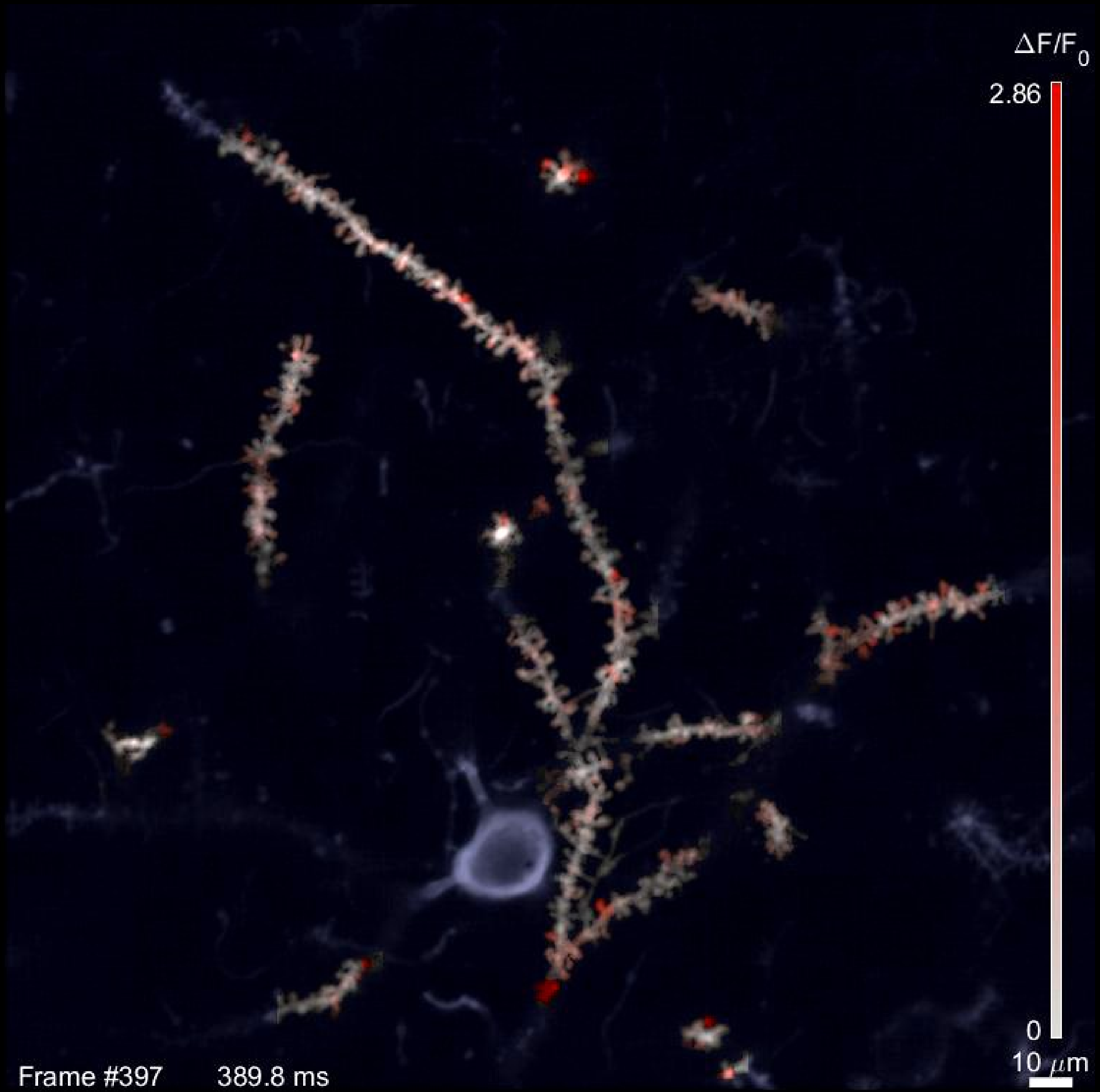


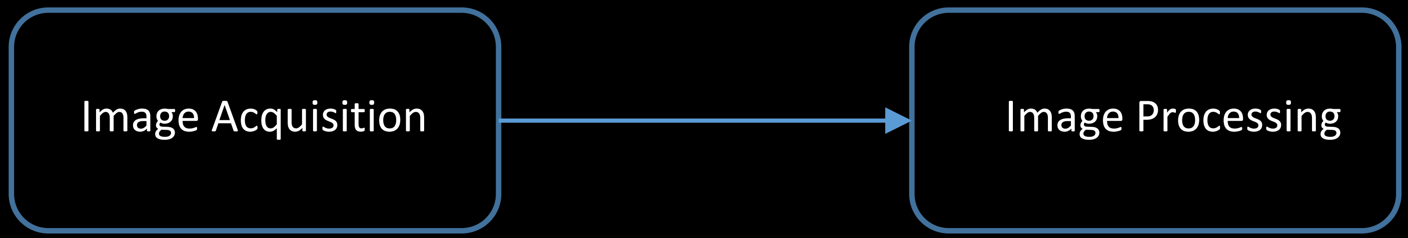
Computational Microscopes for In Vivo Imaging

Kaspar Podgorski

SF-Venus-iGluSnFR.A184S
1016 Hz frame rate
156 μm field of view
130 μm below brain surface
Primary Visual Cortex

What is Computational Microscopy?

What is Computational Microscopy?



What is Computational Microscopy?

Why Computational Microscopy?

Overcome limits on image resolution (e.g. PALM, STORM, SIM)

Overcome limits on measurement speed (e.g. Light Field, multifocal 2P, SLAP)

Overcome limits on measurement modality (e.g. Quantitative phase)

Why Computational Microscopy?

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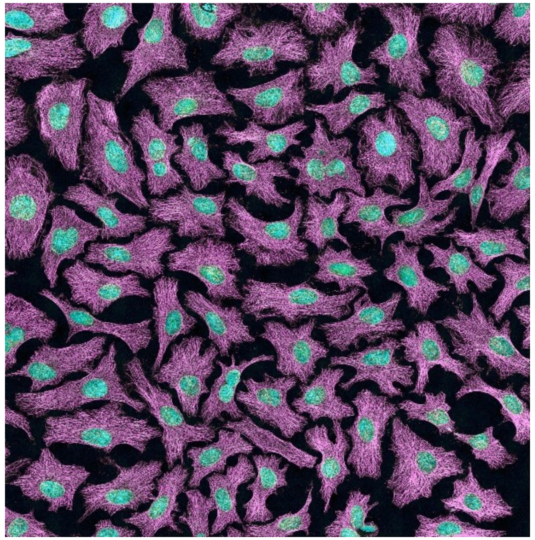
Early Microscopes

2D sample



Microscope

Camera

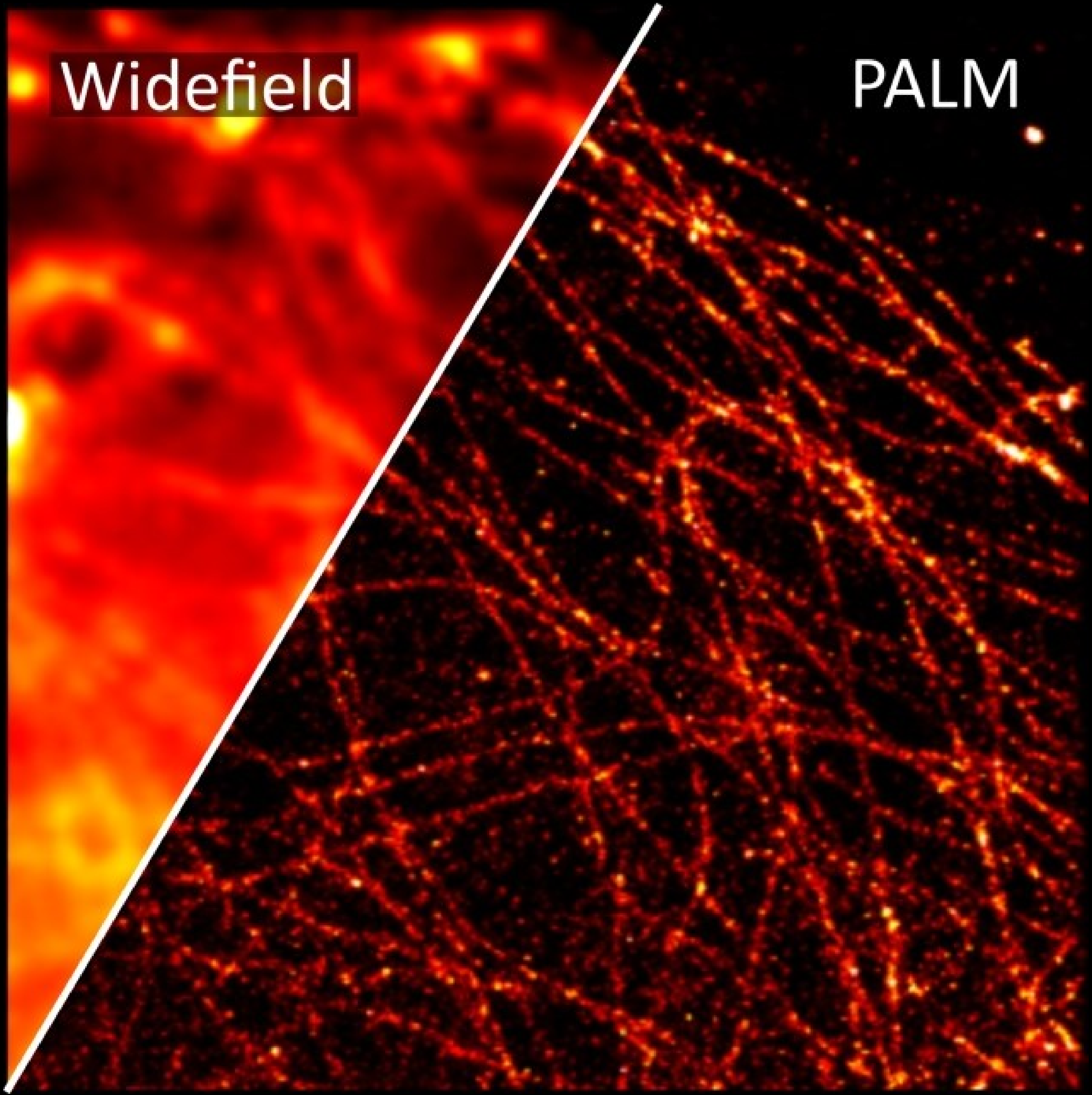
✓

Ernst Abbe

Physicist

Discovered principles of lens and microscope design
Defined the fundamental resolution limit of light microscopy (1873)

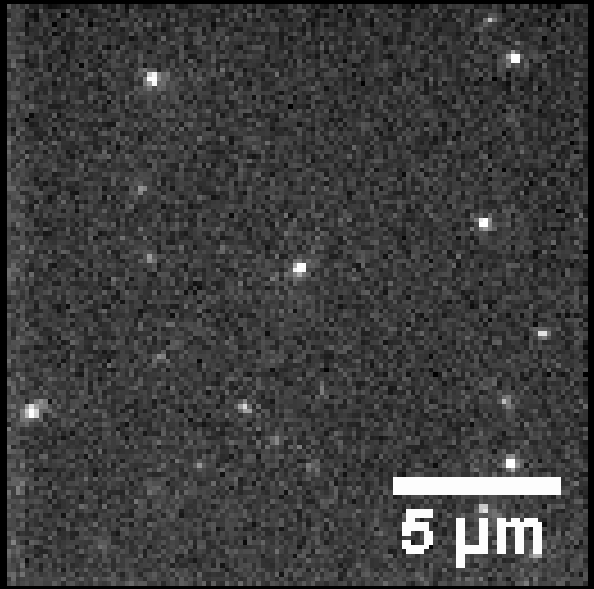
Abbe’s equation, written in stone at Universitat Jena



PALM/STORM microscopy

Computer reconstructed images

Resolution improvement limited only by dye brightness/bleaching
>5x improvement in practice



Prior: sources are single emitters

Why Computational Microscopy?

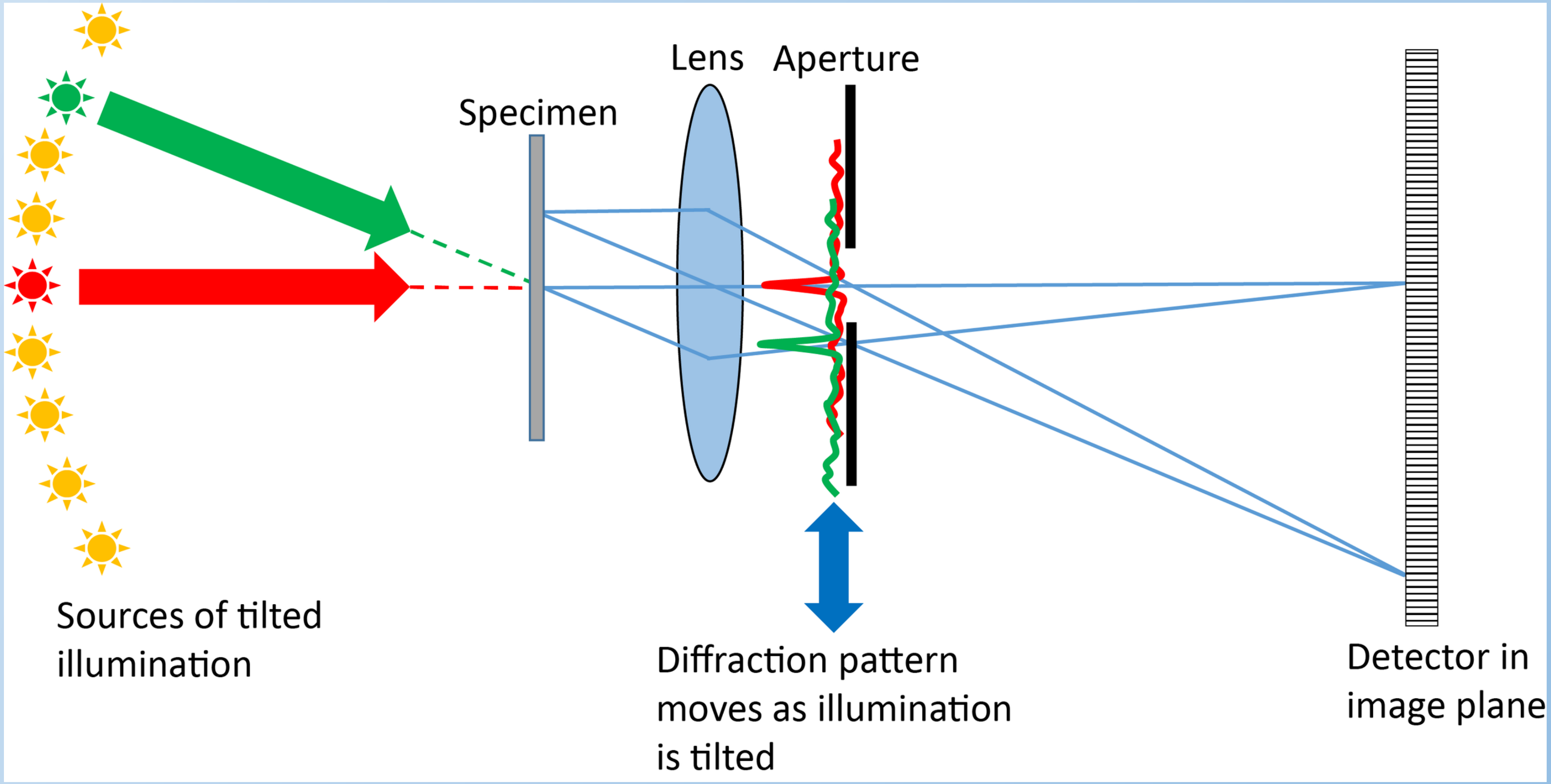
Overcome limits on image resolution (e.g. PALM, STORM, SIM)

Overcome limits on measurement speed (e.g. Light Field, multifocal 2P, SLAP)

Overcome limits on measurement modality (e.g. Quantitative phase)

Fourier Ptychography: Increasing effective measurement aperture, Quantitative Phase retrieval

(Laura Waller lab, others)



Wikipedia

Why Computational Microscopy?

Overcome limits on image resolution (e.g. PALM, STORM, SIM)

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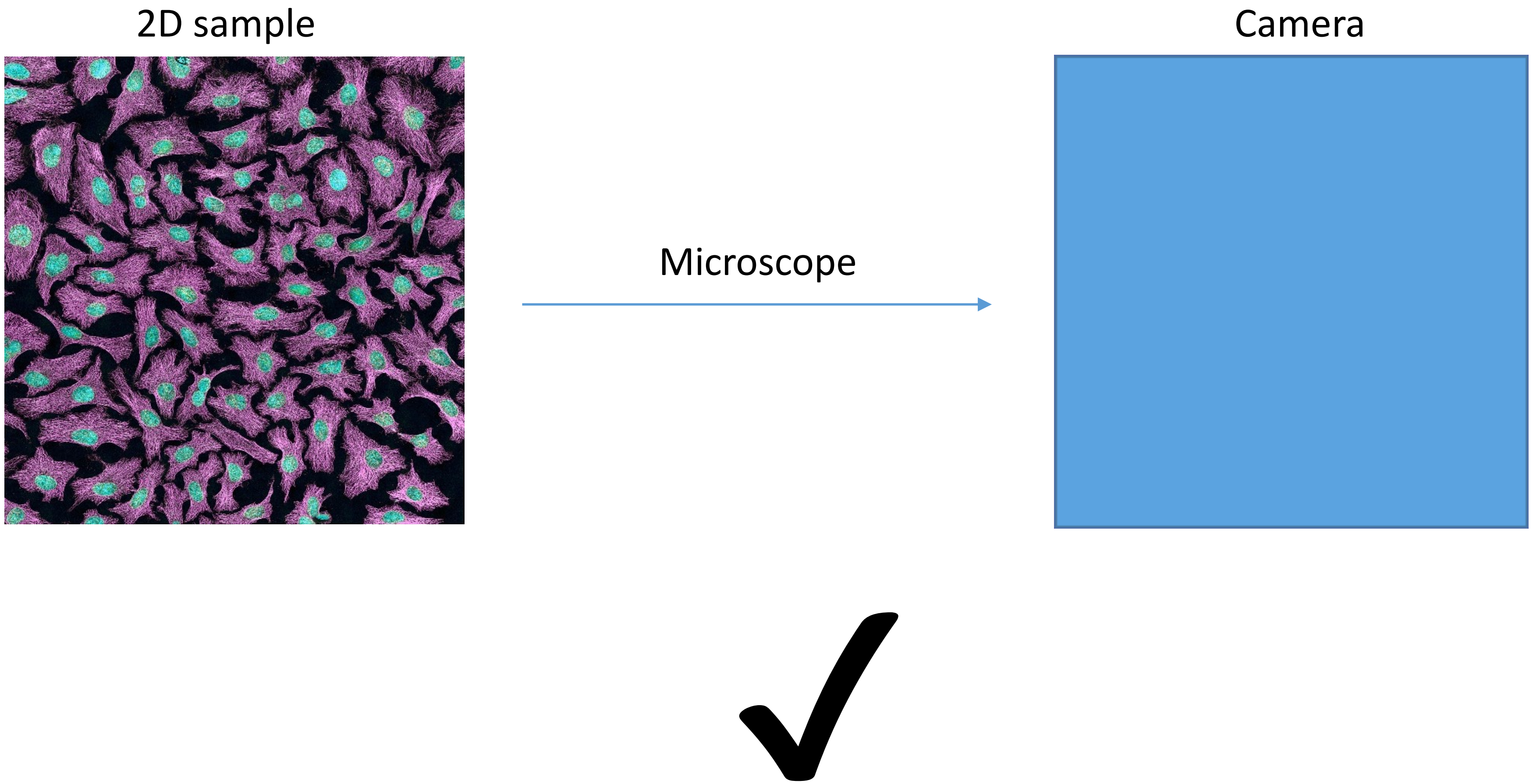
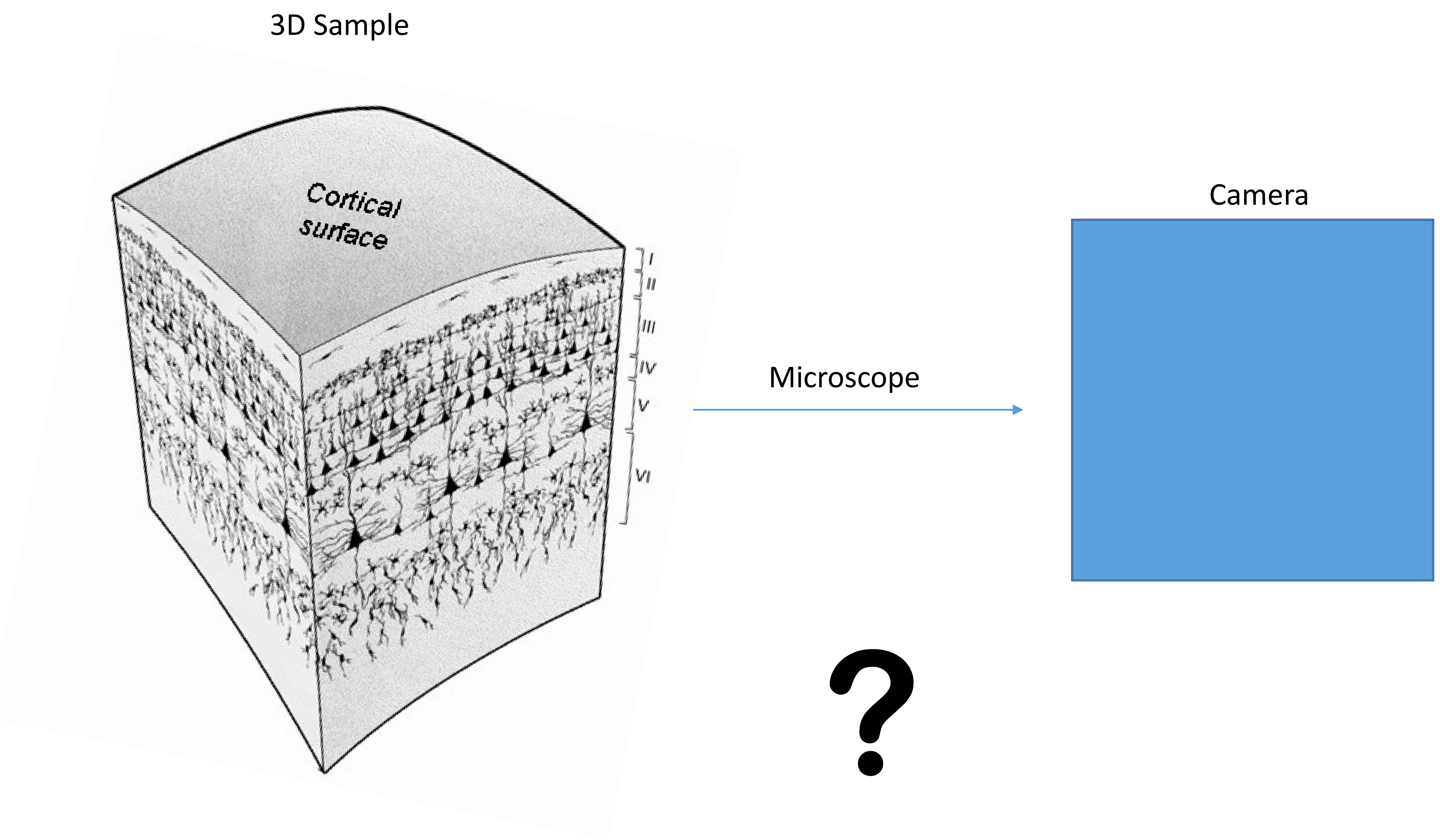
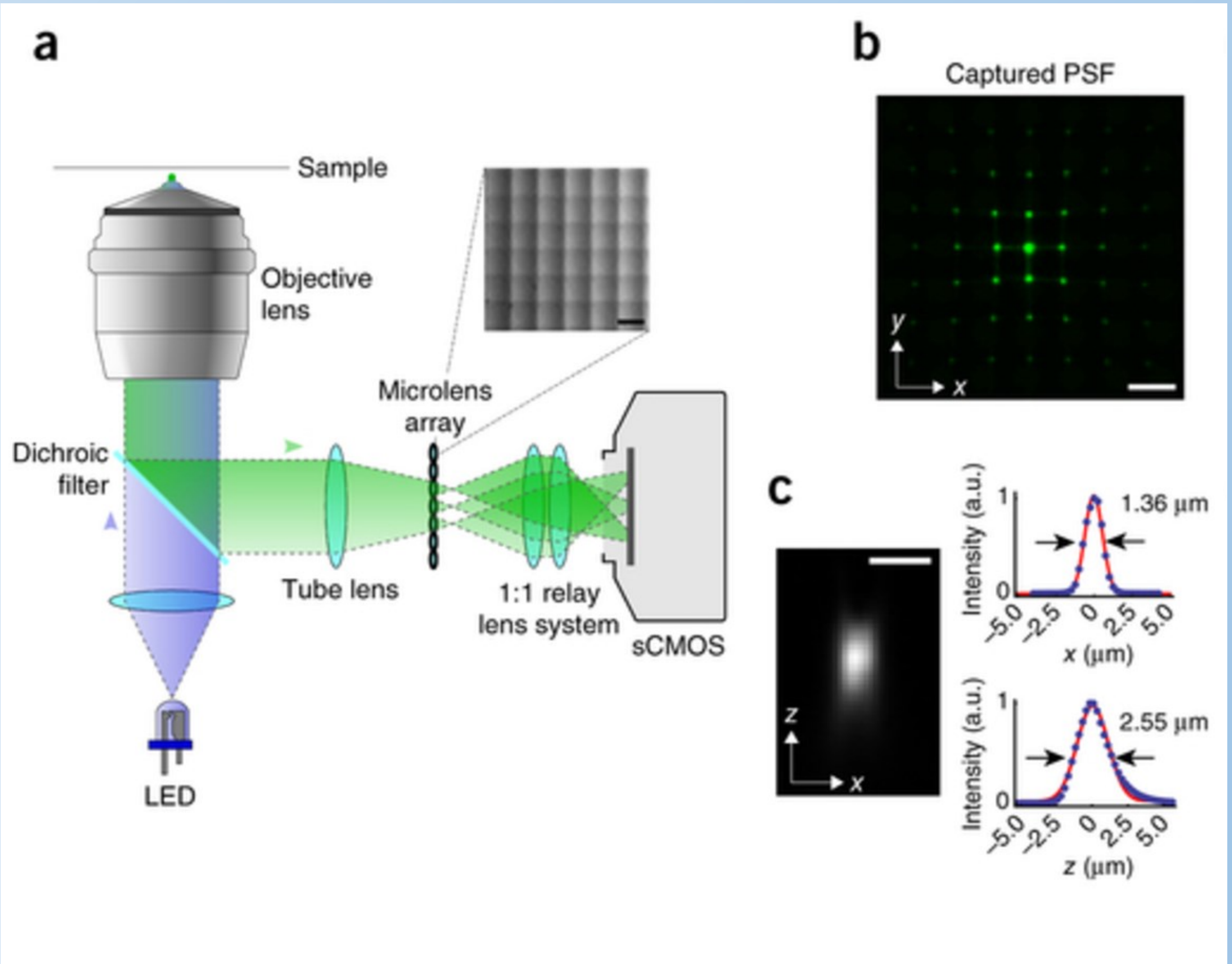
Early Microscopes




3D Sample
Cortical
surface
I
II
III
IV
V
VI
Microscope
Camera
?

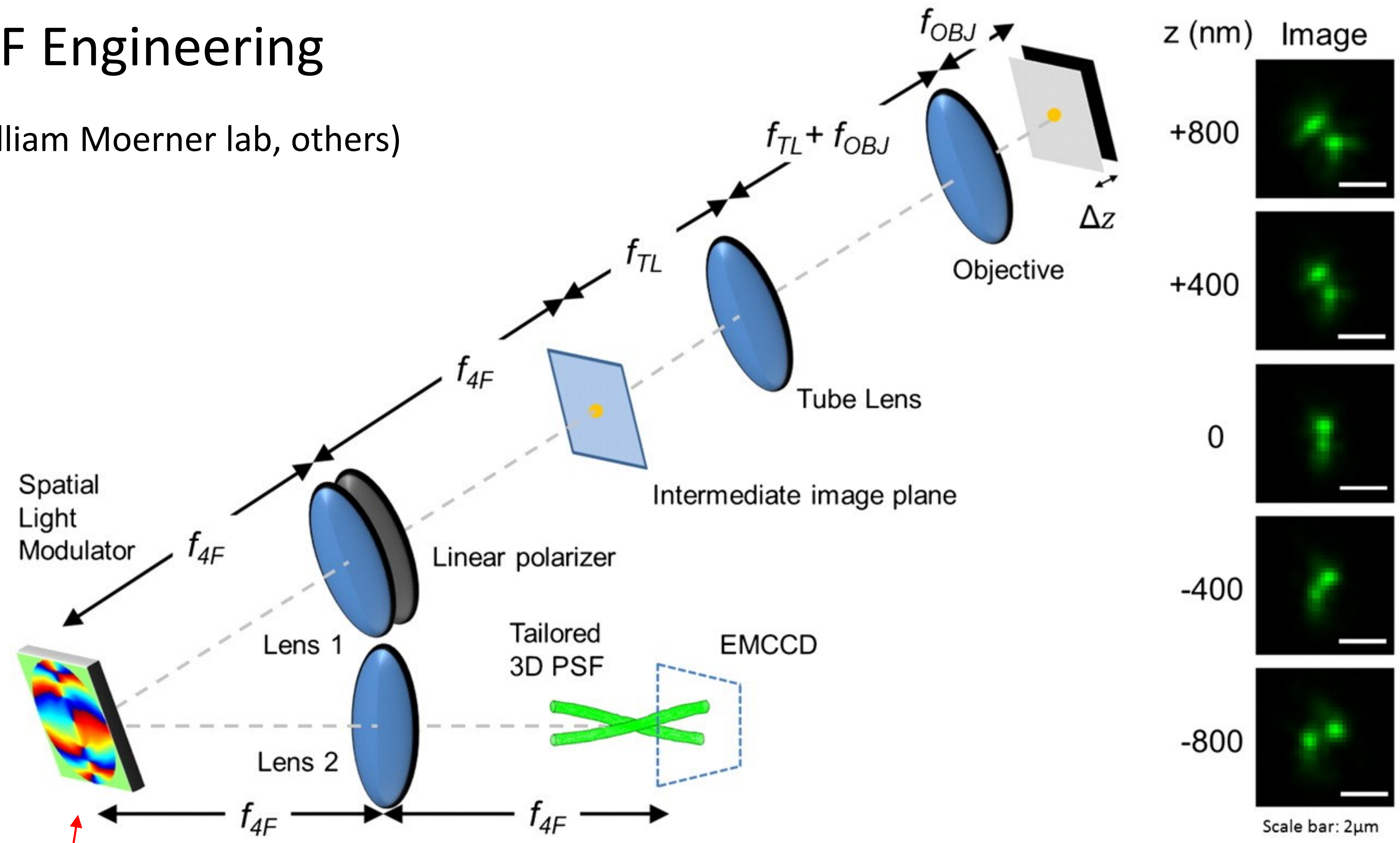
Light Field Microscopy

(Levoy lab, others)



PSF Engineering

(William Moerner lab, others)



Pupil Phase Mask

'Double Helix PSF'

Optimal 3D single-molecule localization for superresolution microscopy with aberrations and engineered point spread functions

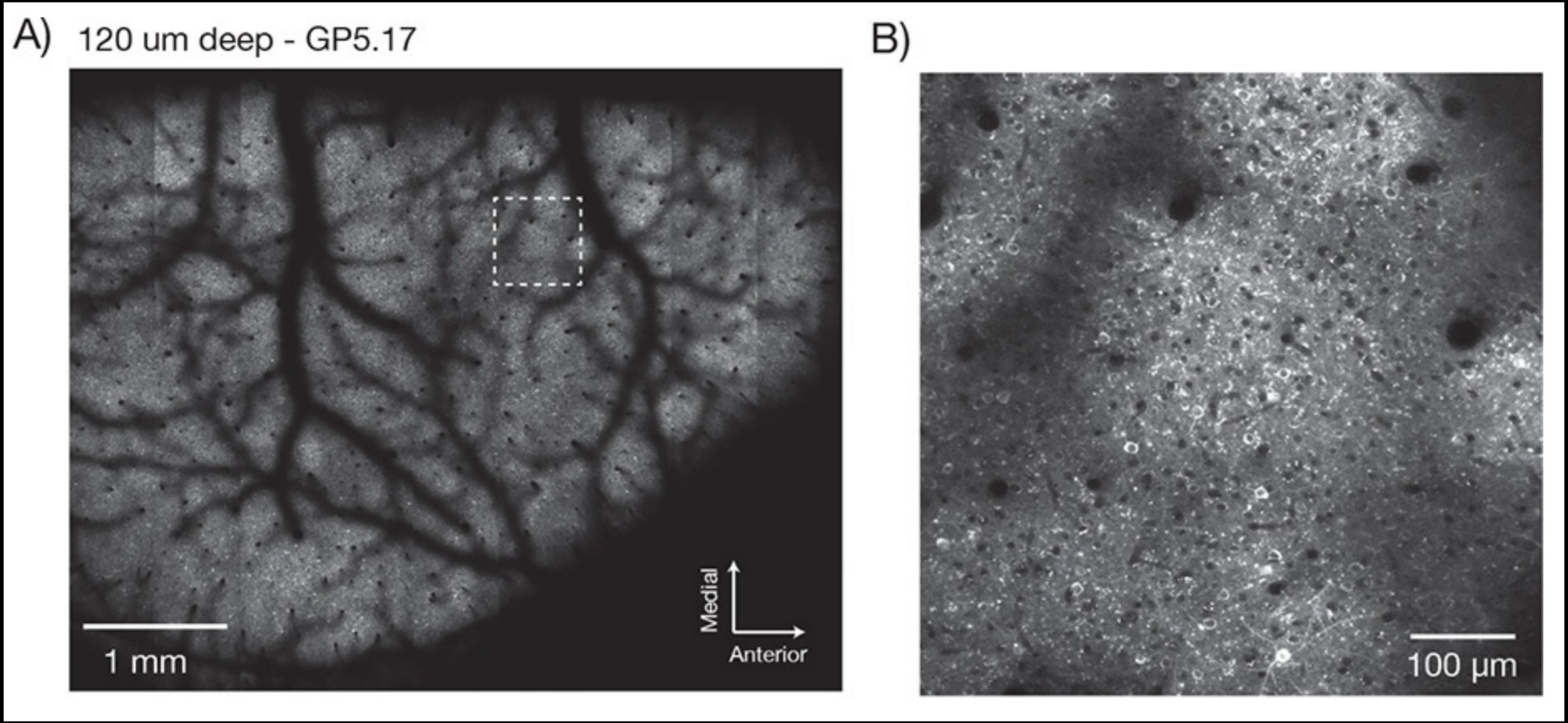
Sean Quirin, Sri Rama Prasanna Pavani, and Rafael Piestun

PNAS January 17, 2012. 109 (3) 675-679; https://doi.org/10.1073/pnas.1109011108

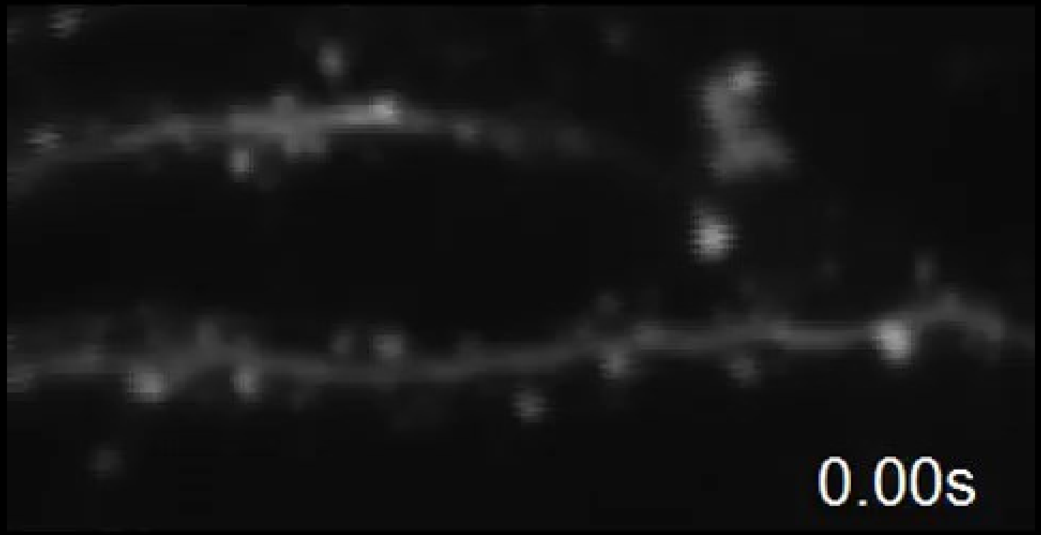
Edited* by Margaret M. Murnane, University of Colorado at Boulder, Boulder, CO, and approved October 27, 2011 (received for review June 3, 2011)

Need for Speed in Two-Photon Imaging:
Larger Volumes, Faster Indicators

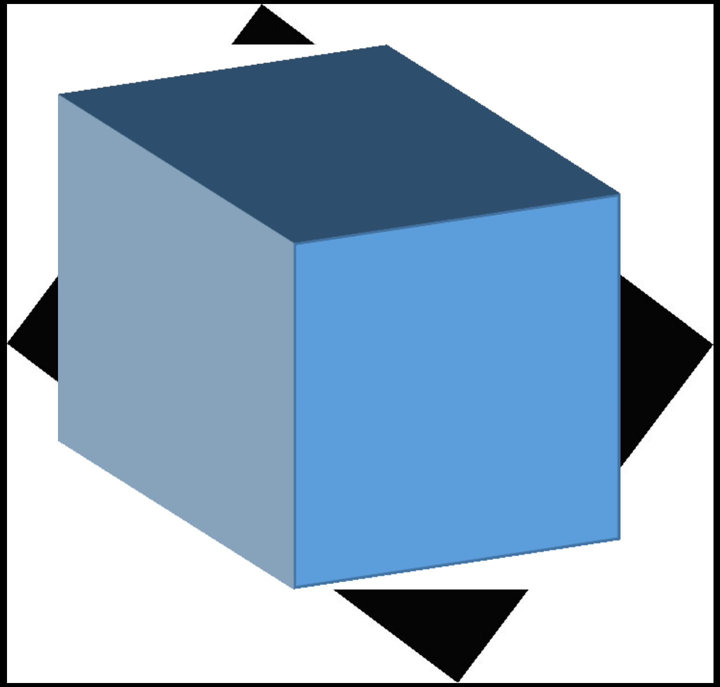
Two-photon Calcium Imaging – GCaMP6f transgenics

