

Databinge: CHIPS for sub-cellular analysis of Ca²⁺ signals

Jeff, 2019 Mar 01

Upcoming databinges...

- Mar 1 - Jeff: CHIPS
- Mar 8 – Aeneas: Serial-Scanning Laser for High-Throughput Optogenetic Stimulation
- Mar 15 – Tristan: Comprehensive Imaging
- Mar 22 – Peter: Meeting report on “Designing and using advanced multiphoton imaging systems in neuroscience”
- Mar 29 – Warren: Jess & the Simple Western platform: why you should (almost) never have to perform a traditional western blot ever again.
- Apr 5– Claire: Jupyter and binder
- Apr 12– Chris: Optical Sensors for Metabolism
- Apr 18 (Thursday) – Teri: Mesoscale Calcium imaging analysis

- Thanks everyone, please let me know if you want to present in the later part of April.

For Neuronal firing:

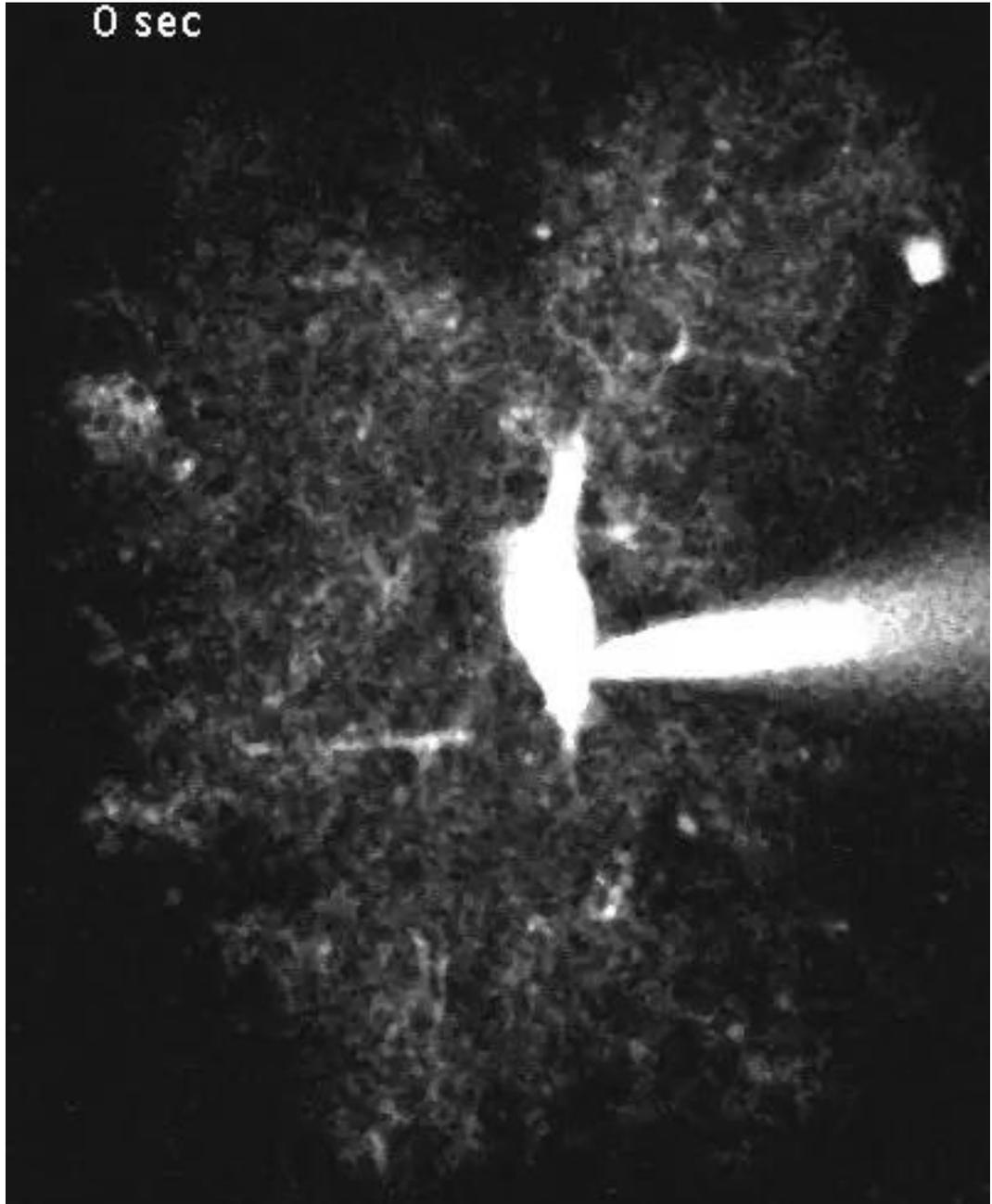
- <https://github.com/cortex-lab/Suite2P>
- Pachitariu et al. “Suite2p: beyond 10,000 neurons with standard two-photon microscopy” bioRxiv 061507
- Excellent topic for future db

Calcium signals in astrocytes.

