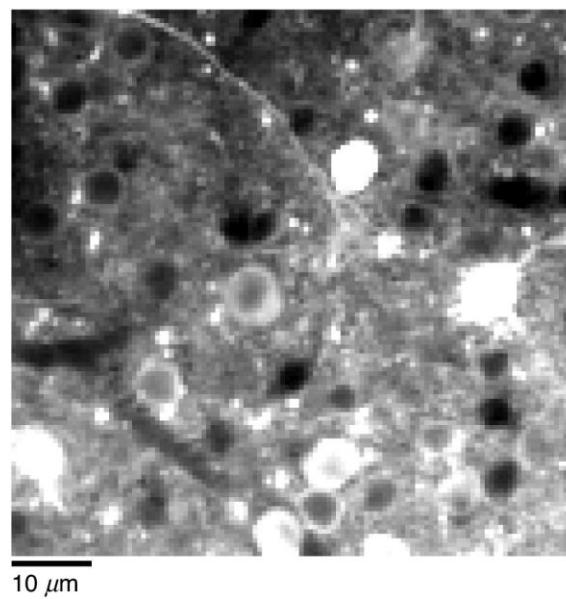


Cell detection

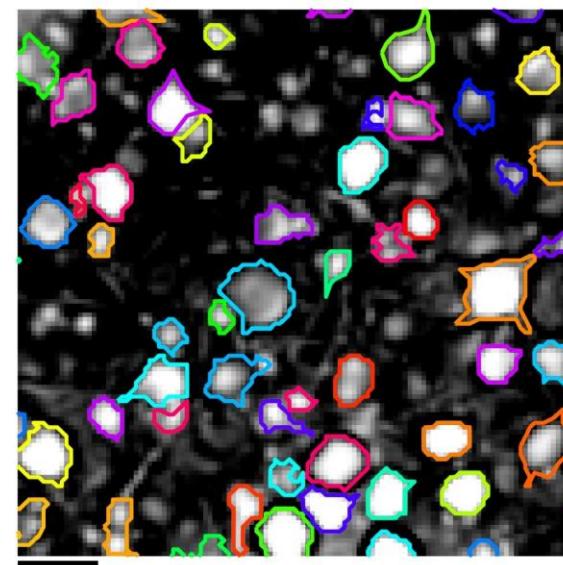
Marius Pachitariu



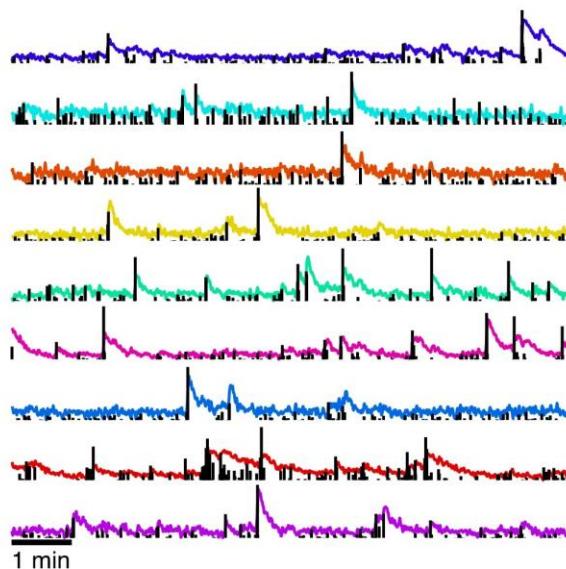
Step 1. Motion registration



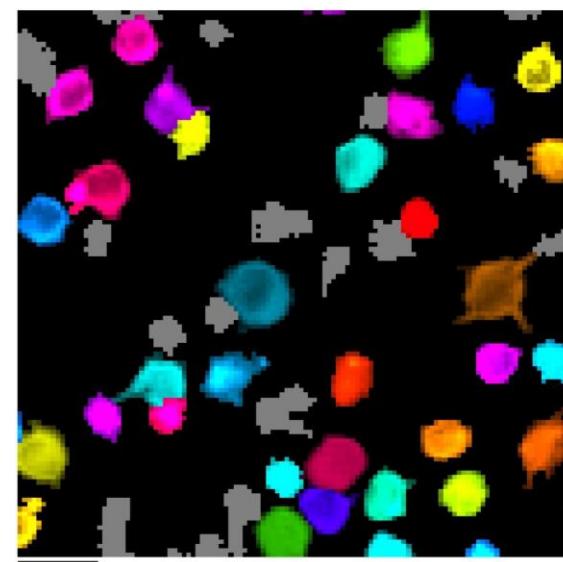
Step 2. ROI extraction

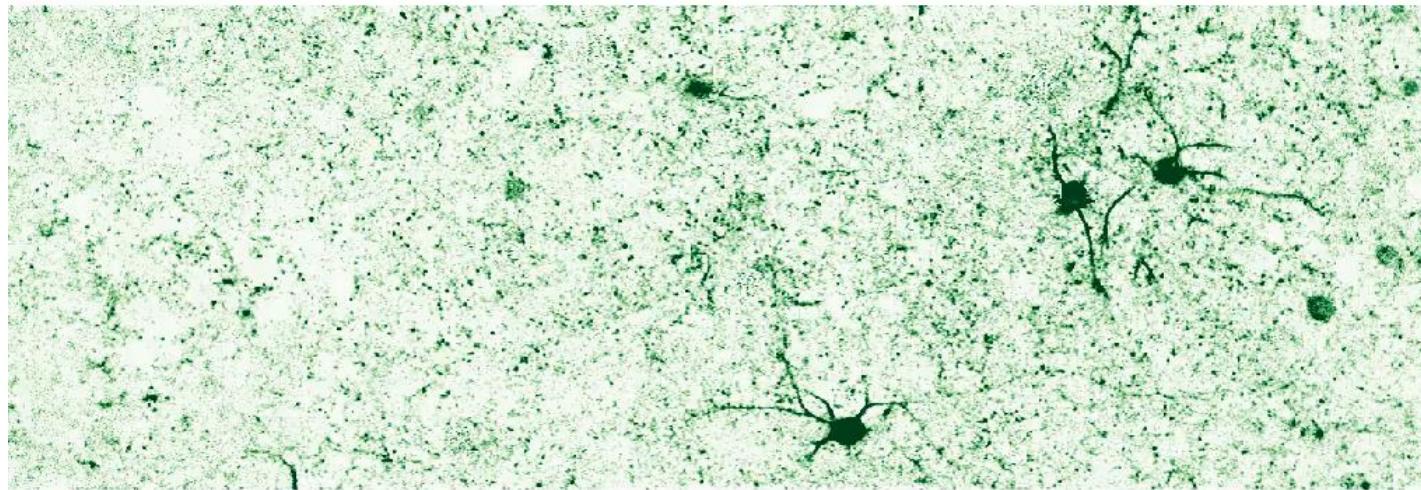


Step 3. Spike deconvolution

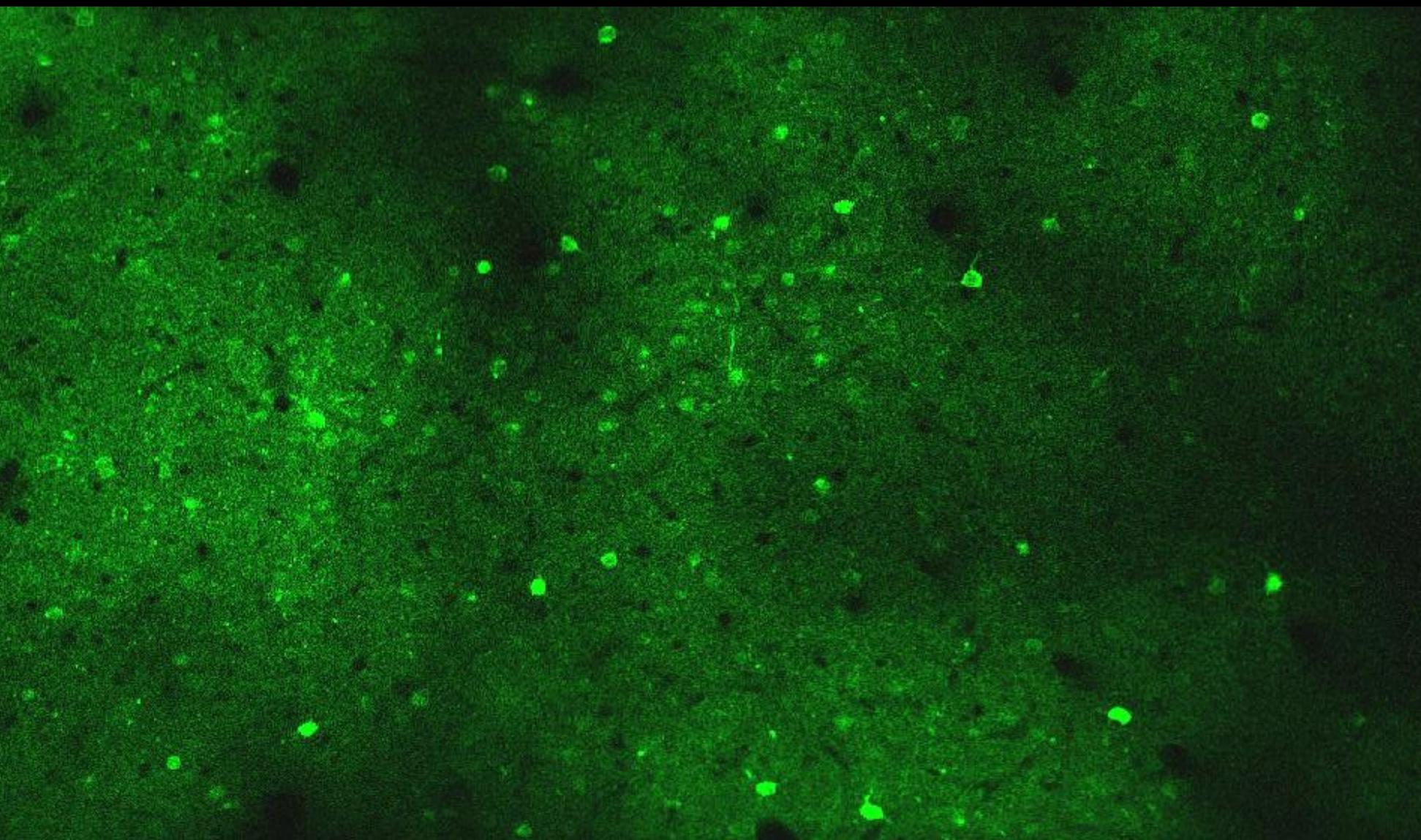


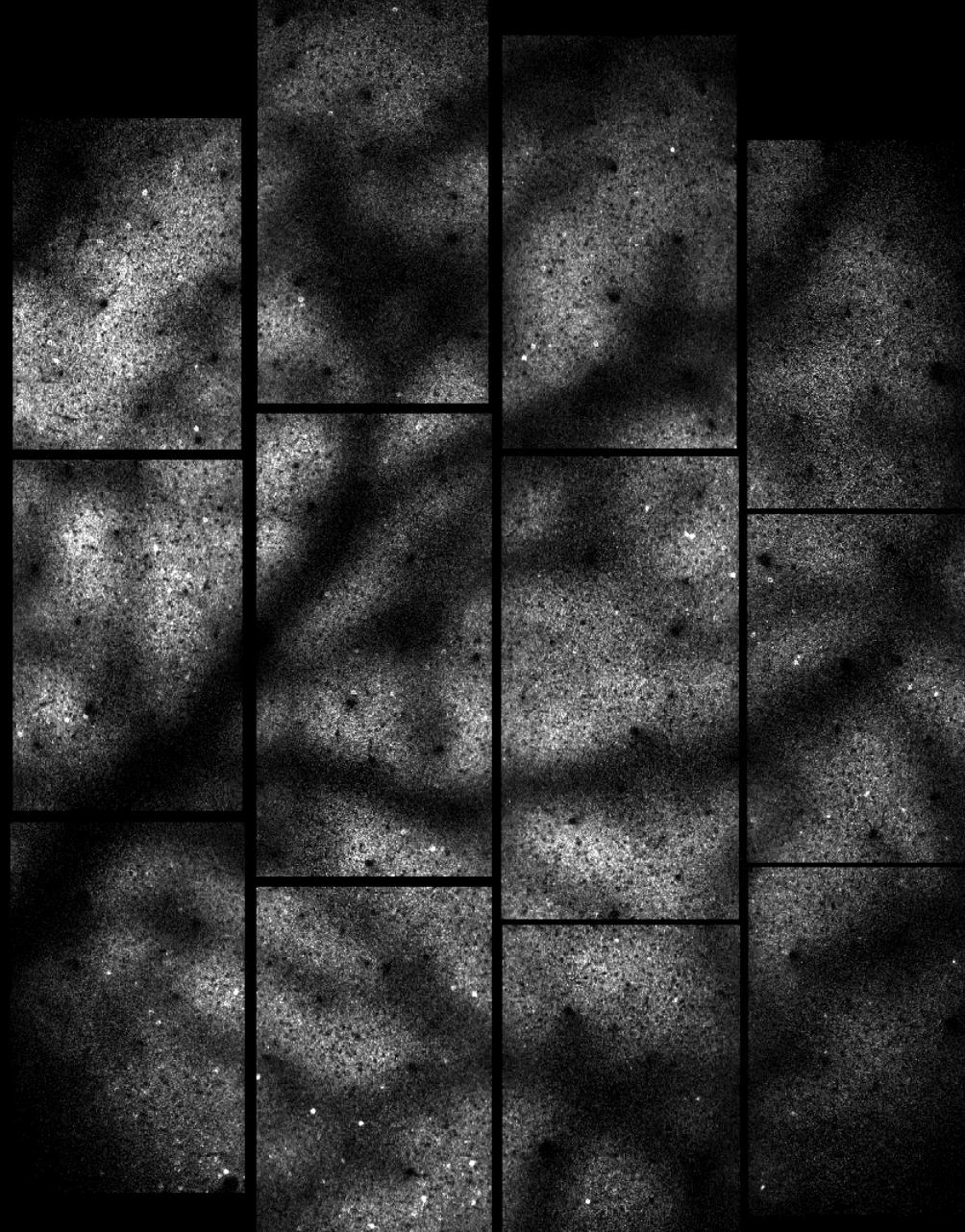
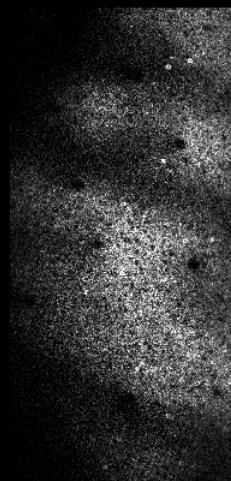
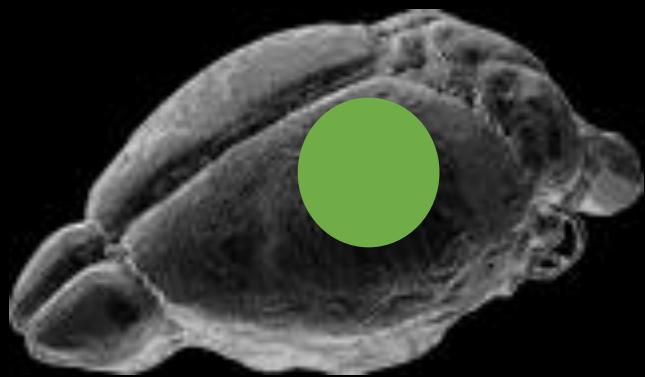
Step 4. Quality control

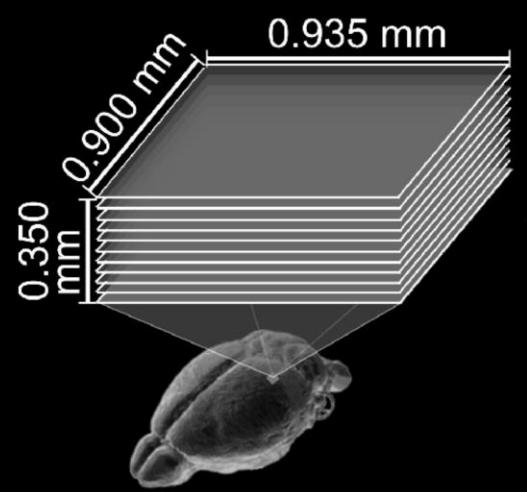




This is the movie you will be using



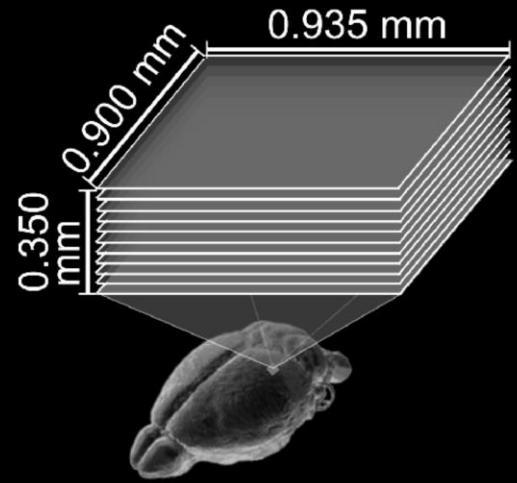




slow acquisition
(3Hz)

