

STEP-3: EXECUTE AND CORRECT TRACKING

(User Guide)

Goal: 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).

Input requirements:

1. The input cell movie needs to have images in 2 channels – fluorescent green and brightfield.
2. Frame names format: should end with **_t00002_ch00.tif** (for the green fluorescent channel) and **_t00002_ch02.tif** (for the bright field channel). In this example, 2 is the number of the frame.
3. Image data type: uint8

Output: After execution is finished, the algorithm generates excel files with numeric information about each tracked cell (such as area, circularity, etc. for each frame) and a number of folders with the visual information. The output layout is explained in detail in [5. Output layout explanation.pdf](#) file.

Soft points: There have been 2 types of cell movies available so far: so called old movies (or Mohammed's movies) and new movies taken by the microscope available in Optical Sciences Centre. The image quality of these two types differ enormously which

The performance of DeepKymoTracker on old movies is very good. There are 2 reasons: 1. The image quality and resolution are very high. 2. The tracking and segmentation neural networks deployed in DeepKymoTracker were trained on old movies, i.e. on similar data.

- As regards the new movies, the image quality of the green fluorescent channel is extremely low in most of the cases, and this unfortunately impacts the quality of segmentation in a negative way. This in turn leads to the necessity to perform a great amount of manual correction by the user (during STEP-4).

The segmentation part of the algorithm will be retrained on the new images in the very near future, so hopefully this drawback will be eliminated.

- Another pitfall about the new movies is that the cells make quite big jumps too often when moving from frame to frame (which happened very rarely in the old movies the tracking neural network was trained on). This leads to the tracking algorithm losing such cells and as a result, the user has to pause the tracking process to make manual corrections. When this happens too many times it gets really frustrating.

The partial solution to the latter problem could be to increase the patch size - the probability of losing the cell inside a bigger patch is somewhat lower. The tool for modifying the patch size manually is provided in the preparatory stage of STEP-3 (see Stage-1: Preparation below).

Instructions

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Upload input movie

Launch DeepKymoTracker and press **GO TO STEP 3** on the title page:

DeepKymoTracker

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You are in the tracking window now. Press flashing **Button1**:

STEP 3: EXECUTE AND CORRECT TRACKING

Frame size : Input movie :
Cell diameter : Initial number of cells :
Patch size :
Frame numbers in this section of movie : from to
Fluorescent frames : Brightfield frames : Red frames :
Number of processed frames :

1. Click to open file menu and then select input movie folder

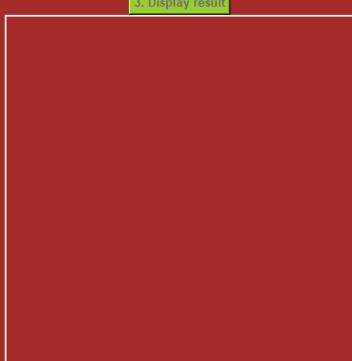
2. Execute

2a. Pause

Edit tools

3. Display result

4. Create final movie and Excel files



Previous Frame

Current Frame

Lineage

1

INSTRUCTIONS FOR USER :

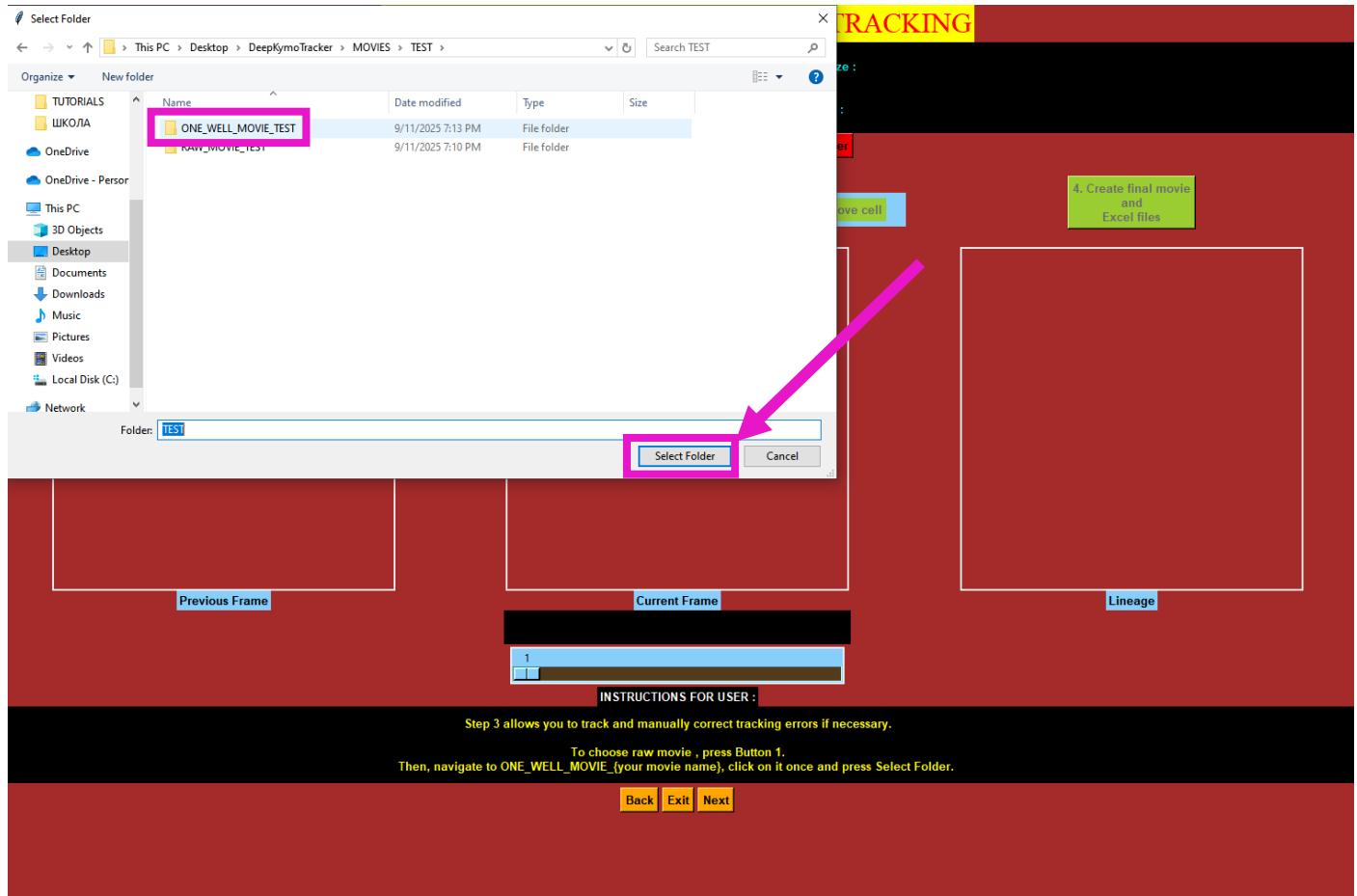
Step 3 allows you to track and manually correct tracking errors if necessary.

To choose raw movie , press Button 1.

Then, navigate to ONE_WELL_MOVIE_(your movie name), click on it once and press Select Folder.

[Back](#) [Exit](#) [Next](#)

In the open file menu, navigate to ONE_WELL_MOVIE_{your movie name} and click on it once (not twice!) and press **Select Folder**.



What happens after that depends on what state your movie is in. There are 3 possible states:

- Untracked movie
- Fully tracked movie
- Partially tracked movie

State-1: Untracked movie

If you have chosen ONE_WELL_MOVIE_{your movie name} that has never been tracked before, the following popup window appears (see the figure below).

In this case, you need to prepare the movie for tracking (Stage-1: Preparation) and, once you are finished, you can proceed to the automated tracking itself (Stage-2: Automated Tracking).

Stage-1: Preparation

As can be seen from the screenshot below, the three windows show all the 3 channels of the movie: brightfield, green and red fluorescent.

As you can see, there are no red channel frames in this example movie. It does not matter though, as DeepKymoTracker needs only brightfield and green channels to perform tracking and segmentation.

You can inspect each frame if necessary, using the provided slide bar.

The main purpose of this stage, however, is to enable the user to manually set up some parameters necessary for successful automatic tracking; these are

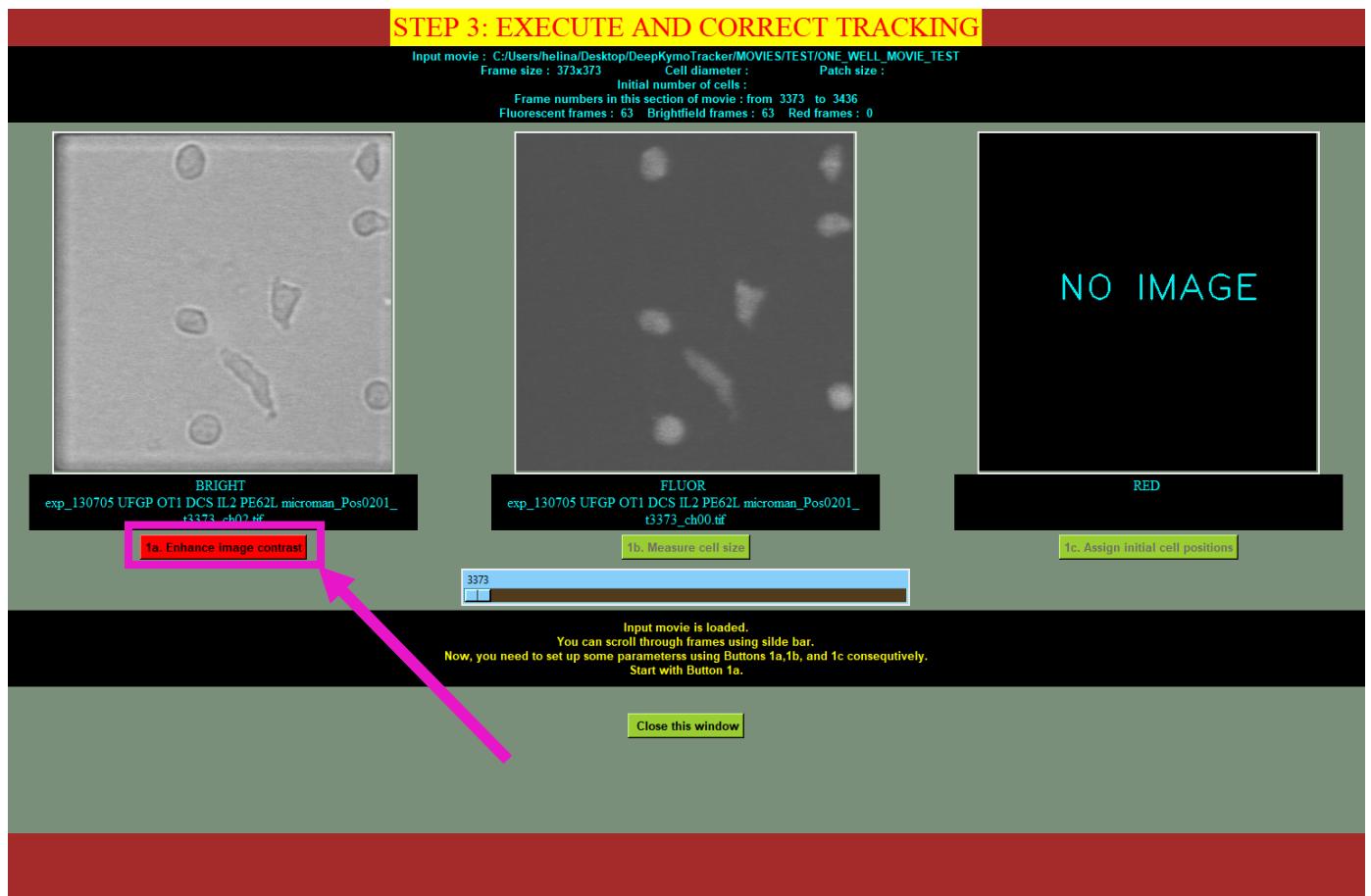
- the average cell diameter (which can vary from movie to movie and therefore, need to be measured for each movie)
- the positions of cells in the very first frame

Apart from these 2 crucial parameters, you will be asked

- to enhance the contrast of the green fluorescent channel
- to change the patch size if necessary

The meaning of these parameters will be explained below.

