STEP-1: EXTRACT MOVIE FROM FOLDER

(User guide for DeepKymoTracker)

(Updated on 27/12/25)

Goal: 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.

Note: You do not need this step if your cell movie is already in the necessary format (this is usually true for so called "old Mohammed` s movies" with the names Pos0201, Pos0304, etc.). In this case, you just directly proceed to Step-2.

.

Input requirements:

- The folder containing multiple movies taken by spinning disc or epi. Examples of folder names: 20181218 DN2B Ep, 20190206 DN2B EpCAM.
- Images file names: they need to contain
    - **_w2FITC_** or **_w3Multi600_** or **_w4Alexa-633_ (**green fluorescent channel),
    - **_w1BF_** (brightfield channel),
    - **_w3TRITC_** (red channel).

  The example name: 5_w1BF_s9_t1173.TIF (brightfield channel), where **s9** is the name of the specific movie you may want to extract from the folder, 1173 is the frame number, w1BF is the indication that this is a brightfield image.
- Images file type: multi-, or single-page TIF files, 16 bit depth.
- Location of the folder: does not matter. The folder with those movies can be anywhere on your computer; the interface will ask you to navigate to this folder in the very beginning.

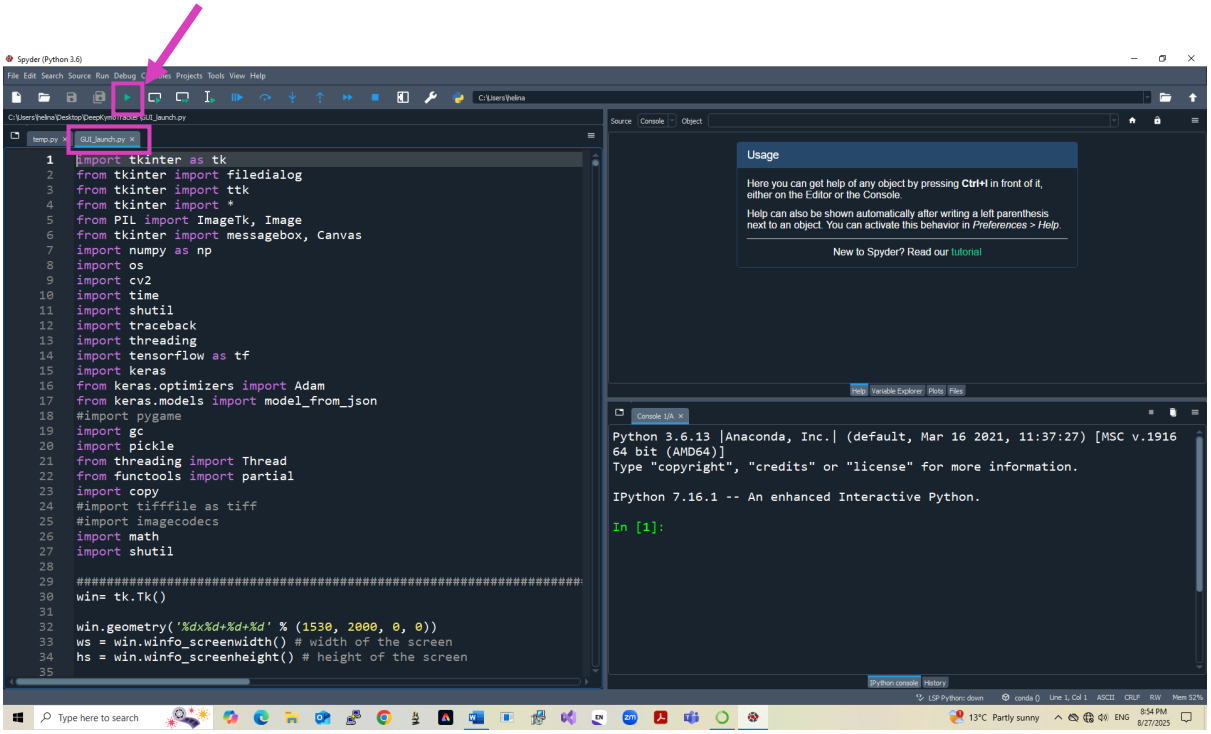
Outcome:

- The resulting movie folder named **s9** (for the example above) will automatically land in the DeepKymoTracker folder.
- Images in all three channels become single-page TIF images.
- The file names are changed slightly: namely,

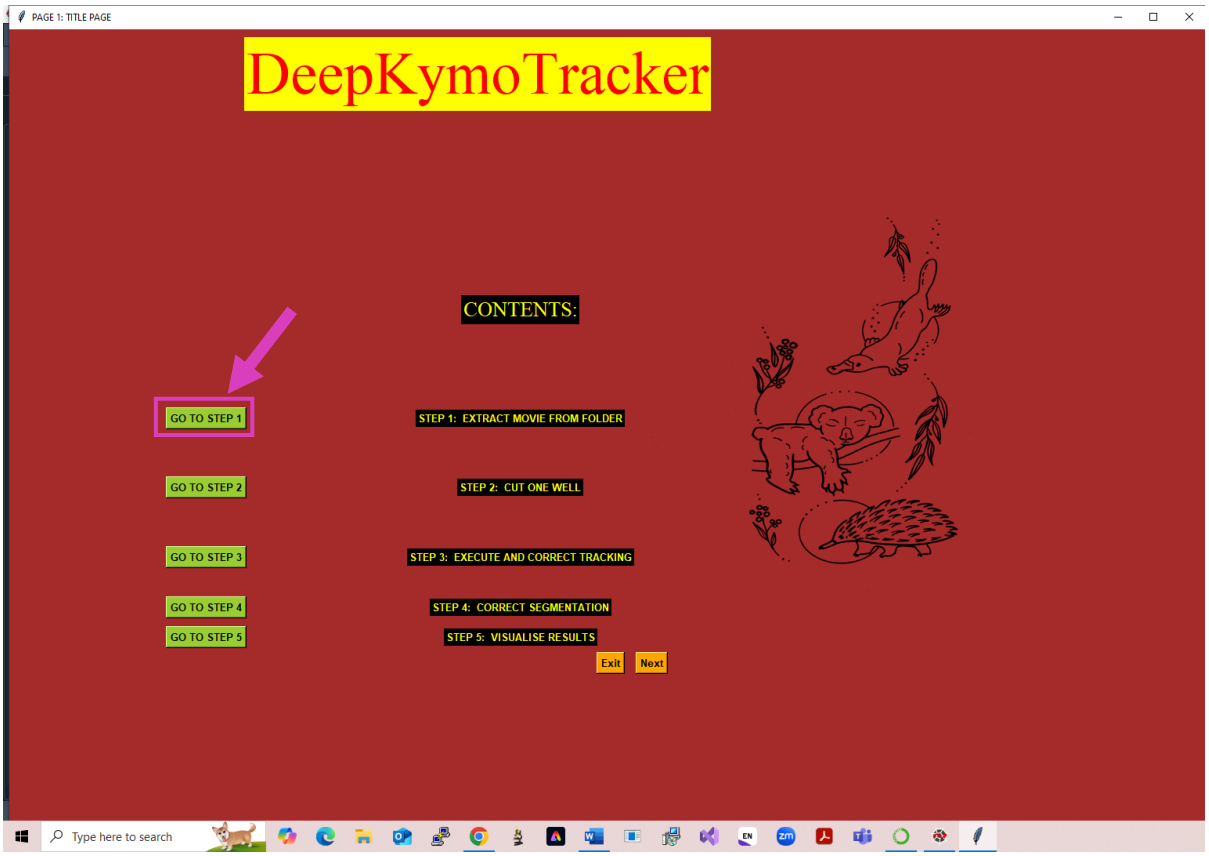
  5_w1BF_s9_t2.TIF (brightfield) is now 5_w1BF_s9_t002_ch00.TIF,
  5_w2FITC_s9_t2.TIF (green fluorescent) is now 5_w2FITC_s9_t002_ch02.TIF,
  5_w3TRITC_s9_t2.TIF (red) is now 5_w3TRITC_s9_t002_ch01.TIF

