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M-Cube — towards correlative multi-scale light-sheet imaging using a compact, modular and moving illuminator

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Abstract. The crossed arrangement of excitation and collection optics is *the* defining feature of selective plane illumination microscopes. It results in an axial sectioning given by the thickness of the light sheet, whereas the lateral resolution depends on the numerical aperture of the collection optics. One disadvantage of this optical scheme is that it has been difficult to image large fields-of-view at high spatial resolution. Yet, it is often not necessary to image the entire sample at high resolution. Instead, a zoom from mm-scale over-views to regions-of-interest that are imaged at µm is often sufficient, e.g., for studying neuronal networks that simultaneously comprise cm-long connections and tiny (sub-µm) synaptic contacts, spanning 6 orders of magnitude. Observations over such different spatial scales typically require the use of different instruments. We here describe our ongoing efforts to build and characterise a compact light-sheet module designed for correlative micro- meso- and macroscopic imaging. Its particularity is that moves with the sample between micro- and macroscopic imaging arms. Based on a compact illuminator and flexible sample carrier the module is mounted on a motorised long-range, high-precision microscope table. The ability to perform seamless back-and-forth multi-scale imaging comes from a strict registering of image coordinates.

1 Introduction

Tissue clearing, along with progress in 3-D fluorescence imaging has prompted a revival of (neuro-) anatomy. The workhorse in such studies is the light-sheet microscope, the defining feature of which is a crossed arrangement of the excitation and collection arms. However, this arrangement trades off field-of-view against spatial resolution. This is problematic, as brain structures, for example, span spatial scales of more than six orders of magnitude — from blood vessels and neuronal networks linking areas centimetres apart to tiny compartments like dendritic spine shafts or astrocyte peripheral processes, which measure little more than a few tens of nm. Imaging such structures at multiple scales, in a continuous manner, from cm-scale overviews to sub-μm detail would provide a powerful tool to neurobiologists, but correlative multi-scale imaging has been difficult to implement on a single instrument.

We here report on our ongoing work [1] reconciling the opposing requirements of large-and small-scale imaging by consistently moving both the sample and a compact, modular and reconfigurable light-sheet module between two independent collection pathways. Our modular design offers a flexible and platform for correlative micro-, meso-, and macroscopic observation (M-Cube) that can be upgraded to accommodate coming modalities.

2 Experimental details

Our light-sheet illuminator consists of two modules and a central sample holder, co-axially mounted on a motorised precision vertical translation stage (Märzhäuser). A 4-line laser combiner (445-, 488-, 561- and 638-nm, respectively, Oxxius) is fibre-coupled to the two arms of the beam-shaping optics, **Fig. 1a**. Each module houses an achromat and a convex cylindrical lens as well as an adjustable horizontal slit aperture (all from Thorlabs), mounted on a x, y, θ, ϕ translation/tip/tilt table, **Fig. 1b**. The L-shaped sample carrier moves manually in x,y (for choosing the precise sample region illuminated) and axially, in z, (motorised, Physik Instrumente). Custom machined holders and cubes of different format can accommodate various samples, **Fig. 1c**. Some holders also offer a rotational degree of freedom around the optical axis (ω).

The entire light-sheet module holds on a 60 cm by 12 cm footprint and it weighs less than 4 kg. It moves rapidly and reproducibly between the micro- and macroscopic collection arms, **Fig. 1d**, on a long-range micro-position-ing table (Märzhäuser) under computer or joystick control, thereby offering a common coordinate system. Illumination can arrive from ±y, either simultaneously or alternately. The two light-sheets are first co-aligned and equilibrated in intensity by aid of a dedicated cuvette bearing an isosceles prism, **Fig. 1e**. A custom

