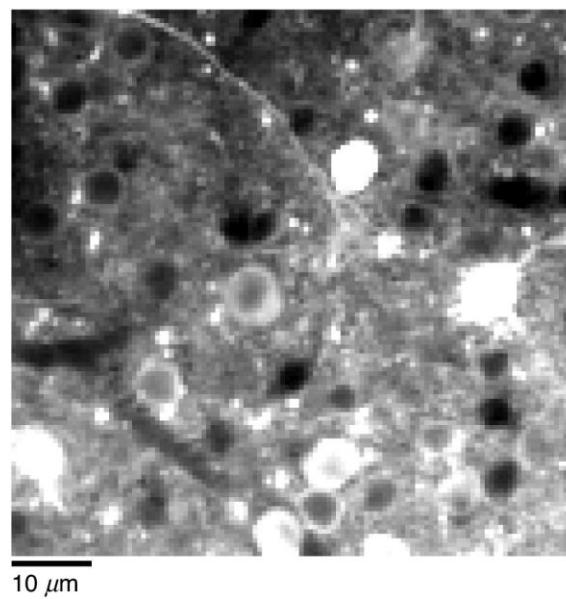


# Cell detection

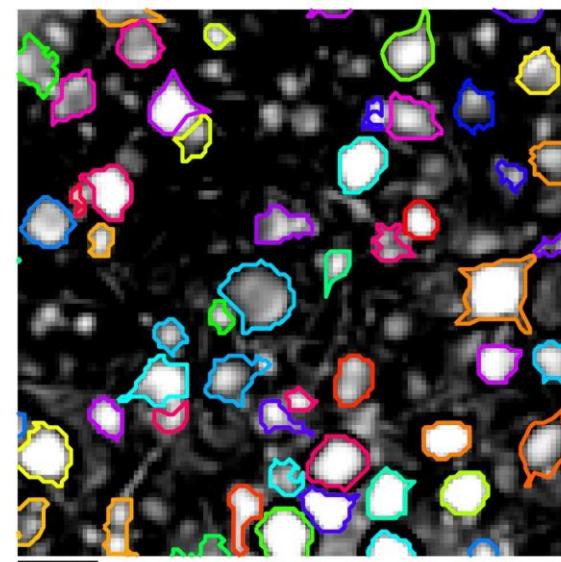
Marius Pachitariu



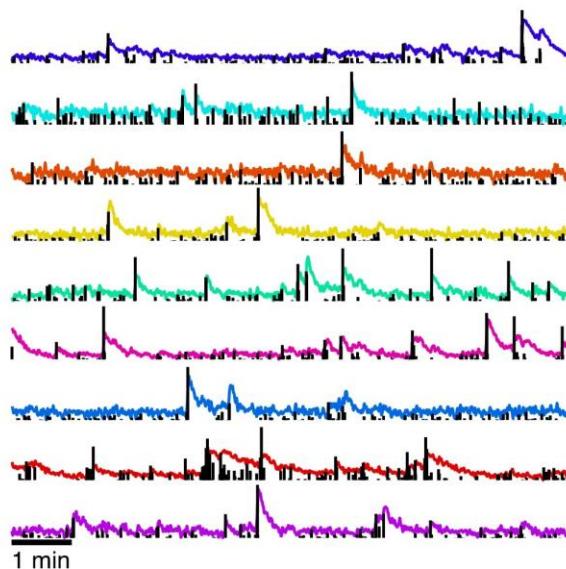
### Step 1. Motion registration



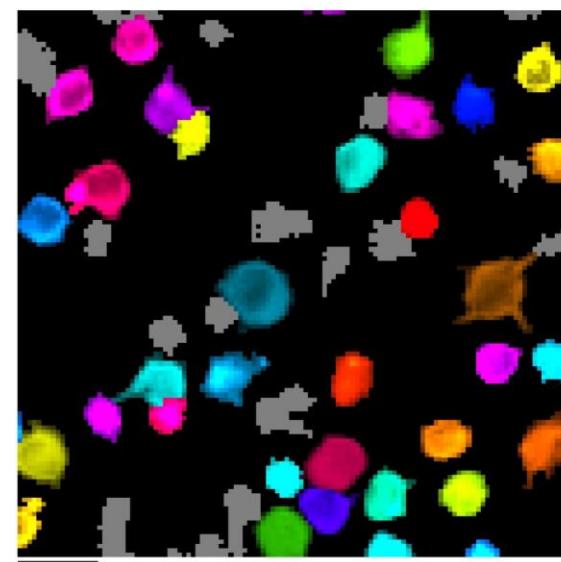
### Step 2. ROI extraction

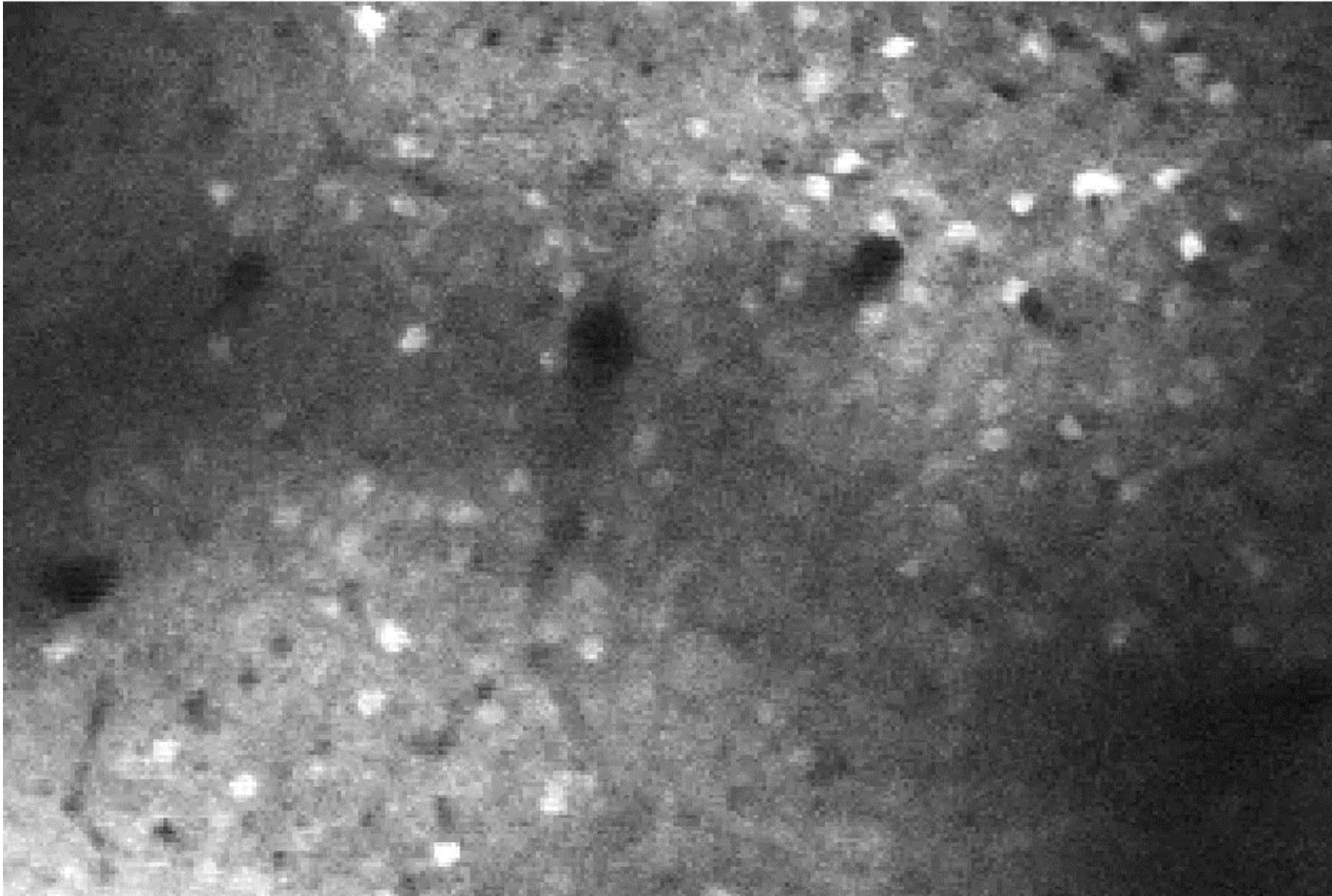


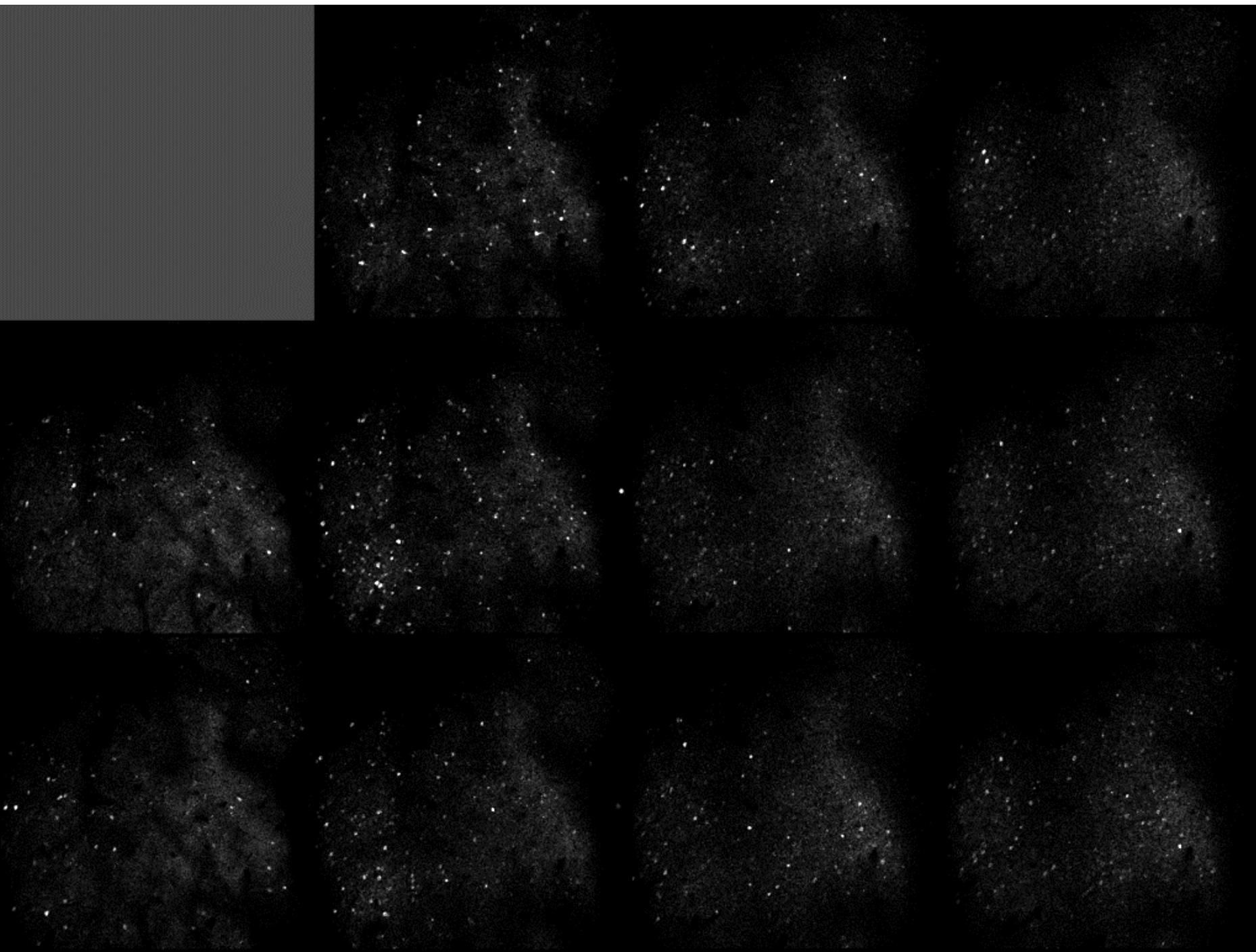
### Step 3. Spike deconvolution

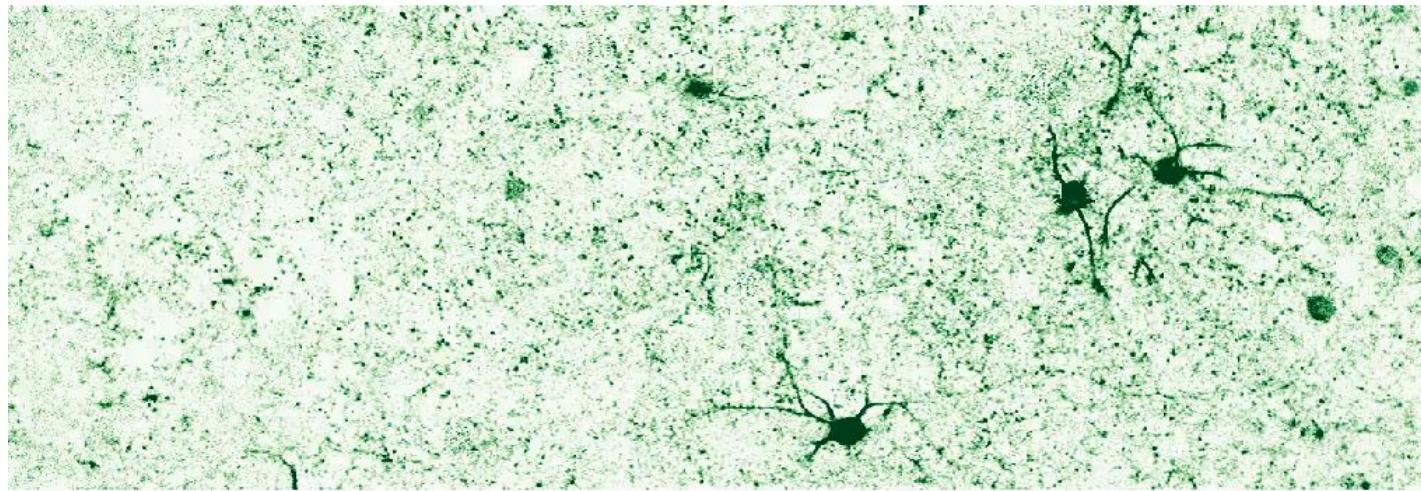


### Step 4. Quality control

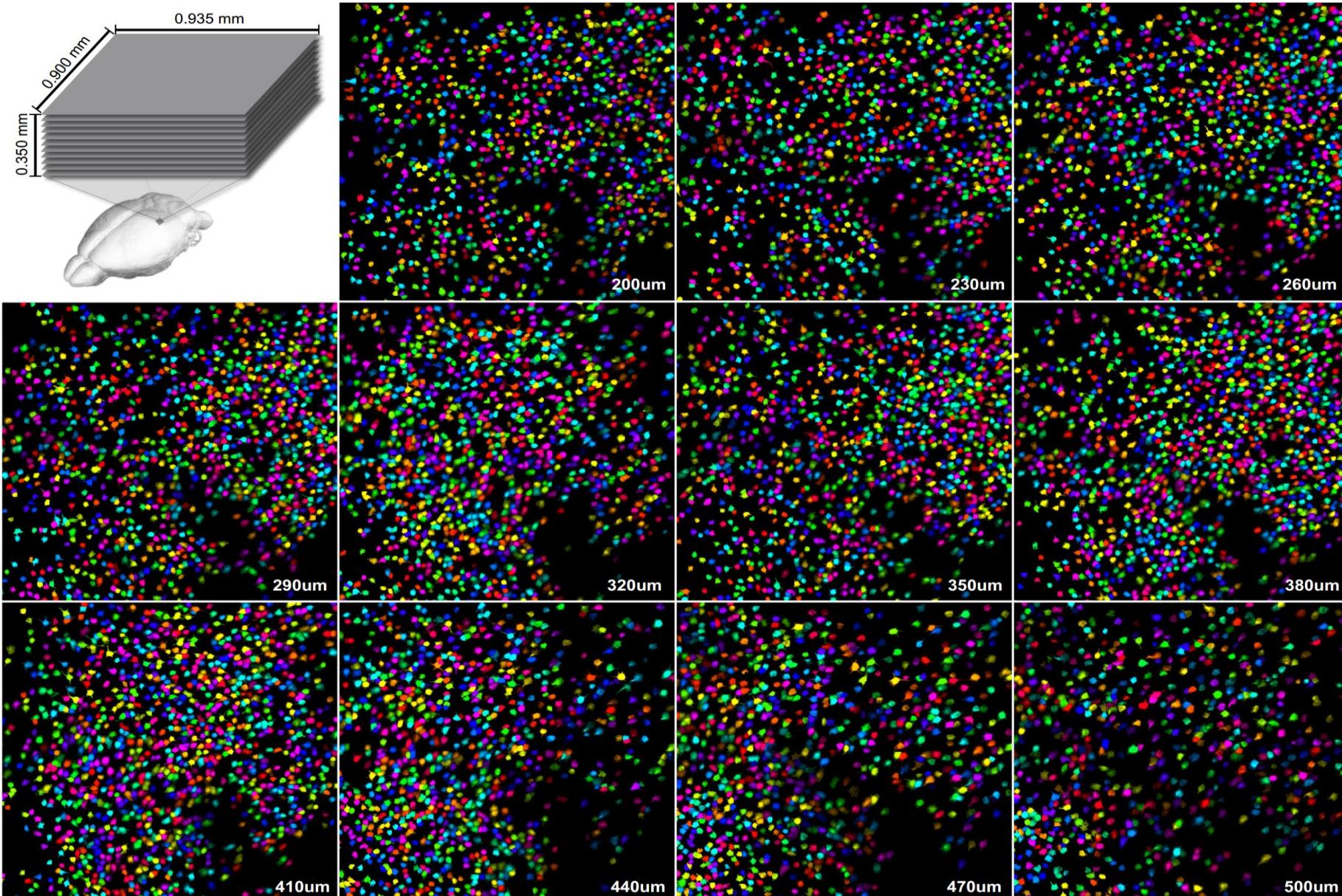








# Suite2p processes all these in 2 hours



# Some ideas for how to detect cells

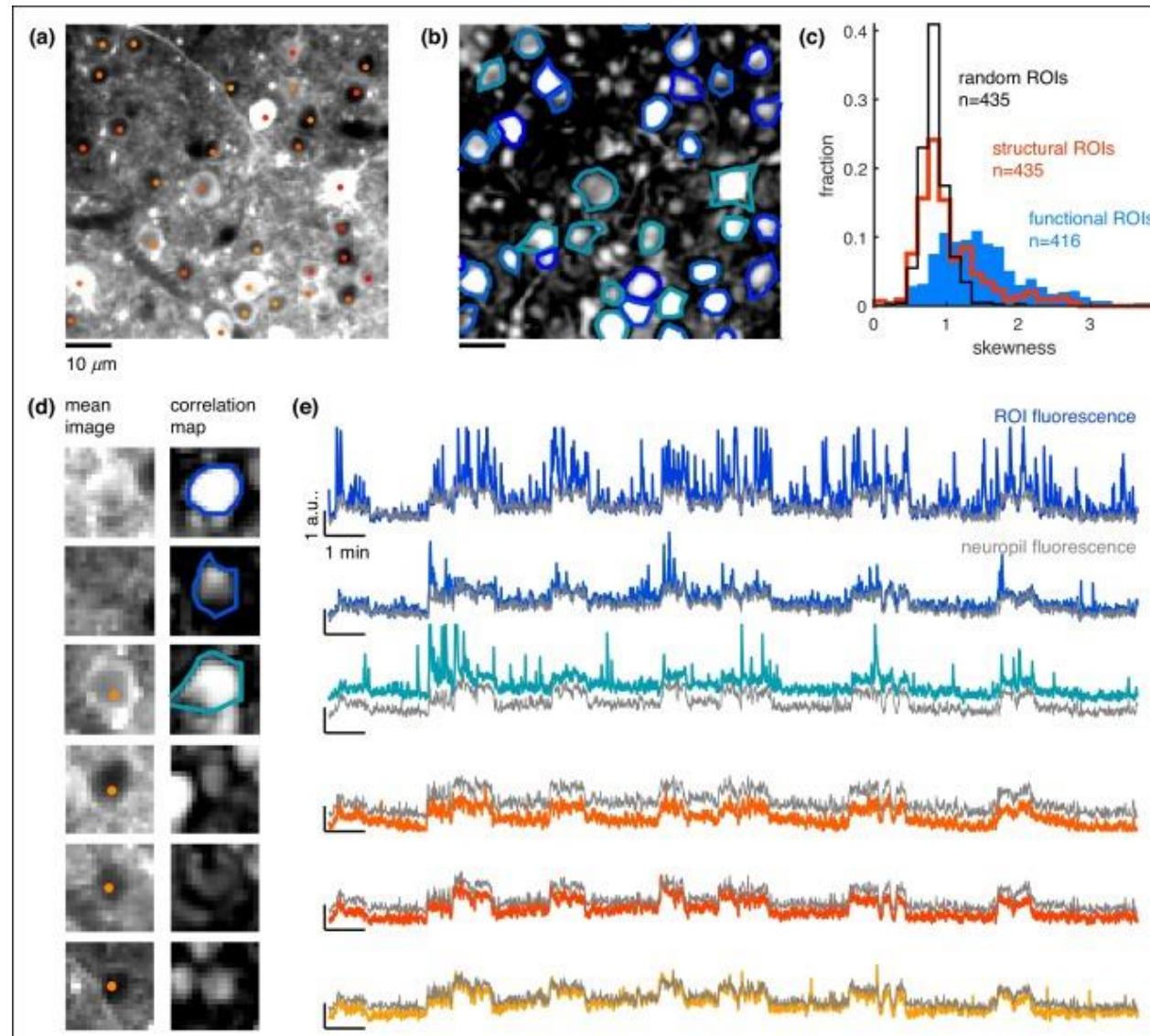
## STRUCTURAL

- mean image
- nuclear tag

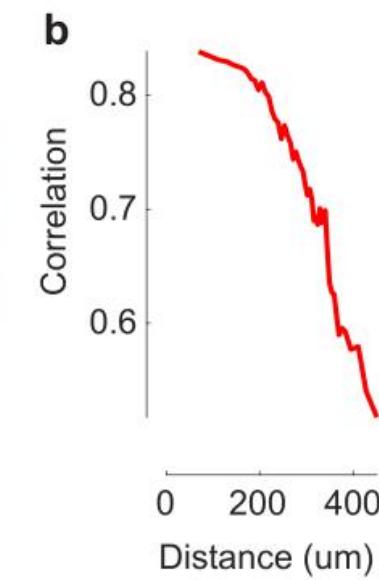
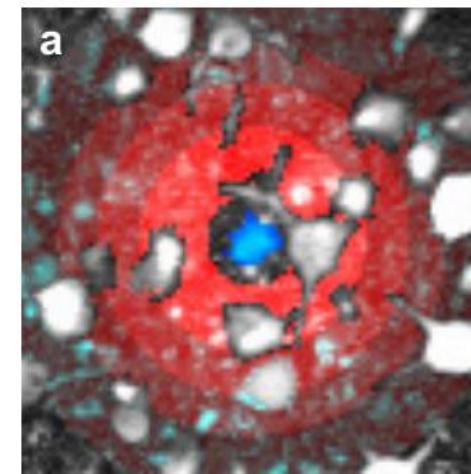
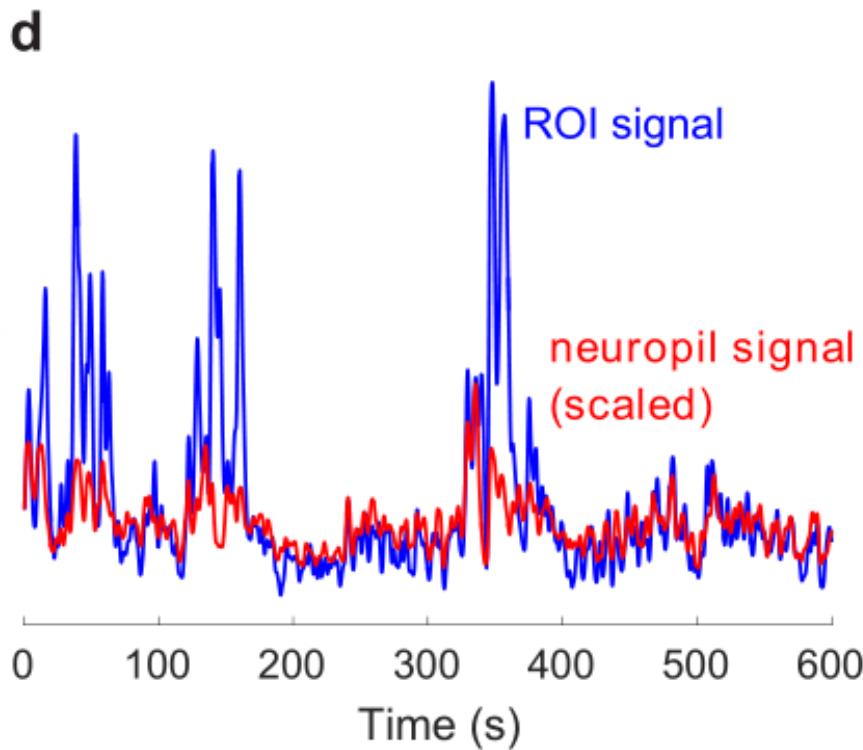
## FUNCTIONAL

- 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

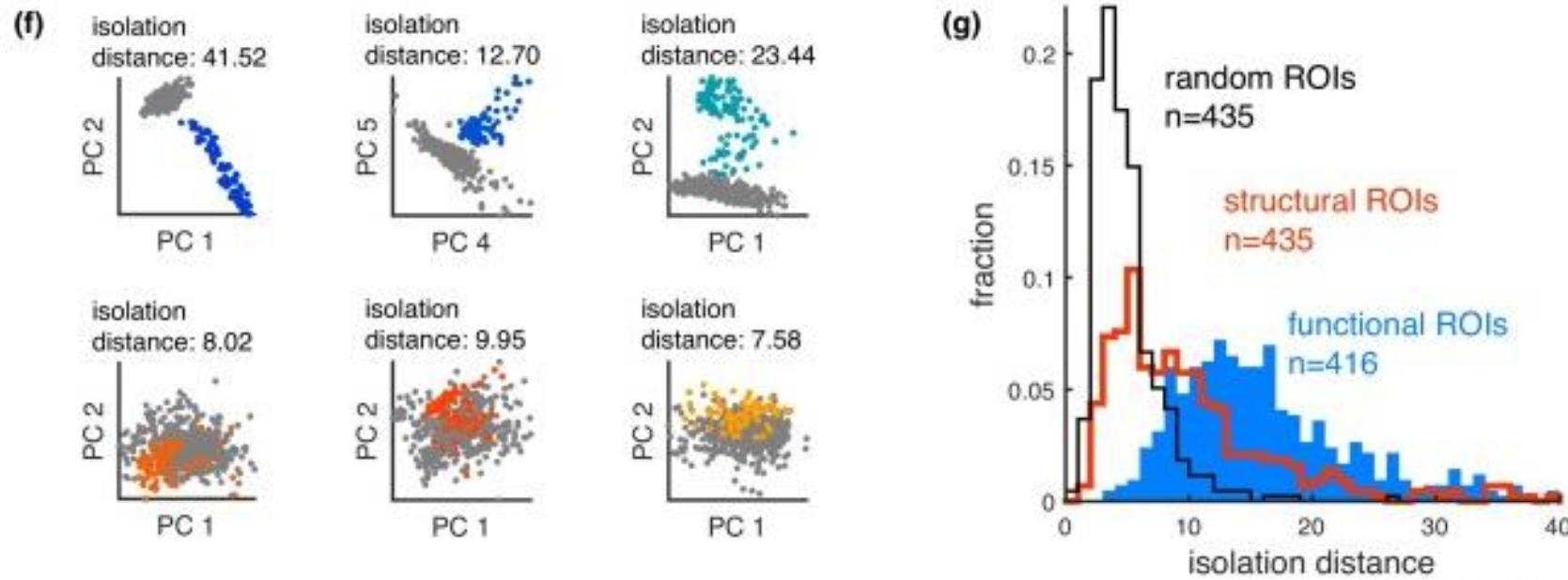
# Why are “structural” methods not enough?



# The neuropil problem



# Functional ROIs are better isolated



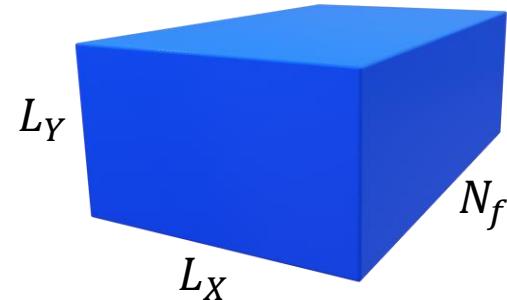
Treat movie  $F$  as a 3D matrix, or “volume”

