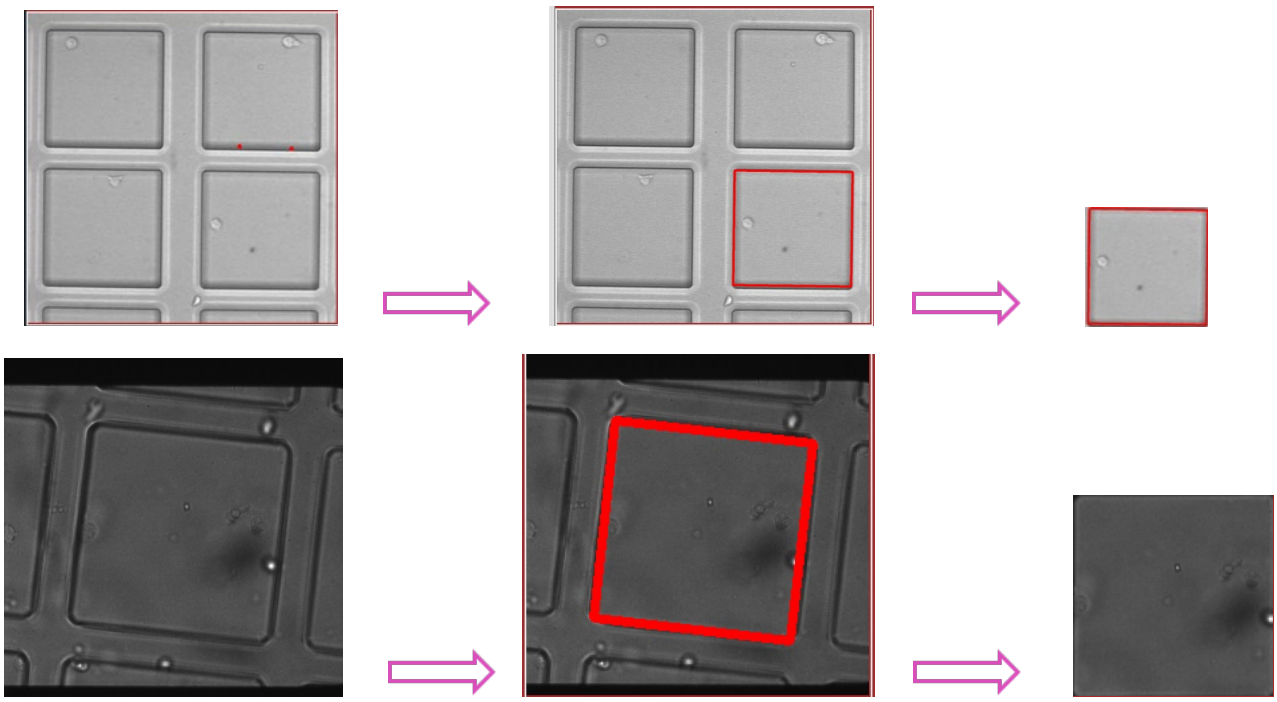
STEP-2: CUT WELL

(User guide for DeepKymoTracker)

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



Soft point: The thresholding method for cutting out a well of interest, employed in this step, requires the border of the well to be a solid, uninterrupted line. However, when a cell/cells gets into the border,the border line gets interrupted by that cell, and the algorithm does not work.

Input requirements:

