STEP-4: CORRECT SEGMENTATION

(User Guide)

Goal: 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).

There are 2 segmentation techniques available:

1. Fast mode: You do not need to draw anything when using this method – you just left-click on the cell and its immediate surroundings an unlimited number of times, and watch how the segmentation changes after every click. Once you are happy with the result, you just save it and move to the next cell.
2. Slow mode: If you are not able to achieve a good segmentation in the fast mode, you switch to the slow mode and start drawing the contour of the cell manually, using the left button of the mouse.

Input requirements:

The input movie should be tracked, i.e. having undergone Step-3. Once you have launched Step-4, you will be asked to choose the tracked movie from the file menu. This folder has the name **TRACKED_MOVIE_{you movie name}** and its path is

\DeepKymoTracker\MOVIES\{your movie_name}\TRACKED_MOVIE_{your movie name}.

For example, for the movie **TEST_SHORT,** used as an example in this tutorial, the path is "C:\Users\helina\Desktop\DeepKymoTracker\MOVIES\TEST_SHORT\TRACKED_MOVIE_TEST_SHORT".

Output: After the manual segmentation is finished, the algorithm updates the results obtained after Step-3, namely, 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.

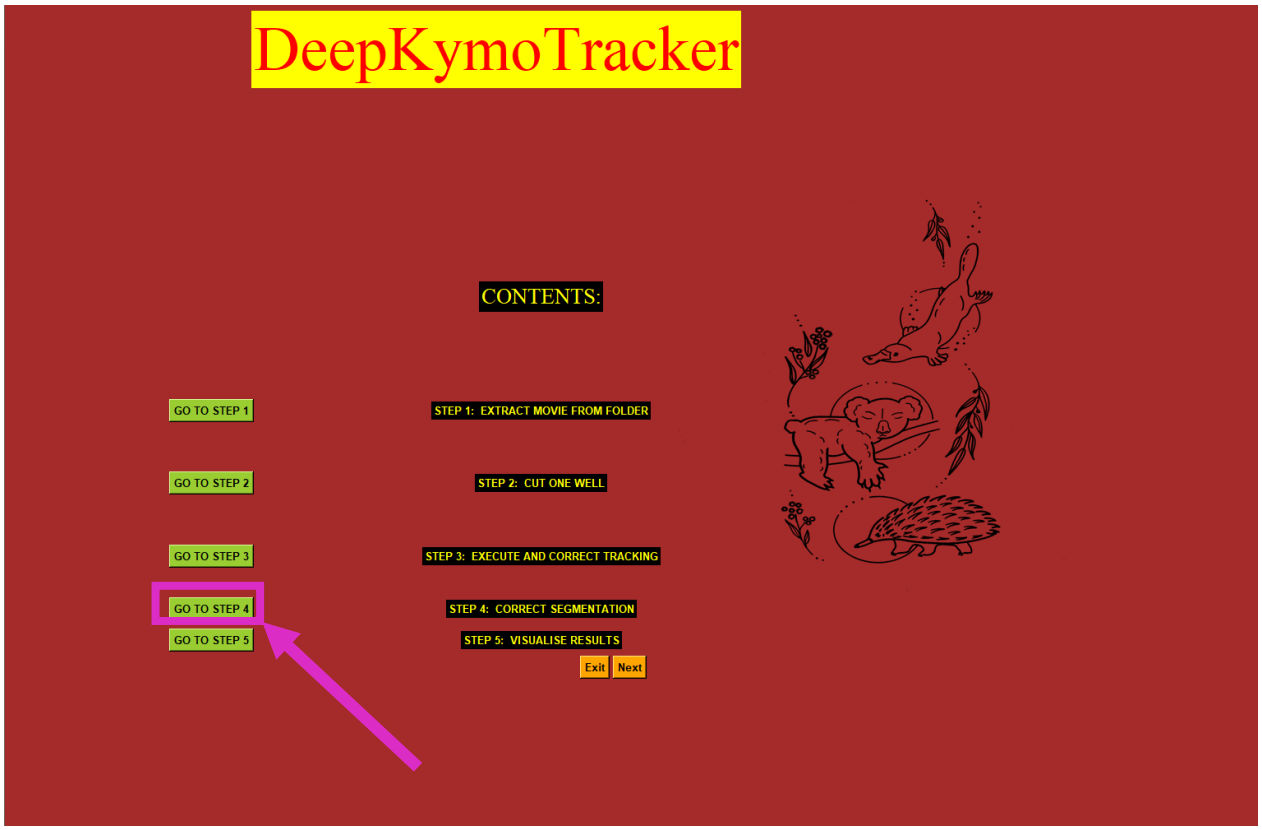
This information will be saved here:

"C:\Users\helina\Desktop\DeepKymoTracker\MOVIES\TEST_SHORT\TRACKED_MOVIE

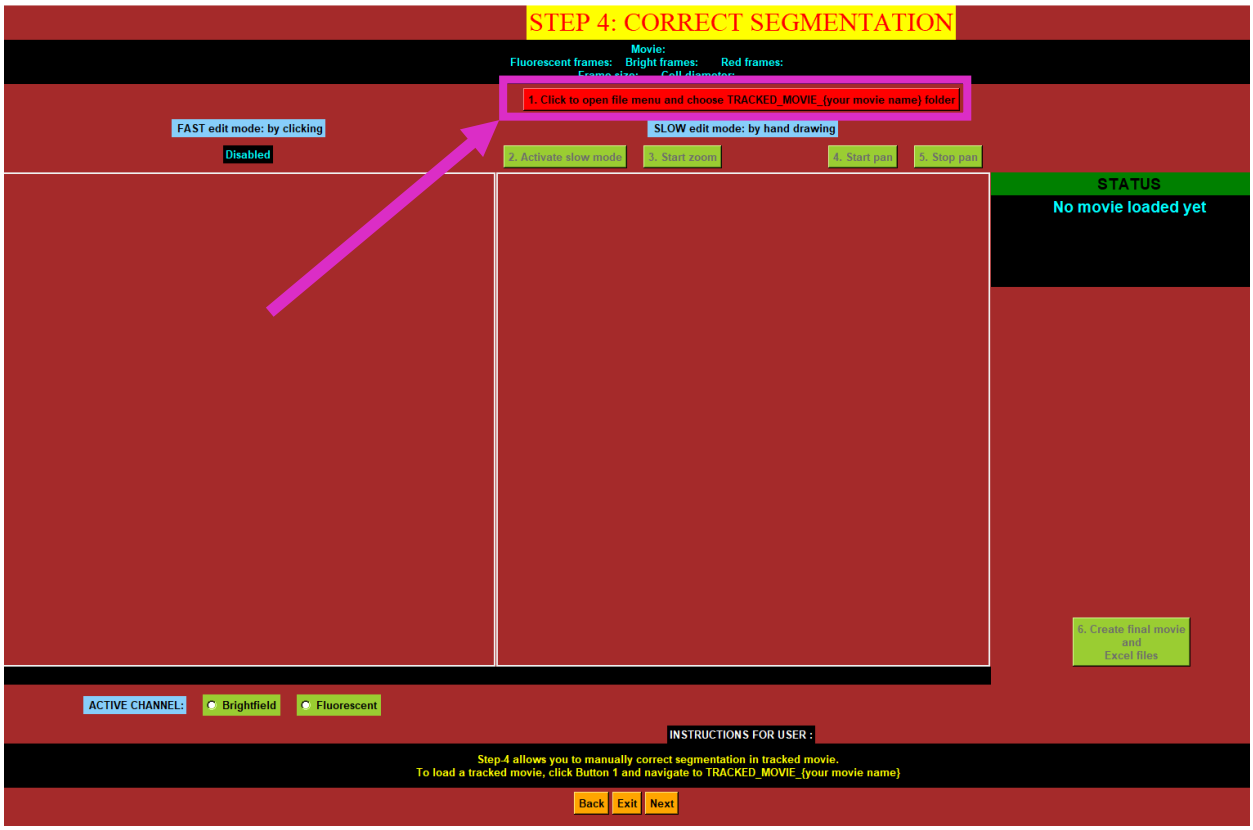
_TEST_SHORT**RESULTS_PER_CELL**".

