Adaptive optics for super-resolution microscopy

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Adaptive optics (AO) has been used in microscopes to compensate the effects of specimen induced aberrations [1]. These distortions of the optical wave front are compensated usually with a deformable mirror (DM) or a liquid crystal spatial light modulator (SLM), restoring image quality. These methods have been applied to different microscopes with various applications in biomedical imaging and other areas. AO has been demonstrated in a range of microscope modalities including conventional widefield microscopes as well as laser scanning systems. Adaptive microscopy is now being developed for super-resolution microscopes – or nanoscopes – which enable resolutions smaller than the diffraction limit of light. We report on specific recent developments in adaptive aberration correction for stochastic optical reconstruction microscopy (STORM) and stimulated emission depletion (STED) microscopy. STORM uses the stochastic switching of fluorophores to permit the localization of individual emitting molecules to a precision for smaller than its diffraction limited image. We have introduced adaptive optics to measure and compensate specimen-induced aberrations showing improved STORM imaging with cells at depths up to 10mm. Both 2D and 3D localization methods have been employed [2]. STED microscopy provides enhanced resolution of sub-cellular structures. Super-resolution is created by restricting the region of fluorescence emission to a size far smaller than the diffraction limited focus of the excitation laser beam. This is achieved by superimposing the excitation focus with a second structured depletion focus that has a region of zero intensity at the centre of a bright ring. This second focus forces excited molecules to undergo stimulated emission and in so doing prevent spontaneous fluorescence emission. The depletion focus is however susceptible to the effects of aberrations, leading to a filling in of the zero intensity or a change in intensity in the bright ring focus. This in turn leads to compromised resolution and a drop in fluorescence signal. We have developed AO methods to overcome these problems by correcting aberrations in tissue specimens [3].

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2. D. Burke *et al.*, “Adaptive optics correction of specimen-induced aberrations in single-molecule switching microscopy,” *Optica* **2**, 177-185 (2015).
3. T. Gould, *et al.*, “Adaptive optics enables 3D STED microscopy in aberrating specimens,” *Opt Express* **20**, 17137–17142, (2012).

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