The mesoSPIM initiative: open-source light-sheet mesoscopes for imaging in cleared tissue

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Over the course of the past decade, tissue clearing methods have reached a high level of sophistication with a wide variety of approaches now available[1]. To image large cleared samples, light-sheet microscopes have proven to be ideal due to their excellent optical sectioning capability in transparent tissue[2]. Such instruments have recently seen extensive technological and commercial development. However, despite this progress, the community is lacking instruments capable of exploring large samples with near-isotropic resolution within minutes. Here, we introduce the mesoscale selective plane-illumination microscopy (mesoSPIM) initiative, an open-hardware project that provides researchers with instructions and software to easily build and operate light-sheet microscopes for centimeter-sized cleared samples (http://www.mesospim.org).

A wide range of commercial light-sheet microscopes have become available since the invention of SPIM[3], most of them optimized towards time-lapse imaging in transparent developing embryos or – if designed for cleared tissue – tailored for only a narrow selection of clearing techniques and immersion media. To overcome these limitations, we set out to design a modular light-sheet mesoscope (Fig. 1a) that combines simple and versatile sample handling with large fields-of-view (FOV) of 2-20 mm. Large-FOV light-sheet microscopes typically suffer from non-uniform axial resolution due to the varying thickness of the light sheet (Fig. 1b). To address this issue, we use a tunable lens to shift the excitation beam waist through the sample in synchrony with the rolling shutter of the camera, a method called 'axially scanned light-sheet microscopy' (ASLM)[4] (Fig. 1b). For whole mouse brains ($\approx$ 1 cm^3), typical datasets are isotropic (6.5 µm sampling), small (12-16 GB), and they are recorded quickly (7-8 minutes) with minimal shadow artifacts. Together with standardized quick-exchange holders, these features allow for the fast screening of samples. With a travel range of 44×44×100 mm, very large samples such as a whole mouse central nervous system can be imaged. After acquiring overview datasets, users can zoom in and record multidimensional

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data at higher resolution by mosaic acquisitions, for example revealing cellular distribution and long-range axonal projections of Purkinje cells in the mouse cerebellum (Fig. 1c-e) or fine neurites in the developing nervous system of a chick embryo (Fig. 1f). The instrument has been tested in combination with all common clearing methods ranging from CLARITY[5], CUBIC[6] to organic solvent approaches such as iDISCO[7] and BABB[2]. Due to the modular design of the mesoSPIM, switching between different imaging media can be done in less than a minute. The mesoSPIM setup, performance characterization, and example applications are presented with detail in Supplementary Notes including Supplementary Figures 1-30. Inspired by the openSPIM[8] and openSPIN[9] projects, documentation for the mesoSPIM hardware and software (written in Python) is freely available (https://github.com/mesoSPIM). Currently, there are 5 mesoSPIM setups in operation across Europe. The mesoSPIM is the ideal instrument to quickly bridge scales from the µm- to the cm-level, and serves as an excellent tool for detailed three-dimensional anatomical investigations in neuroscience and developmental biology.

Code availability

The mesoSPIM software and documentation are available on Github (https://github.com/mesoSPIM). mesoSPIM-control is licensed under the GNU General Public License v3.0 (GPL v3).

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Author information - Contributions

F.F.V.: planned the project, designed the microscope, wrote control software and documentation, set up several mesoSPIM instruments, coordinated the mesoSPIM initiative, imaged samples, analyzed data and wrote the manuscript.