- Bulk loading with organic dyes labelled soma and main processes.
- Ca^{2+} signals in the fine processes were unknown until GECIs could be expressed in astrocytes, which revealed compartmentalized calcium signals.
- In the MacVicar lab, dye loading Fluo4 via a patch pipette reveals similar signals.

Example movie

- Rungta et al. “Ca²⁺ transients in astrocyte fine processes occur via Ca²⁺ influx in the adult mouse hippocampus” 64 (2016) 2093.

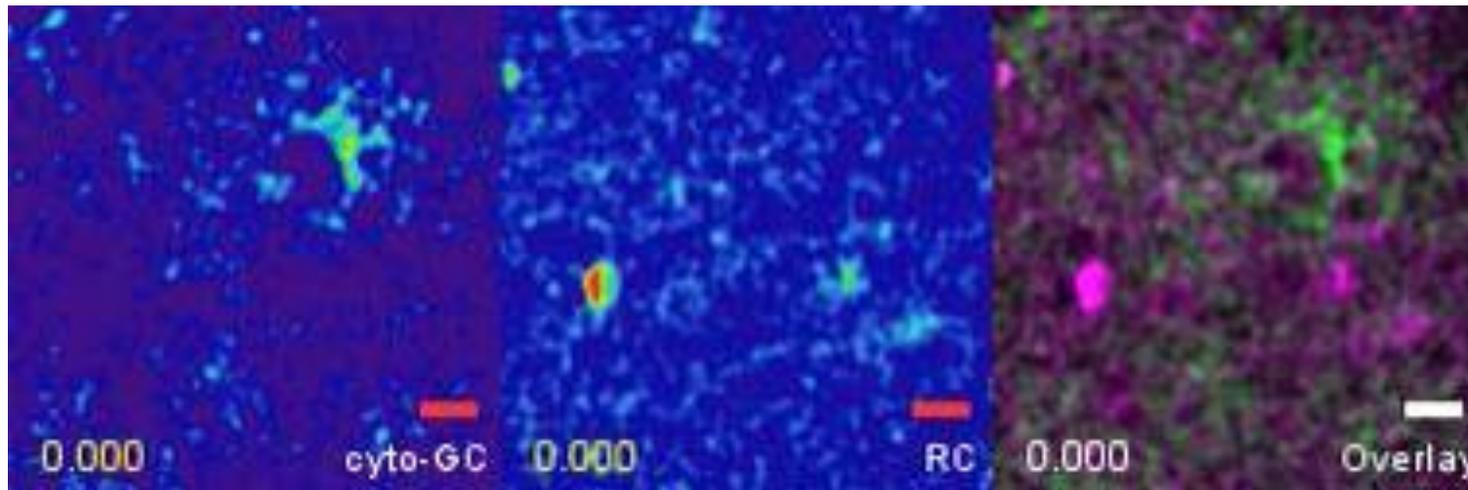


Analysis of the signals.

- Most of the early analysis was done by selecting active regions in ImageJ and then exporting their time courses and quantifying the calcium transients in various ways to assess the impact of any interventions on frequency, amplitude, duration, etc of the peaks.
- Obviously this is time consuming.
- First matlab code was written to handle the quantification of the transients and later to identify active regions.
- Active regions were identified by having a high standard deviations and we would typically rank by activity and study the 50 or 100 most active microdomains.
- ROI's were square, and could be controlled to be separated by a certain number of pixels, but the shape was not adaptive which is advantageous to cover different parts of the astrocyte particularly end feet.

Recently new analysis tools became available...

- Stobart et al. "Cortical Circuit Activity Evokes Rapid Astrocyte Calcium Signals on a Similar Timescale to Neurons" 98 (2018) 726.
- Makes use of a matlab based package called CHIPS to analyze similar signals from astrocytes in awake mice that receive sensory stimulation.
- Initially enthusiastic about this simply because it allowed for identification of arbitrary shaped ROIs



CHIPs: Cellular and Hemodynamic Image Processing Suite

- Available on github: <https://ein-lab.github.io/2p-img-analysis/doc/md/>
- Simple matlab package installer for >2014a
- Allows direct importing of the lsm file from the Zeiss system via bioformats toolbox.
- Does x-y alignment as well as the best imagej plugin.
- Originally published in this paper: Barrett et al. "CHIPS: an Extensible Toolbox for Cellular and Hemodynamic Two-Photon Image Analysis" 16 (2018) 145
- Has code to:
 - characterize blood vessel diameter from line scans or cross-sections
 - RBC blood flow measurements
 - Cellular activity
- Algorithm which detects the Ca²⁺ signals is from another investigator.

FLIKA: YAIAP

- Another project on Ca²⁺ signal analysis: <http://flika-org.github.io>
- Originally published here: Ellefsen et al. “An algorithm for automated detection, localization and measurement of local calcium signals from camera-based imaging.” *Cell Calcium*. 56 (2014) 147.
- Clustering method updated and used currently in this preprint: Ellefsen et al. “Myosin-II mediated traction forces evoke localized Piezo1 Ca²⁺ flickers” bioRxiv: 294611
- This is the method referred to as FLIKA in CHIPs.
- It is based on the clustering algorithm presented in Rodriguez and Laio “Clustering by fast search and find of density of peaks” *Science* 344 (2014) 1492.

