ACTIVE IMAGE OPTIMIZATION FOR LATTICE LIGHT-SHEET MICROSCOPY

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Among the wide family of light-sheet imaging instruments, lattice light-sheet microscopy (LLSM) stands out as the most successful and performant solution to image dynamic cellular mechanisms in 3D in live samples.[1] However, according to our experience[2], the original LLSM design presents two limitations: (1) a very precise (< 0.2 µm) and regular co-alignment of the light sheet and imaging planes is required to optimize spatial resolution, and (2) the imaging depth is limited to a few tens of microns in part because of sample-based optical aberrations. To address these issues Liu *et al* developed an adaptive optics (AO)-LLSM that corrects aberrations both in the excitation and detection paths [3]. They were able to acquire very high quality images in depth in thick biological samples, but to the cost of a very complex and expensive instrument.
Here, we present a simple and cost effective method to maintain high resolution with LLSM independently of imaging depth or duration. First, we added a deformable mirror (DM) in the imaging path. Then, we developed a two-step image optimization procedure that (1) autofocus the light sheet and (2) optimize the DM shape to maximize an image metric using a 3N algorithm [4] (AO step). We tested several metrics and 3N ranges on standard fluorescent samples. We observed that the autofocus step is not sufficient to fully recover image quality. The AO step significantly improves image resolution down to 60 µm inside brain slices (Fig 1). We also quantified the aberration Zernicke modes as a function of depth.

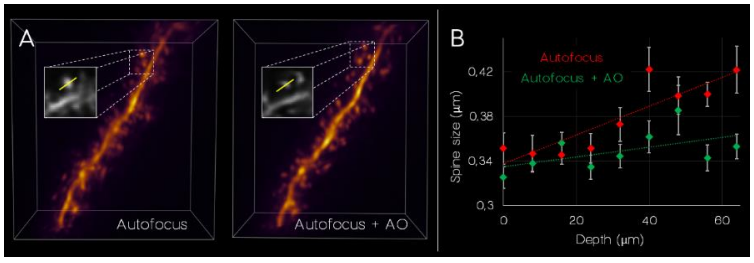

Figure 1: (A) 3D reconstruction of a mCherry labelled dendrite in a live brain slice before (left) and after (right) AO aberration correction at ~40 µm depth. Insets: spines head sizes measured as line intensity profile FWHM. (B) Average spines sizes (n=10), before (red) and after (green) AO, as a function of depth.

Overall, we believe that our simple image optimization method increases the reliability and the range of applications of LLSM.

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