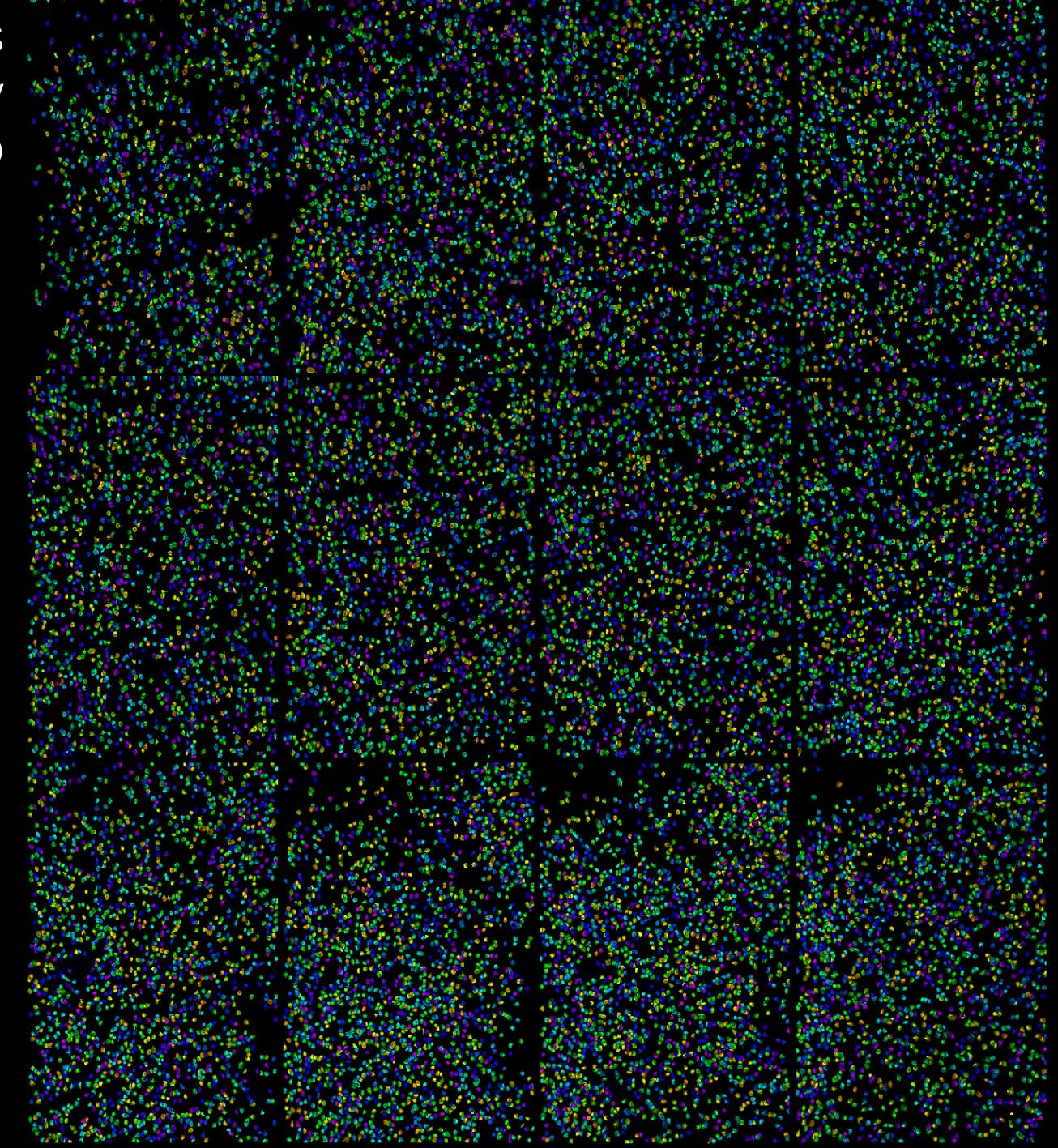
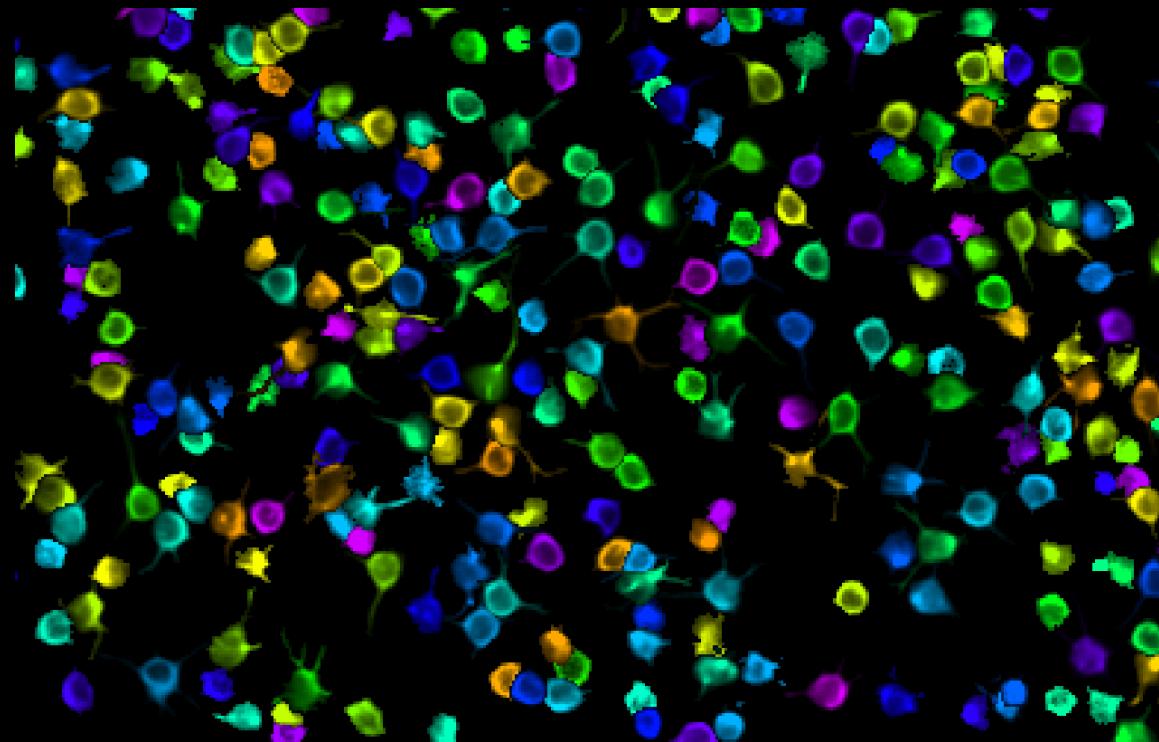
transgenic
gcamp6 mice

visual cortex



~20,000 cells
found by
Suite2p

zoomed in



Some ideas for how to detect cells

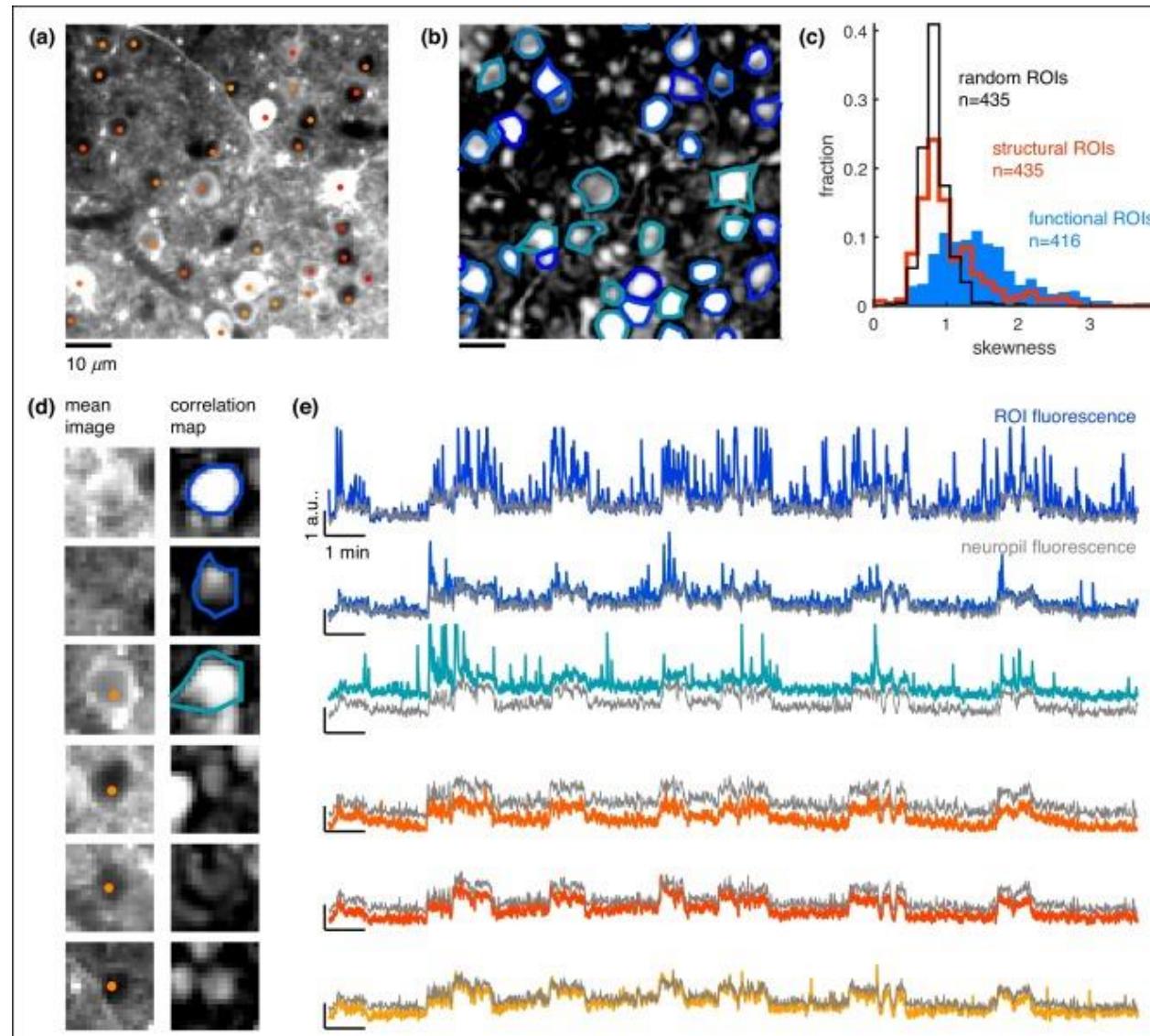
STRUCTURAL

- mean image
- nuclear tag

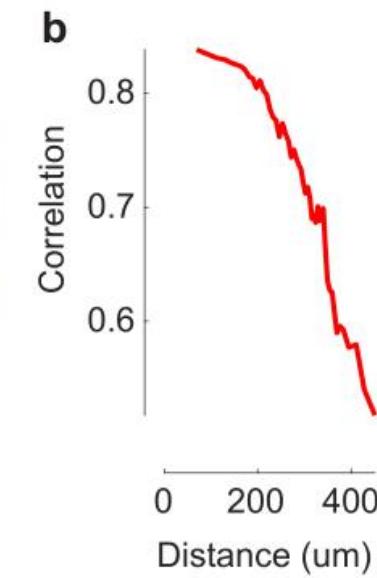
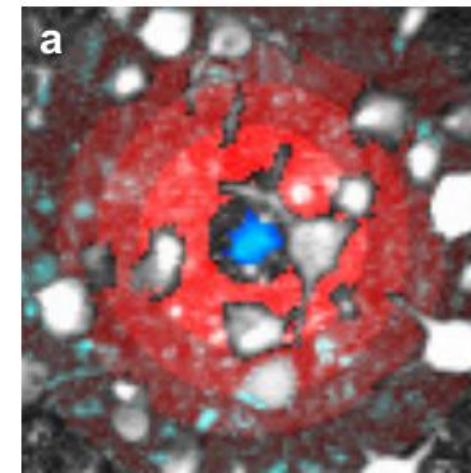
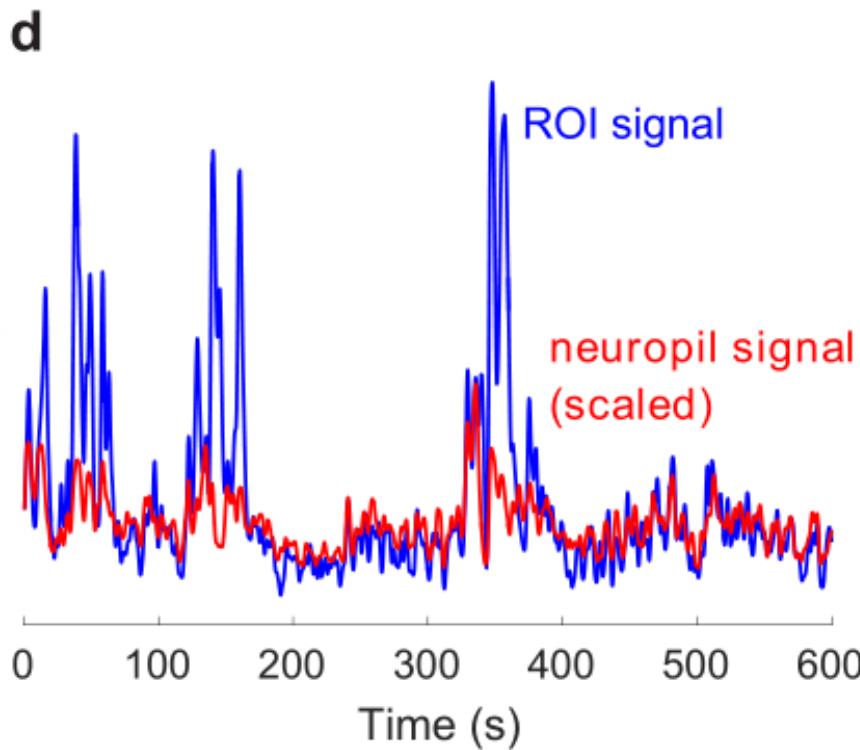
FUNCTIONAL

- using a single image
 - pixel correlation map
 - variance, skewness, max
- full generative model
 - how did the raw data arise?
 - CNMF and others
- partial generative
 - impose the important constraints only
 - Suite2p

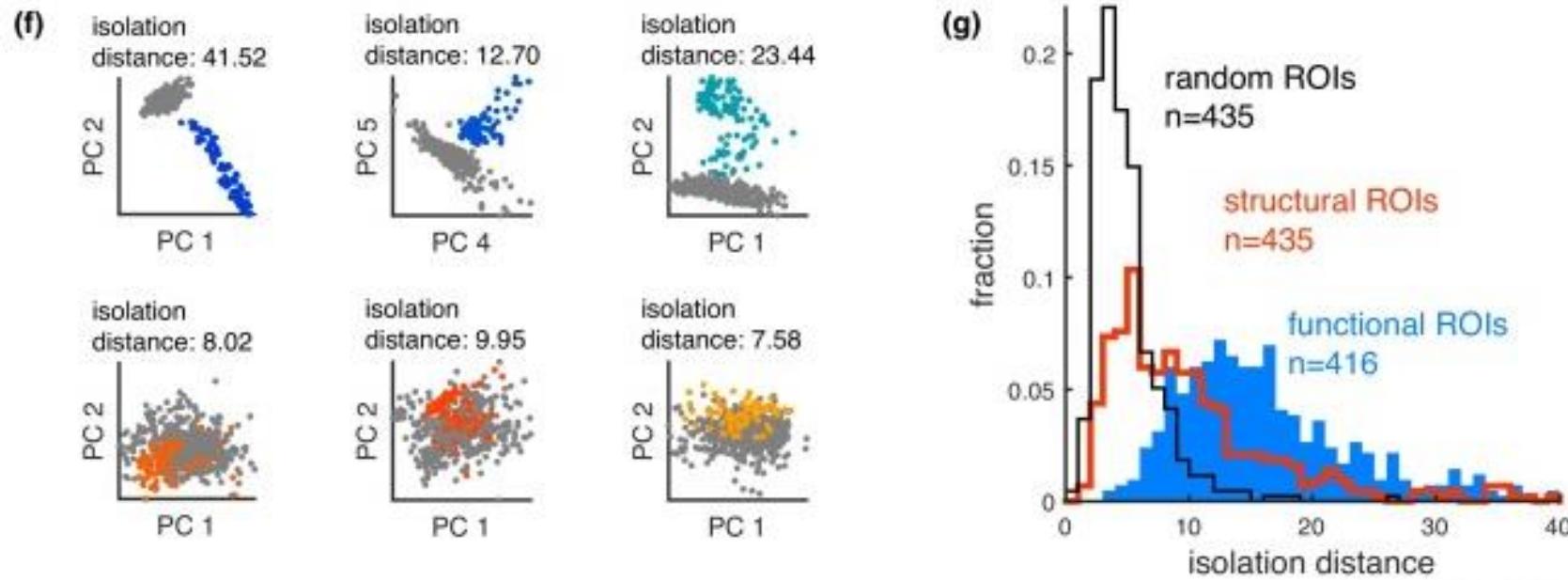
Why are “structural” methods not enough?



The neuropil problem



Functional ROIs are better isolated



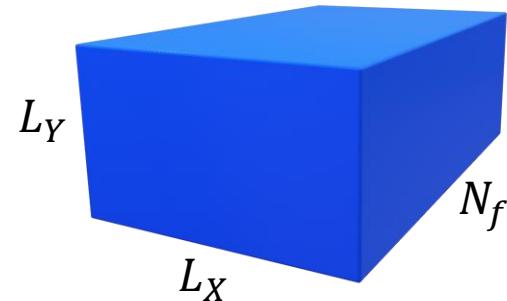
Treat movie F as a 3D matrix, or “volume”

