What can be done with DeepKymoTracker?

(User Guide)

The DeepKymoTracker software was built to track and segment T cells in cell movies, with the ultimate purpose of building the lineage trees of the cells in each movie, along with extracting the numerical information about each cell, such as area, perimeter, circularity, bounding box, and the average intensity of each cell body in each channel (the brightfield, the green, and the red fluorescent).

How does DeepKymoTracker deal with every aspect of cell tracking?

Apart from the two fundamental tasks, tracking and segmentation, there are common bottlenecks in cell tracking: divisions, occlusions, dying or disappearing from the field of view cells, and finally, new cells, appearing in the field of view out of nowhere.
Here is a short description of how DeepKymoTracker deals with each of these tasks.

1. Tracking: automatic + manual correction.

Both approaches are provided in STEP-3.
Once the program is launched, the user is supposed to watch the frame-by-frame progress in real time and, if a tracking error is spotted, they need to pause the algorithm, make manual corrections, and re-launch the program once again.

What are tracking errors?

- Swapping of cell IDs
- Missing a cell division
- Missing a cell death or disappearance
- Missing emergence of new cells

The tools for manual correction of these errors are provided in STEP-3 (these are buttons EDIT IDs, EDIT DIVISION, REMOVE CELL, and ADD CELL).

Warning: you need to manually correct the above-mentioned errors in STEP-3, there will not be a second chance to do it (the next step, i.e. Step-4, is designed to deal with segmentation errors only).

2. Segmentation: automatic + manual correction.

Automatic segmentation happens in STEP-3, simultaneously with tracking. However, manual segmentation correction cannot be done during this step, as STEP-3 is designed to make only tracking error corrections on the fly. So, when watching the progress of the algorithm in STEP-3, you need to ignore segmentation imperfections of cells, if any, and concentrate completely on tracking errors instead. Manual segmentation correction can be done later - the whole STEP-4 is dedicated to this task.
There are 2 correction techniques available, fast and slow:

- **Fast**: it is a semi-automated technique - you just need to click on multiple locations inside the cell itself and the surrounding area, and watch how the segmentation changes with every click, until you find the optimal one.

- **Slow**: it is a traditional manual technique – you just draw the contour of a cell with the mouse. You will have to resort to this approach if you have been unable to achieve a good segmentation with the fast technique.

3. Division detection: automatic (somewhat crude) + manual correction.

Automatic division and the possibility to manually correct missed divisions are both incorporated in STEP-3. Just like tracking errors, the user needs to watch the progress of tracking and, if missed divisions have been spotted, they need to pause the algorithm, make the necessary manual corrections with the tools provided (button EDIT DIVISION), and re-launch the algorithm again.

4. Occlusions: automatic (very crude) + manual correction.

The task of sorting out occlusions can be split into 3 stages:

a) Detecting the occlusion event
b) Determining which cell is which (i.e. determine their IDs)
c) Drawing accurate segmentation of each cell according to their IDs

All the 3 stages are automatic in DeepKymoTracker (STEP-3), but only stage a) is accurate. When it comes to b) and c), they are implemented in a very basic, crude way (the main purpose being to prevent the smooth flow of the algorithm from stopping rather than accurately drawing the occlusions), and, as a consequence, outputs errors quite often.

Therefore, the user needs to watch the progress of the tracking carefully in STEP-3 and, once the swapping of IDs has been detected, pause the algorithm and use the button EDIT IDs to make corrections (this is stage b)).

As for the quality of segmentation of the occluded cells (Stage c)), it should be ignored in STEP-3. Currently, it can be corrected only manually, in the next step (i.e. STEP-4) where you can draw the contours of overlapping cells with the mouse.

Conclusion:

a) Detecting the occlusion event - automatic
b) Determining which cell is which - automatic + manual correction (in STEP-3)
c) Drawing accurate segmentation of occluded cells – only manual (in STEP-4)

5. Detection of dying and disappearing cells: manual only.

The tools for this kind of corrections are provided in STEP-3 (button REMOVE CELL). Again, you need to pause the algorithm to be able to do the correction in the frame where it happened, and then re-launch tracking.