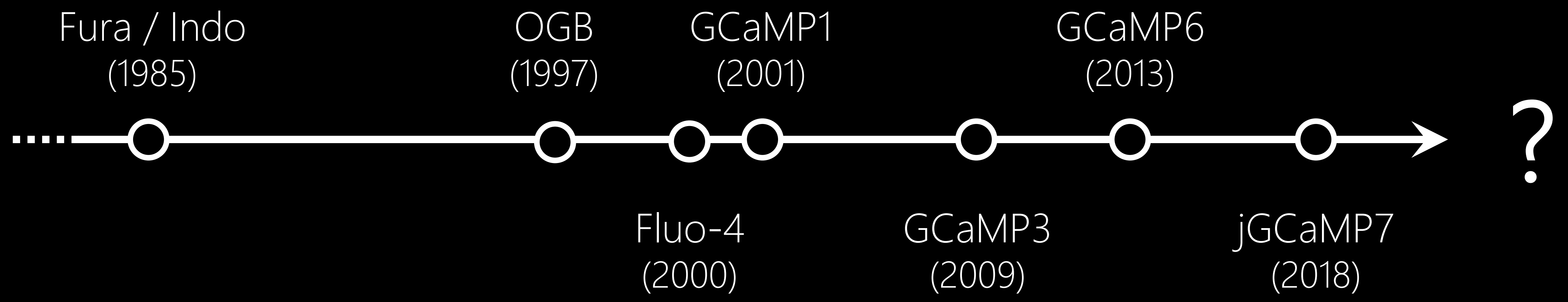


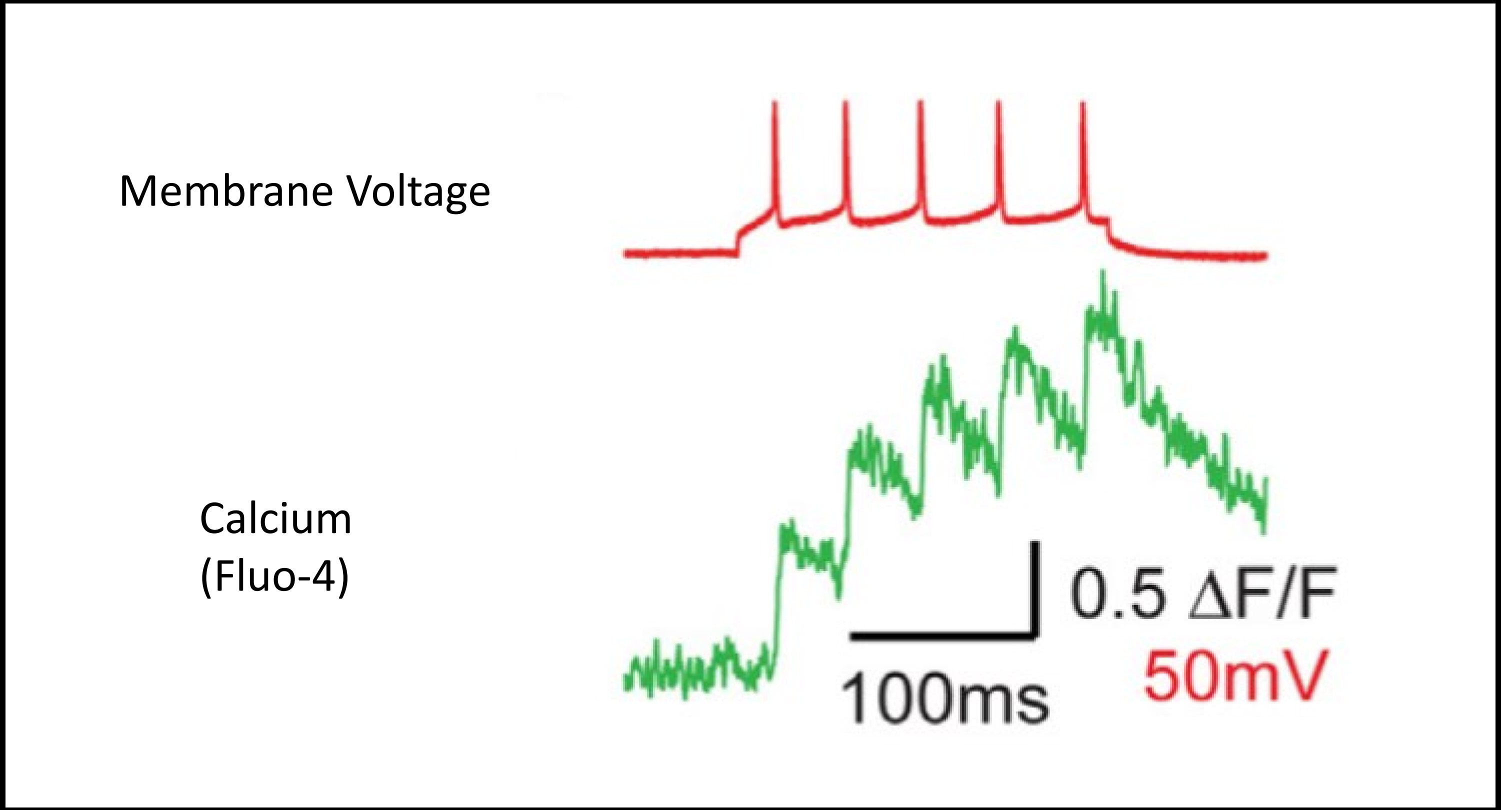
Sofroniew et al. 2016



Chen et al. 2013
Raster scanning, 5Hz
Primary visual cortex
Visual stimulation
Anaesthetized Mouse




Fura / Indo
(1985)
OGB
(1997)
GCaMP1
(2001)
GCaMP6
(2013)
Fluo-4
(2000)
GCaMP3
(2009)
jGCaMP7
(2018)
?



Fernández-Alfonso et al 2013

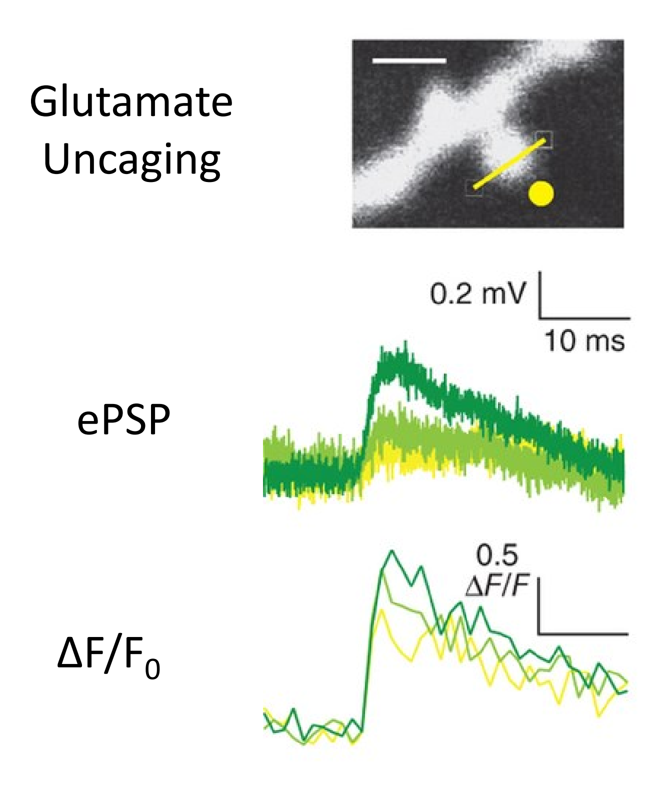
New Indicators

- Neurotransmitters
- Voltage

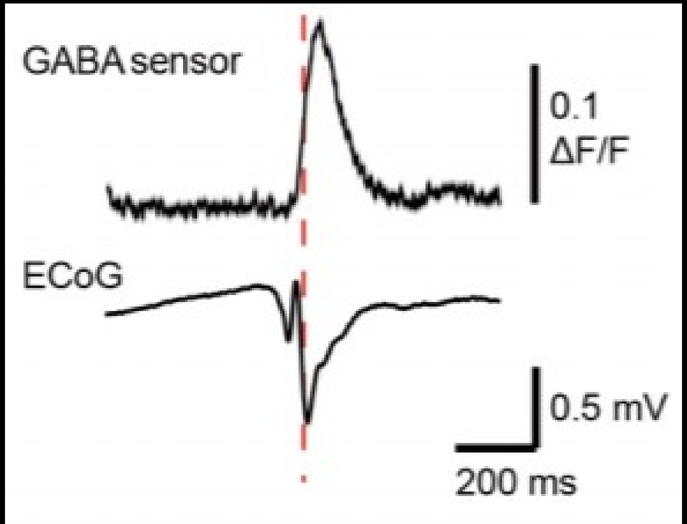
Neurotransmitters

- Glutamate (Marvin et al 2013, Marvin et al 2018a)
- GABA (Marvin et al 2018b)
- Acetylcholine (Borden et al, in prep)

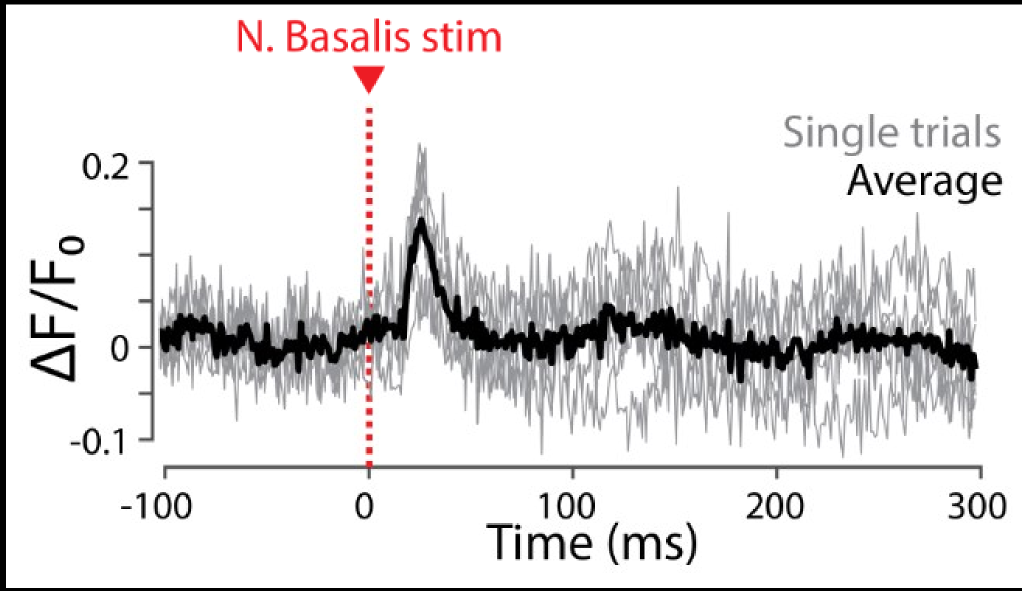
iGluSnFR



iGABASnFR



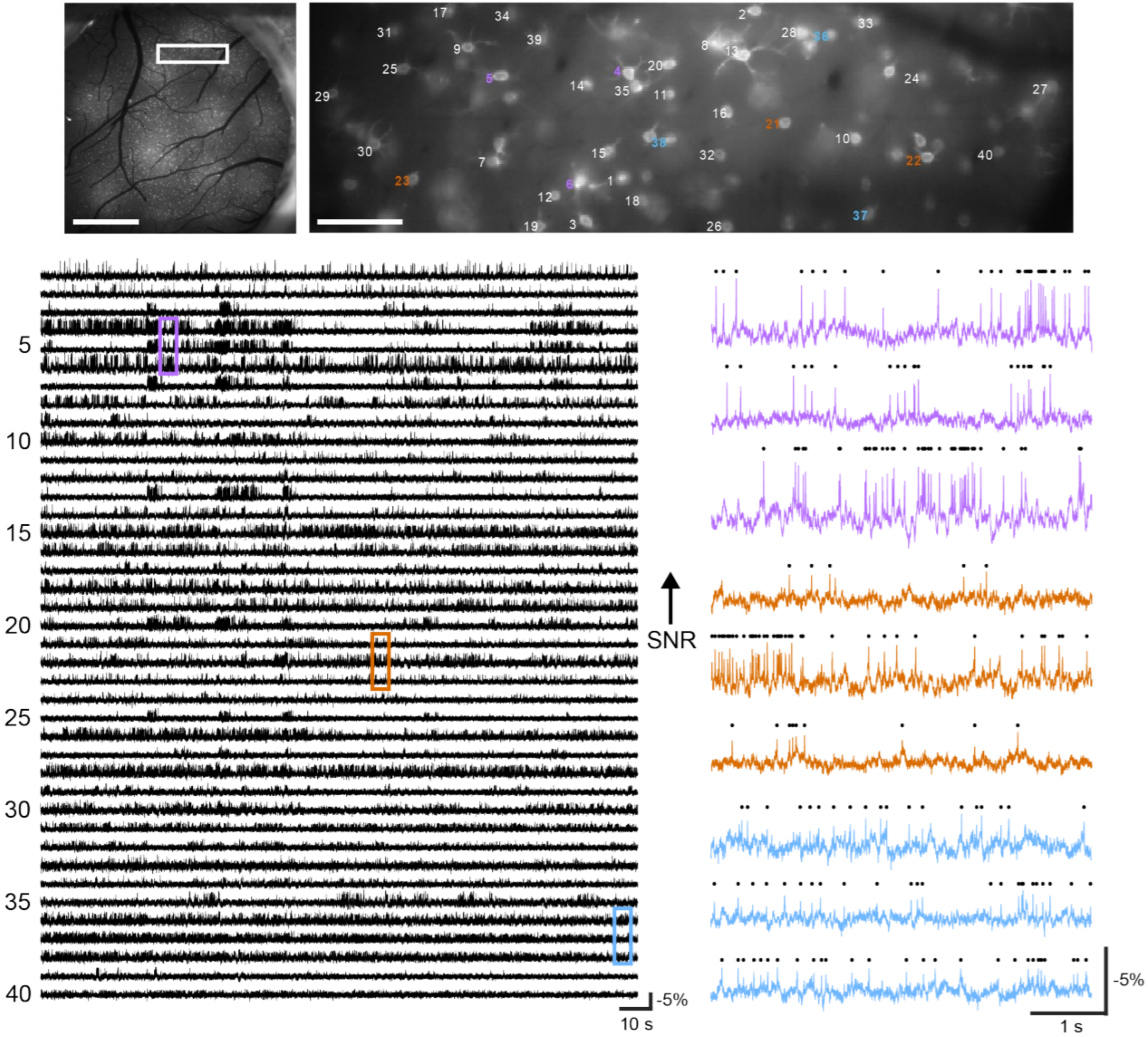
iAChSnFR



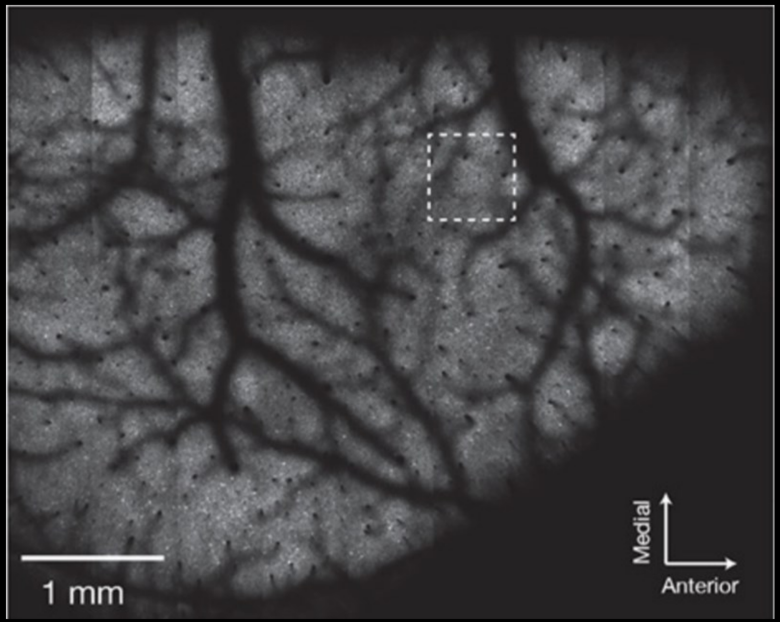
Voltron

Abdelfattah et al 2019

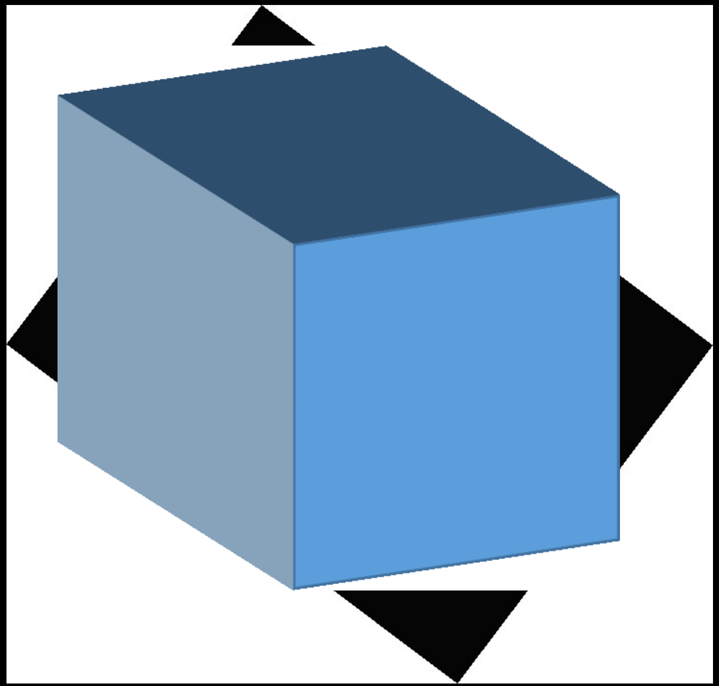
Layer 1 interneurons (NDNF-Cre)



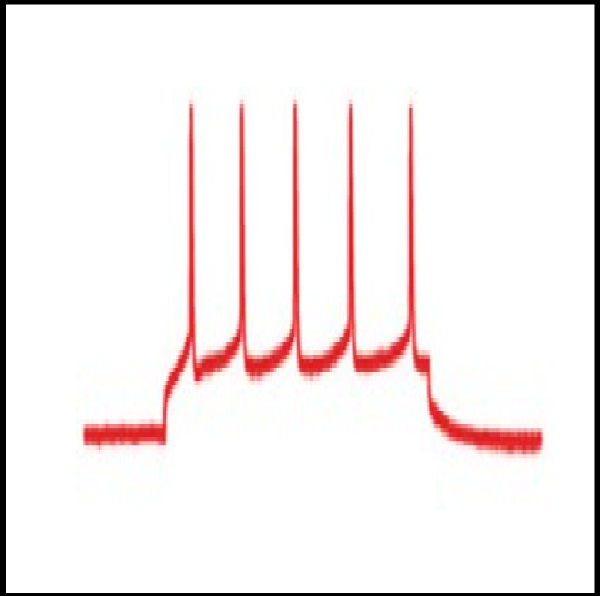
Need for Speed



Larger Areas

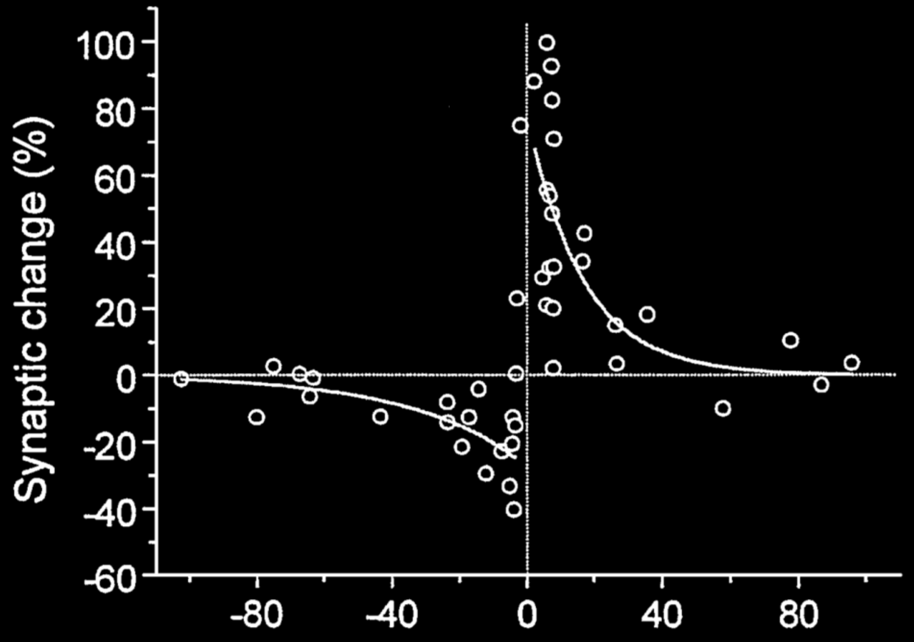


Volume Imaging



Faster Indicators

Milliseconds matter!

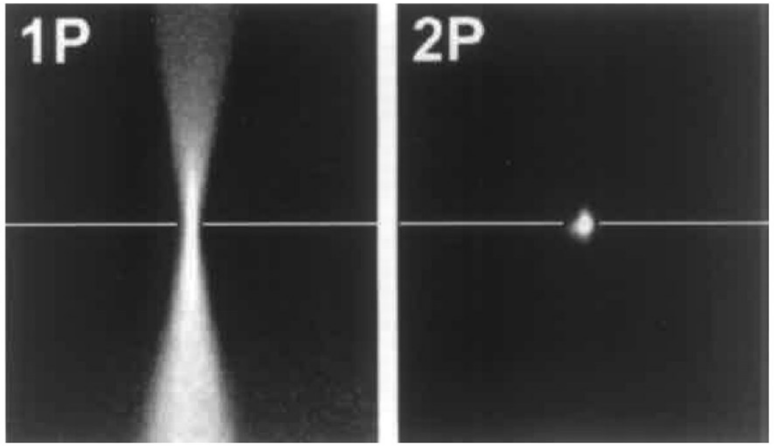


Spike time post-pre (msec)

Spike-Timing-Dependent Plasticity

Bi et al. 2001

Raster-Scanning Two Photon



~I $\quad$ ~I^2

Soeller C, Cannell MB, 1999

Raster Scan

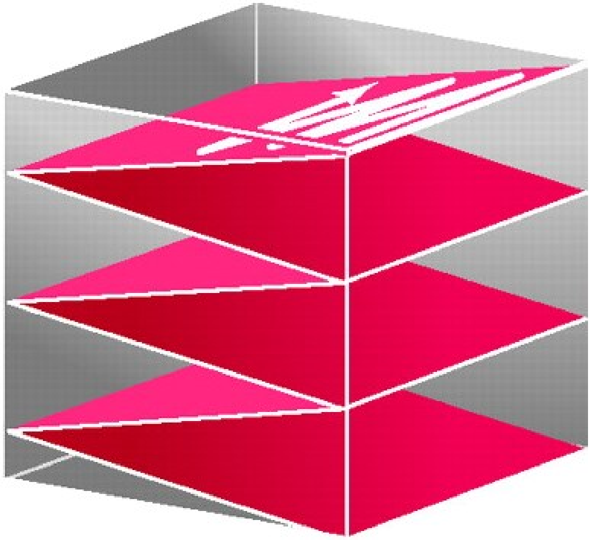
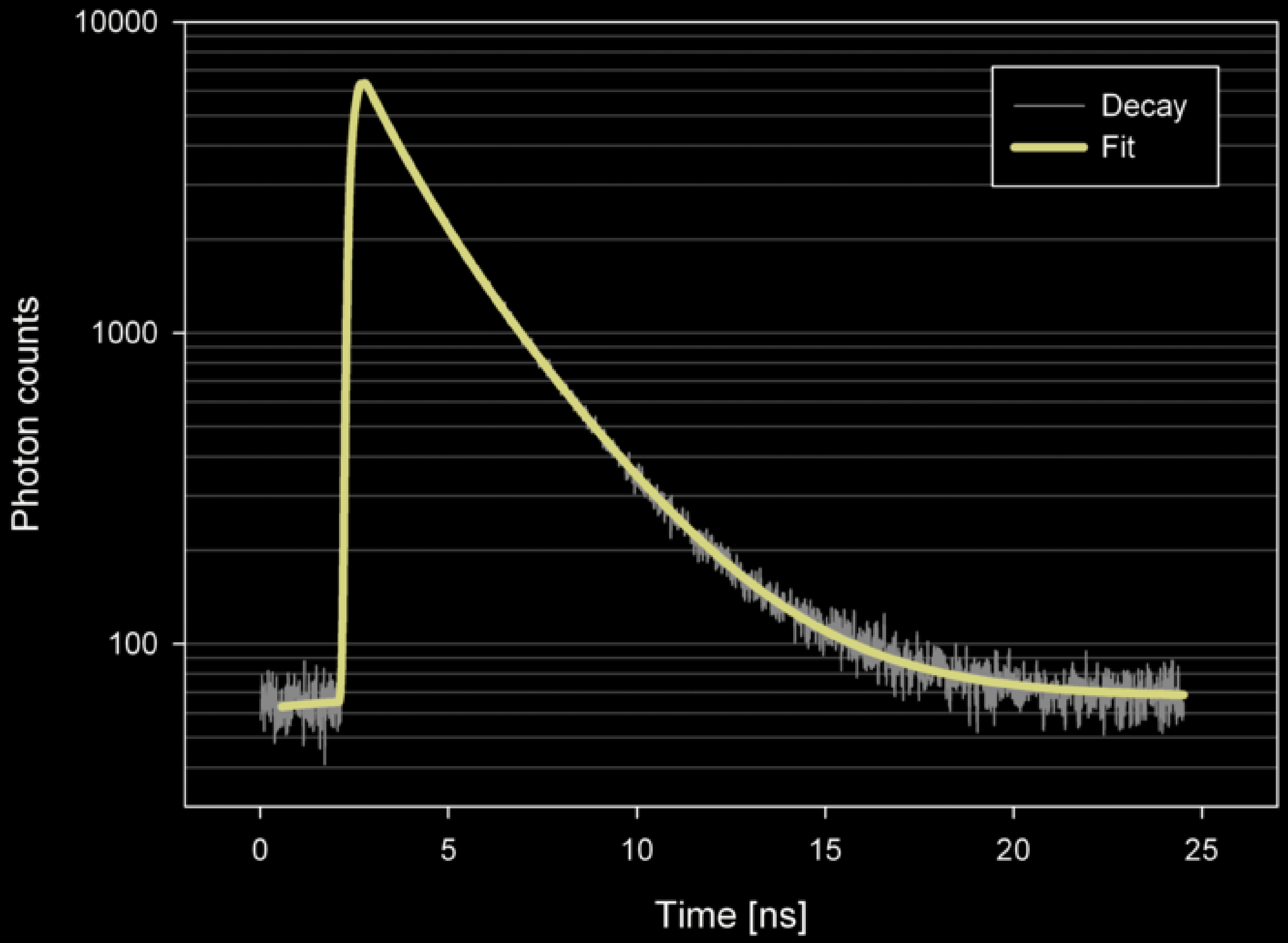


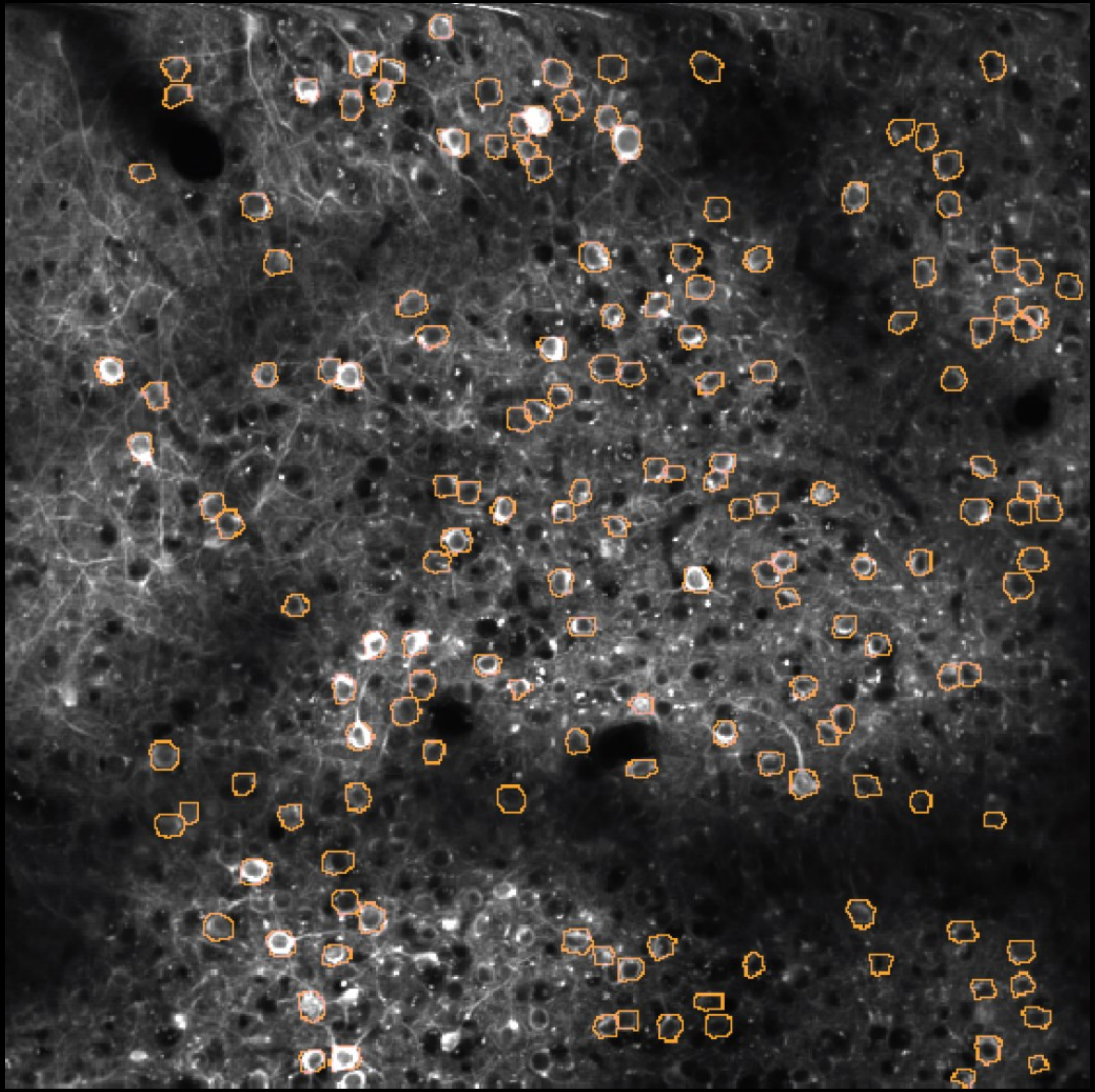
Image: Goebel & Helmchen 2007



Fluorescence half-life ~2.5 ns

~10 ns to emit 95% of photons
Maximum 10^8 sequential measurements/s

1 Megapixel @ 1 kHz = 10^9 measurements/s



Can we match our measurements to the variables we care about?

Image: Nick Sofroniew

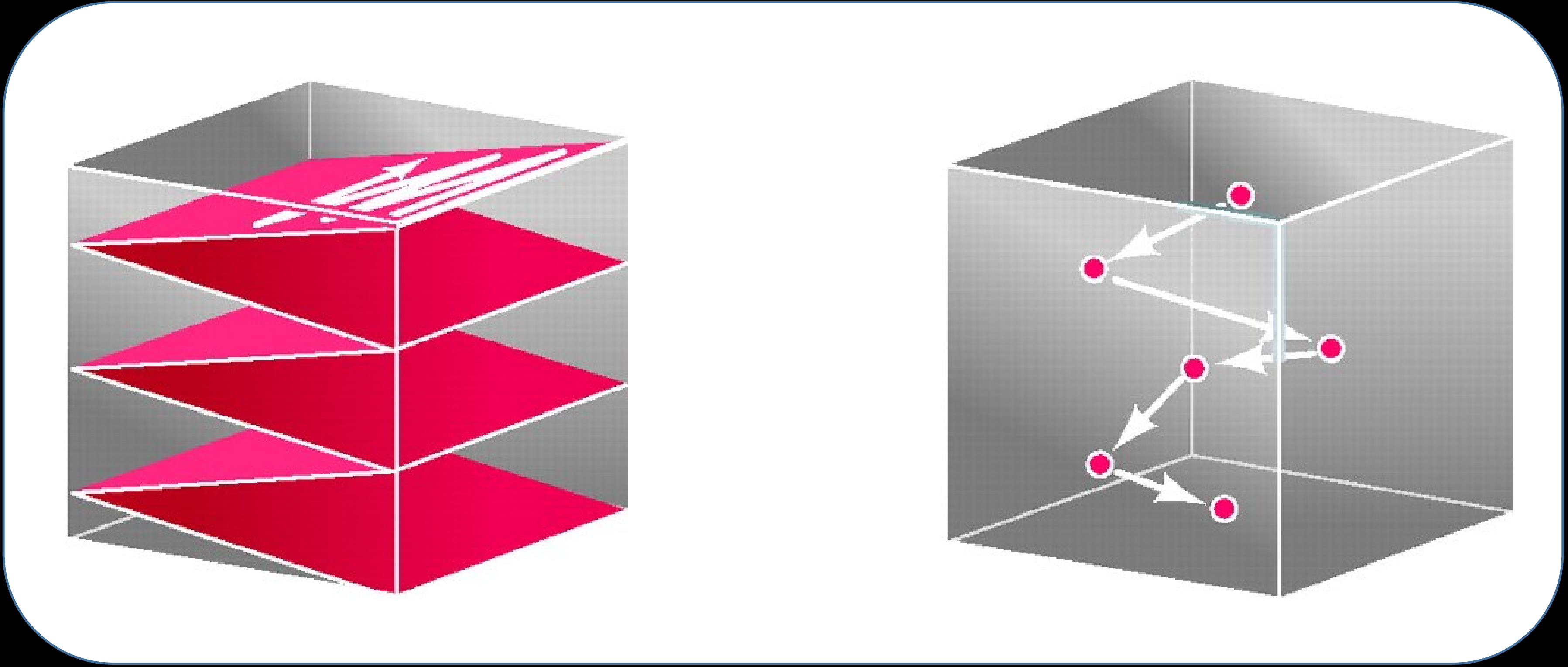
Efficient sampling in laser scanning microscopy

Random Access Imaging	(e.g. S. Dieudonne, P. Saggau, B. Rozsa, A. Silver, K Haas labs)
Projection Microscopy	
Axially-extended beams	(e.g. T. Wilson lab, Y. De Koninck lab, N. Ji lab, D. Tank lab)
Multifocal Multiphoton	(e.g. S. Hell lab, P. So lab, Yuste lab)
Efficient experimental design	(e.g. Vaziri lab, Paninski lab)

Random Access Imaging	Projection Microscopy
Only record spots you're interested in	Record the sum of several images, then unmix images computationally

