4PI ABERRATION CORRECTION FOR DEEP IMAGING WITH A 4PI SINGLE MOLECULE SUPER-RESOLUTION MICROSCOPE

Jingyu Wang[1], Jacopo Antonello[1], Mick Phillips[2], Chao He[1], Ian Dobbie[2], Martin J Booth[1]

[1]Department of Engineering Science, University of Oxford, UK

[2]Micron Oxford Advanced Bioimaging Unit, Department of Biochemistry, University of Oxford

Email: jingyu.wang@eng.ox.ac.uk

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We present a unique 4Pi adaptive optics method on a 4Pi single molecule switching (SMS) microscope for ultra-high spatial resolution single molecule imaging in thick samples.

Single molecule switching (SMS) microscopy can provide nanometre spatial resolution for biological studies in cells; however, the axial resolution of standard SMS microscopes is inferior to the transverse resolution. By using two opposing objective lenses for coherent detection, 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 imaging paths of the 4Pi configuration are different and will vary differently as the imaging position moves in 3D. For these reasons, the axial resolution and imaging efficiency deteriorate significantly in samples, even over the thickness of a single cell. This limits the axial imaging range and thus makes imaging large cells or tissues with uncompromised resolution impossible without compensating the sample induced aberrations[1].

The nature of aberrations in a 4Pi cavity has been described together with the effect on the system performance and a new mathematical representation of these aberrations - 4pi aberration modes - has been introduced[2]. Based on this knowledge, we experimentally demonstrate aberration correction methods using a sensorless Adaptive Optics (AO) approach based for 4Pi aberration modes. A pair of deformable mirrors (DM) are employed in the microscope, one for each objective, and they work together as a single device to control a set of 4Pi aberrated phase that is acquired from the two back pupils of the objectives. We estimate the aberrations based on unique interferometric imaging metrics and compare the performance of this method with correction of separate paths[1] for cell imaging on a 4Pi SMS microscope. This method produces consistent spatial resolutions in a large axial range for volumetric imaging, which will be extremely valuable in imaging thick cells and tissues.

[1] Huang, F., Sirinakis, G., et al., 2016. “Ultra-high resolution 3D imaging of whole cells”. *Cell*, *166*(4), pp.1028-1040 (2016).

[2] Xiang Hao, Jacopo Antonello, Edward S. Allgeyer, Joerg Bewersdorf, and Martin J. Booth, "Aberrations in 4Pi Microscopy," *Opt. Express* 25, 14049-14058 (2017)