High resolution lattice light-sheet microscopy in thick samples

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Lattice light-sheet microscopy (LLSM) stands out as the most successful and performant imaging technique to study 3D sub-cellular dynamic mechanisms in live thin samples[1]. In order to maintain a very high imaging quality in optically thick samples, Liu *et al.* developed an adaptive optics (AO)-LLSM[2] that corrects for sample induced optical aberrations occurring on the illumination and imaging paths. They were able to acquire fast volumetric events at very high spatial resolution within large samples, but to the cost of a complex and expensive instrument.

Here we propose an alternative, simpler, and cost-effective method to maintain sub-micrometric resolution deep into thick samples. We added a deformable mirror (DM) to the detection path of our LLSM[3] and developed a two-step active image optimization procedure: (1) light-sheet autofocus (AF), and (2) optimization of the DM shape to correct wavefront distortion (AO). Both AF and AO steps are based on an indirect, sensorless image-based process. We propose a fast and efficient AO optimization algorithm that minimizes the number of required images to provide an accurate correction. Several image metrics and iterative process were tested on a wide variety of samples. The optimized AO process provides a good robustness to photobleaching.

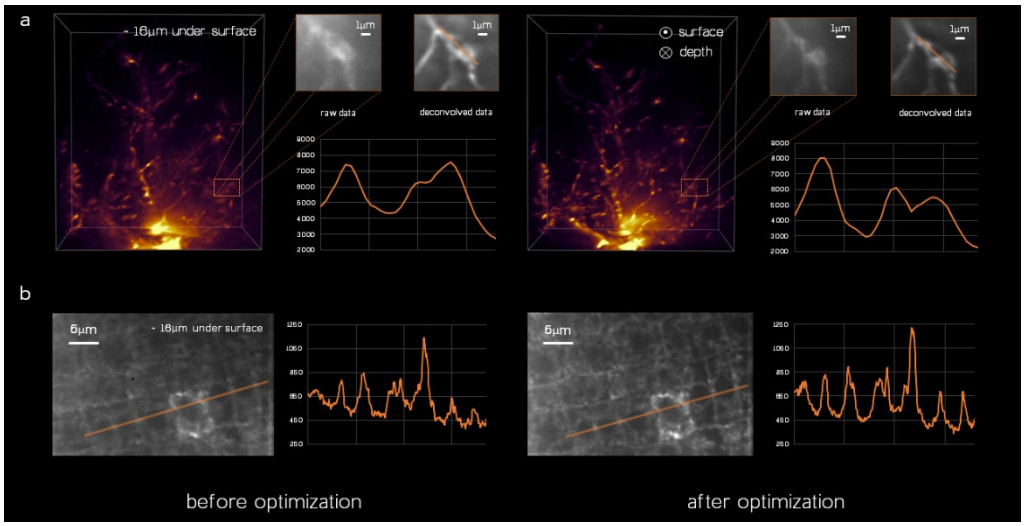

Fig 1: Examples of improved imaging quality on various samples. (a) 3D reconstruction of an astrocyte expressing cytosolic GFP in a fixed organotypic mouse brain slice, ~16 µm below the surface, before (left) and after (right) the optimization. Line intensity profiles show resolution and intensity increases (raw data). (b) 2D images of live Arabidopsis root cells labelled with FM4-64, (before (left) and after (right) optimization (deconvolved data). Line intensity profiles show resolution and intensity increases.

To test our AO technique, we artificially induced known aberrations on various non-aberrating samples (beads, HEK cells, neurons at the surface of brain slices). Our aberration correction method recovered a wavefront flatness of < 50 nm RMS, typically within < 40 s. In thicker samples we demonstrated that both AF and AO steps are required to significantly increase brightness and resolution as shown in brain slices (Fig 1.a) and in Arabidopsis root (Fig 1.b).

Even though our technique does not correct aberrations on the excitation path, we can still significantly improve LLSM image quality and thus extend its range of applications.

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