Raster Scanning

Random Access Imaging



Images: Goebel & Helmchen 2007

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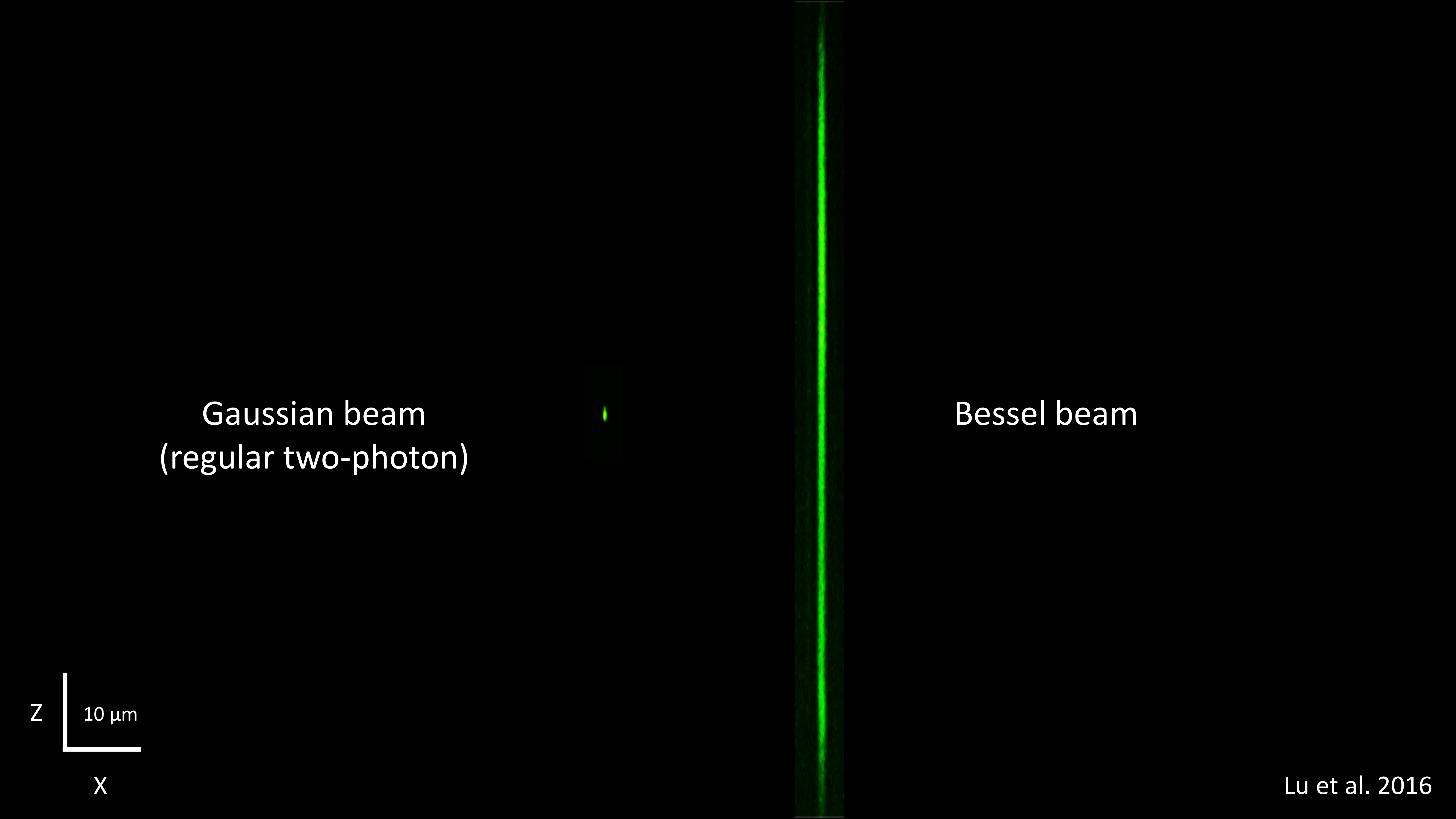
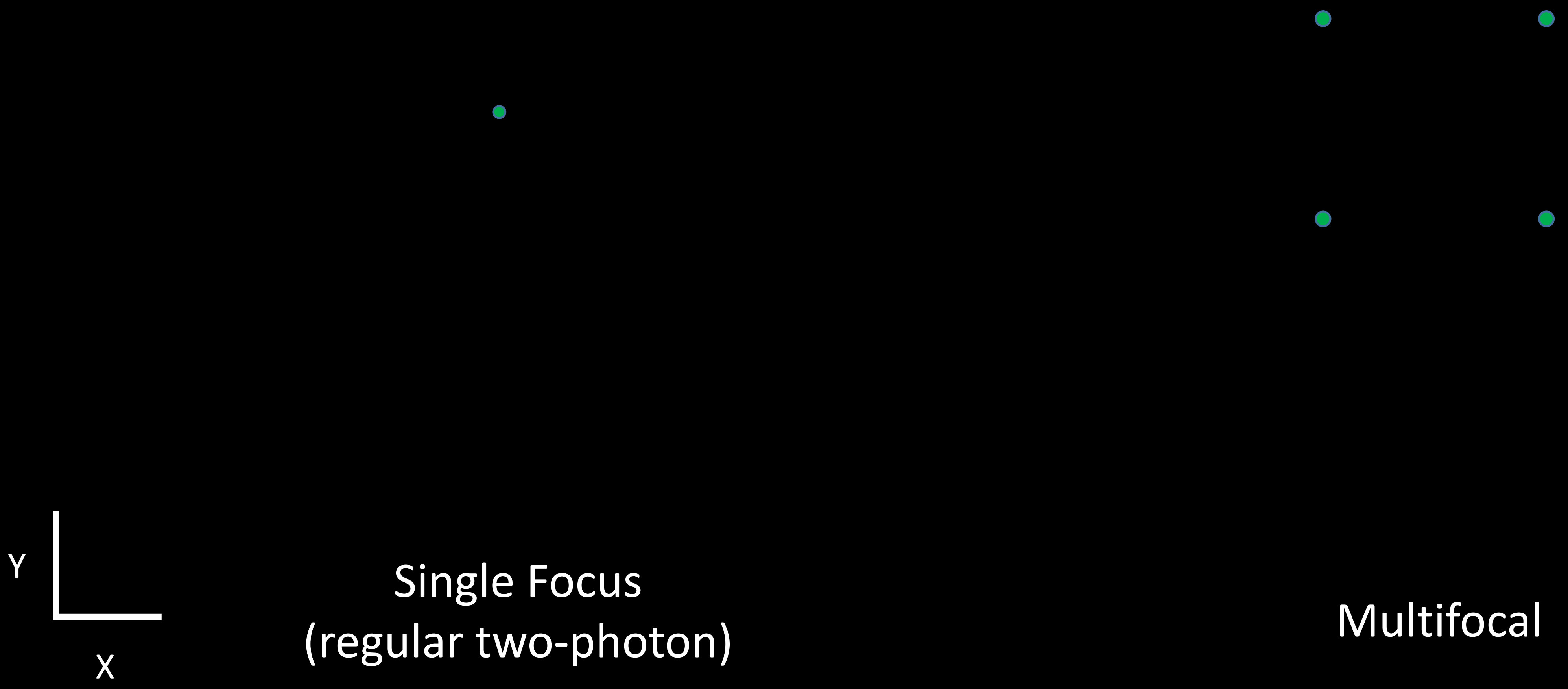
"Projection Microscopy"
Imaging methods that deliberately combine multiple voxels
into each measurement


Gaussian beam
(regular two-photon)
Bessel beam
Z
10 μm
X
Lu et al. 2016


Y
X
Single Focus
(regular two-photon)
Multifocal

Random Access Imaging	Projection Microscopy
Imaging sites must be selected in advance	Records from entire volume
Fast for small numbers of sources, Slow for large numbers of sources	Sample-independent frame rate
Sample motion causes lost data	Post-hoc motion registration
Simple analysis	Requires computational unmixing
Efficient two-photon excitation	Multiple foci require higher powers

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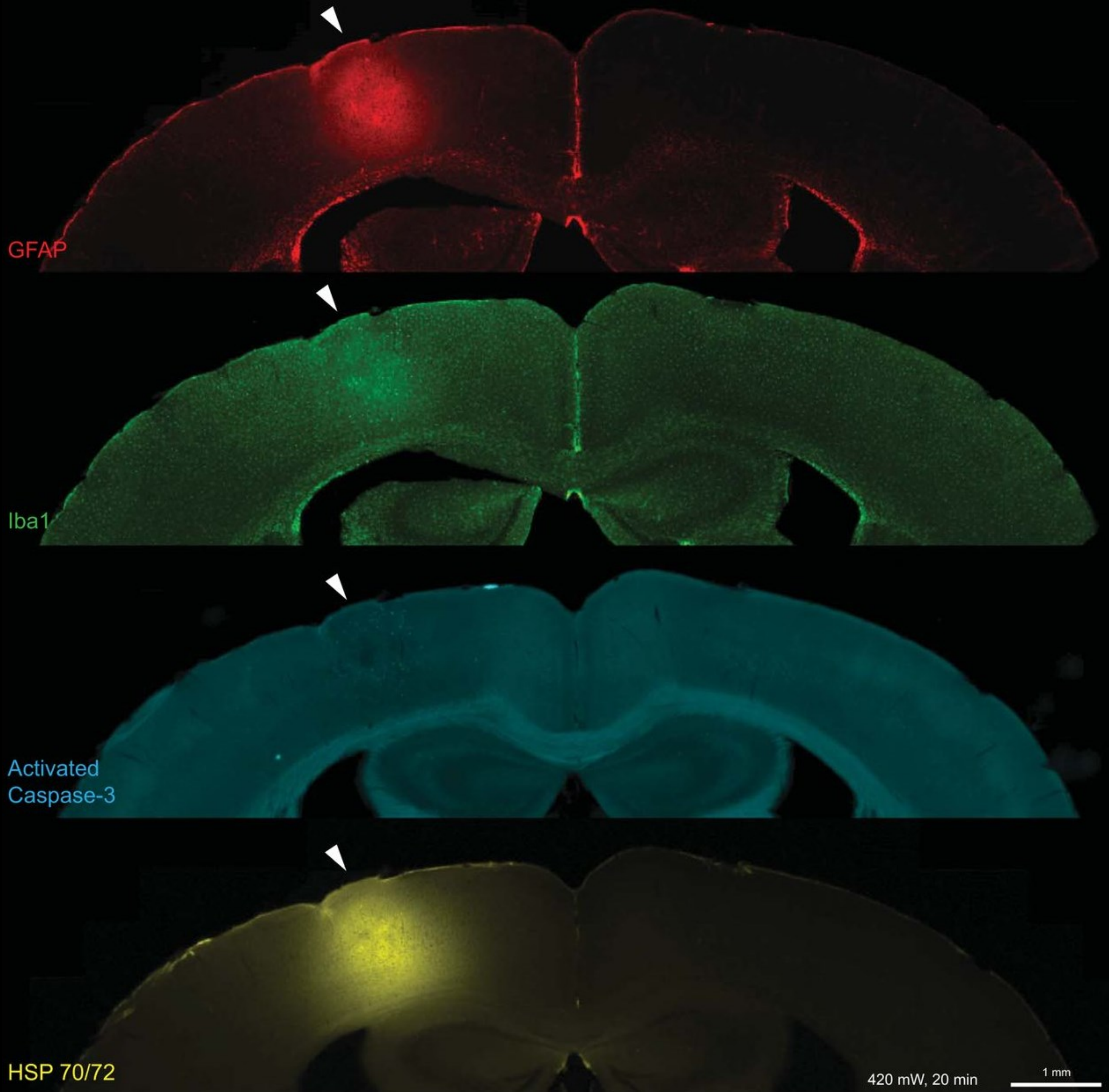
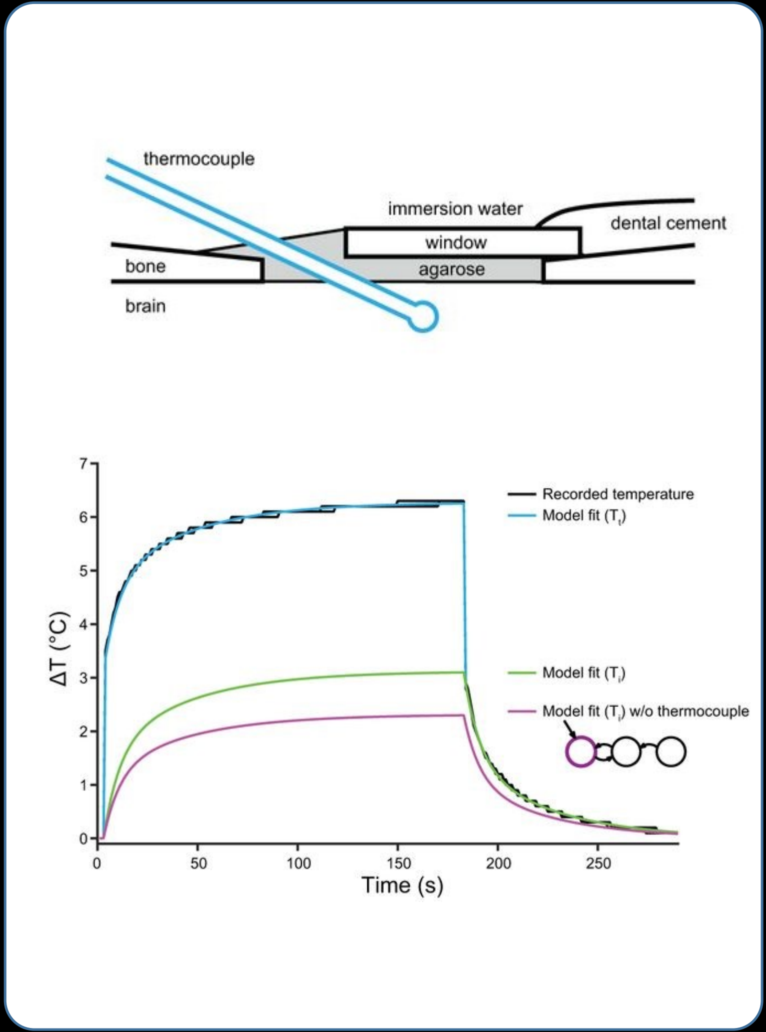


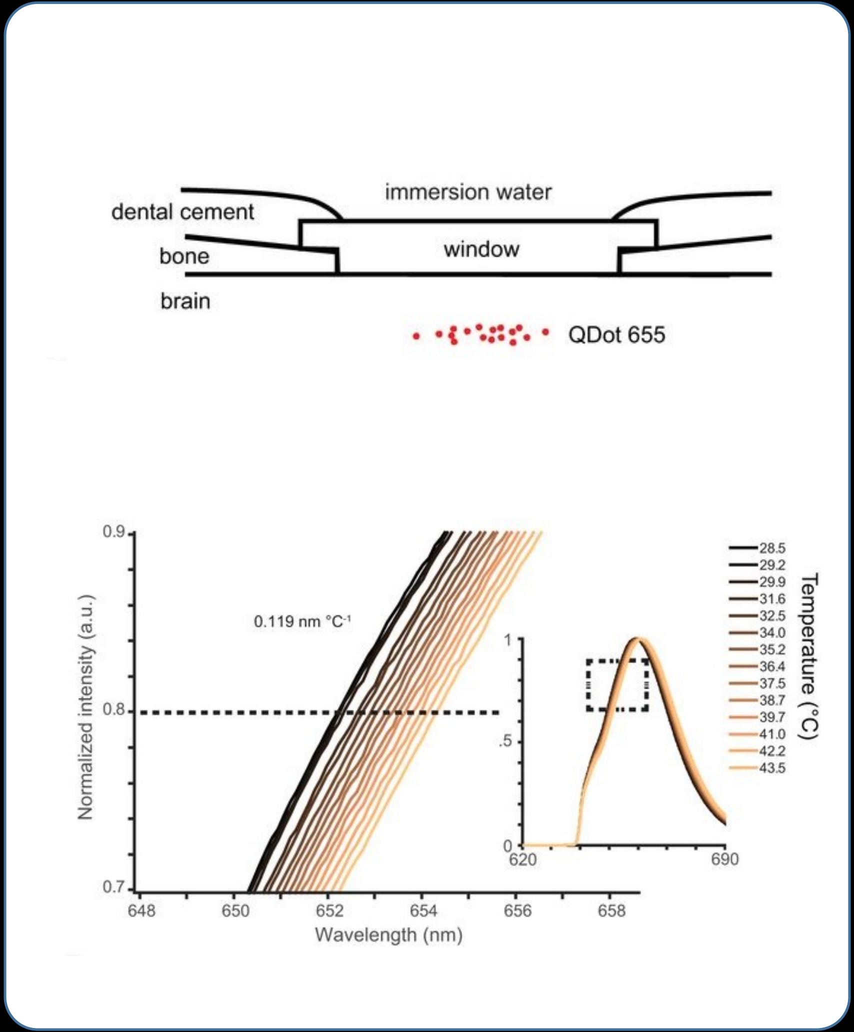
Brain heating by two-photon lasers

Podgorski & Ranganathan 2016

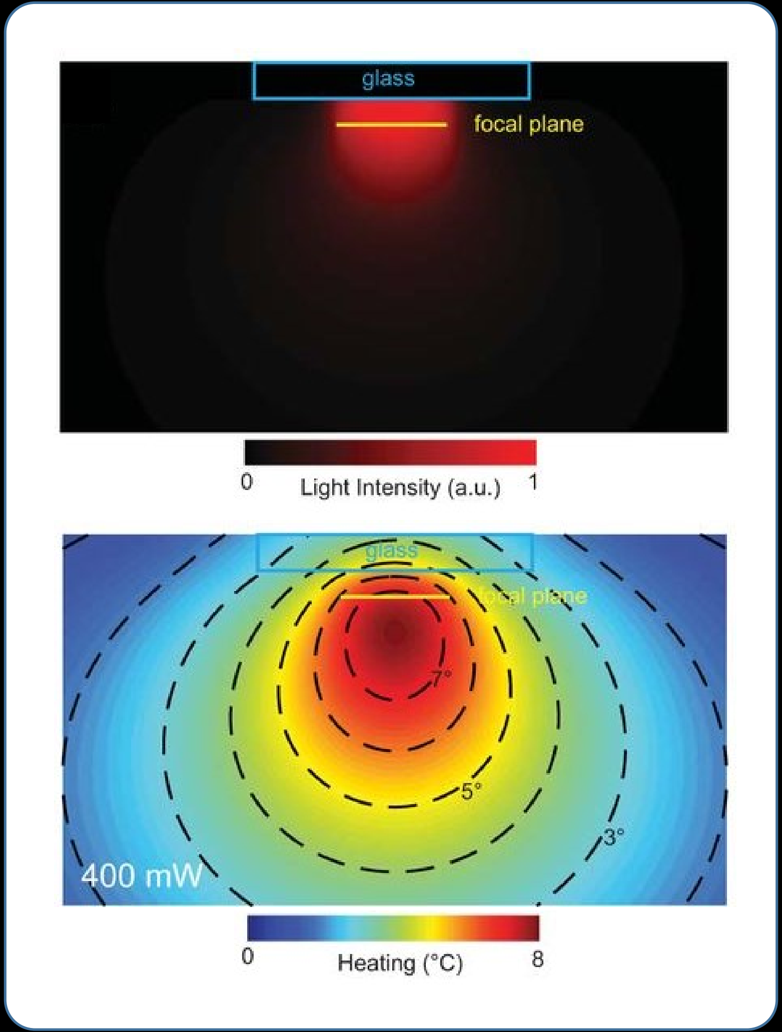
Thermocouple Measurements



Quantum Dot Nanothermometry



Simulations



Podgorski & Ranganathan 2016

Heating = 1.8°C/100 mW

Damage at >250 mW

Continuous illumination
1 mm field of view

Heating is the limiting form of photodamage
in typical 2-photon experiments

Projection microscopy should use
low degrees of parallelization

Podgorski & Ranganathan 2016

Random Access Imaging	Projection Microscopy
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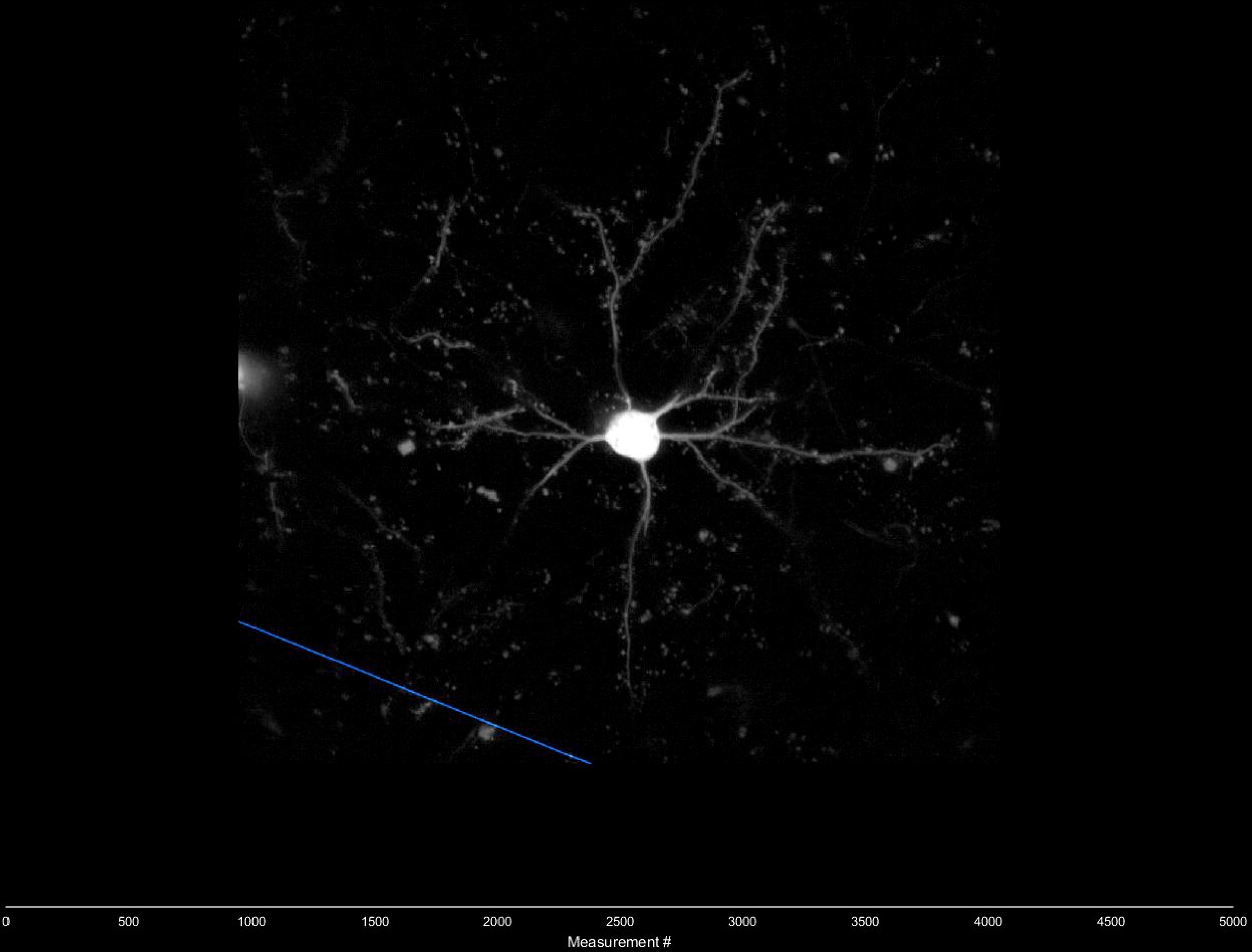
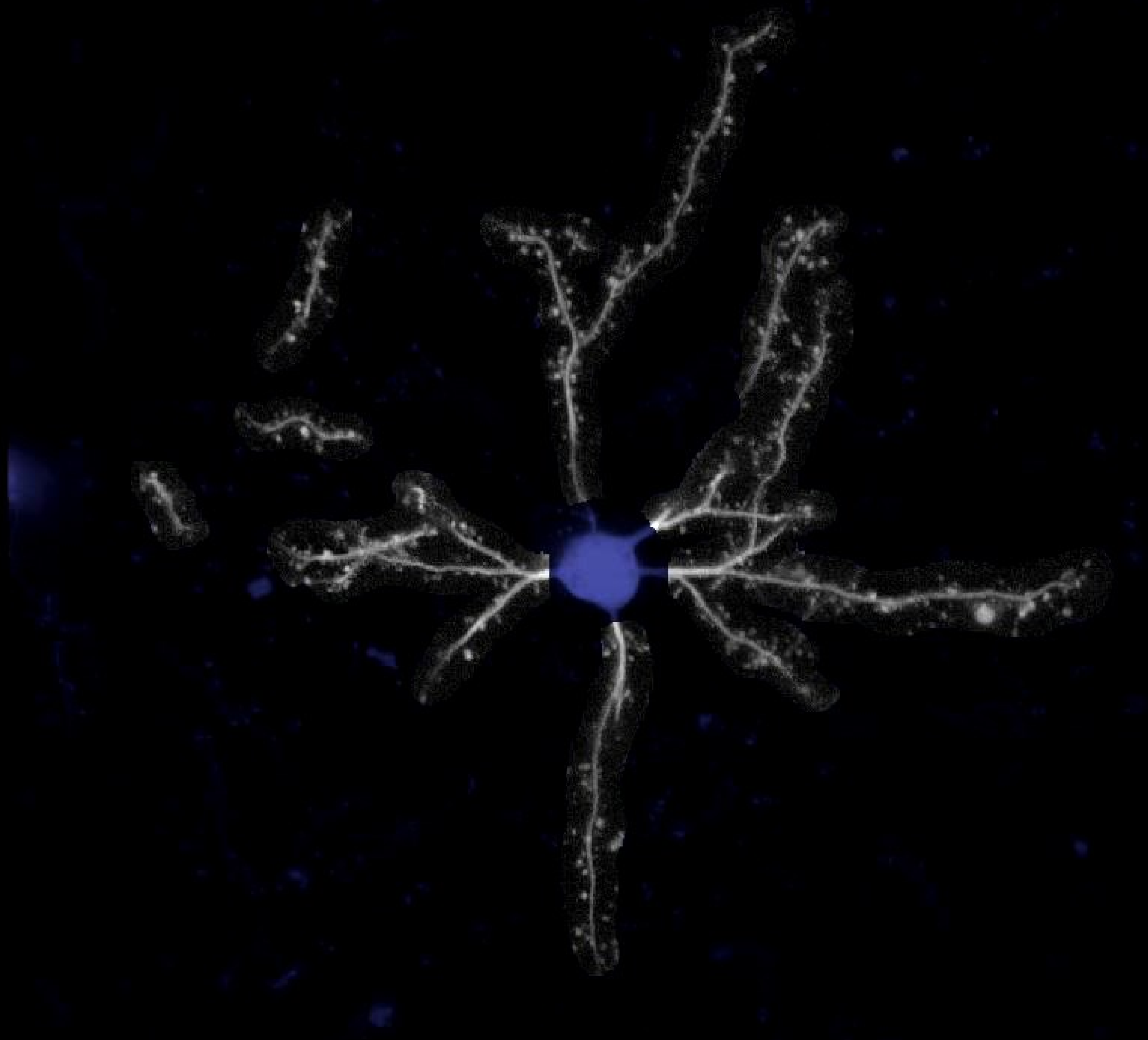


0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Measurement #

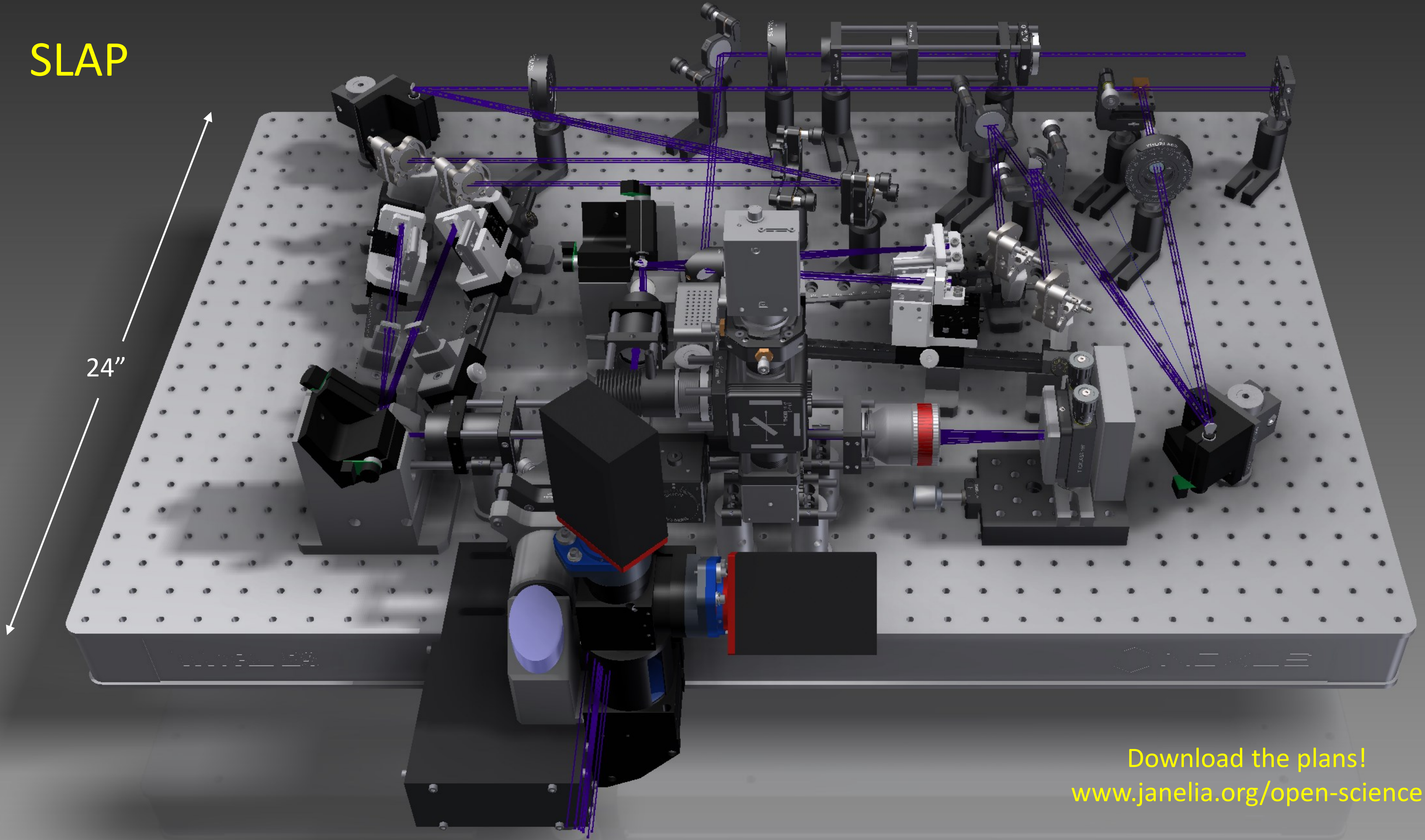


Spatial Light Modulation
Blue tinted regions blocked by SLM
Sparsity = 13%

Scanned Line Angular Projection microscopy

SLAP

SLAP



Download the plans!
www.janelia.org/open-science

SLAP characteristics

• High resolution	matches raster 2P resolution along scan axis
• High speed	$O(N^2)$ voxels acquired in $O(N)$ measurements
• Insensitive to scattering	2P excitation, non-descanned detection
• Insensitive to sample motion	Efficiently records an area surrounding each ROI
• Accurate source unmixing	Tomographic measurements are a low-coherence basis
• Moderate excitation power, below damage thresholds	SLM blocking reduces degree of parallelization Power needed <140 mW *in vivo*, <40 mW *in vitro*

SLAP characteristics

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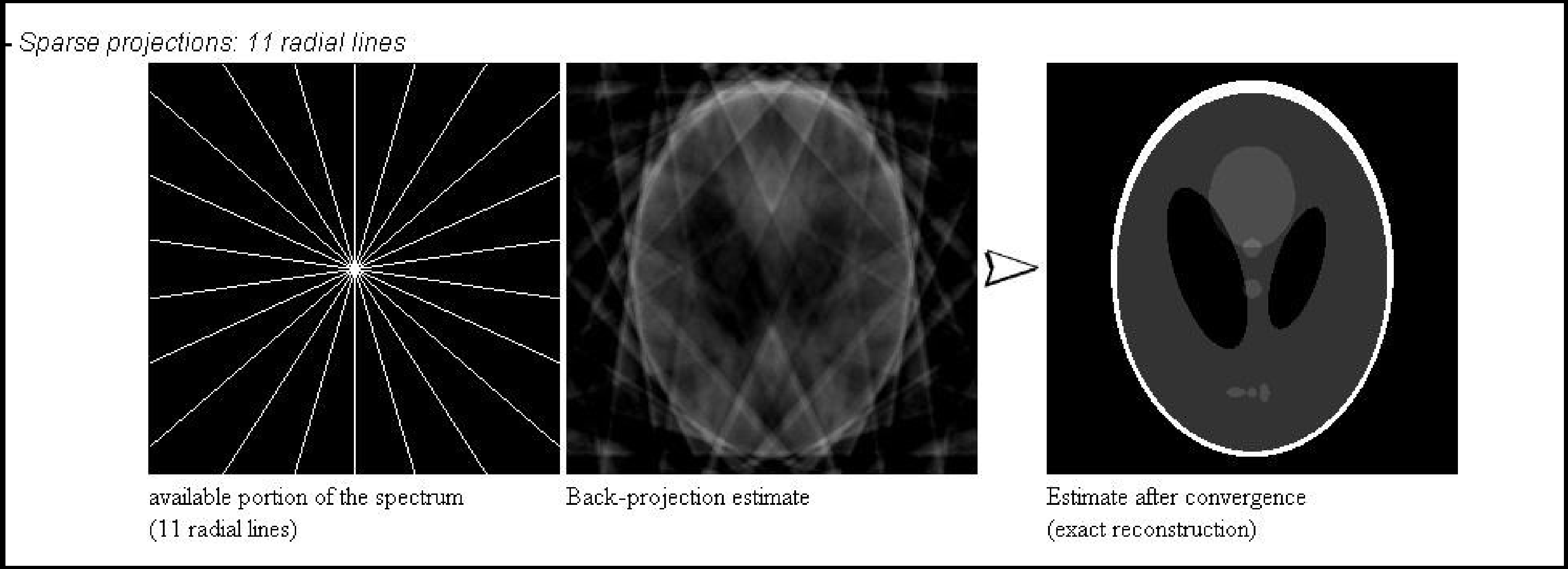
- Moderate excitation power, below damage thresholds — SLM blocking reduces degree of parallelization; Power needed <140 mW *in vivo*, <40 mW *in vitro*

Source Recovery

- 5000 measurements per frame are not enough to recover an arbitrary 1,000,000-pixel image
- However, real images are highly structured
- Prior information allows image recovery from small numbers of structured measurements

