

## Combination single molecule fluorescence and mass photometry

Emanuel Pfitzner<sup>1</sup>, Philipp Kukura<sup>1</sup>

<sup>1</sup>Physical and Theoretical Chemistry Laboratory, Chemistry Department, University of Oxford, Oxford, United Kingdom

Total internal reflection fluorescence microscopy can detect single fluorescently labeled molecules close to a surface with high specificity, temporal, and spatial resolution. Despite the high specificity introduced by the fluorescent label it is not universal, and labels must be attached to individual proteins by means of genetic engineering or designed antibodies. Mass photometry (MP) surpasses this limitation by interferometrically detecting the elastic scattering which allows localization and mass measurement of single proteins. It is, thus, universally applicable to all kinds of biomolecules. MP, however, struggles with detecting species of low molecular mass (< 40 kDa) and its non-specificity prevents particles of similar mass to be distinguished solely by the one-dimensional scattering readout.

Here, we report on the combination of single molecule fluorescence and scattering microscopy. We simultaneously record the position of a particle in time and space *via* fluorescence and determine its mass *via* elastic scattering to improve the sensitivity and specificity of mass photometry.

Firstly, we recorded regular landing assays of various proteins which were labeled with a green fluorescent protein (GFP). The landing events can be separated into fluorescent and non-fluorescent species which effectively suppresses noise at the lower end of the resulting mass distribution and thus enhances the sensitivity of the approach.

Secondly, we investigated protein-protein interactions where one of the binding partners was fluorescently labeled with GFP. The separation into fluorescent and non-fluorescent events

allows to separately determine the mass of two species with an improved precision much like 2D spectroscopy. The enhanced specificity allows differentiation of stoichiometries between binding partners which were otherwise indistinguishable based on the scattering contrast only.