N°46: Bimodal trans-scale imaging of cleared mice embryo with adaptive optics confocal and light-sheet microscopy

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Tissue clearing is nowadays widespread and improves considerably the depth of optical microscopy. However, the high resolution imaging of cellular and matrix structures inside cleared tissues faces two issues: locating a region of interest in large 3D volume and correcting for optical aberrations coming from the tissue geometry and the refractive index mismatch. Here we address them by combining two complementary imaging modalities applied to the cleared mouse embryo: light sheet 3D imaging and adaptive optics in confocal and two-photon (2P) microscopy. The former produces a low spatial resolution (5-10 µm) image, while the latter one corrects for the local geometrical aberrations and approaches the diffraction limit in situ (200-300 nm).

We will first create the "optical landmarks" using the enhancement of endogenous fluorescence around 2P damage sites[1]. These references, imaged by both techniques, allow further triangulation of the structure of interest in 3D, so we will image the whole embryo with the landmarks in a few minutes with the lightsheet microscope. Then we will transfer the specimen to the confocal microscope and perform local optimization of this structure using the adaptive optics[2] in a sensorless mode. We will compare the performance of two metrics: a 2P 'guide star' intensity and molecular brightness measured by the fluorescence fluctuation spectroscopy[3]. In case of using a long working distance water immersion objective in TDE clearing medium (RI 1.5), the obtained in depth isoplanatic patch volume is only few hundreds µm^3. We will calibrate it using a fluorescent clearing solution and acquire a confocal Z-stack of the cranial oculomotor nerve (III) axons fasciculation, revealing how sub-populations of motor axons originate from individual neurons and group to form the main nerve trunk.

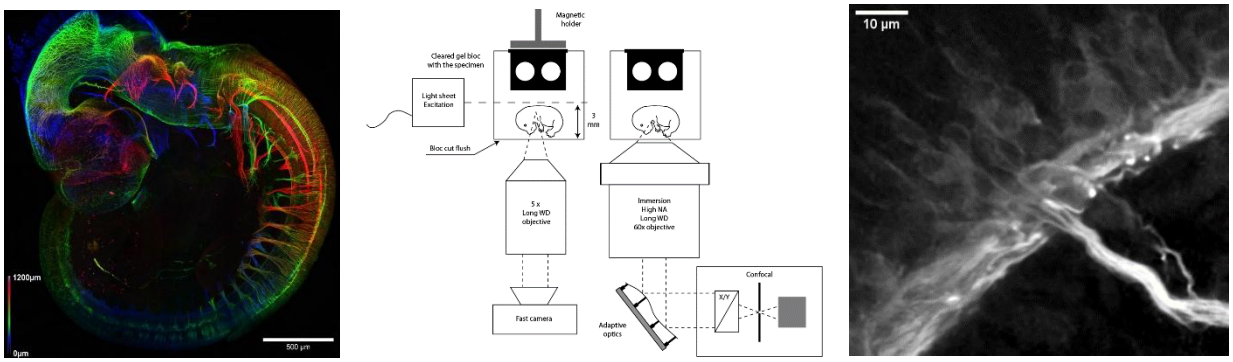

Figure. Schematic presentation of the trans-scale imaging pipeline. A rapid mapping of the fluorescence distribution is performed with a compact light-sheet device (left). The geometric optical aberrations are corrected at the vicinity of the nerve and a high resolution image of the neurons is acquired (right).

The attendees will operate the state of the art light-sheet and adaptive confocal microscope in order to compensate optical aberrations, to calibrate the confocal volume and to image the subcellular tissue structures. Advantages and shortcomings of the used technologies will be discussed.

Prerequisites: basic knowledge in confocal, two-photon & epifluorescence microscopy.

1. Pestov, D., et al. (2010). "Photobleaching and photoenhancement of endogenous fluorescence observed in two-photon microscopy with broadband laser sources." Journal of Optics 12(8).
2. Booth, M. J. (2007). "Adaptive optics in microscopy." Philos Trans A Math Phys Eng Sci 365(1861): 2829-2843.
3. Leroux, C. E., et al. (2011). "Adaptive optics for fluorescence correlation spectroscopy." Opt Express **19**(27): 26839-.
4. Bjorke, B., et al. (2021). "Oculomotor nerve guidance and terminal branching requires interactions with differentiating extraocular muscles." Dev Biol **476**: 272-281.