

A tutorial on how to use the Brain Observatory Toolbox

This tutorial will show you how to access the Brain Observatory data using MATLAB. In particular, this tutorial will show you how to:

1) Instantiate a *sessionfilter* object to:

- A) Get general information on the brain observatory data
- B) Filter sessions by specified criteria such as brain areas, imaging depth and stimuli type

2) Instantiate a *session* object to:

Retrieve and examine kinds of experiment data as listed below of an interested session from its Neurodata Without Borders (NWB) file:

- a) ROI masks
- b) fluorescence traces
- c) running speed
- d) motion correction
- e) eye tracking

3) Call function *convert_fluorescence_trace_into_raster_format* to:

Convert fluorescence trace into data that is in "raster format" for further analysis

Organization of the Brain Observatory Data

Before we start, we first want to describe the organization of the Brain Observatory Data.

An *experiment container* contains three *sessions* (also called *experiments* or *ophys_experiments*) where recordings were made on a single mouse, in a single brain region and at a particular imaging depth. Each of these three sessions consists of a series of "subexperiments" where a particular stimulus set was shown. Neurodata Without Borders (NWB) files downloaded from the Allen Institute API each consist of data from a single session. As described below, all data that we extract into "raster format" consists of data from a single "subexperiment" where a particular stimulus set was shown.

Note: within an experiment container the same stimuli might be repeated in different sessions (i.e., different sessions can have the same "subexperiment type"). For example, Natural Movie 1 is shown in all three sessions in an experiment container. For more information see: http://alleninstitute.github.io/AllenSDK/brain_observatory.html

1) Instantiate a *sessionfilter* object

sessionfilter is a class that resembles BrainObservatoryCache in allensdk (see Python code at: https://github.com/AllenInstitute/AllenSDK/blob/master/allensdk/core/brain_observatory_cache.py).

The MATLAB *sessionfilter* enables you to get information and access data using three types of methods:

- A) Get general information on the brain observatory data using methods that start with "get_"
- B) Filter sessions by different criteria using methods that start with "filter_"

To begin, build a *brain_observatory_cache* object

```
bosf1 = bot.sessionfilter()
```

```
bosf1 =  
sessionfilter with properties:  
  
    valid_session_table: [543x15 table]  
    filtered_session_table: [543x15 table]  
           stimulus: {9x1 cell}  
    targeted_structure: {6x1 cell}  
       imaging_depth: [10x1 double]  
       container_id: [181x1 double]  
        session_id: [543x1 double]  
       session_type: [543x1 categorical]  
        cre_line: {6x1 cell}  
    eye_tracking_avail: [2x1 logical]  
           failed: 0
```

Note: BOT by default excludes all failed experiment containers and their sessions. If you want to have them included, you can pass 1 to the constructor:

```
bosf0 = bot.sessionfilter(1)
```

```
bosf0 =  
sessionfilter with properties:  
  
    valid_session_table: [648x15 table]  
    filtered_session_table: [648x15 table]  
           stimulus: {9x1 cell}  
    targeted_structure: {6x1 cell}  
       imaging_depth: [12x1 double]  
       container_id: [216x1 double]  
        session_id: [648x1 double]  
       session_type: [648x1 categorical]  
        cre_line: {6x1 cell}  
    eye_tracking_avail: [2x1 logical]  
           failed: 1
```

1A) Get general information on the brain observatory data

There are several methods start with "get_" which summarize in the brain observatory data based on particular criteria.

To get the total number of experiment containers we can use:

```
bosf1.get_total_num_of_containers()
```

```
ans = 181
```

Note: Total number of sessions is the number of containers times 3 since there are three sessions per container.

To get all the cortical depths (um) that were ever recorded in any experiment container we can use:

```
bosf1.get_all_imaging_depths()
```

```
ans =  
175  
265  
275  
300  
320  
325  
335  
350  
365  
375
```

•

Note: Recordings didn't take place equally among these depths, only the most common depths are shown on homepage of Brain Observatory: <http://observatory.brain-map.org/visualcoding>

To get all the get all type of cre driver lines from all mice we can use:

```
bosf1.get_all_cre_lines()
```

```
ans = 6x1 cell array  
'Cux2-CreERT2'  
'Emx1-IRES-Cre'  
'Nr5a1-Cre'  
'Rbp4-Cre_KL100'  
'Rorb-IRES2-Cre'  
'Scnn1a-Tg3-Cre'
```

Note: All mice had the same reporter line: Ai93 and tTA driver line: Camk2a-tTA

For more information about transgenic lines: <http://observatory.brain-map.org/visualcoding/transgenic>