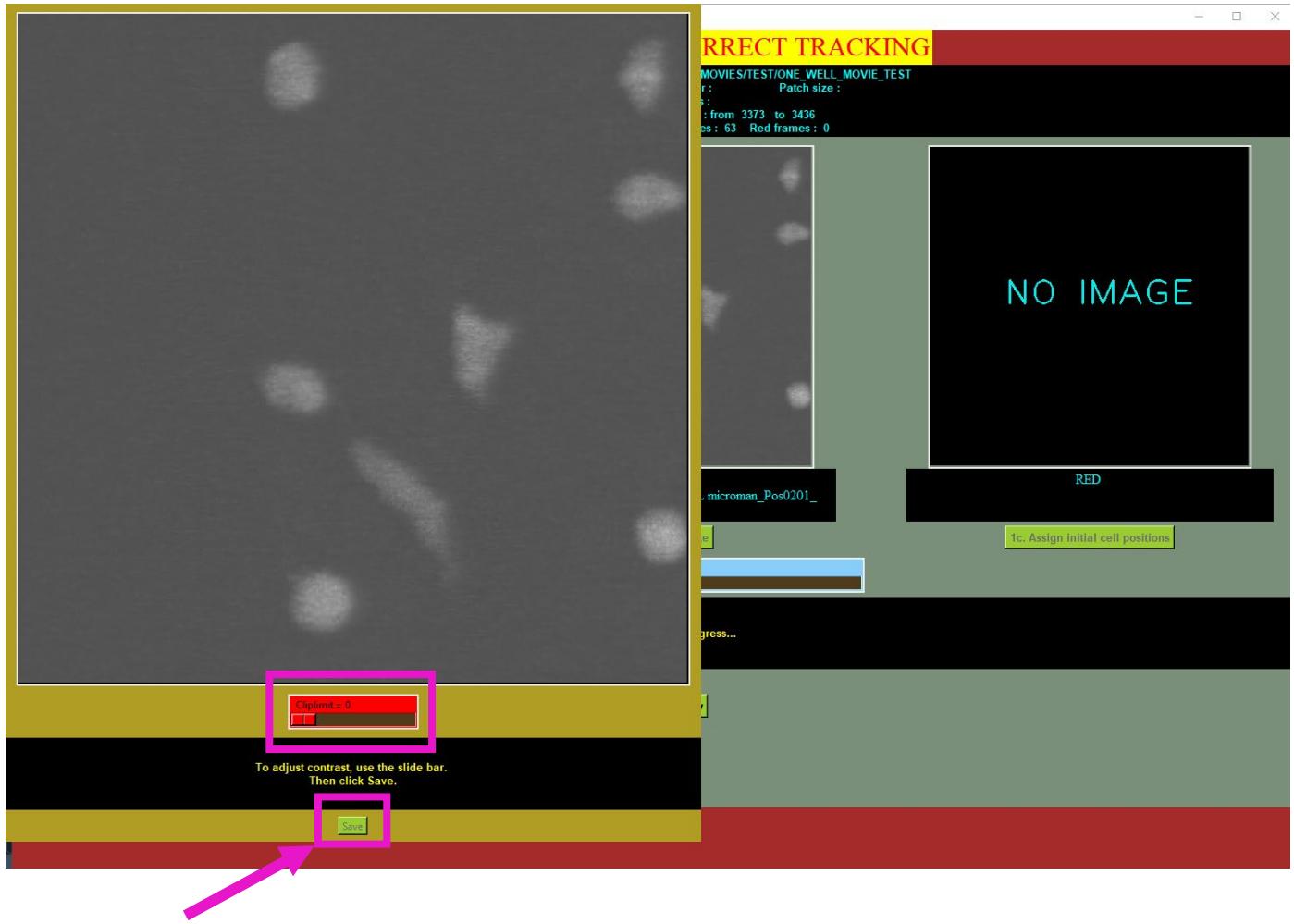
To begin, push button **1a. Enhance image contrast:**



One more popup window appears, with the first frame (green fluorescent channel) in the centre.

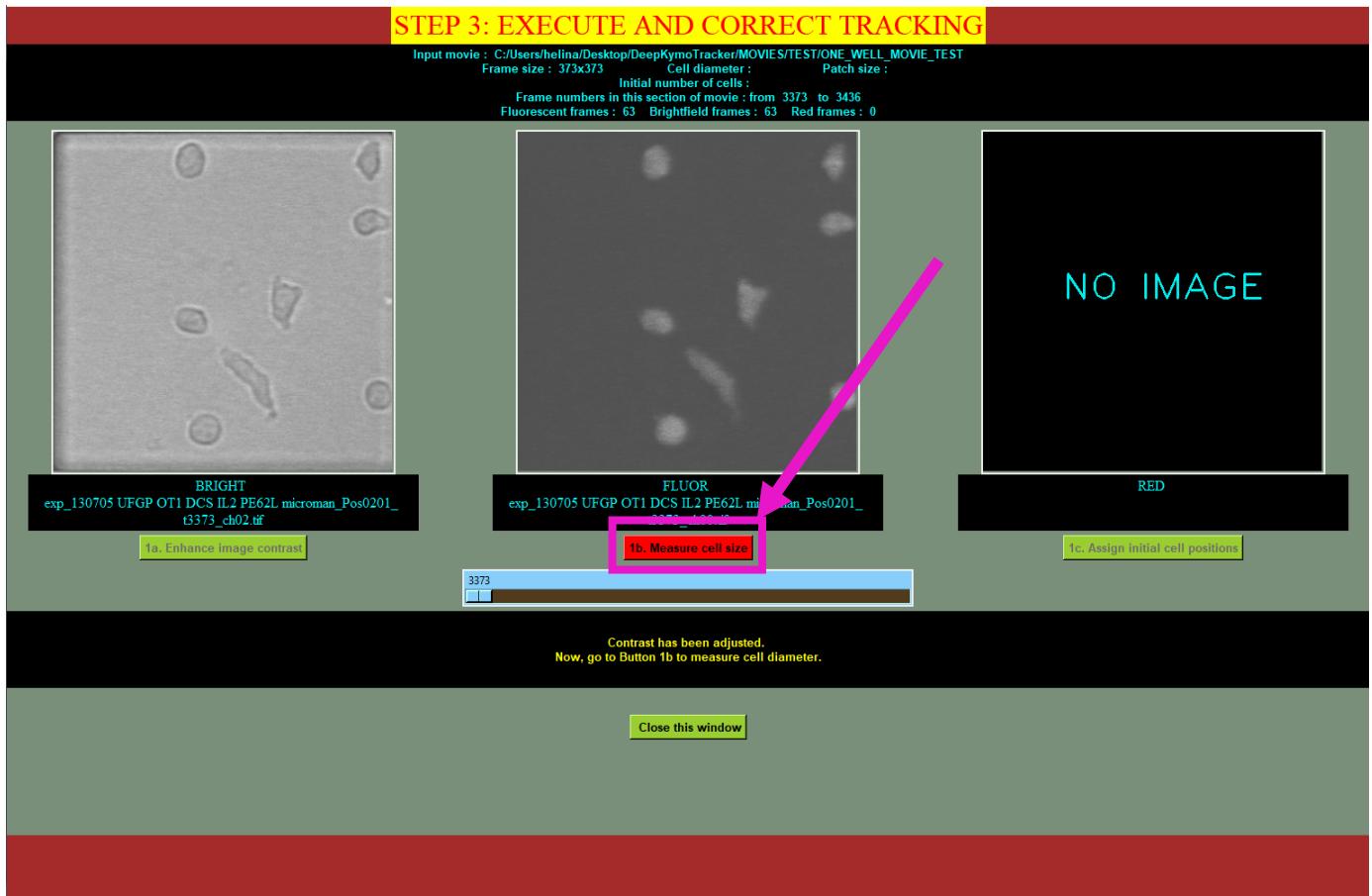
The possibility of changing the contrast was implemented solely for the user's convenience(the tracking algorithm does not need this, it will still be using the original, non-enhanced images anyway). The reason is that in some movies, the green channel is so low contrast that the outlines of cells are barely legible. And this can cause inconvenience as the user is supposed to monitor the progress of tracking in real time.

Play with the slide bar provided to change the contrast, and once you are happy, press **Save.**

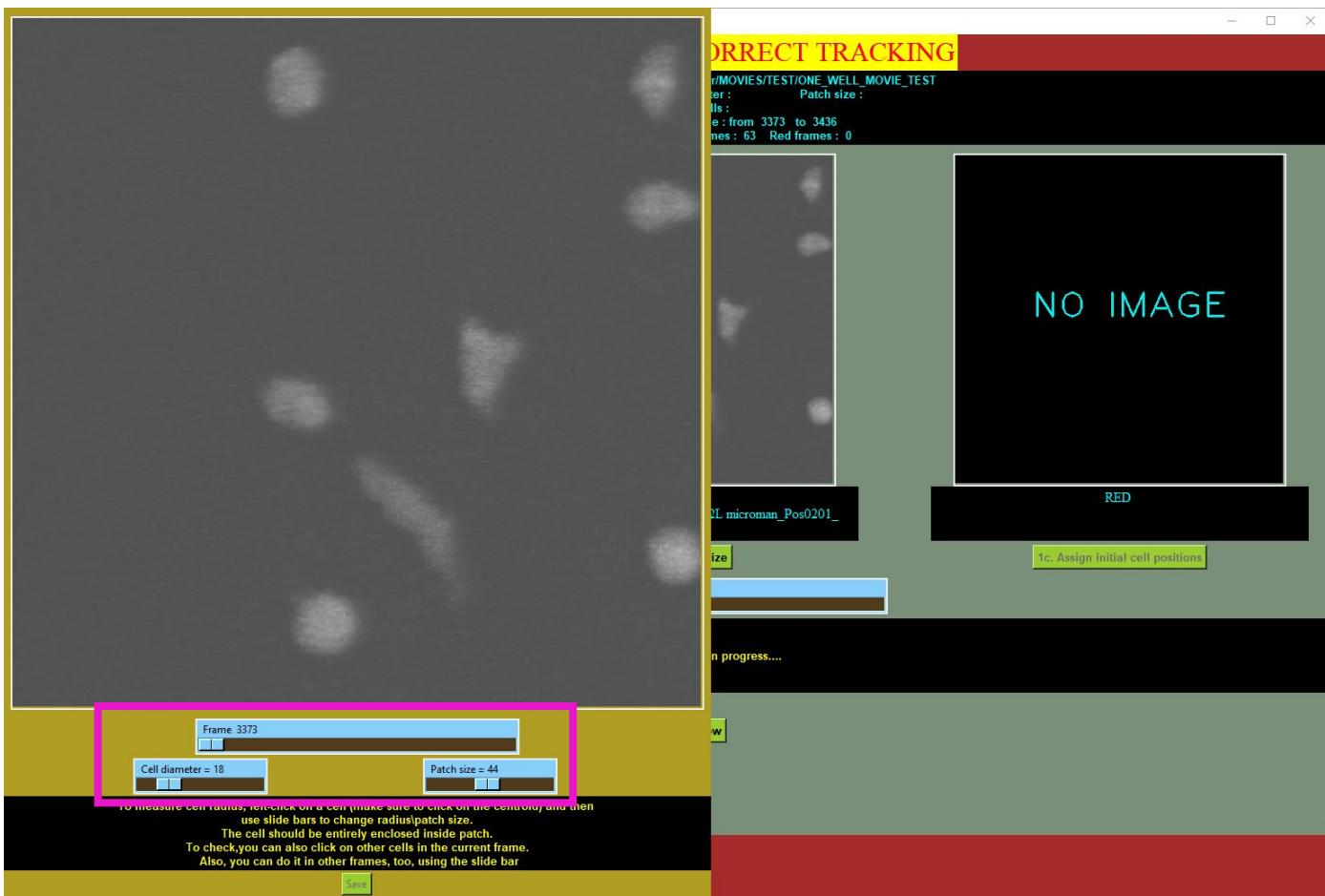


You are back in the preparation window. Now comes the time for the cell diameter measuring. Push

1b. Measure cell size:



Once again, a new popup window appears, with a green fluorescent frame in the centre



Left-click on any cell – the red circle and the yellow square will appear around it. The diameter of the red circle is equal to the average diameter of the cell which will be passed to the tracking algorithm; the size of the yellow square is equal to the patch size which is the second parameter the algorithm needs to know.

Now you can adjust both parameters by using the slide bars.