Instructions

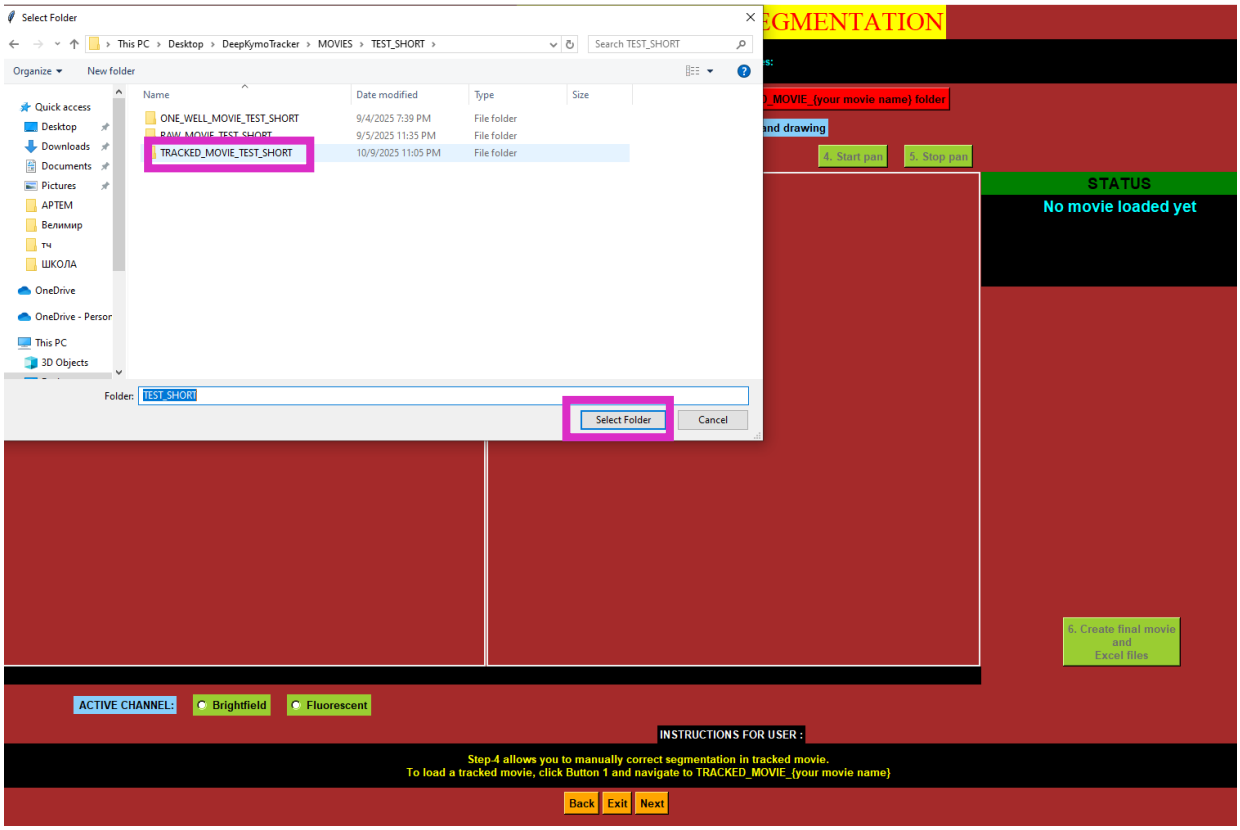
Launch DeepKymoTracker and push button **GO TO STEP 4** in the title page:



The STEP-4 window opens. Push the flashing **Button1**:



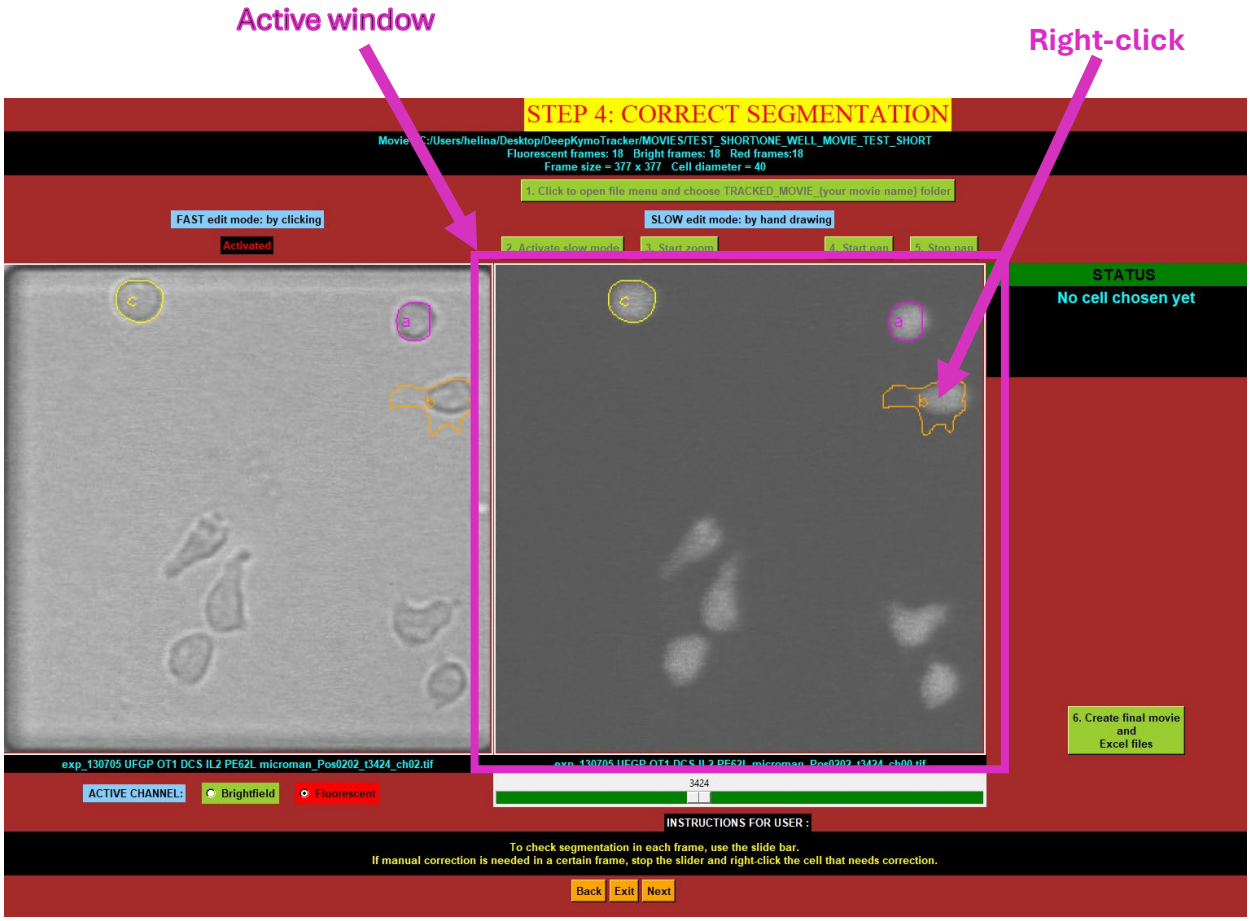
In the open file menu, navigate to **TRACKED_MOVIE_{your movie name}** folder and push **Select Folder**:



