session demonstration script

March 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, if running the notebook locally and an error occurs, make sure to check whether there are updates to the utility, and update your installation as needed, e.g., by running, from the command line: pip install -U util-colleenjg

1.1 1. Setup

1.1.1 Update logging and plot formatting

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

1.1.2 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.

1.1.3 Running in a docker, or specifically in Binder

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

In addition, if the notebook is **running specifically in Binder**, some analyses are slightly altered later, in order to reduce memory use.

If the notebook is **not running in a docker**, the dataset should be downloaded manually.

Be sure to download the dataset, if needed, and update `datadir` and `datadir_nwb` to point the correct location(s).

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

1.1.4 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
- * mouse
id: 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
    838633305
82
                                        13
                                             440889
                                                      20190318
                                                                   175
                                                                        soma
        line runtype sess_n
                                   nrois_tracked nrois_all nrois_allen \
0
     L5-Rbp4
                pilot
                             1
                                               -1
                                                         1468
                                                                        232
1
     L5-Rbp4
                             2
                                               -1
                                                           78
                pilot
                                                                         62
2
                                                           -1
                                                                         -1
     L5-Rbp4
                                               -1
                pilot
                             3
3
     L5-Rbp4
                                               -1
                                                          949
                                                                        458
                pilot
                             4
4
     L5-Rbp4
                                                           79
                                                                         56
                pilot
                                               -1
78
   L23-Cux2
                 prod
                             2
                                              147
                                                          251
                                                                        224
79 L23-Cux2
                 prod
                             3
                                              147
                                                          228
                                                                        210
   L23-Cux2
                             4
                                               -1
                                                                        205
80
                                                          217
                 prod
81 L23-Cux2
                 prod
                             5
                                               -1
                                                          244
                                                                        217
   L23-Cux2
                                                          256
82
                 prod
                             6
                                               -1
                                                                        227
    nrois_allen_all pass_fail all_files
                                             any_files
                                                         incl stim_seed \
0
                 259
                               F
                                          1
                                                                     103
                                                      1
                                                          yes
                  78
                               F
                                          1
                                                      1
                                                                     103
1
                                                          yes
2
                  -1
                               F
                                          0
                                                      1
                                                                     103
                                                          no
3
                 504
                               Ρ
                                          1
                                                      1
                                                                     103
                                                          yes
4
                  79
                               Ρ
                                          1
                                                                     103
                                                      1
                                                          yes
. .
78
                 251
                               Ρ
                                          1
                                                      1
                                                                   16745
                                                          yes
79
                 228
                               Ρ
                                          1
                                                      1
                                                          yes
                                                                   10210
80
                 217
                               Ρ
                                          1
                                                      1
                                                                   24253
                                                          yes
                 244
81
                               F
                                          1
                                                      1
                                                          yes
                                                                   19576
82
                 256
                               F
                                          1
                                                                   30582
                                                      1
                                                           no
                                                   notes
0
    dropped beh and eye tracking frames (7), stim ...
    dropped beh and eye tracking frames (6), stim ...
1
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 ...
80
    FOV shifted (poor alignment with previous sess...
81
                                         z-drift (14 um)
82
                        laser wavelength set to 800 um
```

[83 rows x 21 columns]

1.2 2. Basics of initializing a Session object

Sessions can be intialized with their 9-digit sessid:

or with their mouse_n, sess_n and runtype:

1.2.1 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.

1.2.2 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.

1.2.3 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...

1.2.4 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.

\	<pre>gabor_frame</pre>	unexpected	stimulus_template_name	stimulus_type	[10]:
		NaN	grayscreen	grayscreen	0
	A	0.0	gabors	gabors	1
	В	0.0	gabors	gabors	2

```
3
             gabors
                                      gabors
                                                      0.0
4
                                      gabors
                                                      0.0
                                                                     D
             gabors
                              visflow_right
8839
                                                      0.0
            visflow
8840
            visflow
                              visflow_right
                                                      0.0
8841
            visflow
                              visflow_right
                                                      1.0
8842
            visflow
                              visflow_right
                                                      1.0
8843
        grayscreen
                                 grayscreen
                                                      NaN
                    gabor_mean_orientation
                                              gabor_number
      gabor_kappa
0
               NaN
                                                        NaN
                                         NaN
1
              16.0
                                         0.0
                                                       30.0
2
                                                       30.0
              16.0
                                         0.0
3
              16.0
                                         0.0
                                                       30.0
4
              16.0
                                         0.0
                                                       30.0
8839
               NaN
                                         NaN
                                                        NaN
8840
               NaN
                                         NaN
                                                        NaN
               NaN
                                         NaN
8841
                                                        NaN
8842
               NaN
                                         NaN
                                                        NaN
8843
               NaN
                                         NaN
                                                        {\tt NaN}
                                         gabor_locations_x
0
                                                          Г٦
1
      [-314.2481536790383, 726.6351926350328, -609.4...
2
      [278.93714376420894, -895.0169462360316, 830.4...
      [-694.2565883378384, 458.8415680953749, -472.6...
3
4
      [-631.2261180219028, -600.2310528361336, -887...
                                                          []
8839
8840
                                                          []
8841
                                                          8842
                                                          []
8843
                                         gabor_locations_y
0
                                                          []
1
      [519.3985635606798, 429.54112277826425, 482.75...
2
      [-62.92603512701612, -329.96944361291634, -332...
3
      [162.5089263895926, 433.50619201931613, 567.71...
4
      [-21.003509639097615, -271.4924294875755, 555...
                                                          []
8839
8840
                                                          []
8841
8842
                                                          []
8843
```

С

