session demonstration script

January 31, 2022

1 Example code for using session. Session

Note: This notebook covers several relevant methods of the Session and Stim objects, detailing some of their arguments, as well. For more details, take a look at the docstring associated with a method of interest.

Import notes:

- Any python packages required by the codebase should be installed and available, if the required conda environment, installed from osca.yml, has been activated.
- util is a Github repo of mine, and the correct branch osca_mult is automatically installed from osca.yml.
- Potential updates: Errors internal to the codebase involving util code and occurring after new changes have been pulled from the OpenScope_CA_Analysis repo may be due to an update of the osca_mult branch of util that breaks backwards compatibility. Though I will try to avoid this, check whether there are updates to the utility, if an error occurs, and consider updating your installation, e.g., by running, from the command line: pip install -U util-colleenjg

1.1 Plot formatting

If you wish to use the same formatting style as I do:

1.2 Set paths to main data directory and the mouse dataframe

1.2.1 Data directory

The data directory should contain the session data, either in its **original format** or in **NWB** format.

- * If in NWB format (production data, only), datadir should be a directory that contains the data in NWB format, at any depth.
- * If using the data in its original format, datadir should specifically be the directory right before the data is split into prod (production) and pilot (pilot) data.

1.2.2 Running on Binder

If the notebook is running on Binder, the dataset is downloaded in NWB format from the Dandi archive first, and the data directory is set accordingly.

Be sure to download the dataset, and update `datadir` and `datadir_nwb` to point to its location.

Currently they point to ../../data/OSCA and ../../data/OSCA_NWB, respectively.

1.2.3 Mouse dataframe

The mouse dataframe, contains the metadata for each session, including its 9-digit sessid, the mouse_n, sess_n, etc.

Mouse dataframe columns:

- * sessid: Unique session ID (9-digit)
- * dandi session id: Dandiset session ID for data in NWB format.
- * mouse_n: Mouse number
- * mouseid: Unique mouse ID (6-digit)
- * date: Recording date
- * depth: Recording depth (um)
- * plane: Recording plane ("dend" or "soma")
- * line: Cell line ("L2/3-Cux2" or "L5-Rbp4")
- * runtype: Type of session ("pilot" or "prod"). Only production data is available in NWB dataset.
- * sess_n: Session number
- * nrois: Number of valid ROIs (see *Note*)
- * nrois_tracked: Number of ROIs tracked across sessions (-1 for sessions with no tracking).
- * nrois_all: Same as nrois, but including bad (non valid) ROIs.
- * nrois_allen: Number of valid ROIs when using the allen segmentation for dendritic ROIs, instead of the extr segmentation (see *Note*).
- * nrois_allen_all: Same as nrois_allen, but including bad (non valid) ROIs.
- * pass_fail: Whether the session passed (P) or failed (F) quality control.
- * all_files: Whether all files are available for the session (original data format).
- * any files: Whether any files are available for the session (original data format).
- * incl: Whether the session can be included in analyses (looser criterion than pass fail).
- * stim seed: Seed used to initialize stimuli for the session, during recording.
- * notes: Any notes on the session.

Note: The allen segmentations are used for all **somatic** data. The extr segmentations are preferred for all **dendritic** data. For this reason, the allen segmentation for **dendritic** data is **not included** in the NWB dataset. See **section 6** for details on allen and extr ROI mask types.