6. Detection of new cells: manual only.

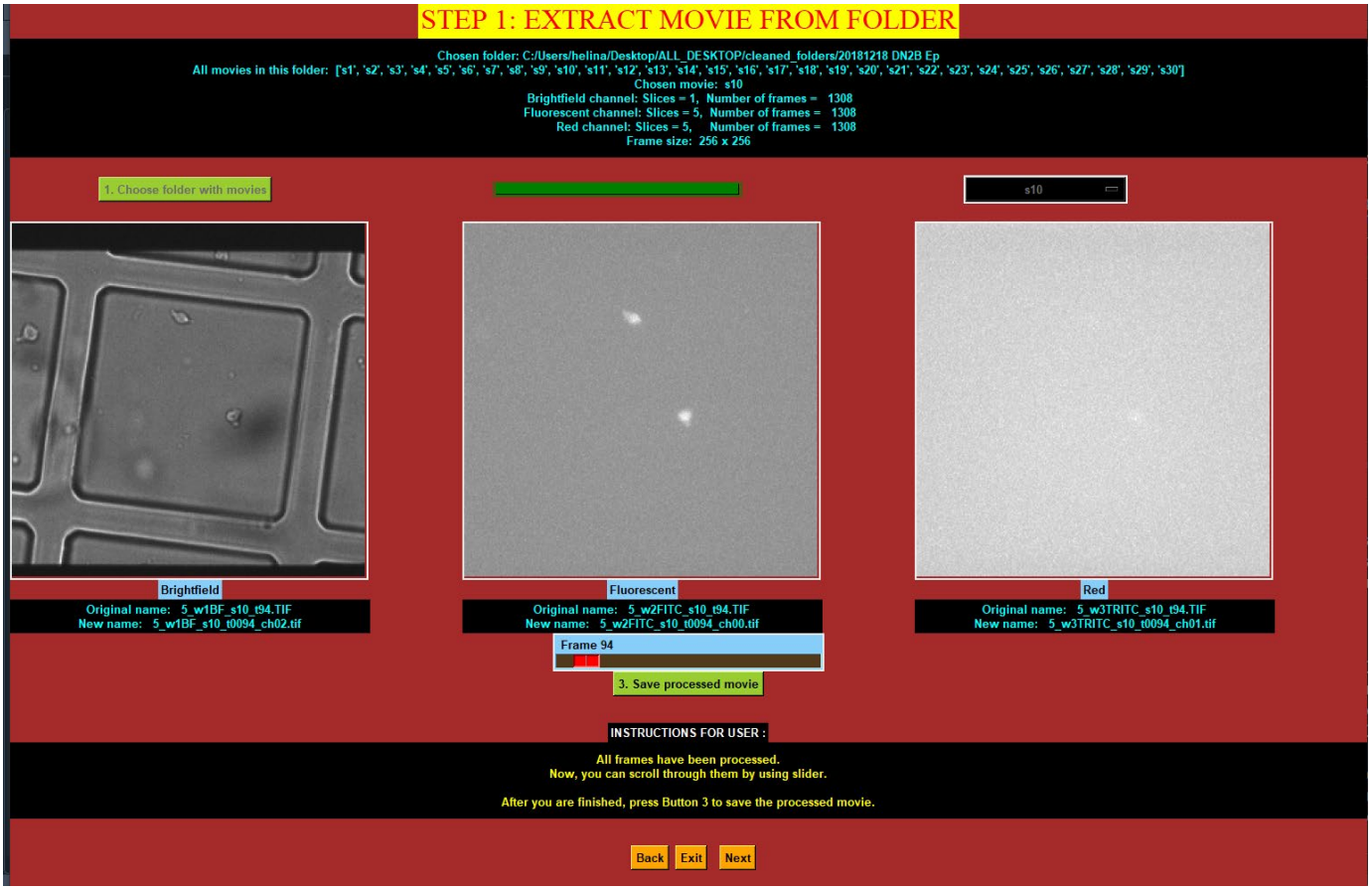
The same approach as for the previous case – the tools are provided in STEP-3 (button ADD CELL).

