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eLife's transparent reporting form

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. If you have any questions, please contact us: <u>editorial@elifesciences.org</u>.

Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., page numbers or figure legends), or explain why this information doesn't apply to your submission:

No statistical method was used to predetermine sample-size. Sample-size estimation was not directly relevant to our submission because we developed a new technique and reported the trends we observed and their significance. Events of interest were identified subsequent to data collection and sample-size was the result of the random occurrence of those events. Wherever possible, we tried to maximize the number of sample events observed to values typically employed in the field for fixed specimens.

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., page numbers or figure legends), or explain why this information doesn't apply to your submission:



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In our submission, biological replication was considered number of times a biological event of interest was captured whereas technical replication was number of times the mounting-to-imaging procedure or digital cell recognition and tracking was performed. Any noted outliers are described in the main text. Criteria for inclusion or exclusion of data is also stated in the main text.

Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., page numbers or figure legends), or explain why this information doesn't apply to your submission:

Any statistical information presented in figures is described in the accompanying figure legend or main text of the submission.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to page numbers in the manuscript.)

Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:



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The additional data files submitted with this manuscript are as follows:

"Martin_SourceData.xlsx" – This Microsoft Excel file contains the source data for all numerical data described in our manuscript. The source data is organized by figure, with each figure delineated by a separate tab. Within figure tabs, data is organized by sub-figure components and where it is presented in the text of the manuscript.

"Martin_RegistrationMacros.ijm" – This is an ImagJ macros file and is supplemental to the correction process described in Figure 1D-F. This registration macros performs correction for a 3-channel stack registration over time.