Instructions:

Launch file **GUI_launch.py** by pressing green arrow:



You will see the title page of DeepKymoTracker. Press button **GO TO STEP 1** (or button **NEXT** at the bottom of the screen).



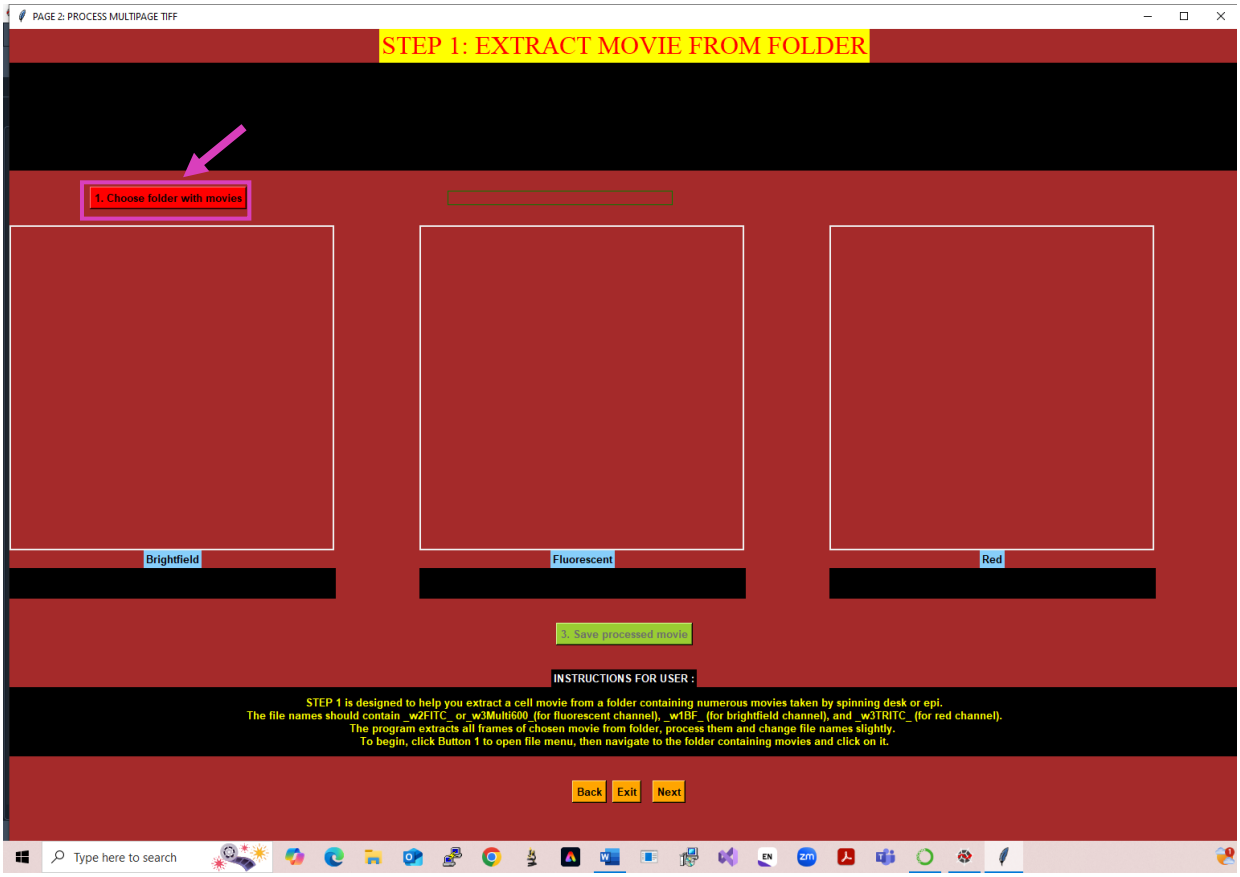
Here is the window you are going to work with. At the botton, you can see the black space (titled INSTRUCTIONS FOR USER:) where the instructions will keep appearing as you proceed. On the top, there is a similar black space where the essential info extracted from the movie being processed will be appearing.

