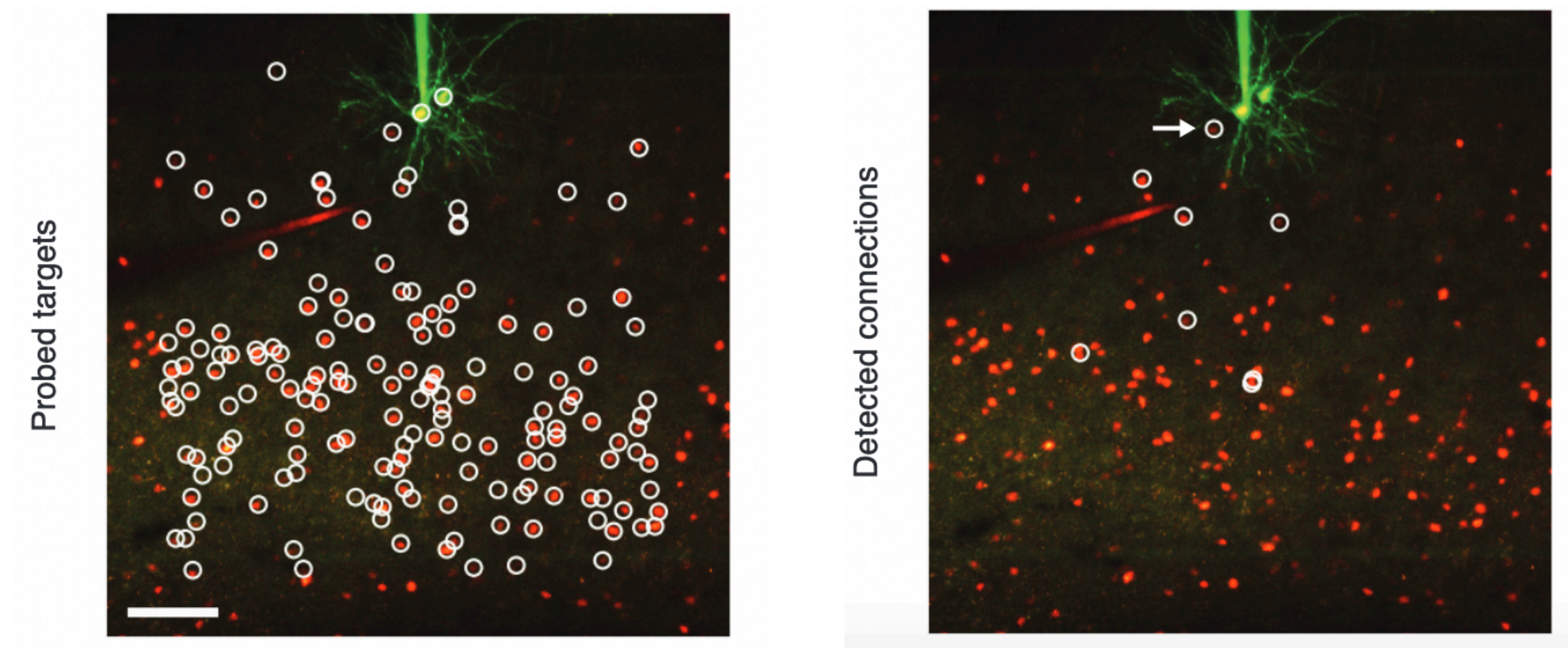


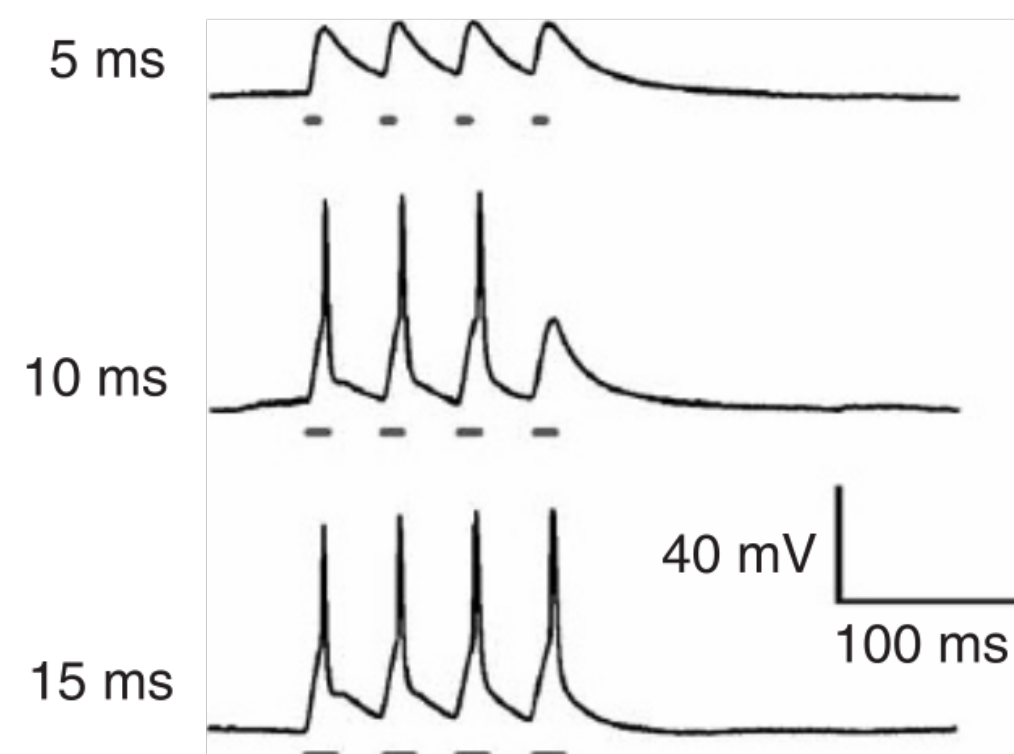
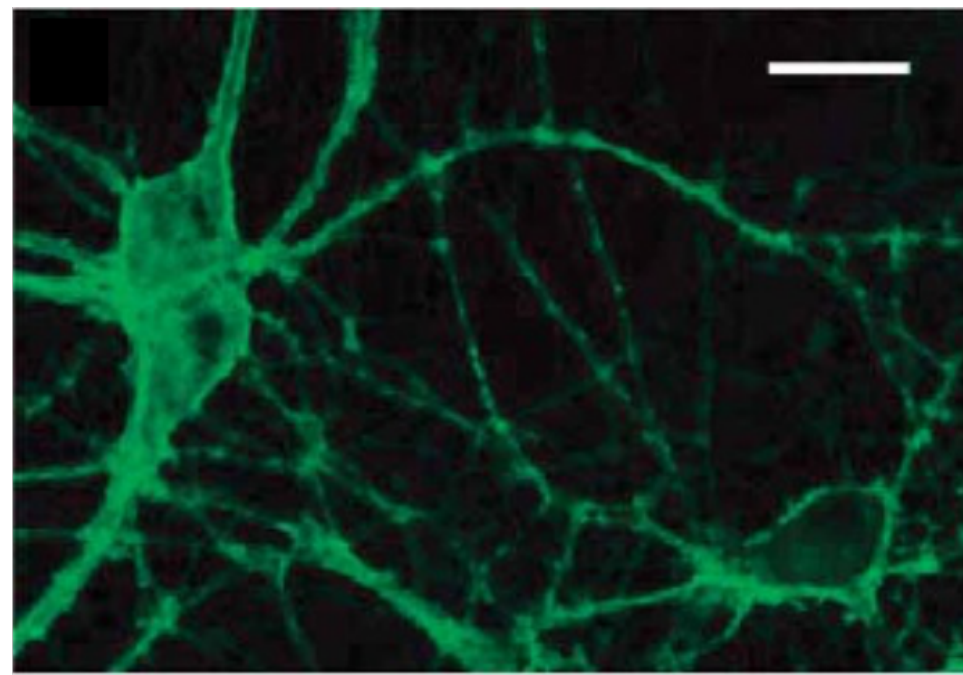
Optogenetic circuit mapping



STATGR8201
Marcus Triplett

Optogenetics

Photostimulation of microbial opsins



Boyden et al (2005)

In green: **channelrhodopsin** (fused to YFP)

- non-specific cation channel, conducts K^+ , H^+ , Na^+ , Ca^{2+}

Illumination of opsin molecules generates an inwards current, induces action potentials.

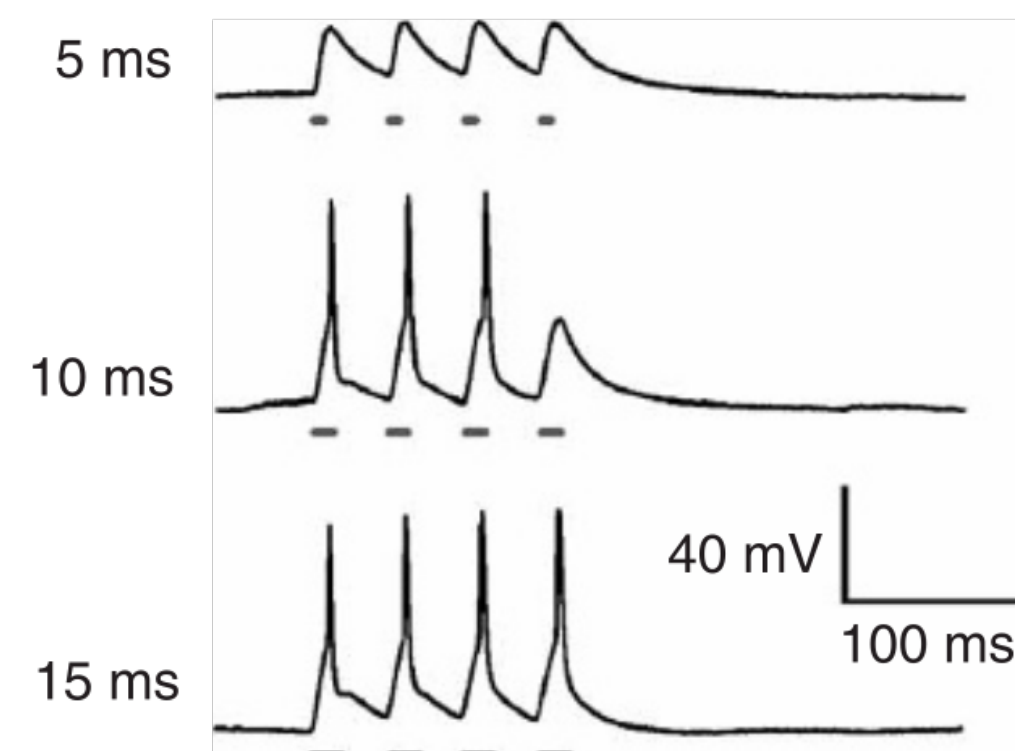
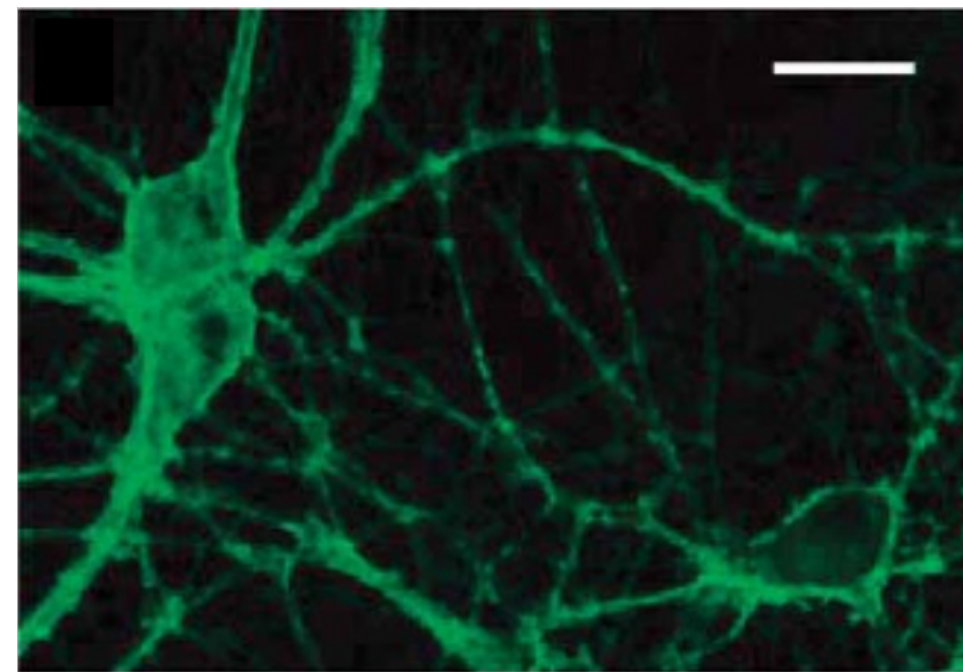
Just one example of an opsin — opsin engineering is a very active research area!

Optogenetics

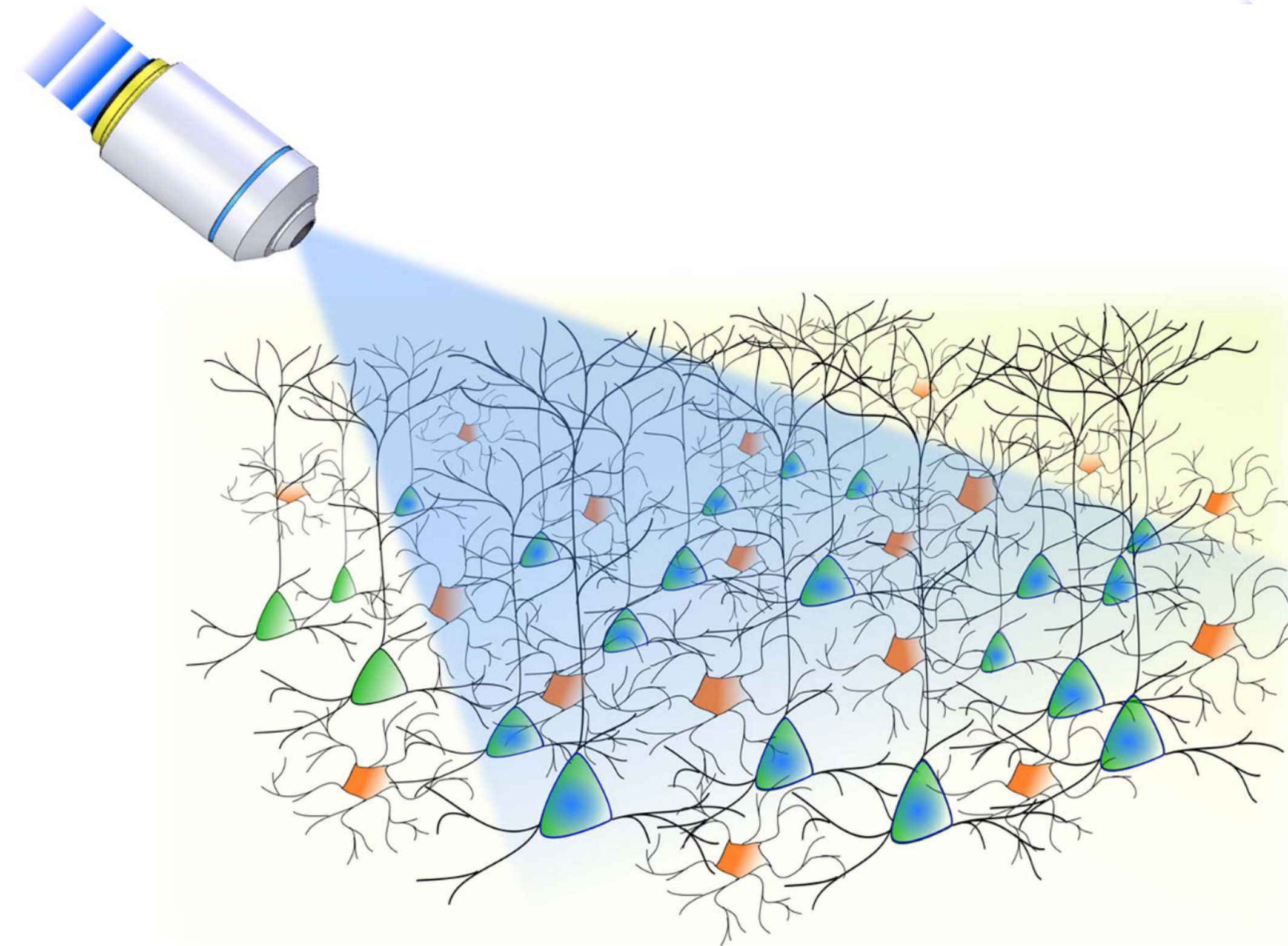
Photostimulation of microbial opsins

Genetic targeting of opsin expression:

- soma rather than neurites
- specific cell-types



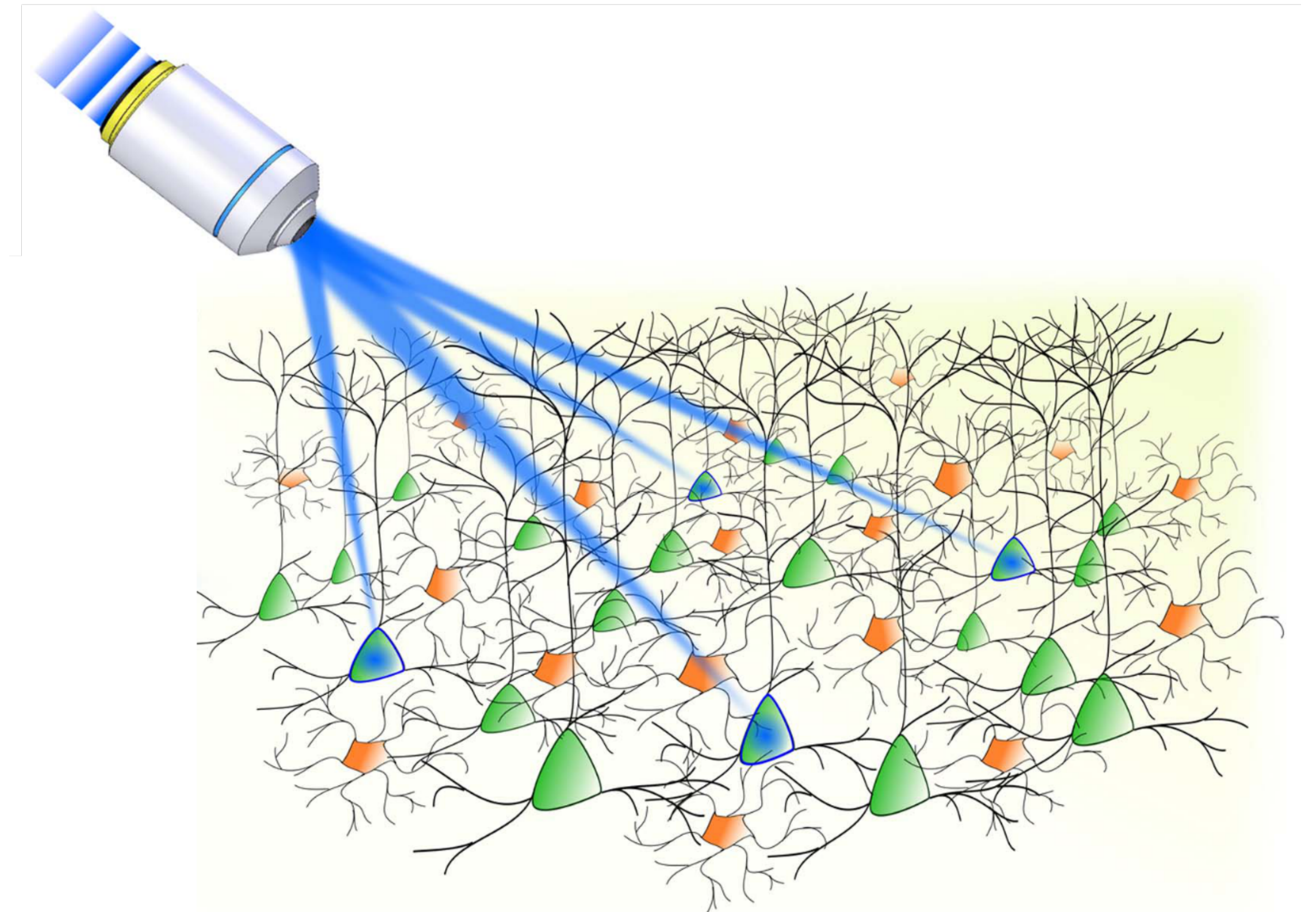
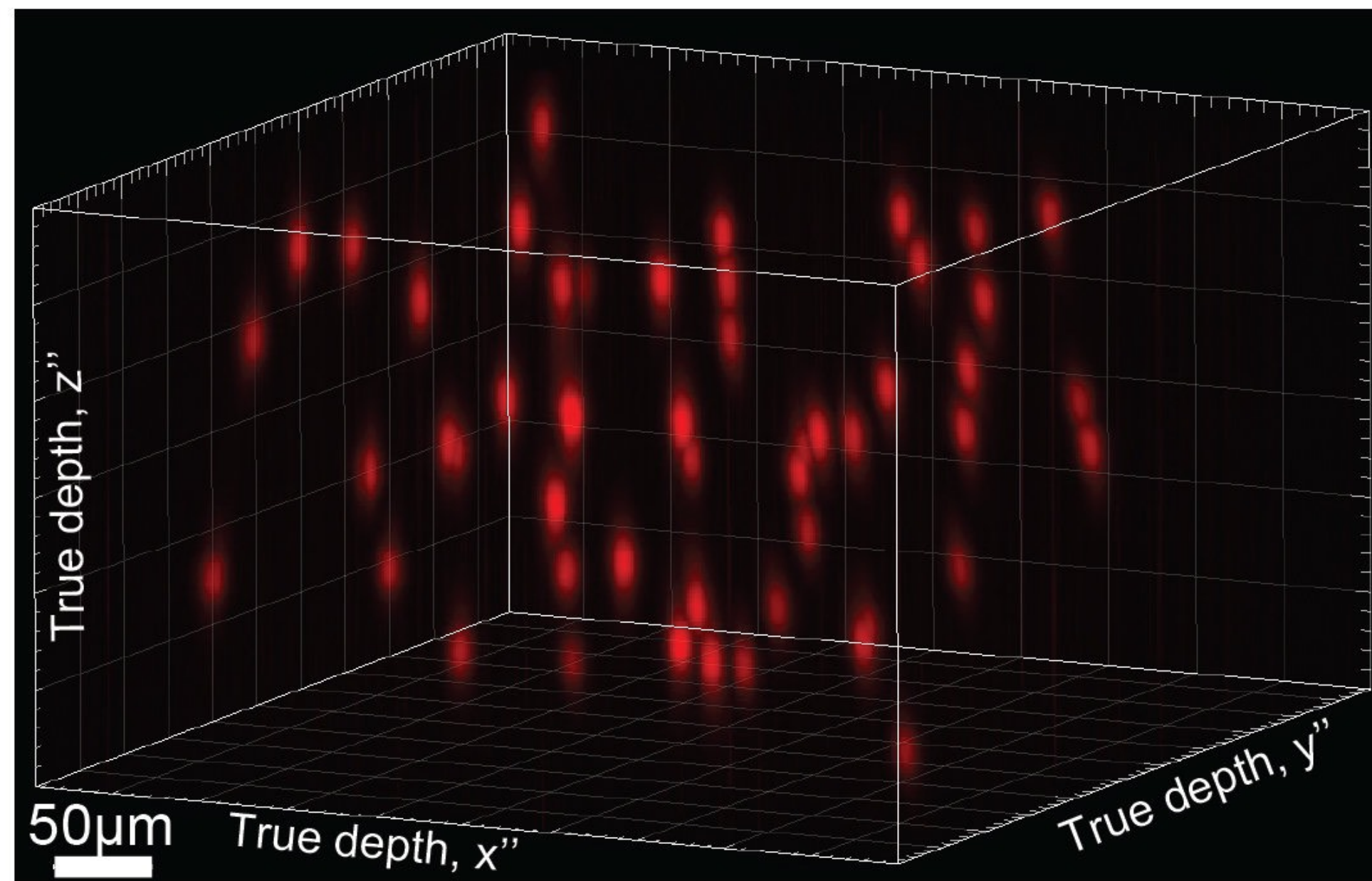
Boyden et al (2005)



Boyden et al (2005), Ronzitti et al (2017)

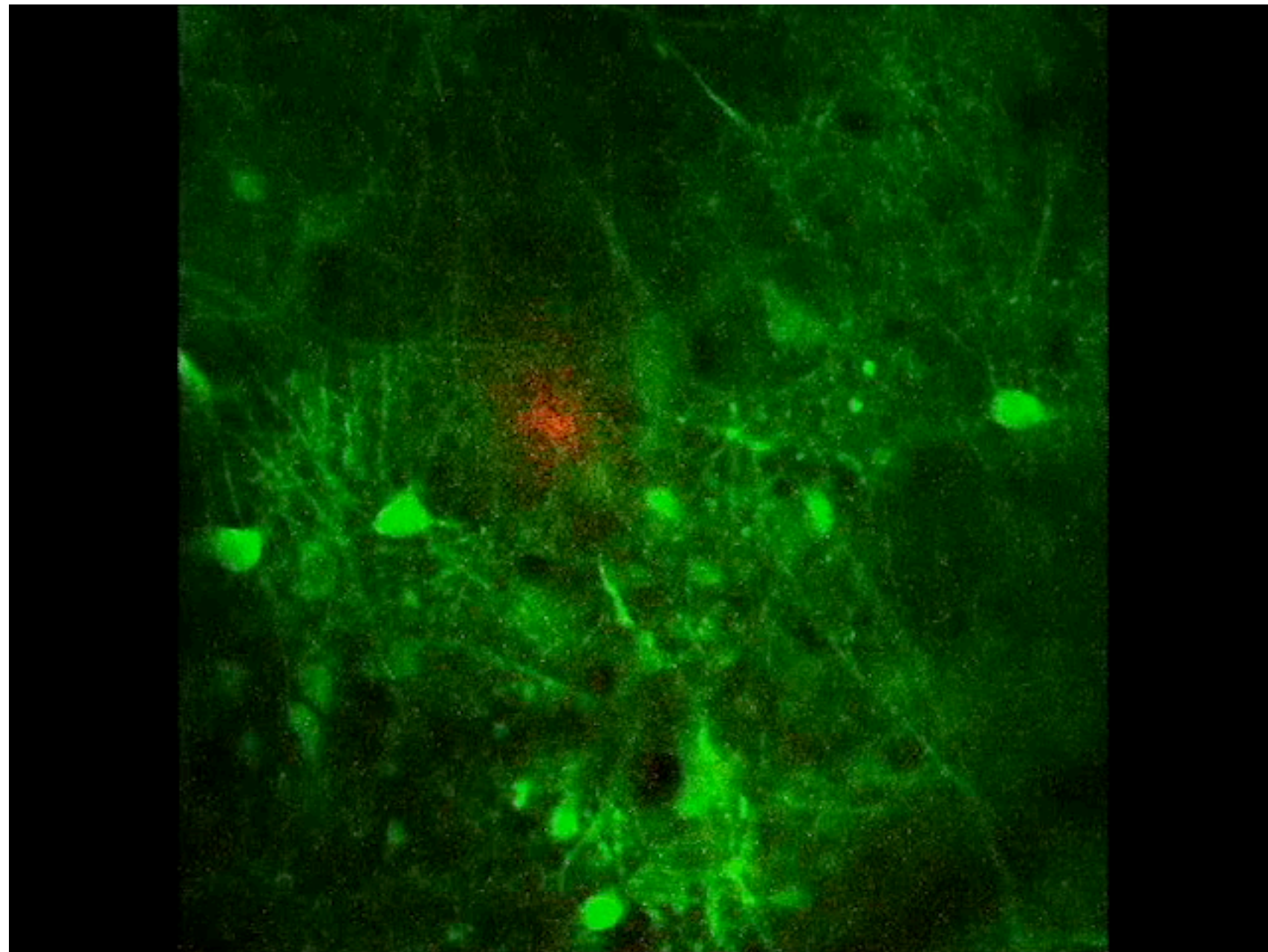
Holographic optogenetics

Sculpted light for cell-specific targeting

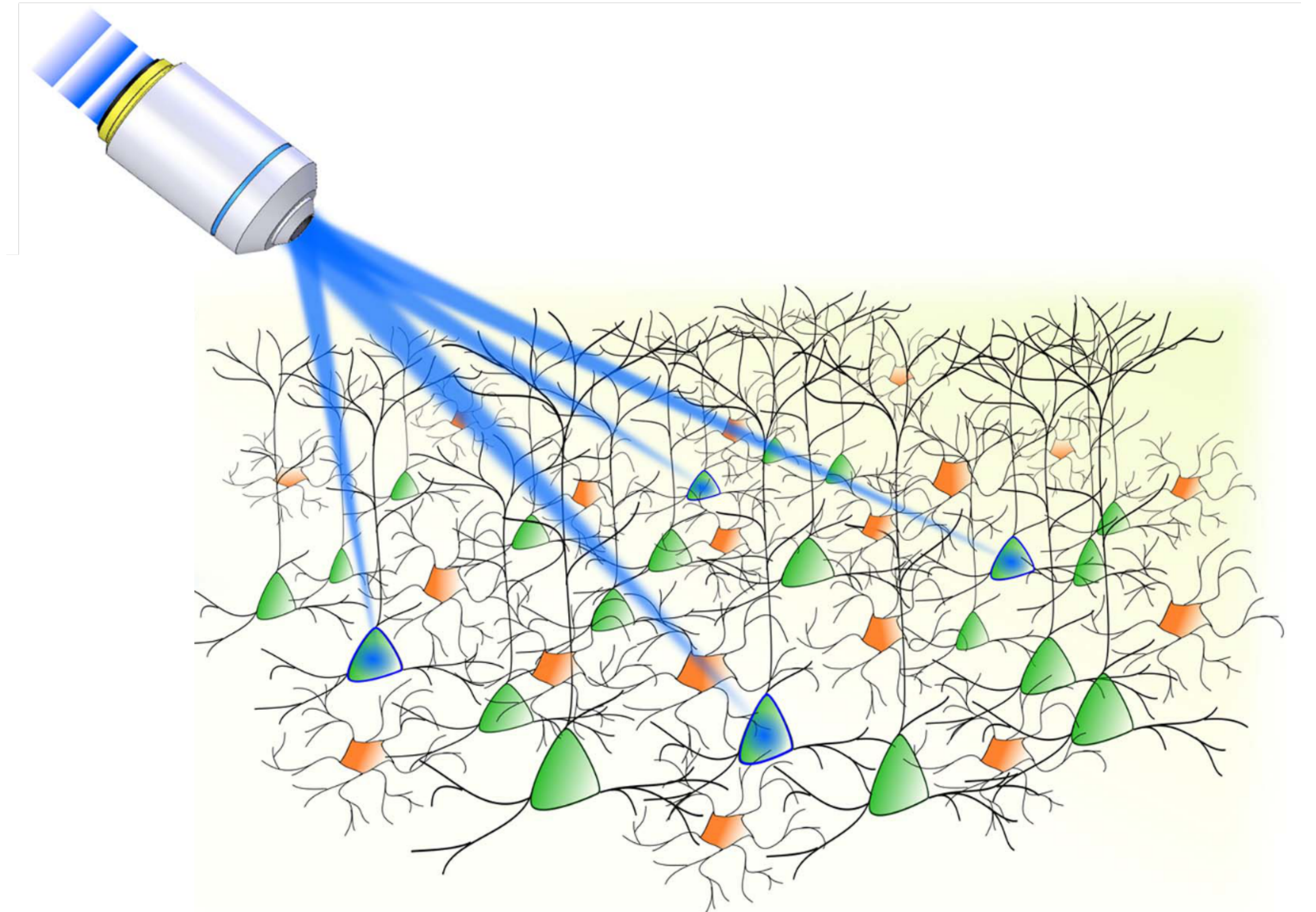


Holographic optogenetics

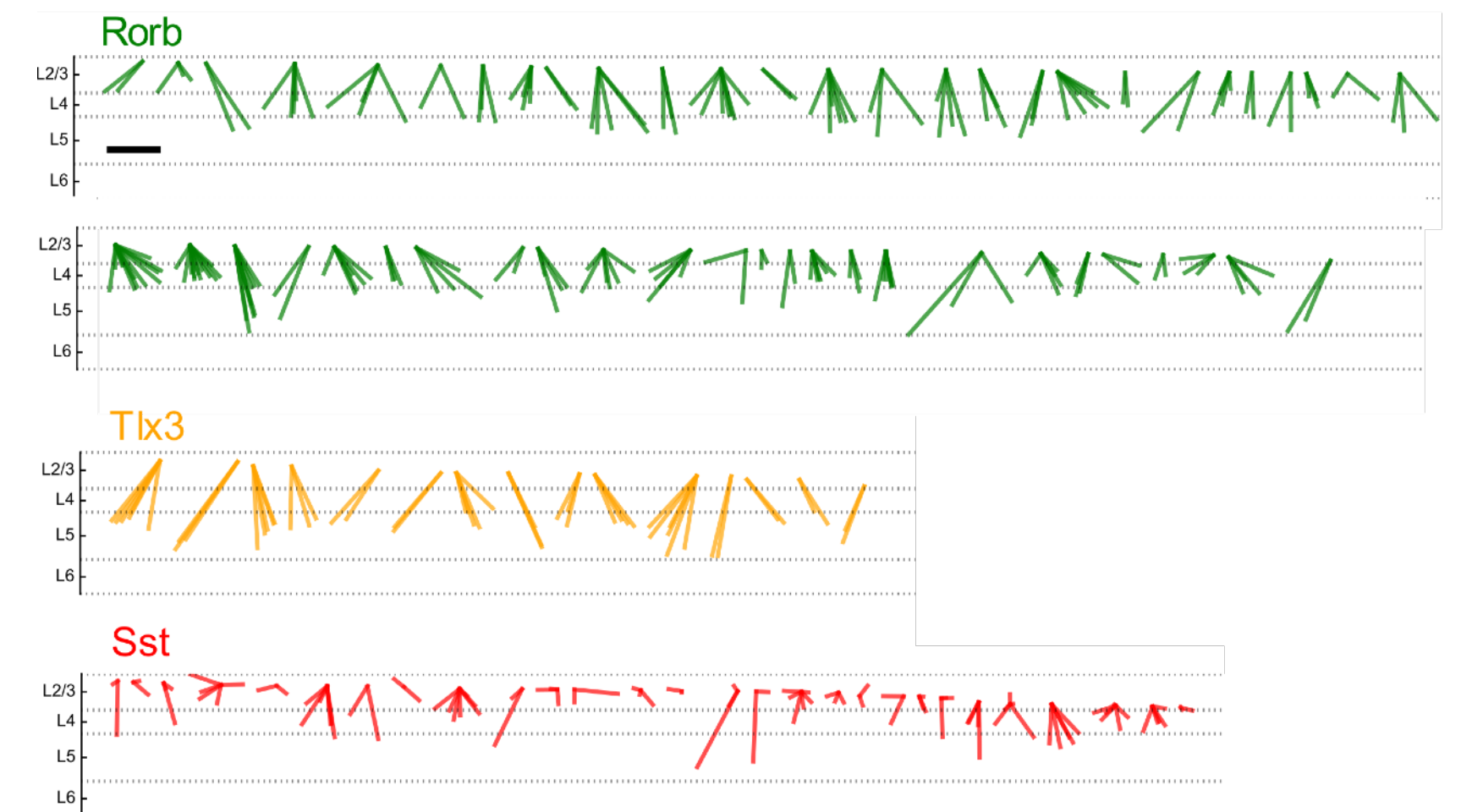
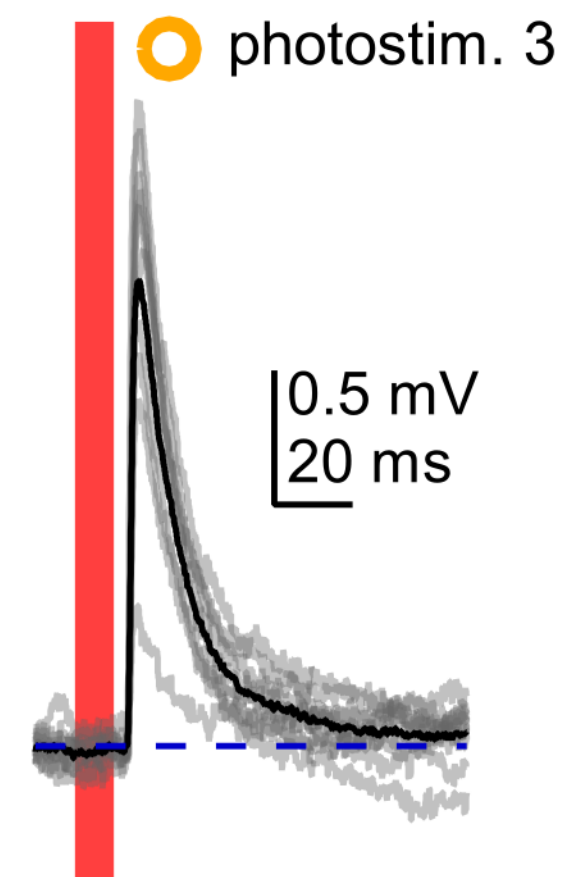
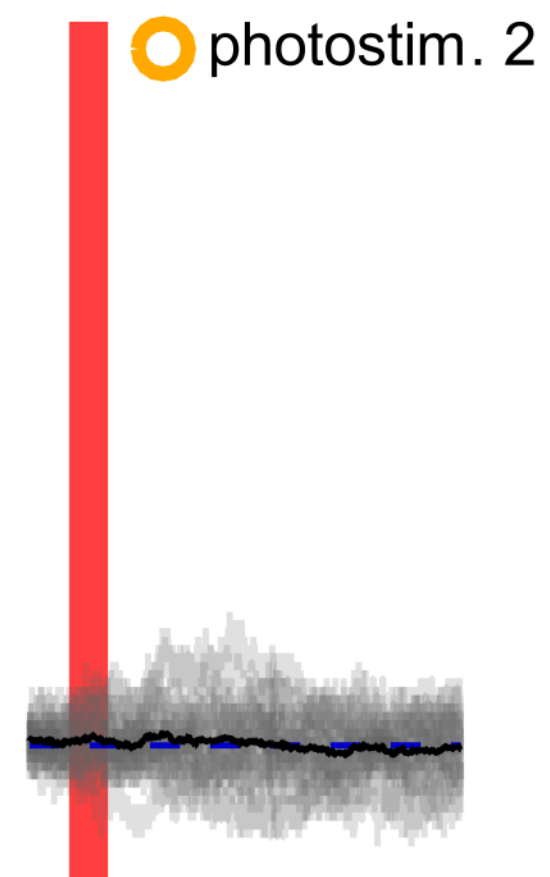
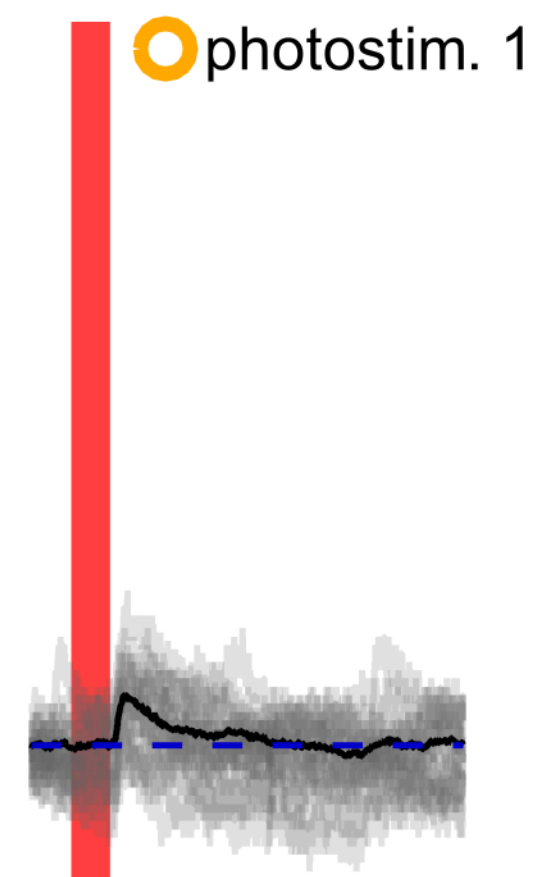
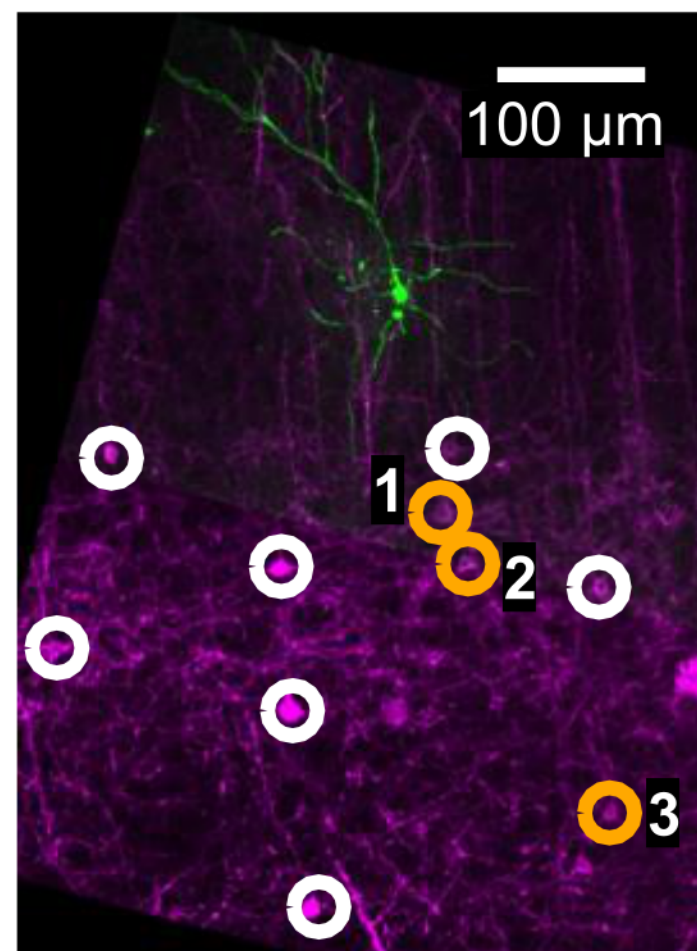
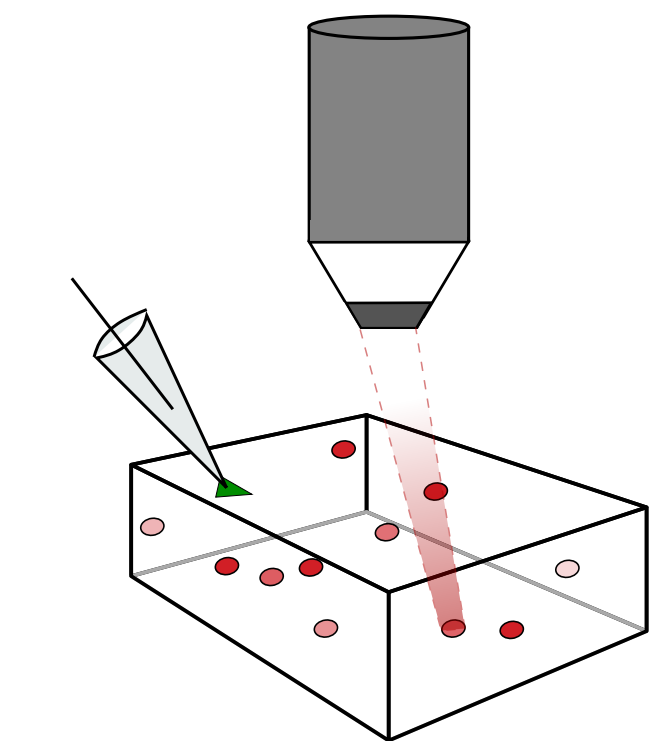
Sculpted light for cell-specific targeting