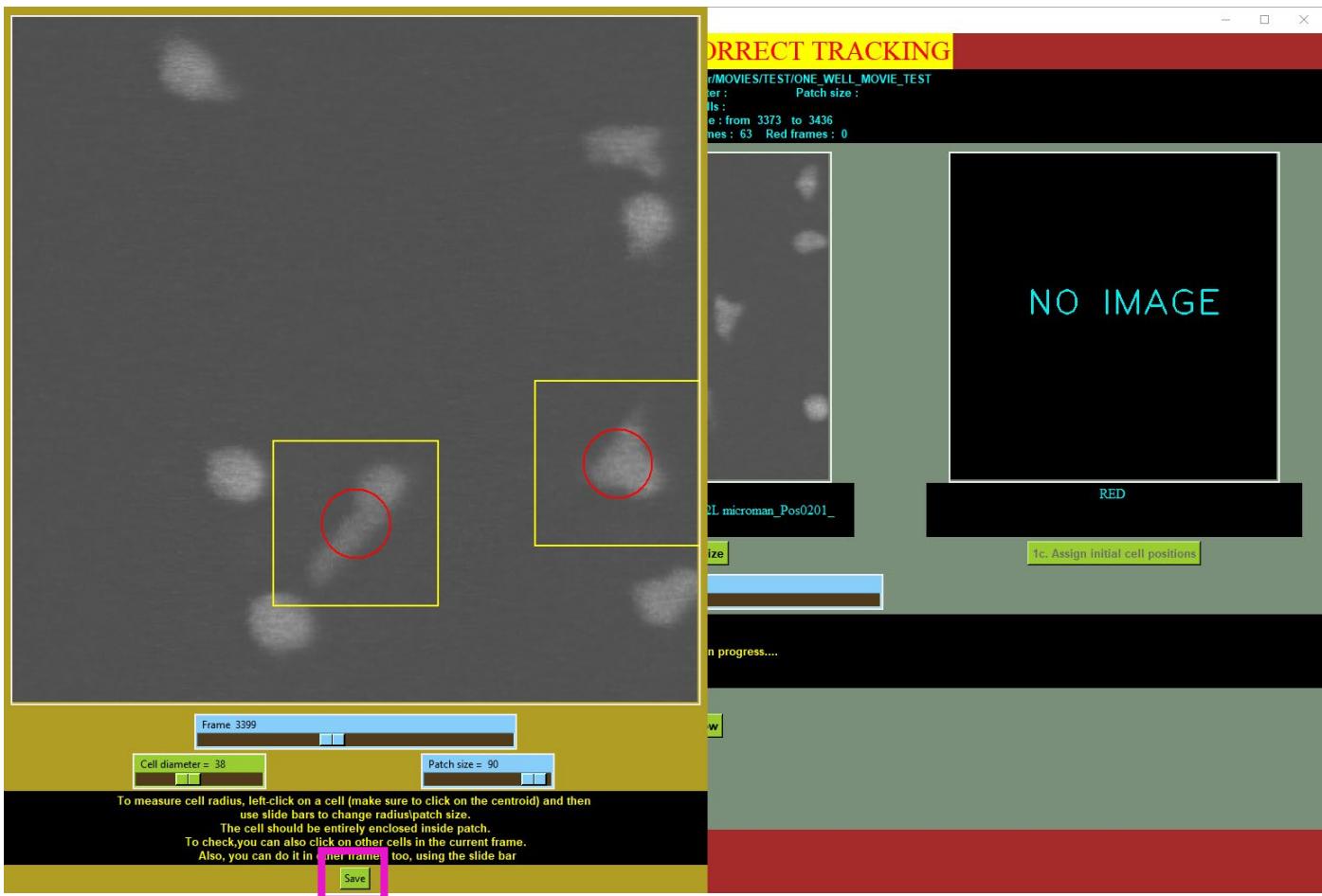
What is the right size?

The main thing you need to watch for is that the patch size is big enough to incorporate the whole body of any cell (the diameter of the cell represented by the red circle diameter is not so crucial – as you can see from the picture below, one of the cells is a bit too long to fit into the red circle, and this is absolutely fine).

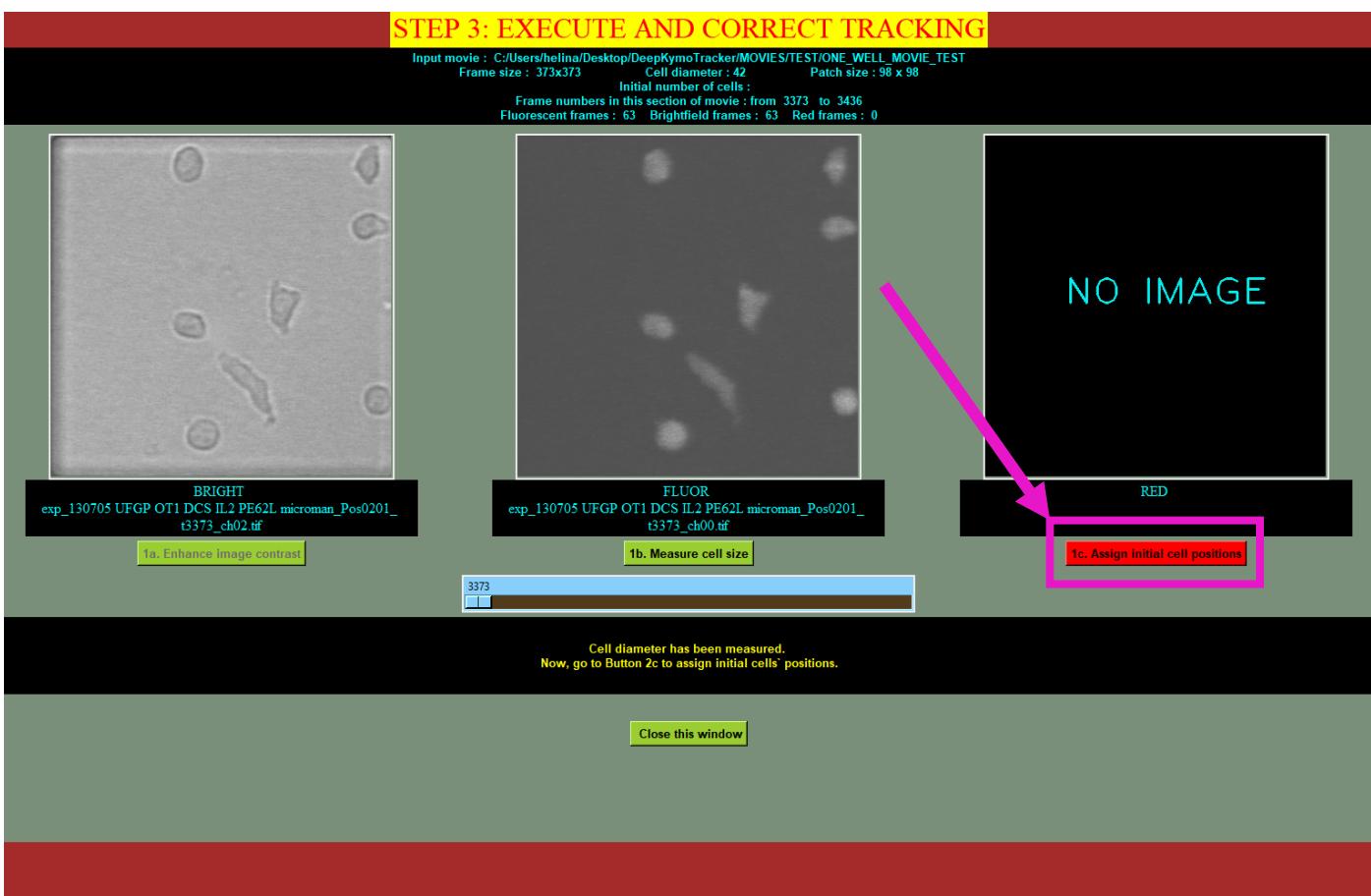
To make sure that your choice of these two parameters is correct, scroll through the frames and keep left clicking on the centroids. Once finished, click **Save**.

Note that the slide bars for the cell diameter and the patch size are programmed in such a way that whichever values you set up by using them, the ratio of the square side to the diameter cannot be less than 2.4 (otherwise the tracking algorithm will break apart).

However, this ratio can be set up bigger than 2.4 – it can be useful for the movies where the cells make really big jumps, because the bigger the patch is the less likely is the algorithm to lose the wildly jumping cell. The perfect ratio in this case can be found only through experimentation though.



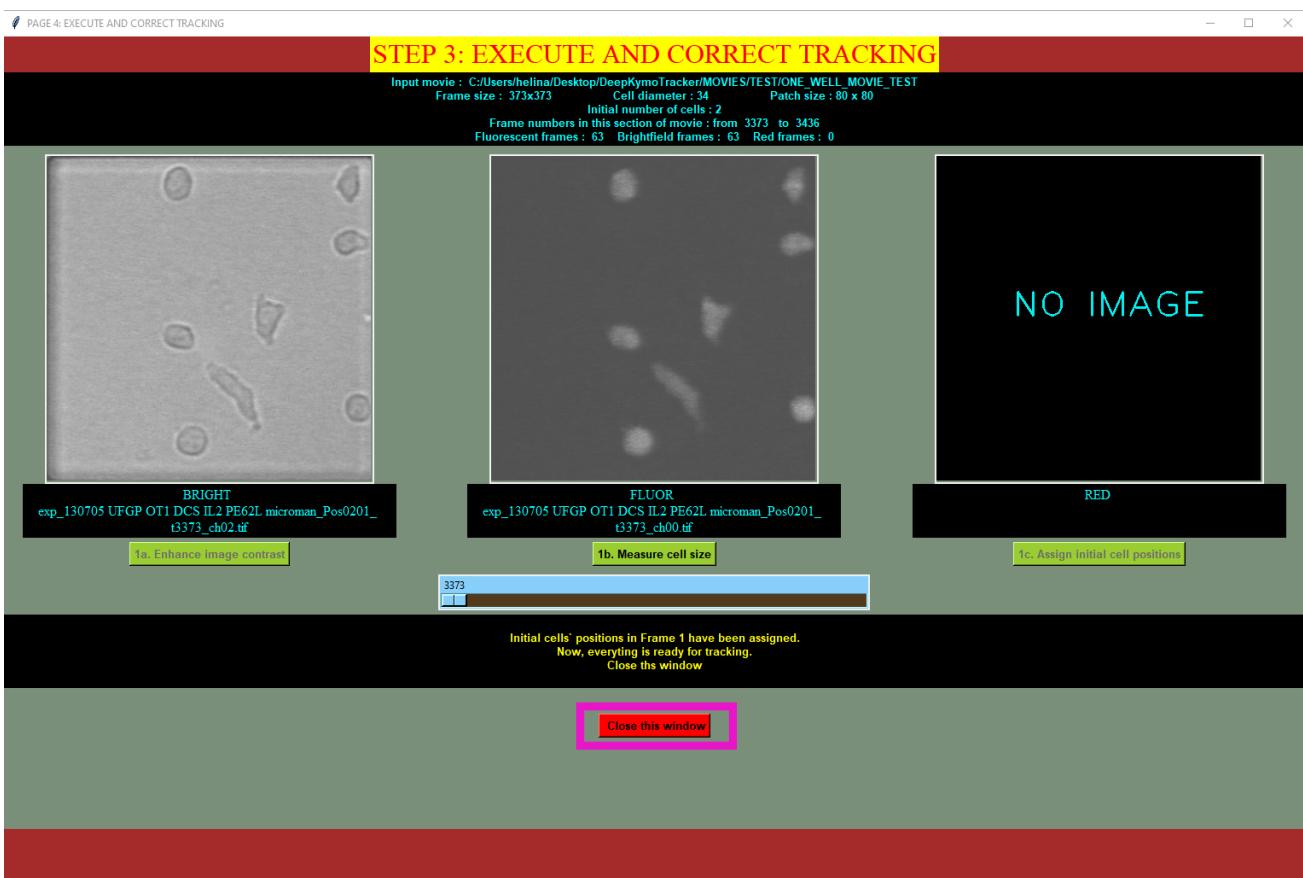
After pressing **Save**, you find yourself in the preparation window once again. Press the last button **1c. Assign initial positions of cells.**



Once again, a new popup window jumps into view. This is the very first frame of your movie from which the tracking will start. Left click on the cells you want the algorithm to track (make sure you click approximately on their centroids) and after that, press **Save**.