To get all the all brain regions that were recorded in any experiment container we can use:

```
bosf1.get_all_targeted_structures()
```

```
ans = 6x1 cell array
    'VISal'
    'VISam'
    'VISl'
    'VISp'
    'VISpm'
    'VISrl'
```

For more information on these locations, see homepage of Brain Observatory: <http://observatory.brain-map.org/visualcoding>

To get all the all types of sessions that appear in any experiment container we can use:

```
bosf1.get_all_session_types()
```

```
ans = 4x1 cell array
    'three_session_A'
    'three_session_B'
    'three_session_C'
    'three_session_C2'
```

Note: There are always three sessions in each container: session A, session B, and session C or session C2

To get all the all type of stimuli that were used in any of the four types of sessions we can use:

```
bosf1.get_all_stimuli()
```

```
ans = 9x1 cell array
    'drifting_gratings'
    'locally_sparse_noise_4deg'
    'locally_sparse_noise_8deg'
    'natural_movie_one'
    'natural_movie_three'
    'natural_movie_two'
    'natural_scenes'
    'spontaneous'
    'static_gratings'
```

Note: For mapping between session type and stimulus type see http://alleninstitute.github.io/AllenSDK/brain_observatory.html

To get the number of experiment containers recorded in each brain region we can use:

```
bosf1.get_summary_of_containers_along_targeted_structures()
```

VISal	35
VISam	25
VISl	38

VISp	66
VISpm	36
VISrl	16

To get the number of experiment containers recorded at each cortical depth we can use:

```
bosfl.get_summary_of_containers_along_imaging_depths()
```

175	53
250	1
265	1
275	75
300	4
320	1
325	3
335	3
350	33
365	1
375	40
435	1

To get the number of experiment containers recorded at each cortical depth in each brain region we can use:

```
bosfl.get_summary_of_containers_along_depths_and_structures()
```

```
ans = 11x7 table
```

	VISal	VISam	VISl	VISp	VISpm	VISrl	total
	-----	-----	-----	-----	-----	-----	-----
175	8	5	10	11	11	5	50
265	1	0	0	0	0	0	1
275	11	9	14	17	9	5	65
300	1	0	1	1	1	0	4
320	0	1	0	0	0	0	1
325	0	1	1	0	1	0	3
335	0	0	0	2	1	0	3
350	2	3	3	10	4	3	25
365	1	0	0	0	0	0	1
375	6	4	5	5	6	2	28
total	30	23	34	46	33	15	181

1B) Filter sessions by specified criteria such as brain areas, imaging depth and stimuli type

sessionfilter.filtered_session_table is an m-by-n table listing the n fields of meta data of m succesful sessions. As different filter methods get called on *sessionfilter*, rows/sessions in *filtered_session_table* are liminated to meet specified criteria, and other properies (e.g., stimuli, targeted_structure, etc) of *sessionfilter*, that are essentially important fields of meta data in *filtered_session_table*, are also updated.

Here we show an example of searching for sessions that Rorb-IRES2-Cre mice had their primary visual cortex recorded at 275 μ m deep, during where drifting gratings were shown and eye tracking went through.

To ensure an "involute" *sessionfilter*:

```
bosf1.clear_filters
```

Eliminate sessions to the ones where drifting gratings were shown:

```
bosf1.filter_sessions_by_stimuli('drifting_gratings')
```

```
ans =  
  sessionfilter with properties:  
  
    valid_session_table: [543×15 table]  
    filtered_session_table: [181×15 table]  
        stimulus: {4×1 cell}  
    targeted_structure: {6×1 cell}  
    imaging_depth: [10×1 double]  
    container_id: [181×1 double]  
    session_id: [181×1 double]  
    session_type: [181×1 categorical]  
    cre_line: {6×1 cell}  
    eye_tracking_avail: [2×1 logical]  
        failed: 0
```

Note that several properties of *bosf* have changed.

