

## Day 1

### ***“Processing and analysis of calcium imaging experiments”***

#### **Instructions**

In the exercises of today, please go through the individual assignments and aim to answer the given questions and/or problems.

Where applicable, document your reasoning using short written answers, screenshots, etc. and collect them in a text document. It is not mandatory to answer all questions.

Please also discuss with your peers and tutors, if you are stuck at a specific question.

Course materials can be found here:

[https://github.com/donatolab/biozentrum\\_block\\_course](https://github.com/donatolab/biozentrum_block_course)

<https://drive.google.com/drive/folders/1fiyQdL55S3kkAa0EcUj8fpo50o3zhQcy>

<https://adam.unibas.ch/>

#### **Data**

The data we are working with in this exercise stems from an imaging session with a trained, adult mouse voluntarily moving on our treadmill apparatus for approx. 50 min.

Example data of this experiment can be found in the course materials:

./behav.mp4:	~30 seconds of mousecam video data of the animal's behavior
./raw.tif:	~30 seconds / 1,000 frames of raw imaging data
./reg.tif:	same, after motion correction
./raw_groupproj.tif:	down-sampled, group-projected raw data (1 frame = 1 sec)
./reg_groupproj.tif:	same, after motion correction
./fov_avg.tif:	average fluorescence projection, after motion correction
./fov_max.tif:	maximum fluorescence projection

...

#### **Task 1: *Pre-processing of raw 2-photon calcium data***

suite2p is a free software tool that we use to extract calcium fluorescence traces, or transients, from imaging time series acquired with a 2-photon microscope.

suite2p has a graphical user interface (GUI) that can be run on windows (which we will be using) and linux based operating systems.

Individual sub-routines can also be called from the programming languages Matlab and Python, to be embedded in user-defined analyses pipelines.

The suite2p documentation can be found here:

<https://suite2p.readthedocs.io/en/latest/index.html>

You may want/need to refer to this documentation during the assignments.

Starting suite2p using *conda* and *python*:

- from the start menu, open an Anaconda Powershell
- type: "conda activate suite2p"
- type: "python -m suite2p"
- ➔ tada!! you have started the suite2p GUI

Running suite2p on "raw" data (a simulation): suite2p operates on several raw file/image formats, including hdf5 and tif. Our raw data is typically converted to tifs before running suite2p.

- in the GUI menu, click on "File/Run suite2p"
- ➔ a new window has popped up
- in the top right half of the new window, observe six columns with eight sections of parameter–value pairs
- ➔ these specify properties of the acquired raw data (e.g. the number of imaging channels, "nchannels", typically a 'green' and a 'red' fluorescence channel; the acquisition frame rate, "fs", typically around 30 fps), as well as several parameters for the suite2p operations (e.g. registration, "do\_registration" and "non\_rigid", for correction of brain movements; ROI detection, "roi\_detect", for detection of regions-of-interest – neurons)
- click on "Load ops file"
- select from the materials repository a file called "ops\_blockkurs.npy" and load it
- ➔ several parameter–value pairs have changed now; these are critical to our recordings
- in the "Registration" column (3rd from left), "do\_registration" is set to "1" (obviously, we want to remove brain motion from the raw data); one column to the right, there is "Nonrigid" motion correction

**Q1.1:** during active behaviors, the animal's brain can actually move a few micrometer in relation to the set imaging field-of-view (e.g. because of the animal's body movements, swallowing, etc.); what do you think are "easy-to-remove" brain movements, what are "difficult-to-remove" ones (think of the 2-photon microscope making thin "optical slices" within the brain)? why is removing brain motion important?

**Q1.2:** what is the consequence of setting the "nonrigid" motion correction parameter to 1 vs setting it to 0 (check the suite2p documentation, if needed)? when do you think non-rigid brain motions could occur?

**Q1.3:** see the parameters "maxregshift", "maxregshiftNR" and "block\_size"; how do they relate to the dimensions of the acquired raw images (check any of the tif files in the course materials)? how do the physical dimensions of the field-of-view play a role here?

- next, we want suite2p to detect neurons (or regions-of-interest, ROIs) for us, in order to extract the calcium transients from these ROIs (see the 5th column, "ROI detection")

**Q1.4:** see parameter "diameter"; what does it mean and how does it relate to the dimensions of acquired raw images?

**Q1.5:** see parameter "anatomical\_only"; what does it do? what happens when you set it to values greater than 0? refer to the documentation; you may want to revisit this question after having looked at the projections in the main GUI (task 2)

## **Task 2:** *Curating suite2p output*

Next, we want to use suite2p to explore the pre-processed data to first assess the quality of the imaged data, and to second curate/improve our cell detection.