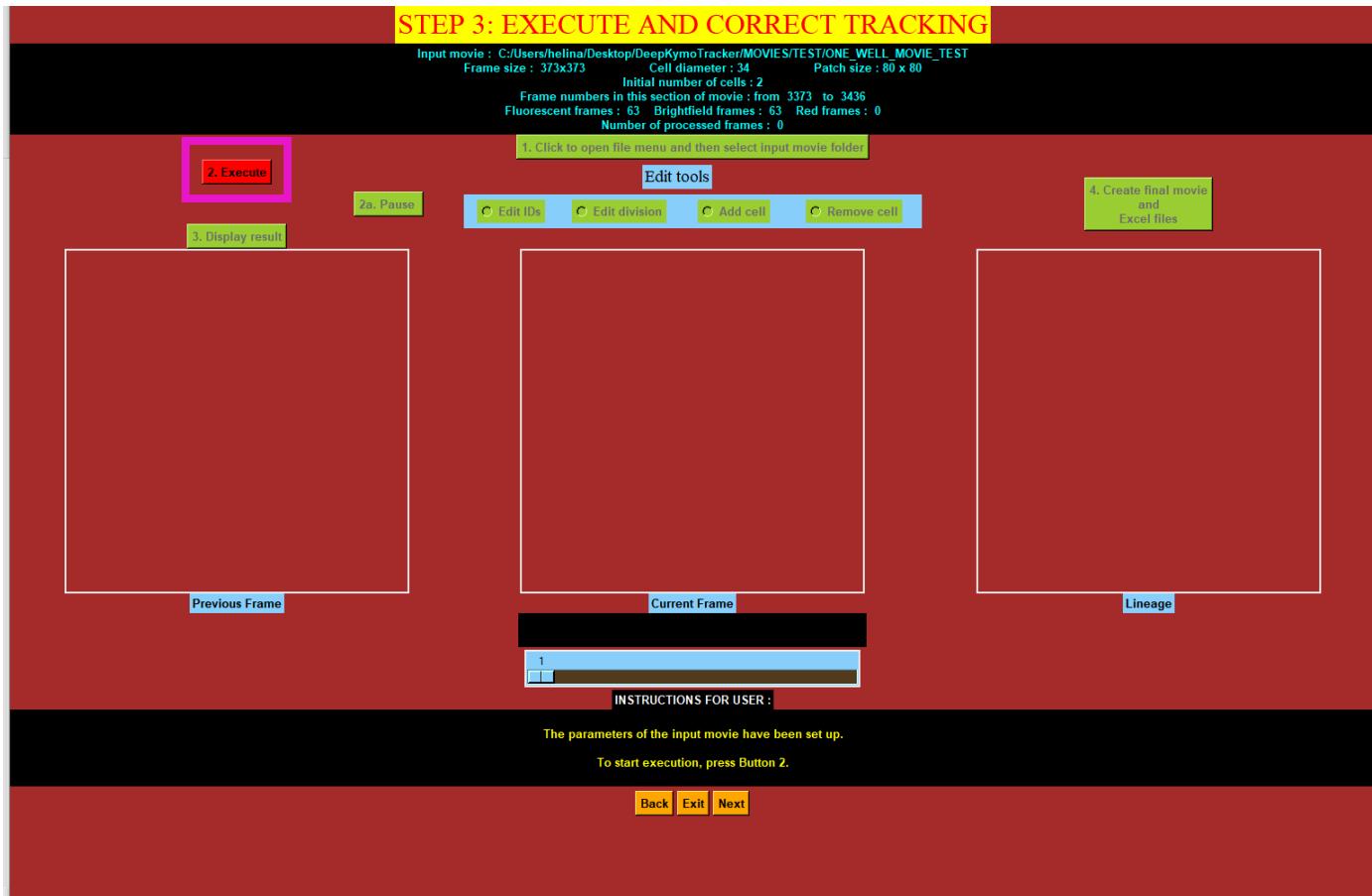


Press **Close this window**:

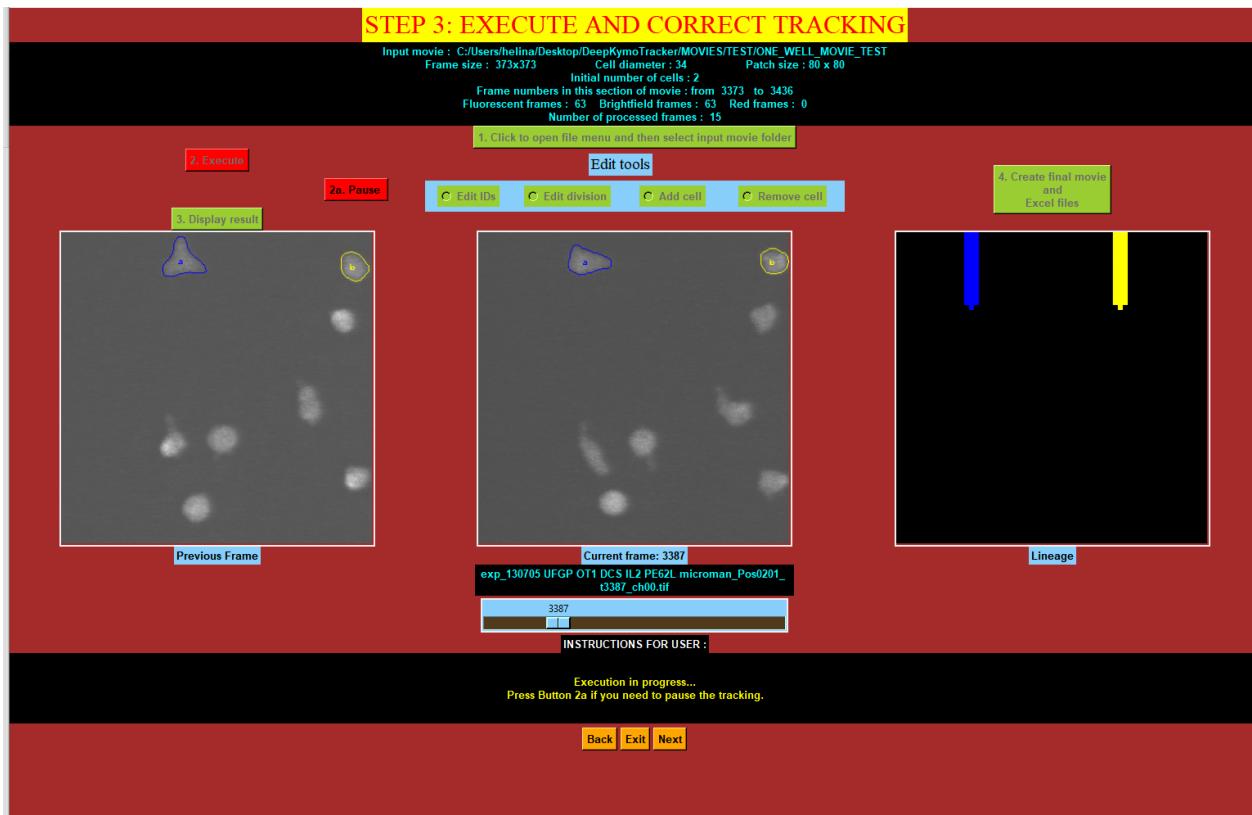


Stage-2: Fully automated execution

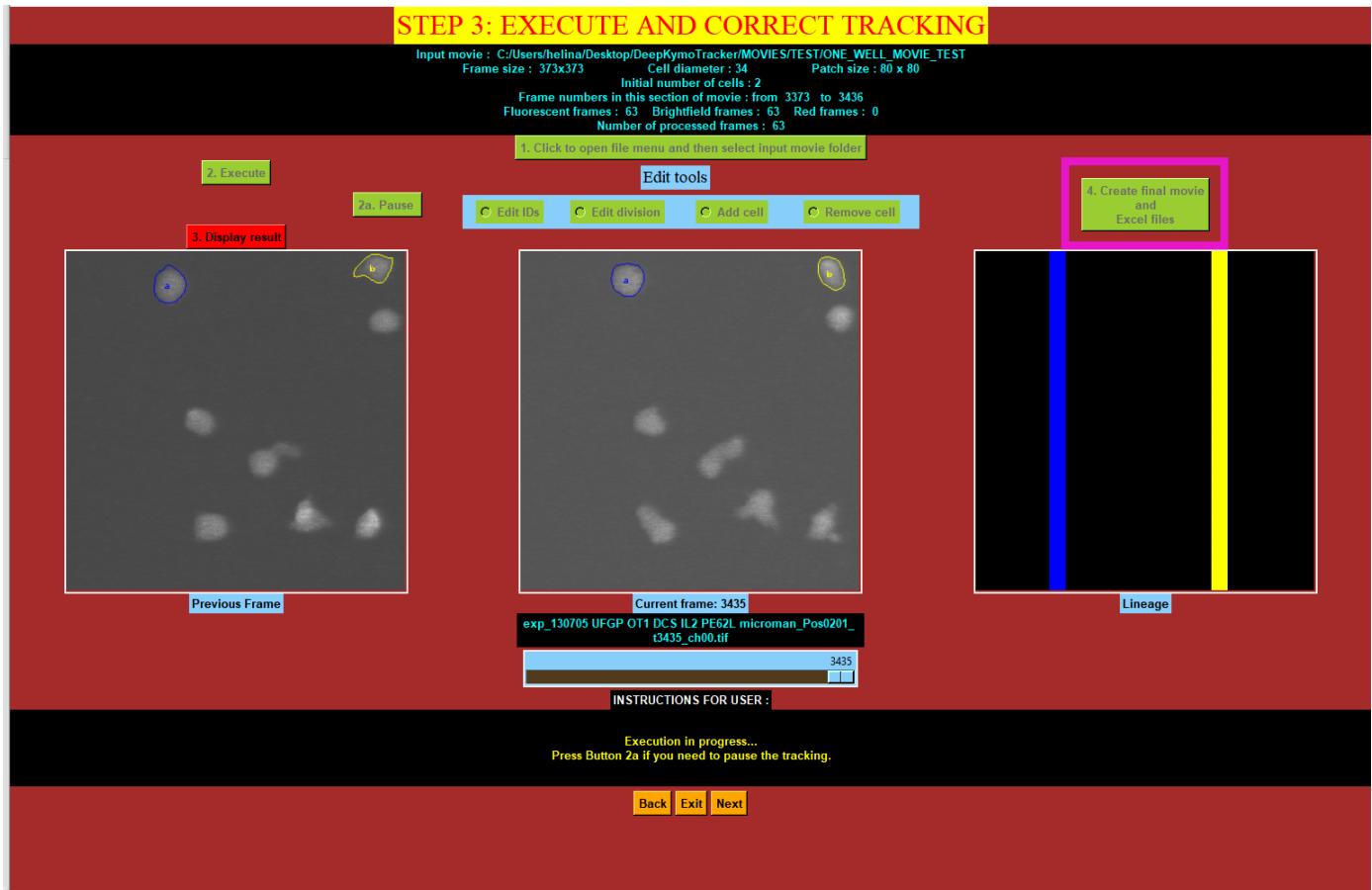
You are back in the main window now. Push **2. Execute**:



The automated tracking starts. You need to monitor the process happening in the middle window and, if something goes wrong you need to pause the algorithm (by pushing button 2a.Pause), make necessary corrections, and re-launch execution by pushing 2. Execute again.



