Measuring Translocating Proteins using Mass Photometry

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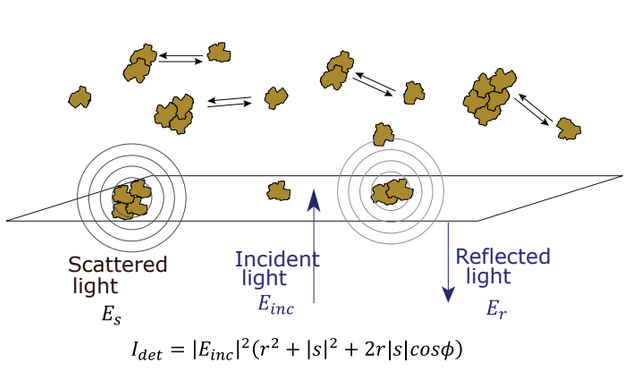
INTRODUCTION

With the vast scale of the proteome better tools are need to improve our understanding. In this vain, we are attempting to combine Mass Photometry (MP) and Nanopores to produce a method to enable this. MP is a optical label-free method to measure the mass of proteins and has been used to measure proteins on supported lipid bilayers [1].

To support MP with nanopores we utilise a microstructure to create suspended bilayer regions which trans-membrane proteins (such as α-hemolysin) can embed themselves into and incorporate the electronics to enable translocation. Part of the challenge is utilise these structures without introducing excess scattering using MP - suspended bilayers on a microstructure etched into CYTOP (water

Principal of Mass Photometry



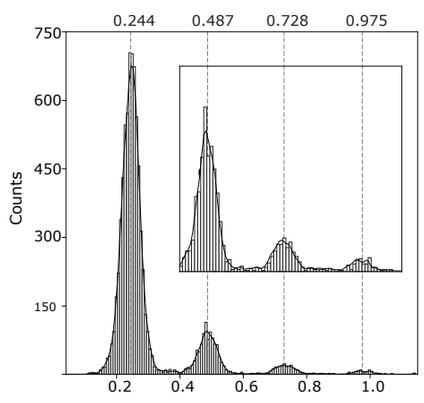
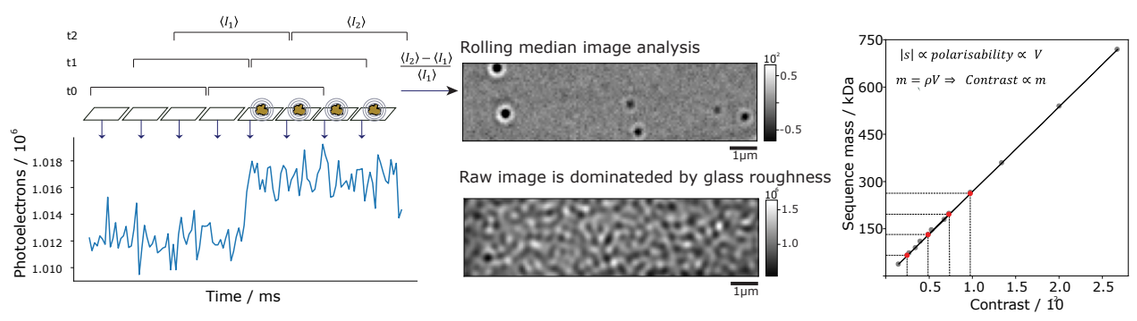