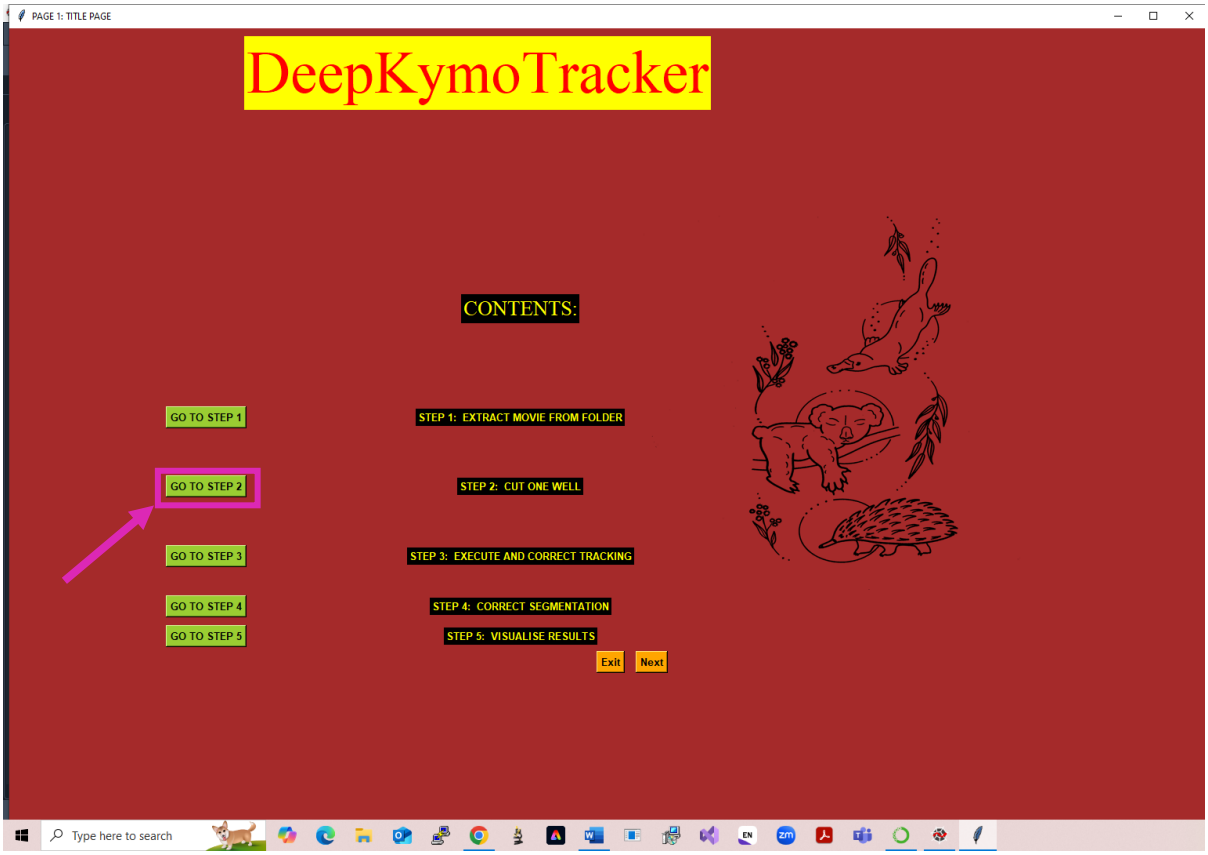
- A cell movie with equal number of green fluorescent and bright field frames.
- As for the red channel, some of the frames, or even all of them, can be missing, it does not matter (the algorithm needs only green fluor and brightfield for tracking).
- Image file names: green fluorescent should end with **_ch00.tif**, brightfield with **_ch02.tif,** red with **_ch01.tif**. Also, numbering of frames should look like **_t0003**, not **_t3**. Example of a correct frame name: exp_130424 UGFP OT1 chAp2a2 DCs IL2 microman_Pos0302**_t00002_ch02.tif.**
- Numbering of frames:the 1st frame of your movies does not necessarily need to be 1, the movie can start with any frame. It means that you can work even with sections of cell movies.
- The folder with the cell movie should be located in DeepKymoTracker folder.If it was obtained from Step-1, it will be placed there automatically. If not, move the folder into it.

Output: .The resulting processed movie will land in your DeepKymoTracker folder. The name will be changed to **INPUT_MOVIE_{the initial name}.** For exmple, if the initial name was **s1**, the output name

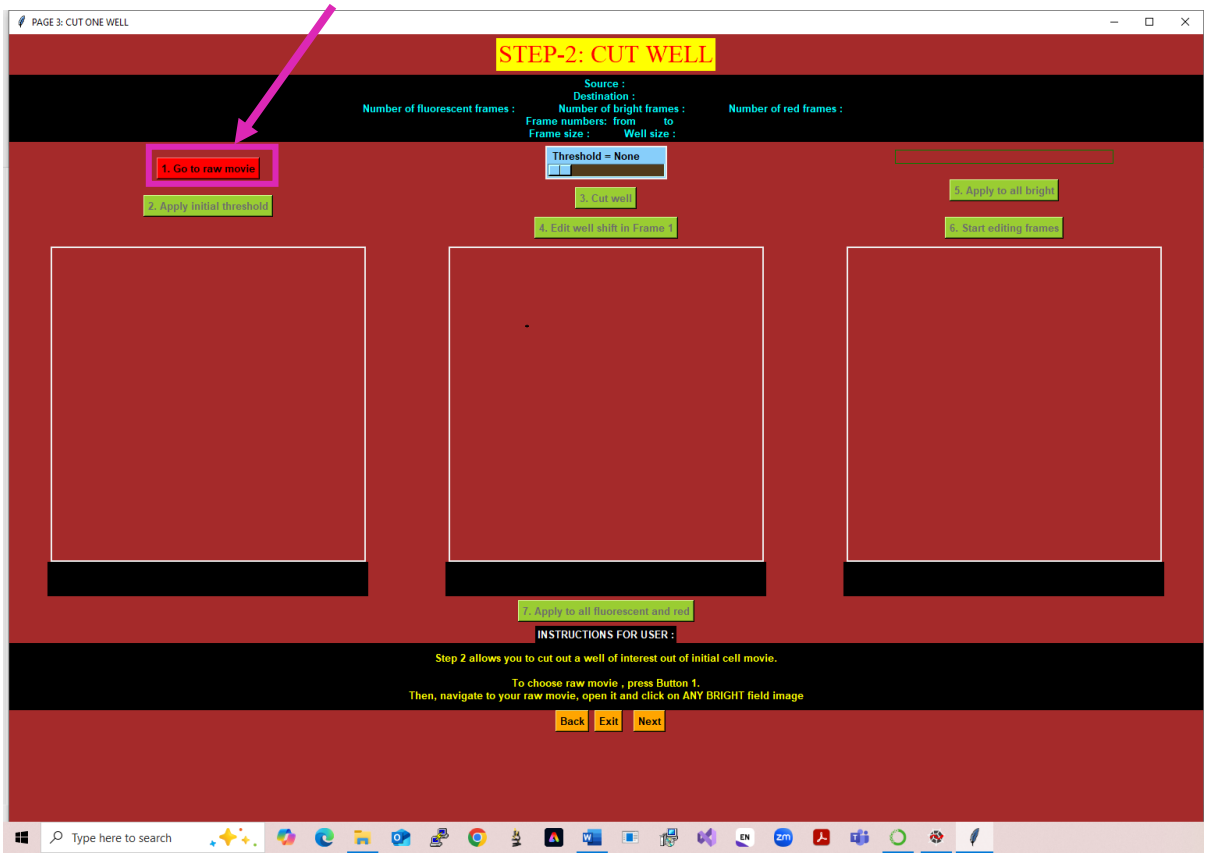
will be **INPUT_MOVIE_s1**. The exact path will be printed on the screen when extraction and processing are finished.