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

$L_Y$  = image height

$L_X$  = image width

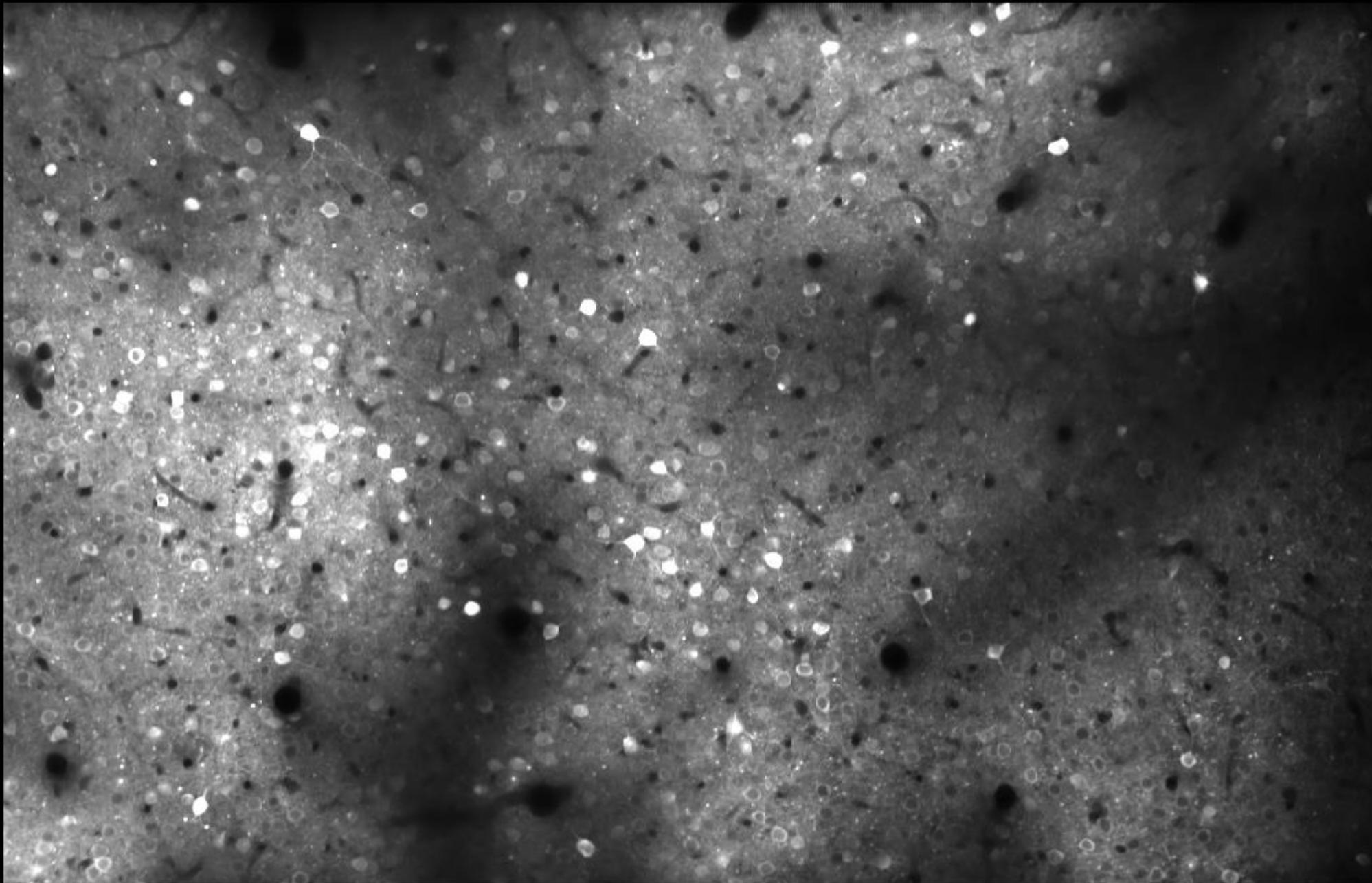
$N_f$  = number of frames



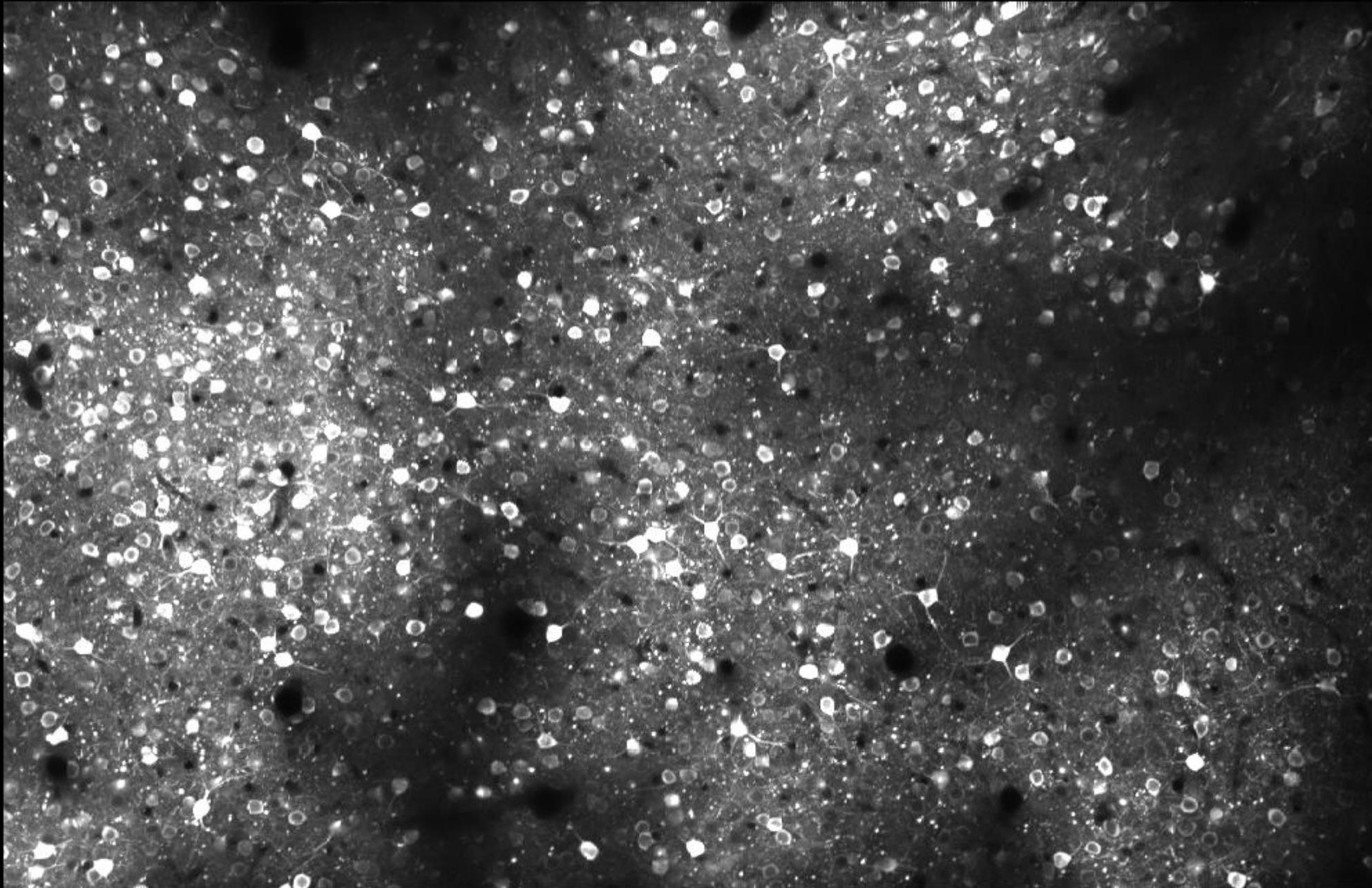
Compare mean and max projections

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

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



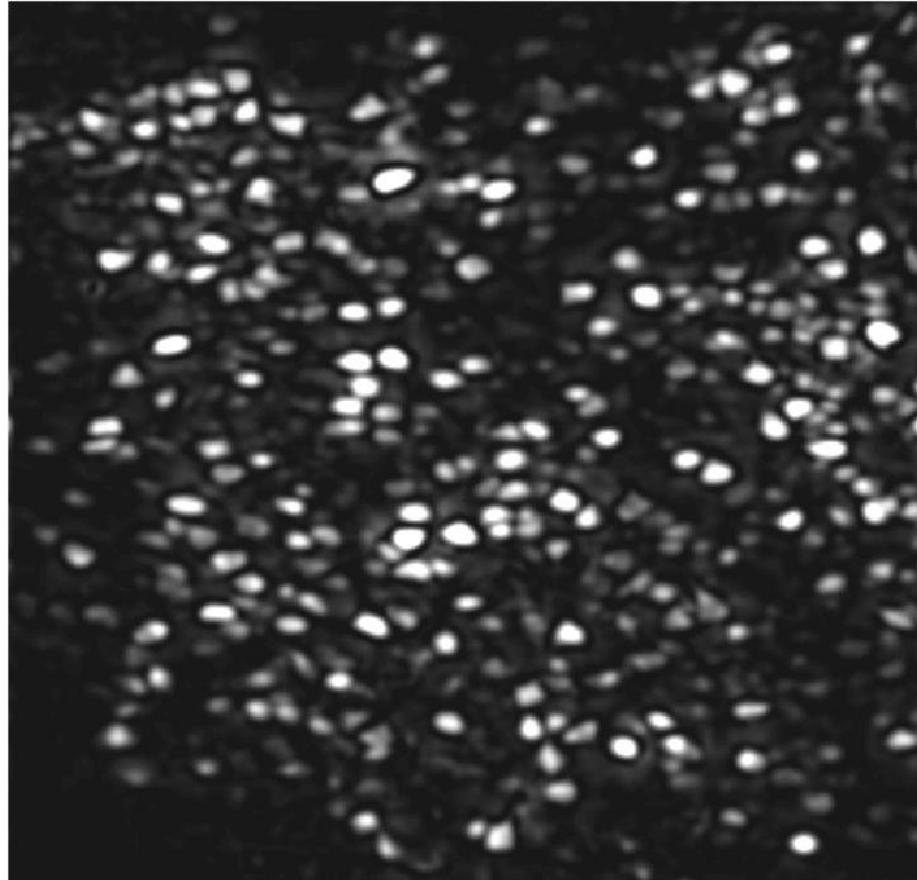
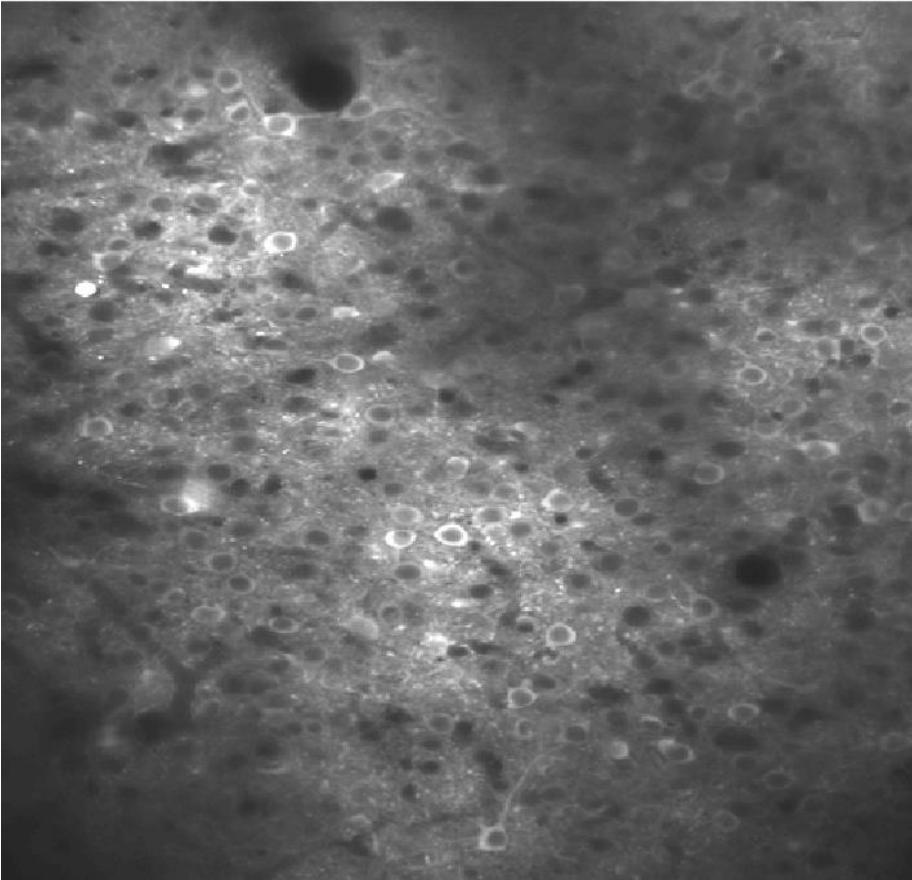
Mean image



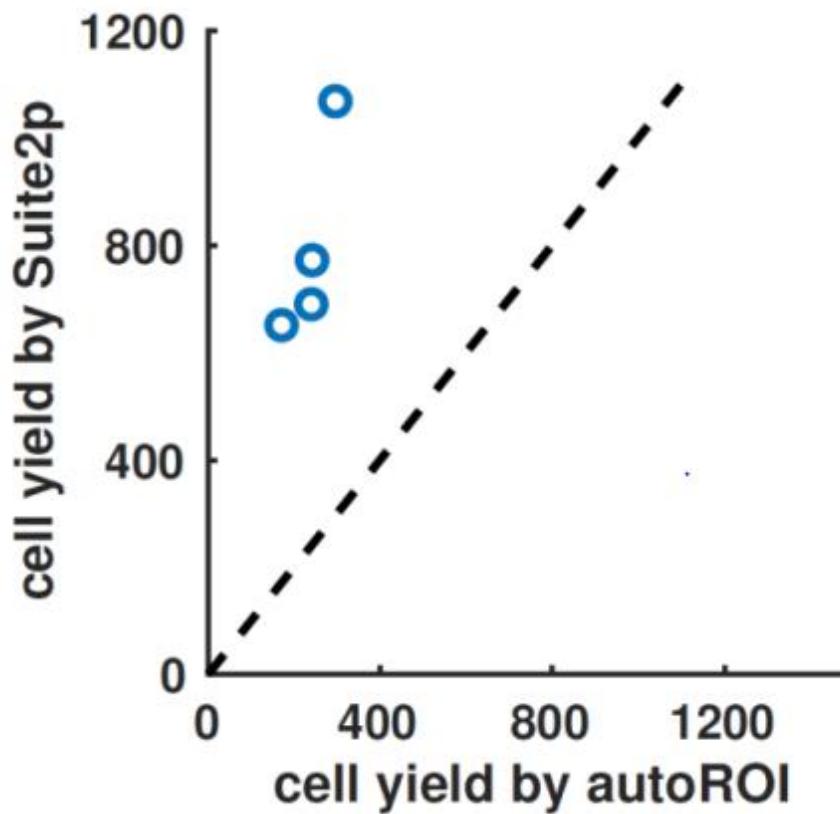
Maximum projection

# What is the correlation map?

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

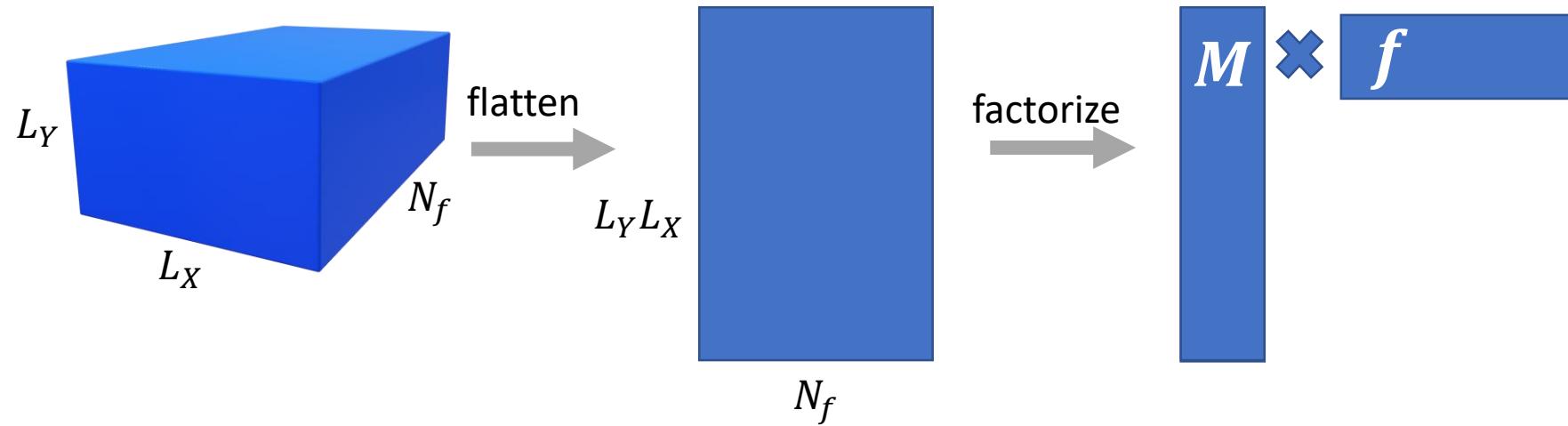


# AutoROI uses only the correlation map



# 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)

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

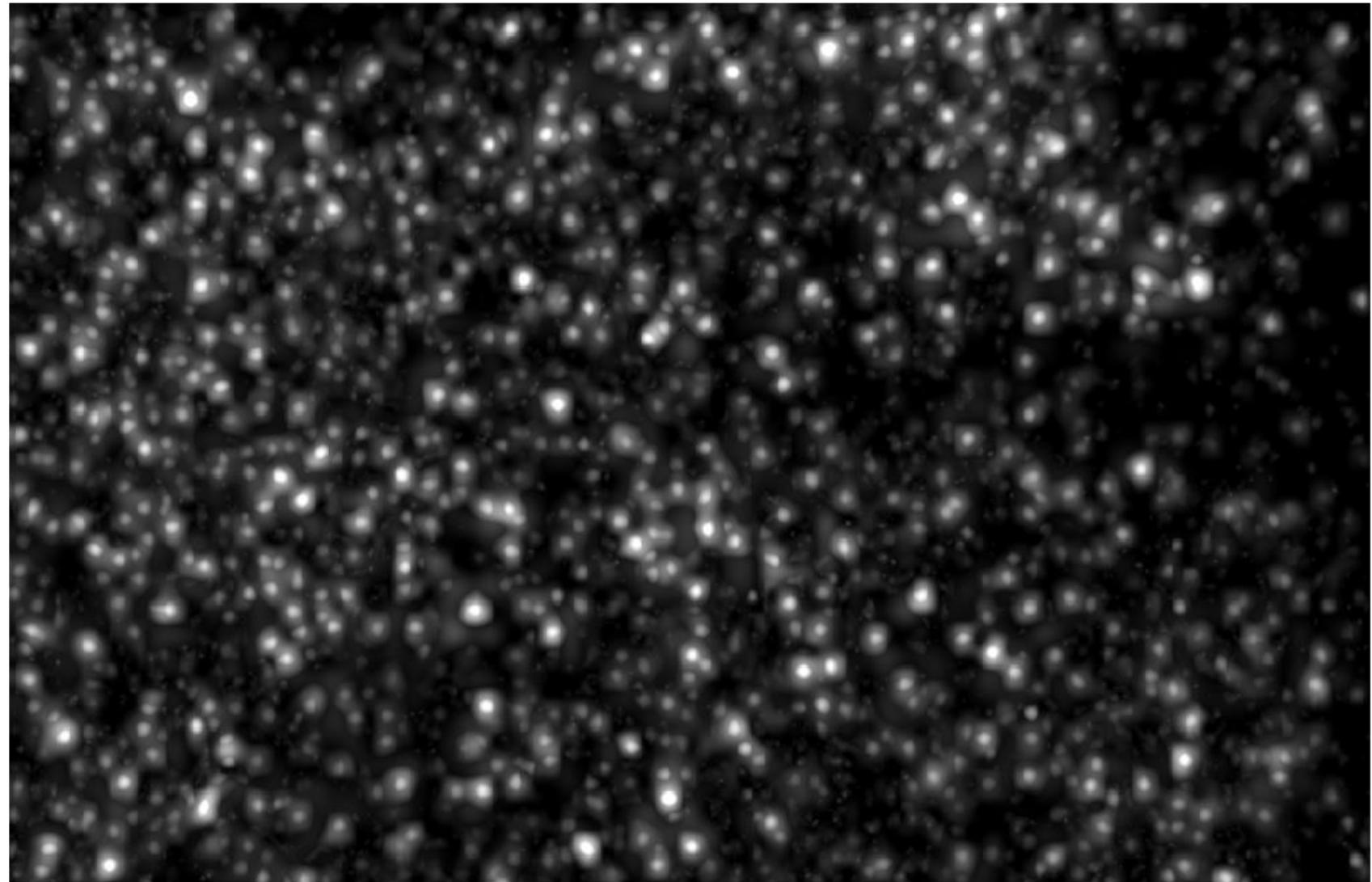
At each iteration  $k$ :

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  $M_k$

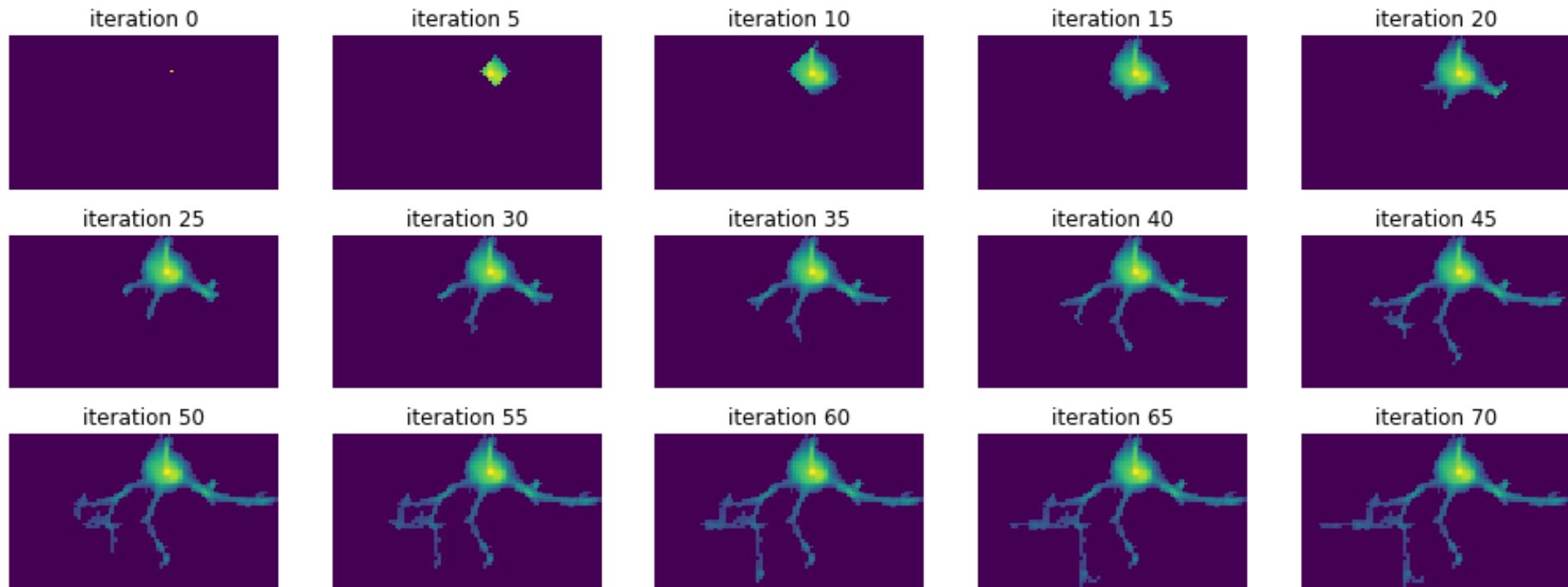
How to hunt for new cells? We need a map.

