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
```

```
         line runtype  sess_n  …  nrois_tracked  nrois_all  nrois_allen  \
0    L5-Rbp4   pilot       1  …             -1       1468          232
1    L5-Rbp4   pilot       2  …             -1         78           62
2    L5-Rbp4   pilot       3  …             -1         -1           -1
3    L5-Rbp4   pilot       4  …             -1        949          458
4    L5-Rbp4   pilot       5  …             -1         79           56
..       …       …     …  …            …        …          …
78  L23-Cux2    prod       2  …            147        251          224
79  L23-Cux2    prod       3  …            147        228          210
80  L23-Cux2    prod       4  …             -1        217          205
81  L23-Cux2    prod       5  …             -1        244          217
82  L23-Cux2    prod       6  …             -1        256          227

    nrois_allen_all  pass_fail all_files  any_files  incl stim_seed  \
0               259          F         1          1   yes       103
1                78          F         1          1   yes       103
2                -1          F         0          1    no       103
3               504          P         1          1   yes       103
4                79          P         1          1   yes       103
..              …         …        …        …   …       …
78              251          P         1          1   yes     16745
79              228          P         1          1   yes     10210
80              217          P         1          1   yes     24253
81              244          F         1          1   yes     19576
82              256          F         1          1    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 …
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]
```

1.3 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 gabor_frame  \
0       grayscreen             grayscreen         NaN
1           gabors                 gabors         0.0           A
2           gabors                 gabors         0.0           B
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       visflow         visflow_right        1.0
8843    grayscreen            grayscreen        NaN

      gabor_kappa  gabor_mean_orientation  gabor_number  \
0             NaN                     NaN           NaN
1            16.0                     0.0          30.0
2            16.0                     0.0          30.0
3            16.0                     0.0          30.0
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                                                     []
1      [-314.2481536790383, 726.6351926350328, -609.4…
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                                                     []
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                                                  []

                                             gabor_sizes  …  \
0                                                     []  …
1      [237, 382, 341, 269, 332, 300, 256, 322, 252, …  …
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                          NaN                0             1800
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  \
0                1800               143             1046              903
1                  18              1046             1055                9
2                  18              1055             1064                9
3                  18              1064             1073                9
4                  18              1073             1082                9
…               …                 …                 …                …
8839               60            125551           125581               30
8840               60            125581           125611               30
8841               60            125611           125641               30
8842               60            125641           125672               31
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]:
```
datatype                         roi_traces
bad_rois_removed                        yes
scaled                                  yes
baseline                                 no
integrated                               no
smoothing                                no
fluorescence                            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()`.

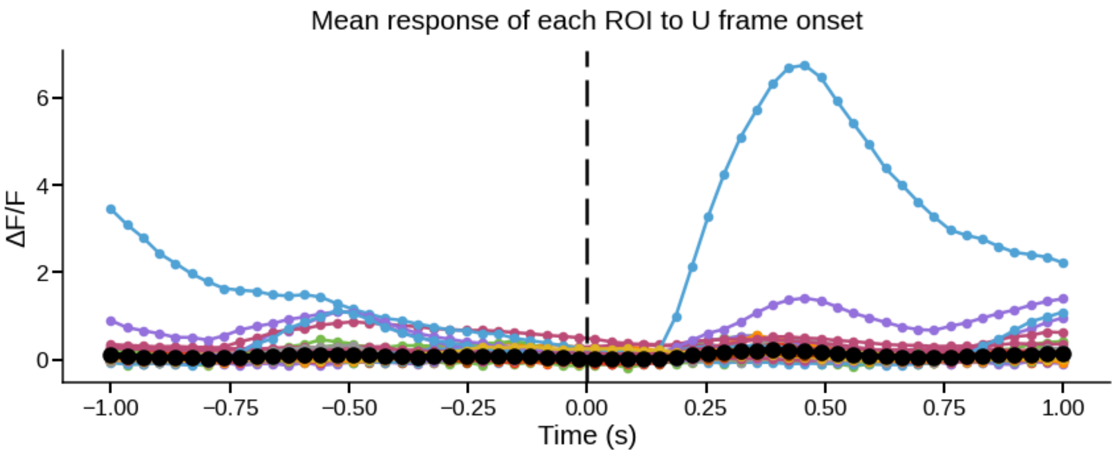
Here, each index level, then column level is turned into a new axis, **i.e. ROIs x sequences x time_values** (In this case, `squeeze_cols` is set to True to prevent each dummy column from becoming its own axis.)