Instructions:

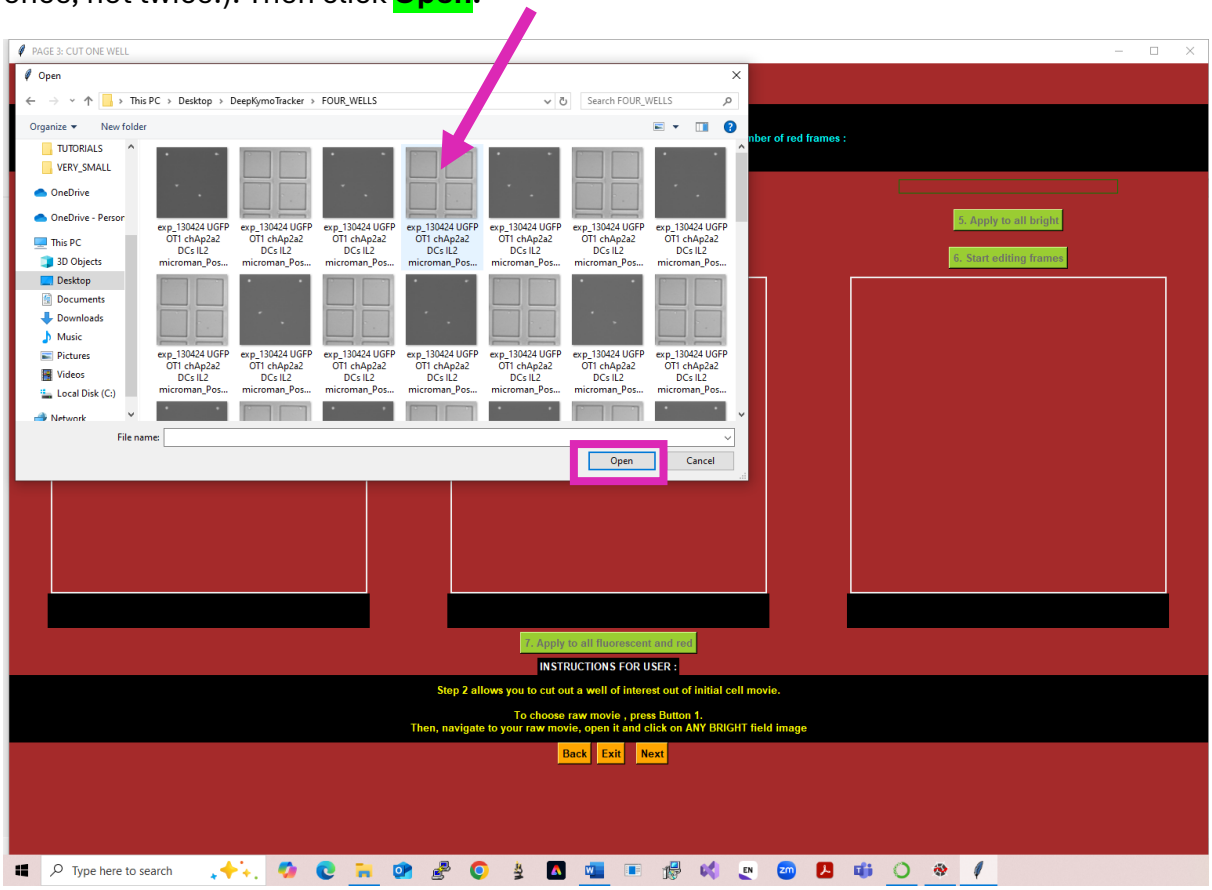
From the title page of DeepKymoTracker, navigate to Step-2, using button **GO TO STEP 2**.



In the open window STEP-2: CUT WELL, you will see the flashing button **1. Go to raw** movie. Push it.



