

1. Parameters for DetectBrightPointSources()

```
%% Specify Parameters for Part 1 (DetectBrightPointSources)
%
% Parameter description
% detectionAlpha
%   - In point source (firing neurons) detection, candidate point sources
%   are tested to be detected by comparing background intensities and maximal
%   intensities at the point source.
%   - 'detectionAlpha' defines the significance threshold in such tests.
%   - A smaller 'detectionAlpha' means more strict detection criterion leading
%   to a smaller number of point sources.
%
% PSFsigma (Point Spread Function Sigma, in pixel)
%   - A cell with three or multiple 2 dim'l vectors, (sigma_XY, sigma_Z),
%   which specify the standard deviation parameters in X/Y- and Z-direction
%   of 3D Gaussian functions. The Gaussian functions are fitted to candidate
%   point sources.
%   - PSFsigma is proportional to the size or radius of point sources. Larger
%   PSFsigma is optimal for detection of bigger point sources.
%   - Typically three PSFsigma vectors are used to detect neurons with
%   different sizes. It can take any number of different sigma vectors.
%
% TopX_ThreshodForBrightness (0 < X < 1)
%   - Among detected point sources, top 100*X% of point sources in brightness
%   or point source intensity are selected as neuron firing events,
%   which are fed into the next tracking step.
%
% makeMov_MIP (true or false)
%   - Flag whether to generate two Maximum Intensity Projection (MIP) videos
%   that display the raw 3D images and detected point source on the images.
%
% figFlag ('on' or 'off')
%   - Whether to display output plots, which are always saved in the output
%   directory in .fig and .png formats.
```

2. Parameters for TrackJitteringFlickering()

```
%% Specify Parameters for Part 2 (TrackJitteringFlickering)
%
% Parameter description
% patchSizeX, patchSizeZ (in pixel)
%   - Edge size or length in X/Y- and Z-direction, respectively,
%   of a local volume around each detected point source, which is utilized
%   to track the jittering and flickering neurons via correlation-based
%   patch-matching.
%
% bandwidthX, bandwidthZ (in pixel)
%   - The radius of the local volume or 3D-patch around each detected point source.
%   It determines the patchSize via  $2 \times \text{bandwidthX} + 1 = \text{patchSizeX}$ .
%
% upbdDeformX, upbdDeformZ (in pixel)
%   - Upper bound of local deformation or jittering in X/Y- and Z-direction,
%   respectively.
%   - Over the whole time frames, we assume that neuron locations can
%   deviate from hypothetical central locations by upbdDeformX and
%   upbdDeformZ (pixels) in X/Y- and Z-direction, respectively.
%
% corrThreshold (0 < r < 1)
%   - Two or multi-dim'l vector specifying multiple thresholds of spatial
%   correlations utilized for patch-matching.
%   - As an ensemble approach, tracking neurons are implemented using each
%   correlation threshold, and then the results are integrated to account
%   for heterogeneity of local image dynamics of individual neurons.
%
% distBtwTracks_toBeMerged (in pixel)
%   - Distance between tracks to be merged.
%   - If two or multiple neuron trajectories are too close, then their
%   segmented ROIs would overlap. Even though the trajectories are correct,
%   overlapping ROIs is what we want to avoid. Thus, the algorithm computes
%   the minimum of euclidean (L2) distance between neuron locations over the
%   frames. If the minimum distance  $\leq \text{distBtwTracks\_toBeMerged}$  for two
%   neurons, then we select only one trajectory that shows a better
%   measure of tracking.
```

```
% minFramesOfFiringEvents (in # of frames)
%   - Minimal number of frames for bright point sources linked in
%     consecutive time frames to be counted as a single firing event.
%
% minFiringFramesOfNeurons (in # of frames)
%   - For a neuron trajectory over the whole time frames to be valid, it
%     should contain bright point sources at the specified minimum number of
%     frames.
%
% imgMarginSizeX, imgMarginSizeZ (in pixel)
%   - Specify a marginal area in X/Y- and Z-direction, which is
%     pre-excluded from the analysis to reduce computational time.
%   - Detected point sources in the specified marginal area will not be
%     tracked.
%
% makeMov_trackedNeurons (true or false)
%   - Flag whether to generate MIP videos displaying obtained neuron
%     trajectories (or firing events) on MIP images.
%
% closeFigs (true or false)
%   - Whether to close output plots while running because the pipeline
%     generates many figures. Output figures are always saved in the output
%     directory in .fig and .png formats. It simply executes 'close all;'
%     after saving the figures.
```

