Ca^{2+} imaging (MATCa) toolbox manual

WORKFLOW
ΨANNOTATIONS: Built-in and custom functions are expressed in Matlab command window coupled with ">>" sign. Workflow notations are consistent with Matlab.]

1. Set matlab working path for the MATCAImage toolbox (One-time only).

    I. >>addpath(uigetdir); %Find the downloaded toolbox folder (Not saved)
    II. >>savepath(); % stored for future sessions.

2. Create you cell_coordinates file (-.mat or –ascii format)
   >>cell_centroids()
    I. Select your reference image path (pop-up).
    II. Enter the desired image range to extract.
    III. Visual check the centroids for entered cell sequence.

    ‡User must select a reference image path that is consistent with other image file names and ends with no numerical parameters. Such as 'hc_images' for hc_image1, hc_images2..., etc. is good but not 'hc_images1'. Also, make sure image folder does not contain any hidden files.

3. Load your data. Here you can apply the sampling rate as well as limits time and cells to be analyzed. Accepted formats are '.xls', '.csv', '.mat', '.txt','-ascii'. Data will be segmented in row-wise (cell) and column-wise (time). 'File directory', 'format' and sampling rate are required inputs. (Add instructions on how to quickly get file directory and format)
   >> [newdata]=dataread(sampling rate,'','','','');
   #Locate your data in the pop-up screen.
   if you would like to limit your time or cells analyzed use this:
   >>[newdata]=dataread(sampling rate, time start, time end, cell start, cell end);
   Example:
   >>[newdata]=dataread(5,300,400,3,256);
   #this will load a 100 sec segment with 254 cells.

   ‡User must use the time entries in seconds.

4. Automated raster, peri-event time, histogram and functional cell calculations.

‡ Dependency: dataread.m

‡ Recommended dataset: Events and cell images.

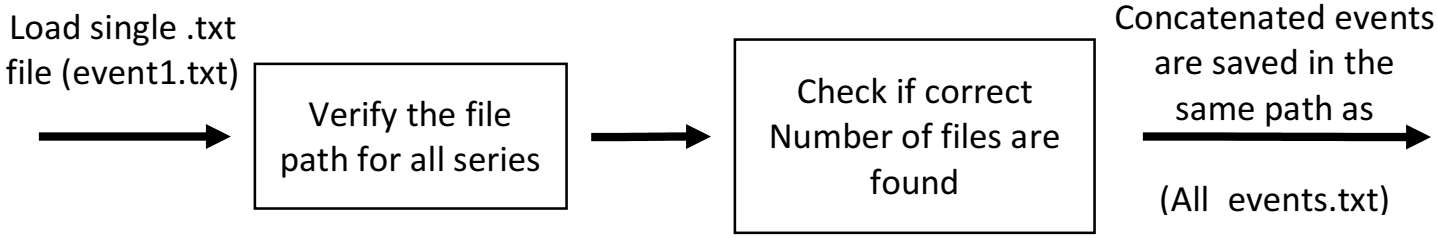
I. Run **>> RasterPlot(sampling rate, start time, end time, first cell, last cell) % Time in seconds. Cells must be in columnar order in the excel or text file. Example:**
   i. **RasterPlot(5,200,600,2,100,5);**
II. Select [Events] or [Events + Transients] (Transients will be used on the original plot only).
III. Locate the dataset(s). Transients are first if user preferred both data.
IV. Events will be used on peri-event and histogram calculations. (Transients will be used on the original plot only).
V. User will be asked for image deposition (Y/N). If yes:
   i. Locate a single image file; e.g. 'ic.tif'
   ii. Program requires non-integer ending on the file path. Thus, user has to confirm the entered image path is correct. For example ('ic2.tif'→ Correct as 'ic')
VI. Illustration of (Original data in cell by time sorting, peri-event, ∑event histogram with respect to entered bin width and functional cell map.

‡ Color code for the functional cell map will be equivalent of #of events/cell for the given time course (start - end time).

5. Concatenation of text files (Events)

>>concatenate_events()

%If events were saved in separate txt files, user can use this to concatenate them in a single text file.