The short overview of each step

STEP-1: EXTRACT MOVIE FROM FOLDER

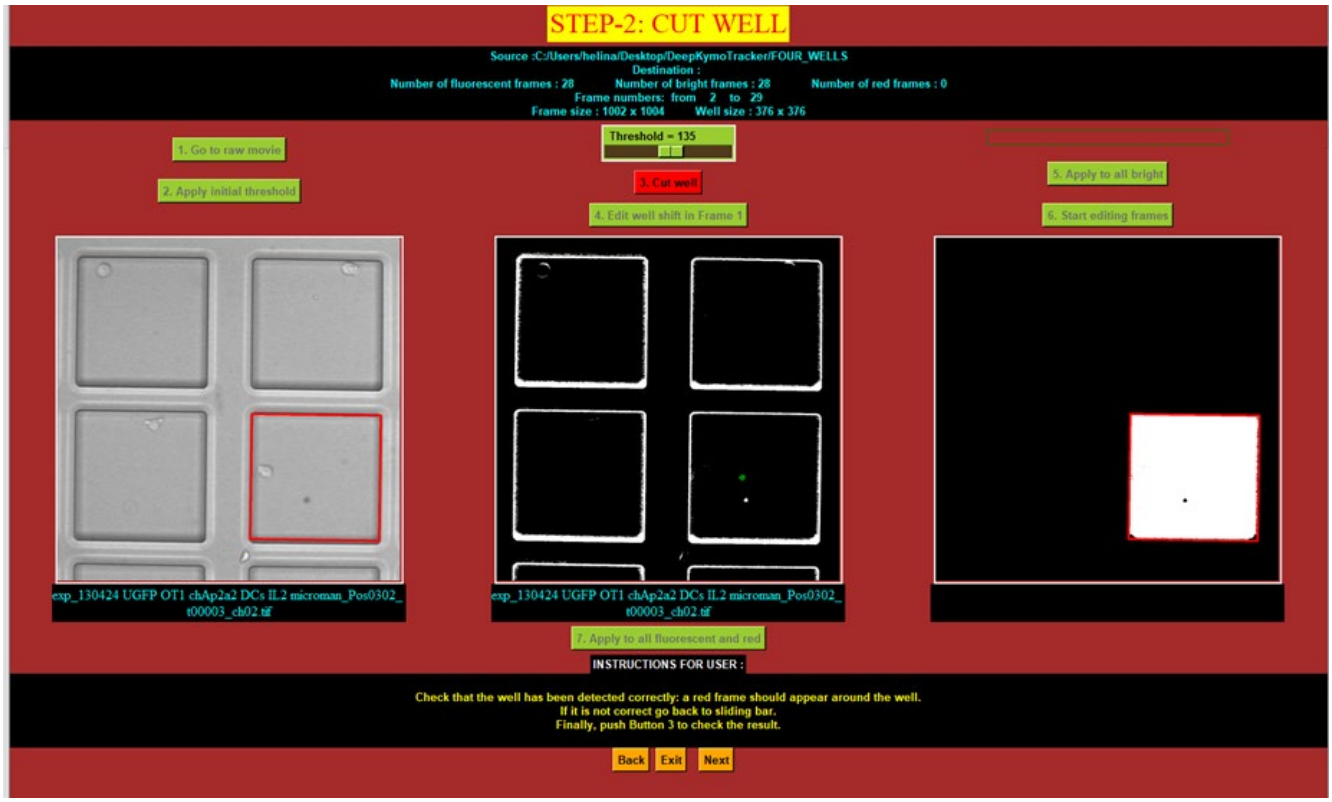
STEP 1 is designed to help you extract a specific cell movie from a folder containing numerous movies taken by spinning desk or epi, bring it to the necessary format suitable for the next step of DeepKymoTracker pipeline, and save the formatted movie in a new folder.

The program extracts all frames of chosen movie from folder, turns each multi-page tiff file into a single-page tiff file (by choosing pixels with the maximum values in each page of the initial multi-page tiff image), and changes files names slightly.



