Adaptive optics two-photon light-sheet microscopy

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There is growing evidence that the three-dimensional microenvironment influences cellular pathways and gene expression, which play critical roles in health and disease. However, studying cellular architecture and dynamics in three-dimensional environments requires microscopes with rapid volumetric acquisition rate, isotropic spatial resolution and high optical penetration depth. Light-sheet microscopy can address some of these challenges, but in its traditional form using Gaussian optics, high axial resolution comes at the cost of small field of views due to beam divergence. Further, light-sheet microscopy uses widefield image formation, which limits its optical penetration depth. Here we address these two challenges by a new type of light-sheet generation and adaptive optics (AO) in both the illumination and detection path.

Bessel beams, especially when coherently superimposed as in Lattice light-sheet microscopy (LLSM), can overcome to a certain extend the limitations imposed by beam divergence. This comes at the cost of sidelobe structures that reduce the excitation confinement. For high aspect ratio light-sheets, this can lead to excessive out-of-focus fluorescence excitation.

Here we present to our knowledge a new class of light-sheets created by a pupil phase filter and two-photon absorption, which offer higher excitation confinement than one photon techniques including LLSM. The use of a spatial light modulator (SLM) allows us to tailor the field of view to a specific imaging application by changing the phase filter. The same SLM is used to compensate optical aberrations via iterative AO. Once the excitation beam is optimized, we correct the wavefront in the detection path by optimizing the observed modulation depth of a stripe pattern that is written with the two-photon beam.

We present imaging results of cancer cells embedded in 3D microenvironments and xenografted into zebrafish embryos (Fig.1). We believe that flexible adjustments of the field of view, while providing high axial resolution and an excellent excitation confinement, and correction of optical aberrations are critical feature for successful imaging in complex 3D environments.

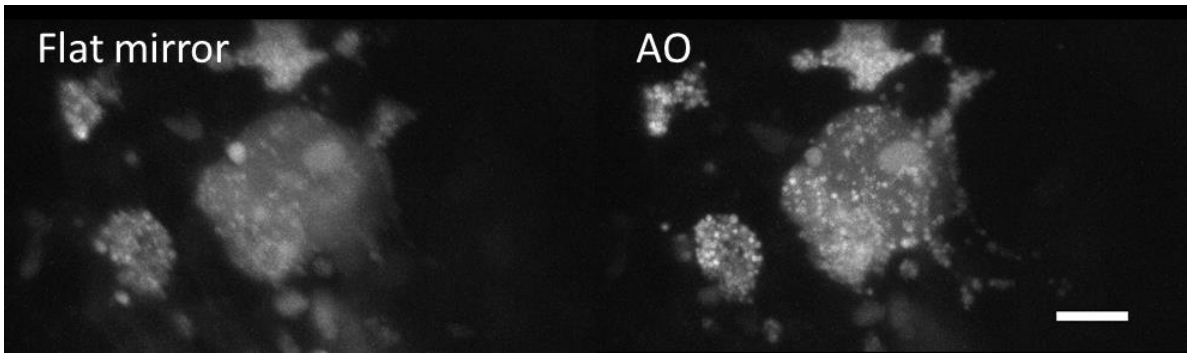

Figure 1: Maximum intensity projection of Melanoma cells in the tail of a Zebrafish as imaged with two-photon light-sheet microscopy without (left) and with (right) adaptive optics. Scale bar: 10 microns