

Life Sciences Reporting Summary

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► Experimental design

1. Sample size

Describe how sample size was determined.

Our manuscript does not report on experimental work. We evaluate and rank the performance of software -cell tracking algorithms on videos- based on a set of performance measures. Therefore, no descriptive statistics have been used and accordingly all the following questions have been answered (N/A).

Regarding the sample size, as explained in the Results section, "Datasets and ground truth" subsection (page 6), we used 52 annotated videos, 4 videos of 13 types, covering a wide range of microscopy and experimental conditions. From each type, two videos were used for training the algorithms and two videos were used to evaluate the performance of the algorithms. The number of videos per dataset (4) was considered appropriate taking into account the labor intense annotation required, the amount of work given to the participants, and the availability of good quality videos of each type.

2. Data exclusions

Describe any data exclusions.

N/A

3. Replication

Describe whether the experimental findings were reliably reproduced.

N/A

4. Randomization

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

N/A

5. Blinding

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

N/A

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.

We have developed code to analyze the performance of the algorithms, and quantify the properties of the videos, to help with the interpretation of the results. A beta version of a Fiji plugin that contains the software is provided as a link in Supplementary Table 3, along with links to the executable versions of the participant's algorithms.

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.

N/A

9. Antibodies

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

M/A

10. Eukaryotic cell lines

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

N/A

b. Describe the method of cell line authentication used.

N/A

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

N/A

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.

N/A

► 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.

N/A

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.

N/A