Multi-scale  
Sparse Projection  
Map

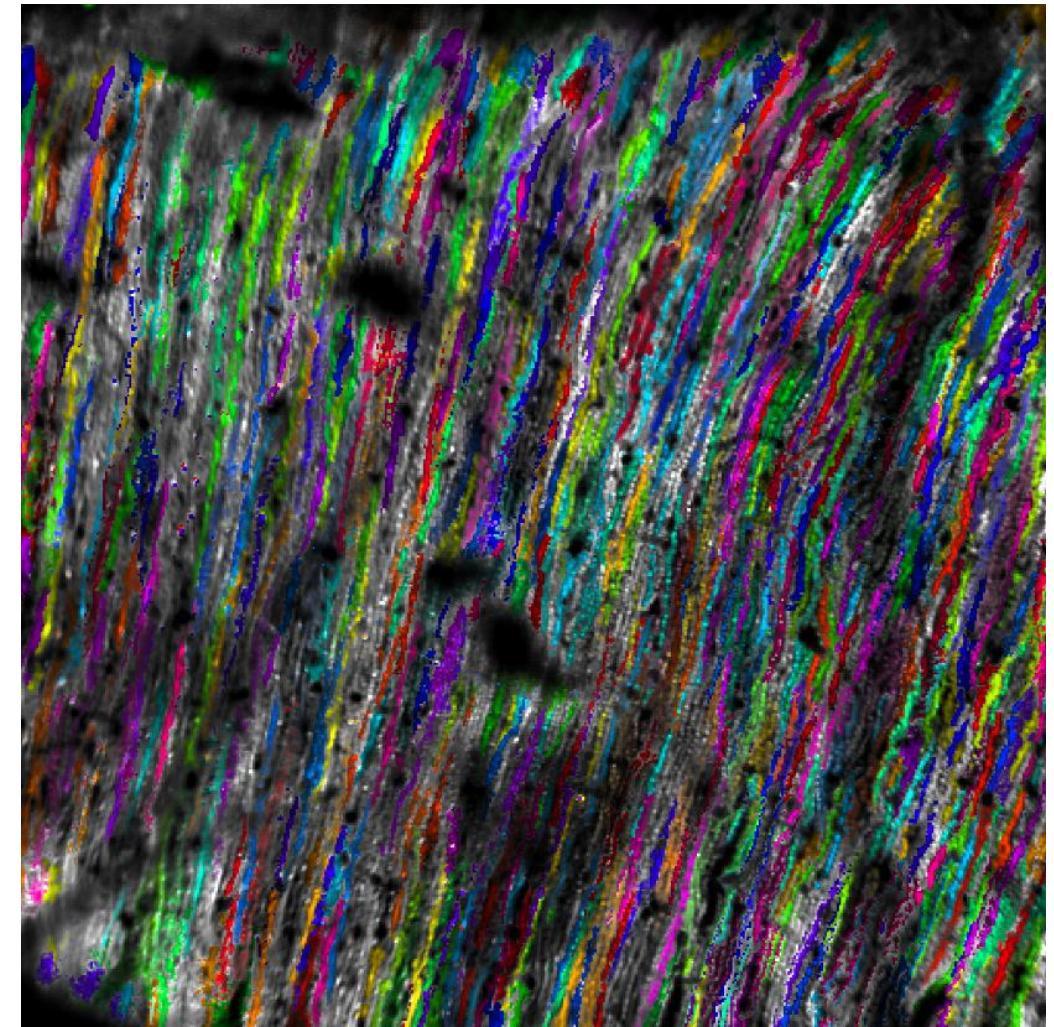
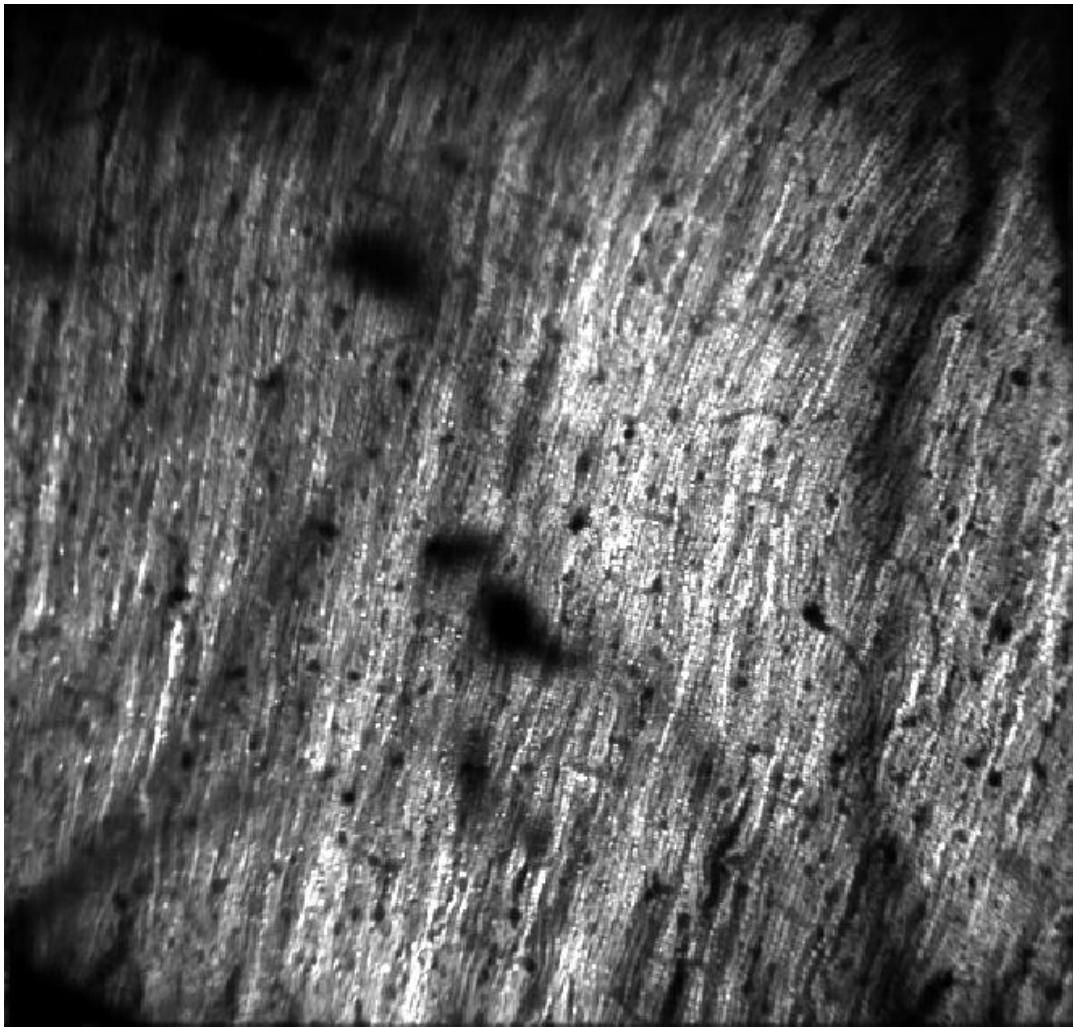
“correlation map”



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



# Cerebellum fibers



Kostadinov et al, 2019

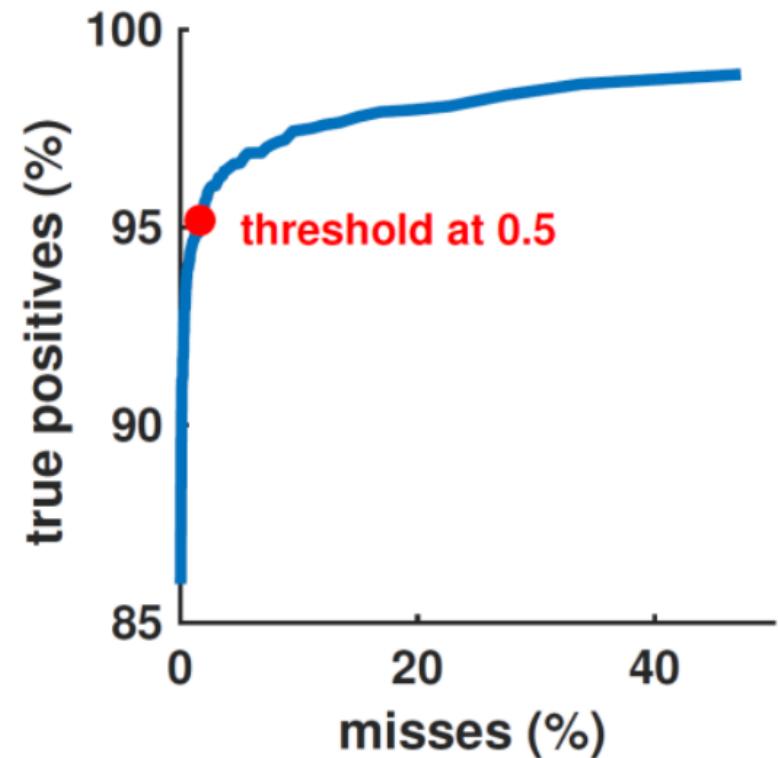
## Some details...

- 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

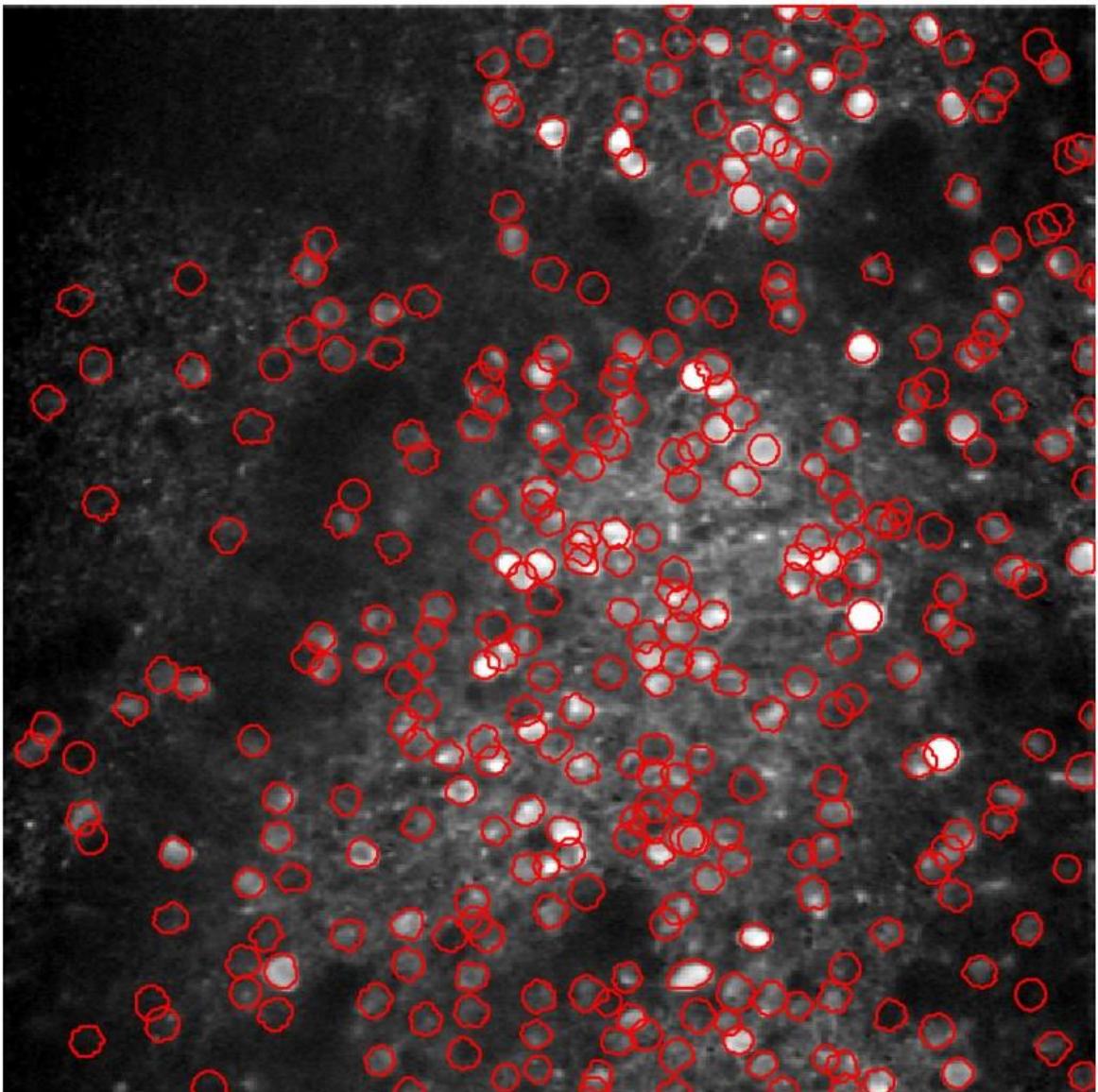
# How to classify if an ROI is a cell?

