

Optical Physiology in NWB



NEURODATA
WITHOUT BORDERS

Optical Physiology in NWB

The community has made calcium imaging analysis tools that support reading and/or writing of NWB files:

- Suite2p (Pachitariu et al., bioRxiv)
- CalmAn (Giovannucci et al., 2019)
- CIAtah (Corder, Ahanonu, et al., 2019)
- EXTRACT (Inan et al., bioRxiv)

We do not have time to fully explain the details of how these packages work...

...so we will simply explain the input-output (I/O) workflow

Input: Storing 2p data in NWB

- The NWB data type for two-photon data is called `TwoPhotonSeries`
 - Recommended to store the imaging data internally instead of externally

```
[5]: nwbfile.acquisition["TwoPhotonSeries"]
```

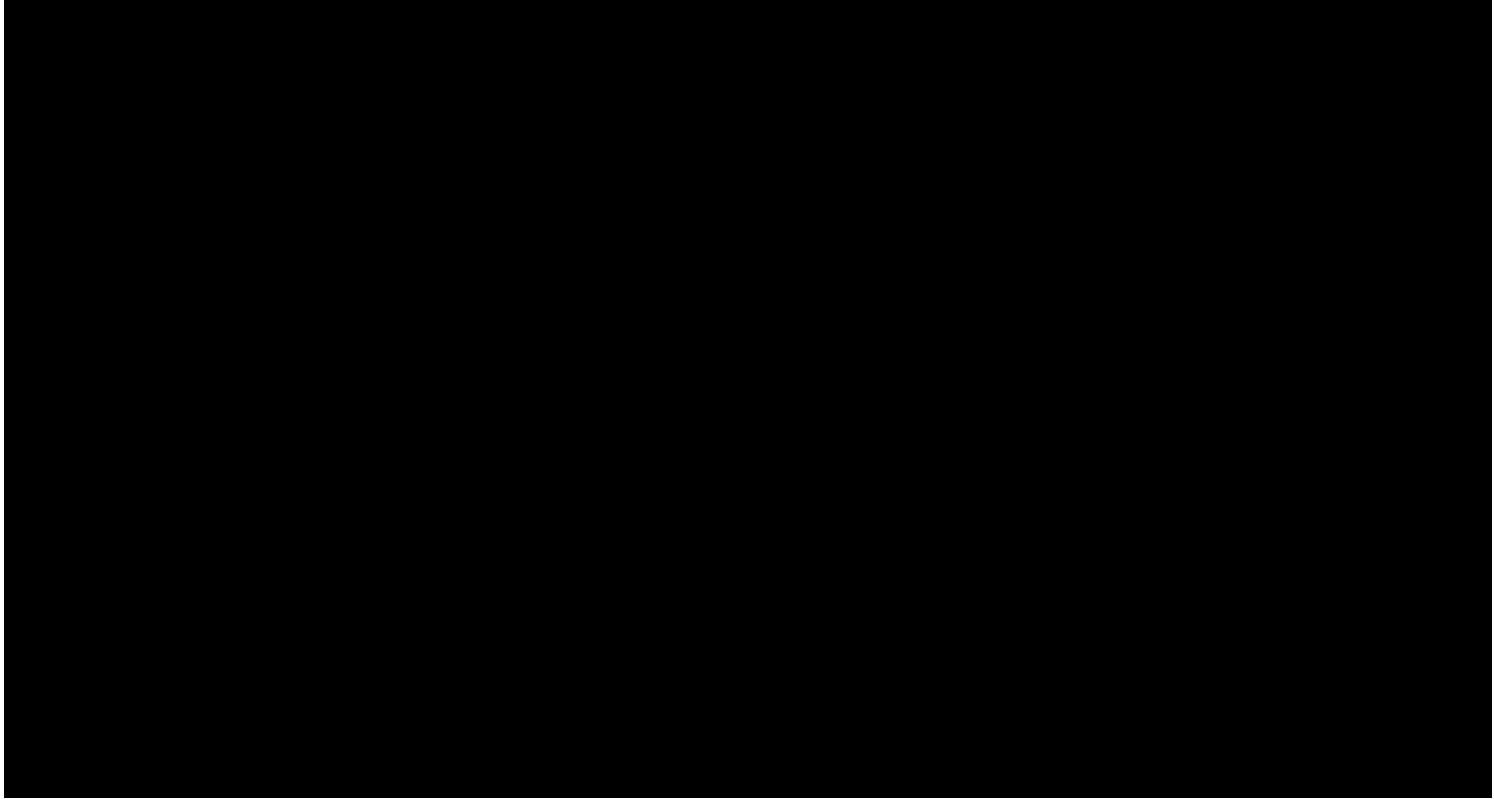
```
[5]: TwoPhotonSeries pynwb.ophys.TwoPhotonSeries at 0x4792728784
Fields:
  comments: Generalized from RoiInterface
  conversion: 1.0
  data: <HDF5 dataset "data": shape (38191, 796, 512), type "<u2">
  description: no description
  dimension: <HDF5 dataset "dimension": shape (2,), type "<i4">
  imaging_plane: ImagingPlane pynwb.ophys.ImagingPlane at 0x4792729120
```