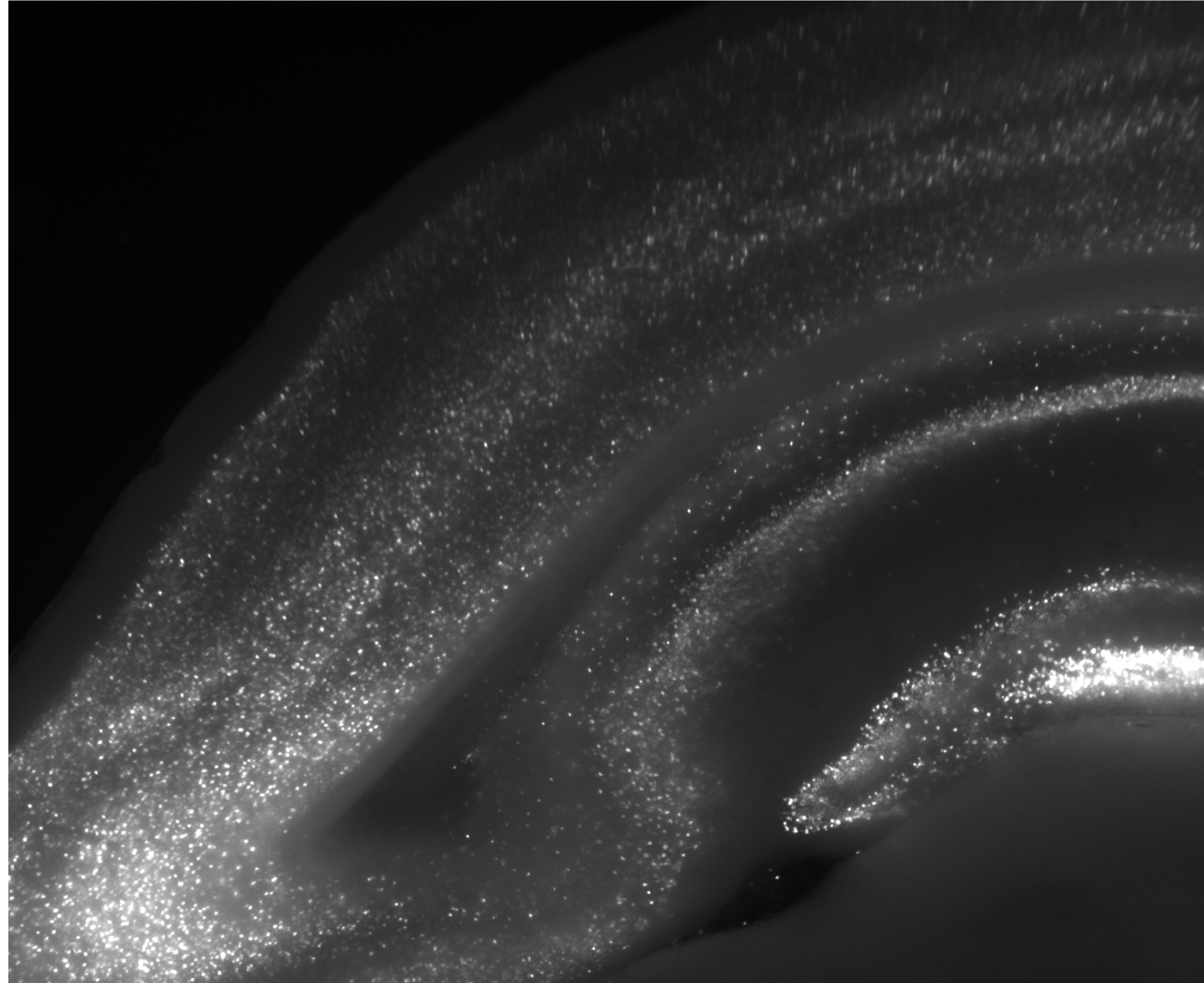
Lerman et al (2019)



Optogenetic circuit mapping



Will it scale?



Optogenetic neural circuit mapping

Sequential stimulation results look good, but how to speed up mapping?

Optogenetic neural circuit mapping

Sequential stimulation results look good, but how to speed up mapping?

- (1) Stimulate faster (don't wait for return to baseline conditions)
- (2) Stimulate many cells at once (test for many synaptic partners per trial)

Optogenetic neural circuit mapping

Sequential stimulation results look good, but how to speed up mapping?

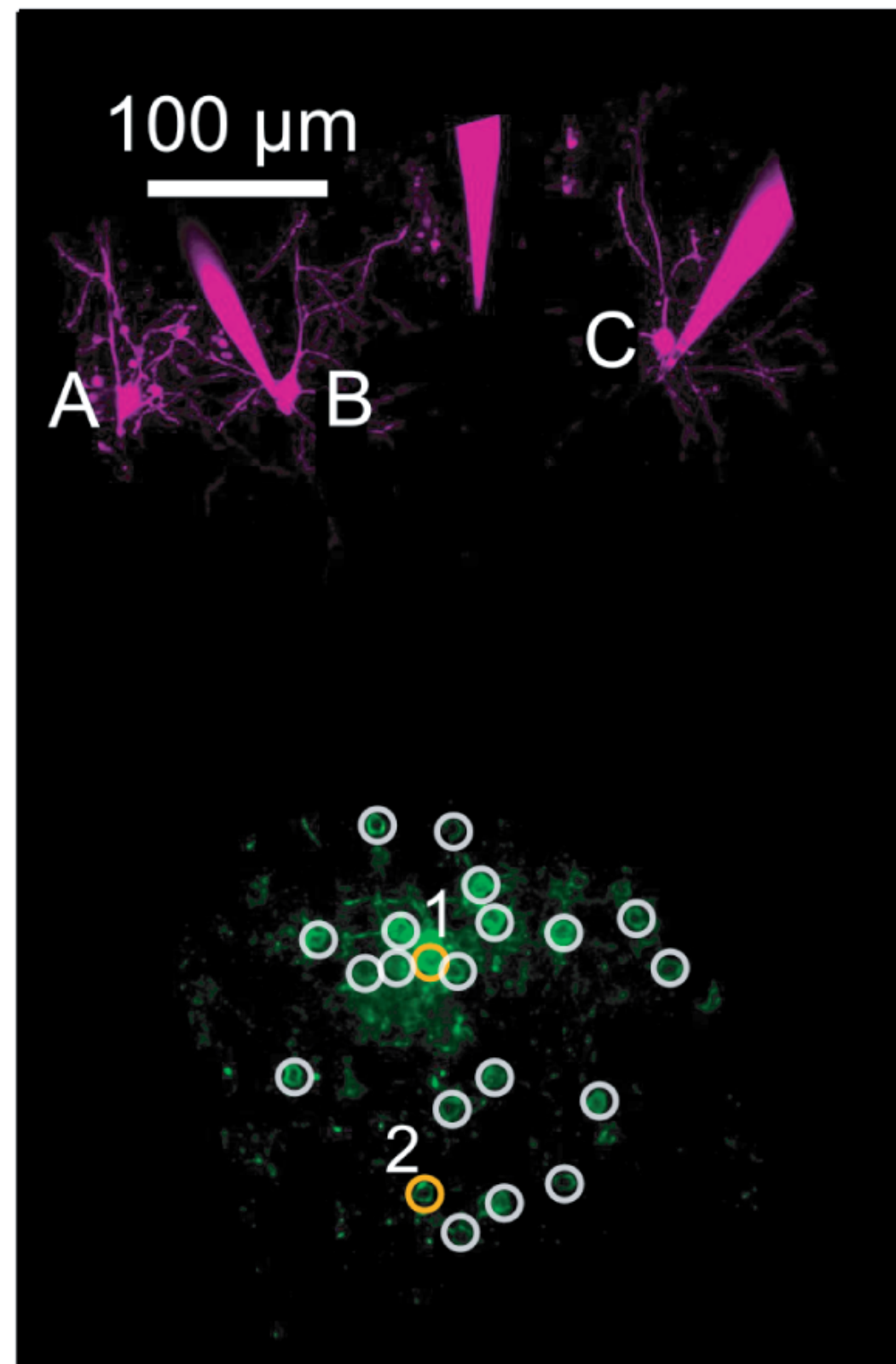
- (1) Stimulate faster (don't wait for return to baseline conditions)
+ computational demixing
- (2) Stimulate many cells at once (test for many synaptic partners per trial)
+ spike inference and compressed sensing

Optogenetic neural circuit mapping

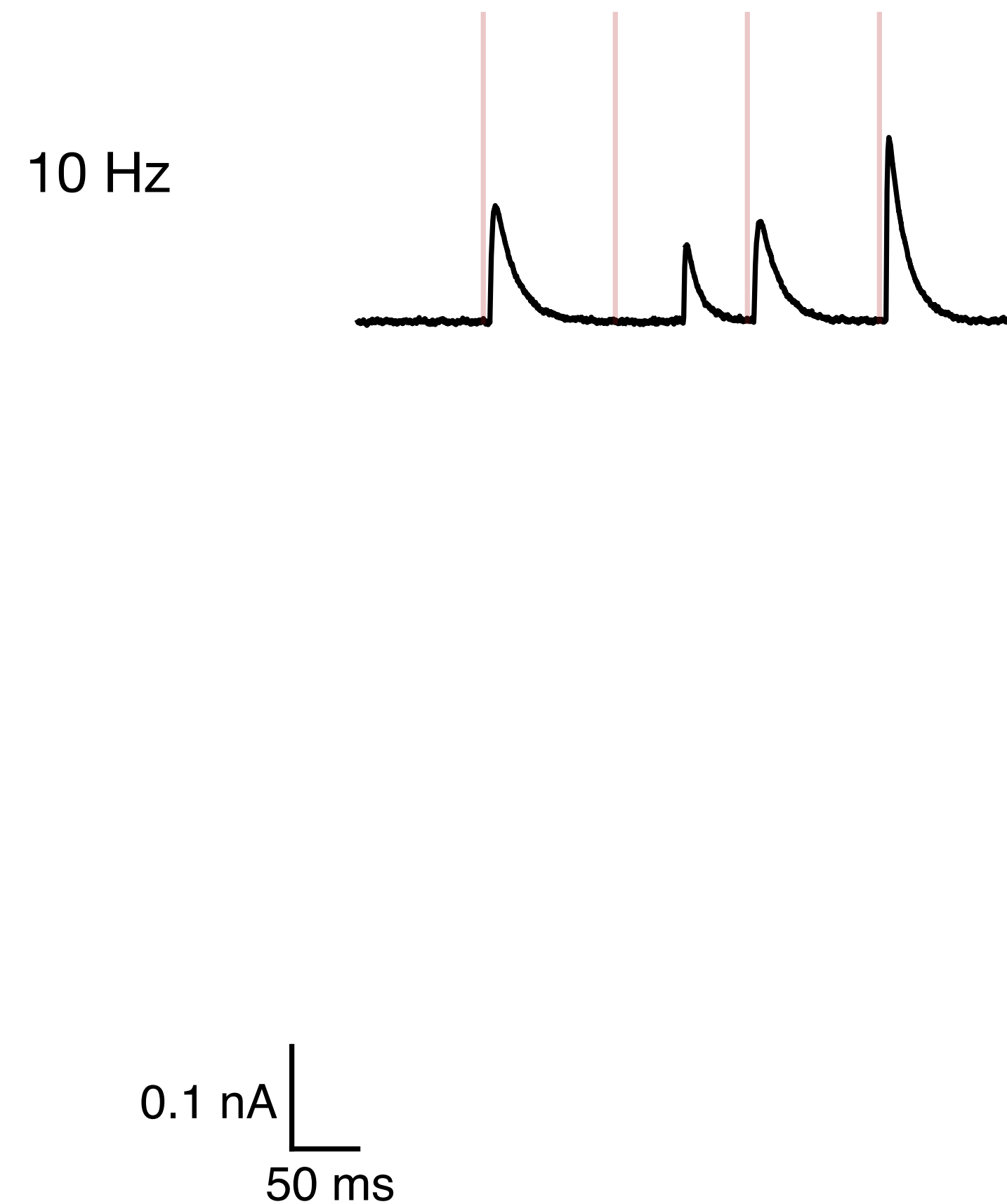
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The trade-off in fast optogenetic circuit mapping

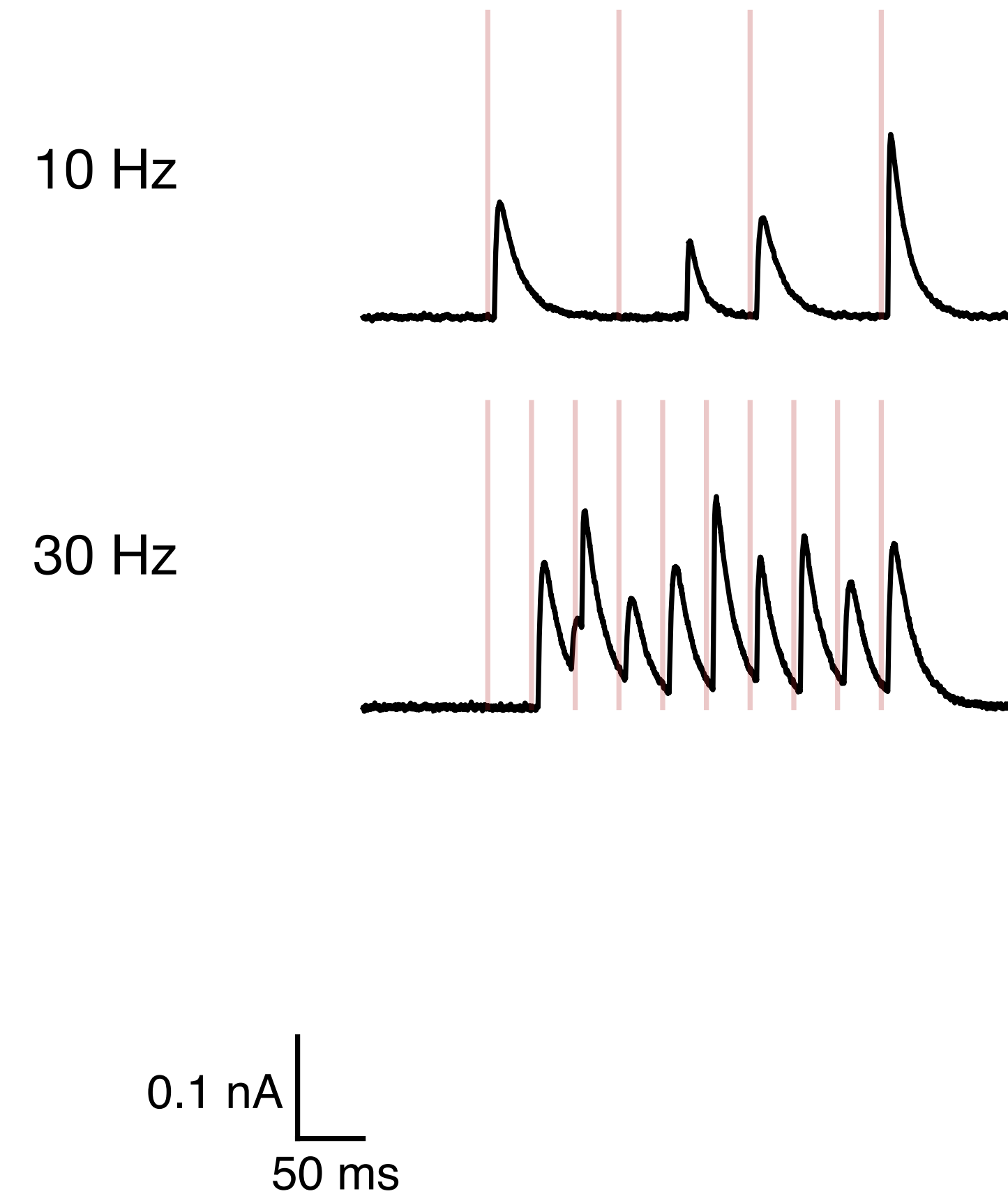
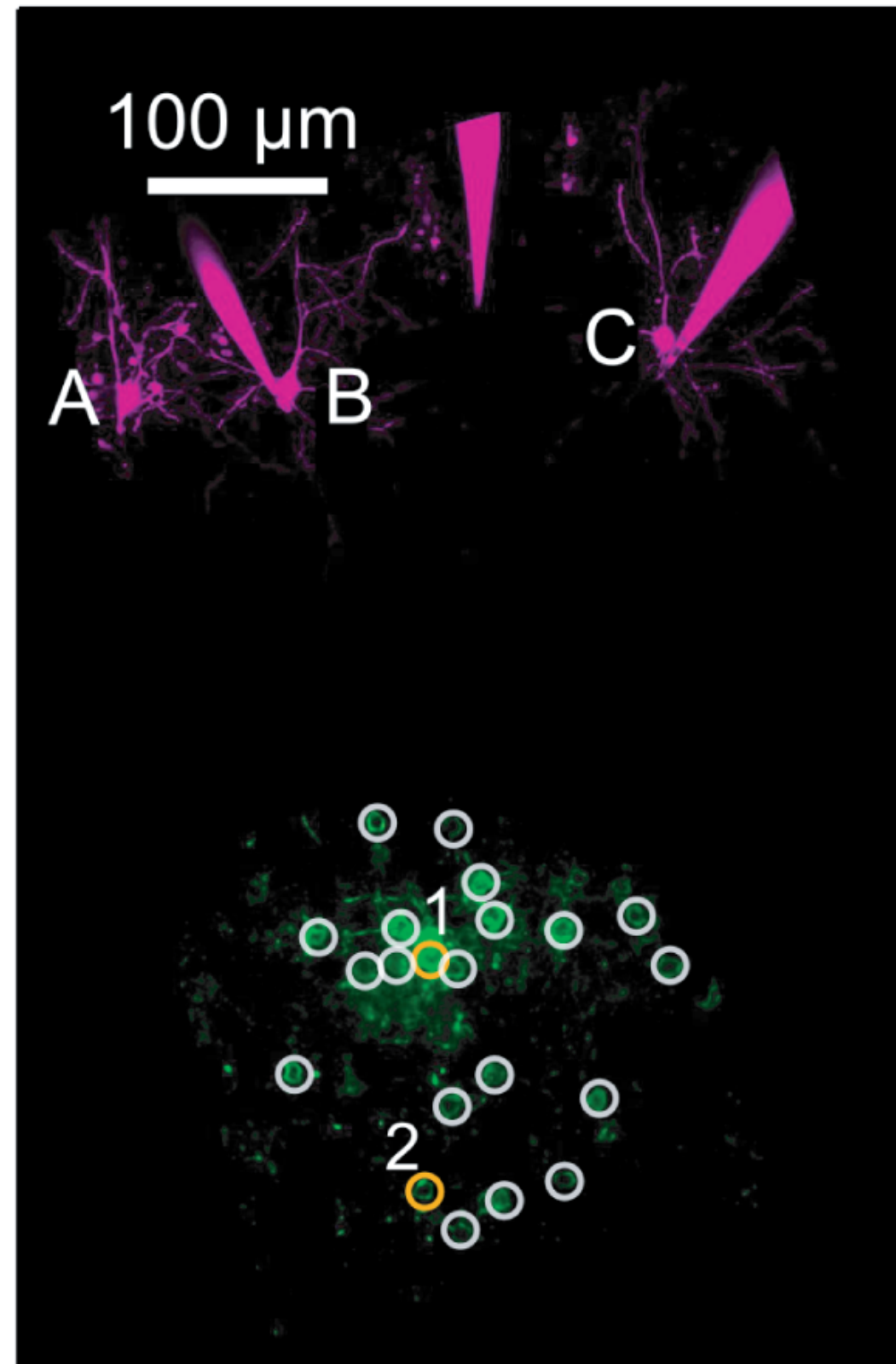


Hage et al (2022), eLife



simulation

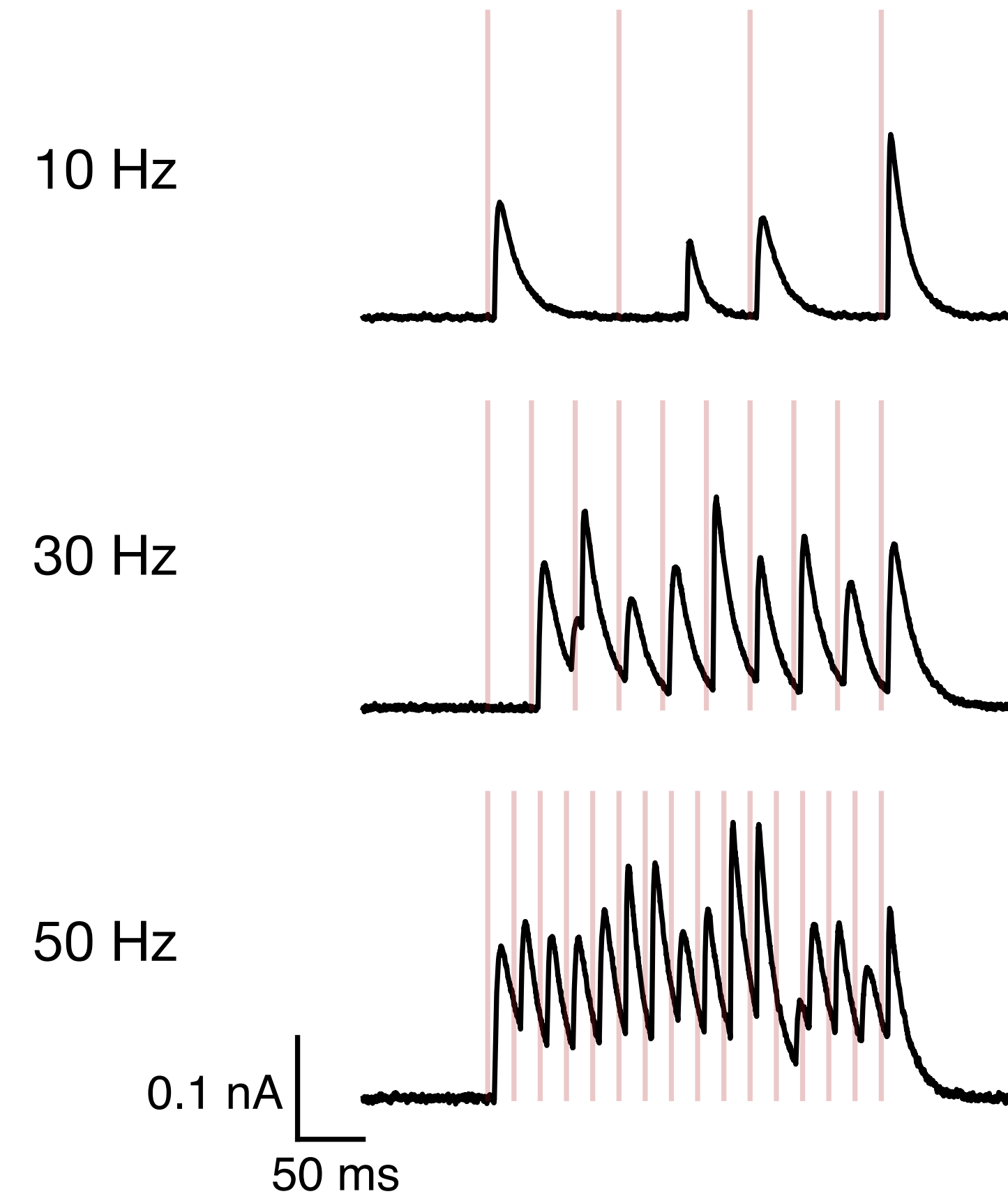
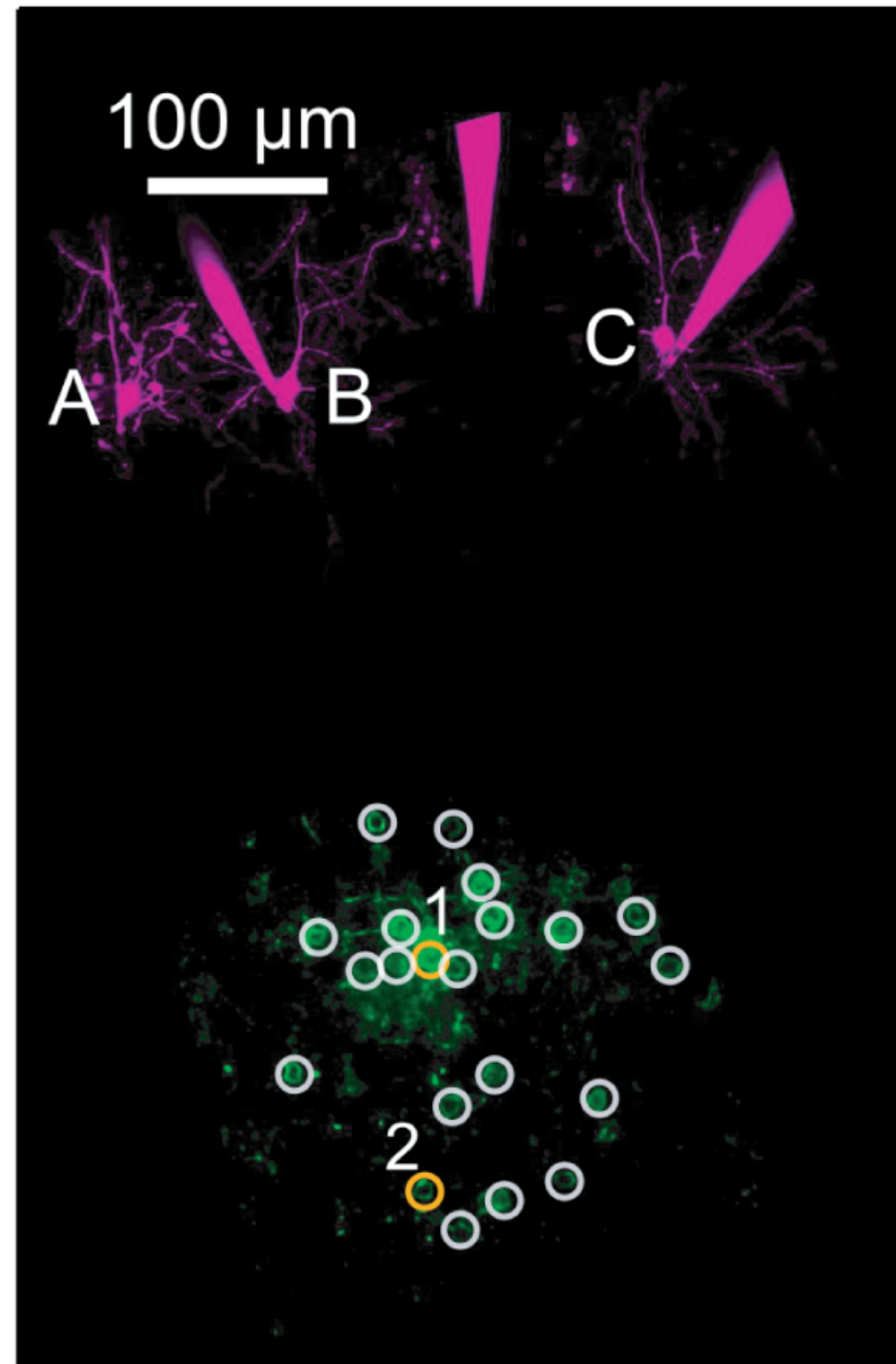
The trade-off in fast optogenetic circuit mapping



Hage et al (2022), eLife

simulation

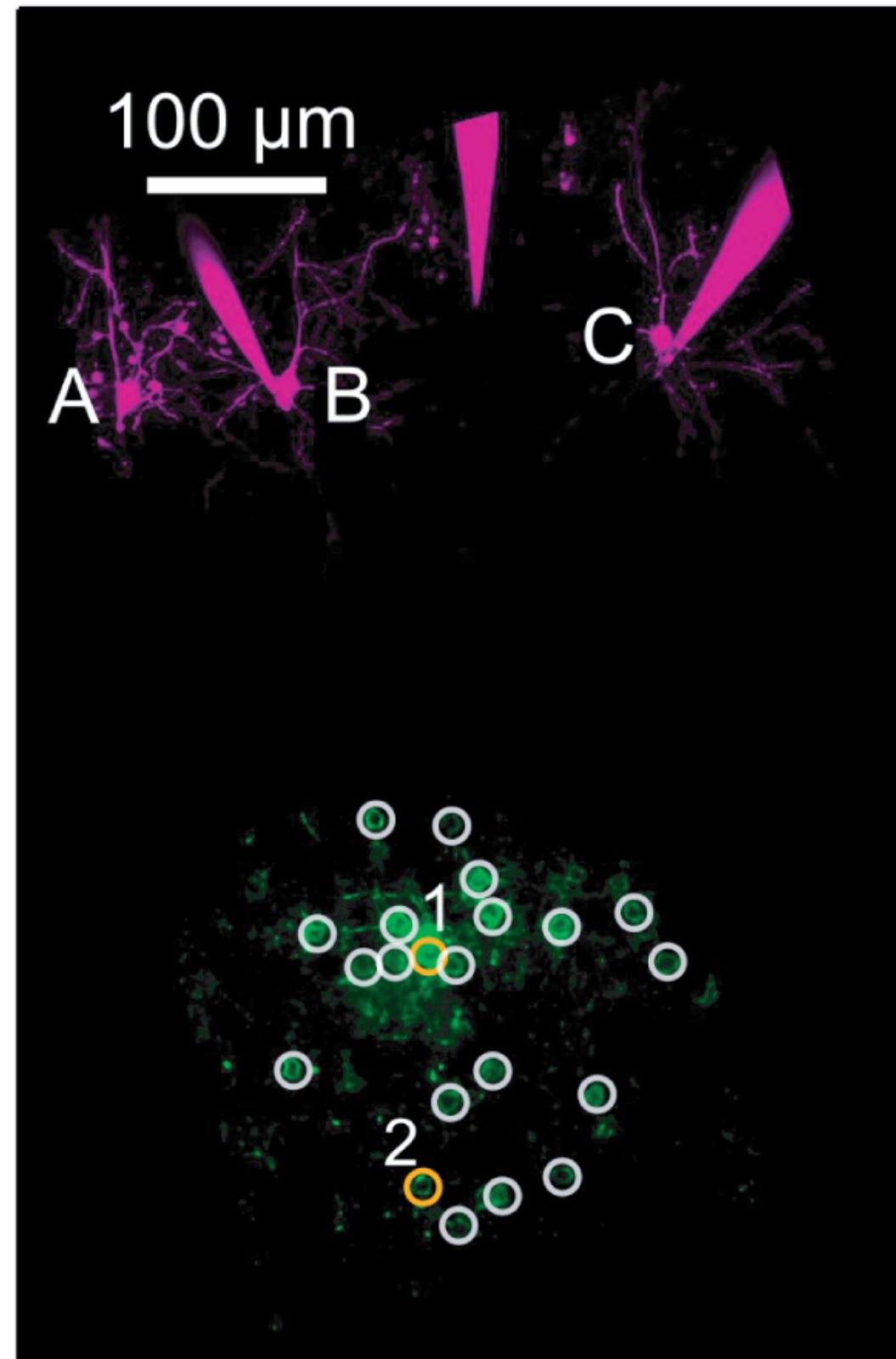
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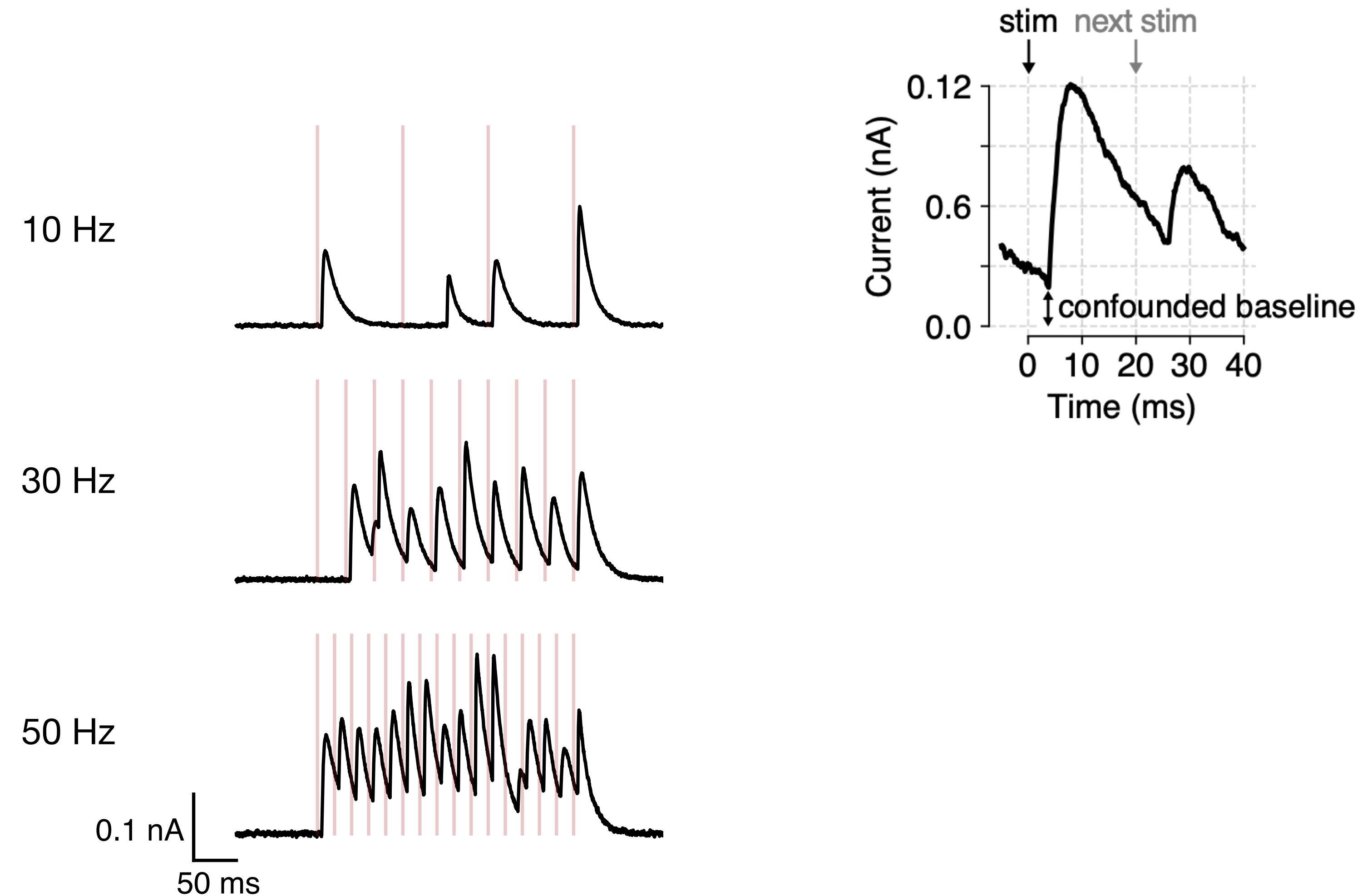
Hage et al (2022), eLife

simulation

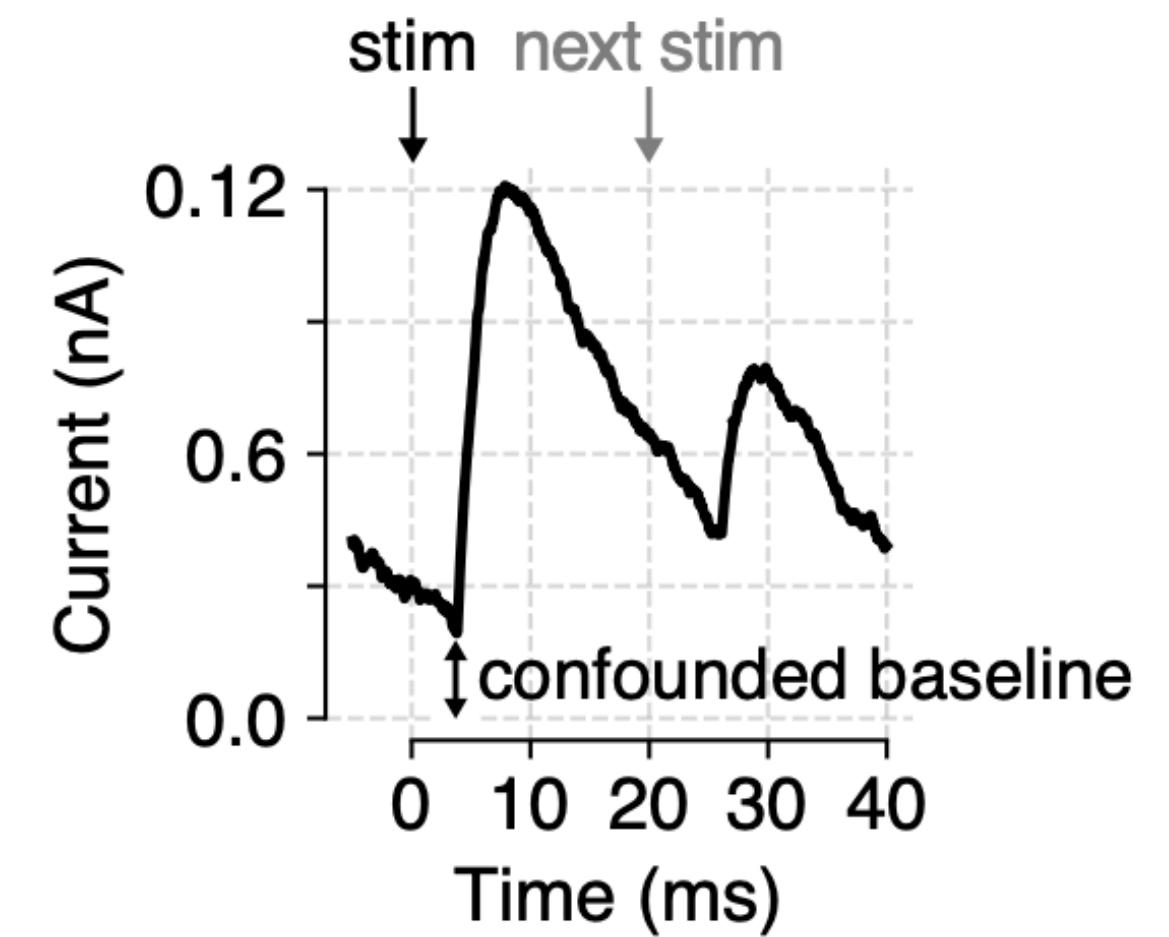
The trade-off in fast optogenetic circuit mapping