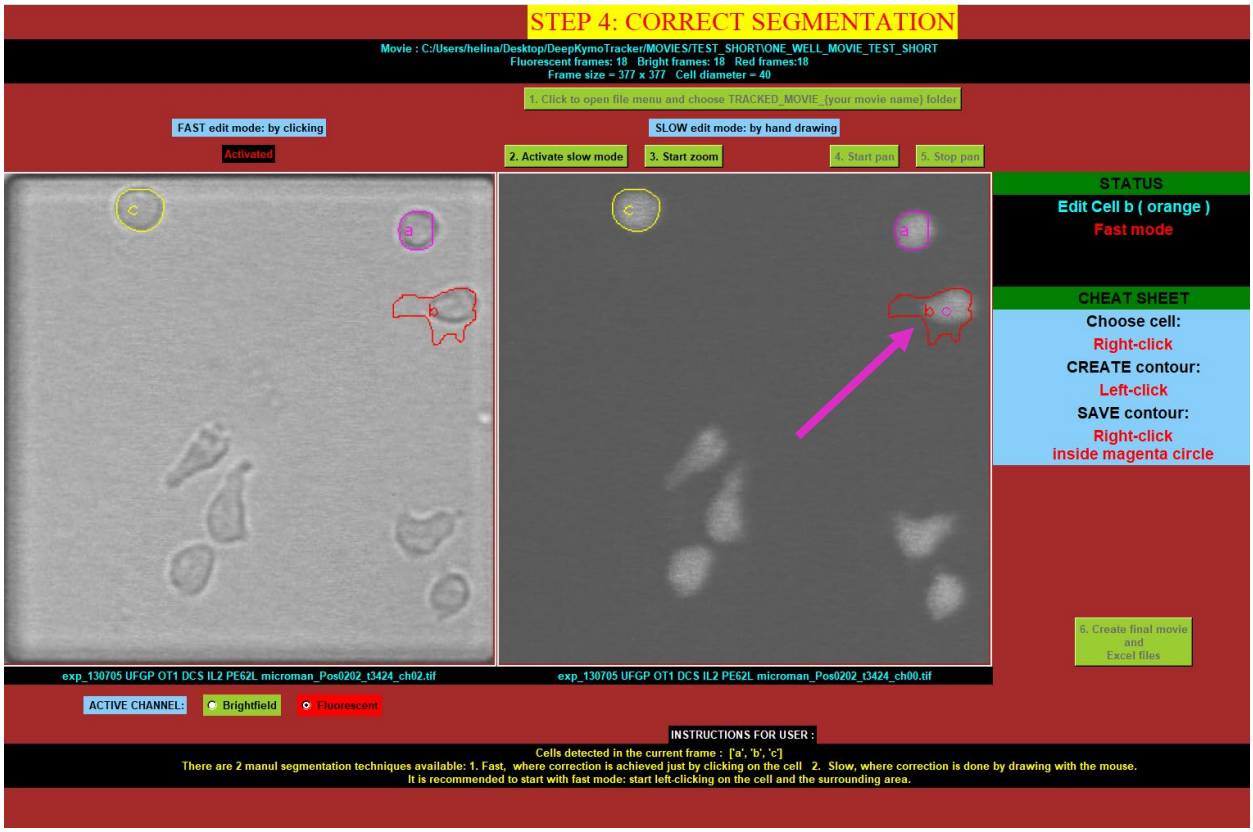
The tracked and segmented movie is uploaded now. You can use the slide-bar to navigate between the frames. The active window, i.e. the window where you are going to make corrections in, is the right window. In the picture below, the right window contains the fluorescent channel, i.e. the fluorescent channel is active now. If you prefer to work with the brightfield images, you can switch to it using button **Brightfield** on the left bottom of the page.

Note that you can switch from one channel to another as many times as you like and any moment you like during the process of manual correction. (The only exception is the pan stage, but you should not worry about it as the buttons **Brightfield** and **Fluorescent** will be disabled at this stage anyway).

To start, you need to right-click on the cell you want to correct. Let it be the orange cell in the picture below:

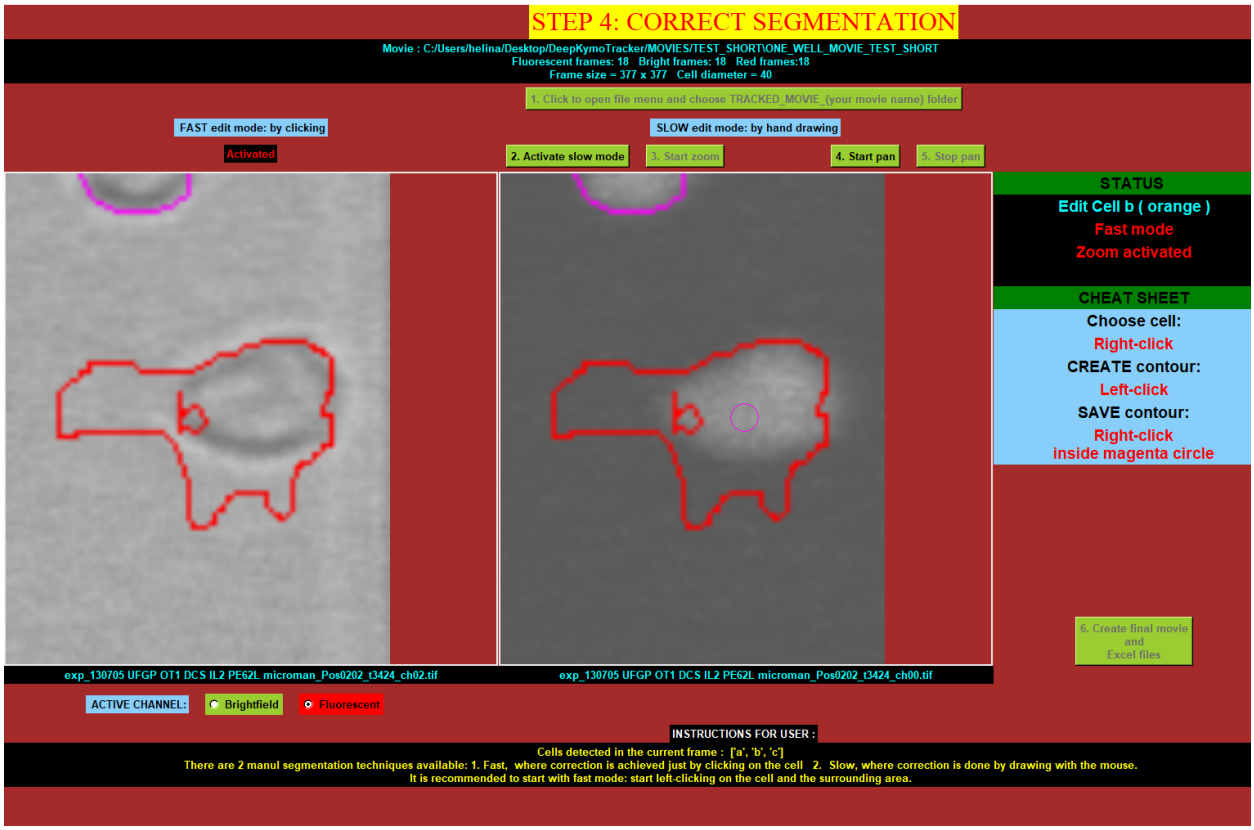


After right-clicking on it, the contour of the cell turns red, and the magenta circle appears where you clicked (the magenta circle will be there throughout the whole process of segmentation correction of that cell and, finally, you will be asked to right-click inside that circle to save your edits).