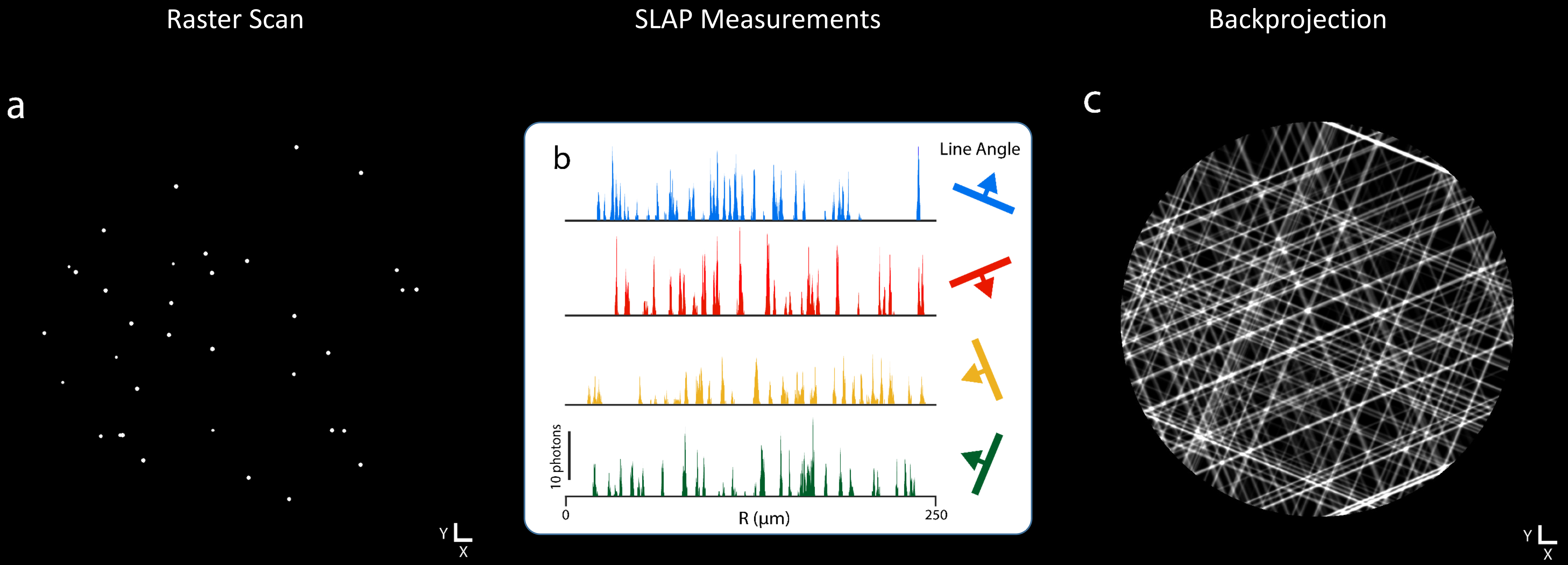


Sparse MRI
Lustig,Donoho, Pauly 2007

Sparse CT
Kudo, Suzuki, Rashed 2013

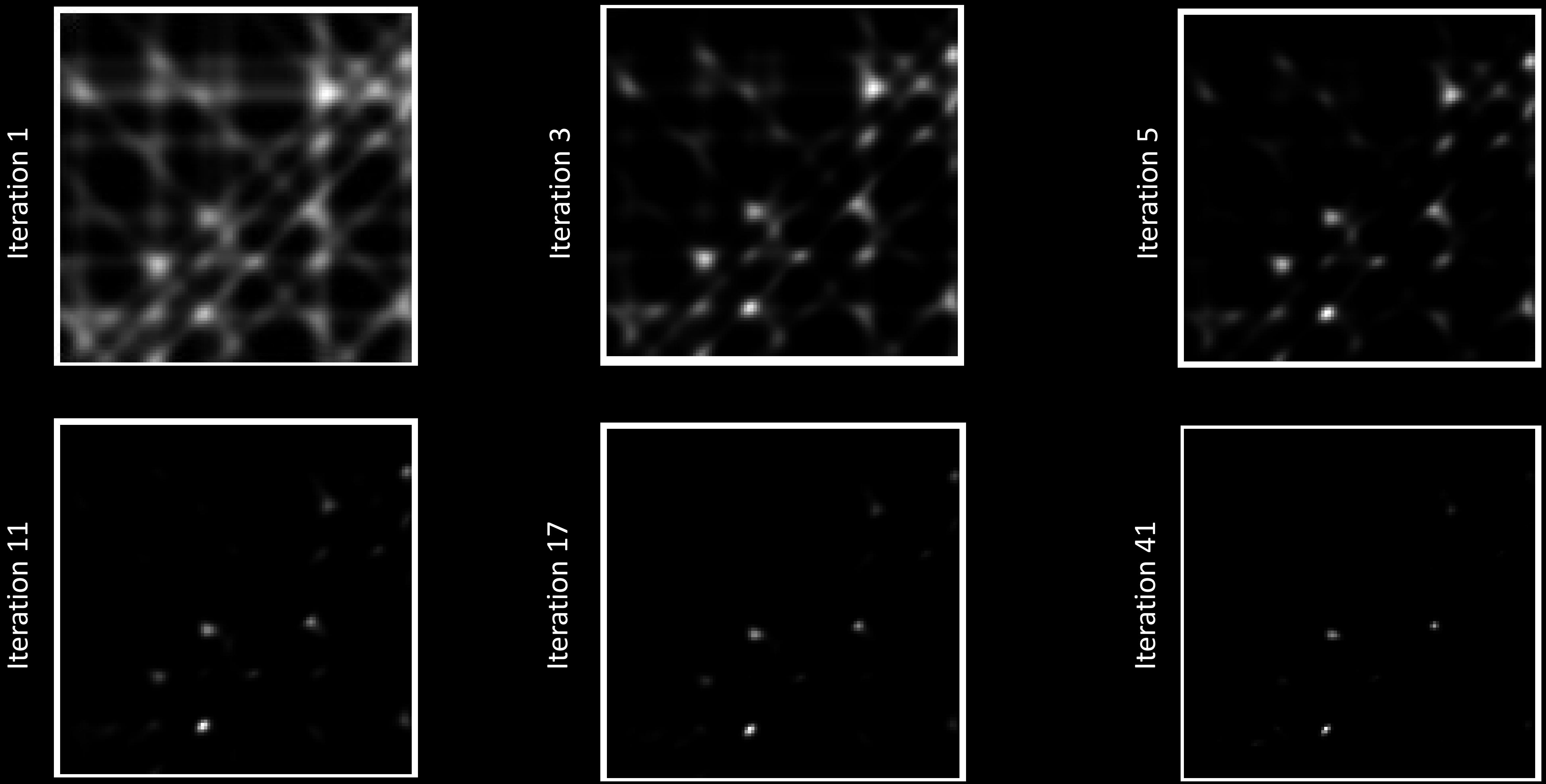
Egiazarian et al 2010

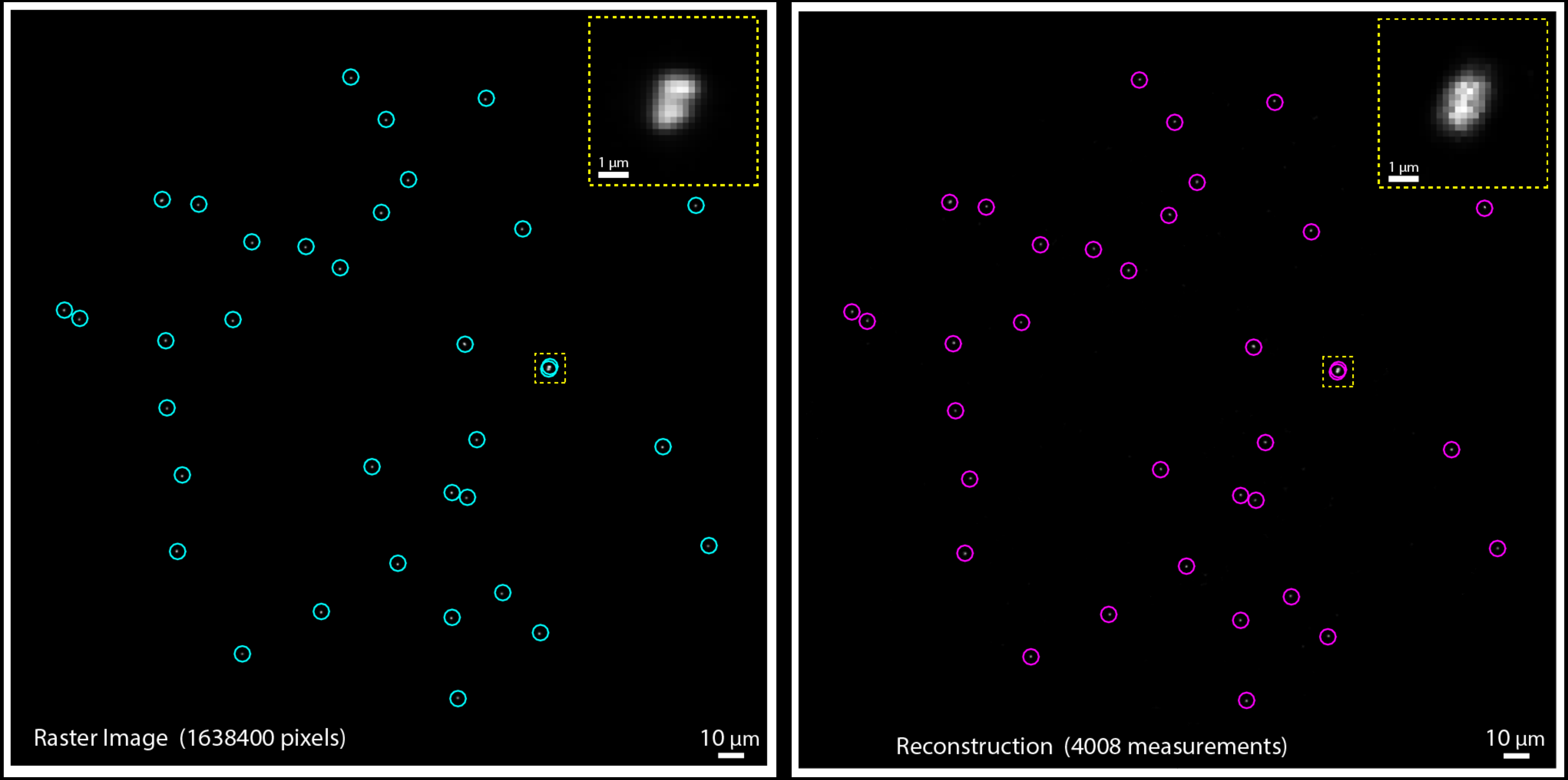
Particle Localization and Tracking

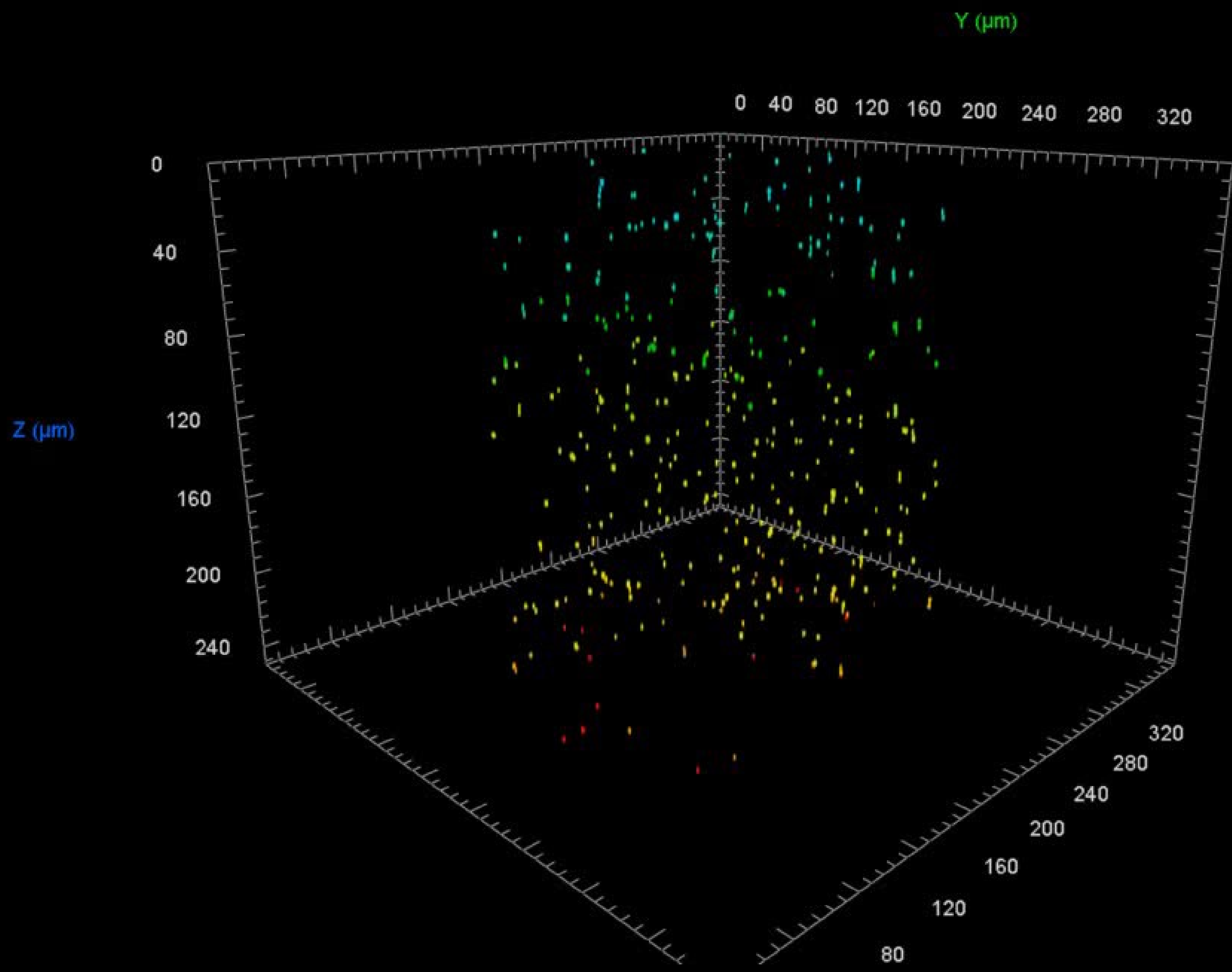


Raster Scan
SLAP Measurements
Backprojection
a
b
c
Line Angle
10 photons
0
250
R (µm)
Y
X
Y
X

Richardson-Lucy Deconvolution




1 µm
Raster Image (1638400 pixels)
10 µm
1 µm
Reconstruction (4008 measurements)
10 µm



1.49x10^{9} voxels/sec

SLAP

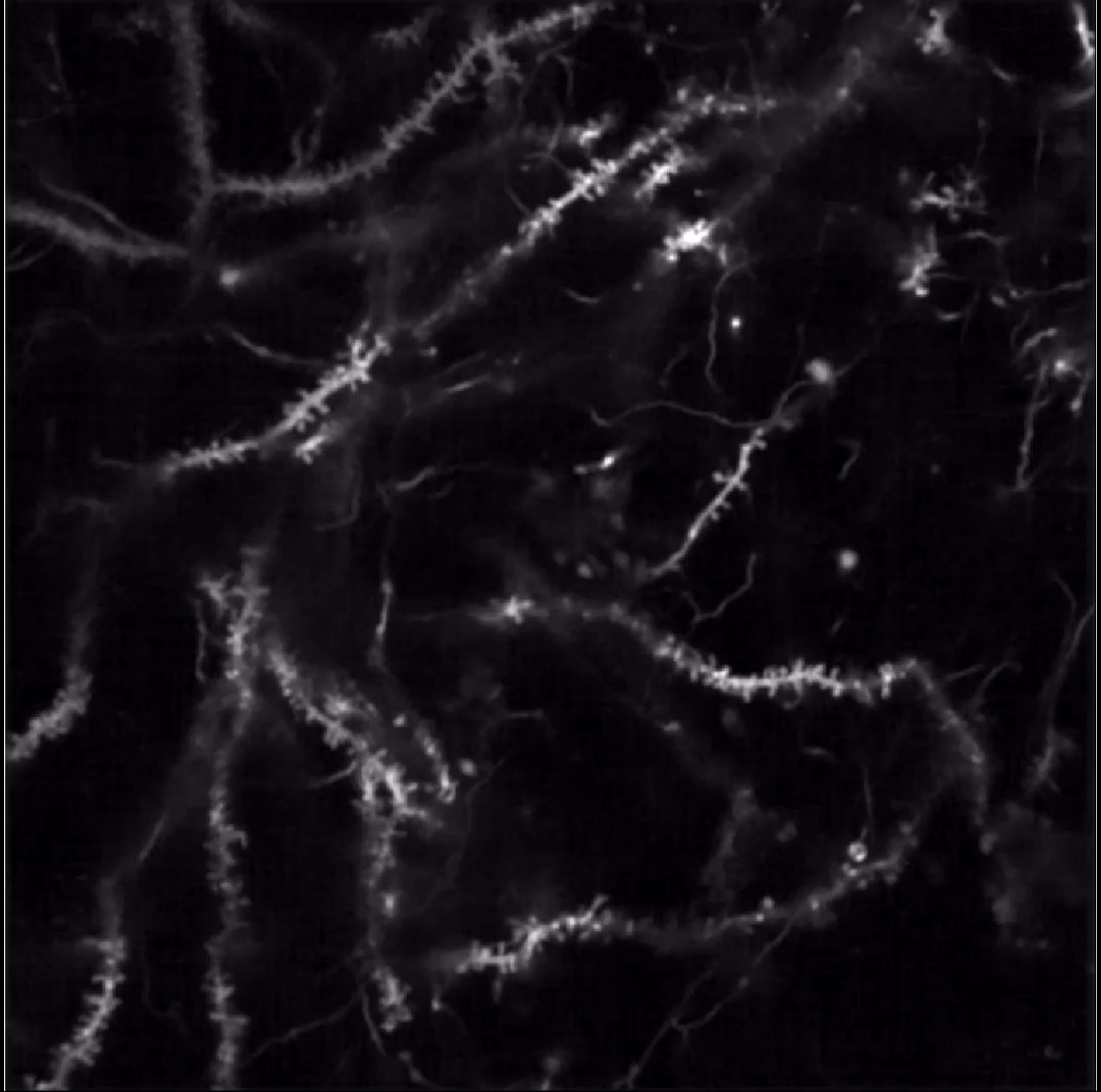
(1250x1250) x 1000 Hz =
1.5 billion voxels per second

5,000 measurements per frame

Raster Scan Speed Limit

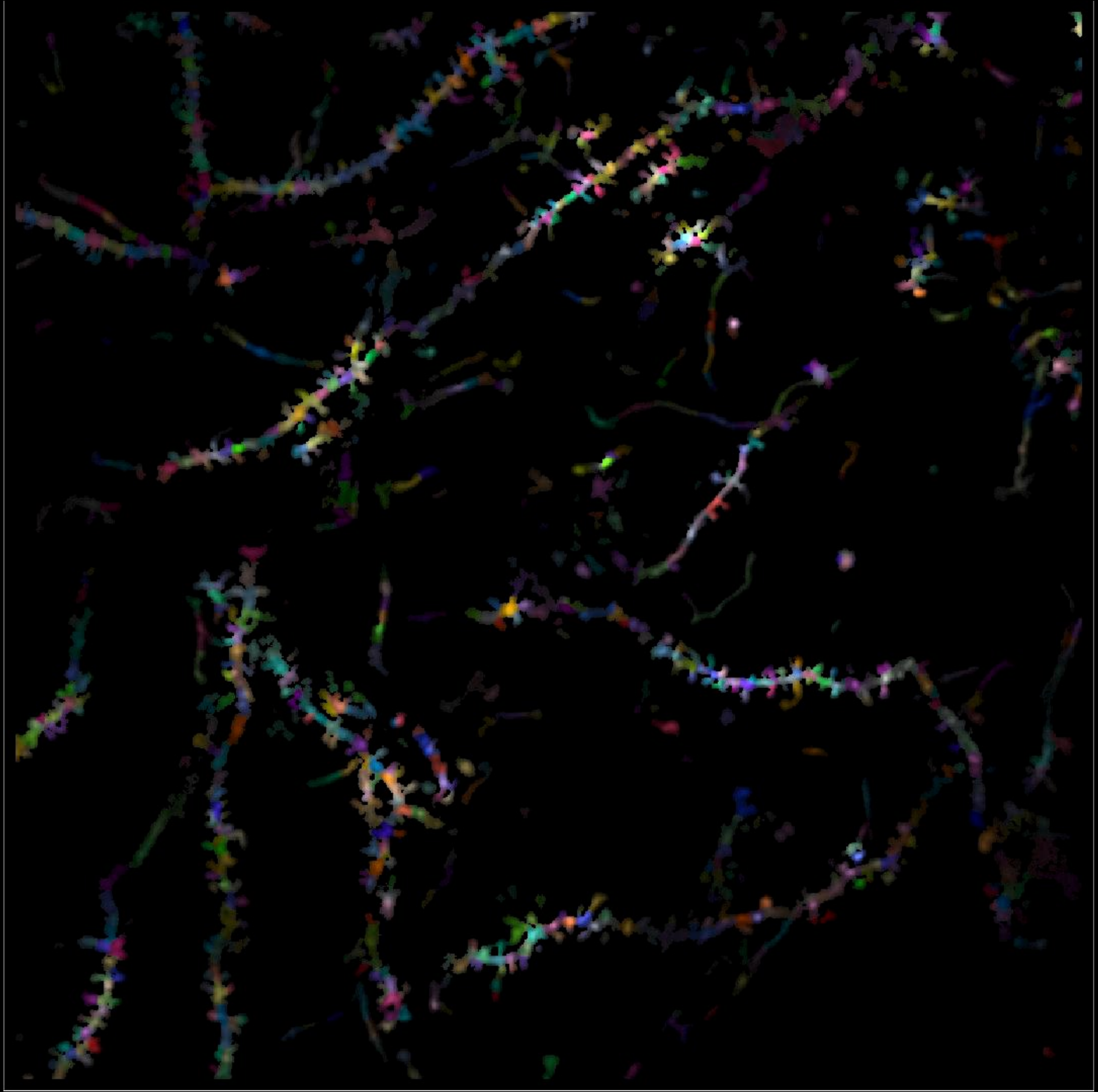
1/(10 ns)= 100 million voxels per second
'Typical' microscopes <10 million voxels/s

Imaging Neural Activity



Raster Reference Volume

Layer 2/3 pyramidal neuron dendrites, mouse neocortex
gamma = 0.5 (dim features emphasized)
11 slices at 0.75 μm spacing
256 μm FOV
120 μm below dura



3D Segmentation
~600 compartments/plane

256 μm FOV
120 μm below dura

Model

Projection Matrix (#Measurements x #Voxels)

Because we measure photons

Segmentation (#Voxels x #Segments)

Measurements

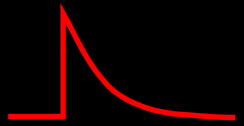
Fluorescence (#Segments x #Frames)

$$\mathbf{y}_t \sim \text{Poisson}\left(\mathbf{PSF}_t + \mathbf{b}_t\right)$$

$$\mathbf{F}_t = \theta \mathbf{F}_{t-1} + \mathbf{w}_t$$

All terms ≥ 0

Dynamics



Baseline

Spikes (#Segments x #Frames)

Estimate this

No explicit regularization needed. **S** is low rank, problem is overdetermined.
Well-conditioned for nearly all samples

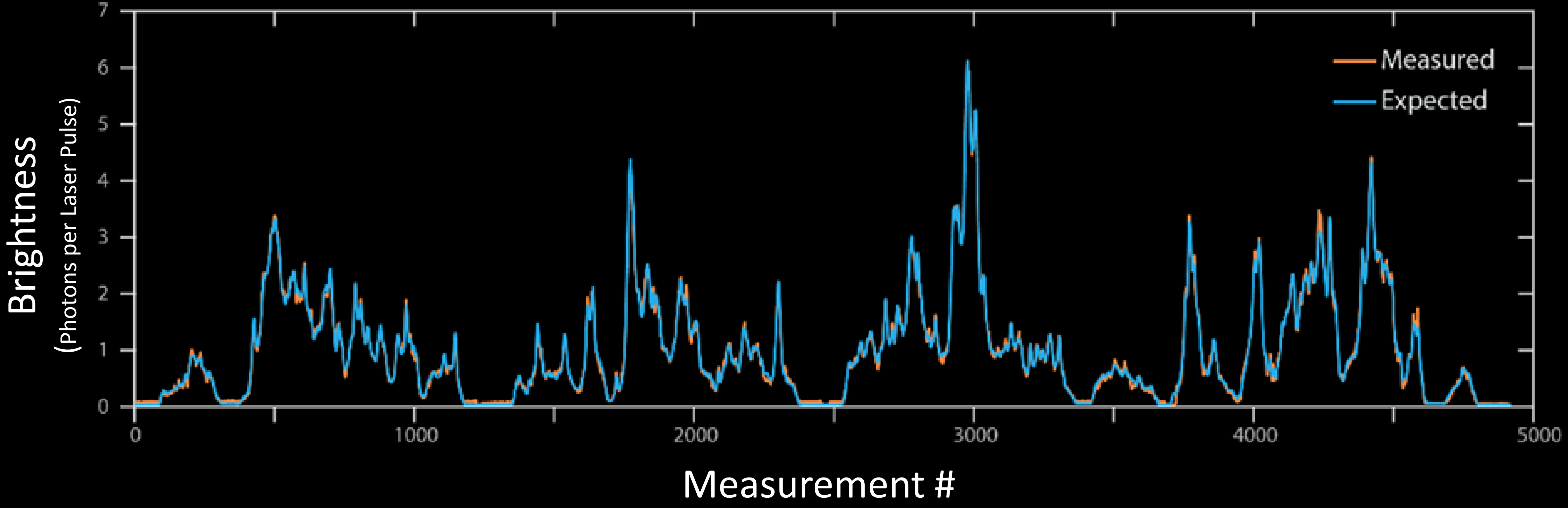
Projection Matrix

$$\mathbf{y}_t \sim \text{Poisson}\left(\mathbf{PSF}_t + \mathbf{b}_t\right)$$

$$\mathbf{F}_t = \theta \mathbf{F}_{t-1} + \mathbf{w}_t$$

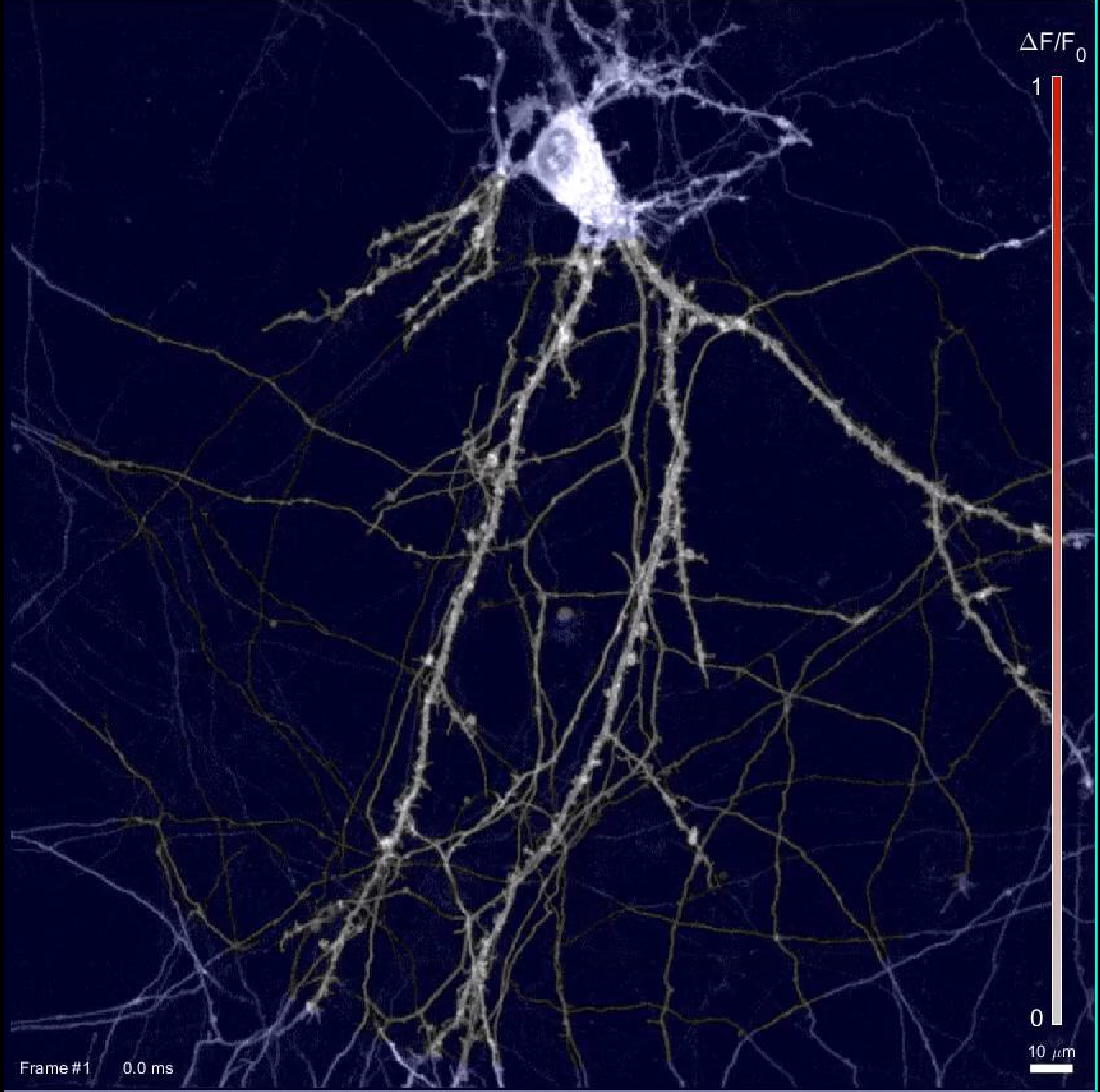
All terms ≥ 0

Need a precise and accurate model of the measurement process

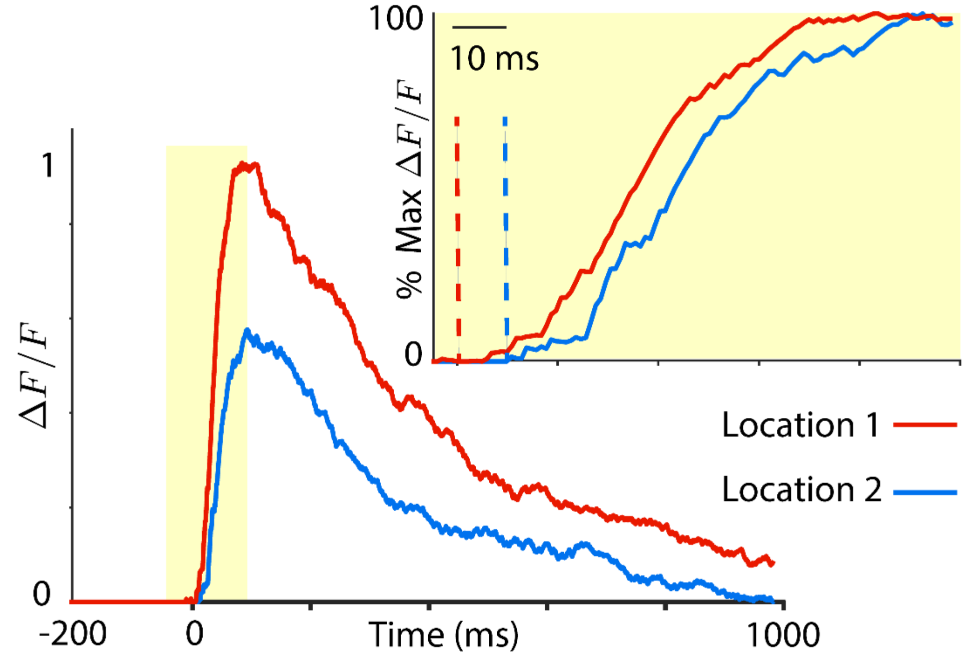


Mouse Cortex
110 um below dura

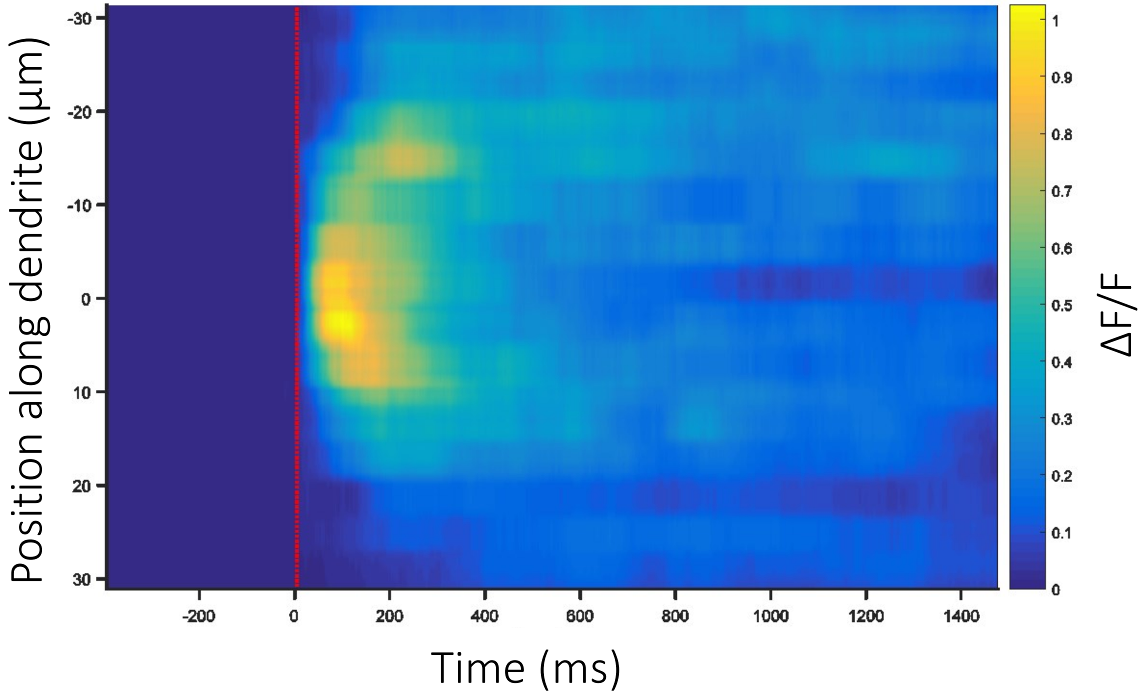
In Vitro Validation



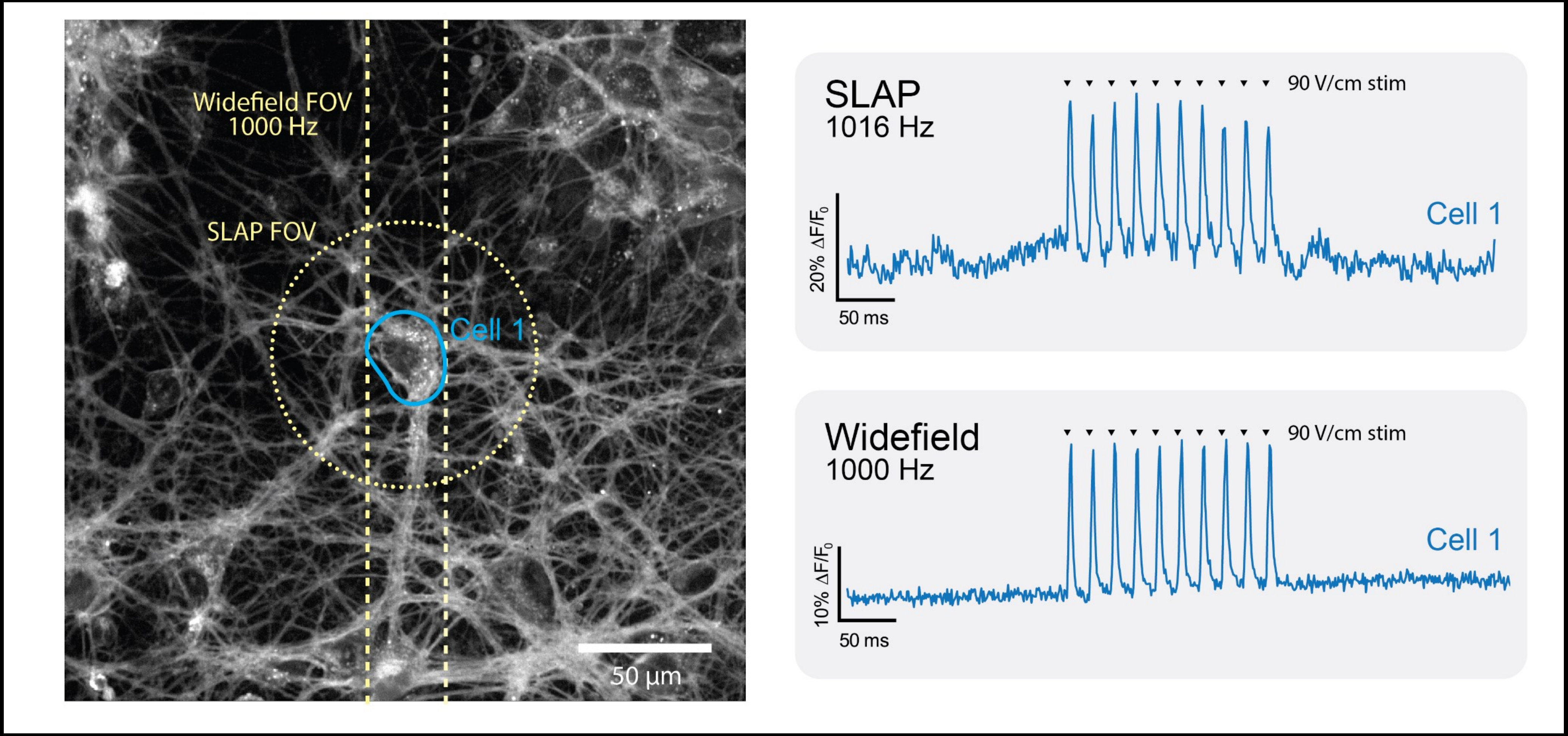
Primary hippocampal culture DIV 19
SF-Venus-iGluSnFR
1016 Hz
Single Trial
Glutamate uncaging at two locations, 10ms apart
Blue-tinted regions are blocked by SLM
256 μm FOV
gamma = 0.5



100
10 ms
% Max ΔF/F
0
1
ΔF/F
0
-200
0
Time (ms)
1000
Location 1
Location 2

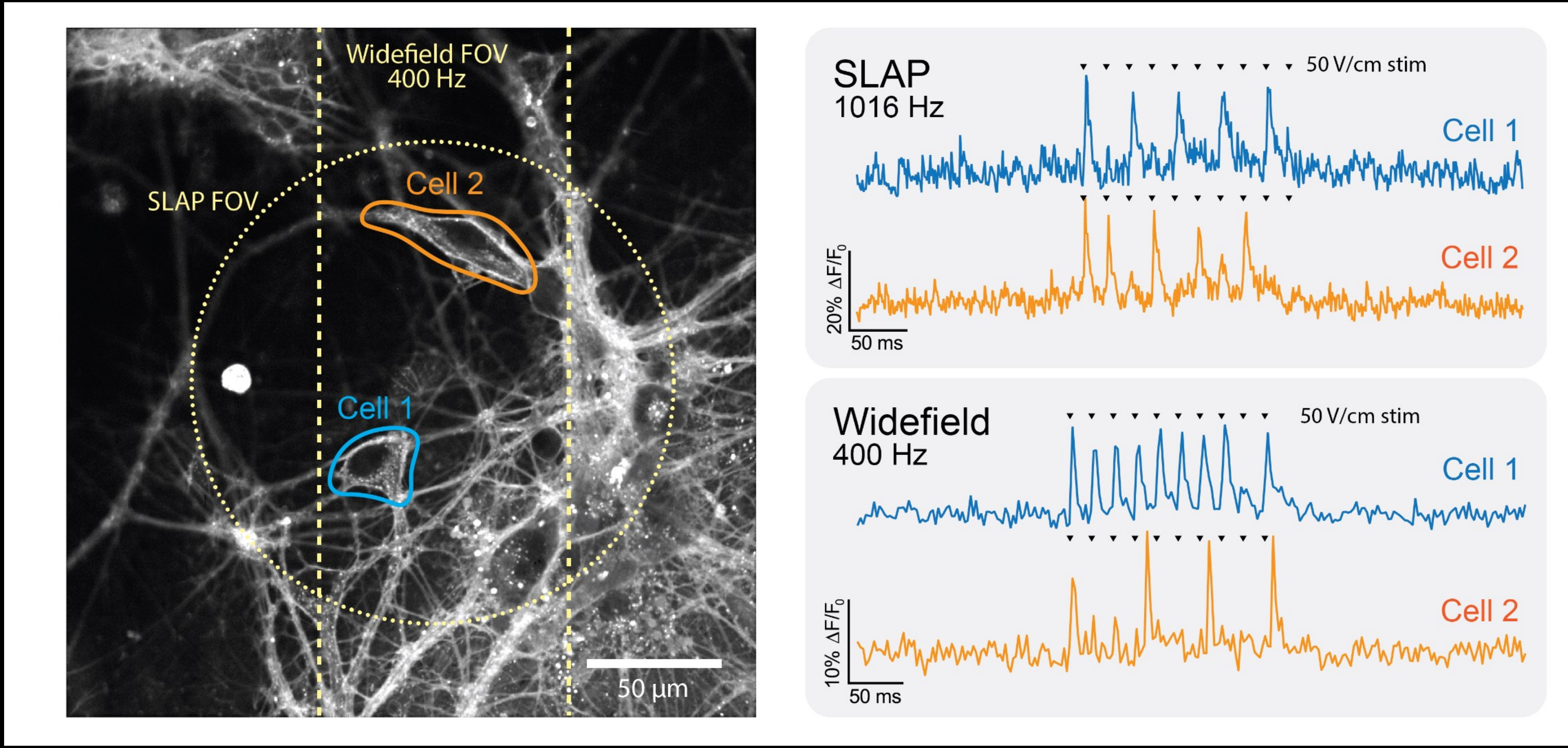


Position along dendrite (μm)
-30
-20
-10
0
10
20
30
-200
0
200
400
600
800
1000
1200
1400
Time (ms)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ΔF/F

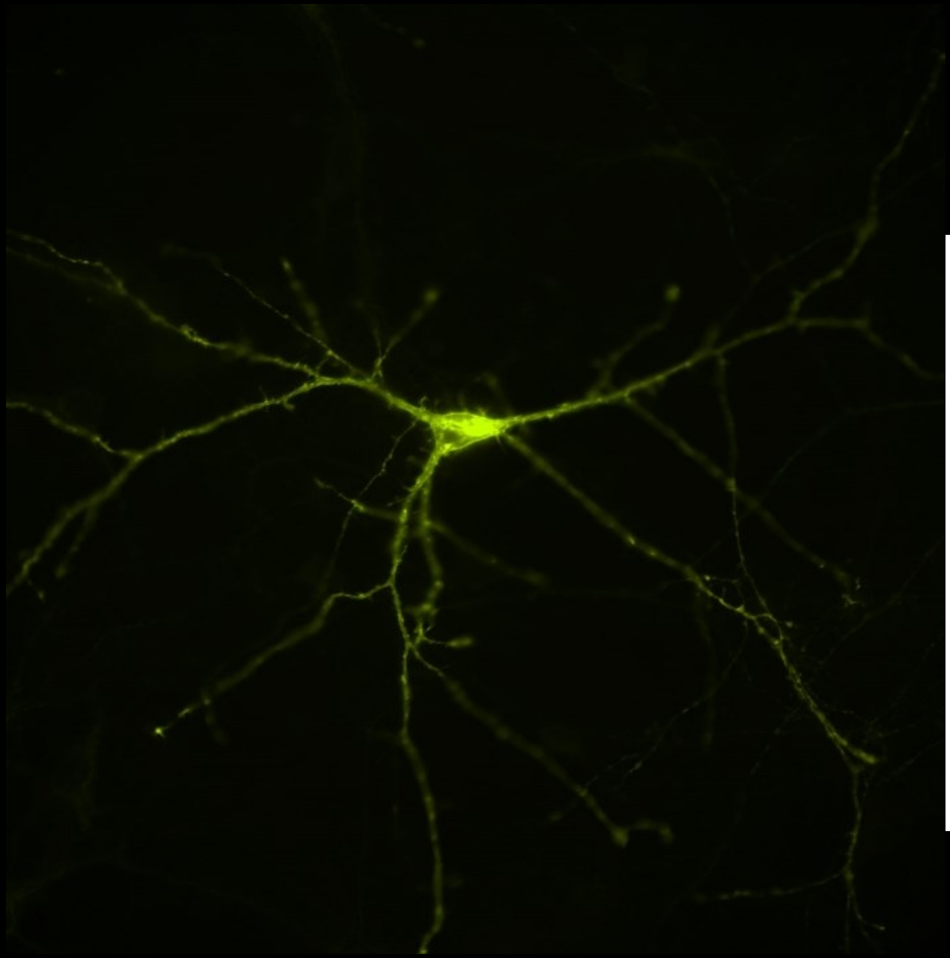


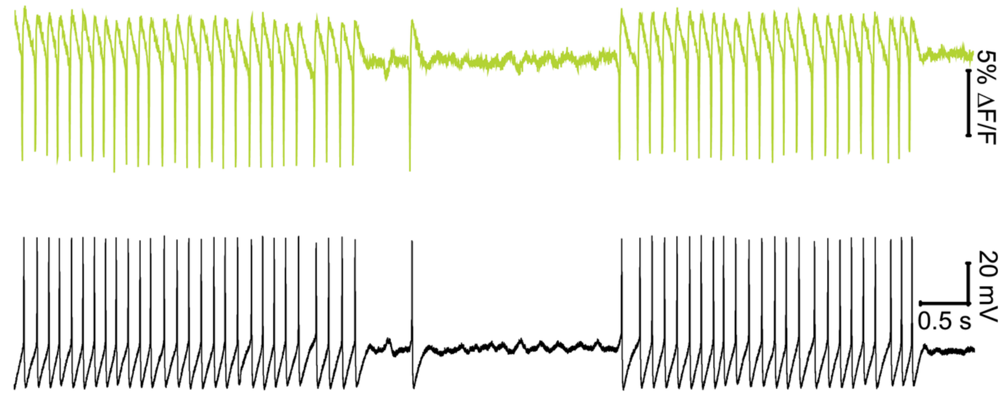
RhoVR1.pip.Sulf (IMO best low-power 2P voltage dye)