$$F = (L_Y, L_X, N_f)$$

L_Y = image height

L_X = image width

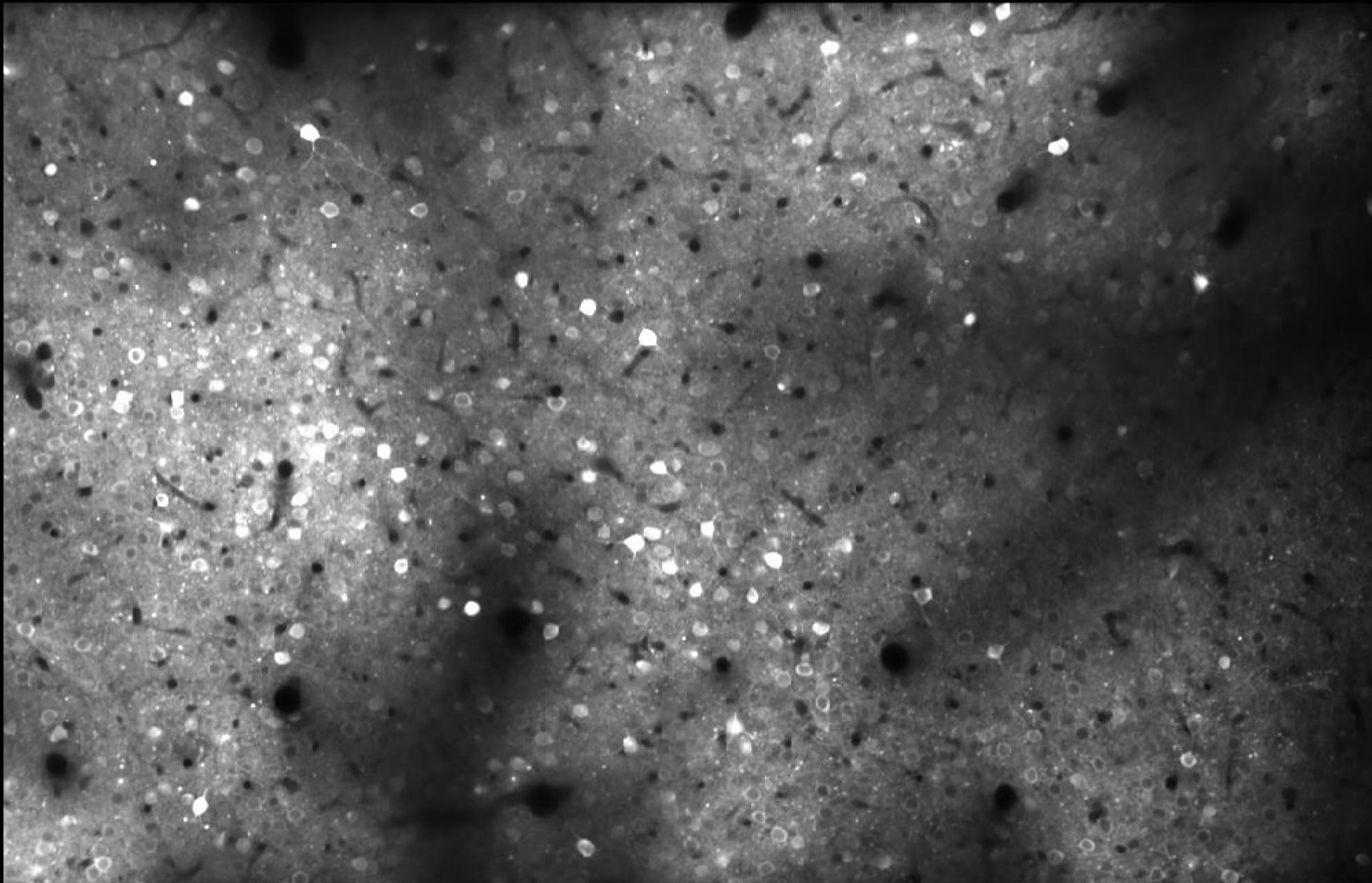
N_f = number of frames



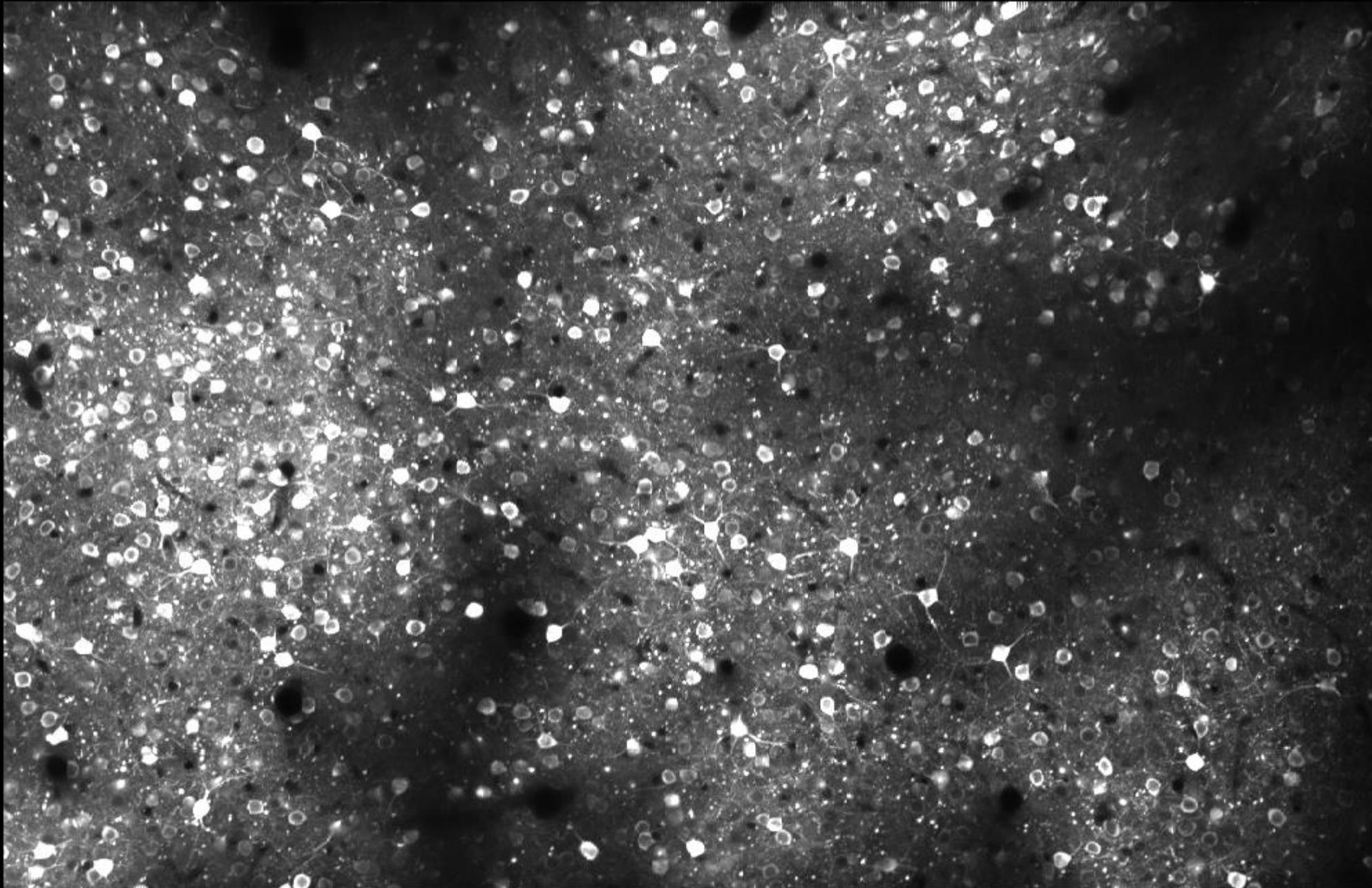
Compare mean and max projections

```
np.mean(F, axis=-1)
```

```
np.max(F, axis=-1)
```



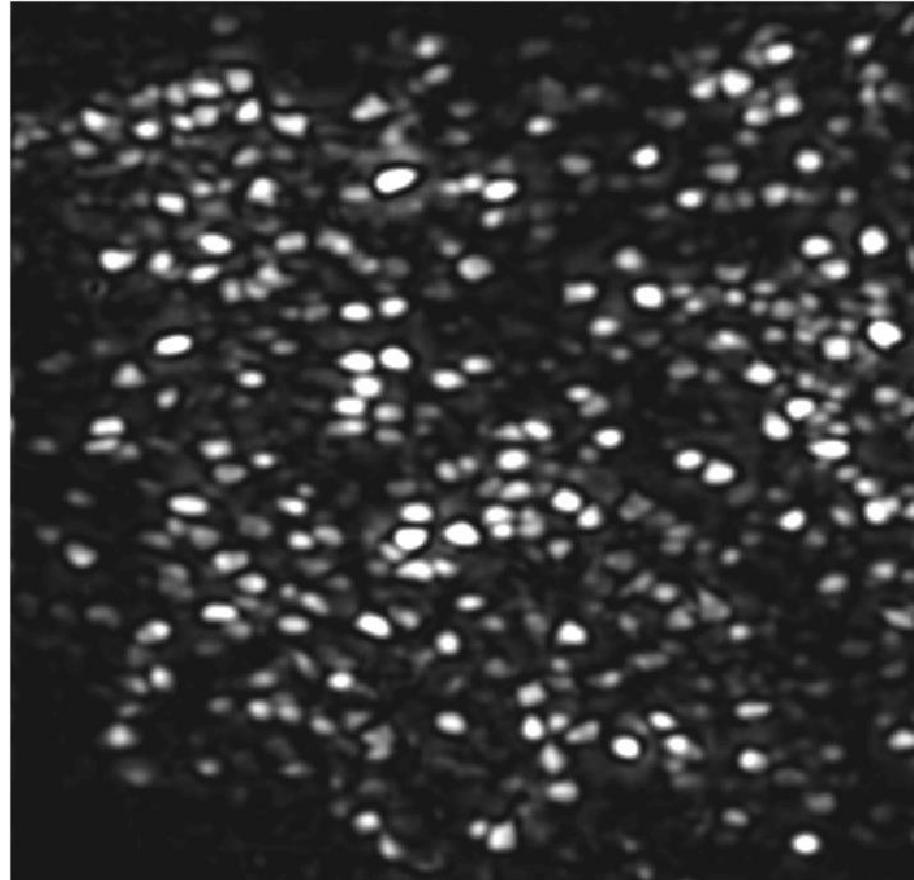
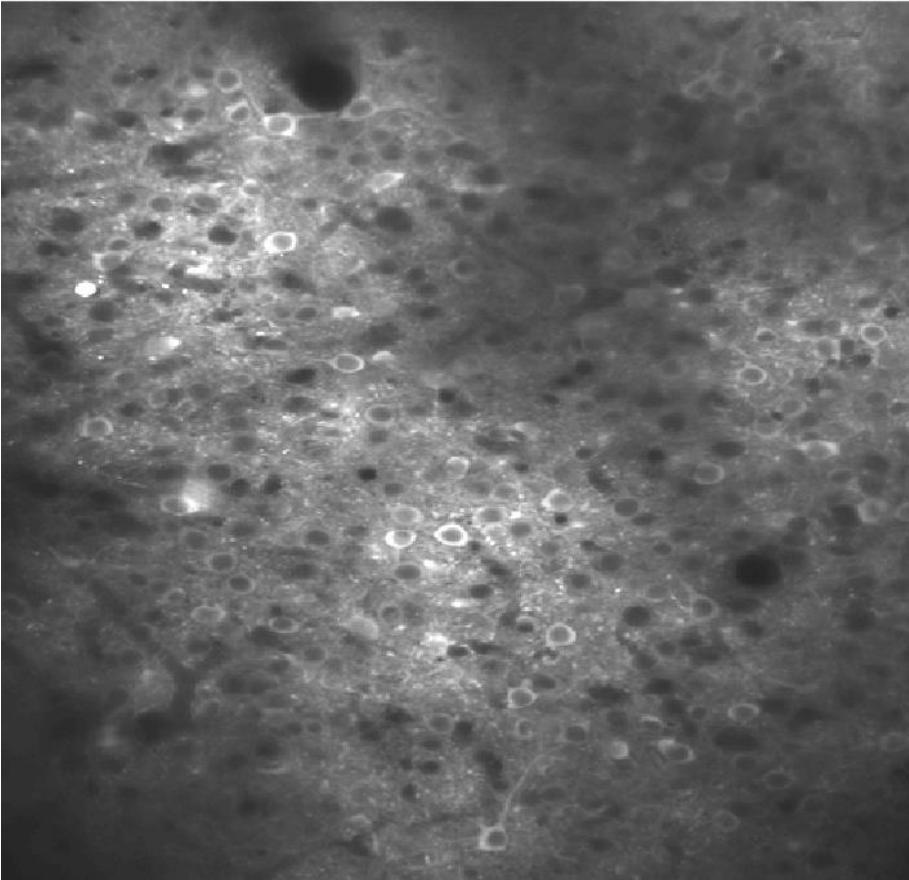
Mean image



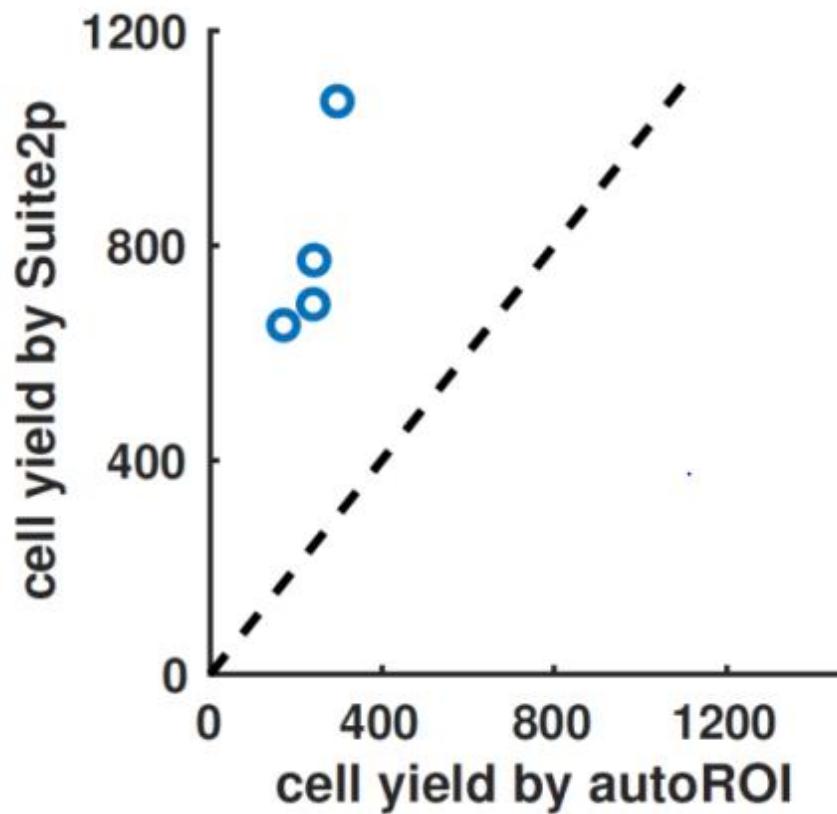
Maximum projection

What is the correlation map?

- $\text{Cmap}(x, y) = \text{corr}(F(x, y, :), F_{\text{blur}}(x, y, :))$
- $F_{\text{blur}}(x, y, t) = \langle F(x_n, y_n, t) \rangle_{x_n, y_n \in \text{Neighbors}(x, y)}$

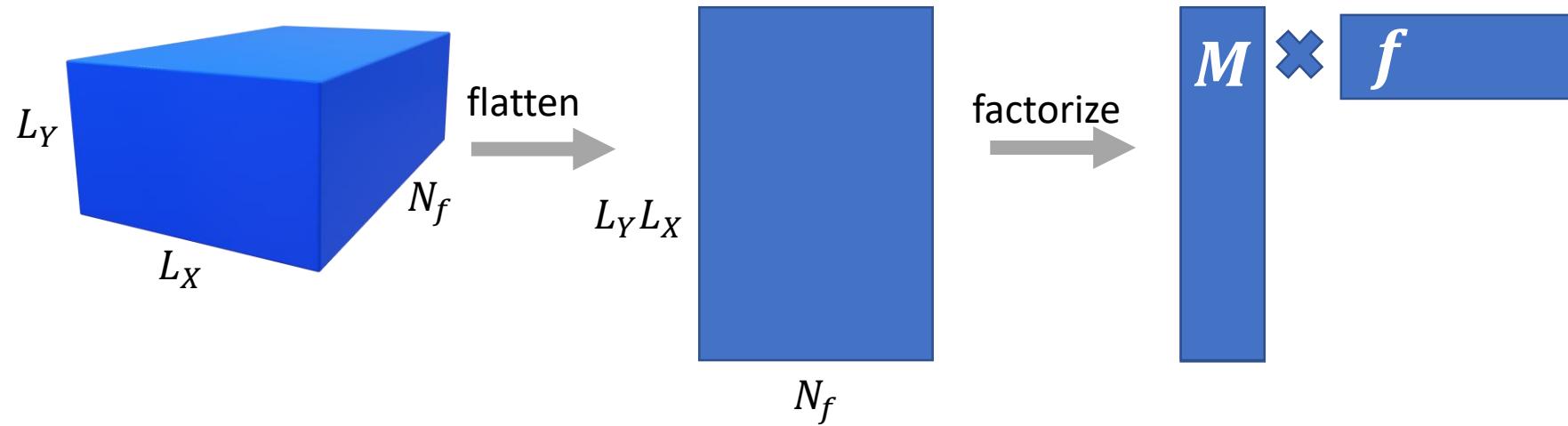


AutoROI uses only the correlation map



Functional cell detection with matrix factorization

$$F(x, y, t) \approx \sum_k M_k(x, y) * f_k(t)$$



What kind of matrix factorization

$$F(x, y, t) \approx \sum_k M_k(x, y) * f_k(t)$$

- ICA
 - Mukamel et al, 2009
 - look for sparsity of f
- CNMF
 - Pnevmatikakis et al, 2016
 - $f, M > 0$
 - f obeys calcium dynamics
- Suite2p (2016)
 - Pachitariu et al, 2016
 - $M > 0$ and local
 - extra term for spatial neuropil
- Suite2p (2019)
 - $M, f > 0$
 - M is connected
 - f is extra sparse

Suite2p (2019 version)

$$F(x, y, t) \approx \sum_k M_k(x, y) * f_k(t)$$

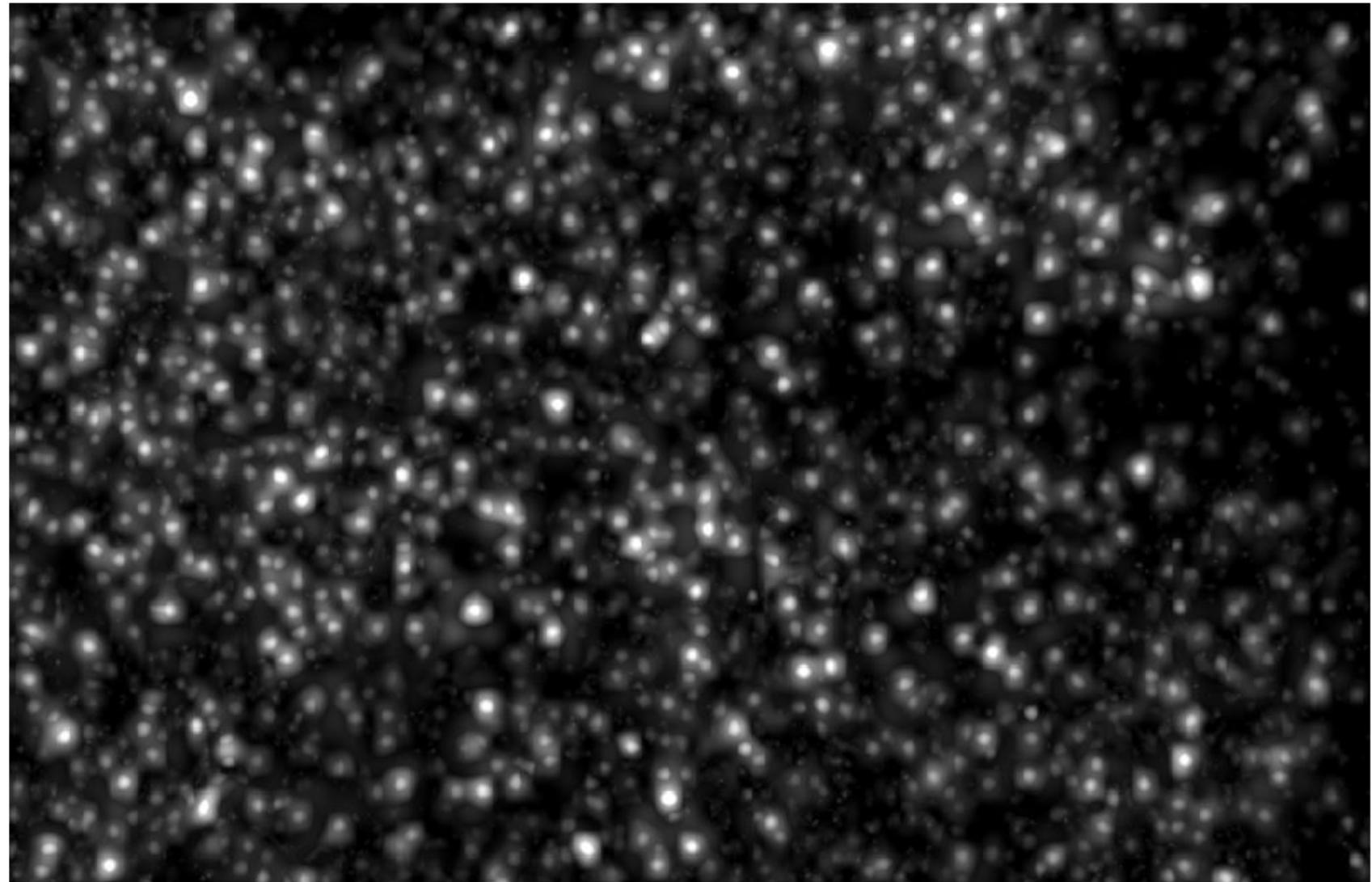
At each iteration k :

1. Look for cells of large relative transients
 - $f_k(t) > T$ for well-chosen T
 - $f_k(t) = \sum_k \frac{M_k(x, y)}{\|M_k(x, y)\|} * F(x, y, t)$
2. Initialize M_k with a *square* of possible size: 3, 6, 12, 24, 48
3. Optimize M_k
4. Peel off from F all the threshold crossings for M_k

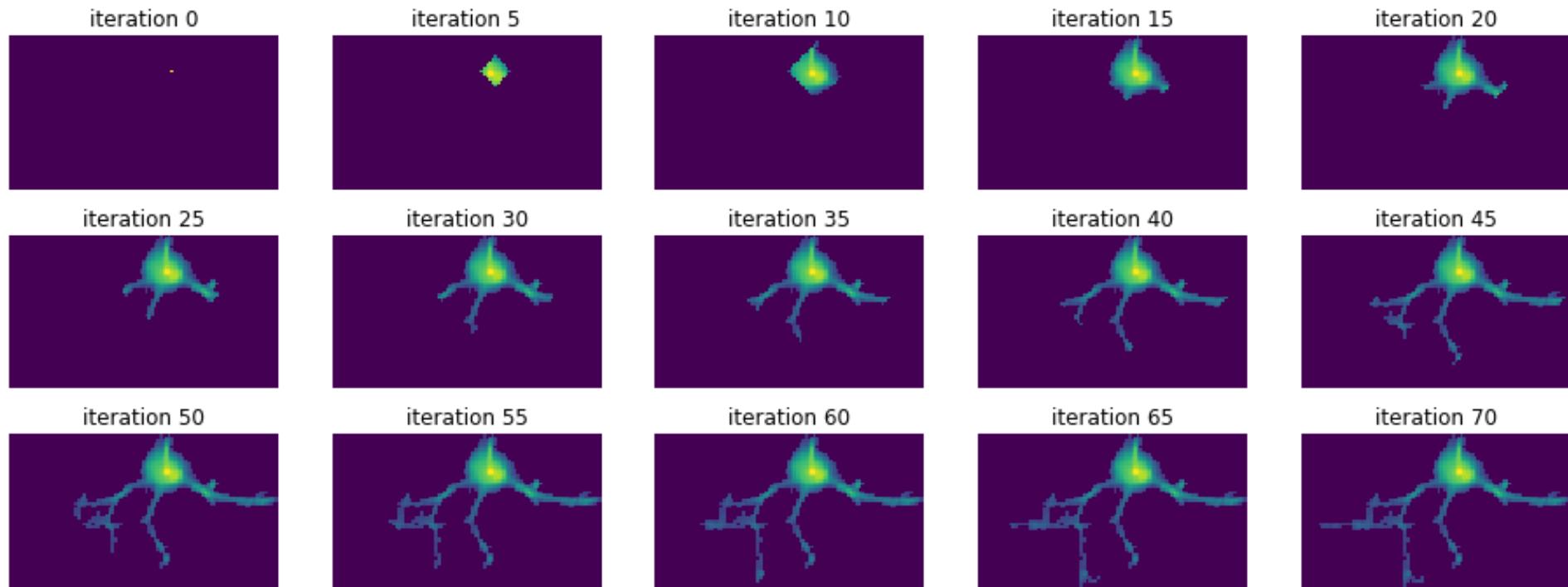
How to hunt for new cells? We need a map.