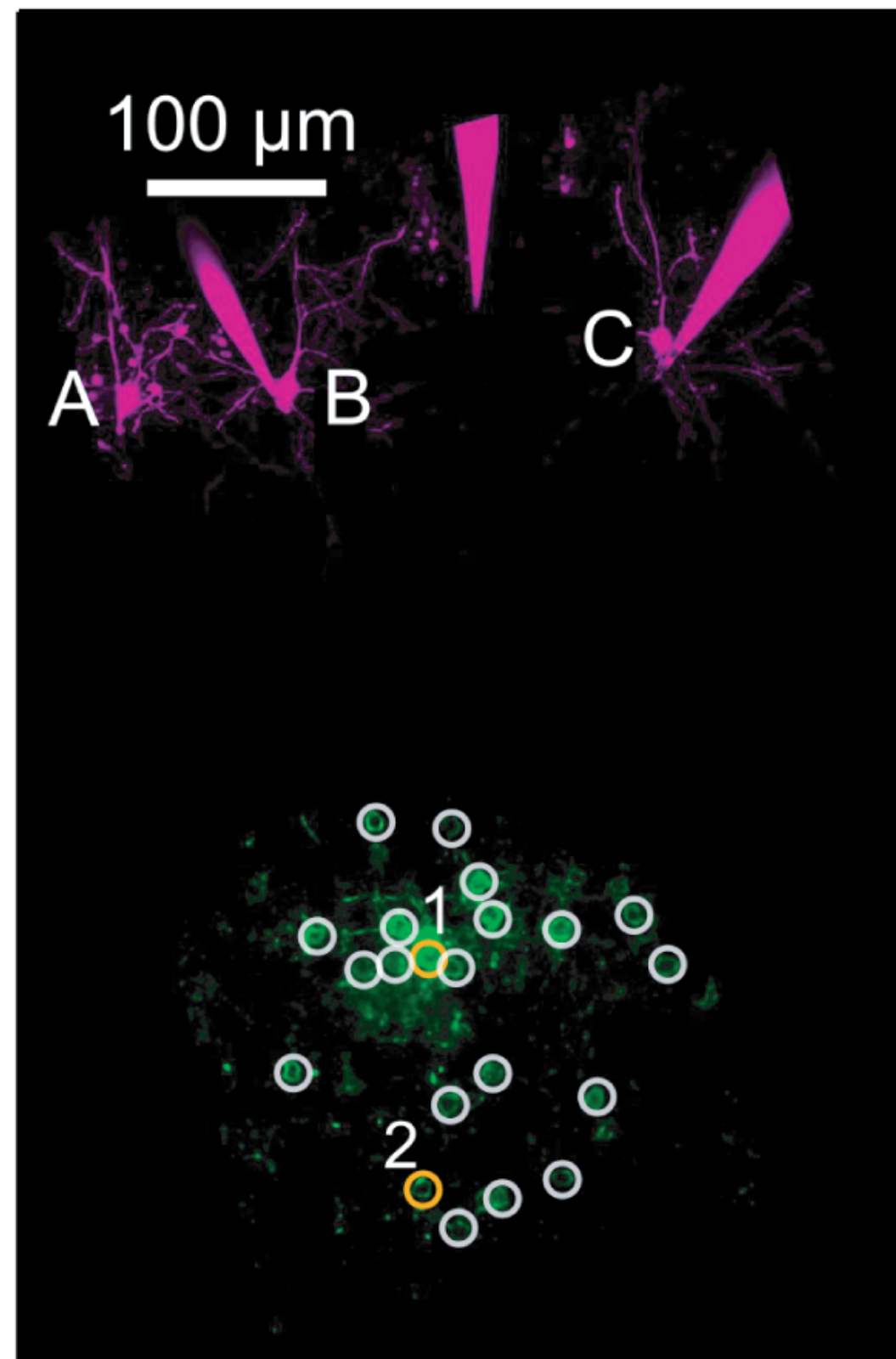
Hage et al (2022), eLife



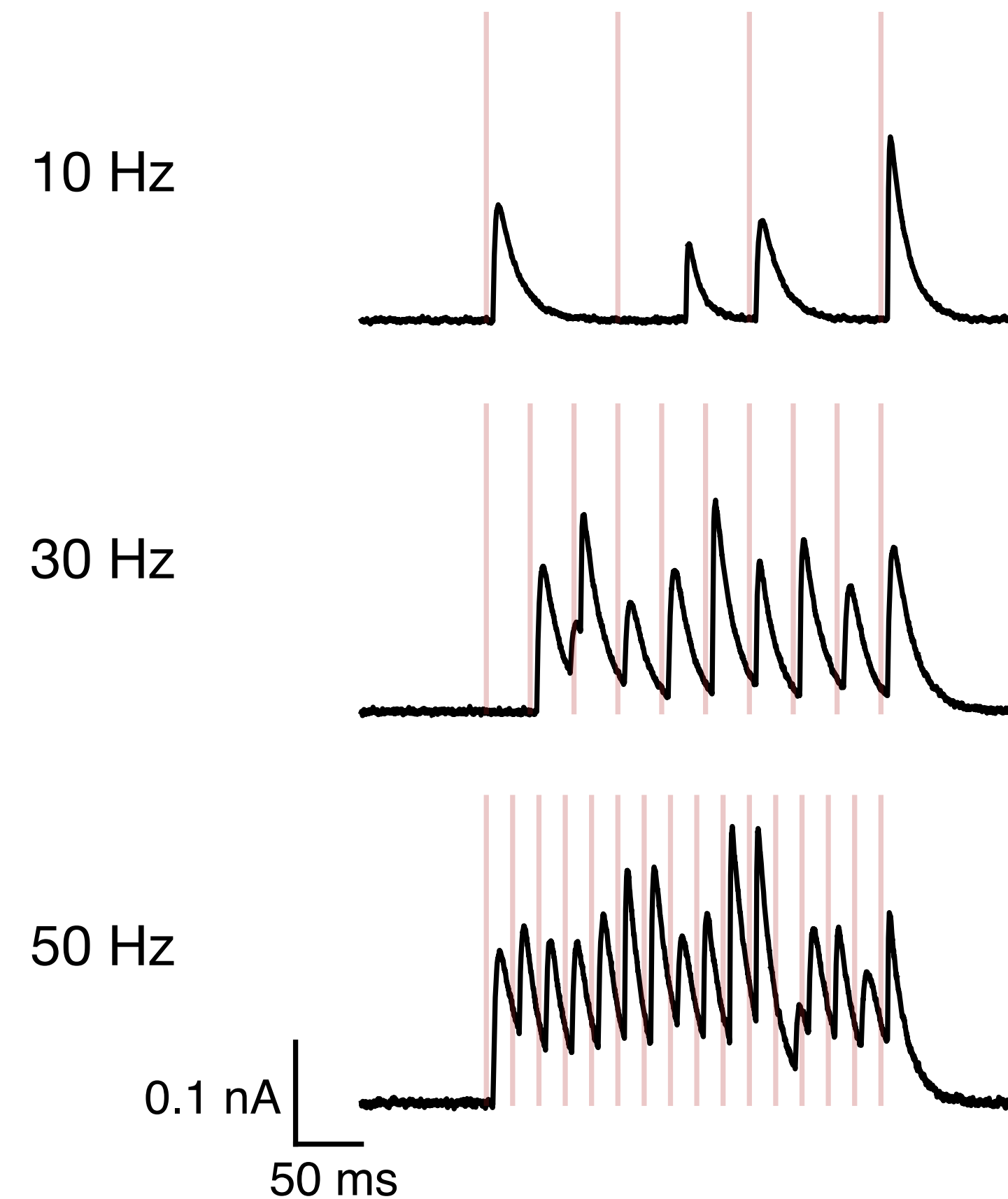
simulation



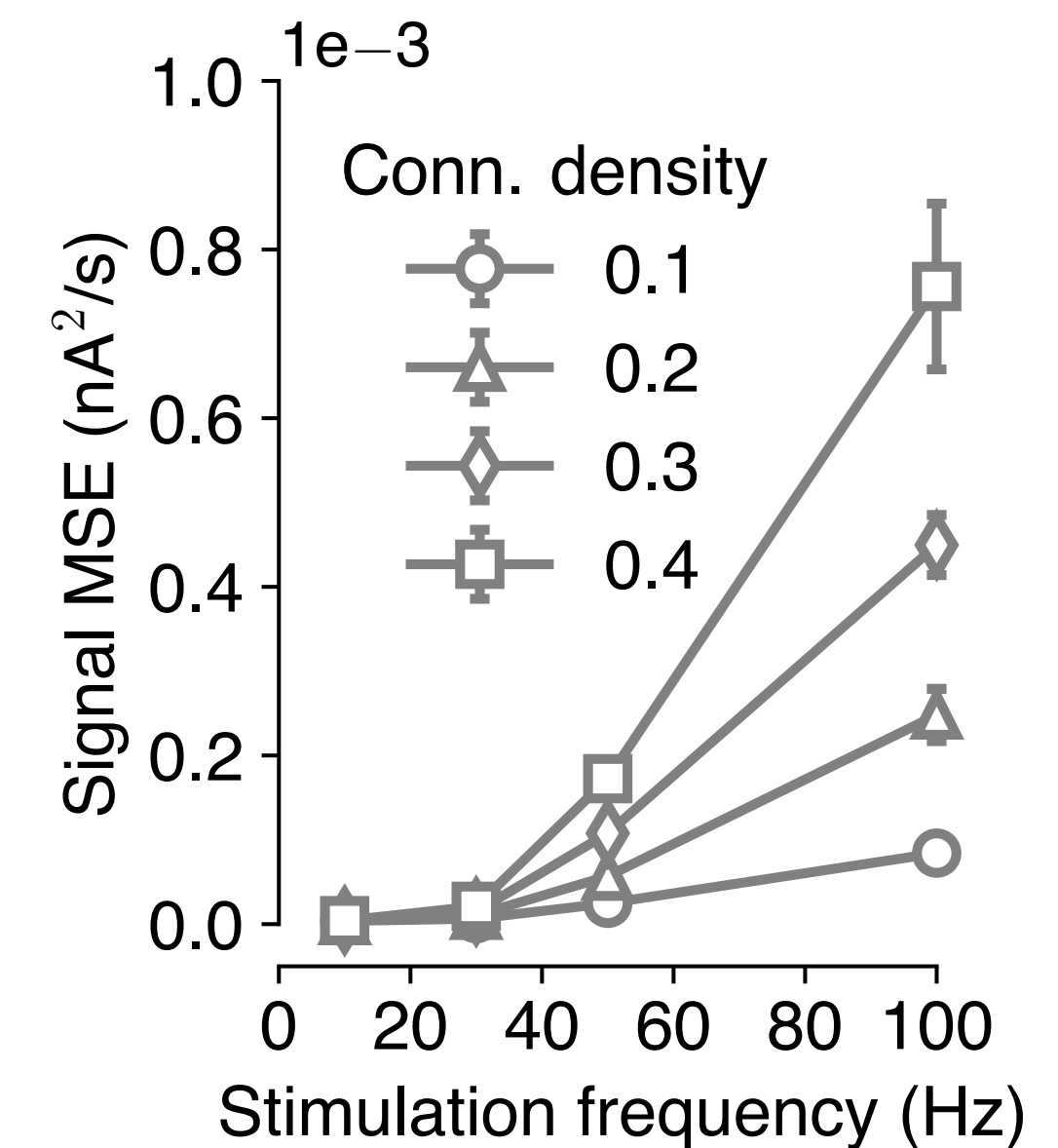
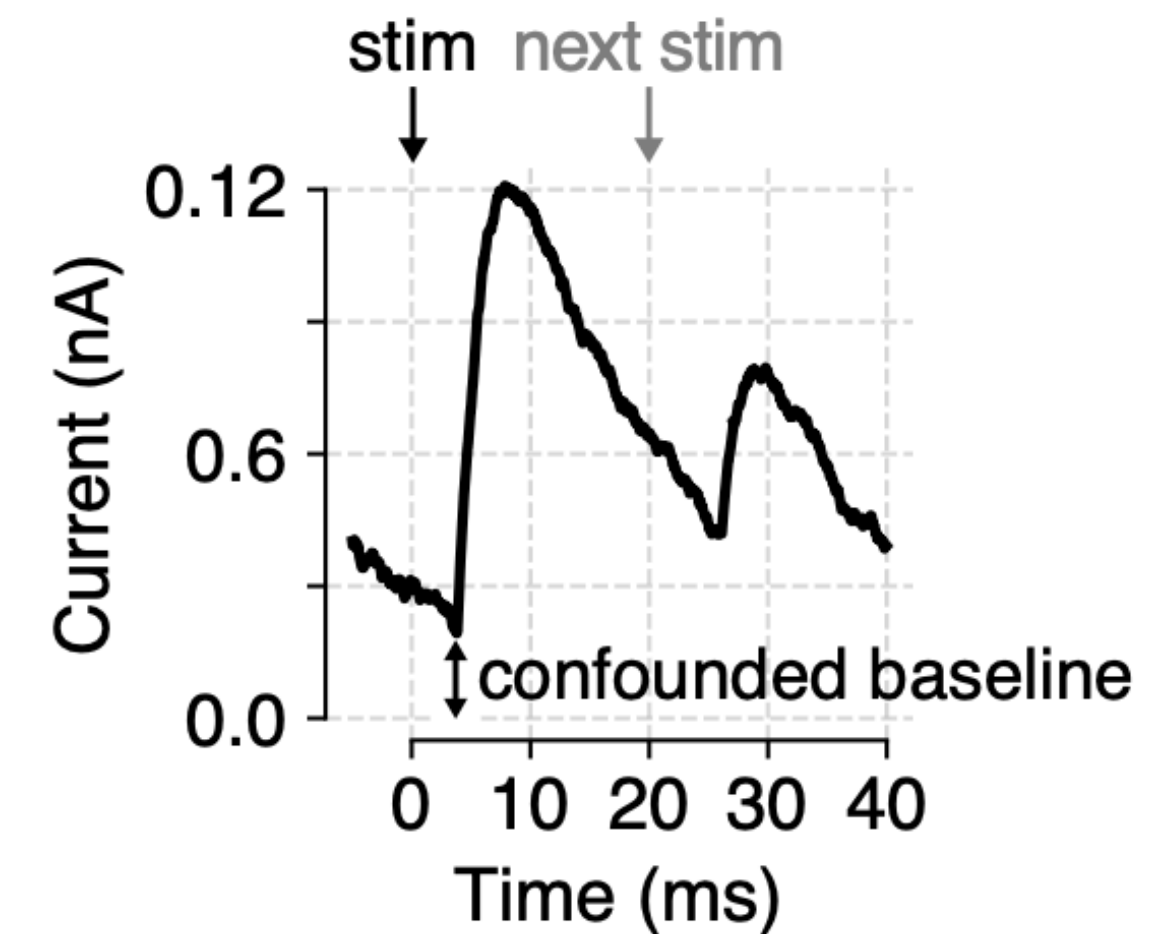
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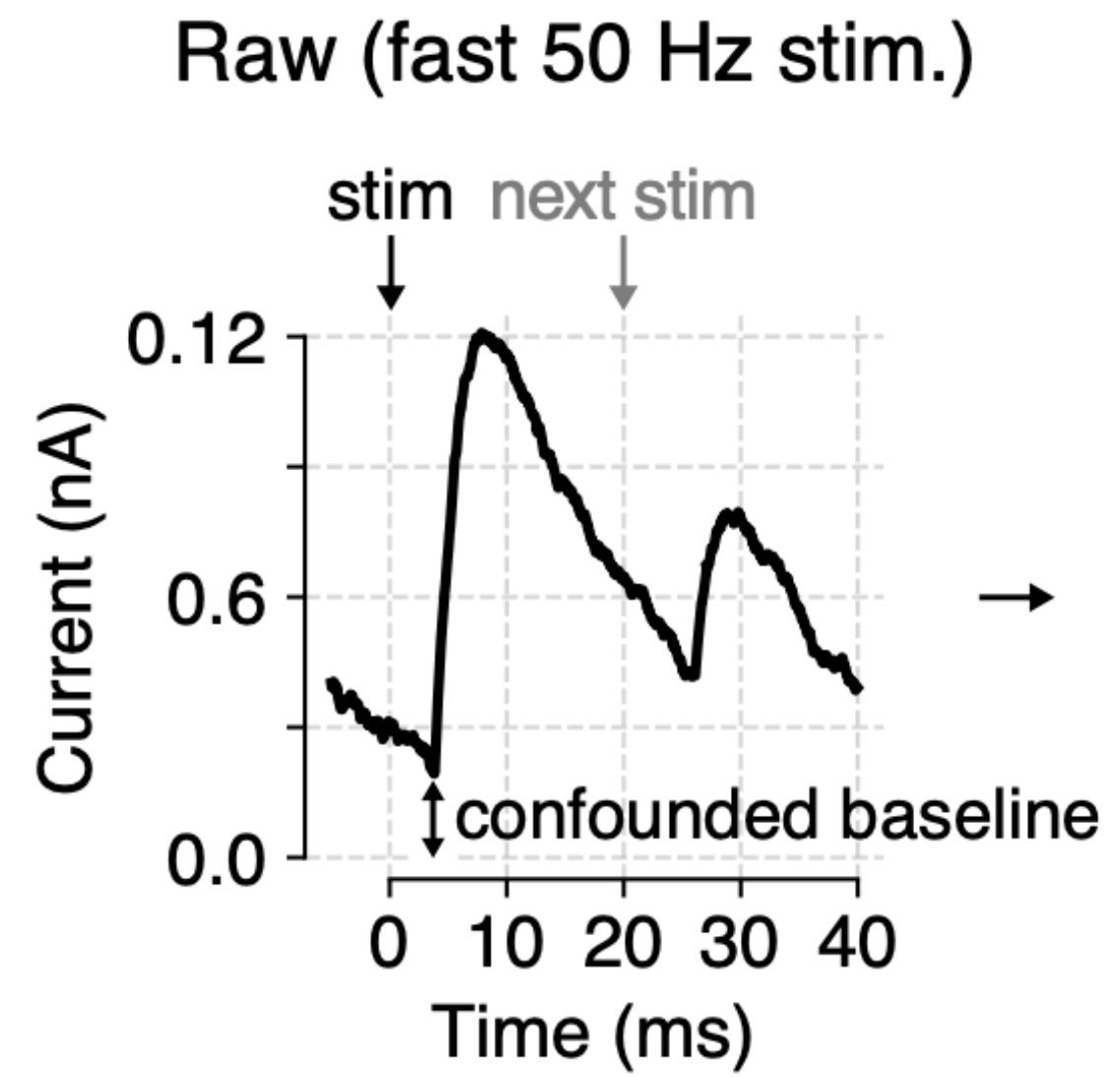
Hage et al (2022), eLife



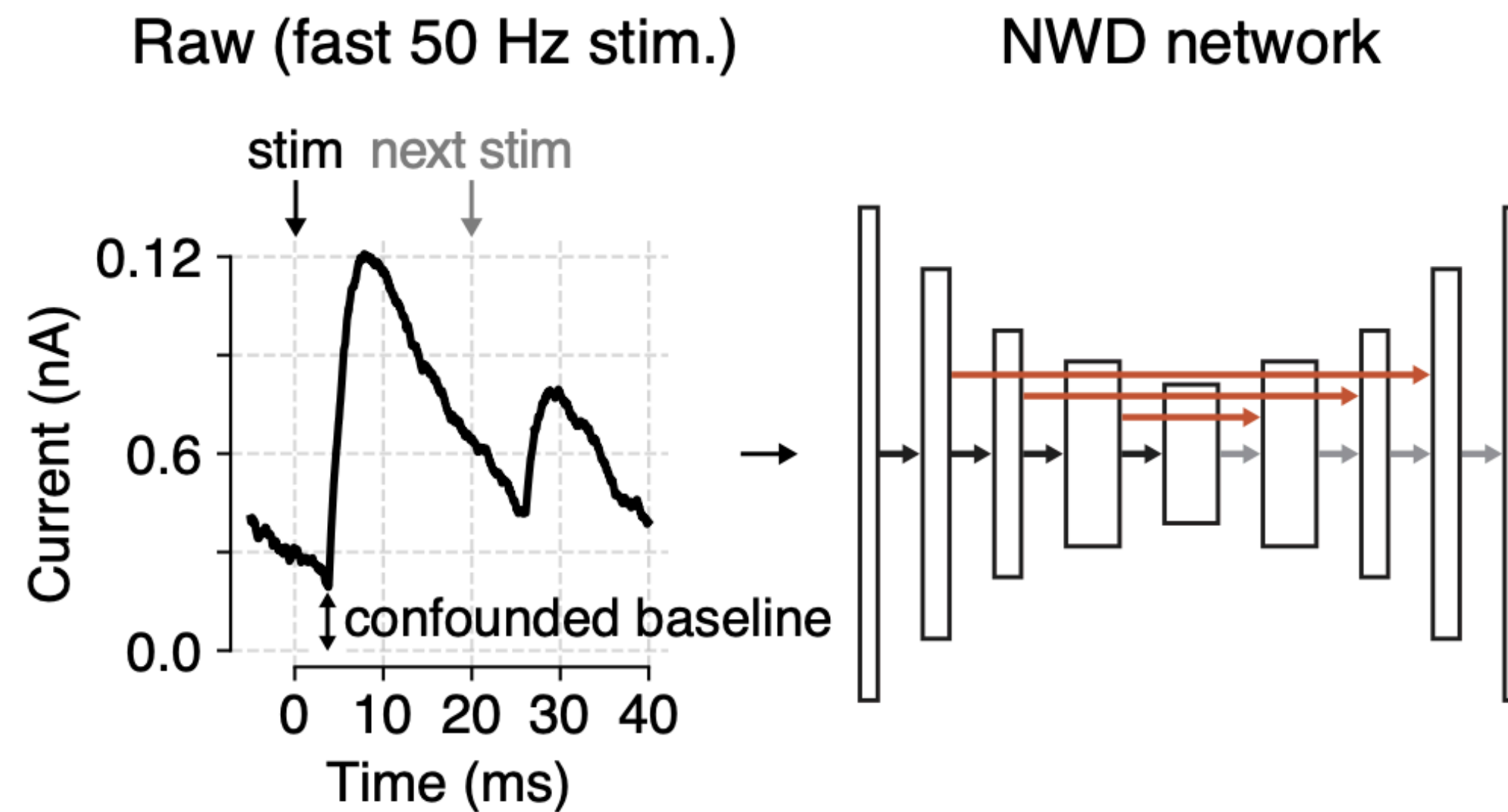
simulation



Neural waveform demixing



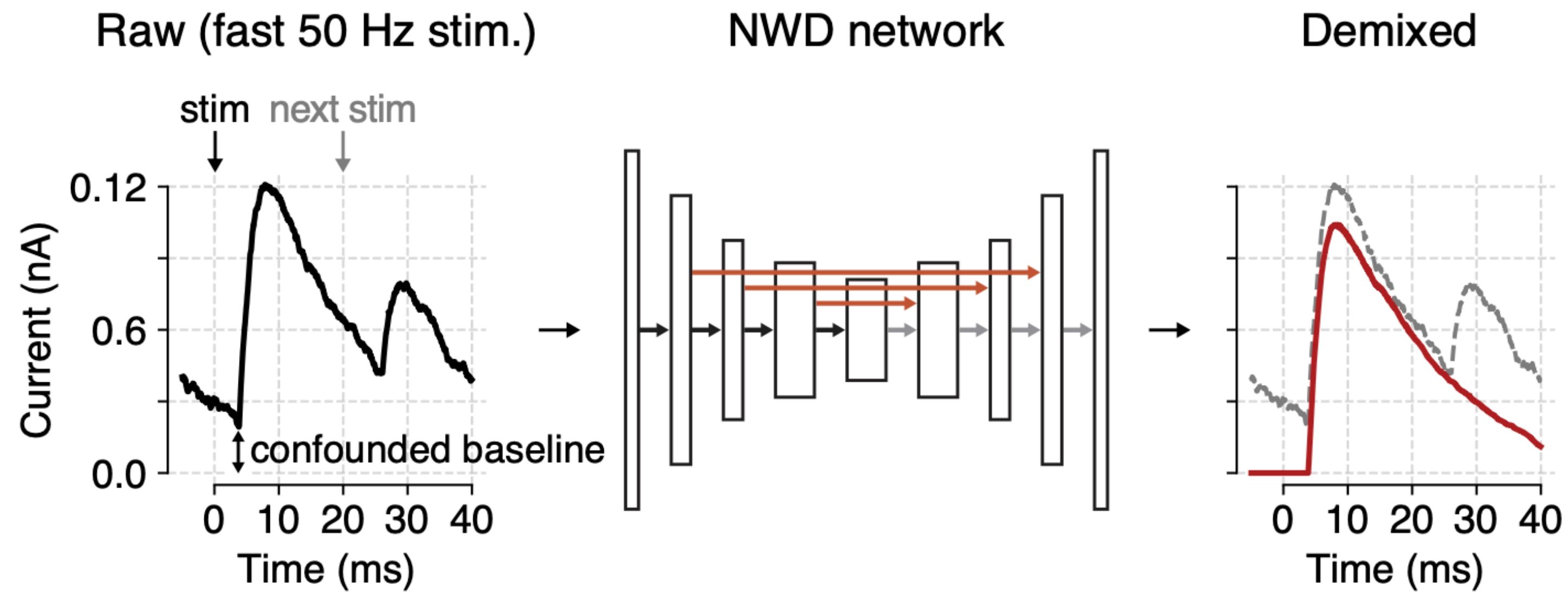
Neural waveform demixing



- ➔ decimate, 1d Conv, BatchNorm, ReLU
- ➡ 1d ConvTranspose, BatchNorm, ReLU, interpolate
- ➞ skip connection

Trained on simulated data, deployed on experimental data

Neural waveform demixing

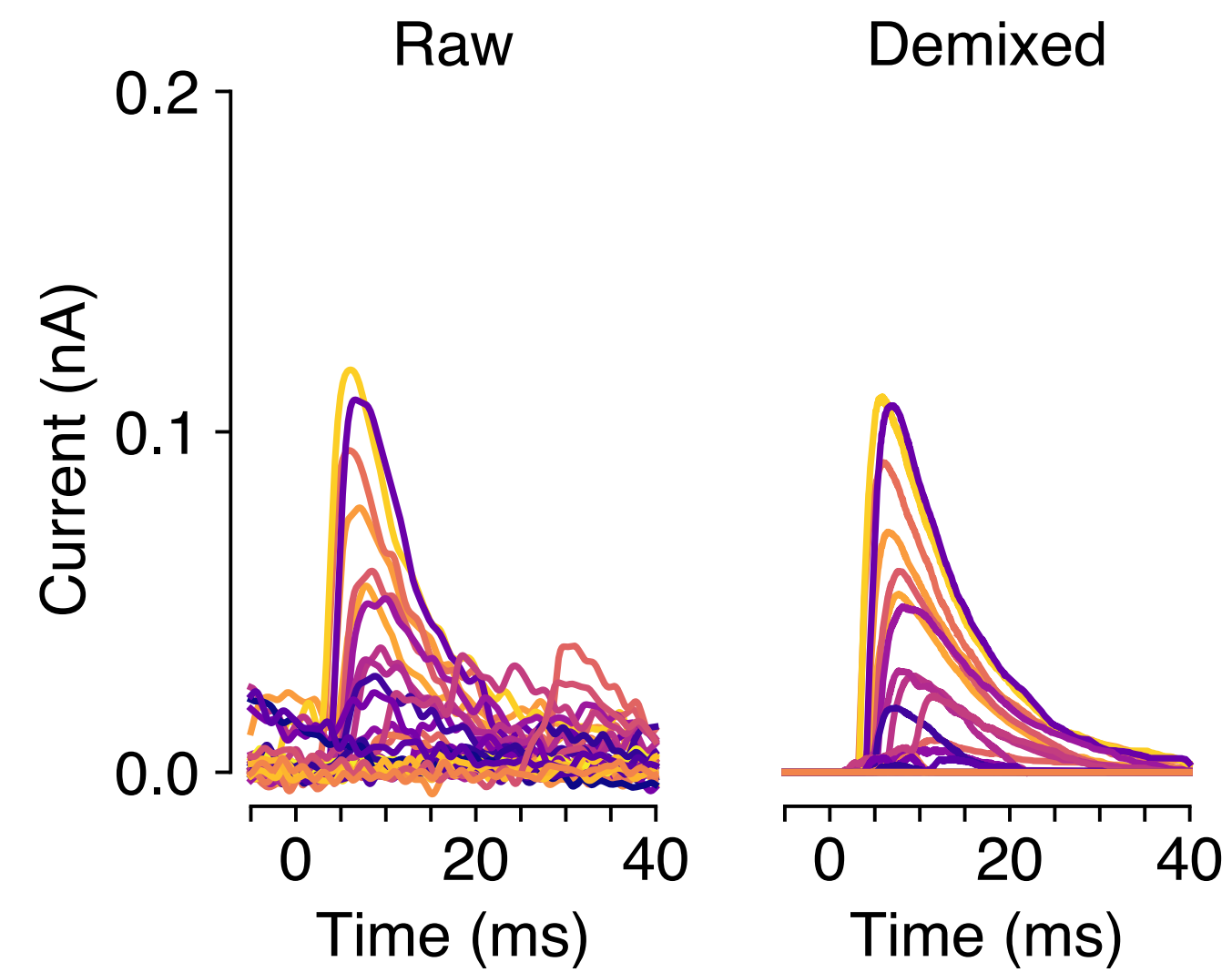
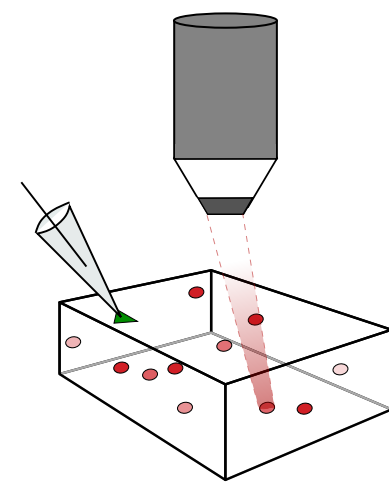


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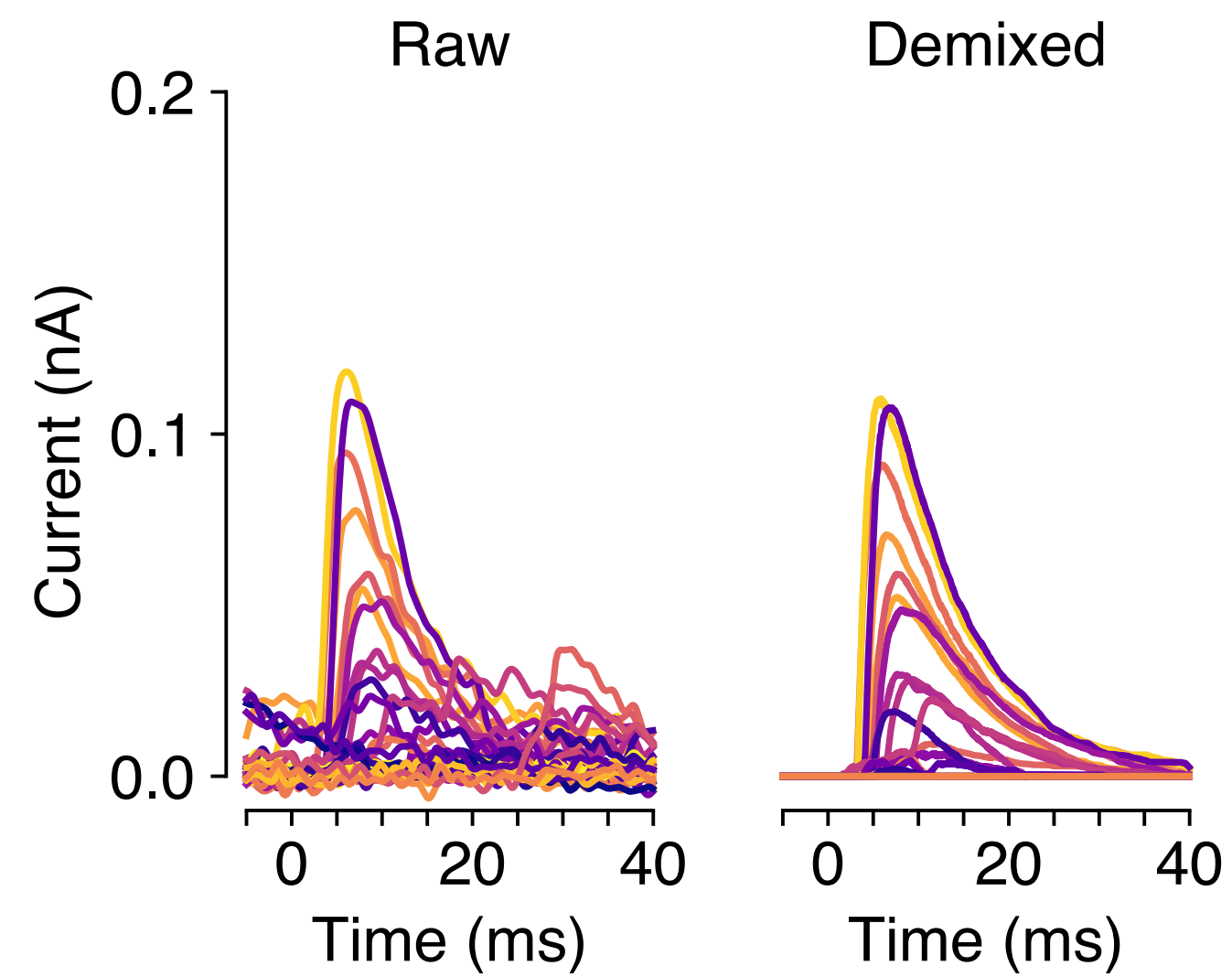
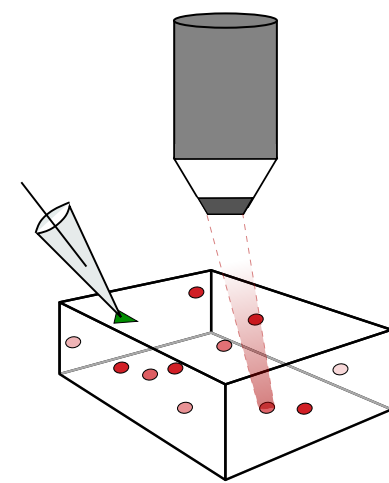
Neural waveform demixing

PV-Pyramidal
single-cell stimulation

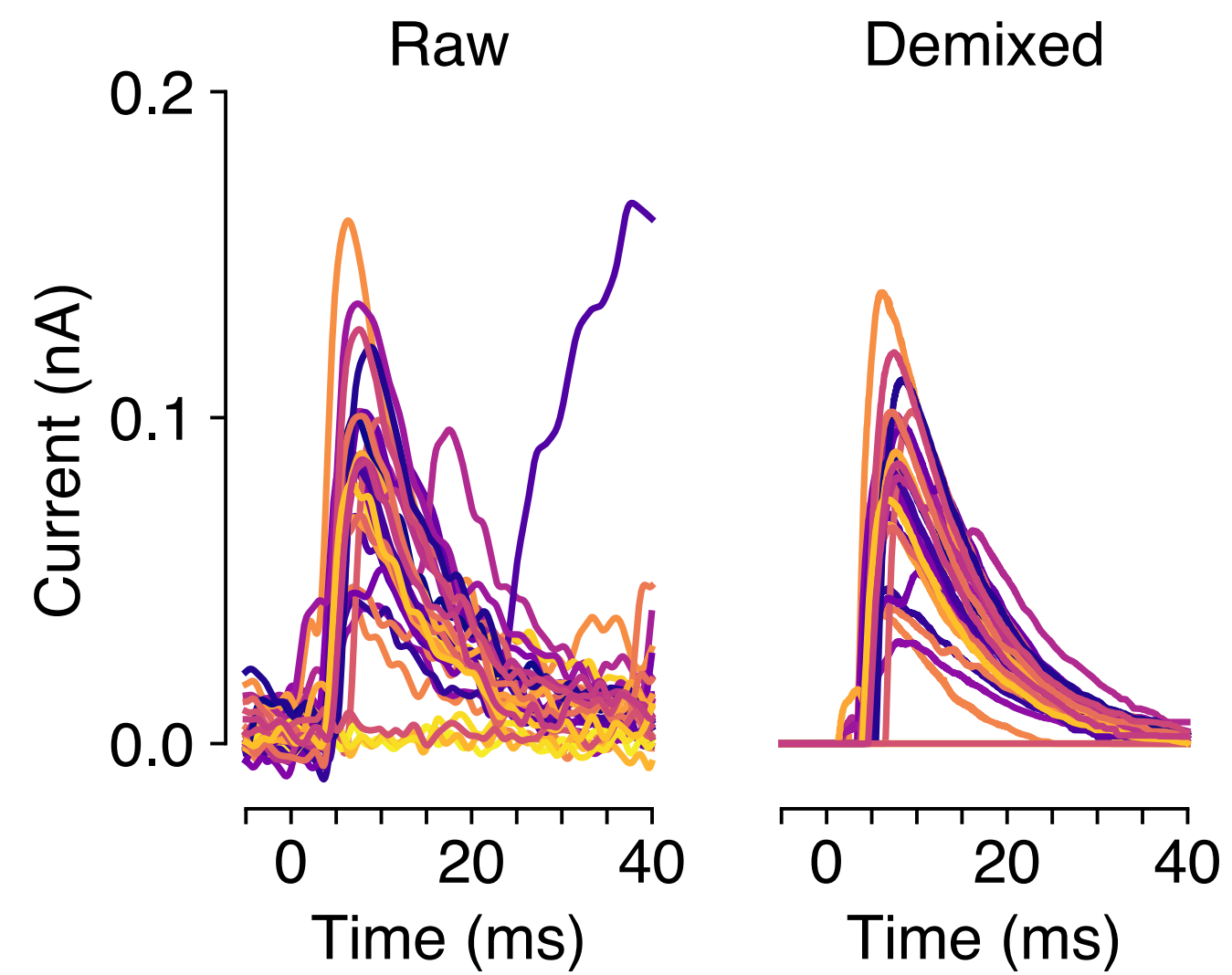
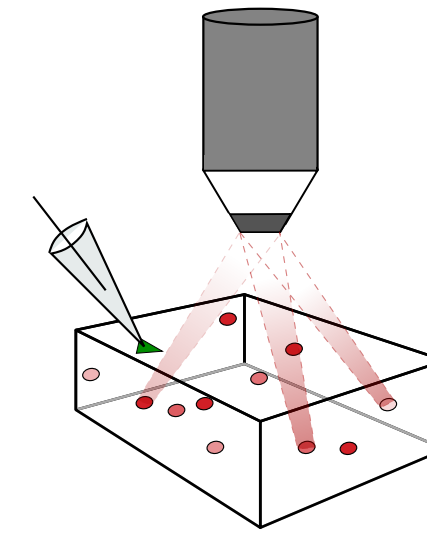


