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

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Light-sheet microscopy is a ideal technique for imaging large cleared samples; however, the community is still lacking instruments capable of producing volumetric images of centimeter-sized cleared samples with near-isotropic resolution within minutes. Here, we introduce the mesoscale selective plane-illumination microscopy initiative, an open-hardware project for building and operating a light-sheet microscope that addresses these challenges and is compatible with any type of cleared or expanded sample (www.mesospim.org).

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. Common clearing techniques fall into two categories: approaches using organic solvents, for example the DISCO family of protocols, and methods using aqueous solutions such as CLARITY and CUBIC. To image samples processed with these methods, a wide range of commercial light-sheet microscopes can be used (Supplementary Note 1). Nonetheless, many users still experience substantial shortcomings when using existing instruments to image cleared samples: for example, the imaging chamber, sample holders and sample stages of microscopes designed for time-lapse imaging in developing embryos are usually undersized for centimeter-sized cleared samples. Even if the setup was specifically designed with clearing in mind, accommodating large samples can be challenging; modern clearing techniques can render a whole mouse central nervous system (CNS) or even entire mice transparent; yet, there are no instruments capable of imaging such samples without cutting. In addition, many instruments achieve optimal image quality only for a limited selection of immersion media, often restricted by the specifications of existing microscope objectives. As typical refractive indices in light-sheet microscopy range from 1.33 (for water) to 1.56 (for a mixture of benzyl alcohol and benzyl benzoate, BABB), this limits several commercial microscopes to a narrow subset of clearing techniques (Supplementary Table 1).

To overcome these limitations, we set out to design a modular light-sheet microscope that combines a large imaging volume, excellent image quality over large fields-of-view (FOV) with simple and versatile sample handling (Fig. 1a, Supplementary Note 2 and Supplementary Video 1). To allow multi-view acquisitions, we adapted an instrument layout similar to the original selective plane-illumination microscope (SPIM)10 with a horizontal detection path and a vertical sample rotation axis (Supplementary Figs. 1 and 2). The instrument is equipped with a zoom microscope in the detection path that allows a FOV of 2–21 mm in combination with a scientific complementary metal-oxide-semiconductor camera (scCMOS). This enables users to view large samples and then zoom in to reveal minute details such as individual axons (Fig. 1c–e). Therefore, we have termed the instrument the mesoscale selective plane-illumination microscope (mesoSPIM).

To streamline sample handling, we use magnetic quick-exchange mounts for the immersion cuvettes and sample holders. These mounts allow rapid switching between different immersion media and samples in less than a minute (Supplementary Video 2). Samples are usually mounted in a cuvette or clamped in a threedimensionally printed holder (Fig. 1a and Supplementary Fig. 3).

The mesoSPIM light-sheet is generated by rapidly scanning a Gaussian beam in the vertical plane using a galvo scanner similar to a digital scanned light-sheet microscope (DSLM)12. This approach has several advantages when imaging large cleared samples. First, it results in uniform image brightness as each part of the FOV is illuminated with the same intensity. Second, when changing the detection FOV using the detection zoom, the height of the light-sheet can be easily adapted by regulating the amplitude of the galvo waveform. Finally, DSLM illumination reduces shadow artefacts13.
In light-sheet microscopy, any sample feature that absorbs or refracts the excitation light casts a shadow across the FOV. The resulting images are full of stripes along the illumination direction that complicate image analysis and visualization. Because in DSLM each part of the sample is illuminated with a scanned cone of excitation light, the shadow zone behind absorbing objects can be shortened by increasing the opening angle of the cone (equivalent to increasing the excitation numerical aperture, NA). In the mesoSPIM, we use an NA of 0.15 to achieve homogenous illumination with minimal shadow artifacts.