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

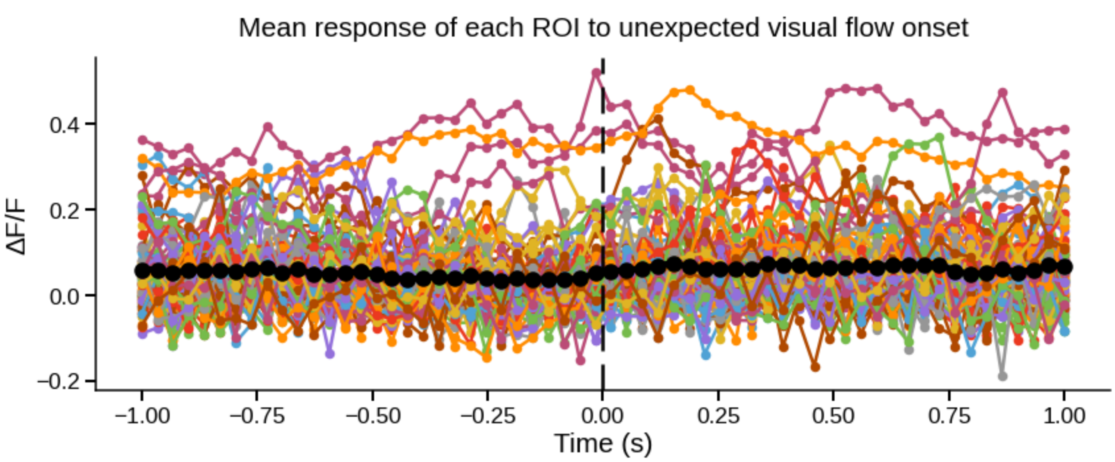
You can also retrieve the time stamps for each frame.

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
bad_rois_removed         yes
scaled                   yes
baseline                  no
integrated               yes
```

```
smoothing                no
fluorescence            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