Neural waveform demixing

PV-Pyramidal
single-cell stimulation

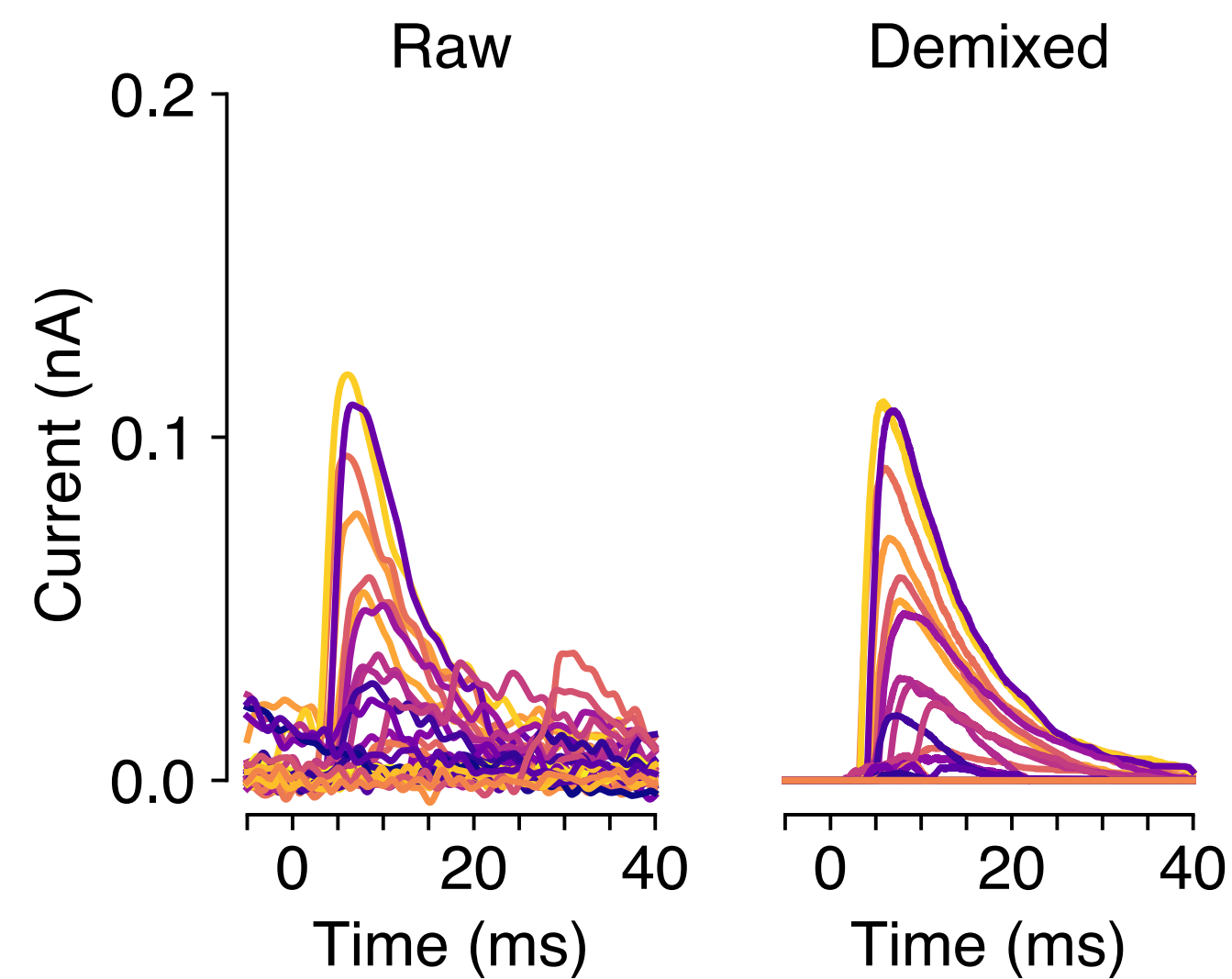
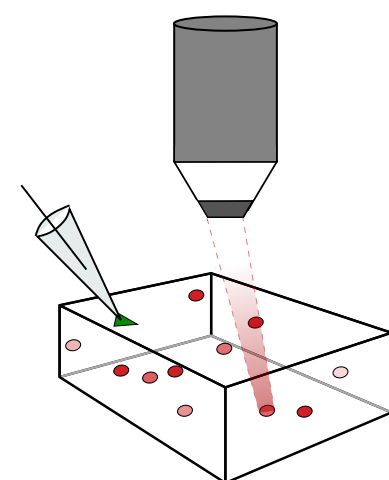


PV-Pyramidal
ensemble stimulation

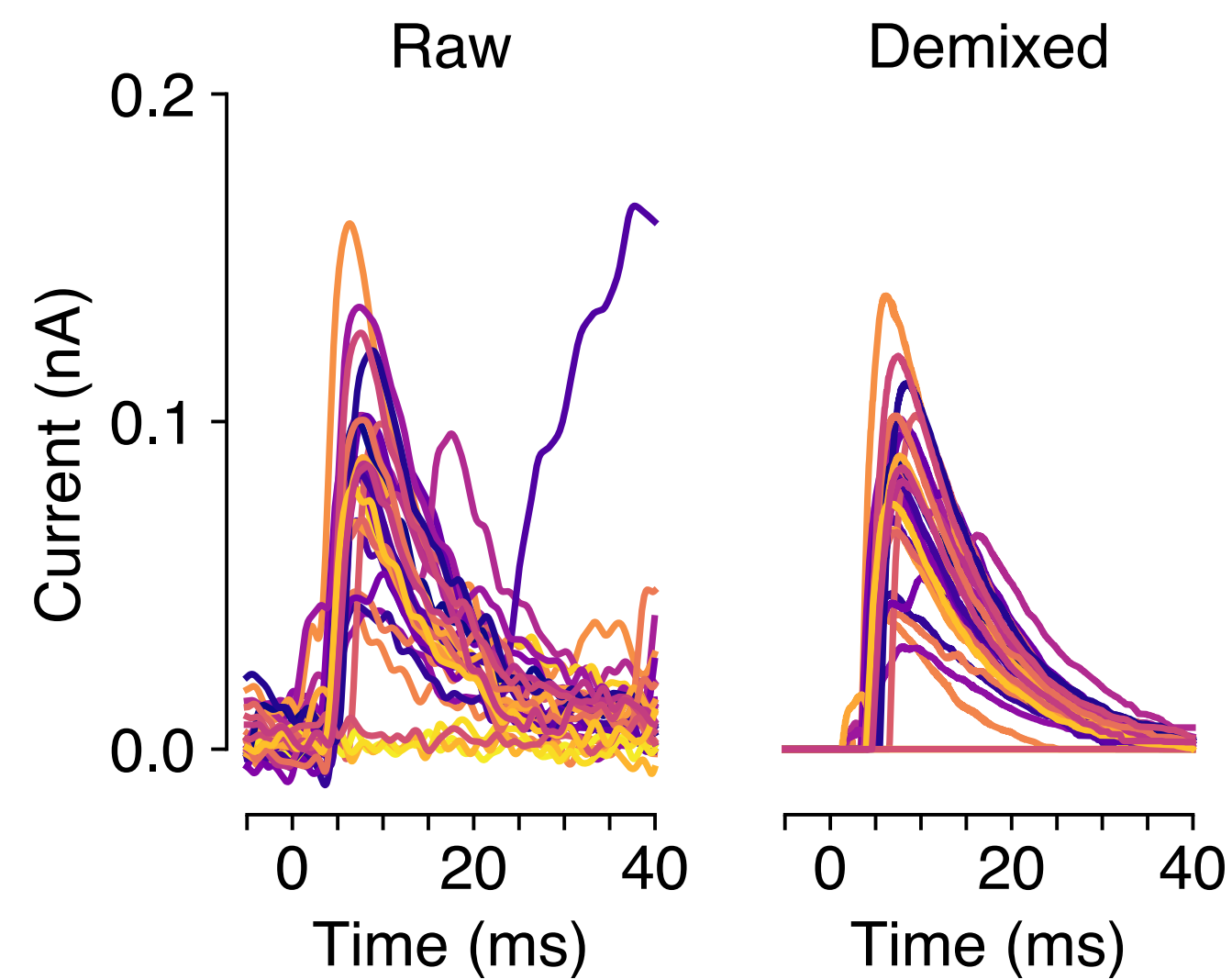
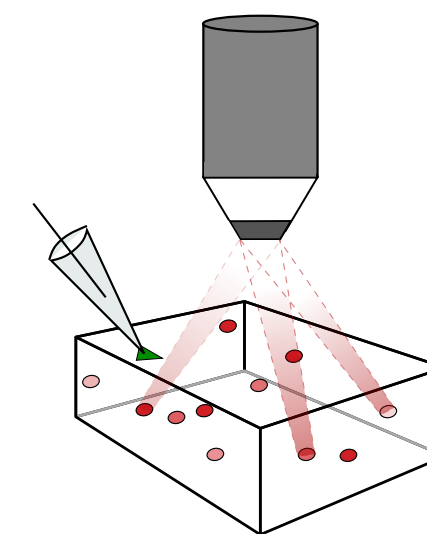


Neural waveform demixing

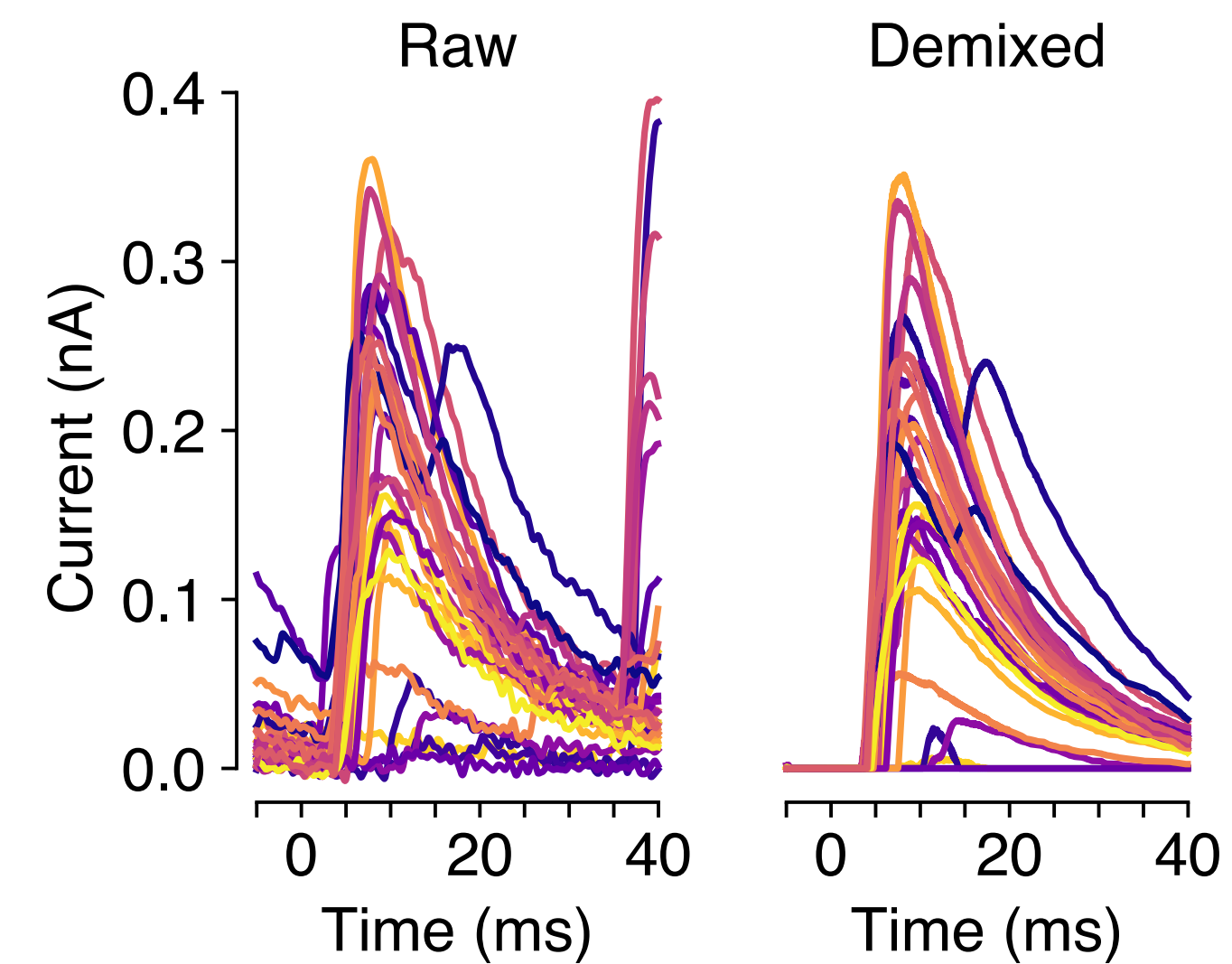
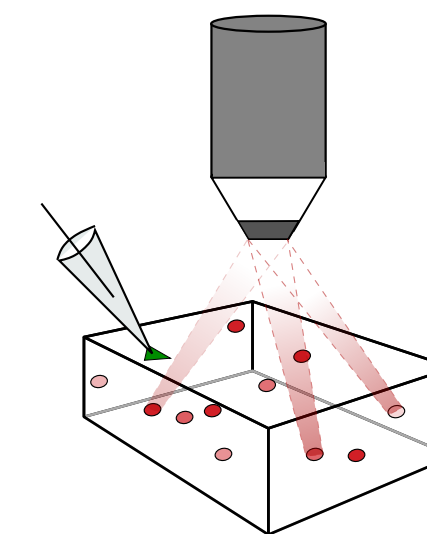
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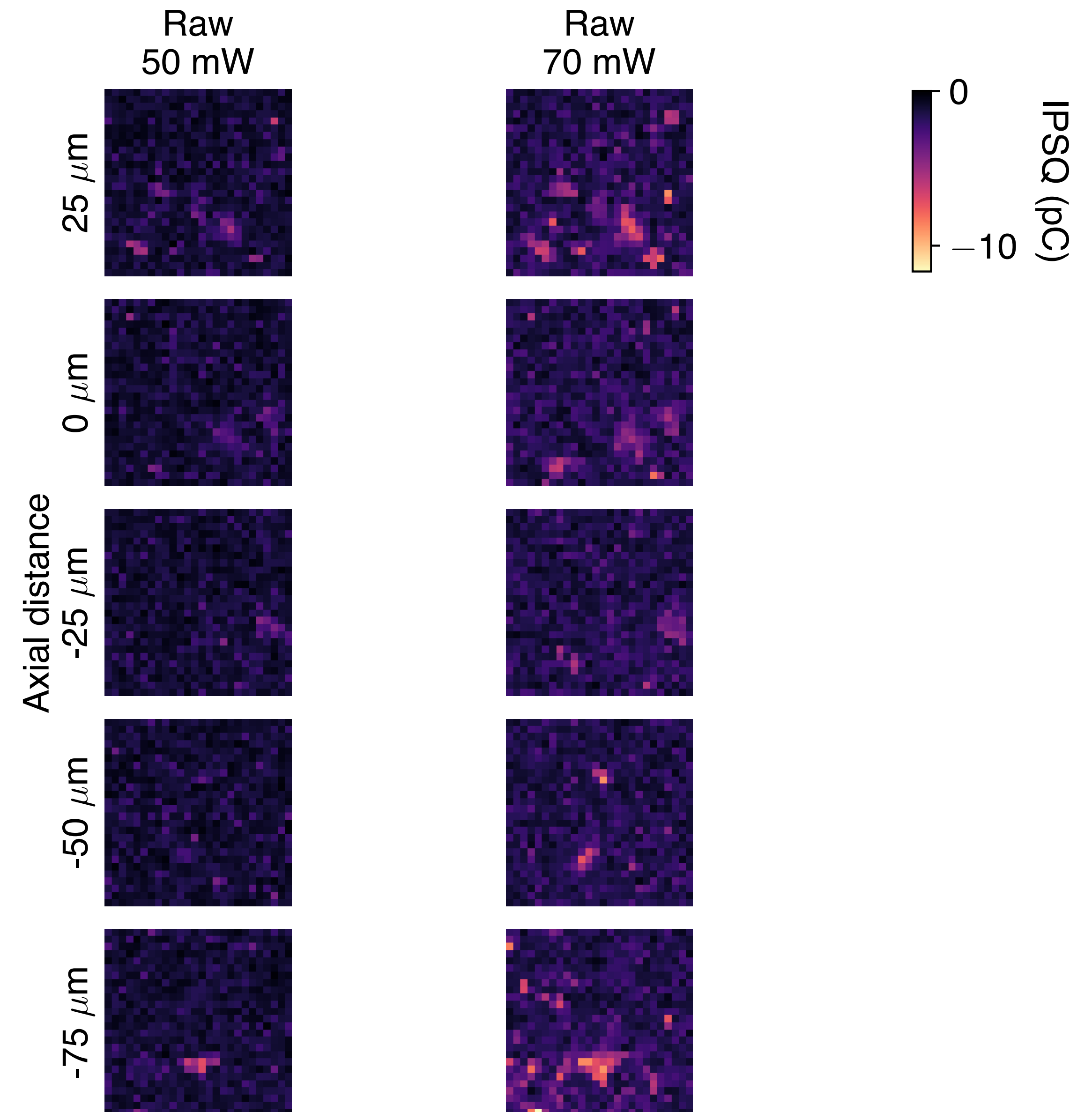
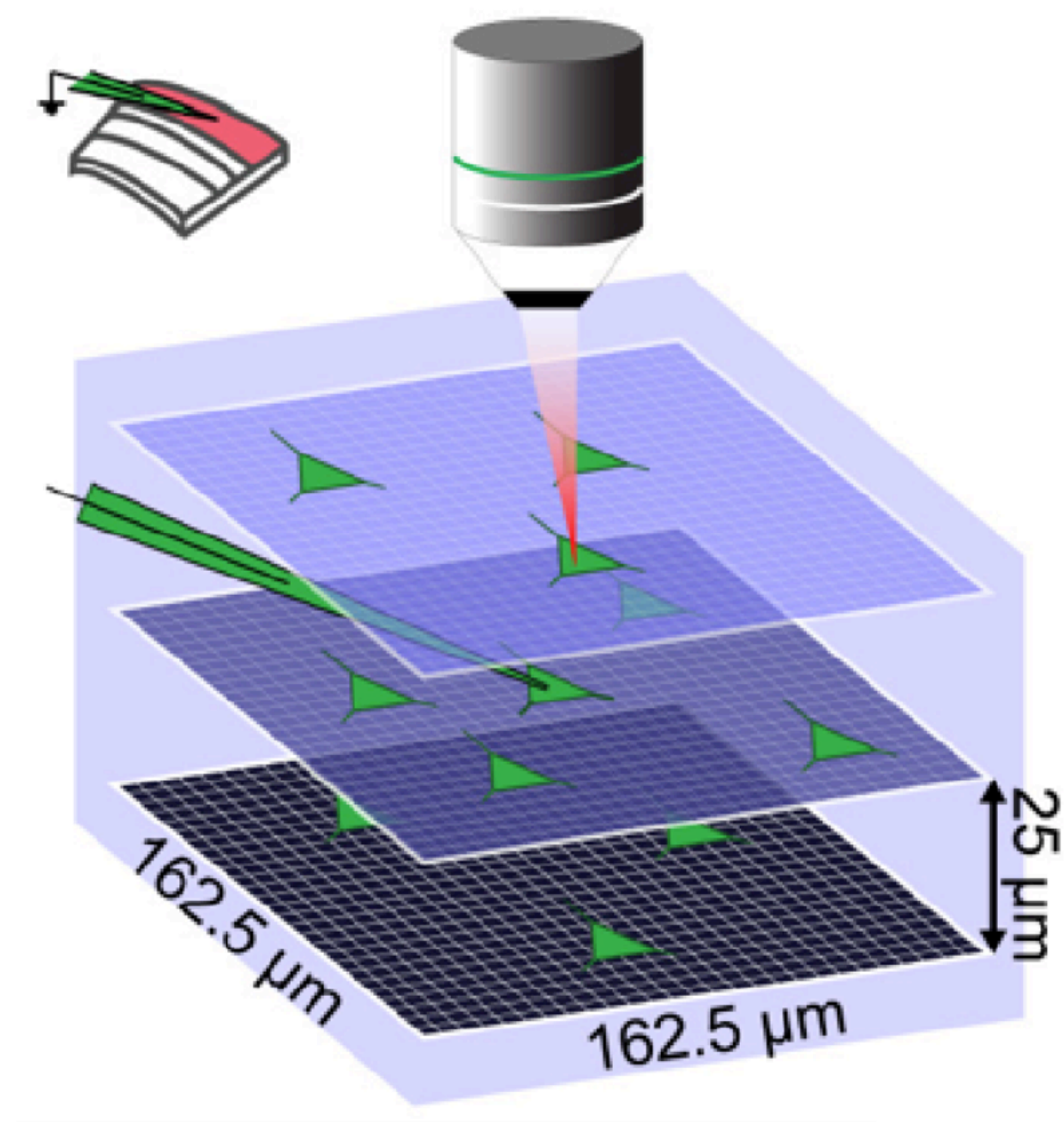
PV-Pyramidal
ensemble stimulation



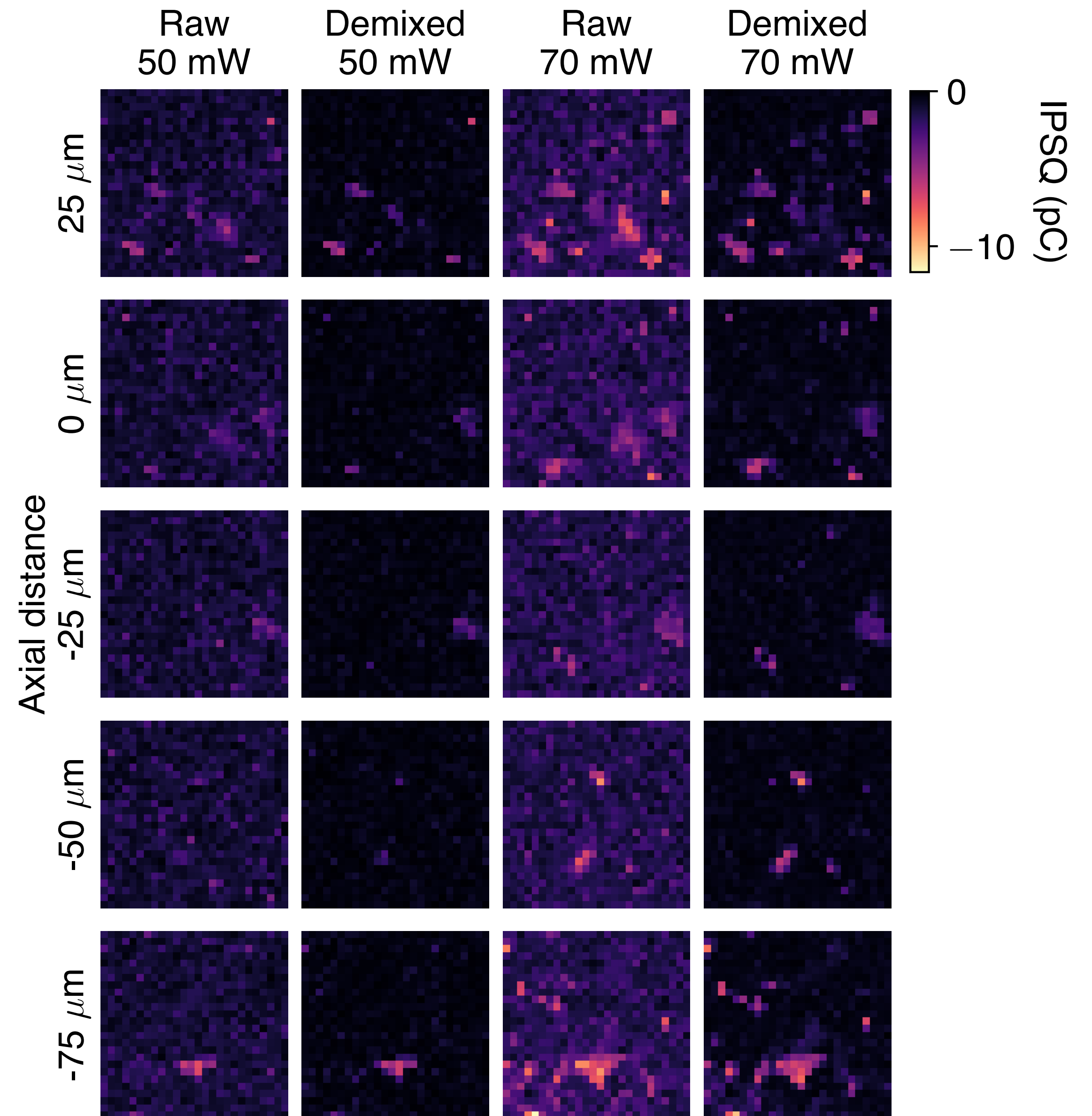
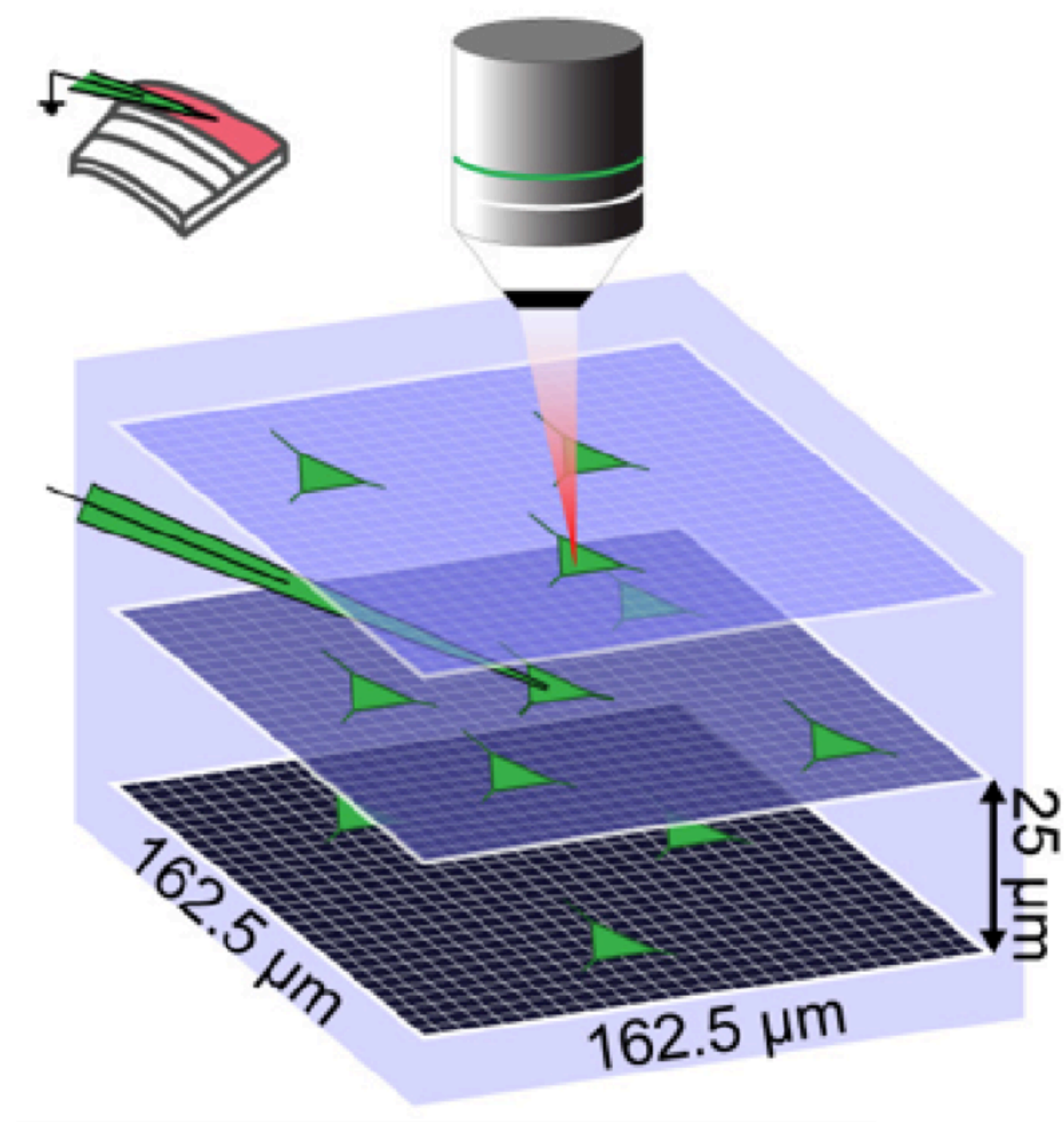
SST-Pyramidal
ensemble stimulation



“Blind” grid mapping



“Blind” grid mapping

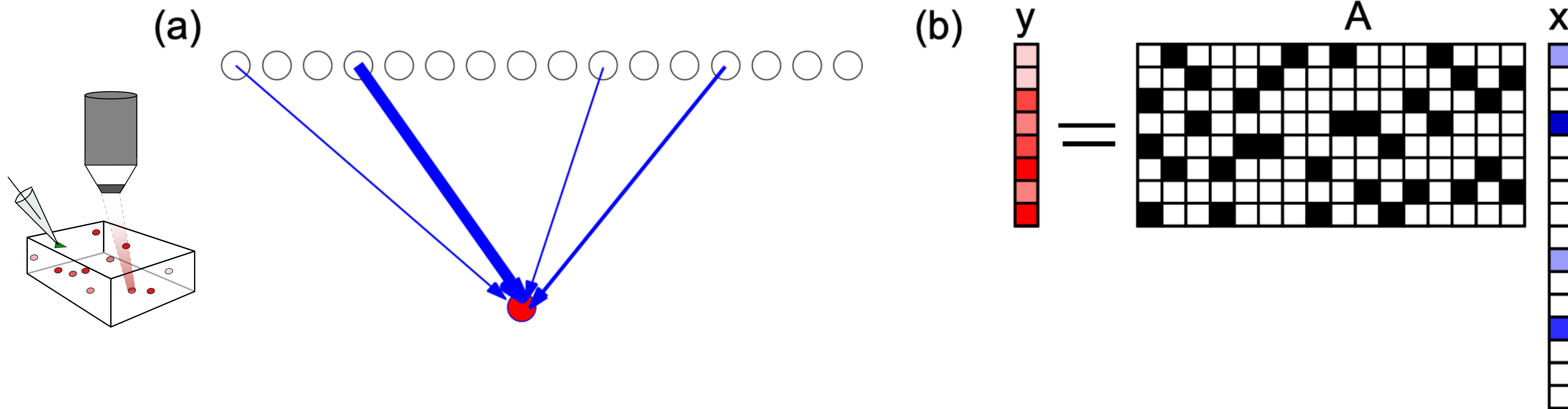


Optogenetic neural circuit mapping

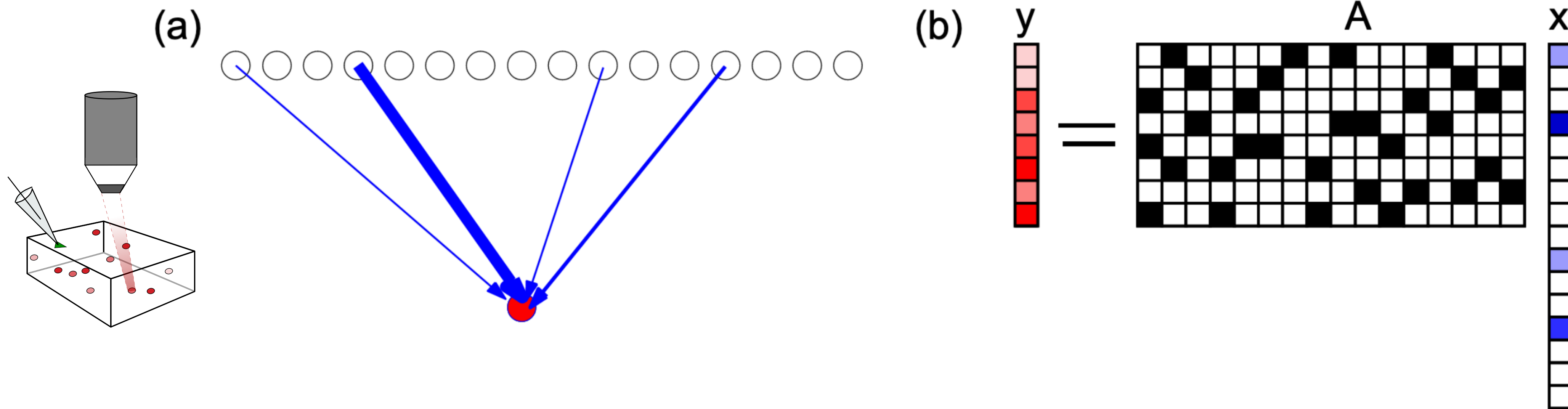
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Compressed sensing-based circuit mapping



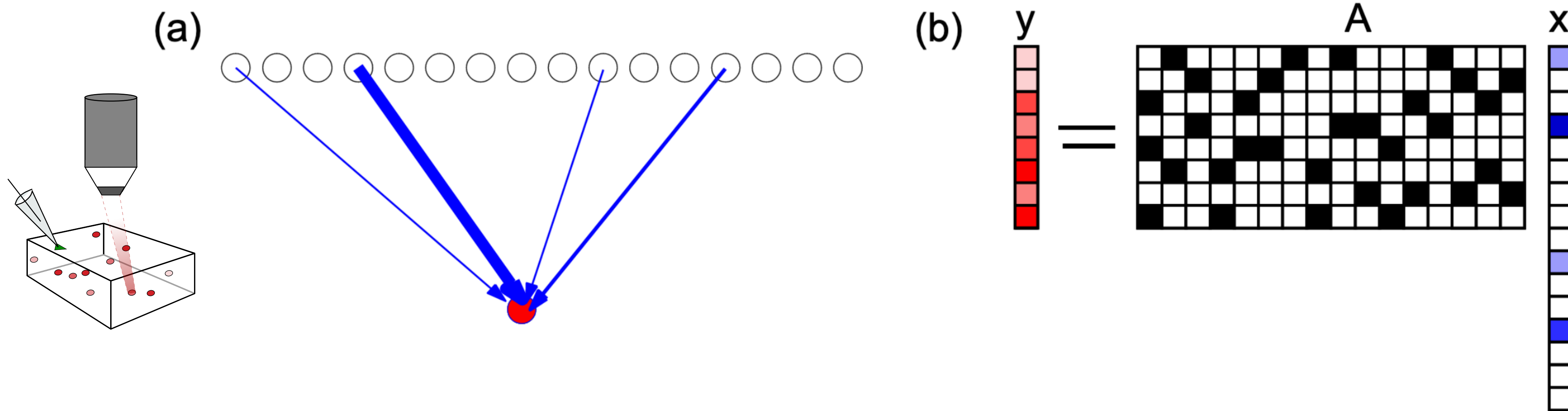
Compressed sensing-based circuit mapping



Model: $\mathbf{y} = \mathbf{A}\mathbf{x}$

Want to minimise $\|\mathbf{x}\|_0$ such that $\|\mathbf{y} - \mathbf{A}\mathbf{x}\|_2 \leq \epsilon$

Compressed sensing-based circuit mapping

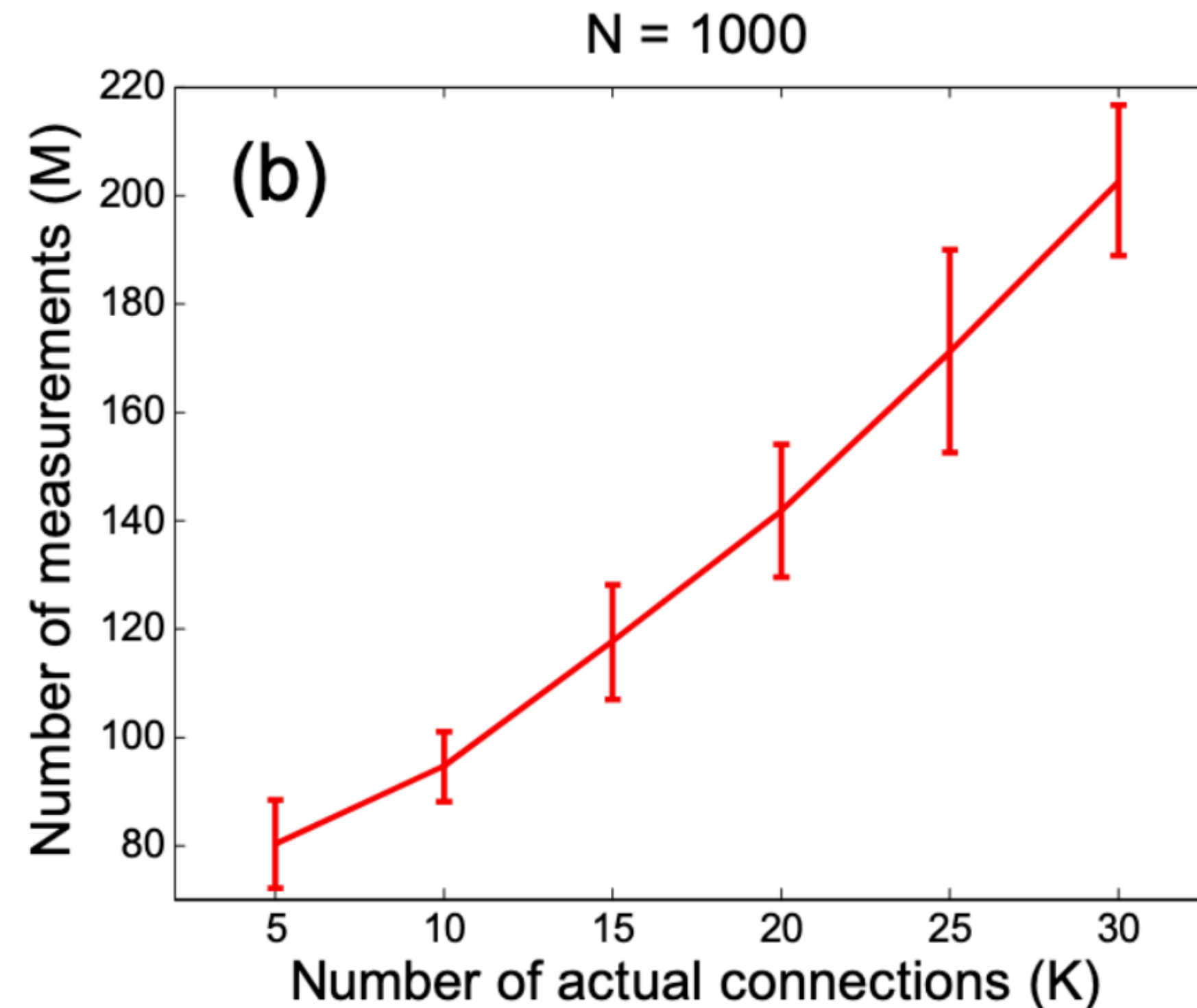


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Want to minimise $\|\mathbf{x}\|_0$ such that $\|\mathbf{y} - \mathbf{A}\mathbf{x}\|_2 \leq \epsilon$

Instead minimise $\|\mathbf{y} - \mathbf{A}\mathbf{x}\|_2 + \gamma\|\mathbf{x}\|_1$ subject to conditions on $\mathbf{A}$ and $\mathbf{x}$

Compressed sensing-based circuit mapping



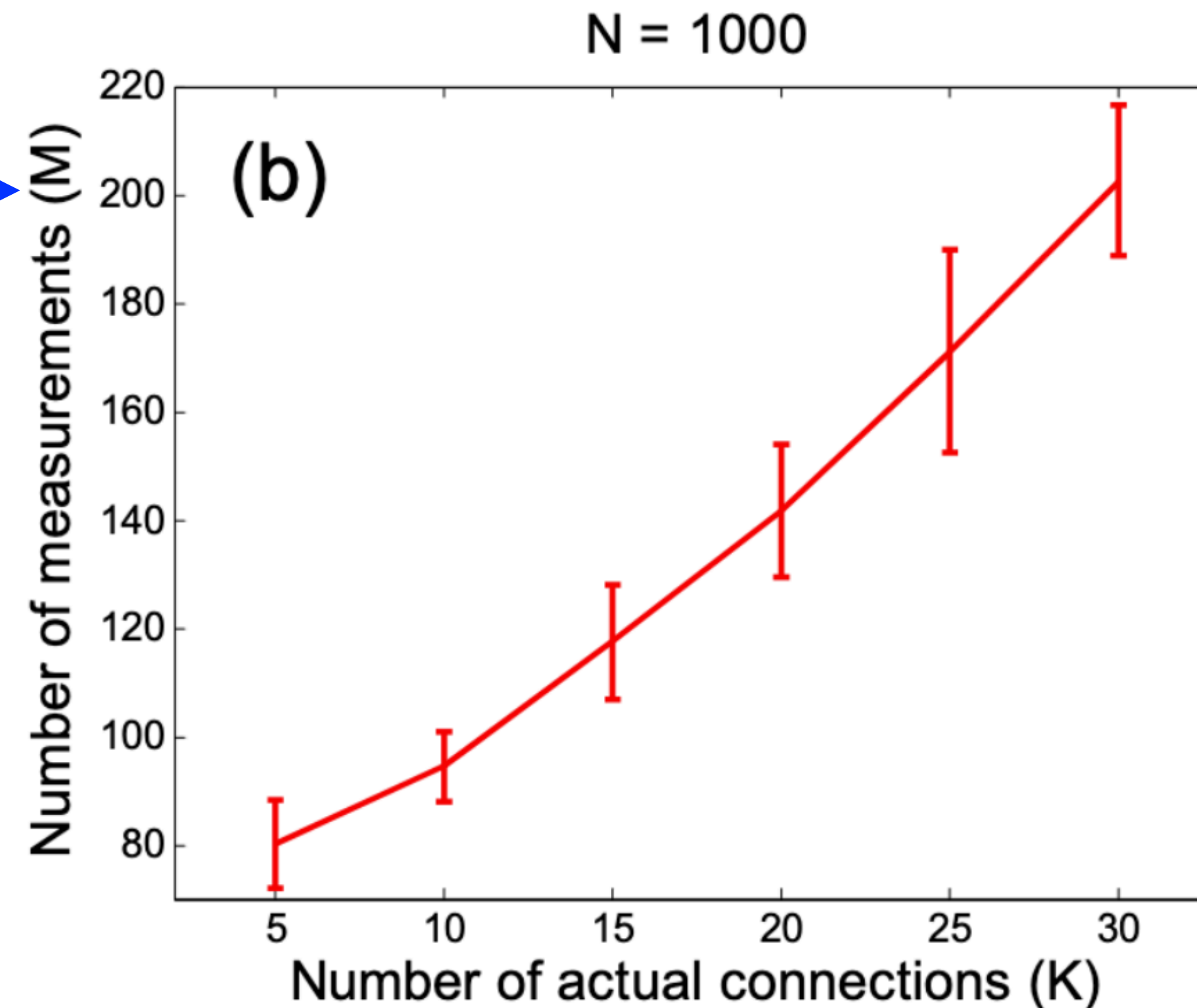
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Compressed sensing-based circuit mapping