STEP-2: CUT WELL

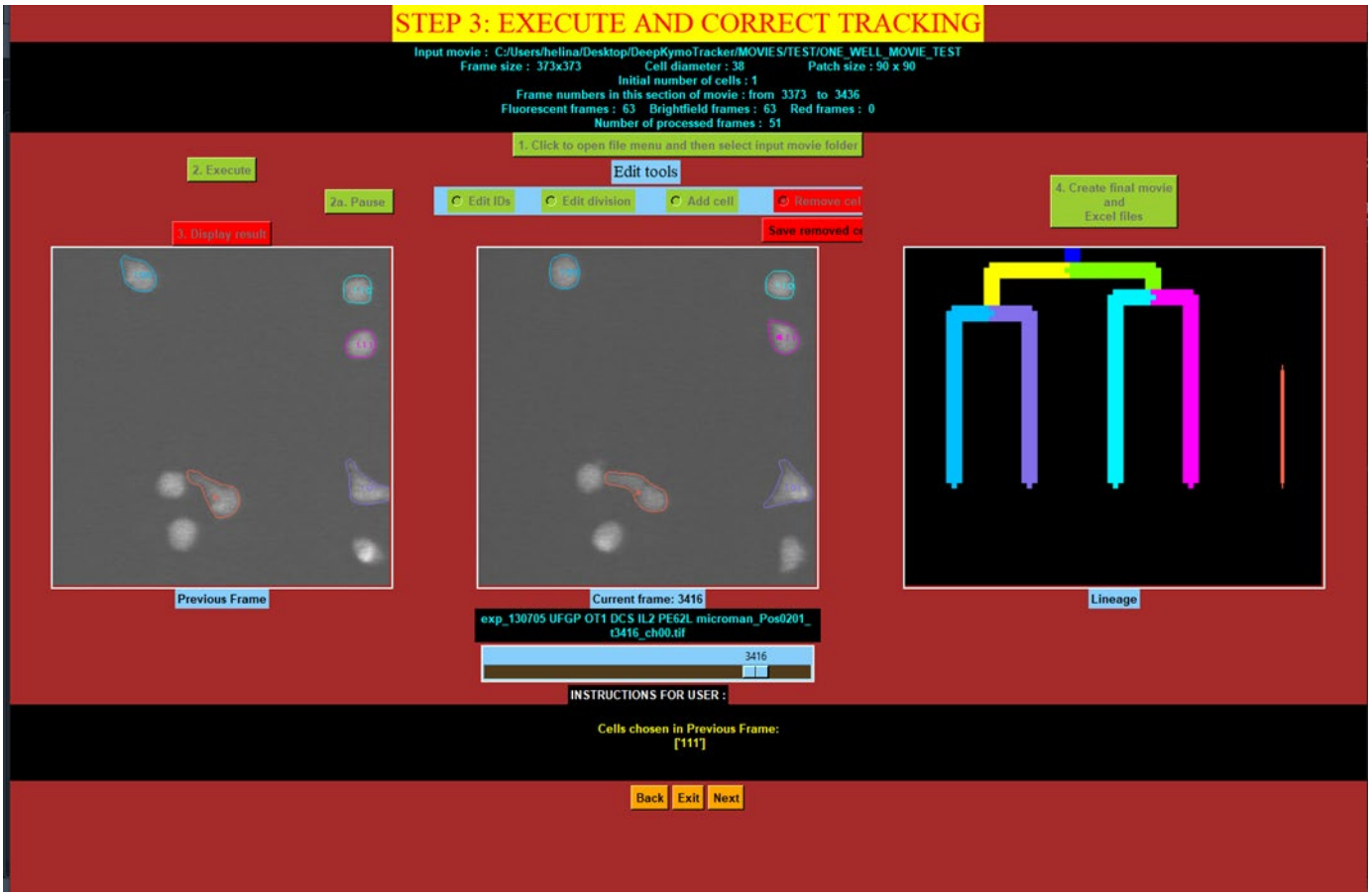
STEP 2 allows you to cut out a well of interest out of an initial cell movie, with the ultimate goal to prepare the movie for the next step, tracking and segmentation (STEP-3: track and correct).



