

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.

No hypothesis based experiment was performed. Therefore, the sample size was not predetermined. For all simulations, the data size was chosen large enough that statistical errors are minimal compared to the effect.

2. Data exclusions

Describe any data exclusions.

For calculation of RMS error for Newton method, single data points for which localization error is more than 10 pixels (<5%) were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All code is open source to help with the reproducibility of computational analysis. Experimental findings were reproduced successfully.

4. Randomization

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

Not relevant for this study as the work presents an image analysis software. Presented data is either illustrative or the result of simulations.

5. Blinding

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

Not relevant for this study as the work presents an image analysis software

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.

Matlab 2017a, Windows Visual Studio 2010, custom Matlab and CUDA code (as supplementary software and available on github), Leica Application Suite X (Version 1.9.0.137), Babcock software (0f05748 2017-09-14), Kirshner Software (<http://bigwww.epfl.ch/kirshner/>). ThunderSTORM(e85c565 2017-01-29), Smith Software (gaussmelv2)

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.

all unique materials are readily available from the authors.

9. Antibodies

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

anti-clathrin: Abcam ab21679, 1:500; Santa Cruz Biotechnology sc-28276, 1:300
anti-rabbit secondary: Jackson ImmunoResearch 711-005-152, 1:300
tubulin: NeoMarkers MS581, 1:300, Sigma-Aldrich T6074, 1:300, Sigma-Aldrich T5293, 1:300; anti-mouse secondary antibody for DNA-PAINT is a custom labeled antibody available upon request from Ingmar Schoen. All antibodies showed a very high specificity for immunolabeling in superresolution microscopy.

10. Eukaryotic cell lines

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

SK-MEL-2, kind gift of David Drubin (Berkeley)
U 2-OS: ATCC (U-2OS ATCC HTB-96)

b. Describe the method of cell line authentication used.

none of the cell lines have been authenticated

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

cell lines were tested 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.

no animals were used

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