DEMONSTRATION OF ADAPTIVE OPTICS METHODS IN A 4PI SINGLE MOLECULE SWITCHING MICROSCOPE

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KEYWORDS: Adaptive optics, 4Pi microscope, single molecule switching, sensorless, wavefront sensing

We present a 4Pi single molecule switching (SMS) microscope for ultra-high spatial resolution single molecule imaging enabled by adaptive optics (AO).

Super-resolution microscopes provide nanometre spatial resolution for cell biological studies; however, the axial resolution of standard SMS microscopes is inferior to the transverse resolution. By using two opposing objective lenses for coherent detection of fluorescent emission, a SMS microscope in 4Pi configuration enables ultra-high axial resolution with an improved signal collection efficiency. Due to the nature of 4Pi imaging, even a moderate sample thickness will inevitably introduce aberrations that affect the focusing performance of the system. More importantly, the aberrations experienced by the two arms of the 4Pi cavity are different and will vary differently as the imaging position moves axially. For these reasons, the axial resolution and imaging efficiency deteriorate quickly with depth in thick samples. This limits the axial imaging range and makes imaging large cells with uncompromised resolution impossible without compensating the depth dependent aberrations [1].

The nature of aberrations in a 4Pi cavity has been described and the effect on the system performance was studied [2]. Based on this knowledge, we demonstrate aberration correction methods using sensorless AO and sensor-based AO scheme. Both AO methods use two deformable mirrors (DM) in the microscope, one for each objective. For the sensorless correction, we devised a compact interferometer for accurate DM calibration and control, and we estimate the aberrations base on imaging metrics. For the sensor-based correction, we employed a wavefront sensor to measure the aberration prior to imaging directly. The AO methods are tested in the 4Pi SMS microscope for imaging whole biological cells. The performance of two methods are compared; the cons and pros of each method are discussed.