Di-4-ANEPPTEA is IMO best high-power dye



Widefield FOV
400 Hz
SLAP FOV
Cell 2
Cell 1
50 µm
SLAP
1016 Hz
50 V/cm stim
Cell 1
Cell 2
20% ΔF/F0
50 ms
Widefield
400 Hz
50 V/cm stim
Cell 1
Cell 2
10% ΔF/F0
50 ms





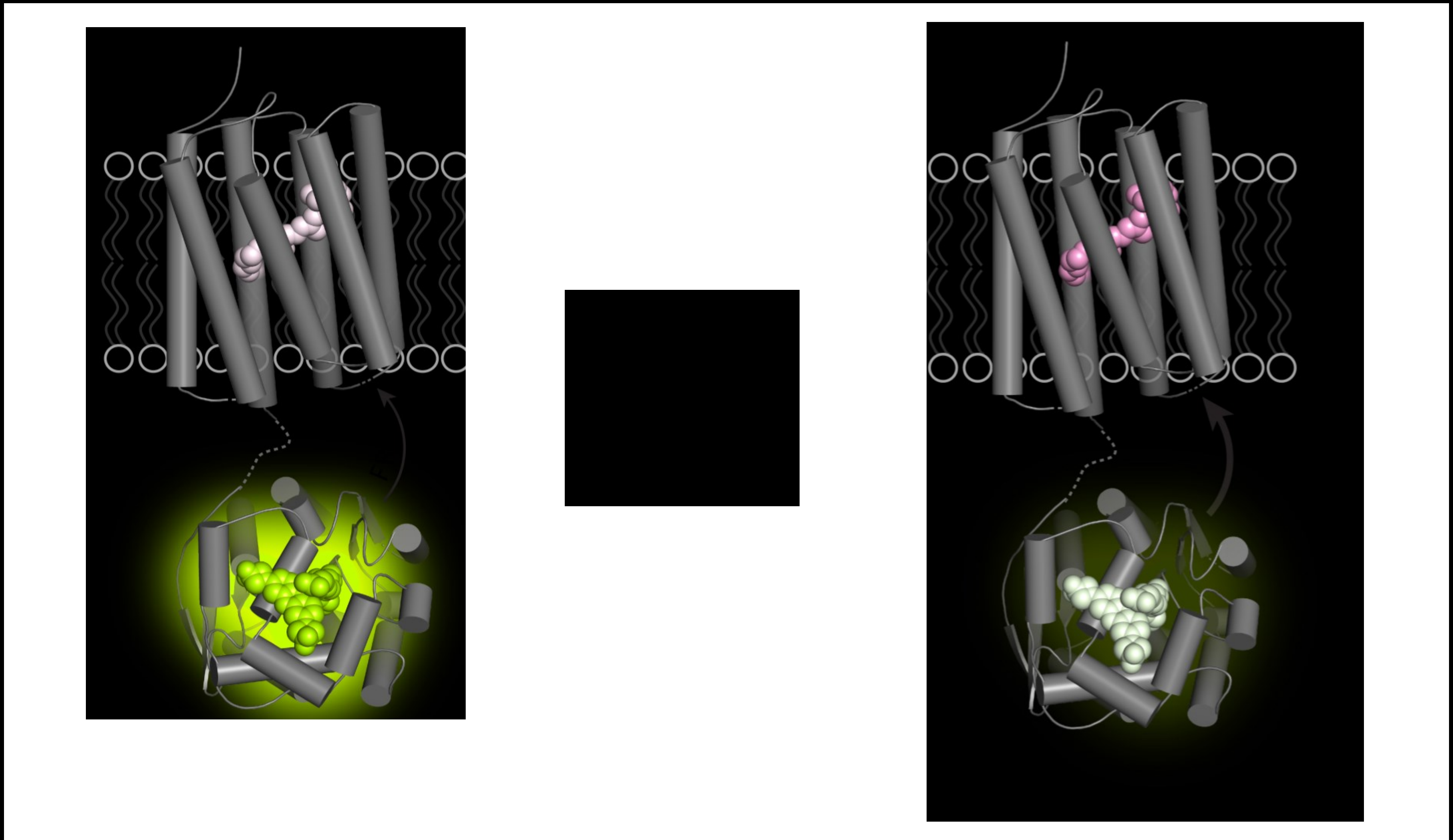
Voltron Fluorescence

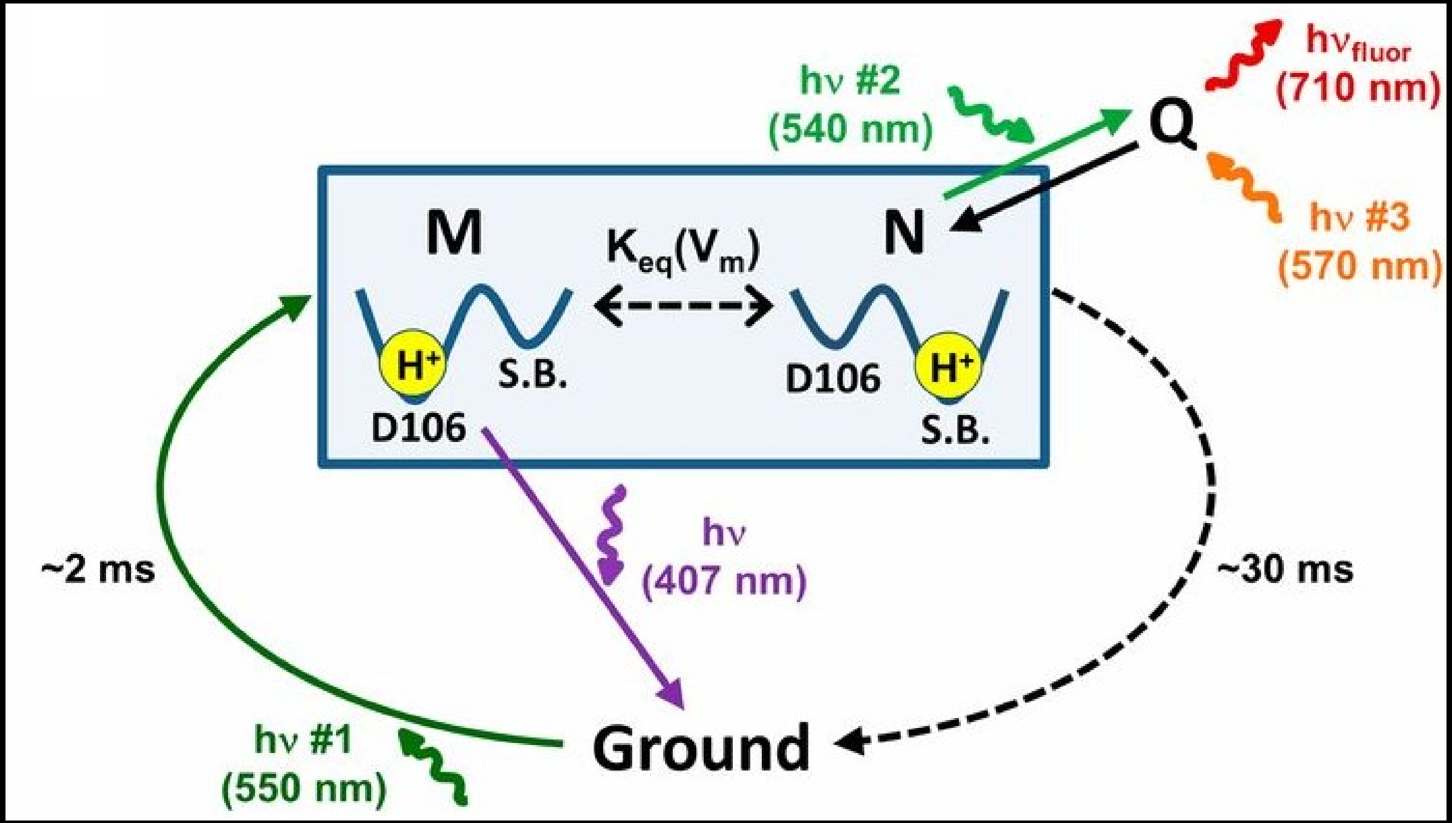
Membrane Voltage

Abdelfattah et al 2018

Opsin domain
(Voltage-sensitive absorbance)

Halo tag
Chemical dye FRET donor

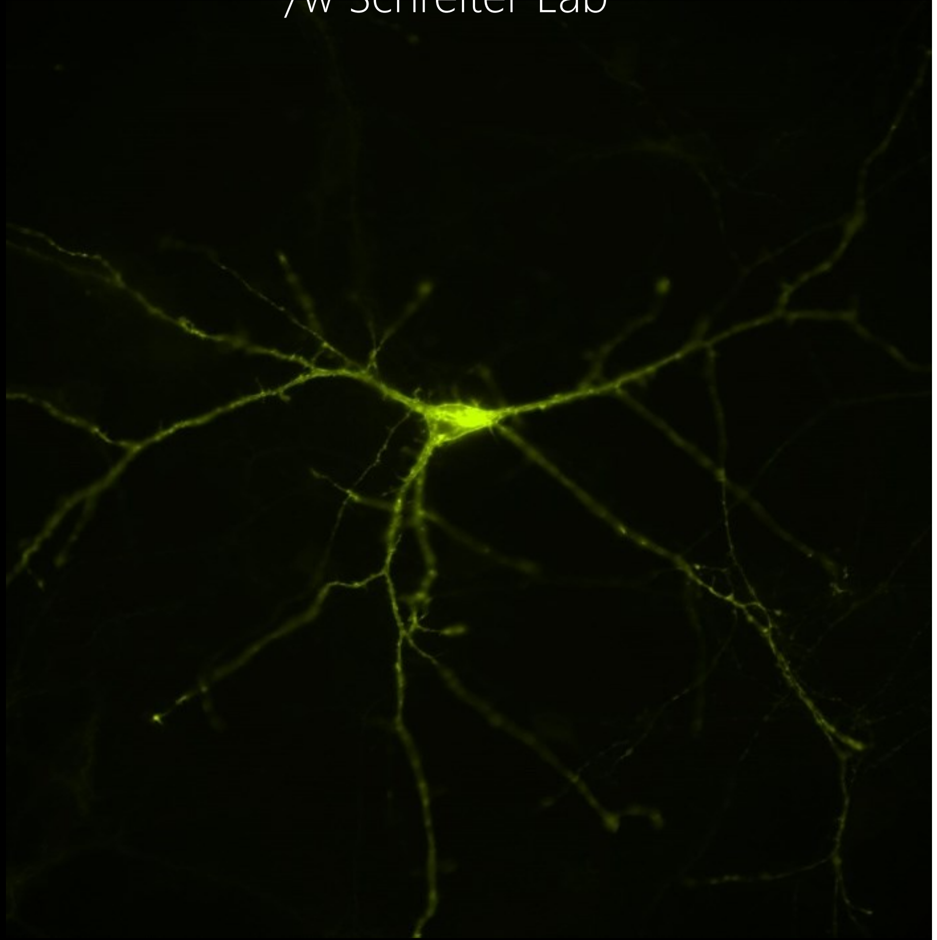


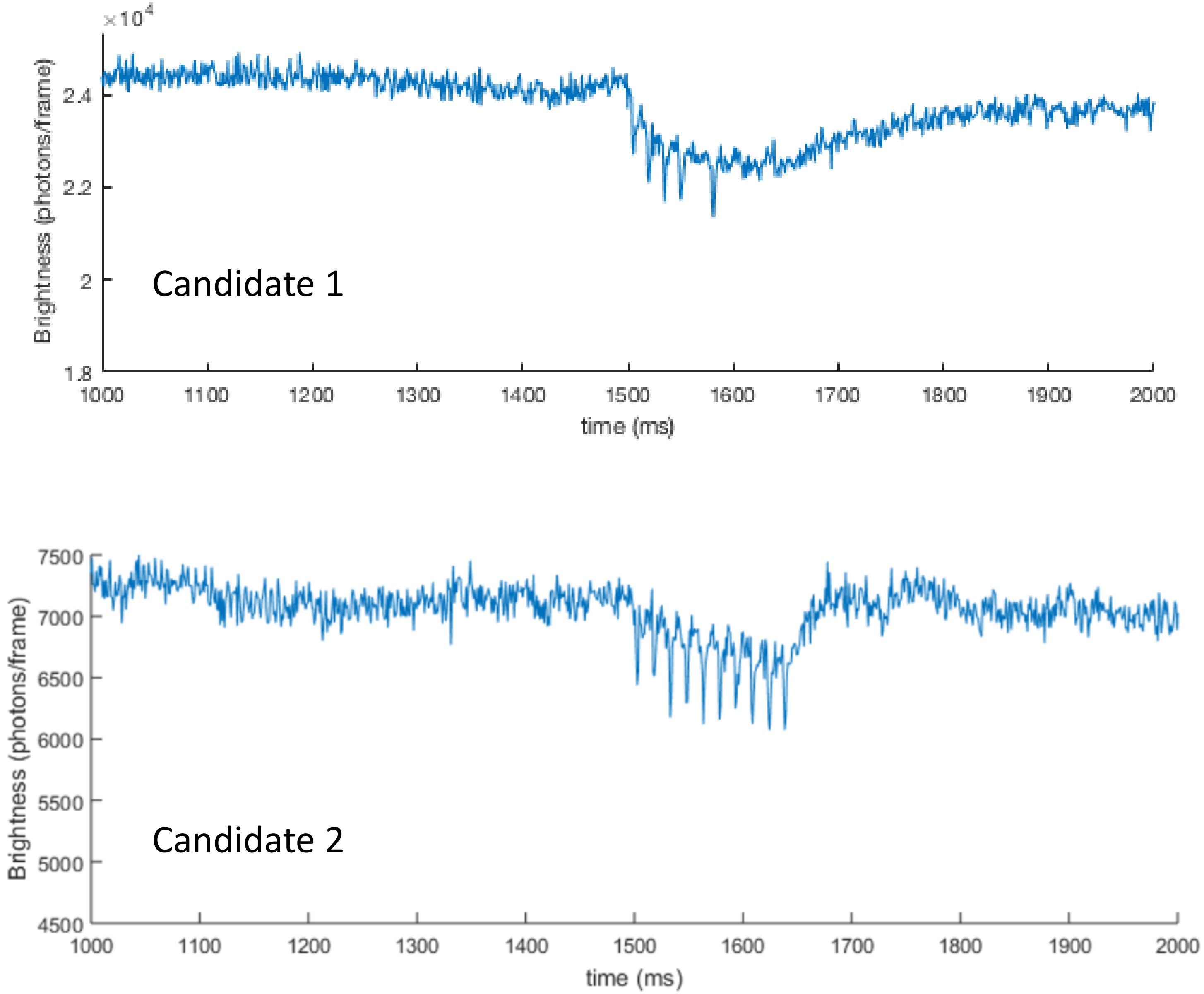


Maclaurin D. *et al*., PNAS, 2013, 110 (15) 5939-5944

Screening for two-photon compatible Voltron variants

/w Schreiter Lab

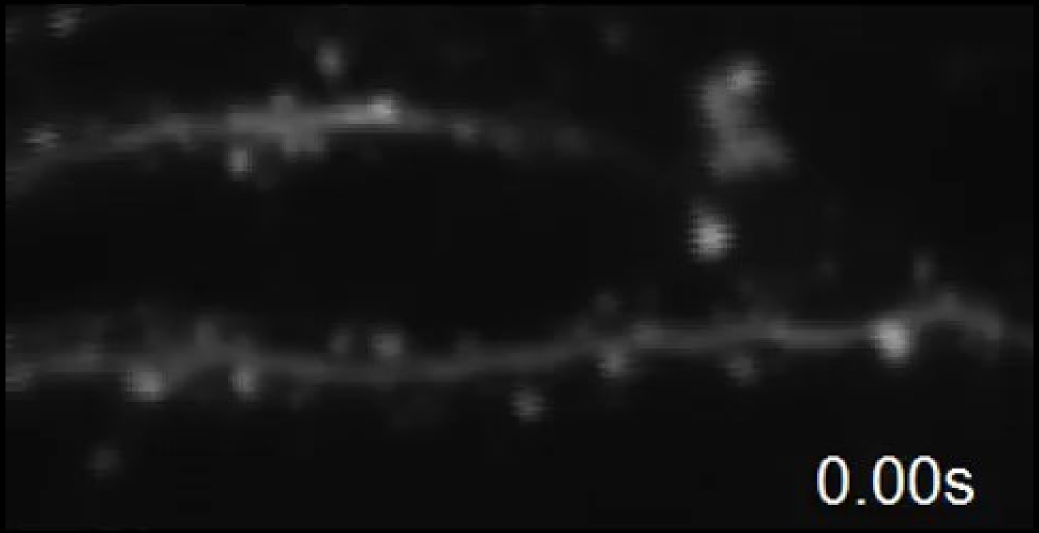




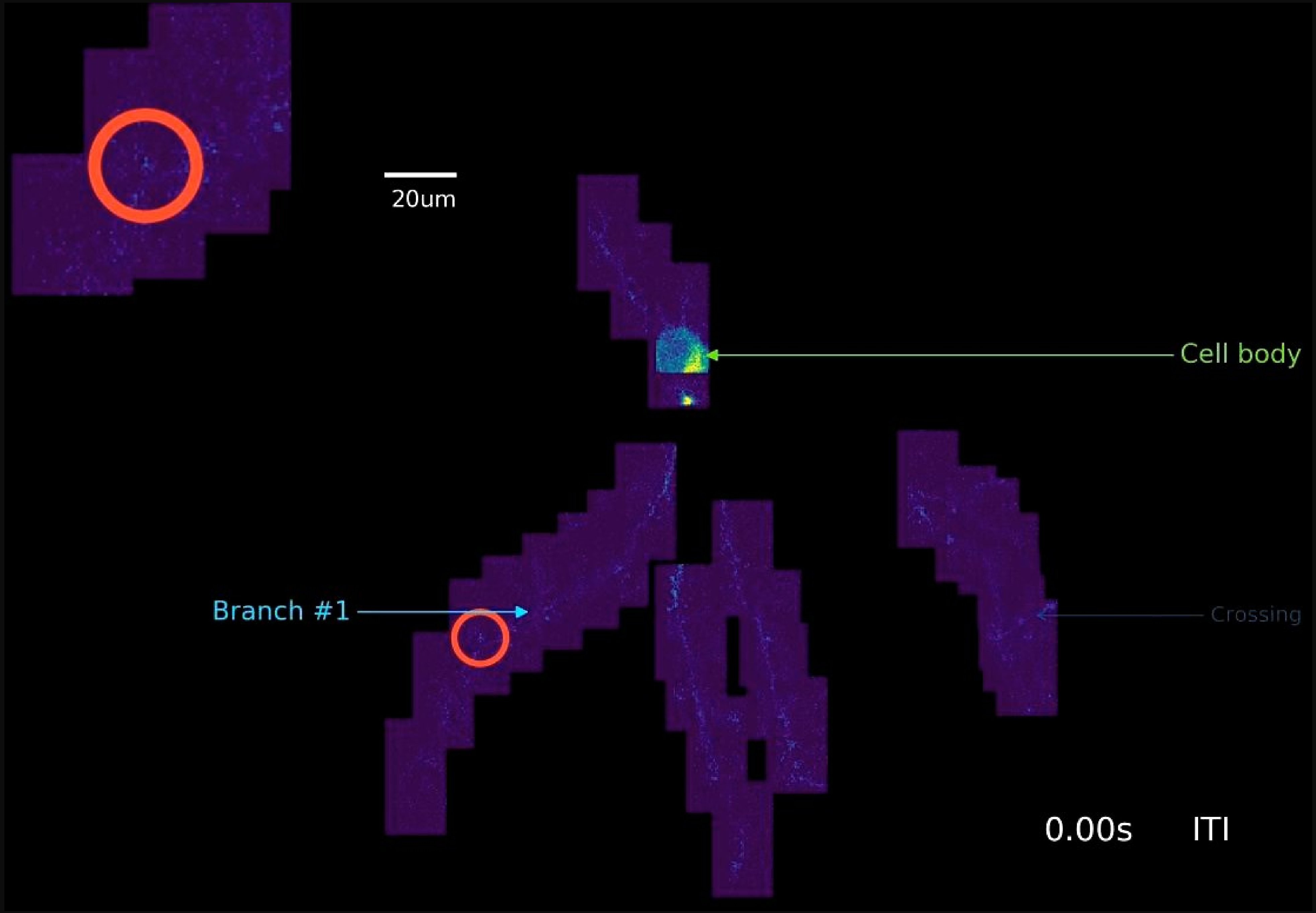
Rosario Valenti + Ahmed Abdelfattah

In Vivo Dendritic Imaging

Calcium imaging as a proxy for synaptic input



Chen et al. 2013
Raster scanning, 5Hz
Primary visual cortex
Visual stimulation
Anaesthetized Mouse



Aaron Kerlin et al 2018, bioRxiv

GCaMP6f

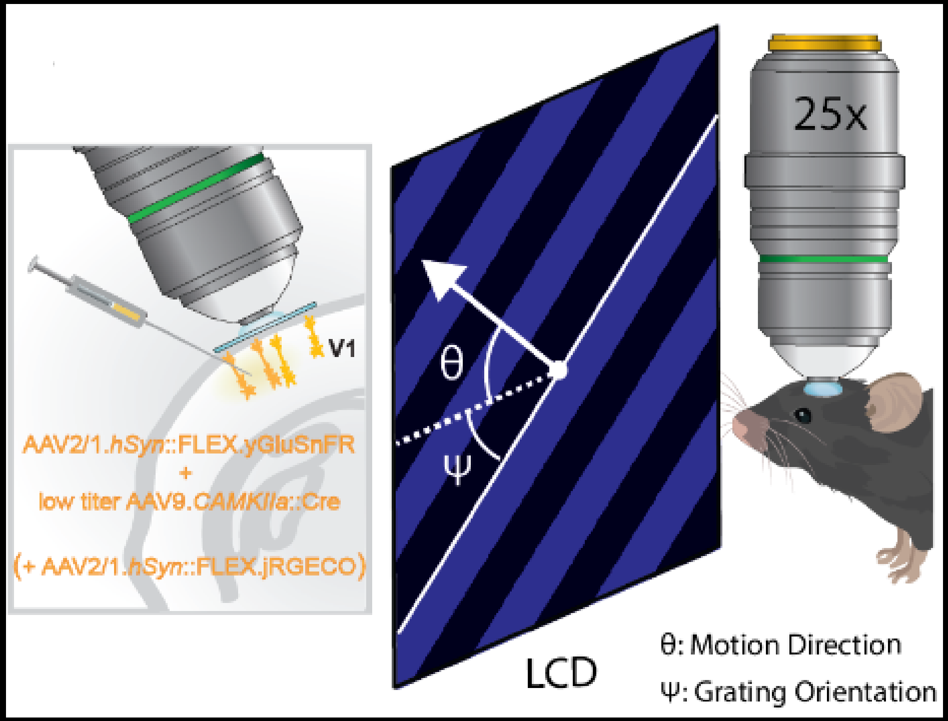
Volumetric patch scanning, 14Hz

Mouse performing motor task

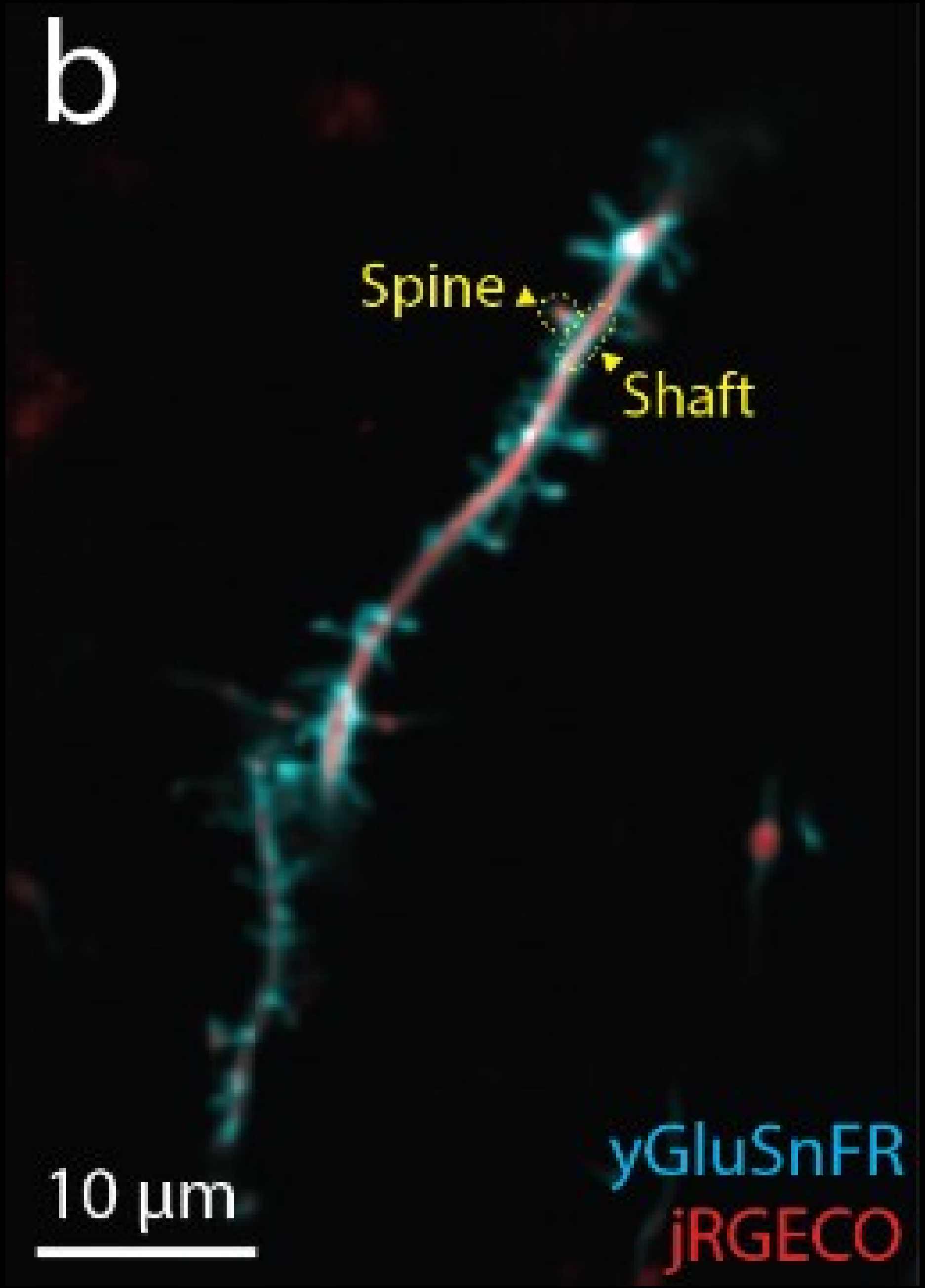
Glutamate *vs* Calcium

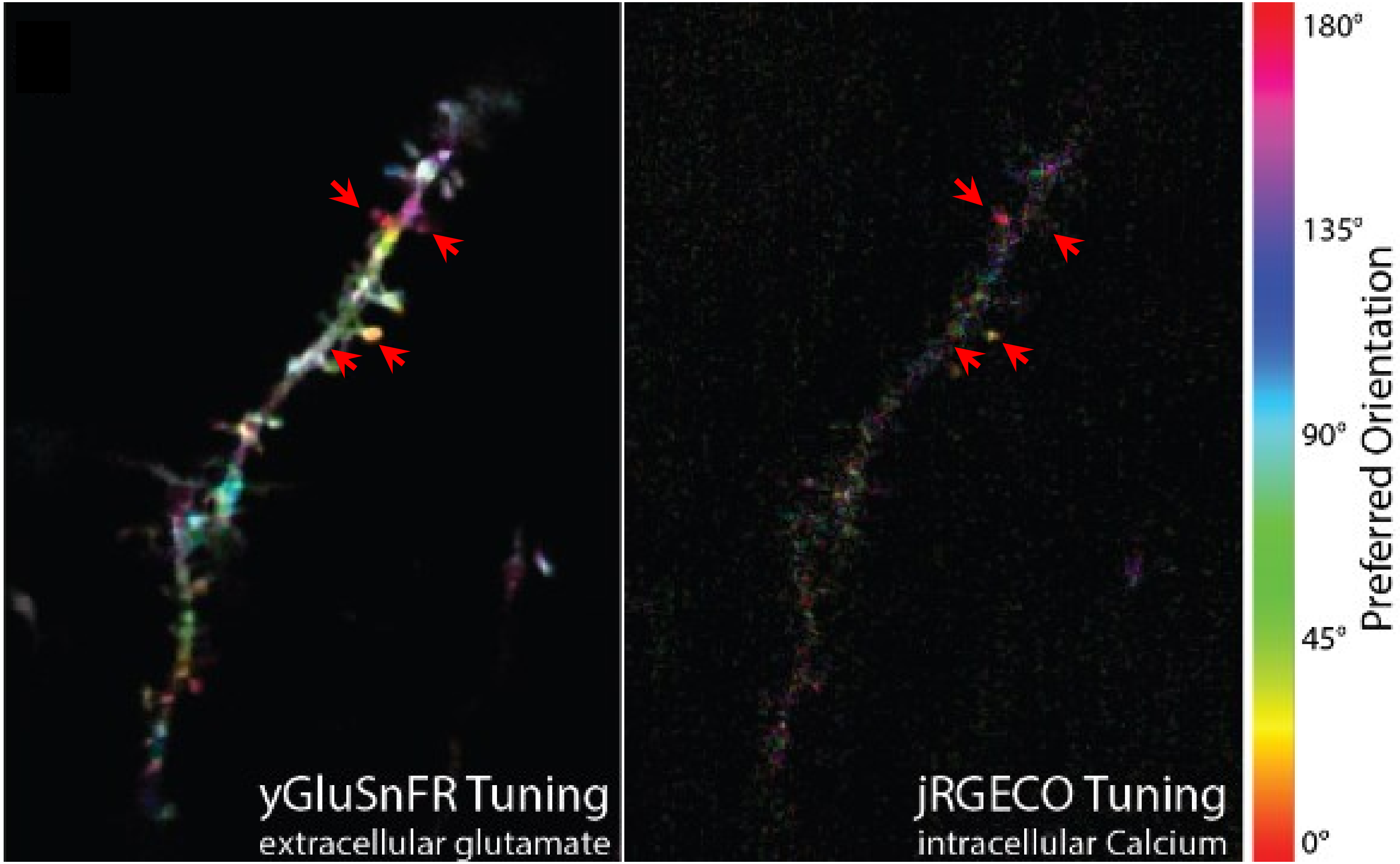
- Distinct Pre- vs. Post- synaptic signals, with different confounds

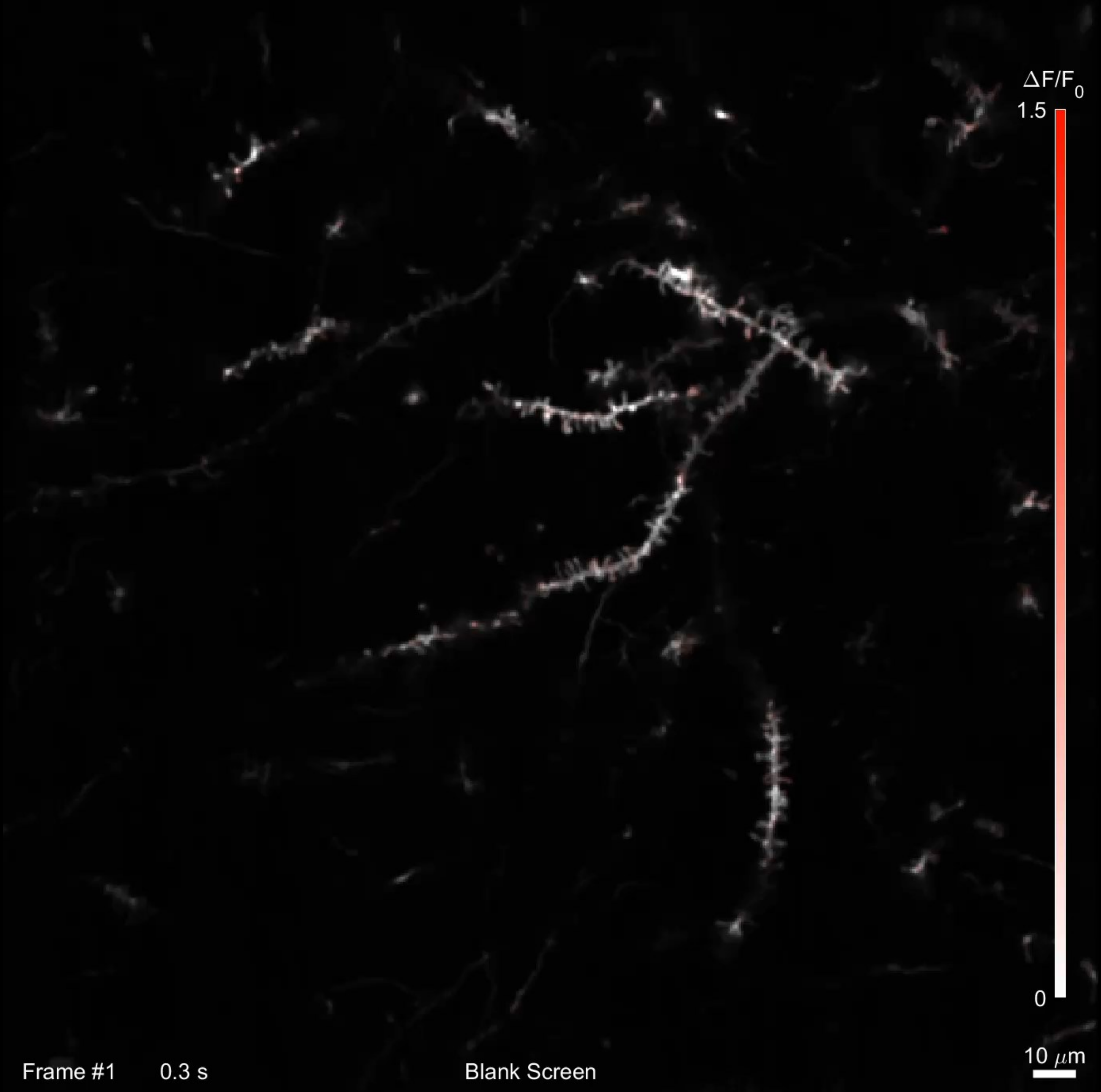
  e.g. NMDAR Mg^{2+} block, glutamate spillover