STEP-3: EXECUTE AND CORRECT TRACKING

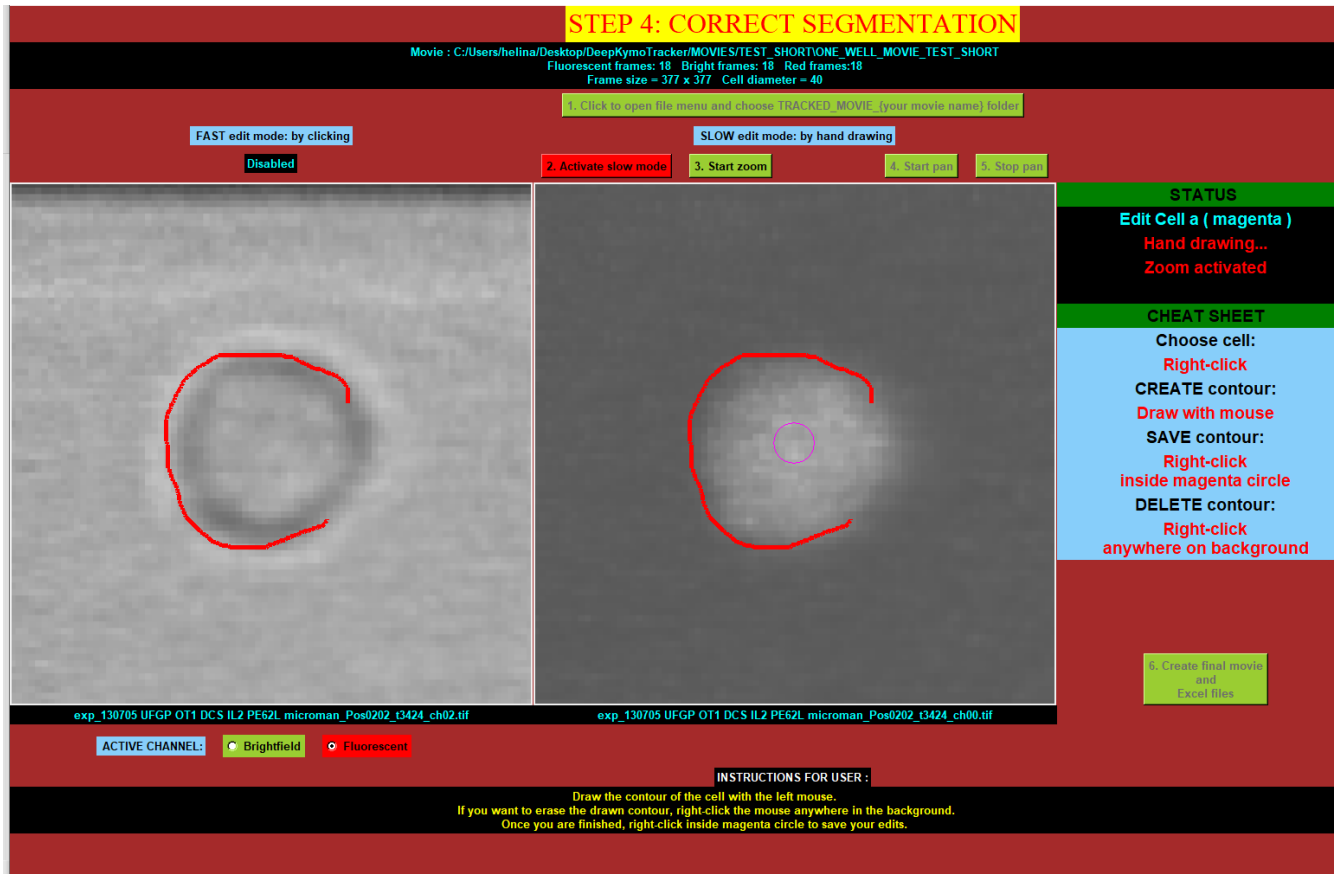
Step-3 is the core part of DeepKymoTracker - it is in this step where the automated tracking, segmentation and division detection happens. The lineage tree is plotted dynamically.

This step also allows you to manually correct tracking errors such as cell ID`s swapping and missed divisions, and to manually add new cells and remove dead cells. **Note:** you can correct only tracking errors here; manual segmentation correction will be conducted at the next step (STEP-4).



