Adaptive Optics for Single Molecule Switching Microscopy

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High resolution microscopy relies on the use of high quality optics with the goal of obtaining diffraction-limited operation, working at the physical limits imposed by the wavelength of the light. Yet in many cases this goal is not achieved as aberrations, distortions in the optical wavefront, blur the focus and reduce the resolution of the system. Aberrations can arise from imperfections in the optics, but are often introduced by the specimen, particularly when imaging thick specimens. Single molecule switching (SMS) super-resolution microscopes (e.g. PALM, STORM, GSDIM, etc.) are particularly susceptible to these effects, which usually limit the observable part of the specimen to a thin region near the surface. The methods of adaptive optics can be used to correct aberrations, increasing the depth at which effective super-resolution images can be obtained.

We investigate efficient methods of implementing adaptive optics in SMS microscopes. SMS microscopy is based on the principle that the location of a single fluorophore can be estimated with a much higher precision than the resolution of the system. Photoswitchable dyes are used such that a small number of well-separated molecules are activated, each of which can be individually located with high resolution. By cycling through a large number of subsets of fluorophores a complete image can be reconstructed. The fluorophores are localised by fitting a model of the microscope PSF to the image data. As aberrations affect the shape and intensity of the PSF, they also affect the fitting procedure in SMS microscopes, resulting in reduced localization precision or rejected fits.

We have implemented adaptive aberration correction in a custom built STORM microscope (Fig. 1). Aberration correction is performed using an efficient sequential image-based method applied to thick cell culture and tissue specimens. Corrected images show improvements in the number of localisations and effective resolution. We expect that correcting for system and sample induced aberrations will be crucial to extending the application of this microscope and related SMS methods to a wider range of specimens.

Fig. 1: STORM images of mouse embryonic stem cells, immuno-stained with Alexa 647. Left: before correction of specimen aberrations. Right: after correction. The resolution improved from 72nm to 63nm after correction. Simultaneously, the number of accepted single emitter fits increased from 20,437 to 48,281. Scale bar 1μm.