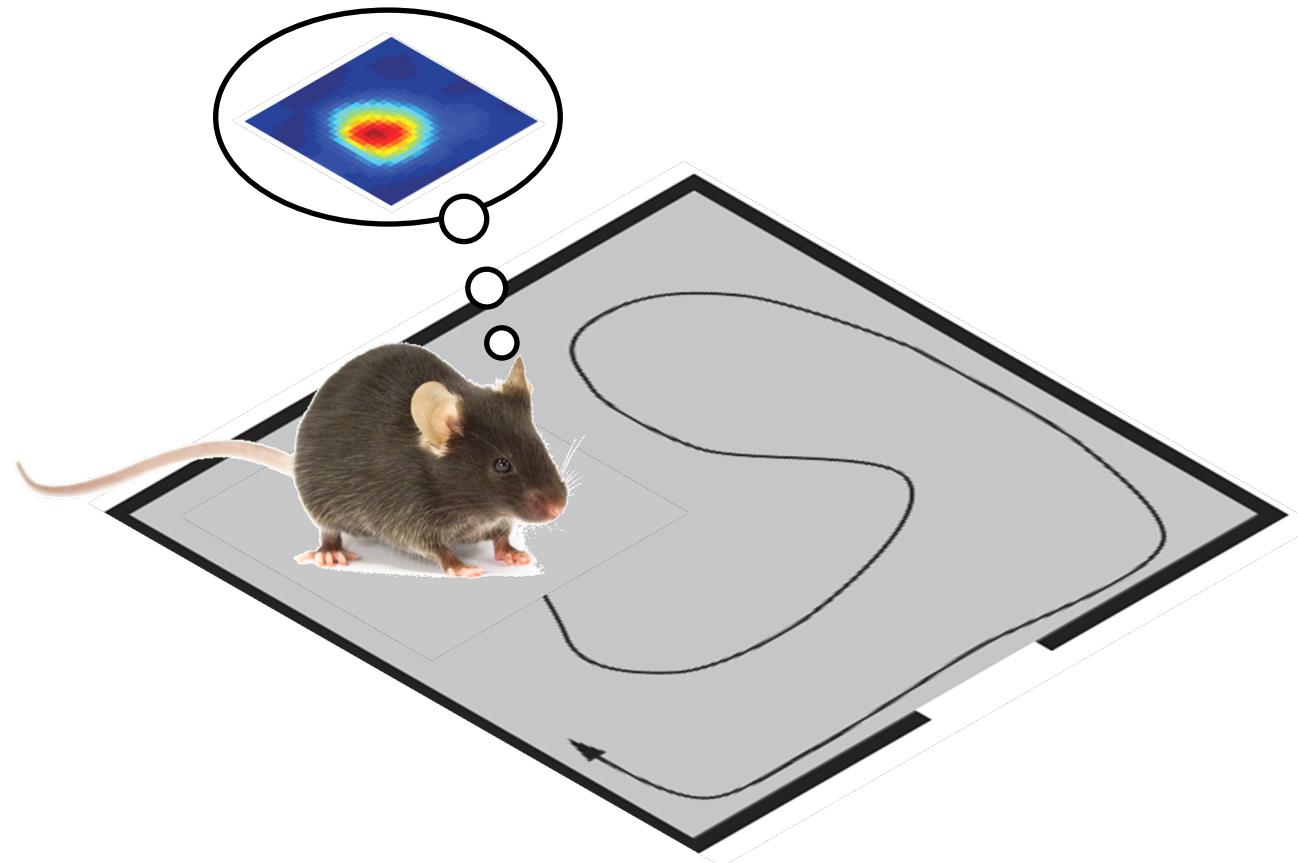


Day 1

“Processing and analysis of calcium imaging experiments”

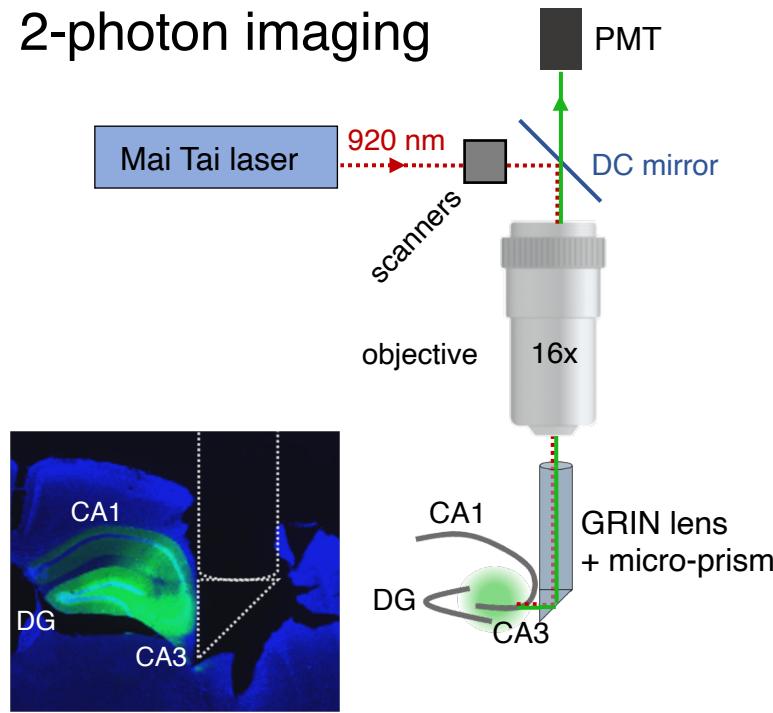
Blockkurs | May 23–25, 2022

Flavio Donato, Laurenz Salm, Catalin Mitelut, Renan Augusto Viana Mendes, Steffen Kandler