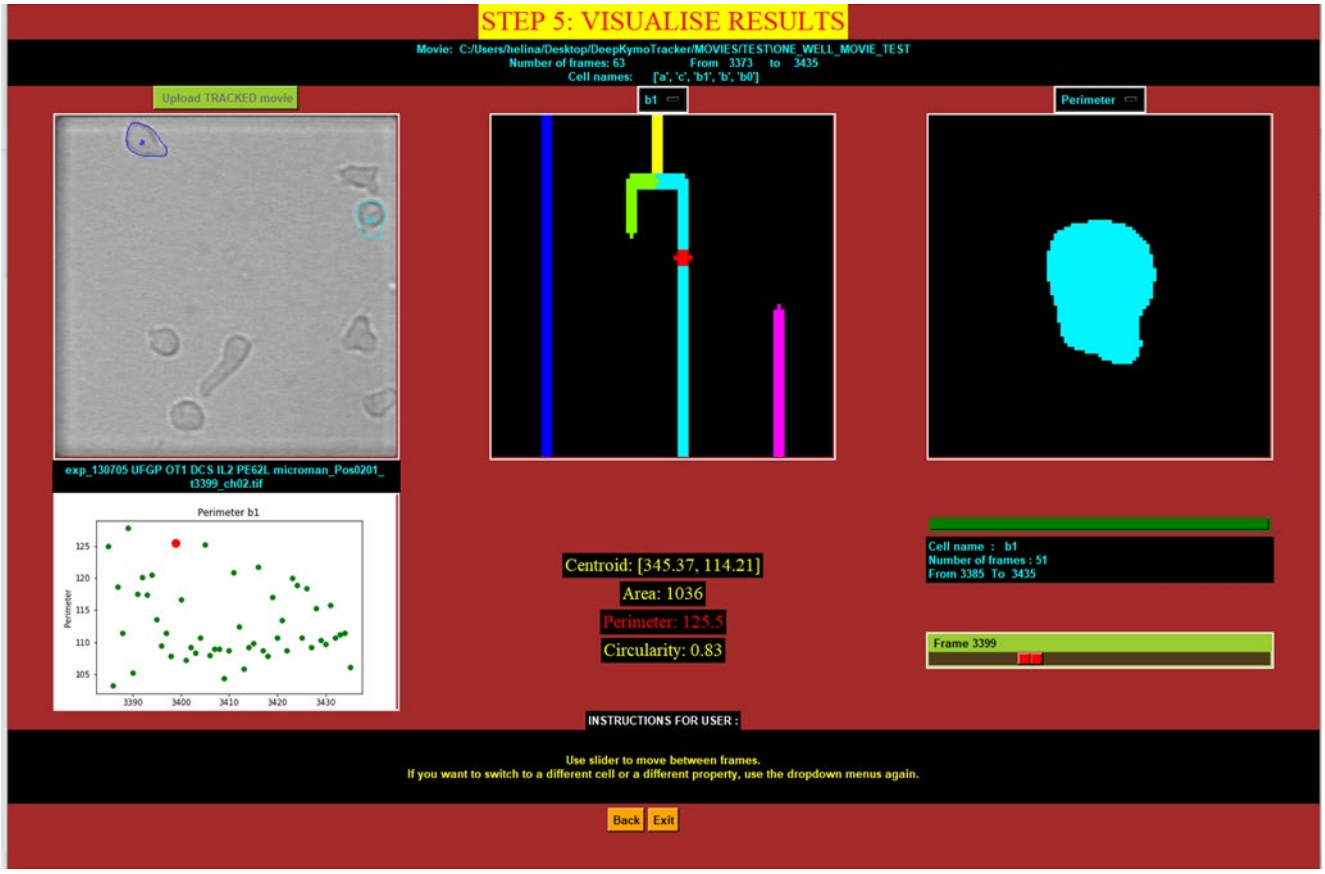
STEP-4: CORRECT SEGMENTATION

Step-4 allows you to manually correct segmentation errors after tracking has been applied to that movie in the previous step (STEP-3: TRACK AND CORRECT).



STEP-5: VISUALISE RESULTS

Step 5 allows you to bring to the screen all main charasteristics (the shapes, the areas, the perimeters, the circularities, the positions in the lineage) of a specific cell for each frame where it is present. By using the provided slide bar, you can see how those characteristics change from frame to frame dynamically.



FAQ

Can DeepKymoTracker cope with the situation where there is more than one cell in the first frame of a cell move?

Absolutely. Moreover, in STEP-3, you will be asked to manually assign the positions of the cells in the first frame by clicking on them.

Can I track a subset of cells present in a cell movie?

Yes. You will have to manually assign the positions of your cells of interest in the first frame (see the pervious question), so click only the cells which you are interested in.

How are cell names (IDs) assigned?

If there is only one cell in the 1st frame, it will be assigned the name 1 (and its daughters will be then 10,11, 101, etc.).

If there are multiple cells in the 1st frame, their names will be a,b,c,... (and the daughters names will then be a0,a1, a00, a10, b0, b1,...).

Is it possible to track only a section, not the whole cell movie?

Yes, you can cut out any number of consecutive frames from your cell movie and apply DeepKymoTracker to it.

Can I interrupt the tracking process (Step-3) and continue sometime later?

Yes, you just pause the algorithm (button PAUSE in Step-3) and then press EXIT button – the tracking results will be saved automatically. When you come back to that movie next time, the interface will inform you that this particular movie has been partially tracked and will ask if you would like to continue. Press OK.

