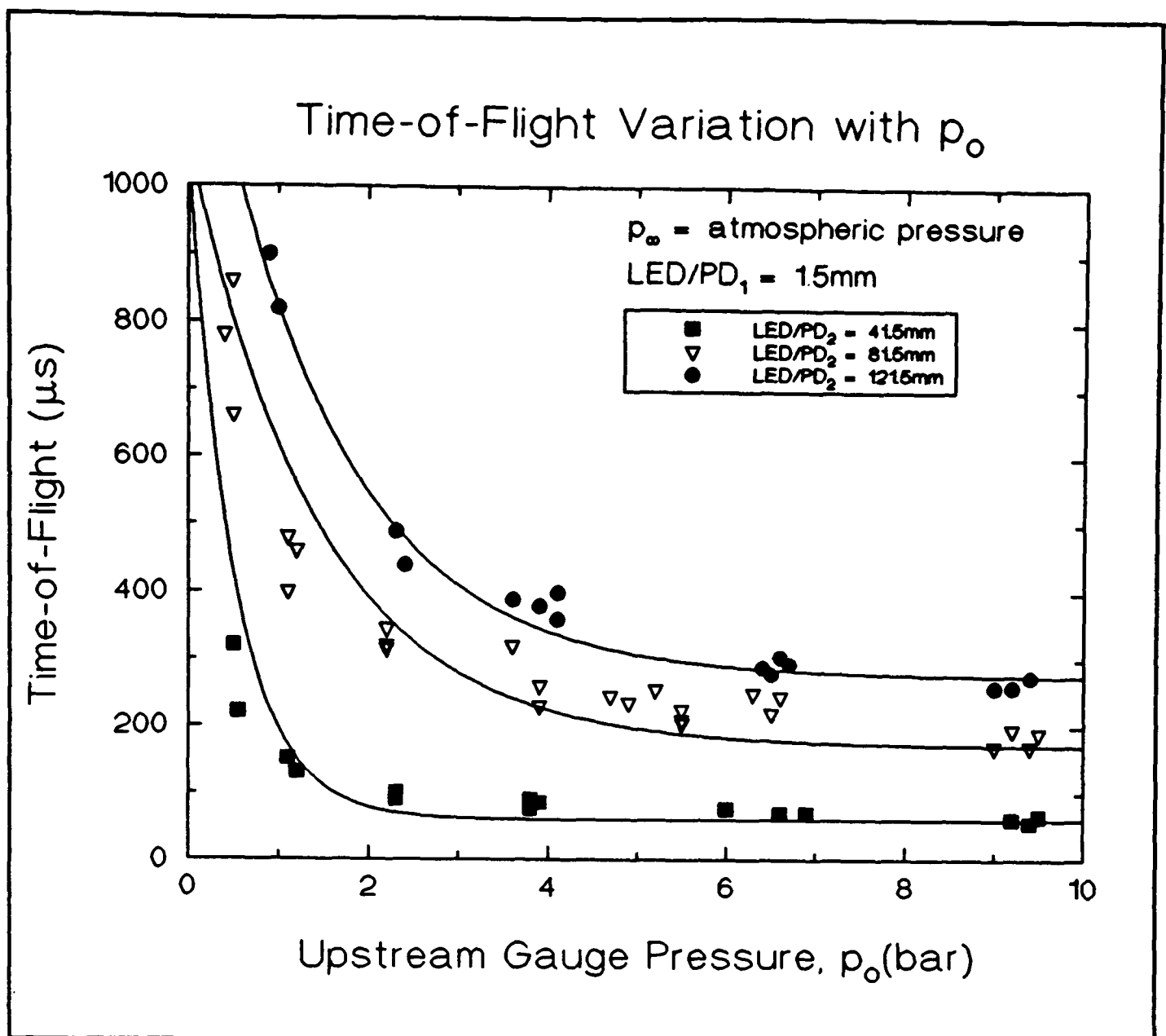


Figure 5.16 Particle time-of-flight variation with upstream stagnation pressure.

2.) Particle t-o-f levels off as p_o increases. This means that further increases in p_o have only marginal effect on particle t-o-f (and, therefore, particle velocities).

3.) Particle t-o-f for a given p_o at three locations of LED/PD₂ show internal consistency (*i.e.*, t-o-f between 1.5mm and 81.5mm is greater than that between 1.5mm and 41.5mm). Also, significant particle deceleration appears to occur between 41.5mm and 81.5mm.

The data from Figure 5.16 were used to construct Figure 5.17, which shows distance-averaged particle velocity as a function of p_o . The particle velocities shown represent the *average* velocity over the entire distance between LED/PD₁ and LED/PD₂. As with Figure 5.17, the *increase* in $\bar{v}_p$ with *increasing* p_o is displayed. This is to be expected, because particle t-o-f and $\bar{v}_p$ are inversely-

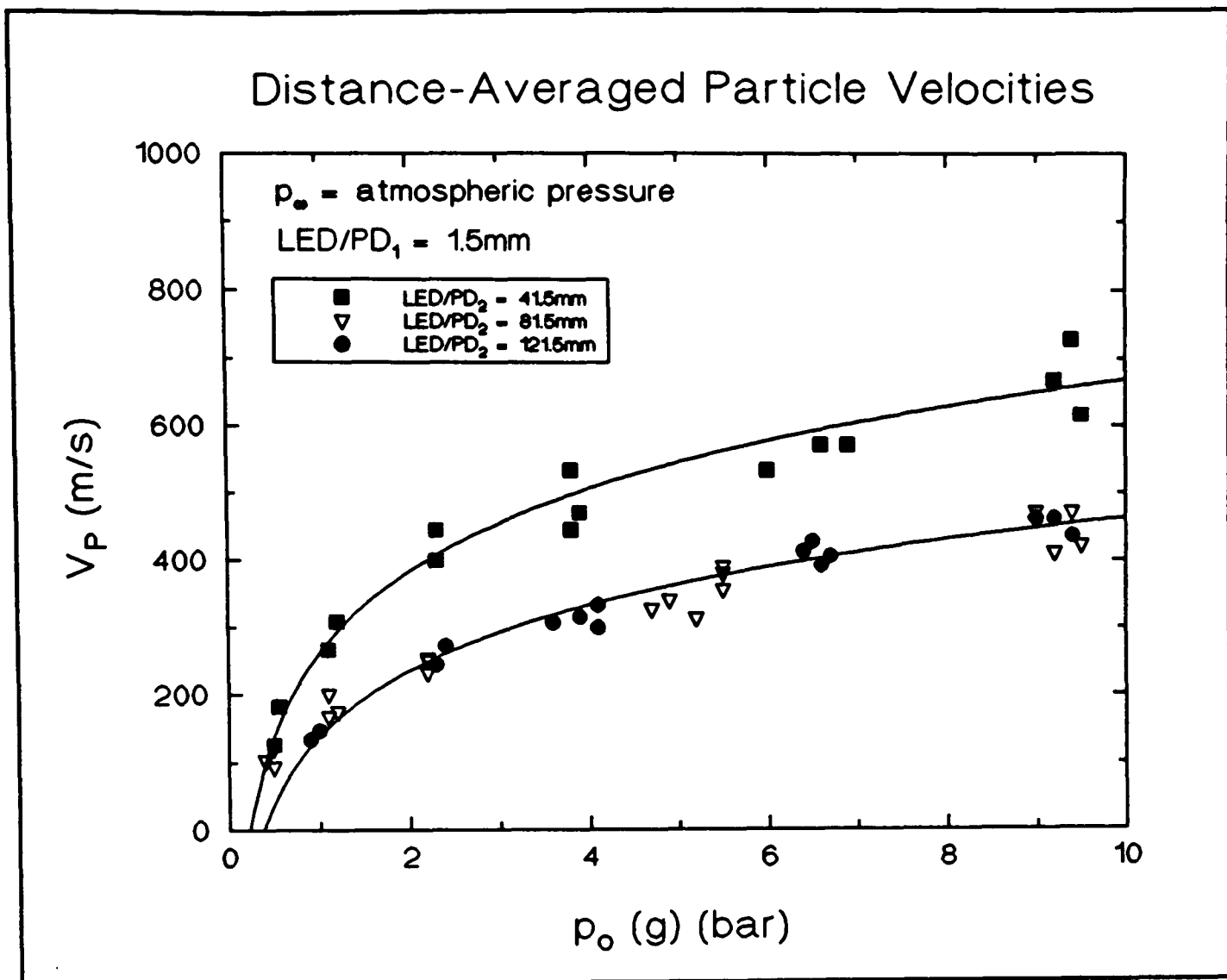


Figure 5.17 Distance-averaged particle velocities vs. upstream pressure, p_o .

related. Also, particle deceleration is apparent between LED/PD₂ = 41.5mm and 81.5mm. Axial variation in $\bar{v}_p$ will be discussed further in **Section 5.6.2**.

In general, Figure 5.17 displays particle velocity results as a function of p_o which appear similar in form to the theoretical velocity of the shock and contact surface in shock tube flow. This again confirms the results found in **Section 5.5**. It should be noted that these initial results came somewhat as a surprise, as it had been thought initially that particle motion in the system under study would be governed by quasi-steady, fully expanded, Mach 2 nozzle flow. Although the particle velocities measured closest to the nozzle exit (LED/PD₁ = 41.5mm) are greater than the theoretical contact surface velocities for a given pressure ratio (see **Section 3.2.1**), the general variation with p_o (or diaphragm pressure ratio, p_4/p_1) is the same. It is necessary to extend these measurements to higher nozzle pressure ratios before a full understanding of the relationship of the measurements to shock and contact surface velocities can be obtained.

5.6.2 Axial Variation in Particle Velocity

The objective of this experiment was the determination of the decay in particle velocity with increasing distance from the nozzle exit plane. A secondary aim was the comparison of present measurements with an empirical correlation for jet velocity decay due to Kleinstein [1964]. Kleinstein analysed the conservation equations for steady-state, compressible flow, presenting an analytical description of the axial and radial variation in concentration, enthalpy, and velocity. Of particular importance is his correlation for axial jet velocity decay:

$$\frac{u_m}{u_e} = 1 - \exp\left(\frac{-1}{K \left(\frac{\rho_\infty}{\rho_e}\right)^{0.5} \frac{x}{r_e} - x_c}\right) \quad (5.10)$$

where K represents a constant related to a compressible eddy viscosity term and x_c is dimensionless core length of the jet. The Kleinstein model, which is shown in Figure 5.18 for three values of K , has been applied by other workers to correlate velocity decay from underexpanded sonic jets (Ewan and Moodie [1986], Birch *et al.* [1987]) and from supersonic jets (Eggers [1966]).

In this experiment, LED/PD₁ was retained at an axial location of 1.5mm, while LED/PD₂ was varied through the target chamber. Again this was necessary to ensure that LED/PD₁ triggered. Figure 5.19 shows a typical particle velocity axial distribution along the jet centreline (the x coordinate has been normalised with nozzle exit radius, r_e .) for a constant p_o of 8.5bar and p_∞ equal to atmospheric pressure. The particle velocity shown in Figure 5.19 is the incremental particle velocity, v_p ². Figure 5.19 shows the rapid decay in v_p with increasing distance from the nozzle exit plane. In general, the trend shown is to be expected. A rapid decay in v_p for $x/r_e < 16$ is followed by a general levelling off of particle velocities. The further decay in velocities for $x/r_e > 37$ is possibly associated with the combined influences of viscous entrainment of slower-moving gas in the target

²Incremental v_p refers to analysis which shows velocity based on t-o-f between two positions of LED/PD₂ in two successive runs.

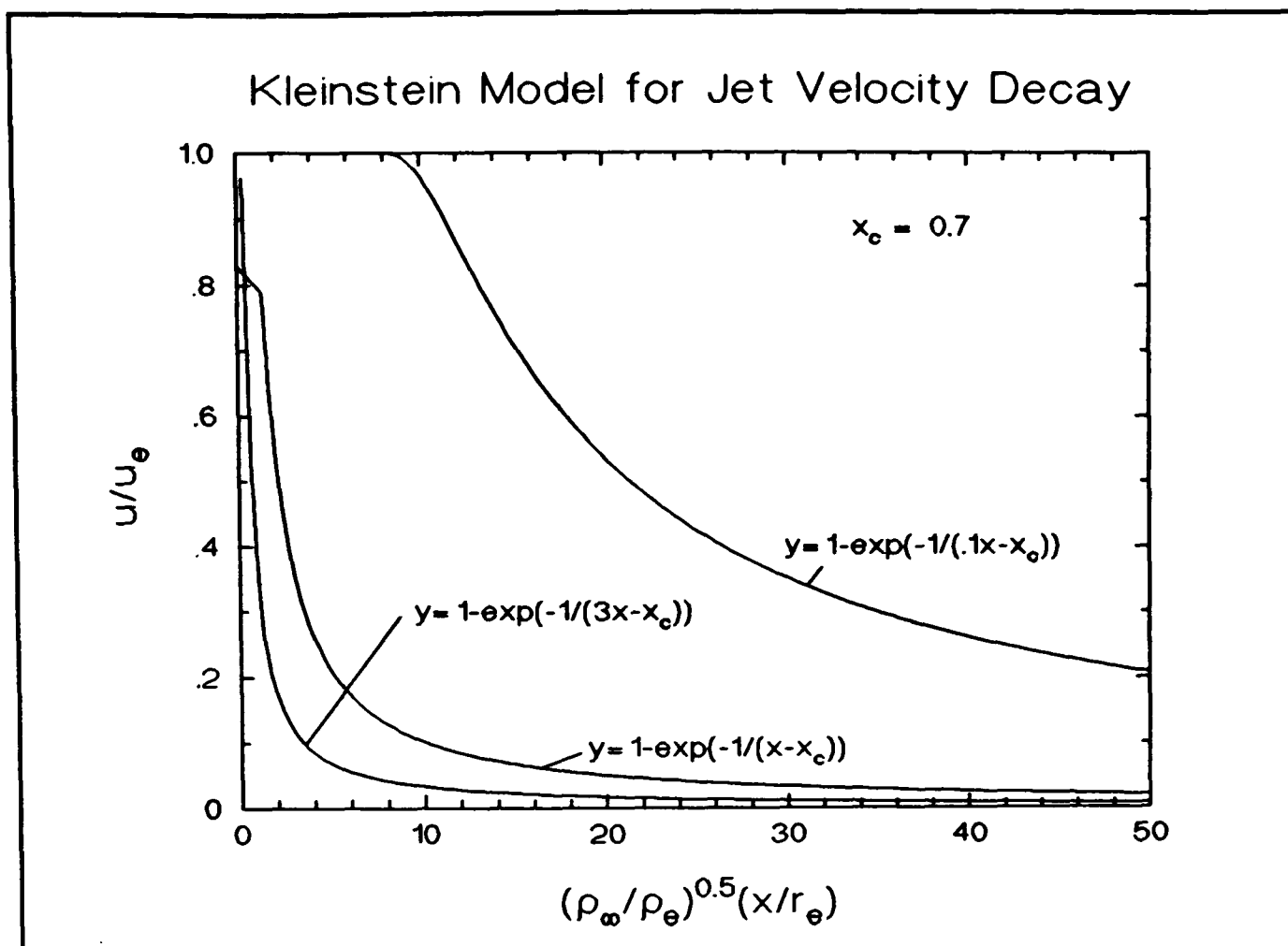


Figure 5.18 Kleinstein model for axial velocity decay.

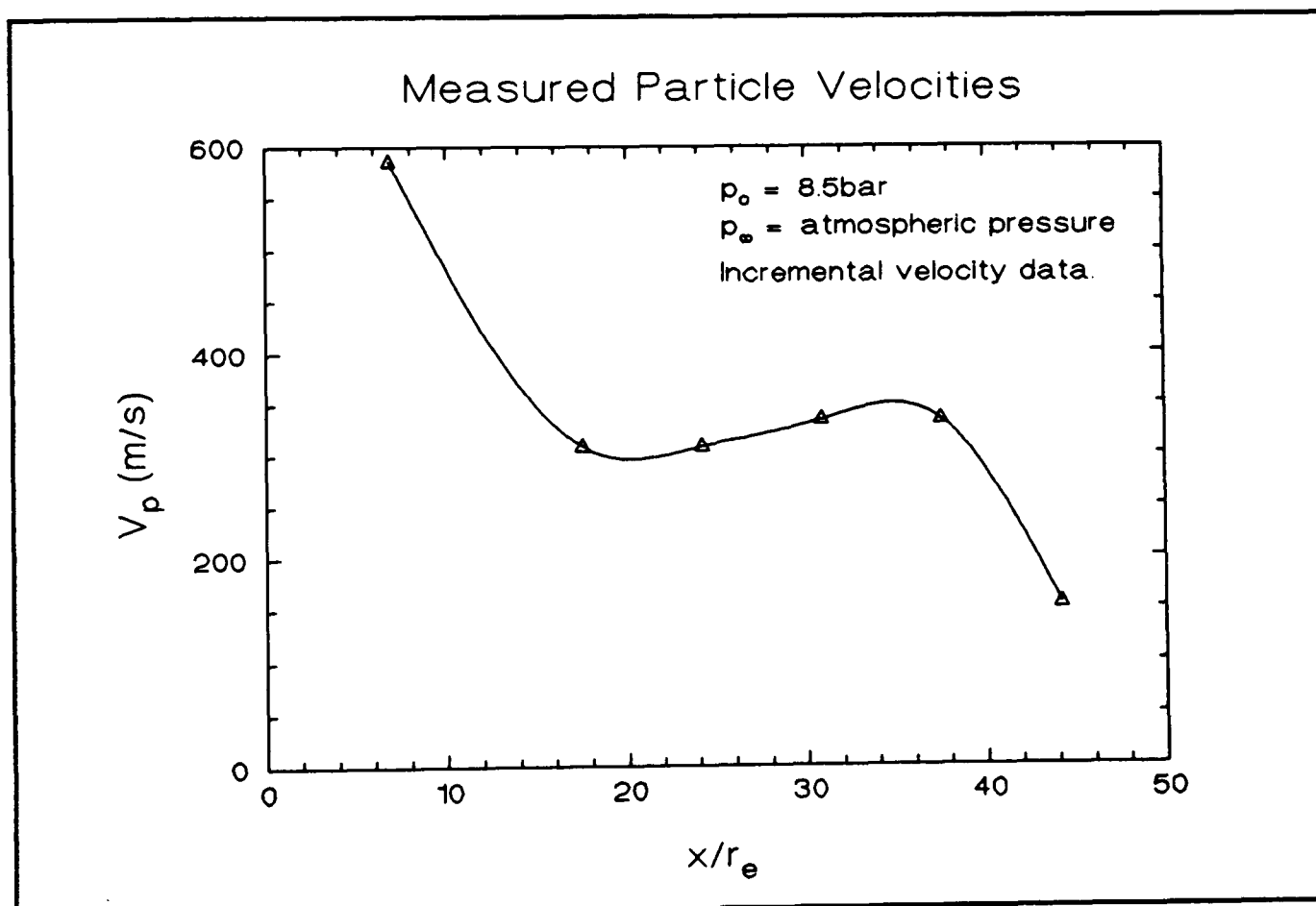


Figure 5.19 Axial decay in particle velocity ($p_o = 8.5\text{bar}$, $p_\infty = \text{atmospheric pressure}$).

chamber as well as the influence of the end wall of the target chamber.

The results of axial particle velocity traversals for three values of upstream pressure, p_o , are brought together in Figure 5.20. In all cases incremental velocity,

v_p , is shown, as that represents the best approximation of local particle velocity. The particle velocities have been normalised with the theoretical shock velocity at the nozzle exit, since that has been shown to be a characteristic velocity associated with the particles at that location (see Section 5.5). Although significant experimental scatter is seen, the general trend in axial particle velocity decay is apparent. This scatter is primarily due to run-to-run variation. Noteworthy is the particle acceleration seen within the first eight nozzle radii downstream of the nozzle exit, caused by the initial jet expansion from the nozzle. This is shown for values of $v_p/v_{p,o} > 1.0$.

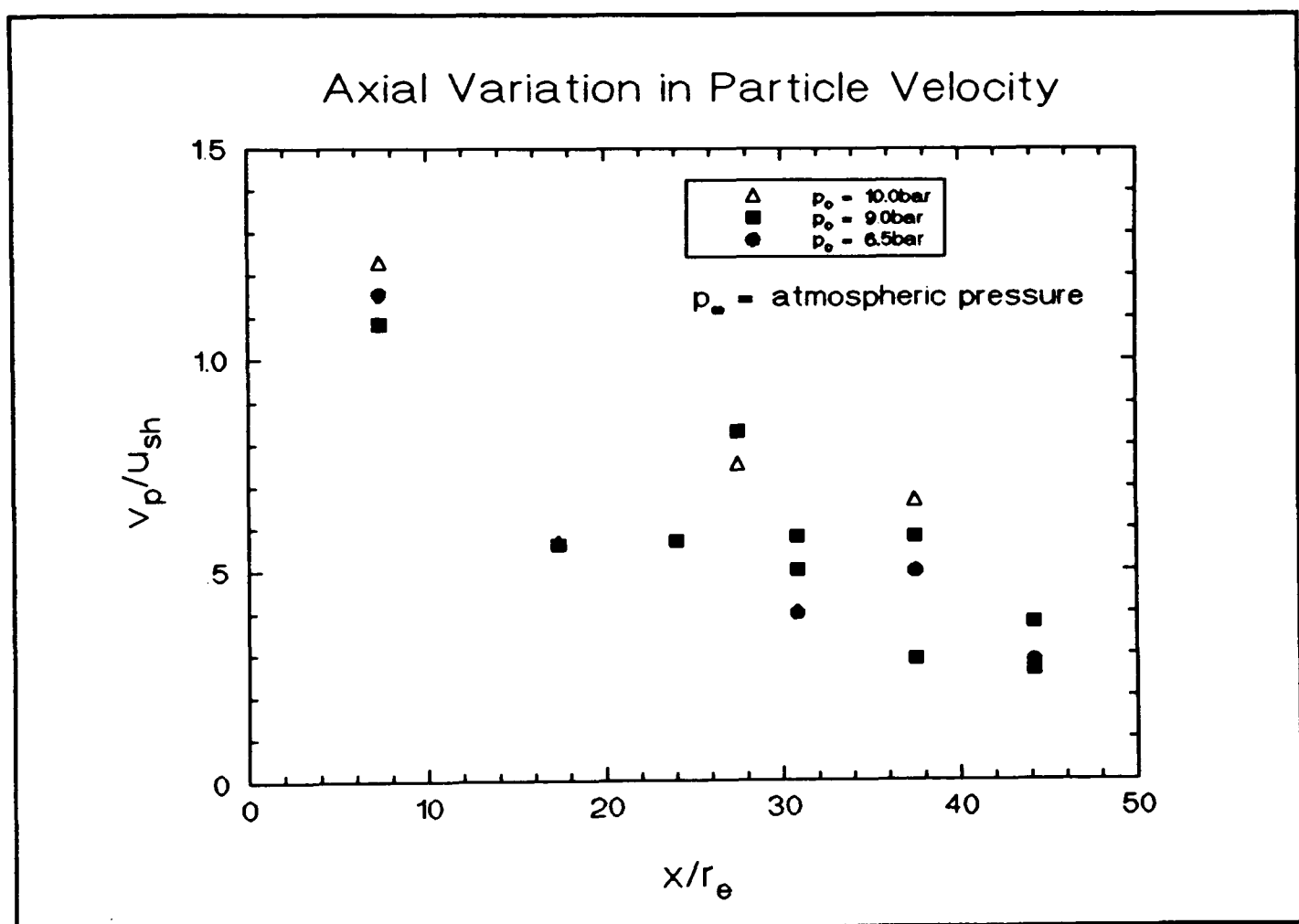


Figure 5.20 Axial particle velocity distributions for three values of upstream pressure.

The results presented in Figure 5.20 have been correlated using the Kleinstein correlation and the resulting experimental data and theoretical curve are compared in Figure 5.21. The values of K and x_c which best fit the data are 0.20 and 2.0, respectively. Despite the fact that these values differ somewhat from those given by Kleinstein for underexpanded sonic flow (0.074 and 0.7, respectively), the good agreement between theory and experiment shown in

Figure 5.21 provides confidence that the velocity decay is well-characterised. An important reason for discrepancies between the correlation values Kleinstein arrived at and those given for the present data is that all of Kleinstein's work was associated with *steady* underexpanded flow, rather than the transient flow seen in the present work.

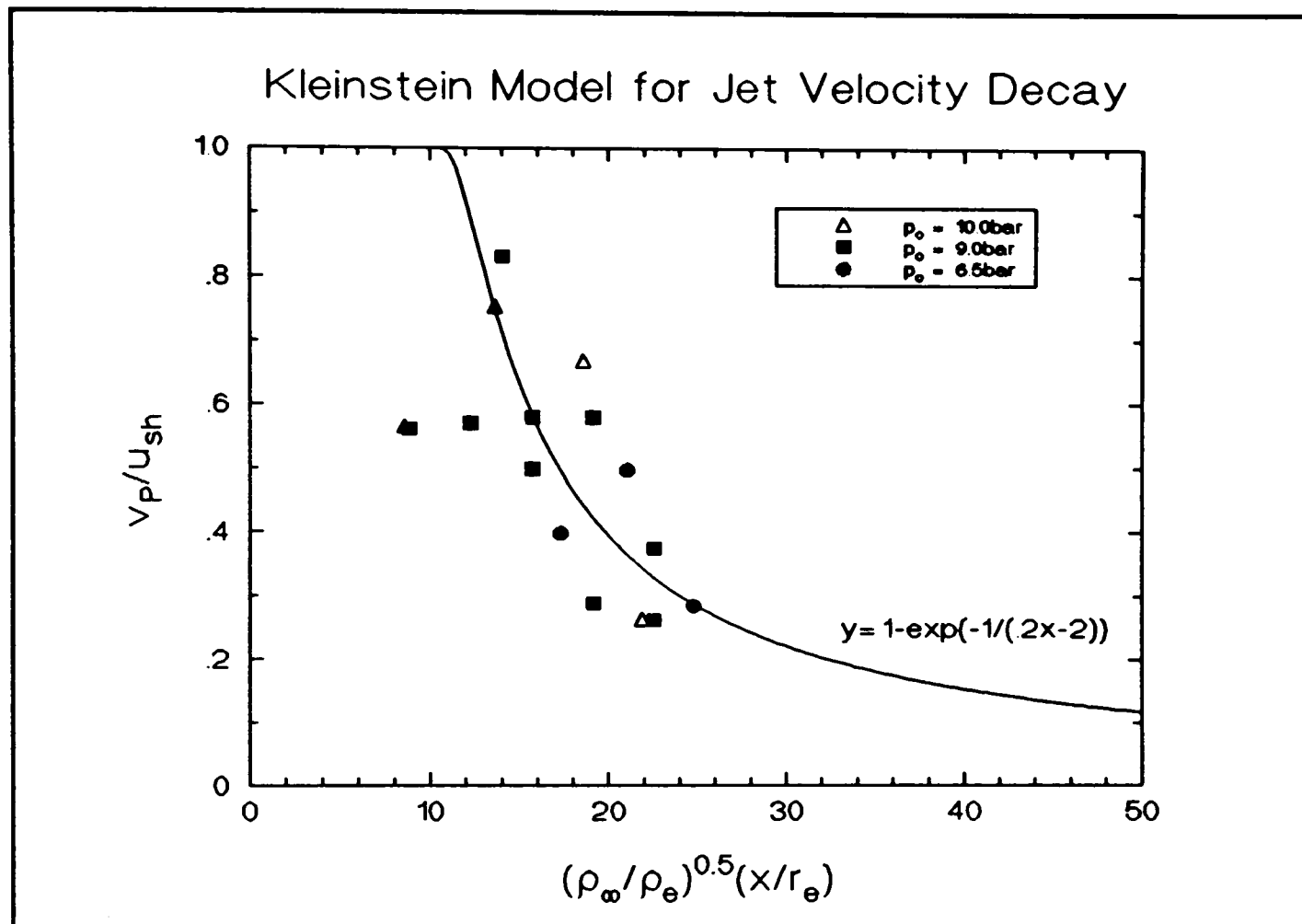


Figure 5.21 Correlation of present axial velocity decay data using Kleinstein [1964] model.

5.6.3 Radial Variation in Particle Velocities

The radial distribution in particle velocities were measured in order to determine the degree to which particle velocities diminish in the viscous mixing layer as axial distance increases. This information was considered useful in determining target placement for biological bombardment experiments. Radial particle velocity profiles were measured by varying the radial position of LED/PD₂ in the target chamber at two axial locations in the target chamber. It should be re-emphasised that the optical technique utilised is incapable of distinguishing

particle velocities at a single radius, as the optical line-of-sight must traverse the chord-length of the axisymmetric jet. This situation is shown in Figure 5.22. The radius shown, however, represents the minimum radius of the jet through which the optical line-of-sight traverses. Despite this caveat, it is still possible to evaluate the general form of the radial particle velocity variation.

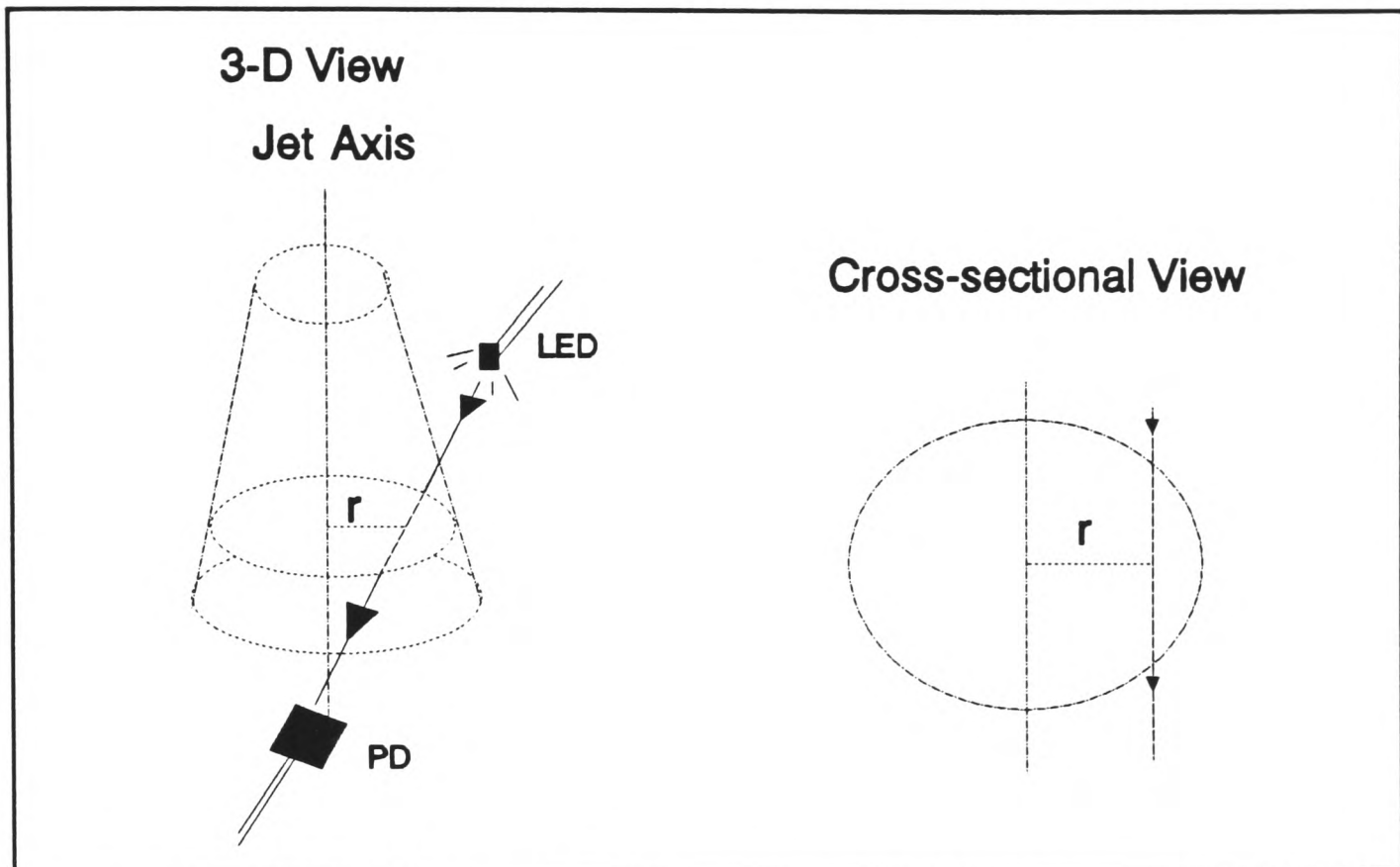


Figure 5.22 Optical line-of-sight through axisymmetric jet.

Figure 5.23 shows the radial variation in particle velocity at two axial locations ($\text{LED/PD}_2 = 42.5\text{mm}$ and 82.5mm) for an approximately constant value of upstream pressure (slight variations in p_o are noted). Again, particle velocities have been normalised with the theoretical shock velocity at the nozzle exit. In general form, Figure 5.23 shows the expected levelling in velocity profile with increasing axial distance. This form has been predicted analytically by many workers (see, for example, Pai [1954]).

The data from Figure 5.23 have been correlated using an analytical correlation for radial velocity variation after Kleinstein [1964] and is shown in Figure 5.24. This analytical correlation uses conservation of jet momentum as a basis for describing a velocity similarity profile for fully-developed compressible jets. Here, $v_{p,cl}$ is particle velocity measured on the jet centreline at that axial

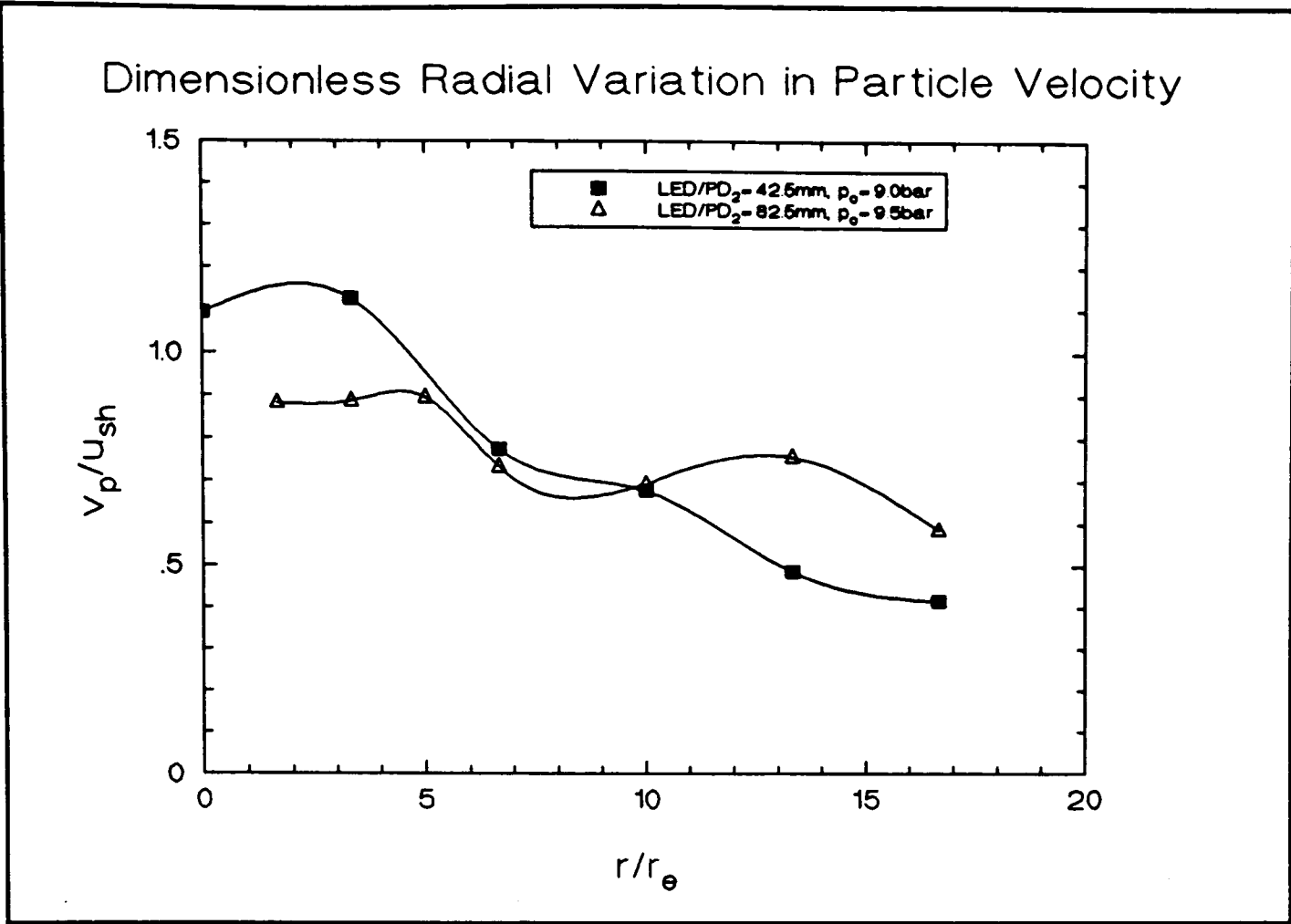


Figure 5.23 Radial variation in particle velocity at two axial locations.