Eliminate sessions to the ones that have posterior Primary Visual Cortex recordings:

```
bosf1.filter_sessions_by_targeted_structure('VISp')
```

```
ans =  
  sessionfilter with properties:  
  
    valid_session_table: [543×15 table]  
    filtered_session_table: [46×15 table]  
        stimulus: {4×1 cell}  
    targeted_structure: {'VISp'}  
    imaging_depth: [6×1 double]  
    container_id: [46×1 double]  
    session_id: [46×1 double]  
    session_type: [46×1 categorical]  
    cre_line: {6×1 cell}  
    eye_tracking_avail: [2×1 logical]  
        failed: 0
```

Eliminate sessions to the ones that were recorded at 275 um deep

```
bosf1.filter_sessions_by_imaging_depth(275)
```

```
ans =  
  sessionfilter with properties:  
  
    valid_session_table: [543×15 table]
```

```

filtered_session_table: [17×15 table]
    stimulus: {4×1 cell}
targeted_structure: {'VISp'}
imaging_depth: 275
container_id: [17×1 double]
session_id: [17×1 double]
session_type: [17×1 categorical]
cre_line: {4×1 cell}
eye_tracking_avail: [2×1 logical]
failed: 0

```

Eliminate sessions to the ones that were operated on mice of Rorb-IRES2-Cre

```
bosf1.filter_session_by_cre_line('Rorb-IRES2-Cre')
```

```

ans =
sessionfilter with properties:

    valid_session_table: [543×15 table]
filtered_session_table: [5×15 table]
    stimulus: {4×1 cell}
targeted_structure: {'VISp'}
imaging_depth: 275
container_id: [5×1 double]
session_id: [5×1 double]
session_type: [5×1 categorical]
cre_line: {'Rorb-IRES2-Cre'}
eye_tracking_avail: [2×1 logical]
failed: 0

```

Eliminate sessions to the ones that eye tracking completely went through

```
bosf1.filter_session_by_eye_tracking(1)
```

```

ans =
sessionfilter with properties:

    valid_session_table: [543×15 table]
filtered_session_table: [2×15 table]
    stimulus: {4×1 cell}
targeted_structure: {'VISp'}
imaging_depth: 275
container_id: [2×1 double]
session_id: [2×1 double]
session_type: [2×1 categorical]
cre_line: {'Rorb-IRES2-Cre'}
eye_tracking_avail: 1
failed: 0

```

As we can see now, there are 2 sessions belonging to 2 different containers that meet all of the criteria given.

Every experiment container in the Brain Observatory has an unique experiment container ID that was created by the Allen Institute. These container IDs are stored in the *experiment_container_id* field

in *filtered_session_table*. For example, to see the experiment container IDs that met our filtering criteria we can run:

```
bosf1.filtered_session_table.experiment_container_id
```

```
ans =  
512124562  
511510989  
•
```

If we want to find out the three sessions in the first experiment container, we can call:

```
bosf2 = bot.sessionfilter
```

```
bosf2 =  
sessionfilter with properties:  
  
  valid_session_table: [543×15 table]  
  filtered_session_table: [543×15 table]  
    stimulus: {9×1 cell}  
  targeted_structure: {6×1 cell}  
    imaging_depth: [10×1 double]  
    container_id: [181×1 double]  
    session_id: [543×1 double]  
    session_type: [543×1 categorical]  
    cre_line: {6×1 cell}  
  eye_tracking_avail: [2×1 logical]  
    failed: 0
```

```
bosf2.filter_sessions_by_container_id(bosf1.filtered_session_table.experiment_container_id(1))
```

```
ans =  
sessionfilter with properties:  
  
  valid_session_table: [543×15 table]  
  filtered_session_table: [3×15 table]  
    stimulus: {8×1 cell}  
  targeted_structure: {'VISp'}  
    imaging_depth: 275  
    container_id: 512124562  
    session_id: [3×1 double]  
    session_type: [3×1 categorical]  
    cre_line: {'Rorb-IRES2-Cre'}  
  eye_tracking_avail: 1  
    failed: 0
```

```
bosf2.session_id
```

```
ans =  
512124564  
512176430  
512270518  
•
```


Now let's dig into the real data.

2) Instantiate a *session* object to:

Retrieve and examine kinds of experiment data as listed below of an interested session from its Neurodata Without Borders (NWB) file:

To illustrate this, let's instantiate a *session* object for the first session in *bosf2*.

```
bos1 = bot.session(bosf2.session_id(3))
```

```
bos1 =  
    session with properties:  
  
        sSessionInfo: [1x1 struct]  
        strLocalNWBFileLocation: 'C:\Users\14868\Documents\GitHub\Brain-Observatory-Toolbox\+bot\Cache\external\NWBFiles\512270518.nwb'
```

