

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ 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	LabVIEW software for microscope control, and MATLAB for subroutines
Data analysis	PetaKit5D (https://github.com/abcucberkeley/PetaKit5D), MATLAB (2019a - 2024a MathWorks), Python (3.8.8), Imaris (10.0, Oxford Instruments), Amira (2023.2, Thermo Fisher), Fiji (1.53t), AGAVE (https://github.com/allen-cell-animated/agave), IndeX (NVIDIA), BaSiC (https://github.com/marrlab/BaSiC), ACME (https://github.com/krm15/ACME/), CellPose (https://github.com/MouseLand/cellpose), CARE (https://github.com/csbdeep/csbdeep), cudasirecon (https://github.com/scopetools/cudasirecon), TrackMate (7.0), MitoGraph (https://github.com/vianamp/MitoGraph).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials. All raw data used in this manuscript are available upon request with a feasible data transfer and storage mechanism (such as a Globus endpoint or cloud bucket). Extensive documentation for the construction of a MOSAIC microscope or our earlier lattice light-sheet microscope can be obtained by execution of a research license agreement with HHMI.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	n/a there is no sex dependent study/statistics
Reporting on race, ethnicity, or other socially relevant groupings	n/a there is no race dependent study/statistics
Population characteristics	n/a
Recruitment	research took place after death and the brain is donated.
Ethics oversight	The Anatomical Gift ACT, Washington Statute Chapter 68.64 RCW informed the brain donation program. We worked with the UW School of Medicine Compliance office on our consent forms and HIPAA compliance. All materials are collected under informed consent.

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

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size	No sample-size calculations were needed in this study. The images we generated with the samples were to demonstrate the performance of the microscope modality.
Data exclusions	No data were excluded in the manuscript.
Replication	No replication imaging experiments were performed.
Randomization	There were no experimental groups, so randomization was not applicable.
Blinding	There were no experimental groups, so blinding was not applicable.

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	Involvement in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Mouse anti-Lamin A/C primary antibody (1:200 dilution, Santa Cruz, sc-376248) DNA-conjugated anti-GFP nanobody (1:200 dilution, Massive Photonics, MASSIVE-TAG-X2 anti-GFP) DNA-conjugated secondary antibody against mouse anti-Lamin A/C (1:200 dilution, Massive Photonics, MASSIVE-sdAB 1-PLEX) Rabbit anti-NF-200 primary antibody (1:200 dilution, Millipore Sigma, N4142-.2ML) Chicken anti-MBP primary antibody (1:200, Thermo Fisher, PA1-10008) Goat Atto 647N-conjugated anti-rabbit secondary antibody (1:200 dilution, Millipore Sigma, 40839-1ML-F) Goat Alexa Fluor 568-conjugated anti-chicken secondary antibody (1:200 dilution, Thermo Fisher, A11011)
Validation	Validation information at the vendor links: Mouse anti-Lamin A/C primary antibody (Santa Cruz, sc-376248): https://datasheets.scbt.com/sc-376248.pdf DNA-conjugated anti-GFP nanobody (Massive Photonics, MASSIVE-TAG-X2 anti-GFP): Rabbit anti-NF-200 primary antibody (Millipore Sigma, N4142-.2ML): https://www.sigmaaldrich.com/US/en/product/sigma/n4142#product-documentation Chicken anti-MBP primary antibody (Thermo Fisher, PA1-10008): https://www.thermofisher.com/antibody/product/MBP-Antibody-Polyclonal/PA1-10008

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	LLC-PK1 cells were gifts from M. Davidson at Florida State University. U2OS, COS-7 and HeLa (CCL-2) cells were purchased from ATCC. mESC Cells were a gift of James Liu at the Janelia Research Campus hTERT-RPE1 Cells were a gift from Michael Rape at UC Berkeley
Authentication	No further authentication was performed for this study.
Mycoplasma contamination	The cell lines were tested for mycoplasma contamination and the results were negative.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used here belongs to commonly misidentified lines.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Zebrafish with 72 hpf or younger, mice (Jackson Laboratories, C57BL6J, stock #:000664; Thy1-GFP line M, stock #:007788) of two to four months old, fruit flies (<i>Drosophila melanogaster</i>), <i>C. elegans</i> at L3 stage larvae.
Wild animals	No wild animals were used in this study.
Reporting on sex	Sex was not considered in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Ethics statements are included in the methods section of the manuscript. The oversight was provided by protocols approved by Animal Care and Use Committee at UC Berkeley, Stony Brook University, and Janelia Research Campus.

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

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a