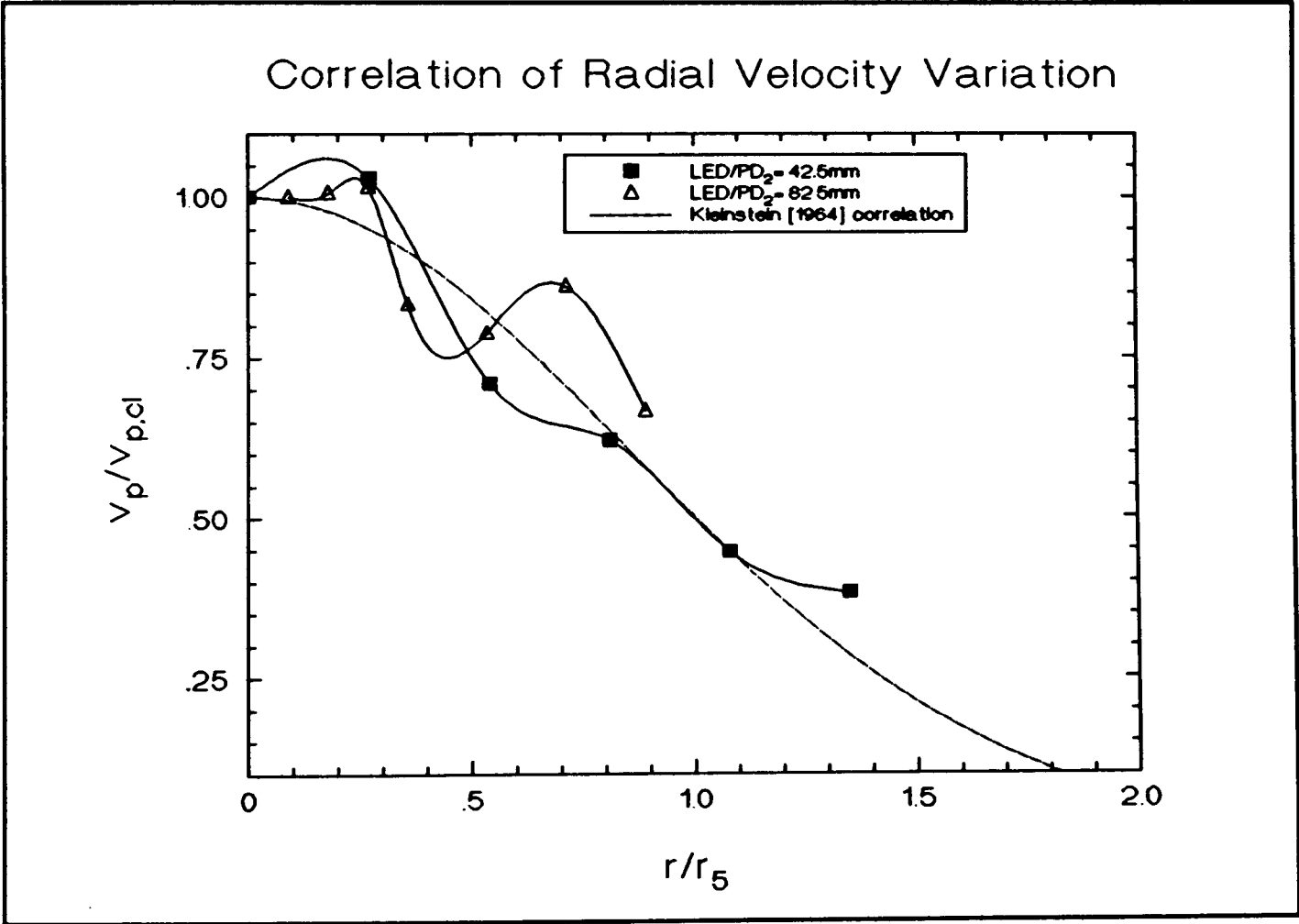


Figure 5.24 Correlation of jet radial velocity variation at two axial locations.

location and r_5 is the jet radius at which particle velocity is equal to one-half the velocity on the jet centreline. Relatively good agreement is seen, despite the

concessions to experimental approximations noted above. The information gained through this experiment will be useful in providing guidelines for target axial positioning in the biological experiments.

5.6.4 Particle Velocity Measurements for Helium

The objective of this experiment was the determination of particle velocities with helium rather than air as a driver gas. The usual particle velocimeter set-up involving two LED/PD pairs - at 1.5mm and 121.5mm from the nozzle exit - was utilised. Upstream pressure p_o was again varied from 0 to 10bar (g), while p_∞ was kept constant at atmospheric pressure. Figure 5.25 shows the resulting particle velocities as a function of upstream (gauge) pressure for helium, and compares these with particle velocities for air over similar axial locations in the target chamber. As the Rankine-Hugoniot relations would predict (see Section 3.2.1), particle velocities with helium as a driver gas are significantly greater than corresponding velocities with air. The use of helium will be shown to be important in biological experiments (see Chapter 7).

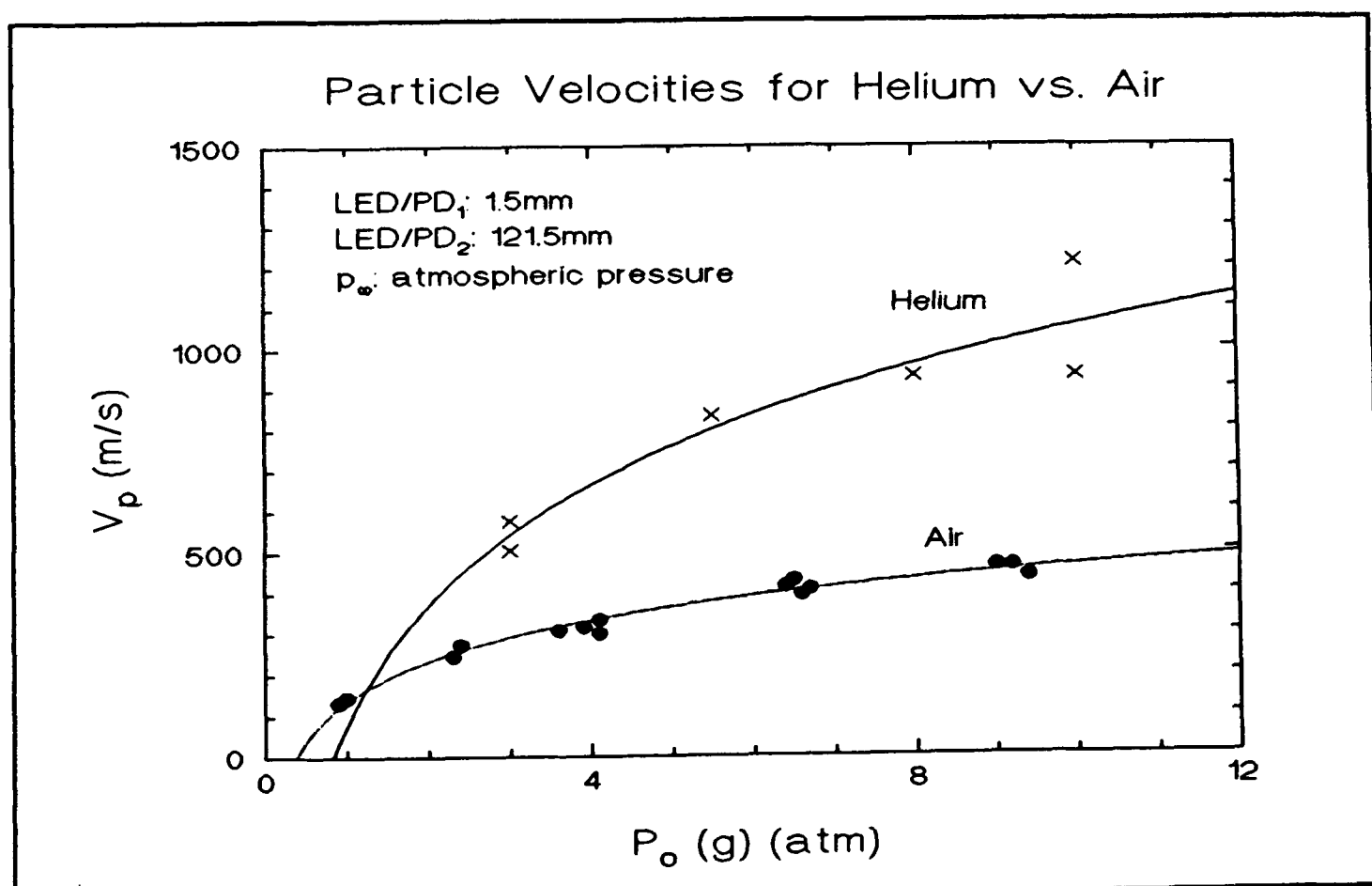


Figure 5.25 Comparison of particle velocities for air and helium.

5.7 Conclusions

Results presented in this chapter have demonstrated that particle motion is governed by transient shock tube flow rather than by fully-expanded, quasi-steady, Mach 2 nozzle flow, as had been thought originally. Using a purpose-built optical particle velocimeter, particle velocities have been shown to vary with diaphragm pressure ratio in a manner analogous to the shock and contact surface velocities of shock tube flow. Experiments utilising a nozzle thin-film gauge in combination with an LED/photodiode pair just downstream of the nozzle exit have confirmed the transient nature of the particle motion, demonstrating that particles are carried on or just behind the primary shock wave which is propagated down the nozzle upon diaphragm rupture. Other experiments involving variation in supply cylinder volume and involving replacement of the convergent-divergent nozzle with a cylindrical shock tube have also been used to substantiate this conclusion.

Calibrations of particle velocities have been carried out in both axial and radial directions, using both air and helium as driver gases, in order to provide guidance in target positioning for biological experiments. Significantly higher particle velocities were achieved with helium rather than air as the driver gas. Correlations due to Kleinstein [1964] have been applied to the present data as a means of characterising the axial and radial variation in particle velocities. Good agreement with these correlations has been demonstrated.

This information will be prove useful in biological bombardment experiments (**Chapter 7**), as an aid in calibrating the apparatus for various types of biological material. Before the information can be applied directly, however, some characterisation of particle target distribution and penetration is necessary. The parameters of target distribution and penetration, like particle velocities, are influenced directly by physical conditions of the apparatus at diaphragm rupture. Knowledge of these parameters must be demonstrated before confident calibration for biological target material can be achieved. Characterising particle target distribution and penetration will be the purpose of the next chapter.

CHAPTER 6. PARTICLE DISTRIBUTION AND PENETRATION

6.1 Introduction

In previous chapters the theoretical control of underexpanded free jets has been explored, and gas dynamics measurements have demonstrated the control of the free jet in the system under study. Particle velocities have been measured and shown to vary in a manner analogous to those velocities characteristic of a shock tube. Control of particle dynamics remains to be proven, however; that will be the purpose of this chapter. Control will be demonstrated through evaluation of two important parameters: particle distribution across a target surface and particle penetration within a target. Recourse will be made to several novel techniques for the evaluation of these two parameters.

The initial part of the chapter will describe three techniques for the visualisation of particle distribution. The first method will look at particle distributions across parafilm targets post-bombardment. Effects of varying nozzle pressure ratio, p_0/p_∞ , and target distance on particle distribution profiles across bombarded targets will be evaluated. A novel optical scanning technique used for these measurements will be described. A second method of visualisation will make use of high-speed video to determine temporal variation in particle-laden jet spread from the nozzle over the first milliseconds of flow. A final technique for evaluation of particle distribution will involve scanning electron microscope (SEM) photography of bombarded targets. The objective of visualisation of highly-magnified targets will be to evaluate particle agglomeration on the bombarded surfaces.

The second portion of the chapter will evaluate penetration of ductile, semi-infinite material by high-velocity, micron-sized, tungsten particles. Of particular importance will be the determination of the dependence of penetration depth on impact velocity. Empirical formulations from the literature for dimensionless penetration depth as a function of impact velocity will be reviewed, and good general agreement will be demonstrated.

6.2 Distribution Measurements

Although a significant amount of effort has been expended previously in the study of jet spread (boundary location) for *steady*, fully-expanded gaseous jets (see, for example, Love *et al.* [1959]), the same cannot be said for the study of *transient* jet expansion - time-dependent effects associated with the emergence of a shock wave from a shock tube, for example. Some work has, however, gone into the evaluation of transient effects at nozzle start-up. Smith [1966] conducted an experimental study of the starting process that occurs in a reflected-shock tunnel. Schlieren photographs of shock wave emergence during the start-up process for a hypersonic nozzle, although useful in understanding transient effects associated with primary and secondary shock propagation, did not adequately display temporal variation in jet structure. Baird [1987] used differential interferometry to visualise the supersonic vortex ring caused by the emergence of a normal shock from a circular tube. Flow field pressures were used to verify the existence of a rearward-facing shock, as demonstrated by a sharp drop in centreline pressure (following in time an equally sharp rise in pressure across the emerging primary shock wave). Smarr [1984] computed shock structure within jets of varying jet Mach number, M_e , and density ratio, ρ_e/ρ_∞ , displaying "snapshots" of the resulting jets as they reached a given axial location. Although the core region for the jet most appropriate to the present study ($M_e = 3.0$, $\rho_e/\rho_\infty = 1.0$), shows jet spread of the order of that seen by workers applying the simpler method of characteristics (Love *et al.* [1959]), the results do suggest the possibility of jet entrainment as a mechanism of importance to jet spread. This mechanism will be visualised in greater detail for the present nozzle conditions in results displayed below.

6.2.1 Preliminary Distribution Measurements

The purpose of the initial study was the evaluation of particle distribution for bombardment of suitable targets. Much preliminary work went into determining ideal conditions for these studies, including target types and quantities

of particles in each run. Ductility, optical translucence, and ready availability made "parafilm" a suitable target for these experiments. The ductile consistency of parafilm is such that particles adhere readily when bombarded at sufficient velocities. The light transparency of parafilm was necessary to permit optical techniques for quantification of particle distribution. In each case the parafilm targets consisted of 10mm radius circles. It was believed that this was the largest target dimension that would not severely block the nozzle exit, especially for conditions where the target was very close to the nozzle exit.

6.2.1.1 Optical Method for Distribution Measurement

An optical method for determining particle distribution across a target surface was utilised in initial experiments. The technique is based on the absorption and scattering of light as it traverses through a dense medium. The fundamentals of the technique are briefly discussed below:

When a beam of light is passed through a translucent or semi-opaque medium, its intensity decreases, primarily due to scattering and absorbing within the medium. Consider a situation in which light of intensity I_o enters a medium, and light emerging on the other side has intensity I . I_o and I are related by:

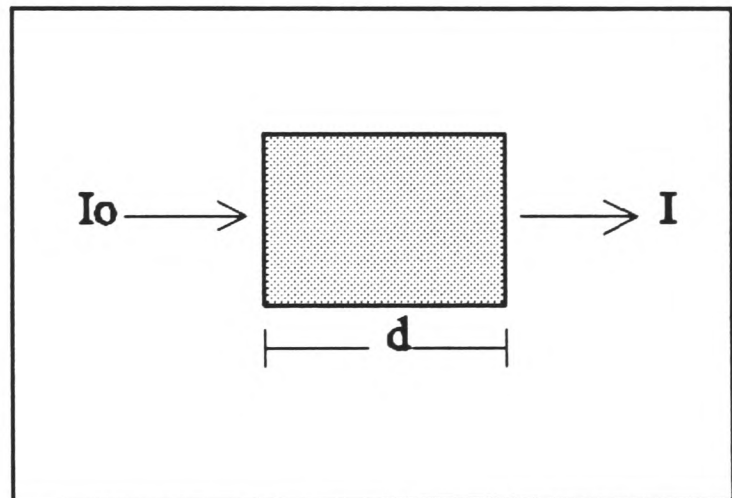


Figure 6.1 Absorption of light.

$$I = I_o e^{-\alpha d} \quad (6.1)$$

where d is the thickness of the medium and α is a material-dependent constant of absorption.

This fundamental idea was used to optically-determine particle distribution over the parafilm targets by measuring $I(x,y)$ across the surface area of the bombarded targets. Tungsten being an optically-opaque substance, presence of tungsten particles at particular locations on the surface decreased the amount of

light passing through the parafilm at those locations through absorption and scatter¹. In the analysis of the transmitted light, $I(x,y)$, the absorption due to parafilm itself was normalised out, by taking I_0 to be the light emerging from a clean sheet of parafilm. This allowed the calculation of the light absorption due only to the presence of tungsten particles.

$$A = \alpha d = -\ln(I/I_0) \quad (6.2)$$

In order to calculate the relative amount of tungsten over a given circular area of target, the absorption was integrated over that area:

$$\frac{\int_0^{\pi} A \pi r dr}{\int_0^{\infty} A \pi r dr} \quad (6.3)$$

In the analysis this was approximated by:

$$\frac{\sum_{i=1}^{\pi} A_i \pi r_i dr}{\sum_{i=1}^{\infty} A_i \pi r_i dr} \quad (6.4)$$

This equation was used to determine the relative radial distribution of tungsten particles over the parafilm targets. The analysis was completed using a LOTUS 1-2-3 spreadsheet program.

In order to measure transmitted light $I(x,y)$ through various bombarded parafilm targets, an optical imaging system at the Oxford Eye Hospital was utilised. The set-up consisted of a charge-coupled device (CCD) video detector, frame grabber, and digitiser, all operating in conjunction with an IBM PC-AT. A sketch of the set-up is shown in Figure 6.2.

¹In the discussion and results that follow, the term *absorption* will refer to both absorption and scatter as mechanisms that decrease transmitted light.

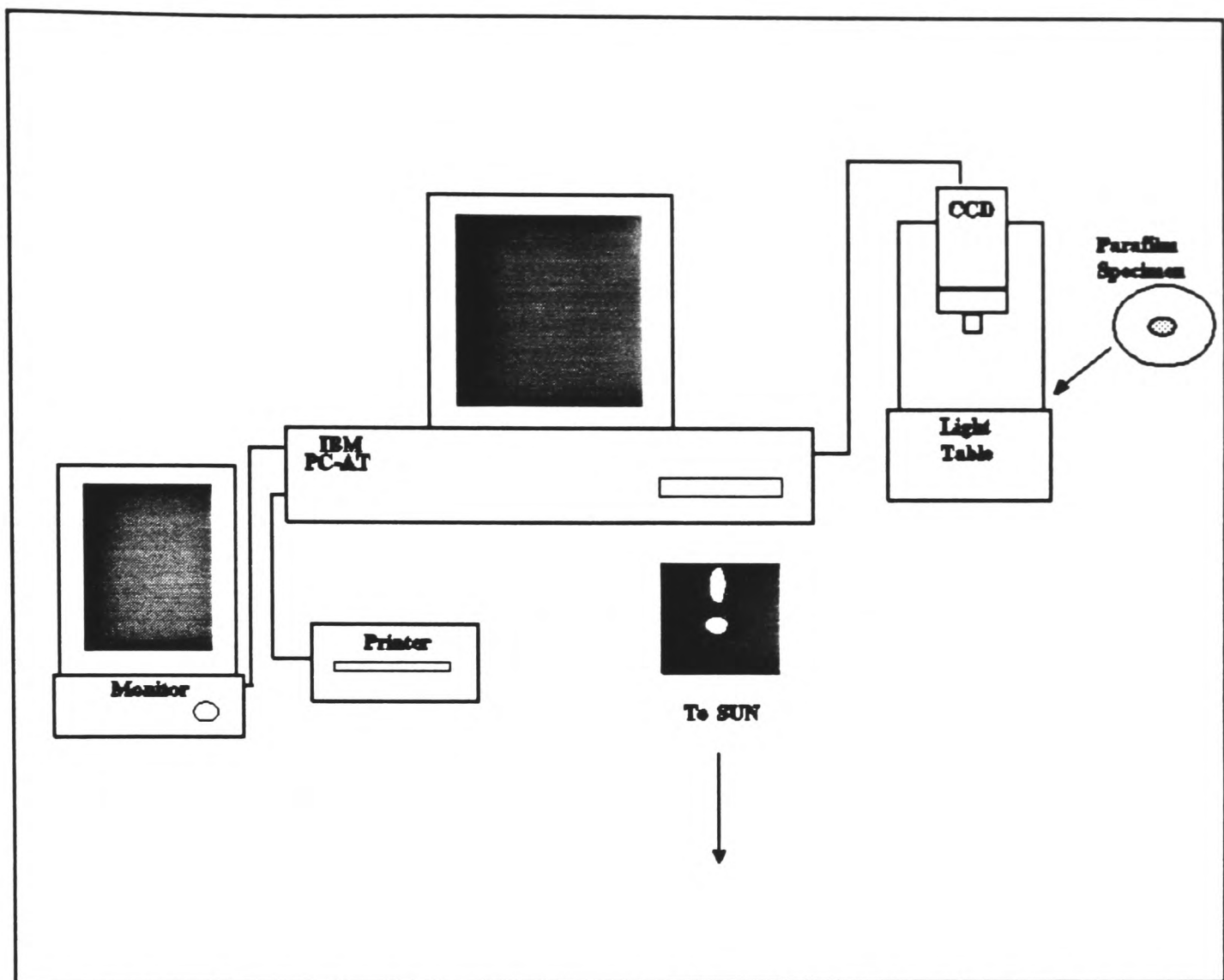


Figure 6.2 Set-up for optical determination of particle distribution across target.

A parafilm target bombarded with tungsten particles under normal operating conditions was placed on a light table which acted as a light source. Light transmitted through the specimen was focused onto the CCD camera. The CCD camera detected the 2-D light intensity distribution on an array of 512 by 512 pixels, each with a grey scale level varying between 0 and 256. A frame grabber operating within an IBM PC-AT collected the intensity distribution data. A digitiser then digitised the image, forming an array of numbers, each representing the light intensity impinging on a particular pixel. Representative specimens were analyzed in this manner using a constant light source. In addition, a clean parafilm sheet was analyzed to act as a control for the distribution experiments.

The raw distribution data produced by this optical technique were collected on floppy disks and transferred to a SUN work station in the Oxford University Robotics Laboratory. Analysis using software kindly provided by the Robotics

Laboratory was used to produce cross-sectional intensity profiles, *i.e.*, intensity profiles along columns of pixels at various X coordinates. These raw intensity data were then transformed to absorption data using the optical transformation technique described in Equations (6.3) and (6.4). The raw absorption data were normalised using the value of transmitted light intensity for a clean parafilm sheet. Finally, the area under each absorption profile curve was numerically integrated (see Equation (6.4)), enabling the determination of the relative number (*i.e.*, percentage of the total) of tungsten particles in a given radial area on the target surface. The total extent of target area impinged upon by tungsten particles could then be determined.

6.2.1.2 Results

Variation with Target Distance

The first series of experiments evaluated the variation in particle distribution for increasing target distance, *TD* - the distance of the target from the exit plane of the nozzle. Approximately 1.5mg of dry tungsten (1 to 5 μ m dia.) particles were immobilised electrostatically on the mylar diaphragm for each shot. Run conditions were: $p_o = 4.5\text{bar}$, $p_\infty = 0.5\text{bar}$. The photograph in Figure 6.3 shows typical results. The parafilm targets were placed at distances of 5mm, 50mm, and 100mm from the nozzle exit. The figure shows that the peak concentration of particles near the target centre decreased and the distribution broadened as *TD* increased. Significantly larger numbers of particles could be found at greater distances from the target centre as *TD* increased.

This effect is quantified in Figure 6.4, which shows absorption profiles from the optical technique discussed above for targets placed at various distances from the nozzle exit plane. High absorption levels correspond to high particle density. *Target Radius* refers to illuminated distance across the light table as imaged by the CCD array. The curves are normalised based on light transmission through a clean sheet of parafilm. Therefore, absorption levels above 0 correspond to the presence of tungsten particles on the target surface. Important here is the sharp

drop-off in the absorption level for the target placed 5mm from the nozzle exit and the high concentration of particles within a disc of radius 2.5mm. In contrast, for the target placed 100mm from the nozzle exit, particle concentrations are significantly lower but are relatively uniform over almost the entire target surface (10mm radius). Intermediate results were obtained with a target placed 50mm from the nozzle exit. It is apparent from Figure 6.4 that some tungsten particles would have impacted that target at radii greater than 10mm had the targets been large enough, particularly for the two larger target distances. It is believed, however, that neglect of those particles at radii greater than 10mm has not substantially altered the results.

The broadening in particle distribution with increasing TD can be further seen in Figure 6.5, which shows the relative radial particle distribution. This represents

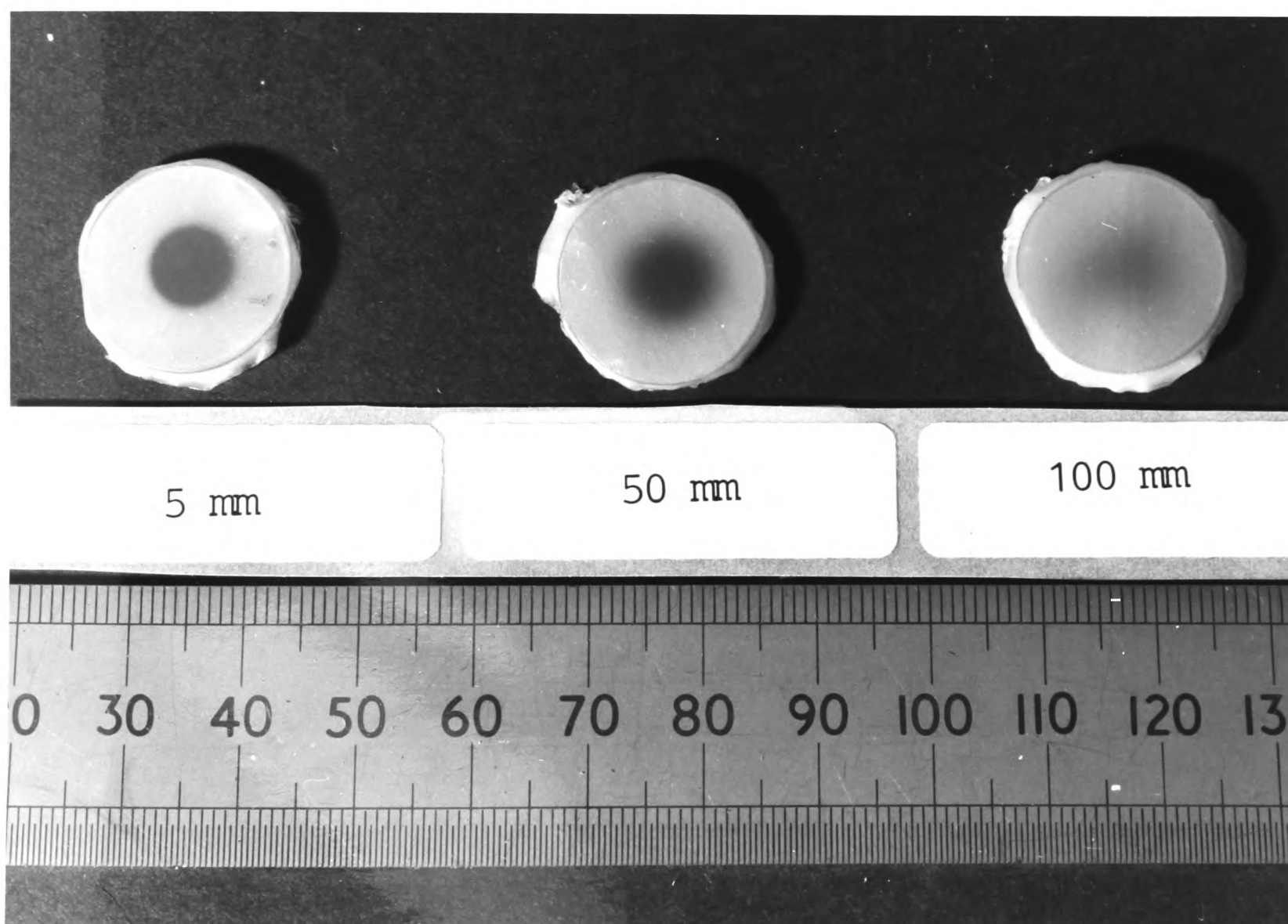


Figure 6.3 Photograph of parafilm targets showing variation in particle distribution with target distance.

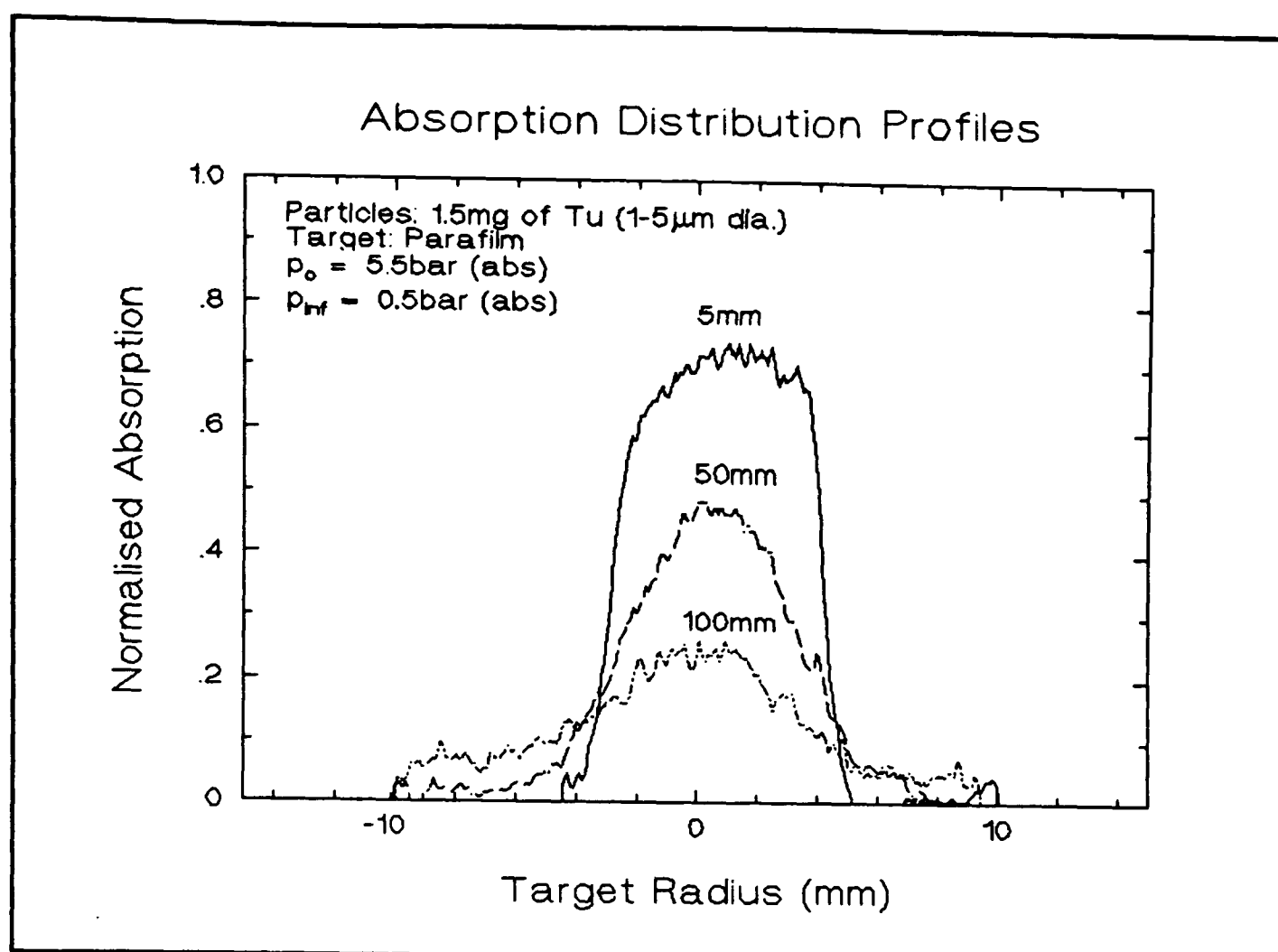


Figure 6.4 Absorption profiles for bombarded targets, quantifying variation in particle distribution for increasing target distance.

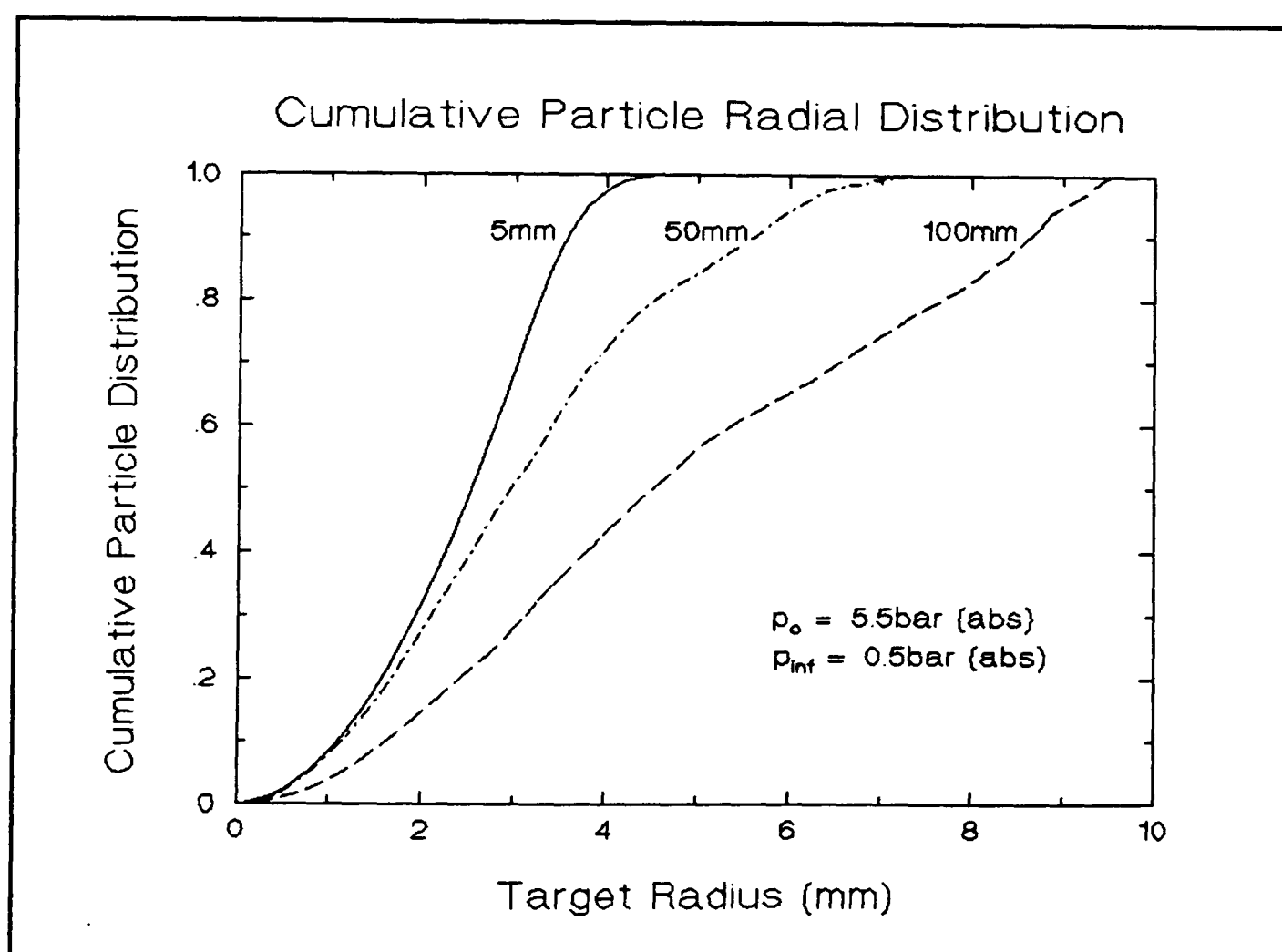


Figure 6.5 Cumulative radial particle distributions for three target distances.

the cumulative area under each curve in Figure 6.4, divided by the total area under that curve. This confirms that particles can be found over a much larger area of the target as TD increases.

Finally, Figure 6.6 shows that the variation in the target radius that contains "100 %" of the tungsten particles is approximately linear with TD. This enables predictions about the TD necessary for a particular radius of target cells and demonstrates the considerable control of particle distribution which can be achieved by changing only the distance of the target from the nozzle exit plane.

