This is a document with instructions on how to use the scripts in the folder “ACVU\_scripts”.

The raw data we want to analyze consist of timelapse fluorescence microscopy images of single chambers. The chambers are (for example) imaged every 20 minutes, in 3 channels (‘CoolLED’, ’488nm’ and ’561nm’). Each single channel image is a Z-stack, each slice of the stack being 4MP, each pixel is unsigned int 16 bit (=2 bytes). That means that a single channel stack consisting of 20 images is about 160MB (2 bytes \* 4MP \* 20 slices). In addition, for each timepoint, a txt file with some parameters is created. Images coming from the same chambers are saved in a folder. All the folders coming from the same experiment are saved in the same directory.

Example:

Experiment Directory (also referred as expDir): ‘160125\_mCherry\_lag2YFP’

Single Worm Directory (also referred as wormName): ‘C01’

Single timepoint files: ‘z001\_488nm.tif’, ‘z001\_561nm.tif’, ‘z001\_CoolLED.tif’, ‘z001\_.txt’

What we want to do is to extract fluorescence instensities in single cells (nuclei) inside the worm. Later on we will need the time relative to hatching and maybe also ecdysis events. So we have data in the form of timeseries.

We proceed as follows:

Function 01: 01downsizedMovie.

Create a movie of the worm (typically in the LED channel, but it’s also possible to do it for the 488 and 561 channels) with AND without timestamps.

Inputs:

* path: this is the full path to the expDir (example: ‘Y:\\Images\\160125\_mCherry\_lag2YFP’)
* worm: wormName
* hatchingTidx: time point at which the worm hatched. **NB:** the number has to be the timepoint minus 1!!! (if the worm hatched in the third image, the input has to be 2). This is because the timelapse starts from z001 and not z000.
* magnification: integer (typically 40 or 60), it’s the same for all the worms in the same experiment. Default: 60.
* channels: channels to take into account when creating the movie. Default: only ‘CoolLED’.
* scaleFactor: compression factor to downsize the images (it has to be power of 2). This is because the images are way too large and for a full chamber movie we don’t actually need such high resolution. Default: 4.

Outputs:

* For each channel two movies are created, one without and one with timestamps on it. The movies are compressed and in 8 bit to save space and special compressed with binning on the drive and make future loading faster. Each frame in the ‘CoolLED’ movie is the mean of the stack, for 488nm and 561nm is the maximum projection.
* A params pickle file containing a pandas Series is created in the expDir. Name: *wormName\_01params.pickle*. It contains the experiment directory (**exp**), worm name (**worm**), **magnification**, **pxlSize**, **tidxHatch** (same as input, so with the minus 1 included) and the scaleFactor used to compress the images (**compression**)
* A times pickle file containing a pandas DataFrame is created in the expDir. Name: *wormName\_01times.pickle*. This is a DataFrame containing the name of the txt file in the first column (**fName**), the time index relative to hatch (**tidxRel**), and the time in hours relative to hatch (**timesRel**).

Txt file creation

Create a txt file named ‘skin.txt’ in the expDir where you right down the timepoint of ecdysis for each worm (again it should be -1, if ecdysis happens in the 25th image, the number should be 24). Example:

Worm hatch L1ecd L2ecd

01 8 51 85

02 6 49 83

Function 02: 02markGonad

With this GUI, the compressed CoolLED movie is loaded and it’s possible, for each timepoint, to mark the position of the gonad with a mouse click. With the radio button on the side, one can switch between different channels. X and Y position are relative to the full size images. No inputs are required. When loading the data, select the “wormaName\_analyzedImages” folder, which is where the compressed movies are saved.

Outputs:

* A gonadPos pickle file containing a pandas DataFrame is created in the expDir. Name: *wormName\_02gonadPos.pickle*. This is a DataFrame containing the X Y pos ( **X** and **Y** ) for each of the time index relative to hatch (**tidxRel**) in which the gonad has been marked.

Function 03: 03cropImages

This function crops a small region around the gonad for all the images of the given worm. Images are saved as new multipage tiff files in the “analyzedImages” folder.

Inputs:

* path: this is the full path to the expDir (example: ‘Y:\\Images\\160125\_mCherry\_lag2YFP’)
* worms: a list of wormNames (example: [‘C01’,’C02’])
* channels: which images to crop (standard = all the channels available)
* size: size of the cropped region. Default: 512

Outputs:

* cropped images and metadata files (these are copied) ONLY for the timepoints where the gonadPos is available

Function 04: 04markCells

GUI designed to mark the position of the cells in all the timepoints where the gonad is marked. For each timepoint, the appropriate channel can be shown with the radio buttons. Left click is to add a cell, right click to remove the closest one. Cells that can be labeled are the ones belonging to the Z1 and Z4 lineage. Names are ‘1.p’,’1.pp’,’1.ppa’,’1.ppp’,’4.a’,’4.aa’,’4.aaa’,’4.aap’.

Also, the background position has to be set for each lineage. Names: ‘b\_1’ and ‘b\_4’.

A popup error message is shown if the cells are not properly labeled (for example if there are two cells with the same name or if one of the background positions is missing).

Output:

* A gonadPos pickle file containing a dictionary is created. Name: *wormName\_04cellPos.pickle*. **The keys of the dictionary are the cell names**. Each element of the dictionary is a data frame containing the **X-Y-Z** positions for each time index relative to hatch (**tidx**).

Function 05: 05outlineCells