All the meta data stored in *bosf2.filtered_session_table* for this session are carried over to *bos1.sSessionInfo*

```
bos1.sSessionInfo
```

```
ans = struct with fields:  
    date_of_acquisition: '2016-04-07T15:29:01Z'  
    experiment_container_id: 512124562  
    fail_eye_tracking: 0  
        id: 512270518  
    imaging_depth: 275  
        name: '20160407_234831_3stimA'  
    specimen_id: 504614281  
    stimulus_name: 'three_session_A'  
    storage_directory: '/external/neuralcoding/prod6/specimen_504614281/ophys_experiment_512270518'  
    targeted_structure_id: 385  
    experiment_container: [1x1 struct]  
        well_known_files: [1x1 struct]  
    targeted_structure: [1x1 struct]  
        specimen: [1x1 struct]  
    cre_line: 'Rorb-IRES2-Cre'
```

2a) ROI Masks

If you want to take a look at specific cells or all cells visually, you can extract and plot their pixel masks. You can also pull out the maximum intensity projection of the movie for context.

```
% get all the cell ids  
cell_ids = bos1.get_cell_specimen_ids()
```

Downloading URL: [http://api.brain-map.org/api/v2/well_known_file_download/516251592]...
Warning: The specified file already exists; overwriting.

cell_ids = 90x1 int64 column vector

517447852
517447402
517447618
517447321
517447330
517447325
517447730
517447755
517447745
517447847

⋮

•

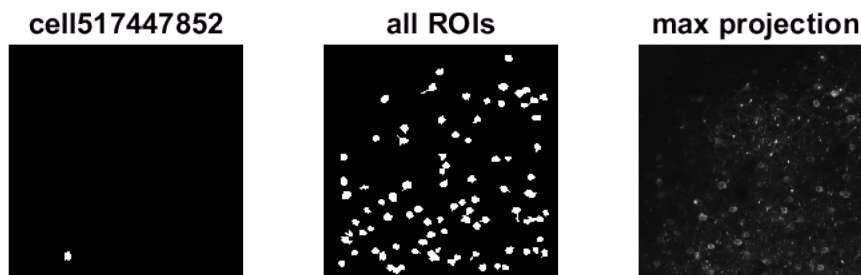
When you call this or any other method of session class, BOT automatically searches for the corresponding NWB file and downloads it if it is not there yet.

Downloading an NWB file from their server can take about 20 min.

```
% plot the ROI mask for the first cell in the list
subplot(1,3,1)
imshow(bos1.get_roi_mask_array(cell_ids(1)))
title(['cell' num2str(cell_ids(1))])

% plot the ROI masks for all cells
all_mask = logical(zeros(512,512));
for iCell = 1: length(cell_ids)
    all_mask = or(all_mask, bos1.get_roi_mask_array(cell_ids(iCell)));
end
subplot(1,3,2)
imshow(all_mask)
title('all ROIs')

% plot the maximum intensity projection
subplot(1,3,3)
imshow(double(bos1.get_max_projection)/max(max(double(bos1.get_max_projection))))
% colormap(gray)
title('max projection')
```



2b) Fluorescence Traces

You can get the fluorescence traces of a specific cell by its index. Let's take cell 589163560 as an example.

```
[time, raw] = bos1.get_fluorescence_traces;
[time, demixed] = bos1.get_demixed_traces;
[time, neuropil] = bos1.get_neuropil_traces;

neuropil_r = bos1.get_neuropil_r;

[time, corrected] = bos1.get_corrected_fluorescence_traces;

%% compute it yourself
% corrected = demixed - neuropil * (neuropil_r .* eye(size(neuropil_r,1)));

[time, dff] = bos1.get_dff_traces;

iCell = bos1.get_cell_specimen_indices(cell_ids(1))

iCell = 1
```

```
subplot(4,1,1)
plot(time, raw (:,iCell))
axis tight
title ('raw')
```