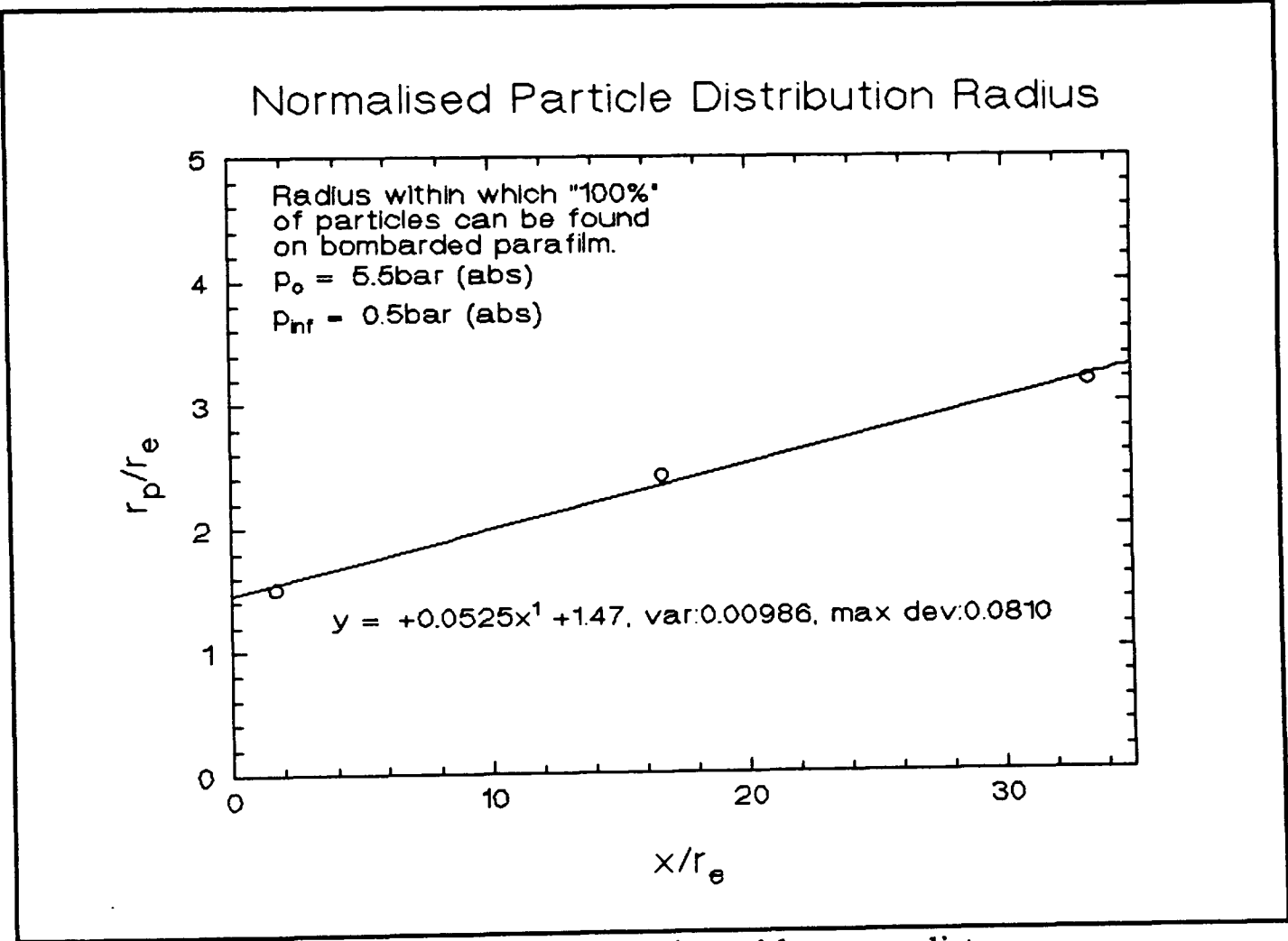


Figure 6.6 Variation in distribution radius with target distance.

Target Distribution Symmetry

The objective of the next series of experiments was the determination of target distribution symmetry. This is an important factor in determining whether tungsten particles will later impinge symmetrically onto a particular area of target plant cells in biological experiments (a desired goal). By comparing absorption profiles in the X- and Y- directions for a particular target specimen, it was

possible to look at the symmetry of the particle distribution. Figure 6.7 shows a representative case for a specimen with a TD equal to 5mm. The normalised absorption profiles represent pixel cross-sections in the X- and Y-directions which traverse the target centre. Although some slight variation in the absorption occurs at peak levels, the comparative drop-offs of each of the profiles are very similar. The drop-offs for each profile occur at approximately the same radius from the target centre, and the drop-offs have approximately equal slopes. For comparison purposes, the absorption level for a clean sheet of parafilm is shown.

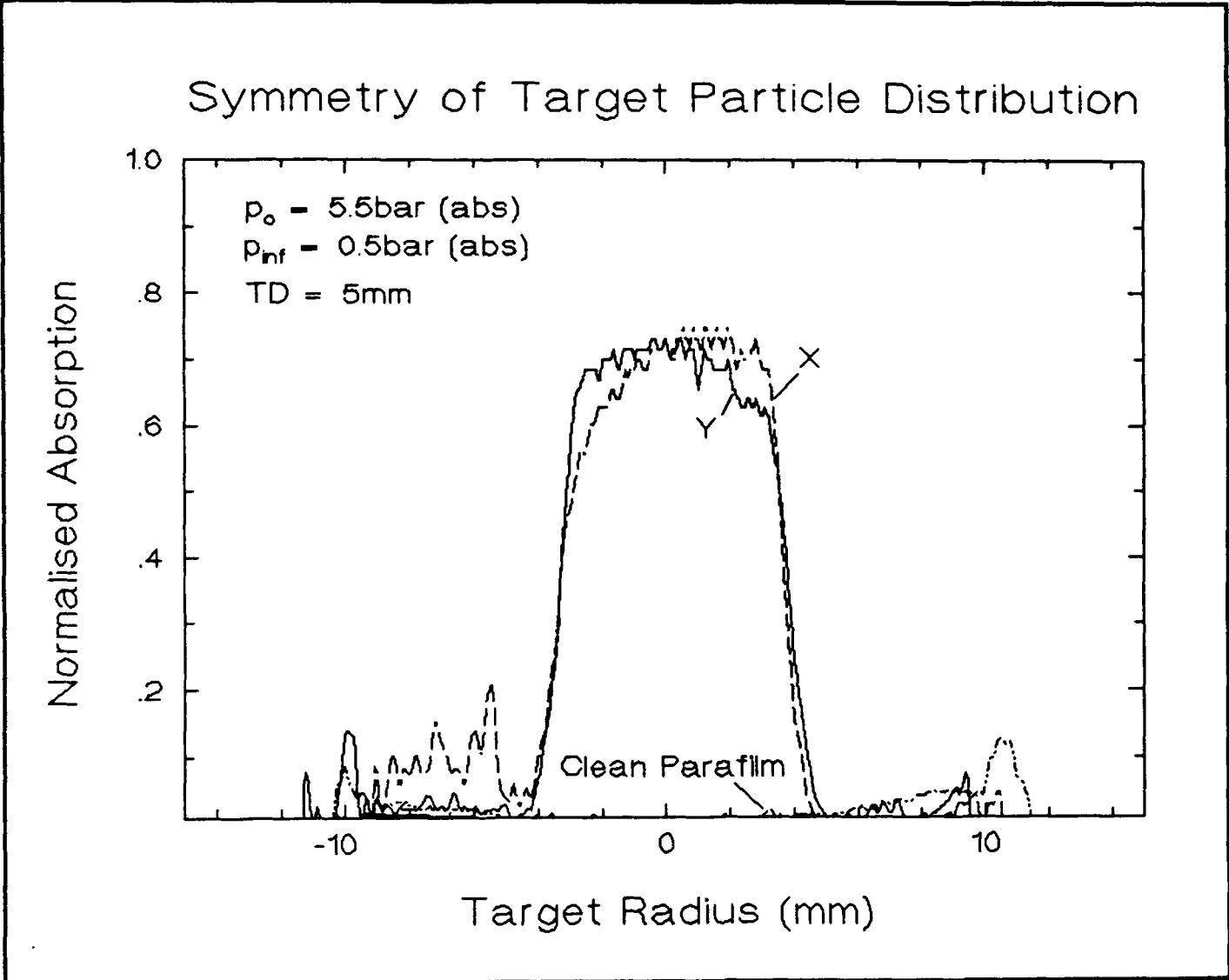


Figure 6.7 Absorption profiles in X- and Y- directions, illustrating symmetry of target distributions.

This experiment shows that the particle target distributions are symmetric about the target centre. This is to be expected, as the nozzle is axisymmetric and has a circular exit plane.

Variation with Nozzle Pressure Ratio

The objective of this series of experiments was the evaluation of the effect of variations in nozzle pressure ratio on particle distribution. In **Chapter 3** the theoretical effect of variation in nozzle pressure ratio on *jet boundary* location was calculated using both an approximate technique and a more exact method of characteristics calculation. Here, primarily through variation in target chamber back pressure, p_{∞} , it was possible to experimentally determine the effect of nozzle pressure ratio on post-bombardment *particle* distribution.

Figure 6.8 shows target particle distributions corresponding to downstream vacuums of "Atmosphere" ($p_{\infty} = 1\text{bar}$), 0.50bar vacuum ($p_{\infty} = 0.50\text{bar}$), and 0.85bar vacuum ($p_{\infty} = 0.15\text{bar}$). Pressure difference ($p_o - p_{\infty}$) was kept constant at 6.0bar in each case. This results in pressure ratios which represent approximately over-expanded, normally-expanded, and under-expanded nozzle exit conditions, respectively. Target distance was constant at 5mm. The photograph shows that the distribution corresponding to the under-expanded condition ($V_o = 0.85\text{bar vacuum}$) is visibly broader than that due to the over-expanded condition ($V_o = \text{Atmosphere}$). This is to be expected, as an over-expanded supersonic nozzle has an oblique shock wave at its exit, which slow the particles and turn their trajectories towards the nozzle axis. On the other hand, an under-expanded supersonic nozzle has expansion waves at its exit which cause the jet (and the particles entrained therein) to turn away from the axis. Also apparent from Figure 6.8, for the under-expanded case, is a darker ring of particles around the primary region of particles. This may represent impingement of the jet barrel shock.

These results tend to show that despite the transient nature of particle propagation discussed in **Chapter 5**, the particle spread (distribution across a target surface) seems to be affected by the nozzle pressure ratio (*i.e.*, the degree to which the nozzle is underexpanded). These arguments would imply that the structure of the free jet is defined transiently as the contact surface carrying the particles exits the nozzle.

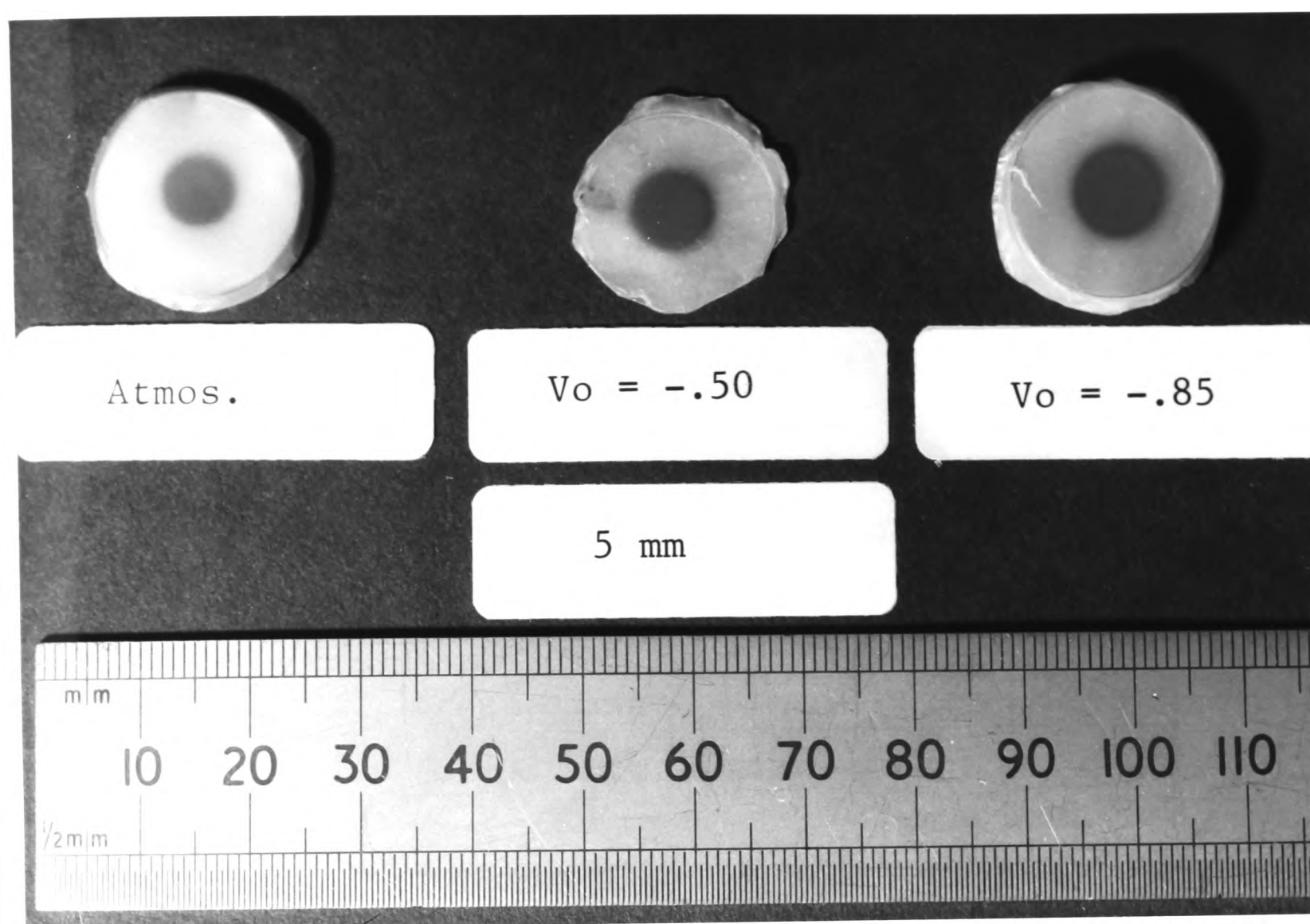


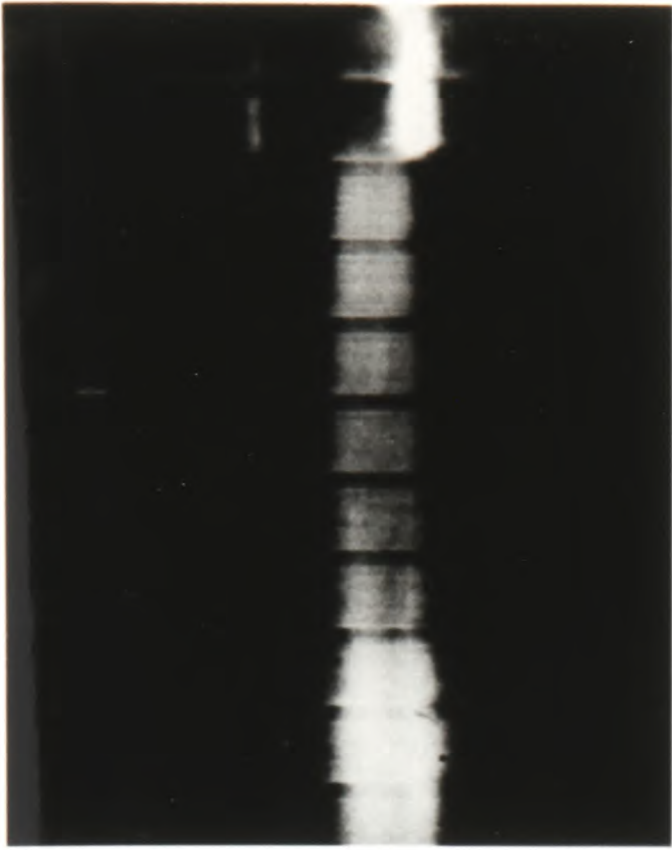
Figure 6.8 Photograph of parafilm targets showing variation in particle distribution with target chamber vacuum.

6.2.2 Particle-laden Jet Visualisation

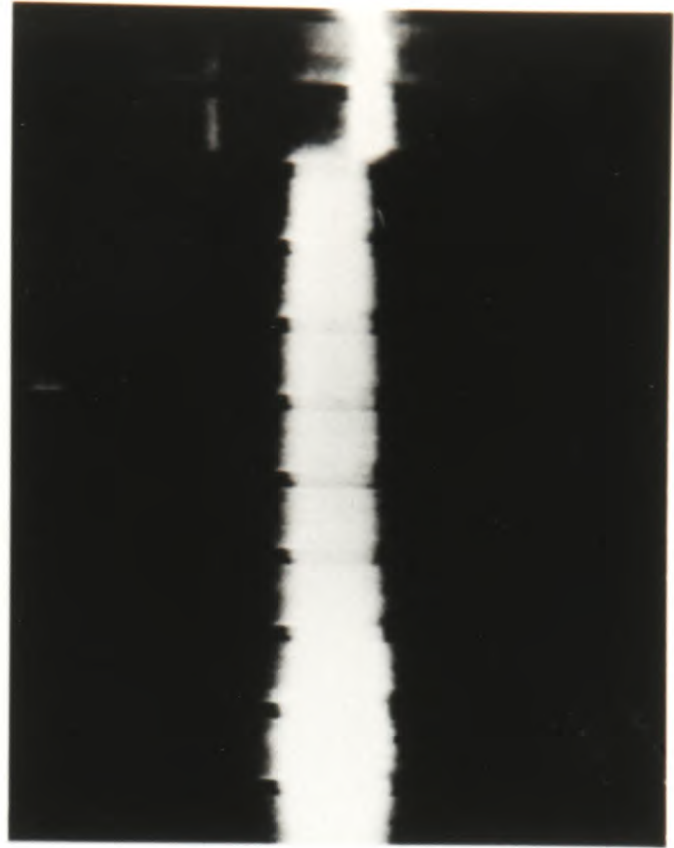
In order to further study the trajectory of particles entrained in the supersonic flow exiting the nozzle, a flow visualisation technique was utilised. This technique involved the use of a high-speed video camera (Kodak Ektapro; 1000 frames/sec; see Section 4.2.1). Preliminary experiments determined that the usual tungsten powder was not sufficiently visible. Finely-ground chalk dust (mean dia.: $10\mu\text{m}$, $\rho = 2\text{kg/m}^3$), however, was determined to be an adequate substitute despite having a Stokes' number approximately 10 times that of micron-sized tungsten particles. This difference, however, should be taken into account when interpreting the photographs which follow. It should be remembered that the chalk particles will show more velocity slippage than would the tungsten particles, and may not follow the gas streamlines as closely. In addition, a volume of chalk much larger than that of tungsten used in the actual biological bombardments was employed to aid visualisation. While this enhanced chalk volume increases the time during which the particle trajectories can be visualised, the smaller volume of tungsten probably does not permit adequate time for the occurrence of all the transient effects seen in the photographs below.

Figure 6.9 shows the resulting expansion of the particle-laden jet at 1ms intervals for $p_o = 4.1\text{bar}$ and $p_\infty = 0.1\text{bar}$. The photographic stills taken from the video screen display the significant change in the jet boundary that occurs over the first four milliseconds of jet formation. As the jet flow develops, the expansion from the nozzle increases, reaching a maximum within 4ms. This temporal variation in jet expansion is still more substantial in Figure 6.10, which shows the particles entrained in a jet resulting from a higher nozzle pressure ratio ($p_o = 6.9\text{bar}$ and $p_\infty = 0.1\text{bar}$). A comparison of Figure 6.9 with Figure 6.10 clearly demonstrates that a higher nozzle pressure ratio (p_o/p_∞) leads to greater jet expansion from the nozzle.

The effect of nozzle pressure ratio on particle-laden jet expansion is quantified in Figure 6.11 and Figure 6.12, which show jet boundary location as a function of time for the two nozzle pressure ratios shown in the preceding figures



1 ms



2 ms

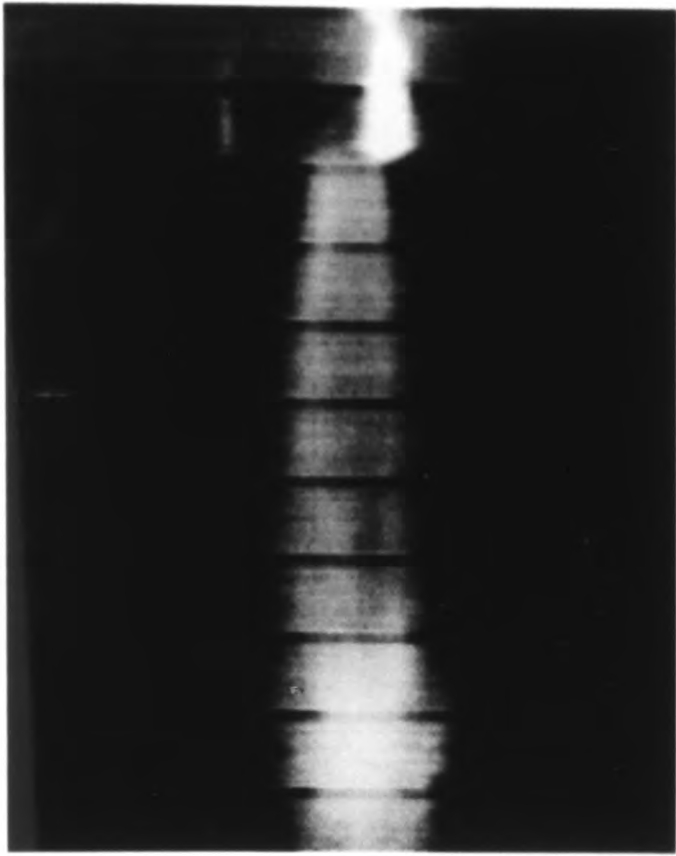


3 ms

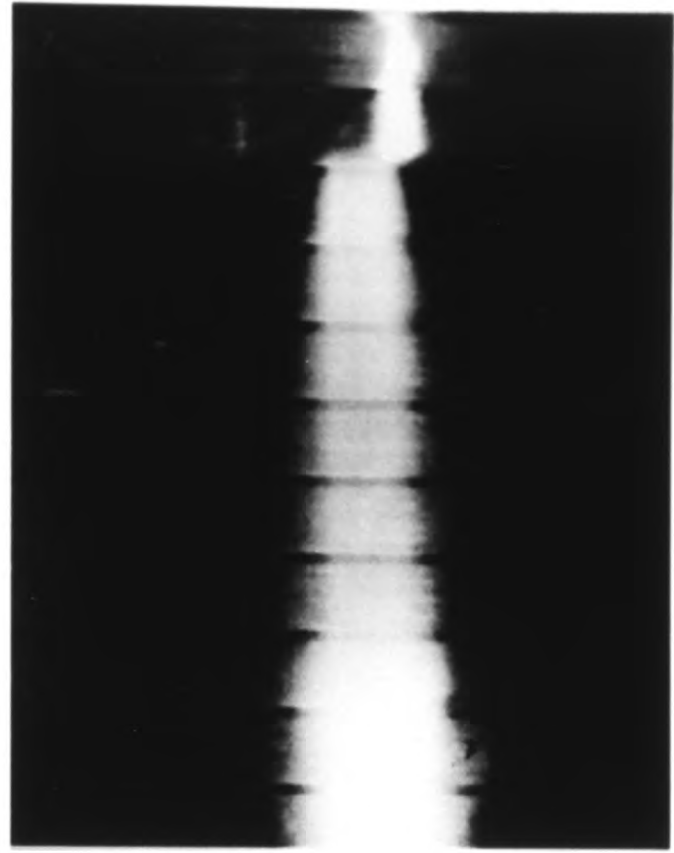


4 ms

Figure 6.9 Photographic stills at 1ms intervals showing particle-laden jet expansion for $p_o = 4.1\text{bar}$, $p_\infty = 0.1\text{bar}$.



1 ms



2 ms



3 ms



4 ms

Figure 6.10 Photographic stills at 1ms intervals showing particle-laden jet expansion for $p_o = 6.9\text{bar}$, $p_\infty = 0.1\text{bar}$.

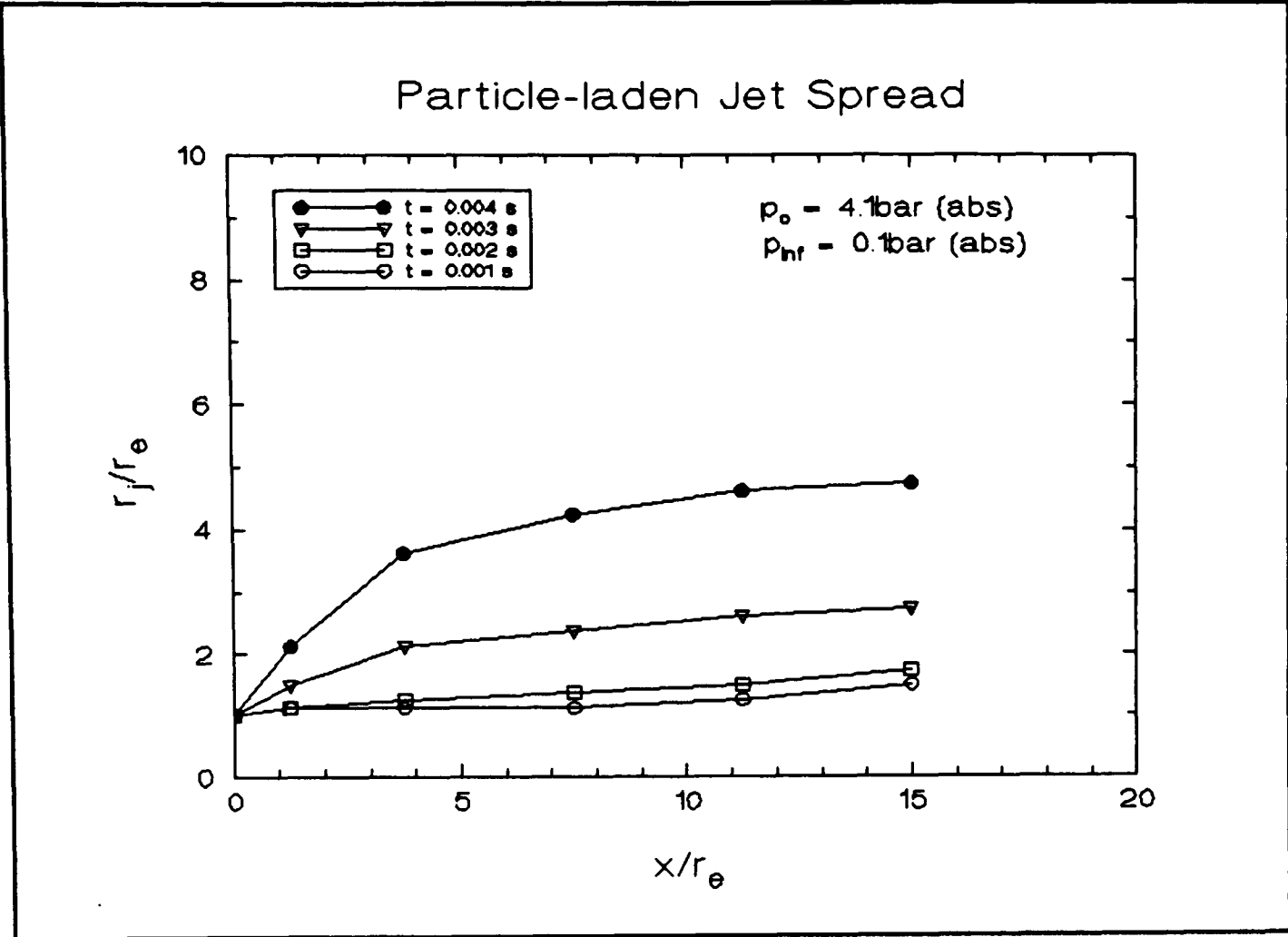


Figure 6.11 Temporal variation in boundary location of particle-laden jet ($p_o = 4.1 \text{ atm}$, $p_\infty = 0.1 \text{ atm}$).

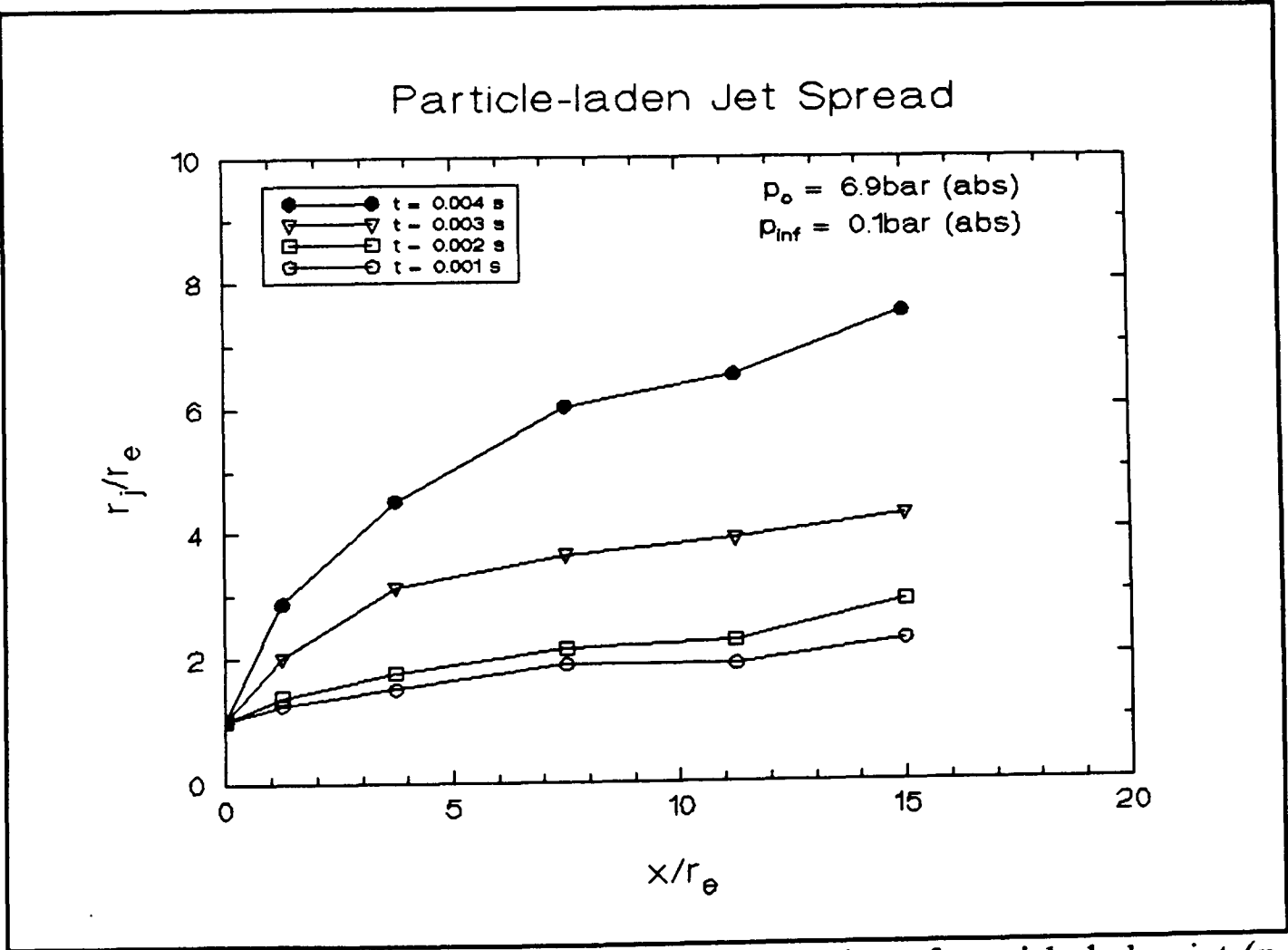


Figure 6.12 Temporal variation in boundary location of particle-laden jet ($p_o = 6.9 \text{ atm}$, $p_\infty = 0.1 \text{ atm}$).

($p_o/p_\infty = 41$ and 69 , respectively). These jet radii represent measurements from the high-speed video photographic stills. As before, jet expansion increases with nozzle pressure ratio.

Measurements of maximum jet boundary expansion attained for a range of nozzle pressure ratios as determined from the high-speed video photographic stills are presented in Figure 6.13. These experimental results are compared with numerical method of characteristics approximations of Love *et al.* [1959] for closely-matched run conditions. Although good agreement is found at the lower nozzle pressure ratio ($p_o/p_\infty = 30$), experimentally-determined measurements of maximum jet expansion exceed calculations for higher nozzle pressure ratios ($p_o/p_\infty = 69$). Although the reason for this underprediction is not clear, presumably it is related to transient development of the jet: the predictions of Love *et al.* [1959] involve only steady-state jets, while the jets in the present study are transiently associated with initial flow from a supersonic nozzle/shock tube.

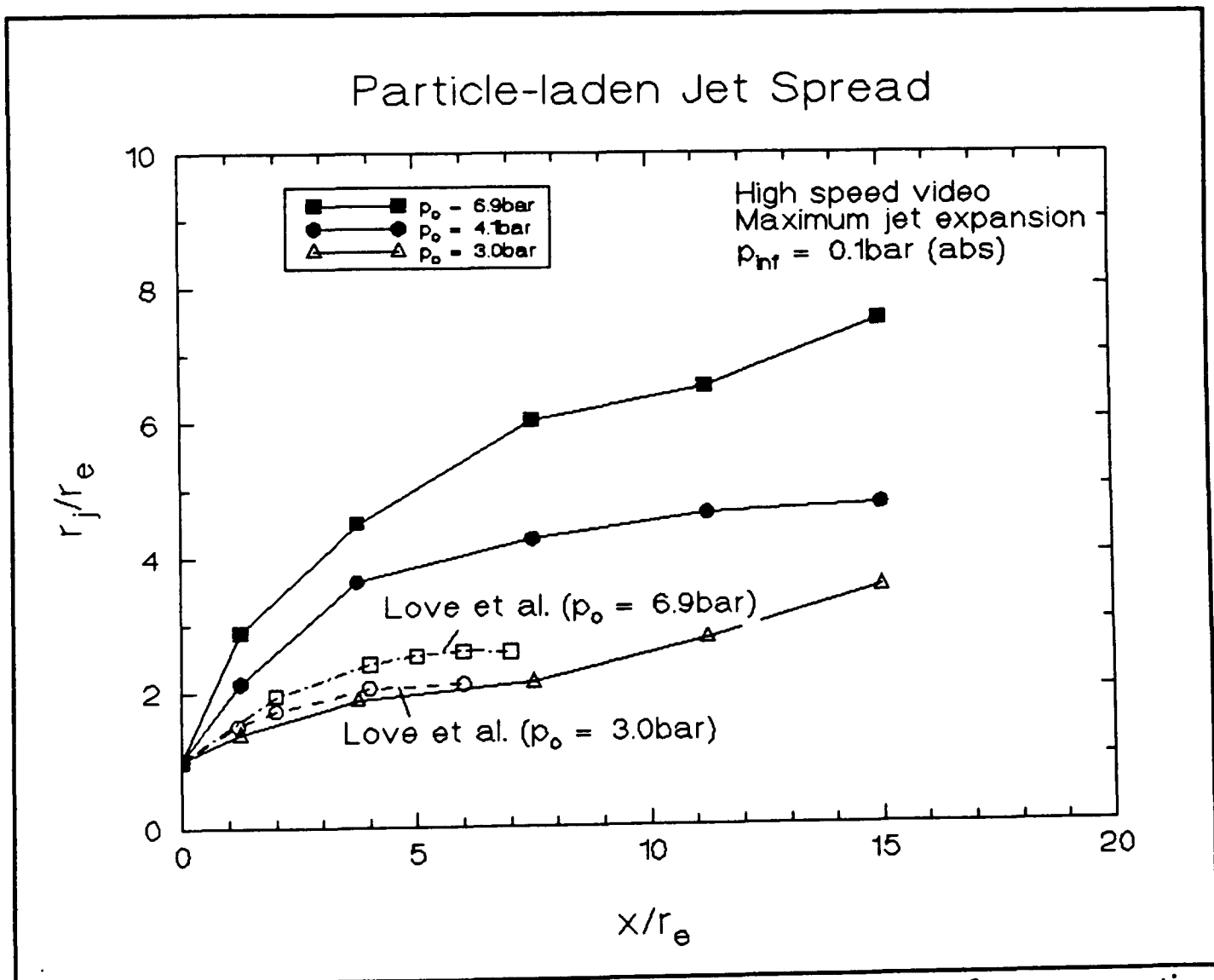


Figure 6.13 Maximum particle-laden jet spread for a range of pressure ratios, compared with M-o-C predictions of Love *et al.* [1959].

A possible explanation for the temporal variation in jet spread seen in the preceding figures relates to variations in pressure at the nozzle exit as a function of time. Badcock [1992] has shown in Figure 3.6 a snapshot of the axial variation in pressure. A sharp pressure rise across the shock is followed several hundred milliseconds later by a gradual expansion front which brings a corresponding increase in static pressure at the nozzle exit. Assuming ambient pressure does not change substantially over the course of the first few milliseconds, changes in static pressure at the nozzle exit will, therefore, lead to changes in pressure ratio, p_e/p_∞ , which transiently alter the ensuing jet structure. This would cause the increase in particle-jet spread seen in Figure 6.9 and Figure 6.10. The increase in jet spread as a function of time would only subside once effects due to blowdown of the upstream supply cylinder become significant.

The formation of a large mixing region surrounding the jet core is probably an important reason that the particle-laden jets visualised here exceed the spread predicted by Love *et al.* [1959]. The predictions of Love were based on an isentropic, inviscid method of characteristics technique which neglected any viscous effects. However, a substantial viscous mixing region exists in the flows of the present study. Results of preliminary experiments with dyes - involving the bombardment of filter paper with 20 μ l of a blue dye in place of tungsten - display the existence of such a mixing region surrounding the axisymmetric core of the jet. The mixing region is seen as the light blue region surrounding the dark blue region (jet core) in Figure 6.14. This mixing region occurs as the jet expanding from the nozzle entrains slower-moving (or stationary) gas from the ambient target chamber.