Naive single-target stimulation
would need more than 10,000
measurements



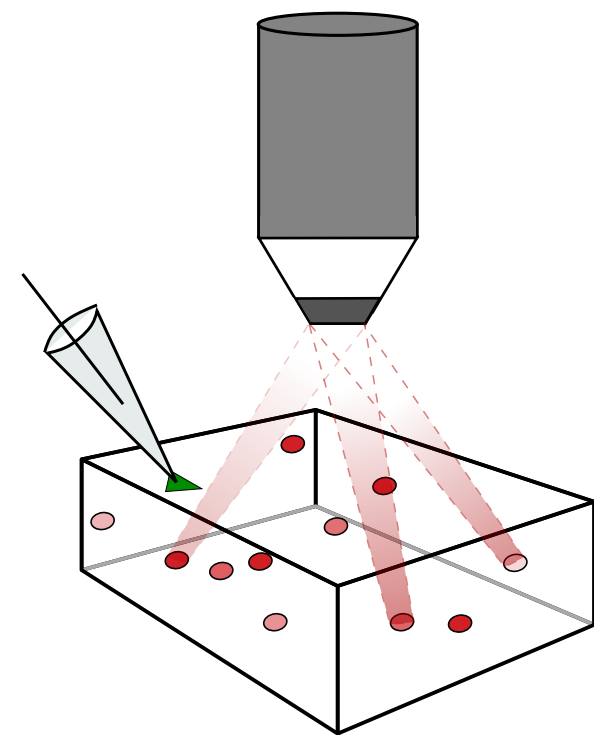
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Compressed sensing-based circuit mapping

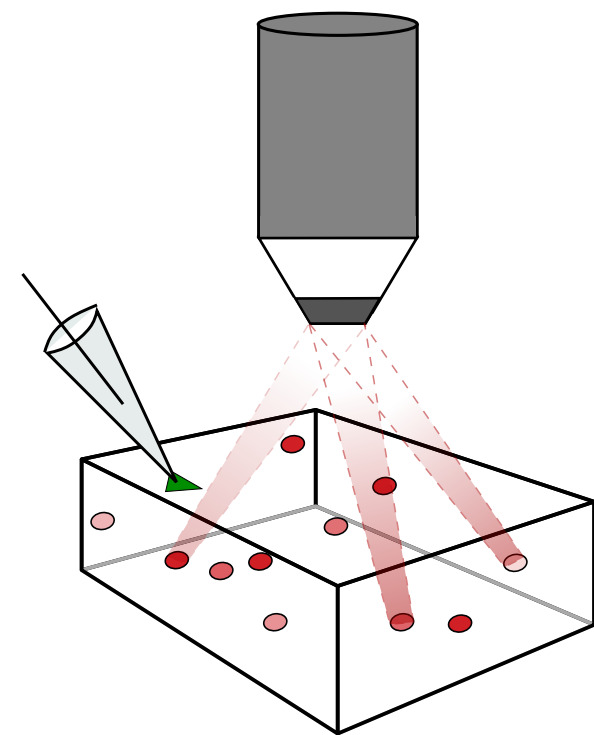
Randomized two-photon holographic
ensemble stimulation



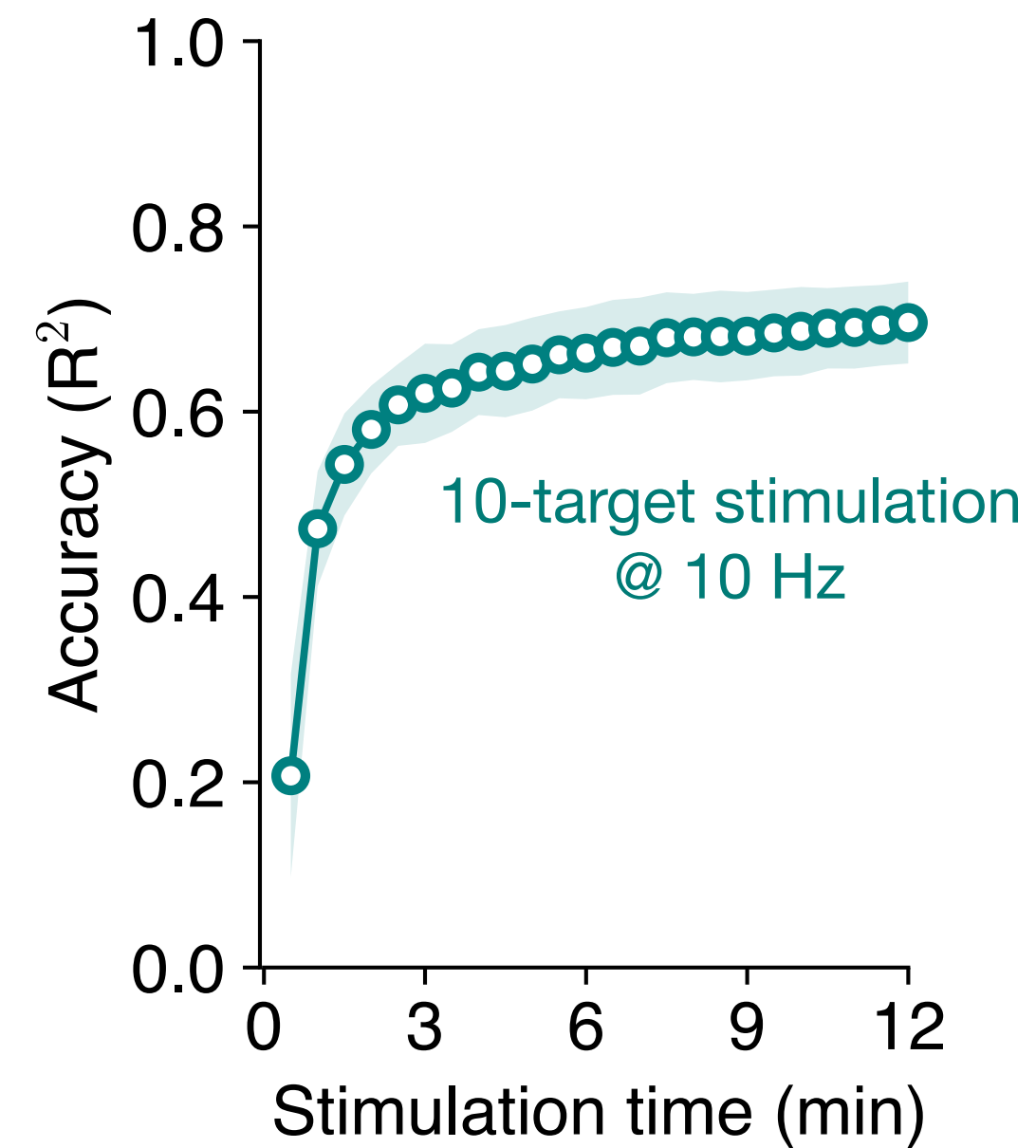
Hu and Chklovskii (2009), NeurlPS
See also Xue et al (2022), eLife for 1p

Compressed sensing-based circuit mapping

Randomized two-photon holographic ensemble stimulation



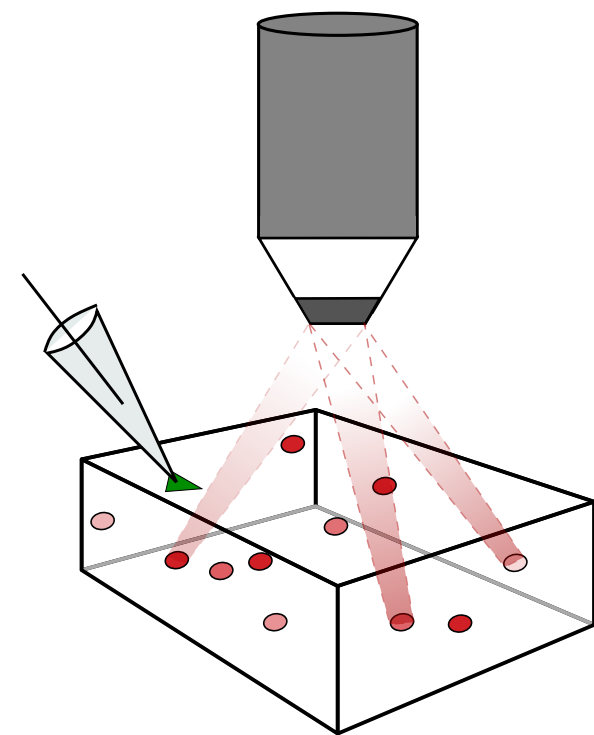
“Vanilla” compressed sensing
(simulation)



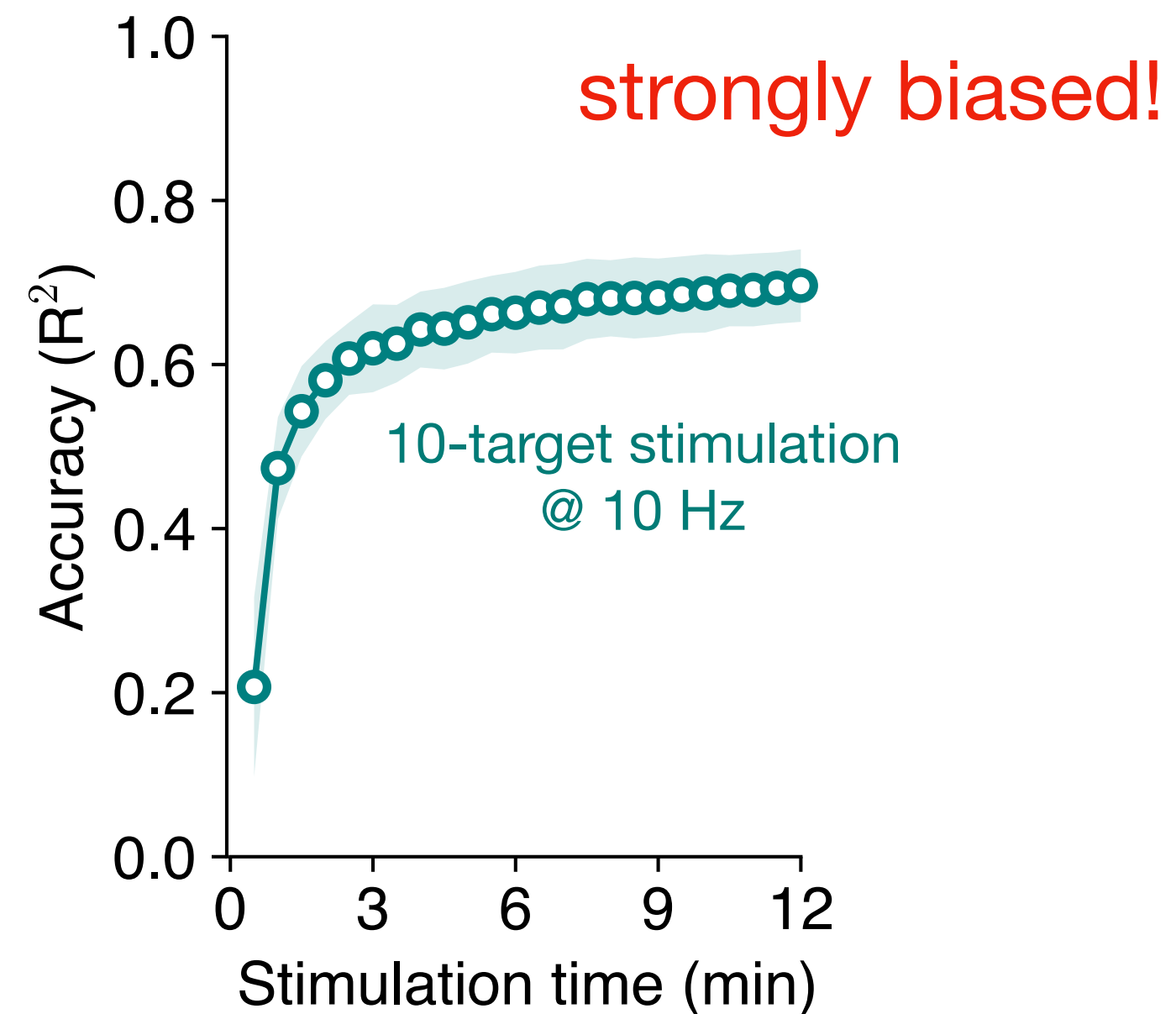
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Compressed sensing-based circuit mapping

Randomized two-photon holographic ensemble stimulation



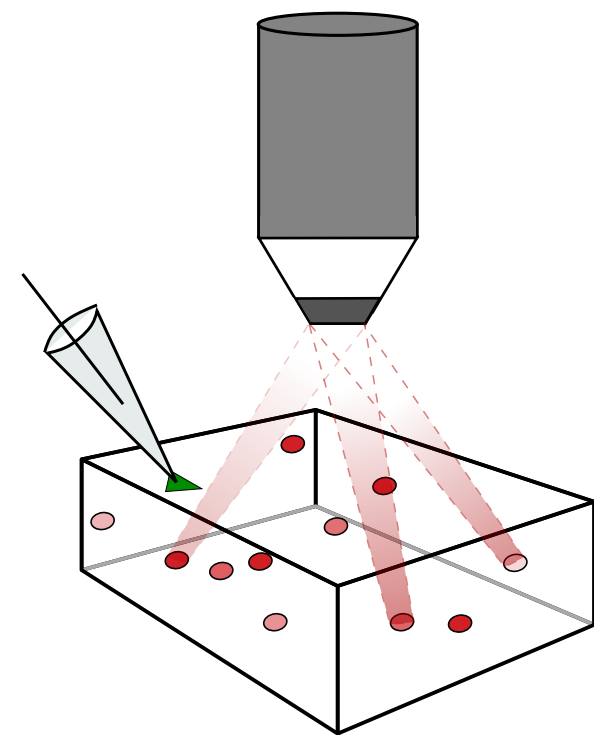
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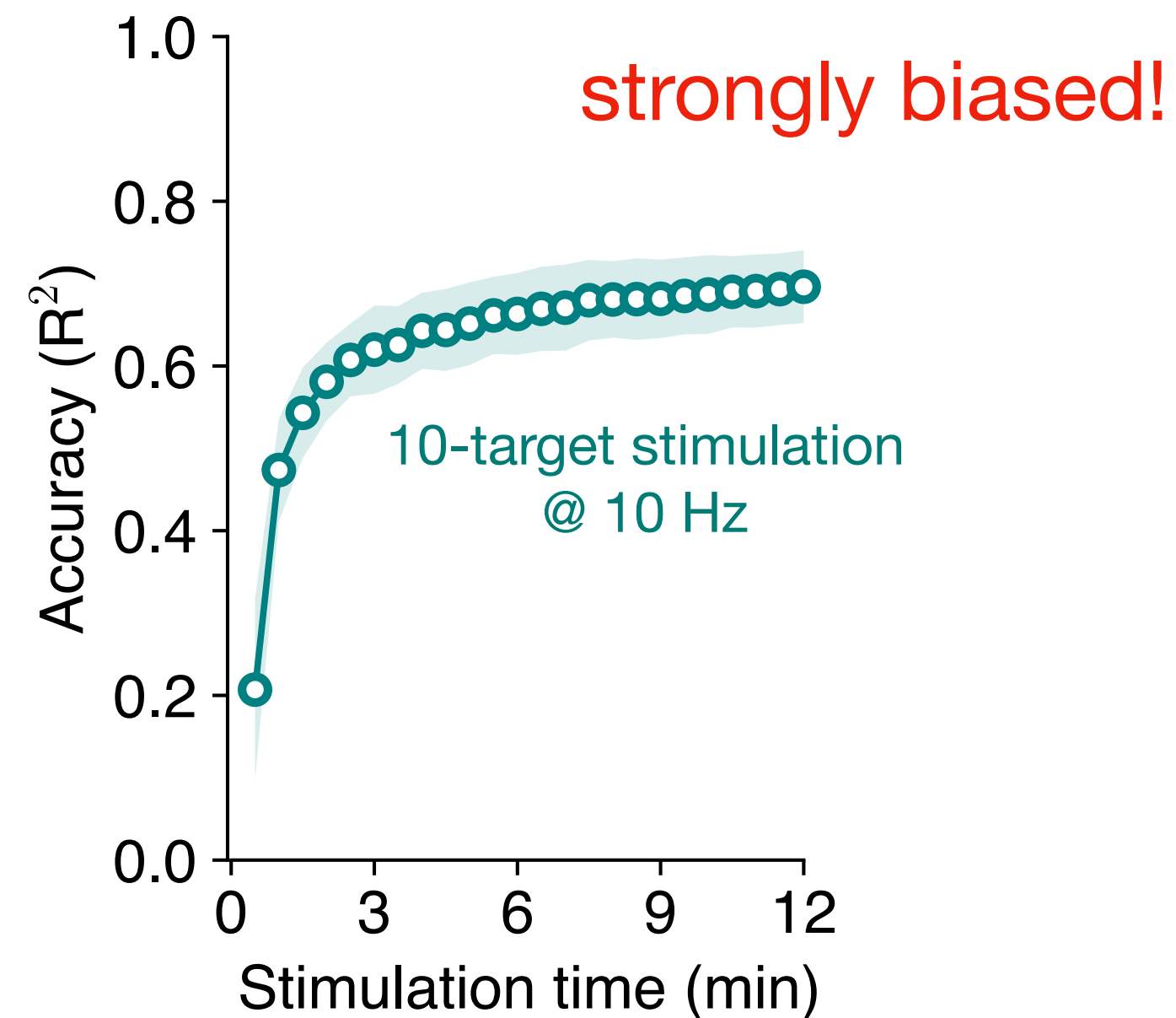
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Compressed sensing-based circuit mapping

Randomized two-photon holographic ensemble stimulation



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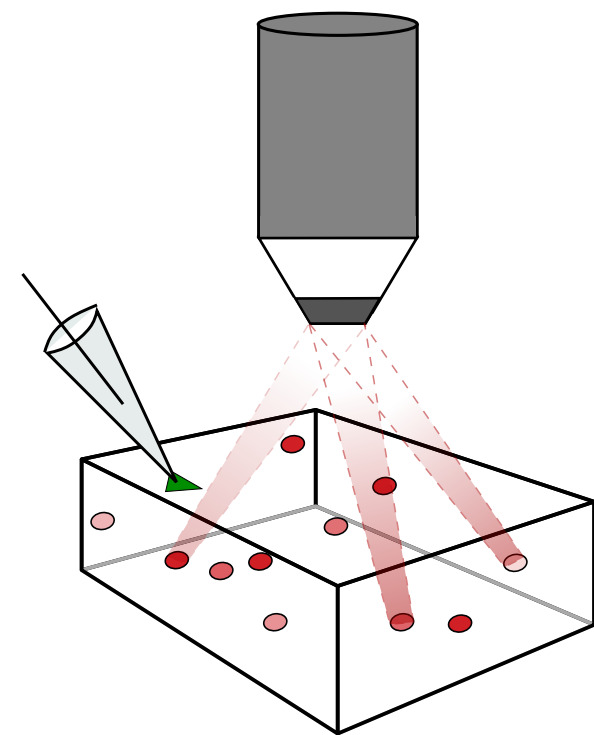


Sources of variability

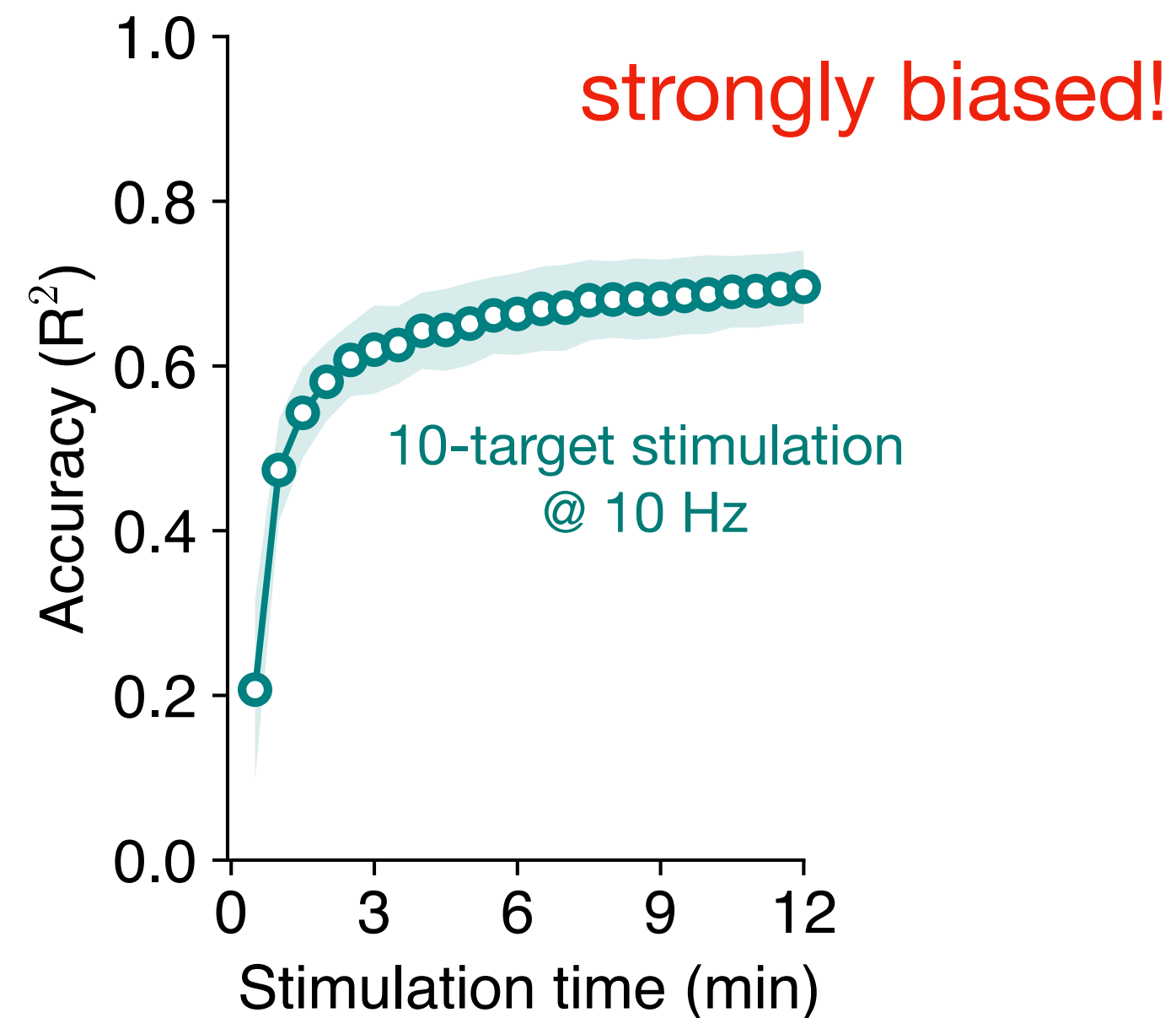
- Power dependence
- Opsin expression
- Synaptic failures
- Spontaneous activity

Compressed sensing-based circuit mapping

Randomized two-photon holographic ensemble stimulation



“Vanilla” compressed sensing
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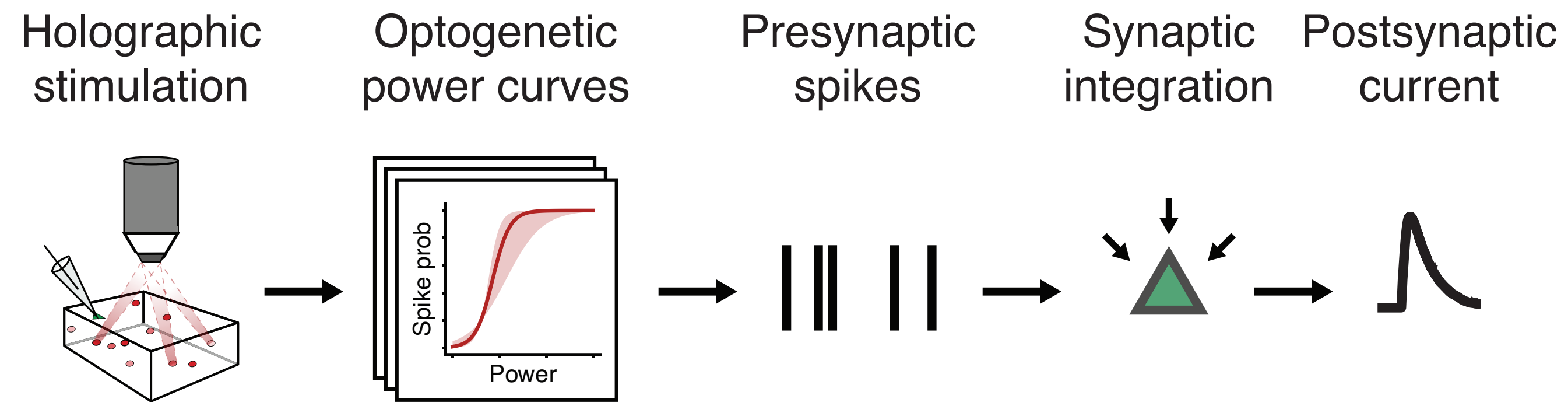
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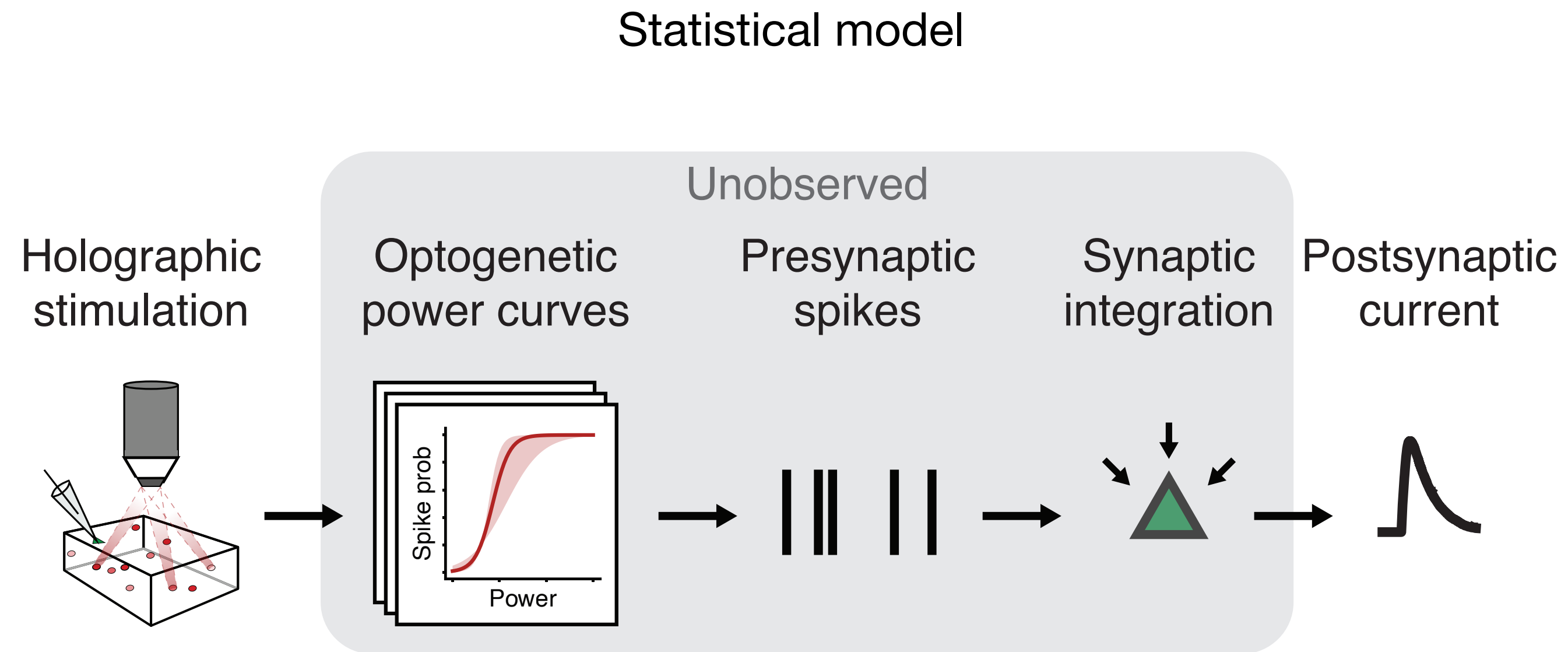
build these into model/
algorithm?

“Model-based” compressed sensing

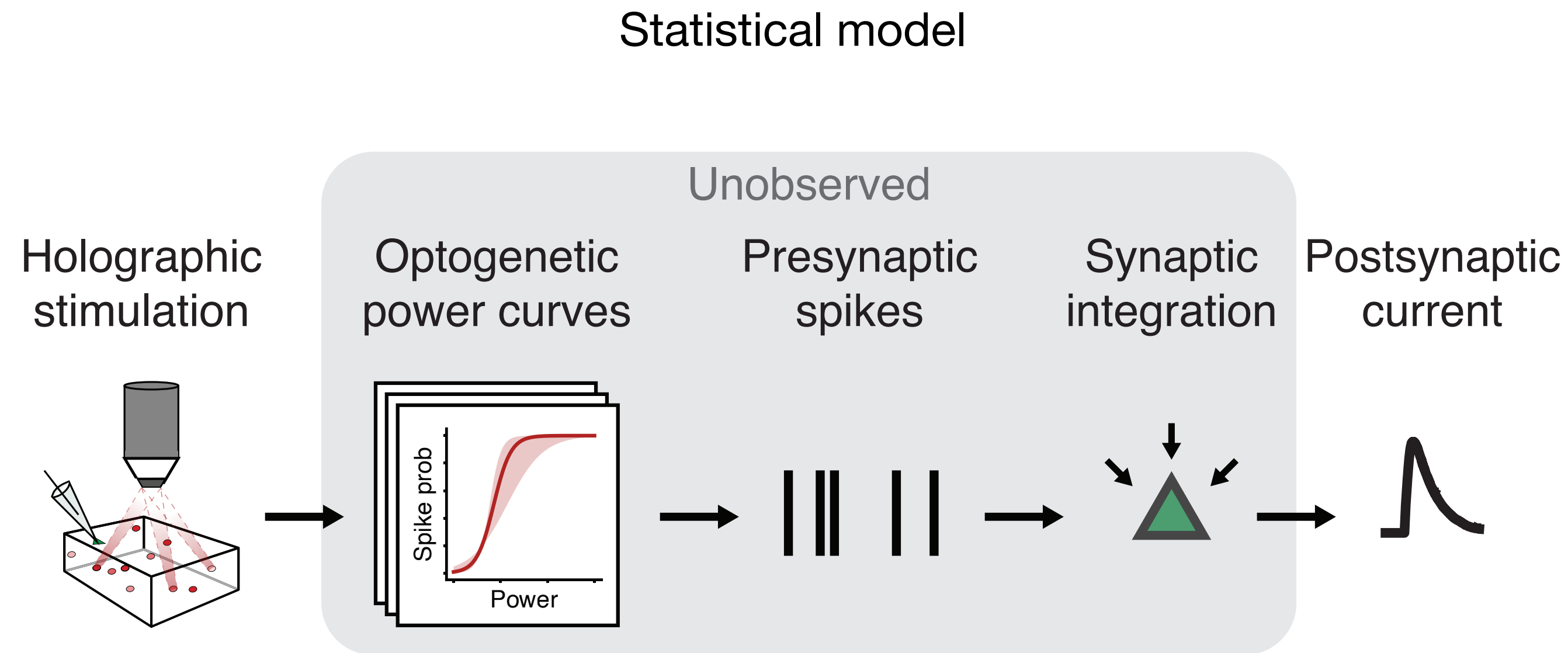
Statistical model



“Model-based” compressed sensing



“Model-based” compressed sensing



Variables to be inferred:

- Synaptic weights
- Presynaptic spikes
- Optogenetic “power curves”
- Electrical noise/PSC variability
- Spontaneous PSCs

Inference

- Many ways to perform inference (e.g., maximum likelihood, MCMC).
- We make a “variational” approximation to the posterior distribution

$$q(\mathbf{Z} \mid \phi) \approx p(\mathbf{Z} \mid \mathbf{y}, \mathbf{x})$$

for latent vars $\mathbf{Z}$.

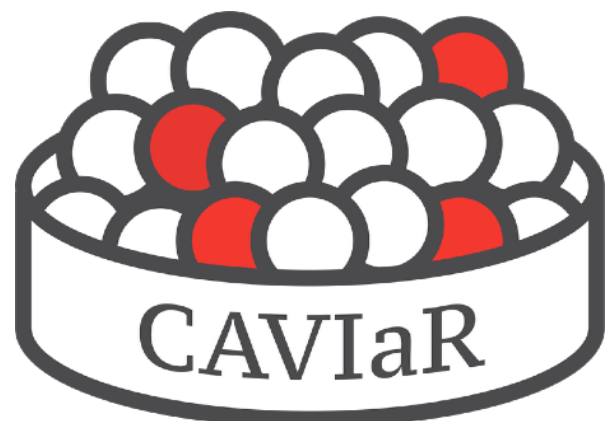
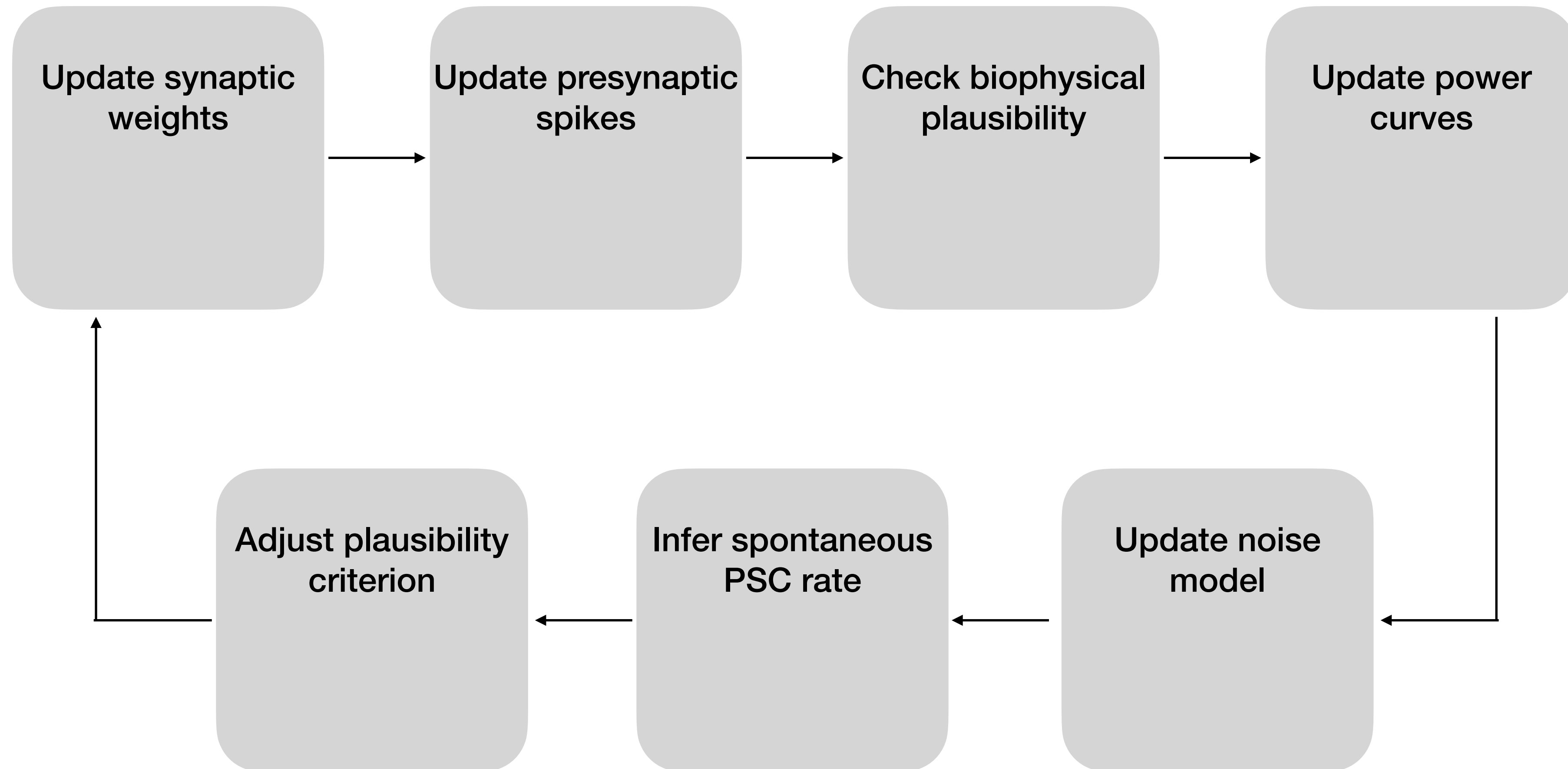
- Assume posterior factorises into a product of simple distributions

$$q(\mathbf{Z} \mid \phi) = \prod_j q(\mathbf{z}_j \mid \phi_j)$$

- Then perform tractable updates of each factor coordinate-wise to make approximation as accurate as possible (e.g., in terms of KL-divergence).

