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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.

Number localization events varies between experiments usually on the order of several hundreds thousands to millions per dataset. These numbers are reported in Supplementary Figures. These sample sizes (hundreds of thousands to millions single molecule localized) depend on imaging and labeling condition of the specimen and, in our opinion, are large enough to provide reliable statistical measurements of precision and resolution.

2. Data exclusions

Describe any data exclusions.

Single molecule localizations were statistically tested and rejected/accepted based on their log-likelihood ratio (as goodness of fit metric), theoretical uncertainty, emitted photon, background as well as their convergence during axial position fitting.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful.

4. Randomization

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

Not relevant. The manuscript describes innovation of a new imaging method.

5. Blinding

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

Not relevant. The manuscript describes innovation of a new imaging method.

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.	Software for characterizing DM, localization single molecules, drift correction and reconstruction are described in previous publications and also summarized in the methods section. The DM control and optimization modules (LabView, instrument control) are included in the Supplementary Software and further updates will be made available at https://github.com/HuanglabPurdue/AO_SMSN.

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 used

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Anti-amyloid beta antibody used for immunohistochemistry is a commercially available antibody from Cell Signaling Technology (Catalogue #2454, Cell Signaling Technology, Danvers, MA) is generated in rabbit and has been validated and reported previously (PMID: 27196974).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	COS-7 cell line; originally from Yale tissue culture facility
b. Describe the method of cell line authentication used.	Cells were not authenticated
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell line was test negative for mycoplasma contamination
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.

4-month-old Trem2 deficient APPPS1-21 mice were reported previously (PMID: 25732305). Briefly, Trem2 deficient mice (Trem2tm1(KOMP)Vlcg) with replacement of exons 2, 3, and part of 4 with LacZ were crossed into the APPPS1-21 (APPPS1) amyloidosis mouse model (kindly provided by Mathias Jucker) expressing the Swedish APP mutation (KM670/671NL) and the L166P mutation in PSEN1 driven under the neuron specific Thy-1 promoter and were reported previously (PMCID: PMC1559665). Both male and female mice were used in this study at 4 months of age and weighed between 20-30 g.

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