| [6]: | | sessid | dandi_session_id | mouse_n | mouseid | date | depth | plane \ | |
|------|----|-----------|------------------|---------|---------|----------|-------|---------|--|
| | 0 | 712483302 | NaN | 1 | 389778 | 20180621 | 20 | dend | |
| | 1 | 712942208 | NaN | 1 | 389778 | 20180622 | 375 | soma | |
| | 2 | 714893802 | NaN | 1 | 389778 | 20180627 | 20 | dend | |
| | 3 | 715244457 | NaN | 1 | 389778 | 20180628 | 20 | dend | |
| | 4 | 716425232 | NaN | 1 | 389778 | 20180702 | 375 | soma | |
| | | ••• | ••• | | | | | | |
| | 78 | 833704570 | 20190307T163524 | 13 | 440889 | 20190307 | 175 | soma | |
| | 79 | 834403597 | 20190308T164555 | 13 | 440889 | 20190308 | 175 | soma | |
| | 80 | 836968429 | 20190314T152429 | 13 | 440889 | 20190314 | 175 | soma | |
| | 81 | 837360280 | 20190315T152224 | 13 | 440889 | 20190315 | 175 | soma | |
| | 82 | 838633305 | NaN | 13 | 440889 | 20190318 | 175 | soma | |

```
... nrois_tracked nrois_all nrois_allen
        line runtype
                      sess_n
0
     L5-Rbp4
                pilot
                             1
                                                -1
                                                          1468
                                                                         232
     L5-Rbp4
1
                pilot
                             2
                                                -1
                                                            78
                                                                          62
2
     L5-Rbp4
                             3
                                                            -1
                                                                          -1
                pilot
                                                -1
3
     L5-Rbp4
                                                -1
                                                           949
                                                                         458
                pilot
                             4
4
     L5-Rbp4
                                                -1
                                                            79
                                                                          56
                pilot
                             5
. .
                             2
                                                                         224
78 L23-Cux2
                                                           251
                 prod
                                               147
79
   L23-Cux2
                                               147
                                                           228
                                                                         210
                 prod
                             3
80 L23-Cux2
                 prod
                             4
                                                -1
                                                           217
                                                                         205
81 L23-Cux2
                             5
                                                -1
                                                           244
                                                                         217
                 prod
82
   L23-Cux2
                 prod
                             6
                                                -1
                                                           256
                                                                         227
    nrois_allen_all pass_fail all_files
                                             any_files
                                                          incl stim_seed \
0
                 259
                               F
                                                          yes
                                                                      103
                               F
1
                  78
                                          1
                                                      1
                                                                      103
                                                          yes
2
                  -1
                               F
                                          0
                                                      1
                                                           no
                                                                      103
3
                               Ρ
                 504
                                          1
                                                      1
                                                                      103
                                                          yes
4
                  79
                               Ρ
                                          1
                                                          yes
                                                                      103
                                                      1
                               Ρ
                                          1
78
                                                      1
                                                                   16745
                 251
                                                          yes
79
                 228
                               Ρ
                                          1
                                                      1
                                                                   10210
                                                          yes
                 217
                               Ρ
                                          1
                                                      1
80
                                                          yes
                                                                   24253
81
                 244
                               F
                                                      1
                                          1
                                                          yes
                                                                   19576
82
                 256
                                                            no
                                                                   30582
                                                    notes
0
    dropped beh and eye tracking frames (7), stim ...
1
    dropped beh and eye tracking frames (6), stim ...
2
                  missing 2P recordings and ROI traces
3
                                                      NaN
4
                                                      NaN
. .
78
    stim2twop alignment shifted corrected with 2nd...
79
    dropped beh and eye tracking frames (6), stim ...
    FOV shifted (poor alignment with previous sess...
80
81
                                         z-drift (14 um)
82
                         laser wavelength set to 800 um
```

[83 rows x 21 columns]

1.3 1. Basics of initializing a Session object

Sessions can be intialized with their 9-digit sessid: or with their mouse_n, sess_n and runtype:

1.3.1 Data format is identified automatically

During initialization, the code looks first for the session data in NWB format, under its dandi_session_id. If it doesn't find it, it looks for the data in its original format. If neither are found, an error is thrown.

1.3.2 Loading the data after initialization.

After creating the session, you must run self.extract_info(). This wasn't amalgamated into the __init__ to reduce the amount of information needed to just create a session object.

1.3.3 Loading ROI/running/pupil info

You can load this information when you call self.extract_info() or manually later by calling self.load_roi_info(), self.load_run_data() and self.load_pup_data().

Loading stimulus and alignment info…

Loading ROI trace info...

Loading running info...

Loading pupil info...

1.3.4 Stimulus dataframe

The stimulus dataframe, stored under sess.stim_df, details the stimulus feature for each segment of the presentation.

A **segment** is the minimal subdivision of the stimulus presentation: **0.3 sec** for the Gabor stimulus, and **1s** for the visual flow, and grayscreen stimuli.

If a feature **does not apply** to certain segments (e.g., gabor_number for visual flow stimulus segments), the values for those segments will be None, NaN or [], depending on the column's datatype.

Missing columns: Note that a few columns are missing, since the session was loaded with full_table=False. * "gabor_orientations": Specific orientation of each Gabor patch, for each segment. * "square_locations_x": Specific x location of each visual flow square, at each frame of each segment. * "square_locations_y": Specific y location of each visual flow square, at each frame of each segment.

This is primarily to save memory, when loading a session, as this information is not typically needed. To load all columns, re-run sess.extract_info() with full_table=True. Data that is already loaded will not be re-loaded.