- 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

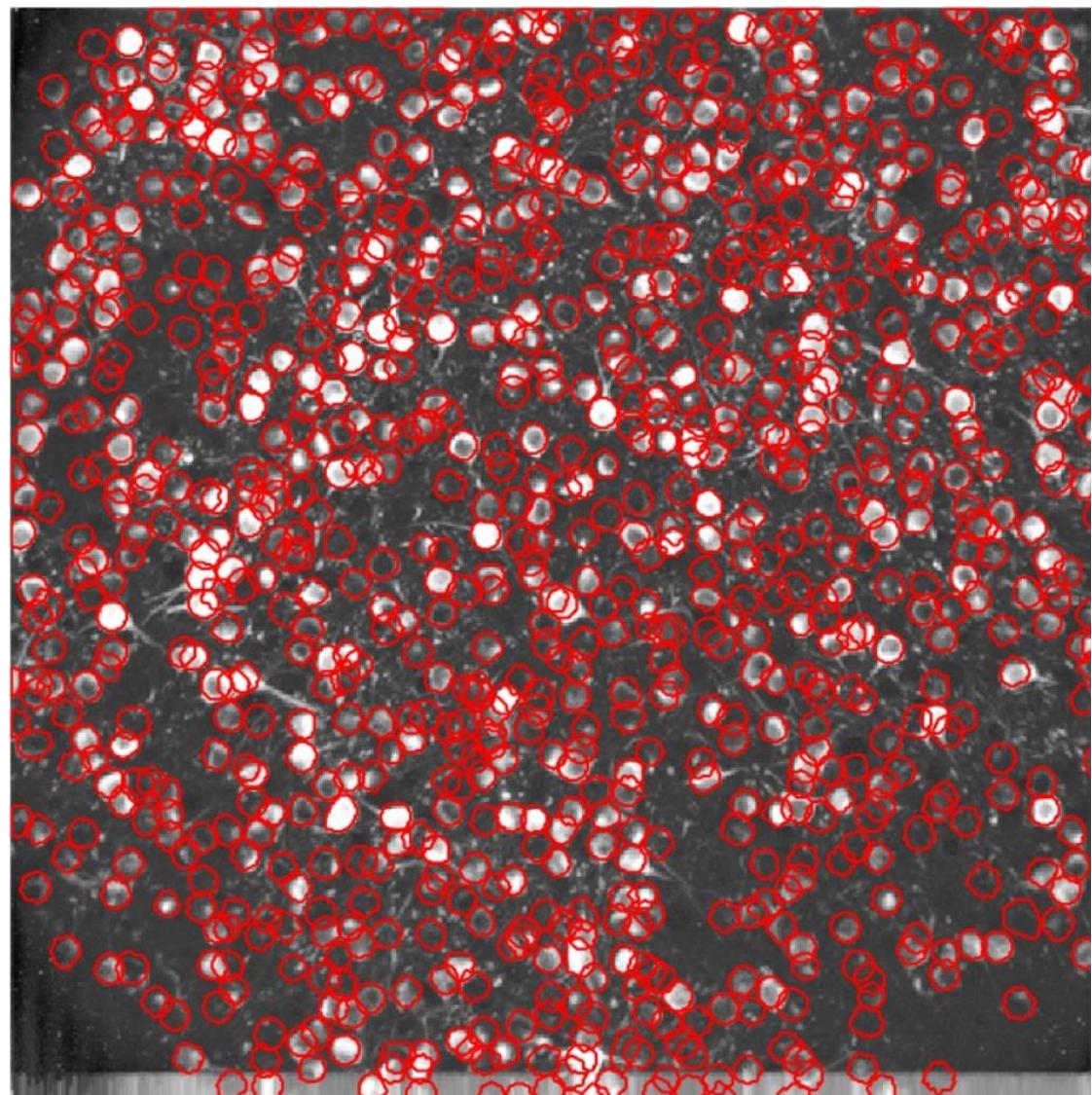
within sample performance



# How to train classifiers?



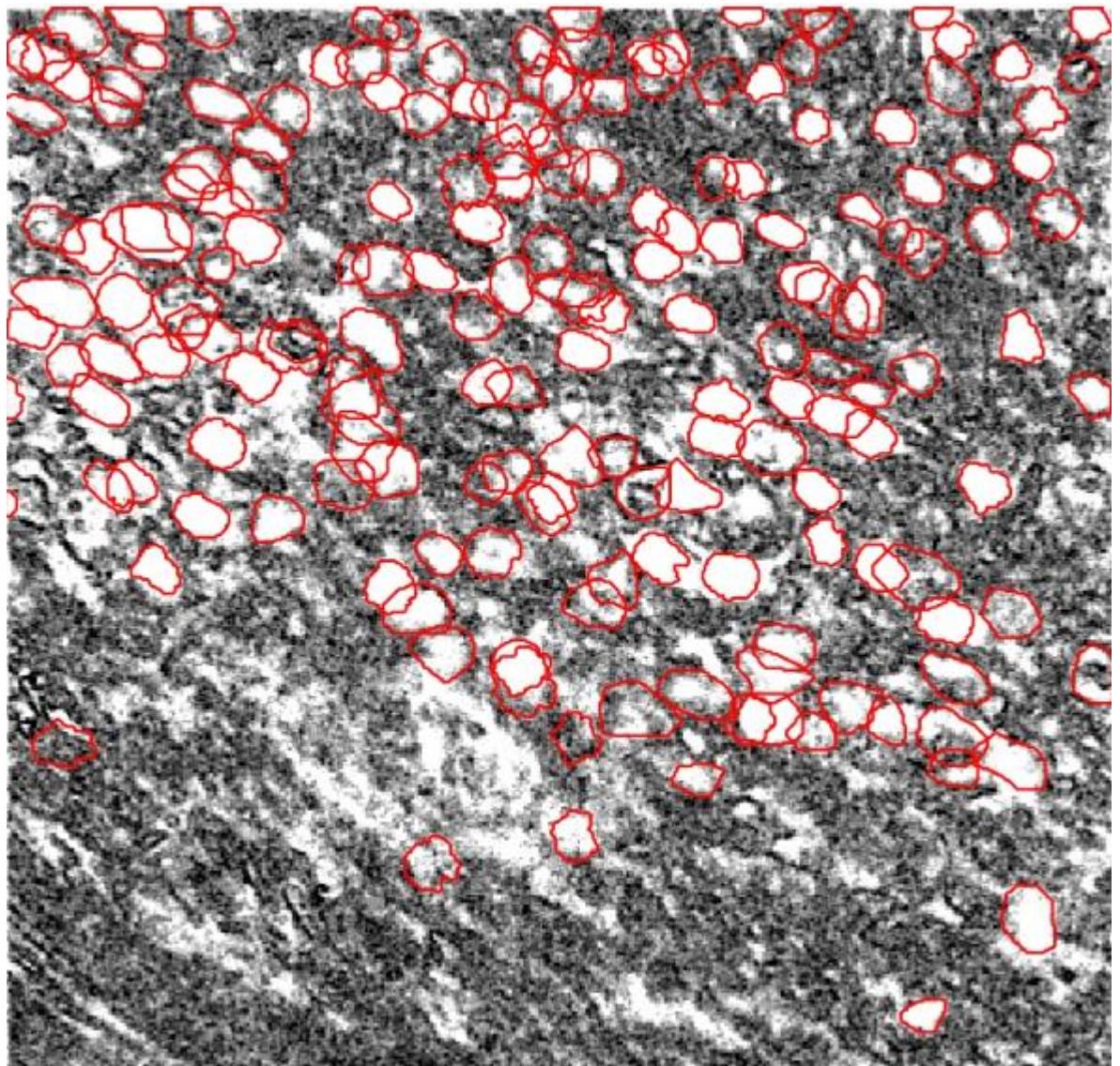
Hausser lab



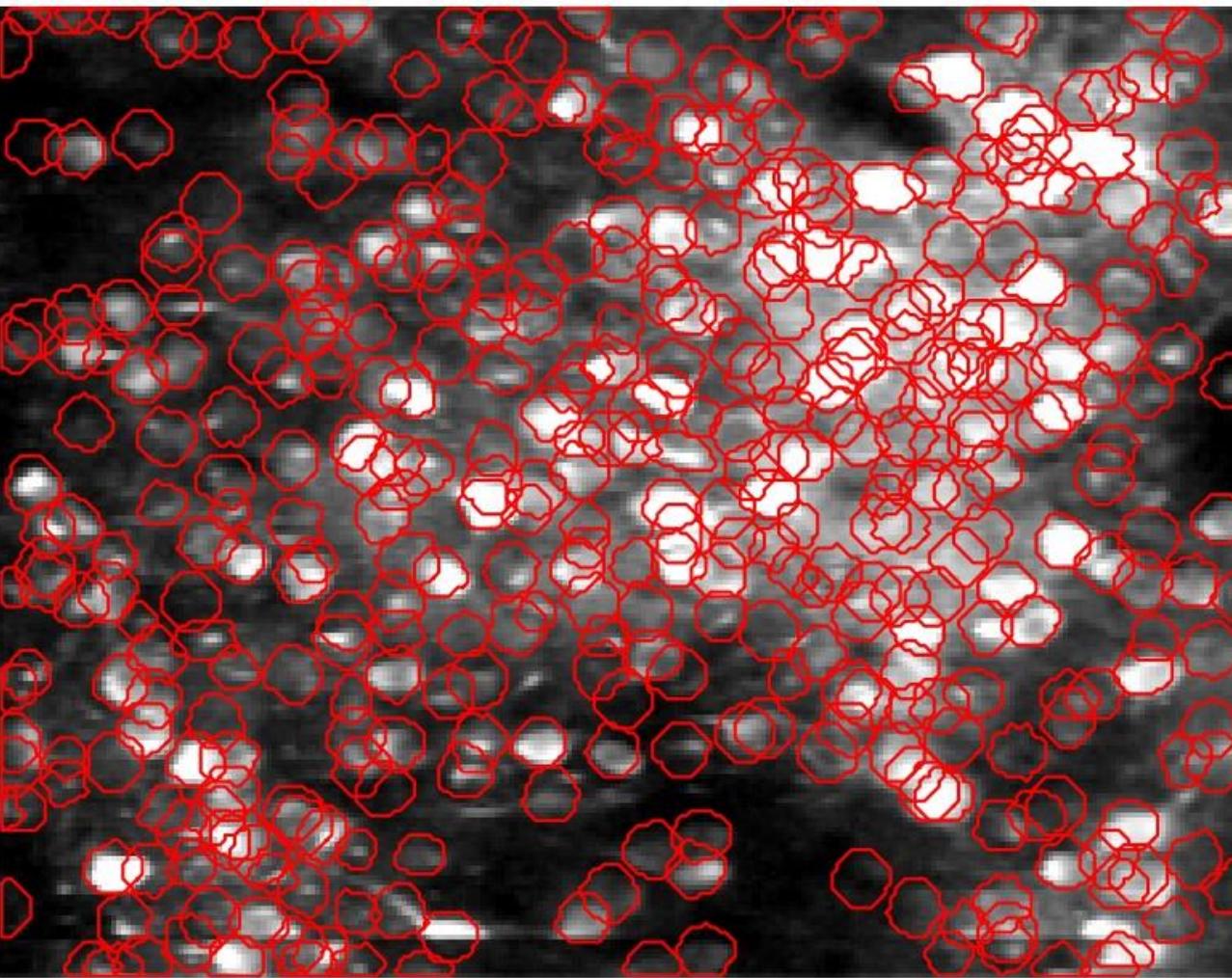
Tank lab

Giovanucci et al, 2019  
(Caiman paper)

# How to train classifiers?

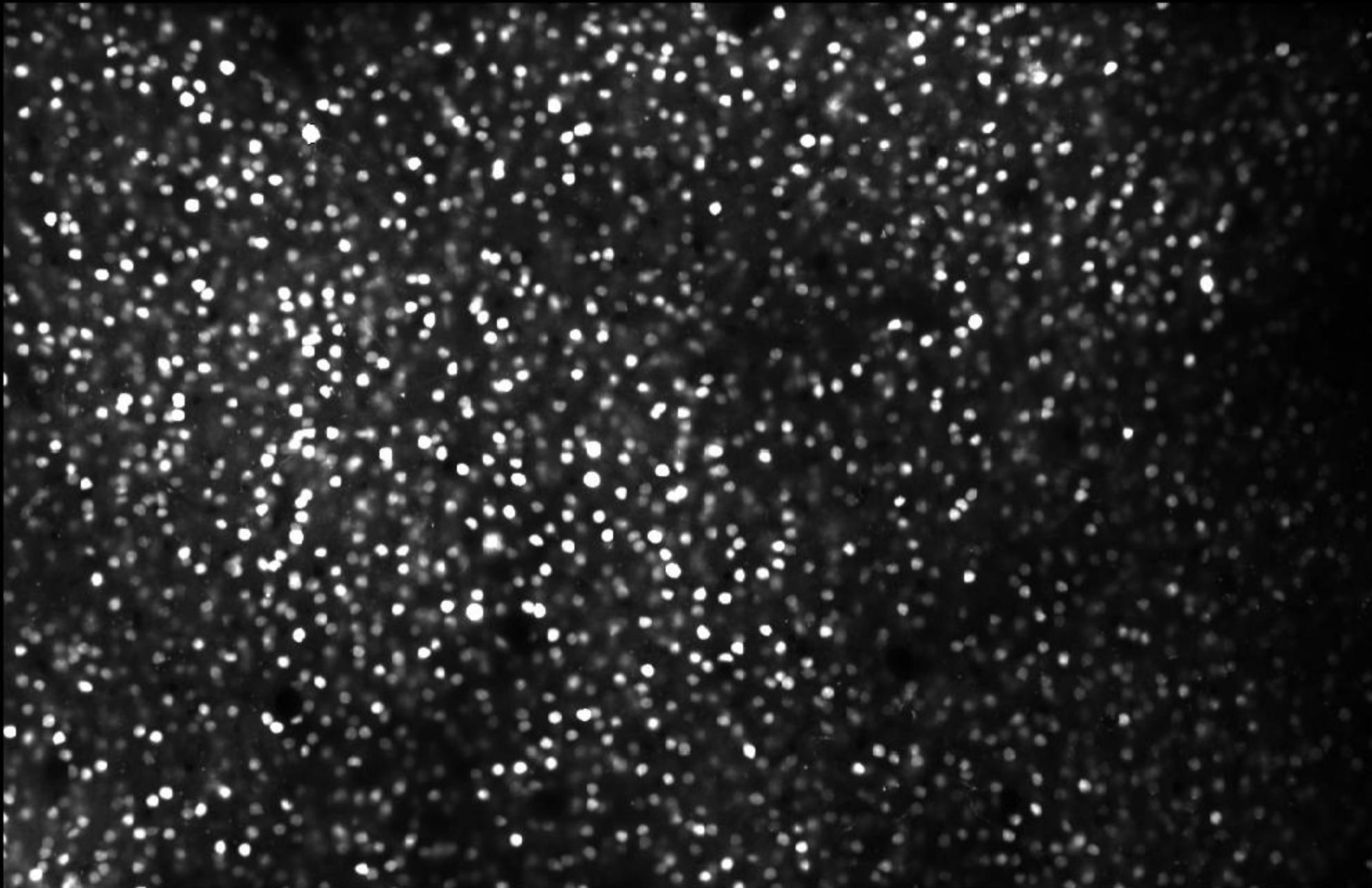


Tank lab

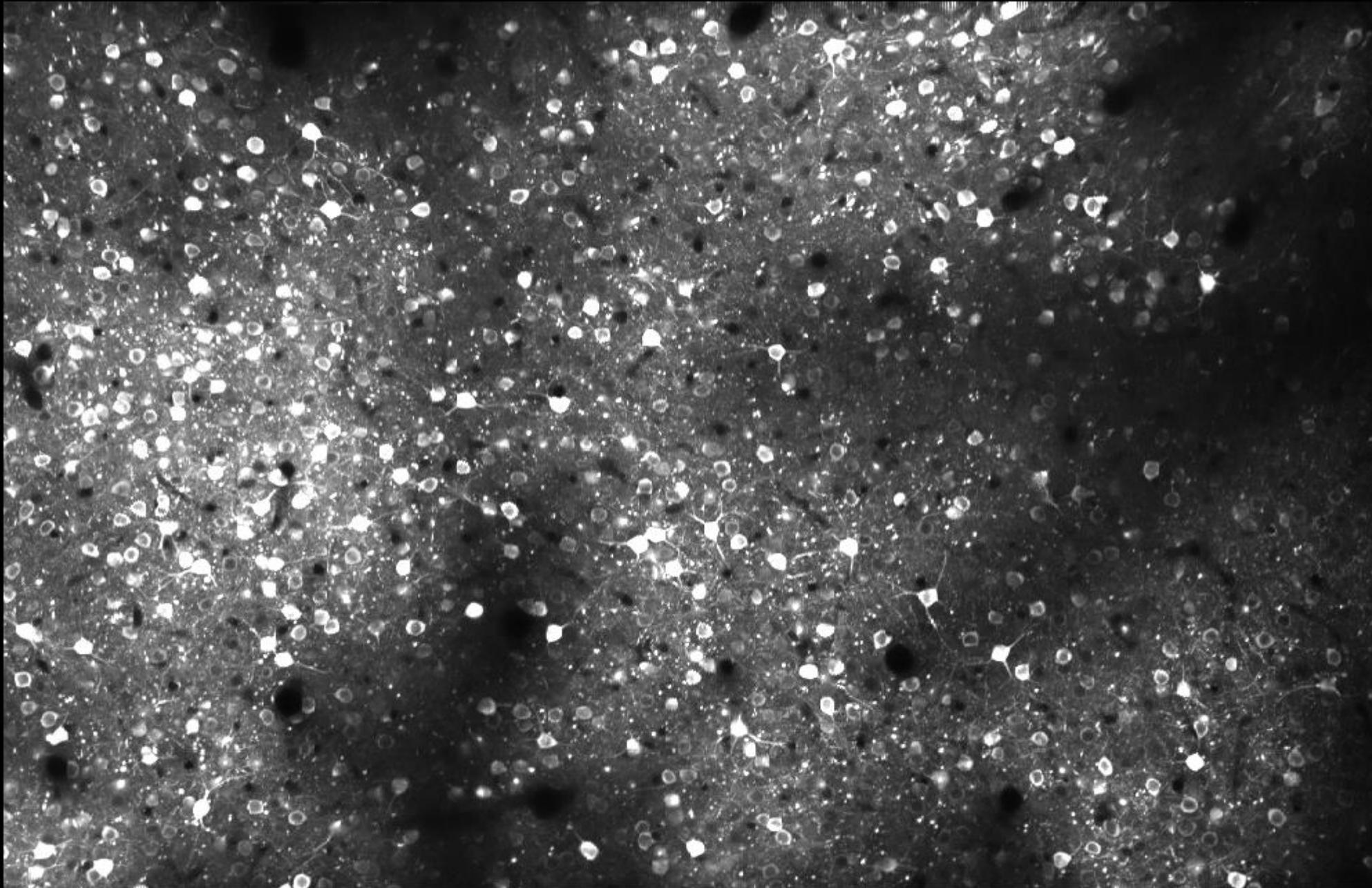


Yuste lab

Giovanucci et al, 2019  
(Caiman paper)