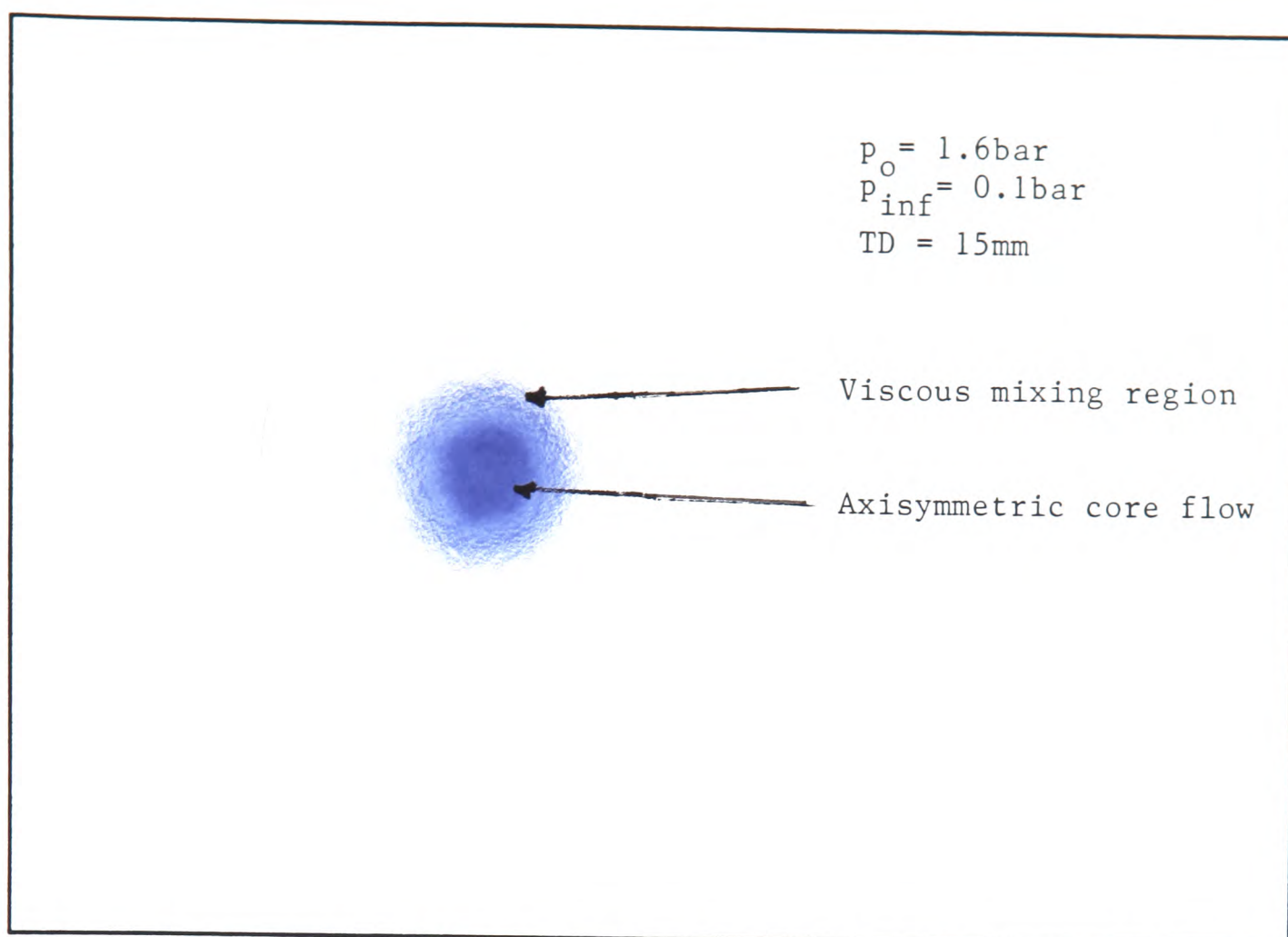


Figure 6.14 Result of dye-bombardment onto filter paper, displaying viscous mixing region surrounding axisymmetric core.

6.2.3 Electron Micrographs of Bombarded Targets

An experiment was conducted to determine the distribution of tungsten particles microscopically after bombardment onto parafilm targets. A scanning electron microscope (SEM) at ICI Seeds (Jealott's Hill, Berkshire) was utilised to visualise the particle distribution at several positions across the bombarded targets. The author is indebted to Cliff Walters of ICI Seeds for making the electron micrographs shown below.

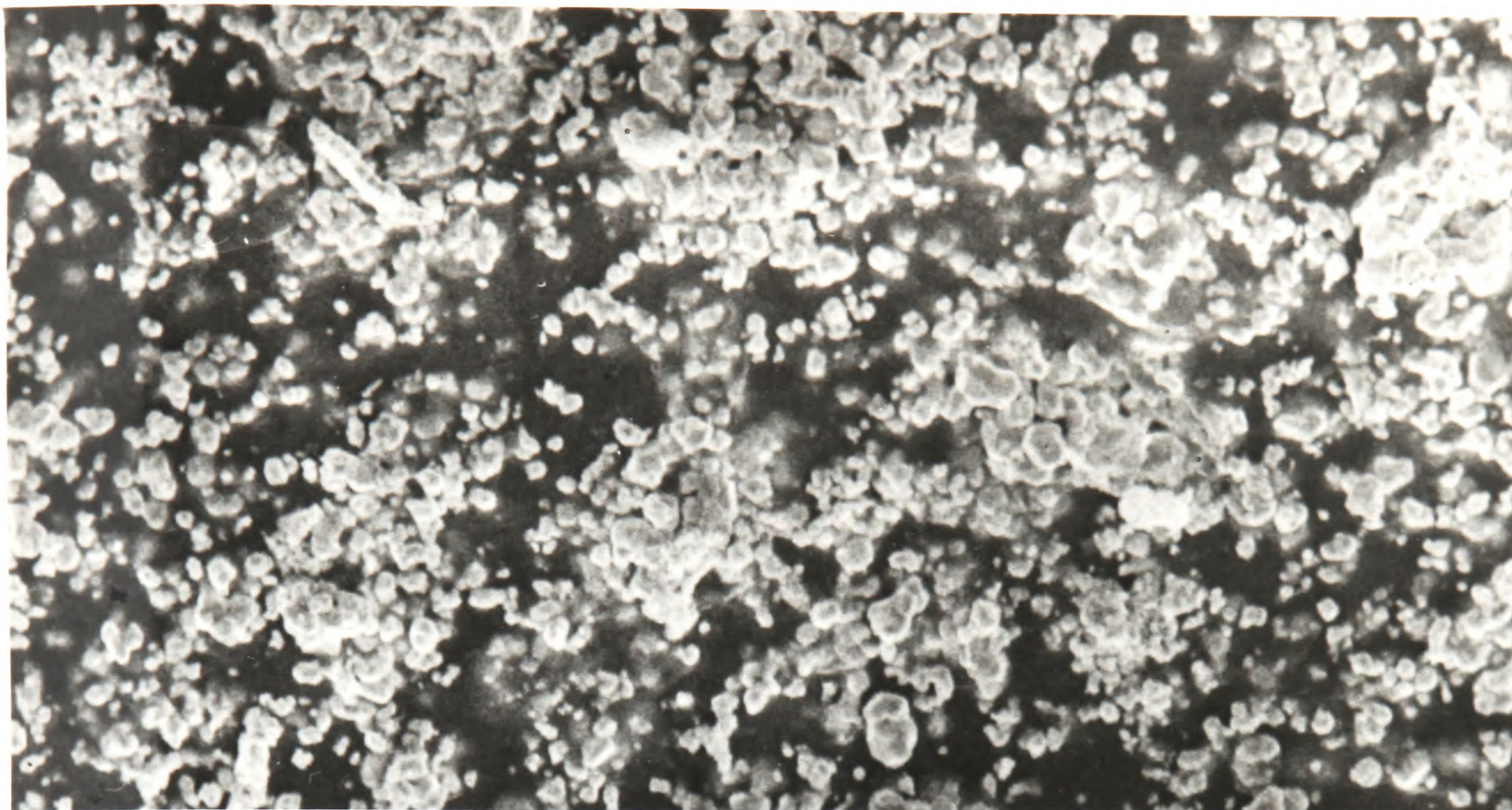
Figure 6.15a shows the distribution of micron-sized tungsten particles at the *centre* of a parafilm target for $TD = 80\text{mm}$ ($p_o = 2.7\text{bar}$, $p_\infty = 0.1\text{bar}$). The photograph is enlarged x 3000. Important here is the uniform distribution of particles at the target centre and the high particle number density. This is contrasted with Figure 6.15b, which displays particle distribution at a location 5mm from the target centre for the same target. A substantial decline in particle number density is apparent, and the formation of several large clumps of particles with many individual particles can be seen. Estimates of particle number density

over the area of the photographs have shown that the number of particles decreases by up to 75% for a location 5mm from the target centre when compared with the number of particles at the target centre.

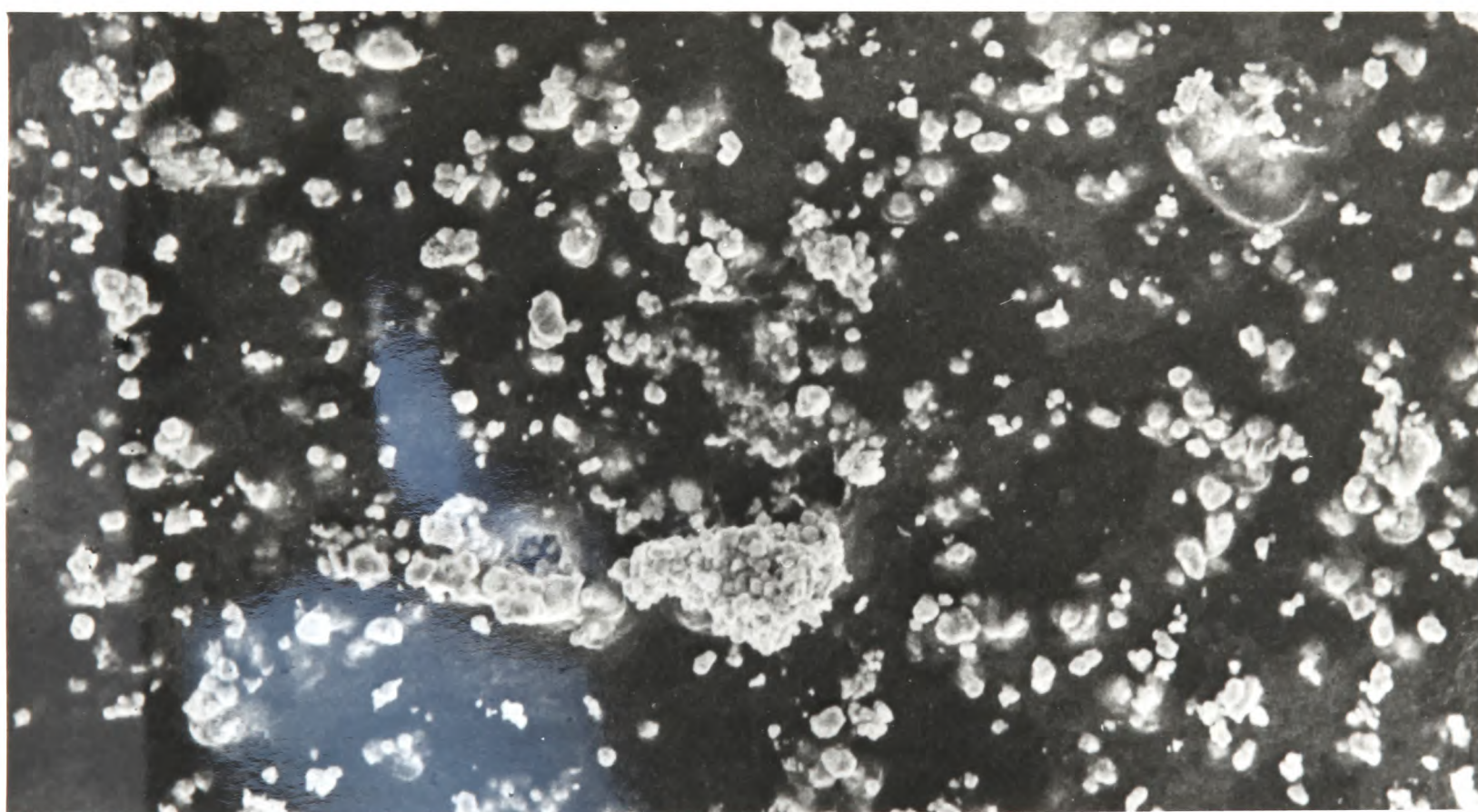
The particle number density drops still further when TD increases to 160mm, as seen in Figure 6.16a, which shows particle distribution at the target centre ($p_o = 2.7\text{bar}$, $p_\infty = 0.1\text{bar}$). Particle clumps appear smaller here; a substantial area of the target remains unimpinged by the tungsten particles. A still further decrease in particle density is seen in Figure 6.16b, which shows particle distribution at a location 5mm from the target centre. At this location few particle clumps are apparent; however, there is substantial target area between the tungsten particles. In evaluating these photographs for biological experiments, it should be remembered that most of the plant cells of interest in the present study (e.g., maize suspension cells) have diameters approximately 25 - 50 times larger than the diameters of the individual tungsten particles. Therefore, even the relatively-low particle number densities seen in Figure 6.16 should be high enough to achieve good transformation efficiencies (this will be confirmed in Chapter 7).

A simple statistical analysis of particle agglomeration (clumping) for two of the photographs (Figure 6.15b and Figure 6.16b) has revealed the extent of particle clumping.² For the analysis, particle clump sizes were divided into small (< 5 particles), medium ($5 < X < 20$ particles), and large (> 20 particles) clumps. The photographs were divided into a gridiron pattern and the particles counted manually. Table 6.1 shows the resulting number frequency for the various clump sizes for a target with $TD = 80\text{mm}$, radius = 5mm. Table 6.2 shows the corresponding number frequency for $TD = 160\text{mm}$, radius = 5mm. It should be noted here that particle counting in the third dimension was not possible. Therefore, particles sitting on top of one another were indistinguishable. Examination of the photographs, however, shows that this should not be a significant source of error.

²Due to the high particle number density, it was not feasible to analyse the other photographs.

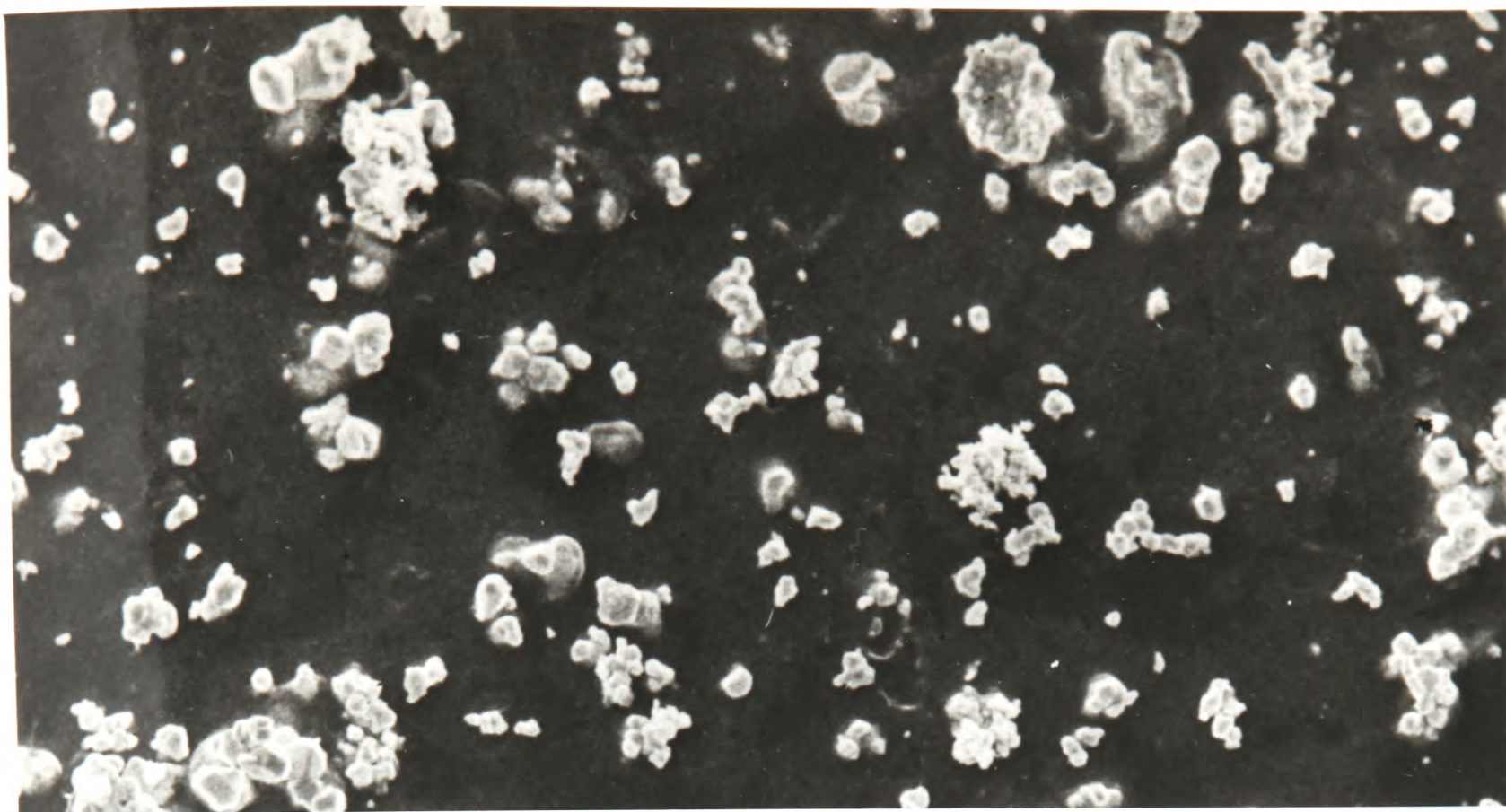


a

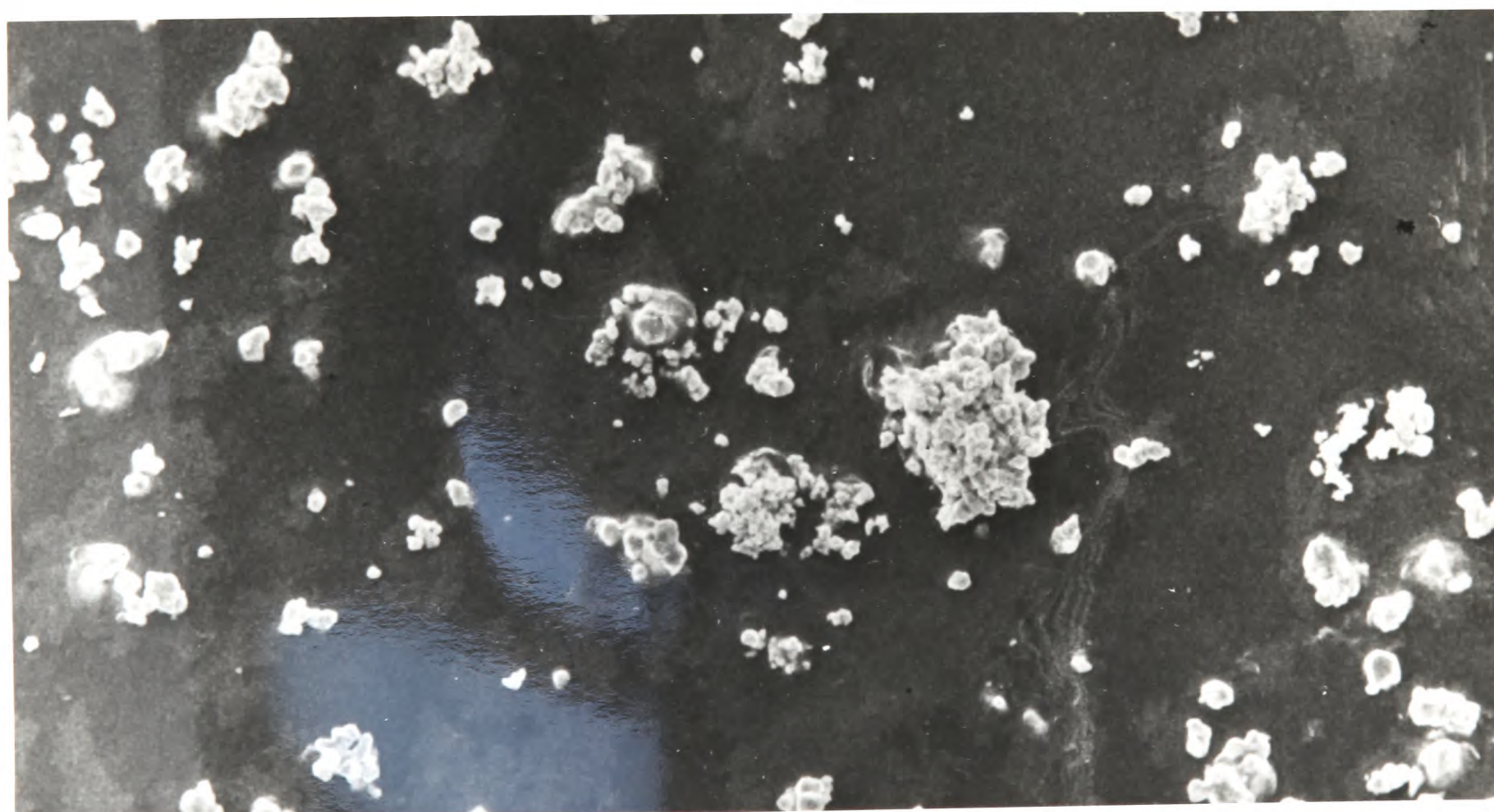


b

Figure 6.15 Scanning electron micrograph of particle distribution on target at $TD = 80\text{mm}$; **a.)** target centre, **b.)** 5mm off-centre (x3000).



a



b

Figure 6.16 Scanning electron micrograph of particle distribution on target at $TD = 160\text{mm}$; a.) target centre, b.) 5mm off-centre (x3000).

Table 6.1 Particle Clumping Analysis: $TD = 80\text{mm}$, radius = 5mm.

Clump Size	No. of Particles	%
< 5	1988	68
$5 < X < 20$	725	24.8
> 20	210	7.2
Total	2923	100

Table 6.2 Particle Clumping Analysis: $TD = 160\text{mm}$, radius = 5mm.

Clump Size	No. of Particles	%
< 5	475	42
$5 < X < 20$	475	42
> 20	180	16
Total	1130	100

Clearly, the great majority of particles are not found to be in large clumps. Instead, most are found as individual particles, or in small to medium-sized clumps, which do far less damage to biological cells than do the large clumps. Note that a greater percentage of particles are in large clumps for $TD = 160\text{mm}$ than for $TD = 80\text{mm}$. This is as intuition would hold, since a 5mm target radius represents a smaller fraction of the total bombarded target area at $TD = 160\text{mm}$ than at $TD = 80\text{mm}$. The percentage of individual and small-size clumps is expected to increase as target radius increases for a given target distance, because smaller clumps (and individual particles) are better able to follow spreading, radially-divergent streamlines. Larger clumps, having greater inertia, presumably follow a relatively-straight path.

6.3 Penetration Measurements

6.3.1 Introduction

The development of analytical and empirical models for the penetration of targets by projectiles has been the objective of much previous research, principally by those with interests in armaments and ballistics. The physical processes involved in such penetration are extremely complex, and the relative importance of the various mechanisms involved depends to a large extent on the material properties, geometry, and relative velocity of the impacting objects. Analytical models for these phenomena have been developed only for highly idealised situations; most have concentrated only on a single mode of deformation or failure, such as elastic or plastic deformation or perforation.

Of particular interest in the current study is the penetration of thick or semi-infinite, ductile media. This will serve as a model for the penetration of biological tissue and will permit the correlation of depth of penetration with certain operator-controllable parameters, most importantly, particle velocity. Before describing the experimental work, it may prove instructive to review appropriate analytical approaches and empirical constructs developed by previous researchers.

6.3.2 Background

Two primary approaches have been adopted to analytically model the problem of projectile impact: use of energy balance and conservation of momentum. In many cases complete analysis requires either an assumption *a priori* or measurement of particular dimensions *a posteriori*. Taylor [1948] applied the energy balance method to study the enlargement of a circular hole by a conical head projectile perforating a thin plate, deriving an equation for total work required for plastic deformation. Thomson [1955] used the energy method to derive expressions for energy dissipation due to plastic deformation, heating, and

inertial resistance of the target material. These methods are restricted to the situations of high-velocity impact in order to justify the assumption of constant projectile velocity during impact and deal primarily with perforation of thin targets rather than penetration into semi-infinite media.

Other investigators have sought development of a force law which could be used to determine a correlation of some penetration parameter involving velocity, v , by solving equations of motion. Allen *et al.* [1957] developed an empirical formulation of the resistive force undergone by a particle during impact. The model they used was:

$$F = C_1 + C_2 v + C_3 v^2 \quad (6.5)$$

The terms on the right-hand side of Equation (6.5) have been subsequently associated with the target's cohesive resistance, frictional effects, and the acceleration of target material near the projectile (added mass effect), respectively. Schulman and Lascher [1974] developed a force law for projectile penetration of a semi-infinite medium incorporating effects of target strength, equivalent normal pressure from fluid dynamics, friction due to the medium, and shear drag integrated over the striker surface.

Awerbuch and Bodner [1974] developed an analytical approach to projectile penetration by considering the mechanics of impact to be divided into two stages. This is shown in Figure 6.17.

Consider the impact of a particle with initial velocity, v_o , impacting a soft, ductile solid. Awerbuch and Bodner argued that in the first stage of the penetration process, only the inertial and compressive forces act on the projectile. The equation of motion for the first stage can, therefore, be written:

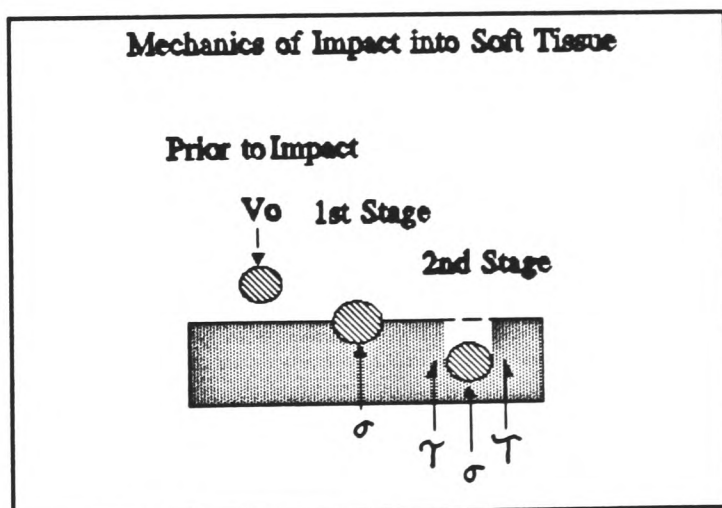


Figure 6.17 Physical mechanisms during particle impact.

$$\frac{d(mv)}{dt} = F_1 = -\frac{1}{2}K\rho A_1 v^2 - \sigma_c A_1 \quad (6.6)$$

where A_1 is projected area, σ_c is the ultimate compressive strength of the target, and K is a constant related to the nose shape. The second stage of particle impact, according to Awerbuch and Bodner, includes an added shearing force acting on the wetted surface.

$$\frac{d(mv)}{dt} = F_2 = F_1 - (\tau_o + \mu \frac{v}{e}) \pi d_p^2 / 2 \quad (6.7)$$

where τ_o is the target shear strength, μ is coefficient of viscosity, and e is the transition distance between projectile and undeformed target material. Although the analysis is of heuristic value, many of the physical parameters involved may not be readily available. A good review of the problem of impact analysis can be found in Backman and Goldsmith [1978].

Other researchers have employed dimensional analysis as a tool to provide physical guidance for the specification of relations which are otherwise empirical. Application of the *Buckingham Pi* theorem to reduce the numbers of physical parameters by consolidating them into dimensionless groups has been successful (Kline [1965], Hoppmann [1974]). Important dimensionless groups in impact problems have included the ratio of impact velocity to target sound velocity, or Mach number v_o/c_t , density ratio of projectile to target ρ_p/ρ_t , dimensionless yield strength $\sigma_{Yt}/\rho_t c_t^2$ (or $\sigma_{Yt}/\rho_t v_o^2$) and dimensionless depth of penetration P/d_p . The exponents of these dimensionless groups are arbitrary and are determined, together with a coefficient, empirically from test data.

A popular functional representation of normal penetration within a ductile, semi-infinite medium has the following form:

$$P/d_p = K_1 \left(\frac{\rho_p}{\rho_t} \right)^{c_4} \left(\frac{v_o}{c_t} \right)^{c_5} \left(\frac{\rho v_o^2}{\sigma_{Yt}} \right)^{c_6} \quad (6.8)$$

Typical values for constants shown in Equation (6.8) used by other researchers are shown in Table 6.3.

Table 6.3 Previous Constants for Correlation of Penetration Data, Equation (6.8)

Investigation	K_1	C_4	C_5	C_6
Charters and Summers [1973]	2.28	0.667	0.667	0
Bruce [1962]	1.96	0.5	0.667	0
Christman <i>et al.</i> [1963]	Arb.	0.333	0	0.333
Herrmann and Jones [1962]	Arb.	0.667	0	0.333

Note: *Arb.*: Arbitrary constant

Important here is the consistent manner in which dimensionless penetration depth varies with impact velocity:

$$\frac{P}{d_p} \sim (v_o)^{0.667} \tag{6.9}$$

This variation will be evaluated in the experiments described below.

6.3.3 Experimental Method

A series of experiments were conducted to evaluate depth of penetration of a projectile within a ductile, semi-infinite medium for a variety of system conditions. Measurements of particle penetration were carried out using targets consisting a 3% (w/v) cured agar solution. Agar has the advantage of being translucent when cured. This facilitated evaluation of particle penetration under an optical microscope. Penetration depth was estimated by slicing the target perpendicular to its surface and measuring particle position in the cross-sections using a light microscope. A Nuebauer scale enabled accurate resolution of particle depth ($\pm 5\mu\text{m}$). Care was taken to ensure that cross-sectional cuts did not affect the position of the particles within the targets. This was achieved by making transverse cuts from the *rear* of the agar targets to prevent driving particles deeper into the agar.

The agar solution was made up by mixing a given amount of agar powder (as measured on a weight/volume percentage basis) with distilled water. The solution was then boiled under pressure for several minutes to ensure thorough mixing, before being poured into target molds. The agar solution was then allowed to cool and harden overnight. Preliminary runs showed that a 3% agar solution (3 g/l) was optimum for penetration experiments. This was strong enough to hold together during a run while still allowing the particles to penetrate.

The penetration depth of dry tungsten particles in a target of 3% cured agar solution (3 g/l) was measured for varying upstream pressure, p_o , up to a maximum of 11bar. Initially, target chamber back pressure was maintained at atmospheric pressure (1bar abs.). These conditions represent variations in flow conditions at the nozzle exit plane (*i.e.*, variation from over-expansion to matched pressure conditions) and represent experimentally-characterised changes in particle velocity, as shown in Section 5.5.1). In the first series of experiments, the target was placed 75mm from the nozzle exit. This is a target distance for which particle velocities had been previously well-characterised.

6.3.4 Results

As shown in Figure 6.18, particle penetration depth in agar varies directly with the upstream total pressure, p_o . Although significant experimental scatter is apparent, the graph confirms that penetration depth increases as particle velocities increase. The particle velocity curve shown in Figure 6.18 is that determined experimentally in independent experiments described previously (see Section 5.5.1).

The actual dependence of non-dimensional penetration depth on particle velocity can be determined by plotting the natural logarithms of these parameters (P/d_p and v_p) against each other. This is seen in Figure 6.19, which consolidates all the particle penetration data. Note that much of the data clusters around coordinates (6,4). A linear regression curve fitted to the data gives:

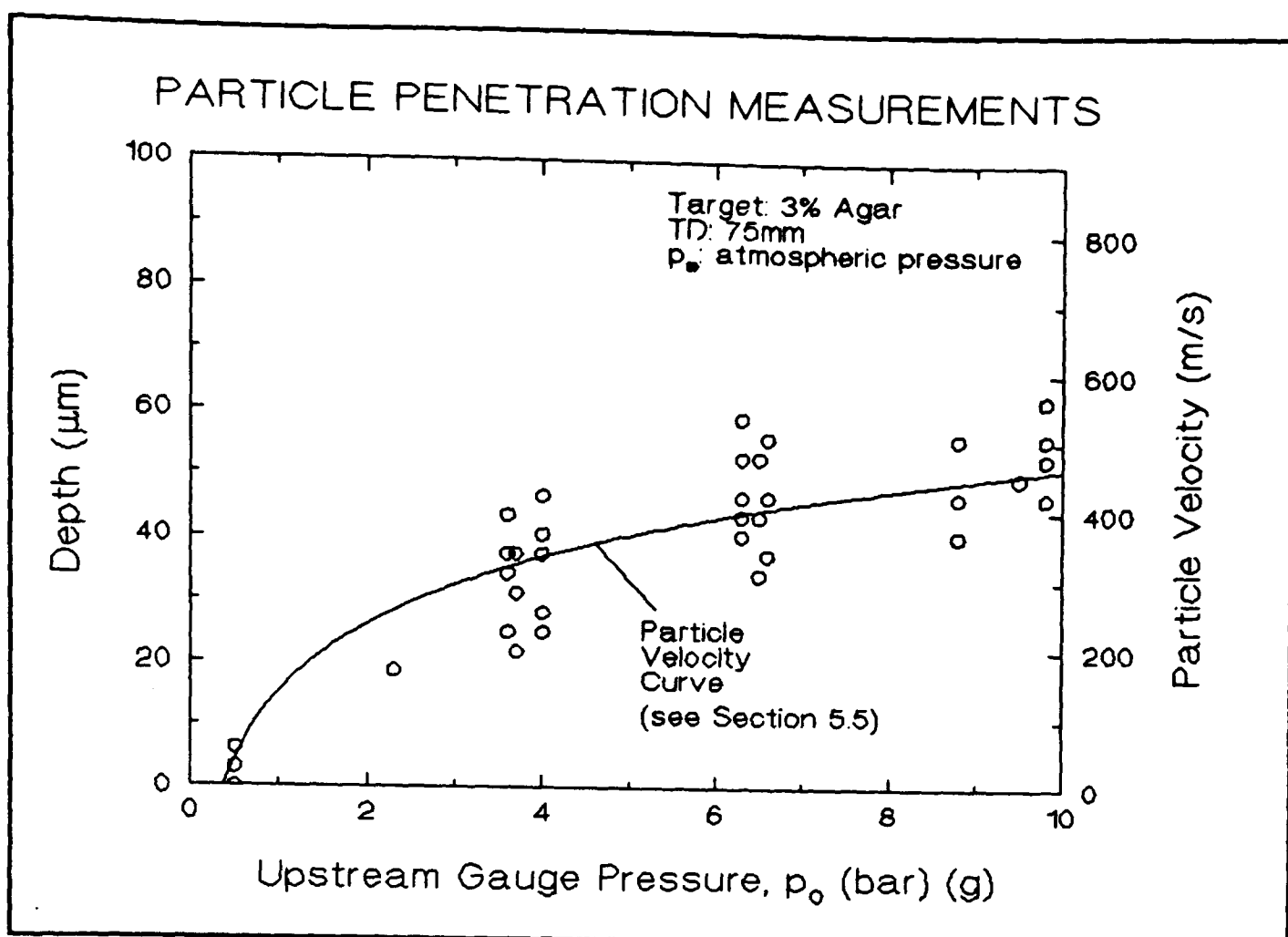


Figure 6.18 Variation of particle penetration with total upstream pressure.

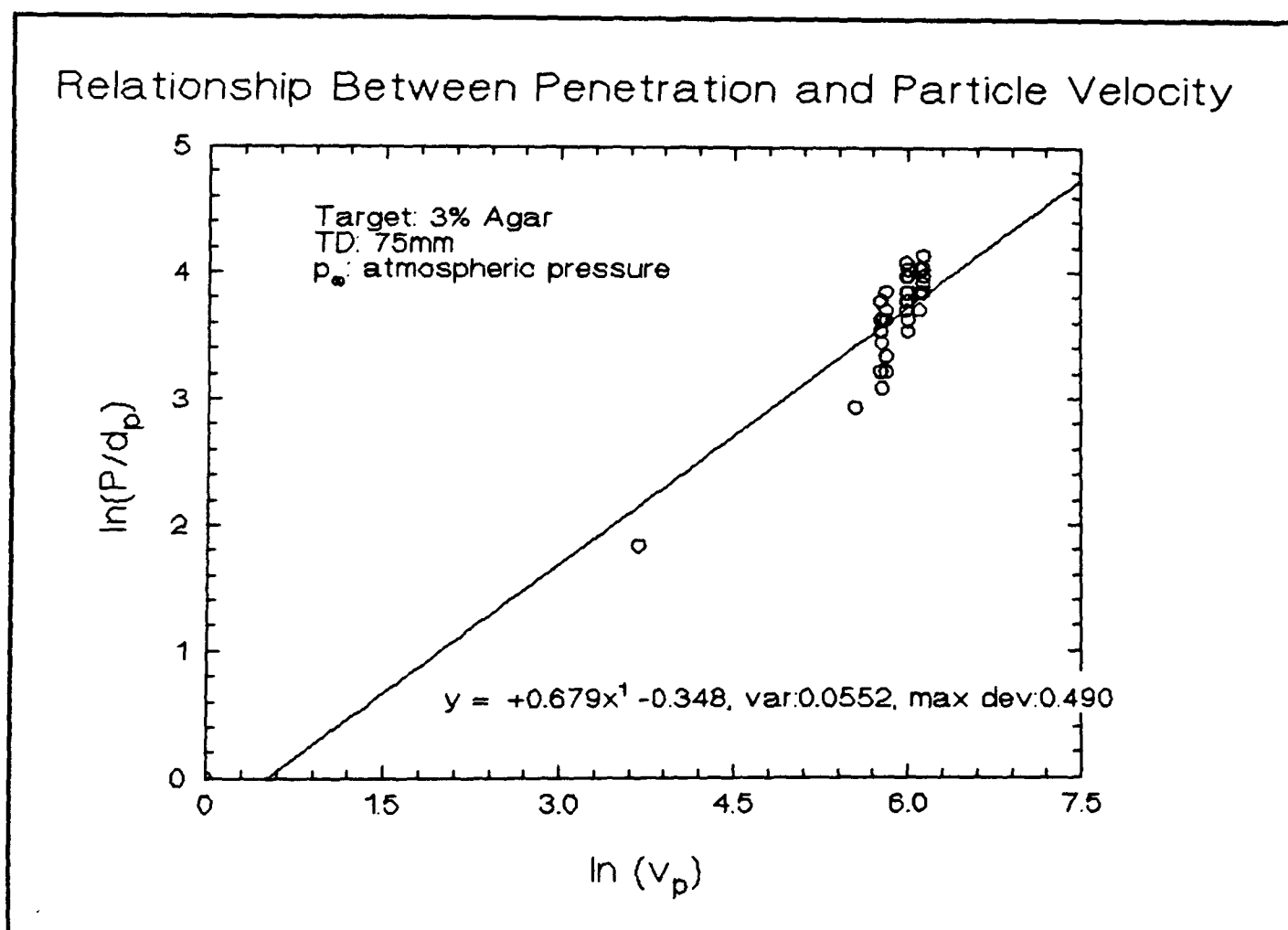


Figure 6.19 Logarithmic dependence of particle penetration on particle velocity.

$$\ln(P/d_p) = 0.679 \ln(v_p) \quad (6.10)$$

Therefore, these data suggest that non-dimensional particle penetration varies to the **0.679** power with particle velocity, not far off the **0.667** value seen in the empirical correlations of previous researchers.

