IN-DEPTH IMAGING OF THICK INHOMOGENEOUS SAMPLES USING AN ADAPTIVE SPIM

Javier Morgado Brajones[1], Grégory Clouvel[2], Guillaume Dovillaire[2], Xavier Levecq[2], Corinne Lorenzo[1]

[1] ITAV, Université de Toulouse, CNRS, UPS, France
[2] Imagine Optic, Orsay, France
E-mail: javier.morgado@itav.fr

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High-quality in-depth imaging of three-dimensional samples remains a major challenge on modern microscopy. Selective Plane Illumination Microscopy (SPIM) is a widely used technique that allows the imaging of living tissues with subcellular resolution. However, scattering, absorption and optical aberrations limit the depth at which useful imaging can be done. In complex samples, refractive index differences in and between cells are the main source of optical aberration. Adaptive optics (AO), originally developed for astronomical telescopes, is a method capable of measuring and correcting aberrations, thereby improving the performance of the optical system. Over the past decade, AO has seen some acceptance in microscopy and has proven a valuable tool for the correction of aberrations in different kinds of fluorescence microscopes.

We have incorporated a wavefront sensor adaptive optics scheme to SPIM ($_{WAO}$SPIM) to correct aberrations induced by optically thick samples such as Multi Cellular Tumor Spheroids (MCTS) [1]. Spatial variations of aberrations within such inhomogeneous, non-transparent samples causes major changes to the light path and limits the quality of AO correction outside a small region around the guide star. Thus, guide star placement plays a critical role in the AO process. Two-photon fluorescence provides us with a tool to produce a non-linear guide star in any region of the field of view by means of focusing a femtosecond NIR laser into the sample. Still, the non-transparency of the sample limits the depth at which corrections can be made. In recent research, this approach has been successfully applied to highly transparent samples [2]. In this work, we show the correction capabilities of $_{WAO}$SPIM using non-linear guide stars in thick, inhomogeneous samples like MCTS.

REFERENCES

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