

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) 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 | Simulated data was created using the net.preibisch.simulation.SimulateMultiViewAberrations class in the multiview-simulation package (release version 0.2.2). Since it is a Maven artifact, the versions of all dependencies are clearly defined and the corresponding version can be built automatically from that source code state (https://github.com/PreibischLab/multiview-simulation/commit/b41b74cce9287f804b670d7de3396605446818a8). |
| Data analysis | The data was reconstructed using BigStitcher (release version 0.3.3). Since it is a Maven artifact, the versions of all dependencies (e.g. multiview-reconstruction) are clearly defined and the corresponding version can be built automatically from that source code state (https://github.com/PreibischLab/BigStitcher/commit/0d7f79a59ab15fb1805157ab72c5bc9802b02fbd). |

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Small example datasets are available online: <https://osf.io/bufza/> (Open Science Foundation). Larger datasets are available on request. When we find ways to host larger datasets, they will be linked from the documentation page: https://imagej.net/BigStitcher#Example_Datasets

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	The sample size of 8 acquired samples was chosen to show a wide variety of samples and sample preparation that can be reconstructed using BigStitcher. Some samples are quite similar (clearing), some were chosen to highlight the generality (expanded, only fixed).
Data exclusions	No data was excluded.
Replication	In total 8 different samples (cleared, expanded, only fixed) were acquired and reconstructed for this publication. Furthermore, one sample was simulated and reconstructed. Additionally, the BigStitcher reconstruction pipeline has been applied to >50 samples in our lab (not part of the publication, most are prepared in collaboration on other projects), which highlights the generality of the method. The tissue sections shown in Fig. 3a + Suppl. Fig. 1 + Suppl. Video 1 as well as Fig. 3b are very similar and highlight reproducibility.
Randomization	Randomization was used for a) the simulation and benchmarking of phase correlation (Supplementary Fig. 12-15), as well as b) for the raytracing (Fig. 2b-e). For a) random overlaps (uniformly distributed) were simulated 300 times for each condition, and the noise for the image generation process is based on Poisson noise. For b), illumination and detection rays in defined areas are sent randomly (uniformly distributed) into the scene.
Blinding	Blinding is not applicable for this study since there is no process that requires it.

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

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	For clearing we used a previously generated BsxH2BeGFP mouse line, where the exon 1 of the bsx gene is replaced starting at the ATG with the coding sequence for histone2B eGFP. Brains from 10-week old female BsxH2BeGFP/+ mice were used for tissue clearing and imaging. C. elegans dauer larvae expressing tagRFP fused to a nuclear localizing sequence under the pan-neuronal rab-3 promotor in all neuron nuclei were obtained by selecting dauer larvae in 1% SDS for 30 minutes. Drosophila larva used for ExM were obtained from the strain w;;attp2, carrying an empty attp2 landing site.
Wild animals	No wild animals were used.
Field-collected samples	No field-collected samples were used.
Ethics oversight	Experiments were conducted according to institutional guidelines of the Max Delbrück Center for Molecular Medicine in the Helmholtz Association after approval from the Berlin State Office for Health and Social Affairs (LAGeSo, Landesamt für Gesundheit und Soziales, Berlin, Germany)

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