Typical light microscope photographs of this penetration shown in Figure 6.20 further illustrate this variation. The specimen in **a)** represents the particle penetration depth for $p_o = 4.3\text{bar}$, while the specimen in **b)** represents the particle penetration depth for $p_o = 8.5\text{bar}$. It is apparent from these photographs that particles have penetrated deeper due to the higher upstream pressures and the corresponding higher particle velocities.

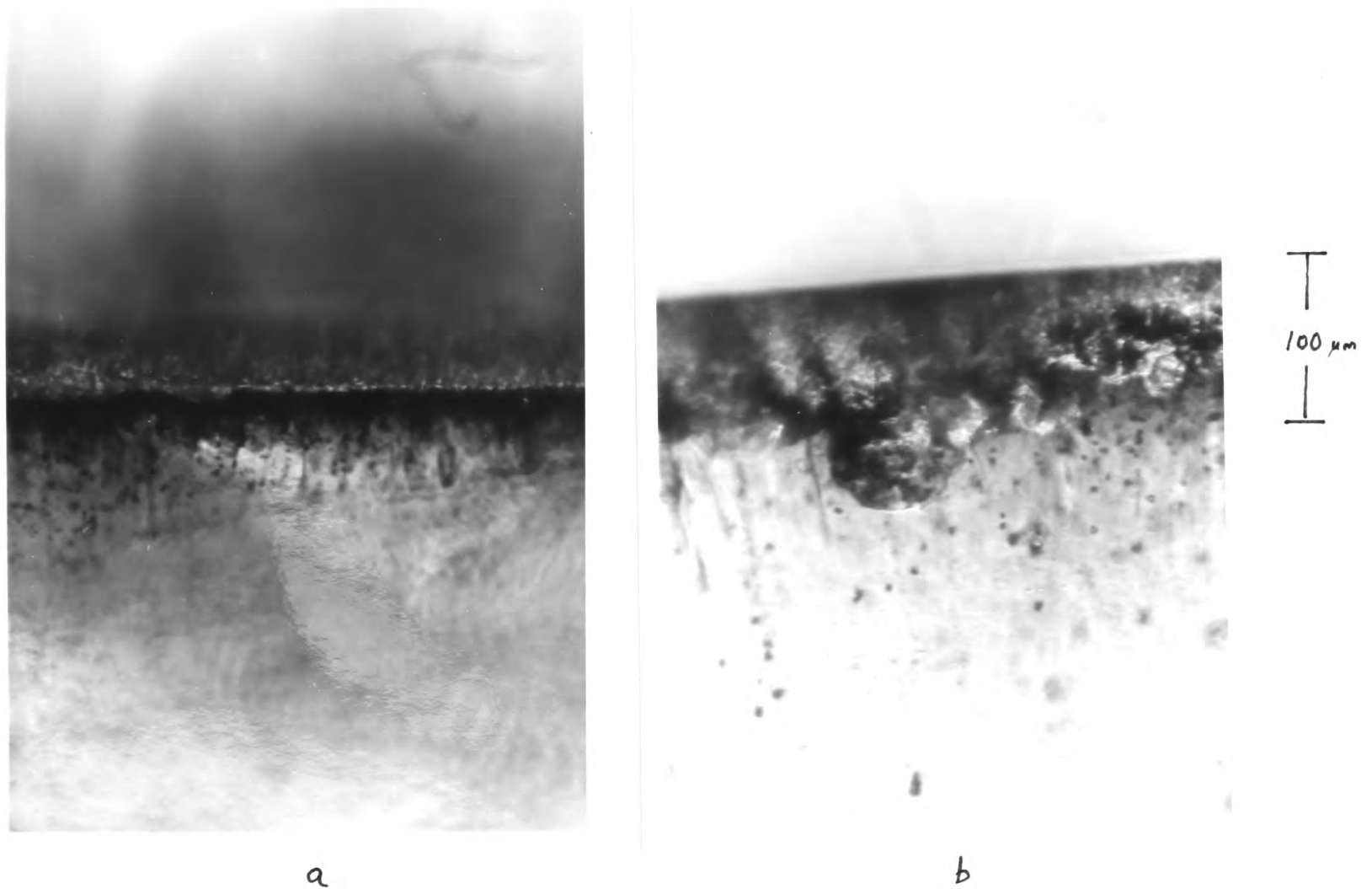


Figure 6.20 Light microscope photographs of particle penetration; **a.)** $p_o = 4.3\text{bar}$, **b.)** $p_o = 8.5\text{bar}$.

6.4 Conclusions

The purpose of this chapter has been to demonstrate the degree of controllability of the present system displays over particle spread (distribution) and particle penetration. This controllability is important if the development of non-target-dependent transformation technique is to be successful.

Particle distribution on the surface of parafilm targets has been evaluated using a novel optical procedure for a variety of target distances. This optical procedure utilises a frame-grabber to store an image of the bombarded target. Variation in grey-scale across the target surface corresponding to the presence of tungsten particles is determined and the results plotted. The results show that particle distribution becomes broader (*i.e.*, maximum levels at the target centre decrease and distribution becomes more uniform) as target distance increases. High speed video photography of the particle-laden jet as it emerges from the nozzle has been utilised to evaluate jet spread as a function of both time and nozzle pressure ratio, p_o/p_∞ . Maximum spread of a particle-laden jet, as visualised using chalk particles, has been shown to increase as p_o/p_∞ increases, as theory holds. It has been speculated that the transient nature of the jet development is related to temporal pressure variation at the nozzle exit associated with shock passage and the propagation of an expansion wave front. Viscous mixing as a mechanism for jet entrainment has been seen to be a prime factor for the finding that maximum spread of jet-particle boundaries exceeds predictions. Scanning electron micrography (SEM) has been used to demonstrate that the agglomeration of particles into large clumps is not a problem for the present system.

Control of the depth of penetration of tungsten particles has been demonstrated, ^{to a limited extent} through control of the total upstream pressure and, therefore, particle velocities. The variation in dimensionless penetration depth with impact velocity has been determined for bombardment of a cured agar solution. The functional relationship between particle penetration depth and impact velocity determined here shows good agreement with previously-determined empirical models.

The controllability over particle trajectories demonstrated here will be utilised in biological experimentation in **Chapter 7**. Important here will be the

determination of the ease with which the present system can be varied for various biological targets.

CHAPTER 7. BIOLOGICAL APPLICATIONS

7.1 Introduction

Bombardment of intact cells and tissue with DNA-coated microprojectiles (Klein [1987]) represents a novel approach to the genetic transformation of plants and avoids inherent disadvantages of methods utilising protoplasts (Cocking *et al.* [1987]) or *agrobacterium* (Klee *et al.* [1987]). Workers have reported success in obtaining transient genetic expression (TGE) through bombardment of various tissue types of maize (Klein *et al.* [1988], Klein *et al.* [1989], Gordon-Kamm *et al.* [1990]), yeast (Johnston *et al.* [1988]), *chlamydomona* chloroplast (Boyton *et al.* [1988]), rice (McElroy *et al.* [1990]), and barley (Kantha *et al.* [1989]).

Each of the cases cited above has involved the use of a biological-ballistic (or "Biolistic")-type particle gun. This direct-delivery approach involves the acceleration of microprojectiles which are seated on the face of a macroprojectile, which, in turn has been accelerated by a shock wave travelling through a gas. The most common method of achieving this shock wave thus far is through use of a chemical explosion (gunpowder charge) (Sanford *et al.* [1987]). This approach involves firing a nylon macroprojectile ("bullet") with a .22 cartridge. The bullet strikes a plastic stopping plate, with which it fuses, stopping its motion. DNA-coated tungsten particles placed at the bullet's tip proceed through a small hole in the centre of the stopping plate toward the target tissue. Other researchers have used an electric explosion of a water droplet (Christou *et al.* [1990], McCabe *et al.* [1988]), a discharge of compressed air (Oard *et al.* [1990], Morikawa *et al.* [1989]) or helium bursting through a ruptured membrane (Russell *et al.* [1991]) to generate this shock wave and, thereby, propel the macroprojectile.

The previous work in the area has concentrated almost exclusively on use of biological techniques for the determination of transient or stable transformation. Transient gene expression implies that DNA-coated tungsten particles have entered a number of the target cells, and that a marker gene from the DNA plasmid has diffused into the cells' nuclei. Use of the GUS-encoding

marker gene (Jefferson [1987]) has predominated among workers in the field. Meanwhile, little work has been done in determining the influence of physical parameters on such system physical characteristics as particle velocity and particle spread. These, presumably, influence to a large degree physical system performance, as measured by particle target distribution and particle target penetration. Finally, this *physical* system performance must have a great influence on the *biological* system performance, as determined by levels of genetic transformation.

The purpose of this chapter is the discussion of experimental results from studies of transient gene expression for various physical system parameters using the gas-propulsion system developed here. A primary objective has been the optimisation of system parameters to achieve maximum TGE for bombardment of a particular cell system, namely maize suspension cells. Other experiments have focused on evaluating the system for penetration depth using maize roots and maize coleoptiles as test systems.

Most of the biological experiments with maize suspension cells discussed herein were conducted in collaboration with Camilla Beech of ICI Seeds, Jealott's Hill, Berkshire. The author is indebted to her for her assistance with bombardments and her patient instruction in appropriate biochemistry techniques.

7.2 Bombardment of Maize Suspension Cells

7.2.1 Materials and Methods

Description of Apparatus

The system under consideration has been fully described in **Chapter 2**. Only a brief description is included here. Figure 7.1 shows schematically the design of the current gas-propulsion system. Primary components of the system include a compressed gas cylinder, diaphragm cassette, convergent-divergent supersonic nozzle, and target chamber. To operate the system, the biological material being genetically transformed (*e.g.*, suspension cells, leaf or root tissue)

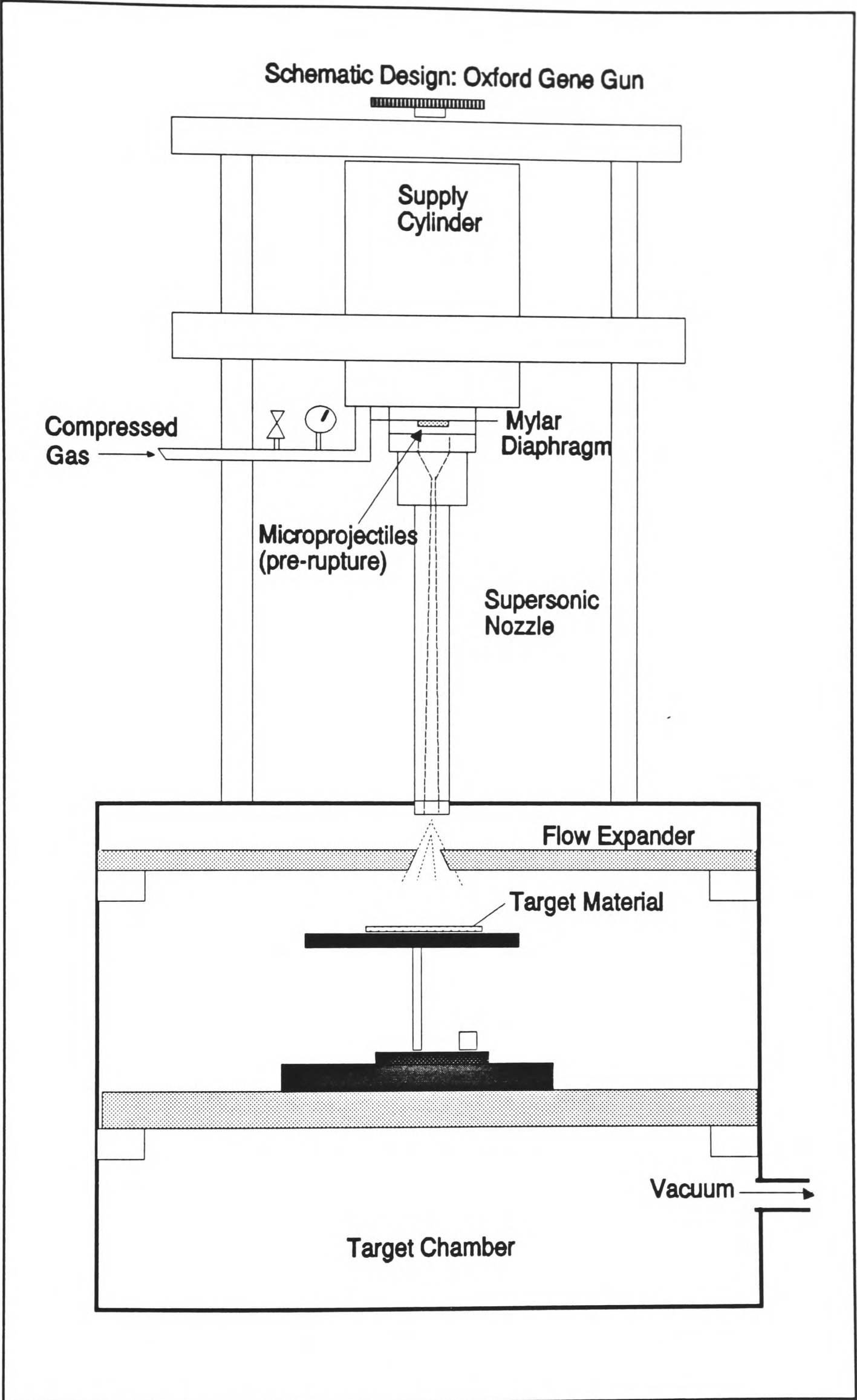


Figure 7.1 Schematic diagram of gas propulsion particle gun.

is placed on one of several shelves in the target chamber. Micron-sized tungsten particles coated with DNA (coating technique described below) are placed on the sterile mylar diaphragm within the diaphragm cassette, and the cassette is slotted into the apparatus. A high-pressure source then pressurises the upstream supply cylinder. A vacuum pump simultaneously evacuates the target chamber (if necessary). The thickness of the diaphragm determines the pressure at which the mylar ruptures. Rupture of the diaphragm causes the propagation of a shock wave downstream through the nozzle and immediate expansion of compressed gas flow behind it. Transient gas flow associated with shock wave propagation accelerates the tungsten particles into the target chamber toward the target tissue. A flow expander is used to diffuse the air flow which follows the particles once steady flow is established.

Particle Coating Methods

Prior to coating and bombardment, tungsten particles (M-17, GTE/Sylvania, Towanda, PA, USA; unless otherwise specified) were sterilised and placed in suspension in the following manner:

- a) A 50 mg sample of $1.048 \mu\text{m}^*$ (median diameter) tungsten microprojectiles (M-17, GTE/Sylvania, Towanda, PA, USA) is weighed into 1.5-ml sterile Eppendorf tube and then sterilized in 1 ml 100% pure ethanol.
- b) In order to disrupt particle aggregates, suspension is sonicated thoroughly by touching the outside of the Eppendorf tube briefly (1 sec) to the probe (horn type) of a sonicator.
- c) Tungsten particles are centrifuged out (and supernatant removed).
- d) Particles are re-suspended in 1 ml sterile distilled water. Tungsten particles are centrifuged out again.
- e) Step d) is repeated twice more. Tungsten particles are then stored in 1 ml sterile dH_2O . Suspension can be stored for up to a week.

Coating of the particles with DNA was done on the morning of bombardment experiments, using the following protocol after Gordon-Kamm [1990]:

a) Plasmid DNA is adsorbed to the tungsten microprojectiles by adding 20 μ l of DNA (1 mg/ml) to 40 μ l of the suspension of tungsten particles described above in a 1.5-ml Eppendorf tube. Suspension is then finger-vortexed (flicked) to ensure mixing.

b) The following are then added in the order shown, finger vortexing after each addition:

253 μ l of 2.5 M CaCl_2

50 μ l of 0.10 M Spermidine (stored frozen)

207 μ l sterile distilled H_2O

c) Vortex for 10 min. at 4°C (in a cold room).

d) Centrifuge DNA/tungsten out at 500g for 5 minutes.

e) Remove 545 μ l of liquid supernatant, leaving 25 μ l in Eppendorf tube for bombardments. This is sufficient for 5 "shots" of 5 μ l each. If more shots are required for that session, this procedure can be repeated in other Eppendorf tubes.

The above coating protocol produces a tungsten/DNA preparation that forms a "droplet" when placed on the mylar diaphragm prior to bombardment. Other researchers (Sanford [1990]) have found that a "dry" particle preparation is useful. This calls for suspending the DNA-coated tungsten particles in ethanol, placing the preparation on the mylar diaphragm, and then allowing the ethanol to dry off (approximately one minute) before bombardment. This protocol is shown below:

a) Particles are sterilized and suspended in distilled water weekly as described above.

b) Follow steps a) - d) above for DNA coating of tungsten particles.

c) Remove **all** supernatant (without removing tungsten).

d) Add 100 μ l of 70% ethanol.

e) Centrifuge out tungsten, remove all supernatant, and resuspend in 30 μ l of 100% pure ethanol.

This method differs from the droplet method above in that after the tungsten/plasmid suspension is placed on the bursting mylar diaphragm, the ethanol solvent is allowed to dry. This seems to prevent large particle agglomerations from forming.

Techniques for Cell Immobilisation

Initial attempts to deliver GUS-encoding plasmid DNA into intact maize cells involved bombardment of suspension cells pulled down only onto filter paper. Therefore, the cells were bombarded without being anchored to the filter paper surface. It soon became apparent that aerodynamic forces related to total pressures within the jet were sufficiently high to detach a large proportion of cells from the filter paper surface. This cell "blow away" problem became a major obstacle to obtaining high levels of transient expression. Considerable time was devoted to finding viable solutions. In the end a biological solution was found to be most successful in retaining target cells onto the filter paper.

This biological technique involved the use of alginate. The author wishes to thank Dr. Theodore Goosen of Queen's University, Ontario for his advice in this area. Alginate is a common organic adhesive which "sets" (hardens) when it comes into contact with calcium. The technique developed here utilised calcium chloride as the setting agent.

Using alginate, the suspension cells were prepared for bombardment in the following manner:

1. Sub-cultured suspension cells (no more than four days since sub-culture) sieved with 500 μm (autoclaved) sieve to remove cell clumps.
2. 5.0 ml packed cell volume (PCV) of LO56 18/B maize cells are taken up in 50ml conditioned medium.
3. Cells mixed with 50ml of a 2.6% (2.6g/100ml) alginate stock at room temperature (Sigma, low viscosity, alginic acid in LO56 medium minus Ca^{2+} , made freshly each week) to give a final concentration of 1.3% alginate.
4. 5.0ml of the cell/alginate mixture is pipetted onto sterile 4.7cm Whatman No. 1 filter paper disc placed on a sterile Buchner funnel/vacuum flask assembly, operated under gentle vacuum. When the majority of the liquid has been pulled through, the vacuum is released.
5. The alginate is "set" by floating firstly the base of the filter disc in 50mM CaCl_2 and then the upper surface containing the cells.

6. Prepared discs are stored on three (3) 4.7cm Whatman No. 1 filter discs, moistened with LO56 liquid media containing additional CaCl_2 (approx. 50mM) in a 9.0cm Petri dish.

An aerodynamic approach to the solution of the cell "blow away" problem was also found to be useful. This entailed the development of a *flow expander* to further expand the free jet flow. The author is indebted to Prof. T. Jones of the Osney Laboratory, Oxford, for the original idea. A more complete description of the approach can be found in **Chapter 2**.

7.2.2 Bombardments

Suspension cells being bombarded were placed on three wetted filter papers. This was found to be beneficial in absorbing much of the shock associated with bombardment. These filter papers were situated on a petri dish lid placed in a purpose-made groove on the target stage in the target chamber. The petri dish lid was oriented "lip down" to alleviate any secondary aerodynamic effects due to flow around the lip. Unless stated otherwise below, standard bombardment conditions were utilised:

$$p_o = 2.7\text{bar}$$

$$p_\infty = 0.1\text{bar}$$

$$\text{Target Distance (TD)} = 160\text{mm}$$

$$\text{Flow Expander In (at 40mm)}$$

7.2.3 Analysis of GUS Expression in Suspension Cells

Histochemical

Subsequent to bombardment, the suspension cells were transferred to solid media and incubated at 27°C. After 48 hours the cells were harvested and prepared for histochemical staining. This staining technique was utilised to detect those cells transiently-expressing the GUS marker gene. A synthetic substrate 5-bromo-4-chloro-3-indoyl-B-glucuronic acid ("X-Gluc", Sigma) was used to detect GUS expression. The substrate mixture contained potassium ferricyanide (5mM), potassium ferrocyanide (5mM), the GUS substrate (0.3%, w/v), sodium phosphate buffer (0.1 M, pH 7.0), Triton x-100 (0.06 % v/v), and DMSO (0.05 % v/v). The substrate mixture (1ml) was pipetted onto the bombarded cells in each petri dish and the dishes resealed with parafilm and incubated in a chamber at 37°C for 24 hours. The samples were then analysed microscopically for cells that had developed the blue precipitate (Jefferson [1987]). Each blue spot (whether a single, isolated cell or an aggregate of cells) was counted as one colour forming unit or "CFU". The variation in transient expression between conditions was determined by evaluation of the number of CFUs for each condition.

7.3 Results and Discussion : Plants

7.3.1 Cell Viability Under Vacuum

An experiment was conducted to determine cell viability under various levels of vacuum for various lengths of time. The purpose of this experiment was to ascertain whether certain vacuums to be used later during bombardments would be detrimental to the suspension cells.

Vacuum level in the target chamber was varied from 0.25bar (570 mm Hg) to 0.05bar (722 mm Hg), and vacuum duration was varied from 62s to 259s. Samples not placed under vacuum were used as controls. In each case a sample was placed in the target chamber, the door sealed, and the chamber evacuated using the vacuum dump tank to speed the vacuum process (minimum 45s). The vacuum was held for a certain length of time before atmospheric pressure was restored in the target chamber. The samples were then incubated at 24°C for one week. The samples were then analysed visually for signs of cell death (necrosis) as displayed by cell browning.

The results showed that for even the maximum vacuum (0.05 bar) and for the longest duration (259s), levels of necrosis were only slightly greater than those seen in the controls. Significant cell growth was apparent in all samples. Based on these results, therefore, this degree or duration of vacuum should not be limiting factors for the bombardment technique.

7.3.2 Coating Methods: "Droplet" vs. "Dry"

The method by which the tungsten particles are coated with plasmid DNA has been shown by other workers (Klein *et al.* [1988]) to be an important factor in transient expression. The purpose of this experiment was the determination of the particular coating technique - "droplet" or "dry" - which optimised the delivery of the GUS-encoding marker gene into maize suspension cells using the gene-delivery system described here. In both cases the volume of the microprojectile-

DNA preparation used to bombard the cells was kept constant at 5 μ l. "Dry" particles were prepared for bombardment by spreading the ethanol/tungsten/DNA droplet with a Gilson tip onto the mylar in the diaphragm cassette. The ethanol was then allowed to evaporate (approximately one minute), leaving dry DNA-coated tungsten particles loosely adhering to the mylar. The particle preparation being used in the "droplet" technique was sonicated immediately prior to the droplet being placed on the mylar in order to disrupt any particle aggregates. Each coating method was evaluated at nominal target distances of 80mm and 160mm.

The results are presented graphically in Figure 7.2. As noted previously, transient gene expression (TGE) is measured by visually counting with the aid of an optical microscope the numbers of cells expressing the GUS gene. Expressing cells stain a deep blue colour several hours after application of a histochemical assay, and these expressing cells (known as colour forming units or CFUs) are counted to determine the level of TGE. As shown in Figure 7.2, levels of transient gene expression for the "dry" tungsten method in this experiment were almost an order of magnitude higher than those for the "droplet" method at a target distance of 160mm. At 80mm levels of transient expression for the "dry" method were twice those for the "droplet" method. One possible explanation for this difference between the coating methods is that the dry method does not allow the formation of particle aggregates as readily as does the droplet method. Even with sonication of the droplet preparation, aggregates form quickly in the droplet as it sits on the mylar prior to bombardment. Unless noted otherwise, the "dry" coating technique was used in all subsequent experiments.

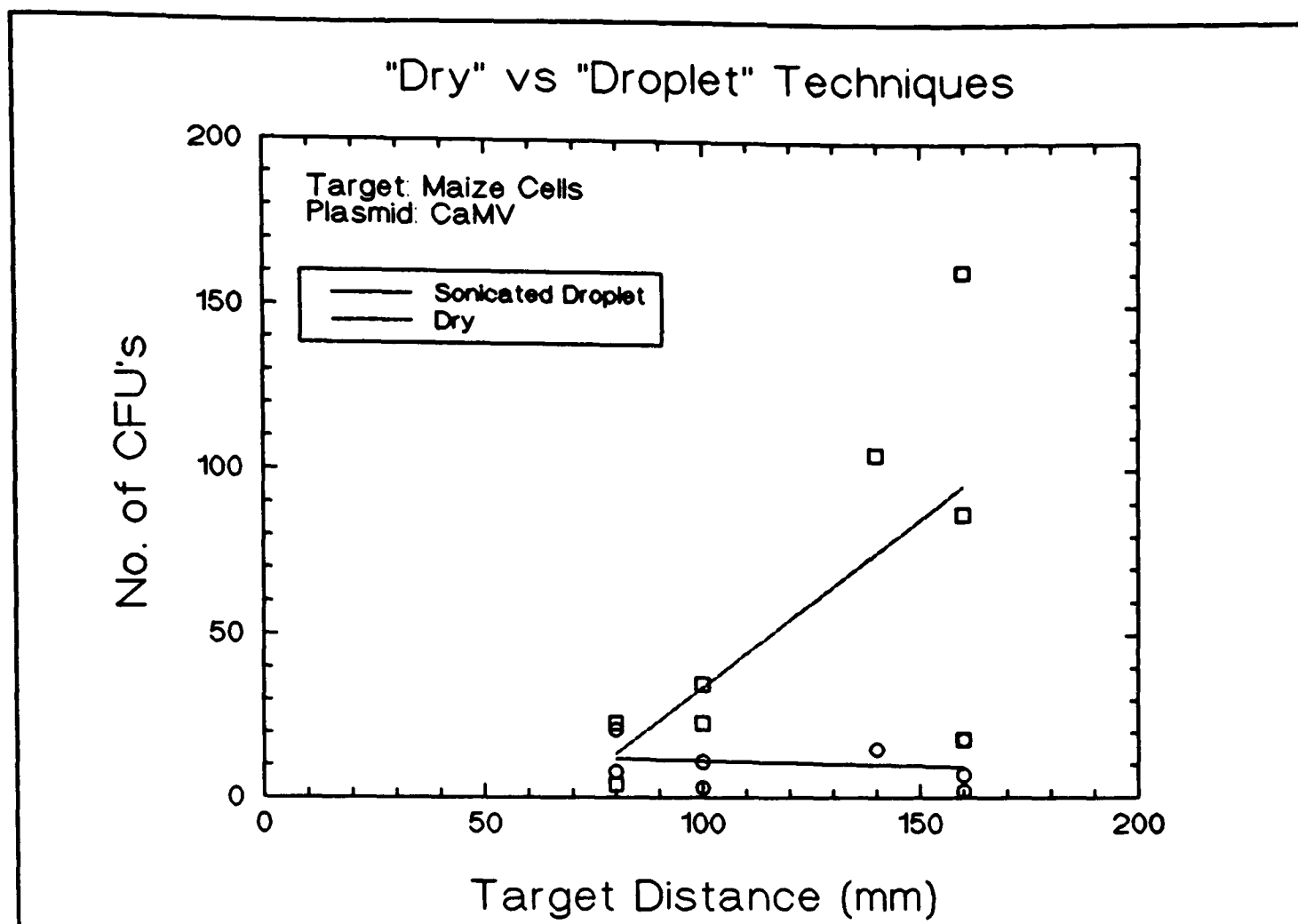


Figure 7.2 Comparison of TGE for "Dry" and "Droplet" coating techniques.

7.3.3 Effect of Microprojectile Volume

An experiment was conducted to determine the optimum volume of DNA-microprojectile preparation for each bombardment, using as a criterion levels of transient GUS-expression. Volumes of DNA-microprojectile preparation were varied between 2.5 and 5 μ l (preliminary experiments had shown that larger volumes caused increased cell death and that smaller volumes were ineffective for GUS expression). Appropriate volumes of tungsten/ethanol suspension were placed onto the mylar diaphragm using a pipette, and the ethanol was allowed to dry. Figure 7.3 shows the results of bombardments on fresh maize suspension cells using standard bombardment conditions.

Although significant run-to-run variation is apparent in the results, as shown by the large standard deviations, the graph shows that a volume of 2.5 μ l is ^{the best recorded} optimum for these conditions. A partial explanation for the substantial scatter

in the data is shown in Figure 7.4, which breaks down the data of Figure 7.3 based on DNA-microprojectile preparation tube. For ease of handling in the experiment two tubes of the same DNA-tungsten preparation were made up as described in **Section 7.2**. These are shown as tubes A and B. Figure 7.4 displays the large tube-to-tube variation that occurred. Although it is still apparent that 2.5 μ l is the optimum volume, Figure 7.4 shows the large variations that can occur between preparation tube for similar bombardment conditions. This variation is difficult to control and accounts for much of the run-to-run variation that has been seen. Unless otherwise noted, a DNA-microprojectile volume of 2.5 μ l was utilised throughout all ensuing experiments.

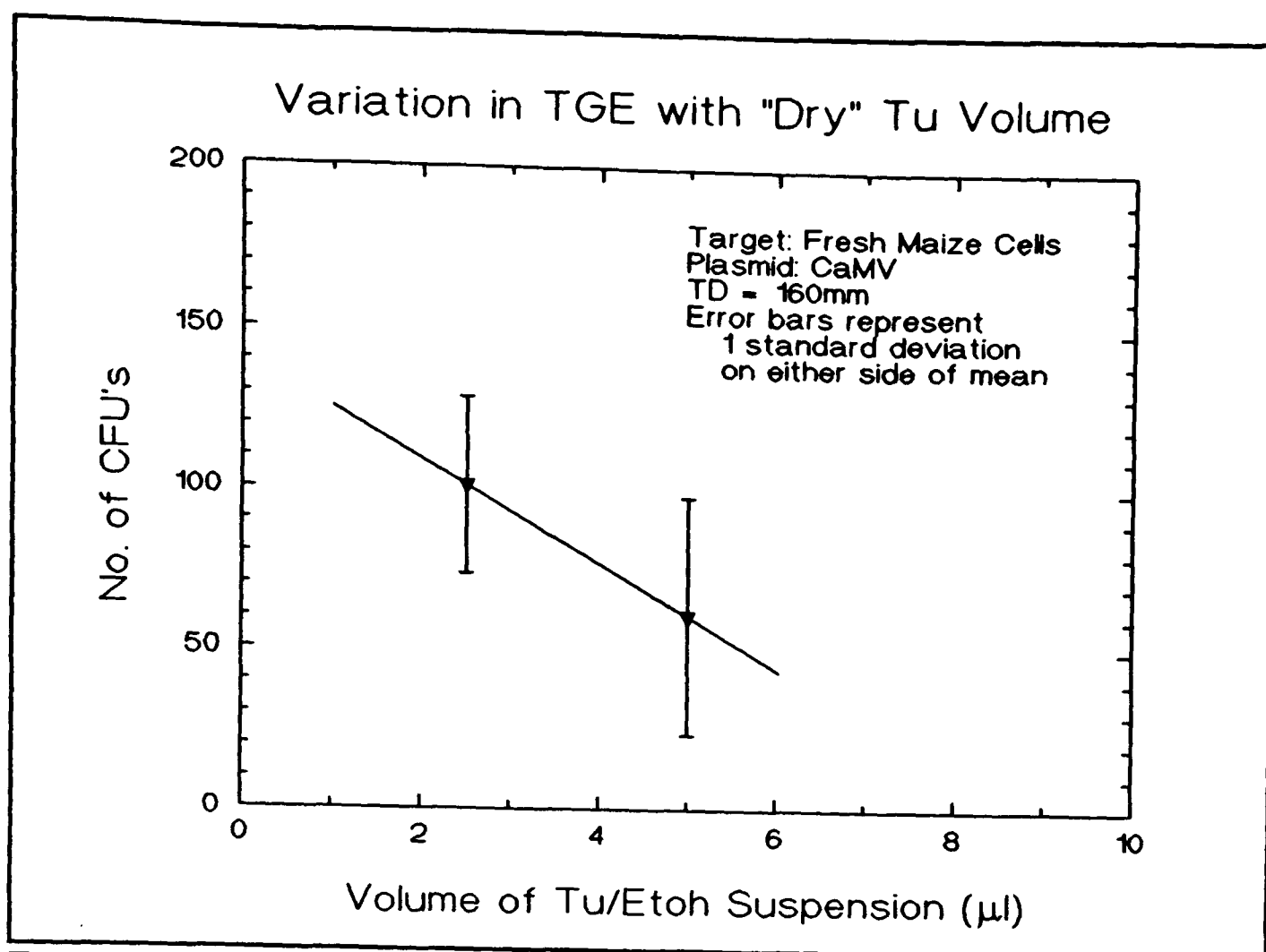


Figure 7.3 Variation in TGE with volume of tungsten/ethanol suspension.

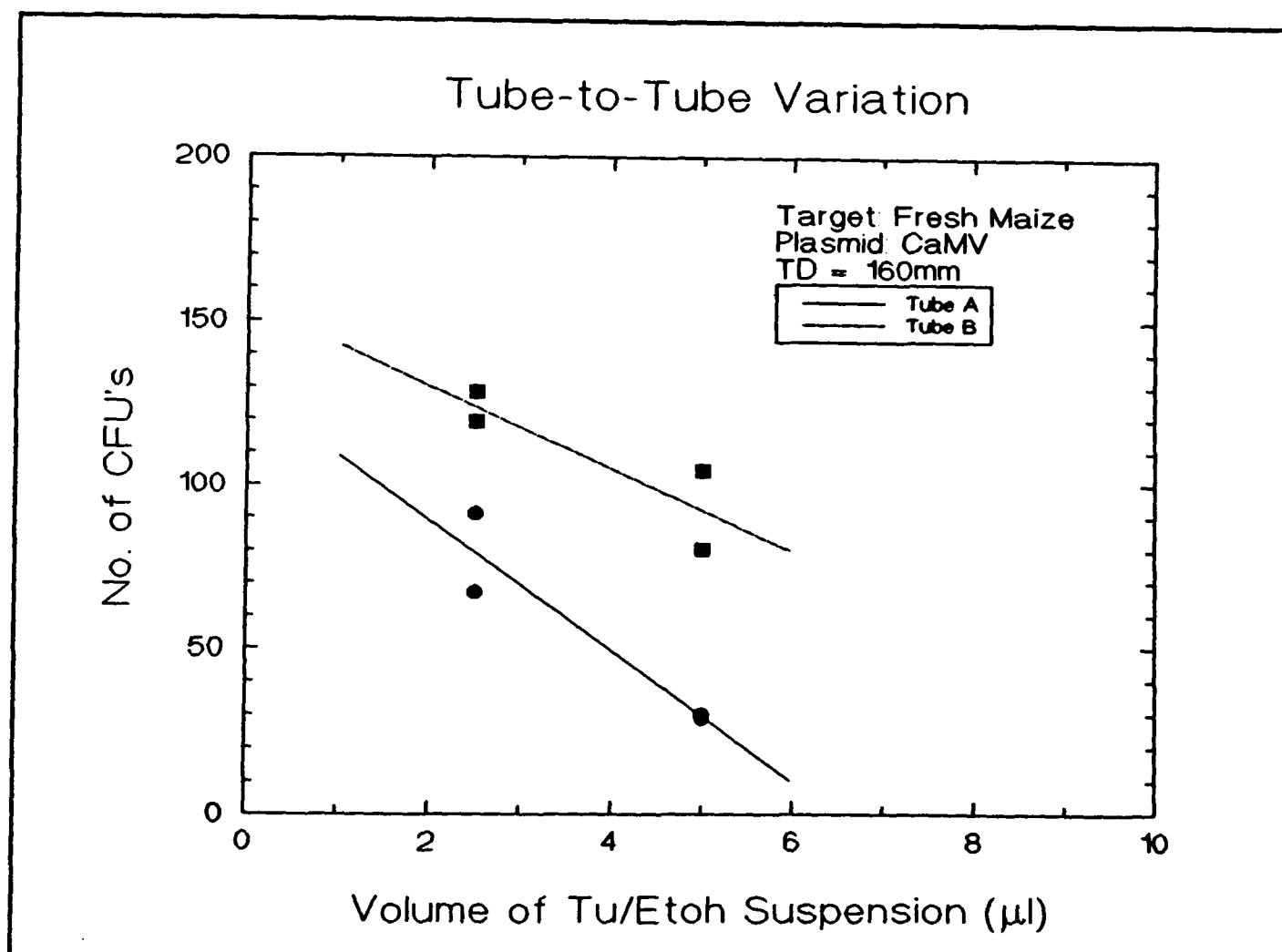


Figure 7.4 Tube-to-tube variation in DNA precipitation.

