The Effect of Biologically Induced Aberrations in Single-Molecule Imaging

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Localisation microscopy (PALM/STORM) is a super-resolution technique that can achieve a precision of ~20 nm in all three spatial dimensions. A significant problem associated with this technique is that of optical aberrations, introduced when imaging beyond the coverslip, resulting in loss of precision [1]. In this study, we model and measure the effect of spherical aberrations, the most commonly occurring aberration in biological samples [2], on the performance of our system. Results were used to generate a protocol for optimising 2D localisation precision and tracking accuracy in measuring dynamics of a DNA-binding transcription factor (TF) for Notch pathway in *Drosophila* salivary glands. The nuclei were imaged at a depth of ~20 µm into live tissue, and improvement in signal to noise ratio was observed using lens of immersion media better matched to the sample and fine adjustment of the correction collar (Figure 1).

We also investigated the effect of spherical aberrations on astigmatic and double helix point spread functions (DHPSF; Figure 2). These PSFs gain 3D information through their axial asymmetry with defocus. Crucial to the precision of 3D localisation is the calibration of such PSFs. The interaction between defocus and spherical aberrations causes significant change to this calibration with error being introduced in all three spatial dimensions [3]. We used fluorescent beads and nanohole array to calibrate 3D PSF of the microscope and analysed precision and accuracy of localisations in the presence of spherical aberrations. We then used these calibration curves to extend the TF study and analyse its dynamics in 3D.

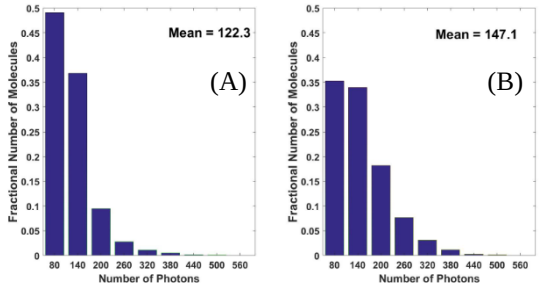

Figure 1: Number of photons per emitter when imaging in Drosophila salivary glands using A) water and B) silicone oil immersion objective

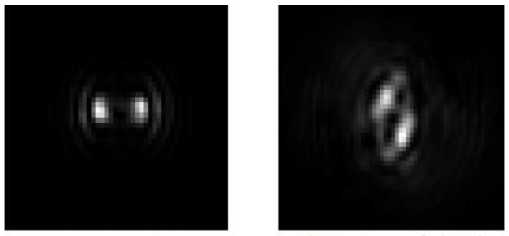

Figure 2: Effect of Spherical aberrations on DHPSF

References

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[3] A. von Diezmann, M. Y. Lee, M. D. Lew, and W. E. Moerner, "Correcting field-dependent aberrations with nanoscale accuracy in three-dimensional single-molecule localization microscopy," *Optica*, vol. 2, no. 11, p. 985, 2015.