You may notice some differences in the appearance of the buttons – some of them are flashing, some are grayed out, etc. This design is not accidental – it aims to making the whole process more convenient to you.

Here is the explanation:

- Flashing buttons will guide you through the pipeline – they are inviting you to press them, so you know what your next move should be.
- If the button remains red for a prolonged period of time,it means that the function associated with this button is in progress – the red color warns you that you should wait until the function is being performed. Once the action is finished, the button color comes back to normal.
- All the buttons except red or flashing ones are usually grayed out which means that they are disabled: even if you try to push one of them they would not react. It was implemented with the purpose to prevent you from taking wrong actions that may interrupt the flow of the program and throw an error.

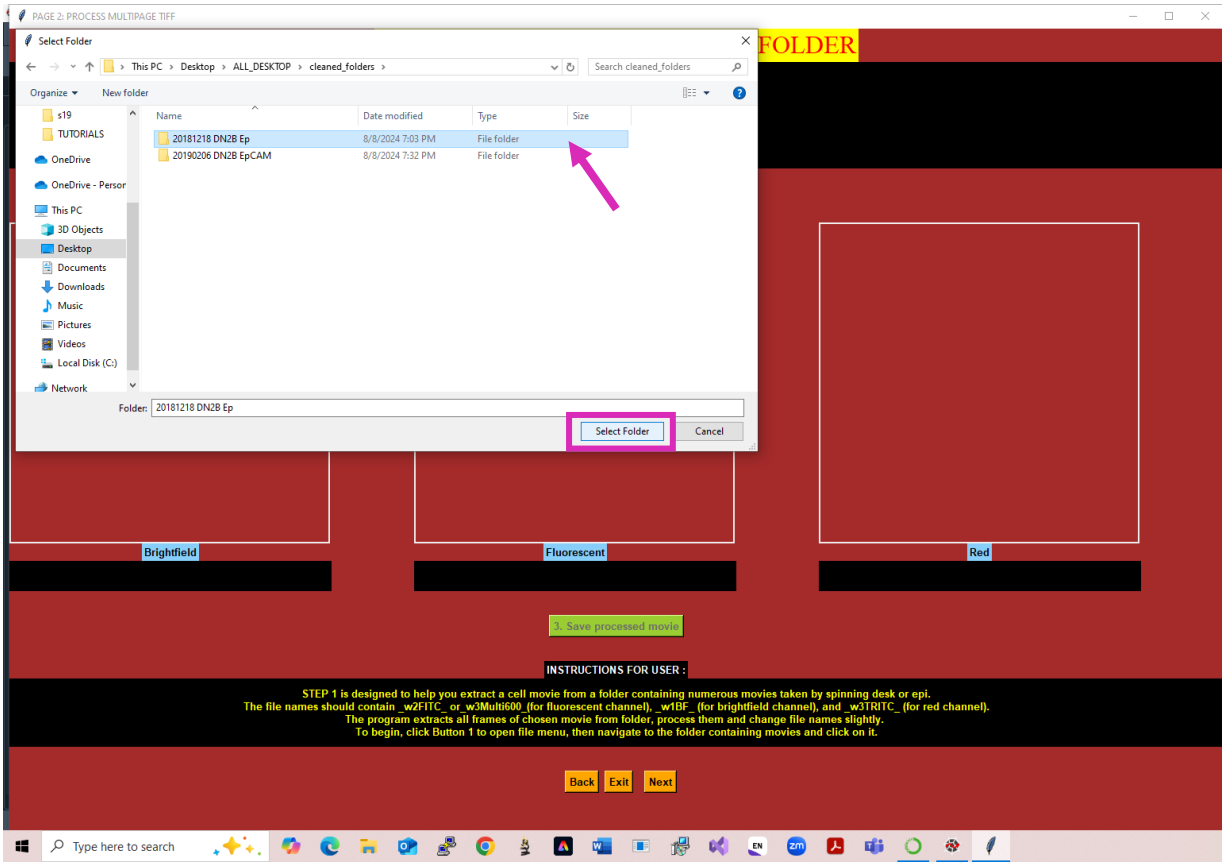
To begin, click flashing button **1. Choose folder with movies**