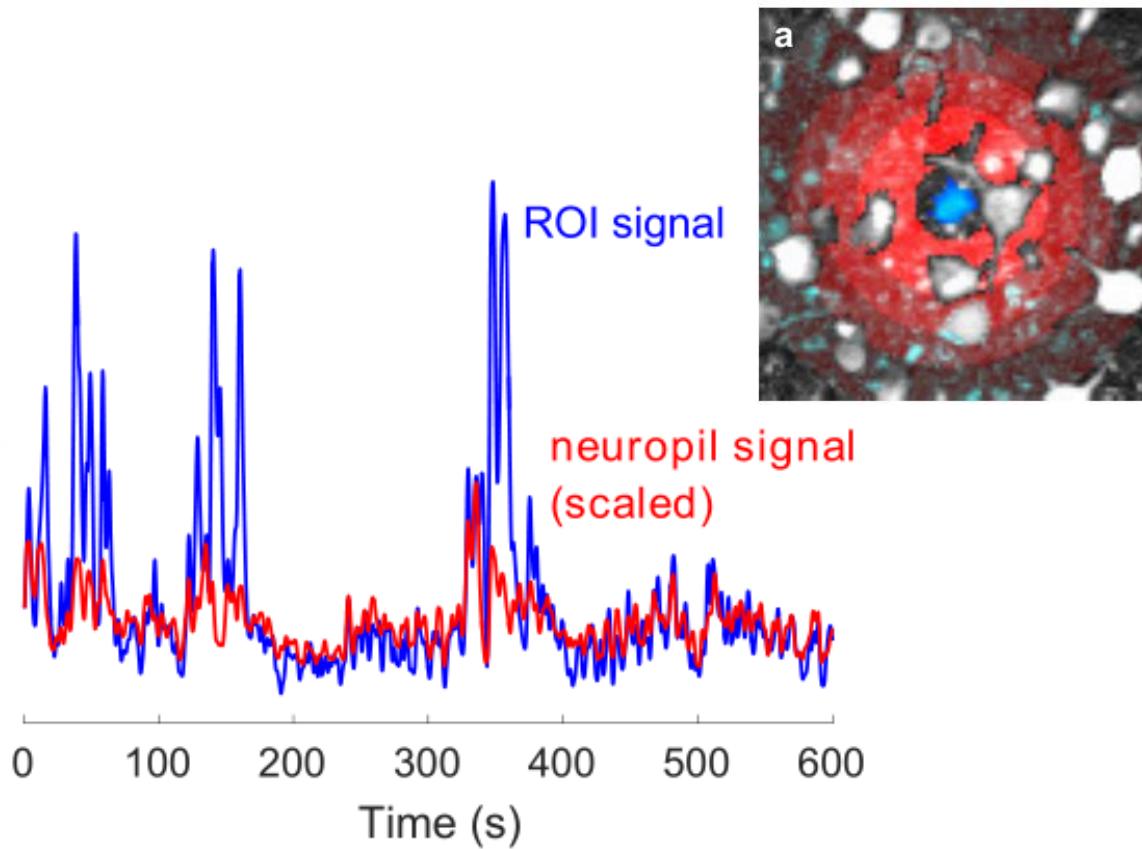


nuclear dtomato, co-localized with gcamp



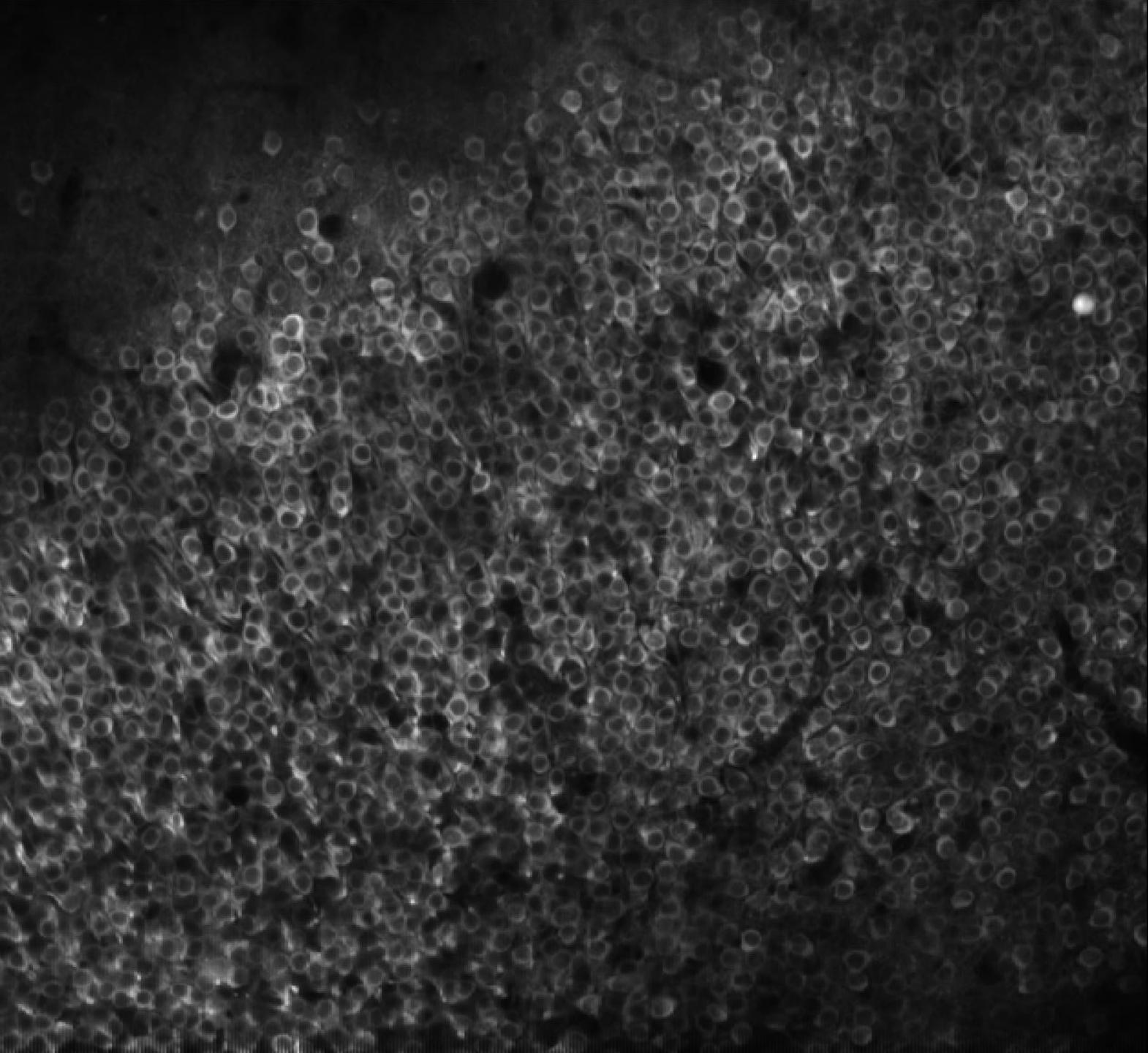
Maximum projection

# How do we extract fluorescence?



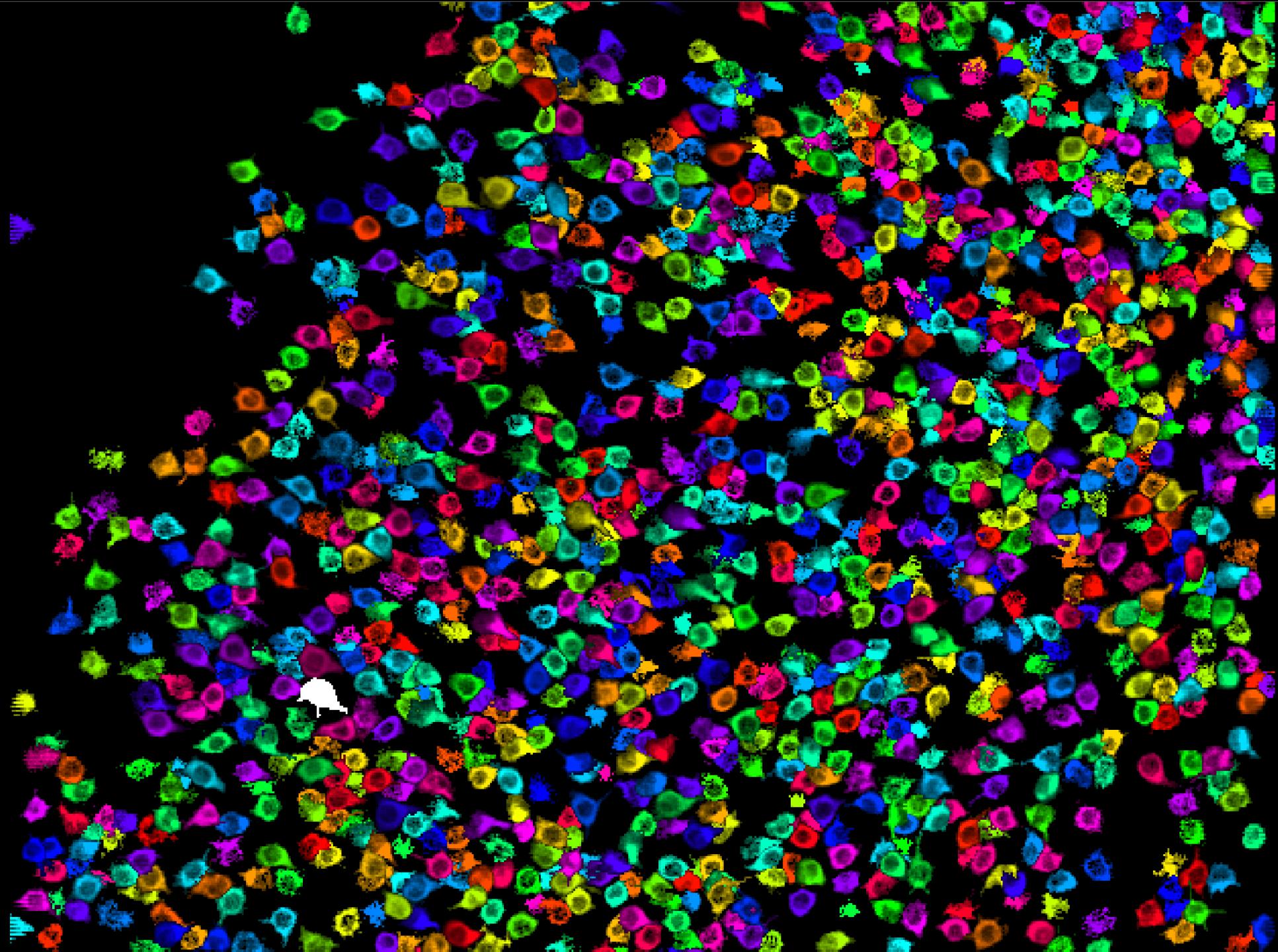
$$F_{corrected} = F - \alpha * F_{neuropil}$$
$$\alpha \sim 0.7$$

What if the recording  
is densely populated?



Weinan Sun

Weinan Sun



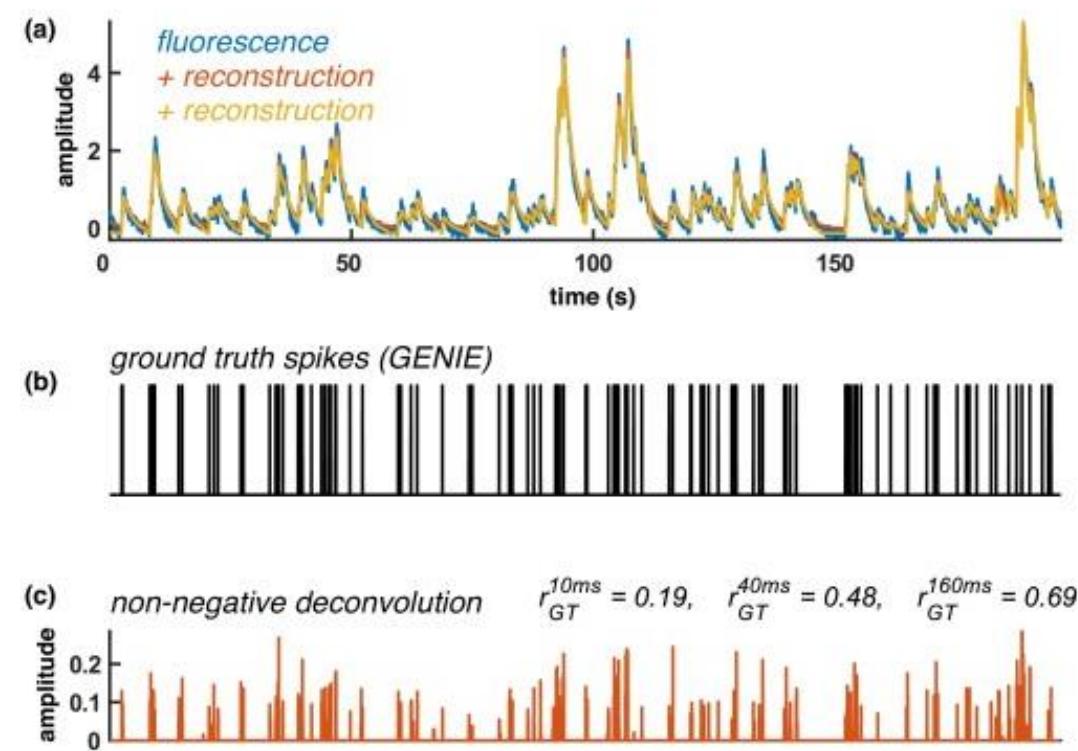
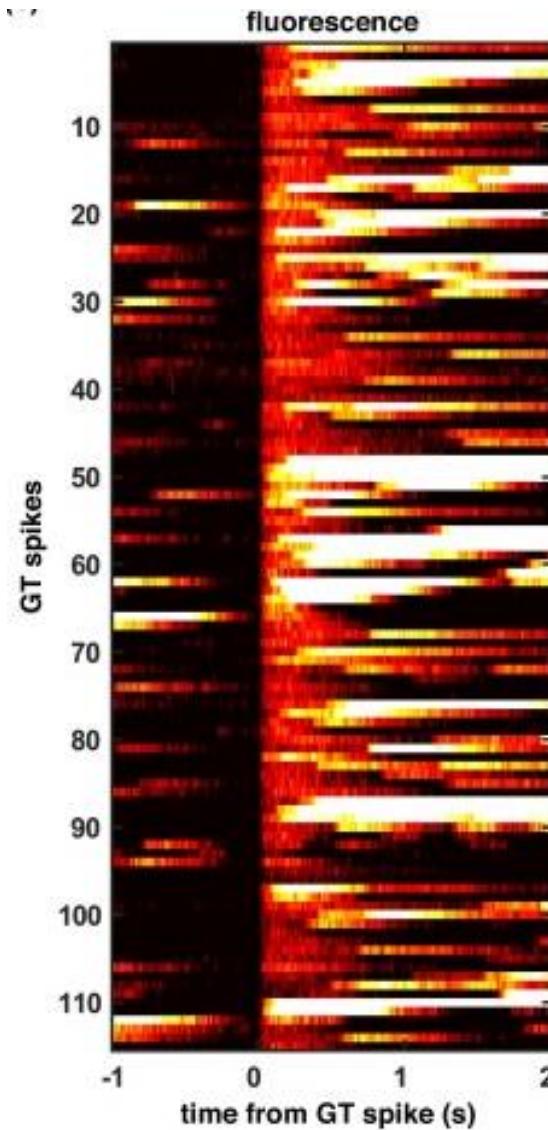
“Demix” the fluorescence by optimizing  
the generative model