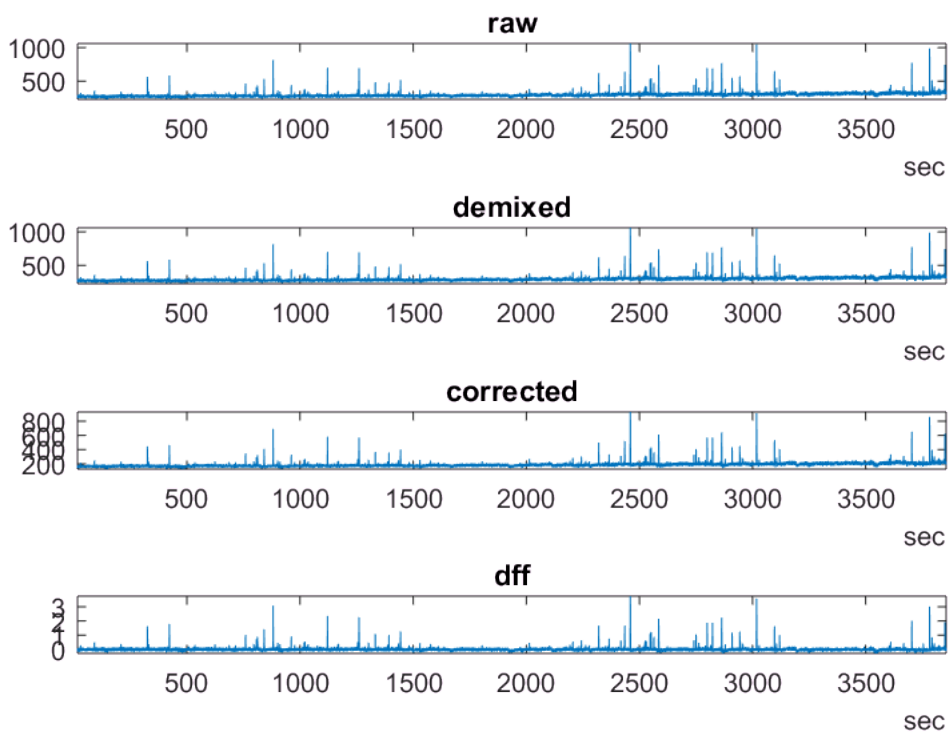
```

subplot(4,1,2)
plot(time, demixed (:,iCell))
axis tight
title ('demixed')

subplot(4,1,3)
plot(time, corrected(:,iCell))
axis tight
title ('corrected')

subplot(4,1,4)
plot(time, dff(:,iCell))
axis tight
title ('dff')

```



You can also examine the fluorescence traces of all cells recorded in a session one cell at a time in sequence. The traces will be plotted in an external figure, hit any key to proceed to the next cell.

```

figure('Position', get(0, 'Screensize'))
set(gcf, 'Visible','on')

for iCell = 1:length(cell_ids)
clf

ft_to_be_plotted = {'raw', 'demixed', 'corrected', 'dff'};

```

```

for ift = 1: length(ft_to_be_plotted)
subplot(length(ft_to_be_plotted),1,ift)
cur_ft = eval(ft_to_be_plotted{ift});
plot(time, cur_ft(:,iCell))
% axis tight
ylim([0,max(max(cur_ft))])
title(ft_to_be_plotted{ift})
end

% figure('KeyPressFcn',@keyDownListener)
%
%     function keyDownListener(

fprintf('Program paused. Press any key to continue to the next cell.\n');
pause;
end

```

Program paused. Press any key to continue to the next cell.
Program paused. Press any key to continue to the next cell.
Program paused. Press any key to continue to the next cell.

```
close all
```