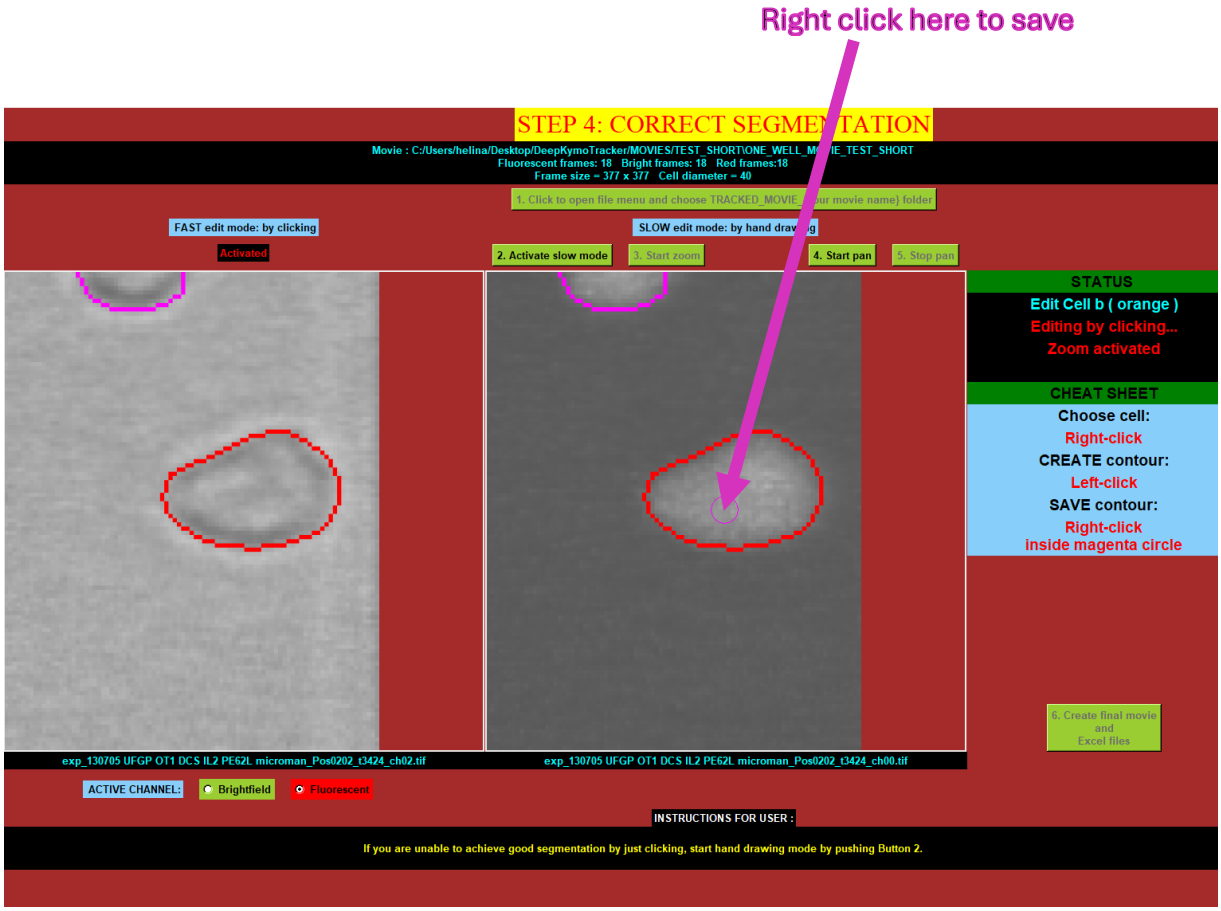
Now you can start left clicking on the cell itself or even on its surroundings to find the optimal segmentation. You can also push button **3. Start zoom** to get a magnified view of the cell. For your convenience, pushing this button immediately gives you the magnified image of the clicked cell, and the cell is positioned right in the centre of the window. To zoom in and zoom out even further, you can use the mouse wheel.

In the picture below, we can see the zoomed-in image with the cell in the centre (that is what happens after pushing **3. Start zoom**):

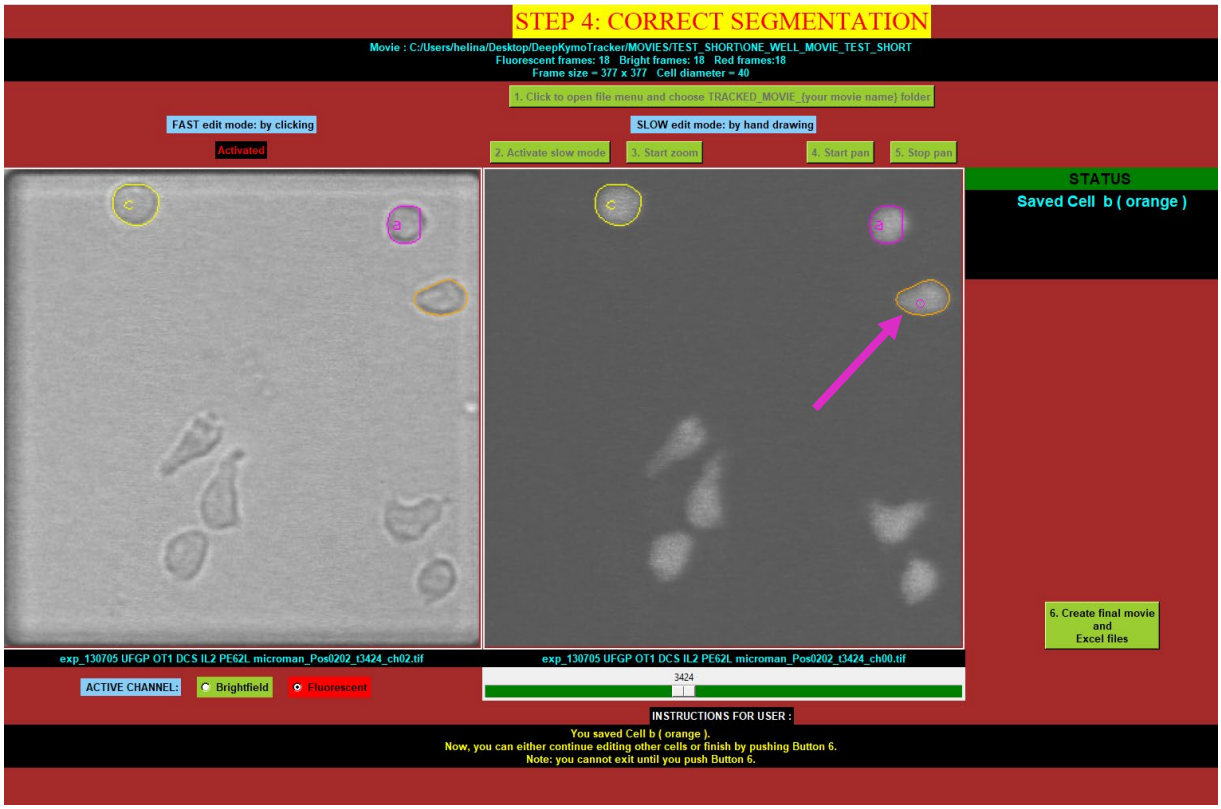


Start left clicking on the cell and around it and see how the shape of the red contour changes at every click. You can also do one or all of the following things:

1. Use the mouse wheel to zoom-in and zoom-out.
2. Swap the active channel by using buttons **Brightfield** and **Fluorescent** as many times as you like.
3. Move the cell from the centre to any other location by using buttons **4. Start pan** and **5. Stop pan**.



