

Reporting Summary

Nature Portfolio 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 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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	Data acquisition was performed using customized setups involving sample stages, Galvanomirrors, data acquisition NI board, and s-CMOS camera, which were controlled by customized MATLAB (available with v2023a,v2023b) acquisition codes.
Data analysis	Data analysis was performed using customized MATLAB (available with v2024a,v2024b,v2025a) analysis codes.

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](#)

The data analysis code used to run and visualize the main demonstration datasets (Figs. 4 and 5) is openly available to the public.

The main demonstration datasets presented (Figs. 4 and 5) are openly available to the public.

Other experimental datasets are not publicly available due to their large size, but can be provided by the corresponding author upon reasonable request.

The data analysis code used to run and visualize the main demonstration datasets with the datasets (Figs. 4 and 5) are openly available to the public at: <https://github.com/CenterForDeepImaging/Dual-Deconvolution>, and <https://doi.org/10.6084/m9.figshare.28600847>.

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
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	Fixed COS-7 cells, mouse brain slices, and whole zebrafish samples used in the experiments were selected from 3–30 fixed samples based on sufficient fluorescence intensity.
Data exclusions	No data was excluded.
Replication	Bead, resolution target, COS-7 cell, mouse brain, and zebrafish experimental conductions were independently repeated 3 times, 2 times, 5 times, 3 times at 130 µm and 180 µm depths, respectively, and 5 times with similar results from different samples.
Randomization	Samples exhibiting sufficient fluorescence intensity were selected for the experiments, and experiments were performed in a randomized manner.
Blinding	Not relevant to our study.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Primary antibody targeting tubulin (ab6046, Abcam, diluted 1:1,000 times), secondary antibody labeled with Alexa Fluor 488 (A-11008, Thermo Fisher, diluted 1:1,000 times).
Validation	Primary antibody (ab6046, Abcam): Anti-beta Tubulin antibody (Loading Control). Validated by the manufacturer using Western blot analysis, confirming a specific band at the predicted size of 50 kDa in various cell lysates under reducing conditions. https://www.abcam.com/en-us/products/primary-antibodies/beta-tubulin-antibody-loading-control-ab6046 Secondary antibody (A-11008, Thermo Fisher): Alexa Fluor 488-conjugated Goat anti-Rabbit IgG (H+L). This cross-adsorbed antibody is validated for specific binding to rabbit IgG heavy and light chains, with minimal cross-reactivity to immunoglobulins from other species. https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008

Eukaryotic cell lines

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

Cell line source(s)	COS-7 [AC28806, Korean Collection for Type Cultures (KCTC)].
Authentication	Cells were not authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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	An adult Thy1-EGFP line M mouse (12 weeks old; Jackson Laboratory, #007788) and zebrafish embryos from the Tg(claudinK:gal4vp16; uas:mEGFP) line (#FRZCC1013) were used.
Wild animals	No wild animals were used in the study.
Reporting on sex	All observed structures in this study were observed regardless of sex. Therefore, sex information was not collected.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All experimental procedures involving animals were approved by the Committee of Animal Research Policy at Korea University (approval number KOREA2021-0037).

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