| [10]: | stimulus_type | stimulus_template_name | unexpected | <pre>gabor_frame</pre> | \ |
|-------|---------------|------------------------|------------|------------------------|---|
| 0 | grayscreen | grayscreen | NaN | | |
| 1 | gabors | gabors | 0.0 | A | |
| 2 | gabors | gabors | 0.0 | В | |
| 3 | gabors | gabors | 0.0 | C | |
| 4 | gabors | gabors | 0.0 | D | |
| ••• | ••• | ••• | ••• | ••• | |
| 8839 | visflow | visflow_right | 0.0 | | |
| 8840 | visflow | visflow_right | 0.0 | | |

```
8841
           visflow
                             visflow_right
                                                     1.0
8842
                                                     1.0
           visflow
                             visflow_right
8843
        grayscreen
                                grayscreen
                                                     NaN
                   gabor_mean_orientation
                                             gabor_number
      gabor_kappa
0
              NaN
                                        NaN
                                                       NaN
1
             16.0
                                        0.0
                                                      30.0
2
             16.0
                                        0.0
                                                      30.0
3
                                                      30.0
              16.0
                                        0.0
4
              16.0
                                        0.0
                                                      30.0
8839
              NaN
                                        NaN
                                                       NaN
8840
              NaN
                                        NaN
                                                       NaN
8841
              NaN
                                        NaN
                                                       NaN
8842
              NaN
                                        NaN
                                                       NaN
8843
              NaN
                                        NaN
                                                       NaN
                                        gabor_locations_x
0
                                                        [-314.2481536790383, 726.6351926350328, -609.4...
1
2
      [278.93714376420894, -895.0169462360316, 830.4...
3
      [-694.2565883378384, 458.8415680953749, -472.6...
4
      [-631.2261180219028, -600.2310528361336, -887...
8839
                                                        8840
                                                        8841
8842
                                                        8843
                                                        gabor_locations_y
0
      [519.3985635606798, 429.54112277826425, 482.75...
1
2
      [-62.92603512701612, -329.96944361291634, -332...
3
      [162.5089263895926, 433.50619201931613, 567.71...
4
      [-21.003509639097615, -271.4924294875755, 555...
8839
                                                        []
8840
                                                        8841
                                                        8842
                                                        8843
                                              gabor_sizes ...
0
                                                        [237, 382, 341, 269, 332, 300, 256, 322, 252, ... ...
1
2
      [355, 245, 207, 246, 209, 371, 209, 400, 214, ... ...
```

```
3
      [270, 274, 369, 230, 364, 205, 360, 315, 396, ... ...
4
      [228, 332, 237, 248, 346, 308, 333, 277, 232, ... ...
                                                          8839
8840
                                                          []
                                                          []
8841
8842
                                                          []
8843
     square_proportion_flipped start_frame_stim
                                                     stop_frame_stim \
0
                                                                 1800
                             NaN
1
                             NaN
                                               1800
                                                                 1818
2
                             NaN
                                               1818
                                                                 1836
3
                             NaN
                                               1836
                                                                 1854
4
                             NaN
                                               1854
                                                                 1872
8839
                            0.00
                                             249960
                                                               250020
8840
                            0.00
                                             250020
                                                               250080
8841
                            0.25
                                             250080
                                                               250140
8842
                            0.25
                                             250140
                                                               250200
8843
                             NaN
                                             250200
                                                               251999
      num_frames_stim
                         start_frame_twop
                                             stop_frame_twop
                                                               num_frames_twop
                  1800
                                                                            903
0
                                       143
                                                         1046
1
                    18
                                                                               9
                                      1046
                                                         1055
2
                    18
                                      1055
                                                         1064
                                                                               9
                                                                               9
3
                    18
                                      1064
                                                         1073
4
                                                         1082
                                                                               9
                    18
                                      1073
                                                                              30
8839
                                    125551
                                                      125581
                    60
                                                                              30
8840
                    60
                                    125581
                                                      125611
                                                                             30
8841
                    60
                                                       125641
                                    125611
8842
                                                                             31
                    60
                                    125641
                                                       125672
8843
                  1799
                                    125672
                                                       126575
                                                                            903
      start_time_sec
                       stop_time_sec
                                        duration_sec
0
             14.30646
                            44.332150
                                           30.025690
1
             44.33215
                            44.639380
                                             0.307230
2
             44.63938
                            44.939040
                                             0.299660
3
             44.93904
                            45.232430
                                             0.293390
4
             45.23243
                            45.526750
                                             0.294320
8839
           4183.68954
                          4184.690500
                                             1.000960
8840
           4184.69050
                          4185.691070
                                             1.000570
8841
           4185.69107
                          4186.692190
                                             1.001120
8842
           4186.69219
                          4187.690570
                                            0.998380
8843
           4187.69057
                          4217.673903
                                           29.983333
```

[8844 rows x 24 columns]

1.3.5 Stimulus objects

Once sess.extract_info(), each Session object now contains Stim objects.

These come in one of three subclasses: Gabors, Visflow, Grayscr, and can be accessed with: sess.stims, sess.gabors, sess.visflow, sess.grayscr.

The the Stim object stim, the Session object can be accessed with stim.sess.

number of rois : 90 mouse number : 4 mouse ID : 411771

gabor object : Gabors (stimulus from session 760260459)

2p frames per sec : 30.08 stimulus frames per sec: 59.95

1.4 2. Retrieving data of interest

1.4.1 Identifying stimulus segments of interest

From a Session's Stim, you can get a list of segments that fit a specific criterion, e.g. U segments (unexpected, 3rd Gabor frame).

1.4.2 Identifying frame numbers of interest, to index the data

Then, you can retrieve the exact frame numbers that match these segments.

Specifically, you can access: * twop frame numbers, which index the two-photon data and pupil data, and * stim frame numbers, which index the running data.

Note: When retrieving the frame numbers, specifying ch_fl (check flanks) ensures that only frame numbers whose flanks are within the recording are returned. In other words, any frame number too close to the start or end of the recording (based on pre/post values), will be dropped.

1.4.3 Retrieving the data of interest

You can now get the **ROI** / **running** / **pupil** data corresponding to these reference frames and the specified pre / post periods (in sec).

1.4.4 Retrieving data statistics of interest

You can also directly obtain statistics on the data of interest.

| [15]: | datatype | roi_traces |
|-------|------------------|------------|
| | bad_rois_removed | yes |
| | scaled | no |
| | baseline | no |
| | integrated | yes |
| | smoothing | no |

```
fluorescence dff
general ROIs sequences
stats None stat_mean 0.062516
error_SEM 0.017370
```

1.4.5 Using hierarchical dataframes

Data and statistics are returned in a hierarchical dataframe with **columns** and **indices**.