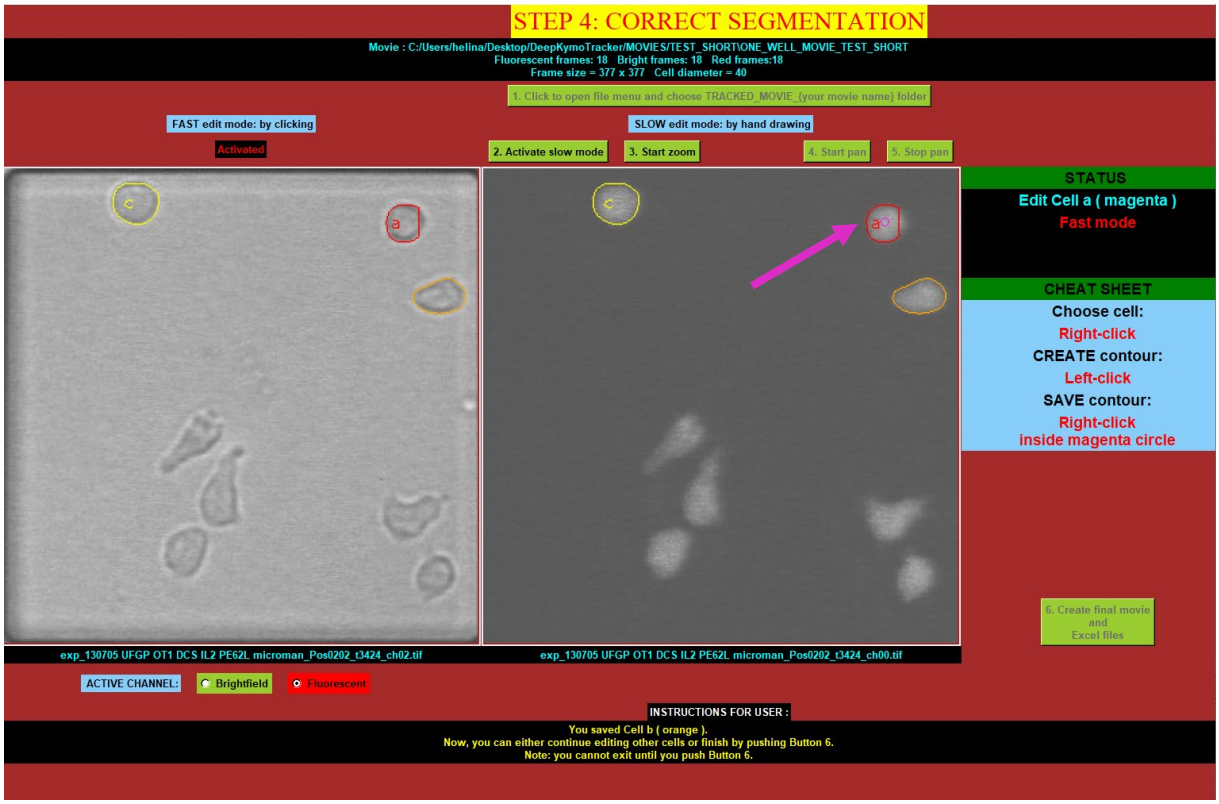
Finally, once you are happy with the result, right-click inside the magenta circle: this immediately brings you back to the initial view where the improved orange cell segmentation is saved now:



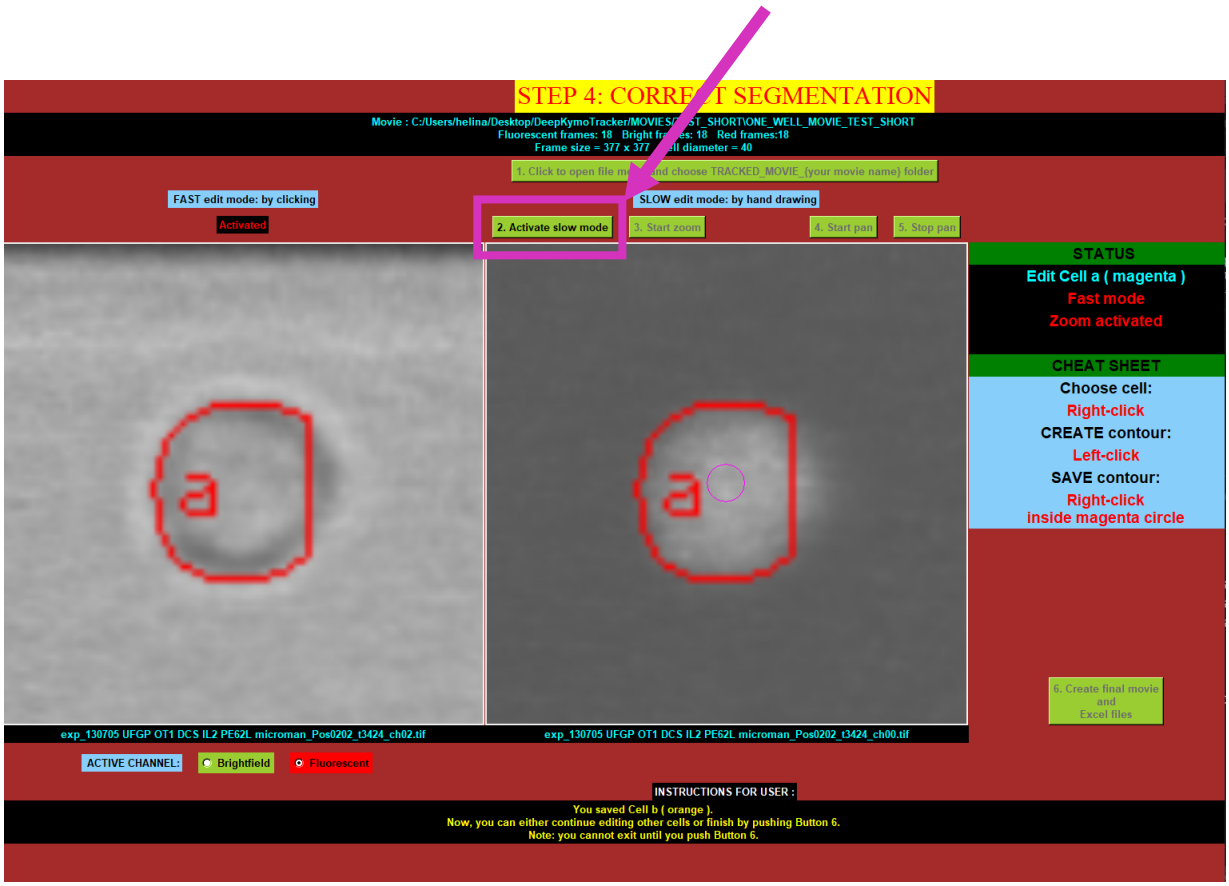
You can repeat this procedure as many times as you like, moving from cell to cell in the same frame, and also from frame to frame using the slide-bar.