7.3.4 Effect of Plasmid Promoter

Several experiments were conducted to evaluate the effect of varying plasmid promoter on levels of transient GUS-expression. The promoter sits ahead of the gene construct on the plasmid and causes activation of gene expression. Because it acts as a catalyst for gene expression, the plasmid promoter can have a large effect on measurements of effectiveness of gene delivery. The first experiment compared two promoters: pCaMVI₁GUS, the plasmid used in the earliest experiments, and pWI₁₁GUS, a wheat dwarf viral promoter. Standard bombardment conditions were used. The particles were coated using the "dry" method. The DNA-microprojectile volume was kept constant at 2.5 µl. Half the samples were stained with the X-Gluc substrate after 48 hours incubation. The other half were stained after a total of 120 hours incubation to determine the variation in the level of GUS-expression with time.

The results, which are presented in Figure 7.5, show the importance of plasmid type on GUS-expression. For four replicates each, the level of GUS-expression for the plasmid with the wheat dwarf viral promoter, pWI₁₁GUS, averaged almost 12 times higher than those levels recorded with pCaMVI₁GUS when histochemically assayed 48 hours post-bombardment (350.25 ± 70.8 vs. 29.25 ± 20.7). When assayed 120 hours post-bombardment the relative superiority of the wheat promoter over the previous promoter was even more significant (30 times higher; 93.50 ± 53.2 vs. 3.0 ± 4.7), although the absolute levels of expression were not as great.

A second experiment was conducted to evaluate transient GUS-expression for three plasmid promoters: pCaMVI₁GUS, pWI₁₁GUS, and pAdhI₁GUS, a GUS-encoding plasmid using an alcohol dehydrogenase promoter. This experiment again used the histochemical staining technique to measure levels of GUS expression. The results are presented in Figure 7.6 and Figure 7.7 and Tables 7.1 and 7.2. "CFUs" refers to the number of blue cells counted visually under a light microscope. Based on the number of CFUs counted, it is apparent that Adh and WI promoters are superior for this particular line of maize

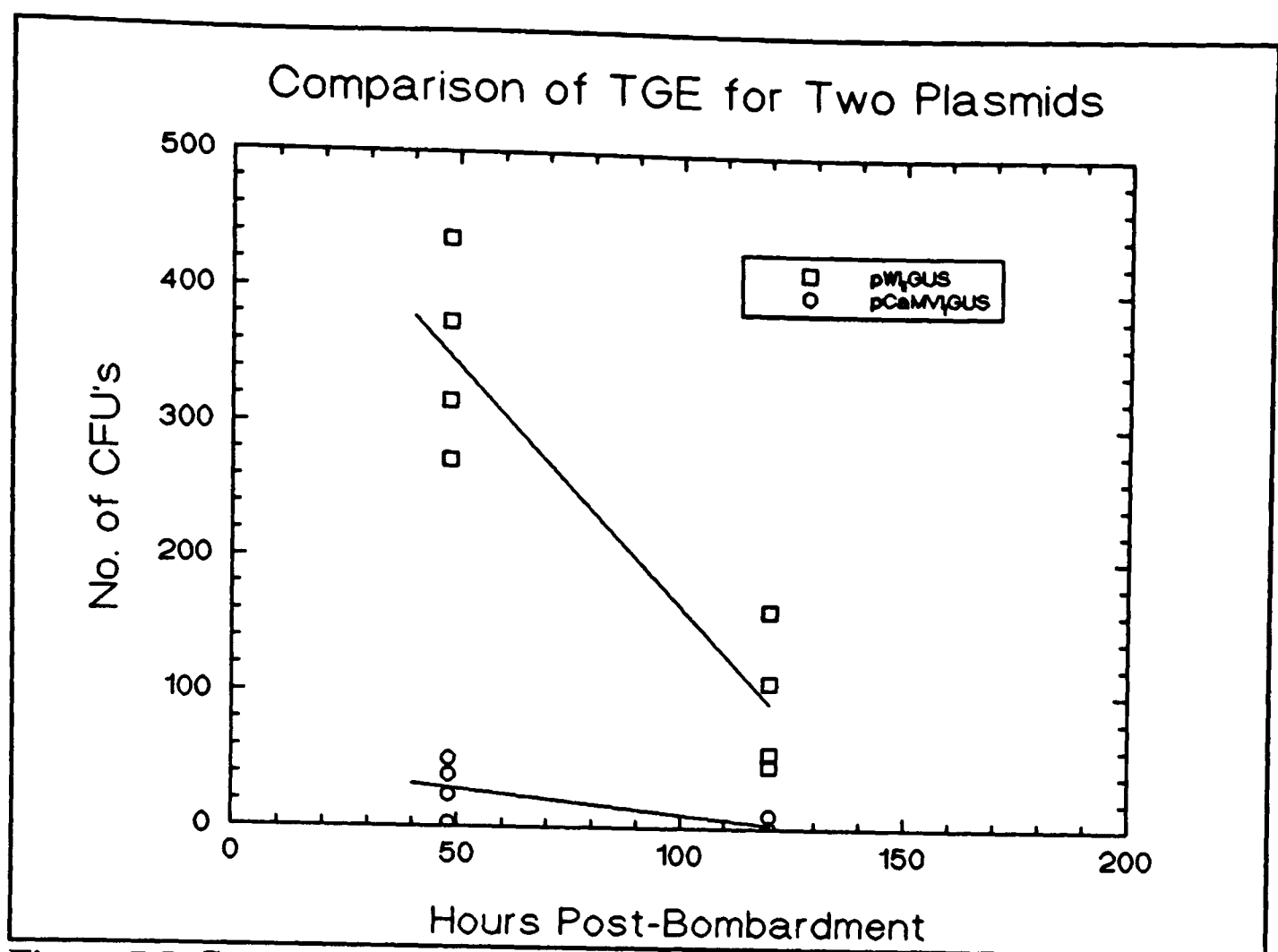


Figure 7.5 Comparison of TGE for two plasmids over 5-day incubation period.

suspension cells. Especially in Table 7.2, the number of CFUs (counted on one-half of the normal filter paper) were among the highest levels of transient genetic expression achieved for the transformation of maize cells with the gas propulsion particle gun. In fact, the WI plasmid had an average of 450 CFUs (over half a filter paper), inferring that full filter papers would have averaged approximately 900. One sample, experiment No 16, showed 613 blue spots over one-half a filter paper (or, presumably, 1226 for a full filter paper). A photograph in Figure 7.8 of a sample specimen, taken with the aid of a light microscope, shows the CFU distribution.

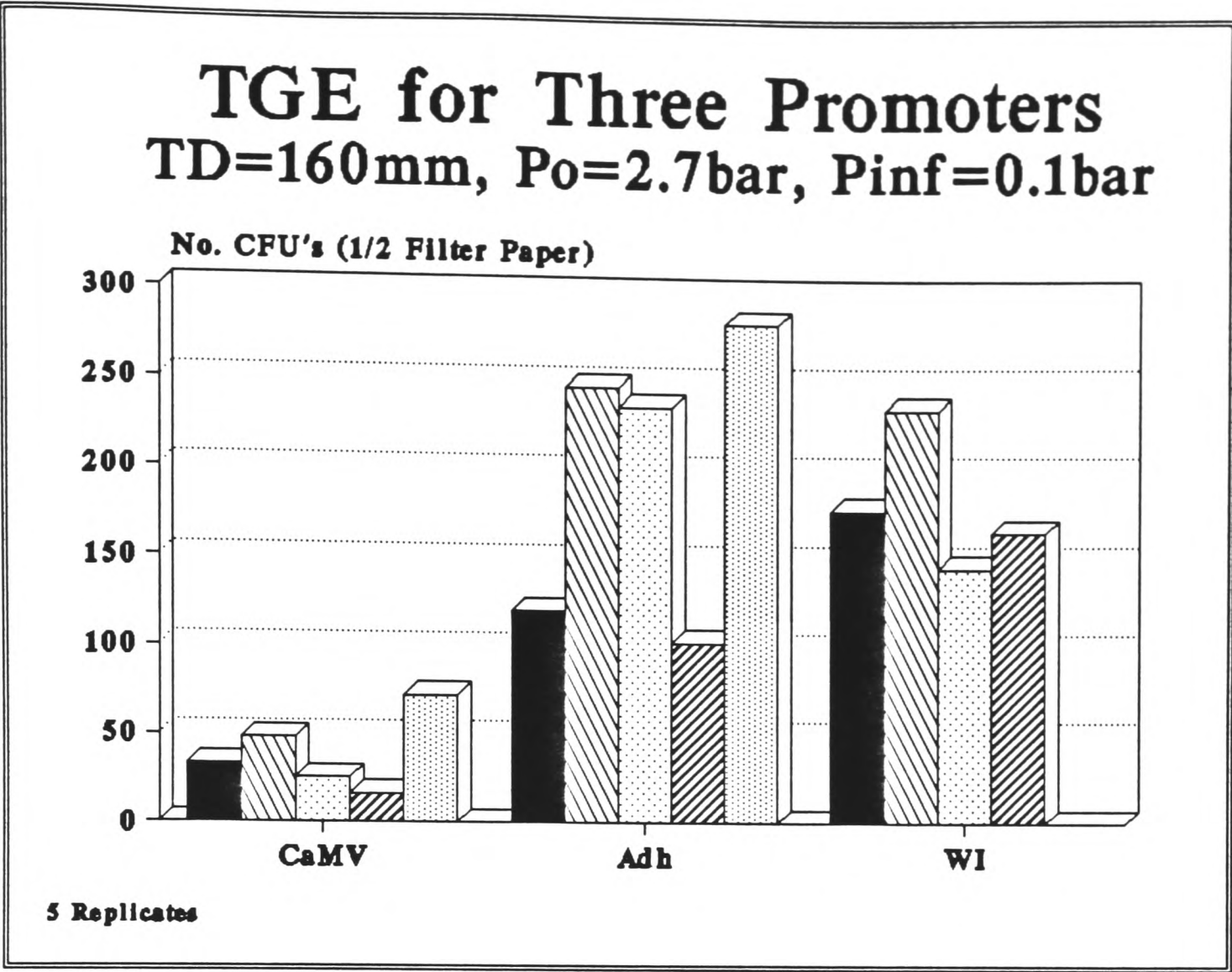


Figure 7.6 TGE for three promoters.

Table 7.1 Comparison of TGE results for Three Plasmid Promoters

No.	Promoter	TGE, Histochemical (No. CFUs)
1	-ve Control	0
2	-ve Control	0
3	-ve Control	0
4	CaMV	33
5	CaMV	48
6	CaMV	25
7	CaMV	16
8	CaMV	72
9	Adh	120
10	Adh	246
11	Adh	235
12	Adh	102
13	Adh	282
14	WI	177
15	WI	234
16	WI	145
17	WI	165
18	WI	52*
19	-ve Control	3
20	-ve Control	0

* - Target off-center

TGE for 3 Promoters (Repeat)

TD=160mm, Po=2.7bar, Pinf=0.1bar

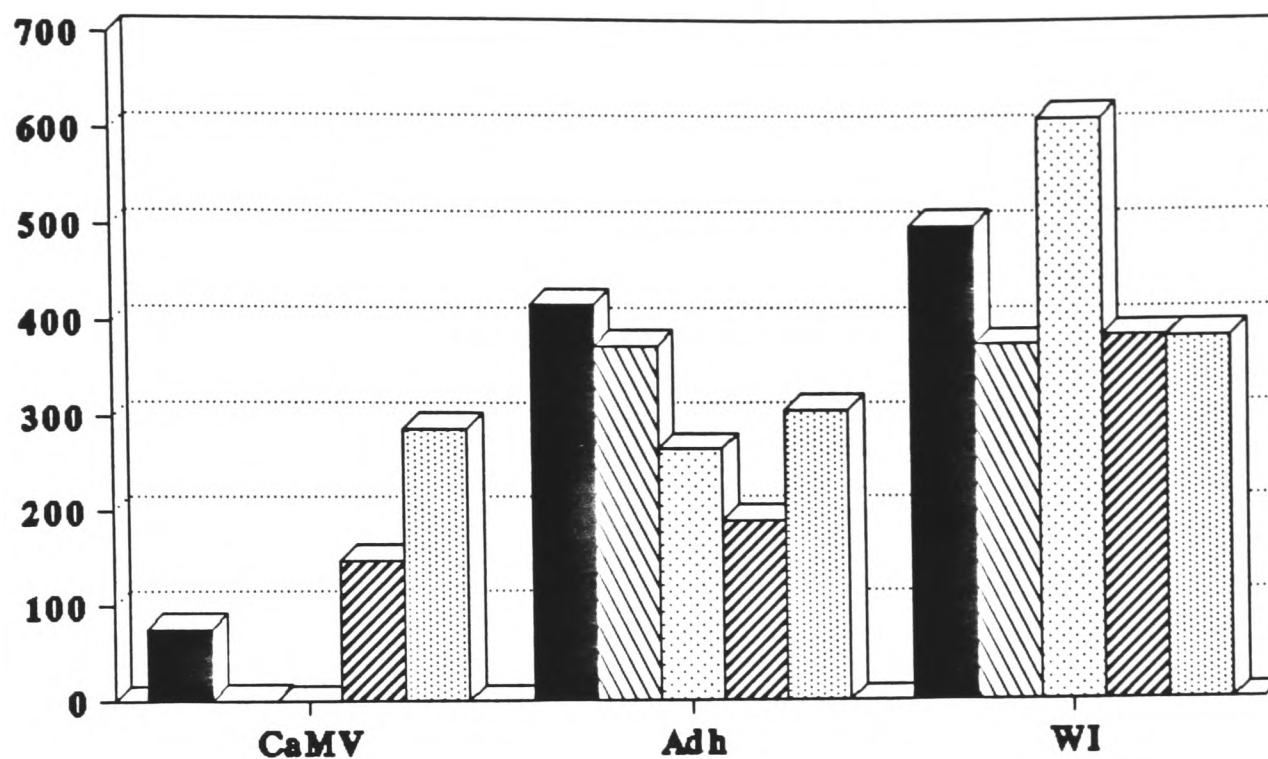


Figure 7.7 TGE for three promoters (repeat).

Table 7.2 Comparison of TGE results for 3 Promoters (Repeat)

No.	Promoter	TGE, Histochemical (No. CFUs)
1	-ve Control	0
2	-ve Control	0
3	-ve Control	0
4	CaMV	76
5	CaMV	1
6	CaMV	0
7	CaMV	148
8	CaMV	289
9	Adh	419
10	Adh	376
11	Adh	267
12	Adh	190
13	Adh	308
14	WI	501
15	WI	377
16	WI	613
17	WI	386
18	WI	385
19	-ve Control	3
20	-ve Control	6
21	+ve Control	> 1000

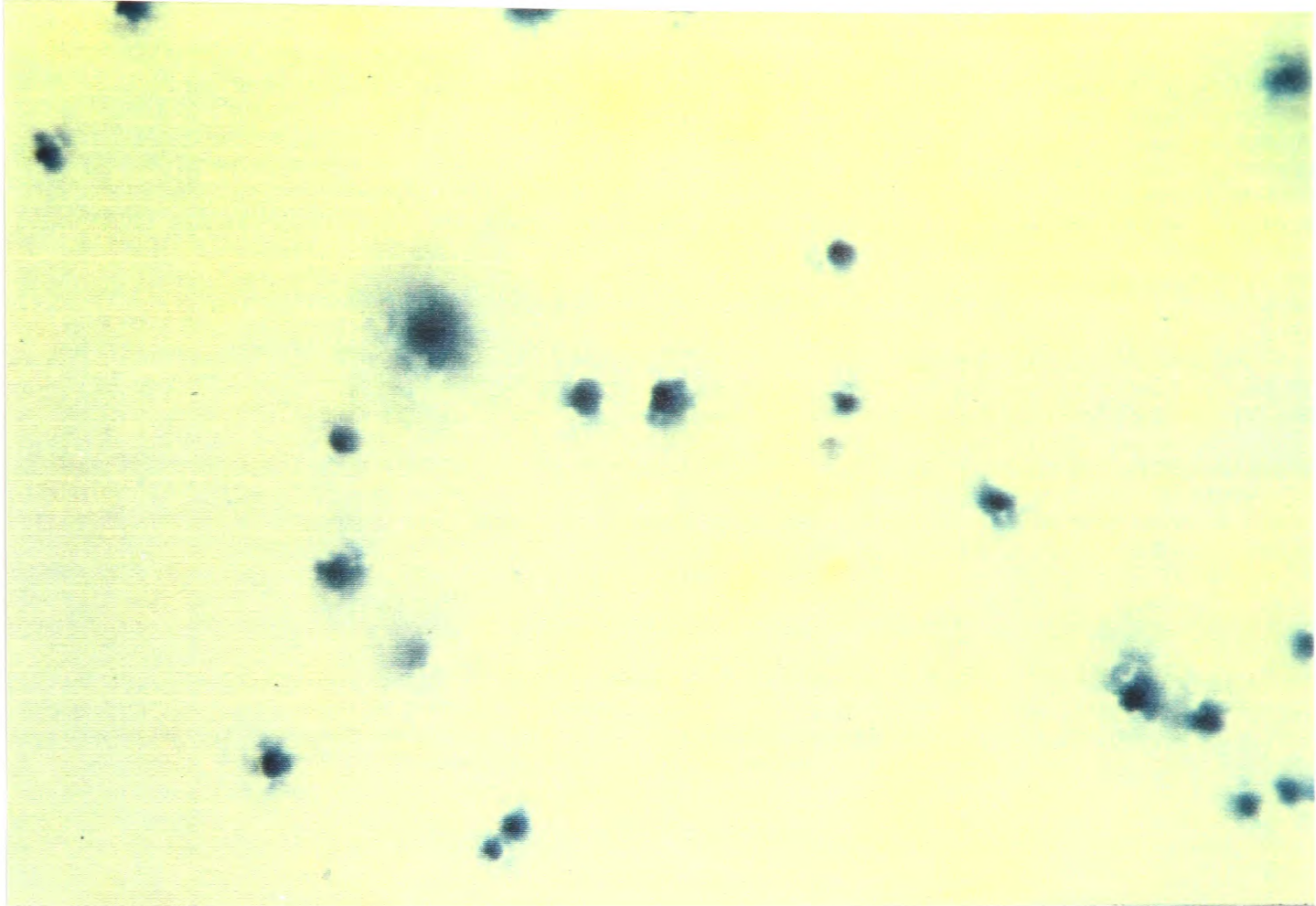


Figure 7.8 Light microscope photograph showing transient expression of pWI₁₁GUS 48 hours post-bombardment after histochemical staining with X-gluc (x32).

7.3.5 Effect of Target Mesh

The objective of this experiment was the determination of the effect of placement of a protective mesh over the target on rates of transient gene expression. In this experiment a 0.5mm X 0.5mm nylon mesh was placed 20mm above the target cells to reduce detrimental effects of the jet's shock wave and to disrupt large particle aggregates. A preliminary study had shown this mesh to be effective in disrupting the shock wave expanding from the nozzle exit and preventing cell blow away, thereby increasing transient expression. This effect due to the presence of the mesh was particularly noticeable at target distances less than 160mm. All other bombardment conditions were standard. The plasmid used was pAdhI₁GUS. Results from this experiment are shown in Figure 7.9.

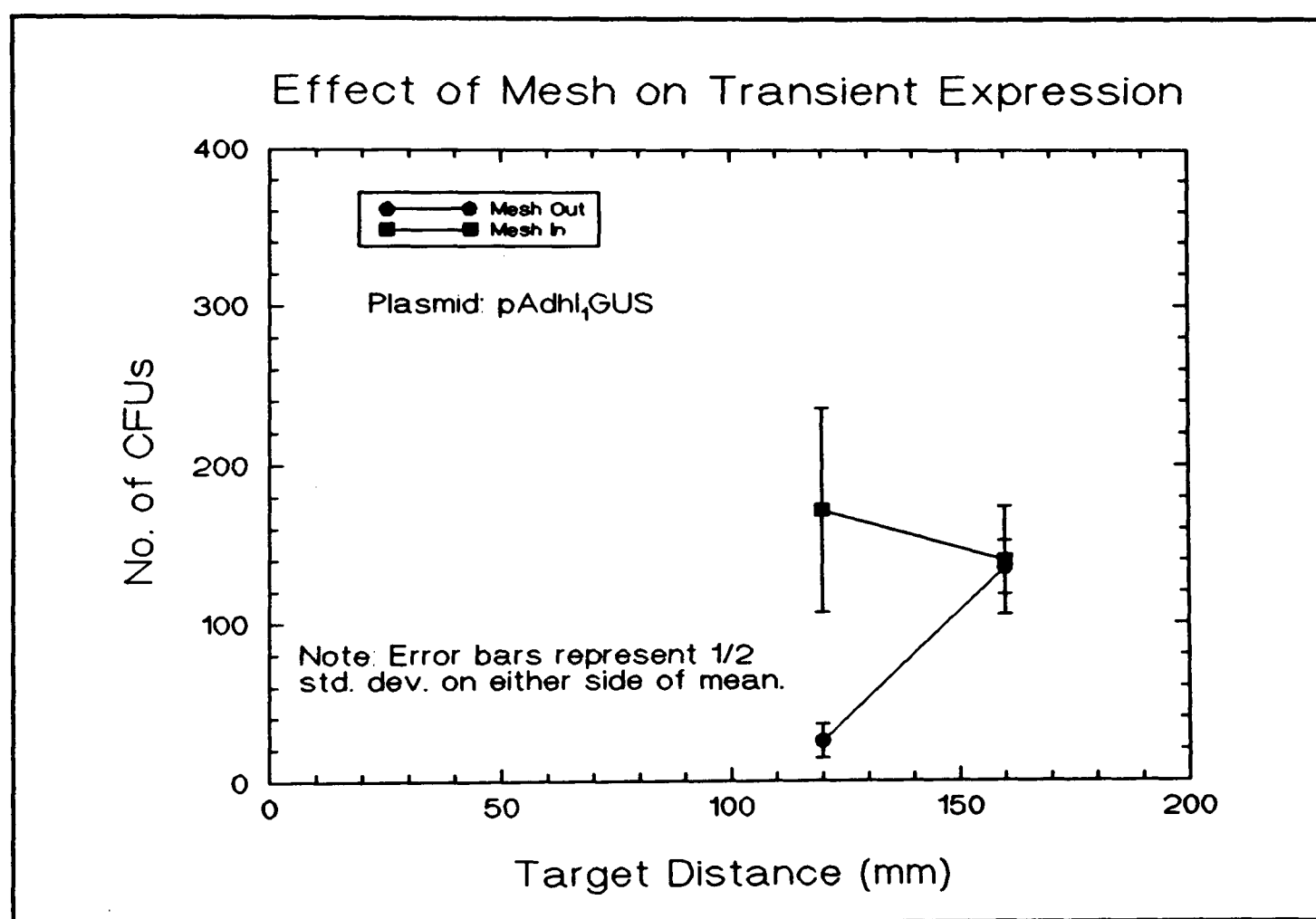


Figure 7.9 Effect of protective target mesh on TGE.

The figure shows that the mesh has a beneficial effect on TGE at $TD = 120\text{mm}$. Little effect is seen on rates of TGE at $TD = 160\text{mm}$. Presumably, this

is because viscous effects reduce the effects of the expanding jet as target distance increases.

7.3.6 Effect of Target Distance

The objective of this experiment was the determination of the effect of variation in target distance on transient gene expression. Bombardments were conducted using standard bombardment conditions. In addition, the 0.5mm X 0.5mm nylon mesh was kept 20mm above the target cells, again to reduce detrimental effects of the jet's shock wave and to disrupt large particle aggregates. Plasmid (pAdhI₁GUS) was precipitated onto 1 μ m tungsten using the Gordon-Kamm (1990) technique. Target cells consisted of maize suspension cells (LO56) stiffened with alginate.

Levels of transient expression resulting from these bombardments (as determined histochemically using an X-gluc substrate) are shown in Figure 7.10. Note that use of the mesh allows the target distance to be reduced from 160mm to 120mm with no resulting loss in transient expression. Also note that levels of transient expression vary considerably from run to run. Figure 7.10 graphically displays the variation in transient expression with target distance, showing the mean and standard deviation of the three bombardments at each condition. The highest levels of transient expression occur at a target distance of 120mm, when the mesh is utilised.

7.3.7 Effect of Driver Gas

An experiment was conducted to look at the transformation efficiency with helium as a driver gas instead of air. The potential enhanced effectiveness of the bombardment procedure associated with the higher particle velocities seen with helium was evaluated. A secondary purpose was to determine the effect of not using alginate to immobilise the cells. Target cells were LO56 maize suspension cells. Plasmid was p35SPAC. All other conditions were as previously stated. The results are shown in Table 7.3.

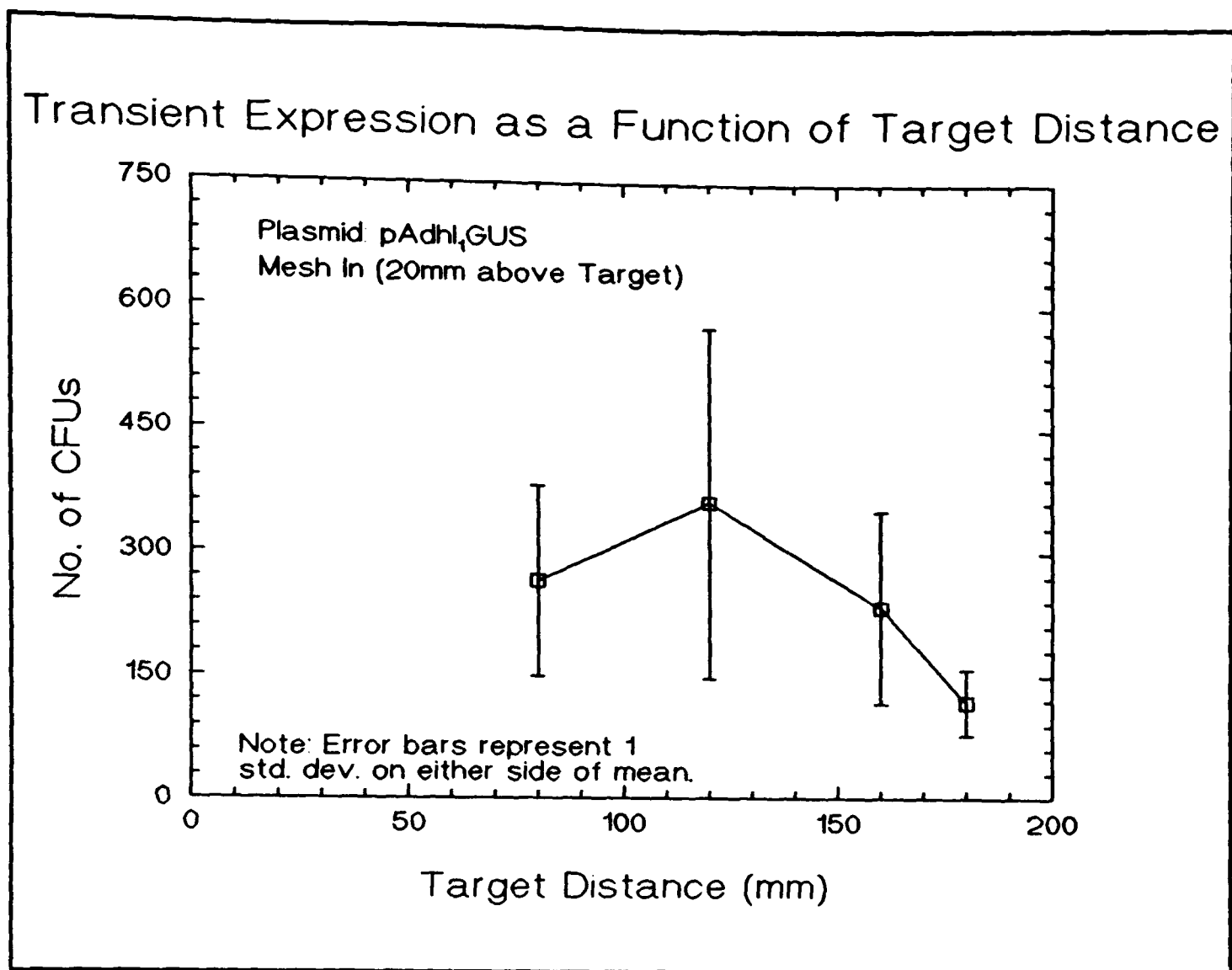


Figure 7.10 Variation in TGE with target distance.

Table 7.3 displays the significant increase in transformation efficiency that occurs when alginate is not used as an immobilising agent. It was believed that the reduced density of helium would permit bombardment without an immobilising agent. Because the density of helium is only one-seventh that of air at standard atmospheric conditions, blow away effects due to the jet were expected to be reduced with helium. This was found to be the case, although some cell blow away did occur. A corresponding order-of-magnitude increase in numbers of transformed cells results when alginate is not necessary. Presumably, this is because alginate forms a sticky layer over the target cells - a layer which acts to slow the microprojectiles before they are able to reach the cells themselves. When alginate is not present, no sticky layer is formed, and the microprojectiles have a better chance of piercing the tough wall of the suspension cells.

From the results of this experiment the effect of higher particle velocities associated with helium as a driver gas is not immediately apparent. Because of week-to-week biological variation in the state of the cells - a variation that cannot be easily controlled in the laboratory - it is difficult to compare results of transient genetic expression for experiments conducted on differing days. However, the results shown in Table 7.3, especially when no alginate is present, show that helium does offer some tangible advantages as a driver gas.

Table 7.3 TGE of Maize Suspension Cells using Helium as Driver Gas

Plasmid	Alginate?	No. of CFUs
-ve Control	Yes	1
p35SPAC	Yes	101
p35SPAC	Yes	121
p35SPAC	Yes	30
p35SPAC	No	1113

7.3.8 Results of Bombardment of Roots and Coleoptiles

A series of experiments was conducted to determine bombardment effectiveness for root systems. A secondary purpose was the comparison of transformation efficiencies for roots with those for coleoptiles¹. A final objective was the evaluation of two *root-specific* plasmids (plasmids specifically designed for the transformation of that particular tissue).

Method

Maize cultivars (ICI, UE95) were surface sterilised and incubated at 28° for three days prior to isolation of roots and coleoptiles. The root and coleoptile of each kernel were then detached and collected in a petri dish containing liquid media (1 x KNOPS) prior to bombardment. Previous experiments had shown that preculturing of the target tissue was not necessary. Separate bombardments were carried out on roots and coleoptiles. For bombardment roots or coleoptiles were placed in the centre of a petri dish filled with liquid media (1/2 KNOPs), solidified with an alginate (1.2% SPA). Target tissue was placed in each dish just prior to alginate setting in order to embed the lower surface of the roots or coleoptiles, thereby preventing blow away of the target tissue during bombardment. An average of nine roots or coleoptiles were bombarded simultaneously in any given bombardment.

Tungsten microprojectiles (M-17, GTE) were sterilised and coated as described in Section 7.2.1. Three plasmids were utilised in the experiments: p35SPAC (which had been used successfully in bombardment of maize suspension cells) and two root-specific promoters, pRMP3 and pRMP4. Coated particles were placed on the diaphragm membrane using the *dry* technique, also described in that section. Bombardments were conducted at the nominal operating conditions: $p_o = 2.7\text{bar}$, $p_{\infty} = 0.1\text{bar}$, $TD = 160\text{mm}$, Driver Gas: Air.

¹The "shoots" which form at the top of the seed.

The target tissue was cultured post-bombardment on the media on which it was bombarded for 48 to 72 hours at 25°. After incubation bombarded target tissue was placed on 4.7cm filter paper in 5cm petri dishes. 1.5ml X-Gluc solution was pipetted on top of the tissue in each dish. A separate petri dish was used for the tissue associated with each bombardment. Roots were then incubated at 37° overnight. As previously, cells expressing the GUS gene stained blue. These were counted and photographed the following morning. An LO56 "GUS positive" callus was used as a positive control. Bombardment with uncoated tungsten microprojectiles constituted the negative control.

Results

As shown in Tables 7.4 and 7.5, significantly higher levels of TGE were found for the coleoptiles (N6) than for the roots (MMRM). In several experiments over 100 CFUs were found on a particular bombarded coleoptile in a bombardment involving approximately eight coleoptiles simultaneously². Transient gene expression for coleoptiles was approximately an order of magnitude greater than that for maize roots. This disparity between effectiveness with roots and coleoptiles is thought to be related to the relative toughness of the outer tissues of each, with the tissue of roots being somewhat tougher. It should be noted, however, that with both roots and coleoptiles some genetic expression was seen in cells several layers beneath the surface tissue, indicating that some tungsten particles (and their associated DNA) had penetrated to the important cortical layer. Light microscope photographs of TGE in coleoptiles after bombardment (plasmid: p35SPAC) are shown in Figure 7.11.

²Frontal surface area of each coleoptile averages 10mm².