This has the advantage of allowing metadata to be stored in dummy columns, however extracting data from these dataframes can be tricky, syntactically.

| [16]: | data | type | | roi_traces |
|-------|-------|-------------|-------------|------------|
| | bad_1 | rois_remove | yes | |
| | scale | ed | | yes |
| | base | line | | no |
| | integ | grated | | no |
| | smoot | thing | | no |
| | fluo | rescence | dff | |
| | ROIs | sequences | time_values | |
| | 0 | 0 | -1.000000 | -0.338172 |
| | | | -0.966102 | 0.155122 |
| | | | -0.932203 | 0.150821 |
| | | | -0.898305 | 0.053135 |
| | | | -0.864407 | -0.100729 |
| | ••• | | | ••• |
| | 102 | 81 | 0.864407 | 0.297796 |
| | | | 0.898305 | -0.031714 |
| | | | 0.932203 | 0.339743 |
| | | | 0.966102 | 0.524661 |
| | | | 1.000000 | -0.289968 |
| | | | | |

[442800 rows x 1 columns]

To extract a numpy array with the correct dimensions from a hierarchical dataframe, you can use the following utility function: gen_util.reshape_df_data().

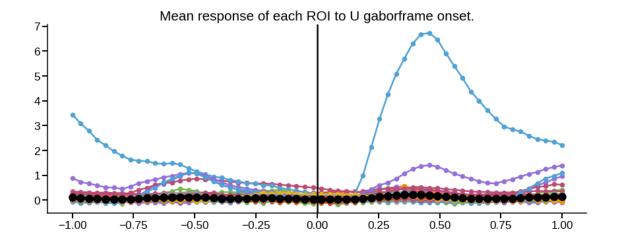
Here, each index level, then column level is turned into a new axis, i.e. $ROIs\ x\ sequences\ x\ time_values$ (In this case, squeeze_cols is set to True to prevent each dummy column from becoming its own axis.)

ROI data shape: 90 ROIs x 82 sequences x 60 time values

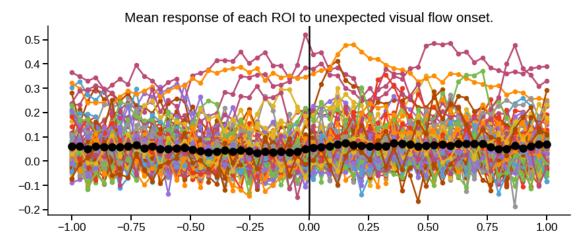
You can also retrieve the time stamps for each frame.

1.4.6 Visualizing the data

Finally, we can plot each ROIs mean activity across sequences, as well as a mean across ROIs.



1.4.7 The same steps apply for Visflow



1.5 3. Tracked ROIs

ROI tracking was performed on the production data.

At any point, it is possible to **restrict the data returned** to only the tracked ROIs, called sess.set_only_tracked_rois(True).

Here, we retrieve the data, integrated over each sequence.

The dataframe returned contains data only for tracked ROIs.

| [23]: | datatype | roi_traces |
|-------|-----------------------------|------------|
| | <pre>bad_rois_removed</pre> | yes |
| | scaled | yes |
| | baseline | no |

| integ | grated | yes | | |
|--------------|-----------|----------------------|--|--|
| smoot | thing | no | | |
| fluoi | rescence | dff | | |
| ${\tt ROIs}$ | sequences | | | |
| 28 | 0 | 0.101591 | | |
| | 1 | 0.188843 | | |
| | 2 | -0.072082 | | |
| | 3 | 0.685275 0.033439 | | |
| | 4 | | | |
| ••• | | ••• | | |
| 22 | 27 | 0.026887 | | |
| | 28 | 0.523182 | | |
| | 29 | -0.039192 | | |
| 30 | | -0.059955 | | |
| | 31 | 0.080472 | | |

[1504 rows x 1 columns]

1.5.1 Extracting tracked ROI data correctly (!)

Importantly, the ROIs are now sorted in their tracking order, which ensures that they are correctly aligned across sessions.

As a result, the "ROIs" index may no longer be in increasing order, like in this example.

```
ROI numbers, ordered for tracking: 28, 69, 90, 2, 55, 7, 5, 73, 101, 16, 93, 85, 84, 17, 15, 18, 29, 26, 13, 48, 1, 74, 25, 87, 72, 88, 89, 76, 0, 78, 67, 57, 62, 50, 42, 41, 43, 14, 32, 6, 45, 46, 98, 44, 27, 34, 22
```

To ensure that the tracked ROI order is preserved when extracting the data, the safest option is to use the utility function introduced above, i.e. gen_util.reshape_data_df(). It will ensure that the order is preserved.

```
Tracked ROI data shape using the correct method, i.e., gen_util.reshape_df_data()
47 ROIs x 32 sequences
```

Do not use the .unstack() method for hierarchical dataframes!