- return to the main suite2p GUI
- in the menu, click on "File/Load processed data"
- from the materials repository, selected file "../suite2p/plane0/stat.npy"
- ➔ this is a typical preprocessing result of suite2p, based on the parameter settings you played with in task 1
- on the top center of the GUI, click on "cells", "not cells", then "both"
- ➔ observe, side-by-side, what suite2p has detected as ROIs, and classified as potential neuron ("cell", left) and as potential "garbage" ("not cell", right)
- on the top left, observe the different "Backgrounds" (ignore the "ROIs" for now);

**Q2.1:** what are these different 'background' projections? which one do you consider more suitable to assess the 'quality' of the recording? which is useable for detecting neurons/ROIs if you were asked to do this manually (e.g. by clicking on or drawing circles around potential neurons' ROIs)?

- below the "Backgrounds", check out the "Colors" section; the different selections highlight specific properties of the individual ROIs (e.g. shape), with their values encoded in a colormap of your choice (drop-down menu), and on which their classification into the "cells" and "not cells" groups was based
- in both groups, select a range of ROIs (by clicking on them; by cycling through them with the keyboard cursors) and observe their raw calcium transients shown as a cyan trace in the bottom section of the GUI (for clarity, you may want to uncheck the boxes "deconv" and "neuropil" in the very bottom of the GUI)

**Q2.2:** describe the raw calcium fluorescence of select "cells" ROIs; what is their range of amplitudes? at which values are their baselines at? what is the level of "noise" (i.e. baseline fluctuations)? what is the shape of a calcium events (=neural activity)? take screenshots, if needed; zoom in/out with the mouse wheel

**Q2.3:** do the same for "not cells" ROIs; what are the 'spatial' features of the ROIs in this group? are their differences in their location within the field-of-view?

**Q2.4:** are there false-positive and false-negative classified ROIs? curate them and assign them to their correct group (by right-clicking the ROI); when done, build your revised neuron classifier: in the menu, click on "Classifier/Build"; in the pop-up menu, click on "Load iscell.npy"; select from the materials the file "./suite2p/plane0/iscell.npy"; click on "build classifier" and save yours as e.g. "cell\_classifier\_<YOURNAME>.npy"

**Q2.5:** what happens if you do the procedure you just did in Q2.4 iteratively, across several imaging sessions?

**Q2.6:** if you have time (i.e. do this at the end of ), search for ROIs with flat, zero baselines; can you find out the reason/cause why their baselines are zero-ed?

### **Task 3:** *Visualization of neural population dynamics*

suite2p has few built-in tools to visualize the population activity of select ROIs.

- click on "Visualizations/Visualize selected cells"  
➔ a new windows has popped up
- click on "compute raster maps + PCs" (this may take a few seconds)
- select "PC" and/or "rastermap" to do a first visualisation of neuronal population activity

**Q3.1:** describe your observation; is there any structure in the data? use the sliders (the red boxes) to zoom in and out in x and y

## **Session #2:** *Calcium transients and event detection*

For this session we need to load the jupyter lab environment and run methods from the Calcium() class. The answers for the questions below come mostly from the presentation but also a few from running the jupyter lab notebooks. You can find the required instructions for loading jupyter lab here:

[https://github.com/donatolab/biozentrum\\_block\\_course/tree/main/Day\\_1/binarization](https://github.com/donatolab/biozentrum_block_course/tree/main/Day_1/binarization)

Tasks and questions for session #2:

**Q4.1:** What is conda and what do we use it for?

**Q4.2:** How did we use github in this course?

**Q4.3:** What is one advantage of interactive coding such as used in jupyter lab?

**Q4.4:** Name any two python packages (not just those used in the course) and briefly describe their use.

**Q4.5:** What did we use the Calcium() class for today? (hint: think about input and output)

**Q4.6:** Name the 2 types of spiking (or boolean) data we generated and briefly describe their differences.

**Q4.7:** What is a sign of an “bad/weird/unhealthy” [Ca] signal in a single cell?

**Q4.8:** How did we reduce the effect of bleaching in the single cell traces?

**Q4.9:** What is “pip” and how did you use it for this session? (hint: if lost, try googling it)

**Q4.10:** What is the effect of lowering the “min\_thresh\_std\_onphase/upphase” on the # of spikes per cell? What is the effect of increasing it?

**Q4.11:** What is the effect of setting “high\_cutoff” parameter to 5Hz on the cell spikes and overall rasters? What about 0.1Hz?

**Q4.12:** Describe the differences you see in single cell [ca] activity and spikes for cells with low ids (e.g. cell 0,1,2...) vs. cells with high id (e.g. 500, 800,1000)?

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