Multi-scale
Sparse Projection
Map

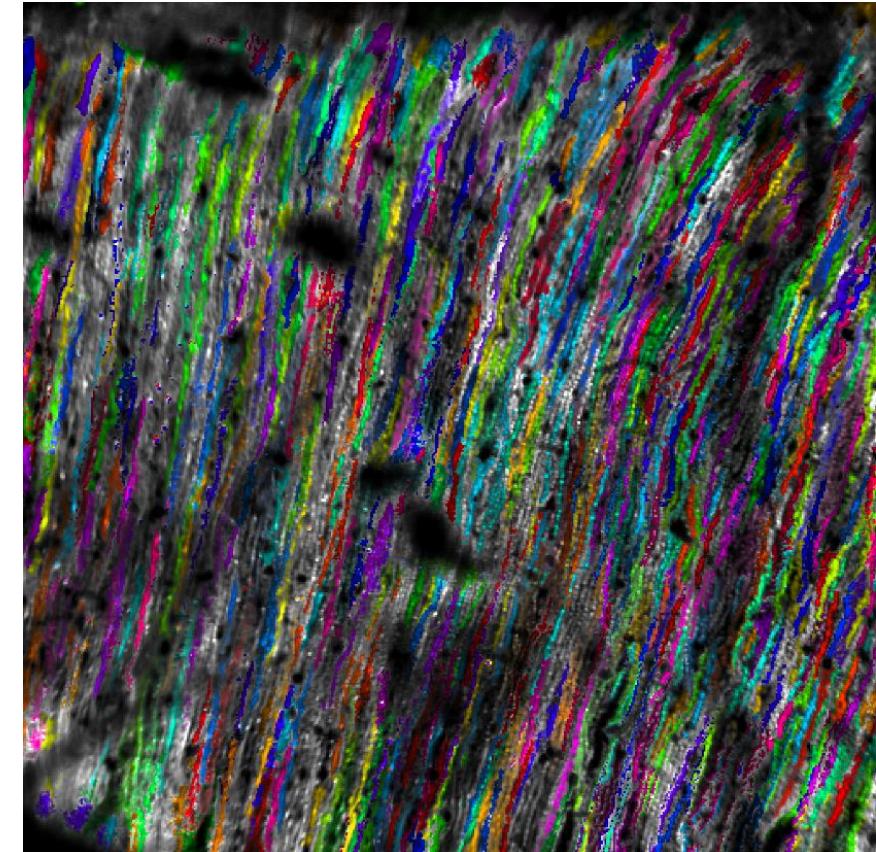
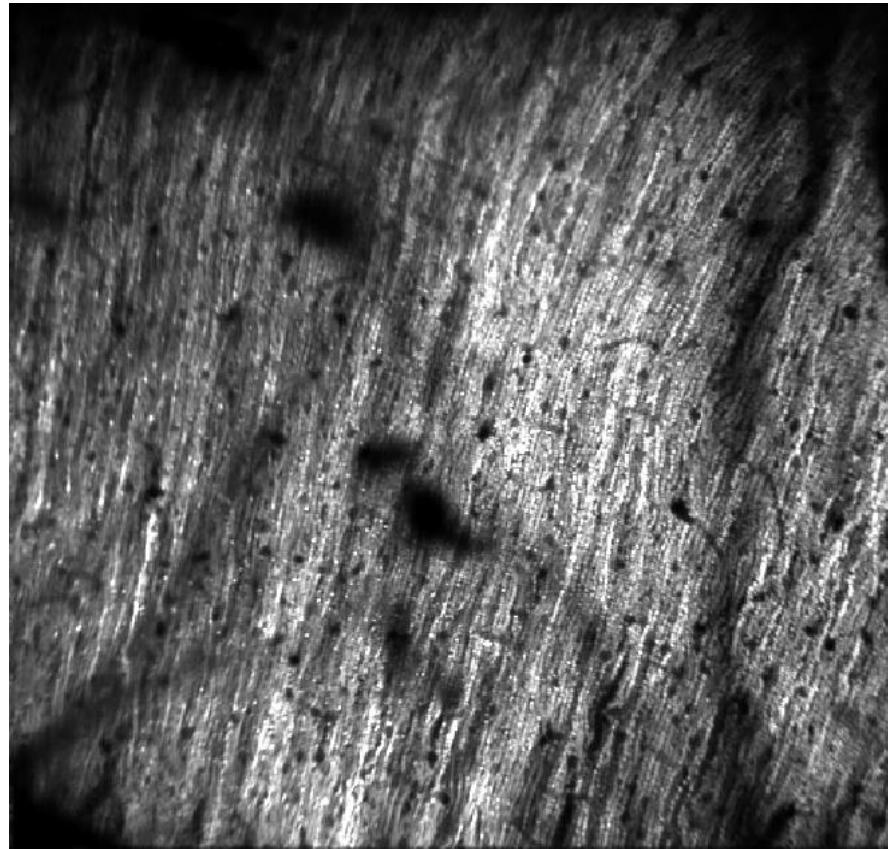
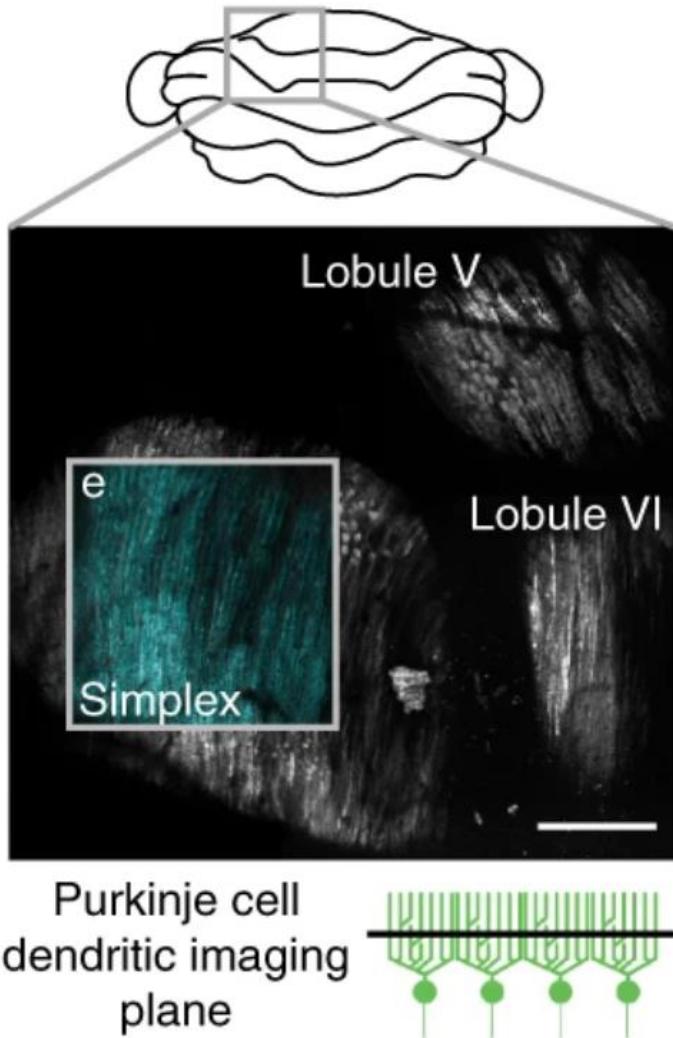
“correlation map”



We initialized a new cell, now how do we grow it?



Purkinje cell dendrites (cerebellum)



File Classifier Visualizations

 Q: ROIs On

select cells

draw selection

select top n
select bottom n

n= 40

858

cells

both

not cells

0

Background

Q: ROIs

W: mean img

E: mean img (enhanced)

R: correlation map

Colors

A: random

S: skew

D: compact

F: footprint

G: aspect_ratio

H: classifier

J: correlations, bin= 15

0.00 0.50 1.00

0.500 cell probability

apply

Classifier

not loaded

add current data to classifier

Selected ROI:

315

med: [209, 326]

npix: 165

skew: 2.26

compact: 2.99

footprint: 2.00

aspect_ratio: 1.88

Activity mode:

+

▲

F - 0.7*Fneu

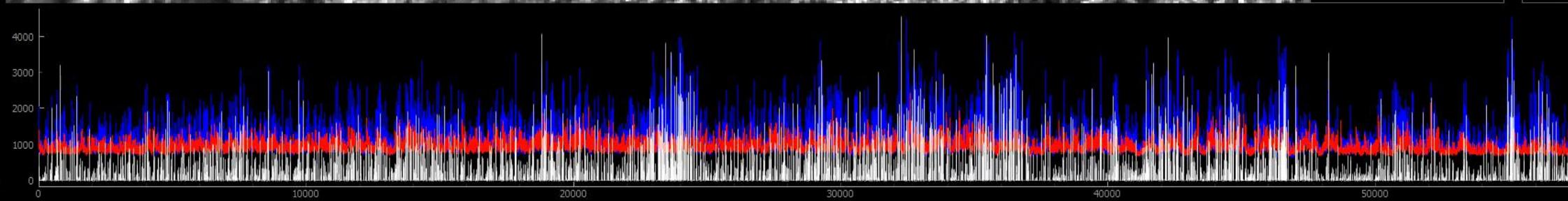
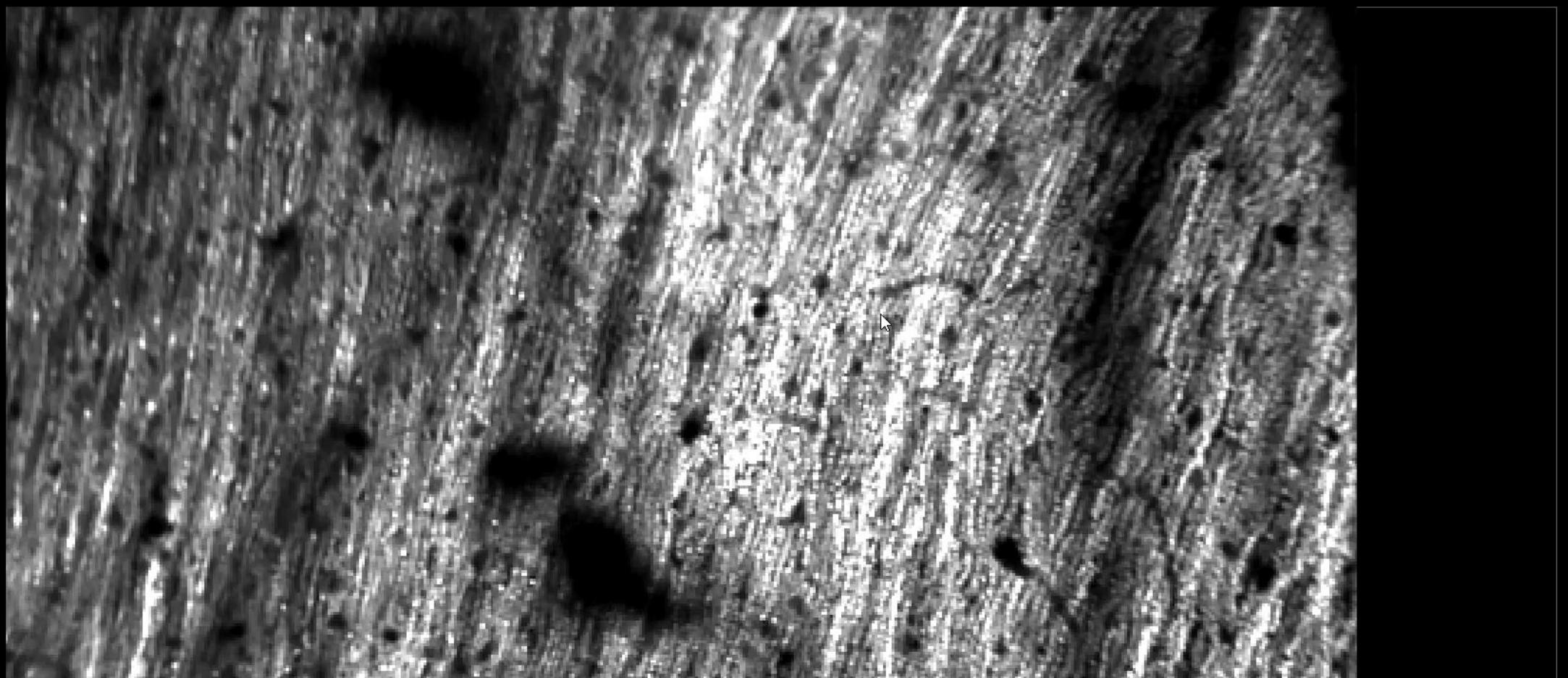
-

▼

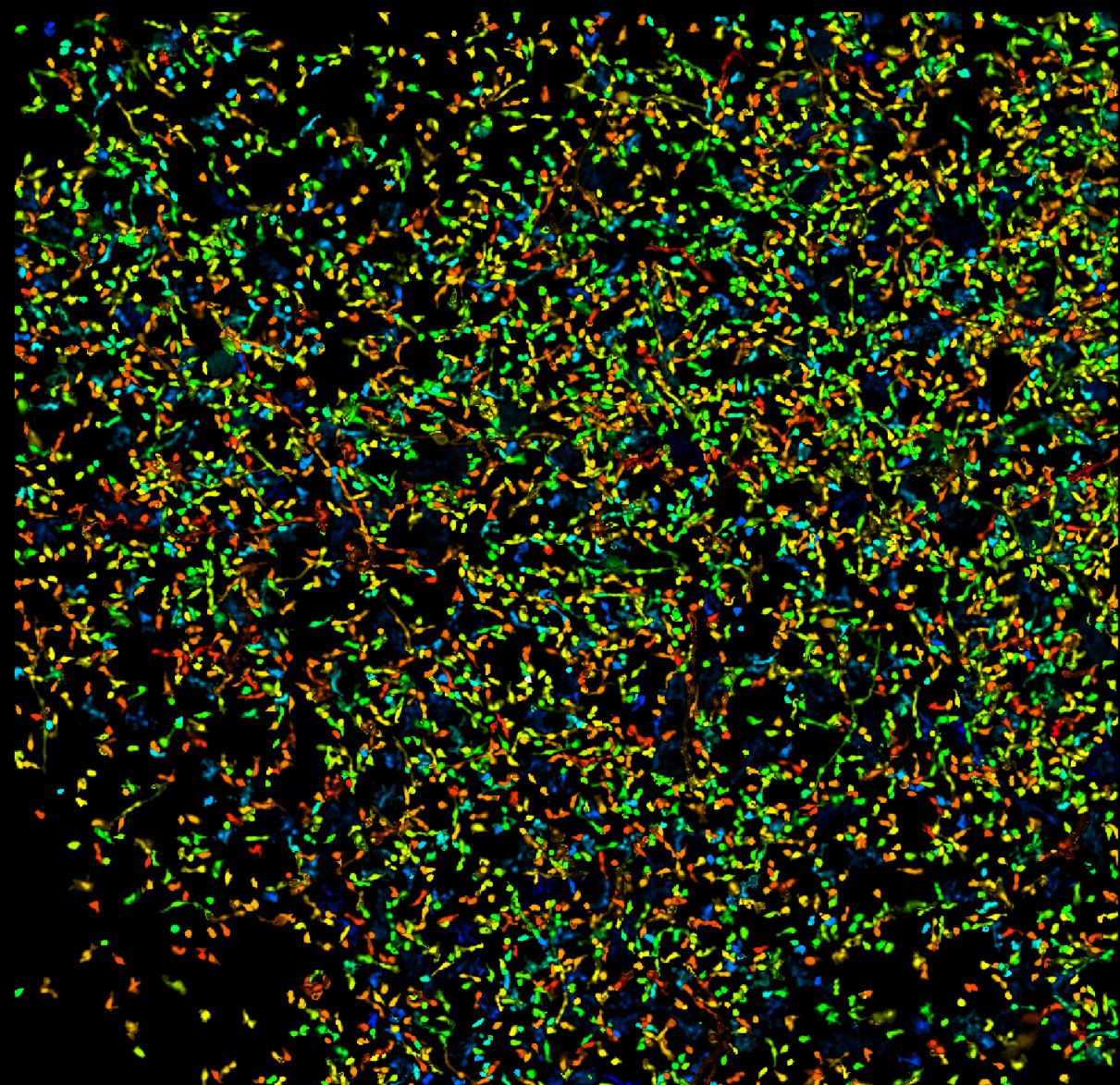
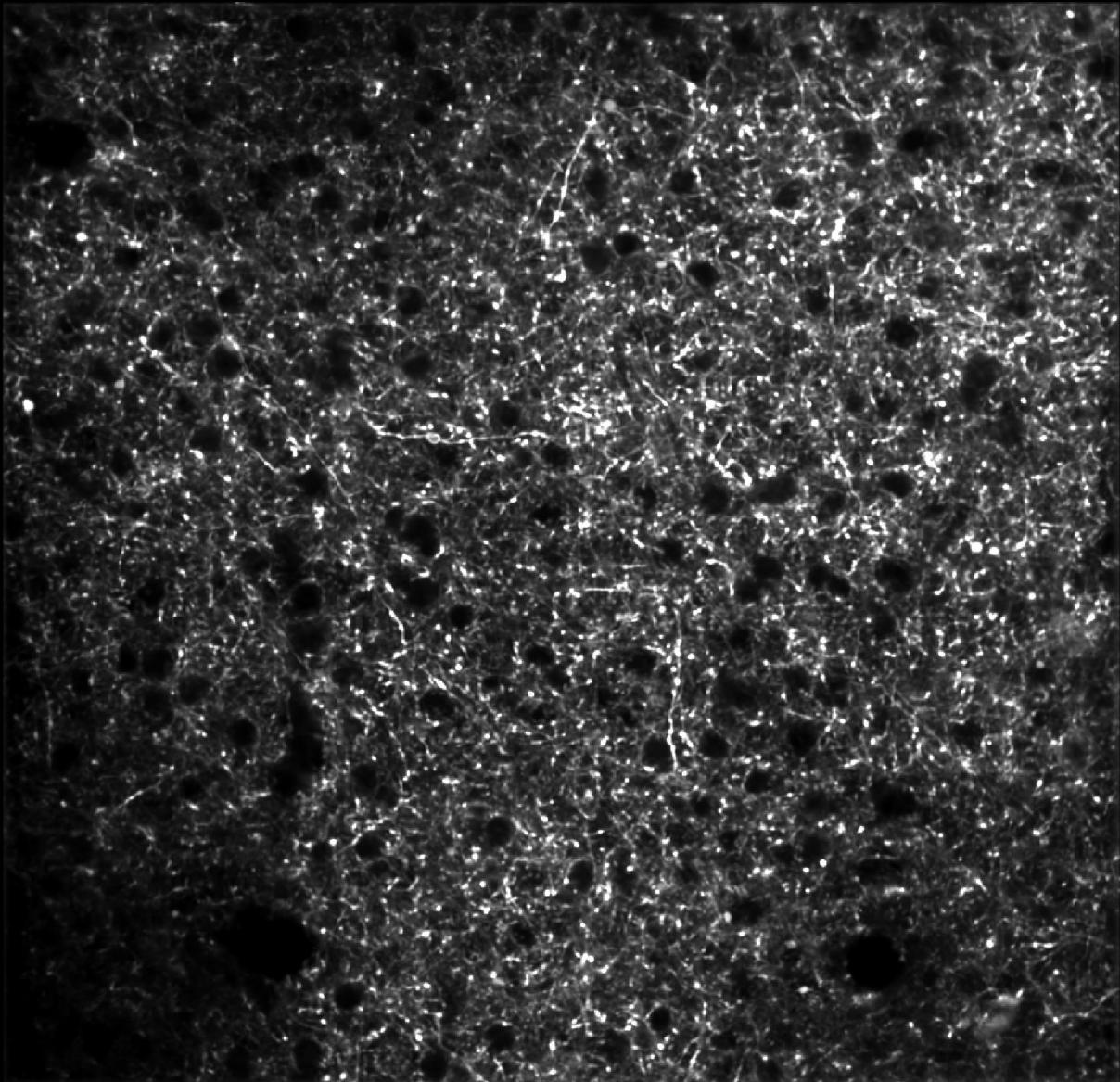
Fluorescence