Imaging of hippocampal CA3 region

2-photon imaging



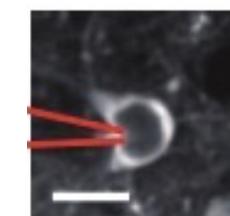
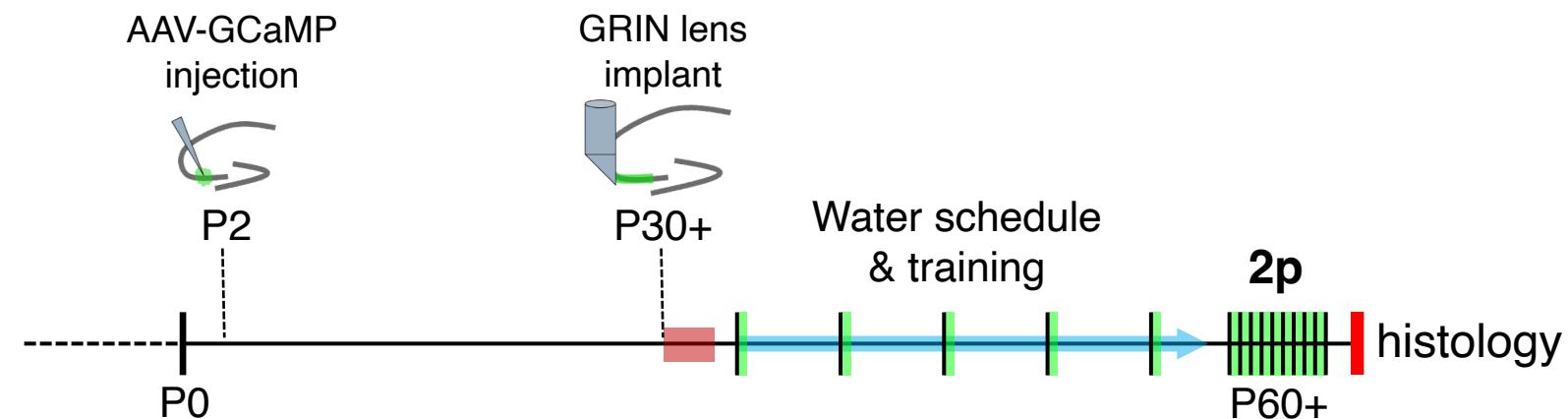
DAPI (cell nuclei)
GCaMP6