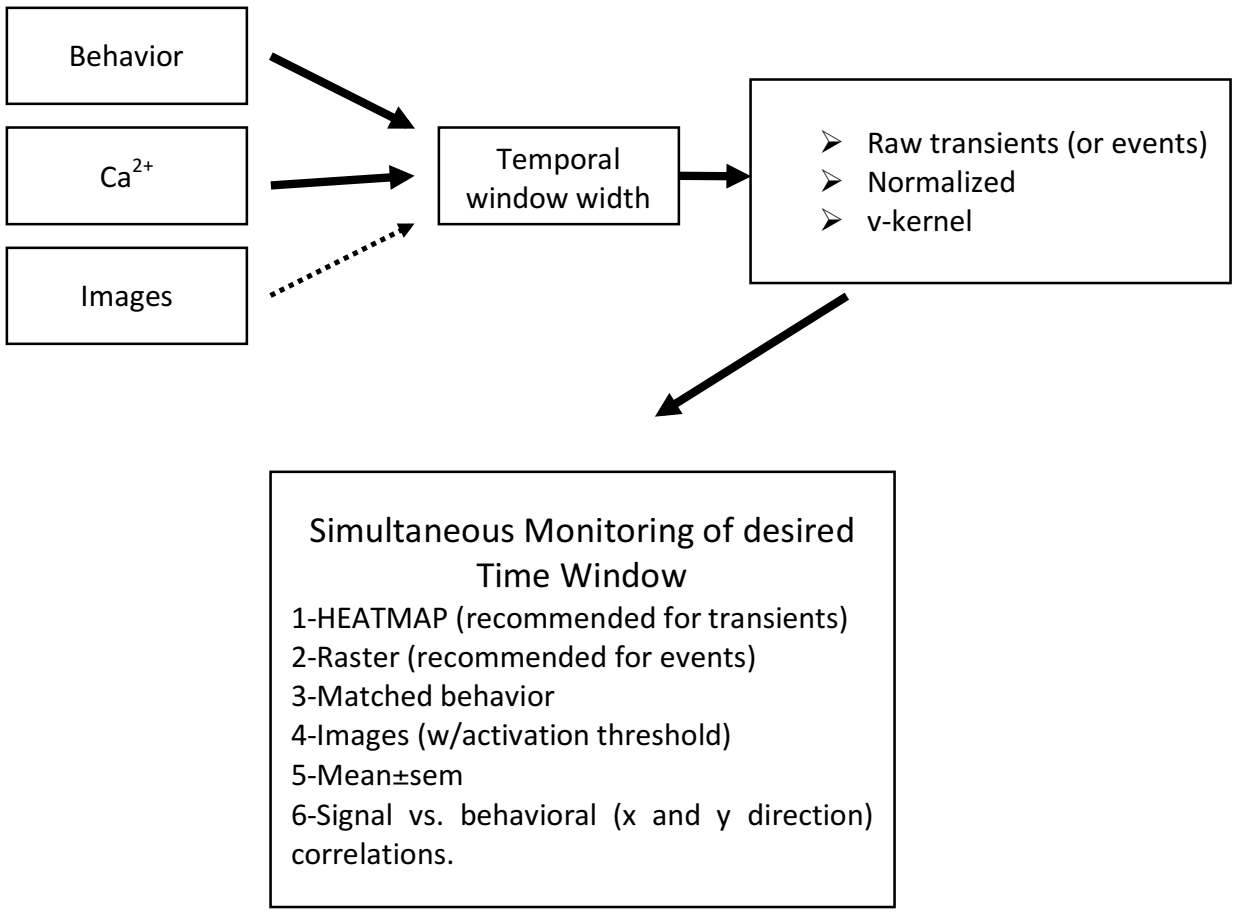
6. Functional Imaging

‡Dependencies: dataread.m, Conv.kernel.m

‡ A computer with high memory and fast processor is recommended.

User can load a matching behavioral and calcium dataset to analyze desired time window(s) by a 'slide' cursor. Users also have option to load actual images (.tif,.tiff or .jpeg) option for users to monitor active cell(s) in the same time course. In addition to raw format (transients or events), core data can also be analyzed via normalized amplitudes or v-Kernel transformations. Population activity as a function of user-defined time window is served to determine a behavioral relationship with the calcium data.