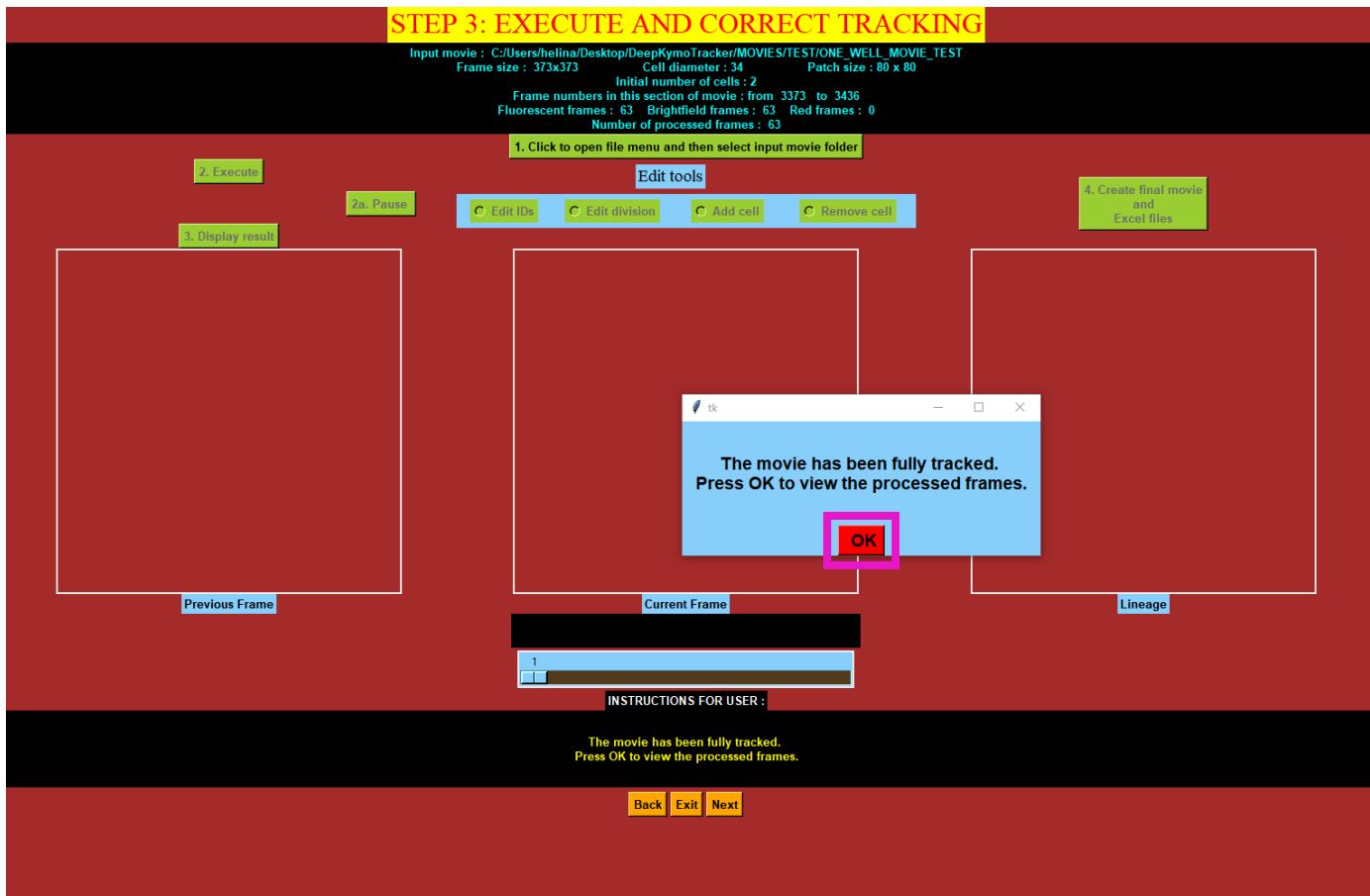
If everything goes fine, however, you just wait to the end and then push button **4. Create final movie and Excel files**.



State-2: Fully tracked movie

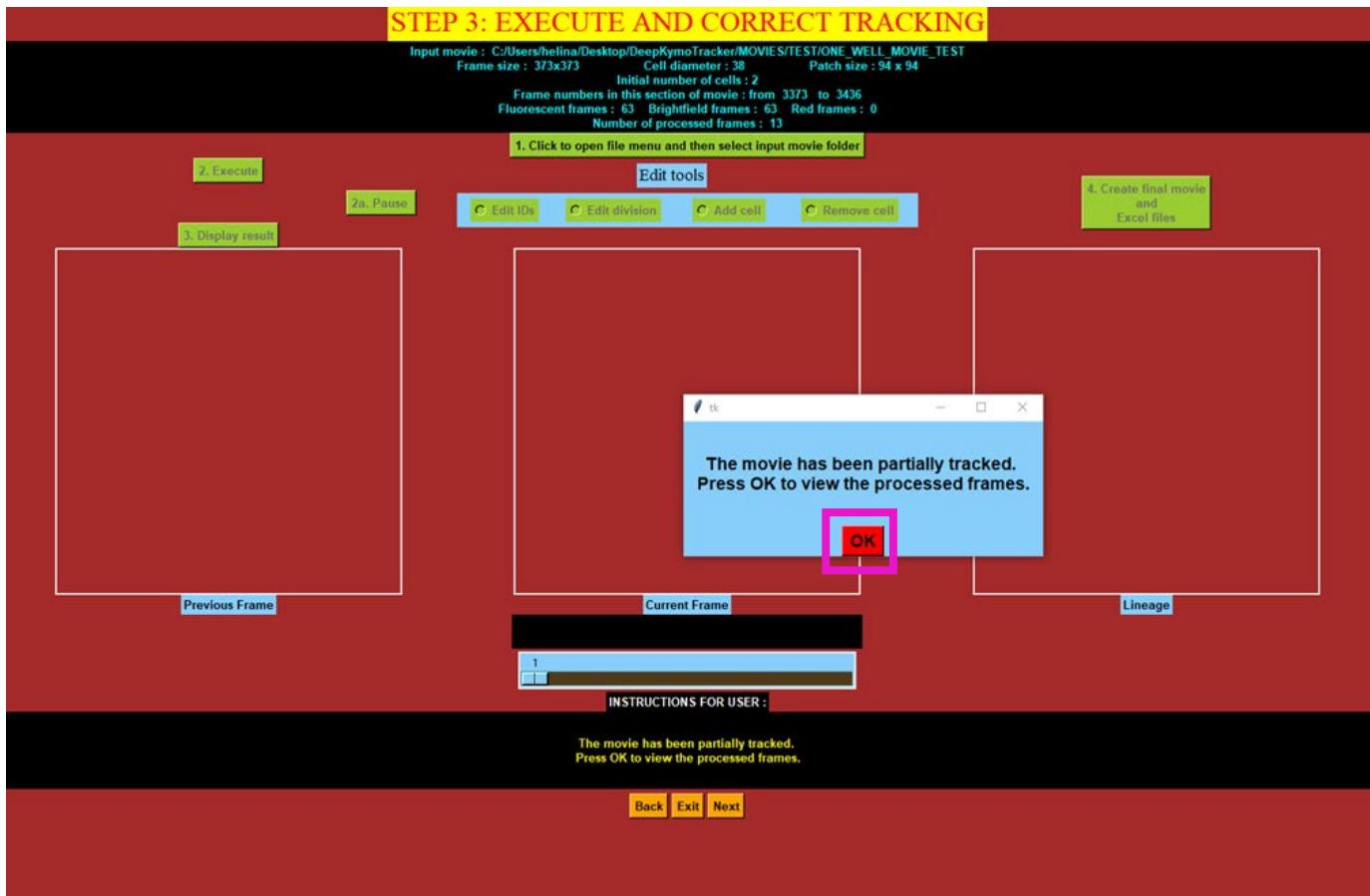
If after choosing ONE_WELL_MOVIE_{your move name} you get this notice, it means that the movie has already been fully tracked. You can press **OK**, then **3. Display results**, and finally use the slide bar to look through the tracked frames.

If you notice some tracking errors you need to stop the slider at this frame, apply one of the editing tools that will be described below, and push **2.Execute**. The tracking will start with the frame you just corrected.



State-3: Partially tracked movie

If you received this message, it means that you force interrupted the tracking in the past. And the movie was left unfinished as a result. To resume tracking, all you need to do now is to push button **OK** and then **2. Execute**.



So far, we have not used button Pause. Now is the time to describe its functionality.

What can you do with Pause button?

1. Pause to inspect results
2. Pause to interrupt execution
3. Pause to edit tracking errors

1. Pause to inspect results

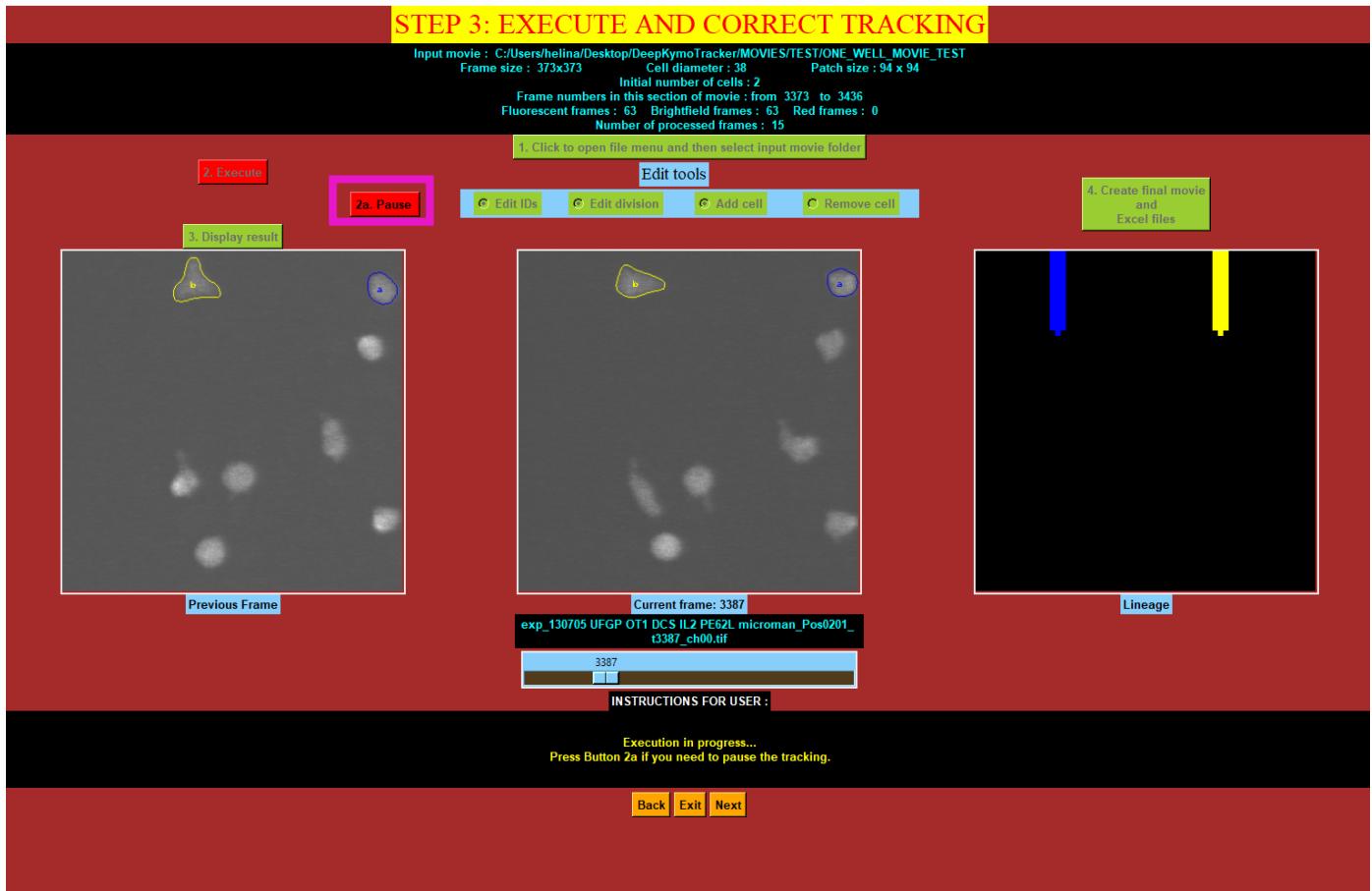
When tracking cells with DeepKymoTracker, the user is supposed to monitor the progress (in the middle window) and, if a tracking error has been spotted, they need to pause the algorithm and correct the error manually.

However, sometimes you are not sure whether you have failed to spot an error or not, due to a lapse of concentration.