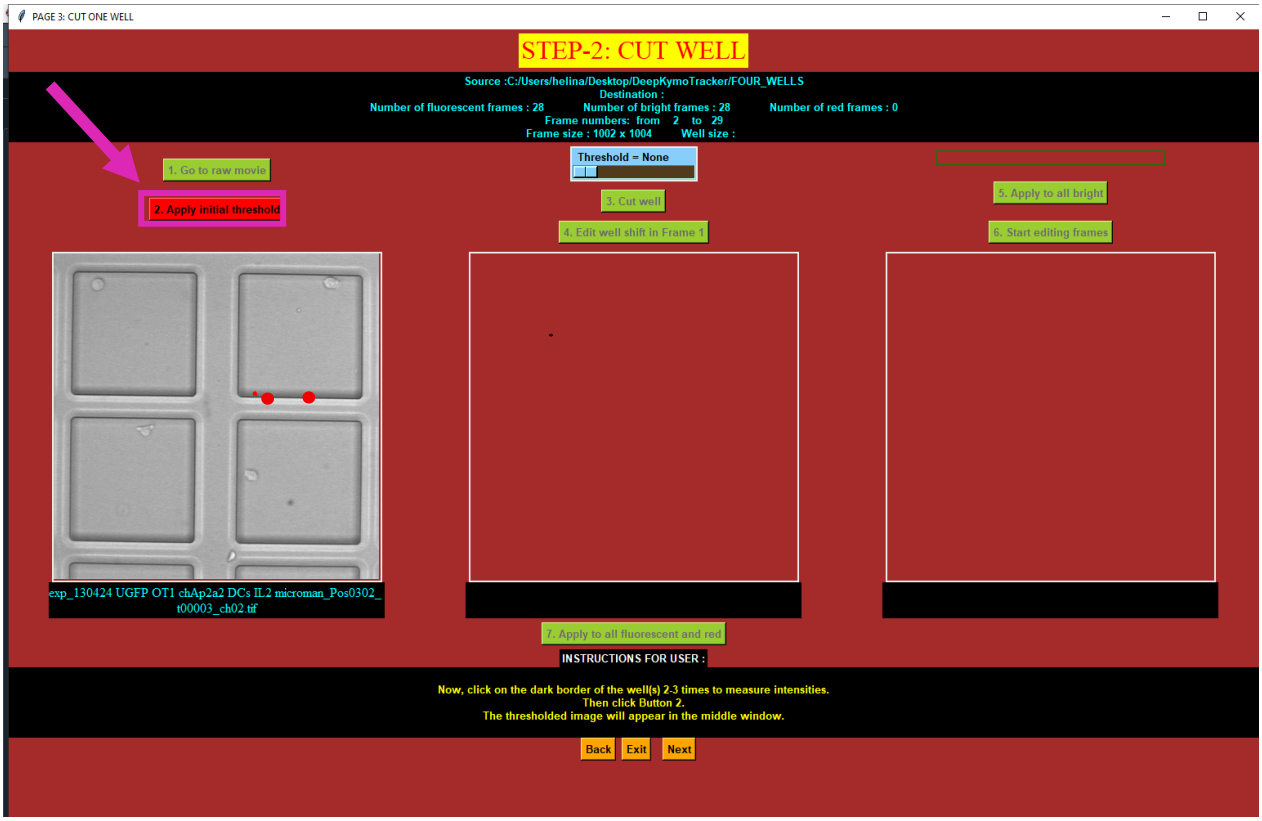
In the file menu, navigate to the folder with your movie, open it and click on any brightfield frame (click once, not twice!). Then click **Open**.



In the left window, you will see the clicked brightfield frame. We are going to cut a well of interest out of this particular frame first.

