ADAPTIVE OPTICS WITH A DEFORMABLE MIRROR FOR CONFOCAL AND STED MICROSCOPY

Jörn Heine, Janina Hanne, Tobias Weihs, Christian Wurm, Benjamin Harke, Markus Köhler, Andreas Schönle, Matthias Reuss
Abberior Instruments GmbH
Hans-Adolf-Krebs-Weg 1
37077 Göttingen, Germany,
Email: m.reuss@abberior-instruments.com

KEY WORDS: Adaptive Optics, Deformable Mirror, Aberration Correction, Fluorescence Microscopy

It is often overlooked that the last part of the optics of a microscope is the sample itself. While the optical train of a light microscope up to and including the objective lens is carefully engineered to ensure perfect imaging, the sample itself is usually less controlled and in general not optimal for imaging. A number of measures [1, 2] can be taken to make sure that the index of refraction of the immersion medium matches the one of the sample as close as possible, but they are experimentally intense and might generate artefacts.

Here, we present an adaptive optics system [3] based on a deformable mirror. It corrects for spherical aberrations (usually the source of imaging imperfections) without the need to perfectly match embedding media. Thus, the adaptive optics system allows to perform imaging with any objective lens (air, water, oil, …) on any sample. Once set up, the system automatically follows along the change in focusing depth in order to ensure perfect conditions during refocusing or when recording z-stacks. Other aberrations such as coma and astigmatism are corrected as well.

With this, we record confocal, 2D- and 3D-STED stacks hundreds of microns deep inside the sample. In particular 3D-STED is highly susceptible to spherical aberrations. Where imaging without adaptive optics is possible only a few microns deep [4], with our adaptive optics systems we achieve excellent 3D-STED resolution and signal up to 120 µm deep inside the sample. For confocal imaging, we increase brightness dramatically, e.g. by up to five-fold when focusing 250 µm deep.

[1] Staudt, Thorsten, et al. "2, 2′-thiodiethanol: a new water soluble mounting medium for high resolution optical microscopy." Microscopy research and technique 70.1 (2007): 1-9.

[2] Richardson, D. S., and Lichtman, J. W. “Clarifying tissue clearing.” Cell 162 (2015), 246–257.

[3] Booth, Martin J. "Adaptive optics in microscopy." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 365.1861 (2007): 2829-2843.

[4] Heine, Jörn, et al. "Three dimensional live-cell STED microscopy at increased depth using a water immersion objective." Review of Scientific Instruments 89.5 (2018): 053701.