Even though the .unstack() method is typically a convenient way to extract a 2D array from a hierarchical dataframe, it will cause major problems here. Specifically, .unstack() internally triggers a resorting of the hierarchical indices. Thus, using it will completely mess up the tracked ROI order.

```
Tracked ROI data shape using the wrong method, i.e., .unstack() 47\ \text{ROIs}\ \text{x}\ 32\ \text{sequences}
```

As you can see, the dimensions are still correct. However, the ROI sorting is actually lost!

For example, **ROI** #5, which should appear at index 6 in the array, is now at index 3.

```
Data for the tracked ROI at index 6, when using the correct method: i.e., gen_util.reshape_df_data() 0.005, 0.024, -0.050, -0.553, 0.629, -0.051, -0.057, 0.071, -0.003, -0.067 ... Data for the tracked ROI at index 6, when using the wrong method: i.e., .unstack() 0.053, 0.191, 0.232, -0.004, -0.008, 0.060, 0.083, -0.031, 0.015, 0.024 ... Data for the tracked ROI that should be at index 6 is instead at index 3, when using the wrong method: i.e., .unstack() 0.005, 0.024, -0.050, -0.553, 0.629, -0.051, -0.057, 0.071, -0.003, -0.067 ...
```

1.5.2 Reset the session to start using all ROIs, again

1.6 4. Additional tips on indexing a hierarchical dataframe

| [31]: | scale | ed | | yes |
|-------|--------------|-----------|-------------|-----------|
| | base | line | no | |
| | integ | grated | no | |
| | smoot | thing | no | |
| | fluorescence | | | dff |
| | ROIs | sequences | time_values | |
| | 0 | 1 | -1.0 | -0.183646 |
| | | 20 | -1.0 | 0.013693 |
| | | 21 | -1.0 | -0.091127 |
| | 3 | 1 | -1.0 | 0.221201 |
| | | 20 | -1.0 | 0.347209 |
| | | 21 | -1.0 | -0.163844 |
| | 5 | 1 | -1.0 | -0.243460 |
| | | 20 | -1.0 | -0.422120 |
| | | 21 | -1.0 | 0.203985 |

1.7 5. Retrieving several Session objects, based on criteria

1.7.1 Identifying mice or session IDs to omit (pilot data only)

sess_gen_util.all_omit() allows keeping track of which session IDs or mice must be left out.

This actually **only applies to pilot data**, where some mice did not see all the stimuli of interst, and one session has incomplete data.

For the prod data, the lists are empy.

1.7.2 Retrieving mouse / session numbers and IDs that fit specific criteria

sess_gen_util.get_sess_vals() can be used to retrieve information for sessions that meet certain criteria.

e.g., session number 1, 2 or 3, production, dendritic plane

mouse 1: 758519303 (session 1) mouse 1: 759189643 (session 2)

```
mouse 1: 759660390 (session 3)
mouse 3: 761624763 (session 1)
mouse 3: 761944562 (session 2)
mouse 3: 762250376 (session 3)
mouse 4: 760260459 (session 1)
mouse 4: 760659782 (session 2)
mouse 4: 761269197 (session 3)
mouse 7: 777496949 (session 1)
mouse 7: 778374308 (session 2)
mouse 7: 779152062 (session 3)
mouse 12: 826659257 (session 1)
mouse 12: 827300090 (session 2)
mouse 12: 828475005 (session 3)
mouse 13: 832883243 (session 1)
mouse 13: 833704570 (session 2)
mouse 13: 834403597 (session 3)
```

1.7.3 Loading the sessions

sess_gen_util.init_sessions() can be used to initialize the sessions and extract the requested data.

Creating session 758519303...

Loading stimulus and alignment info...

Loading ROI trace info...

Loading running info...

Finished creating session 758519303.

Creating session 759189643...
Loading stimulus and alignment info...
Loading ROI trace info...
Loading running info...
Finished creating session 759189643.

Creating session 759660390...
Loading stimulus and alignment info...
Loading ROI trace info...
Loading running info...
Finished creating session 759660390.

Creating session 764704289...
Loading stimulus and alignment info...
Loading ROI trace info...
Loading running info...
Finished creating session 764704289.

1.7.4 Using the loaded sessions

Now, one can run through the sessions, and run whatever analysis is needed.

Note here that, when calling stim.get_segs_by_criteria(), features that do not apply to the stimulus (e.g., gabfr for the visflow stimulus) are simply ignored.

```
Session ID: 758519303 (mouse 1, session 1)
   visflow: 31 sequences
   gabors: 94 sequences
Session ID: 759189643 (mouse 1, session 2)
   visflow: 34 sequences
   gabors: 90 sequences
Session ID: 759660390 (mouse 1, session 3)
   visflow: 33 sequences
   gabors: 105 sequences
Session ID: 764704289 (mouse 6, session 1)
   visflow: 33 sequences
   gabors: 96 sequences
```

1.8 6. Retrieving ROI masks from a session

Boolean ROI masks can be obtained for each Session.

1.8.1 Dendritic mask types

For dendritic sessions, the Session is built to assume that extr (not allen) ROI data is to be used. This can be checked by checking self.dend. As long as self.dend is properly set, the correct ROI data and masks will be loaded.

The allen masks were extracted with a pipeline tailored to somatic ROIs, and are therefore not preferred for dendritic data.

In contrast, the extr masks were extracted with the EXTRACT pipeline, which specifically enables dendrite-shaped ROIs to be identified.

