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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

A total of 12 neuronal recordings from 5 animals were analyzed (including simultaneous 2PM-MiniLFM verification recordings). Only animals were included in the study for which all animal procedures (as described in Online Methods) worked successfully to allow for signal detection (see next question). Provided that animal procedures (surgeries and viral injections/GECI expression) were successful as verified using a standard two-photon microscope, we found imaging results and data quality to be reliably reproducible and consistent, both across imaging sessions with the same animal, and across animals. Since the subject of our manuscript is to establish a neural recording method rather than any biological findings, we consider this sample size sufficient to verify the performance of our method.

2. Data exclusions

Describe any data exclusions.

Only animals were included in the study for which all animal procedures (as described in Online Methods) worked successfully to allow for signal detection (i.e., GECI expression observable, correct implantation of the GRIN lens), as verified using a standard two-photon microscope. Of these animals, none were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

For all animals in which animal procedures (surgeries and viral injections/GECI expression, see Online Methods) were successful (as verified using a standard two-photon microscope), imaging and data analysis results were reliably reproduced, both across imaging sessions with the same animal, and across animals.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization was not relevant to this study, because no experimental groups were formed.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not relevant to this study, because no group allocation was performed.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Custom-written Java (ImageJ/Fiji, release 2017-05-30) and R (v3.x) code implementing focal spot analysis for LFM alignment, as well as Matlab (2017a) code implementing the signal extraction and motion detection pipeline, as described in the Main Text and Online Methods are included as Supplementary Software. Other than than, the SID Matlab package published as Supplementary Software with our previous manuscript doi:10.1038/nmeth.4341 is required, as well as the dependencies listed in the README file accompanying that package.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukariotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukariotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukariotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Adult (P90+) male and female C57Bl/6J wild-type mice, obtained from The Jackson Laboratory

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	The study did not involve human research participants.