PMT: photo-multiplier tube

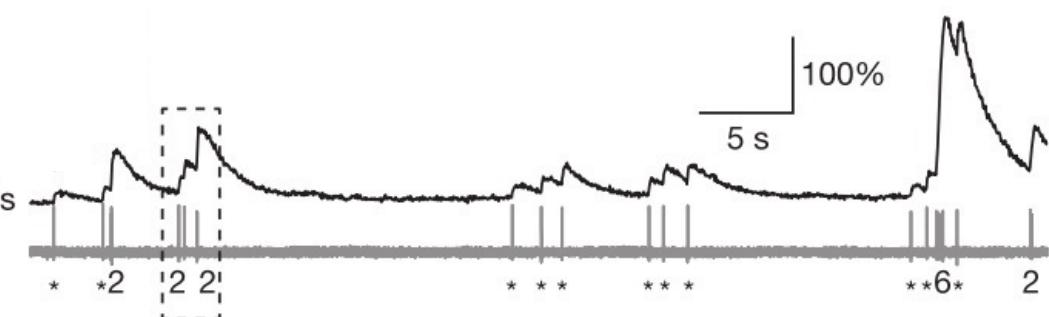
GRIN: gradient-index

DC: dichroic

Experimental timeline

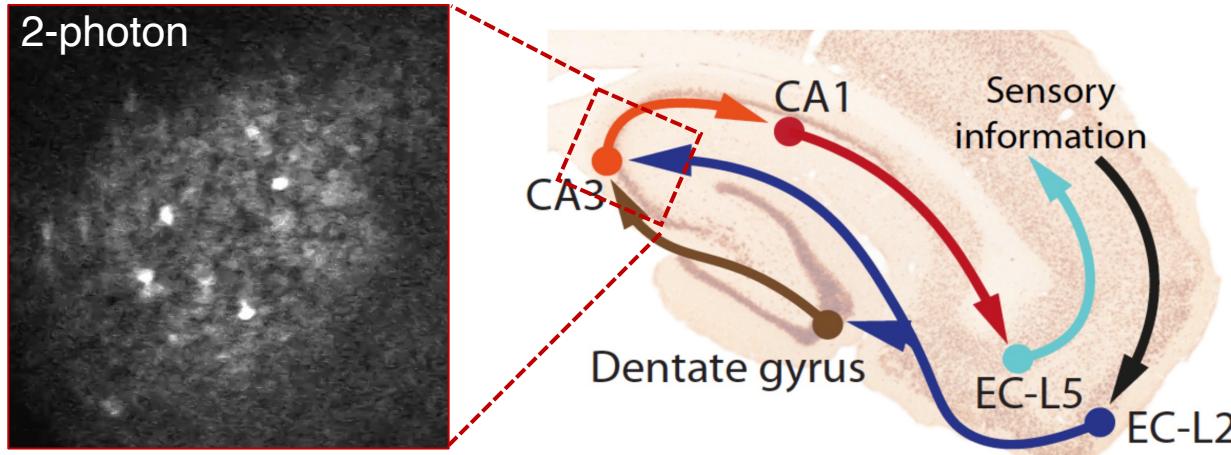


Imaging / Fluorescence
Ephys / Voltage



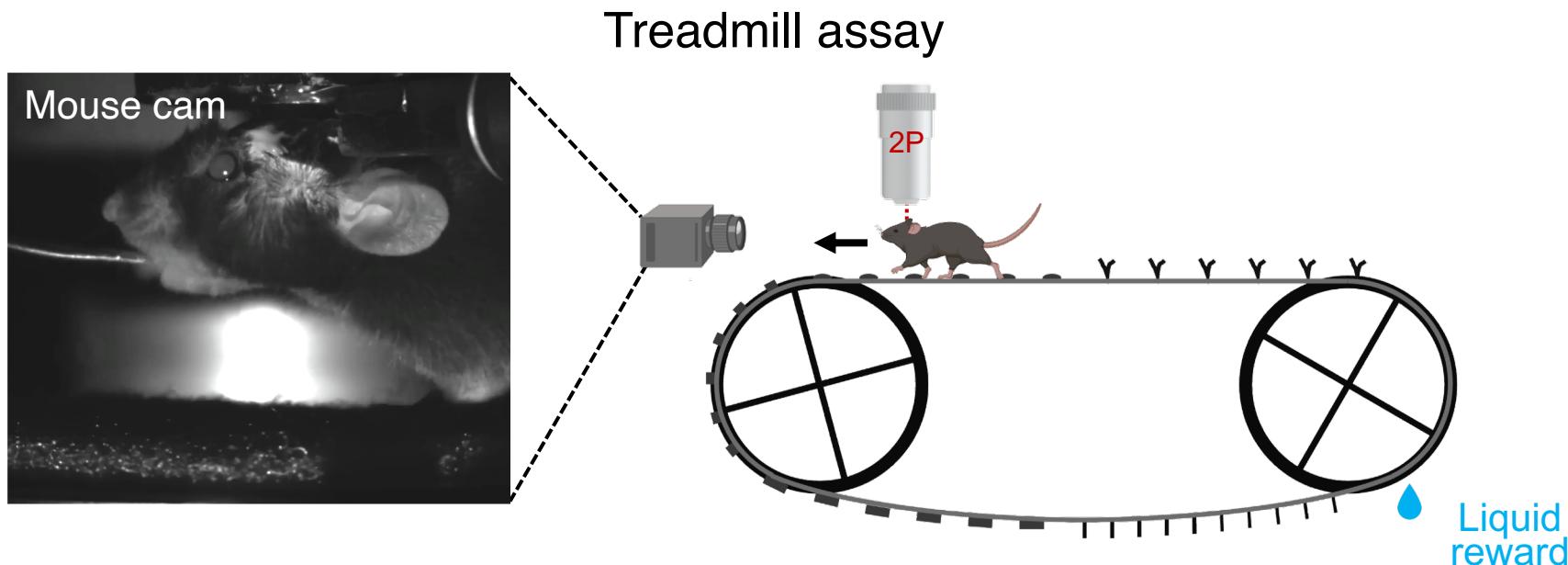
Chen et al., Nature 2013

Emergence and refinement of ‘structure’ in hippocampal CA3 populations



Q1: How does a population code emerge and evolve in the maturing brain?

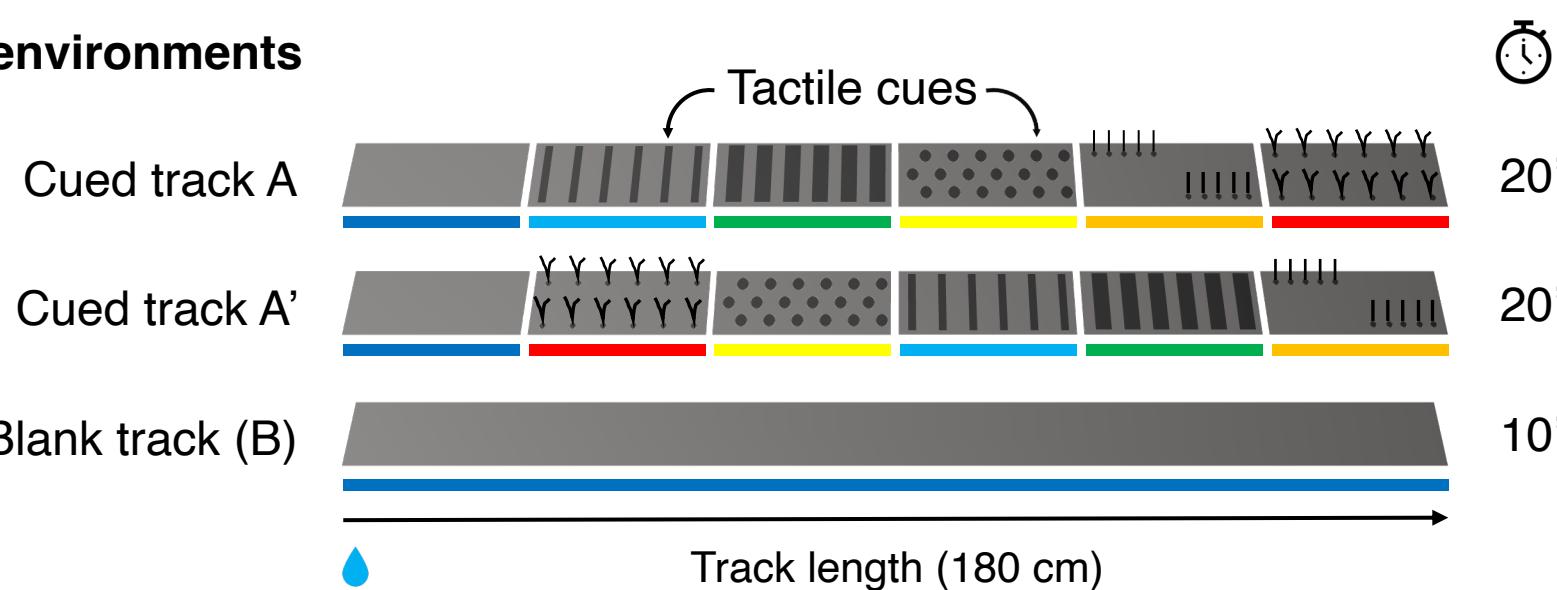
Q2: How does it reflect general and specific spatial features of environments?