Note that, for this reason, *only the extr dendritic ROIs and masks* are included in the data in NWB formatted data.

```
Dendritic session, ROI type: extr
Somatic session, ROI type: allen
```

1.8.2 Loading masks

Masks can be loaded as follows, with dimensions: **ROI** \mathbf{x} **height** \mathbf{x} **width**, retrieving only masks for ROIs that are valid (when evaluated by their dF/F traces).

Notes: - If sessions are set to use only tracked ROIs, as described above, only masks for the tracked ROIs (sorted in the tracking order) will be returned. - If running this notebook on **Binder**, the dendritic masks are not loaded, as the memory requirements are too high (~2-3GB).

In most functions, by default, ROIs that are considered **bad (non valid)** are automatically removed (rem_bad=True).

Note that, for the NWB data, the bad ROIs were removed altogether.

These ROIs either:

- (1) contain NaN/Infs values or
- (2) have been deemed too noisy.

If, for whatever reason, all masks are needed, including those for the bad ROIs,

- (1) ensure that the session is currently set to return all ROI data, with sess.only_tracked_rois(False), then
- (2) call self.get_roi_masks(rem_bad=False).

Of course, as explained above, if using the NWB data, there are no bad ROIs.

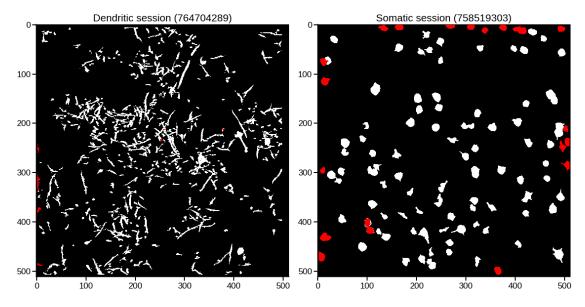
1.8.3 Bad ROIs

When using the data in its **original format**, one can get a list of bad ROIs, by using self.get_bad_rois().

If the data is in **NWB format**, there should be **no bad ROIs**, as they have already been removed.

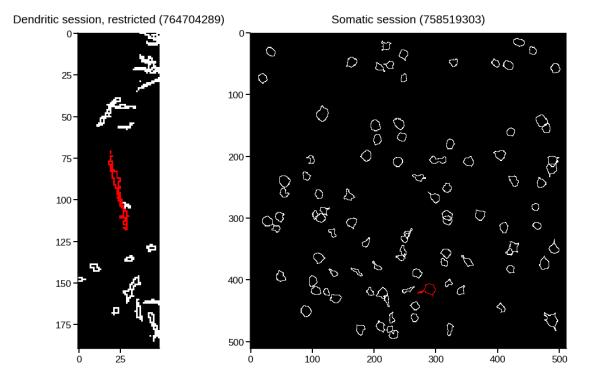
1.8.4 Visualizing ROI masks

sess_plot_util.plot_ROIs() can be used to visualize ROIs, where specific ROIs can be set to red using a valid_mask.



1.8.5 Visualizing ROI mask contours

sess_plot_util.plot_ROI_contours() can be used to visualize ROI contours, optionally restricted to around an ROI of interest.



1.9 7. Visualizing stimulus templates (NWB data only)

If using the NWB versions of the data, one should note that different NWB versions are available for each session, on the Dandi archive.

The basic versions are the smallest ones (~130 MB to 1.7 GB each), and contain all the data needed for most analyses. In contrast, the versions with +image in the name also contain the stimulus templates, i.e. all unique stimulus frame images. They are typically ~1.5 GB larger than the corresponding basic versions.

We can load an example session: mouse 1, session 1, downloaded from the Dandi archive: sub-408021/sub-408021_ses-20180926T172917_behavior+image+ophys.nwb.

Be sure to download the file, and place it in the `datadir_nwb` directory: ../../data/OSCA_NWB.

/home/colleen/Documents/OpenScope_CA_Analysis/analysis/session.py:287: UserWarning:

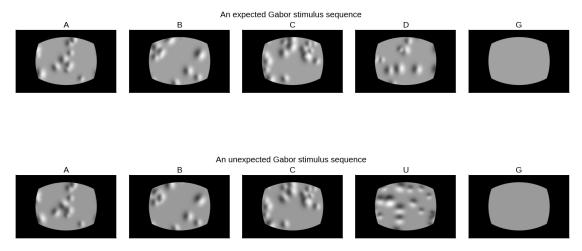
Several NWB files were found for this session. When loading data, the first file listed that contains the required data will be used.

Loading stimulus and alignment info...

As the warning indicates, the Session object has found both the basic version of the data for this session, and the version that also contains the stimulus template (+image) in the specified data directory. At any step where data must be loaded, the Session object will load it from the first listed version (alphabetically) that contains the required data.

1.9.1 Gabor sequence images

We can now identify the frame numbers for the **first Gabor sequence**, and **visualize** the corresponding stimulus images.



As we can see, whereas the Gabor patch orientations are consistent across frames in the expected sequence, they are rotated by 90° in the U frame of the unexpected sequence.