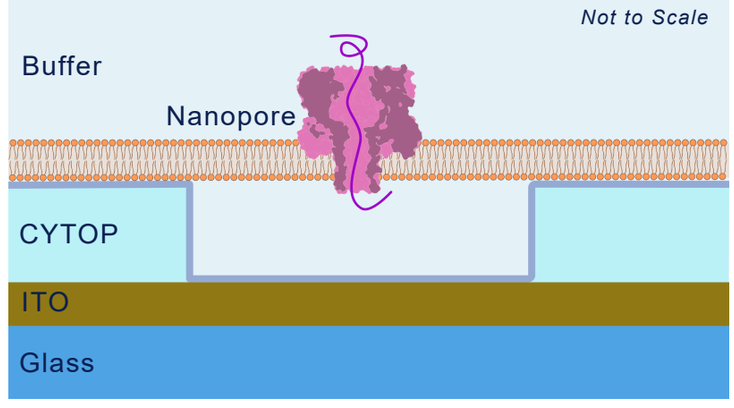
Image Processing



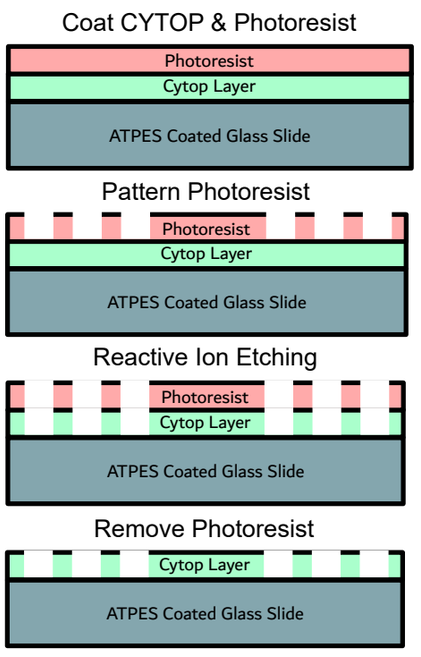
AIMS

1) Form suspended bilayers to perform label-free tracking
2) Measure mass and diffusion of proteins in the suspended bilayer
3) Develop electronics to enable translocation