```
gabor_sizes ...
0
                                                         [237, 382, 341, 269, 332, 300, 256, 322, 252, ... ...
1
2
      [355, 245, 207, 246, 209, 371, 209, 400, 214, ...
      [270, 274, 369, 230, 364, 205, 360, 315, 396, ... ...
3
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
                             NaN
                                                                 1800
1
                             NaN
                                              1800
                                                                 1818
2
                             NaN
                                              1818
                                                                 1836
3
                             NaN
                                              1836
                                                                 1854
4
                                              1854
                                                                 1872
                             NaN
                            0.00
                                                               250020
8839
                                            249960
8840
                            0.00
                                            250020
                                                               250080
8841
                            0.25
                                            250080
                                                               250140
8842
                            0.25
                                            250140
                                                               250200
8843
                             NaN
                                            250200
                                                               251999
                        start_frame_twop
                                            stop_frame_twop
      num_frames_stim
                                                              num_frames_twop
0
                  1800
                                       143
                                                        1046
                                                                            903
1
                                                                              9
                    18
                                      1046
                                                        1055
2
                                                                              9
                    18
                                      1055
                                                        1064
3
                                                                              9
                    18
                                                        1073
                                      1064
4
                                                                              9
                    18
                                      1073
                                                        1082
8839
                    60
                                   125551
                                                      125581
                                                                             30
8840
                    60
                                   125581
                                                      125611
                                                                             30
8841
                                                                             30
                    60
                                    125611
                                                      125641
8842
                    60
                                    125641
                                                                             31
                                                      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]

1.2.5 Stimulus objects

Once sess.extract_info() is run, each Session object also 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

1.3 3. Retrieving data of interest

1.3.1 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).

1.3.2 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.

1.3.3 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).

1.3.4 Retrieving data statistics of interest

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

[15]:	datatype	9		roi_traces
	bad_rois	s_remo	oved	yes
	scaled			no
	baseline	Э		no
	integrat	ted		yes
	smoothin	ng		no
	fluores	cence		dff
	general	${\tt ROIs}$	sequences	
	stats	None	stat_mean	0.062516
			error_SEM	0.017370

1.3.5 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	ed	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().

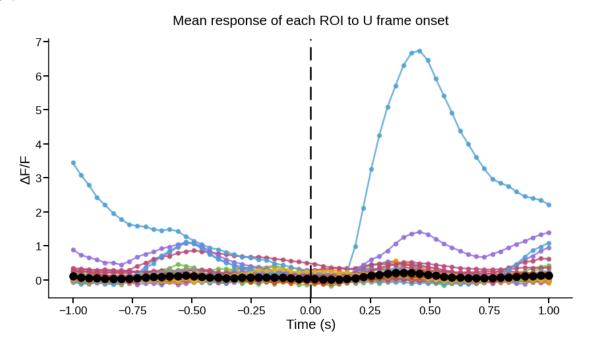
Here, each index level, then column level is turned into a new axis, i.e. ROIs x sequences x time_values (In this case, squeeze_cols is set to True to prevent each dummy column from becoming its own axis.)

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

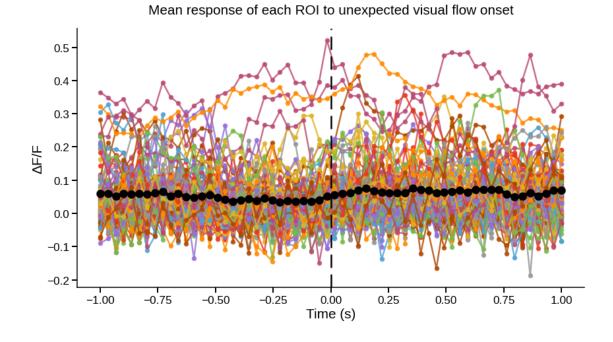
You can also retrieve the time stamps for each frame.

