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IT-3-O-2173 Effects of optical aberrations in single molecule and super resolution imaging

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The images created in a fluorescence microscope are imperfect because of aberrations caused by the various objects in the optical path. These aberrations may be due to both the microscope optics and the sample itself. More specifically, they may be due to imperfect optic design and manufacturing processes, imprecise alignment of optical components and mismatched and inhomogeneous refractive indices within the sample specimen. Aberrations caused by the sample are necessarily greater as we go deeper, particularly in heterogeneous biological samples. As a consequence, most single-molecule tracking and single-molecule based super-resolution imaging is performed in microscopes equipped with total internal reflection illumination, which limits fluorescence excitation to a thin layer a few hundred nanometres thick. The aberrations impose limitations on the resolving power of a fluorescence microscope, the localisation precision of super resolution imaging and the proportion of features detected in single molecule tracking analysis. Achieving the same high resolution throughout a 3D sample such as biological cells depends on correcting these aberrations and recovering a high signal-to-noise ratio in deeper layers, which can be achieved using adaptive optics. Correcting the aberrations is particularly important for measuring accurate distances within a 3D volume. Note that it is not, however, trivial to determine what the aberrations from each point in the sample are, particularly in widefield microscopes.

There are approximately ten significant low-order Zernike modes of aberration present in a standard fluorescence microscope. We have investigated the effects of these different aberrations on data analysis in single molecule imaging techniques such as tracking in live cells. In particular, we have studied the consequences of aberrations on single molecule detection rates and apparent intensities in the poor signal-to-noise ratio environment of biological cells. These have wide consequences for the accurate determination of, for example, stoichiometry, FRET and diffusion rates from single-molecule measurements.

We have also studied the impact of aberrations on data analysis in single molecule localisation super-resolution techniques, such as STORM/PALM imaging, with varying levels of noise. Aberrations directly affect the signal-to-noise ratio and hence the achievable localisation precision.

Lastly, both Gaussian and astigmatic point spread functions were considered in order to extend the improvements to three dimensional super resolution imaging and single molecule tracking.

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