However appealing the fast segmentation technique might seem, sometimes you need to resort to the traditional hand-drawing , which is called **slow mode** here.

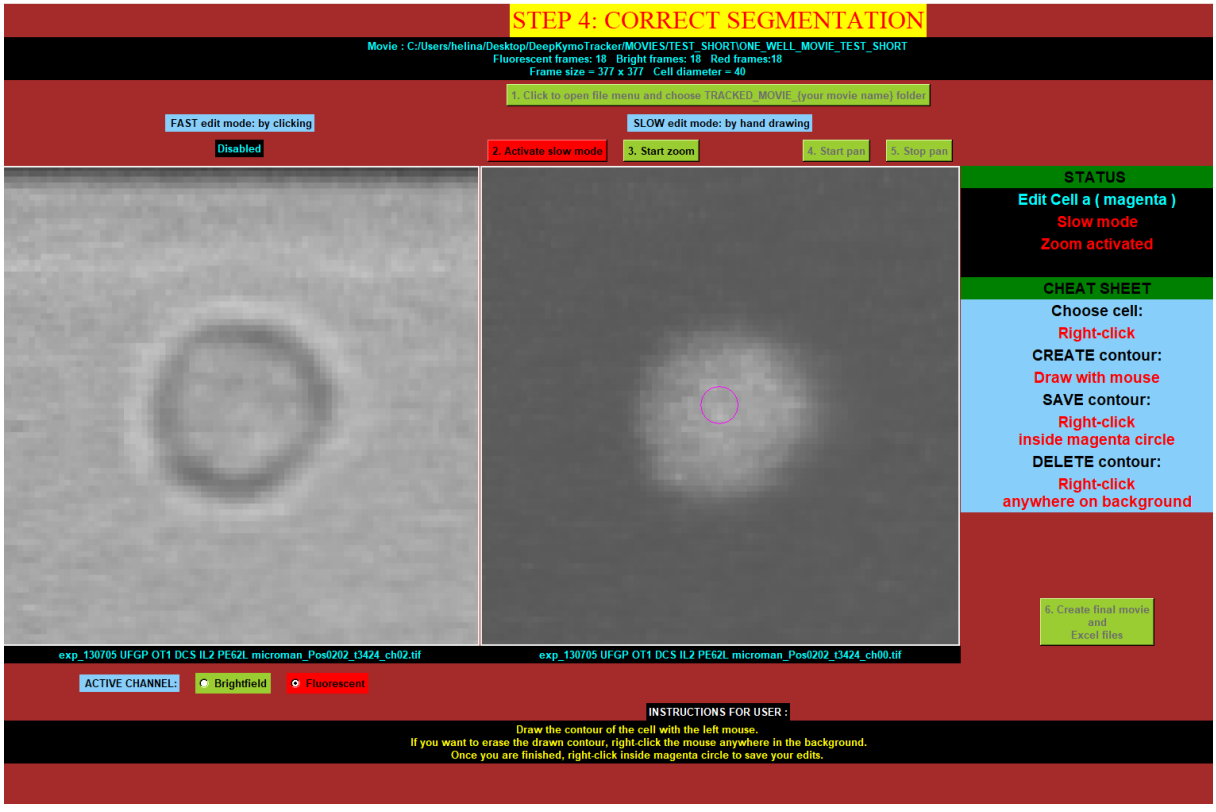
Let us suppose that we want to improve the segmentation of the magenta cell (which is red in the picture below because we have already right clicked on it):



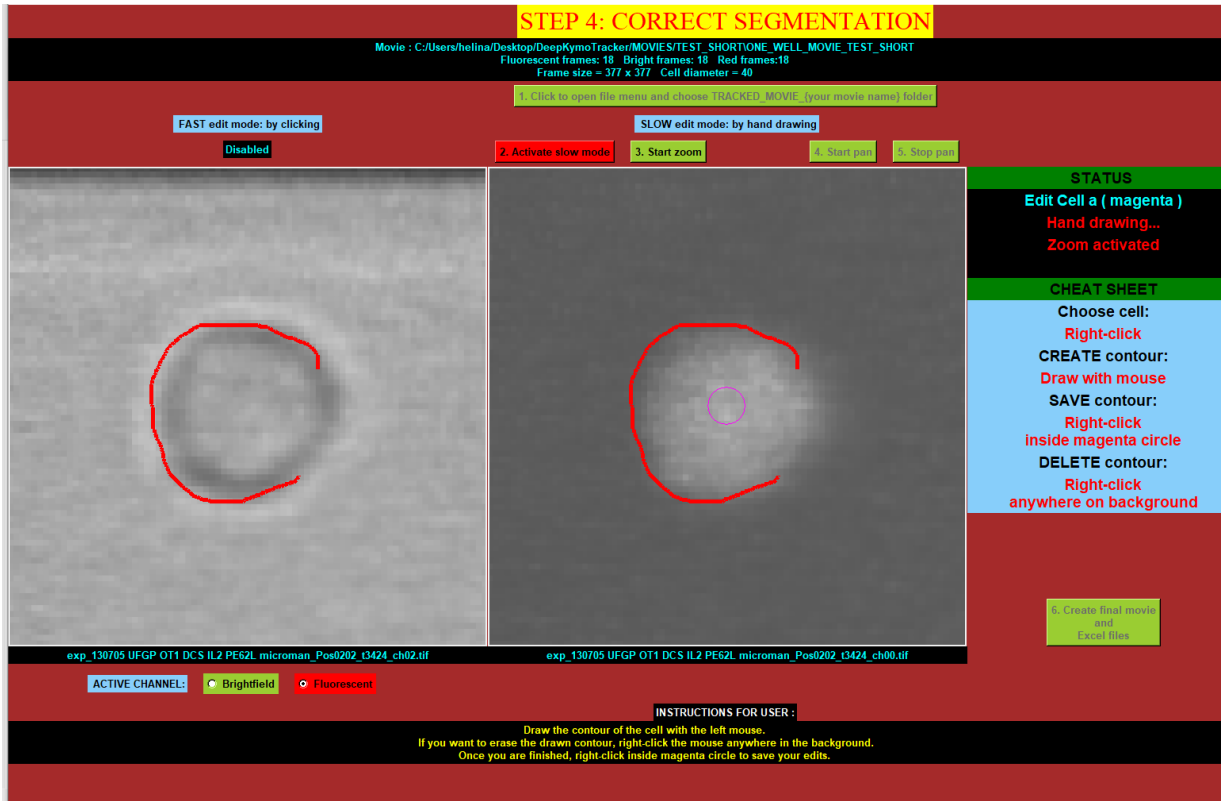
The best approach is to push **3. Start zoom** first and maybe even zoom-in further by **using mouse wheel**, it is up to you. Here is what you can get after pushing **3. Start zoom**:



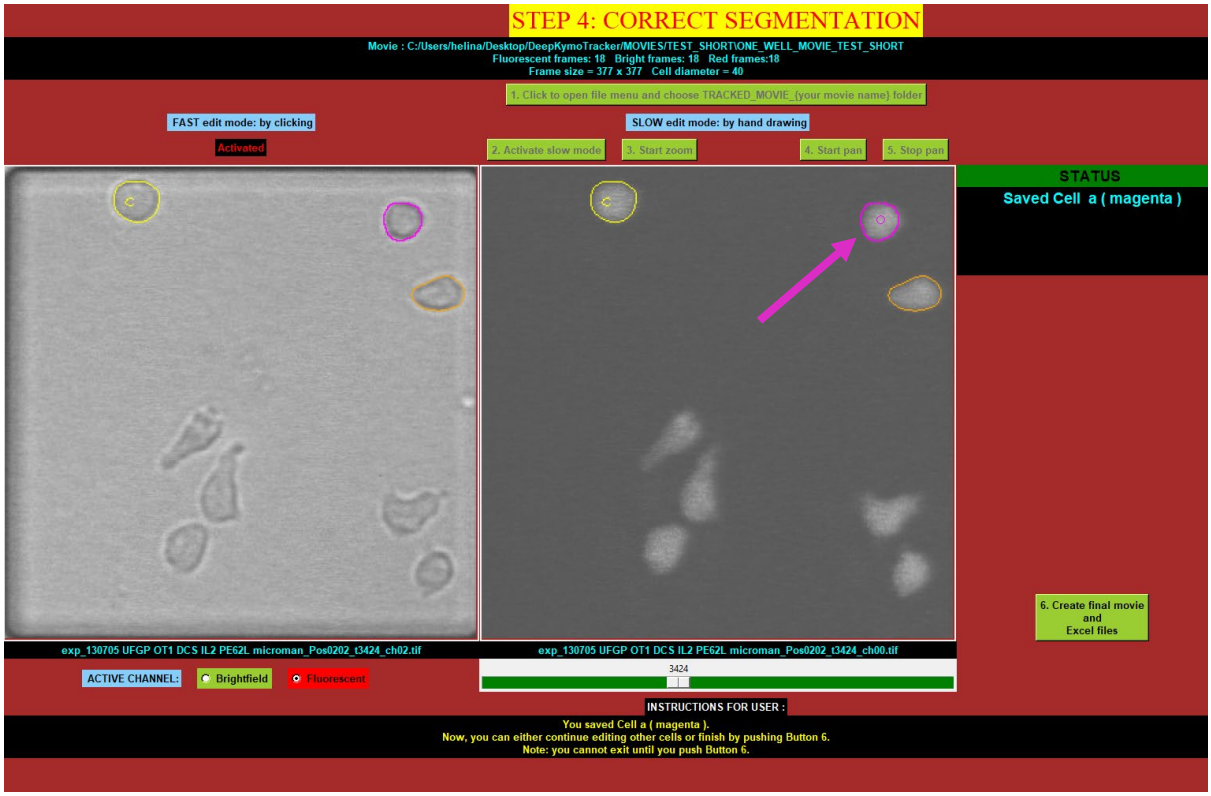
To launch the slow segmentation mode, push **2. Activate slow mode**. The red contour of the cell disappears:



Start drawing with the left mouse button. As well as in the fast mode, feel free to apply **more zoom with the mouse wheel** and to swap the active channel and even to pan if needed.

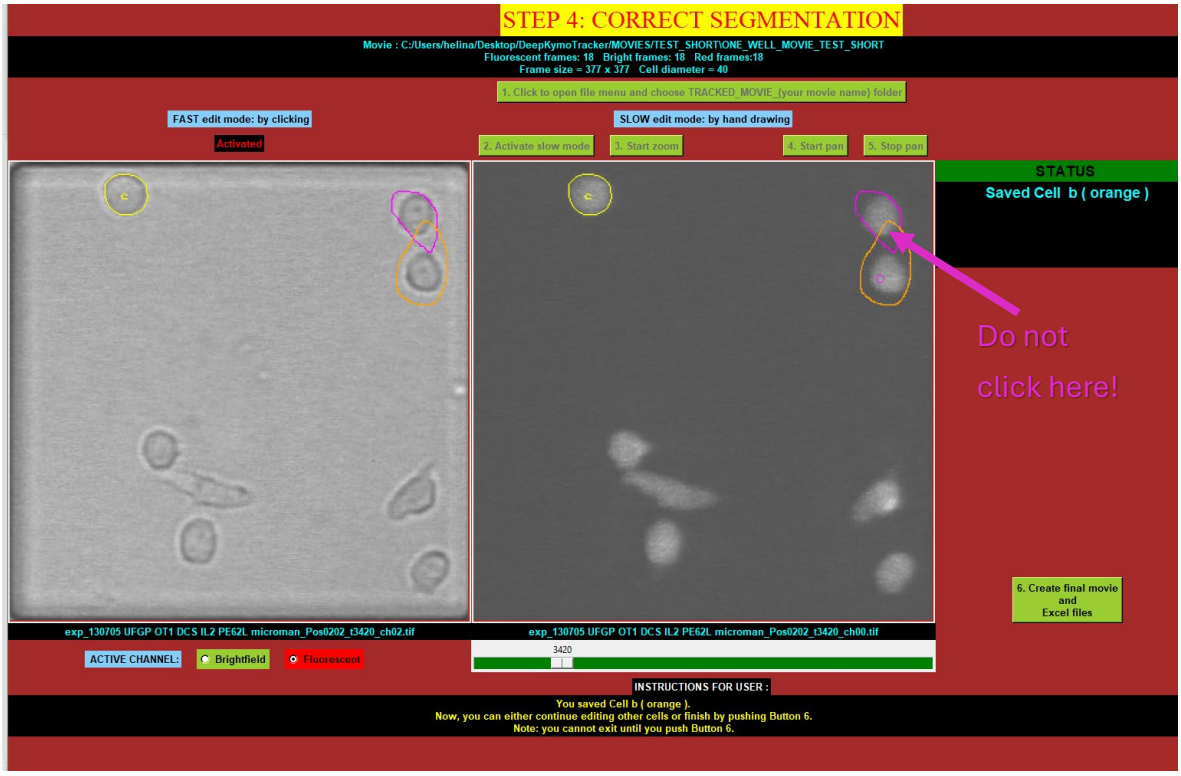


Finally, click inside the magenta circle to save the edits: it will bring you back to the original view of the frame, with the improved magenta cell segmentation:



Overlapping cells

Note that the hand-drawing tool (slow mode) allows you to draw contours of the occluding cells, too (see the screenshot below). Warning: if you want to re-edit the segmentations of occluded cells later, make sure you do not click on the intersection - the algorithm will not be able to recognise the cell you want to edit!