1.3.6 Visualizing the data

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



1.3.7 The same steps apply to analysing the visual flow stimulus



1.4 4. 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.

datat	суре	roi_traces
bad_1	cois_removed	yes
scale	ed	yes
basel	line	no
integ	grated	yes
smoot	thing	no
fluoi	rescence	dff
ROIs	sequences	
28	0	0.101591
	1	0.188843
	2	-0.072082
	3	0.685275
	4	0.033439
		•••
22	27	0.026887
	28	0.523182
	29	-0.039192
	30	-0.059955
	31	0.080472
	bad_1 scale basel integ smoot fluon ROIs 28	1 2 3 4 22 27 28 29 30

[1504 rows x 1 columns]

1.4.1 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
```

Note that these **ROI numbers** are assigned for each session separately. Thus, e.g., ROI #28 from session 1 is most likely not the same neuron/dendrite as the ROI #28 from session 2, for the same mouse. For this reason, **the tracking ordering** is important: it allows ROI traces from different sessions to be lined up correctly with one another.

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 ...
```

1.4.2 Reset the session to start using all ROIs, again

1.5 5. Additional tips on indexing a hierarchical dataframe

[31]:	scale	ed		yes
	base.	line		no
	integ	grated		no
	smoot	thing		no
	fluo	rescence		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

1.6 6. Retrieving several Session objects, based on criteria

1.6.1 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 interest, and one session has incomplete data.

For the prod data, the lists are empy.

1.6.2 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

```
1: 758519303 (session 1)
mouse
mouse
      1: 759189643 (session 2)
     1: 759660390 (session 3)
mouse
mouse 3: 761624763 (session 1)
mouse 3: 761944562 (session 2)
      3: 762250376 (session 3)
mouse
     4: 760260459 (session 1)
mouse
      4: 760659782 (session 2)
mouse
     4: 761269197 (session 3)
mouse
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)
```

1.6.3 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.

1.6.4 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

1.7 7. Retrieving ROI masks from a session

Boolean ROI masks can be obtained for each Session.

1.7.1 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

1.7.2 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 in **Binder**, the dendritic masks will 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.

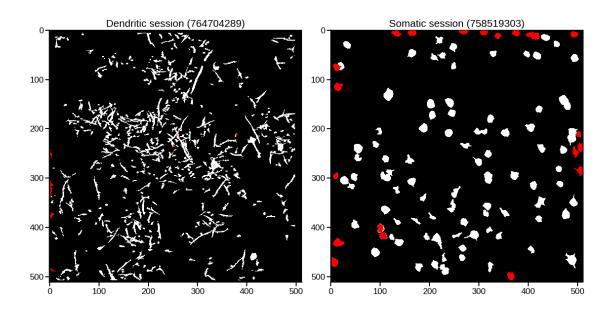
1.7.3 Bad ROIs

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.

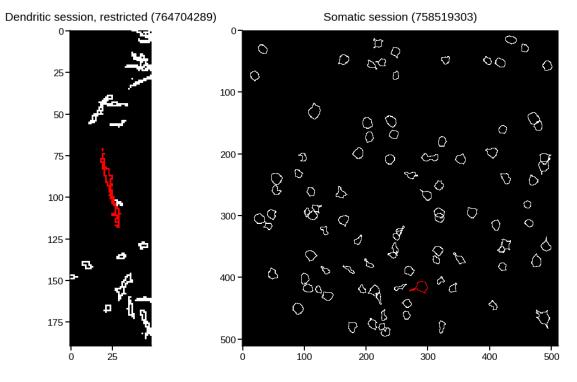
1.7.4 Visualizing ROI masks

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



1.7.5 Visualizing ROI mask contours

 ${\tt sess_plot_util.plot_ROI_contours}$ () can be used to visualize ROI contours, optionally restricted to around an ROI of interest.



1.8 8. 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:

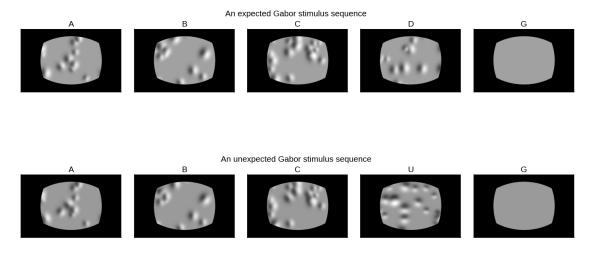
Several NWB files were found for this session. When loading data, the first file listed that contains the required data will be used.

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.

1.8.1 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.

1.8.2 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.

1.8.3 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

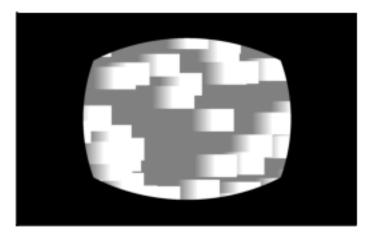












An unexpected visual flow sequence

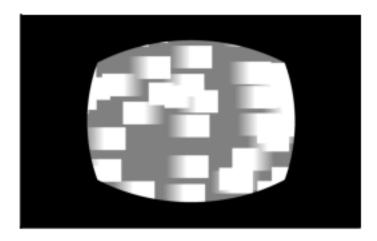












We plot the first few frames in each sequence separately. We then also plot all the frames retained for each sequence, **overlayed in a graded way**, in order to visualize the squares **in motion**.

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, ~25% of them are moving in the opposite direction, i.e., leftward.

1.8.4 Stimulus generating code and examples

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

1.9 9. 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

1.9.1 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()
```

1.9.2 Example Session and Stim object property values.

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

```
Public property values:
self.all_files: True
self.any_files: True
```

Session (758519303)

```
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]
    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
```