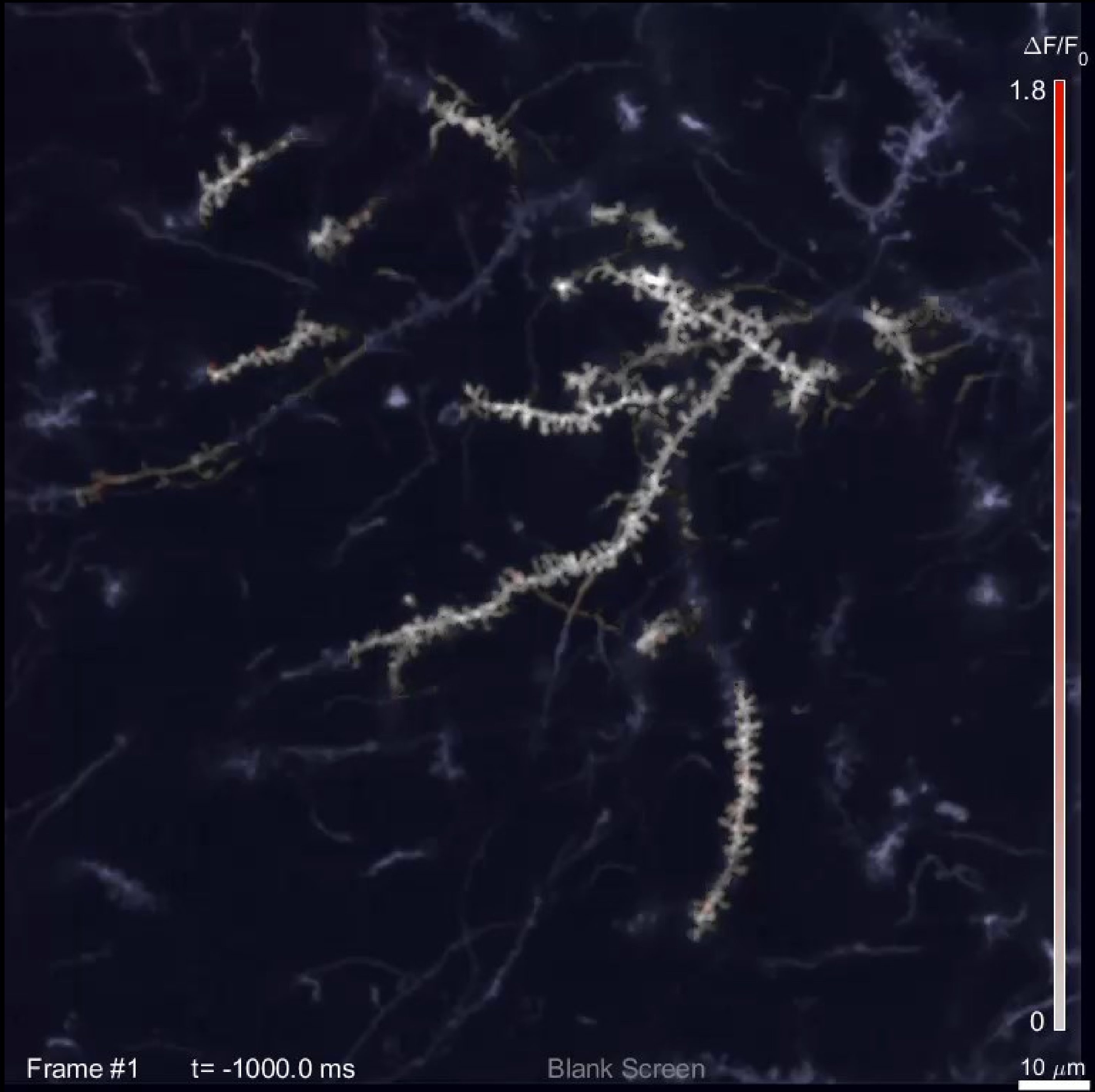
Anaesthetized mice, viewing moving gratings
Imaging L2/3 neurons in primary visual cortex



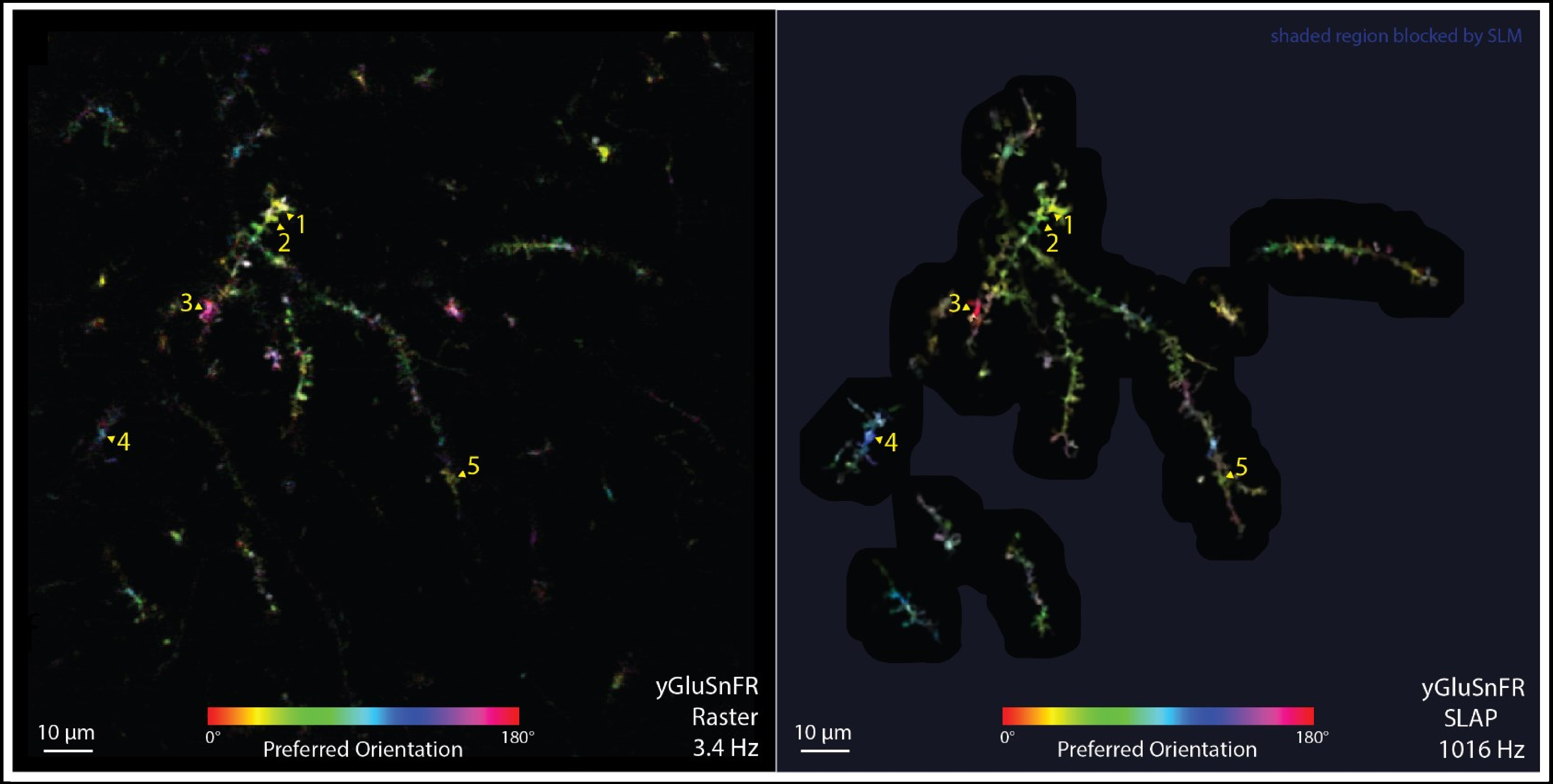

yGluSnFR Tuning
extracellular glutamate
jRGECO Tuning
intracellular Calcium
180°
135°
90°
45°
0°
Preferred Orientation

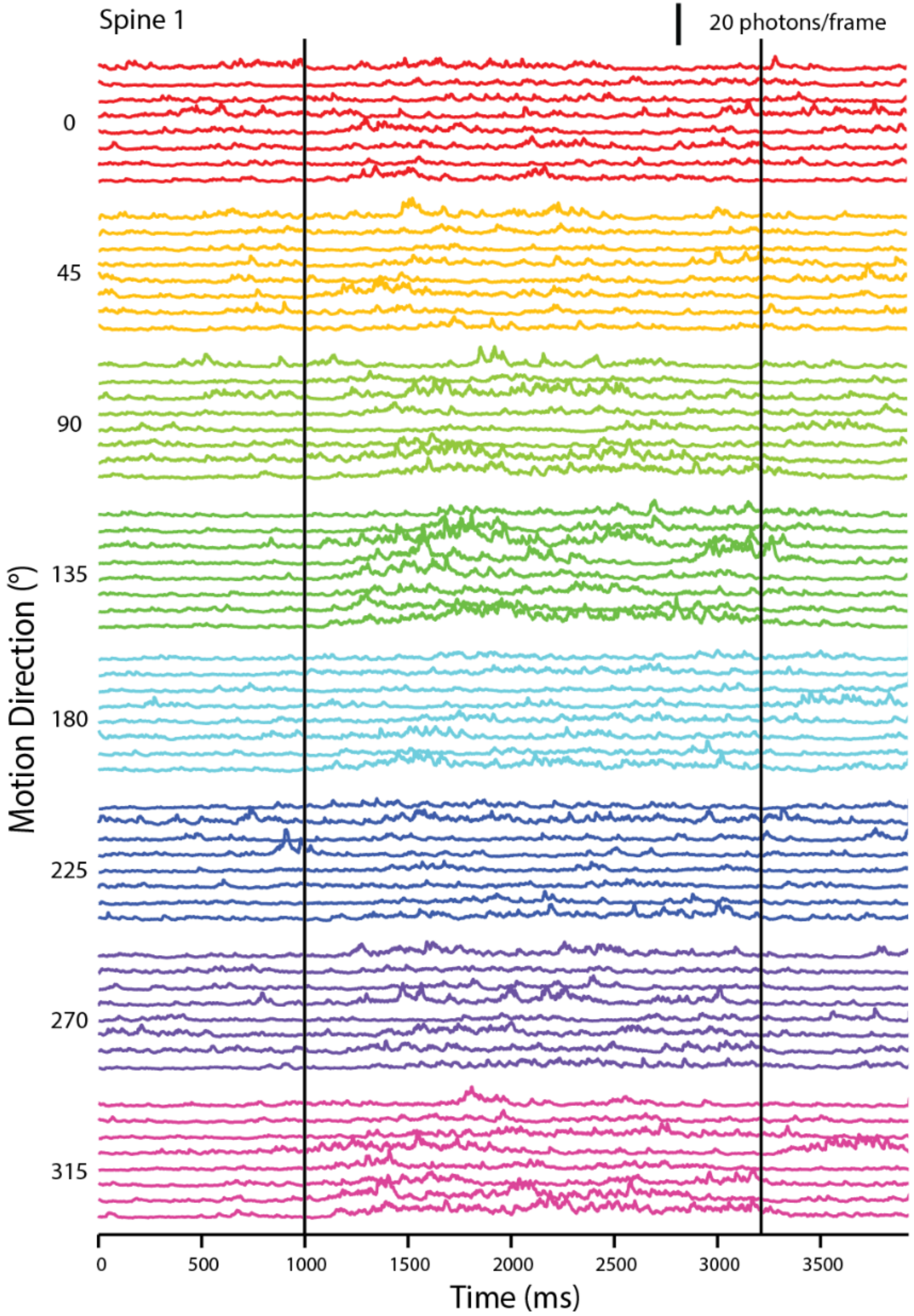


Raster Scanning
3.4 Hz
Visual Cortex
Anaesthetized mouse
Visual Stimulation



SLAP
1016 Hz
Visual Cortex
Anaesthetized mouse
Visual Stimulation


shaded region blocked by SLM
1
2
3
4
5
yGluSnFR
Raster
3.4 Hz
10 µm
0°
180°
Preferred Orientation
yGluSnFR
SLAP
1016 Hz
10 µm
0°
180°
Preferred Orientation



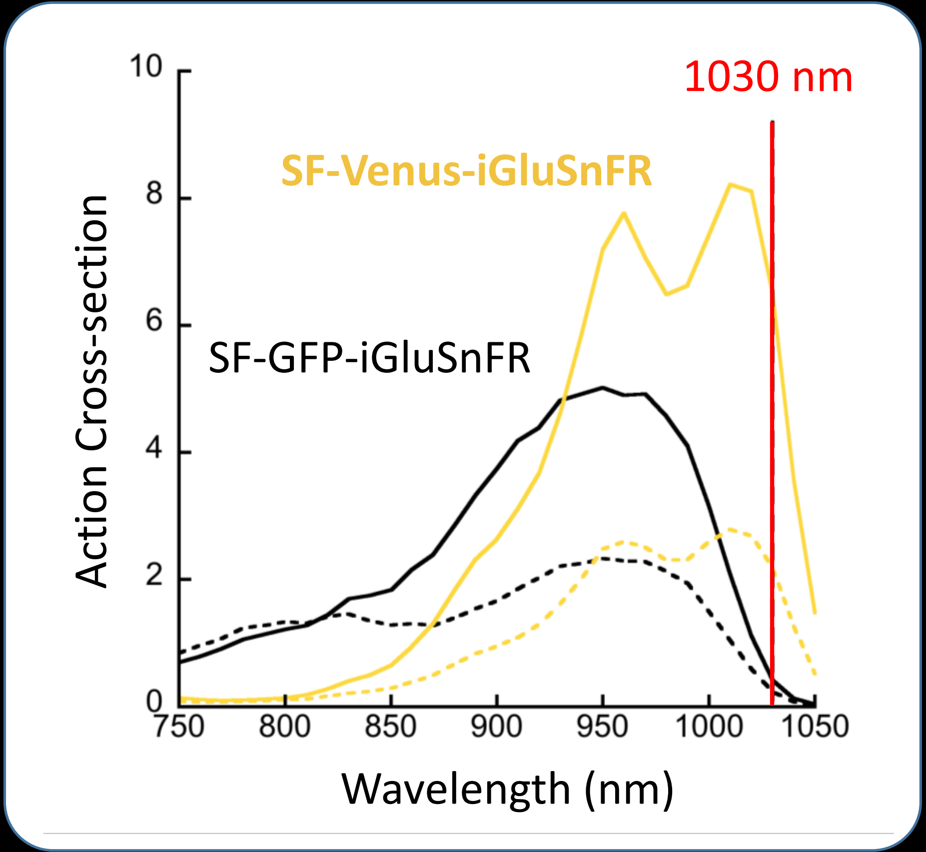
Spine 1
20 photons/frame
Motion Direction (°)
0
45
90
135
180
225
270
315
0
500
1000
1500
2000
2500
3000
3500
Time (ms)

Making Better Indicators

Making Indicators compatible with 1030 nm lasers

Low cost
High power
Stable
Compact
Dual-color imaging of YFPs and RFPs

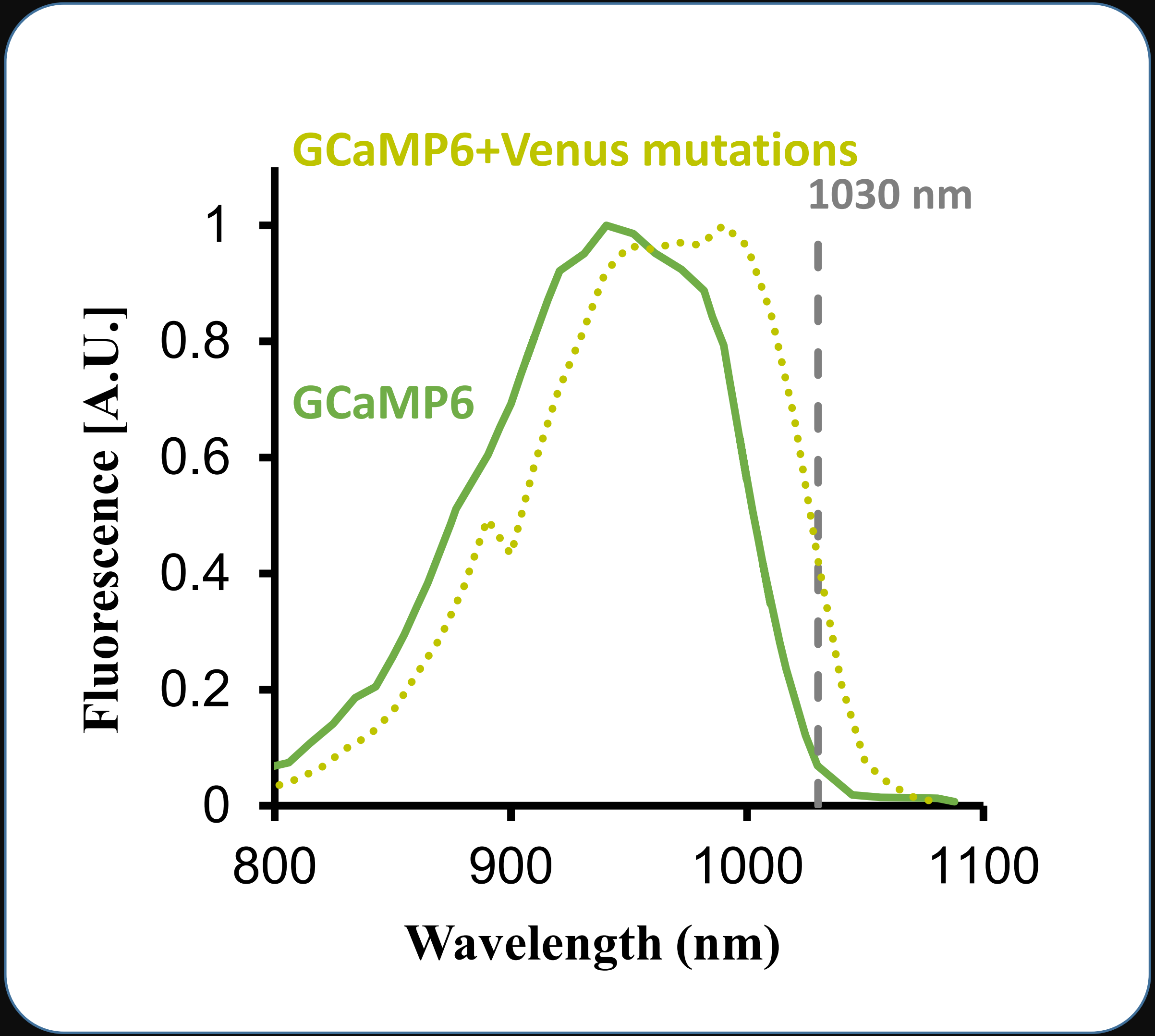
iGluSnFR –> yGluSnFR



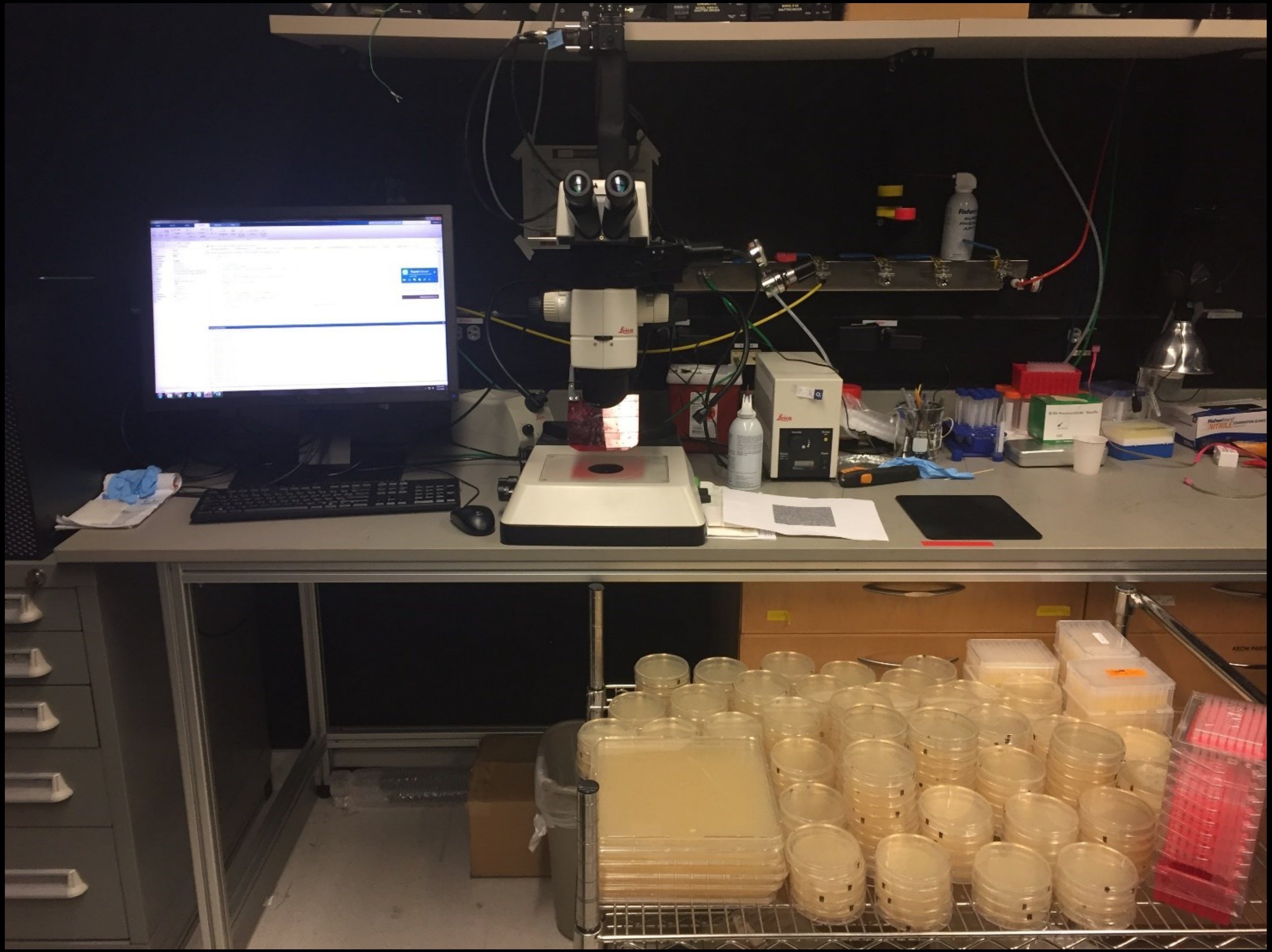
Marvin et al. 2018

Improved brightness, color + affinity variants

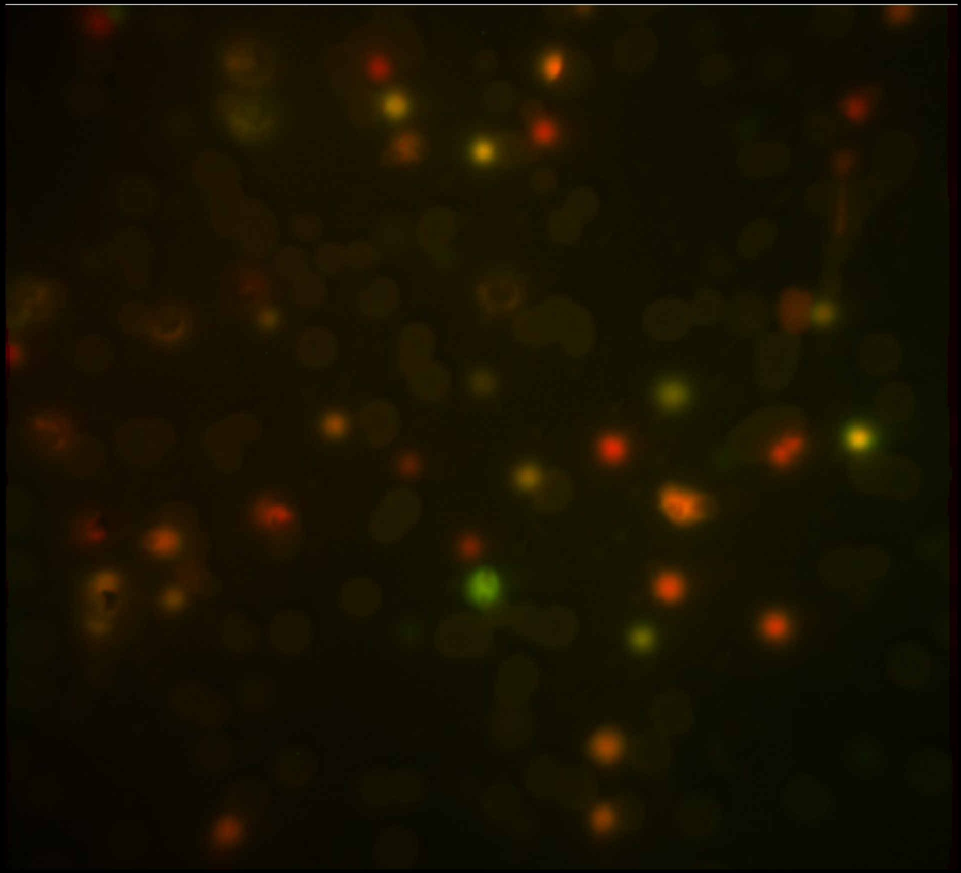
GCaMP –> YCaMP?



/w Looger Lab

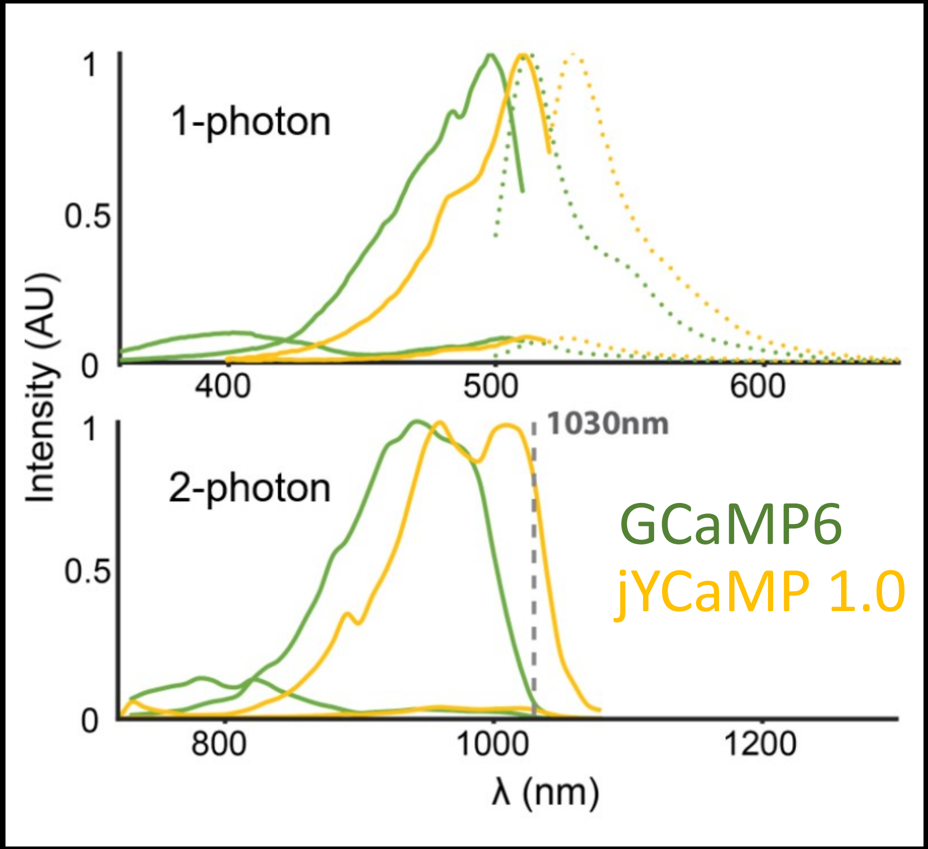


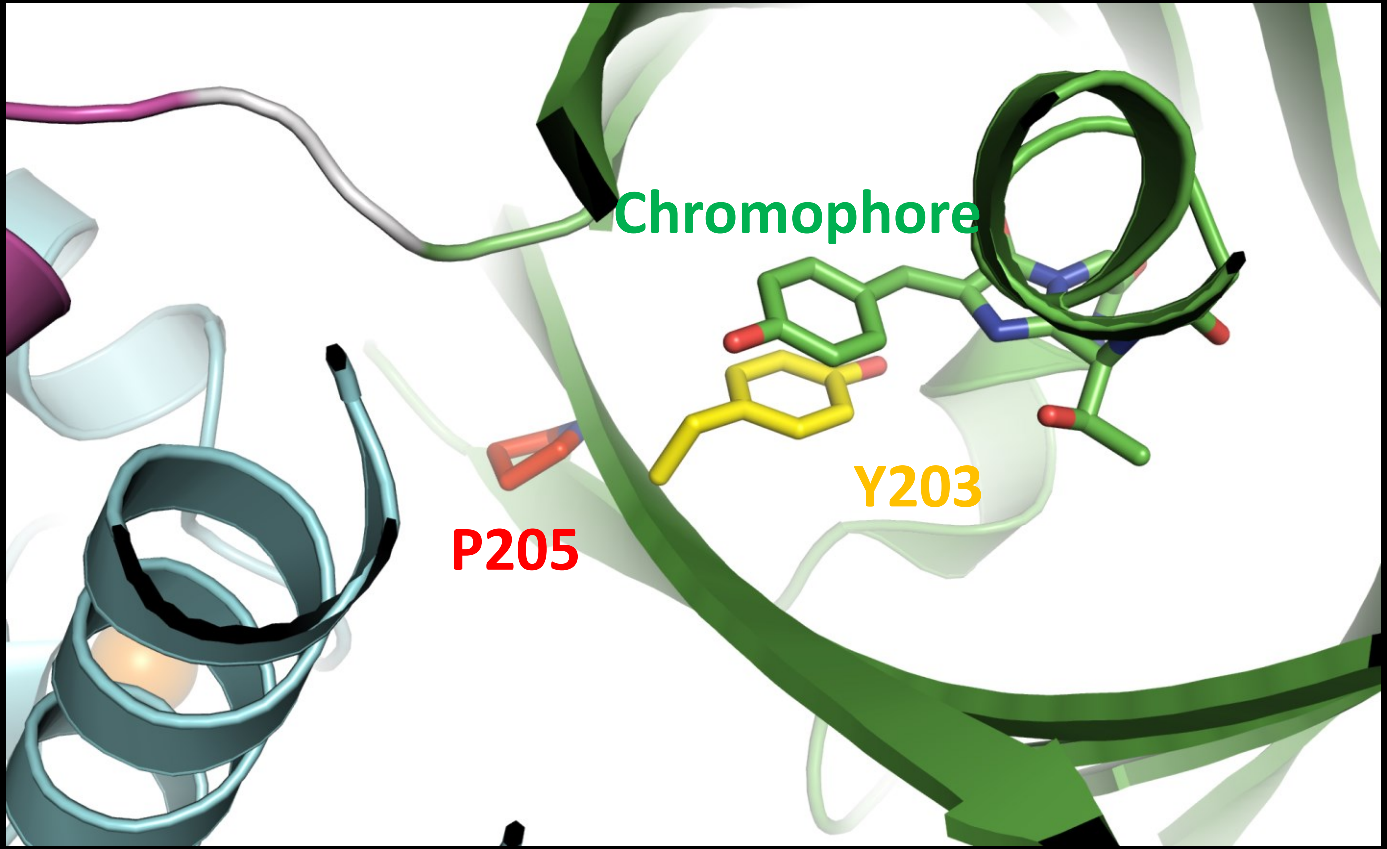
Screening apparatus
(lightly modified dissection scope + PC)



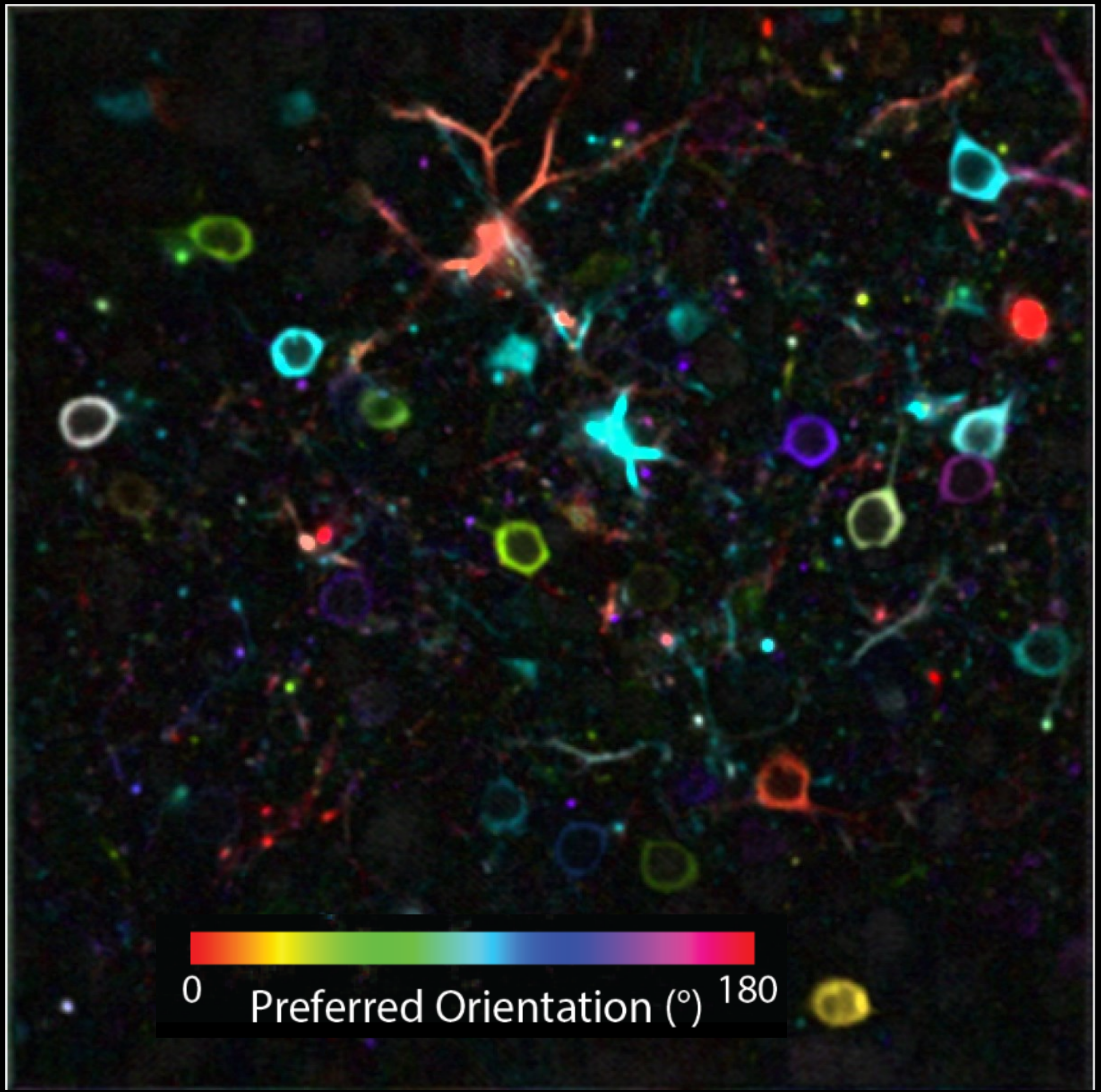
Bacterial colonies pseudocolored by emission spectrum
(sensitive to <1nm shift)

jYCaMP

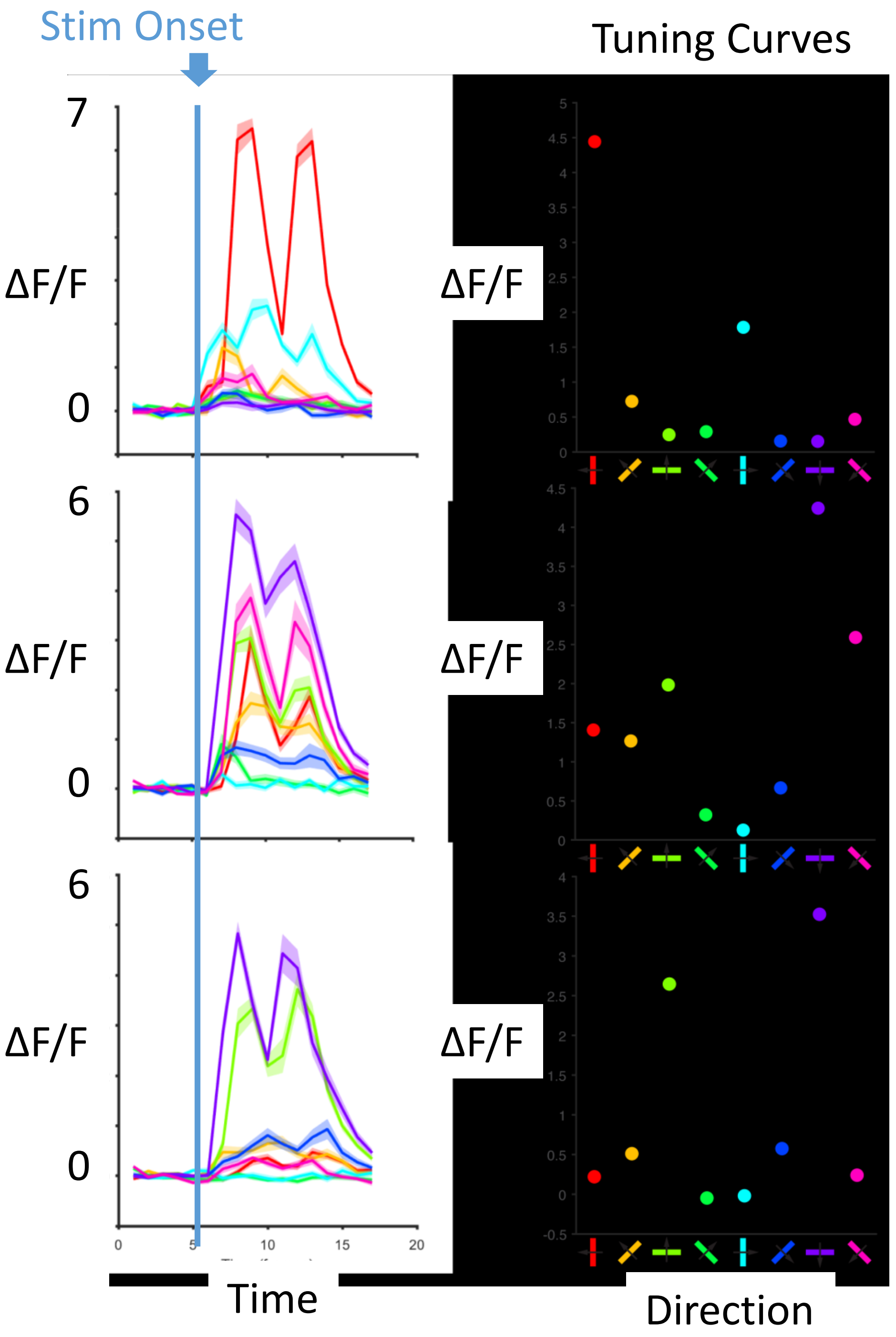




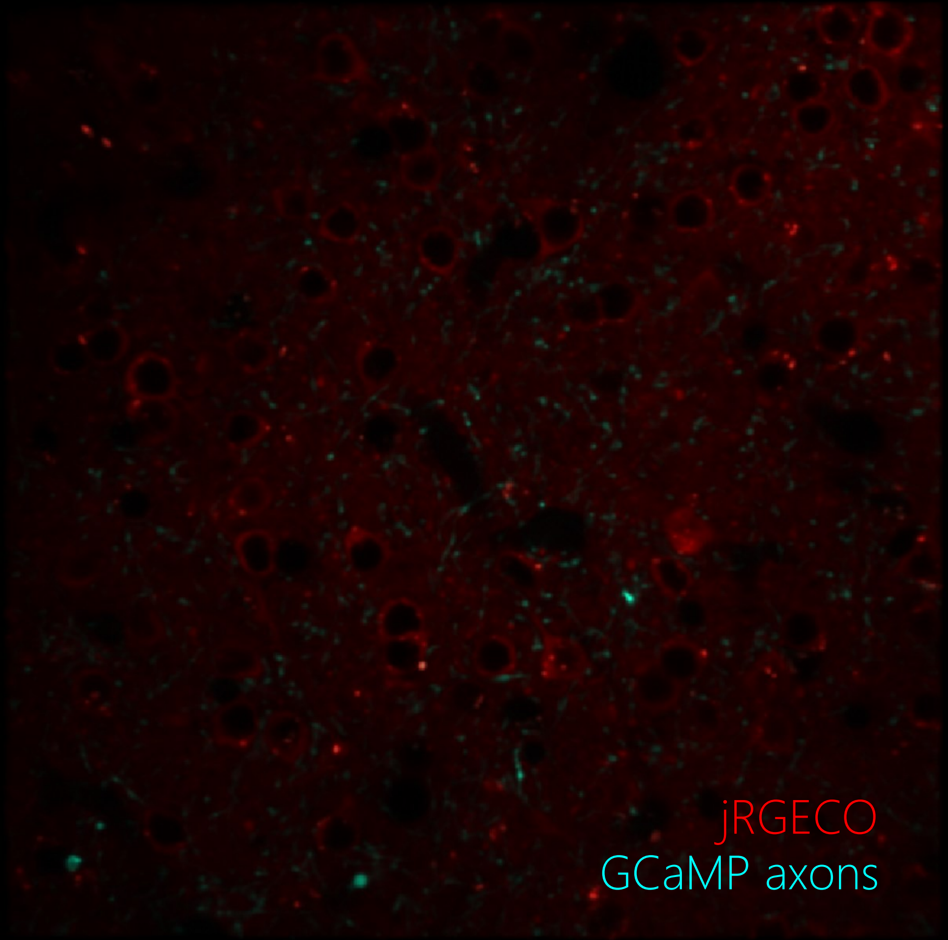
/w Manuel Mohr (Schreiter/Looger Labs) + Abhi Aggarwal (Podgorski Lab) + Eric Schreiter