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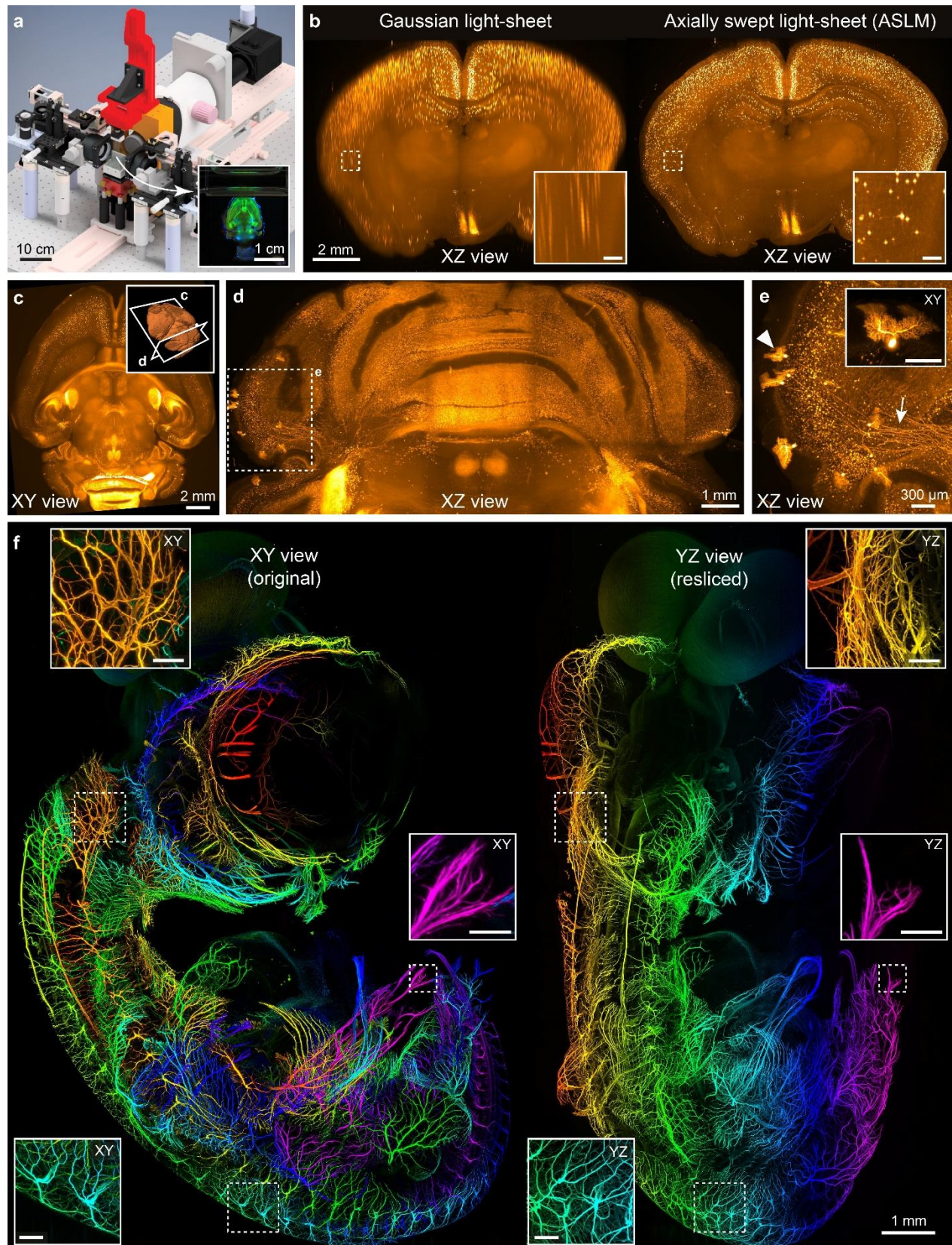
Figure 1: Example demonstrations of the mesoSPIM light-sheet mesoscope. **a**) Overview of the mesoSPIM instrument. Inset: Photograph of a Thy1-YFP mouse brain during image acquisition. **b**) Comparison of axial image quality achieved in a CLARITY-cleared VIP-tdTomato mouse brain for scanning with a Gaussian beam (left) and for the axially swept light-sheet mode, ASLM (right).   Images are maximum intensity projections over 250-µm range. **c**) Overview image of a CLARITY-cleared TPH2Cre-tdTomato mouse brain. Inset shows 3D orientation of views in c and d. **d**) XZ maximum intensity projection of a high-resolution dataset (4× zoom, range: 500 µm) taken in the cerebellum of the sample in c. **e**) Volume rendering of sparsely labeled Purkinje cells and their axonal projections (arrow). Inset: individual Purkinje cell (arrow head, scale bar: 200 µm). **f**) Depth-coded original XY (left) and resliced YZ (right) projections of a dataset taken from a 7-day old chicken embryo (neurofilament labeling) cleared using BABB. Throughout the dataset (acquired at $1.6 \times 1.6 \times 2$ µm^3 sampling), neurites are visible in great detail. Because of the ASLM mode this same holds true for the original (transverse) and the resliced (axial) direction. The assignment of color to Z-position is similar for both the XY and YZ view. Scale bars of all insets: 200 µm.