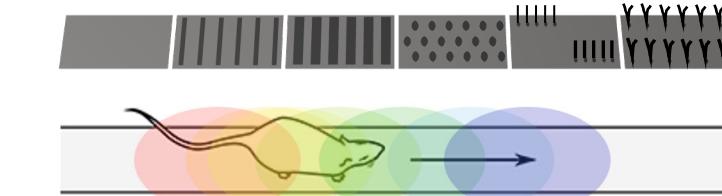
- Tracking of
- Movement
 - Laps
 - Behavior
 - Reward licks

Treadmill assay to probe spatial activity in CA3

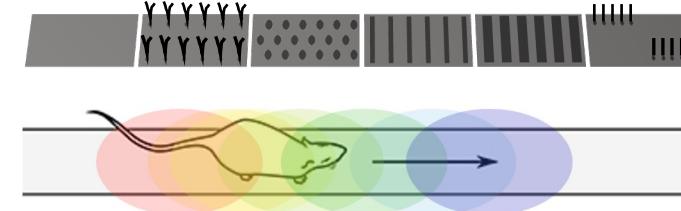
Haptic environments



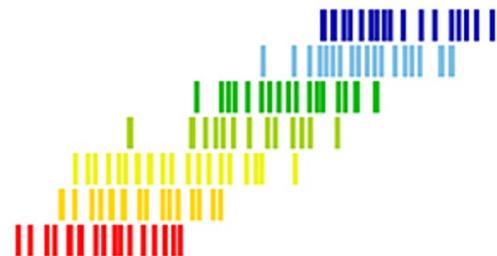
A



A'



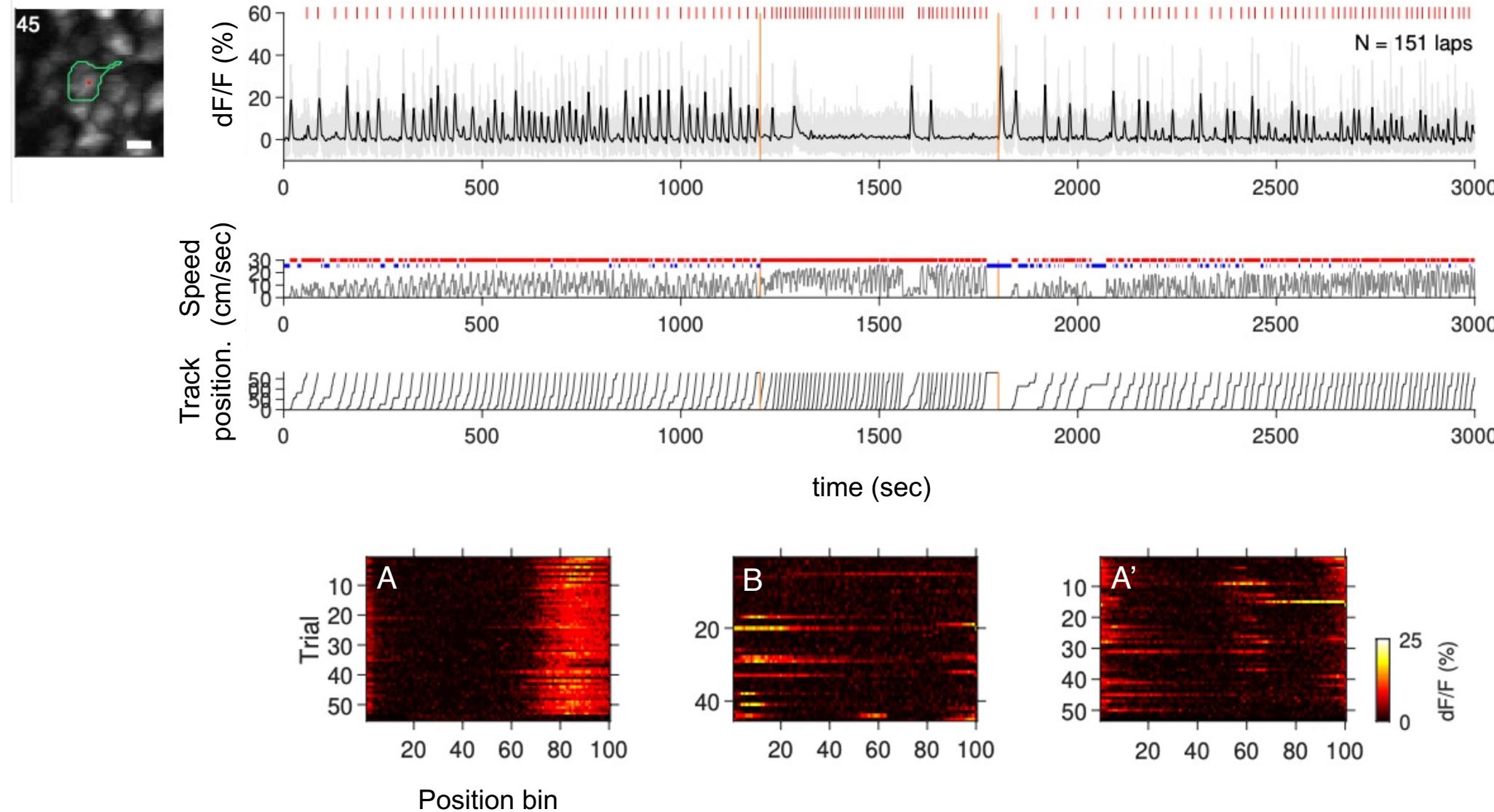
Place cells



?