Buttons **4. Start pan** and **5. Stop pan**

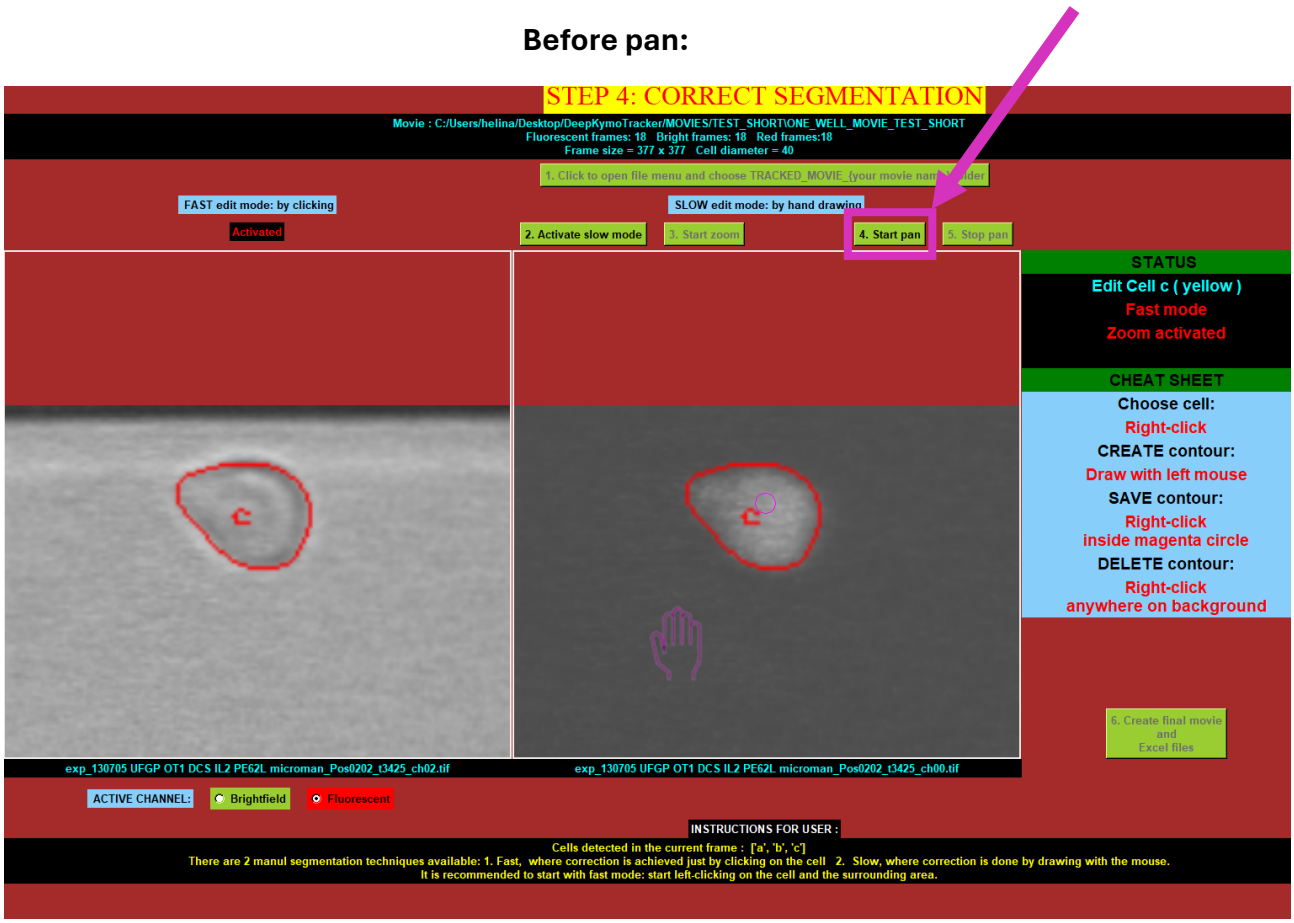
In 99% of cases, you will not need this tool as applying zoom tool leaves your cell in the centre of the window which is already very convenient.

The pan tool is included just in case you find it more convenient to shift the position of the cell from the centre to somewhere else – it maybe useful when editing multiple occluded cells, for instance.

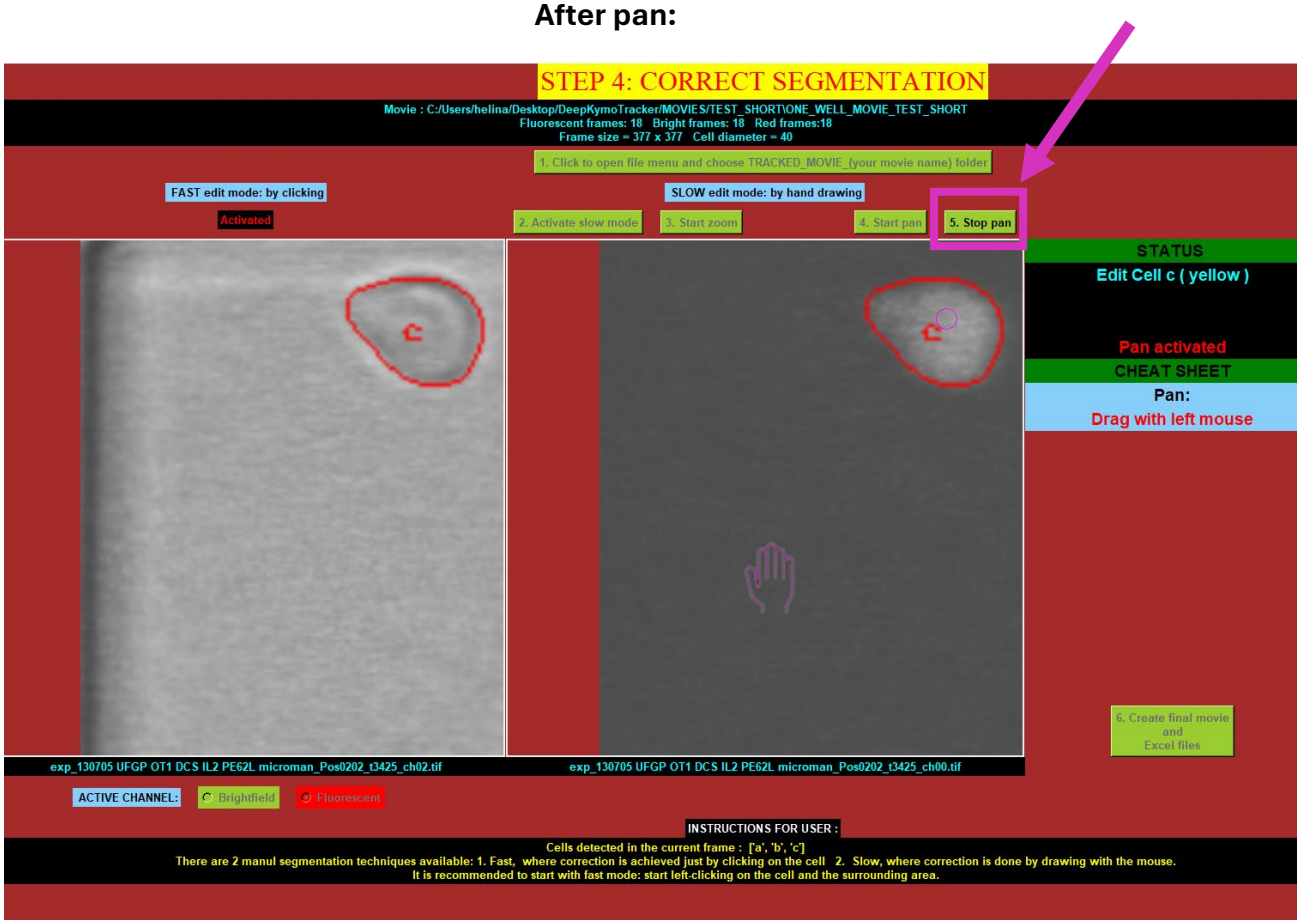
How to use this tool? First of all, note that button **4. Start pan** becomes active only when you have already applied zoom, i.e. 1) have pushed button **3. Start zoom** and 2) have played with a mouse wheel a bit.

Once the button **4. Start pan** is activated, you can start dragging the zoomed image with the left mouse. Then, push **5. Stop pan**.

Before pan:



After pan:



How to exit

As you probably noticed, the buttons **Back**, **Exit** and **Next** were hidden most of the time in Step-4. This is done on purpose: you need to save your edits and update all excel files before exiting. So, push button **6. Create final movie and Excel files**, and after all the updates are recorded, the above-mentioned buttons appear on the screen again and you can now exit the page.