Neuropil

Deconvolved



Axons and boutons (thalamus → V1)



File Classifier Visualizations

 Q: ROIs On

select cells

draw selection

select top n

select bottom n

n= 40

1968 cells

both

not cells

0

Background

Q: ROIs

W: mean img

E: mean img (enhanced)

R: correlation map

Colors

A: random

S: skew

D: compact

F: footprint

G: aspect_ratio

H: classifier

J: correlations, bin= 2

0.00 0.50 1.00

0.000 cell probability

apply

Classifier

not loaded

add current data to classifier

Selected ROI:

0

med: [666, 212]

npix: 60

skew: 2.28

compact: 1.02

footprint: 1.21

aspect_ratio: 1.21

Activity mode:

+

▲

-

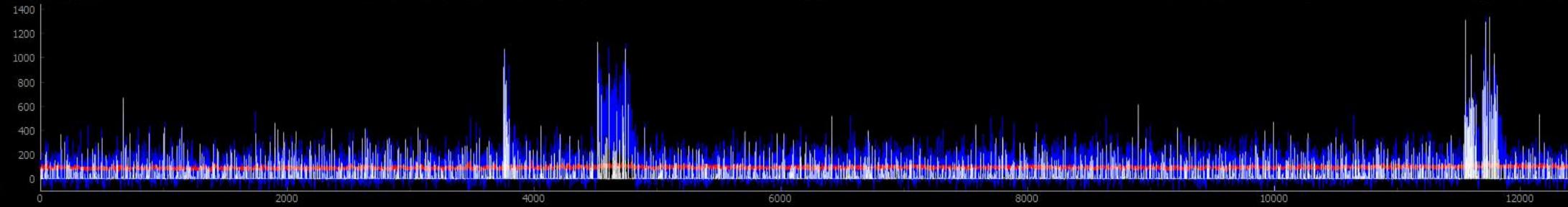
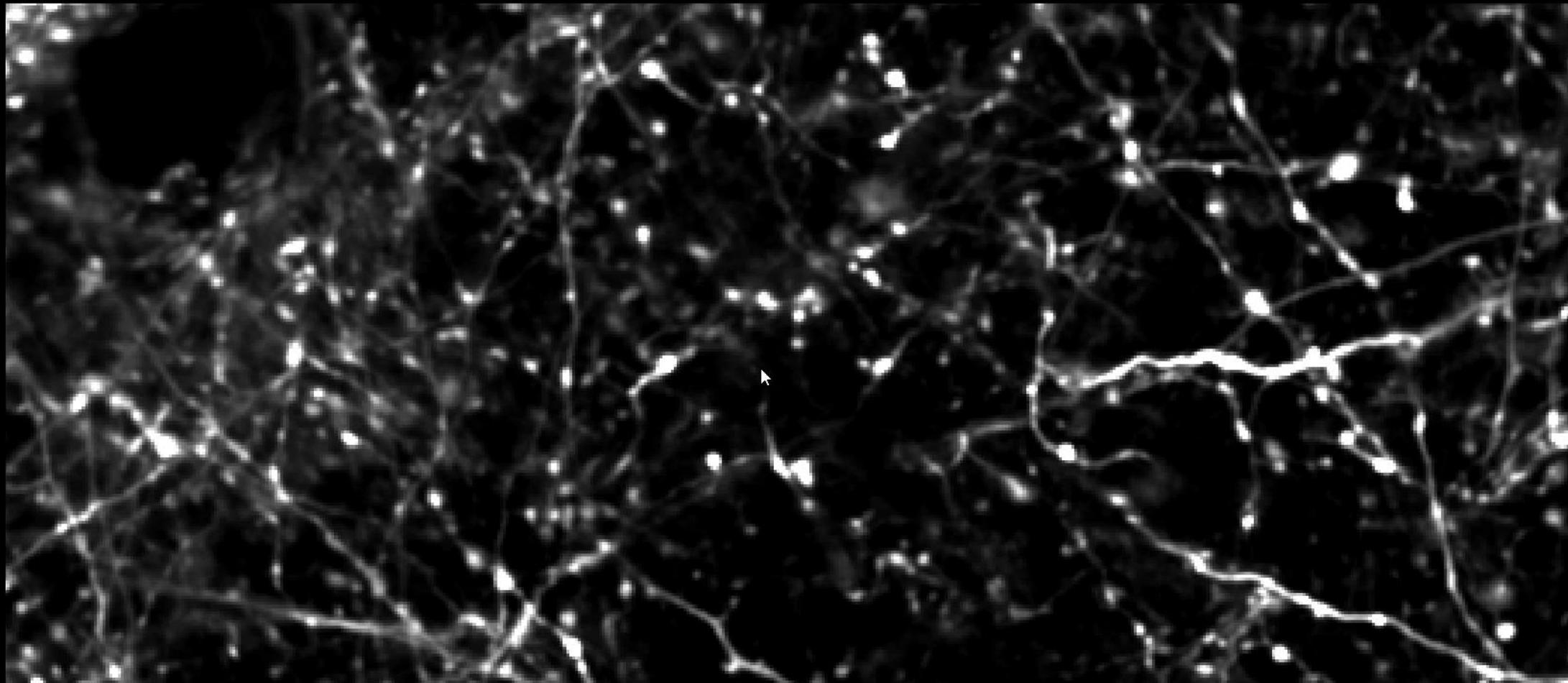
▼

F - 0.7*Fneu

Fluorescence

Neuropil

Deconvolved



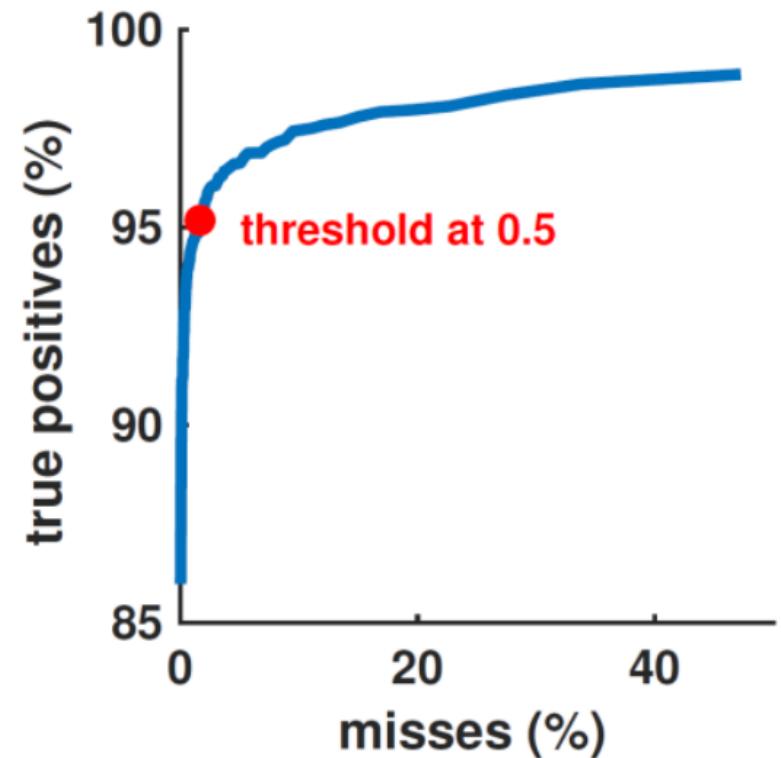
Some details...

- How to avoid effects of background fluctuations?
 - high-pass filter
- How to avoid over-merges?
 - test each new component for possible splits
- How to avoid splits?
 - that almost never happens
- What do we with components that are not cells?
 - make classifiers

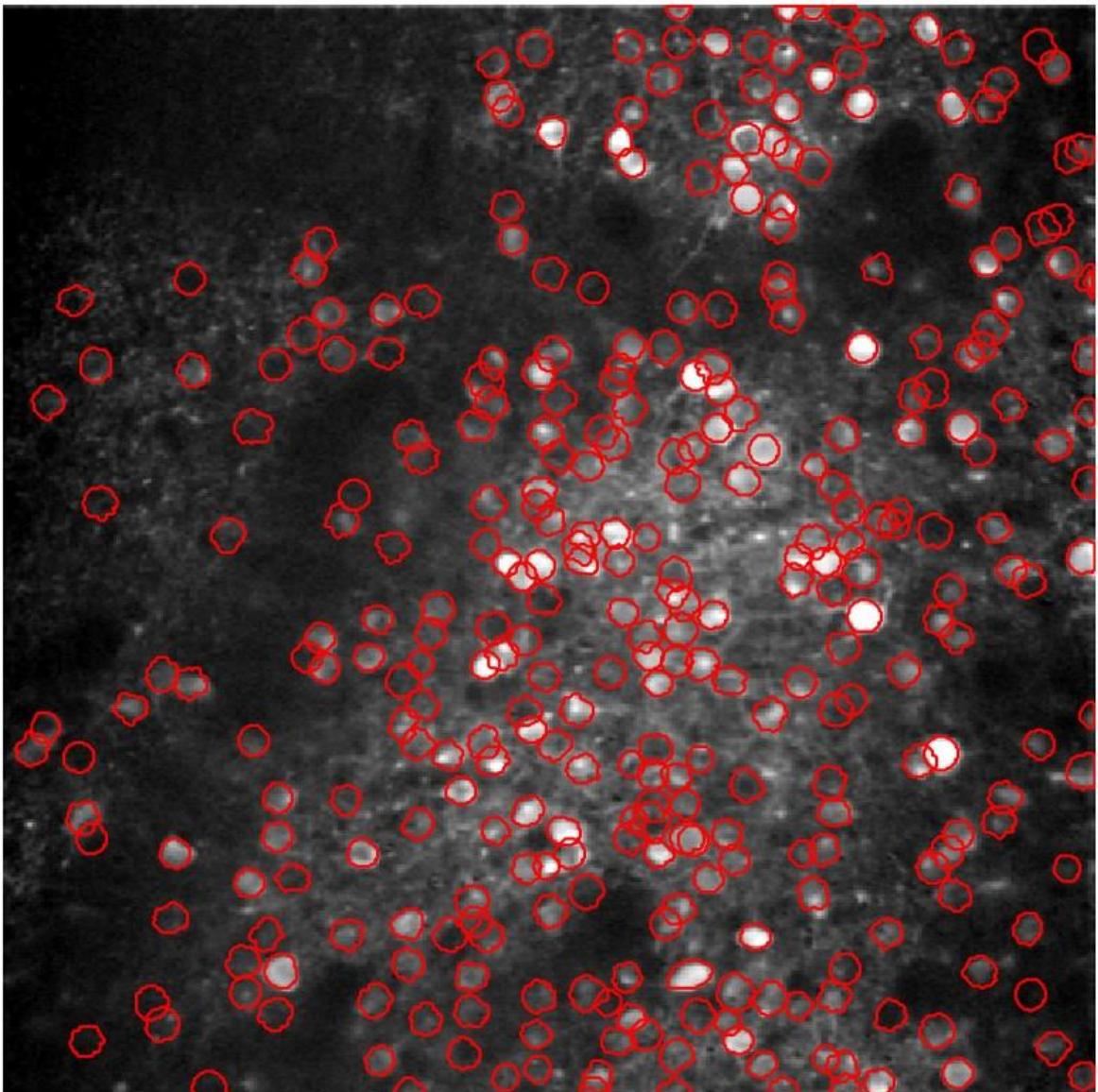
How to classify if an ROI is a cell?

- Basic feature predictors
 - Number of pixels
 - Compactness of ROI
 - Skewness of ROI
 - ...
- Shape predictors
 - Use CNNs on the masks
 - Aphorpe et al, 2013, Giovanucci et al, 2013

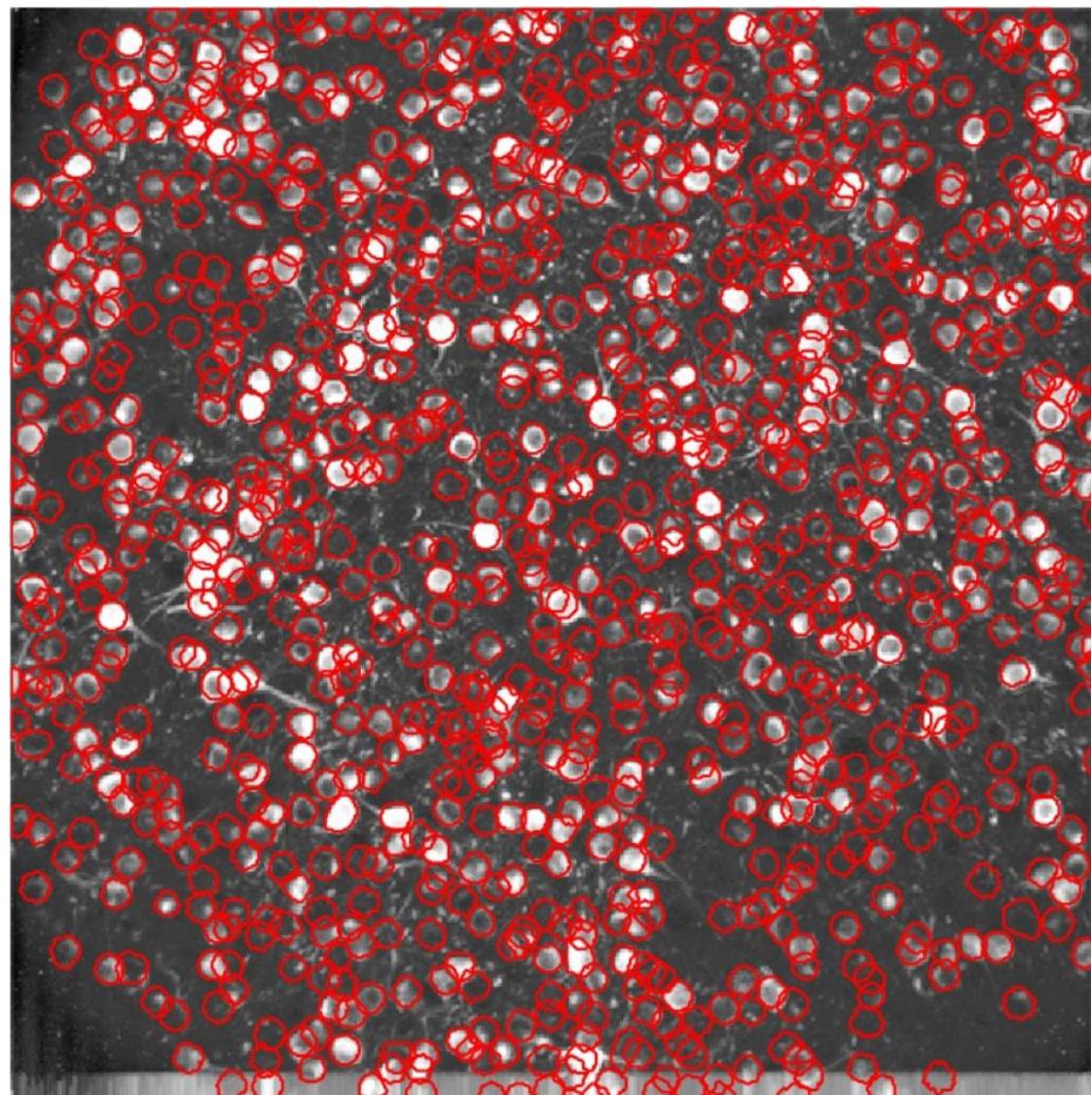
within sample performance



How to train classifiers?