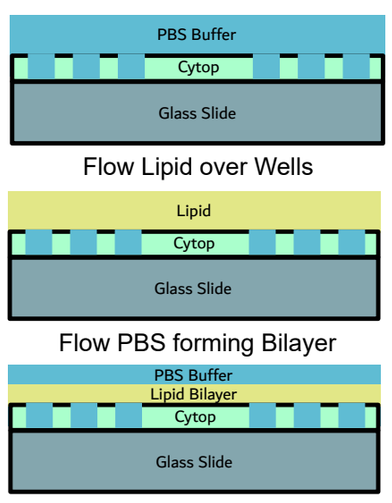
Design



Fabrication



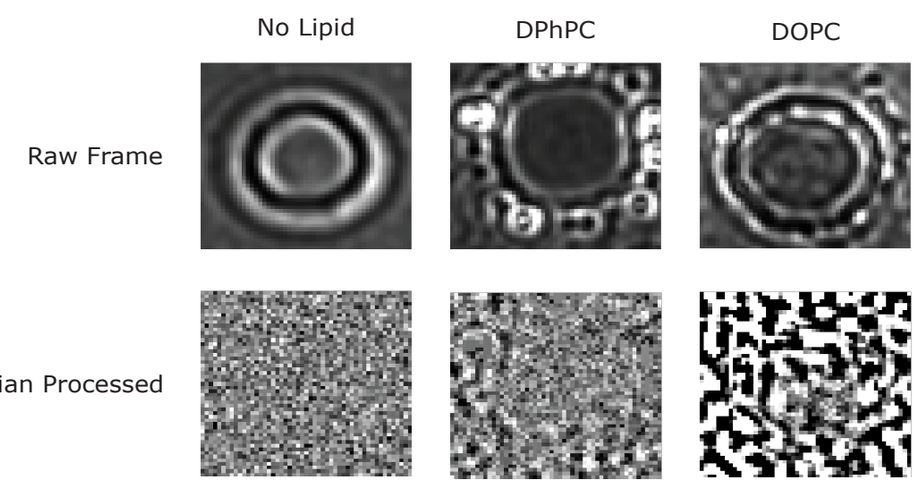
Bilayer Formation



RESULTS

Imaging Suspended Bilayer with MP

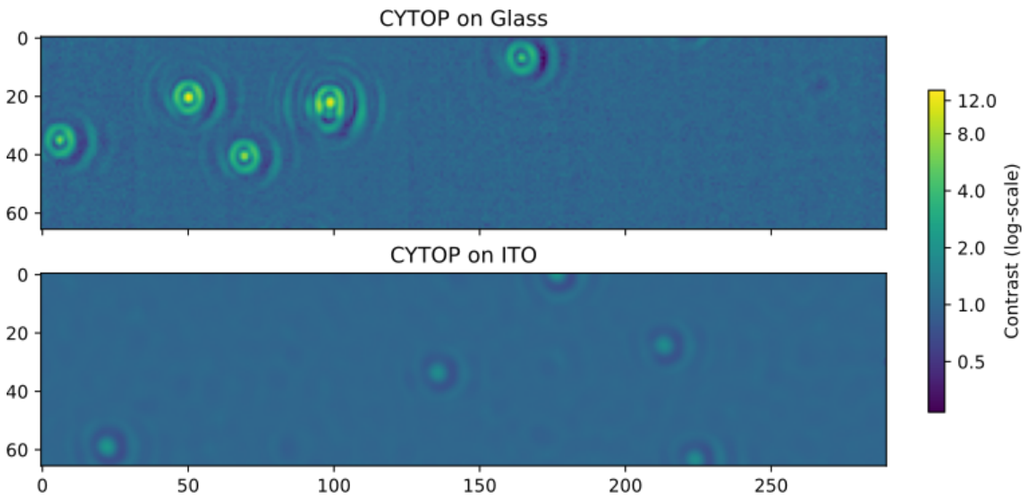
Suspended bilayer formed using 2-phase approach
While DOPC appears cleaner in raw image, the higher fluidity means it exhibited greater temporal noise evident after median processing
DPhPC althrough noisier in the raw frame, with large vesicles present, resulted in a better signal after median processing



Coating ITO with CYTOP

Coated glass and ITO with APTES > Then coated with CYTOP
ITO maintained conductivity after APTES treatment

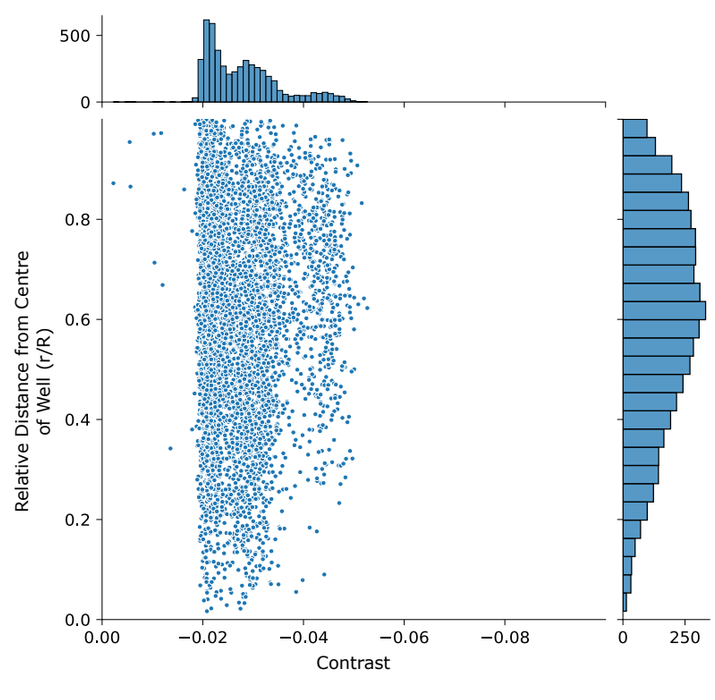
Contrast of Gold Nanoparticles measured on the CYTOP-ITO is 6 times lower than CYTOP-glass due the increased scattering from