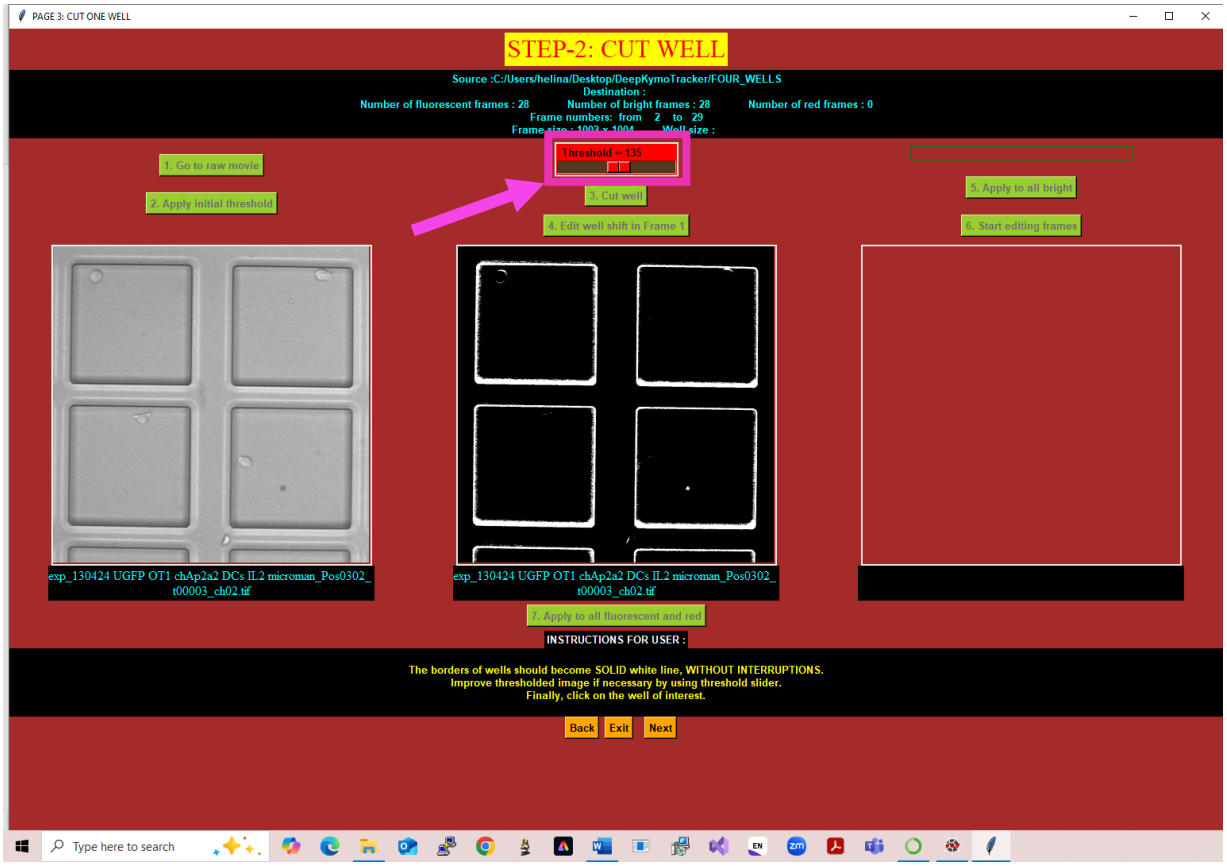
The first step is to threshold the image. As you can see, all the buttons are disabled, so it is of no use trying to push any of them. What you should do instead is to click on the border of your well of interest

at least twice (red dots will appear on the image at each click). You are actually measuring the intensity of the well border in this way. Immediately, the button **2. Apply initial threshold** starts flashing. Push it.

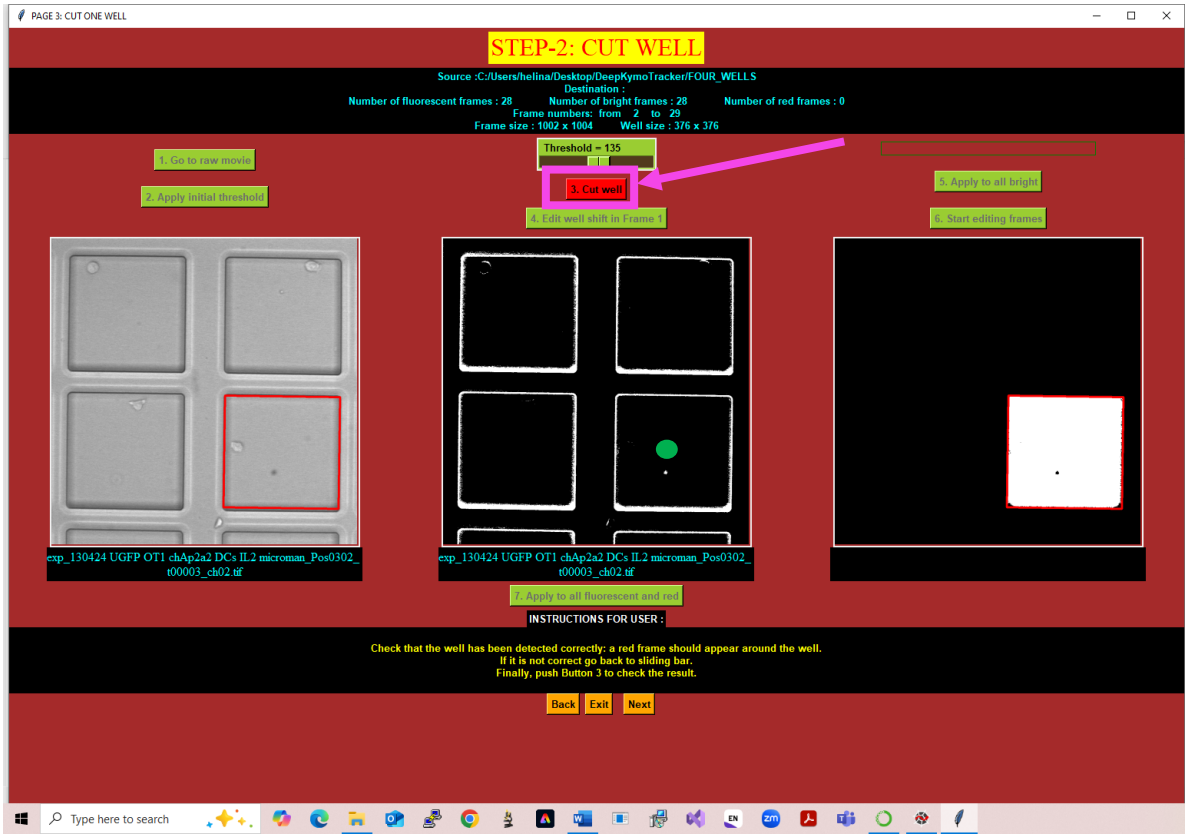


The thresholded image pops up in the middle window and the threshold slider starts flashing. Use the slider to improve the thresholded image: the border of the well of interest needs to be one solid line, without interruptions.