Suite2p on NWB 2p imaging data

1. Install suite2p: <https://github.com/MouseLand/suite2p>
2. Select an NWB file. Explore NWB file using nwbwidgets to make sure that the file contains a `TwoPhotonSeries` with internally stored imaging data
3. Rename NWB file from `*.nwb` to `*.h5`
4. In suite2p, select File > Run suite2p
5. Select input format “h5”
6. Enter data path `“/acquisition/TwoPhotonSeries/data”`
7. Click “Add directory to data_path” and select path of the directory with the data
8. Change “save_NWB” output setting from 0 to 1
9. Click “RUN SUITE2P” button

Main settings	Output settings	Registration
nplanes <input type="text" value="1"/>	preclassify <input type="text" value="0.0"/>	do_registration <input type="text" value="1"/>
nchannels <input type="text" value="1"/>	save_mat <input type="text" value="0"/>	align_by_chan <input type="text" value="1"/>
functional_chan <input type="text" value="1"/>	save_NWB <input type="text" value="1"/>	nimg_init <input type="text" value="300"/>
tau <input type="text" value="1.0"/>	combined <input type="text" value="1"/>	batch_size <input type="text" value="500"/>
fs <input type="text" value="10.0"/>	reg_tif <input type="text" value="0"/>	smooth_sigma <input type="text" value="1.15"/>
do_bidiphase <input type="text" value="0"/>	reg_tif_chan2 <input type="text" value="0"/>	smooth_sigma_time <input type="text" value="0"/>
bidiphase <input type="text" value="0"/>	aspect <input type="text" value="1.0"/>	maxregshift <input type="text" value="0.1"/>
multiplane_parallel <input type="text" value="0"/>	delete_bin <input type="text" value="0"/>	th_badframes <input type="text" value="1.0"/>
ignore_flyback <input type="text" value="-1"/>	move_bin <input type="text" value="0"/>	keep_movie_raw <input type="text" value="0"/>
		two_step_registration <input type="text" value="0"/>

Suite2p: Load ROIs from NWB file



CalmAn on NWB 2p imaging data

1. Install CalmAn: <https://github.com/flatironinstitute/CalmAn>
2. Use NWB file and name of `TwoPhotonSeries` object (usually “TwoPhotonSeries”)

```
opts_dict = {'fnames': ['/Users/rly/example_movies/Sue_2x_3000_40_-46.nwb'],  
            'var_name_hdf5': 'TwoPhotonSeries',  
            # ...
```

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            # ...
```

3. Use CalmAn to do motion correction, CNMF fitting, and plot and review results
4. Save estimates back to NWB file

```
cnm2.estimated.save_NWB(  
    "/Users/rly/example_movies/Sue_2x_3000_40_-46.nwb",  
    imaging_rate=fr  
)
```