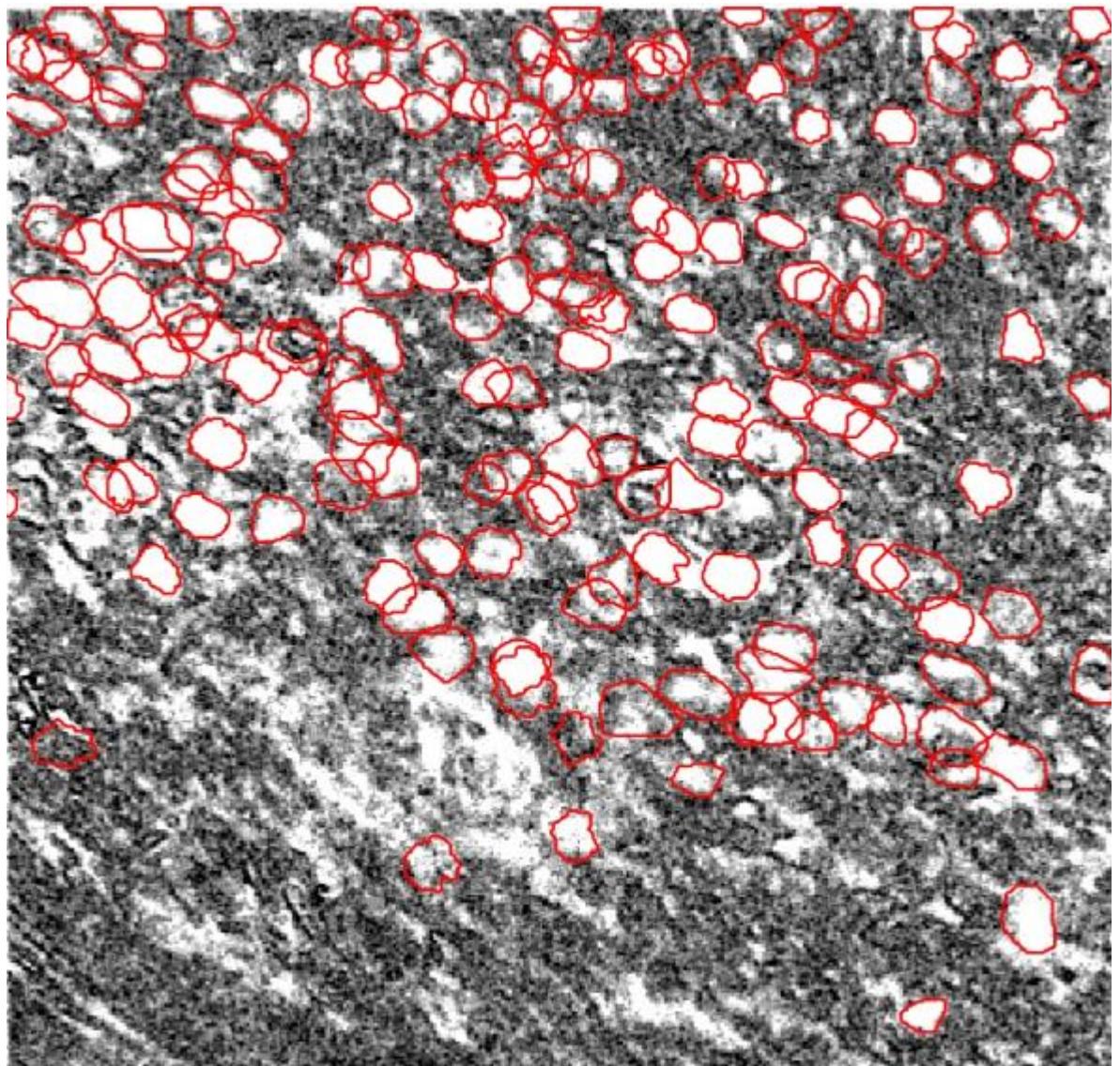
Hausser lab



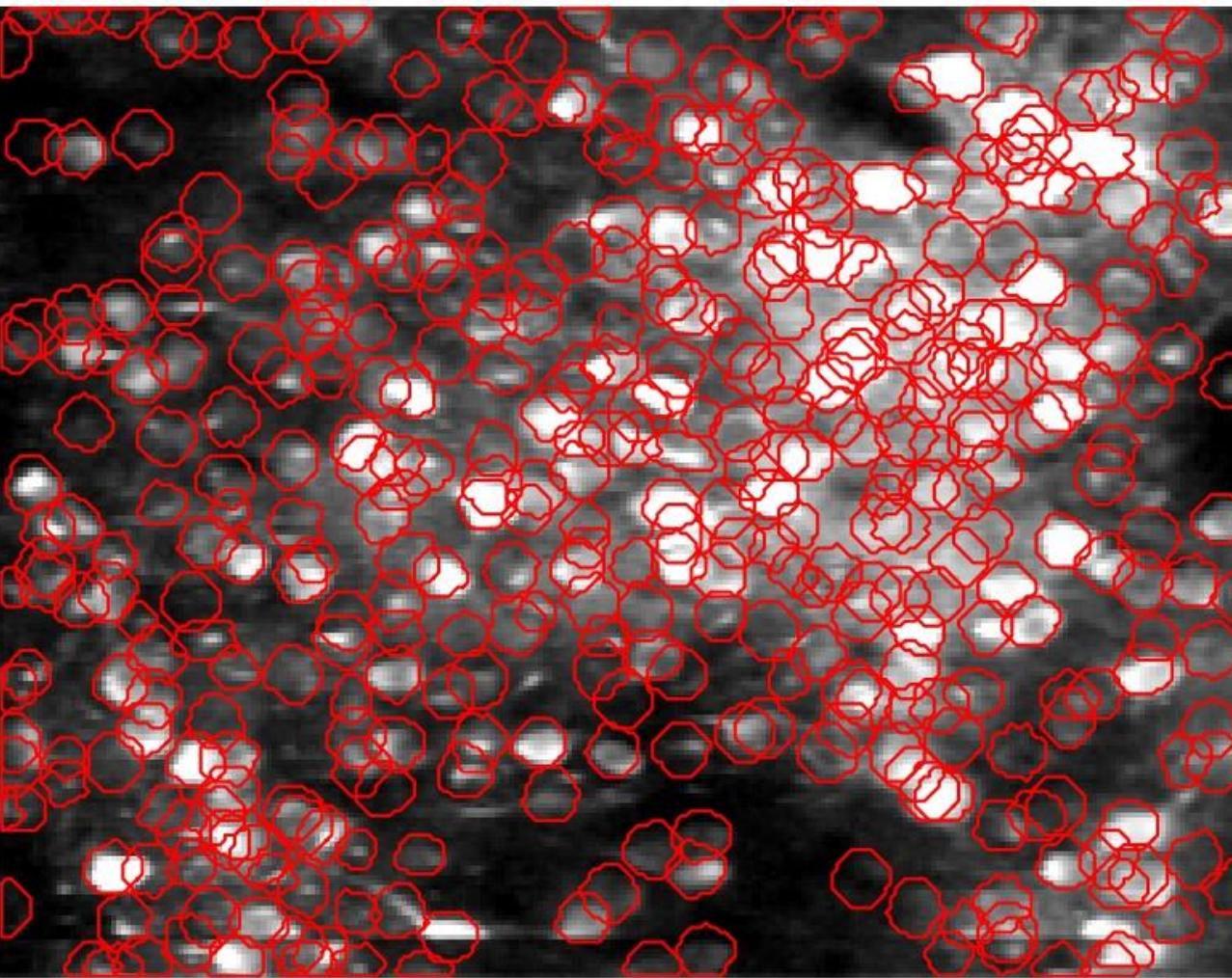
Tank lab

Giovanucci et al, 2019
(Caiman paper)

How to train classifiers?

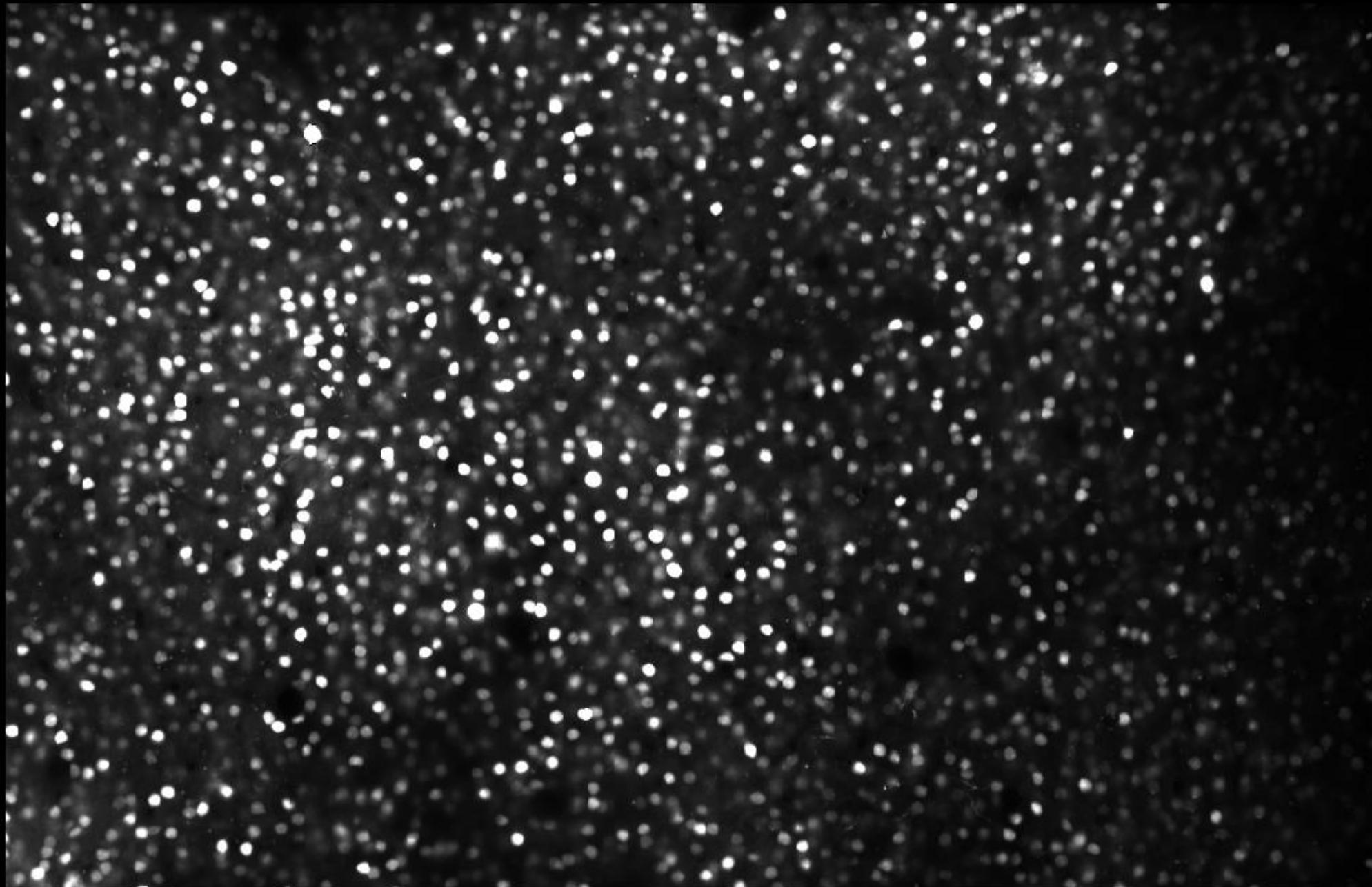


Tank lab

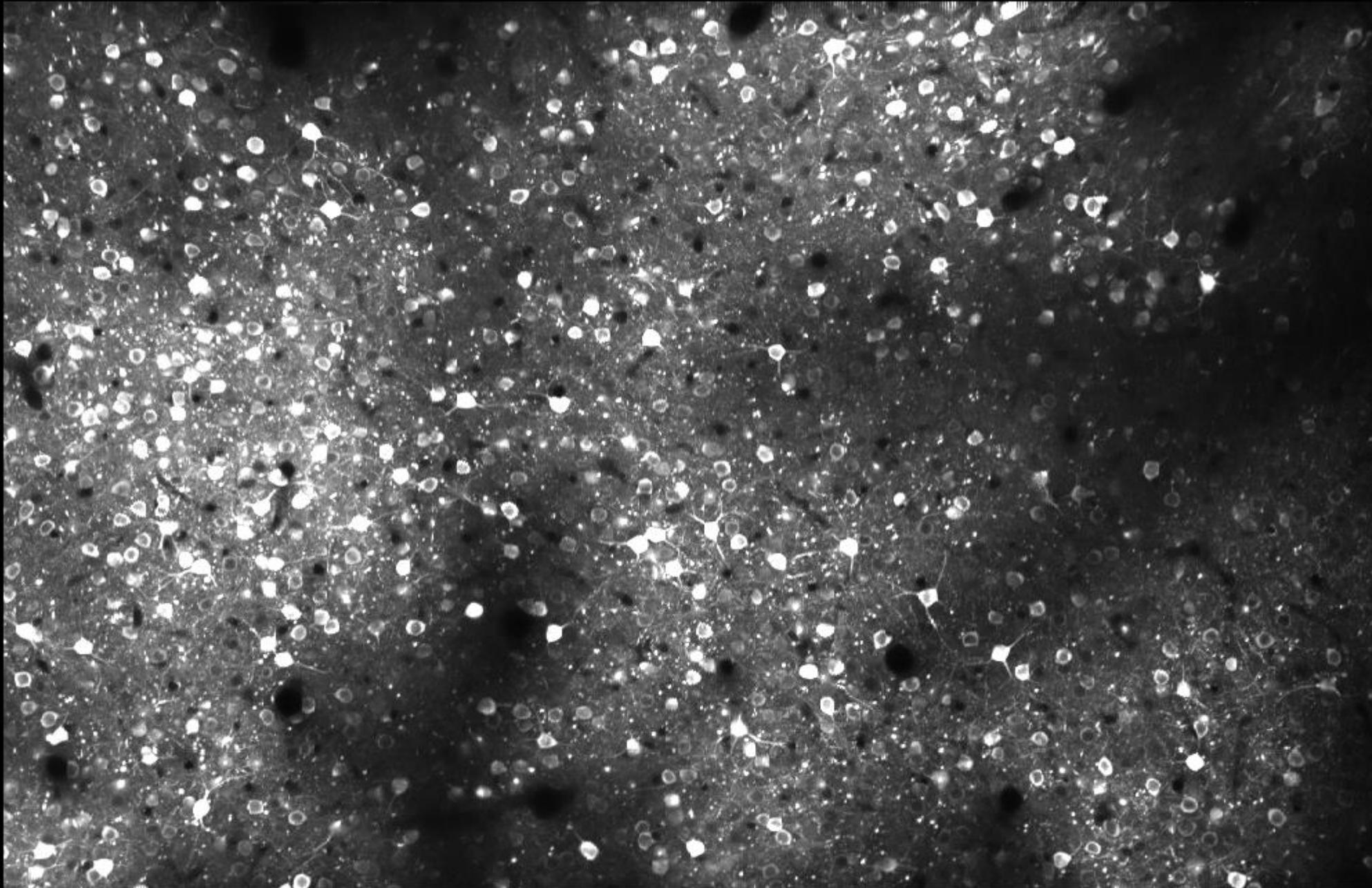


Yuste lab

Giovanucci et al, 2019
(Caiman paper)

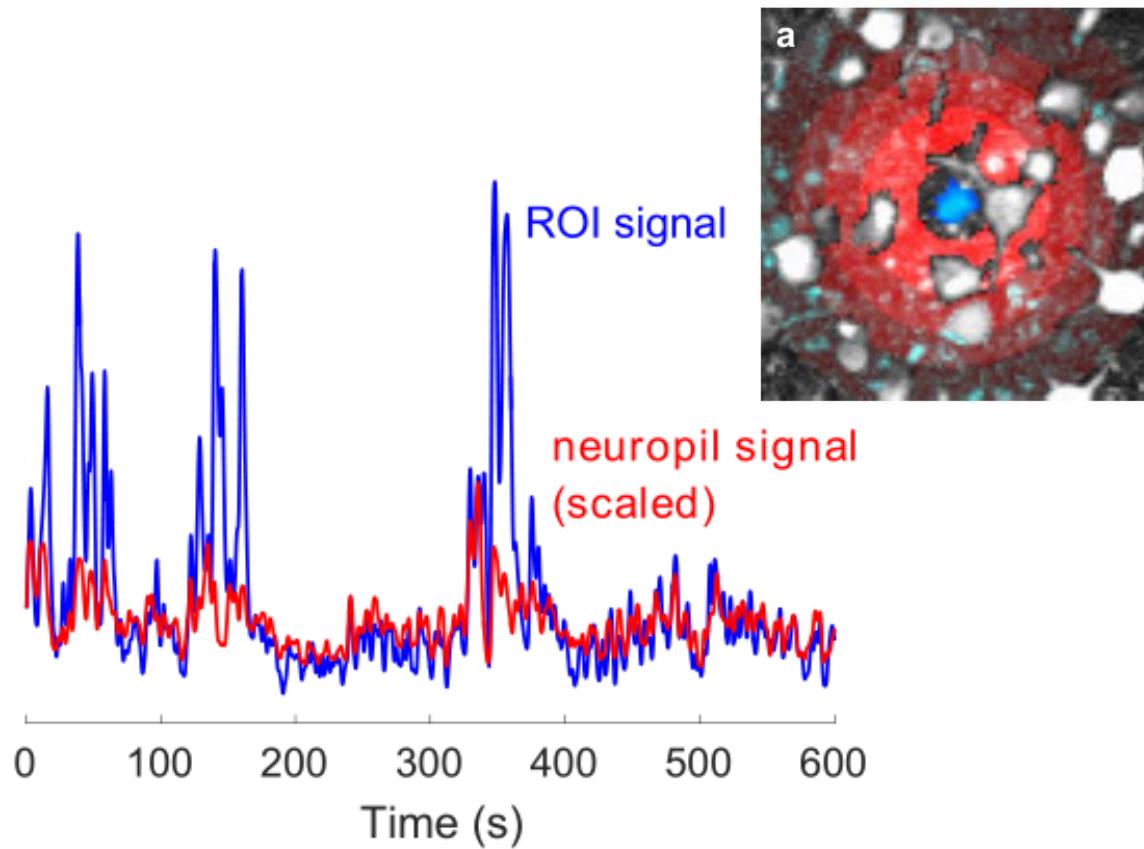


nuclear dtomato, co-localized with gcamp



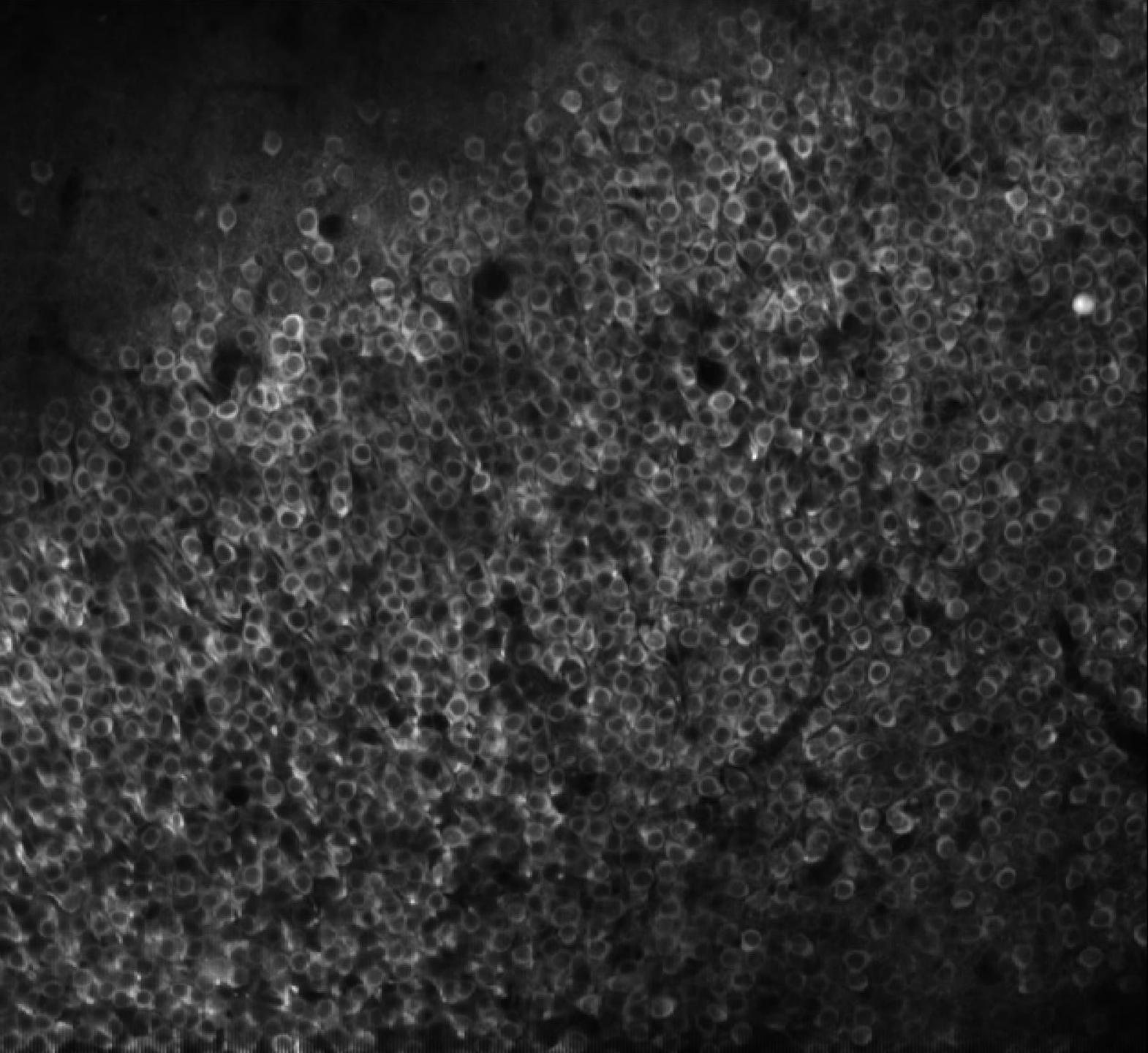
Maximum projection

How do we extract fluorescence?