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

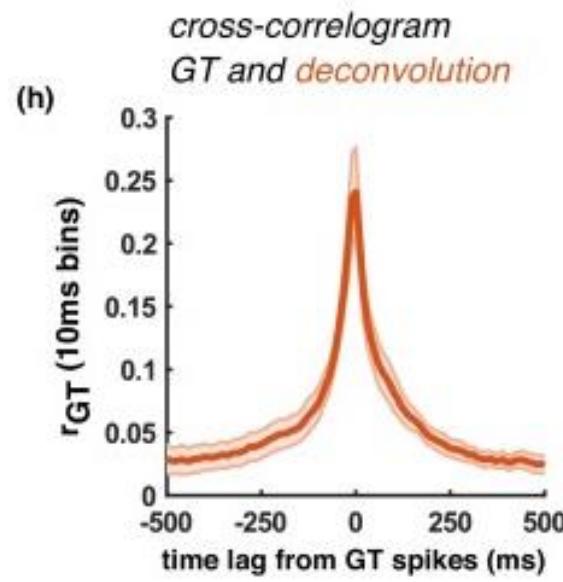
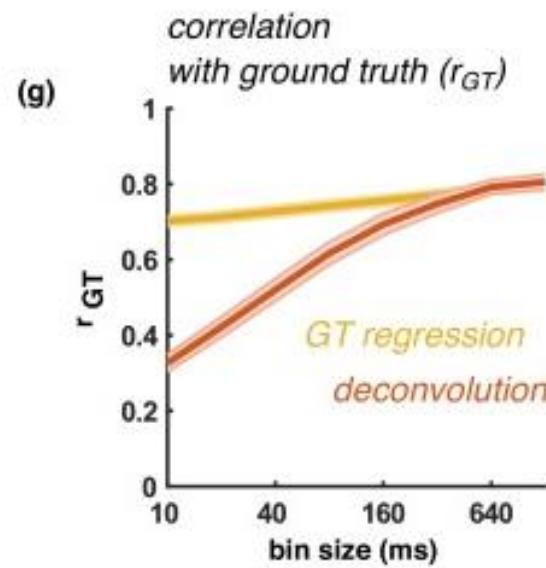
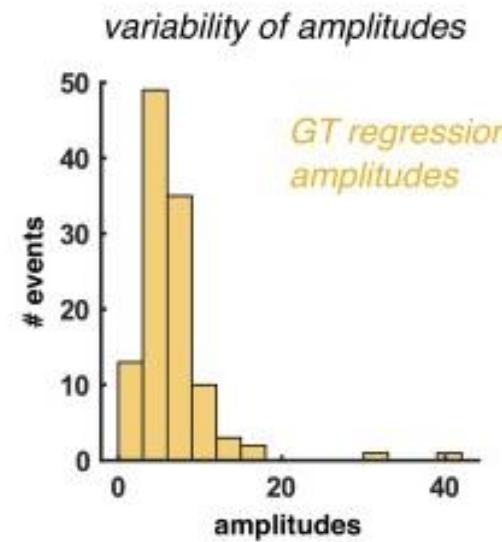
Pnevmatikakis et al, 2016