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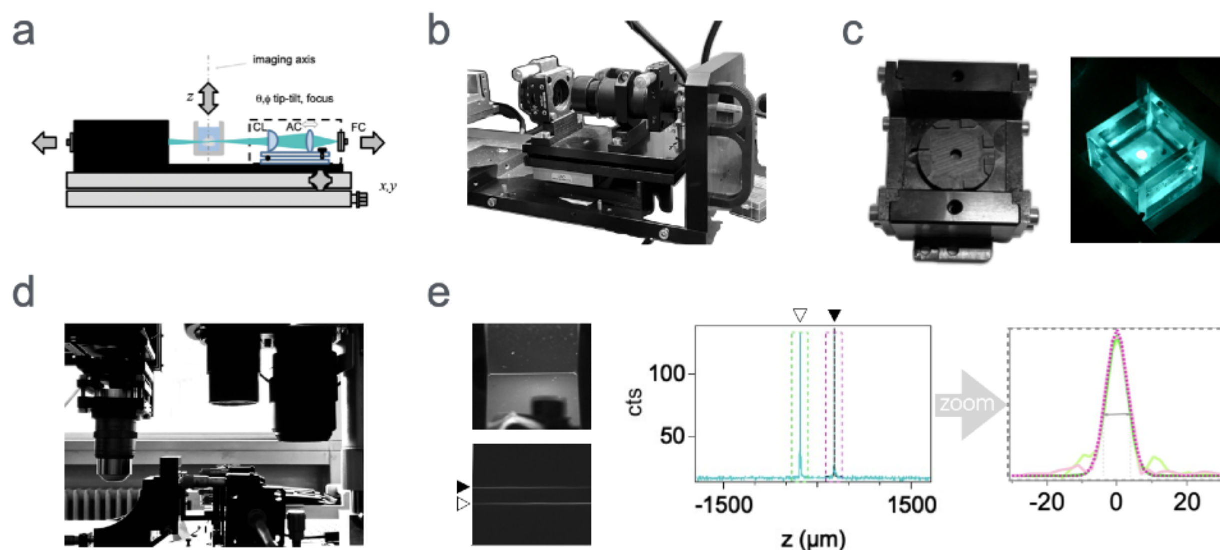

Fig. 1. *A modular light-sheet illuminator.* (a), coordinate system and scheme of the excitation optical path. CL - cylindrical lens, AC - achromatic lens, FC - fibre coupler. (b) photograph of one of the illumination modules. (c), sample holders. (d), arrangement of the illuminator (optical axis orthogonal to the page plane) and the micro (*left*) and two macro-objectives (*right*). (e), *Top left*, top view of the isosceles prism used for light-sheet alignement. *Bottom left*, two co-linear light-sheets. *Right*, intensity profiles and Gaussian fits on the zoomed image. FWHM shown is 10.1±0.2 µm. Green and turquoise pseudo colour label the left and right lightsheet, respectively.

IMAGEJ macro allows the user to overlay and blend the acquired images obtained by alternate illumination.

For the low-magnification imaging arm, we used a commercial upright macroscope (MVX10 MacroView, Olympus), with a ×1/NA0.25 or ×2/NA0.5 objective, a sCMOS camera (edge, PCO), and a motorised focus drive mounted on the fine focus wheel (Märzhäuser).

An upright microscope was assembled from optical bench components, based on the Thorlabs 2” rail system and custom pieces. For the images shown, we employed a ×25/NA1.05 water objective (Nikon) and ZEISS tube-lens assembly. A multi-thread adaptor allows other choices. Images are captured synchronizing the focus with the sample *z*-stage, on a sCMOS Camera (edge, PCO). Peripherals are controlled by μMANAGER [2].

3 Results

We first imaged a 45°-oblique pyranine-labeled tissue paper [3] and compared the image quality obtained upon epifluorescence and light-sheet illumination, **Fig. 2a**. LS illumination increased Michelson’s contrast on macro-images from C_M = 0.65 for EPI (t_{exp} = 50 ms) to 0.995 for light-sheet illumination (20 ms). We then chemically clarified a stretch of mouse gut using our novel, in-house developed universal, ultrafast clearing technique [4]. Due to its optical density and multi-layered cytoarchitecture, the digestive tube wall is challenging to image, and 3-D imaging of non-sectioned tissue is essential to appreciate its full complexity [5]. **Fig. 2b** shows mucosal micro vessels after dye perfusion (FITC).

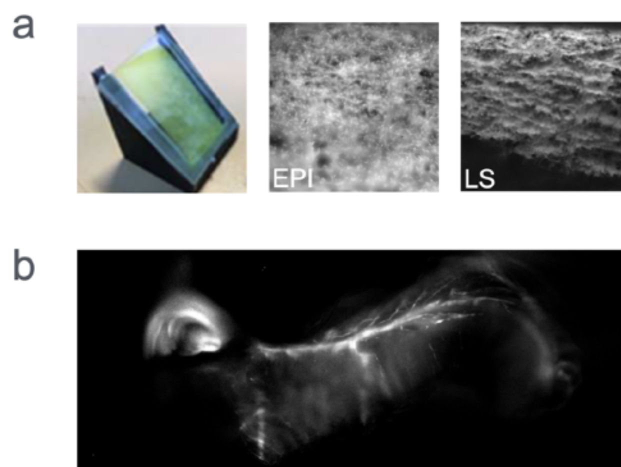

Fig. 2. *Examples*. (a), *left*, fluorescent oblique paper, *middle* and *right*, max-projections of EPI and light-sheet image stacks. (b), max-projection of FITC-dextran labelled intestinal blood vasculature. λ_{ex} = 488 nm, dz = 5 μm, total z-range 1 mm.

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