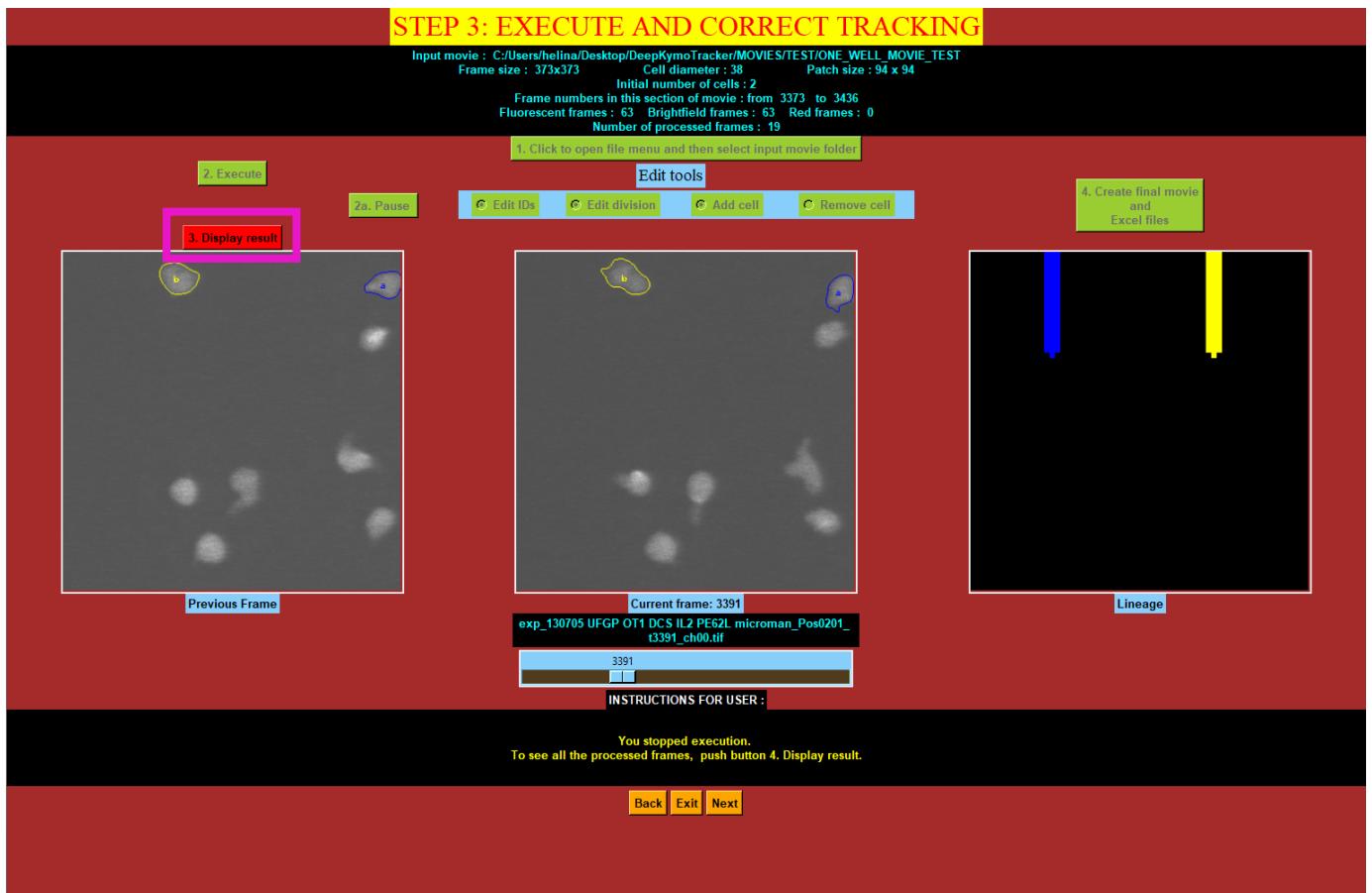
In this case, you can pause the automated tracking, inspect the results and, if everything is fine, re-launch tracking.

How exactly can it be done?

First of all, push the flashing **Pause** button.



The tracking stops and **Display result** button starts flashing. Press it.



Now you can use the slide bar to move between the tracked frames and look for errors.

If everything is fine, push **Execute**. Tracking will resume, starting with the frame which is the next after the one you paused at.

2. Pause to interrupt execution

You might want to interrupt execution if you do not have time to monitor the progress to the end of the movie and therefore, want to leave it for another time. Push the buttons in the following order:

Pause → **Create final movie** → **Exit**

Pushing Button **4. Create final movie** is optional – it is, probably, too early to create the output information if the movie is not fully tracked yet, but on the other hand, might be useful at this stage as well.

This procedure will make your movie qualified as partially tracked and therefore, when you come back to the movie next time (with the purpose of resuming tracking), you will have to deal with **State-2: partially tracked movie** described above.

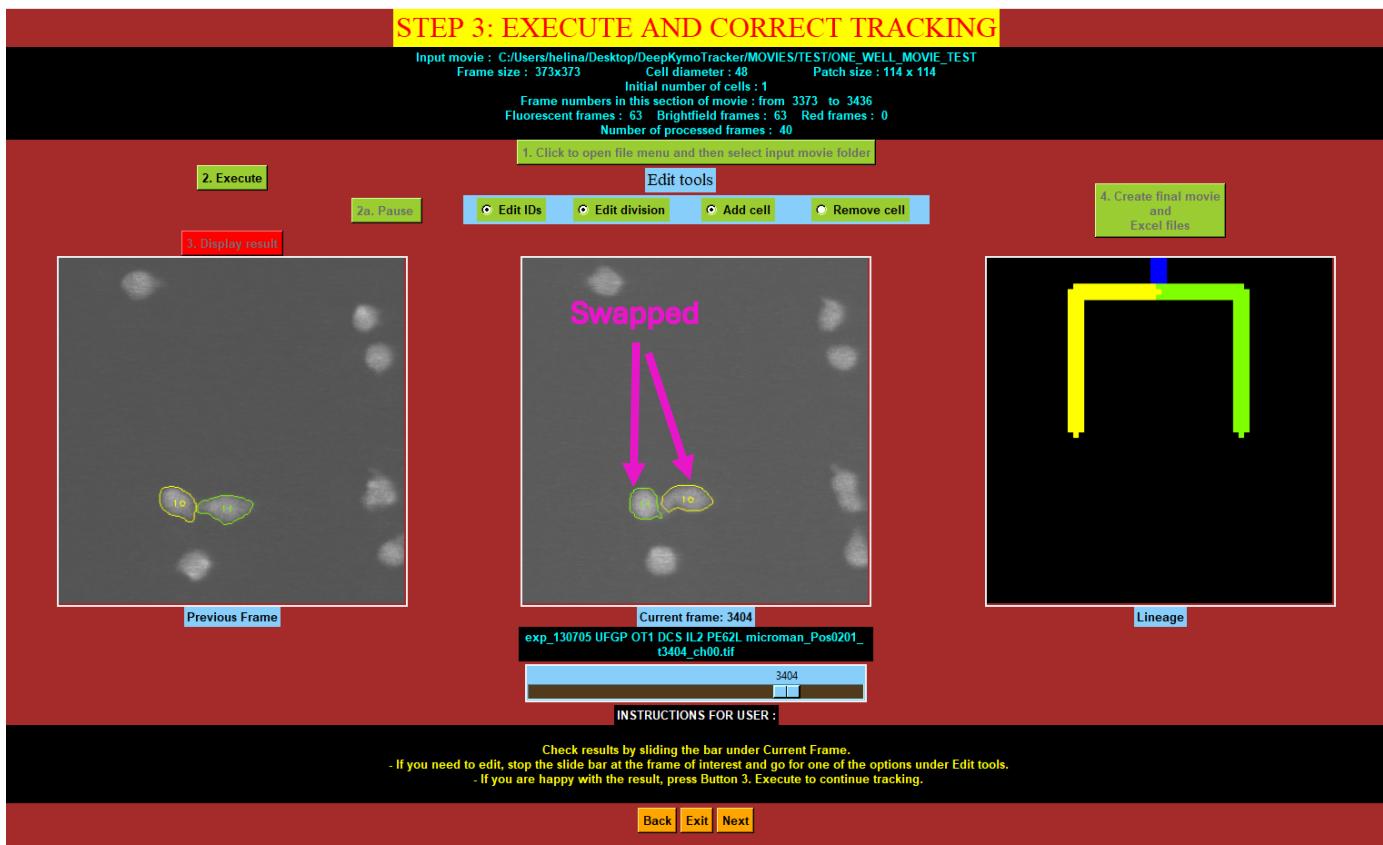
3. Pause to edit tracking errors

1. Edit IDs

Let us suppose that you spotted cells swapping while monitoring the automated tracking.

Push the button **2a. Pause**, then **3. Display results**. Using the slide bar, navigate to the frame where the error occurred. The frame with the error needs to be positioned in the middle window, i.e. Current Frame.

In the example below, two close cells, yellow and green, got swapped in Current Frame (they were correct in Previous Frame):



Now, push **Edit IDs**. A new button (Save ID edits) appears and starts flashing, but we are going to ignore it for a while.

What we need to do now is to correct the swapped IDs of the two cells in Current Frame: the yellow cell needs to become green, and vice versa.

For each cell that we are going to correct, we need to inform the algorithm of 2 things: 1) the ID of the cell 2) its newly assigned centroid.

The idea is that you obtain the ID from Previous Frame as the first step, and the new centroid from Current Frame, as the second step.

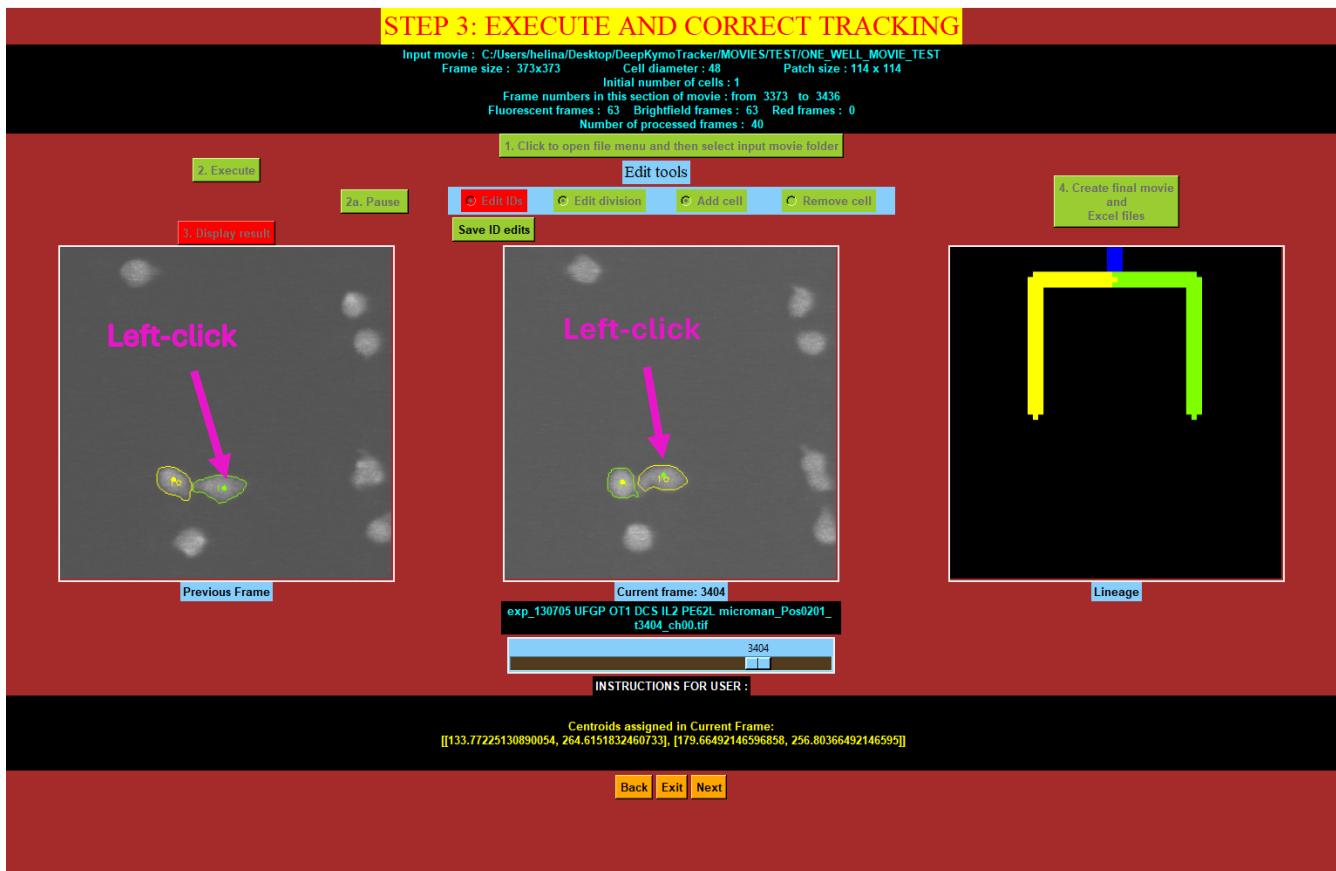
Let us start with the green cell in Current Frame. We want to make it yellow.

First, left click on the yellow cell in Previous Frame – the algorithm knows now that the ID is that of the yellow cell.