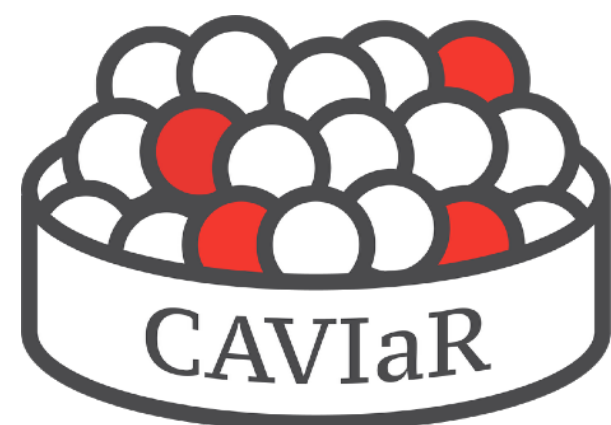
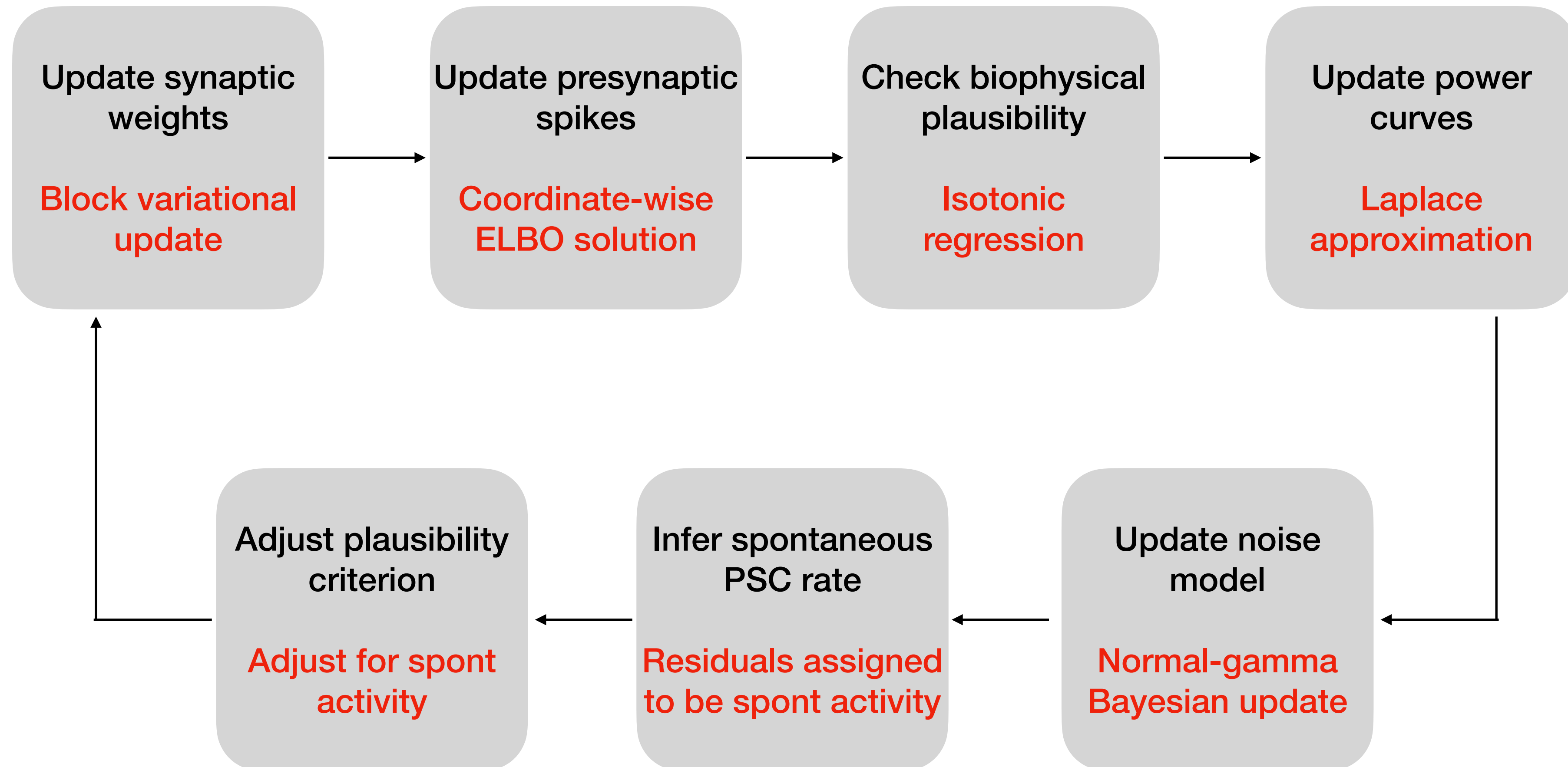
CAVIaR

(coordinate-ascent variational inference and isotonic regularisation)



CAVIaR

(coordinate-ascent variational inference and isotonic regularisation)



CAVlaR

(coordinate-ascent variational inference and isotonic regularisation)

Algorithm 1: Coordinate-ascent variational inference and isotonic regularization (CAVlaR)

input: PSC traces $\mathbf{c}$, stimulus information $\mathcal{I}$, PAVA threshold θ_{PAVA} , spontaneous penalty backtracking scalar α , soft orthogonality threshold θ_{orthog} , minimal test statistic τ_{min} , number of iterations $iters$

```

1 initialise  $\lambda_{nk} \leftarrow 1$  for all  $n, k$  such that  $I_{nk} > 0$  and  $\tau_{\text{test}}(\mathbf{c}_k) \geq \tau_{\text{min}}$ 
2  $\lambda_{\text{spont}} \leftarrow 0$  // initialize spontaneous rate to 0
3  $i \leftarrow 1$ 
4 while  $i \leq iters$  do
5   update  $q(\mathbf{w} \mid \boldsymbol{\mu}, \boldsymbol{\Omega}) \propto \exp \mathbb{E}_{q(\mathcal{Z} \setminus \mathbf{w})} [\ln p(\mathbf{y}, \mathcal{Z} \mid \mathcal{I})]$  // variational solution for synaptic weights
6   for  $n = 1, \dots, N$  do // infer spikes via Monte Carlo ELBO solution
7     sample  $\phi_n[m] \sim q(\phi_n \mid \boldsymbol{\nu}, \boldsymbol{\Sigma})$  for  $m = 1, \dots, M$ 
8     for  $k = 1, \dots, K$  do
9        $\lambda_{nk} \leftarrow \operatorname{argmax}_{\lambda_{nk}} \text{ELBO}(\lambda_{nk} \mid \{\phi_n[m]\}_{m=1}^M)$ 
10    end
11     $\hat{F}_n \leftarrow \text{PAVA}(\mathcal{I}, \lambda_n)$  // estimate optogenetic power curve
12    if  $\hat{F}_n(\max_k I_{nk}) < \theta_{\text{PAVA}} + \lambda_{\text{spont}}$  then // check plausibility criterion
13       $\mu_n \leftarrow 0, \lambda_n \leftarrow \mathbf{0}$ 
14    end
15  end
16  for  $n = 1, \dots, N$  do // Laplace approx of receptive field posterior
17     $\boldsymbol{\nu}_n, \boldsymbol{\Sigma}_n \leftarrow \text{RECEPTIVEFIELDLAPLACE}(\mathbf{s}_n, \phi_n, \mathbf{v}, \mathbf{L})$ 
18  end
19  update  $q(\sigma^{-2} \mid \theta_{\text{sh}}, \theta_{\text{ra}}) \propto \exp \mathbb{E}_{q(\mathcal{Z} \setminus \sigma^{-2})} [\ln p(\mathbf{y}, \mathcal{Z} \mid \mathcal{I})]$  // variational solution for noise precision
20  while  $\|\mathbf{y} - \boldsymbol{\mu}^\top \boldsymbol{\Lambda} - \mathbf{z}\|_2^2 / \|\mathbf{y}\|_2^2 \geq \epsilon$  do // begin spontaneous PSC inference
21    for  $k = 1, \dots, K$  do
22      if  $\sum_{n=1}^N \lambda_{nk} \leq \theta_{\text{orthog}}$  and  $\tau_{\text{test}}(\mathbf{c}_k) \geq \tau_{\text{min}}$  then
23         $z_k \leftarrow S([y_k - \boldsymbol{\mu}^\top \boldsymbol{\Lambda}_{:,k}]_+, \gamma)$  // soft-threshold residual with penalty  $\gamma$ 
24      end
25    end
26     $\gamma \leftarrow \alpha \gamma$  // shrink penalty
27  end
28   $\lambda_{\text{spont}} \leftarrow \frac{1}{K} \sum_{k=1}^K \mathbb{1}_{[z_k \neq 0]}$  // update spontaneous rate
29   $i \leftarrow i + 1$ 
30 end
31  $\boldsymbol{\mu}, \boldsymbol{\Omega}, \boldsymbol{\Lambda}, \boldsymbol{\phi}, \mathbf{z} \leftarrow \text{FNSCAN}(\boldsymbol{\mu}, \mathbf{z})$  // scan resulting spontaneous PSCs for potential false negatives

```

Algorithm 2: RECEPTIVEFIELDLAPLACE

```

1 for  $n = 1, \dots, N$  do
2   for  $t = 1, \dots, T_{\text{max}}$  do
3      $\kappa \leftarrow 1$  // Newton stepsize
4      $\Psi_n(\phi_n) = -\mathbb{E}_{q(\mathbf{s}_n \mid \lambda_n)} \left[ \sum_{k=1}^K \ln p(\mathbf{s}_{nk} \mid \phi_n, I_{nk}) + \ln p(\phi_n \mid \mathbf{v}, \mathbf{L}) \right] - \frac{1}{\alpha_{\text{barrier}}} \sum_{i=0}^1 \ln(\phi_n^i)$ 
5      $\mathbf{J} = \nabla_{\phi_n} \Psi_n, \mathbf{H} = \nabla \nabla_{\phi_n} \Psi_n$ 
6      $\mathbf{d} = \mathbf{H}_n^{-1} \mathbf{J}_n$  // search direction
7     while  $\Psi_n(\phi_n + \kappa \mathbf{d}) > \Psi_n(\phi_n) + \alpha_{\text{backtrack}} \kappa \mathbf{J}_n^\top \mathbf{d}$  do // backtrack
8        $\kappa \leftarrow \beta_{\text{backtrack}} \kappa$ 
9     end
10     $\phi_n \leftarrow \phi_n - \kappa \mathbf{d}$  // make step
11  end
12   $\boldsymbol{\nu}_n \leftarrow \phi_n$ 
13   $\boldsymbol{\Sigma}_n \leftarrow \mathbf{H}_n^{-1}$ 
14 end
15  $q(\phi \mid \boldsymbol{\nu}_n, \boldsymbol{\Sigma}_n) = \text{TruncNormal}(\boldsymbol{\nu}_n, \boldsymbol{\Sigma}, 0, \infty)$  // truncate support to  $(0, \infty)$ 

```

Algorithm 3: FNSCAN

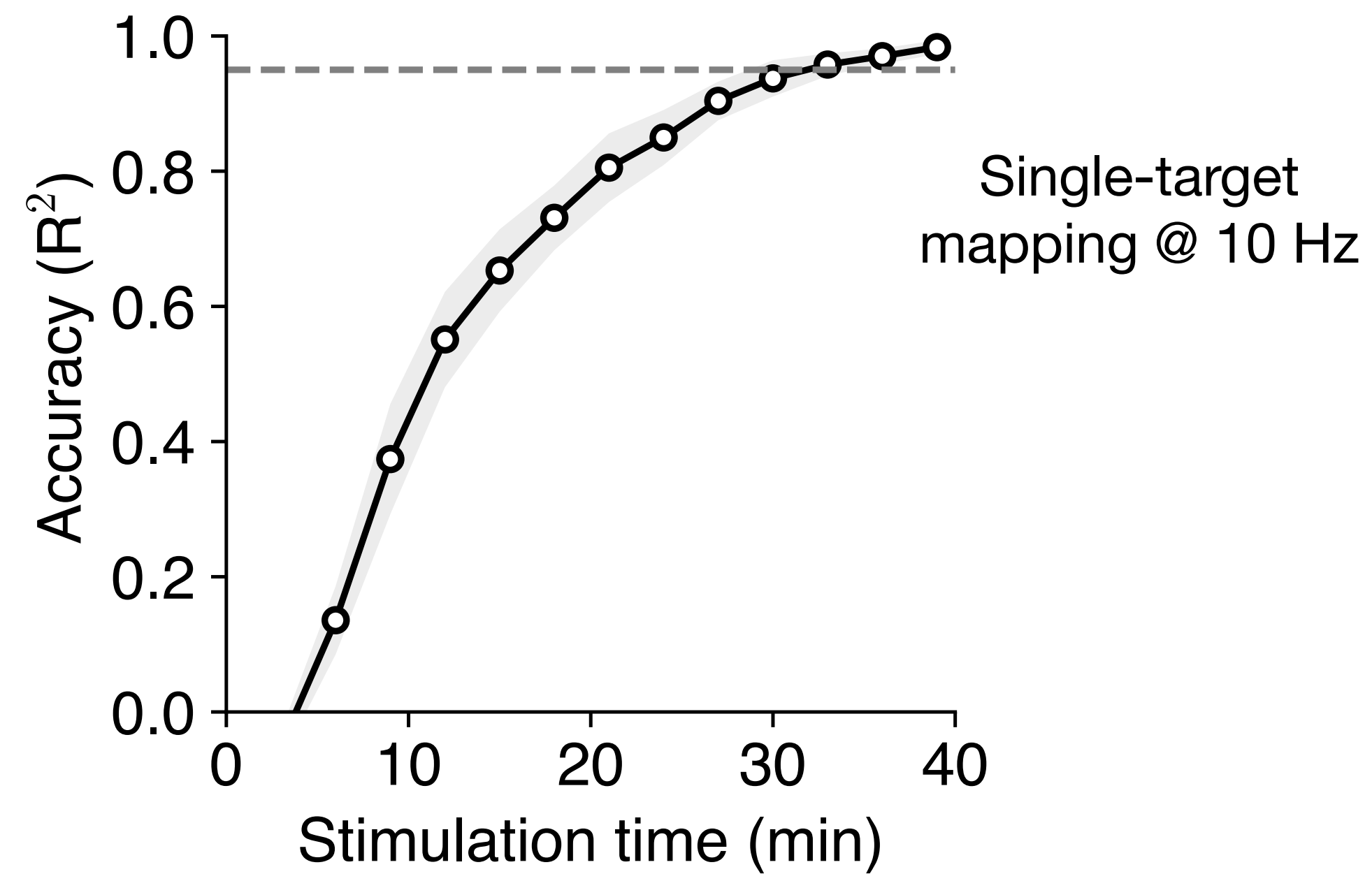
input: Synaptic weights $\boldsymbol{\mu}$, spontaneous synaptic currents $\mathbf{z}$, stimulus information $\mathcal{I}$, PAVA threshold θ_{PAVA}

```

1  $S_{\text{disc}} \leftarrow \{n \in \{1, \dots, N\} : \mu_n = 0\}$  // initialize pool of candidate neurons
2 while  $|S_{\text{disc}}| > 0$  do
3   for  $n = 1, \dots, N$  do // collect spontaneous PSC indices aligning with neuron stim times
4      $\text{spont}_n \leftarrow \{k \in \{1, \dots, K\} : z_k \neq 0 \text{ and } I_{nk} > 0\}$ 
5   end
6    $n^* \leftarrow \operatorname{argmax}_n |\text{spont}_n|$  // select neuron with most coincidental spontaneous PSCs
7    $\hat{F}_{n^*}^{\text{spont}} \leftarrow \text{PAVA}((I_{n^*k})_{k \in \text{spont}_{n^*}}, (z_k)_{k \in \text{spont}_{n^*}})$  // estimate putative power curve
8   if  $\hat{F}_{n^*}^{\text{spont}}(\max_k I_{n^*k}) \geq \theta_{\text{PAVA}}$  then
9      $\mu_{n^*} \leftarrow \text{mean}(\{z_k : k \in \text{spont}_{n^*}\})$  // neuron passes PAVA criterion, declare connected
10     $\beta_{n^*} \leftarrow \text{s.e.m.}(\{z_k : k \in \text{spont}_{n^*}\})$ 
11    for  $k \in \text{spont}_{n^*}$  do
12       $\lambda_{n^*k} \leftarrow 1$  // declare spontaneous PSC to be spike from neuron  $n^*$ 
13       $z_k \leftarrow 0$  // remove spontaneous PSC from vector  $\mathbf{z}$ 
14    end
15  end
16   $S_{\text{disc}} \leftarrow S_{\text{disc}} \setminus \{n^*\}$  // remove  $n^*$  from pool of disconnected neurons
17 end

```

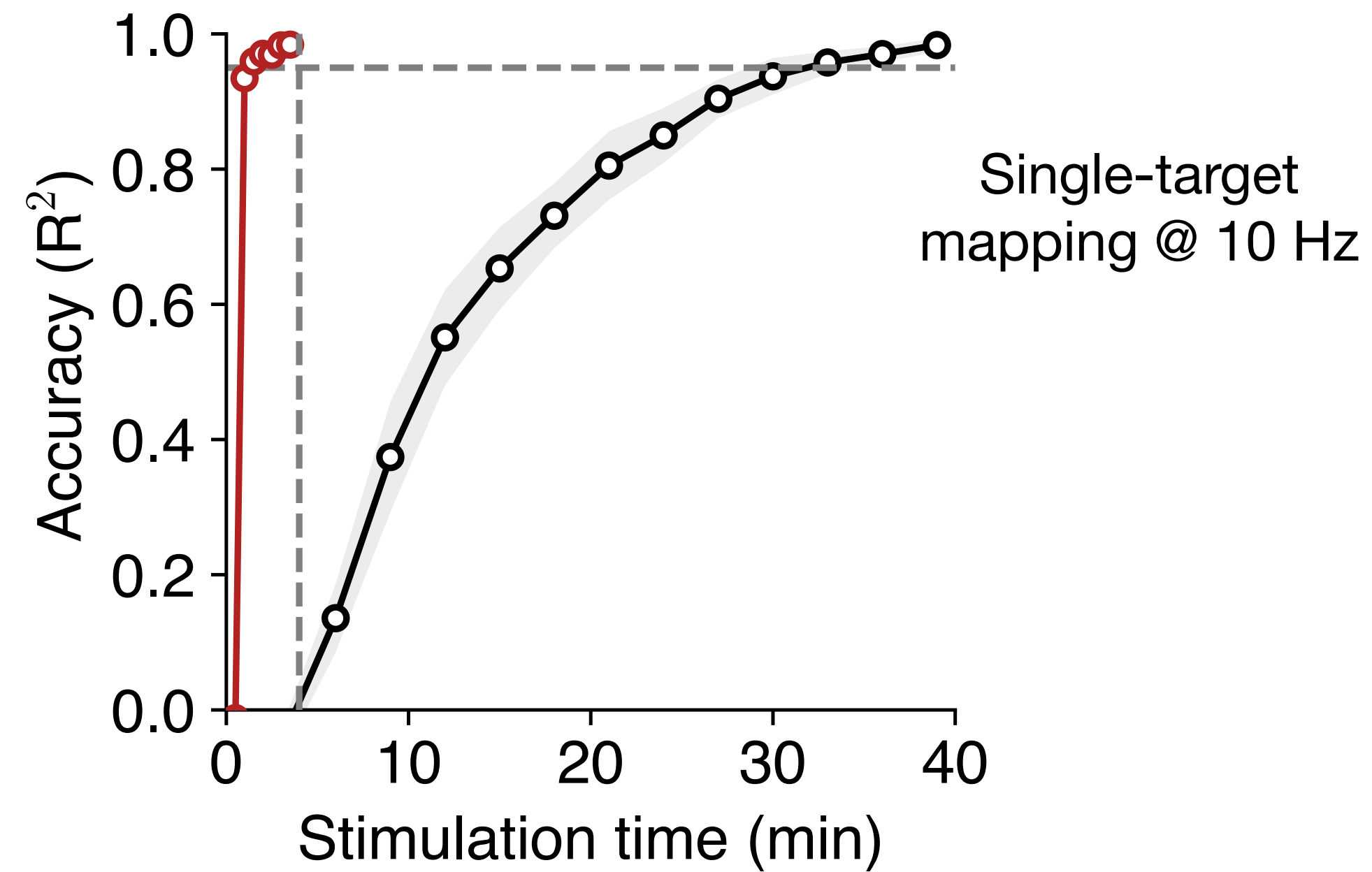
Order-of-magnitude mapping speedup



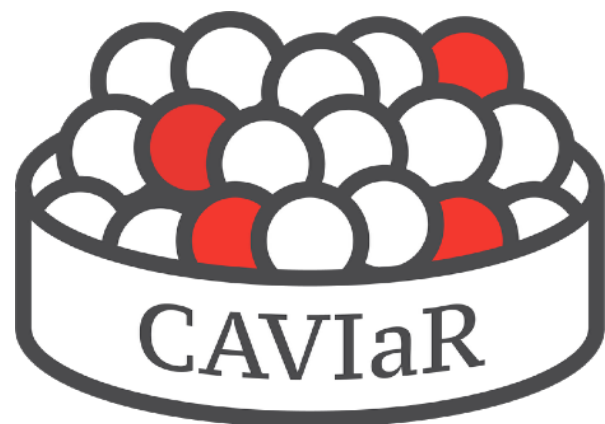
Simulation: 1000 neurons, 10% connectivity

Order-of-magnitude mapping speedup

Model-based compressive mapping
(20 targets @ 50 Hz, demixed)



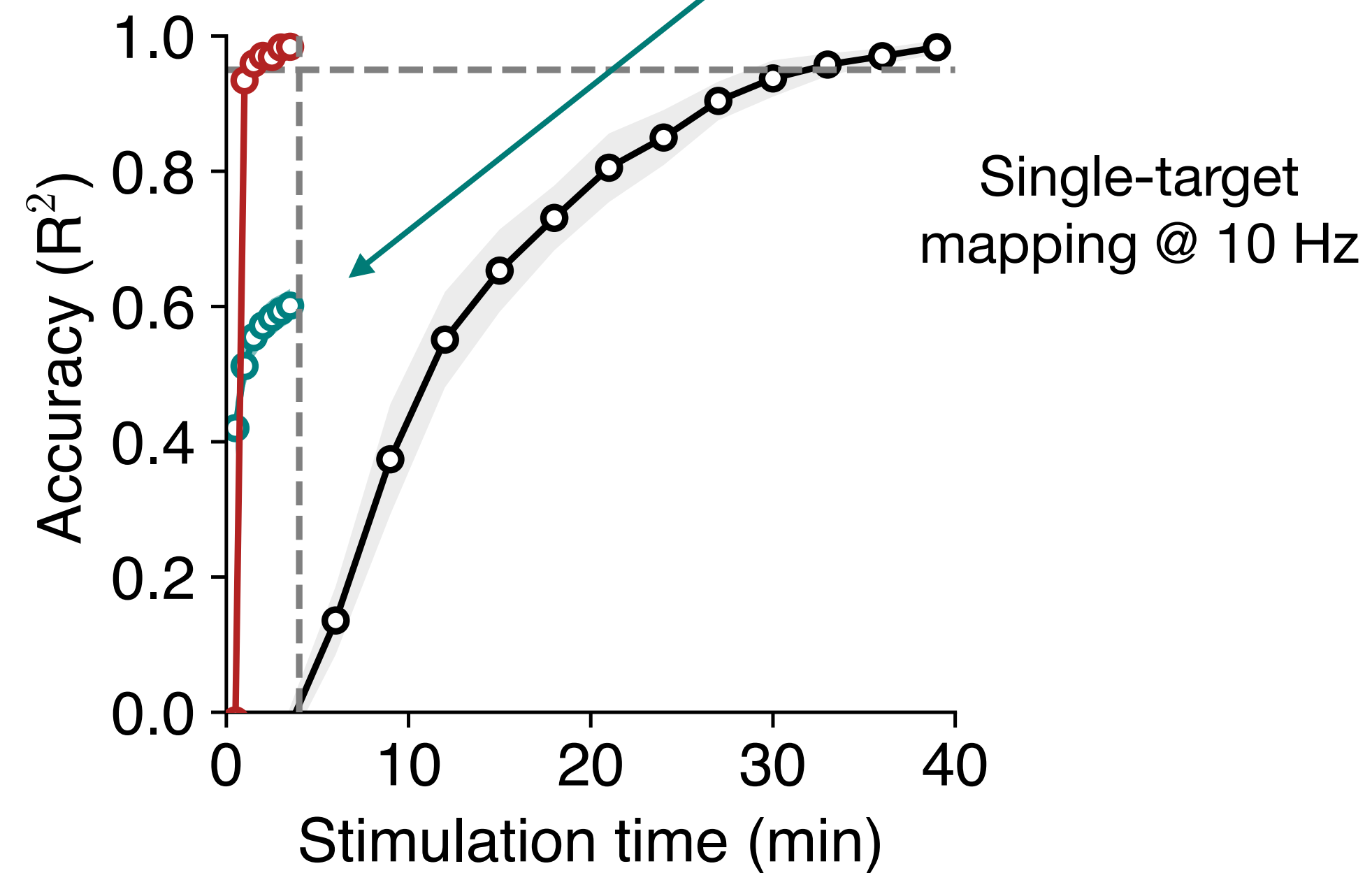
Simulation: 1000 neurons, 10% connectivity



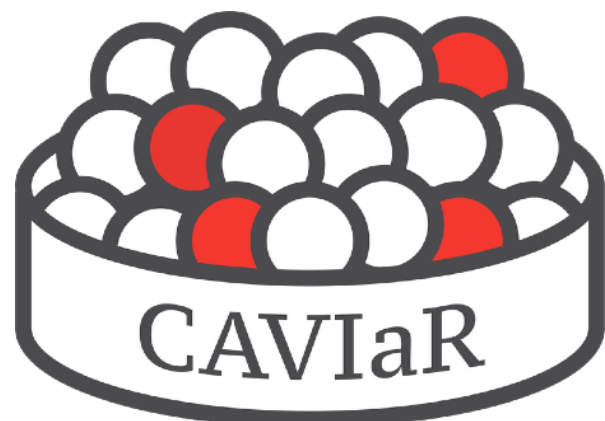
Order-of-magnitude mapping speedup

Model-based compressive mapping
(20 targets @ 50 Hz, demixed)

Vanilla compressed sensing
(20 targets @ 50 Hz, demixed)



Simulation: 1000 neurons, 10% connectivity



Experimental implementation

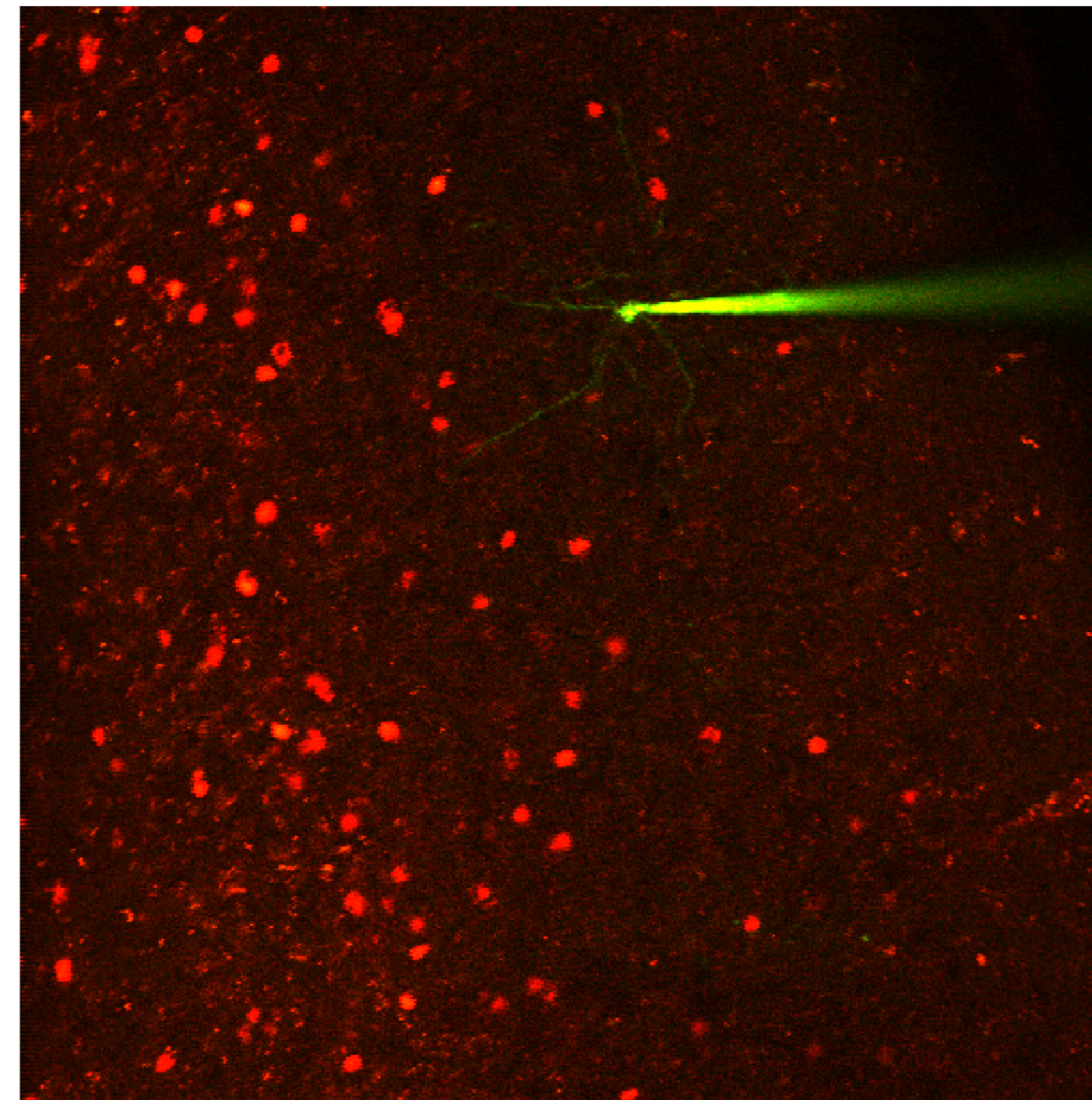
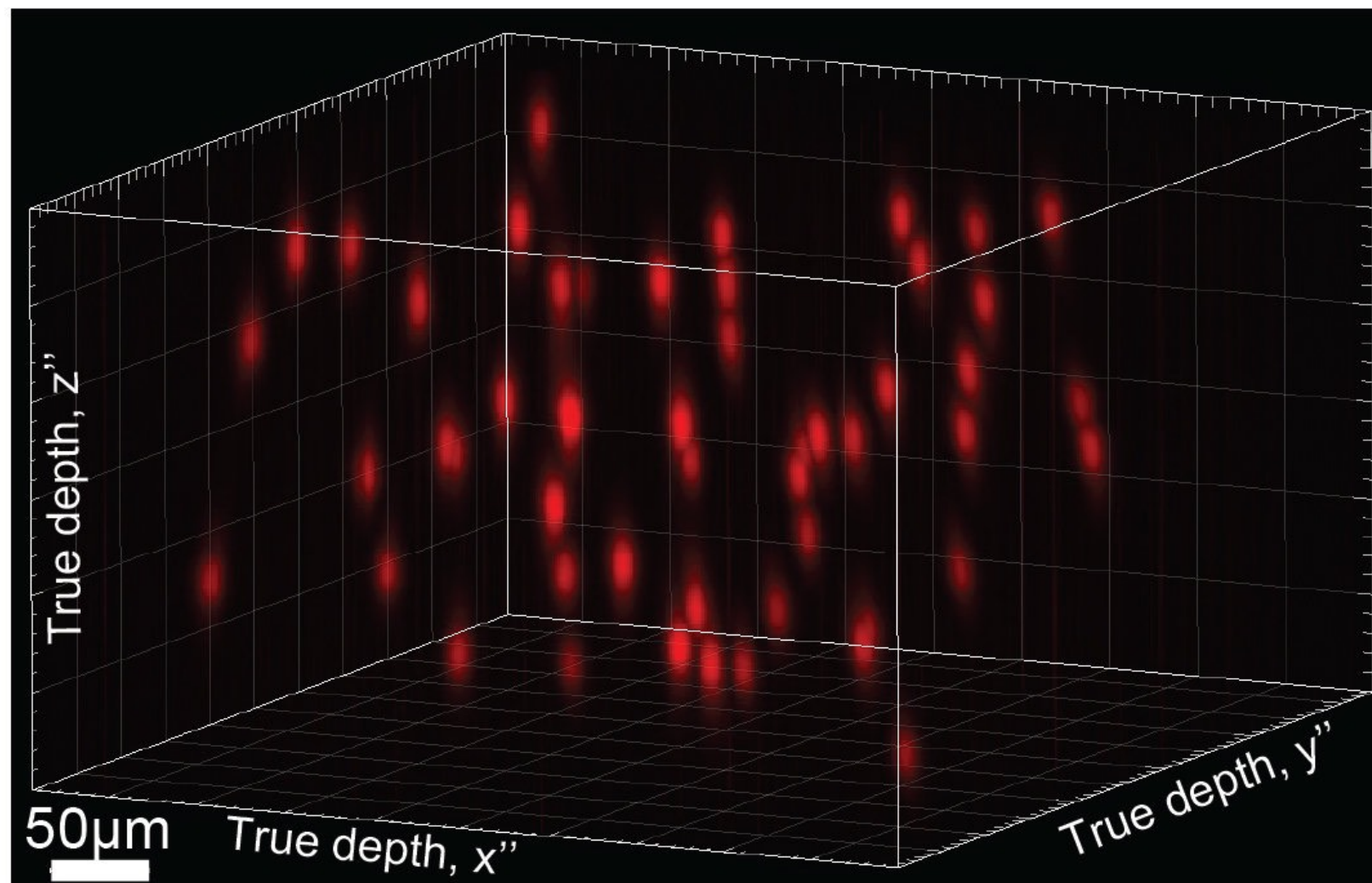
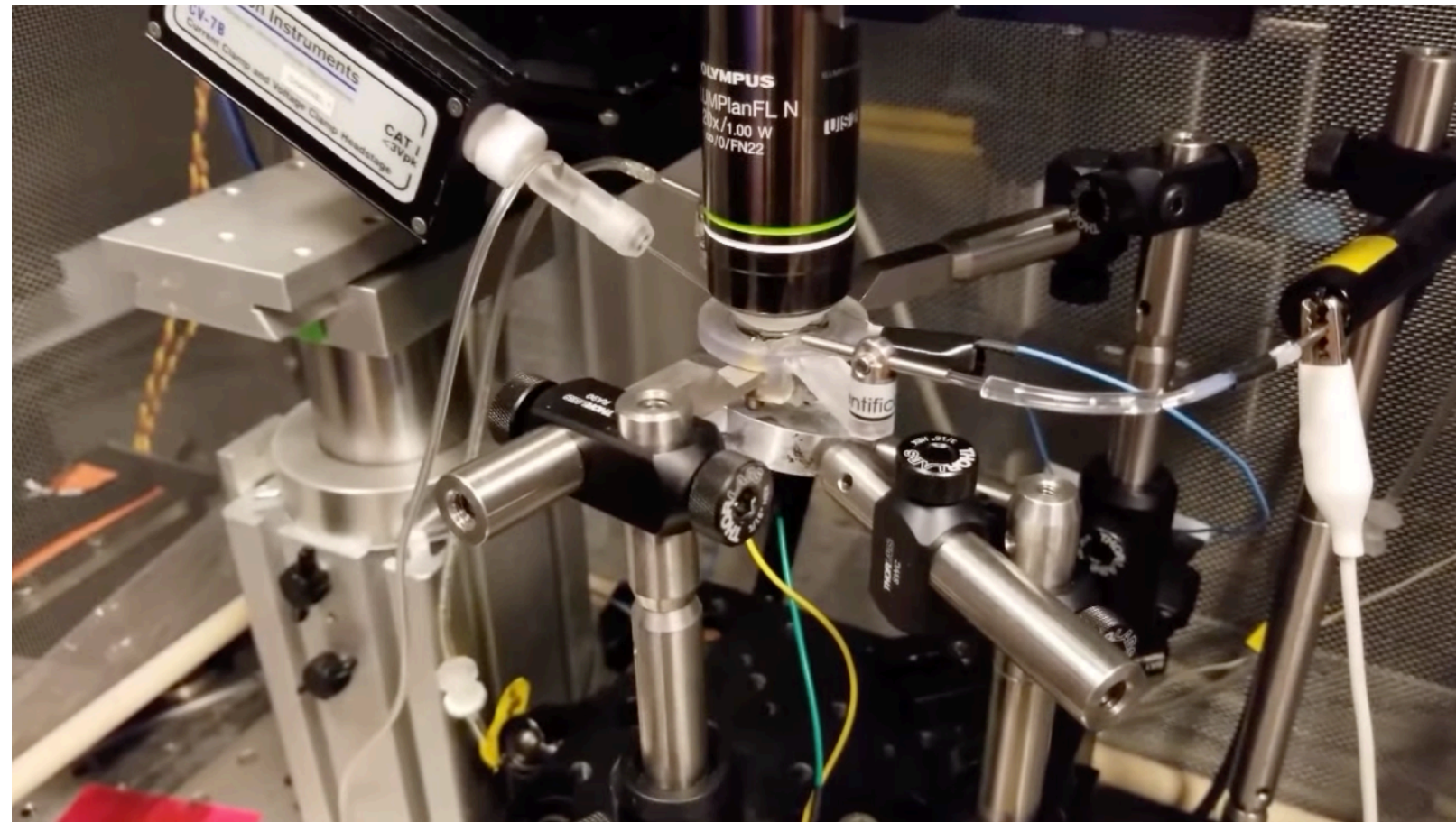
Marta Gajowa (UCB)



Hillel Adesnik (UCB)



Experimental implementation



680x680x100 μm^3 volume
(5 planes)

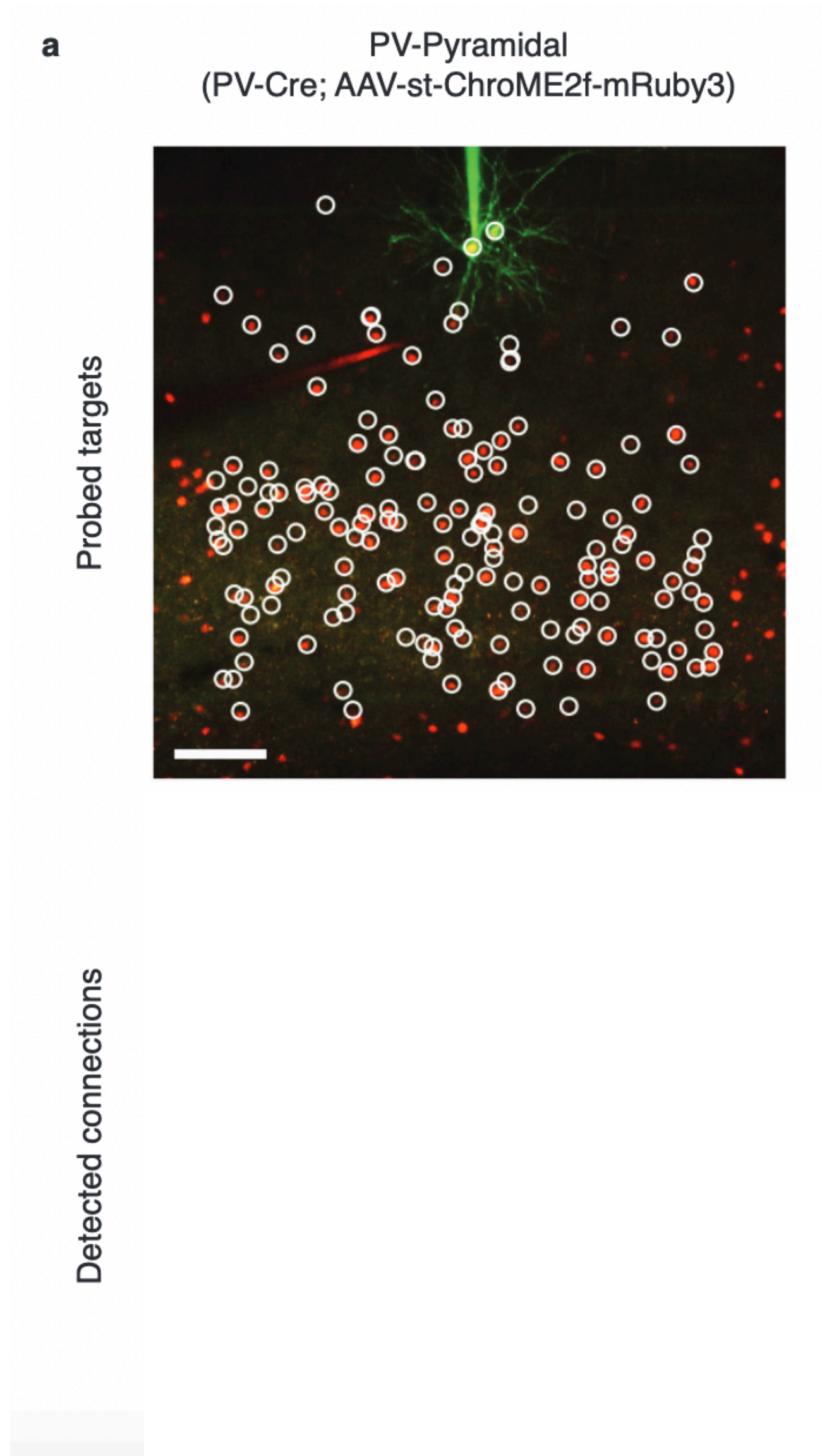
Marta Gajowa (UCB)



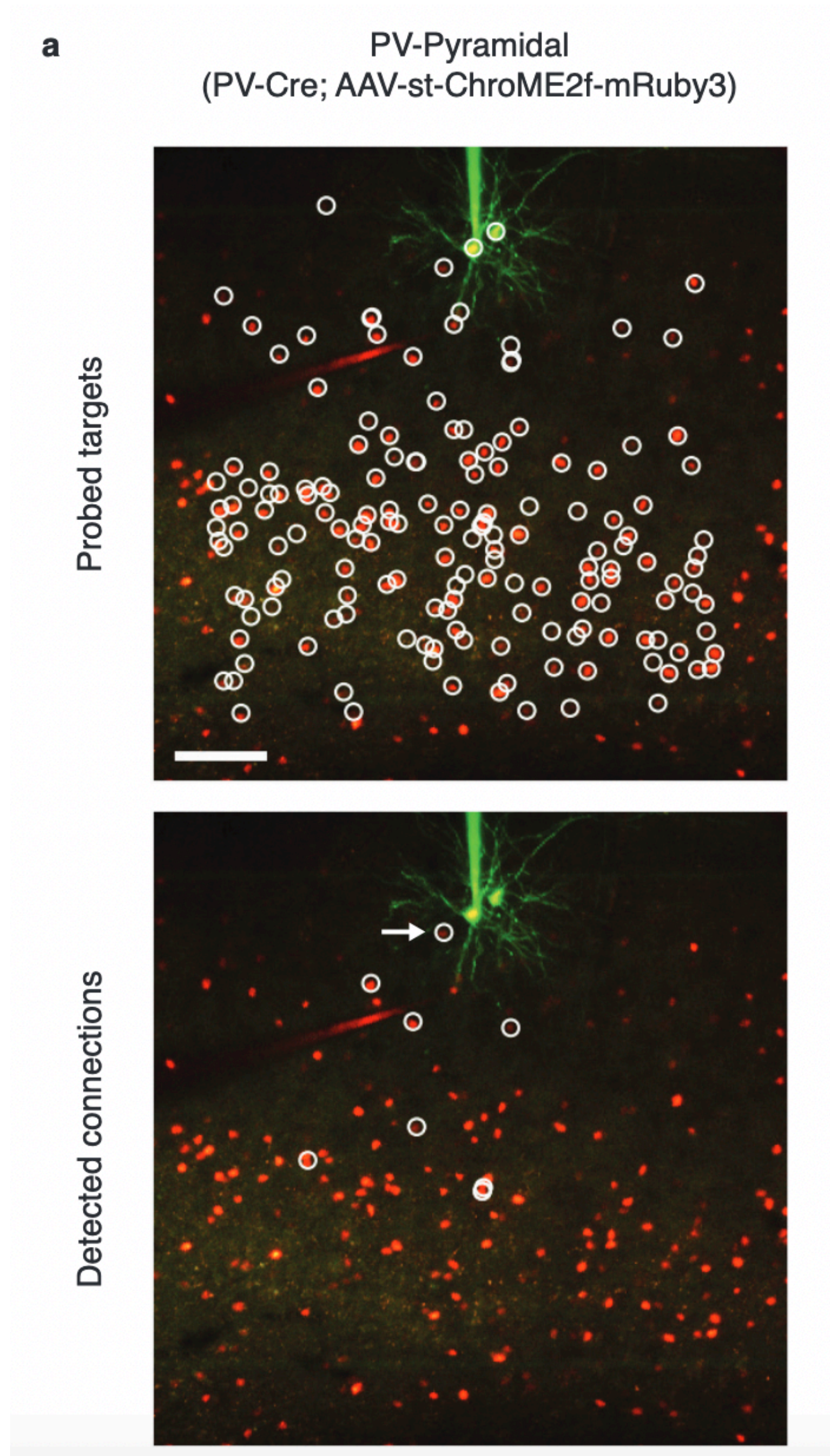
Hillel Adesnik (UCB)