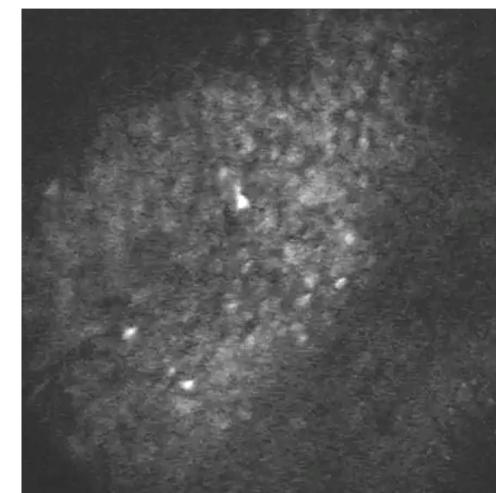
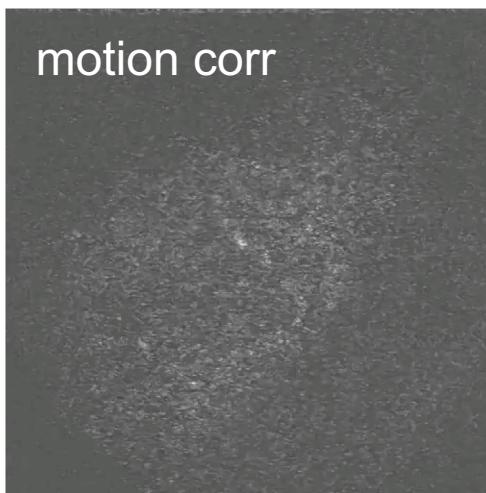
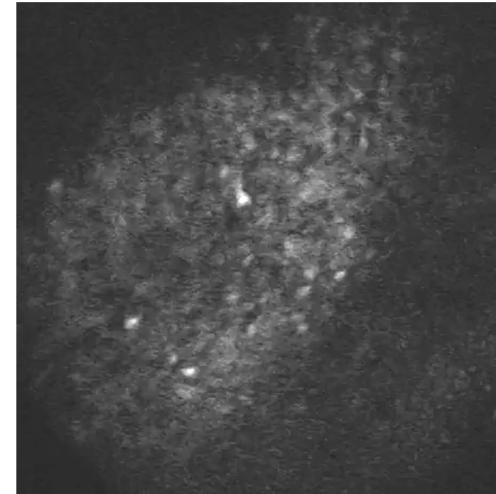
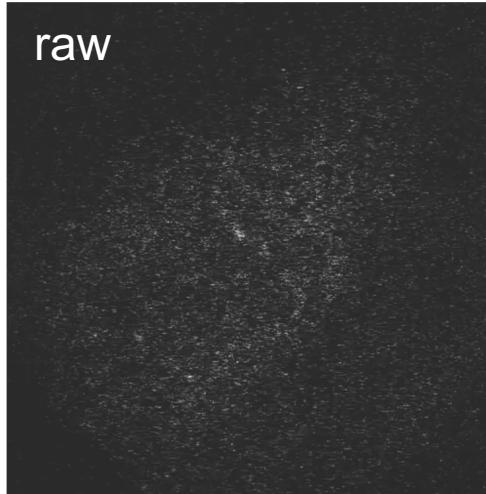
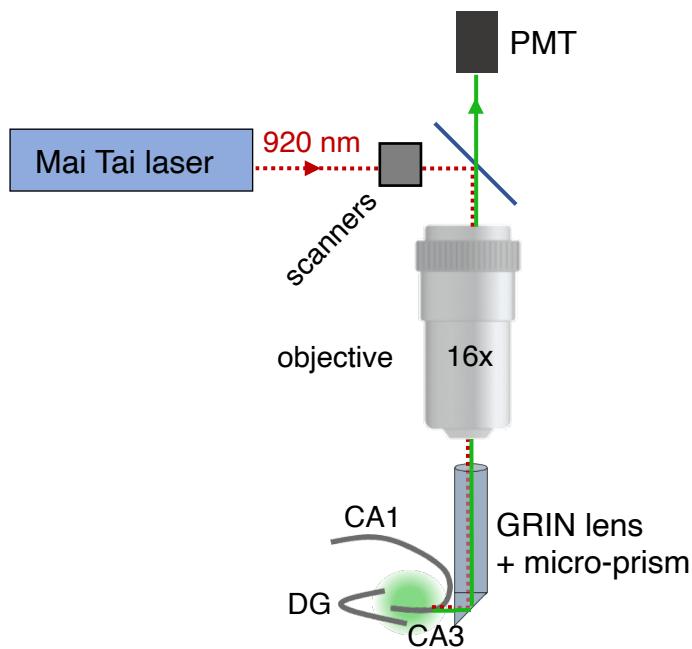


