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

☐ 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

☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ 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

☐ Give P values as exact values whenever suitable.

☐ 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: Commercial software: Zeiss Zen was utilized for collecting microscopy data

Data analysis: Data was preprocessed utilizing custom python algorithms now integrated in HySP (http://bioimaging.usc.edu/software.html#HySP), data was rendered using commercial software Imaris 9.2.0. Simulations were made using custom Python code starting from real data.

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

All the relevant data are available from the corresponding author upon reasonable request. Datasets for figures 1, 2-3, 4, 5, 6, 7 are available for download at http://bioimaging.usc.edu/software.html#sampledatasets in the samples section.

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 study design

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

**Sample size**
No sample size was predetermined. The work here presented describes a pre-analysis visualization method which is run against 4 different fluorescent sample types starting from autofluorescence, single- and multiple-fluorescent labels, combinatorial labels. We then validate multiple scenarios with simulated data.

**Data exclusions**
No data was excluded from analysis.

**Replication**
Reproducibility was verified across different samples. Datasets are available for download at http://bioimaging.usc.edu/software.html#sampleddatasets in the samples section while software can be downloaded from http://bioimaging.usc.edu/software.html#HysP.

**Randomization**
Sample order was not considered in this work as this is a pre-analysis visualization method which runs on one sample per time. Order does not affect how the method works.

**Blinding**
Blinding was not relevant to this study as this method returns an image as output which requires no interpretation from the user beside the capability of distinguishing colors on a screen.

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**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

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
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<tbody>
<tr>
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<td>Antibodies</td>
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<td>Eukaryotic cell lines</td>
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<td>Palaeontology</td>
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<td>Animals and other organisms</td>
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<td>x</td>
<td>Human research participants</td>
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<td>Clinical data</td>
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### Methods

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<td>Flow cytometry</td>
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<tr>
<td>x</td>
<td>MRI-based neuroimaging</td>
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**Animals and other organisms**

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

**Laboratory animals**

8-weeks-old C57Bl mice, 20-32 hours-post-fertilization zebrafish embryos (ubi:Zebrabow, Gt(desm-Citrine)ct122a/+, Tg(kdr:leGFPS843, Tg(hsp70I:Creulean-P2A-CreERT2), Tg(hsp70I:Creulean-P2A-CreERT2), Tg(fli1:miK2). All zebrafish lines are available from the authors.

**Wild animals**

This study did not involve wild animals.

**Field-collected samples**

This study did not involve field-collected samples.

**Ethics oversight**

For Mouse Lines: Institutional Animal Care and Use Committee (IACUC) of the Children’s Hospital of Los Angeles (permit number: 38616) and of the University of Southern California (permit number: 20685)

For Zebrafish lines: IACUC of University of Southern California (permit number: 12007)

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