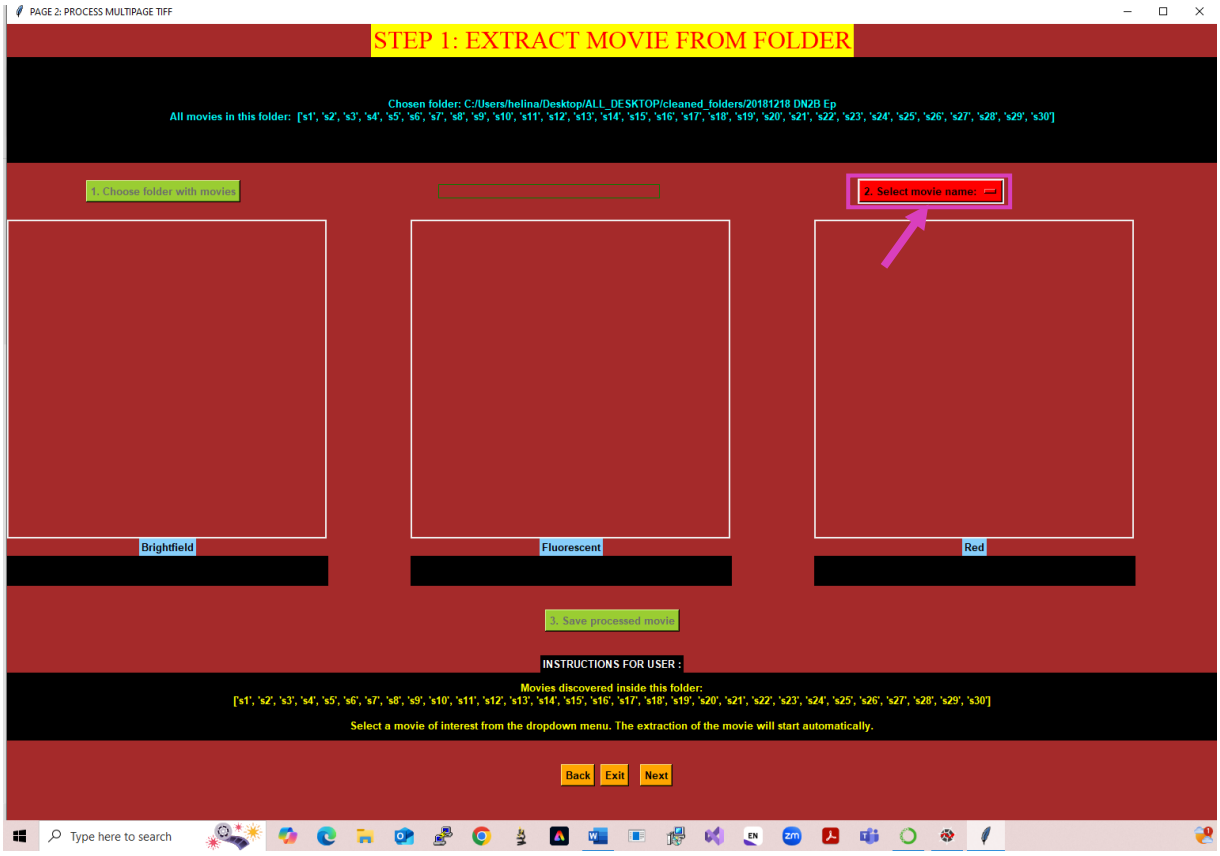
In the open menu, navigate to your folder with multiple movies , highlight it and press **Select Folder**.