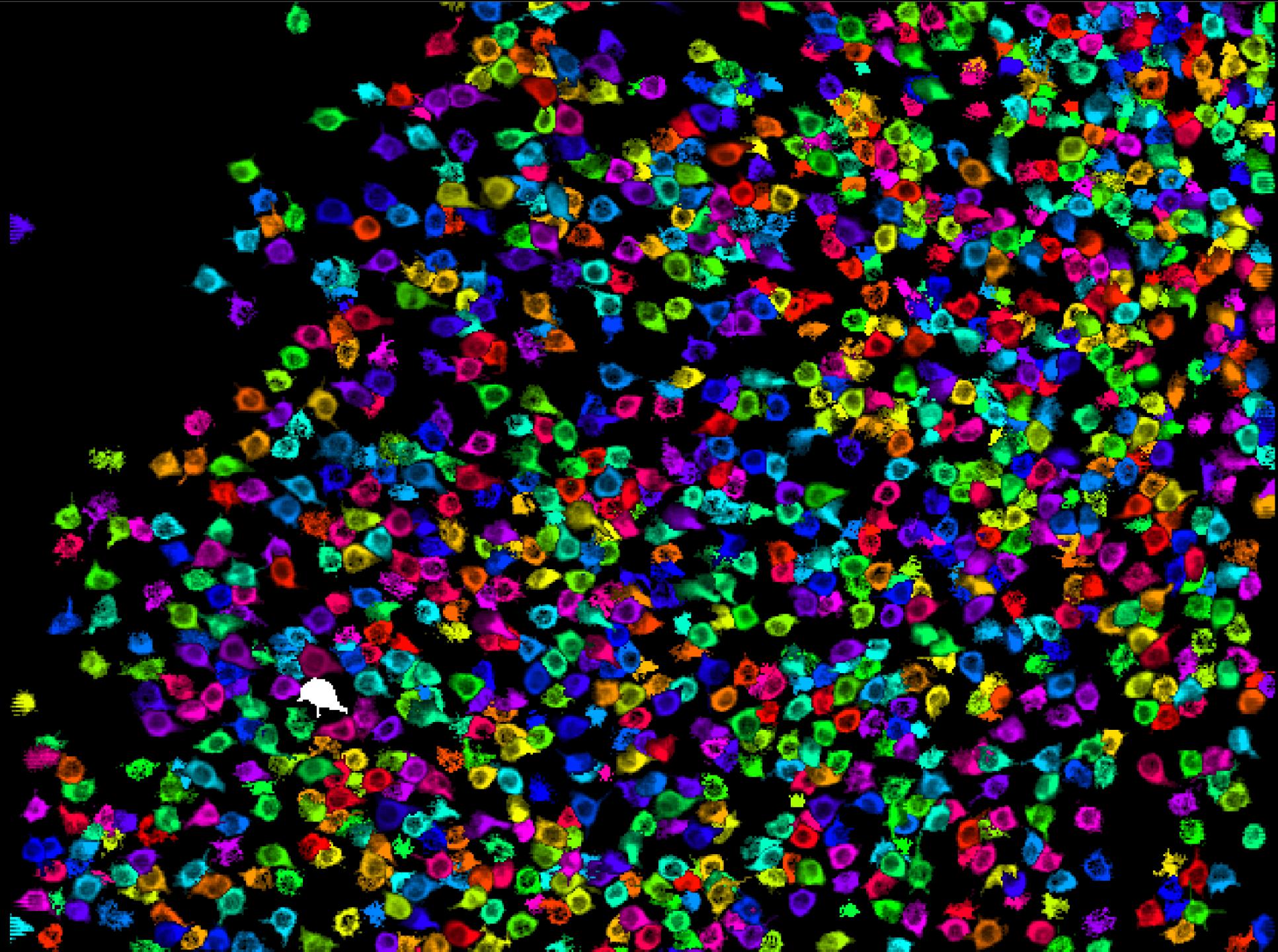
$$F_{corrected} = F - \alpha * F_{neuropil}$$
$$\alpha \sim 0.7$$

What if the recording
is densely populated?



Weinan Sun

Weinan Sun



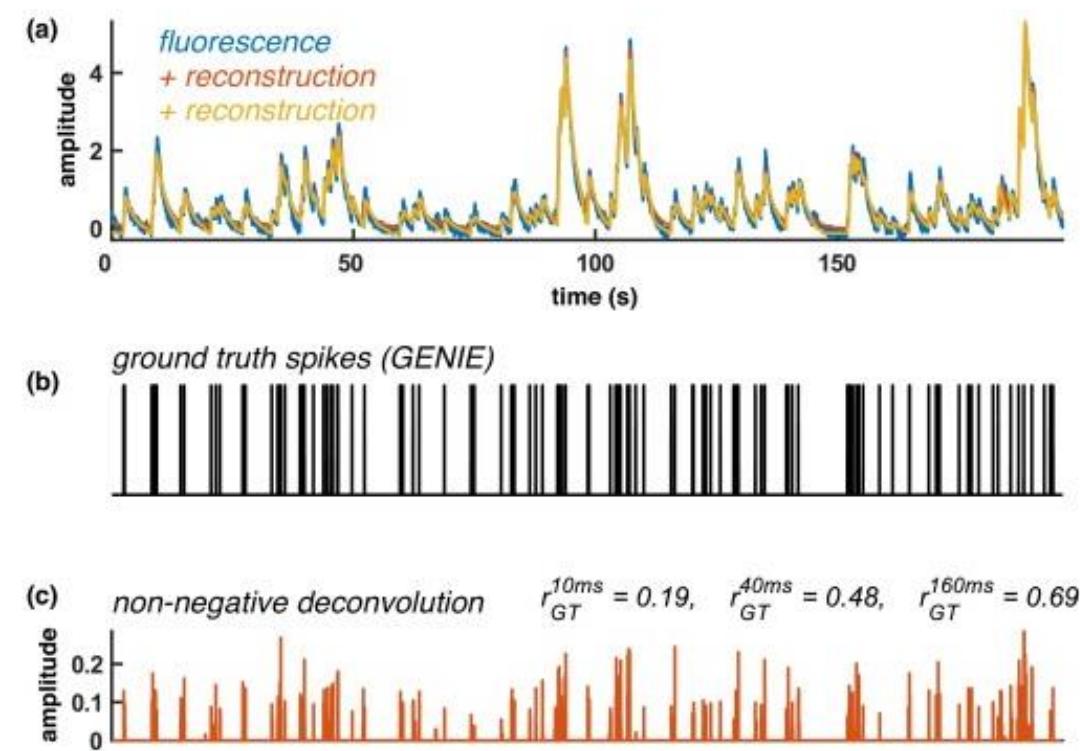
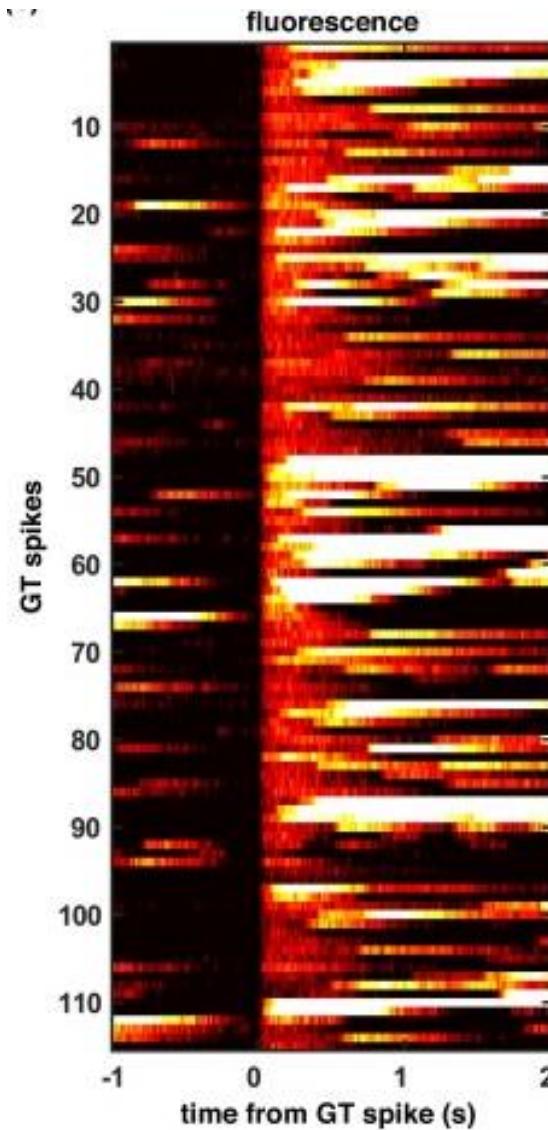
“Demix” the fluorescence by optimizing
the generative model

$$F(x, y, t) \approx \sum_k M_k(x, y) * f_k(t)$$

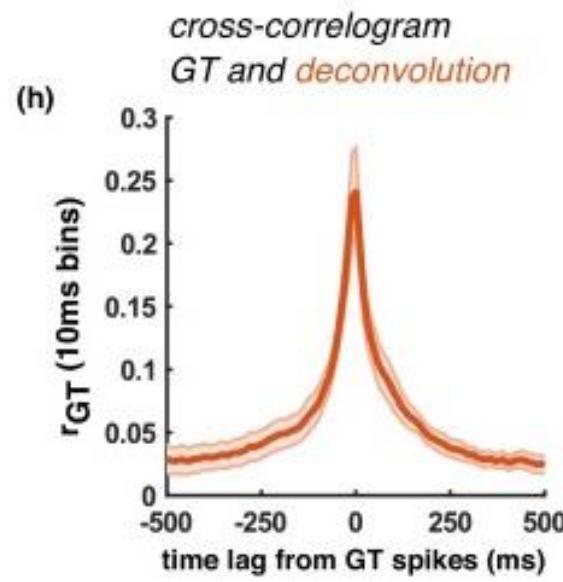
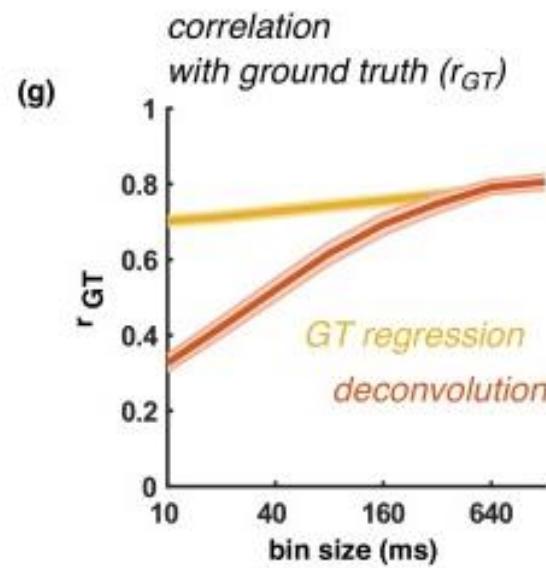
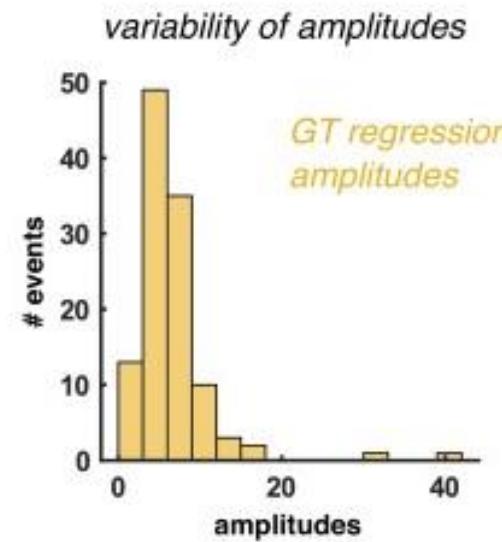
Pnevmatikakis et al, 2016