Simultaneous Denoising, Deconvolution, and Demixing  
of Calcium Imaging Data

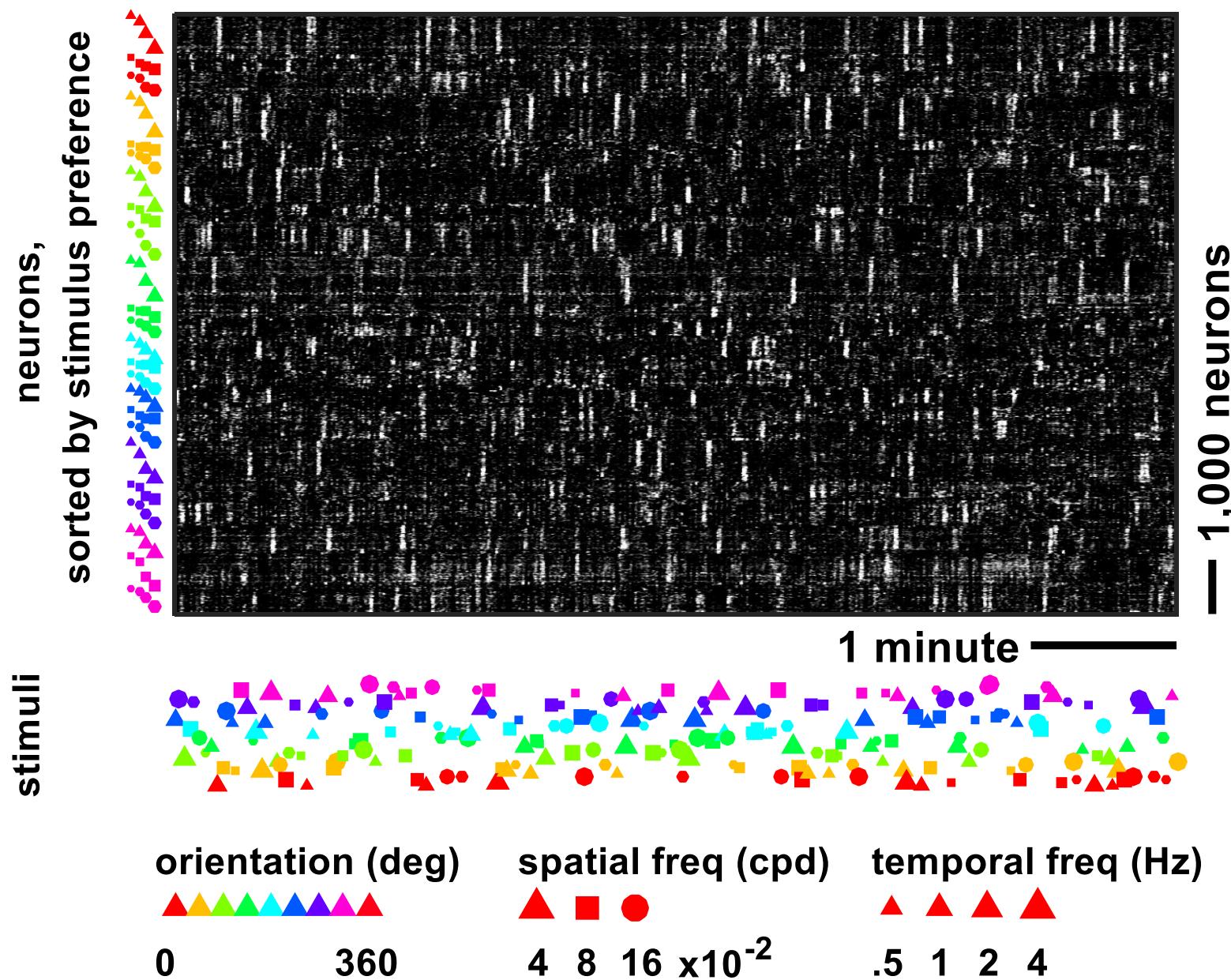
# Spike deconvolution



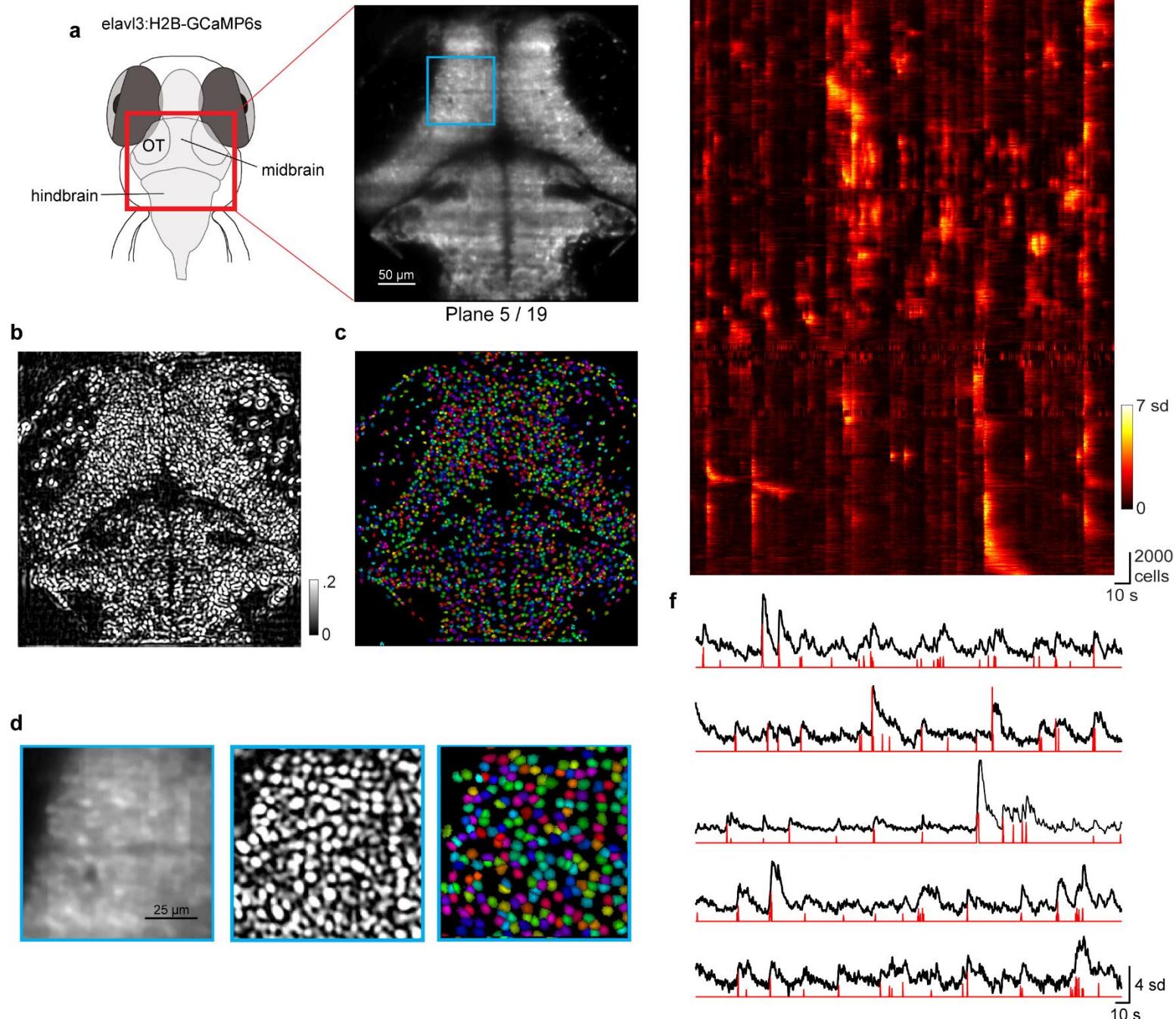
# Spike deconvolution



If you deconvolve, you get higher temporal precision

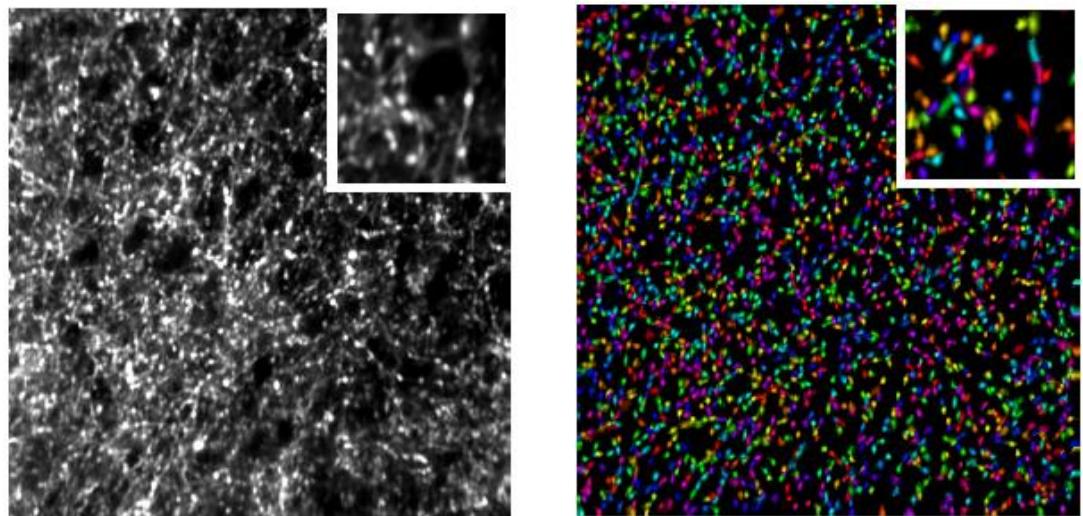


# Other data: zebrafish

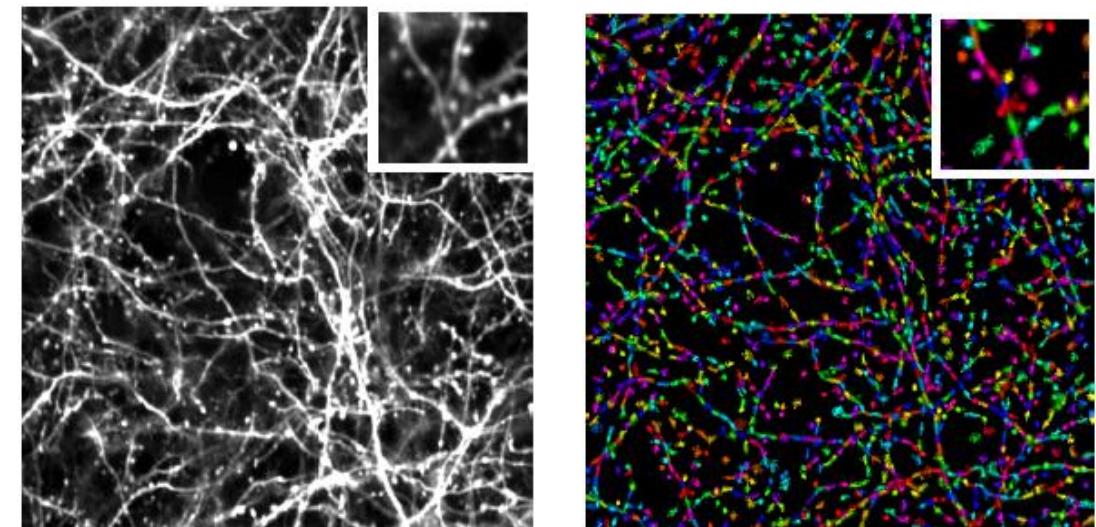


Asaph Zylbertal  
& Isaac Bianco

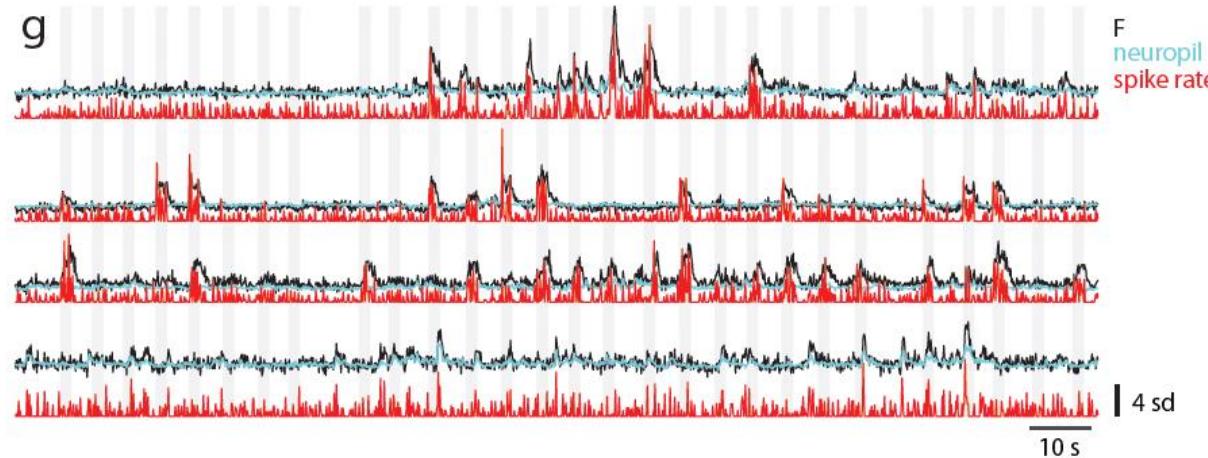
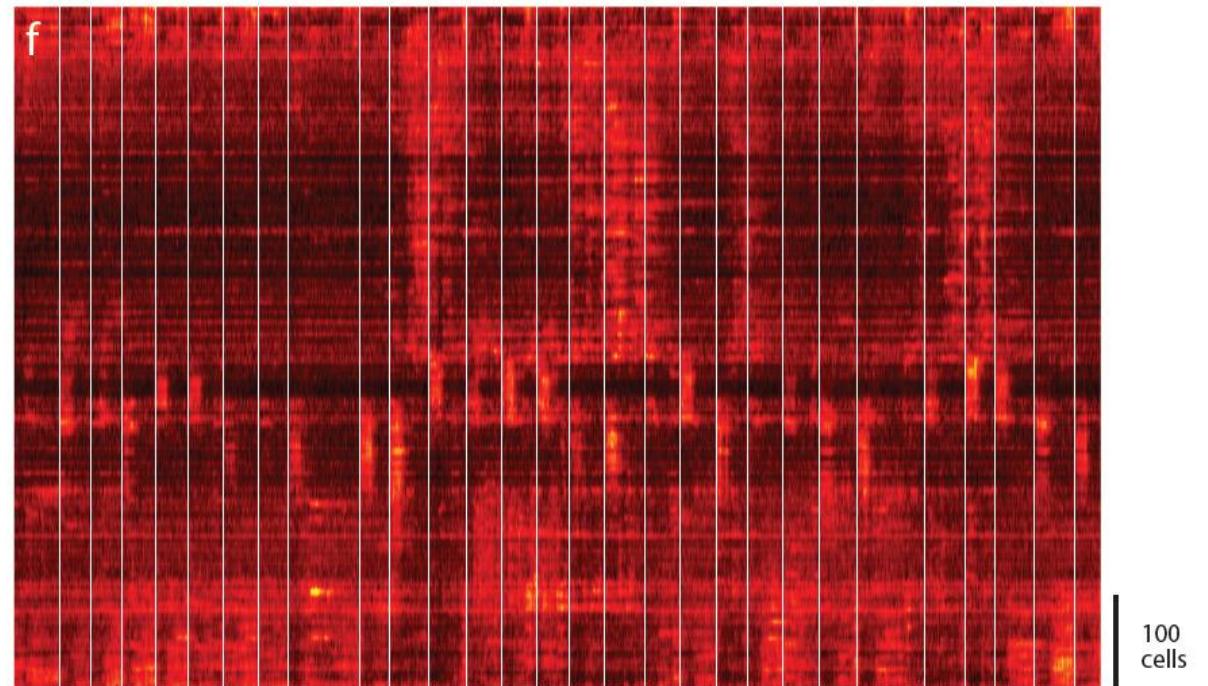
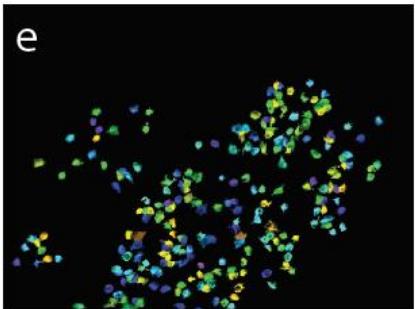
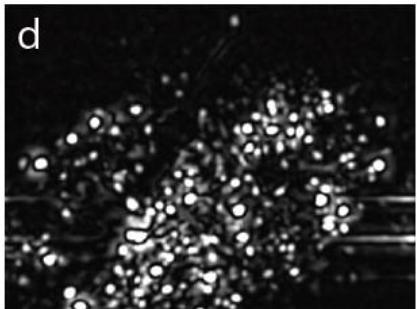
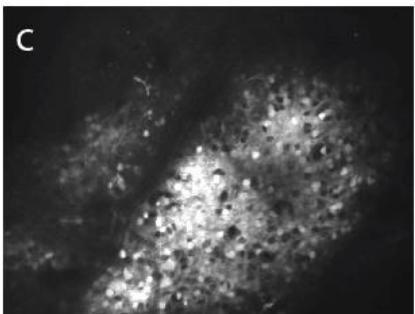
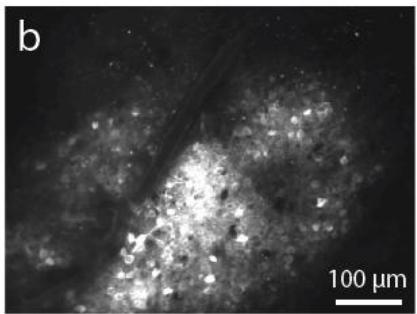
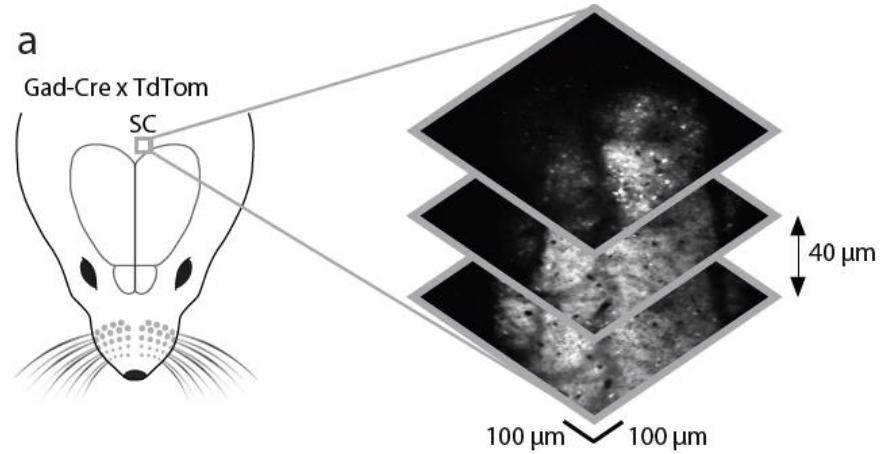
**Large-scale bouton recordings in V1**



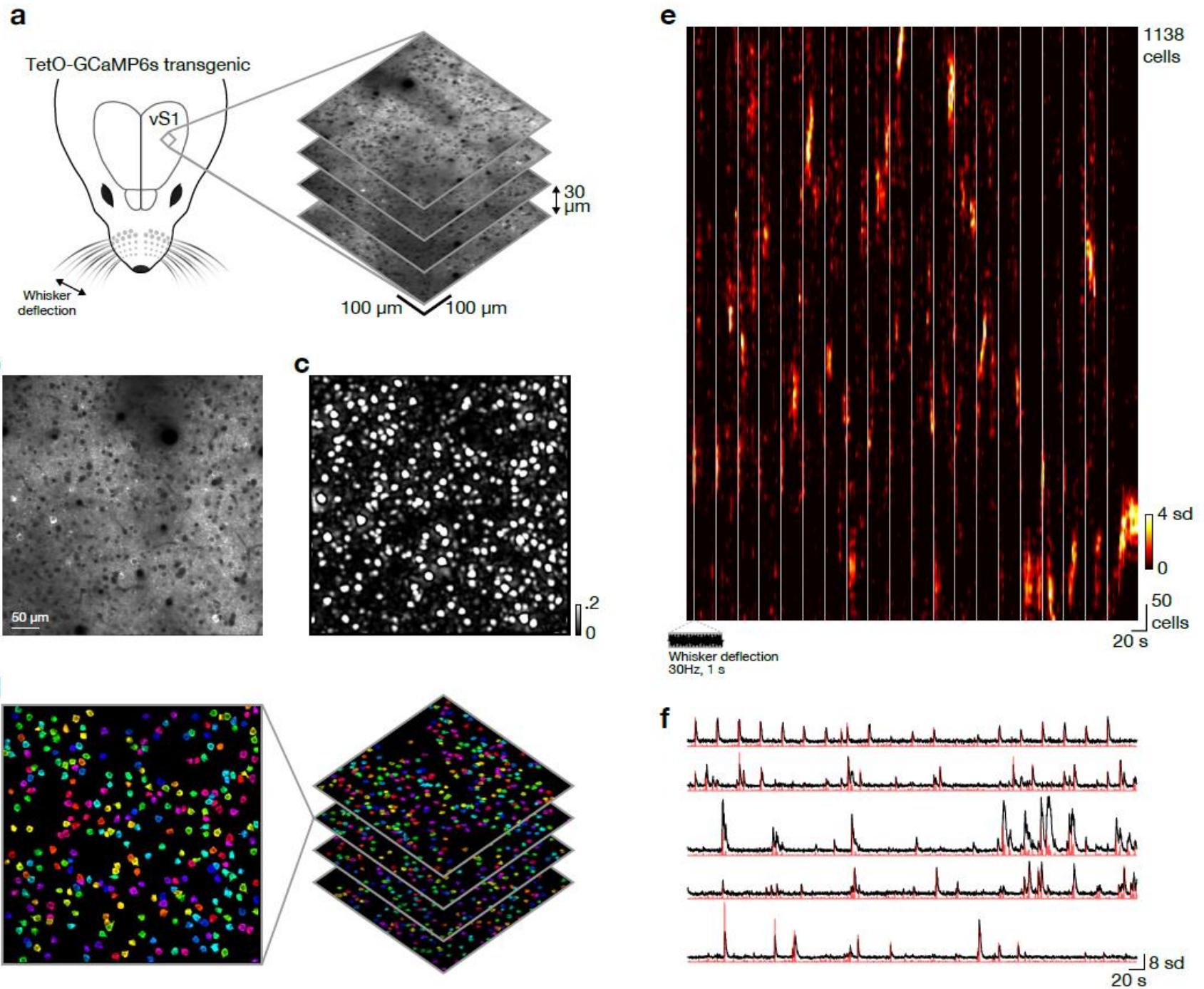