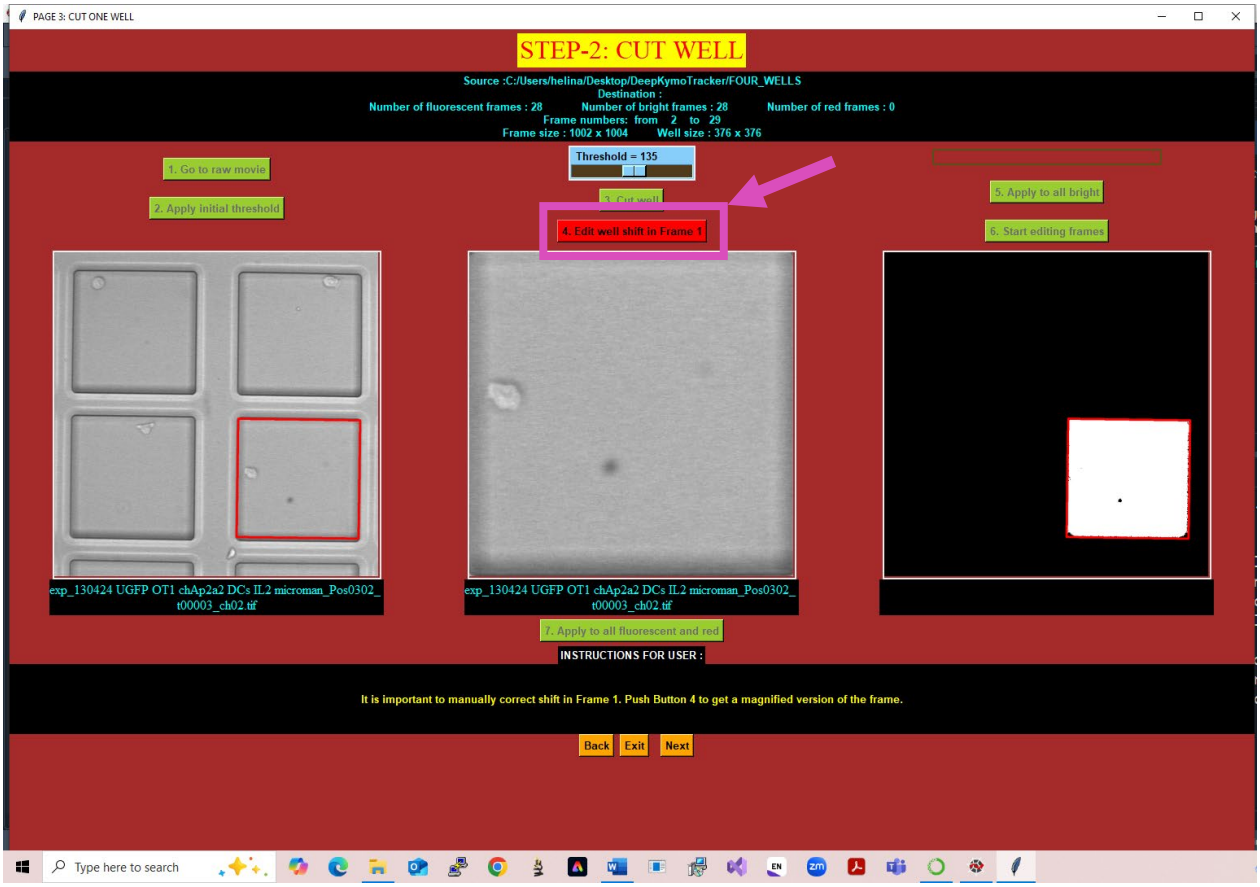
Warning: if your well contains cell/s located on the well border, the threshold technique will not work however hard you try (because such cells interrupt the border line). What you need to do is launch Step-2 again and click on another frame where the border is not interrupted by a cell.



