LIGHT SHEET FLUORESCENCE MICROSCOPY WITH AN LED

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Light emitting Diodes (LED) are usually not considered a good light source for Light Sheet Fluorescence Microcopy because their spatial incoherence makes it difficult to focus the light tightly along one direction. We propose to illuminate a selective volume of the sample with LEDs and we spatially modulate light over said volume using a spatial light modulator: by acquiring N illumination patterns and solving an inverse problem, we reconstruct N planes of the sample, as in LSFM.

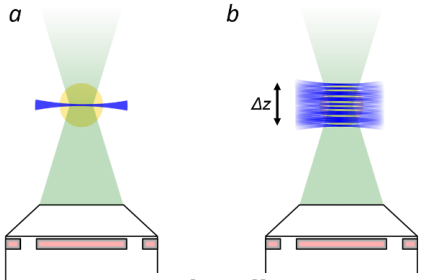

Figure 1: The illumination is confined to a single plane in LSFM (a). Illuminated volume in our setup: the entire depth of field of the detection objective is illuminated and axially modulated

Besides the advantage of color availability and low cost, the LEDs do not form speckle patterns on the sample, avoiding the shadowing artifacts typical of light sheet microscopy. We demonstrate this by imaging chemically cleared fluorescent mouse and adult zebrafish brains and zebrafish embryos in vivo. This approach can only reconstruct a thin volume of the sample [1]. In order to extend the reconstructed volume we scan the illumination pattern along the direction of illumination axis, using a fast translating stage. By synchronizing the motion with the acquisition of a CMOS camera, we show a reconstruction of a 3mmX3mmX0.1mm with isotropic resolution (2.5µm). Furthermore, we propose the use of the spatial modulation for Compressive Sensing, a signal analysis technique that reduces the number of modulation patterns to be aquired. The technique yields an accurate reconstruction of the sample anatomy even at significant compression ratios, achieving higher volumetric acquisition rate and eventually reducing photodamage on the biological samples.

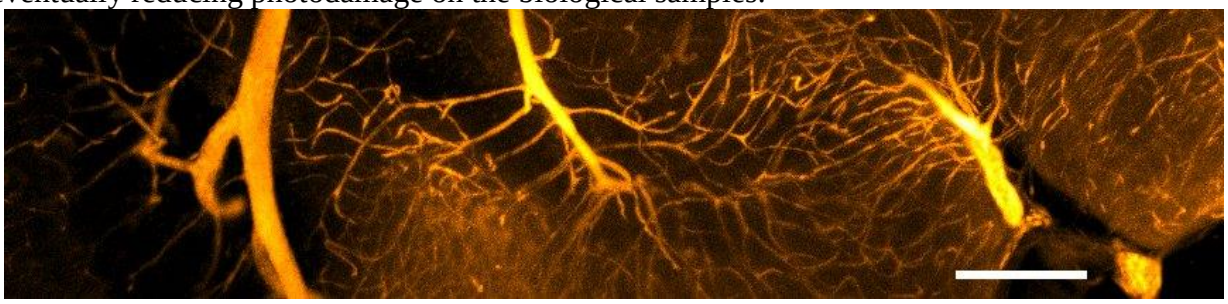
Figure 2: Reconstruction of a region of a chemically cleared (3DISCO) adult zebrafish brain. Scalebar is 200µm

[1] Calisesi, Gianmaria, et al. "Spatially modulated illumination allows for light sheet fluorescence microscopy with an incoherent source and compressive sensing." *Biomedical Optics Express* 10.11 (2019): 5776-5788.