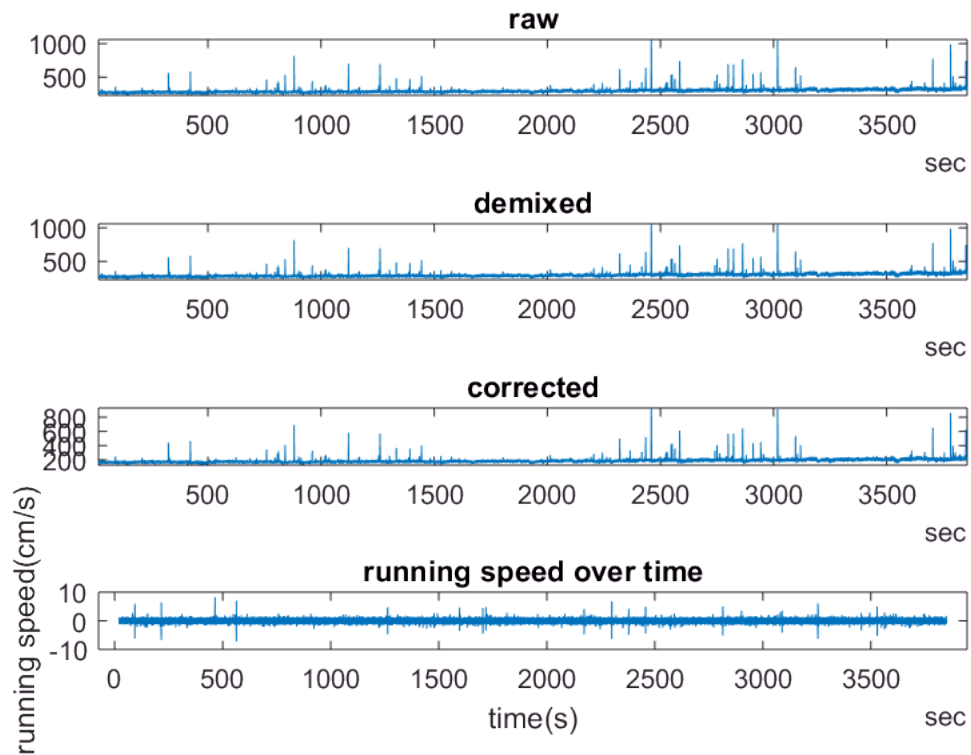
2c) Running Speed

All experiments contain running speed. Extreme outliers from the tracking have been removed and replaced with NaN, which will appear as gaps in the plotted data.

```

[time, runningSpeed] = bos1.get_running_speed;
plot(time, runningSpeed)
title('running speed over time')
xlabel('time(s)')
ylabel('running speed(cm/s)')

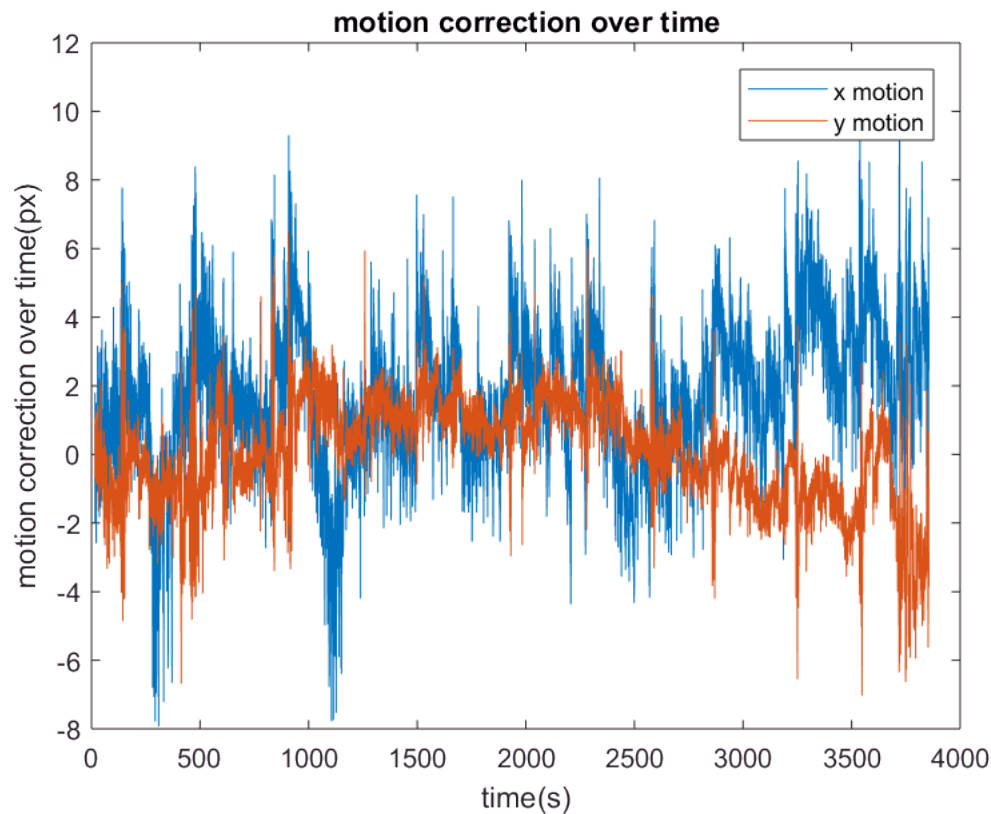
```



2d) Motion Correction

X and Y translation values in pixels required to correct for motion artifacts during the experiment are available as well.

```
close all
mc = bosl.get_motion_correction;
plot(table2array(mc(:, 'timestamp')), table2array(mc(:, 'x_motion')), table2array(mc(:, 'timestamp')), table2array(mc(:, 'y_motion')));
title('motion correction over time')
xlabel('time(s)')
ylabel('motion correction over time(px)')
legend('x motion', 'y motion')
xlabel('time(s)')
```