Can I interrupt manual segmentation (Step-4) and continue sometime later?

Yes. It is designed to be used this way.

Monitoring tracking visually: is it absolutely essential to do it on the fly?

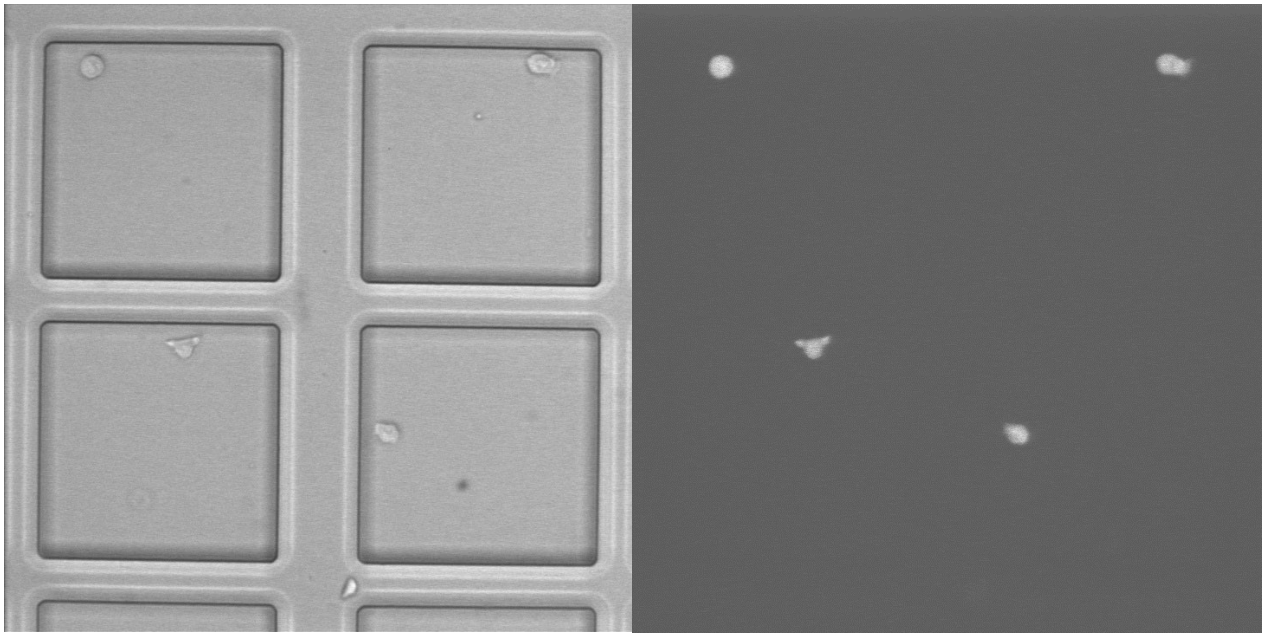
It is just the most efficient way in terms of time. You can leave the algorithm running by itself and come back later to check the results, no doubt. But if the algorithm has tracked say 3,000

frames by the time you got back and you discover an error in Frame 1000, it means that after manual correction in that frame, the algorithm will start tracking from Frame 1000, i.e. it will have to track the remaining frames all over again.

Therefore, if you are unable to finish monitoring the tracking of the whole movie in one go, it is recommended to interrupt the process by pushing buttons PAUSE and EXIT– the tracking results will be automatically saved, and next time the algorithm will resume tracking from where it left off.

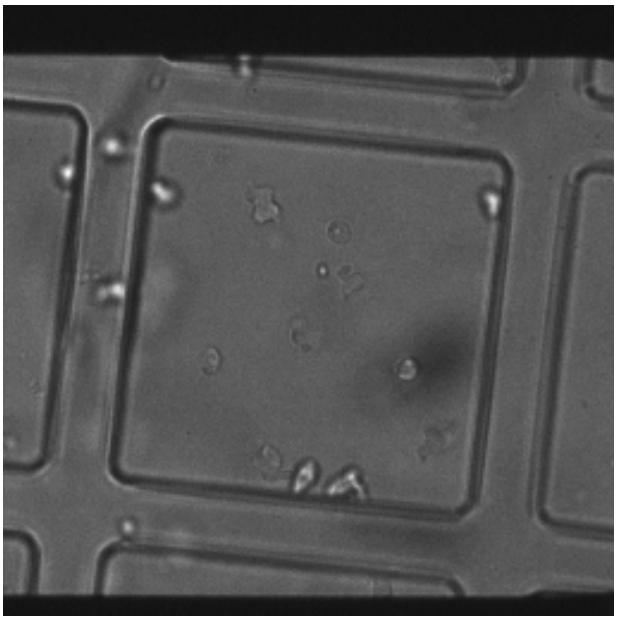
What is the difference between the "old" and "new" cell movies?