**Large-scale synapse recordings in V1**



# Other data: superior colliculus



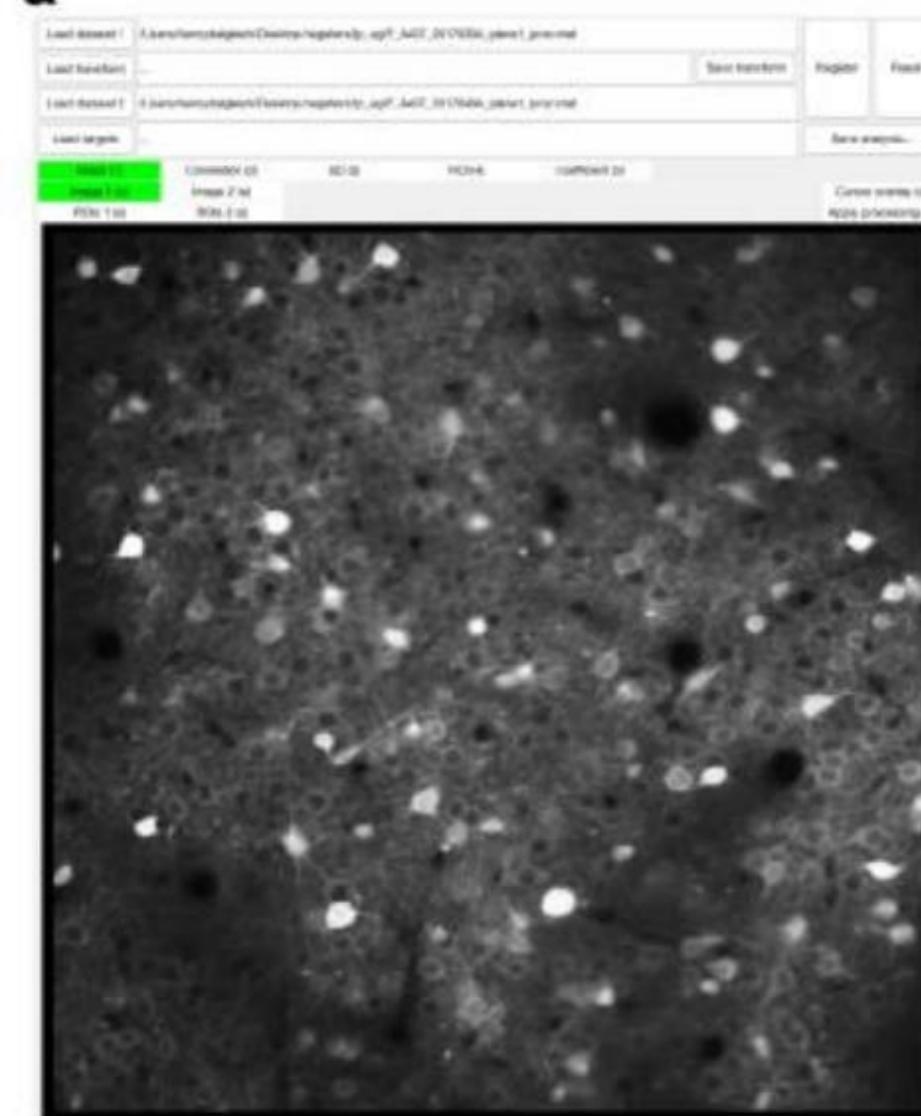
# Other data: barrel cortex



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****b**

Workflow:

Suite2P output

Manual selection of consistent image features

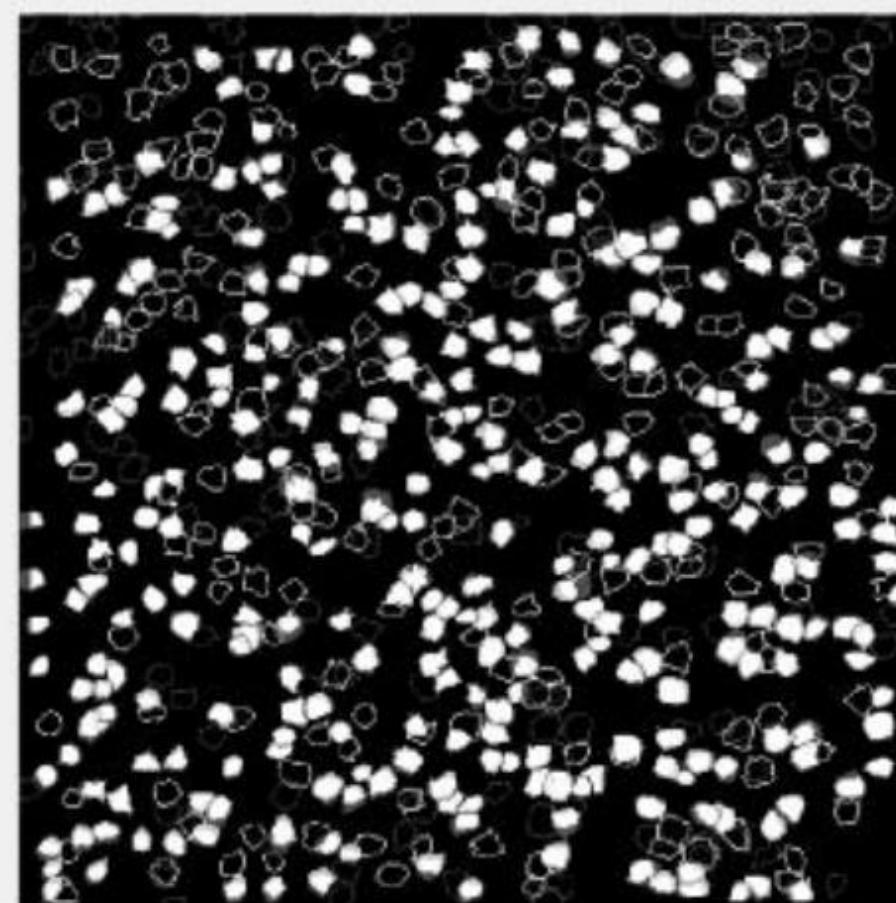
Calculation of affine transformation

Transformation of ROIs and images

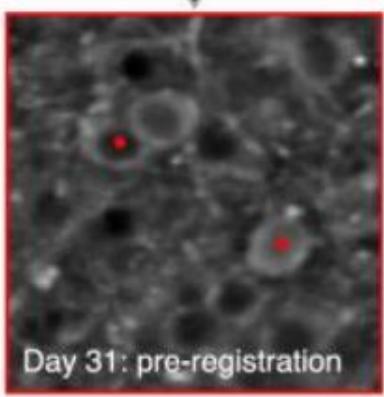
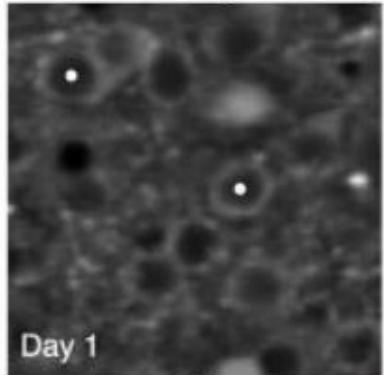
Auto-detection of ROI overlap

Manual curation of ROI overlap statuses

ROI overlap matrix



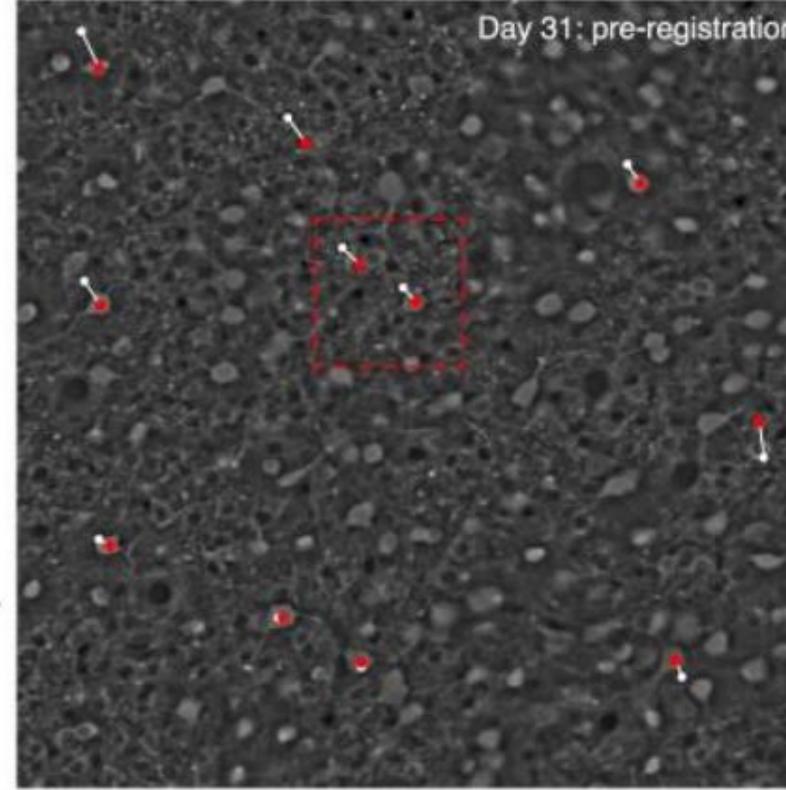
**c** Manual selection of consistent image features



**d**

Calculation of affine transformation

Day 31: pre-registration



**e** Transform images and ROIs