Example:



STEPS:

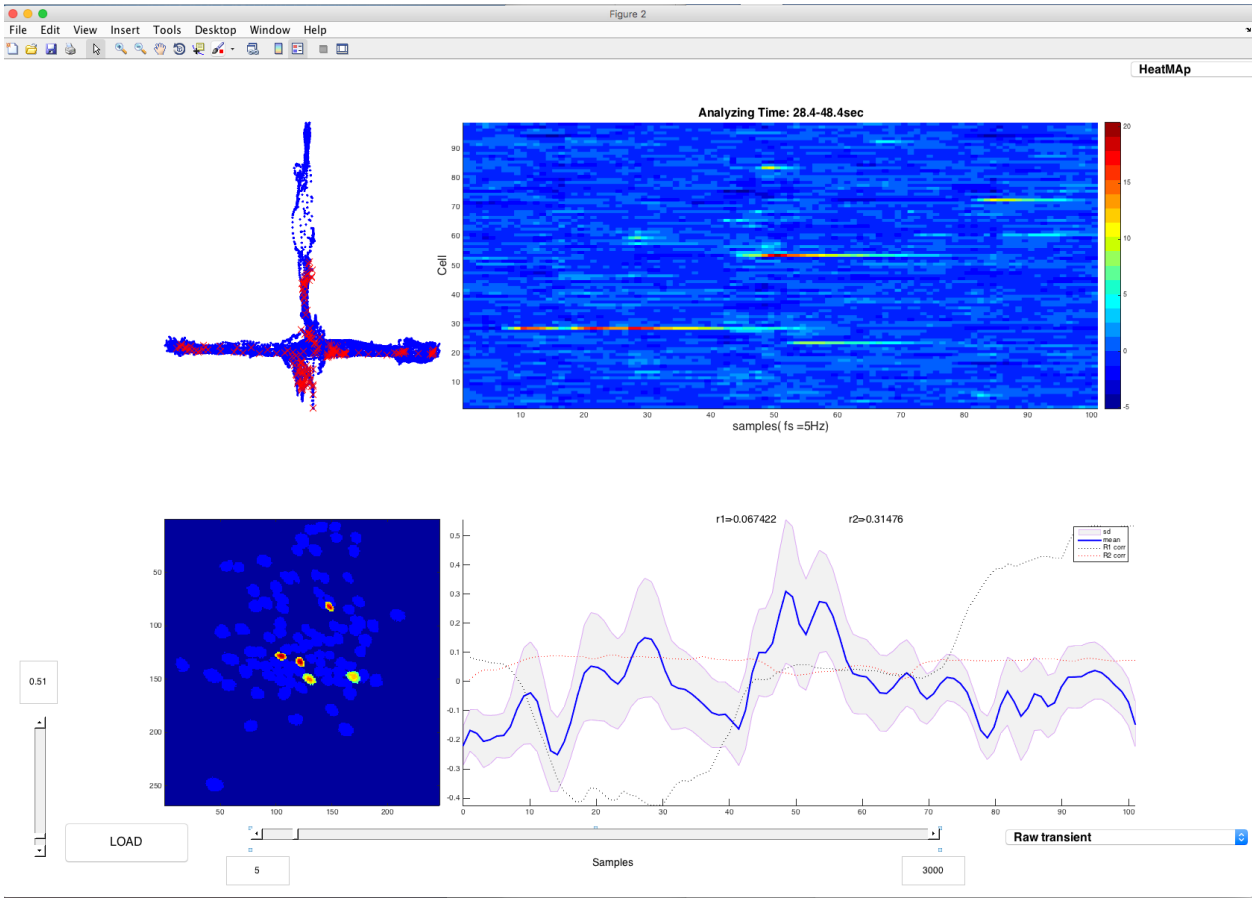
1. Make sure you have access to server or your local folder that contains core and dependencies.
2. User must have following datasets:
    I. Calcium data (transients are recommended)
    II. Matched behavior (Excel sheet with N(time) X M(different tracking points)
    III. (Actual cell images are optional)

3. **>>CalciumBehaviorPair(5,10,1,600,2,100,3,4);**
   % CalciumBehaviorPair('Ca^{2+} sampling rate ','Video sampling rate', 'start time, 'end time', 'first cell', 'last cell', 'behavior_x','behavior_y')
4. Click LOAD button → First, locate your behavioral data then calcium data
5. Enter a bin width (in seconds) in the pop-up window.
6. Select (yes/no) for images deposition.
    a. Click a single image file in your image file
    b. ‡ Make sure path of the images are ending without a number!!!
7. Select a desired data format from the tab-down window at the bottom right of the GUI (Raw, normalized or v-kernel)
8. If there is no error;
    a. Click sliding cursor towards right.
    b. You should see calcium and behavioral datasets for the set time window by multiple drawings.
    c. Make sure your sampling rate and analyzed time indicator matches.
    d. Change your Heatmap to Raster if your calcium data is event data.
    e. You can also change the data format from raw⇔normalized⇔v-kernel.
    f. Pull up your activation threshold cursor(bottom left) if you have images.