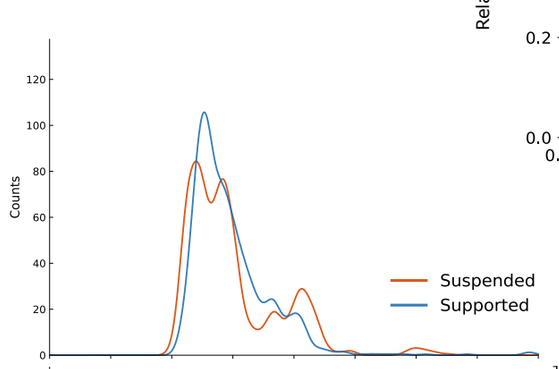


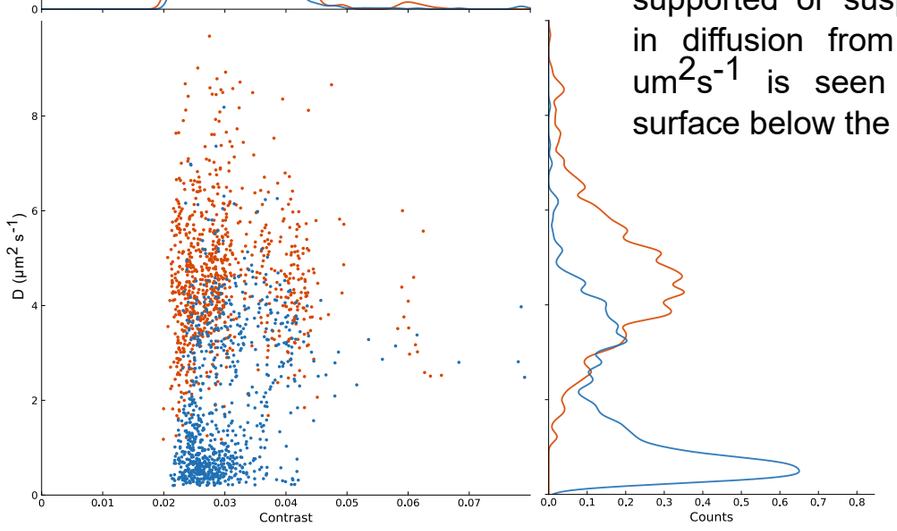
Label-free Tracking of DNA Origami on Suspended Bilayer

1MDa cholesterol linked DNA oligos inserted into suspended DPhPC bilayer and recorded at 1450 FPS within the CYTOP well.

Contrast of the particles remains consistent with radial position. However, they show a slight bias towards being



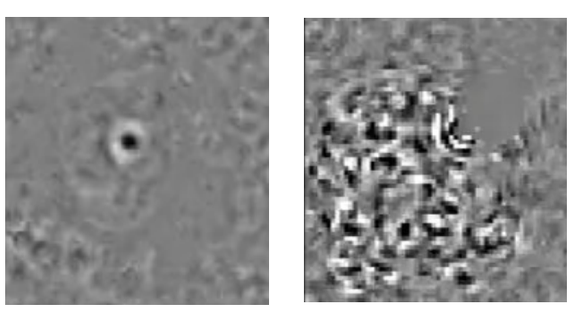




When trajectories segmented to supported or suspended, an increase in diffusion from 0.25 um^2s^{-1} to 5 um^2s^{-1} is seen due to removal of surface below the bilayer

Imagine of Alpha Hemolysin Clusters on Suspended Bilayer

Alpha hemolysin (aHL) was inserted into a suspended DPhPC bilayer and recorded
While unable to measure individual proteins, it would form transient measurable clusters
aHL also only inserted into the well



SUMMARY

1) Demonstrated ability to use CYTOP microstructures in MP experiments
2) Investigated how suspended lipid stiffness increases temporal noise
3) Tracked DNA origmi on suspended bilayer
4) Measured clusters of alpha hemolysin on a suspended bilayer

References

[1] Foley, E.D.B. et al. Nat. Methods 18, 1247-1252 (2021)
[2] Watanabe, R. et al. Nat. Commun. 5, 4519 (2014)

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