3. Parameters for SegmentDynamicROIs()

```
%% Specify Parameters for Part 3 (SegmentDynamicROIs)
%
% Parameter description
% PSFsigma_forROI (Point Spread Function Sigma, in pixel)
%   - 2 dim'1 vectors, (sigma_XY, sigma_Z),
%   which specify the standard deviation parameters in X/Y- and Z-direction
%   of 3D Gaussian functions.
%   - This sigma vector is just an initial value in fitting 3D Gaussian
%   function for neuron segmentation purpose.
%   - Set to be a more typical value among the specified PSFsigma values in
%   Part 1.
%
% levelOf3DGaussianDist_toSegment (0< X <1)
%   - To get a mask for a tracked neuron, images when the neuron is firing
%   (detected as bright point sources) are first averaged. After fitting a
%   3D Gaussian function to the averaged neuron firing image, a central X
%   portion of the Gaussian function is defined to be the mask of the
%   neuron (X = levelOf3DGaussianDist_toSegment).
%   - Larger X leads to a larger mask.
%
% levelOf3DGaussianDist_toSegment_small (0< X <1)
%   - The algorithm automatically checks and reports overlaps between the
%   neuron masks segmented using levelOf3DGaussianDist_toSegment.
%   - If some pairs of masks overlap with each other in at least 10 frames,
%   then those neurons are automatically re-segmented using a smaller threshold
%   (= levelOf3DGaussianDist_toSegment_small).
%   - After this adjustment, overlaps between the masks are reported again.
%   If it is severe, users can adjust these parameters.
%
% frameLengthForMovingMedian (in # of frames)
%   - Extracted Ca2+ activity time courses of tracked neurons (mean intensities within
%   masks) are normalized, by following  $(F - F_{base})/F_{base}$ .
%   - This algorithm utilizes moving medians as the base activity ( $F_{base}$ ).
%   - 'frameLengthForMovingMedian' specifies a time period length to
%   compute the moving medians.
```

```
% allROIsOutput (true or false)
%   - Whether or not to generate/save visualization output of maximum
%   intensity projection of averaged images of tracked neurons and their
%   segmented masks.
%   - Setting 'true' may cost some computational time for plotting.
%
% makeMov_MIPofROIs (true or false)
%   - Flag whether to generate three MIP videos visualizaing detailed
%   outcomes of the dynamic ROIs.
%
% closeFigs (true or false)
%   - Whether to close output plots while running because the pipeline
%   generates many figures. Output figures are always saved in the output
%   directory in .fig and .png formats. It simply executes 'close all;'
%   after saving the figures.
```