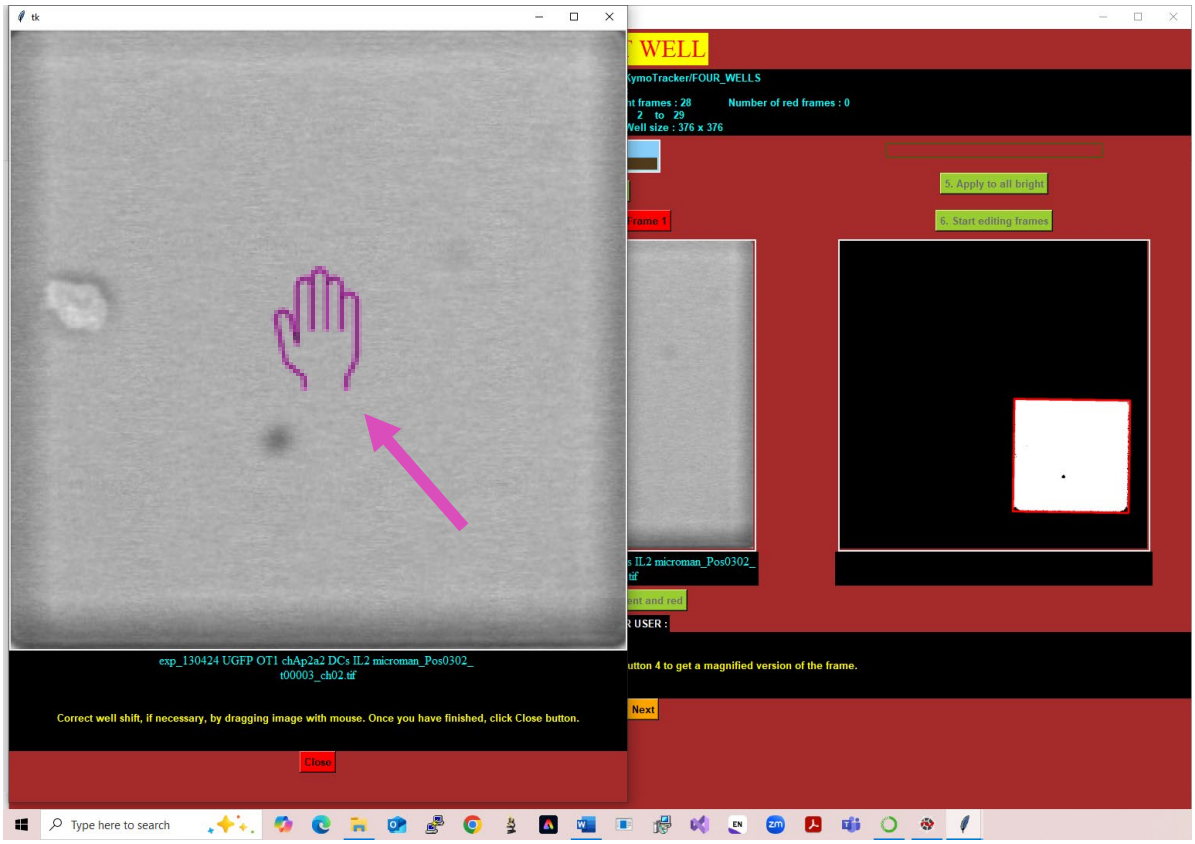
Click with the mouse inside the well of interest after that: the cropped well should appear in the right window. If you are happy with it, push button **3. Cut well.**



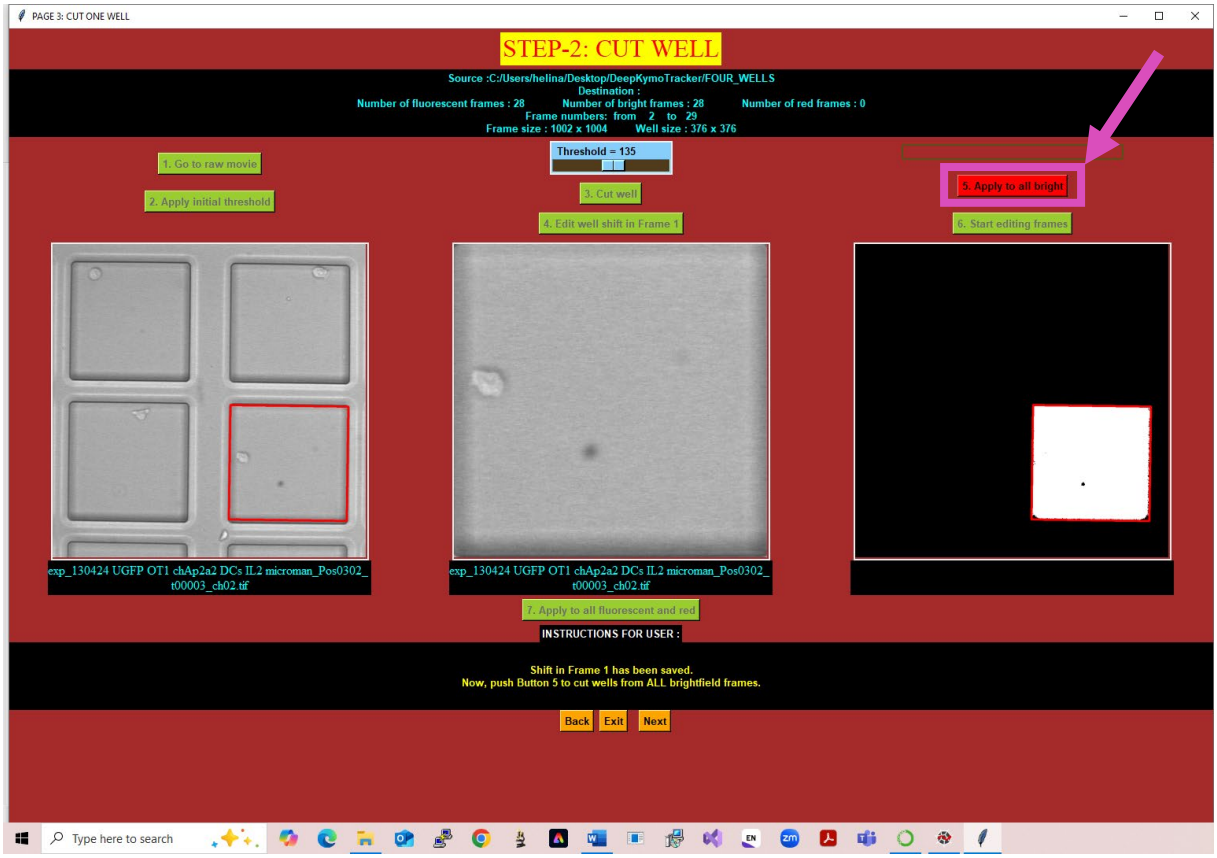
The cropped well (brightfield) will appear in the middle window. To check more closely the quality of the cropping, press button **4. Edit well shift in Frame 1**.