9. Monitor the correlation changes between calcium data vs. x-direction (behavior-1) and y-direction (behavior-2).

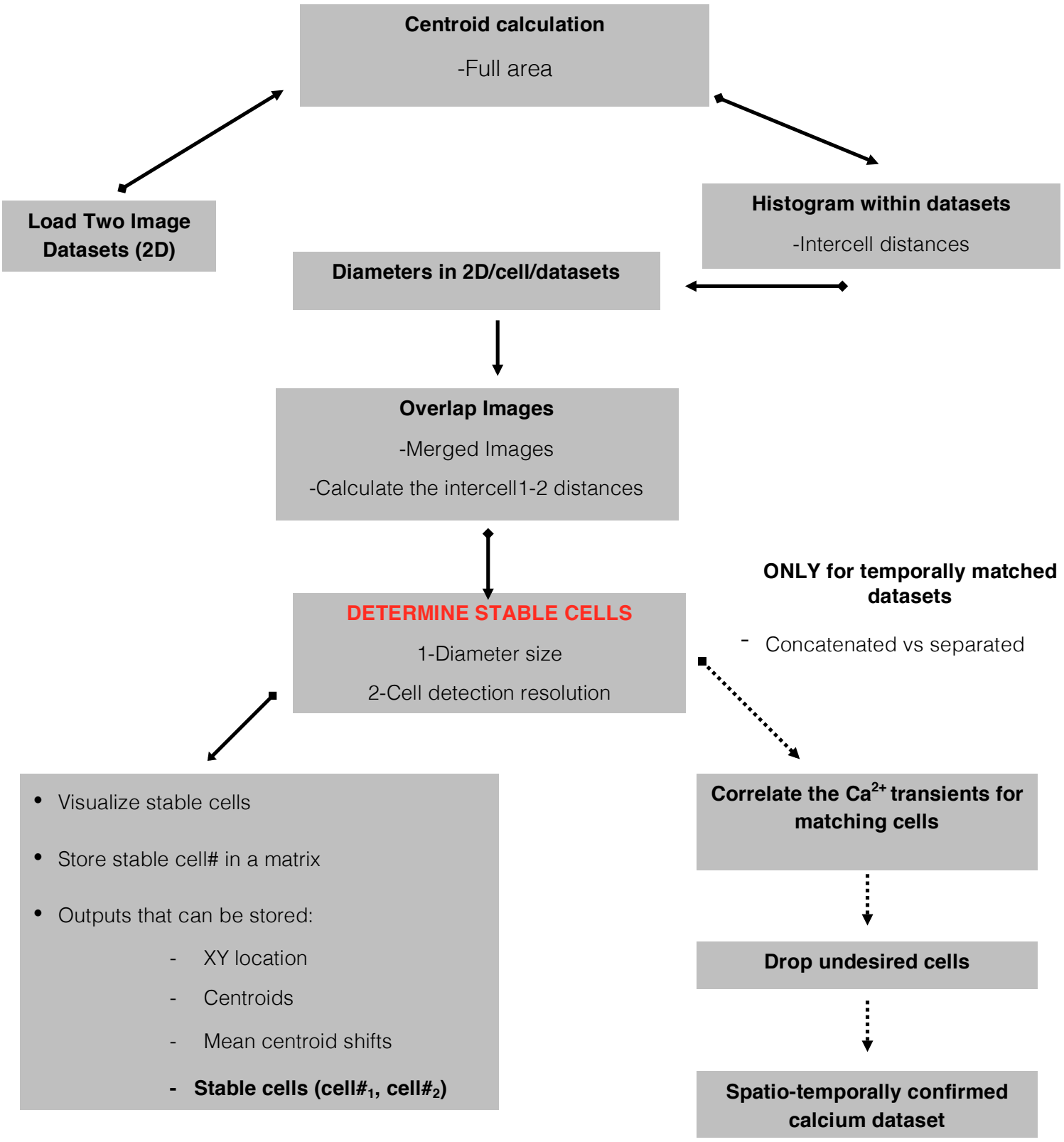
‡ If all features are included in GUI, an average computation step is <200ms with a high-speed computer.

A snapshot for an example computation:



7. Stable Cell imaging

- Find the identical cell population imaged in two different sessions.
- Determine cell centroids in two different imaging sessions.
- N of stable cells from first to second session.
- Centroid shifts with actual pixel values
- Manual or automated qualification for shifts.
- Option to include Ca^{2+} dynamics.