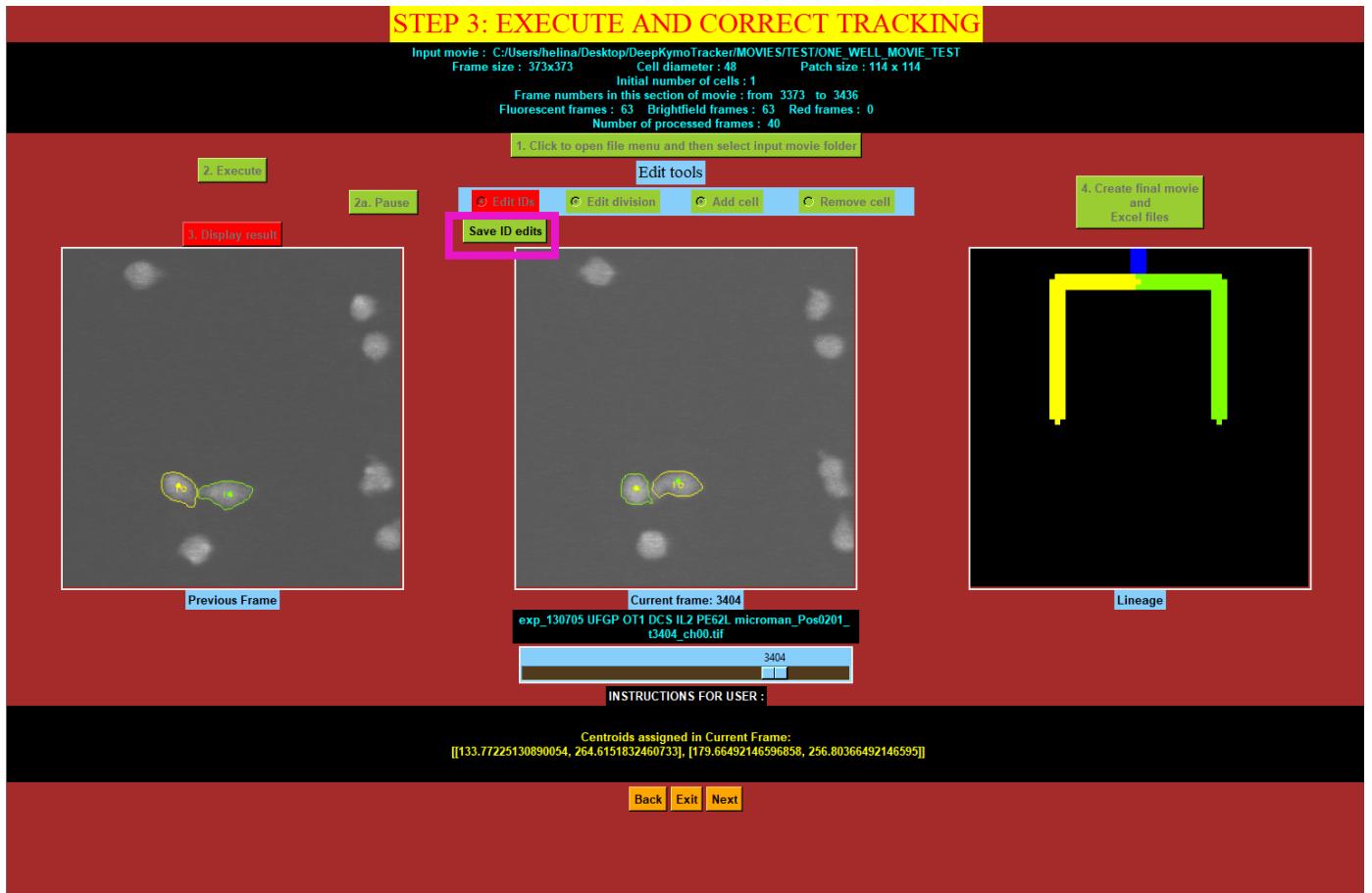
Second, left click on the centroid of the green cell in Current Frame – the algorithm knows now the yellow cell centroid needs to be where you clicked in Current Frame.



Repeat the procedure for the second cell: first, click on the green cell in Previous Frame (you extract the ID of the green cell) and, second, click on the centroid of the yellow cell in Current Frame (you show where the centroid of the green cell in Current Frame needs to be).



Push button **Save ID edits** and then, **2.Execute**. The tracking will resume from the Current Frame, i.e. the frame where you have just made ID corrections.

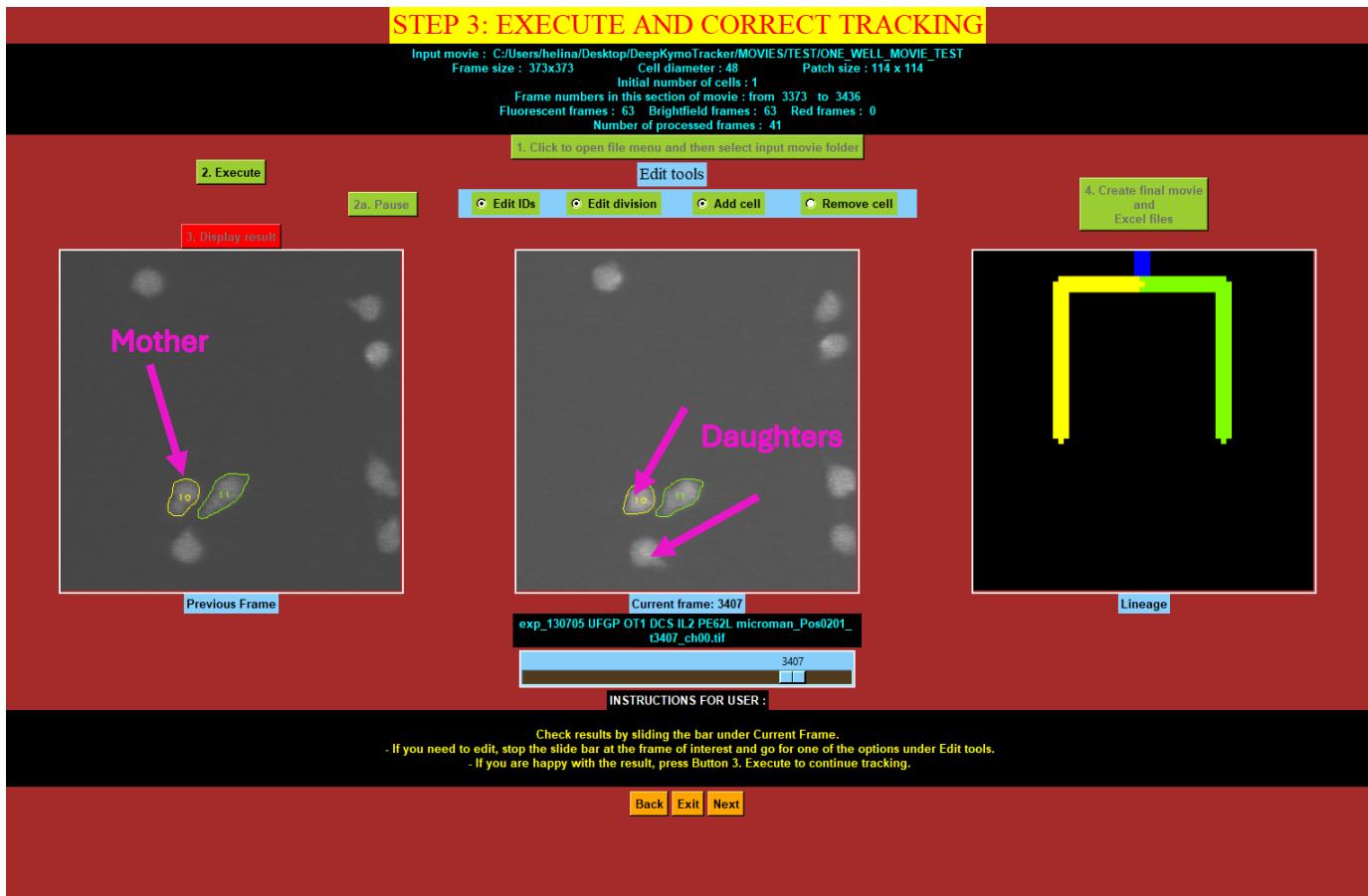


Note: you can correct IDs of multiple cells in Current Frame simultaneously (as we just did for 2 cells).

2. Edit division

If you spotted a missed division during the automated tracking, you need to perform the same procedure: **Pause** → **Display results** → **navigate to the frame with the error** (i.e. position it in Current Frame window).

Let us suppose (for the learning purposes) that the yellow cell in Previous Frame was supposed to divide into 2 daughters: the yellow cell and the cell at the bottom of Current Frame, but the algorithm missed this division and therefore, we want to correct it:



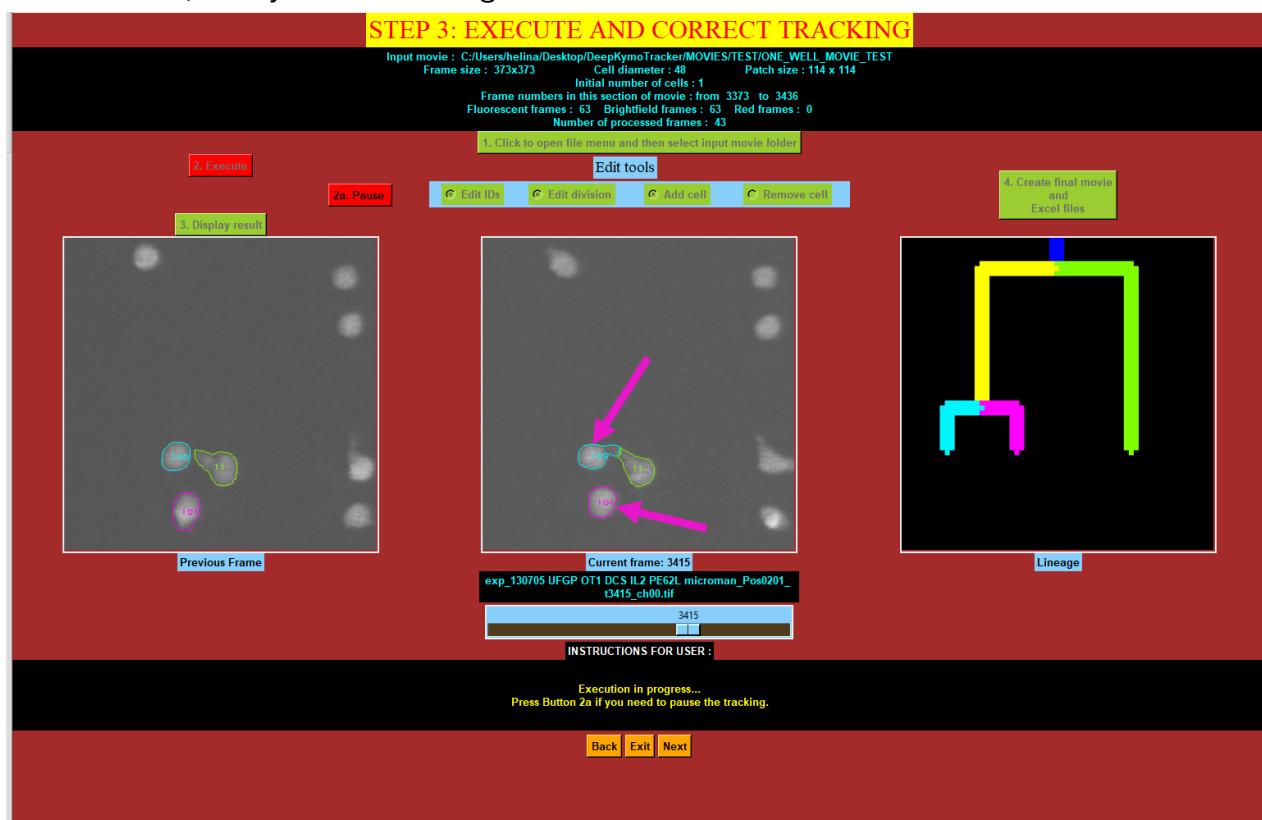
First, you need to inform the algorithm of who Mother Cell is. To this end, you left-click on the yellow cell in Previous Frame (remember: just as before, we obtain IDs from Previous Frame):



The next step is to specify the daughter cells. We do it by left-clicking on both daughters in Current Frame (remember: we obtain centroids from Current Frame). After that, you push **Save division edits** button and **Execute**. The tracking resumes starting with Current Frame.



After a while, you should see the following picture (the screenshot below): the yellow (mother) cell disappeared and two new cells (daughters), the cyan and the magenta ones, came to existence instead. The division can be clearly seen in the lineage tree: the yellow branch has split into 2 subbranches, the cyan and the magenta ones.