Note: do not double click on the chosen folder as we all normally do - it is not working with DeepKymoTracker! Instead, click (once!) on the folder to highlight it and then press **Select Folder**.

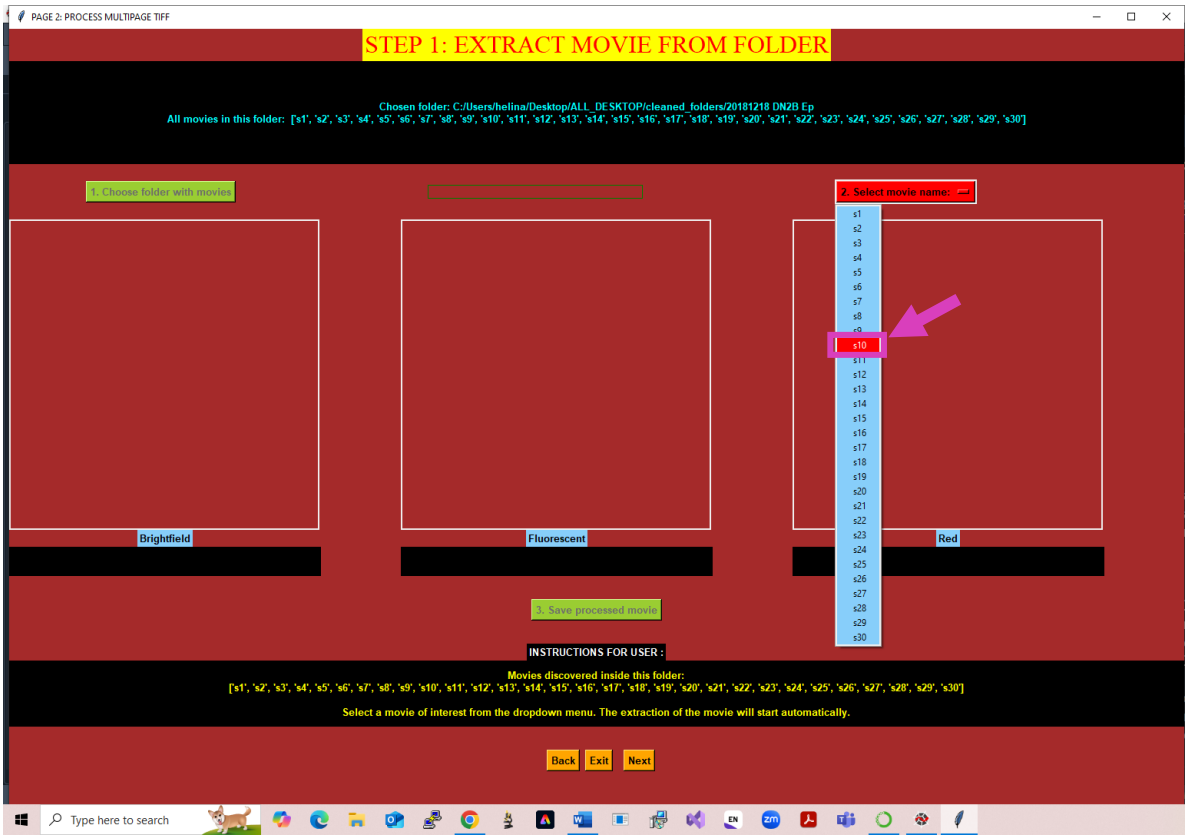
Internal



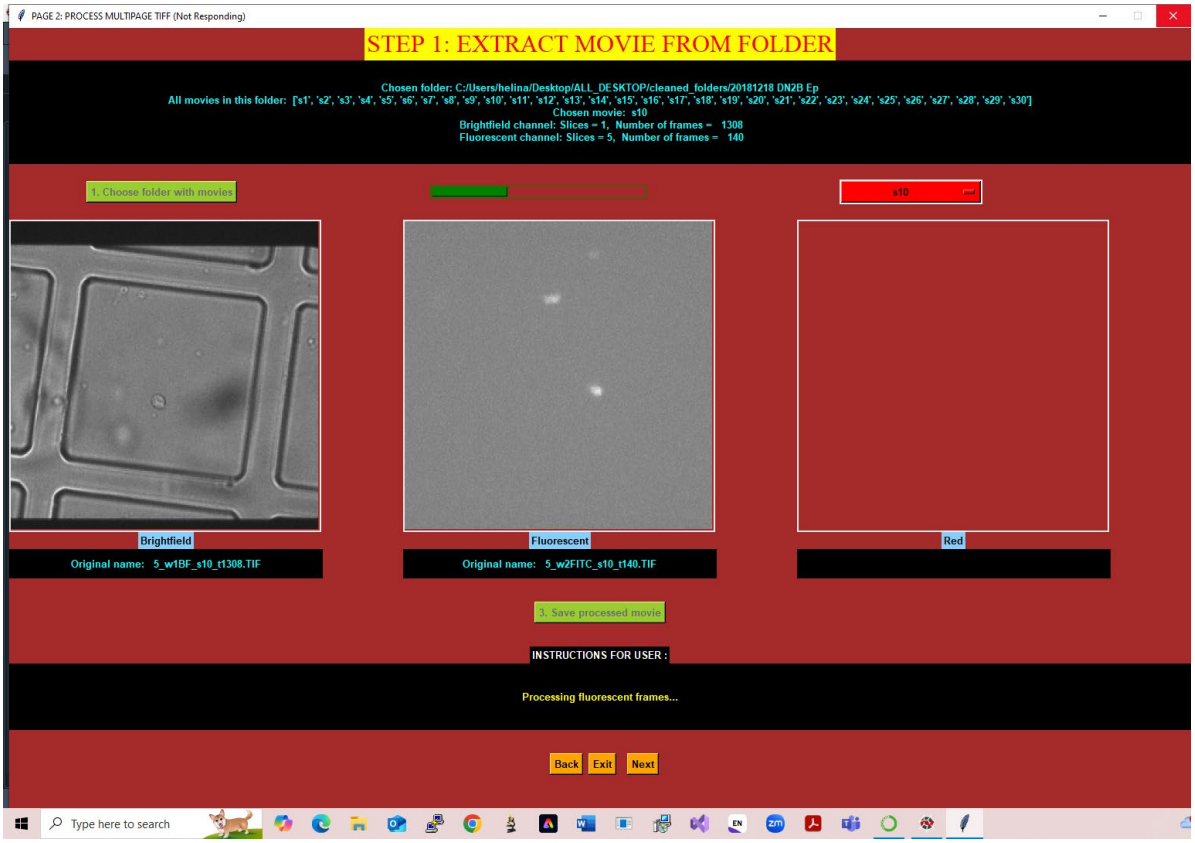
After that, a new flashing button appears, **2. Select movie name**. Press it to open dropdown menu with the movie names discovered inside the folder.



In the dropdown menu, choose a movie of interest and click on it. The extraction of the movie will start immediately.



The extraction and processing of the movie happens consequentially: first, you will see processed bright frames in the left window, then – fluorescent green channel in the middle window, and finally, red channel images in the right window.



When the process is finished, a flashing slide bar appears below the middle window inviting you to scroll through the images to check.

Finally, you can either save the processed movie (by pressing button **3. Save processed movie**) or, if you lost interest in that movie for some reason 😊, you can just press **Exit**.