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Trackosome: a computational toolbox to study the spatiotemporal dynamics of centrosomes, nuclear envelope and cellular membrane

Domingos Castro, Vanessa Nunes, Joana Lima, Jorge Ferreira and Paulo Aguiar
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Original submission

First decision letter

MS ID#: JOCES/2020/252254

MS TITLE: Trackosome: a computational toolbox to study the spatiotemporal dynamics of centrosomes, nucleus and cellular membrane

AUTHORS: Domingos Castro, Vanessa Nunes, Joana Lima, Jorge Ferreira, and Paulo Aguiar
ARTICLE TYPE: Tools and Resources

We have now reached a decision on the above manuscript which is to request some minor revisions. First of all, I would like to apologise for the time taken in review. It has been a challenge to obtain reviewers for this work (as is often the case for such manuscripts, this is not a reflection on you or your work!).

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

The majority of the comments can be addressed by changes to the text. I do agree though with the comment that it would be very good to know how effective the tool is when analysing cells that are not constrained on micropatterns. I hope that you will be able to address this easily.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In this MS Castro et al describe computational tools to track centrosomes and measure the dynamic shape changes of the nucleus and cell cortex. Overall the MS is well written and provides a useful toolbox that can be applied by other scientists for centrosome tracking and measuring the changes of centrosome position with regards to nucleus and cortex.
Apart from a few clarifications and an additional data set (see below) I would recommend this MS for publication in JCS.

Comments for the author

I would encourage the authors to improve the description of the image processing and tracking tools in the material and methods section. Some of the relevant information can be found in the supplement, but this is often not appropriately referenced. For example, on page 17 a "developed search algorithm" is mentioned but not further explained.
"Gaussian" to my knowledge is not a stand-alone noun. I presume the authors mean Gaussian curves, or functions.
How does the algorithm deal with centrosomes that are coming close to each other so that they are both segmented in the same ROI? Can the identity of each individual centrosome be maintained?
All the experimental analysis was performed on cells that are immobilised by micropatterns. Does this tool also work under more physiological conditions with cells that are continuously moving and changing shape? It would be good to see an example of how the software deals with this scenario. If this tool works exclusively for cells on micropatterns then this should be discussed more prominently, I would even suggest adding this information to the title. Otherwise, readers will be misled into applying this image processing and tracking tool without using immobilised cells.

Reviewer 2

Advance summary and potential significance to field

In this paper, Castro et al. present a novel computational toolbox to study the spatiotemporal dynamics of centrosomes, nuclear membrane and cell membrane. This image analysis toolbox can reliably track centrosomes in relation to the nucleus and the cell membrane, and can also identify nuclear membrane fluctuations during the onset of mitosis. The toolbox is developed in MATLAB, has a complete graphical user interface (GUI) and is open-source allowing access to the algorithms should the user wish to modify the codes.
Importantly it will be made available to freely download upon publication.

Trackosome provides a large amount of options to the user for editing the raw data before quantification, and also during the segmentation and tracking. The GUI contains a multitude of options to visualise and manipulate the results.

Using Trackosome, the authors are able to track centrosomes in 3D, even in noisy datasets, and show that centrosomes trajectories during early mitosis are not independent, by calculating their movements in relation to the nuclear envelope and using elliptical coordinates, a novelty compared to more classical tracking approaches where cartesian coordinates are used. Trackosome can also be used to track other organelles in 3D.

When looking at nuclear membrane fluctuations, Trackosome is able to reliably identify small fluctuations in membrane shape with sub-pixel precision, even when using a low-quality synthetic videos with added noise. The module also works in batch mode. In cells un-treated and treated

with nocodazole, the authors are able to show, for the first time, the effect of microtubules on the fluctuations of nuclear shape in early mitosis.

Overall, Trackosome will allow users to investigate centrosome movement and nuclear envelope fluctuations with a precision not previously available, using one toolbox.