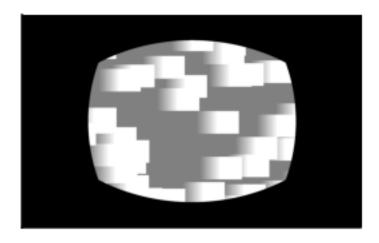
1.9.2 Warping

Note that the periphery of the images is masked in black. This is because, during the actual stimulus presentation, the images were presented on a **flat screen**, **and spherically warped**. This ensured that the apparent properties of the stimuli (size, speed, spatial frequency, etc.) were constant across the monitor, as seen from the mice's perspectives. The black masks overlayed on the unwarped stimuli stored in the NWB file, therefore, **mask the parts of the stimuli that were** outside the edges of the screen, due to warping, and thus **not visible** to the mice during the experiments.

1.9.3 Visual flow sequence images

We can also visualize the **visual flow stimulus**. It is important to note that, whereas the Gabor images are static for each segment, the visual flow stimulus is in motion, and therefore changes at each frame. For this reason, we will simply identify the first visual flow segment in a sequence, and visualize the first few frames in that follow it.

An expected visual flow sequence



An unexpected visual flow sequence

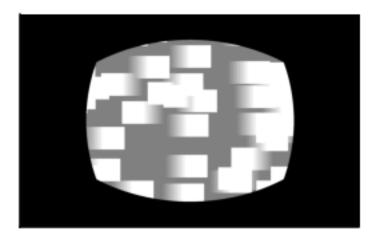












We plot the first few frames in each sequence separately. We then also plot all the frames retained for each sequence, **overlayed in a graded way**, in order to visualize the squares **in motion**.

As we can see, in the **expected** sequence, all of the squares are moving rightward, uniformly. In contrast, in the **unexpected** sequence, although most squares are still moving rightward, $\sim 25\%$ of them are moving in the opposite direction, i.e., leftward.

1.9.4 Stimulus generating code and examples

This repository contains the code to generate these stimuli, as well as some example videos.

1.10 8. Last notes

There is much more to the codebase, and even to the Session and Stim objects, and almost all functions and methods are thoroughly documented.

When looking to implement a new analysis, consider checking to see whether relevant functions have already been implemented in:

```
* analysis/session.py
* analysis/basic_analys.py
* sess_util/sess_gen_util.py
```

1.10.1 Methods/properties attached to Session and Stim objects.

```
Loading stimulus and alignment info...
Loading ROI trace info...
Loading running info...
Loading pupil info...
Session (758519303)
 Public properties:
    self.all_files
    self.any_files
    self.dandi_id
    self.date
    self.dend
    self.depth
    self.drop_tol
    self.gabors
    self.grayscr
    self.home
    self.line
    self.max_proj
    self.mouse df
    self.mouse n
    self.mouseid
    self.n_stims
    self.notes
    self.nwb
    self.only_tracked_rois
    self.pass_fail
    self.plane
    self.pup_data
    self.pup_data_available
    self.roi_facts_df
    self.roi_masks
```

```
self.roi_names
  self.run_data
  self.runtype
  self.sess_files
  self.sess n
  self.sessid
  self.stim2twopfr
  self.stim_df
  self.stim_fps
  self.stim_seed
  self.stims
  self.stimtypes
  self.tot_stim_fr
  self.tot_twop_fr
  self.tracked_rois
  self.twop2stimfr
  self.twop_fps
  self.visflow
Public methods:
  self.check flanks()
  self.convert frames()
  self.data_loaded()
  self.extract_info()
  self.get_active_rois()
  self.get_bad_rois()
  self.get_fr_ran()
  self.get_frames_timestamps()
  self.get_local_nway_match_path()
  self.get_nrois()
  self.get_plateau_roi_traces()
  self.get_pup_data()
  self.get_registered_max_proj()
  self.get_registered_roi_masks()
  self.get roi masks()
  self.get_roi_seqs()
  self.get_roi_trace_path()
  self.get_roi_traces()
  self.get_roi_traces_by_ns()
  self.get_run_velocity()
  self.get_run_velocity_by_fr()
  self.get_single_roi_trace()
  self.get_stim()
  self.load_pup_data()
  self.load_roi_info()
  self.load_run_data()
  self.set_only_tracked_rois()
```