Table 7.4 Transient Genetic Expression for Maize Roots and Coleoptiles

Target	Plasmid	Total No. of CFUs	Avg. CFUs/Explant
MMRM	-ve Control	0	0
MMRM	-ve Control	0	0
N6	-ve Control	0	0
N6	-ve Control	0	0
MMRM	-ve Control	0	0
MMRM	-ve Control	0	0
MMRM	p35SPAC	79	7.9
MMRM	p35SPAC	31	3.4
N6	p35SPAC	> 213	> 26.6
N6	p35SPAC	> 198	> 18.0
MMRM	p35SPAC	24	2.4
MMRM	p35SPAC	24	2.7
MMRM	pRMP3	24	2.4
MMRM	pRMP3	0	0.0
N6	pRMP3	149	18.6
N6	pRMP3	269	24.5
MMRM	pRMP3	0	0.0
MMRM	pRMP3	43	3.9
N6	pRMP4	231	23.1
N6	pRMP4	---	---
MMRM	pRMP4	26	2.6
MMRM	pRMP4	23	2.3
MMRM	pRMP4	17	1.7
MMRM	pRMP4	26	2.6

MMRM - Maize roots
N6 - Maize coleoptiles

Table 7.5 Transient Genetic Expression for Maize Roots and Coleoptiles

Target	Plasmid	Total No. of CFUs	Avg. CFUs/Explant
MMRM	-ve Control	1	0.1
N6	-ve Control	7	0.8
MMRM	p35SPAC	49	4.9
N6	p35SPAC	> 189	> 23.6
MMRM	p35SPAC	77	8.6
N6	p35SPAC	> 482	> 60.3
MMRM	p35SPAC	114	10.4
N6	p35SPAC	> 318	> 35.3
MMRM	pRMP3	61	6.1
N6	pRMP3	> 353	> 39.2
MMRM	pRMP3	48	4.8
N6	pRMP3	> 409	> 40.9
MMRM	pRMP3	32	3.2
N6	pRMP3	> 503	> 50.3
MMRM	pRMP3	72	7.2
N6	pRMP3	> 310	> 38.8
MMRM	pRMP4	55	5.5
N6	pRMP4	> 254	> 23.1
MMRM	pRMP4	44	5.5
N6	pRMP4	> 330	> 33.0
MMRM	pRMP4	16	1.5
N6	pRMP4	> 223	> 24.8
MMRM	pRMP4	111	12.3
N6	pRMP4	> 302	> 33.6

MMRM - Maize roots

N6 - Maize coleoptiles

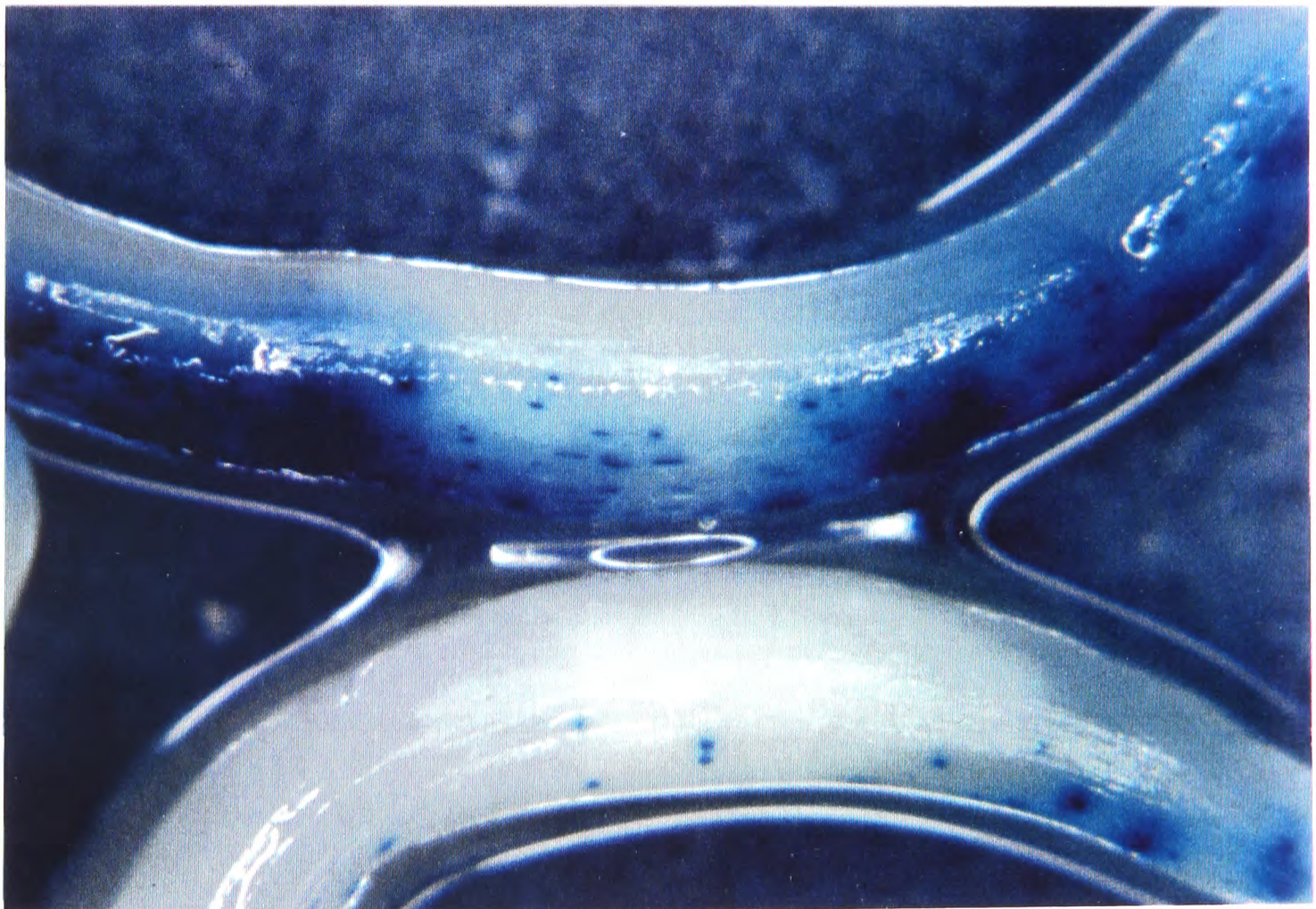
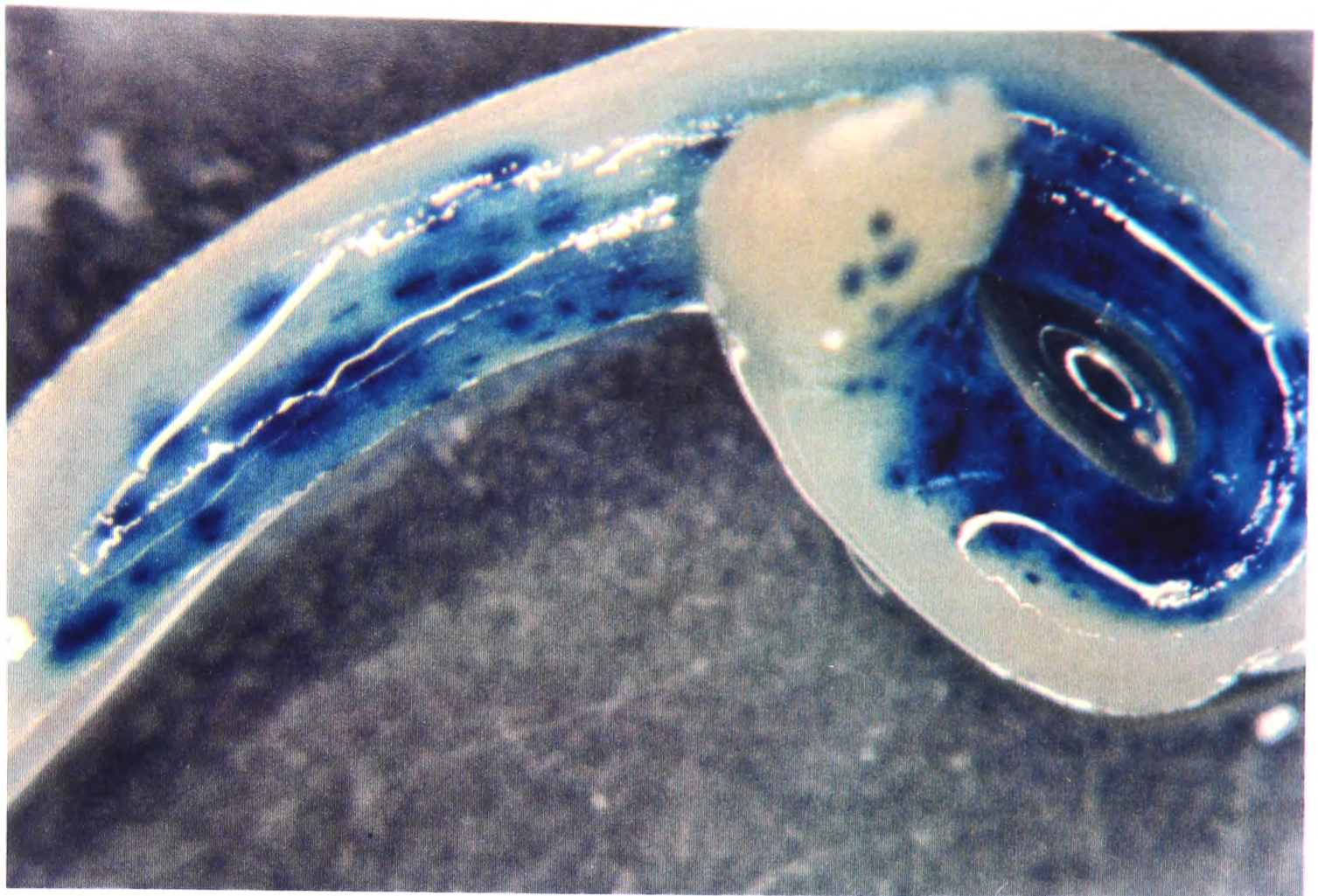


Figure 7.11 Light microscope photographs of TGE in coleoptile, x32.

7.3.9 Results of Bombardment of Leafy Tissue

The purpose of this experiment was the evaluation of the apparatus in bombardments on intact (leafy) tissue. The work was conducted in collaboration with Dr Ian Graham and Dr Joe Ross of Oxford University, Department of Plant Sciences and Dr Bernard Pelissier of Rhône Poulenc, Lyon, France.

Targets included leaves of egg plant, tobacco (*Nicotan Plumhaginifolia*), cucumber, maize, as well as maize embryos and cotyledon. Plasmids consisted primarily of Actin or 35 S promoters and GUS sequences and were precipitated onto 1µm tungsten particles using standard techniques described above. Standard bombardment conditions were evaluated: total pressure (p_o) varied from 2.5 to 7.0bar; target chamber pressure (vacuum) varied from p_{∞} = 0.5bar to 0.1bar; target distance varied from TD = 80 to 160 mm; the target-protecting mesh was used for various conditions; driver gas was air.

Results

Levels of transient genetic expression were again measured by X-gluc staining and visual counting of expressing cells (colour forming units (CFUs): blue spots). Intact leafy tissue showed significant levels of transient expression, as seen in Table 7.6. A photograph of the egg plant leaf is shown in Figure 7.12.

Table 7.6 TGE of Intact Leafy Tissue from Various Species

TD(mm)	Other	Plasmid	Target	No. of CFUs
160	Mesh	368	Egg plant Lf	155
160	Mesh	35D	Cucumber Lf	> 150
120	Mesh	35D	Cucumber Lf	> 150

Results in Table 7.6 display the relatively high levels of transformation attained through bombardment of intact leafy tissues. Evidence of this nature is important in demonstrating that the device and technique of concern here are *species-independent*.

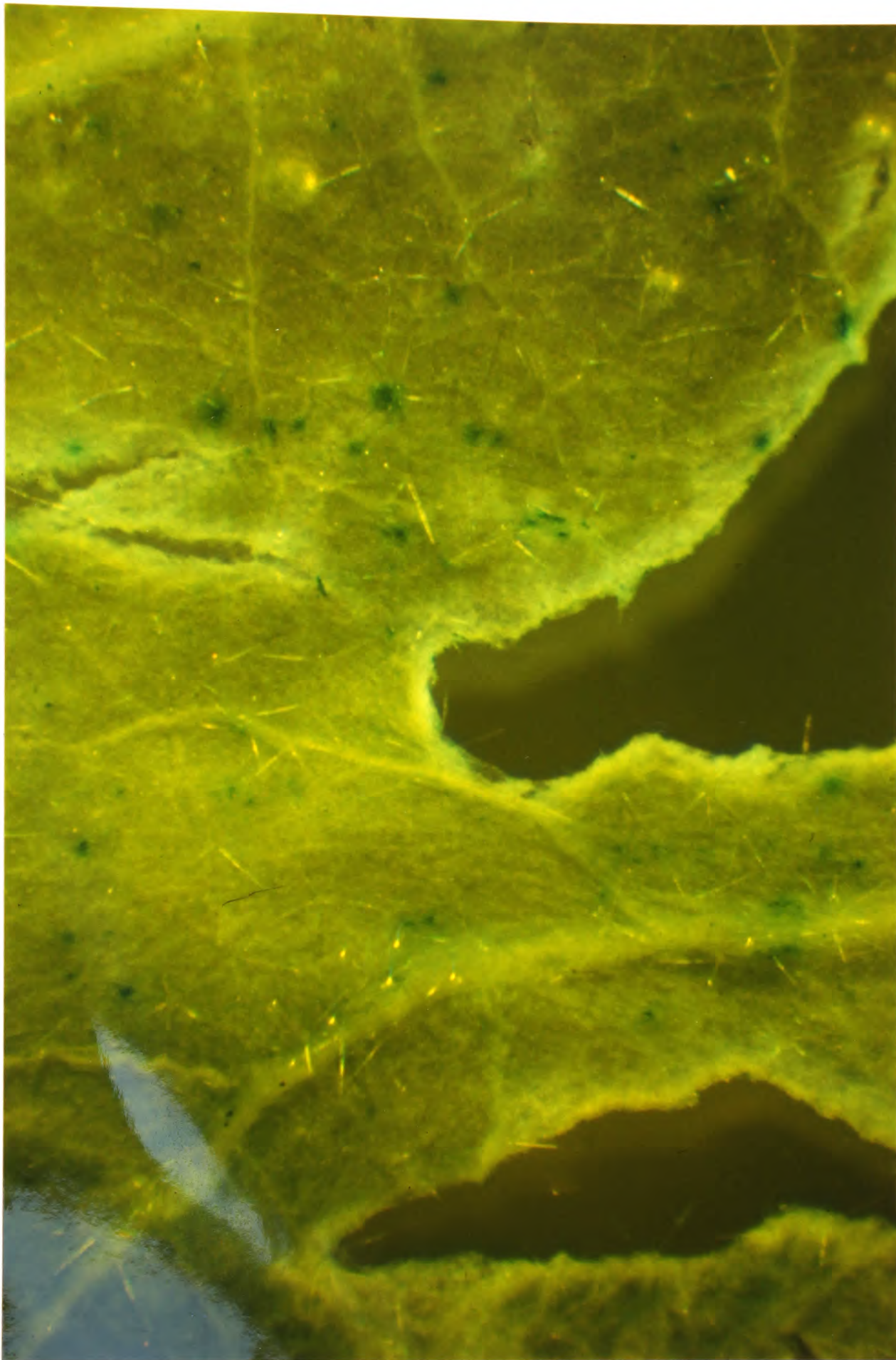


Figure 7.12 Photograph of eggplant leaf, showing significant levels of transient expression (plasmid: 368), x10.

7.4 Preliminary Results and Discussion: Mammalian Cells

7.4.1 Bombardment as a Technique for Gene Therapy

Particle bombardment has lately been evaluated as a useful technique for delivering genes to mammalian tissue as a first step toward gene therapy (Johnston *et al.* [1991]). Gene therapy represents a potentially effective method of therapy for single gene diseases. In these disorders, a specific genetic alteration results in the absence of a key protein or the production of an abnormal protein, leading to a characteristic manifestation (phenotype). The object of gene therapy would be to replace the product of a defective or missing gene with that of a normal gene. With the development of recombinant DNA technology, there is now great potential for innovative medical treatment using gene therapy, whereby normal genes are transferred in efficient expression vectors into the genome, resulting in the production of a normal protein to correct the specific defects. At present over 2000 single gene diseases are known, of which 300 are well-characterised (Cooper *et al.* [1989]). Since germline insertion of genetic material into embryos is rightfully considered unethical, researchers interested in gene therapy have targeted somatic cells (*e.g.*, stem cells) which not only proliferate but also have the capacity for self-renewal. A limiting step in gene therapy remains direct delivery of the therapeutic gene to cells *in vivo*.³

Preliminary experiments were carried out in association with Dr Yvonne Alexander of the Department of Medical Genetics, University of Glasgow, to determine whether the Oxford gas propulsion particle gun could deliver genetic material into mammalian tissue. Preliminary experiments involving histological determination of depth of particle penetration showed that 1 μm tungsten "bullets" could be delivered to sufficient depths in mouse skin. A second series of experiments was then carried out to evaluate genetic transformation of mammalian cells. Live albino mice bred specifically for such purposes were used as test subjects. The animals were prepared by shaving the hind legs; the animals

³At the moment most cells are genetically modified *ex vivo/in vitro* using viral vectors and then transplanted back into the patient.

were then placed under anaesthesia. Bombardments were conducted using a special platform in the target chamber specifically designed for animal bombardments. This platform allowed full freedom of movement of the animal to ensure target area was aligned squarely with the nozzle exit. Target areas on each animal consisted of two hind legs and two ears.

Bombardments were carried out using helium as a driver gas. Total pressure was varied between 4.0bar and 6.0bar. A plasmid containing the marker gene β -galactosidase was precipitated onto the tungsten particles using the previously-stated "dry" technique. To prevent animal suffocation, a vacuum was not used. Test subjects were "sacrificed" 48 hours post-bombardment and the bombarded tissue removed. Tissue was then fixed, embedded in paraffin wax, sectioned and stained with a standard histochemical assay (McEvoy *et al.* [1988]) to highlight the histology of the skin. Tissue was incubated at 37° overnight. Samples were then visualised and photographed under a light microscope the following morning.

Results displayed in Figure 7.13 and Figure 7.14 show that significant transient expression of the β -galactosidase gene in mouse skin cells does occur using the gas propulsion gene delivery method. The particular tissue samples shown here resulted from bombardment at 4.0bar, with helium as the driver gas. In the photograph of Figure 7.13 (enlargement: x 200), an insoluble blue precipitate is seen in cells expressing the β -galactosidase enzyme as a result of the histochemical assay. This can be compared with the photograph in Figure 7.14 of the negative control bombardment using uncoated tungsten particles. No enzyme expression is seen in the photograph of the negative control tissue. This provides confidence that the expression seen with the DNA-coated particles is actual and not artifactual.



Figure 7.13 Photograph of TGE in mouse skin (hind-leg) after bombardment with DNA-coated particles at $p_o = 4.0\text{bar}$ (x 167).

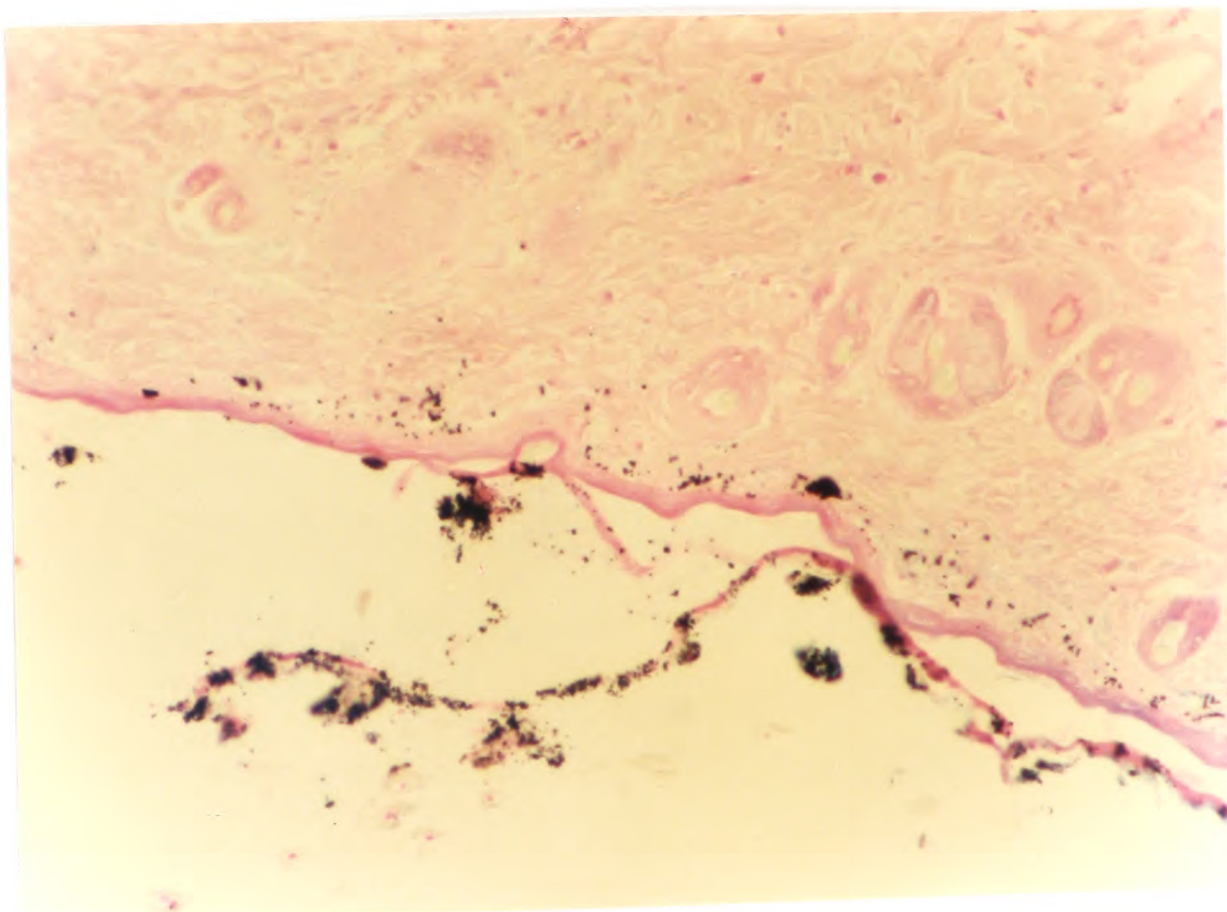


Figure 7.14 Photograph of negative control tissue after bombardment of mouse skin (hind-leg) with uncoated particles at $p_o = 4.0\text{bar}$ (x 167).

7.4.2 Bombardment of Human Leukaemia Cells

Introduction

The study of the genetic structure of human leukaemia cells is vital if that disease is ever to be eradicated. One cell line in particular (HL60) has proven useful in the past for the study of leukaemia (Birnie [1988]). The HL60 cell line was established from a female suffering from a form of acute non-lymphoblastic leukaemia, known as promyelocytic leukaemia (Collins *et al.* [1977]). Studies of this cell line, and other human cell lines derived from leukaemia patients, have yielded a great deal of information about the normal processes of white blood cell formation as well as the aberrations in these processes that cause leukaemias. The predominant characteristic of leukaemia cells is the phenomenon of "maturation arrest", whereby the programme of differentiation leading to the development of mature cells is prematurely arrested at a stage at which proliferation still occurs. This leads to an explosive expansion of a clone of immature cells and a corresponding lack of mature cells in the patient's peripheral blood (Dacie and Lewis [1975]).

Maturation arrest in HL60 cells, and in various other leukaemic cell lines, can be halted by the addition of certain reagents or growth factors to cultures of the cells (Birnie [1988]). This leads to the possibility of determining the role of particular genes in the mechanisms controlling normal differentiation and maturation, and in those causing maturation arrest leading to leukaemia. The most direct method of studying this is to introduce the candidate genes in both sense and anti-sense orientations, and under the control of promoters of varying strength, into the cells, and then to determine the effects on the differentiation and maturation of these cells in culture (Collins and Groudine [1982]). However, this approach has largely been frustrated in the past by the resistance of HL60 and similar cells to transformation procedures. Successful transformations of HL60 have been limited to efficiencies of approximately 10^{-5} (*i.e.*, 0.001%) (Holt *et al.* [1988]).

Therefore, the objective of this series of experiments was to determine whether an increase in transformation efficiency could be achieved with the gas-propulsion particle gun described here. Cultured HL60 cells were prepared as described in Birnie [1988] and placed in 1.5ml Nunc tube. It was believed that the steep walls of this tube would prevent significant cell blow away during the blast. The target chamber platform was modified to allow the Nunc tube to be held near the nozzle exit, ensuring full bombardment of the tube opening. 1 μ m tungsten particles (M-17, GTE) were coated with a plasmid DNA (marker gene: β -galactosidase) using the "dry" technique described in Section 7.2.1. Helium was used as a driver gas. Preliminary experiments showed that a stagnation pressure of $p_o = 4.1\text{bar}$ was satisfactory. Target chamber pressure during bombardments was atmospheric pressure.

Results

Photographs in Figure 7.15 show HL60 cells that have been transfected with a β -galactosidase gene regulated by a strong promoter. The cells were cultured for three days in complete medium, then fixed in formaldehyde and glutaraldehyde and incubated with a substrate for β -galactosidase that produces an insoluble blue precipitate in cells expressing the enzyme. Non-expressing cells are seen to be pink; variations in the intensity of the blue stain reflect differences in the amounts of β -galactosidase, possibly as a result of differences in the amounts of the gene in individual cells. This is similar to the range of gene expression seen in maize suspension cells, also thought to be related to the levels of genetic material within individual cells. Transformation efficiencies for the HL60 cell line have ranged between 10 and 50%, significantly higher than that achieved to date with any other transfer technique (Birnie, personal communication). Negative controls using uncoated particles (not shown) proved that cell expression was not an artifact of the assay procedure.

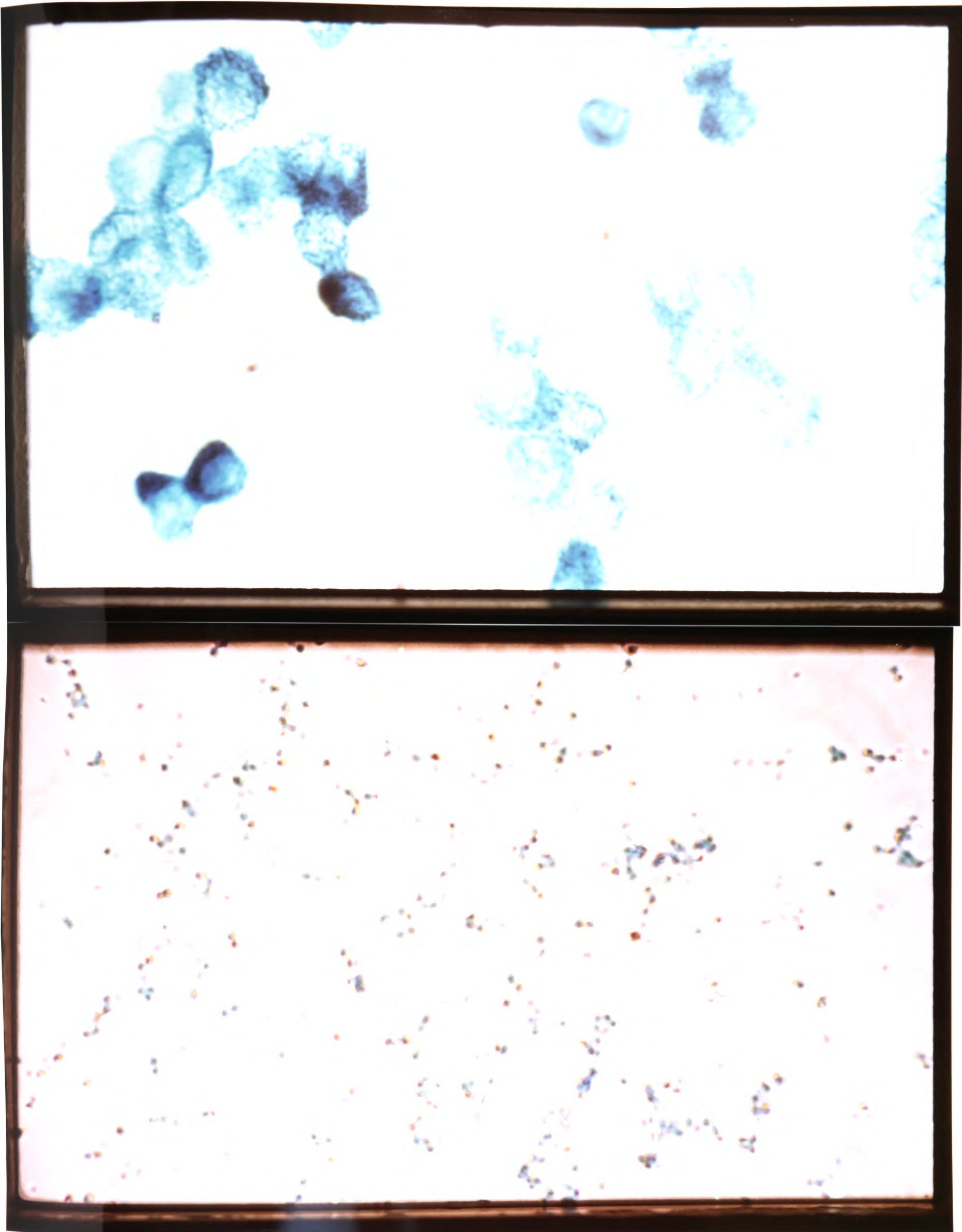


Figure 7.15 Light Microscope photographs of HL60 cells, 72 hours post-bombardment, showing transformed cells (blue) and non-expressing cells (pink); x60 (upper), x10 (lower).

7.5 Conclusions

Experimental results from studies of transient gene expression in various species of *flora* and *fauna* resulting from bombardment using the gas propulsion particle gun have been discussed in this chapter. A primary objective has been the optimisation of physical and biological system parameters to achieve maximum TGE for bombardment of a particular cell system, namely maize suspension cells. Other experiments have focused on evaluating the system for penetration depth using maize roots and maize coleoptiles as test systems.

Physical bombardment conditions that have been optimised include coating technique, tungsten suspension volume, target distance, effect of target mesh and upstream and target chamber pressures. Plasmid (or promoter) type has comprised the primary biological bombardment condition that has been optimised. The resulting values are shown in Table 7.7.

Table 7.7 Optimised Conditions for Bombardment of Maize Suspension Cells

Condition	Optimised Value (with Units)
Coating Technique	Dry
Suspension Volume	2.5 μ l
Target Distance	120mm (with Mesh), 160mm (no Mesh)
Upstream Pressure, p_o	2.7bar
Target Chamber Pressure, p_∞	0.1bar
Promoter	pWI ₁₁ GUS, p35SPAC

Significant levels of TGE have additionally been achieved with intact plant tissue, including maize roots and coleoptiles and leaves of egg plant and cucumber.

Finally, promising results for TGE in mammalian tissue (mouse and human) have been obtained using the particle bombardment device, although no optimisation has been attempted, as yet. These results show that the bombardment technique may hold promise as a method for gene delivery in gene therapy, while providing a novel (and in some cases, the sole) method of transfecting cell lines for cancer research.

CHAPTER 8. CONCLUSIONS

8.1 Thesis Review

Progress has been achieved toward the development of a species-independent technique which would allow geneticists to directly deliver specific exogenous DNA to plant and animal tissue, both *in vitro* and *in situ*. Direct delivery of DNA to plants represents the first step along paths to many goals: conferral or enhancement of disease- and virus-resistance, improvement of crop yields, and dispensation of beneficial qualities - extended shelf life of fruits or prolonged periods of ripeness, for example. Correspondingly, DNA delivery to animal tissue may permit the eventual therapeutic treatment of genetic disorders and may hold promise as a mechanism for the study of cancer.

A method of direct DNA delivery based on particle bombardment has been advanced here. Particle bombardment has its roots in the work done by John Sanford at Cornell University in the late Eighties (Sanford *et al.* [1987]). In the original system, a .22 charge fired a plastic macroprojectile downstream. Movement of the macroprojectile was halted by a stopping plate, but DNA-coated microprojectiles proceeded toward the biological target. Advantages of this original system included high levels of transient genetic expression (TGE), the ability to transfect many target cells at once (thereby making the procedure less labour-intensive), and relative species-independence. An important disadvantage of the system, however, was the inability to control microprojectile trajectories, such that microprojectiles showed run-to-run inconsistencies in the area of the target location actually bombarded; this represented a severe drawback, especially for smaller targets. Run-to-run differences in the amounts gunpowder charge utilised also caused inconsistencies in actual particle velocities achieved; this was made apparent by run-to-run variations in TGE. Therefore, although biologists were obtaining hopeful levels of TGE and even starting to achieve stable

integration of exogenous DNA into the plant genome with the previous bombardment devices, certain necessary improvements were apparent.

The novel bombardment device developed here to overcome these disadvantages represents a major shift in philosophy, away from the instantaneous, random detachment of microprojectiles from a macroprojectile carrier through impact with a stopping plate and toward utilisation of a more controlled acceleration scheme. The device described in the preceding chapters has employed techniques from classical gas dynamics for the acceleration of DNA-coated microprojectiles. Initially, a convergent-divergent supersonic nozzle, contoured using the method of characteristics (**Chapter 2**), was designed and fabricated, because particles were believed to be accelerated by the steady gas flow accelerating through the nozzle. However, the actual physical mechanism by which the microprojectiles were being accelerated was soon called into question after surprising observations that particles appeared to be delivered to the target well before the main force of the accelerating air. This was initially made apparent when target cells dislodged by the force of the air "blast" were seen to be imbedded with tungsten particles. Therefore, work began to be concentrated on determining the physical mechanism of particle acceleration.

The two hypotheses proposed to answer the question of particle acceleration mechanism are evident from the above discussion: the first considers particle acceleration to be related to quasi-steady flow associated with an underexpanded nozzle, while the second advances the theory that particle acceleration in the gas propulsion particle gun is related to transient shock tube physics. The case for each hypothesis has been explored experimentally. For the first case, the work of previous researchers evaluating expansion of underexpanded, supersonic free jets was reviewed; empirical correlations from the literature for first Mach disc location and jet characteristic wavelength (**Chapter 3**) were compared with experimental results from schlieren and pitot probe measurements (**Chapter 4**). Good correlation was demonstrated: this was seen as proof that well-characterised nozzle flow does occur at some point during the bombardment process, although it did not prove that it is this nozzle flow which carries the particles. The Mach number at the nozzle exit as a function of nozzle pressure ratio (p_o/p_∞) was then measured with a pitot tube; initial gas flow

velocity (as determined by peak pitot pressure) showed good correlation with the theoretical contact surface velocity consistent with shock tube flow (derived in Chapter 3). This demonstrated that shock tube flow occurs at the beginning of the bombardment process, as the initial shock wave and slug of air propagates downstream. Again, it did not prove that it is this initial flow which carries the particles. Therefore, evidence for either hypothesis could be found. It was left to particle velocity measurements to determine which of the two hypotheses was closest to the truth.

A novel particle velocimeter utilising two light emitting diode / photodiode (LED/PD) pairs placed in parallel in the target chamber was employed to determine particle time-of-flight (TOF) between the two axial locations (Chapter 5). A variation on this set-up involved placing a thin-film temperature gauge along the inner surface of the nozzle near the nozzle exit in order to trigger the passage of the shock wave. These set-ups enabled several experiments which, it was believed, would be instrumental in determining the important particle-acceleration mechanism: i.) evaluation of shock-to-particles time delay; ii.) variation in particle TOF for different volumes of upstream supply cylinder (and, correspondingly, different quasi-steady-state "run times"); iii.) determination of particle velocities for replacement of convergent-divergent nozzle with constant-bore, cylindrical (shock) tube; and iv.) evaluation of particle velocities for a range of nozzle pressure ratios. The results from each of these experiments showed conclusively that transient, shock tube mechanisms were of greatest importance in particle acceleration: i.) the time between passage of the shock at the nozzle exit and detection of the particles was measured and shown to be of the order of $100\mu\text{s}$. By utilising the results of these measurements and accounting for shock propagation time down the length of the nozzle, it was possible to demonstrate that particles were detected at the nozzle exit between 400 and $800\mu\text{s}$ after diaphragm rupture (depending on p_0/p_∞). These measured values were shown to be significantly less than the approximately $1800\mu\text{s}$ theoretically necessary for the attainment of steady flow (Smith [1966]); ii.) particle TOF between two locations in the target chamber was shown to be insensitive to variations in the volume of the upstream supply cylinder. If steady flow had been the underlying mechanism for particle acceleration (hypothesis I),

it was believed this value of particle TOF would vary; iii.) particle velocities were shown to exceed sonic velocity when a cylindrical tube replaced the convergent-divergent nozzle; this could only be the case if transient mechanisms were responsible for particle acceleration; iv.) particle velocities measured in the target chamber varied with p_o/p_∞ in a manner consistent with theoretical contact surface/shock velocity variation. The above results were conclusive in confirming that transient effects predominate for particle acceleration in the gas propulsion particle gun.

Another important aspect of the work was the development of physical experiments which would aid in setting up and optimising conditions for biological experiments. Because of the novelty of the technique, there was little information from the literature that could be directly applied to this optimisation. Although some correlations and approximate techniques were found to be useful (e.g., the approximate method of Adamson and Nicholls [1959] and the method of characteristics - both for jet boundary prediction (see **Chapter 3**)), most important parameters had to be evaluated experimentally. Some of the more useful results are discussed below:

a.) Measurements utilising a Kistler impact transducer (**Chapter 4**) demonstrated the expected decline in impact pressures with increasing distance from the nozzle exit - a measurement evaluating the strength of the shock wave emerging from the nozzle exit. A further decrease in impact pressures was seen with the introduction of a flow expander in the target chamber. This information facilitated target placement for the plant cell experiments in order to reduce the effect of target cell blow away. In the biological bombardment experiments it was shown that TGE for maize suspension cells was maximised at a target distance of 160mm (for conditions without a target mesh) and 120mm (with a mesh). Placement of targets closer than this to the nozzle exit showed reduction in TGE due to target cell blow away; target placement at greater distances showed reduction in TGE, presumably due to reduced particle velocities (see **Chapter 5**).

b.) High-speed video visualisation of particle spread from the nozzle exit showed spatial and temporal variation. The temporal variation was assumed to be related to transient variations in static pressure at the nozzle exit, as predicted numerically by Badcock [1992] (**Chapter 3**). Because significantly more particles were utilised in these visualisation experiments than is normally used for bombardment experiments, it was not clear whether this temporal variation actually occurs in biological bombardment experiments. The increase in particle spread seen with axial distance, however, does occur in biological bombardments, as demonstrated by optical evaluation of bombarded parafilm targets (**Chapter 6**). Therefore, the reduction in particle velocities that occurs at greater target distances is seemingly offset by an increase in particle spread and, therefore, an increase in the area of the target bombarded, so that maximum TGE occurs at the axial locations mentioned in a.) above.

c.) Schlieren visualisation and pitot pressure measurements (**Chapter 4**) demonstrated the temporal variation in jet structure, displaying the fall-off in supersonic flow and the onset of subsonic flow. This was found to be useful in determining the percentage of time that the "blast" is supersonic and the percentage that is subsonic - helpful, especially, for designing aerodynamic flow expanders to reduce target cell blow away. Also, these measurements were employed to aid in determining the minimum amount of gas necessary in the upstream supply cylinder; particle velocity measurements (**Chapter 5**) confirmed that a reduction from a 500ml volume supply cylinder to 50ml supply cylinder would not significantly affect particle velocities.

8.2 Future Work

Results of biological experiments discussed in **Chapter 7** have demonstrated that the gas propulsion particle gun does hold promise for the genetic transformation of both plant and animal tissue. Of particular note are the unexpectedly high rates of transfection of leukaemia (HL60) suspension cells *in vitro*. This provides hope that the range of applicability of the technique can be extended to other cell and tissue types being used currently by plant biologists,

geneticists, and even medical researchers. In conjunction with the extension of the device's applicability, further physical quantification is necessary. For example, the use of the device with other driver gases should be explored further. In particular, the particle velocities associated with helium require additional calibration; this additional calibration is important because helium appears to be useful as a driver gas for a range of biological applications, especially where reduced gas densities are important (*e.g.*, to reduce cell blow away). Particle velocity measurements with helium as the driver gas have been shown to be significantly higher than with air as a driver gas. This work should be extended to fuller calibration throughout the target chamber and at higher spatial resolution.

As mentioned above, one area of intense interest for application of the particle gun is in the medical field. It has been postulated that gas propulsion of small polymer-encapsulated drugs in particulate form may hold the key for transcutaneous drug-delivery. This would alleviate the need for invasive needles and reduce the chances of the spread of contagious diseases (AIDS, for example). The technique may hold particular merit for mass immunisation schemes in developing countries and may be of use to patients who currently require frequent injections each day (diabetes sufferers, for example).