[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 ROIs x 32 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]:
```
scaled                              yes
baseline                             no
integrated                           no
smoothing                            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 x height 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.

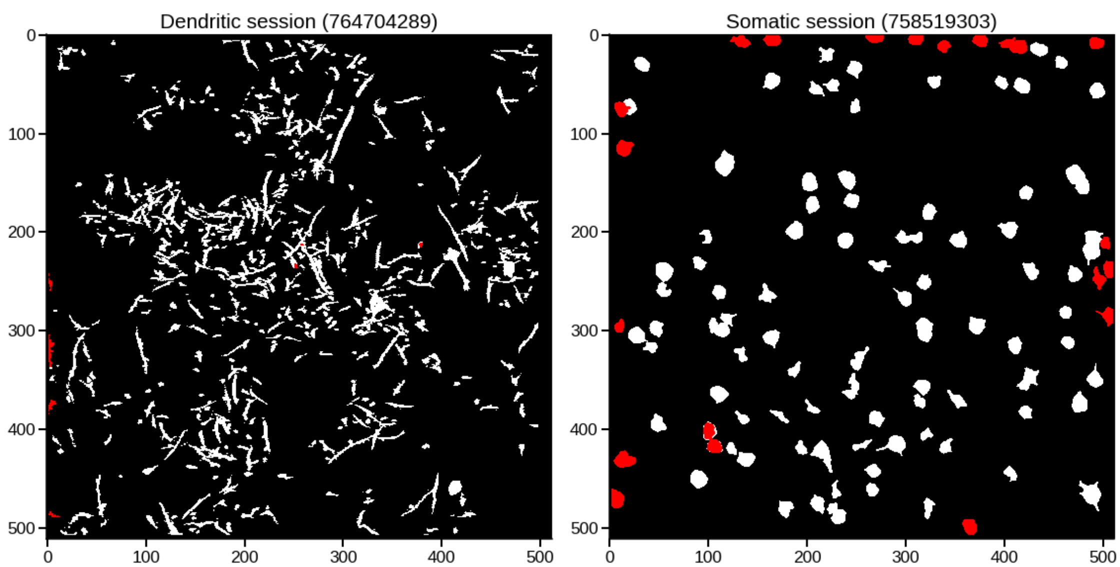
1.8.3 Bad ROIs

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.

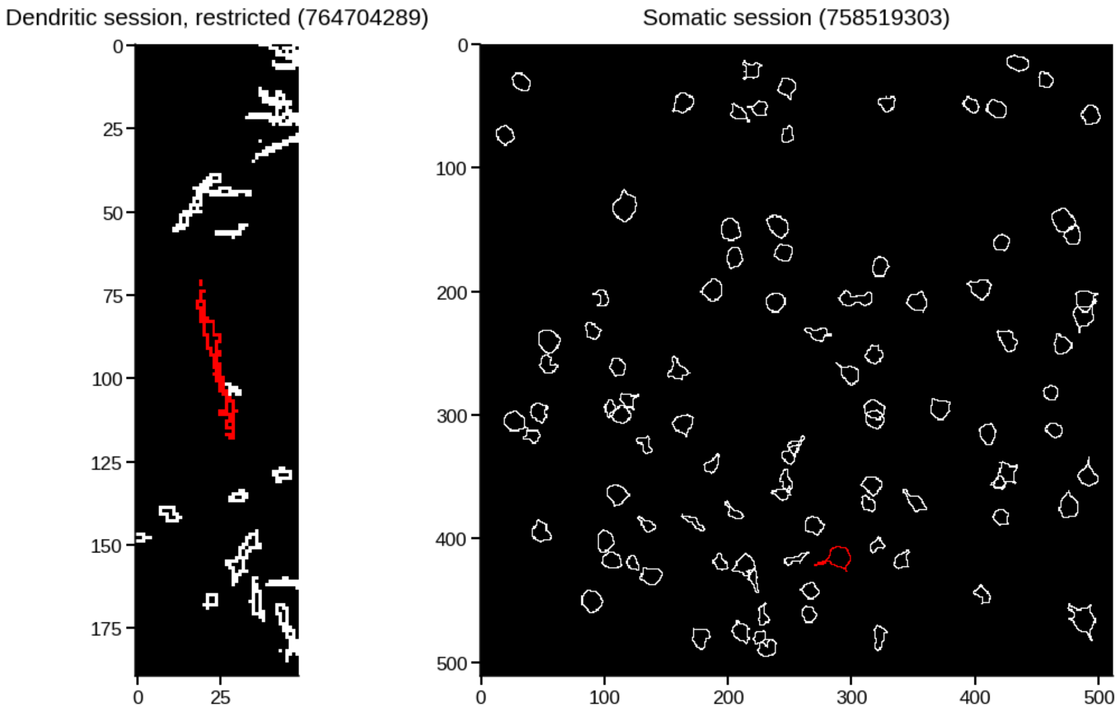
1.8.4 Visualizing ROI masks

`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.
```

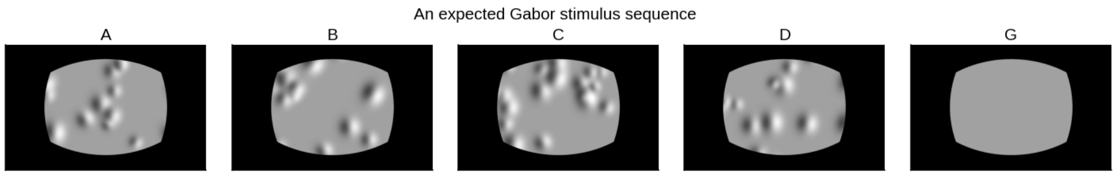
```
/home/colleen/Documents/OpenScope_CA_Analysis/analysis/session.py:287:
UserWarning:
Several NWB files were found for this session. When loading data, the first file
listed that contains the required data will be used.
```

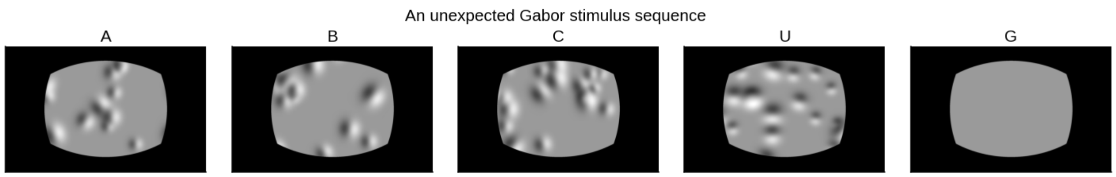
```
Loading stimulus and alignment info…
```

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.