Centroid calculation
-Full area
Load Two Image Datasets (2D)
Histogram within datasets
-Intercell distances
Diameters in 2D/cell/datasets
Overlap Images
-Merged Images
-Calculate the intercell1-2 distances
DETERMINE STABLE CELLS
1-Diameter size
2-Cell detection resolution
ONLY for temporally matched datasets
- Concatenated vs separated
• Visualize stable cells
• Store stable cell# in a matrix
• Outputs that can be stored:
- XY location
- Centroids
- Mean centroid shifts
- Stable cells (cell#1, cell#2)
Correlate the Ca2+ transients for matching cells
Drop undesired cells
Spatio-temporally confirmed calcium dataset

8. Calcium dynamics
   Goal: Calcium transients are exponential decay like waveforms that are often rated as only peak values with a threshold (s.d.) or normalization (df/f). Despite the mean lifetime of a transient is generally centered around the peak values, prolonged activations (bursts) can be underrated by discarding the temporal content. To complete the information, we calculate the envelopes of transients and compared it with the peak values of each transient (this part not has not been added yet).

   Usage:
   1->>ClassifyWave (sampling rate,'start_time','end_time','first_cell','last cell'); %basically same with dataread() function.
   2-Select a desired data file.
   3-Move top left cursor to select a single cell response (each cell will be analyzed separately).
   4-Adjust the middle cursor from sharp to smooth depending on peak intervals (sharper→closer to original, smoother→more LP filtered)
   5- In order to qualify only interested peaks, amplitude and time width entries must be entered. Time width determines the required time interval between qualified peaks in terms of seconds. For instance, if you enter 2 sec and there is bursting activity with same amplitude within 2 seconds, only first peak will be rated (Envelope will contain the entire information though).
   6- Enter Threshold button.
   7-Return to cell# cursor to select another cell and follow the same workflow.

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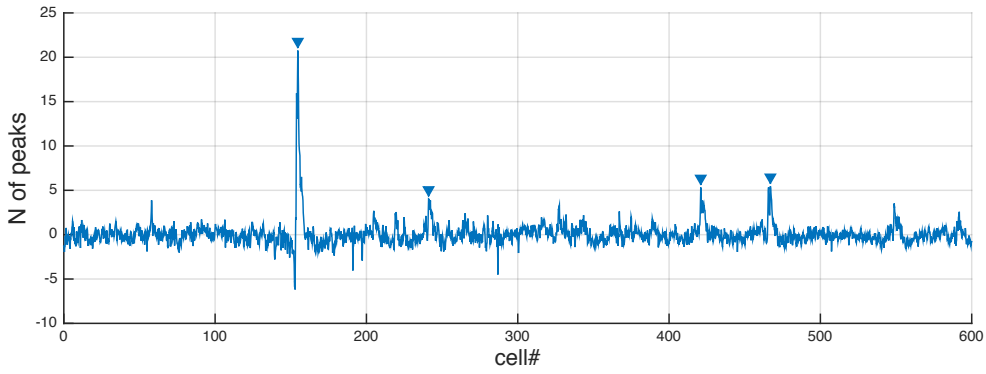
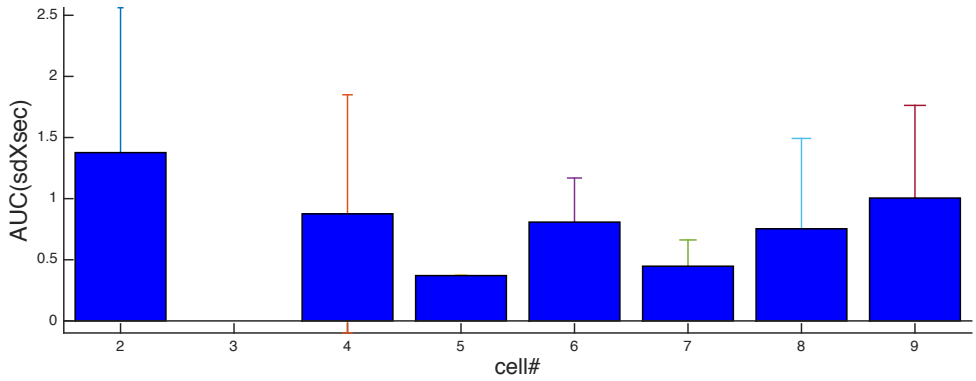