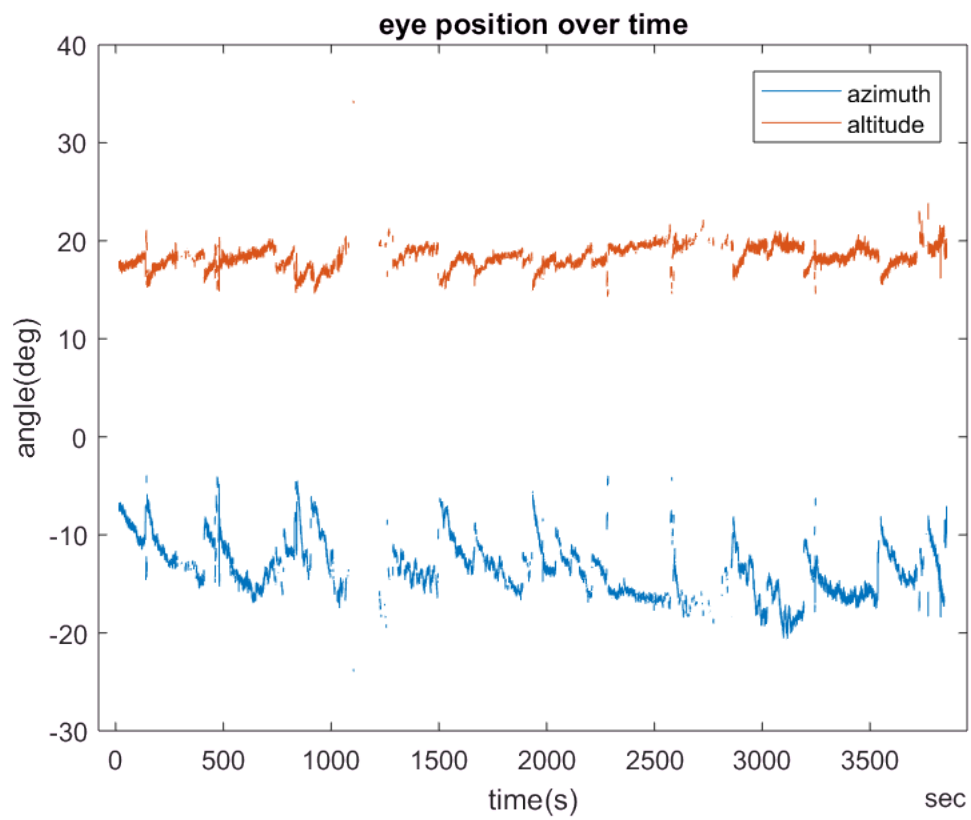


Eye Tracking

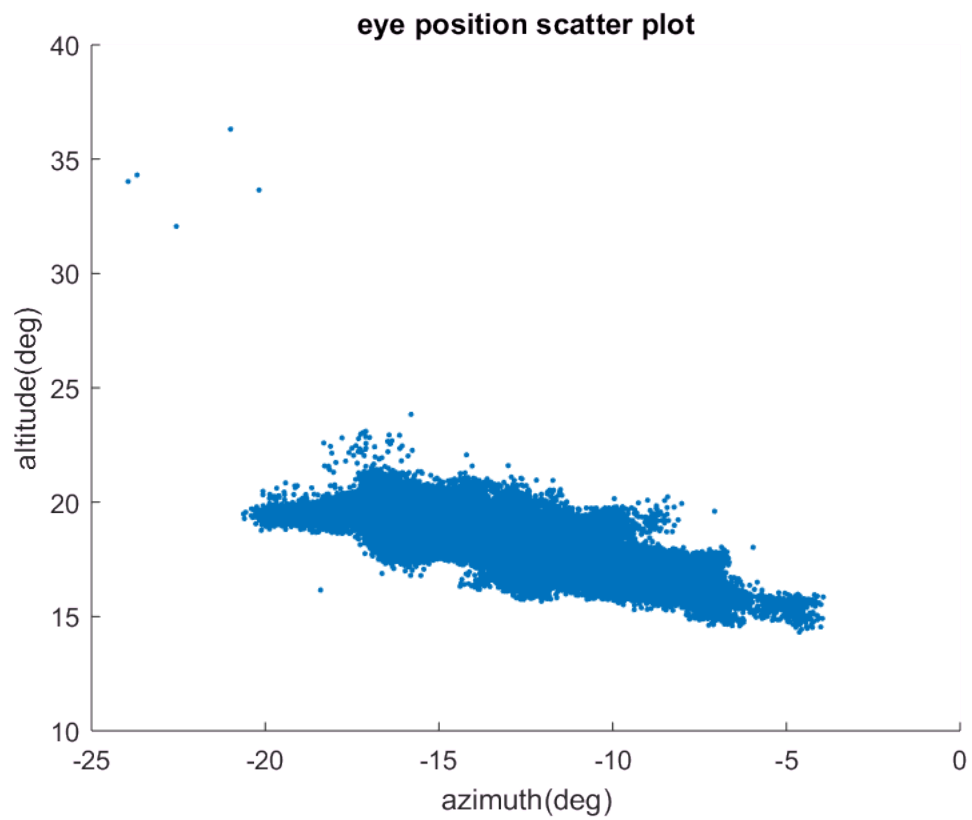
Many experiments contain pupil position and pupil size from eye tracking. Extreme outliers from the tracking have been removed and replaced with NaN, which will appear as gaps in the plotted data. If an experiment does not have eye tracking data, a `NoEyeTrackingException` will be raised.