Example place cell activity in the task



The first set of assignments

- Processing pipeline: Raw imaging data → Calcium transients

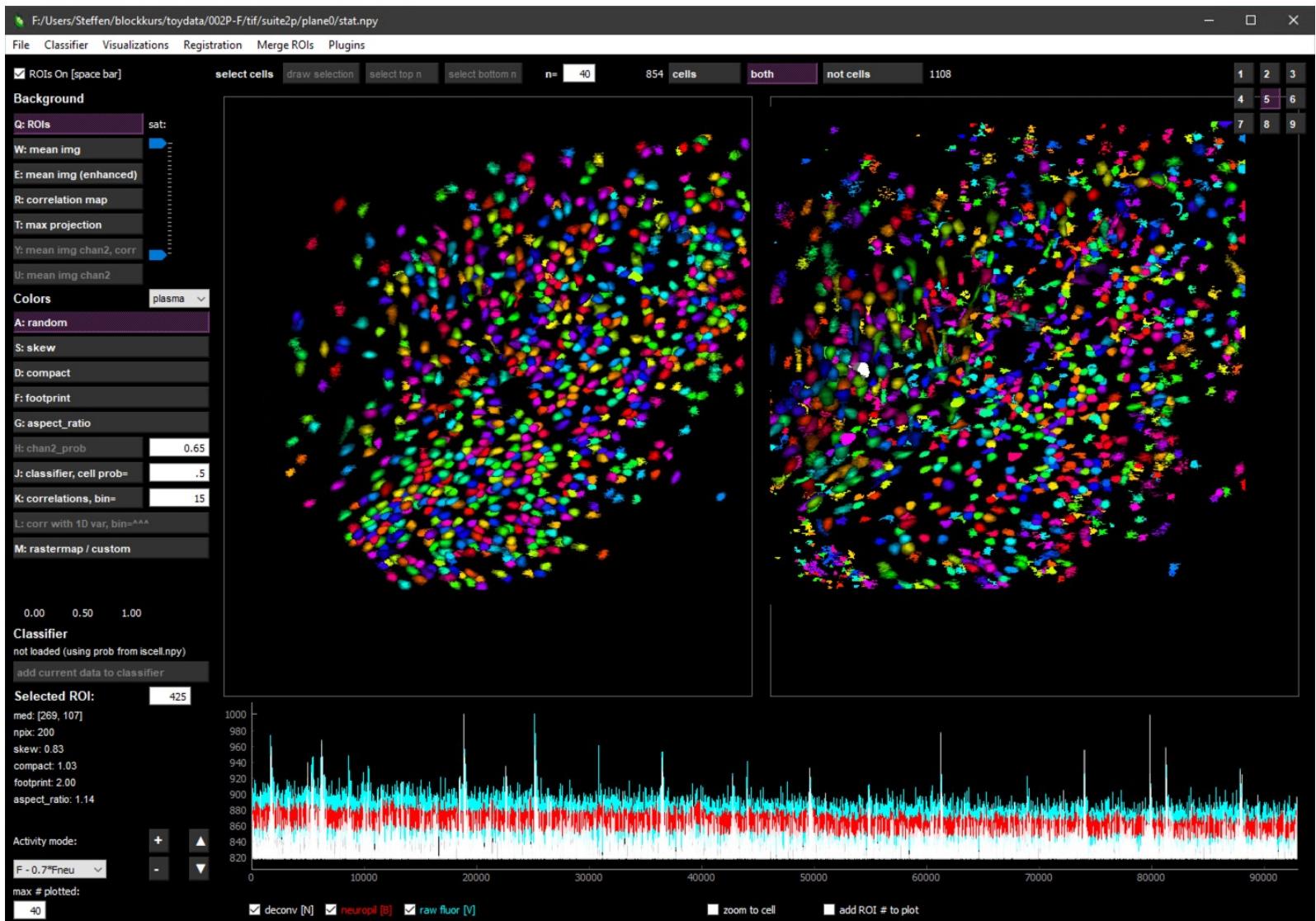


group-projected

group-projected

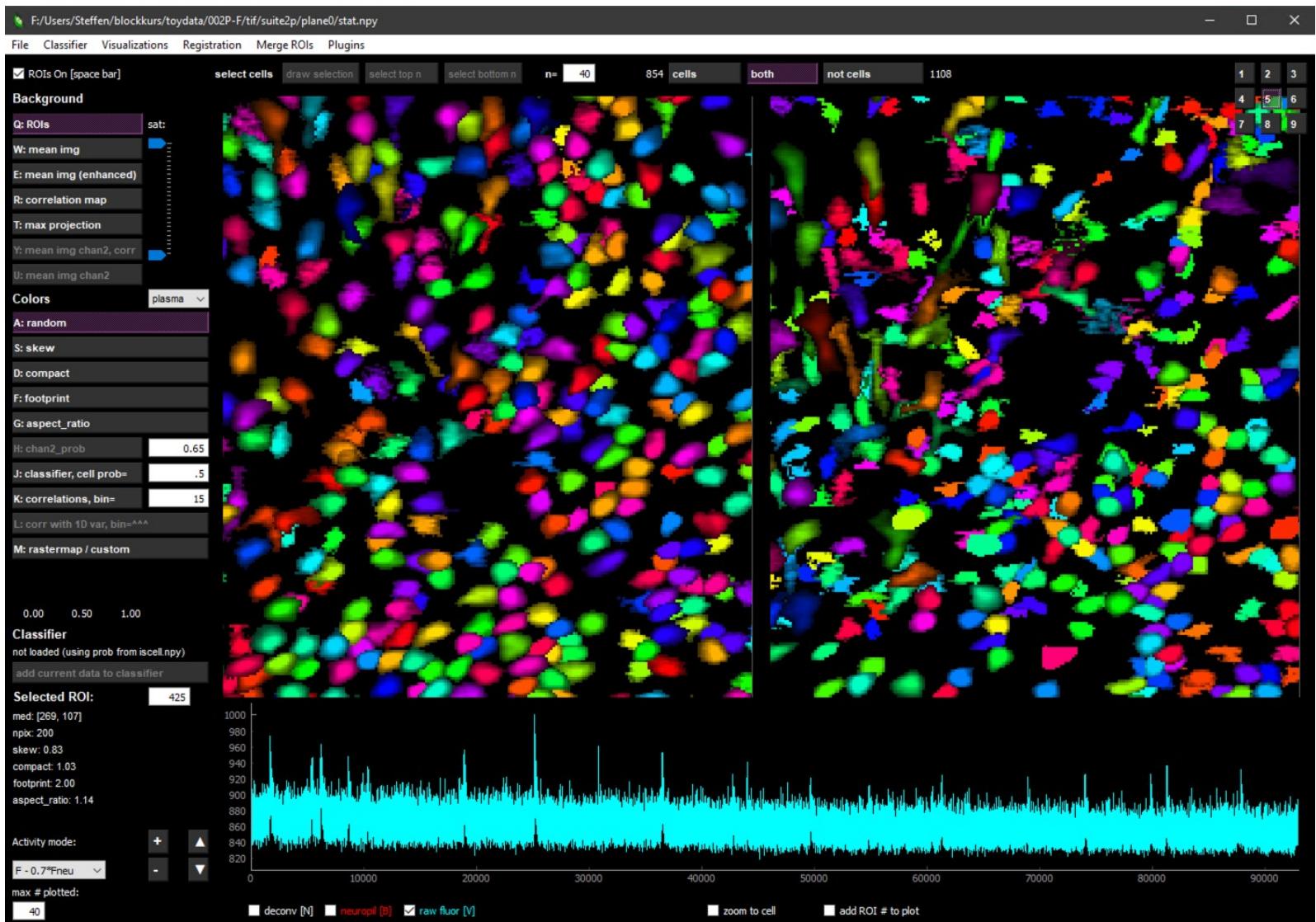


The GUI



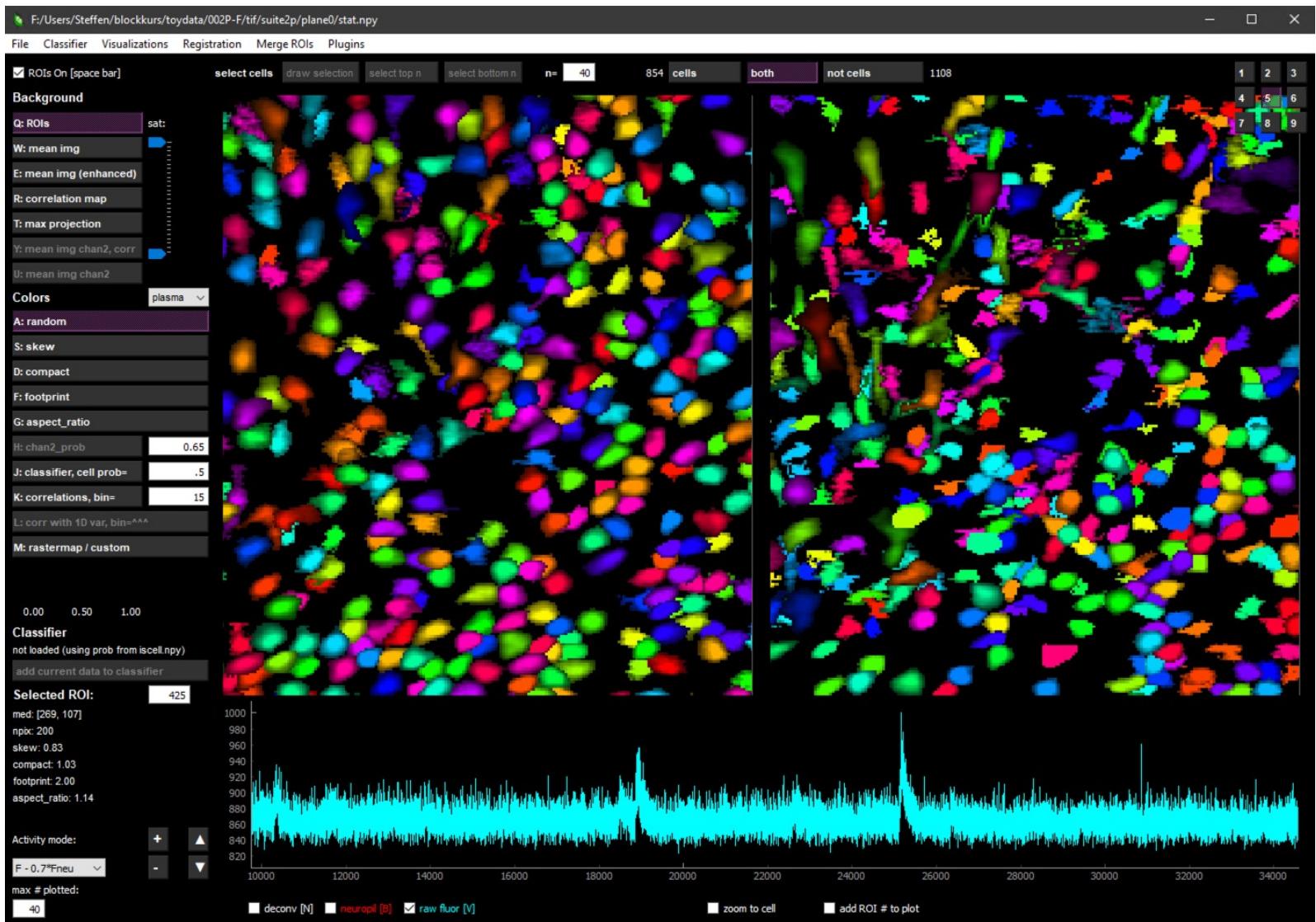


The GUI





The GUI



Suite2p



The GUI

F:/Users/Steffen/blockkurs/toydata/002P-F/tif/suite2p/plane0/stat.npy

File Classifier Visualizations Registration Merge ROIs Plugins

Choose run options (hold mouse over parameters to see descriptions)

File paths

input_format: tif
look_one_level_down: 0
Add directory to data_path

data_path

Add save_path (default is 1st data_path)
Add fast_disk (default is save_path)