A project to miniaturise the device as a first step toward the evaluation of the technique for drug-delivery is under way. It is believed that significantly higher stagnation pressures may be required for this application. Also, methods to reduce the sharp "bang" that accompanies bombardment into atmosphere will be necessary if the technique is to be clinically-viable.

Many other valuable applications may be found in the future for the device. If used appropriately, the technique could be of tremendous benefit to researchers in genetics and to clinicians in need of alternative drug-delivery schemes. Of course, if used unscrupulously, the method could lead to genetic tampering with animals or worse - this aspect must be understood early and researchers made fully aware of the responsibilities that accompany the application of the technique.

APPENDIX A. SUPERSONIC NOZZLE DESIGN

A.1 Method of Characteristics

A two-dimensional¹ method of characteristics routine after Cheers [1963] was utilised to design the internal contour of the diverging section of the supersonic nozzle. It should be noted that most of the classical aerodynamics texts (*e.g.* Liepmann and Roshko [1957]) include a section on supersonic nozzle design. The design is made possible by division of the flow field into a number of discrete cells, in each of which conditions are assumed to be uniform. It is preferable to arrange the waves such that they produce a constant flow deflection, δ . Because the waves are considered simple, a relation exists between the flow deflection, $\Delta\theta$, and the Prandtl-Meyer angle, Δv :

$$\Delta v = \pm \Delta \theta \quad (\text{A.1})$$

where the positive sign is used for intersection with down-going expansion waves and the negative sign for up-going expansion waves. In this two-dimensional system, it is possible (indeed, necessary) to assume that the strength of a wave (*i.e.*, the flow deflection $\Delta\theta$ caused by the wave) is not altered by intersection with other (weak) waves). Based on the above assumptions, it is not difficult to show that the flow conditions in any particular flow cell are given by:

$$\theta_x - \theta_1 = (m - n) \delta \quad (\text{A.2})$$

and

$$v_x - v_1 = (m + n) \delta \quad (\text{A.3})$$

where m and n represent the number of down-going and up-going expansion waves crossed, respectively. The number in any cell, then, is simply (m, n) .

Reference will be made to the original design for a Mach 2 nozzle. From common gas tables (see *e.g.*, Anderson [1985]), it can be seen that for a Prandtl-

¹Axisymmetric effects for such a slowly-diverging nozzle were not expected to be significant.

Meyer angle of 28° , the corresponding Mach number is 2.0585. Therefore, the design should be such that the cell at the end of the diverging section of the nozzle will have flow parallel to that at the throat, with $\nu = 28^\circ$ and $\theta = 0^\circ$. This requires 14° expansion through up-going waves and 14° expansion through down-going. To avoid compression waves, the maximum possible wall angle is 14° . Assuming that the sonic line at the throat is straight and choosing 2° as the standard wall deflection, a pattern as in Figure A.1 emerges. Note that reflection from the centre line has the effect of symmetry. The upper wall here makes 7 turns of 2° each; therefore 7 expansion waves of $\delta = 2^\circ$ are necessary. Where these strike the upper wall at a point downstream, the flow must be turned through -2° to cancel the waves. Waves are drawn at an angle midway between the angles of the Mach waves on either side; for example, the wave between (0,0) and (2,0) is drawn at:

$$\frac{1}{2}[(\mu_{0,0} - \theta_{0,0}) + (\mu_{2,0} - \theta_{2,0})] = \frac{1}{2}[(90^\circ - 0^\circ) + (61.96^\circ - 2^\circ)] = 74.98^\circ \quad (\text{A.4})$$

where μ represents the Mach angle. This procedure can then be utilised to complete the network.

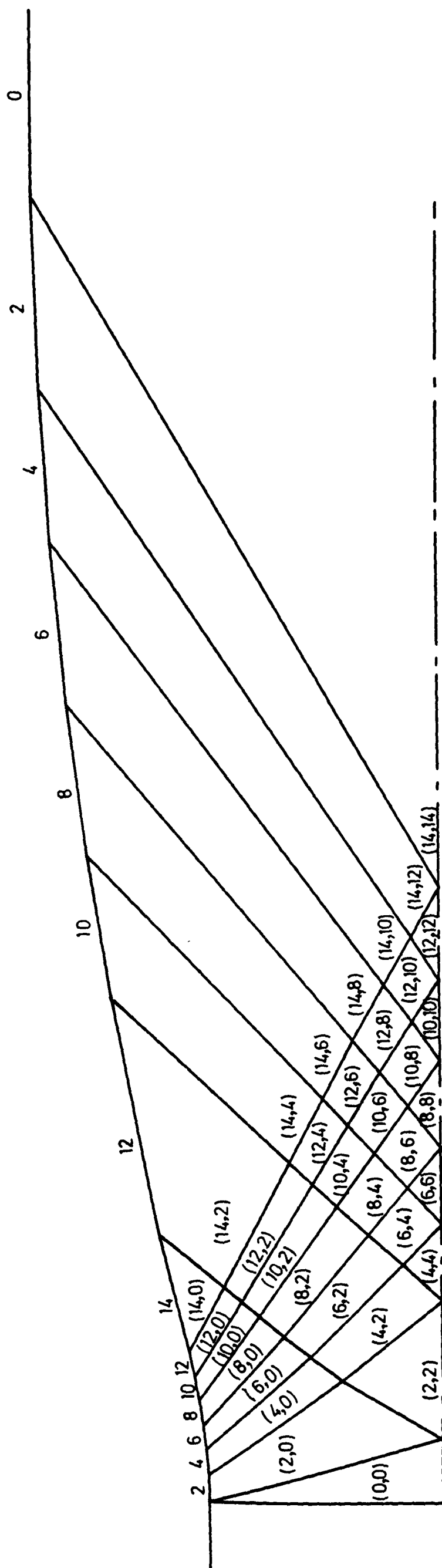


Figure A.1. Two-dimensional nozzle design using the MOC.

A.2 Nozzle Boundary Layer Analysis

To ensure boundary layer growth within the nozzle does not significantly affect free stream flow carrying the majority of the particles, a check was made of the boundary layer thickness. Because originally it was not clear whether quasi-steady, supersonic flow or transient, shock tube flow was of greatest importance in affecting particle trajectories, the boundary layer growth associated with both types of flow were evaluated. The results of the calculations are discussed in the succeeding sections.

Quasi-Steady Flow

The development of a compressible boundary layer along the design nozzle for steady-state conditions at $M_e = 2.33$ was computed using two boundary layer computer programs developed at Stanford University: STANLAM for laminar boundary layers and STANTURB for turbulent. Both use integral methods for the boundary layer calculations (see, *e.g.*, Cohen and Reshotko [1955]), producing density and velocity profiles at the nozzle exit. The displacement thickness (*i.e.*, the amount by which the flow is displaced due to the presence of the slower-moving boundary layer) can be calculated by the following equation:

$$\delta^* = \int_0^{\infty} \left(1 - \frac{u \rho}{u_{\infty} \rho_{\infty}} \right) dy \quad (\text{A.5})$$

A numerical integration of Equation (A.5) with a routine involving the trapezoid rule was utilised with the profiles output from the program. The results of the two calculations are show in Table A.1 below.

Table A.1 Boundary Layer Displacement Thickness: Quasi-Steady

Boundary Layer Type	Displacement Thickness (mm)
Laminar	0.08
Turbulent	0.43

Because the approximate nozzle exit Reynolds for standard conditions (1.64×10^7) implies that the flow is most likely in the turbulent regime, the turbulent value is probably the best to use. Nonetheless, the turbulent value shows that the displacement boundary layer thickness is less than 15% of the 3mm exit radius.

Transient Shock Tube Flow

Mirels [1966] has developed boundary layer correlations for the laminar boundary layer behind a moving shock in air and argon. Mirels assumes an ideal shock in the working section of a shock tube, such that the density increase across the shock is related to the shock Mach number as (Liepmann and Roshko [1957]):

$$\frac{\rho_2}{\rho_1} = \frac{u_1}{u_2} = \frac{(\gamma + 1)M_1^2}{(\gamma - 1)M_1^2 + 2} \tag{A.6}$$

Applying the law of conservation of momentum and correcting for real gas effects on gas properties, Mirels shows that for air ($\gamma = 1.4$), the laminar boundary layer displacement thickness in a shock tube varies with M_s and ρ_2/ρ_1 as:

Table A.2 Boundary Layer Displacement Thickness for Air: Transient

M_s	ρ_2/ρ_1	$\delta^* \times 10_4 \text{ (ft}^{-1/2}\text{)}$
1.25	1.429	2.12
1.50	1.862	3.62
1.75	2.279	4.76
2.00	2.667	5.66
2.50	3.333	7.00
3.00	3.857	7.95

For bombardment conditions ($M_s = 2$), Table A.2 shows that the transient boundary layer displacement thickness is 0.1725mm at the exit of the design nozzle. This should not be of great significance for the 3mm exit radius.

Therefore, boundary layer growth should not be a significant problem at the nozzle exit, regardless of the analytical approach used.

APPENDIX B. APPROXIMATE JET BOUNDARY CALCULATION

An approximate technique after Adamson and Nicholls [1959] (see also Adamson [1964]) can be utilised to predict the location of the boundary for an underexpanded jet with an nozzle exit pressure ratio, p_e/p_∞ , not far above 1.0.

Consider flow at the nozzle exit as seen in Figure B.1. It can be seen that the flow turns through an angle $\nu_{ex} - \nu_e$ relative to the nozzle wall, where ν_{ex} and ν_e are expansion and exit Prandtl-Meyer angles, respectively.² The overall flow expansion (with respect to the jet axis), α , can then be seen to be:

$$\alpha = \nu_{ex} - \nu_e + \delta_e \quad (\text{B.1})$$

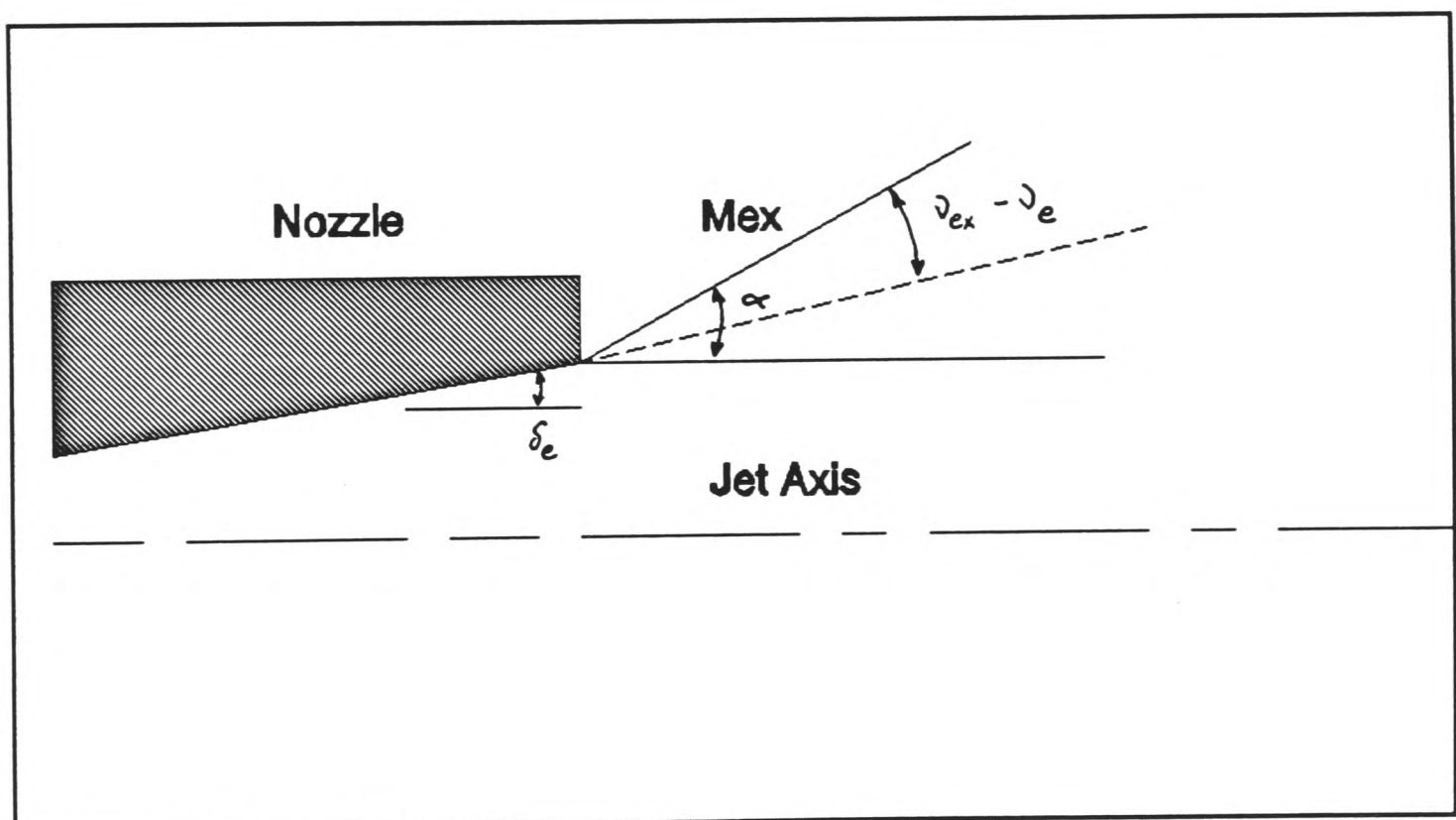


Figure B.1 Turning of flow at nozzle exit.

The flow is turned from this initial expansion angle by intersection of expansion waves with the jet boundary, reflecting as compression waves, so that the condition of constant pressure along the jet boundary is satisfied. The present approximation replaces the effect of expansion waves intersecting the flow near

²Parameters subscripted with $_{ex}$ refer to conditions at the jet boundary immediately after expansion to atmospheric conditions.

the jet boundary with effects of a quasi-one-dimensional area increase. In other words an area ratio calculation is used to estimate average Mach number and pressure at downstream axial points.

The jet continues to expand after exiting the nozzle: in any infinitesimal downstream increment, dx , the flow radius, dr , increases, with a corresponding increase in flow area, dA , and decrease in pressure, $\frac{\partial P}{\partial A} * dA$. $\frac{\partial P}{\partial A}$ can be calculated from quasi-one-dimensional (area ratio) relations if one makes the assumption that a stream tube along the jet boundary forms a "nozzle extension". This decrease in pressure must be balanced by an equivalent pressure increase, gained by turning the flow through an infinitesimal angle, $d\theta$, in order to prevent any pressure change along the jet boundary. This is shown in Figure B.2.

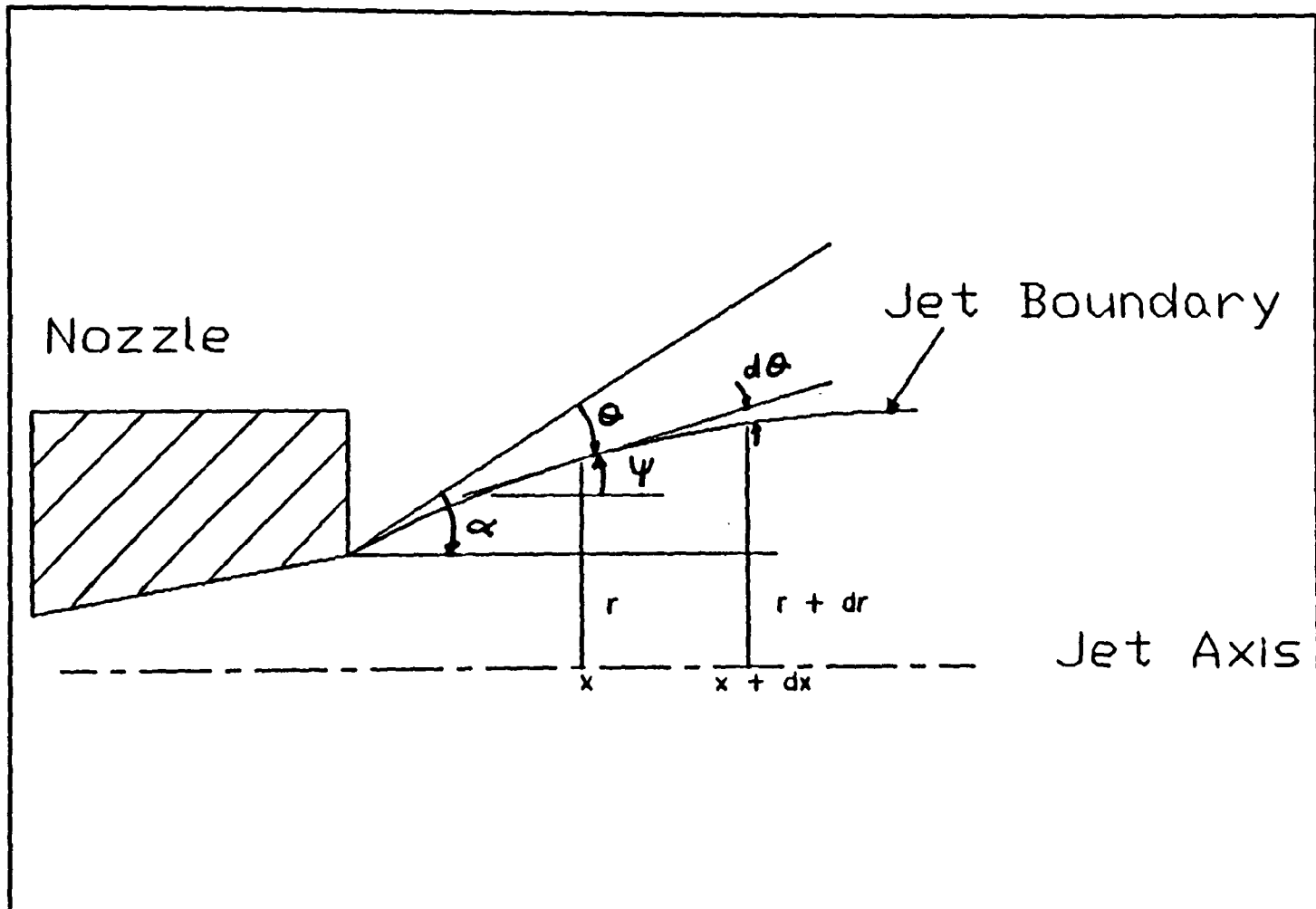


Figure B.2 Diagram of incremental flow expansion.

The equation for the pressure along the jet boundary may be written:

$$dp = 0 = \left(\frac{\partial p}{\partial A}\right)dA + \left(\frac{\partial p}{\partial \theta}\right)d\theta \quad (\text{B.2})$$

Isentropic relations can be used to relate dA to dM , the change in Mach number. Letting $\hat{p} = p/p_o$ (dimensionless static-to-total pressure ratio), Equation (B.2) becomes:

$$\left(\frac{\partial \hat{p}}{\partial M}\right)dM + \left(\frac{\partial \hat{p}}{\partial \theta}\right)d\theta = 0 \quad (\text{B.3})$$

p_o is assumed constant along the jet boundary, since waves are of infinitesimal strength. For isentropic flow $\hat{p}$ is related to M by (Liepmann and Roshko [1957]):

$$\hat{p} = [1 + (\gamma - 1)M^2/2]^{-\gamma/(\gamma - 1)} \quad (\text{B.4})$$

so that, differentiating Equation (B.4):

$$\left(\frac{\partial \hat{p}}{\partial M}\right)dM = \left(\frac{\partial \hat{p}}{\partial M^2}\right)dM^2 = -\gamma/2 \left[\hat{p}(dM^2)/(1 + \frac{\gamma-1}{2}M^2) \right] \quad (\text{B.5})$$

From linearised theory, the change in pressure due to a small change in direction is (Liepmann and Roshko [1957]):

$$\Delta \hat{p} = (\gamma \hat{p} M^2 / \sqrt{M^2 - 1}) \Delta \theta \quad (\text{B.6})$$

This shows that the strength of the wave is proportional to the deflection angle. For infinitesimal changes in pressure and direction, Equation (B.6) may be substituted together with (B.5) into (B.3) to obtain a relation between θ (the total angle measured between the tangent to the jet boundary at any point and the original jet flow direction, $\nu - \nu_e$) and M , the Mach number at any point along the boundary:

$$\frac{-\gamma}{2} \left[\hat{p} / (1 + \frac{\gamma-1}{2}M^2) \right] dM^2 + (\gamma \hat{p} M^2 / \sqrt{M^2 - 1}) d\theta = 0 \quad (\text{B.7})$$

Letting $\beta = (M^2 - 1)^{1/2}$ and rearranging:

$$d\theta = \frac{2}{\gamma - 1} \left[\frac{\beta^2}{\beta^2 + 1} \right] \left[\frac{\gamma + 1}{\gamma - 1} + \beta^2 \right]^{-1} d\beta \quad (\text{B.8})$$

Equation (B.8) can now be integrated from nozzle exit conditions after expansion, to any point on the boundary. At the nozzle exit, $\beta = \beta_{\alpha}$ ($M = M_{\alpha}$) and $\theta = 0$. Therefore:

$$\theta = \sqrt{(\gamma + 1)/(\gamma - 1)} \tan^{-1}[\sqrt{(\gamma - 1)/(\gamma + 1)} \beta] - \tan^{-1} \beta - [\sqrt{(\gamma + 1)/(\gamma - 1)} \tan^{-1}(\sqrt{(\gamma - 1)/(\gamma + 1)} \beta_{\alpha}) - \tan^{-1} \beta_{\alpha}] \quad (\text{B.9})$$

In order for constant pressure conditions to hold, θ at any point is simply the difference between the Prandtl-Meyer angle associated with the Mach number which corresponds to the area ratio at that point and the Prandtl-Meyer angle associated with expansion Mach number, M_{α} .

With θ known, one may calculate the jet boundary in terms of r and x , since if ψ is angle made by the tangent to the jet boundary with the axis, then:

$$\tan \psi = \frac{dr}{dx} \quad (\text{B.10})$$

but,

$$\psi = \alpha - \theta \quad (\text{B.11})$$

and if one defines dimensionless r and x in terms of the radius of the exit section of the nozzle, then Equation (B.10) becomes:

$$d(x/r_e) = \frac{d(r/r_e)}{\tan(\alpha - \theta)} \quad (\text{B.12})$$

Therefore, x/r_e at any point (assuming $x = 0$ at the nozzle exit) is:

$$\frac{x}{r_e} = \int_1^{r/r_e} \frac{d(r/r_e)}{\tan(\alpha - \theta)} \quad (\text{B.13})$$

The integral in this equation may be calculated numerically, using Simpson's (or trapezoidal) rule. At any r/r_e , the corresponding area ratio A/A^* may be

calculated, using as A/A^* ($r/r_e = 1$) the value which corresponds to M_α .
Therefore,

$$(A/A^*)_{r/r_e} = (r/r_e)^2 (A/A^*)_{M=M_\alpha} \quad (\text{B.14})$$

With the area ratio known, Mach number and θ can be found from compressible flow tables. For any given set of nozzle parameters, α can be calculated using Equation (B.1).

This approximate technique was used to calculate jet boundary location for a range of pressure ratios, nozzle divergence angles and exit Mach numbers. The technique was implemented using a LOTUS 1-2-3 spreadsheet program. A Newton-Raphson algorithm was utilised to find Mach number as a function of area ratio. Results were verified with calculations presented by Adamson and Nicholls [1959], as shown in the text.

APPENDIX C. METHOD OF CHARACTERISTICS FOR FREE JET

The application of the method of characteristics (MOC) to free jet expansion has been covered in many aerodynamics texts (*e.g.*, Liepmann and Roshko [1957]). The usual starting line for the calculation is a right-running ξ characteristic which, in this case, is a Mach line from the lip of the nozzle to the jet centreline. For a nominal exit Mach number, $M_e = 2.33$, Prandtl-Meyer angle, $\nu_e = 35.03^\circ$, the angle of the Mach line can be calculated:

$$\mu_1 = \sin^{-1}(2.33)^{-1} = 25.416^\circ \quad (\text{C.1})$$

This allows the establishment of a starting line; additional ξ characteristics are generated through the assumption that the jet expands to the chamber back pressure by means of a Prandtl-Meyer expansion fan. A finite number of waves may be assumed to comprise this centred expansion fan: this analysis assumes 6 expansion waves comprise the expansion fan. For a free jet expanding to the given back pressure for the present analysis ($p_e/p_\infty = 0.5$), the flow can be seen to accelerate, such that at a downstream location: $M = 2.777$; the corresponding Prandtl-Meyer angle for this M is: $\nu = 45.269^\circ$. Therefore, the flow is turned such that: $\theta = 10.238^\circ$. The intersections of right-running ξ characteristics with left-running ν characteristics from arbitrary points along the Mach line are found by assuming straight characteristic lines between each successive point. The properties at any intersection point may be found from compatibility relations for axisymmetric flow:

$$\frac{\delta}{\delta \eta} (\nu - \theta) = \sin \mu \frac{\sin \theta}{r} \quad (\text{C.2})$$

$$\frac{\delta}{\delta \xi} (\nu + \theta) = \sin \mu \frac{\sin \theta}{r} \quad (\text{C.3})$$

Equations (C.2) and (C.3) were programmed on a LOTUS 1-2-3 spreadsheet to enable the development of the characteristic net seen in Figure 3.17 in the text. In the spreadsheet, $\alpha = \sin (1/v)$. Also, the inclusion of the r term causes the algorithm to break down near the jet centreline, although this has little effect on the calculation of the jet boundary. The spreadsheet is included below.

Axisymmetric M-o-C Routine

Given: Me = 2.33
 Pe/Pinf = 0.5
 gamma = 1.4

Calc: nu1 = 35.03103 Prandtl-Meyer Function
 Pe/Po = 0.076306 Isentropic Expansion

Method: P4/Po = 0.038153
 M4 = 2.777234 Newton-Raphson (=M4,M8,...)
 nu4 = 45.26938 Newton-Raphson (=nu8,nu12,...)
 theta4 = 10.23835 del(theta)=del(nu)

Lattice Point	nu	theta	M=f(nu)	alpha	alpha + theta	theta - alpha
1,0	35.0310	0	2.33	25.415	25.41582	-25.4158
2,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7374	1.706392	2.399476	24.630	26.33644	-22.9236
c	38.4438	3.412785	2.470575	23.876	27.28912	-20.4635
d	40.1502	5.119178	2.541675	23.168	28.28797	-18.0496
e	41.8566	6.825570	2.614600	22.486	29.31218	-15.6610
f	43.5630	8.531963	2.695856	21.773	30.30553	-13.2416
2,z	45.2694	10.238	2.777113	21.105	31.34383	-10.8671
3,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7397	1.709	2.399570	24.629	26.33767	-22.9203
c	38.4486	3.417	2.470776	23.874	27.29103	-20.4575
d	40.1579	5.124	2.533708	23.245	28.37027	-18.1215
e	41.8674	6.831	2.615112	22.481	29.31341	-15.6505
f	43.5770	8.538	2.696526	21.767	30.30593	-13.2298
y	45.2975	10.233	2.778450	21.094	31.32738	-10.8622
z	45.3200	8.532	2.779523	21.086	29.61827	-12.5543
4,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7424	1.711	2.399683	24.627	26.33915	-22.9164
c	38.4540	3.422	2.470999	23.871	27.29390	-20.4500
d	40.1658	5.132	2.545036	23.136	28.26816	-18.0046
e	41.8778	6.841	2.619469	22.442	29.28333	-15.6017
f	43.5899	8.549	2.697139	21.762	30.31182	-13.2135
x	45.3121	10.242	2.779146	21.089	31.33158	-10.8470
y	44.4829	9.386	2.739661	21.408	30.79425	-12.0219
z	44.4937	6.825570	2.740176	21.403	28.22945	-14.5783
5,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7457	1.715	2.399821	24.626	26.34095	-22.9115
c	38.4606	3.428	2.470894	23.873	27.30128	-20.4448
d	40.1756	5.141	2.545462	23.132	28.27301	-17.9916
e	41.8908	6.852	2.620033	22.437	29.28953	-15.5853
f	43.6060	8.562	2.697904	21.756	30.31867	-13.1937
w	45.3286	10.253	2.779935	21.083	31.33569	-10.8303
x	44.4984	9.392	2.740399	21.402	30.79434	-12.0097
y	43.2307	8.102	2.680034	21.908	30.01067	-13.8068
z	43.2395	5.119178	2.680451	21.905	27.02434	-16.7859

6,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7500	1.719	2.399998	24.624	26.34326	-22.9054
c	38.4700	3.435	2.471304	23.868	27.30414	-20.4335
d	40.1911	5.149	2.546133	23.125	28.27509	-17.9766
e	41.9130	6.861	2.621001	22.428	29.28953	-15.5678
f	43.6358	8.570	2.699324	21.744	30.31438	-13.1739
v	45.3569	10.255	2.781282	21.072	31.32684	-10.8178
w	44.5250	9.388	2.741664	21.391	30.78010	-12.0032
x	43.2559	8.094	2.681232	21.898	29.99273	-13.8041
y	41.7740	6.594	2.616091	22.473	29.06701	-15.8791
z	41.7813	3.412785	2.616424	22.470	25.88287	-19.0573
7,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7558	1.725	2.400243	24.621	26.34646	-22.8968
c	38.4827	3.445	2.471856	23.863	27.30859	-20.4177
d	40.2115	5.162	2.547020	23.117	28.27935	-17.9553
e	41.9419	6.875	2.623724	22.404	29.27898	-15.5293
f	43.6740	8.584	2.701140	21.728	30.31283	-13.1447
u	45.3858	10.256	2.782658	21.061	31.31713	-10.8057
v	44.5494	9.387	2.742828	21.382	30.76946	-11.9948
w	43.2776	8.090	2.682266	21.889	29.98002	-13.7991
x	41.7938	6.588	2.615818	22.475	29.06316	-15.8879
y	40.2107	4.990	2.546988	23.117	28.10754	-18.1277
z	40.2162	1.706392	2.547225	23.115	24.82176	-21.4089
8,0	35.0310	0	2.33	25.415	25.41582	-25.4158
b	36.7659	1.735	2.400661	24.617	26.35192	-22.8822
c	38.5080	3.459	2.472957	23.851	27.31088	-20.3929
d	40.2569	5.173	2.548997	23.098	28.27141	-17.9253
e	42.0121	6.878	2.626913	22.375	29.25315	-15.4978
f	43.7730	8.573	2.705856	21.689	30.26244	-13.1156
t	45.4586	10.215	2.786123	21.033	31.24894	-10.8190
u	44.6197	9.342	2.746174	21.354	30.69692	-12.0127
v	43.3463	8.041	2.685535	21.861	29.90241	-13.8206
w	41.8611	6.535	2.618745	22.449	28.98399	-15.9141
x	40.2762	4.935	2.549836	23.090	28.02523	-18.1554
y	38.6391	3.287	2.478657	23.793	27.08042	-20.5069
z	38.6425	0	2.478806	23.792	23.79215	-23.7921

APPENDIX D. CROSS-CORRELATION TECHNIQUE

Consider two signal sequences $x(n)$ and $y(n)$, each of finite amplitude. The cross-correlation of $x(n)$ and $y(n)$ is a sequence $r_{xy}(l)$, which is defined as:

$$r_{xy}(l) = \sum_{n=-\infty}^{\infty} x(n) y(n-l); \quad l=0, \pm 1, \pm 2, \dots \quad (\text{D.1})$$

or, equivalently:

$$r_{xy}(l) = \sum_{n=-\infty}^{\infty} x(n+l) y(n); \quad l=0, \pm 1, \pm 2, \dots \quad (\text{D.2})$$

The index l is the time shift or (*lag*) parameter and the subscripts xy on the cross-correlation sequence $r_{xy}(l)$ indicate the sequences being correlated. The order of the subscripts, with x preceding y , indicates the direction in which one sequence is shifted, *relative to the other*. As an example, in (D.1), the sequence $x(n)$ remains unshifted and $y(n)$ is shifted by l units in time, to the right for l positive, and to the left for l negative.

The procedure for computing the cross-correlation function between $x(n)$ and $y(n)$ involves shifting one of the sequences, $x(n)$ say, to obtain $x(n-l)$, multiplying the shifted sequence by $y(n)$ to obtain the product function $y(n)x(n-l)$ and then summing all the values of the product function to obtain $r_{xy}(l)$. The procedure is repeated for different values of lag, l .

An algorithm to compute $r_{xy}(l)$ for positive lags can be developed using the above procedure. In order to use the same algorithm for negative lags, the following relationship (see *e.g.*, Proakis and Manolakis [1989] for a proof) can be used:

$$r_{xy}(-l) = r_{yx}(l) \quad (\text{D.3})$$

With this relationship, the values for negative lags can be obtained using the same algorithm for positive lags and interchanging the roles of $x(n)$ and $y(n)$.

The above procedure was utilised to develop a FORTRAN computer program, operational on the OUCS VAX. A program listing is presented below.

```

C*****C
C*
C*  CROSS-CORRELATION ROUTINE FOR TWO SIGNALS
C*  TO DETERMINE PHASE LAG
C*
C*****C
    program xcorr
    integer iread
    character*13 in1,in2,out1
    parameter (itotal=300)
    parameter (lm=2000)
    parameter (n=2000)
    parameter (m=2000)
    dimension v1(2000), v2(2000), vmult(2000), r(2000)

C*****C
C**
C**  itotal = number of lag time pts written
C**  iread  = number of data pts read for each signal
C**  lm     = length of correlation
C**  n      = length of first sequence in subroutine
C**  m      = length of second sequence in subroutine
C**
C*****C

    write (6,*)'enter no. of values to be read'
    read (5,*) iread
    write (6,*)'enter 2 input files, output file (separate lines)'
    read (5,26) in1,in2,out1
26  format(a13/a13/a13)

    open (unit=7,file=in1)
    open (unit=9,file=in2)
    open (unit=8,file=out1)

C** Zero data matrices **C
    do 50 i=1,2000
        v1(i)=0.0
50    v2(i)=0.0

C** Read in full data set for two signals **C

    do 75 i=1,iread

```

```

    read (7,*) x, v1(i)
75    continue

    do 100 i=1,iread
100    read (9,*) y, v2(i)

C*****C
C*
C** Normalize two signals between 0 and 100
C*
C*****C

C** Find minimum of the two signals **C

    vmin1 = v1(1)
    vmin2 = v2(1)
    do 102 i=1,iread
        if (v1(i).le.vmin1) vmin1 = v1(i)
        if (v2(i).le.vmin2) vmin2 = v2(i)
102    continue
    write (6,*) vmin1,vmin2

C** Find maximum of the two signals **C

    vmax1 = vmin1
    vmax2 = vmin2
    do 104 i=1,iread
        if (v1(i).ge.vmax1) vmax1 = v1(i)
        if (v2(i).ge.vmax2) vmax2 = v2(i)
104    continue
    write (6,*) vmax1,vmax2

C*****C
C**
C** Offset signals so that each begins at V = 0.0 volts
C** no need to assume v1(1) and v2(1) are both negative
C**
C*****C
    vmin1 = v1(1)
    vmin2 = v2(1)

    do 105 i=1,iread
        v1(i) = v1(i) - vmin1
        v2(i) = v2(i) - vmin2
105    continue

C** If offset signal is less than 0, make it equal to zero

```

```

do 106 i=1,iread
  if (v1(i).lt.0.0) v1(i)=0.0
  if (v2(i).lt.0.0) v2(i)=0.0
106 continue

```

```

vmax1 = vmax1 - vmin1
vmax2 = vmax2 - vmin2

```

```

write (6,*) vmax1,vmax2

```

```

fact1 = 100./vmax1
fact2 = 100./vmax2

```

C** Fact1 and fact2 are factors to normalize signals

```

do 107 i=1,iread
  v1(i) = v1(i)*fact1
  v2(i) = v2(i)*fact2
107 continue

```

C** Multiply template for zero phase lag **C

```

do 110 i=1,iread
  vmult(i) = v1(i)*v2(i)
110 continue

```

```

c    do 120 i=1,itotal
c 120 write (6,*) v1(i), v2(i), vmult(i)

```

```

r(0) = 0.0

```

```

do 125 k=1,iread
125  r(0)=r(0)+vmult(k)
write (6,*) r(0)

```

```

call cros (v2,n,v1,m,r,lm)

```

C** Here v1 is being shifted to the right relative to v2

```

ntotal = -1 * itotal
do 200 i=itotal,1,-1
200  write (8,*) i,r(i)

```

```

call cros (v1,n,v2,m,r,lm)

```

C** Here v2 is shifted to the right relative to v1

```
do 300 i=0,ntotal,-1
300  write (8,*) i,r(-i)
```

```
stop
end
```

```
SUBROUTINE CROS(x,n,y,m,r,lmax)
DIMENSION x(1),y(1),r(1)
DO 10 L=1,LMAX
NL=M+1-L
IF (NL.GE.N-1) NL=N-1
R(L)=0.0
DO 10 K=L,NL
R(L)=R(L)+X(K)*Y(K-L)
10 CONTINUE
RETURN
END
```

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(57) Abstract

Apparatus for, particularly, penetrating living cells with genetic material by bombarding the cells with DNA coated microprojectiles comprises a gas pressurizable reservoir (1) connected to a nozzle (N) which leads to a target chamber (10) containing a target station (28). A membrane seals the upper end of the nozzle from the reservoir and microparticles are supported on the upstream side of the membrane. Upon increase in the gas pressure in the reservoir, the membrane ruptures and the particles are projected at supersonic speeds through the nozzle (N), into the chamber (10), and bombard a target at the station (28). Target disturbance is minimised by a flow expander plate (17) and an obstruction (29).