Experimental implementation



Experimental implementation



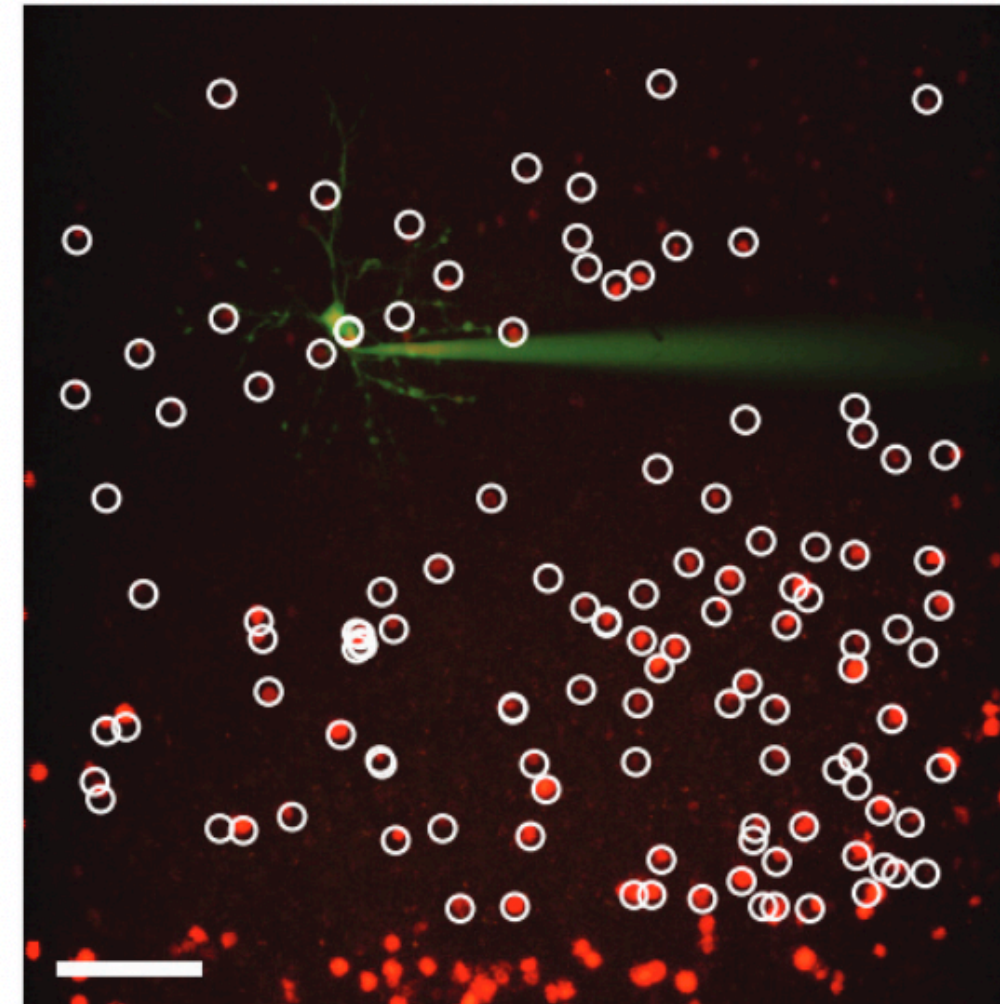
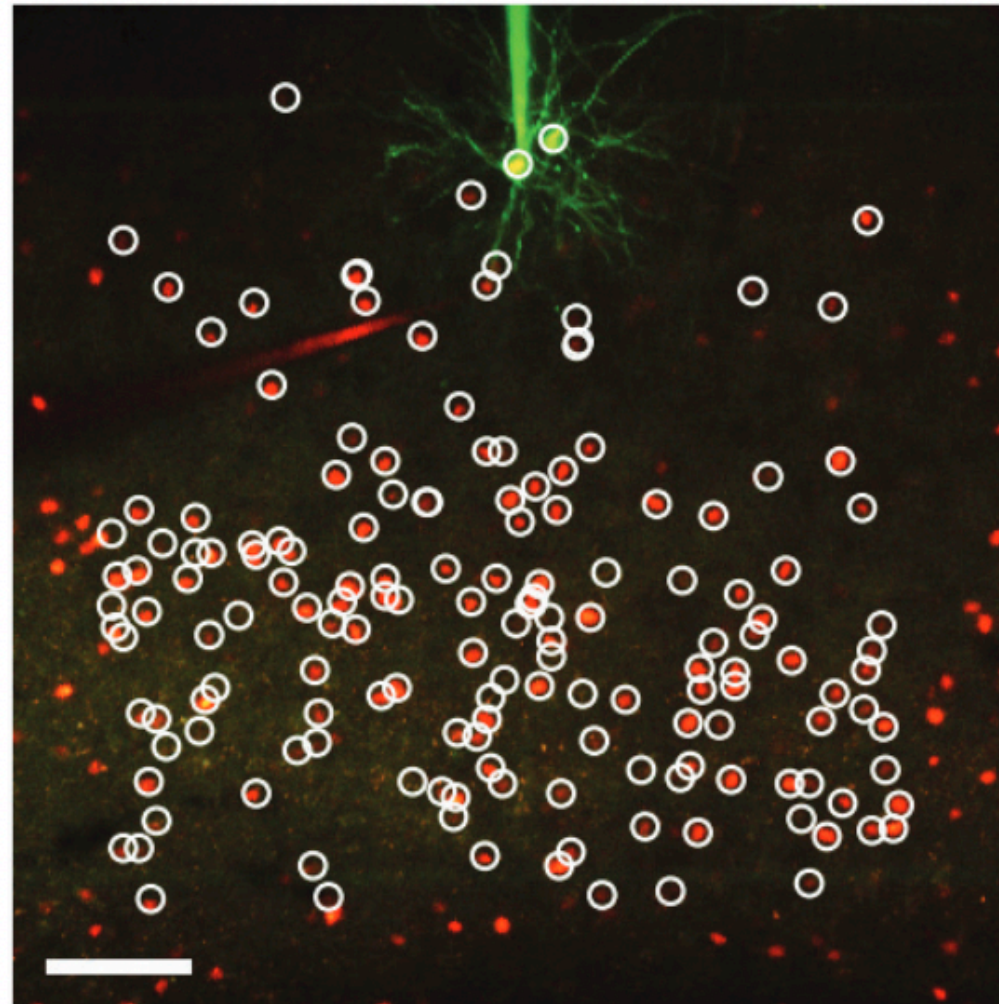
Experimental implementation

a

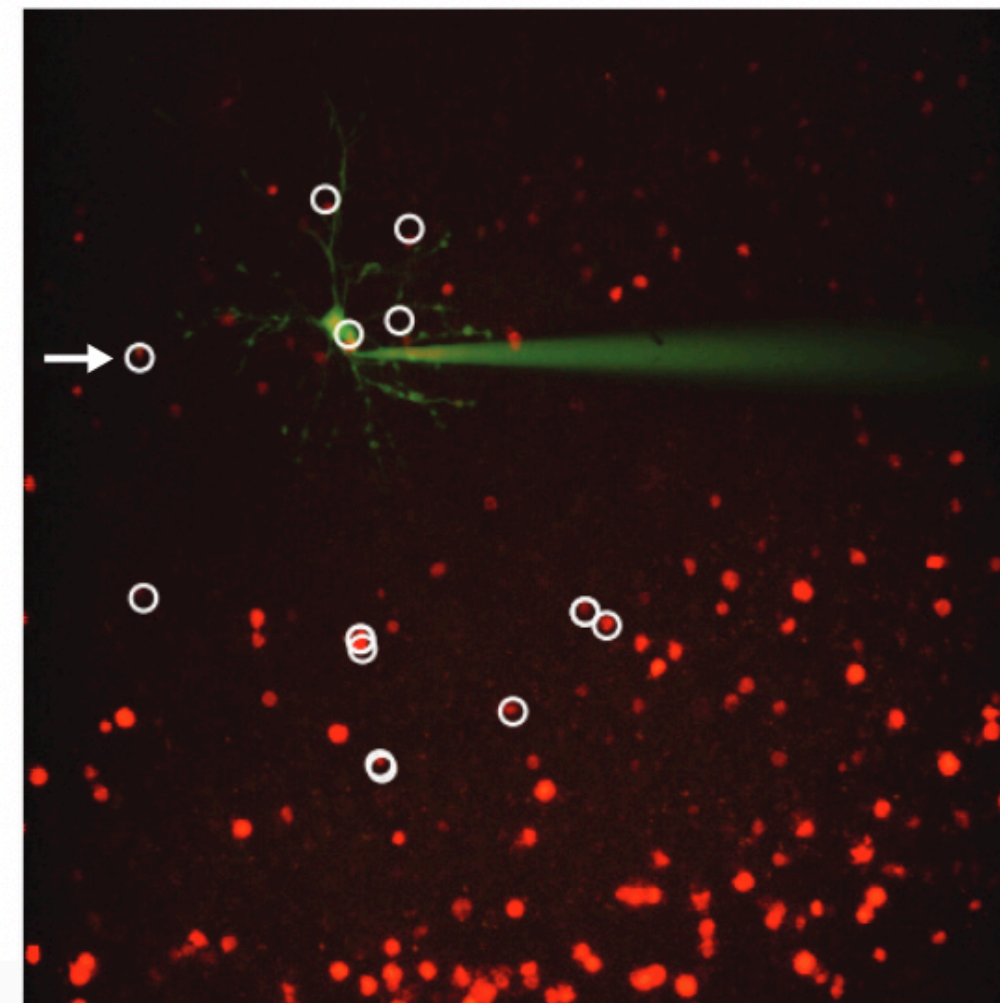
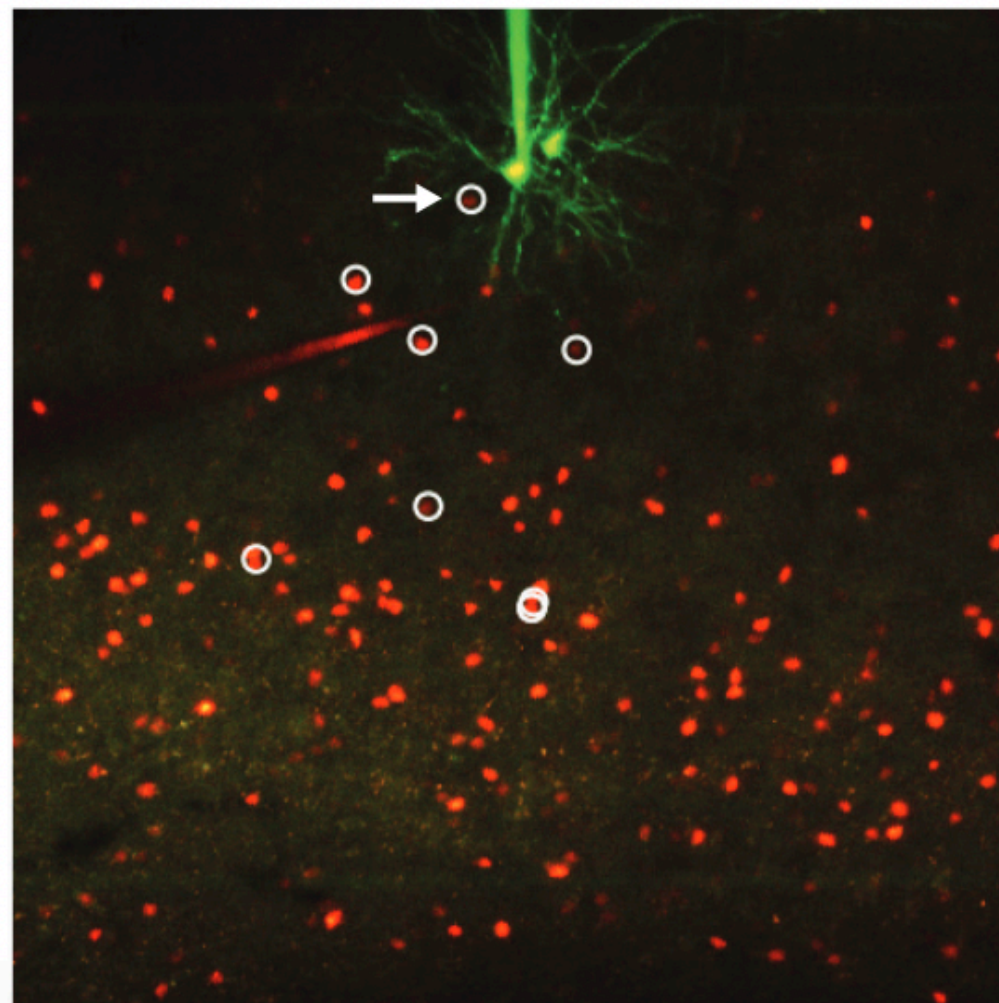
PV-Pyramidal
(PV-Cre; AAV-st-ChroME2f-mRuby3)

SST-Pyramidal
(SST-Cre; AAV-st-ChroME2f-mRuby3)

Probed targets



Detected connections

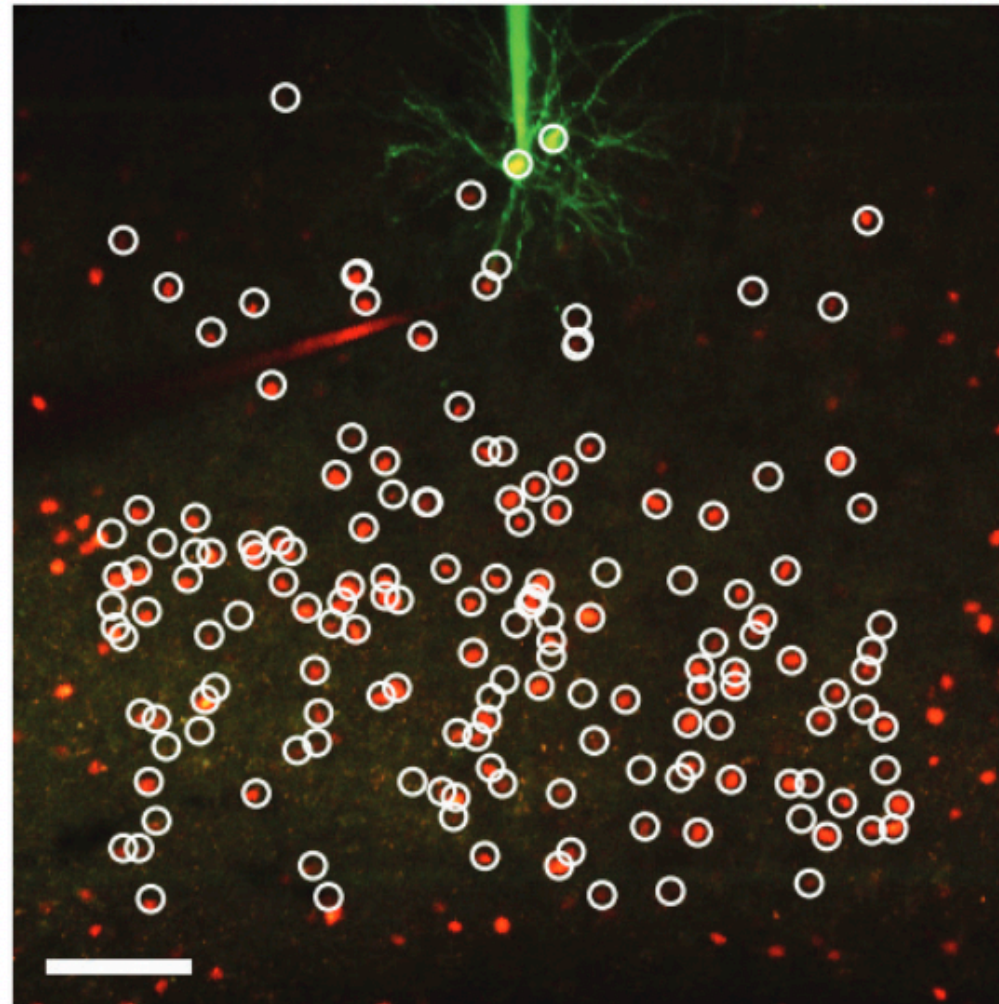


Experimental implementation

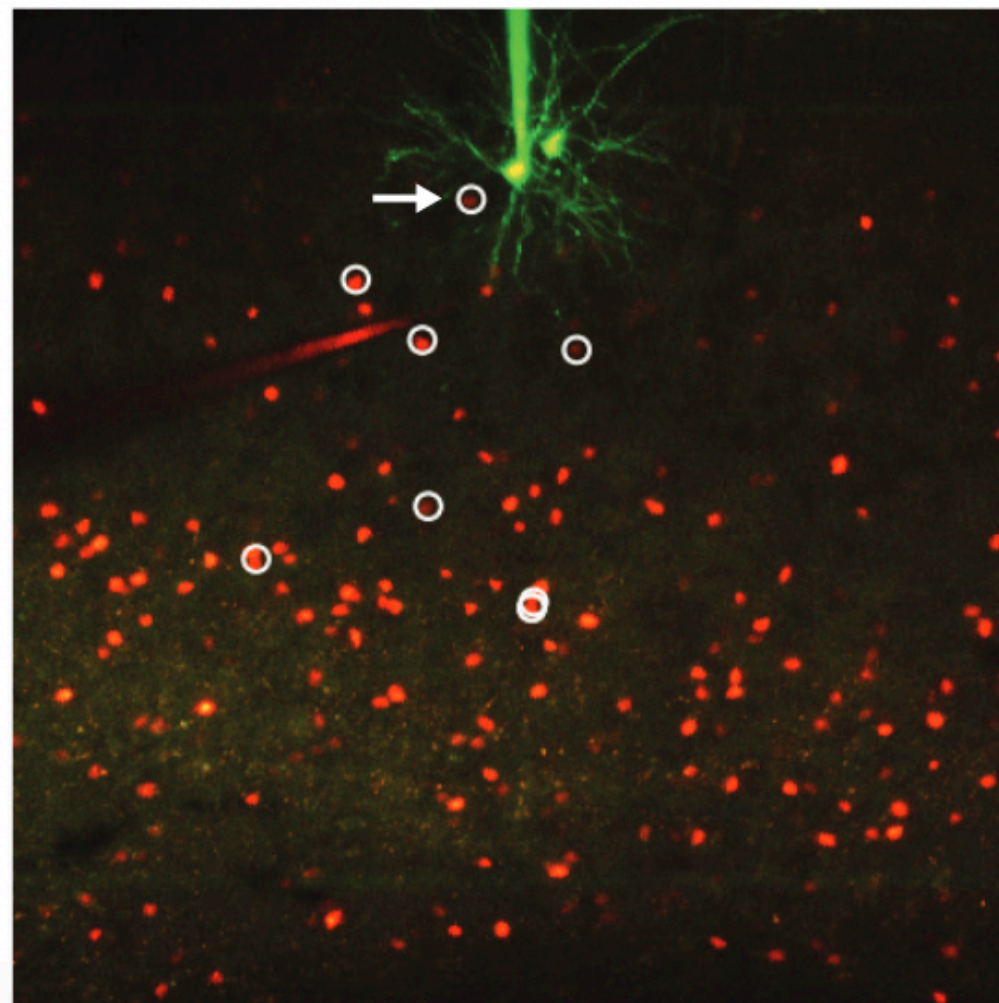
a

PV-Pyramidal
(PV-Cre; AAV-st-ChroME2f-mRuby3)

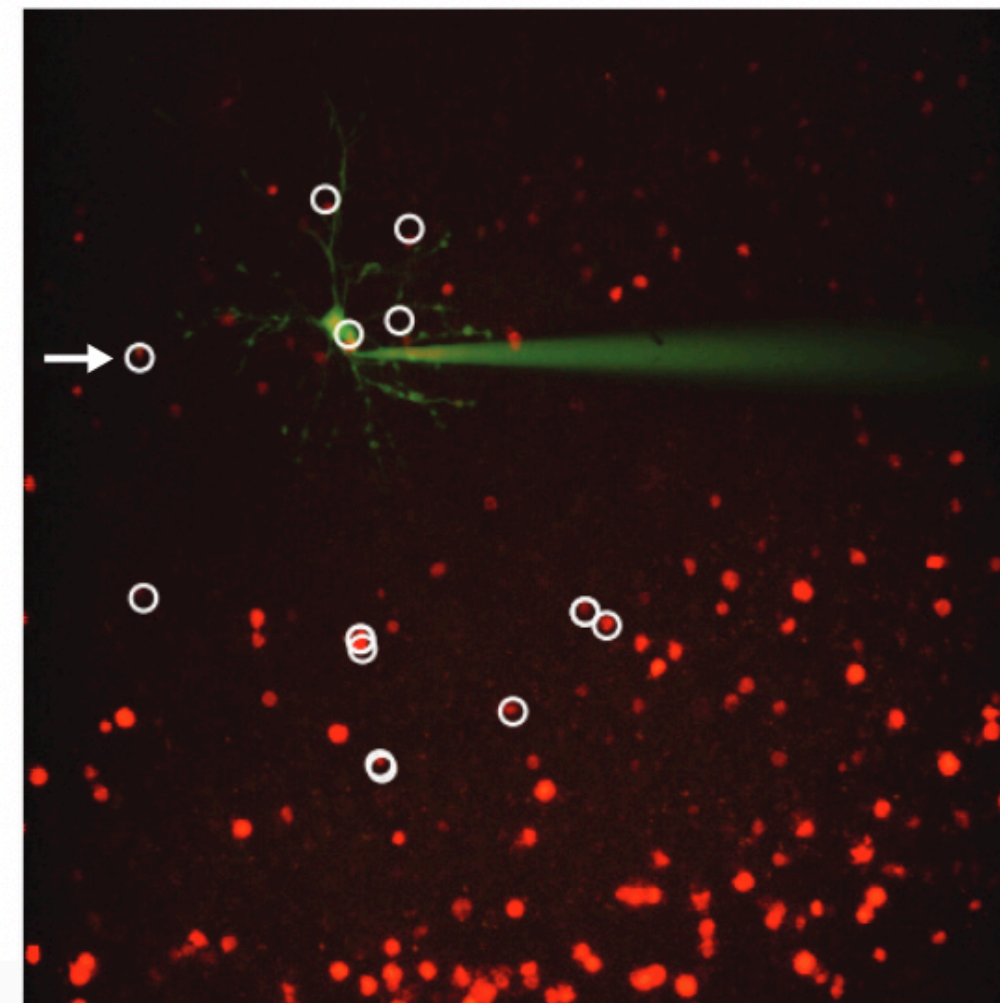
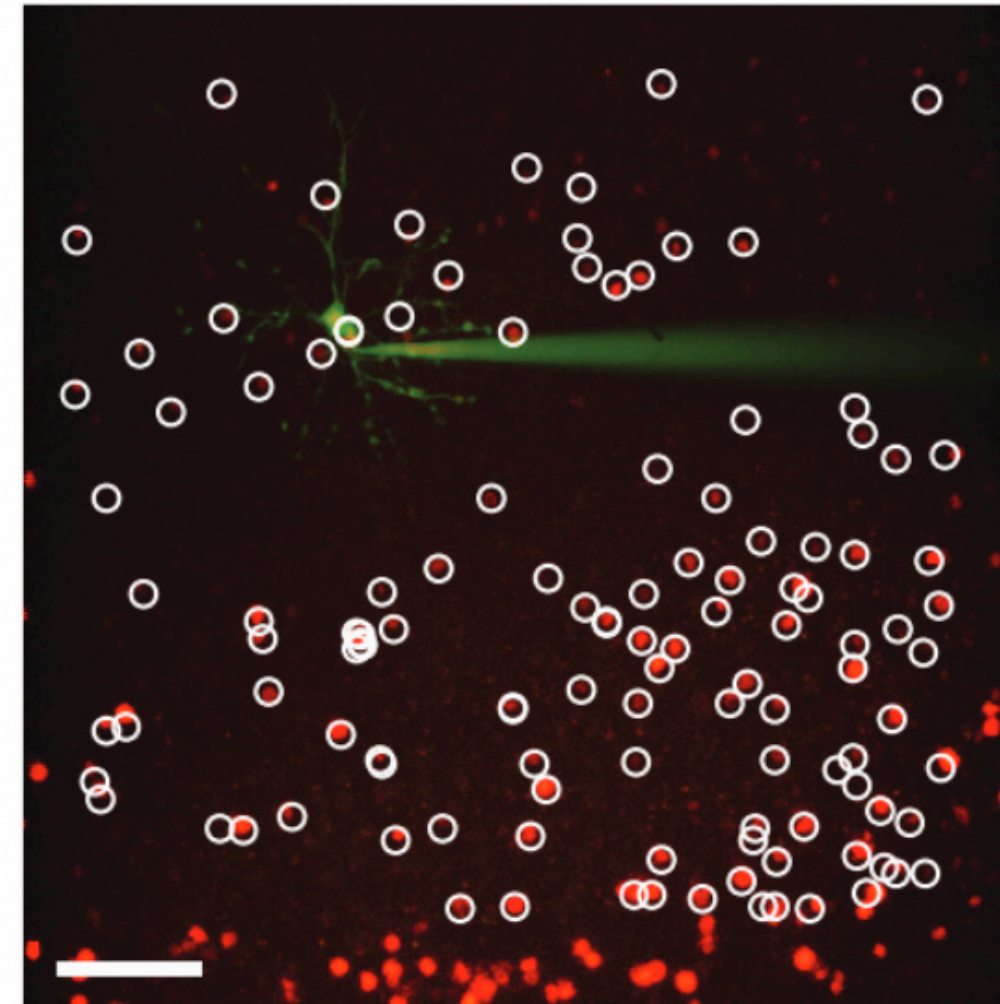
Probed targets



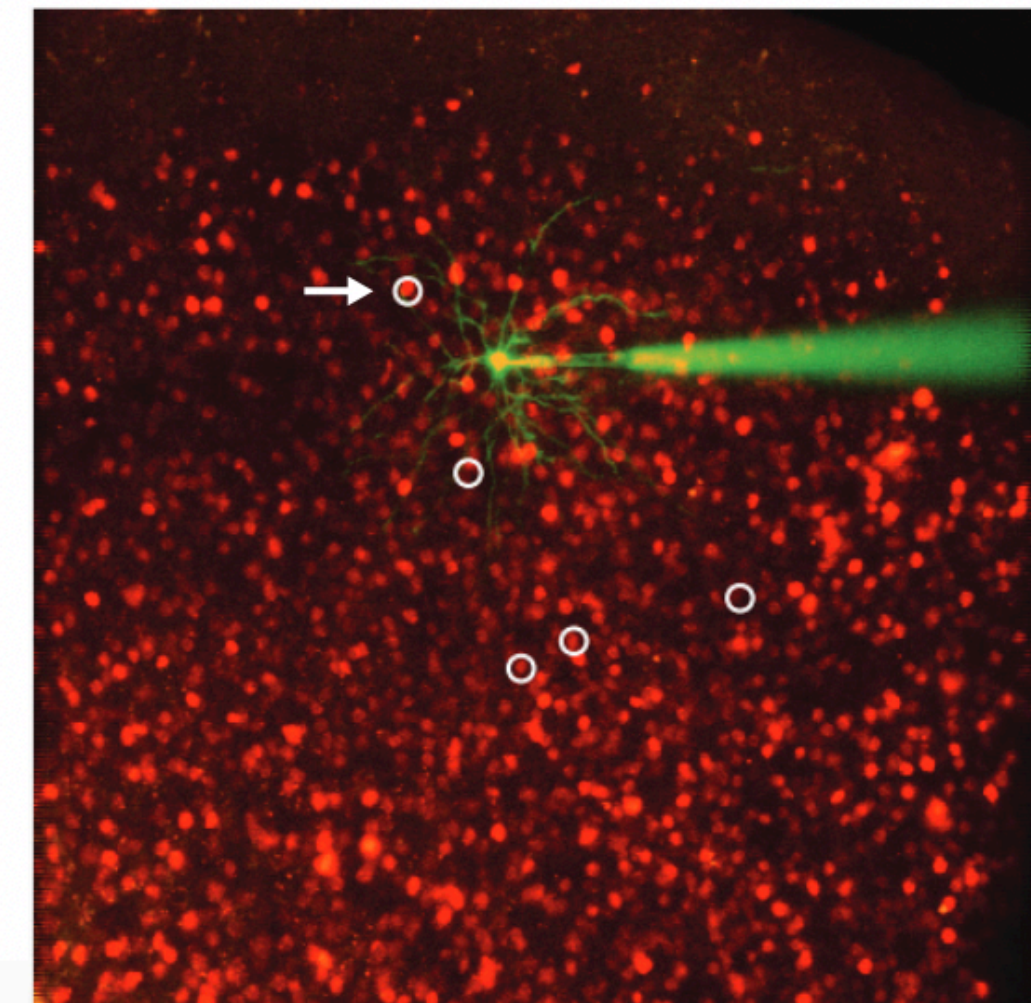
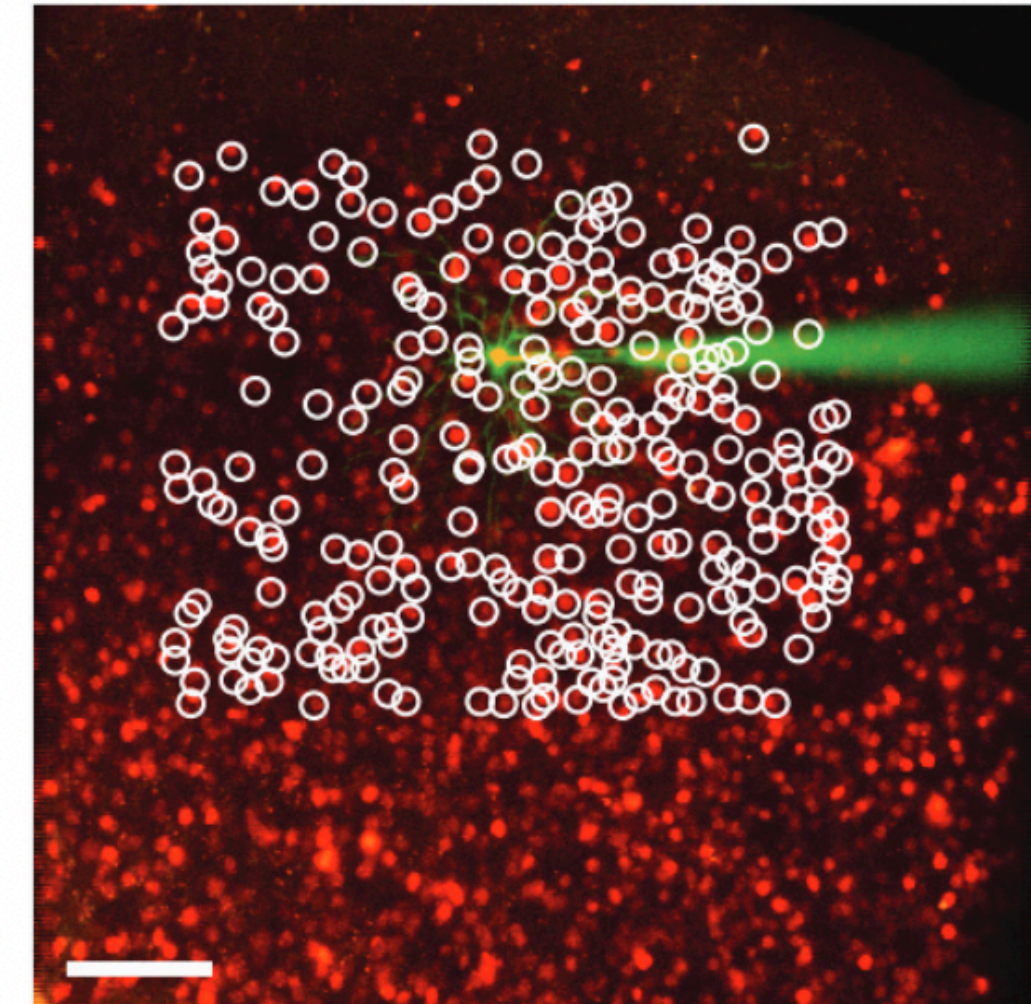
Detected connections



SST-Pyramidal
(SST-Cre; AAV-st-ChroME2f-mRuby3)



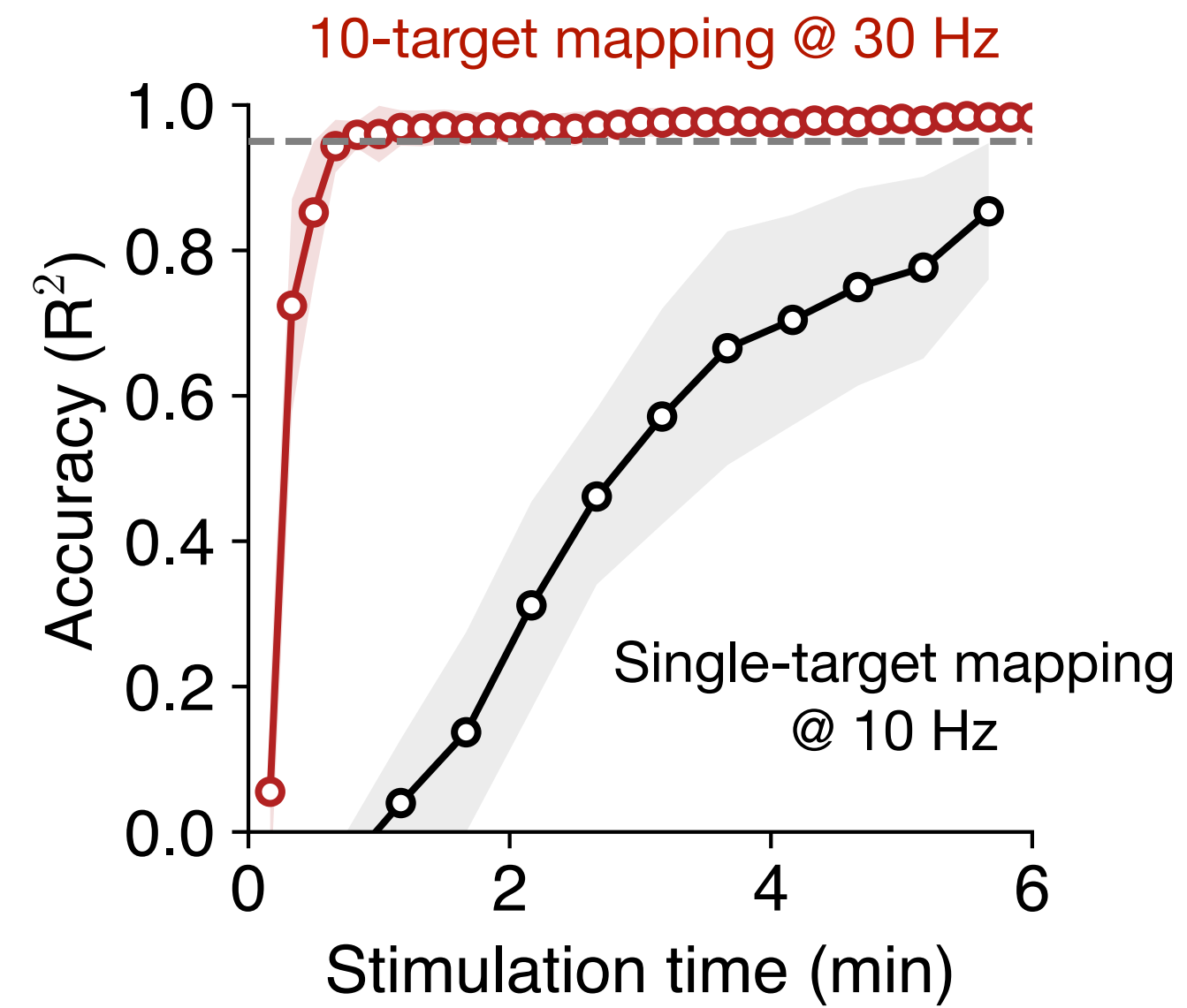
Pyramidal-Pyramidal
(Emx-Cre; AAV-st-ChroME2s-mRuby3)



Experimental implementation

Simulation (279 neurons)

Pilot experiment (279 neurons)

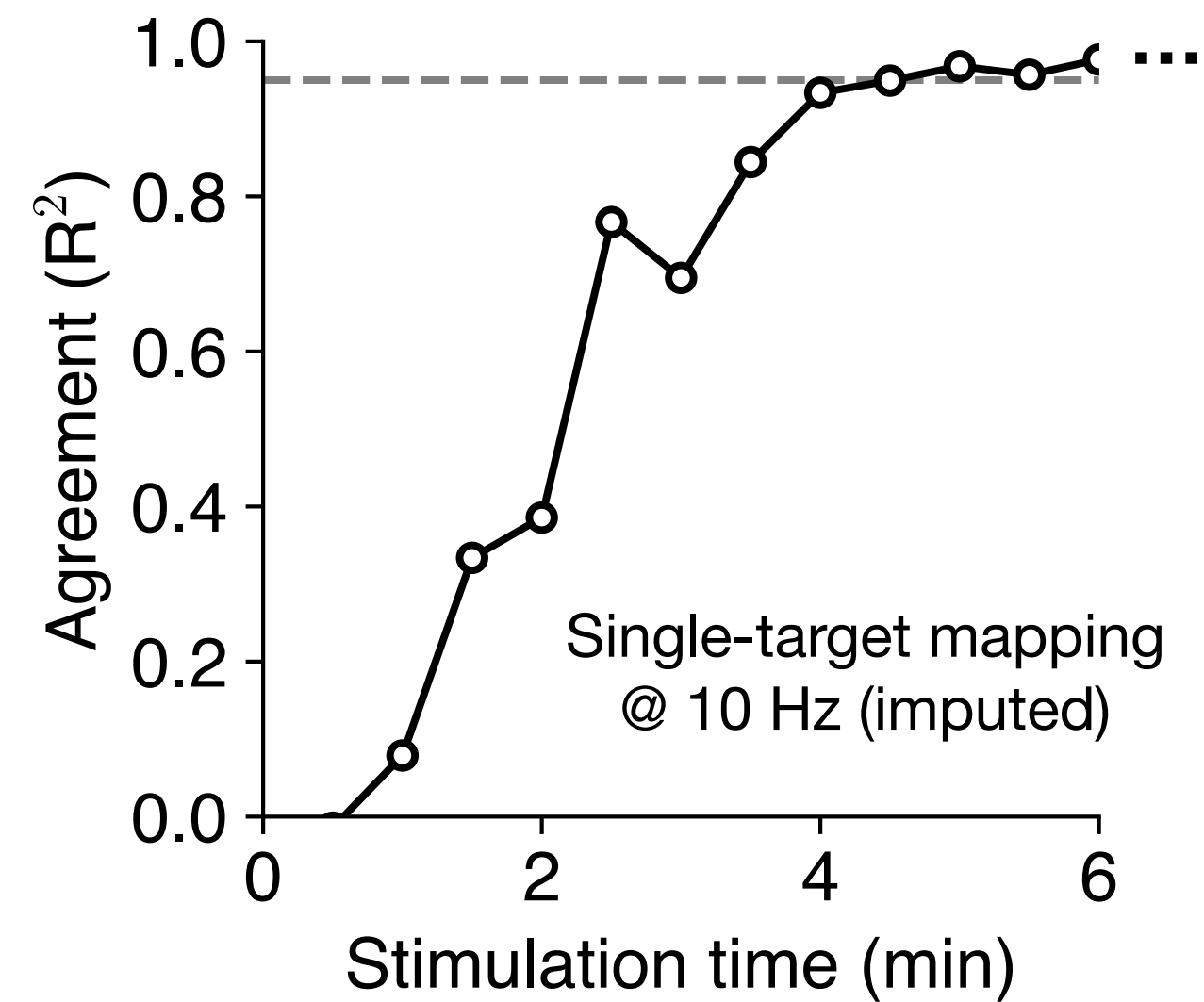
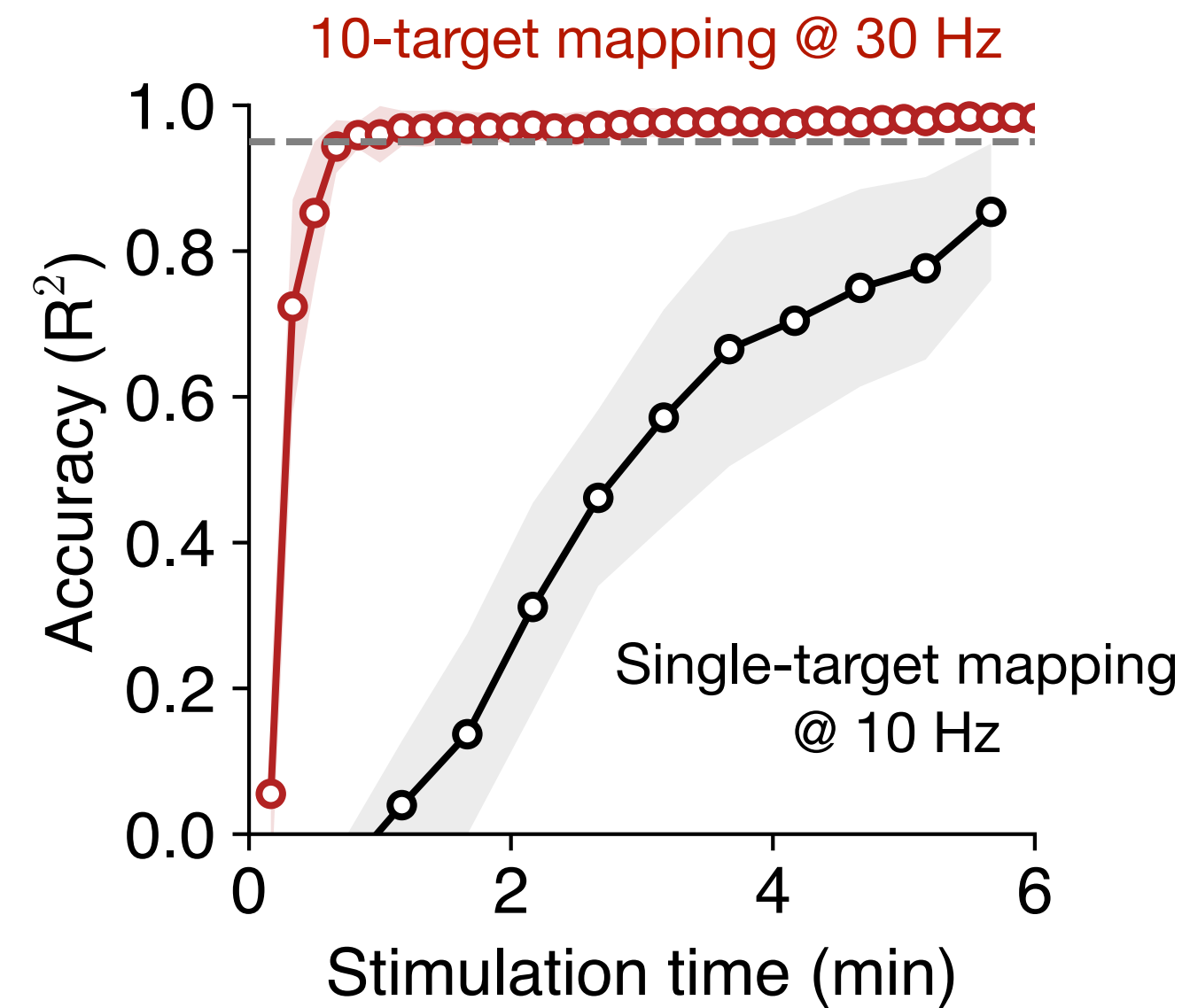