As shown in `bos1.sSessionInfo`, the session in `bos1` doesn't have eye tracking data, so let's filter out all sessions with eye tracking data and takes the first session in the list to demonstrate this functionality.

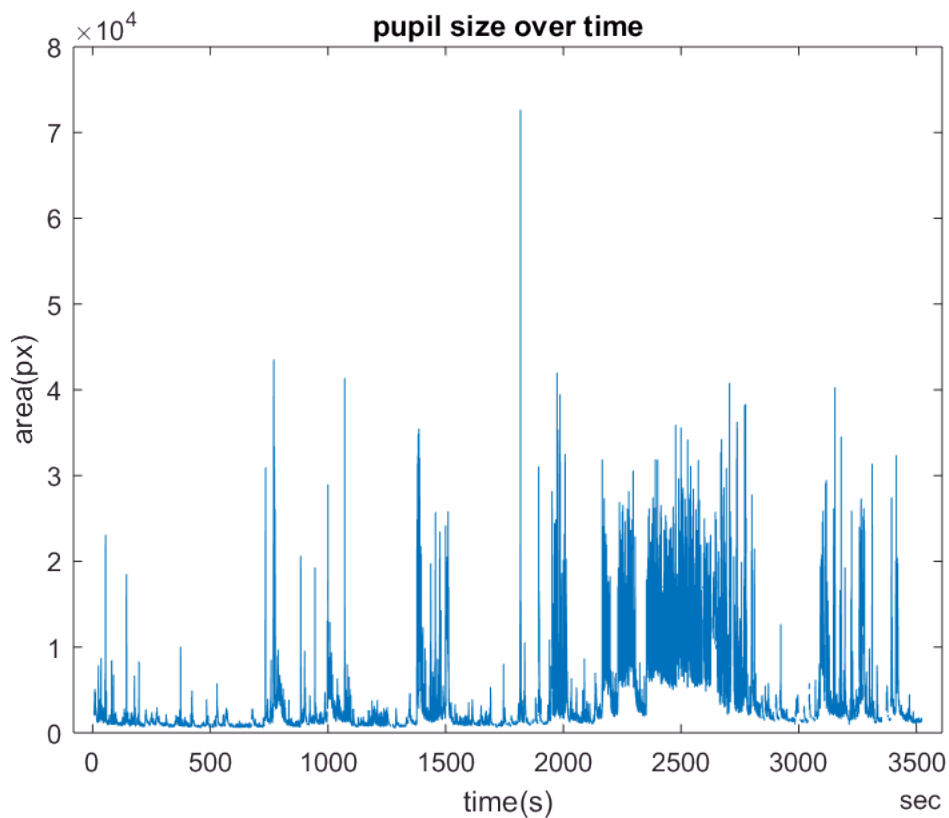
```
[time, pupilLocation] = bos1.get_pupil_location;
azimuth = pupilLocation(:,1);
altitude = pupilLocation(:,2);
plot(time, azimuth, time, altitude)
title('eye position over time')
legend('azimuth', 'altitude')
xlabel('time(s)')
ylabel('angle(deg)')
```



```
scatter(azimuth, altitude, '.')  
title('eye position scatter plot')  
xlabel('azimuth(deg)')  
ylabel('altitude(deg)')
```

```
[time, pupilArea] = bos2.get_pupil_size;  
plot(time, pupilArea)  
title('pupil size over time')  
xlabel('time(s)')  
ylabel('area(px)')
```



3) Call function `convert_fluorescence_trace_into_raster_format` to:

Convert fluorescence trace into data that is in "raster format" for further analysis

```
convert_fluorescence_trace_into_raster_format('Df0verF', bos1.sSessionInfo.id, 'drifting_grati
```

[90] cells converted into raster format.

There are [90] raster files in directory [raster\drifting_gratings\drifting_gratings_512270518].