Rodriguez and Laio Science 2014 clustering method

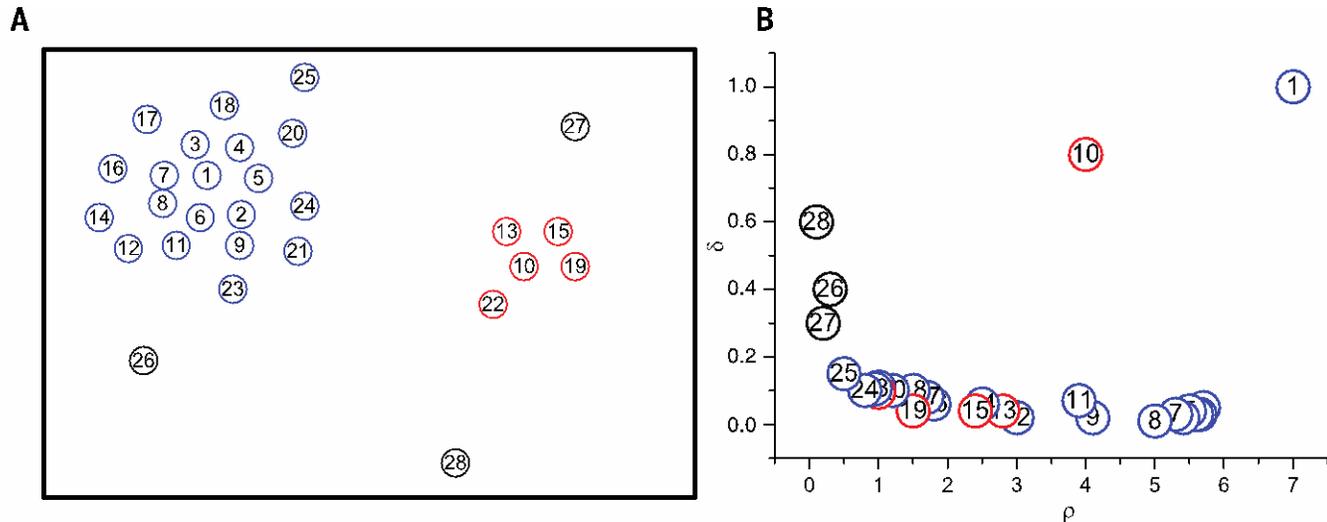
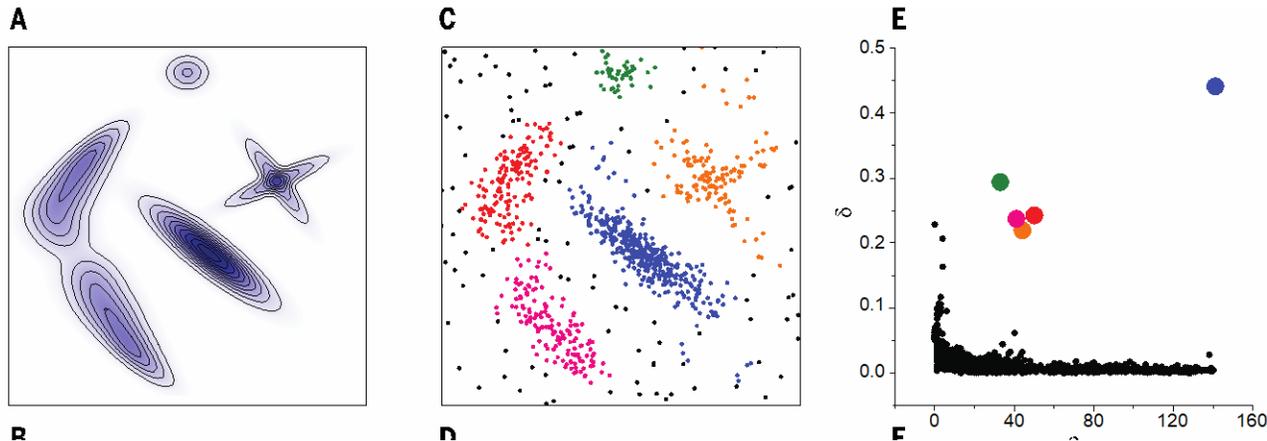


Fig. 1. The algorithm in two dimensions. (A) Point distribution. Data points are ranked in order of decreasing density. (B) Decision graph for the data (A). Different colors correspond to different clusters.

- Identifies the number of clusters
- Assumptions made:
 - cluster centers are surrounded by neighbors with lower local density
 - they are a relatively large distance from any points with higher local density.
- For each point compute rho (the local density) and delta (the distance to the point with higher local density)
- Plot this to get the decision graph (B)
- After finding cluster centers each remaining point is assigned to the same cluster as its nearest neighbor of higher density.
- Reliability of cluster assignment is established by defining a cluster border: What points are within a distance cutoff of points in another cluster?
- Find highest density within the border and use this to define the core VS halo.



Do examples

- Interactive loading of lsm
- Non-interactive with saving of plots/data
- Ali iglusnfr?