4. Parameters for VisualizeActivityMapMasksIndexedByHCL()

```
%% Specify Parameters for Part 4 (VisualizeActivityMapMasksIndexedByHCL)
%
% Parameter description
% activityWeightedByImgCorr (true or false)
%   - Whether to apply a weighting scheme when collecting calcium
%     activities from the ROIs. If true, spatial correlation-based weights
%     are multiplied to normalized calcium activities to reduce spurious
%     peaks in the signals.
%
% upSamplingFactor_forMaskContourImg (>= 1)
%   - Specify an image-resizing factor to visualize the masks of tracked
%     neurons, particularly when neuron masks are too small for contour
%     visualization.
%   - The mask contours when the neurons are firing are visualized on top
%     of maximum intensity projection images.
%
% makeMov_ROIsAtHighActivities (true or false)
%   - Flag whether to generate a MIP video displaying ROIs. ROIs are shown
%     only when activities are determined to be high by K-means clustering.
%
% makeMov_ROIsFiringAnnotated (true or false)
%   - Flag whether to generate a MIP video displaying ROIs. ROIs are shown
%     only when ROIs are determined to be firing via spatial correlation.
%
% makeMov_singleROIs_AllFrames_firingAnnotated (true or false)
%   - Flag whether to generate all of the single ROI local MIP videos. They
%     display all time frames and ROIs are shown when they are determined to
%     be firing.
%
% makeMov_singleROIs_Snapshots_atHighActivities (true or false)
%   - Flag whether to generate all of the single ROI local MIP videos. They
%     display a subset of time frames when the target ROI's activities are
%     determined to be high by K-means clustering.
%
% closeFigs (true or false)
%   - Whether to close output plots while running because the pipeline
%     generates many figures. Output figures are always saved in the output
%     directory in .fig and .png formats. It simply executes 'close all;'
%     after saving the figures.
```

5. Parameters for DetermineResponsiveness()

```
%% Specify Parameters for Part 5 (DetermineResponsiveness)
%
% Parameter description
% stimulationFile
%   - A file path and name for the stimulation information (.csv file). The file
%     is typically located at the same folder with the movieData object (.mat)
%     file.
%
% stimulusInterval (in # of frames)
%   - A time interval (in frames) between consecutive two different stimuli.
%
% negCtrlStimulusName
%   - 'stimulusLabel' for a negative control condition. To determine the
%     responsiveness, the statistical test requires a negative control.
%
% activityWeightedByImgCorr (true or false)
%   - Whether to analyze 'weighted' or 'unweighted' calcium activities.
%   - This option depends on which type was specified in Part 4
%     (VisualizeActivityMapMasksIndexedByHCL).
%
% make_AllTSPlots (true or false)
%   - Flag whether to generate time series plots for all ROIs.
%
% make_BoxplotsOfTtests (true or false)
%   - Flag whether to generate boxplots of t-tests for all ROIs.
%
% make_AllResponseCurves_perStimulus (true or false)
%   - Flag whether to generate plots of across-trial response curves and their mean
%     curves per-stimulus for all ROIs.
%
% makeROIIMovieWithStimulusLabel_atHighActivities (true or false)
%   - Flag whether to generate a MIP video displaying ROIs and stimulation
%     status. ROIs are shown only when activities are determined to be high
%     by K-means clustering.
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% makeROIMovieWithStimulusLabel_firingAnnotated (true or false)
%   - The same as the above except that ROIs are shown only when ROIs are
%     determined to be firing via spatial correlation.
%
% makeAllFramesOfSingleROIs_firingAnnotated (true or false)
%   - Flag whether to generate all of the single ROI local MIP videos that
%     also show stimulation status. The MIP videos display all time frames
%     and ROIs are shown when they are determined to be firing.
%
% makeSnapshotsOfSingleROIs_atHighActivities (true or false)
%   - Flag whether to generate all of the single ROI local MIP videos that
%     also show stimulation status. The MIP videos display a subset of time
%     frames when the target ROI's activities are determined to be high by
%     K-means clustering.
%
% FalseDiscoveryRate_threshold
%   - The level of significance for adjusted P-values.
%
% thresholdForMinimalNumberOfNeuronsForEachStimulusCombination (X: integer)
%   - After t-tests and FDR controls, we select significant stimulus
%     subsets that activated at least a certain number of neurons (= X). See
%     the paper.
%
% numNaNRows_btwnNeuronClusters
%   - When mean activities per stimulus are visualized along with
%     identified stimulus subsets, numNaNRows_btwnNeuronClusters specifies
%     empty spaces between the stimulus subsets for visualization purposes.
%
% closeFigs (true or false)
%   - Whether to close output plots while running because the pipeline
%     generates many figures. Output figures are always saved in the output
%     directory in .fig and .png formats. It simply executes 'close all;'
%     after saving the figures.

```