Load ops file

Save ops as default
Revert default ops to built-in
Save ops to file

Load example ops

1P imaging
dendrites/axons

Main settings

nplanes: 1
preclassify: 0.0
nchannels: 1
functional_chan: 1
tau: combined
fs: 1.0
do_bidiphase: 0
bidiphase: 0
multiplane_parallel: 0
ignore_flyback: -1

Output settings

save_mat: 0
align_by_chan: 1
save_NWB: 300
combined: 1
reg.tif: 0
reg.tif_chan2: 0
aspect: 1.0
delete_bin: 0
move_bin: 0

Registration

do_registration: 1
nimg_init: 300
batch_size: 500
smooth_sigma: 1.15
smooth_sigma_time: 0
maxregshift: 0.1
th_badframes: 1.0
keep_movie_raw: 0
two_step_registration: 0

Nonrigid

nonrigid: 1
block_size: 128, 128
snr_thresh: 1.2
maxregshiftNR: 5
1P: 0
1Preg: 0
spatial_hp_reg: 42
maxOverlap: 0.75
pre_smooth: 20
spatial_taper: 40
high_pass: 100

ROI detection

roidetect: 1
denoise: 0
anatomical_only: 0
diameter: 0
min_neuropil_pixels: 350

Extraction/Neuropil

neuropil_extract: 1
allow_overlap: 0
inner_neuropil_radius: 2
Classification/Deconvolution

soma_crop: 1
spikedetect: 1
max_overlap: 0.75
max_iterations: 20
win_baseline: 60.0
sig_baseline: 10.0
neucoeff: 0.7

RUN SUITE2P STOP Add a clean-up *.py

save settings and add more (batch) remove last added

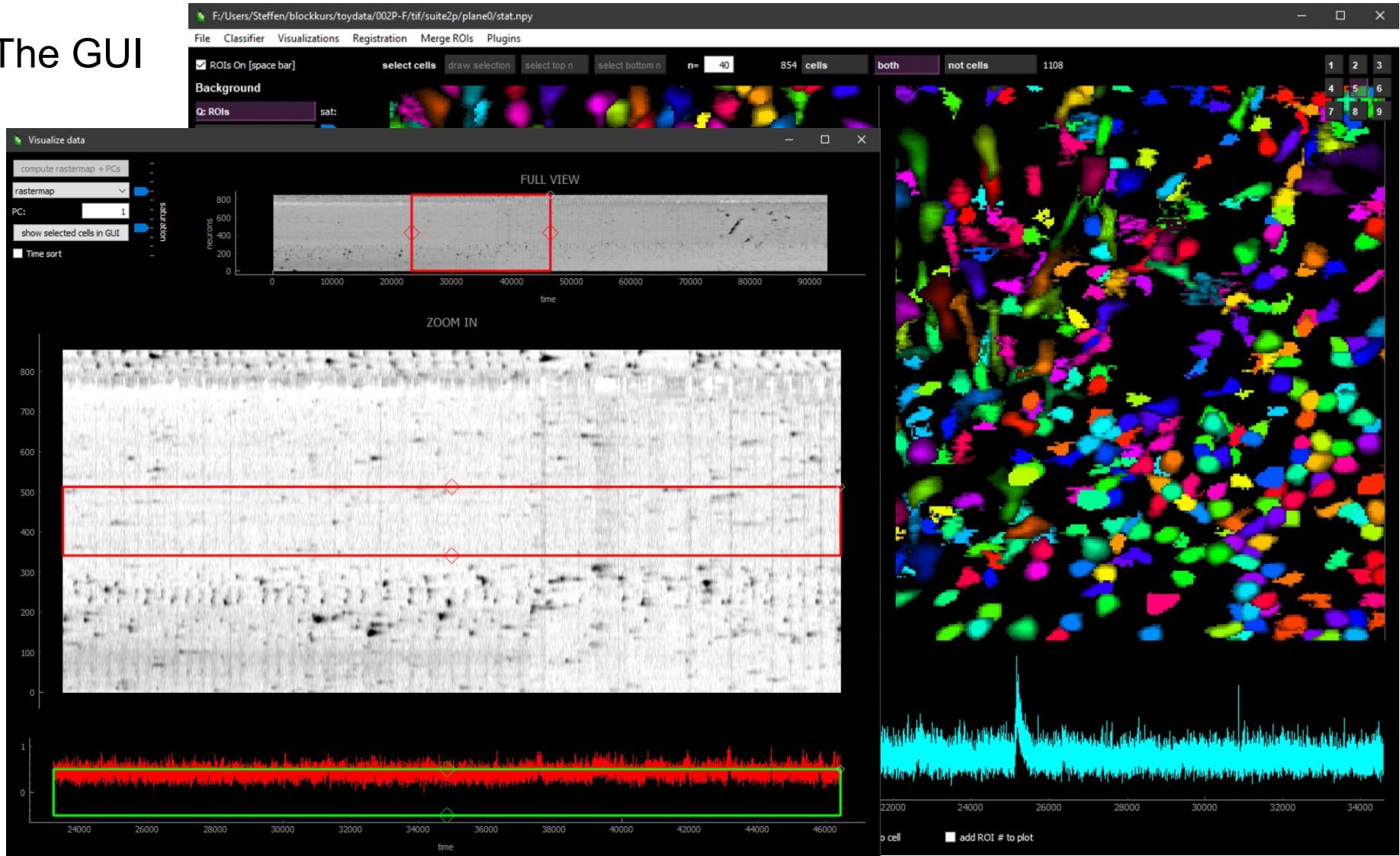
30000 32000 34000

<https://suite2p.readthedocs.io>, bioRxiv 2017

Suite2p



The GUI



Exercises

- We would like you to think about the data, the preprocessing steps, what it does to the data, ...
- Discuss among yourselves and with the tutors
- Document your thinking
- Try to get as far as you can with the assignments. You don't have to finish everything.

Good luck !!!