Gabors (stimulus from session 758519303)

```
Public properties:
  self.all_gabfr
  self.all gabfr mean oris
  self.block_params
  self.deg_per_pix
  self.exp_gabfr
  self.exp_gabfr_mean_oris
  self.exp_max_s
  self.exp_min_s
  self.kappas
  self.n_patches
  self.n_segs_per_seq
  self.ori_ran
  self.phase
  self.seg_len_s
  self.sess
  self.sf
  self.size ran
  self.stim_fps
  self.stim params
  self.stimtype
  self.unexp_gabfr
  self.unexp_gabfr_mean_oris
  self.unexp_max_s
  self.unexp_min_s
  self.win_size
Public methods:
  self.get_A_frame_1s()
  self.get_A_segs()
  self.get_all_unexp_segs()
  self.get_all_unexp_stim_fr()
  self.get fr by seg()
  self.get_frames_by_criteria()
  self.get_n_fr_by_seg()
  self.get_pup_diam_data()
  self.get_pup_diam_stats_df()
  self.get_roi_data()
  self.get_roi_stats_df()
  self.get_run()
  self.get_run_data()
  self.get_run_stats_df()
  self.get_segs_by_criteria()
  self.get_segs_by_frame()
  self.get_start_unexp_segs()
  self.get_start_unexp_stim_fr_trans()
```

```
self.get_stats_df()
    self.get_stim_beh_sub_df()
    self.get_stim_df_by_criteria()
    self.get_stim_images_by_frame()
    self.get stim par by frame()
    self.get_stim_par_by_seg()
Visflow (stimulus from session 758519303)
 Public properties:
    self.block_params
    self.deg_per_pix
    self.exp_max_s
    self.exp_min_s
    self.main_flow_direcs
    self.n_squares
    self.prop_flipped
    self.seg_len_s
    self.sess
    self.speed
    self.square_sizes
    self.stim_fps
    self.stim params
    self.stimtype
    self.unexp max s
    self.unexp_min_s
    self.win size
  Public methods:
    self.get_all_unexp_segs()
    self.get_all_unexp_stim_fr()
    self.get_dir_segs_exp()
    self.get_fr_by_seg()
    self.get_frames_by_criteria()
    self.get_n_fr_by_seg()
    self.get_pup_diam_data()
    self.get_pup_diam_stats_df()
    self.get_roi_data()
    self.get_roi_stats_df()
    self.get run()
    self.get_run_data()
    self.get_run_stats_df()
    self.get_segs_by_criteria()
    self.get_segs_by_frame()
    self.get_start_unexp_segs()
    self.get_start_unexp_stim_fr_trans()
    self.get_stats_df()
    self.get_stim_beh_sub_df()
```

```
self.get_stim_df_by_criteria()
    self.get_stim_images_by_frame()

Grayscr (session 758519303)

Public properties:
    self.sess
    self.stimtype

Public methods:
    self.get_all_fr()
    self.get_start_fr()
    self.get_stim_images_by_frame()
    self.get_stop_fr()
```

1.10.2 Example Session and Stim object property values.

Properties with long values (e.g., long dataframes, arrays, lists, strings) are omitted, for brevity.

```
Session (758519303)
```

```
Public property values:
  self.all_files: True
  self.any_files: True
  self.dandi id: 20180926T172917
  self.date: 20180926
  self.dend: extr
  self.depth: 175
  self.drop_tol: 0.0003
  self.gabors: Gabors (stimulus from session 758519303)
  self.grayscr: Grayscr (session 758519303)
  self.home: ../../data/OSCA_NWB
  self.line: L23-Cux2
  self.mouse_n: 1
  self.mouseid: 408021
  self.n stims: 2
  self.notes: nan
  self.nwb: True
  self.only_tracked_rois: False
  self.pass_fail: P
  self.plane: soma
  self.pup_data_available: True
  self.runtype: prod
  self.sess n: 1
  self.sessid: 758519303
  self.stim_fps: 59.951703429774675
  self.stim_seed: 30587
  self.stimtypes: ['gabors', 'visflow']
  self.tot_stim_fr: 251999
```

```
self.tot_twop_fr: 126741
    self.twop2stimfr: [nan nan nan nan nan nan nan]
    self.twop_fps: 30.078983328254086
Gabors (stimulus from session 758519303)
 Public property values:
    self.all_gabfr: ['A', 'B', 'C', 'D', 'G', 'U']
    self.all_gabfr_mean_oris: [0.0, 45.0, 90.0, 135.0, 180.0, 225.0]
    self.deg_per_pix: 0.06251912565744862
    self.exp_gabfr: ['A', 'B', 'C', 'D', 'G']
    self.exp_gabfr_mean_oris: [0.0, 45.0, 90.0, 135.0]
    self.exp_max_s: 90
    self.exp_min_s: 30
    self.kappas: [16]
    self.n_patches: 30
    self.n_segs_per_seq: 5
    self.ori_ran: [0, 360]
    self.phase: 0.25
    self.seg_len_s: 0.3
    self.sess: Session (758519303)
    self.sf: 0.04
    self.size_ran: [204, 408]
    self.stim_fps: 59.951703429774675
    self.stim_params: ['gabor_kappa']
    self.stimtype: gabors
    self.unexp_gabfr: ['U']
    self.unexp_gabfr_mean_oris: [90.0, 135.0, 180.0, 225.0]
    self.unexp_max_s: 6
    self.unexp_min_s: 3
    self.win_size: [1920, 1200]
Visflow (stimulus from session 758519303)
 Public property values:
    self.deg per pix: 0.06251912565744862
    self.exp_max_s: 90
    self.exp min s: 30
    self.main_flow_direcs: ['left (nasal)', 'right (temp)']
    self.n_squares: [105]
    self.prop_flipped: 0.25
    self.seg_len_s: 1
    self.sess: Session (758519303)
    self.speed: 799.7552664756905
    self.square_sizes: [128]
    self.stim_fps: 59.951703429774675
    self.stimtype: visflow
    self.unexp_max_s: 4
    self.unexp_min_s: 2
```

self.win_size: [1920, 1200]

Grayscr (session 758519303)

Public property values:

self.sess: Session (758519303)

self.stimtype: grayscreen