Sharp

Smooth



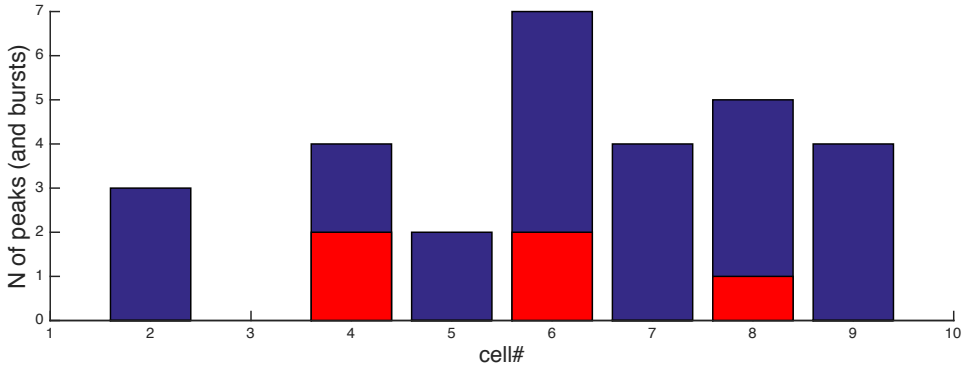
Amp

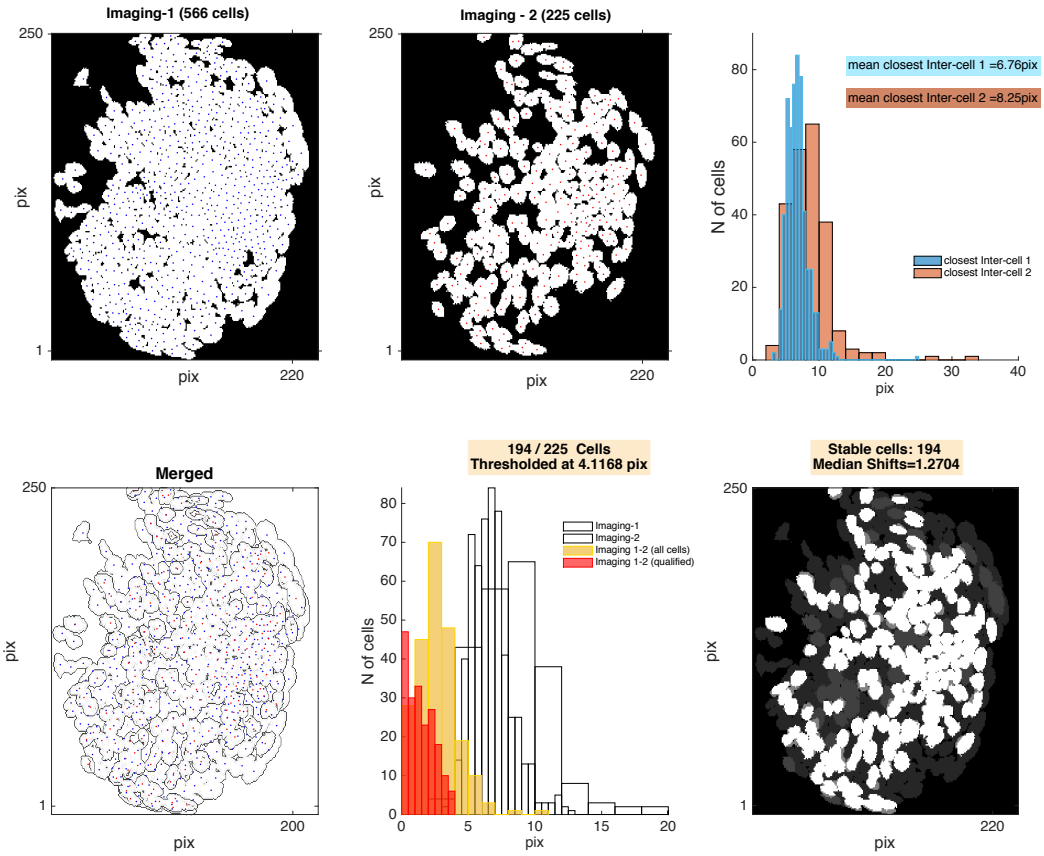
Widt
h

6

1

Threshold




Imaging-1 (566 cells)
Imaging - 2 (225 cells)
mean closest Inter-cell 1 =6.76pix
mean closest Inter-cell 2 =8.25pix
closest Inter-cell 1
closest Inter-cell 2
N of cells
pix
Merged
194 / 225 Cells
Thresholded at 4.1168 pix
Imaging-1
Imaging-2
Imaging 1-2 (all cells)
Imaging 1-2 (qualified)
Stable cells: 194
Median Shifts=1.2704