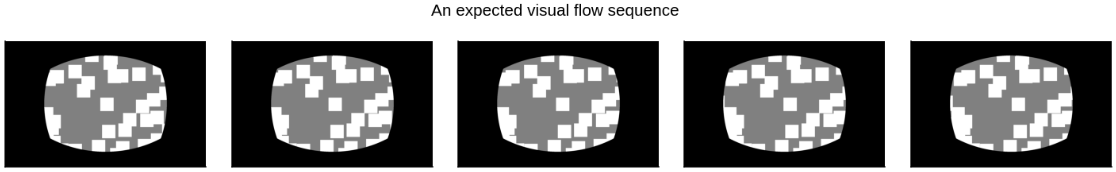
As we can see, whereas the Gabor patch orientations are consistent across frames in the expected sequence, they are rotated by 90° in the U frame of the unexpected sequence.

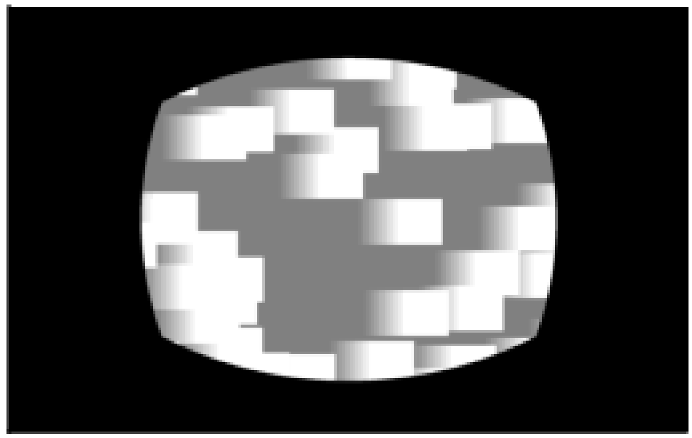
1.9.2 Warping

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.

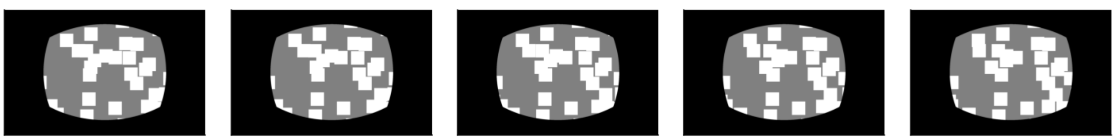
1.9.3 Visual flow sequence images

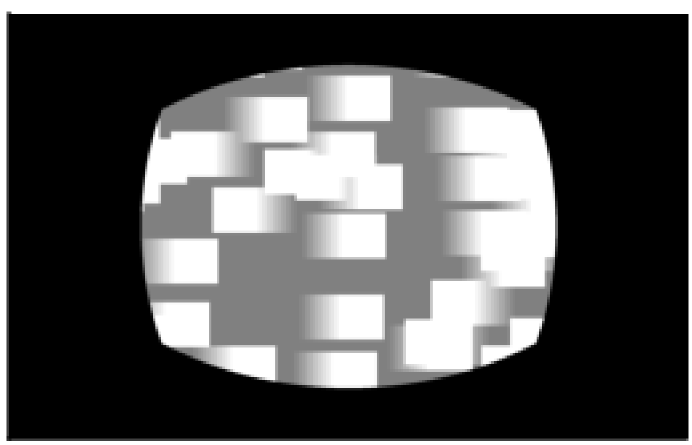
We can also visualize the **visual flow stimulus**. It is important to note that, whereas the Gabor images are static for each segment, the visual flow stimulus is in motion, and therefore changes at each frame. For this reason, we will simply identify the first visual flow segment in a sequence, and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





An 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.

1.9.4 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]
    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
```