Experimental implementation

Simulation (279 neurons)

Pilot experiment (279 neurons)

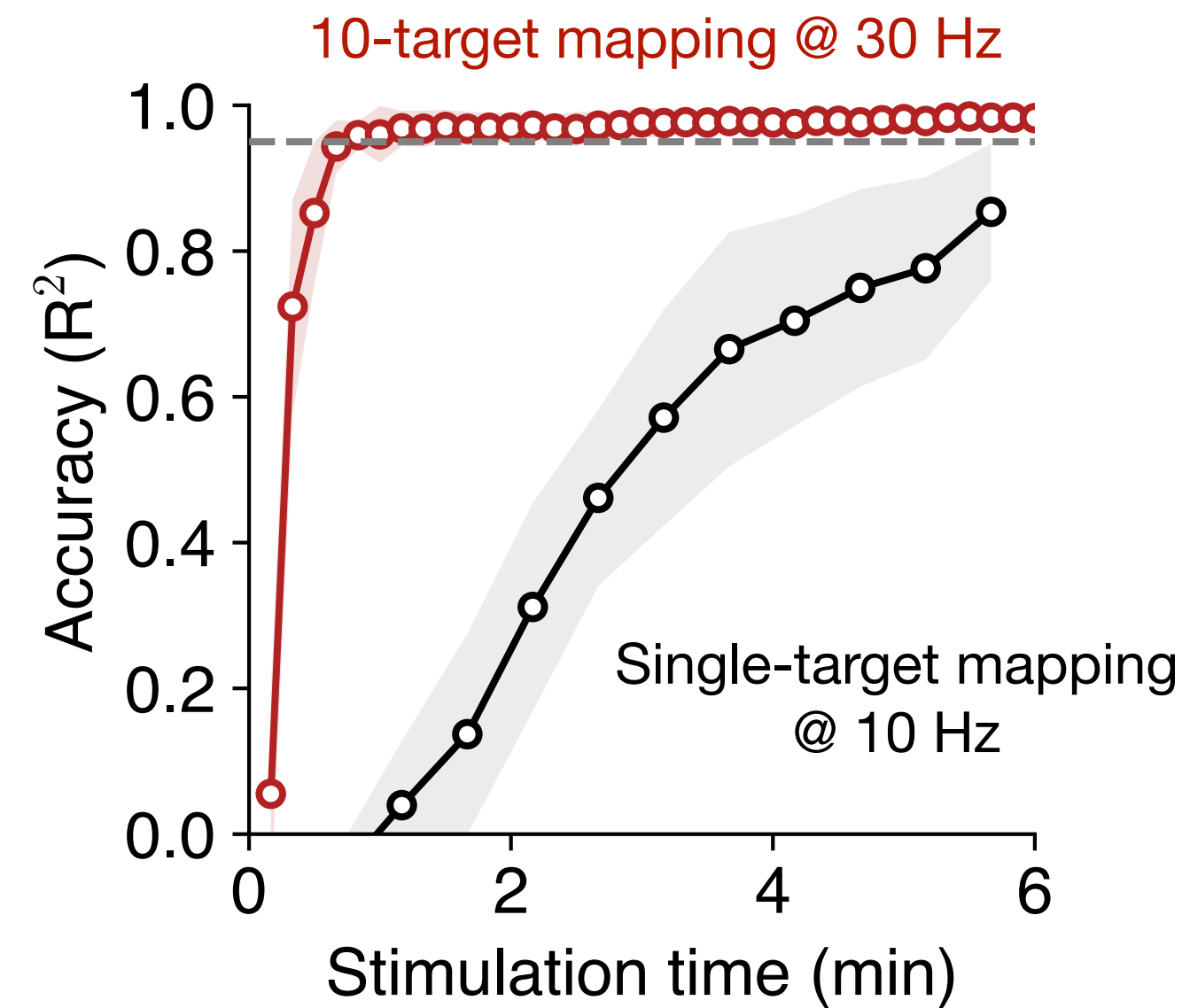
Convergence to final solution



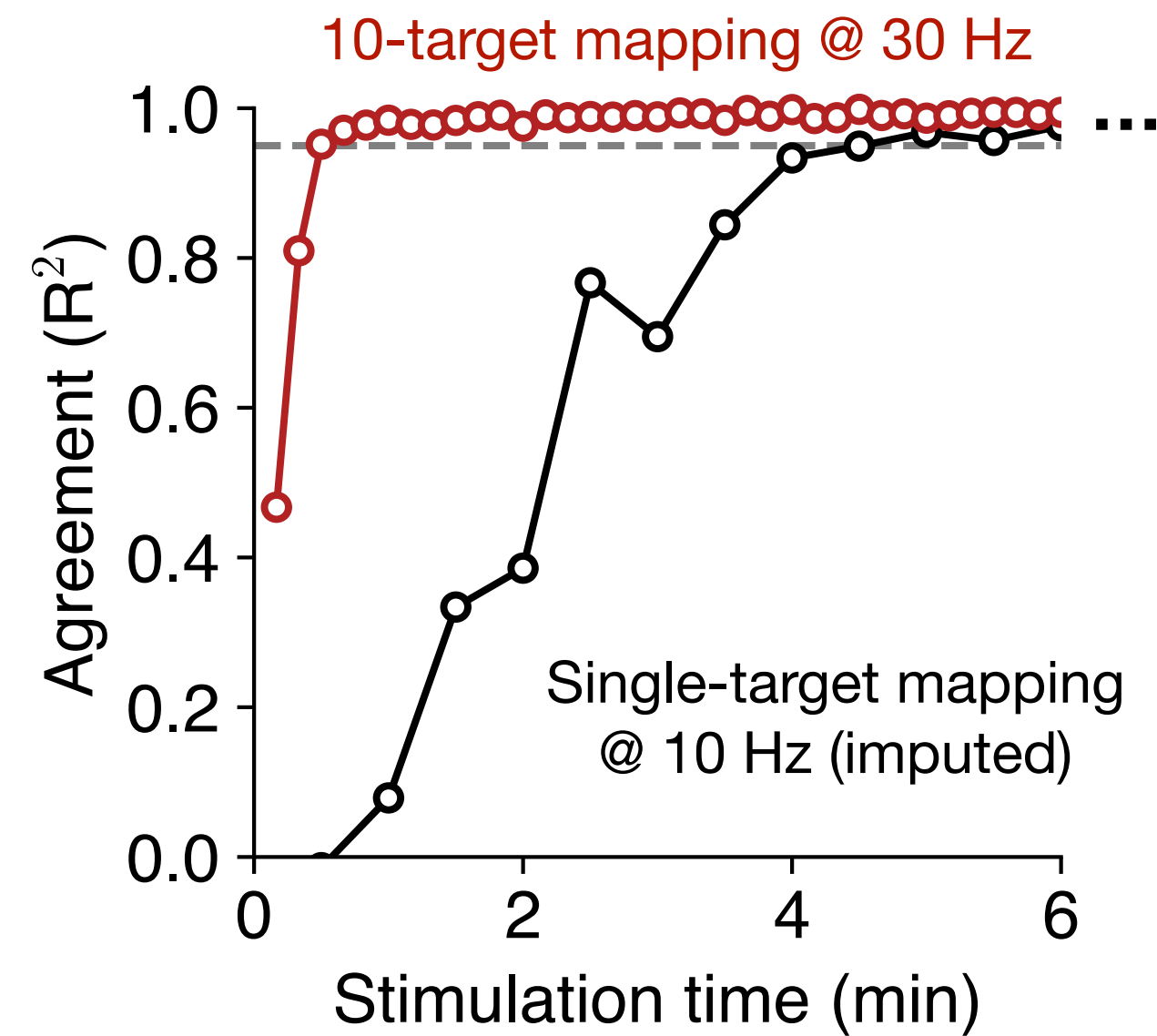
Experimental implementation

Simulation (279 neurons)

Pilot experiment (279 neurons)



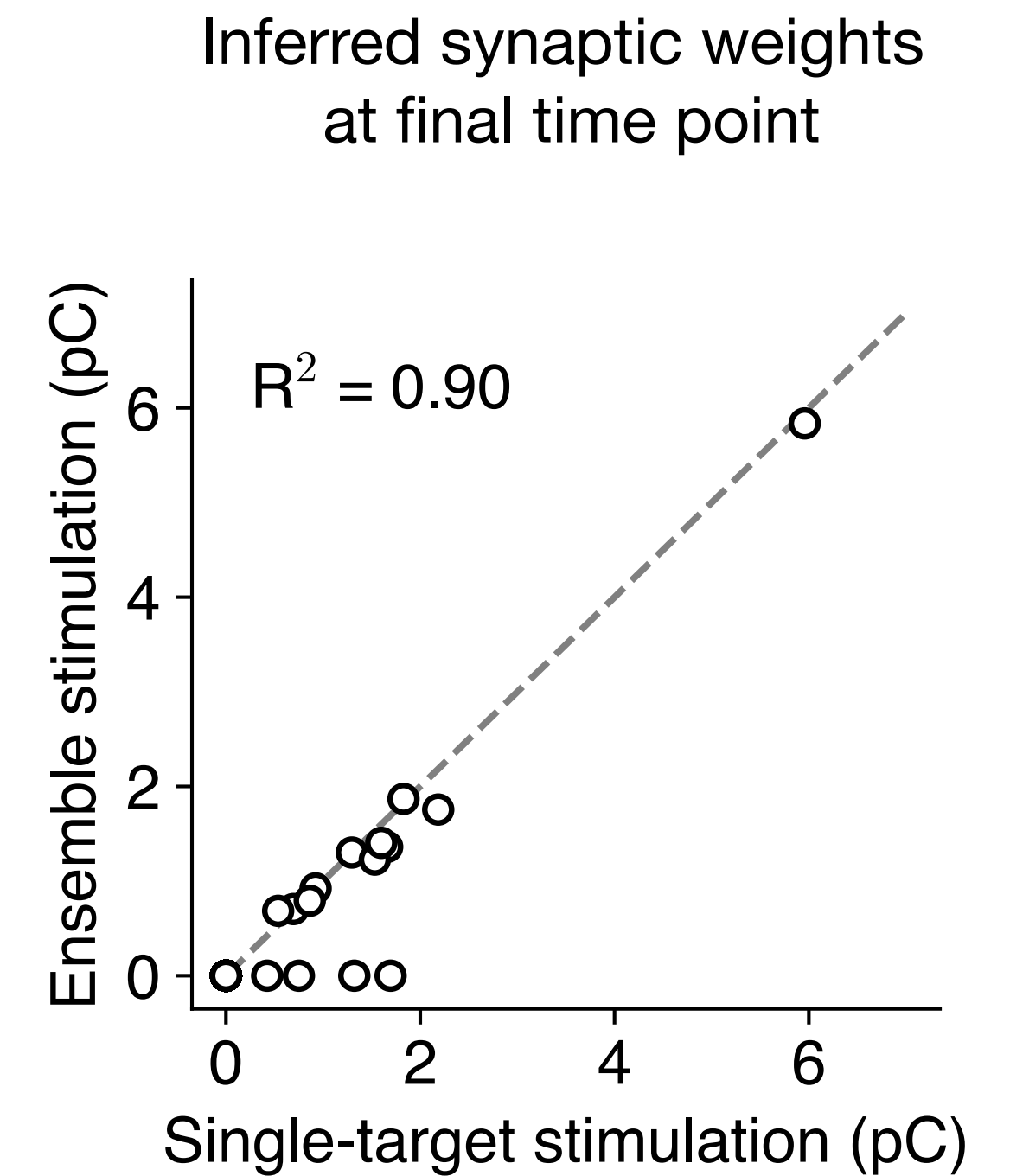
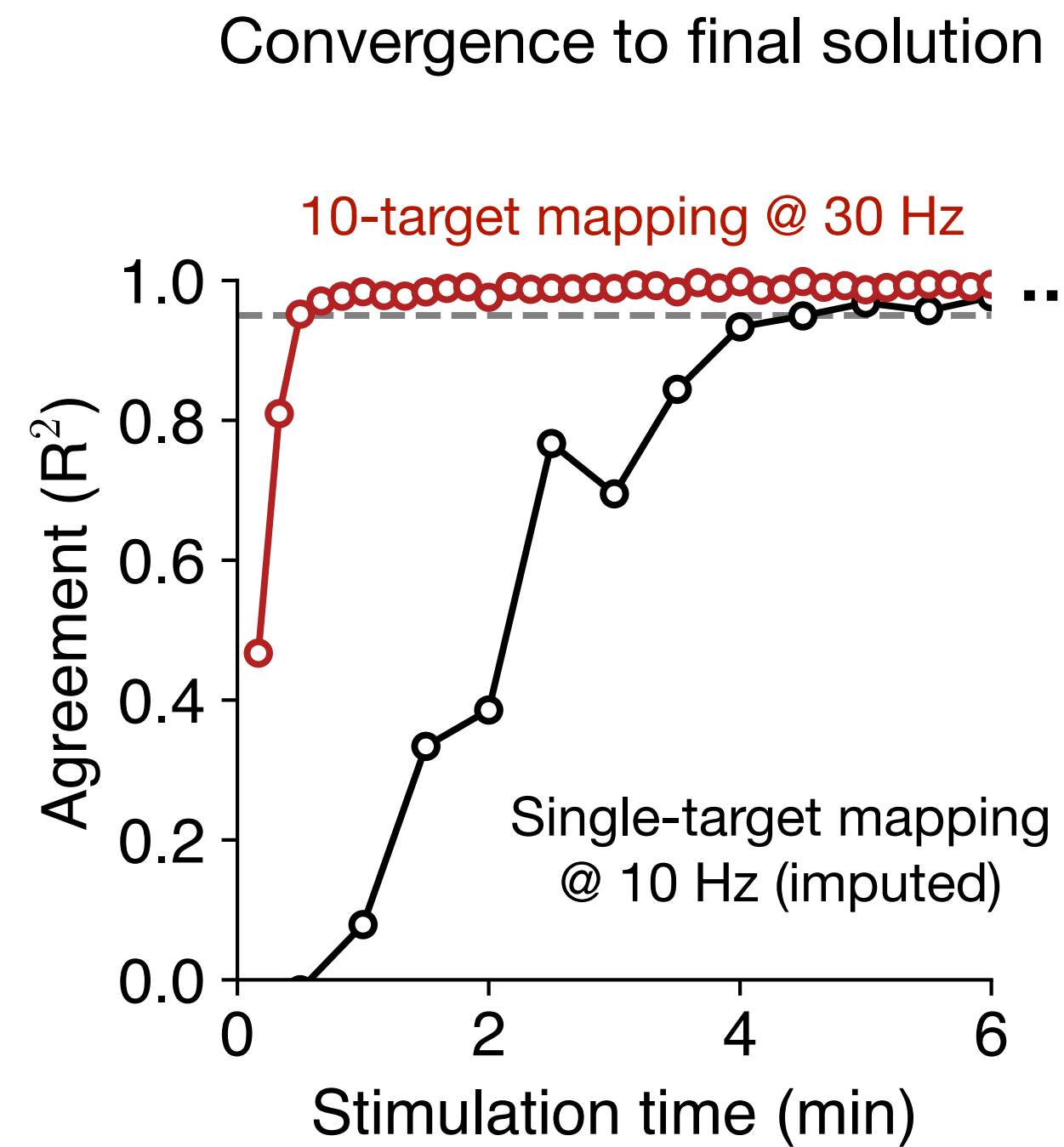
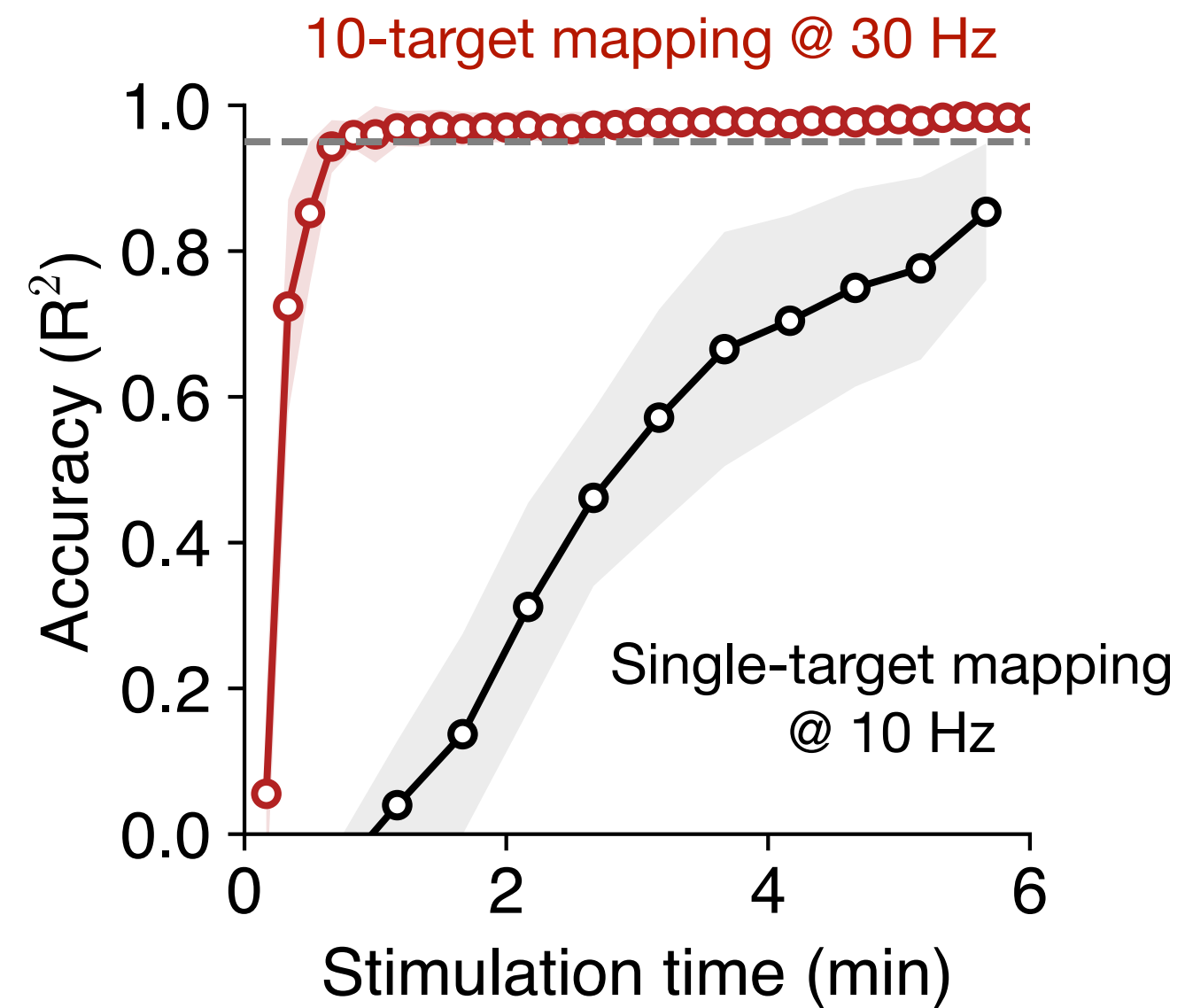
Convergence to final solution



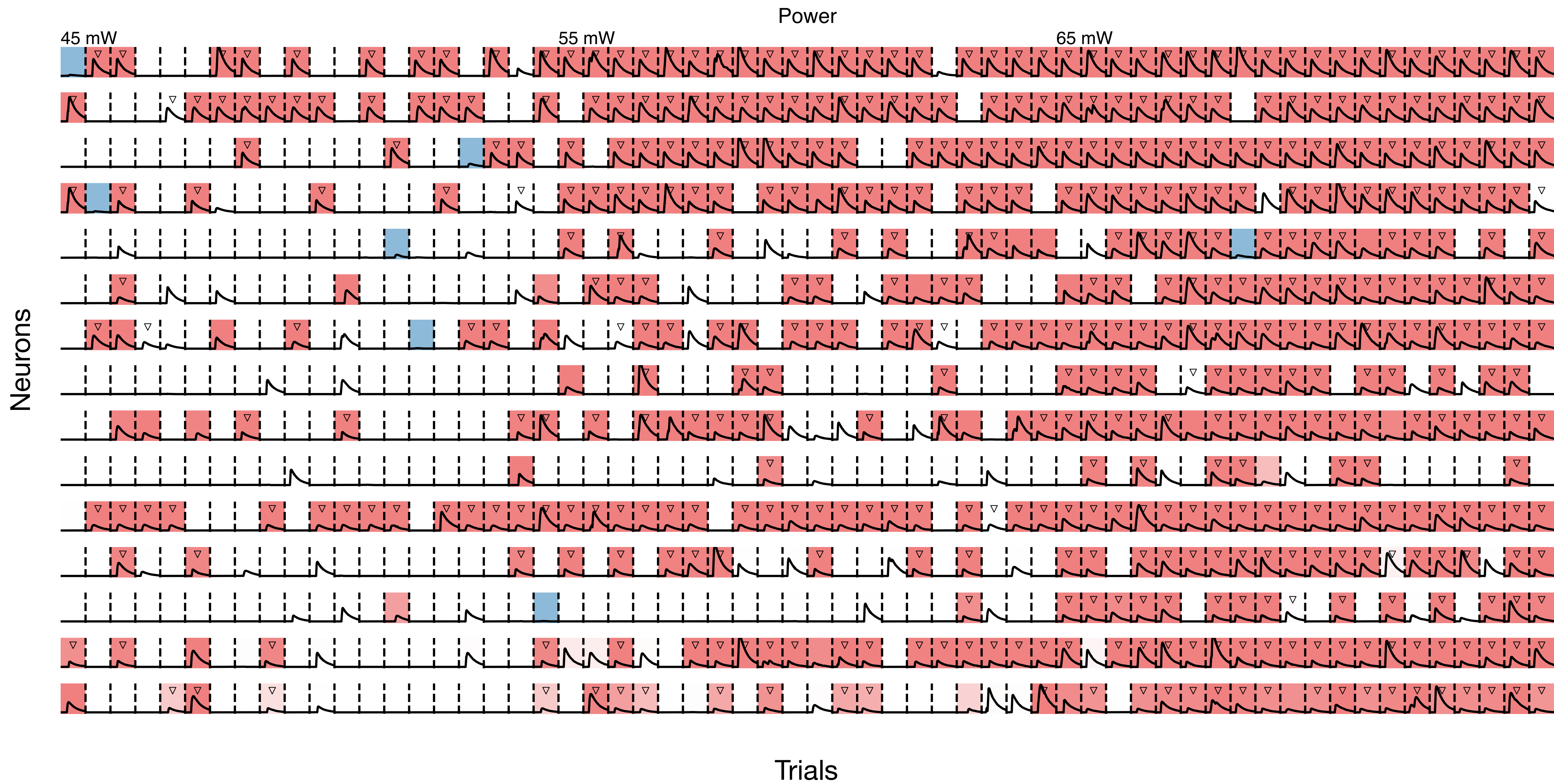
Experimental implementation

Simulation (279 neurons)

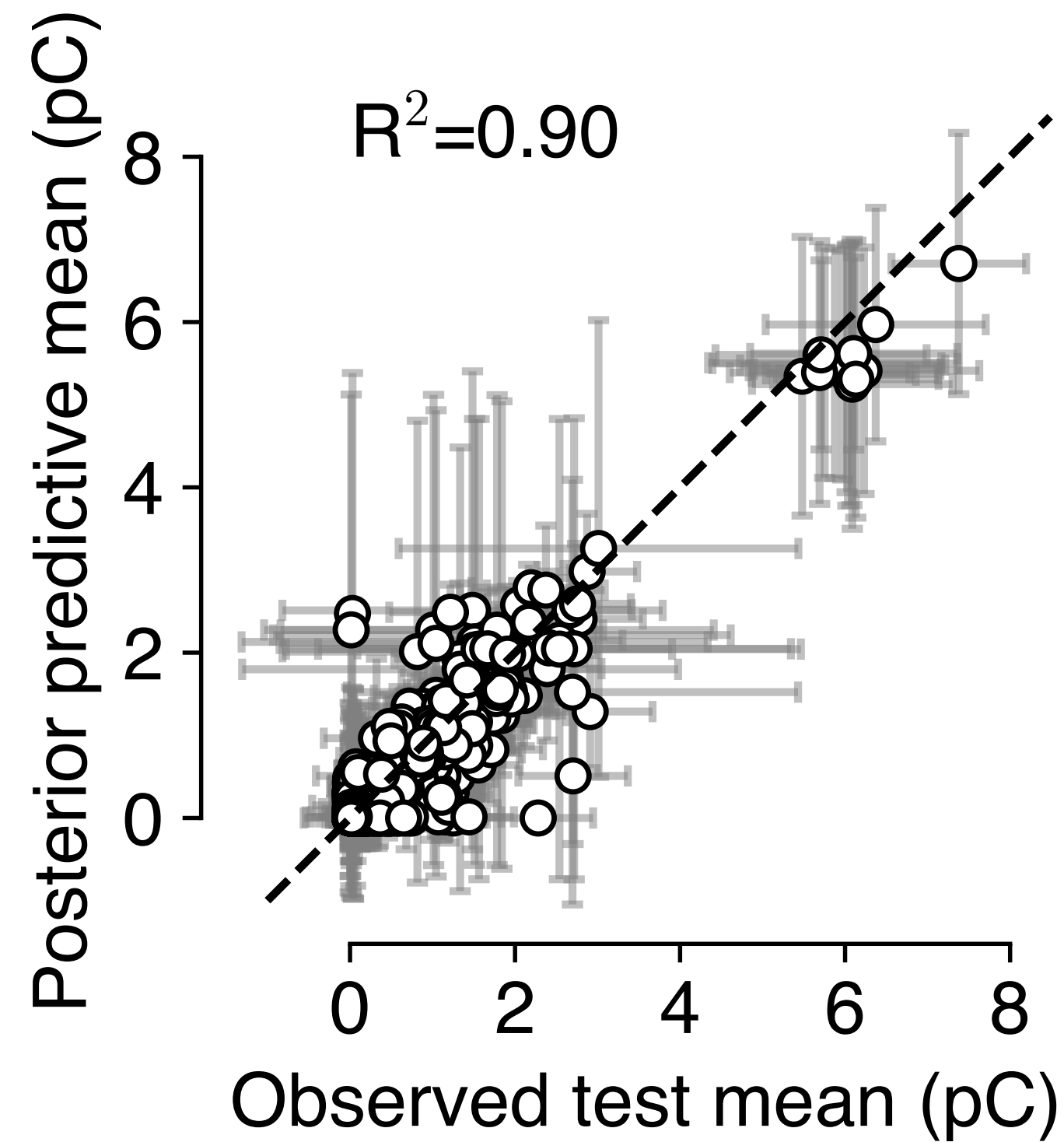
Pilot experiment (279 neurons)



“Checkerboard” visualisation



Leave-one-hologram-out cross-validation



Fit model to H-1 holograms (out of H total).

Then predict response to Hth hologram (i.e., a combination of neurons never seen before).

Future directions

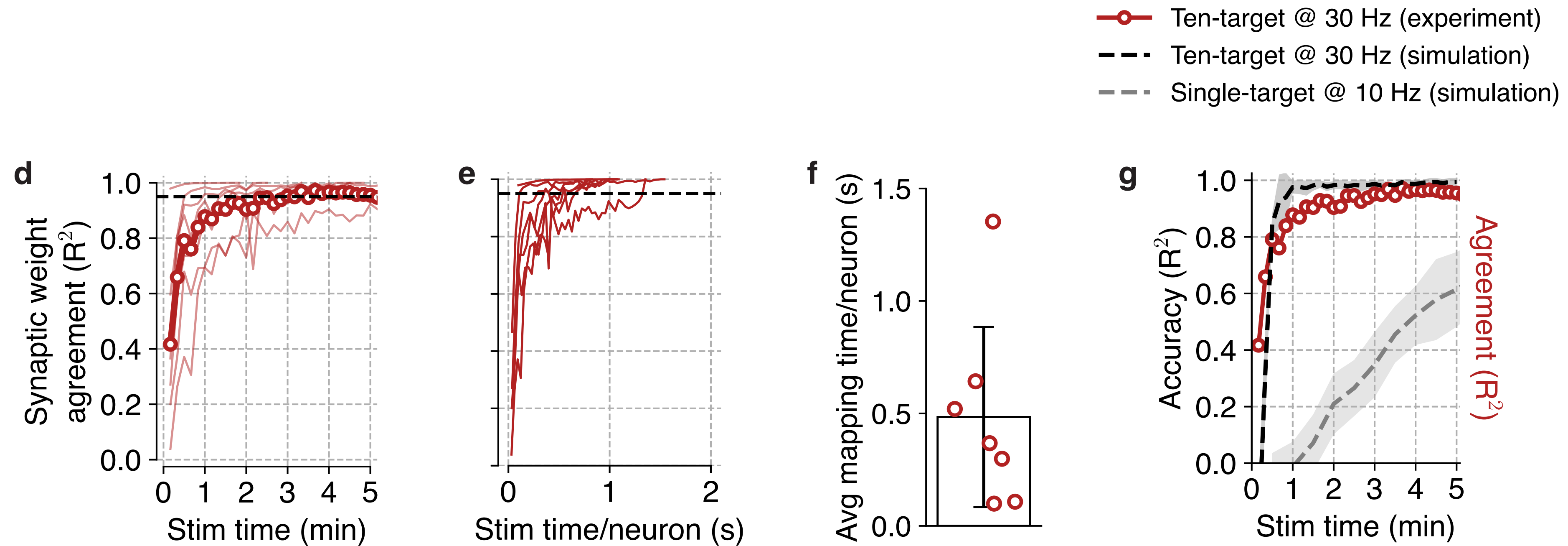
- Subtraction of direct photocurrents
- Simultaneous calcium imaging of presynaptic activity
- Voltage imaging of pre and/or postsynaptic activity
- Online experimental design/active learning

Rapid learning of neural circuitry from holographic ensemble stimulation enabled by model-based compressed sensing. (2022). Triplett*, Gajowa*, ..., Paninski. bioRxiv 2022.09.14.507926

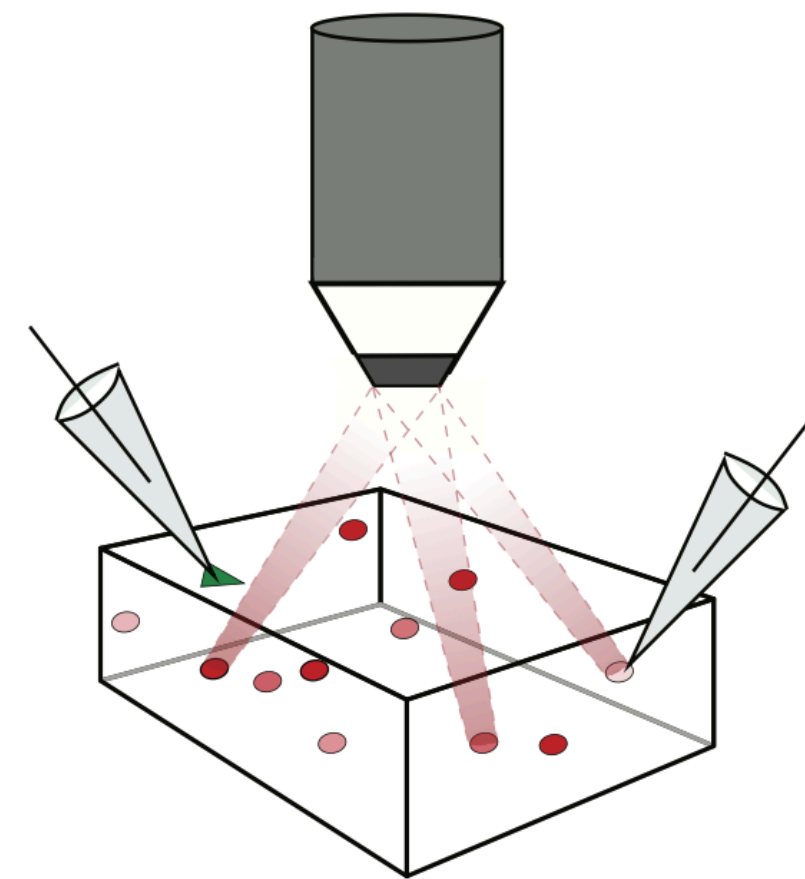
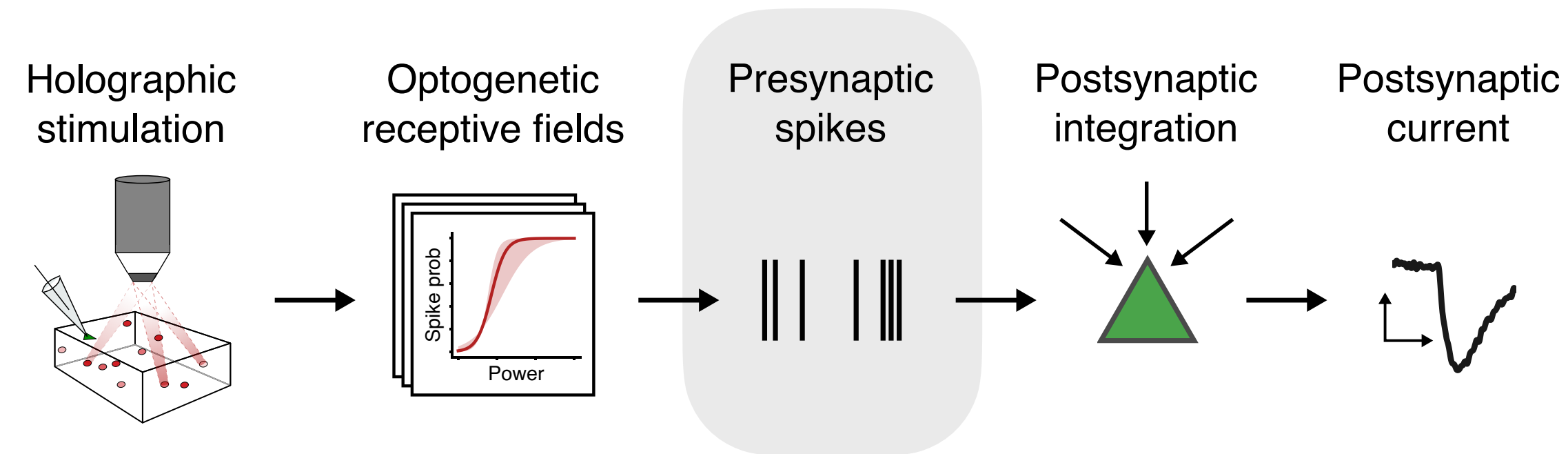
Software: <https://github.com/marcustriplett/circuitmap>



Summary over experiments



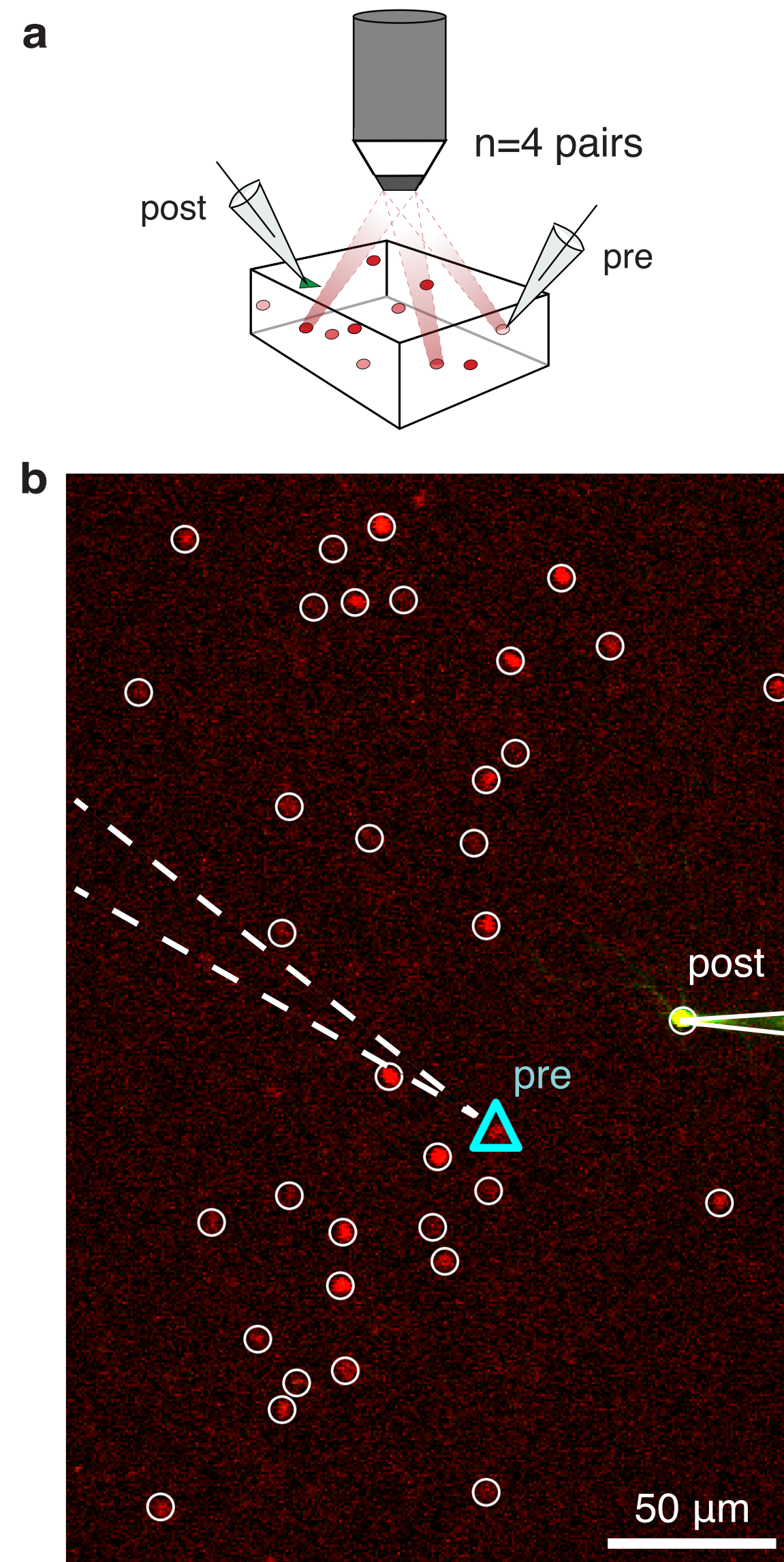
Validating spike inference



Paired patch experiment

1. Measurements from both presynaptic and postsynaptic neurons
2. Interleave single-target and ensemble stimulation
3. Infer presynaptic spikes from postsynaptic measurements

Validating spike inference



c Example ground-truth spiking scenarios