Simultaneous Denoising, Deconvolution, and Demixing
of Calcium Imaging Data

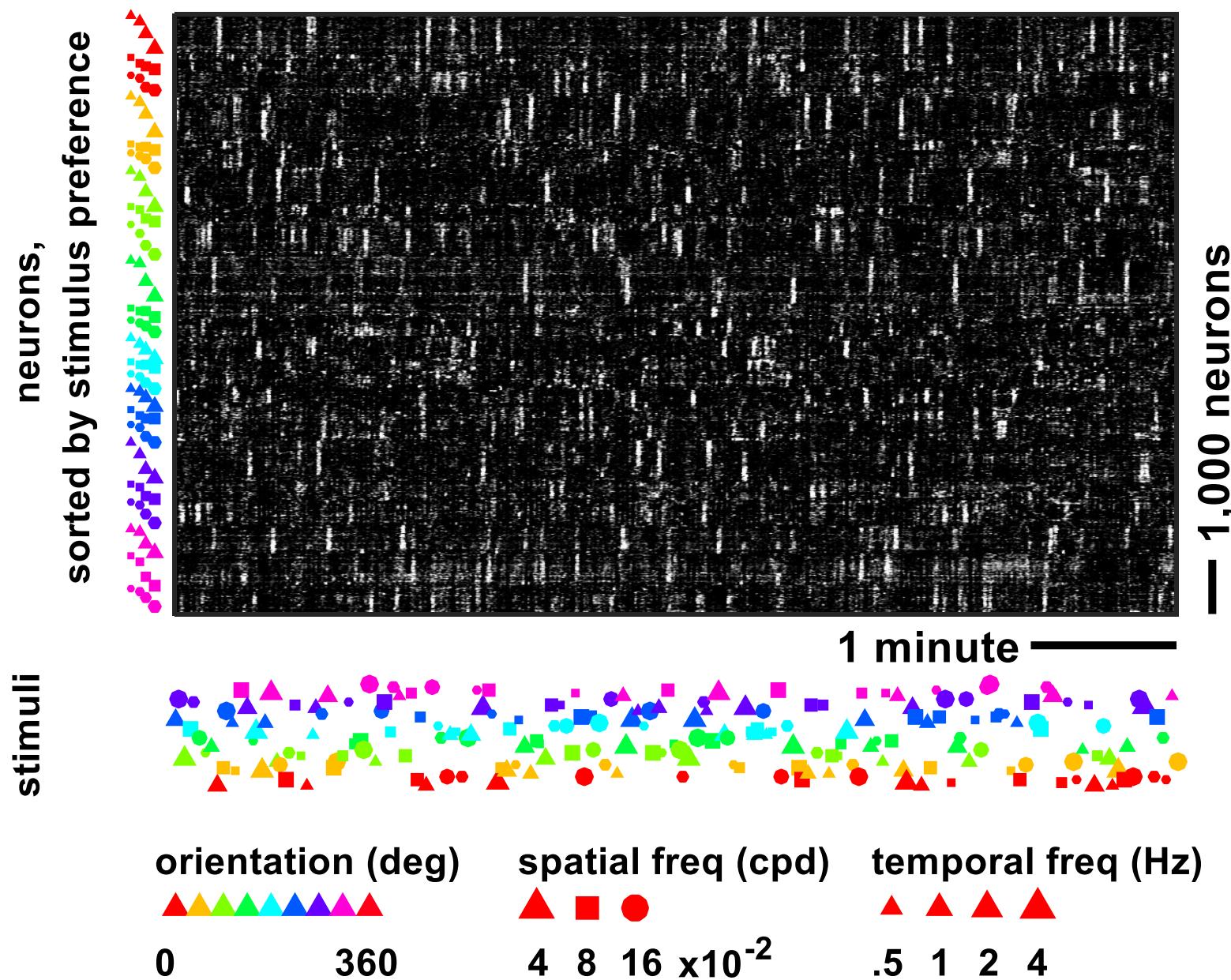
Spike deconvolution



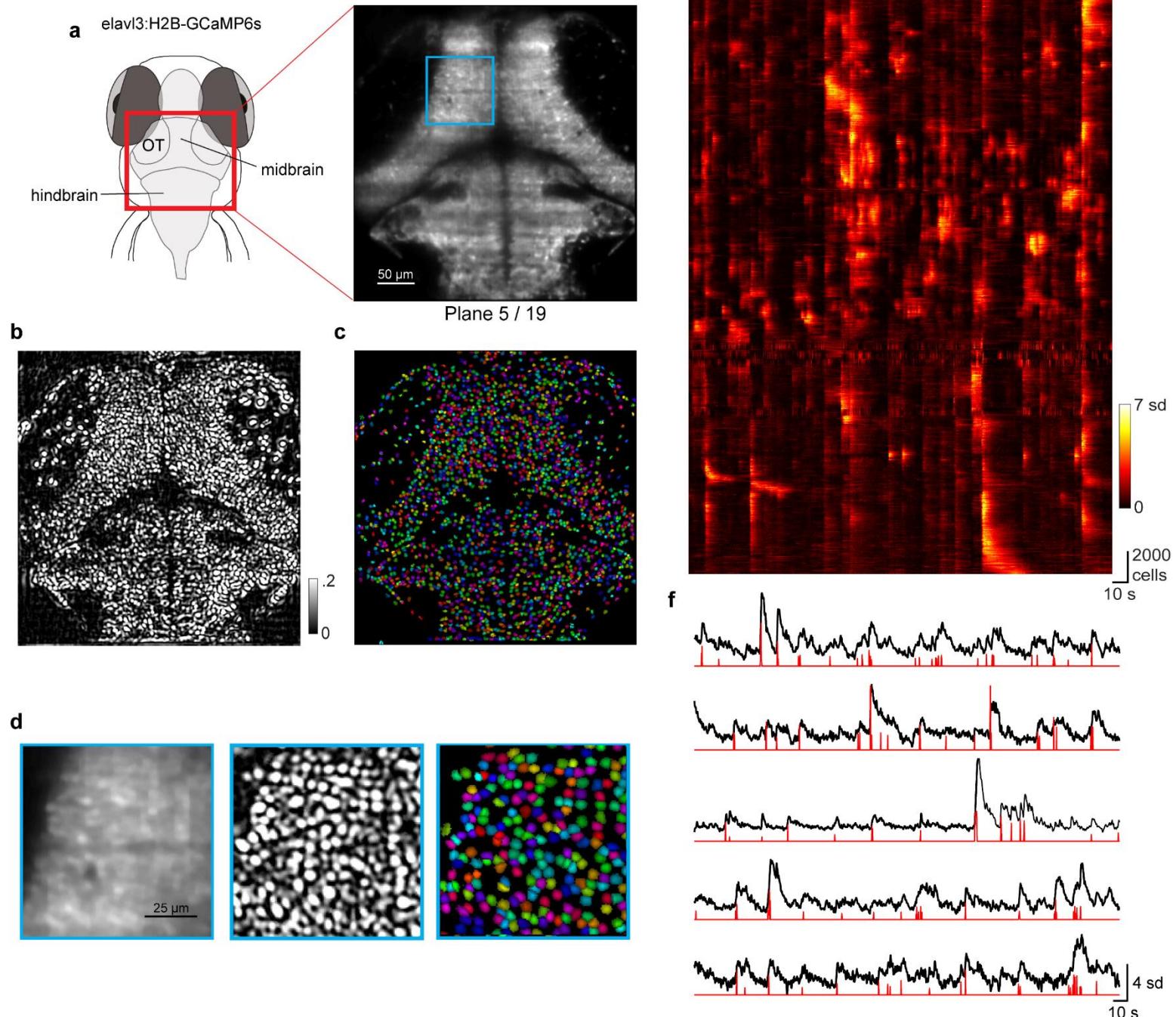
Spike deconvolution



If you deconvolve, you get higher temporal precision

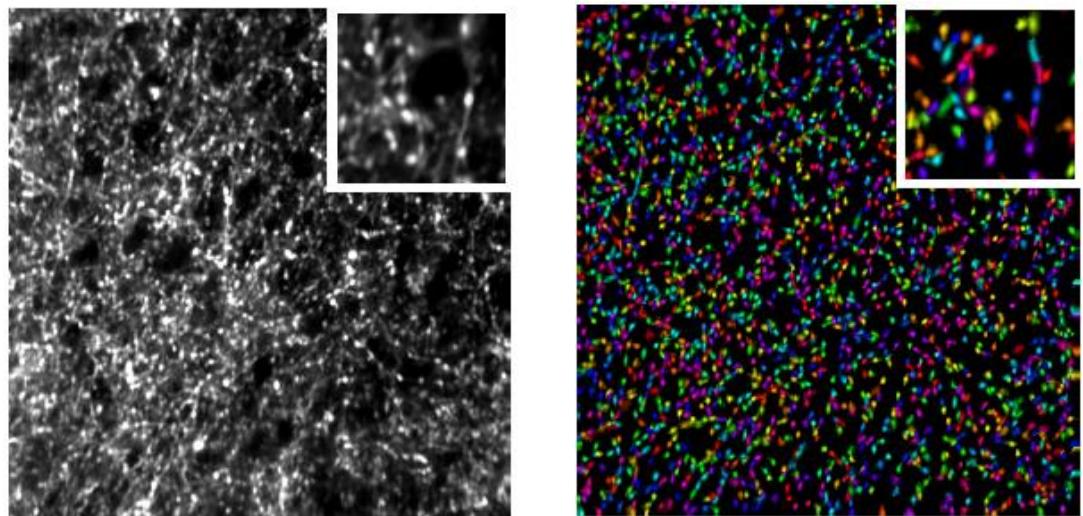


Other data: zebrafish

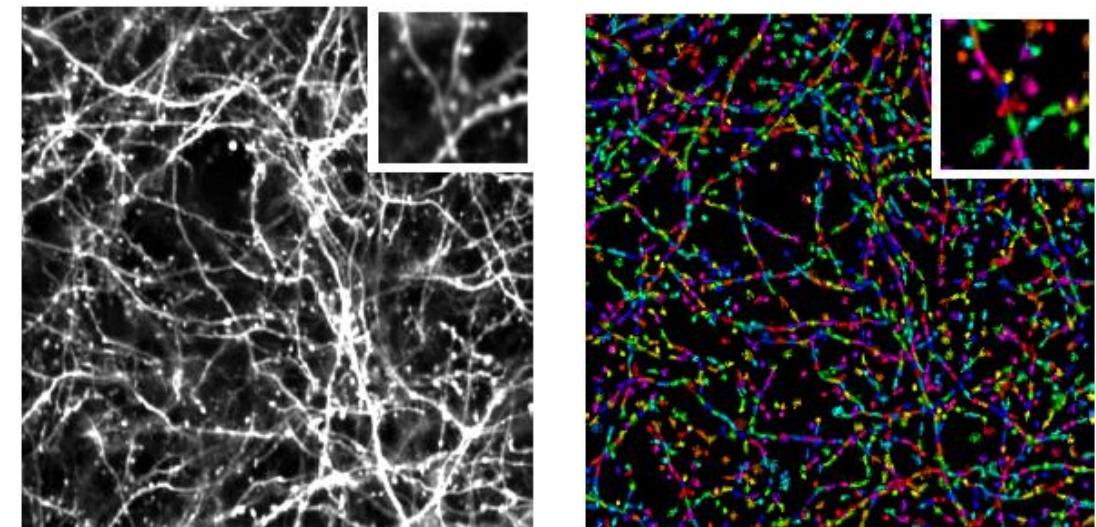


Asaph Zylbertal
& Isaac Bianco

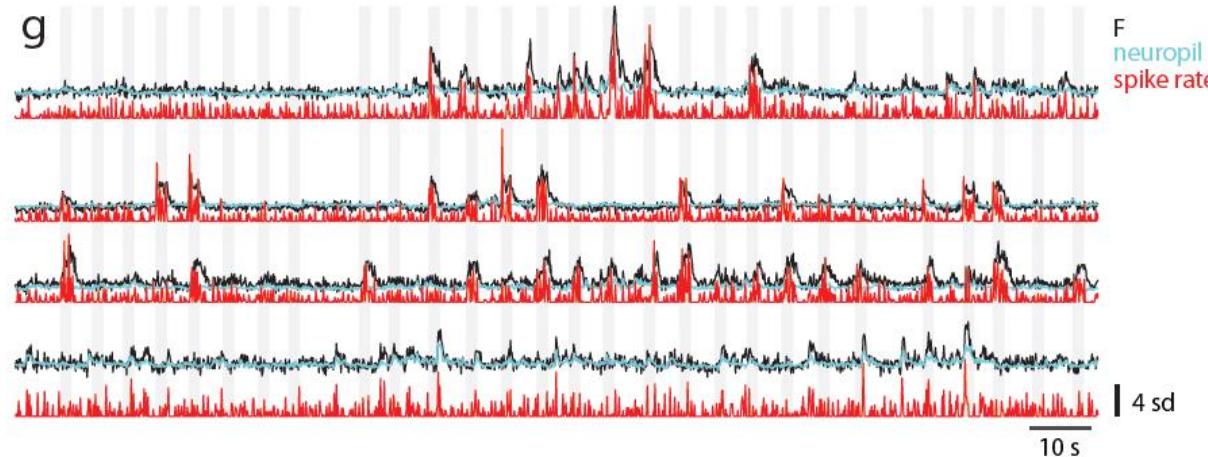
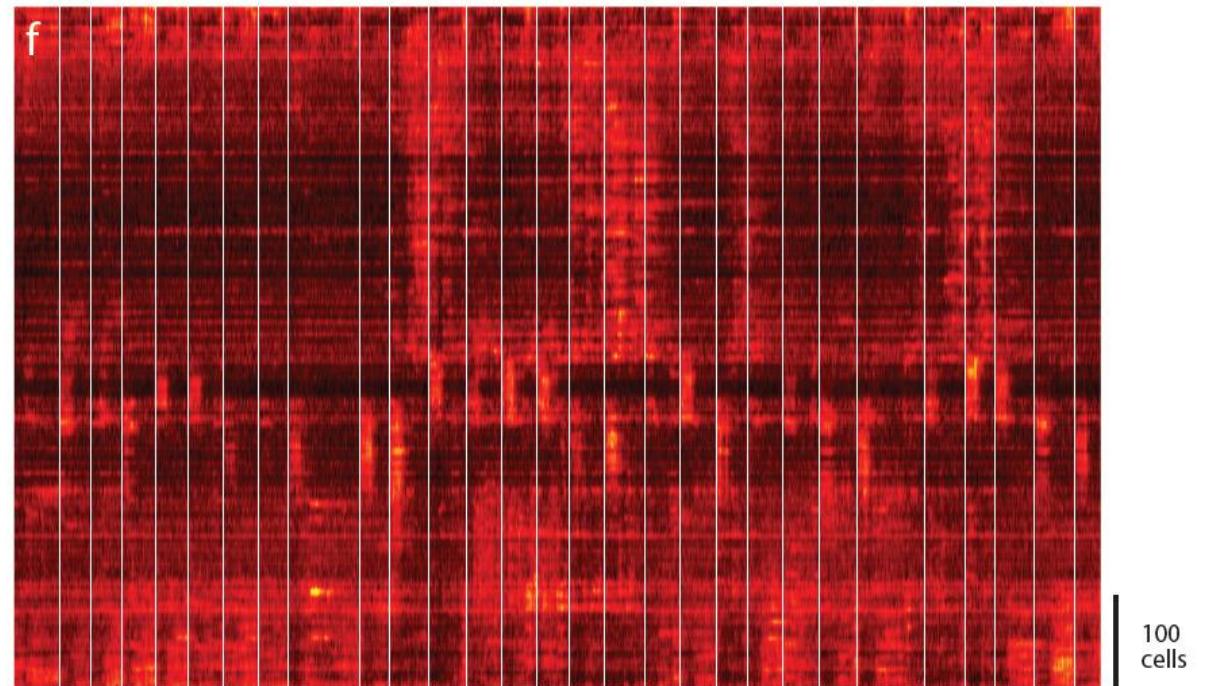
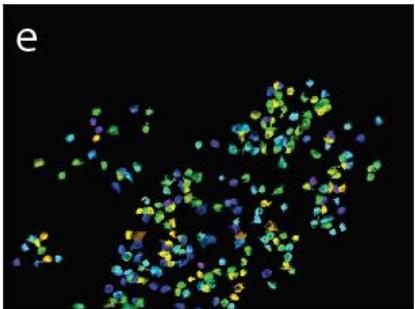
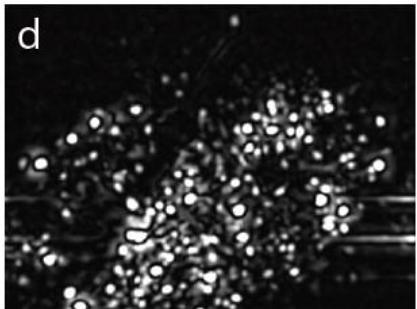
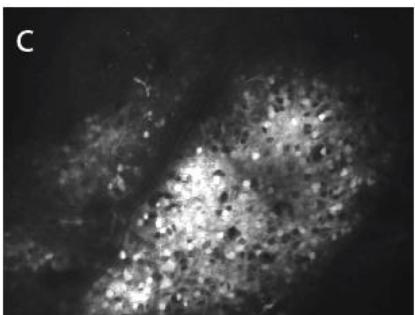
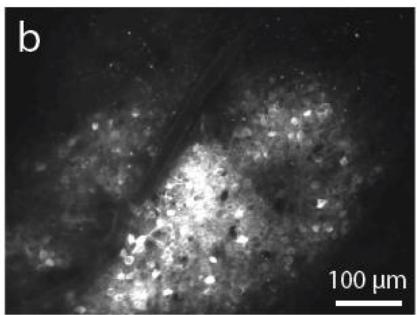
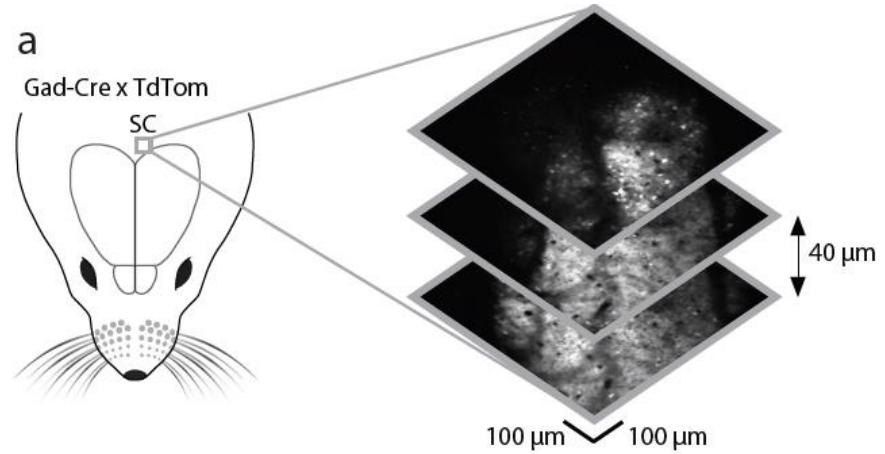
Large-scale bouton recordings in V1



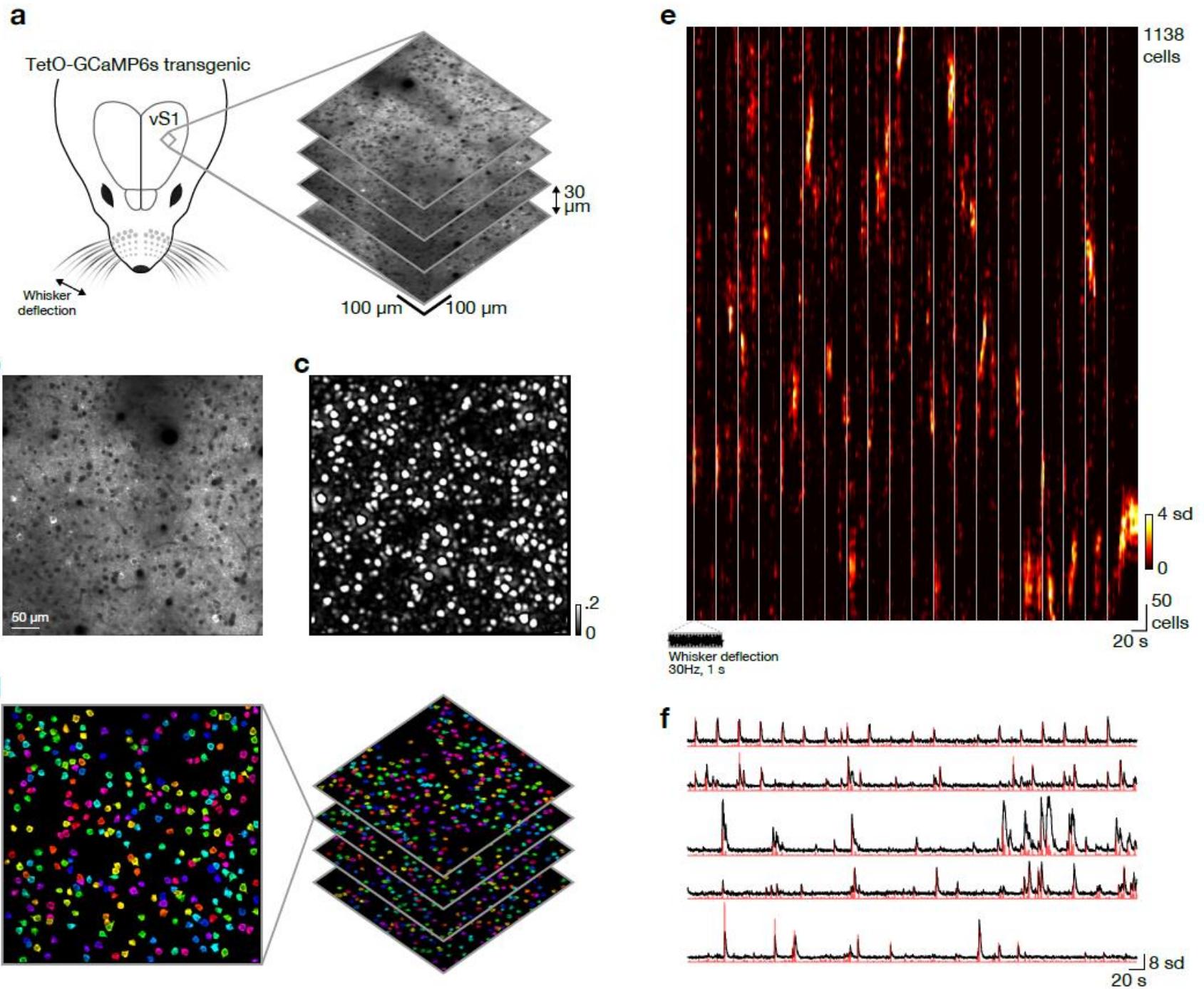
Large-scale synapse recordings in V1



Other data: superior colliculus



Other data: barrel cortex



Henry Dalgleish &
Michael Häusser

Conclusions

- Inspect your data closely
 - data is a movie; how to visualize?
 - summary statistics are useful, but not sufficient
- general approach
 - use a map to find candidate cells
 - have a model
- vertical integration
 - denoising, deconvolution, demixing
- use posthoc classification
 - train a classifier on YOUR data

a

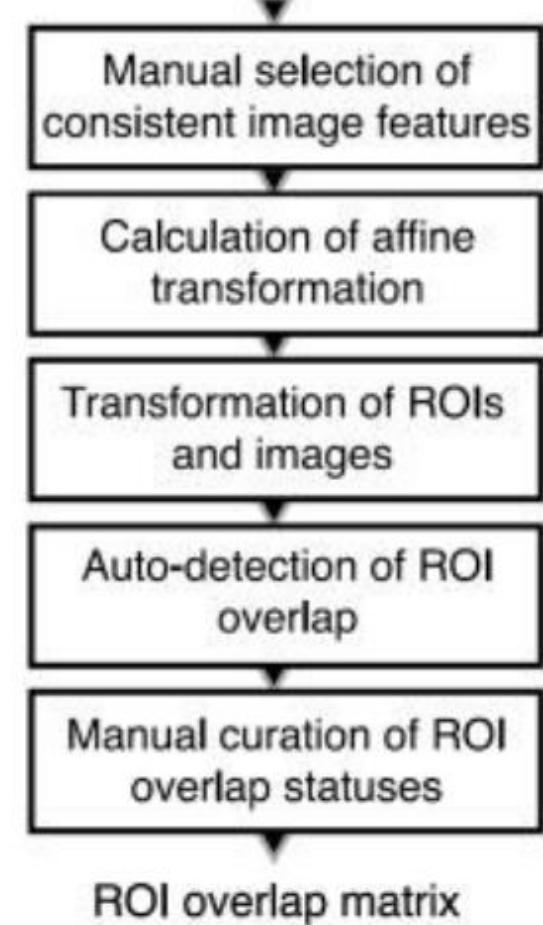
A fluorescence microscopy image showing a tissue section. The image is split vertically: the left side shows brightfield illumination, and the right side shows DAPI staining, which highlights nuclei as bright spots against a dark background. The overall image is grainy and shows cellular structures.



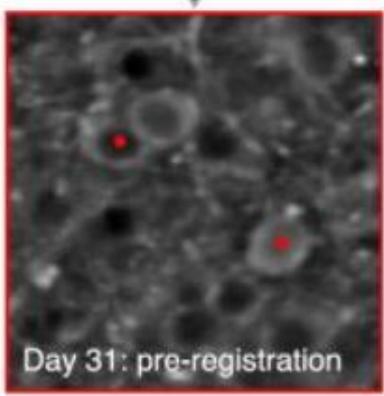
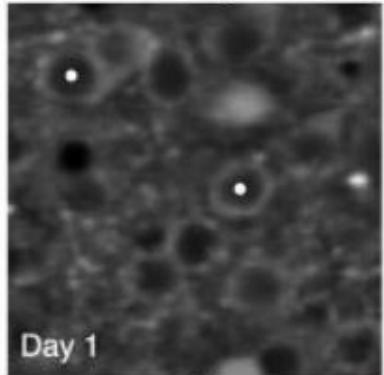
b

Workflow:

Suite2P output



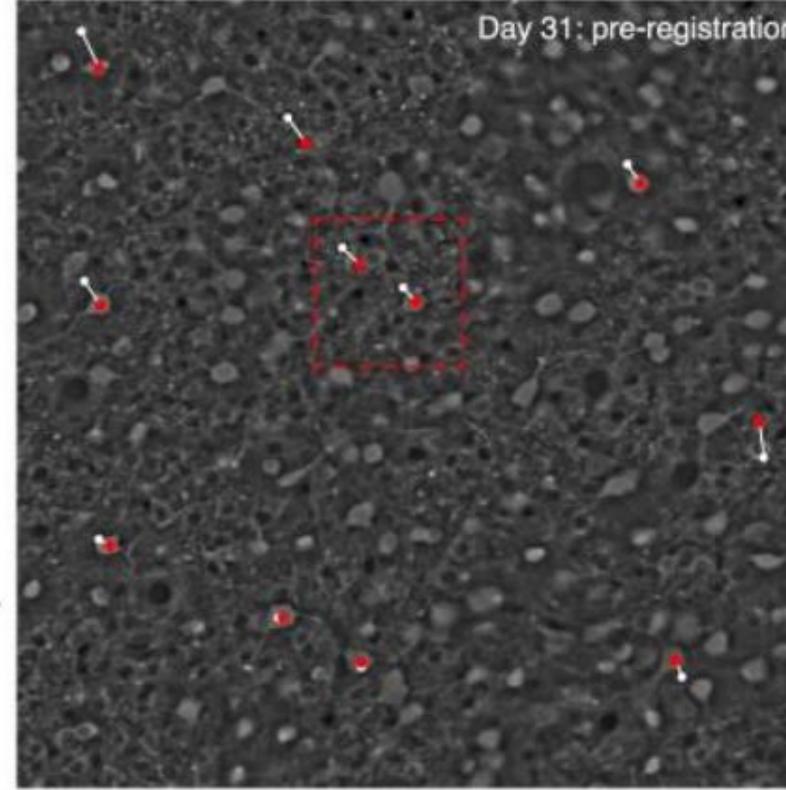
c Manual selection of consistent image features



d

Calculation of affine transformation

Day 31: pre-registration



e Transform images and ROIs