A Gaussian beam with increased excitation NA does, however, have a reduced Rayleigh range, which leads to axially blurred images outside of the narrow waist region of the light-sheet (Fig. 1b). As we deemed uniform axial resolution to be absolutely necessary to achieve the highest possible data quality, we integrated axially scanned light-sheet microscopy (ASLM) in the mesoSPIM (Fig. 1b, see Supplementary Note 3 and Supplementary Videos 3–5). In our ASLM implementation, we shift the excitation beam waist through the sample using an electrically tunable lens (ETL) and synchronize this motion with the rolling shutter of the sCMOS camera. Therefore, only the axially most confined region of the light-sheet contributes to image formation comparable to earlier approaches using mechanical translation of the sample. In ASLM mode, the mesoSPIM achieves an axial resolution of 5.57 ± 0.03 µm (FWHM, n = 2,170 beads, n̄ = 1.45) across a 3.29 mm FOV and 6.52 ± 0.07 µm (FWHM, n = 322 beads, n̄ = 1.45) across a FOV of 13.29 mm (Supplementary Note 4 and Supplementary Fig. 4). These features enable us to image a whole mouse brain (≈ 1 cm³) with isotropic sampling (6.5 µm) within 7–8 min resulting in a relatively small dataset (12–16 GB).

The microscope software (mesoSPIM-control, see Supplementary Software) is written in Python and allows users to specify sequences of z-stacks using a table-based acquisition manager (Supplementary Note 5, Supplementary Fig. 5 and Supplementary Video 6). The software can also be used to acquire large-scale tiling acquisitions; for example, to visualize fine neurites in the developing nervous system of a 7-day-old chicken embryo resulting in a 880-GB dataset (Fig. 2 and Supplementary Video 7). To achieve optimum optical sectioning in ASLM, the amplitude and offset of the ETL waveform need to be adapted when changing the excitation wavelength, zoom or the immersion medium and can even depend on the local refractive properties of the sample. Therefore, mesoSPIM-control allows users to select configuration files with default ASLM settings for different immersion media and to manually optimize ASLM parameters (Supplementary Video 8).

With a travel range of 44 × 44 × 100 mm, large samples such as a whole mouse CNS can be imaged in their entirety (Fig. 1c) and Supplementary Video 9). After acquiring overview datasets, users can zoom in and record multidimensional 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. 1d,e and Supplementary Videos 10–12).

We tested the instrument in combination with all major clearing techniques (Supplementary Note 6) including active and...
passive\textsuperscript{11} CLARITY (Fig. 1b–e, Supplementary Figs. 6 and 7 and Supplementary Video 13) and CUBIC-X\textsuperscript{1} (Supplementary Fig. 8). Among organic solvent methods, we tested iDISCO\textsuperscript{3} (Supplementary Fig. 9 and Supplementary Video 14) and BABB\textsuperscript{9} (Fig. 2 and Supplementary Fig. 10). To demonstrate multi-view acquisitions with the mesoSPIM, we imaged a BABB-cleared chicken embryo from multiple directions (Supplementary Fig. 11) and fused the resulting datasets using BigStitcher\textsuperscript{16} (Supplementary Fig. 12). Given its flexible sample holders, the mesoSPIM is compatible with a wide range of sample types ranging from \textit{Drosophila melanogaster} (Supplementary Fig. 13 and Supplementary Video 15) to cleared human cortex processed using MASH\textsuperscript{17} (Supplementary Fig. 14 and Supplementary Videos 16 and 17).

Inspired by the openSPIM\textsuperscript{18} and openSPIN\textsuperscript{19} projects, the mesoSPIM hardware documentation and software are freely available. Depending on the configuration, a mesoSPIM requires a budget of $170,000–240,000 (Supplementary Table 2) and can be installed in a day if all parts are available (Supplementary Video 18). Currently, five mesoSPIM setups are in operation across Europe and several more instruments are under construction. The mesoSPIM is the ideal instrument to quickly bridge scales from the $\mu$m- to the cm-level, which enables it to serve as an excellent tool for detailed three-dimensional anatomical investigations in neuroscience and developmental biology. We have designed the mesoSPIM as a versatile and modular imaging platform and expect that it will be extended toward even larger samples, combined with novel clearing methods, and integrated with other imaging modalities such as optical projection tomography\textsuperscript{20}.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
Data was deposited to the Image Data Resource (http://idr.openmicroscopy.org) under accession number idr0066.

**Code availability**
The mesoSPIM software and documentation are available as Supplementary Software. Updated versions can be found on Github (https://github.com/mesoSPIM). mesoSPIM-control is licensed under the GNU General Public License v.3.0 (GPL v.3).