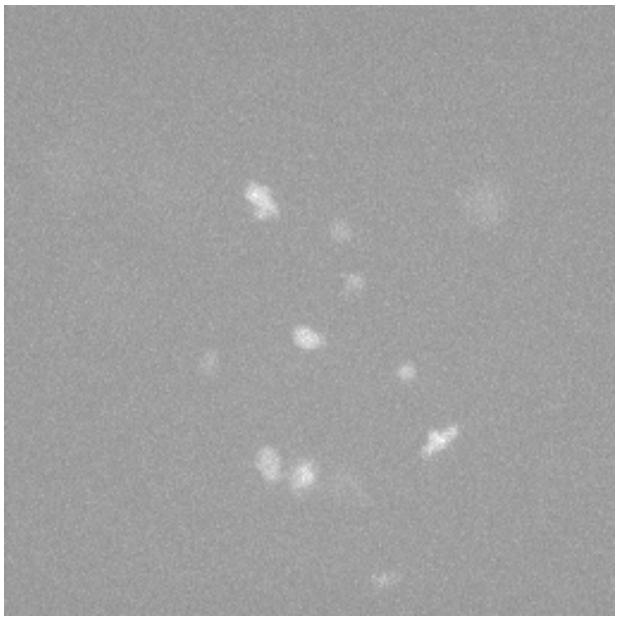
The "old" movies (or Mohammed`s movies) typically have names Pos0201, Pos0402, etc. and typically have 4 wells in each frame:



They are of a very good quality and resolution, and the tracking and segmentation neural networks which lay at the core of DeepKymoTracker have been trained on those movies. As a consequence, the performance of the software is very good on those movies.

The "new" movies were taken by a different type of microscope, and the quality is much worse, especially when it comes to the green fluorescent channel: the resolution is poor, and the background is not as homogenous as in the "old" movies. This leads to the algorithm producing much poorer cell segmentation results and consequently, entails a lot of manual post-correction.





To improve the situation with the poor segmentation, it is planned to retrain the segmentation neural network on the new movies. Once it has been finished, this tutorial will be updated, so you will be notified.

Another issue with the “new” movies is that the cells make quite big jumps too often, and as the tracking neural network was trained on “old” movies where this happened extremely rarely, the neural network is unable to cope with such situations, and therefore, the user has to stop the tracking quite often to make manual corrections.

One of the remedies, if you notice those big jumps, would be to go to the very beginning of Step-3 and increase the patch size – it should improve tracking results, but only to some extent.

Is it a good idea to move between the steps using buttons Back and Next at the bottom of each page?

On the one hand, these buttons were designed to enable you to do so. However, there is no guarantee that you will not run into OOM errors as no precautions have been implemented yet to prevent this from happening.

Therefore, the best recommendation is to use EXIT button once you are finished with one step, and then to launch DeepKymoTracker once again and go to the next step from the title window.

Once all the necessary techniques are implemented, this tutorial will be updated, so you will know.

Where can I look at the tracked movie?

There are 3 ways available:

1. Go to folders TRACKED_BRIGHTFIELD_CHANNEL, TRACKED_GREEN_FL_CHANNEL, TRACKED_RED_FL_CHANNEL. Here you can look through every frame and every channel of the cell movie.

2. The more dynamic way is to watch **lineage_movie.avi.** In this movie, the brightfield channel and the lineage tree are coupled.
3. Finally, you can go to Step-3 of DeepKymoTracker, push Button 1 and choose ONE_WELL_MOVIE_{your movie name}. If it is tracked, you will get a notification from the interface “The movie has been fully tracked. Press OK to view the processed frames”. After that, you can use the slide bar to scroll through each frame of the tracked movie.
   You will see only the green fluorescent channel and the lineage tree here.

What should I do if I need help or notice a bug?

Feel free to write to helinafedorchuk@yahoo.co.uk (personal email address) or kfedorchuk@swin.edu.au (expires on 10/06/2026).