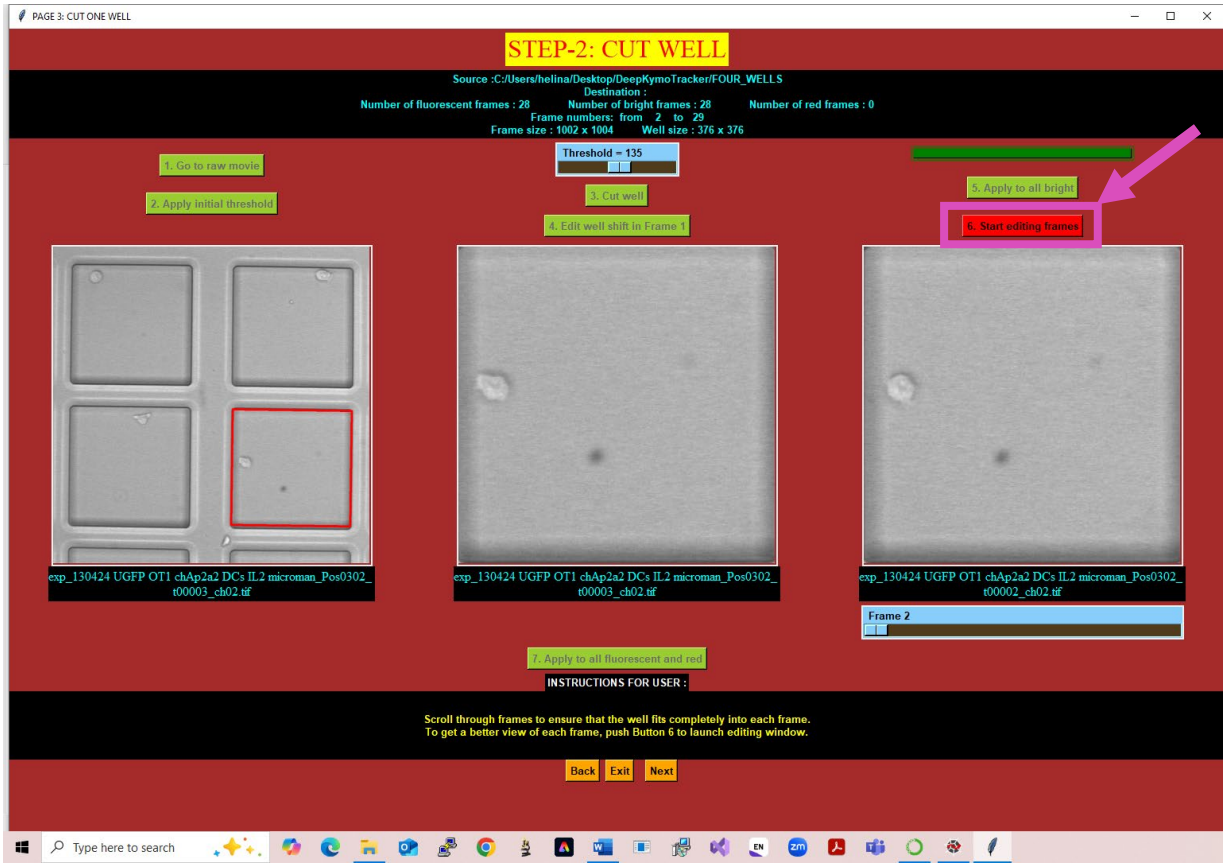
The magnified cropped well appears in a new popup window. You can inspect the quality of the cropped image more closely now. To correct the possible shift, drag the image with mouse. Once finished, press **Close**. The popup window will close.



Now ,it is time to cut the well from all bright frames. To start the process, click **5. Apply to all bright**.



The processed brightfield frames will appear in the right window in real time. Once the process is completed, the slide bar for scrolling the processed bright frames will appear below and button **5. Start editing frames** starts flashing.



To check the quality of the cropping in every single frame, press **6. Start editing frames.**

A popup window appears again, but this time it has a scroll bar which allows you to go through every single frame and make manual shift corrections (using mouse to drag the image) if necessary. Once finished, close the window.

Finally, click **7. Apply to all fluorescent and red**. The well will be cropped out of all green fluorescent and red frames.