This was a very enjoyable paper to review, congratulations to the authors. A huge amount of thought has been put into developing this toolbox, which will be of great use to the cell biology and image analysis community. I am happy to recommend publication after correction of the minor points below.

Comments for the author

As a general point, is there a plan for toolbox maintenance in the future?
What will you use to post updated versions of Trackosome?

In Fig 4:
Please mention how many cells were analysed for each group.

In Methods 4.1, Time-lapse microscopy:
Z-stacks are acquired with a 63x or 100x objective, and z-step spacing is 0.5 micron, which is above Nyquist sampling. Did you have to compromise z resolution for time for centrosome tracking? Did you try deconvolving the stacks or did you find that wasn't needed?

In Methods 4.2, Tracking Algorithm, the authors mention 'centrosomes dislocations in-between frames'. Could you please clarify? Do you mean a movement by a larger than usual number of pixels?

In Methods 4.4, Trackosome toolbox: membrane fluctuations:
Could the same analysis of nuclear membrane fluctuations be applied to the cell membrane too?

In Supplementaty Information:
In the legend of Fig S5, in B) part, the legend for the sub-panels 1.1 to 1.4 should be updated, as it is currently confusing:
1.2) instead of 2), 1.3) instead of 3) and 1.4) instead of 4).

Finally, thank you to the authors for providing the demonstration videos, they are great and really showcase the power of the toolbox.

First revision

Author response to reviewers' comments

Reviewer #1
Reviewer 1 Advance Summary and Potential Significance to Field:

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Apart from a few clarifications and an additional data set (see below) I would recommend this MS for publication in JCS.

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in the material and methods section. Some of the relevant information can be found in the supplement, but this is often not appropriately referenced. For example, on page 17 a "developed search algorithm" is mentioned, but not further explained.

R: The expression “developed search algorithm” was not very fortunate indeed and potentially generates confusion. In fact, we were only referring to a specific part of the algorithm where each large ROI is iteratively shortened to create a short ROI that contains only the centrosome. We have modified the manuscript to correct this issue and we have also added more information to section 4.2 Trackosome toolbox: Centrosome Dynamics, in the Materials and Methods.

"Gaussian" to my knowledge is not a stand-alone noun. I presume the authors mean Gaussian curves, or functions.

R: Indeed, at the end of Section 4.2 we have "fitting a Gaussian to each intensity profile", which may not be clear. We meant "Gaussian curve". The manuscript has been corrected accordingly.

How does the algorithm deal with centrosomes that are coming close to each other so that they are both segmented in the same ROI? Can the identity of each individual centrosome be maintained?

R: In this scenario, the identity of the two centrosomes may not be maintained. But, as presented in the manuscript, we have incorporated in the *centrosome dynamics’* GUI visual feedback regarding the automatic tracking status, and tools allowing manual or semi-automatic corrections whenever needed. In the case of centrosomes coming too close and identity being switched, the user can correct the positions of misplaced centrosomes, and let the algorithm proceed automatically from the corrected positions.

All the experimental analysis was performed on cells that are immobilised by micropatterns. Does this tool also work under more physiological conditions with cells that are continuously moving and changing shape? It would be good to see an example of how the software deals with this scenario. If this tool works exclusively for cells on micropatterns then this should be discussed more prominently, I would even suggest adding this information to the title. Otherwise, readers will be misled into applying this image processing and tracking tool without using immobilised cells.

R: Trackosome works equally well on non-patterned substrates. It was misfortunate that this was not clear in the original manuscript. Anxious to demonstrate the relevance and usefulness of the new tool in non-conventional situations (such as substrates with micro-patterns), we now realized we have not made clear that Trackosome can, of course, be used with cells on non- patterned substrates. We have now modified the manuscript to clarify this very important point, and we have added a supplementary figure (S3,A-D) on the analysis of cells in conventional, non- patterned substrates.