**Online content**
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41592-019-0554-0.
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Author contributions
FFV and F.H. designed the project. FFV designed the microscope, wrote control software and documentation, coordinated the mesoSPIM initiative and analyzed data. FFV, EP and PB imaged samples. D.K., EP, R.A., M.S., L.E., A.d.B., K.H., N.F., T.T., N.R., H.U.Z., T.K., P.P., D.H., R.R., S.H., A.S. and A.R. prepared samples for imaging. FFV, EP, D.K., R.A.A.C., FM, U.Z., L.B., A.H., C.L. and A.A. set up the mesoSPIM instruments. FFV and F.H. wrote the manuscript with input from all coauthors.

Competing interests
The authors declare no competing interests.

Additional information
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

- **Data collection**
  - Datasets were acquired using mesospim-control (https://github.com/mesoSPIM/mesoSPIM-control), a custom open-source image acquisition software.

- **Data analysis**
  - Data preprocessing (conversion to .tif, cropping, downsampling etc) was done using Fiji (fiji-2.0.0-pre-7). Volume renderings and supplemental videos were done using Imaris 9.2.0 (Bitplane). The color projections in Figure 2 were done using a set of custom Matlab R2018a scripts (github.com/mesoSPIM/mesoSPIM-color-projection). Analysis of point spread functions for resolution measurements (Supplementary Figure 4) were done in Python 3.6.0 (github.com/mesoSPIM/mesoSPIM-PSFanalysis).

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Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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For the main figures was deposited to the Image Data Resource (http://idr.openmicroscopy.org) under accession number IDR0066. Data associated with Supplementary Notes is available from the authors upon reasonable request.
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size  We did not calculate sample sizes beforehand. Generally speaking, microscope point spread-functions are highly reproducible as they are caused by deterministic physical processes such as wavefront propagation and aberrations. Therefore, typical publications estimate PSFs with small sample sizes (sometimes only 5-20 beads). To estimate PSF parameters reliably over the FOV, we chose the bead concentration such that with ASLM mode enabled, at least 1000 beads could be measured across the FOV.

Data exclusions  For the PSF measurements at 1x zoom, only the central region of the FOV (13.29 mm in X × 1.3 mm in Y or 2048 × 200 pixels) was used for the estimation of the average axial FWHM as other parts of the FOV were affected by shadow artifacts in the sample.

Replication  In ASLM mode, fitting a PSF (for FWHM estimation) to each individual bead represents an individual measurement. As we estimated hundreds to thousands of PSFs for each zoom, we consider the results consistent.

Randomization  We chose random subregions inside the agarose blocks for PSF measurements.

Blinding  Does not apply. The manuscript describes an imaging method.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

| Antibodies used                  |
|----------------------------------|
| Mouse anti-neurofilament (RMO270, Invitrogen 13-0700) |
| Goat anti-mouse IgG-Cy3 (Jackson ImmunoResearch 115-165-003) |
| Donkey anti-mouse IgG Alexa Fluor 647 (Invitrogen A-31571, Lot 1900251) |

Validation

Antibody validation information for the Invitrogen 13-0700 mouse anti-neurofilament antibody is available from Thermo-Fisher Scientific under the Catalog-Number #13-0700.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The following mouse strains were used:
- Viptm1[cre]Jh/J
- B6.Cg-Tg(Rosa26Sortm14(CAG-tdTomato)Hze/J
- Tg(Tph2-cre)RH35Gsat/Mmucd
- B6.Cg-Tg(Camk2a-tTA)1Mmay/DboJ
- B6.Cg-Igs7tm92.1(tetO-ECFP*/Venus*)Hze/J
- Tg(Rbp4-cre)K100Gsat/Mmucd
The study used female and male mice aged 4-17 weeks (indicated in the corresponding figure caption).

Wild animals
The study did not involve any wild animals.

Field-collected samples
The study did not involve any field-collected samples.

Ethics oversight
Cantonal Veterinary Office of the Canton of Zurich
Cantonal Veterinary Office of the Canton of Basel-Stadt
UPMC University ethic committee

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics
Brain tissue samples were taken from one human body donor (no known neuropathological diseases)

Recruitment
Brain tissue samples were taken from one human body donor (no known neuropathological diseases) of the body donation program of the Department of Anatomy and Embryology, Maastricht University. The tissue donor gave informed and written consent to the donation of their body for teaching and research purposes as regulated by the Dutch law for the use of human remains for scientific research and education ("Wet op de Lijkbezorging"). Accordingly, a handwritten and signed codicil from the donor posed when still alive and well, is kept at the Department of Anatomy and Embryology Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands.

Ethics oversight
Department of Anatomy and Embryology, Maastricht University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.