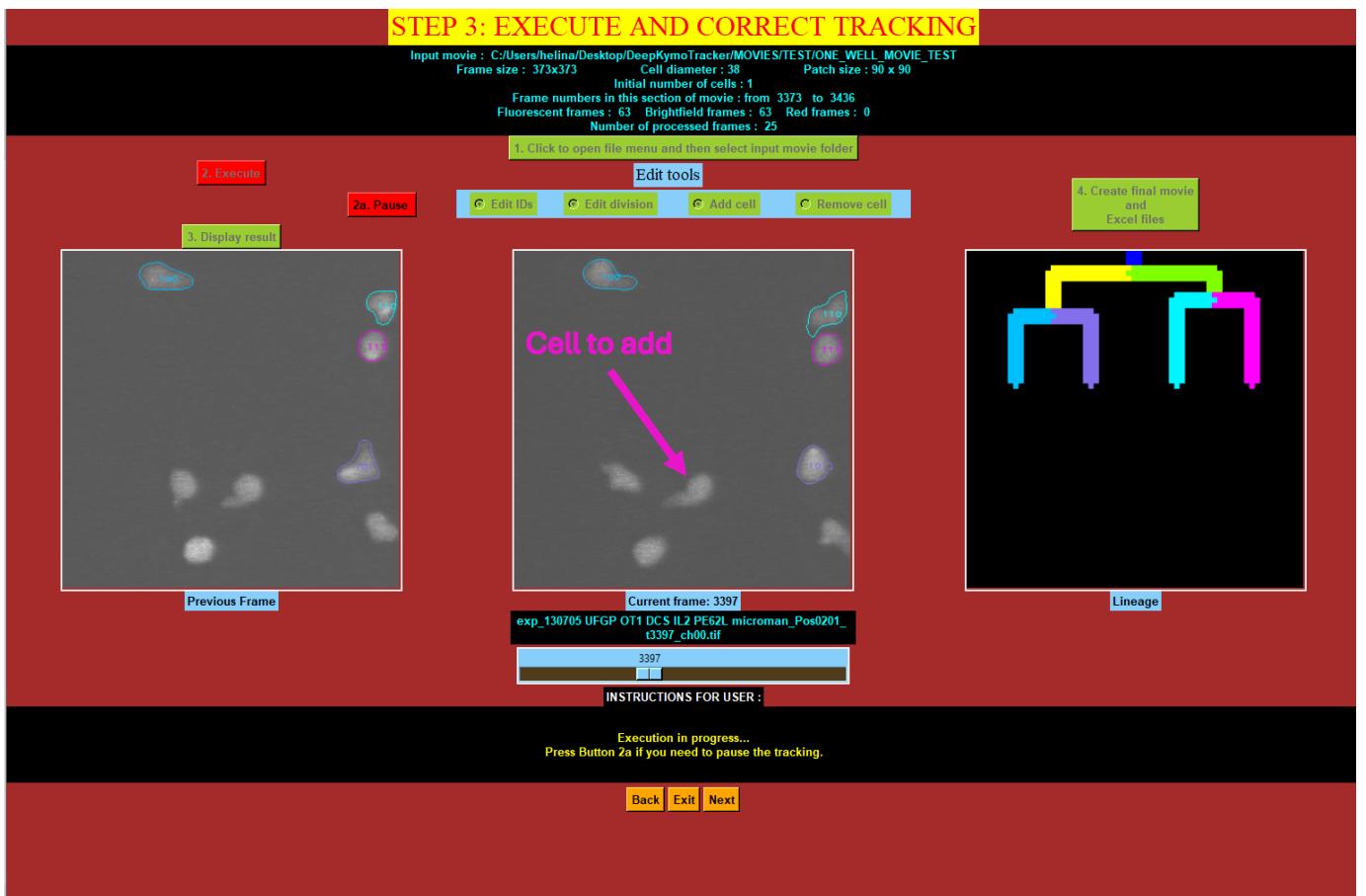
Note: you can correct only one division in Current Frame! The pipeline is not programmed to tackle more than one division per frame. (it will probably be implemented in the future).

3. Add cell

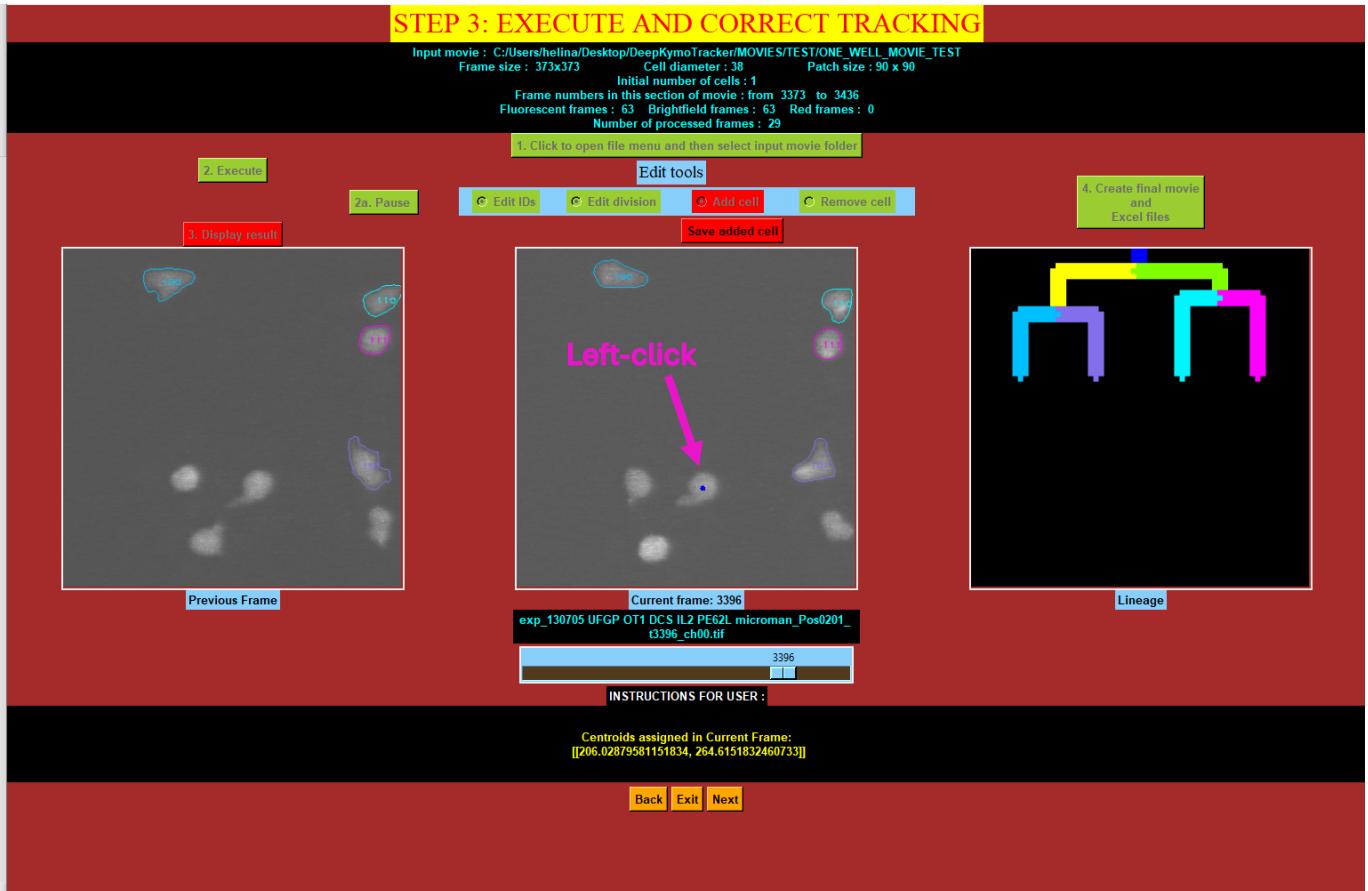
If you want to add a new cell (or multiple cells) in a frame, you need perform the same procedure:

Pause → **Display results** → **navigate to the frame** (i.e. position it in Current Frame window).

Let us suppose (for learning purposes) that we want to add the cell indicated in the screenshot below:



All we need to do to achieve this is to push **Add cell**, then left-click on the centroid of this cell in Current Frame, push **Save added cells** and finally, push Execute. The tracking will resume starting from Current Frame.



After a while, you will see the following picture (the screenshot below): the orange cell appeared, and the orange branch corresponding to this cell came to existence in the lineage.



Note: you can add as many cells as you wish in Current frame – just click on each of them (try to click on their centroids) and then push **Save added cell**.

4. Remove cell

Cells in a movie can die or leave the field of view. In this case, you need to remove them manually. If you spotted one of these events, you need perform the same procedure: **Pause** → **Display results** → **navigate to the frame in which you want to make corrections** (i.e. position it in Current Frame window).

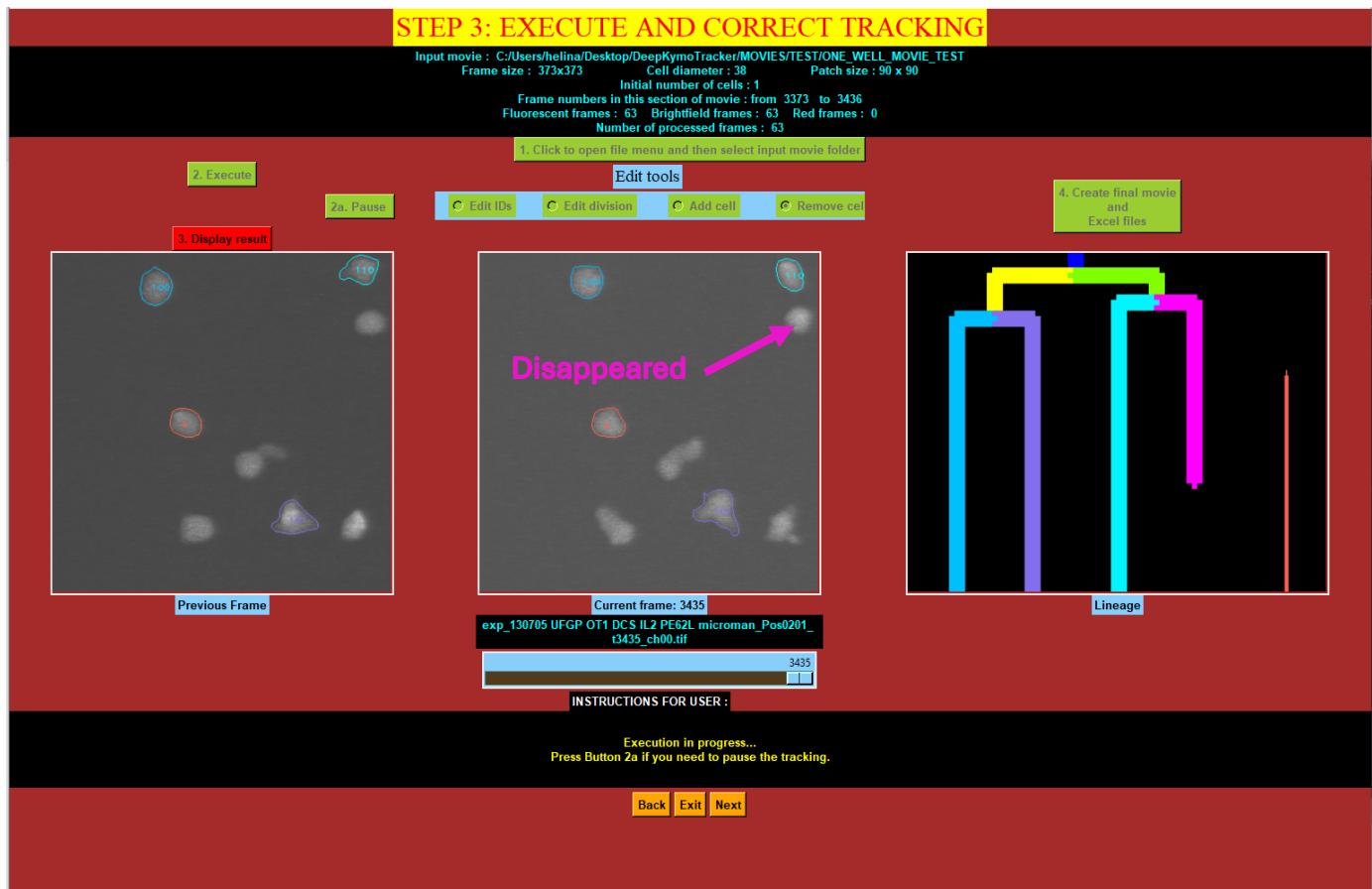
Let us say (for learning purposes) that we want to remove the magenta cell in Current Frame (see the screenshot below):



Push **Remove cell**, left-click on the magenta cell, push **Save removed cell** and, finally, push **Execute**.



After a while, you will see the following picture (the screenshot below): the magenta cell disappeared and the magenta branch in the lineage tree stopped growing.



Note: you can remove as many cells as you wish in Current Frame – just click on each of them and then push button Save **removed cell**.