Reviewer #2
Reviewer 2 Advance Summary and Potential Significance to Field:

In this paper, Castro et al. present a novel computational toolbox to study the spatiotemporal dynamics of centrosomes, nuclear membrane and cell membrane. This image analysis toolbox can reliably track centrosomes in relation to the nucleus and the cell membrane, and can also identify nuclear membrane fluctuations during the onset of mitosis. The toolbox is developed in MATLAB, has a complete graphical user interface (GUI) and is open-source, allowing access to the algorithms should the user wish to modify the codes. Importantly it will be made available to freely download upon publication.

Trackosome provides a large amount of options to the user for editing the raw data before quantification, and also during the segmentation and tracking. The GUI contains a multitude of options to visualise and manipulate the results.

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Overall, Trackosome will allow users to investigate centrosome movement and nuclear envelope fluctuations with a precision not previously available, using one toolbox.

This was a very enjoyable paper to review, congratulations to the authors. A huge amount of thought has been put into developing this toolbox, which will be of great use to the cell biology and image analysis community. I am happy to recommend publication after correction of the minor points below.

Reviewer 2 Comments for the Author:

As a general point, is there a plan for toolbox maintenance in the future? What will you use to post updated versions of Trackosome?

R: Code updates will be posted in the GitHub repository of Trackosome. These will be motivated by eventual bugs reports and feature requests by the users. For example, we are currently working on an additional feature to improve the 3D segmentation of nuclear and cellular membranes, ideal for videos with poor contrast. GitHub is a well-established collaborative platform for software development and version control, used in many scientific computing projects. Noteworthy, being open-source and hosted at GitHub, makes it easy other colleagues to contribute with new features/improvements to Trackosome.

In Fig 4:

Please mention how many cells were analysed for each group.

R: The number of cells for each group have now been added to the legend of Fig 4. in the revised version of the manuscript.

In Methods 4.1, Time-lapse microscopy:

Z-stacks are acquired with a 63x or 100x objective, and z-step spacing is 0.5 micron, which is above Nyquist sampling. Did you have to compromise z resolution for time for centrosome tracking? Did you try deconvolving the stacks or did you find that wasn't needed?

R: As our imaging conditions were very demanding, we found it necessary to adjust both the temporal resolution and z-step spacing. Indeed, we observed that our imaging conditions decreased phototoxicity and ensured that cells still entered mitosis. Deconvolution was not necessary, since the tracking algorithm was efficient even in low SNR conditions.

In Methods 4.2, Tracking Algorithm, the authors mention 'centrosomes dislocations in-between frames'. Could you please clarify? Do you mean a movement by a larger than usual number of pixels?

R: The use of the word "dislocations" was not ideal. We have now replaced it by "displacement". With this sentence we wanted to point to the fact that centrosomes move a certain number of pixels between each frame. In the context of the algorithm description, this justifies the use of "large" ROIs: these must be large enough to ensure centrosomes are not "lost" from one frame to the next. This is important because, for each frame, the ROIs are centered at the positions of the centrosomes from the previous frame. We updated this section to make it more clear.

In Methods 4.4, Trackosome toolbox: membrane fluctuations:

Could the same analysis of nuclear membrane fluctuations be applied to the cell membrane too?

R: Yes, and, in principle, any other membrane structure.

In Supplementaty Information:

In the legend of Fig S5, in B) part, the legend for the sub-panels 1.1 to 1.4 should be updated, as it is currently confusing: 1.2) instead of 2), 1.3) instead of 3) and 1.4) instead of 4).

R: They have now been updated.

Finally, thank you to the authors for providing the demonstration videos, they are great and really showcase the power of the toolbox.

R: From our previous experience these videos can really help new users to start easily/effectively using the new computational tools. Thank you also for the very constructive comments.

Second decision letter

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ARTICLE TYPE: Tools and Resources

Thank you for your careful revisions to your manuscript. I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.