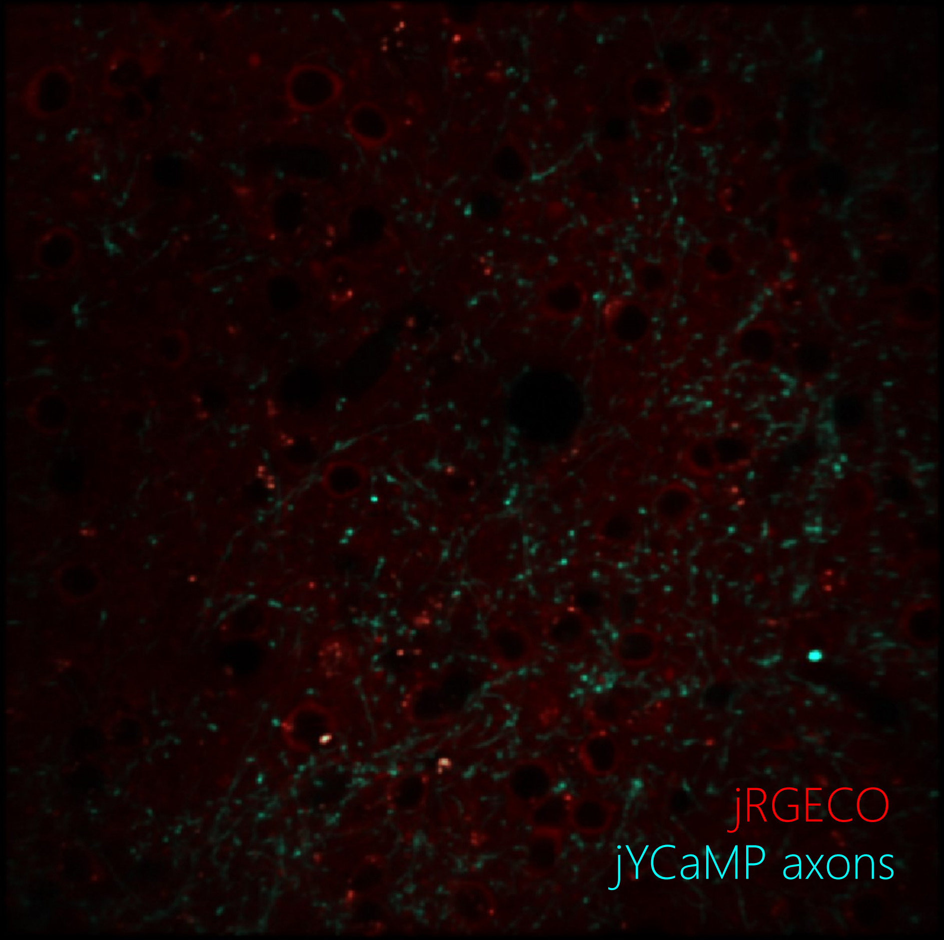


jYCaMP 1.0 L2/3 Visual Cortex 1030 nm



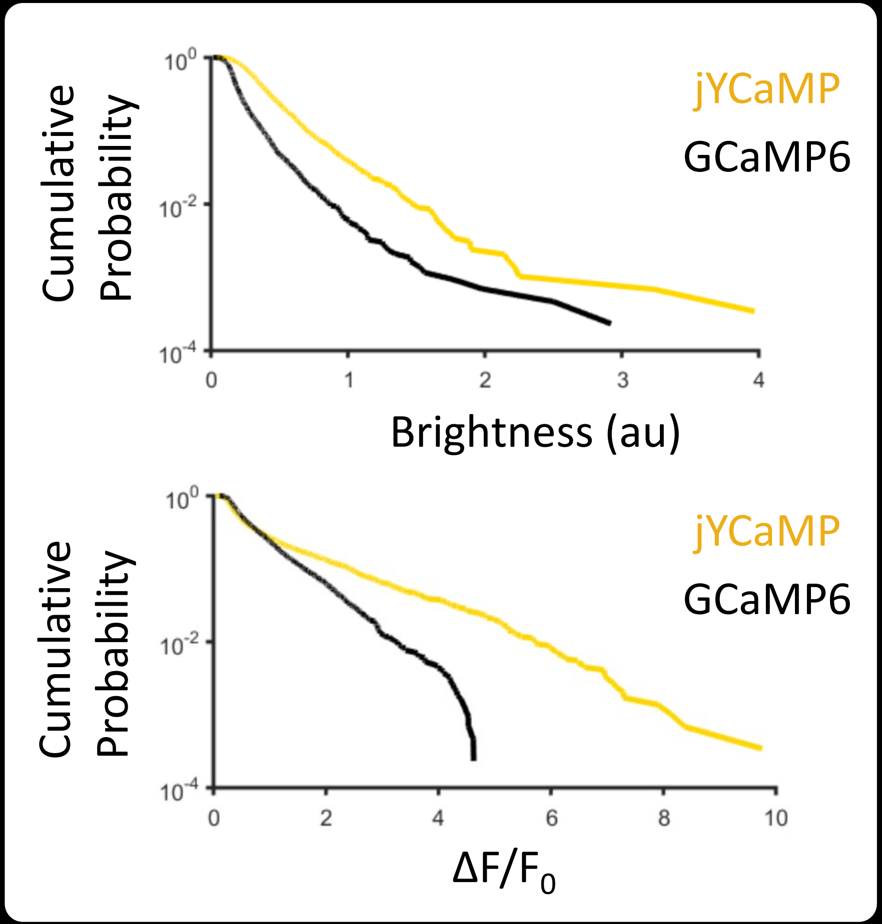
Better two-color imaging along with red GECIs



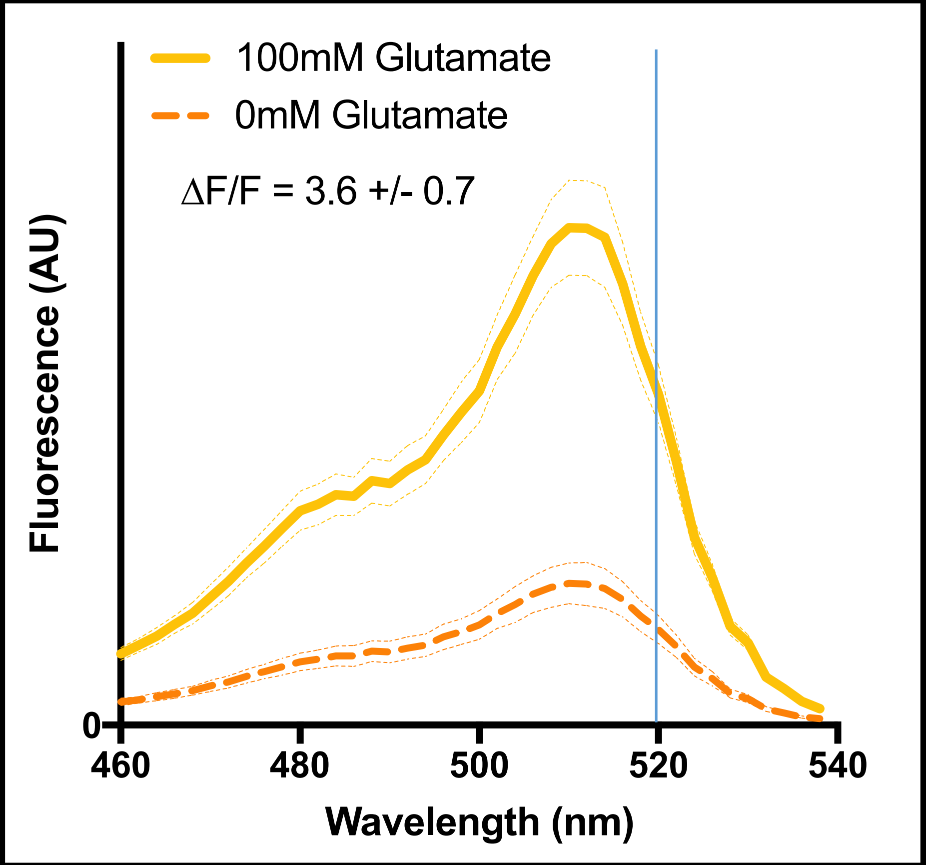


with Manuel Mohr, Abhi Aggarwal, Schreiter Lab, Looger Lab, GENIE

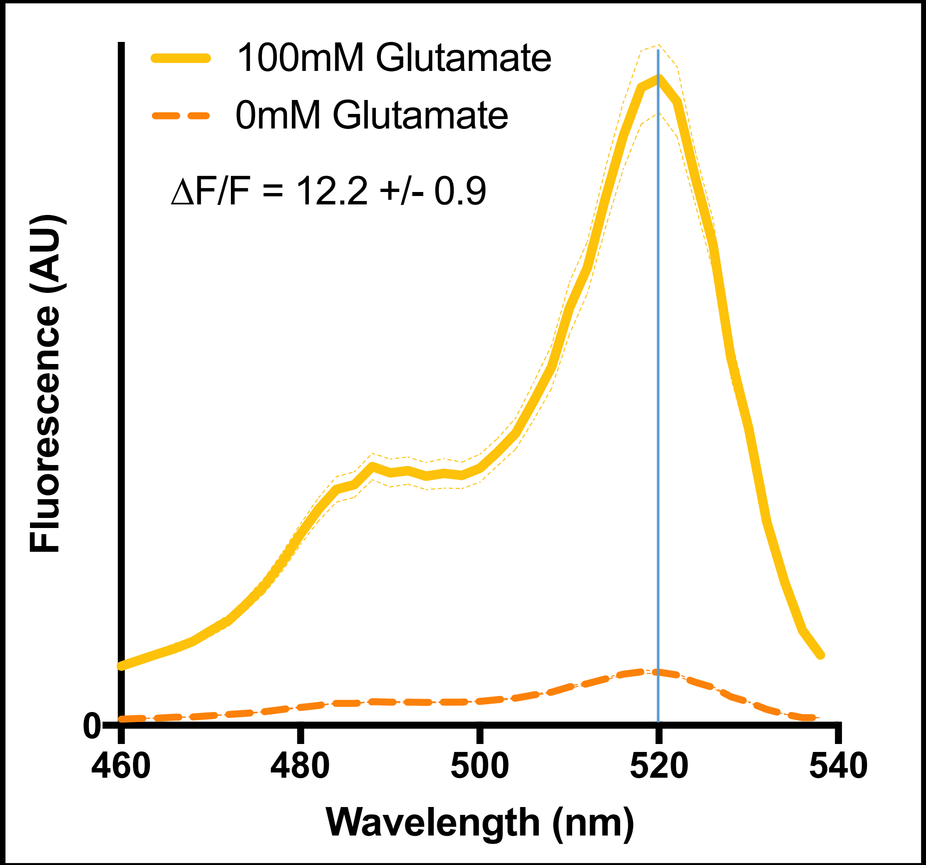
Higher brightness and sensitivity at 1030 nm:



SuperFolder-yGluSnFR (Marvin 2018)

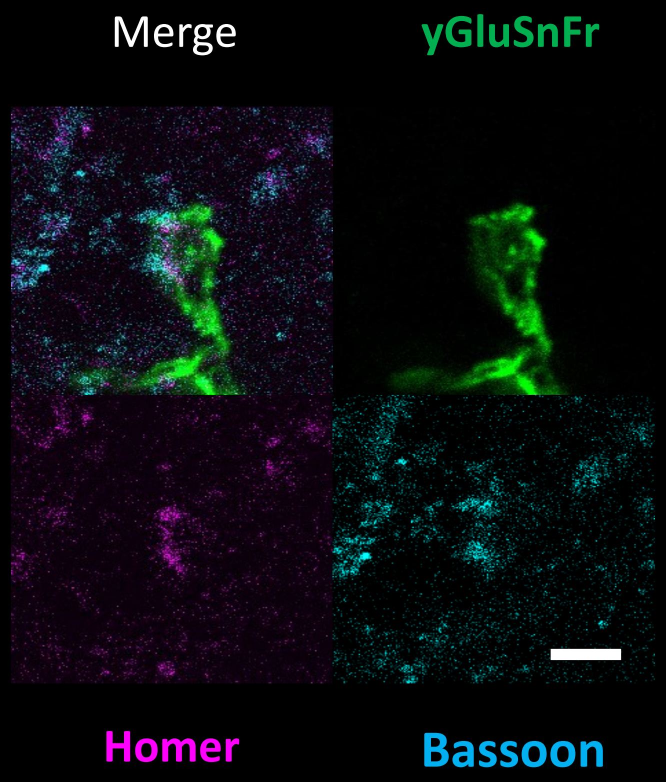


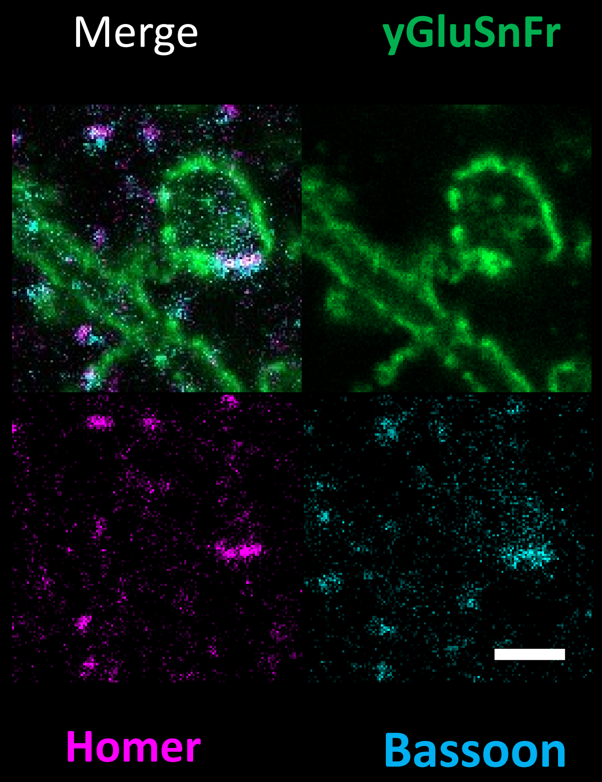
New yGluSnFR variant

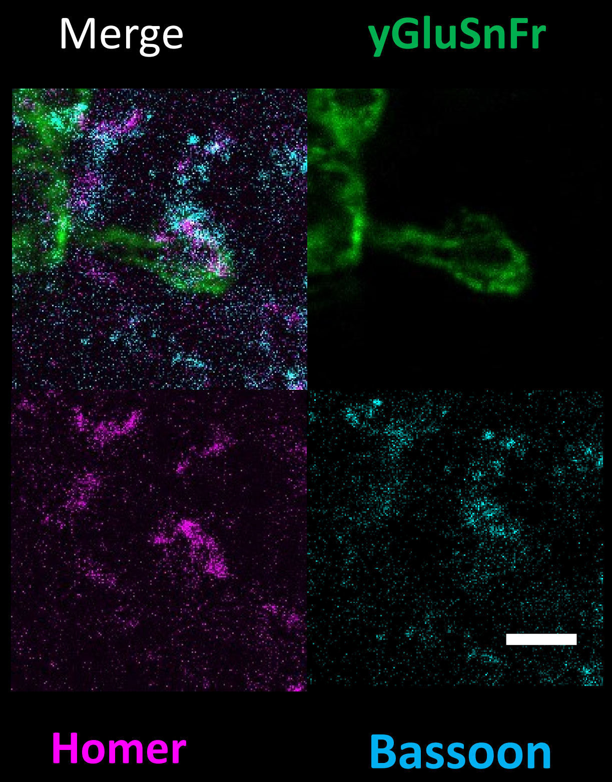


Abhi Aggarwal

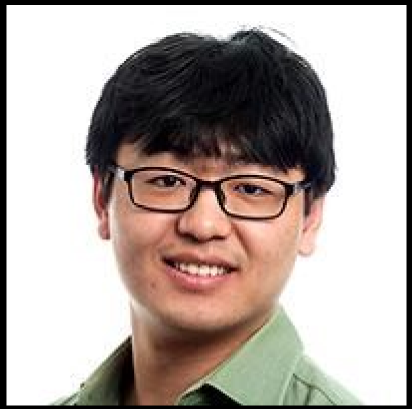
	λ_{ex} (nm)	λ_{em} (nm)	EC	QY	Brightness	Max/min
mVenus	515	528	92,200	0.57	52.5	--
yGluSnFR A184V	512	522	90,600	0.492	44.6	3.58 (at 512nm)
New Variant	520	530	52.2K	0.77	40.2	12.18 (at 520nm)



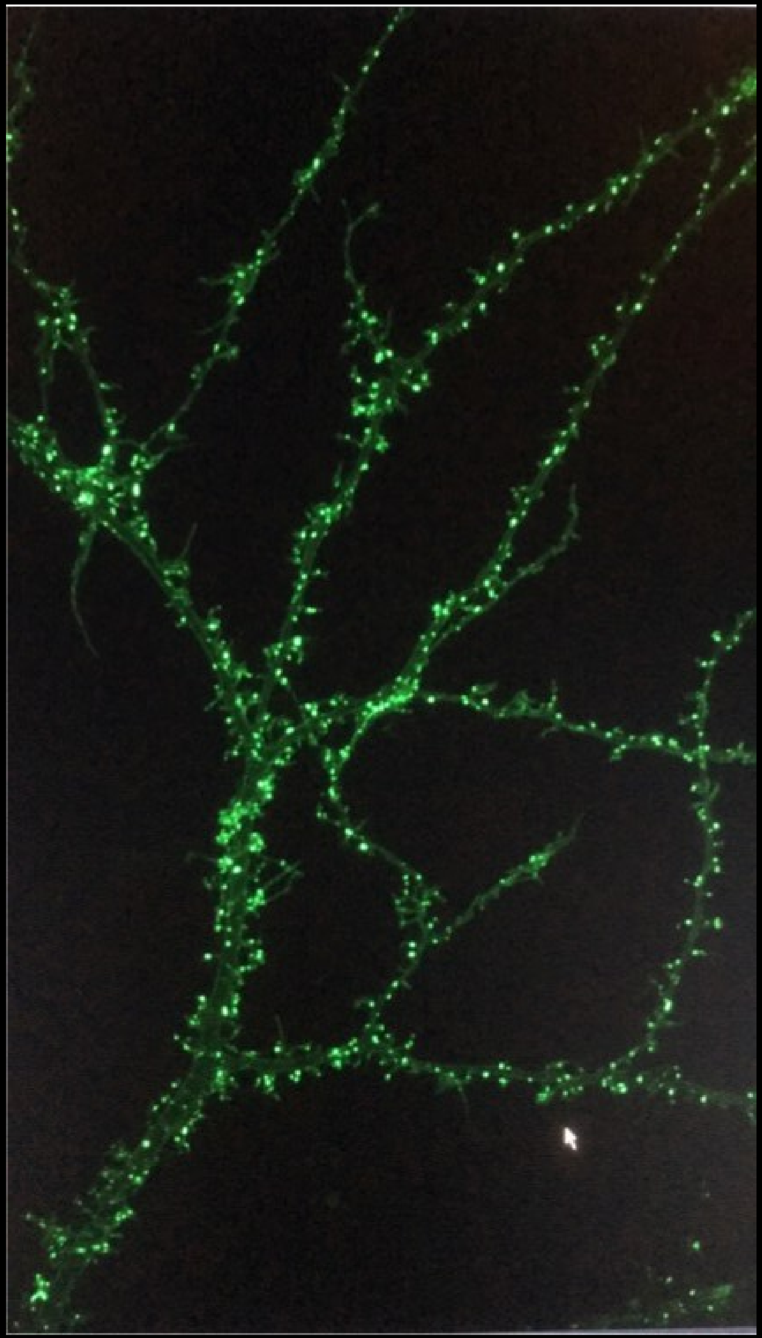




7x Expansion microscopy



JJ Kim



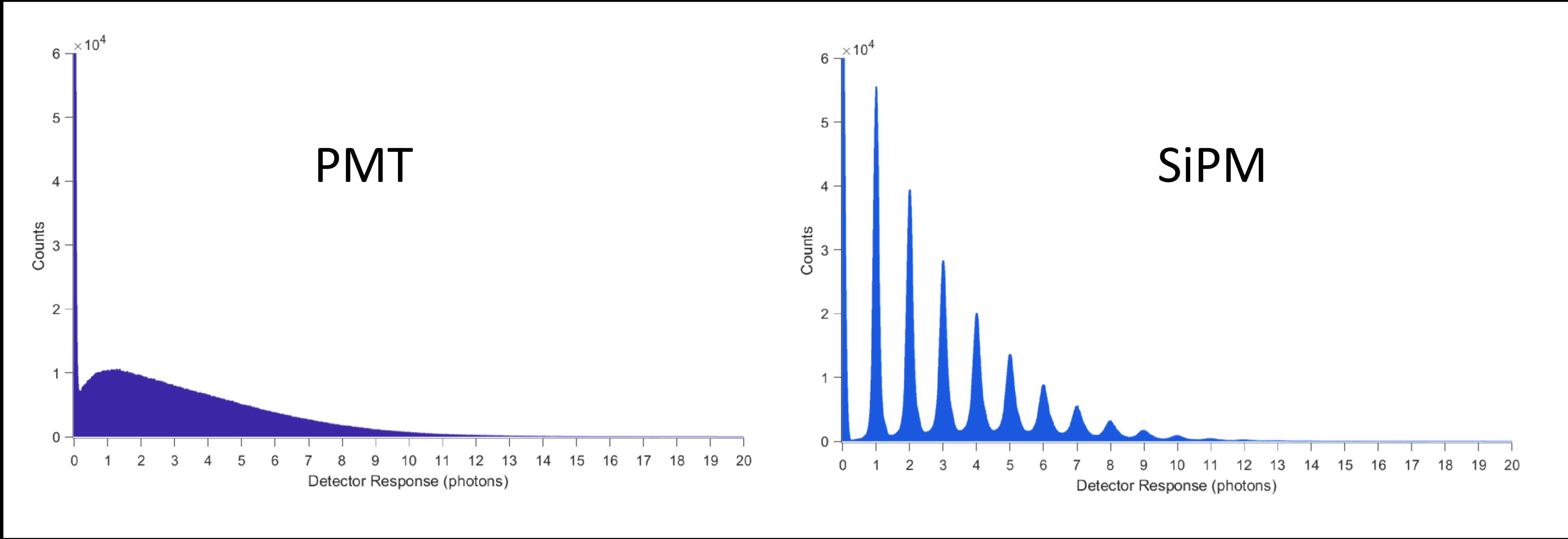
Localizing yGluSnFR to synapses using TARP-y subunit c-tails

- Works, but very cell-type specific
- Spine intensity comparable to pMinDis-yGluSnFR
- SNR better, bleaching worse…

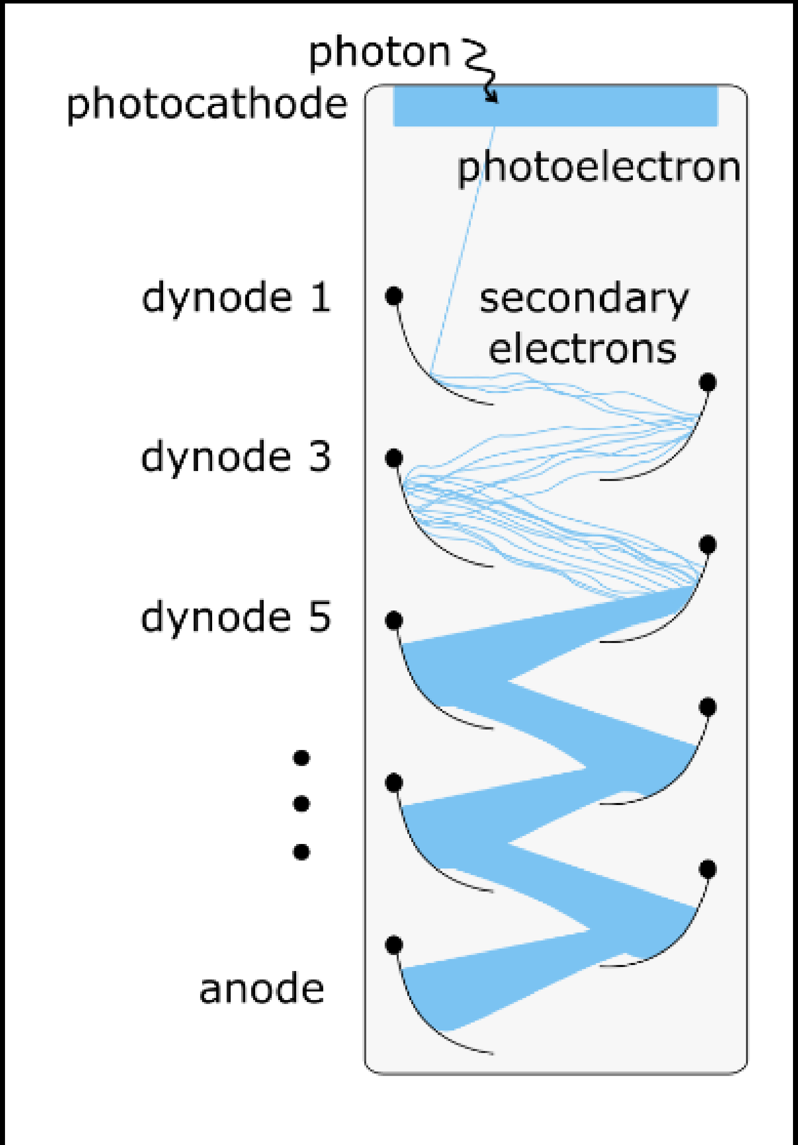
Manuel Mohr
(Schreiter lab)

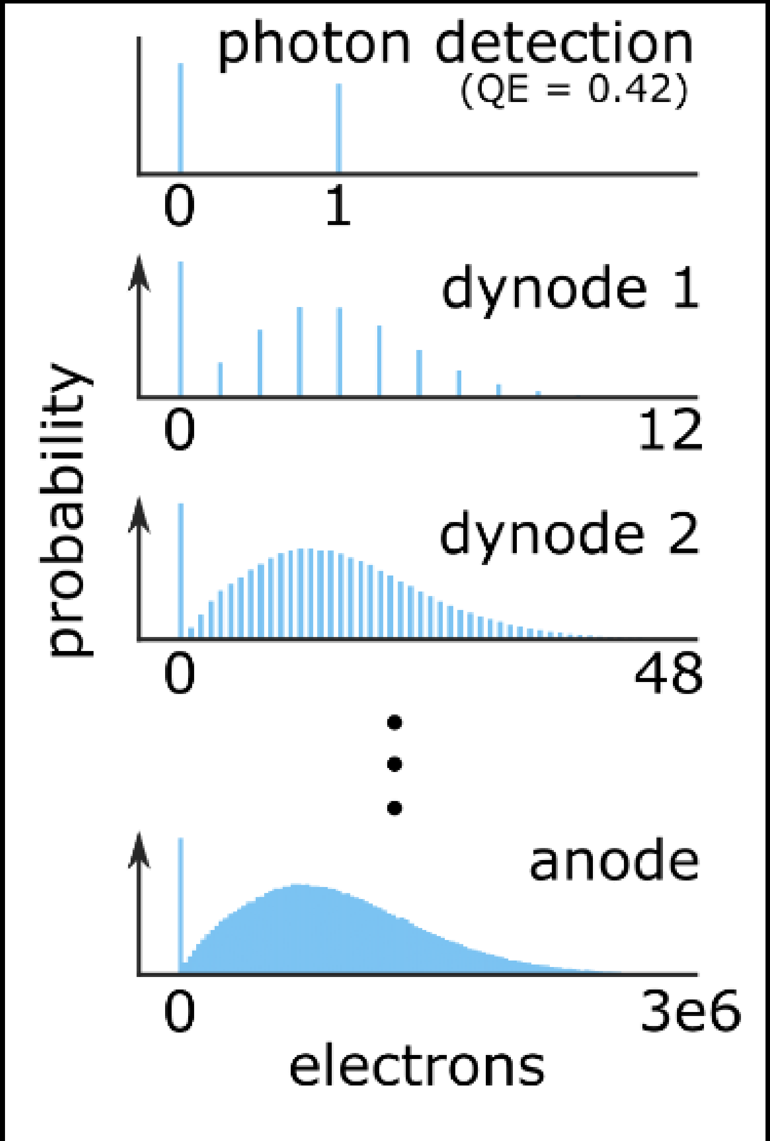
Silicon Photomultipliers (SiPMs)

Measured pulse height distributions at equal photon rates:

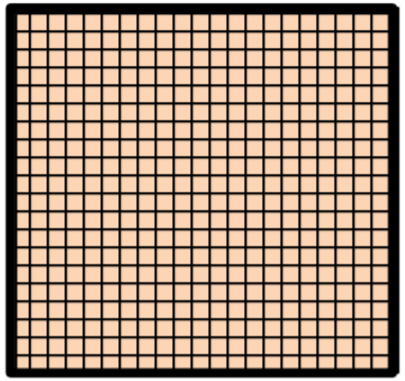


PMT operating principles

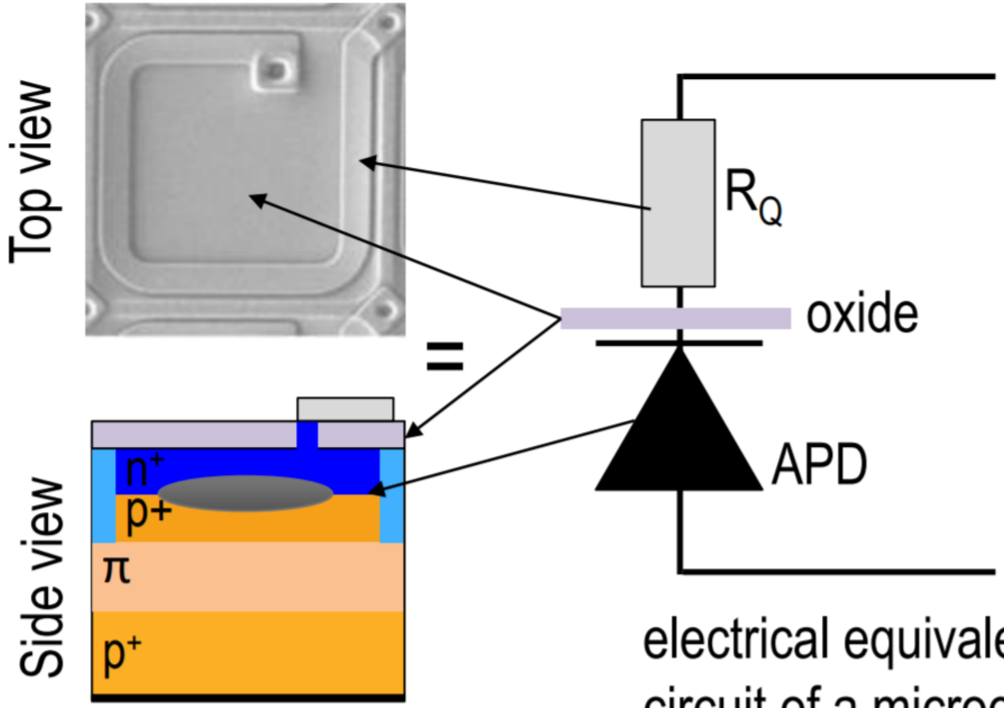




SiPM structure



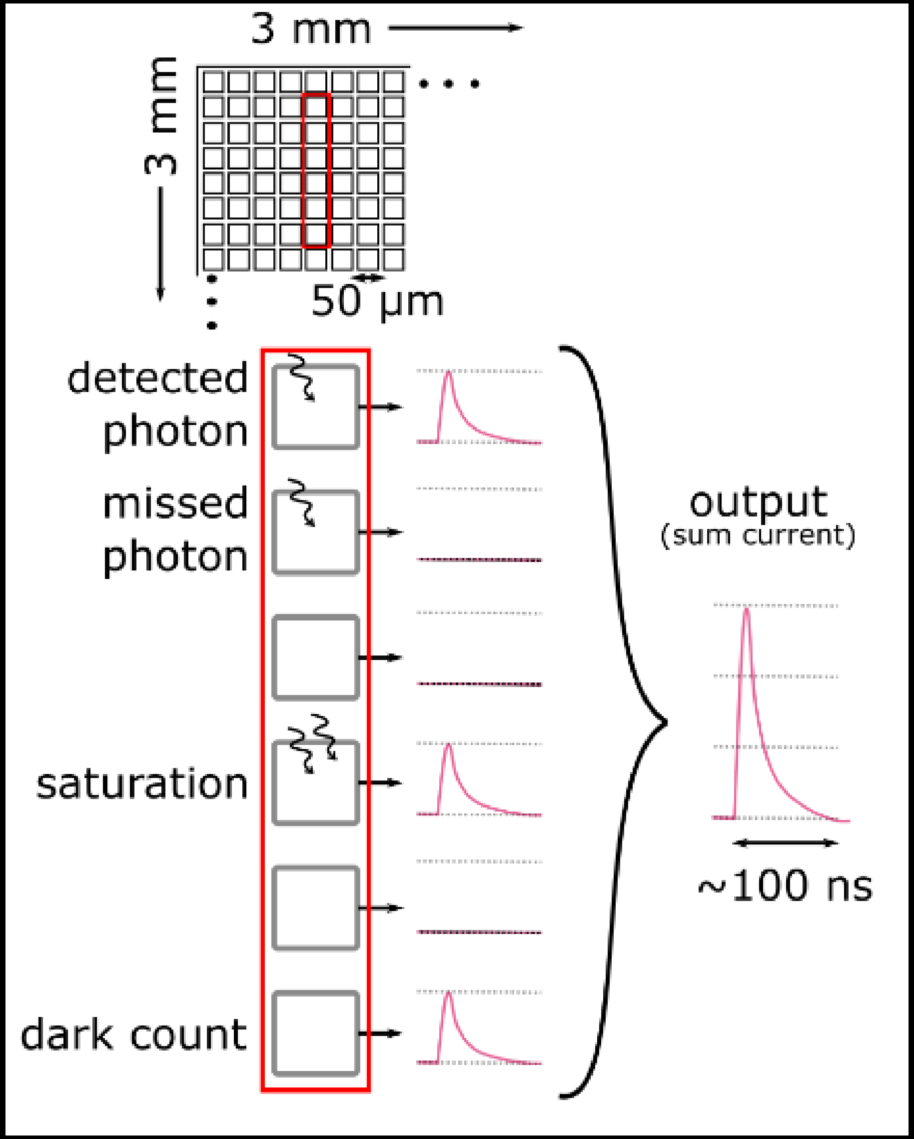
SiPM is an array of microcells

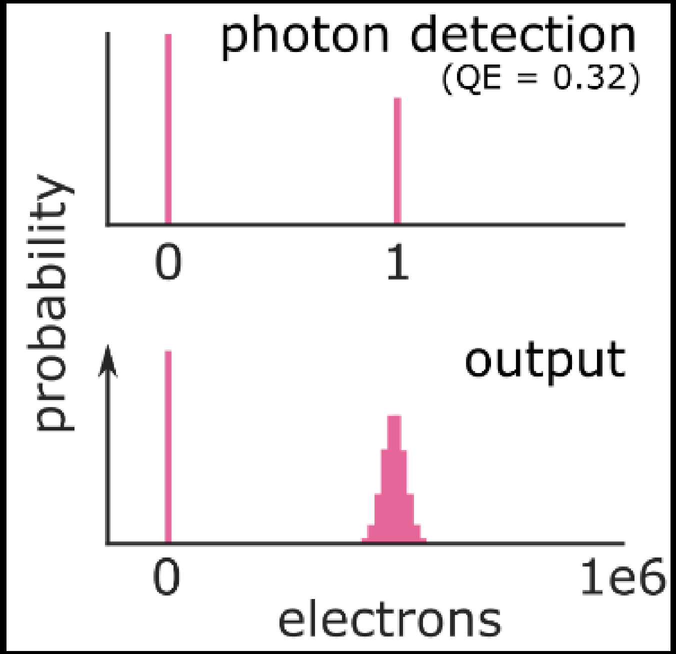


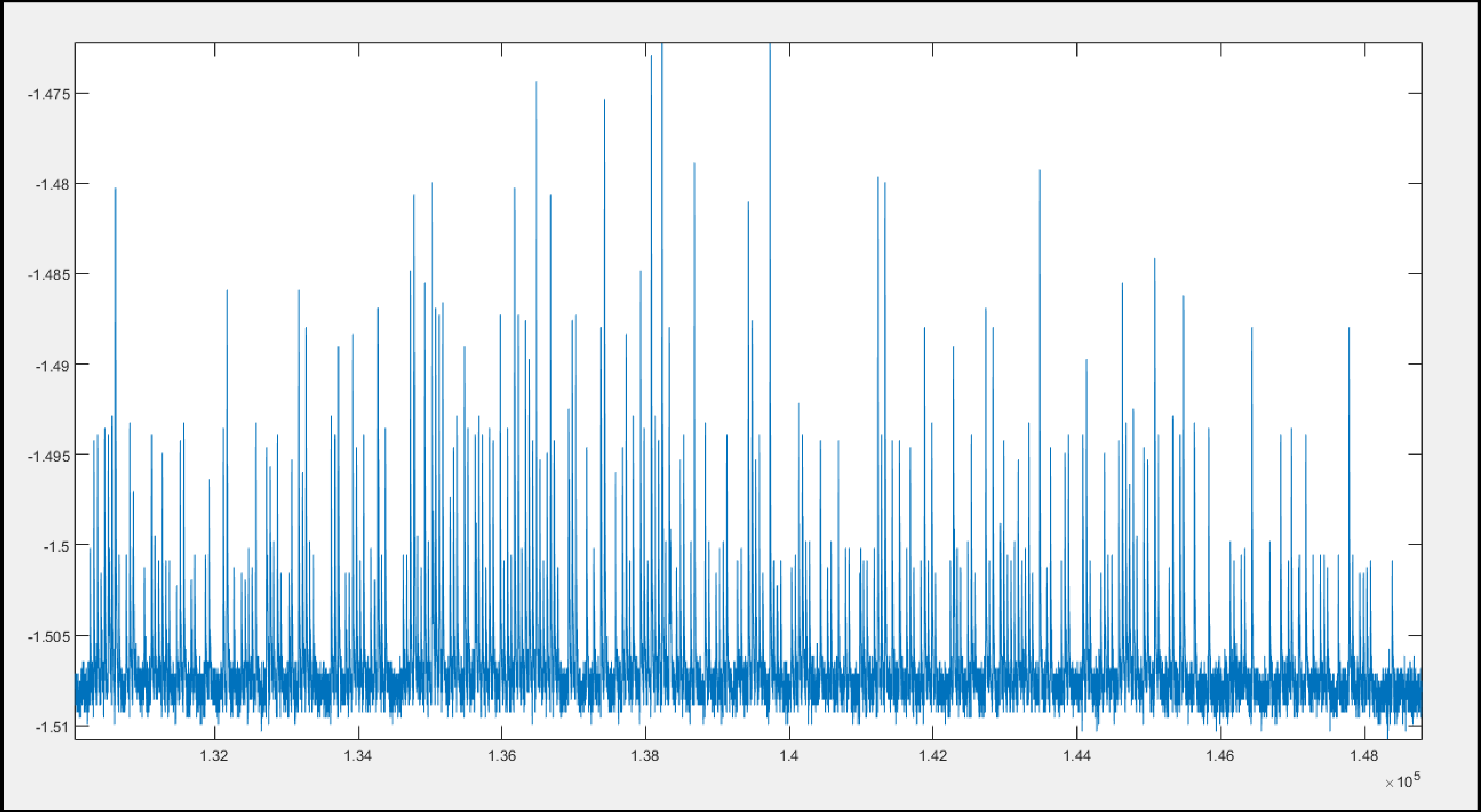
Single microcell

electrical equivalent circuit of a microcell

SiPM Operating principles

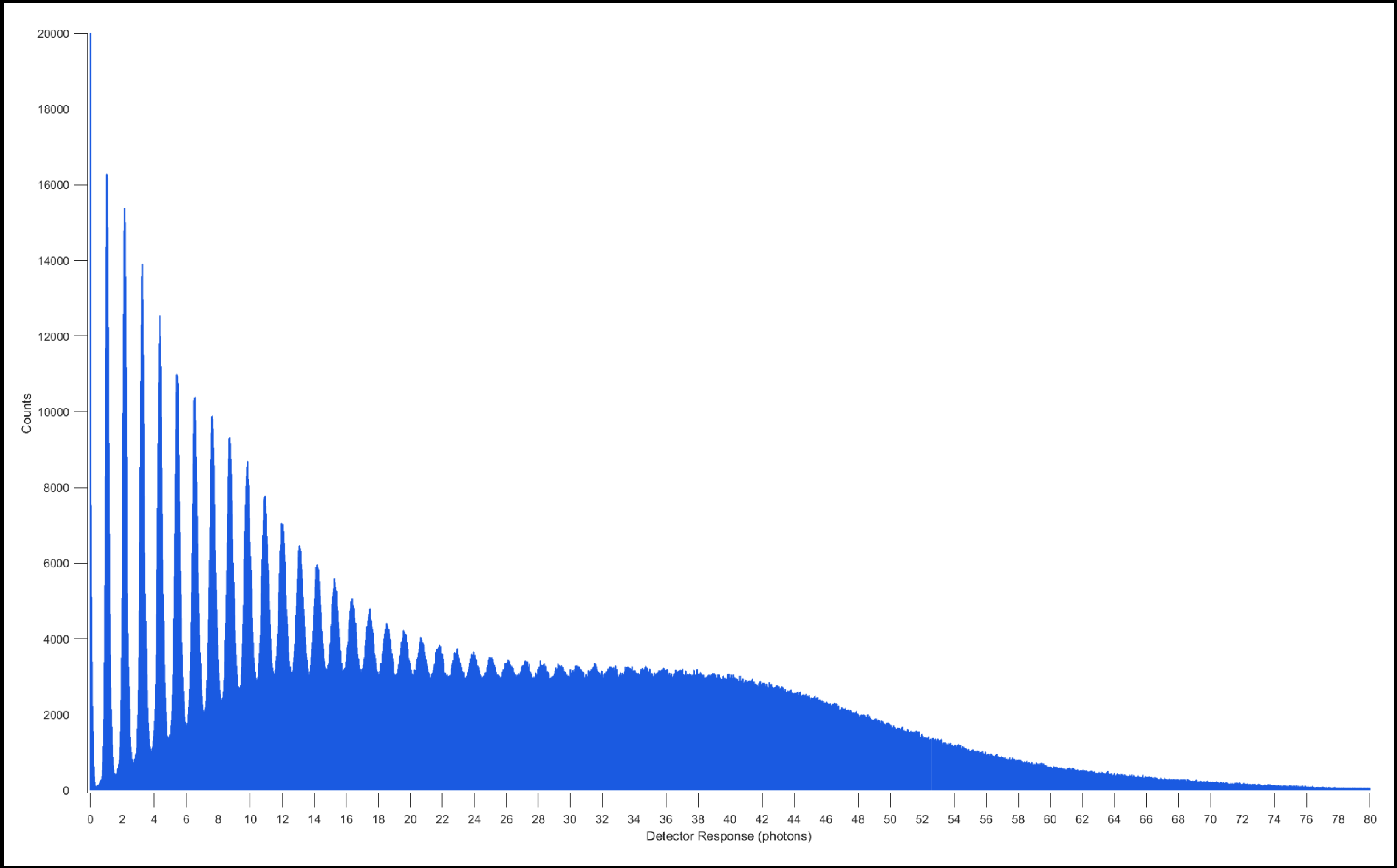






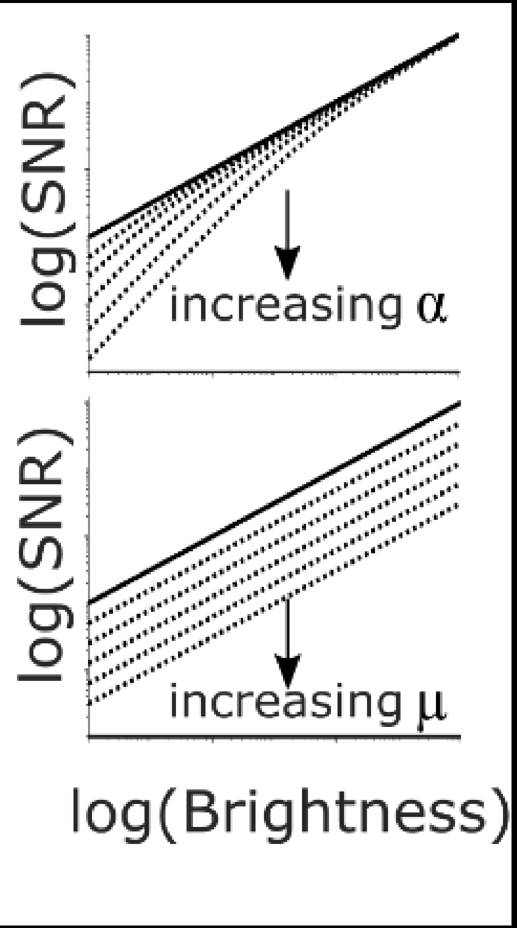
Voltage

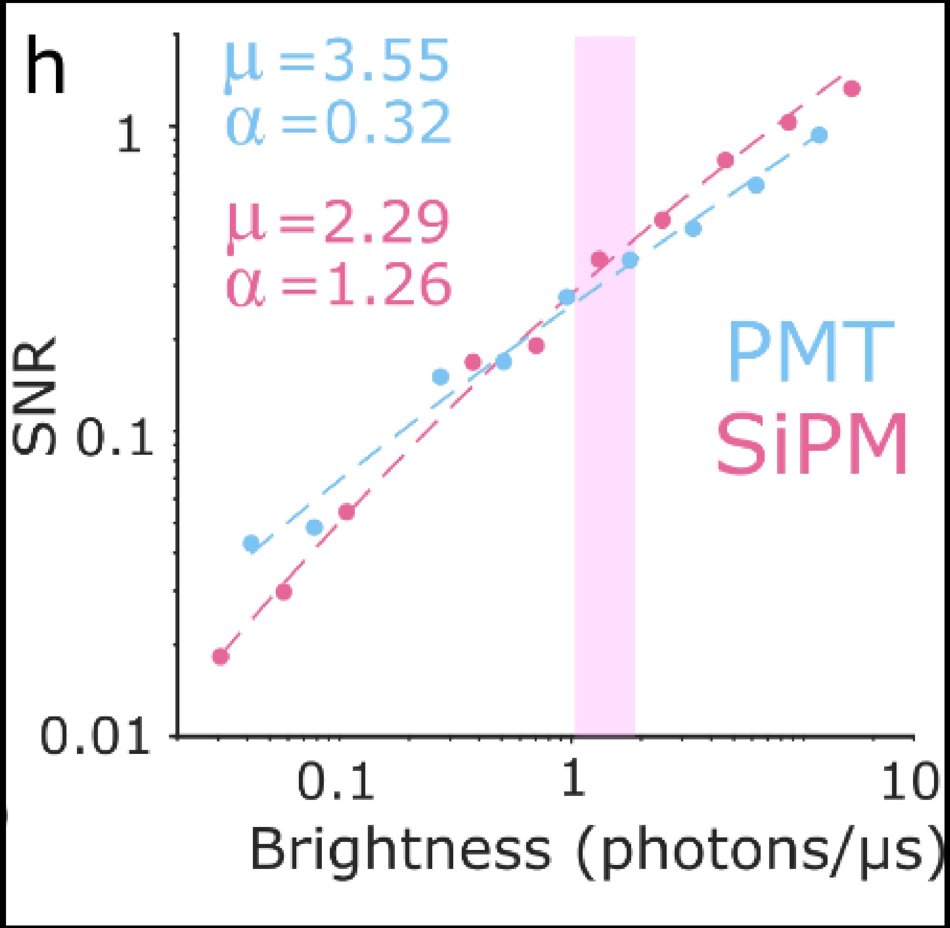
Time (digitizer samples)

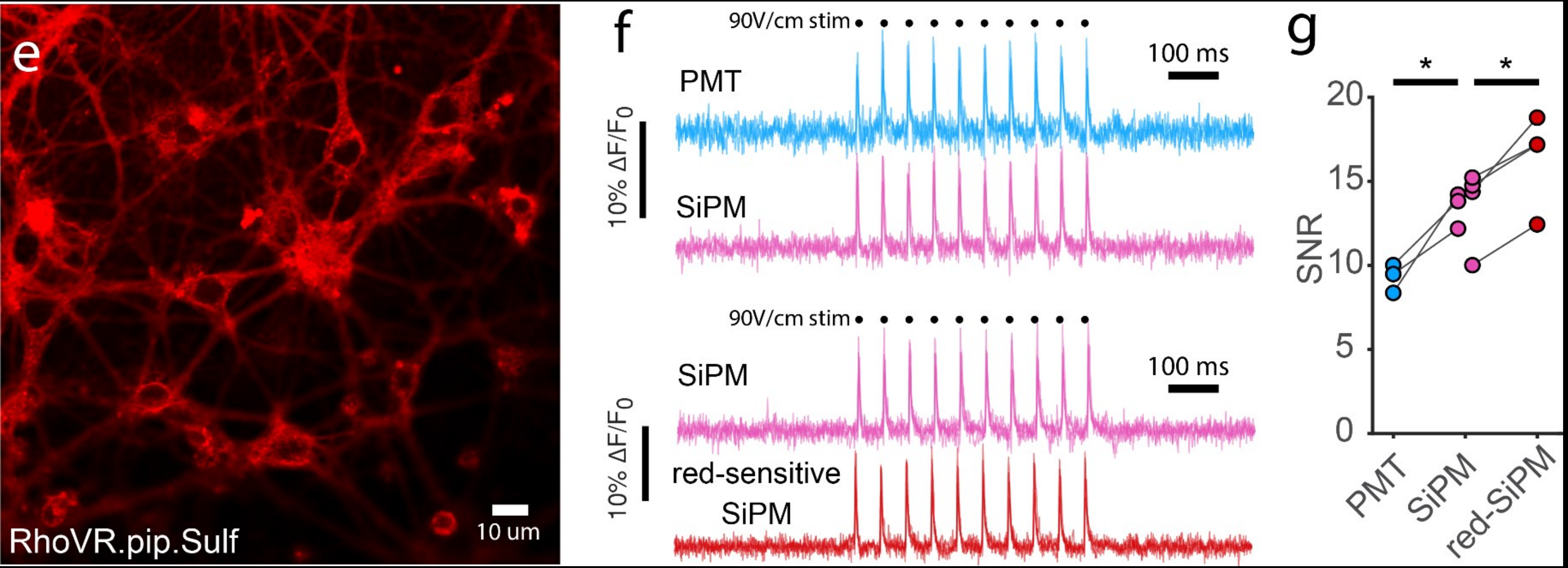


Counts
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80
Detector Response (photons)

Additive and Multiplicative Noise







Modi et al, 2019, BioRxiv

Thank You

Heather Davies
Abbas Kazemipour
Ondrej Novak
Emiliano Jimenez
JJ Kim
Abhi Aggarwal
Ondrej Zelenka
Dan Flickinger
Jonathan Marvin
Justin Little
Philip Borden
Ahmed Abdelfattah

Eric Schreiter
Loren Looger
Karel Svoboda
Na Ji
Shaul Druckmann
Takashi Kawashima
Sachin Vaidya
Gayathri Ranganathan
Jeff Magee
Hersh Bhargava
Claire Deo
John Heddleston
Srini Turaga
Philipp Keller
Misha Ahrens
Manuel Mohr
Aaron Kerlin
Boaz Mohar
Sal DiLisio

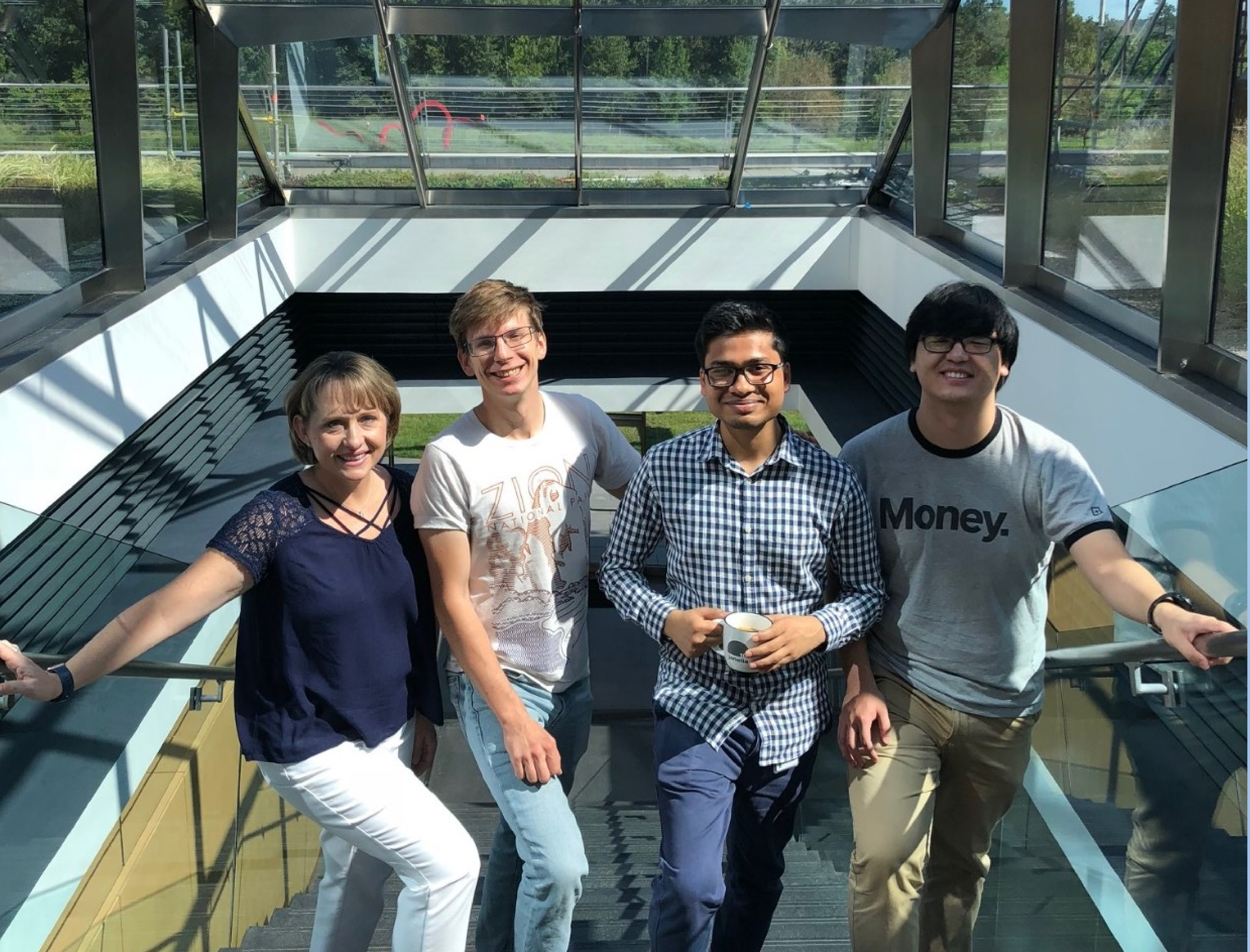
Doug Kim
Hod Dana
John Macklin
Ronak Patel
Steve Sawtelle
Mladen Barbic
Chris McRaven
Bill Biddle
Bruce Bowers
Roger Rogers
Vasily Goncharov
Amy Hu
Brenda Shields
Jared Rouchard
Miriam Rose
Sara Barnes
Sarah Erwin
Kim Ritola
Melissa Ramirez

<u>Vidrio Technologies</u>

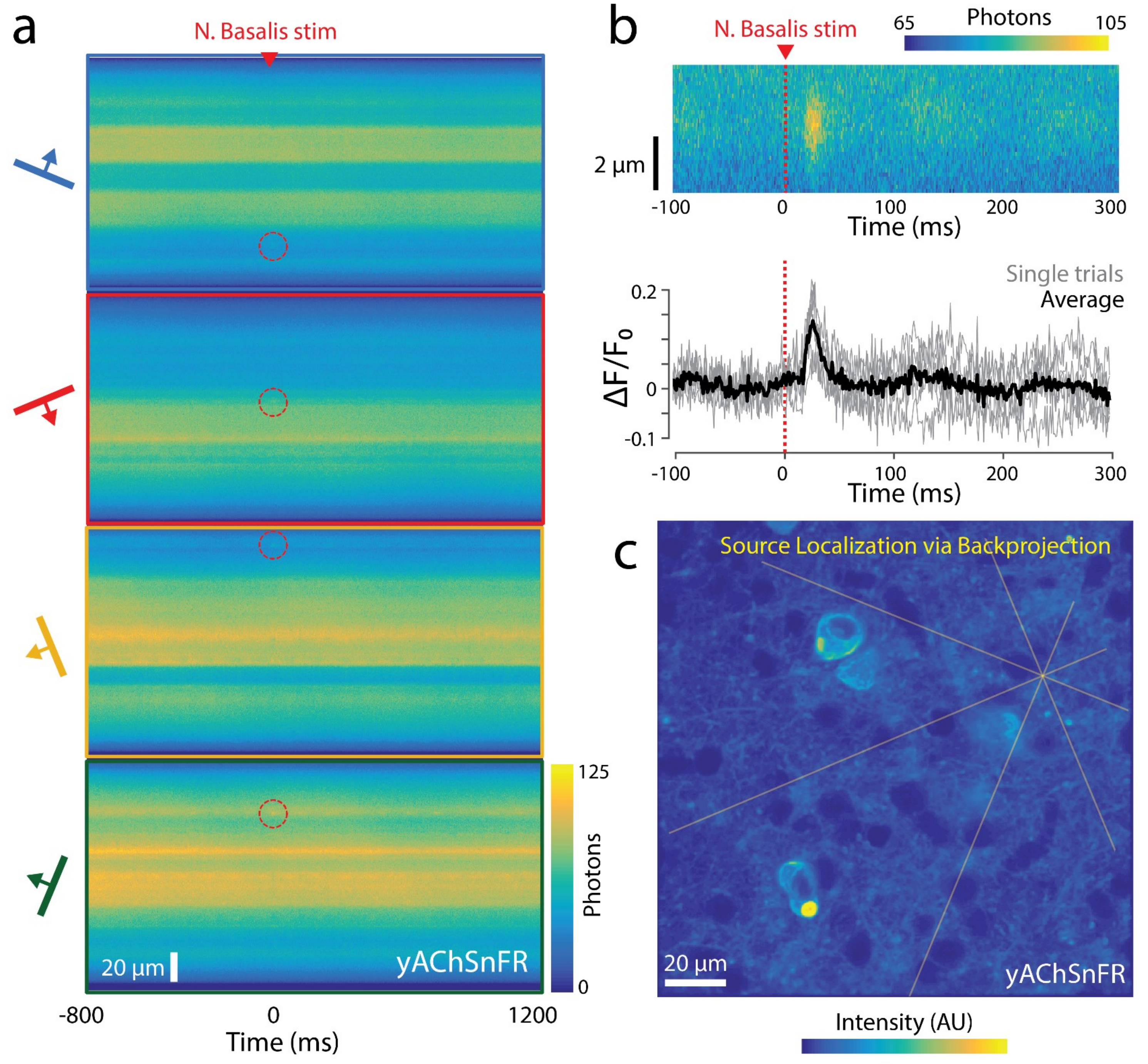
Jonathan King
Georg Jaindl

<u>UC Berkeley</u>

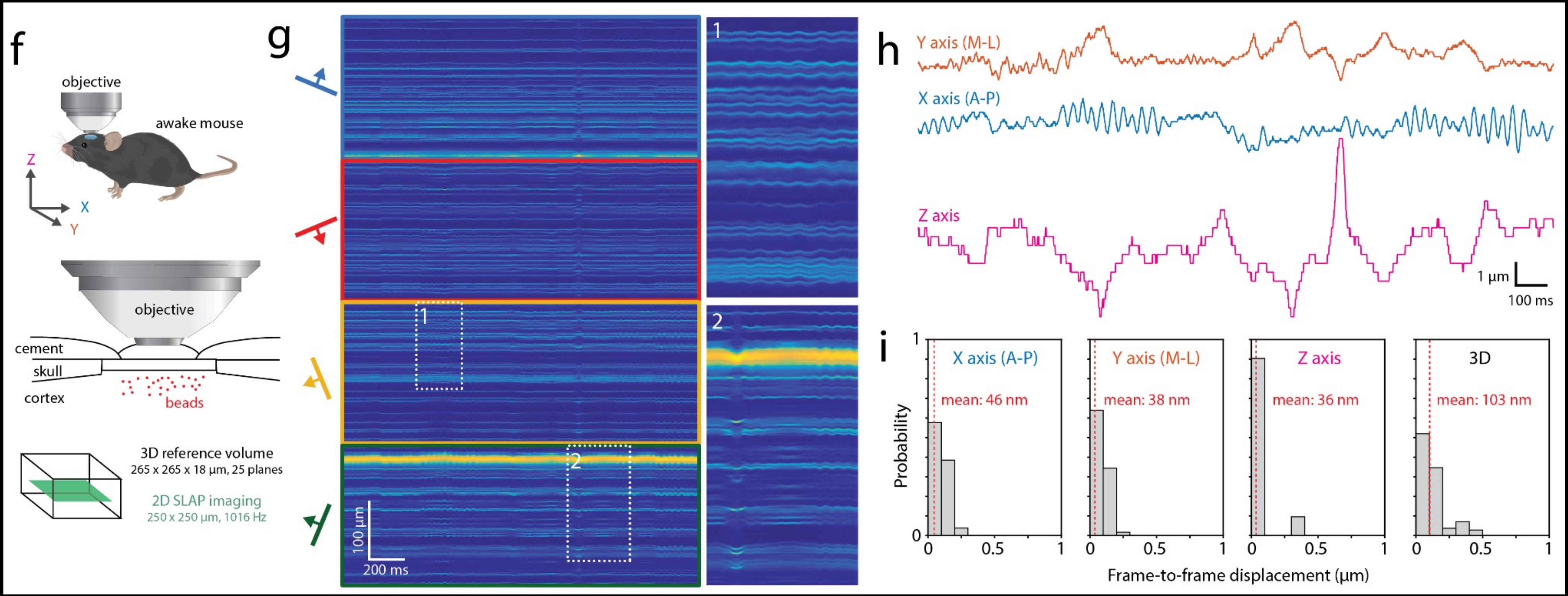
Evan Miller
Parker Deal
Sarah Abdullatif



Questions?


a
N. Basalis stim
20 µm
yAChSnFR
125
Photons
0
-800
0
1200
Time (ms)
b
N. Basalis stim
65
Photons
105
2 µm
-100
0
100
200
300
Time (ms)
Single trials
Average
0.2
ΔF/F0
0
-0.1
-100
0
100
200
300
Time (ms)
c
Source Localization via Backprojection
20 µm
yAChSnFR
Intensity (AU)

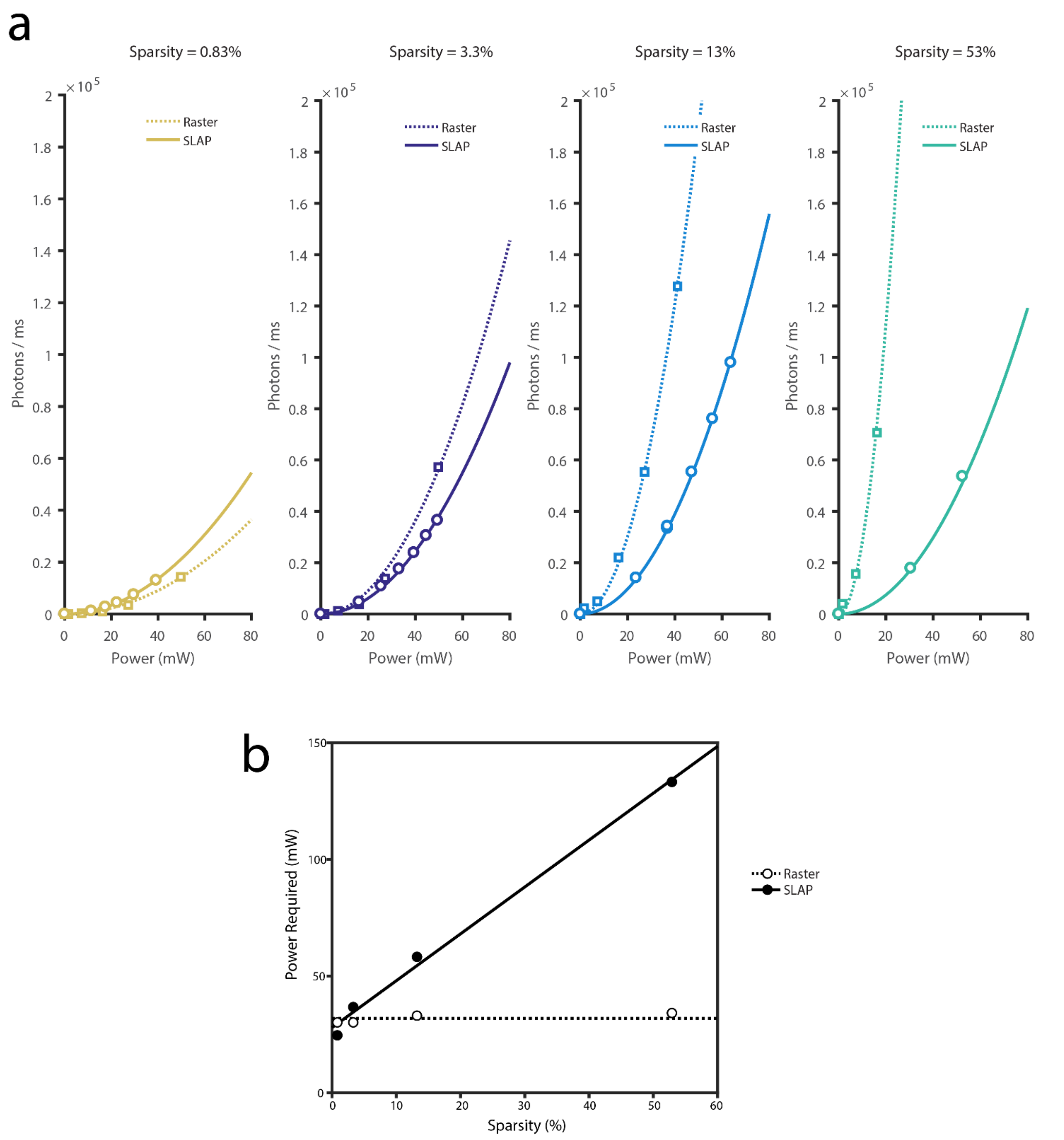
Kilohertz, submicron motion tracking in awake mice

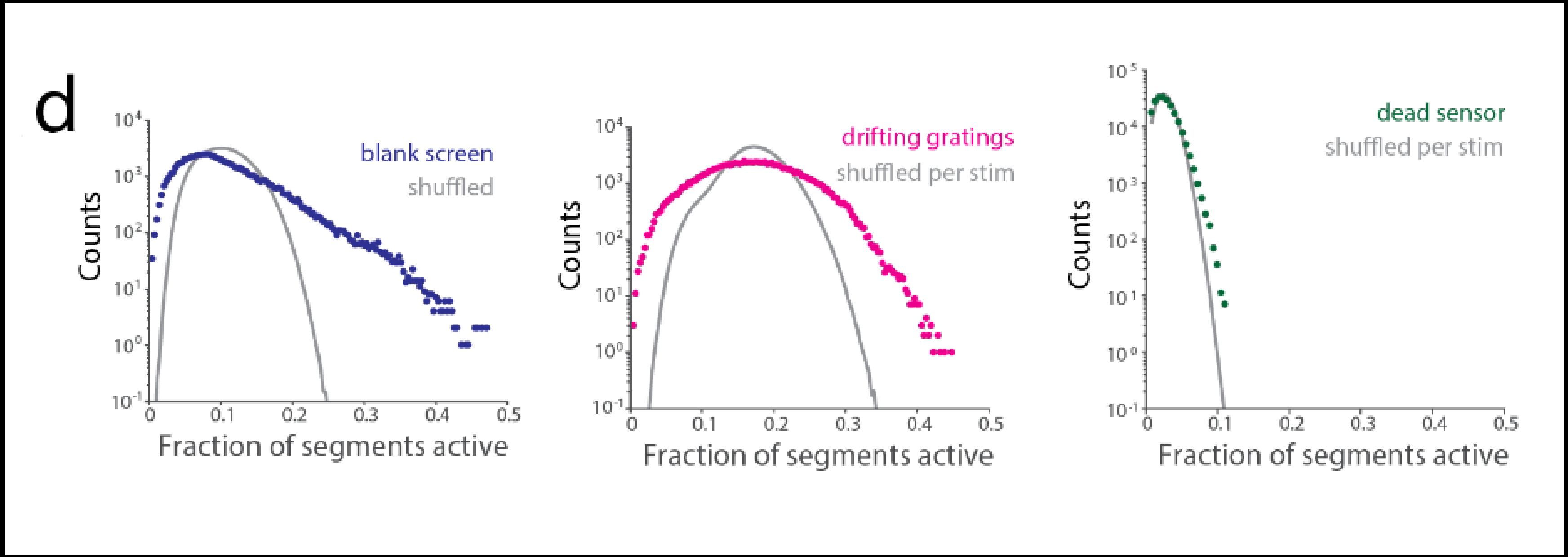


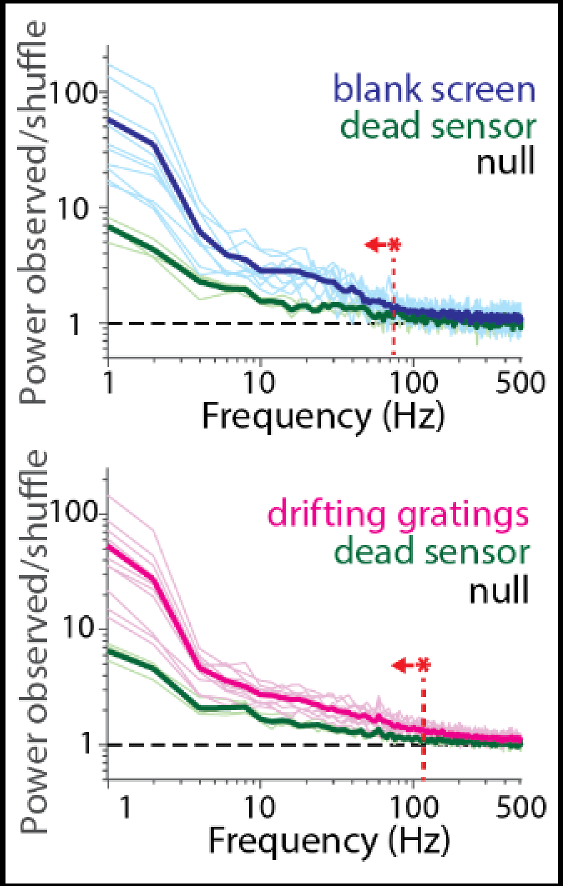
Laser Power Usage

vs

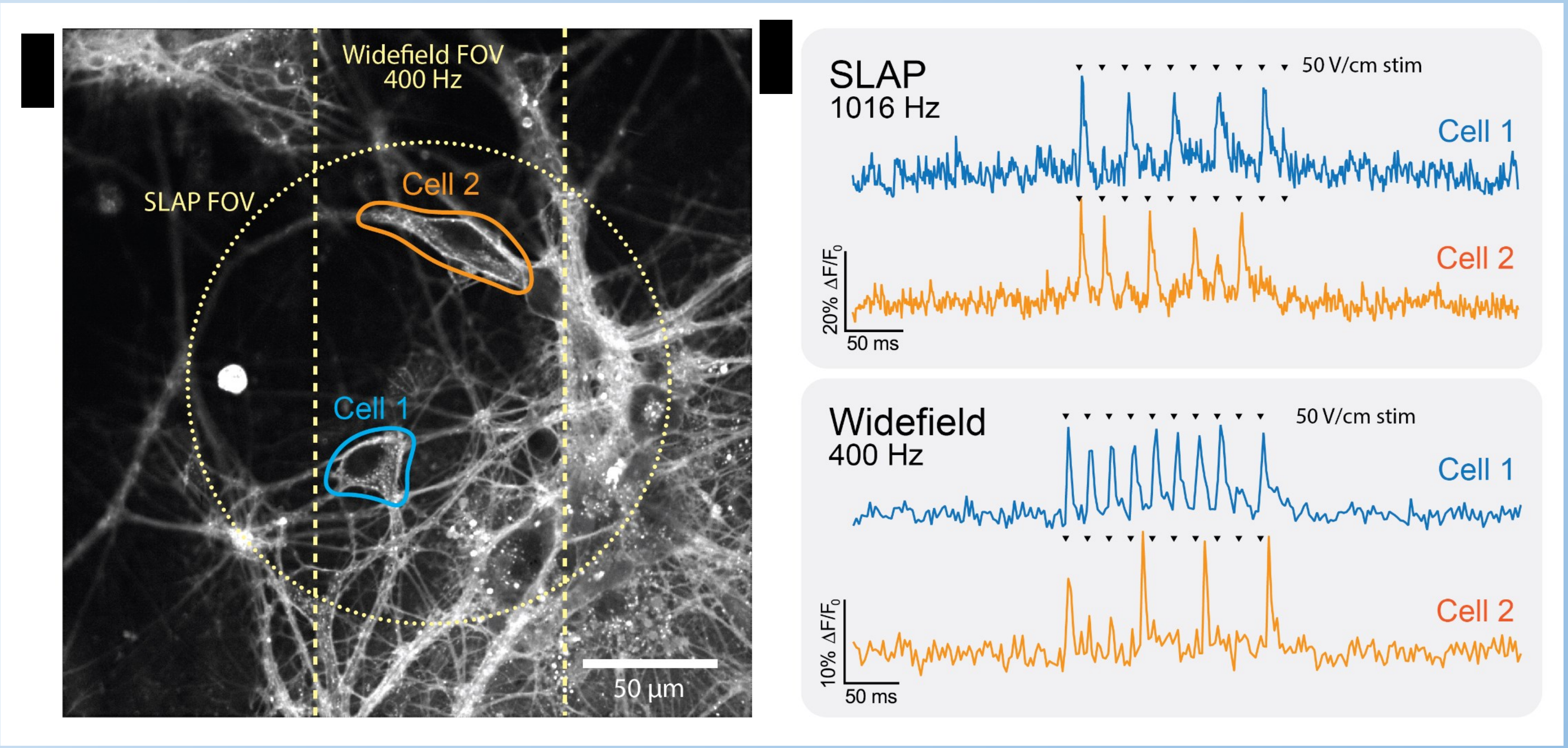
SLM open fraction
("Sparsity")







Dendritic activity is highly synchronized at frequencies up to ~100Hz


[redacted]
Widefield FOV
400 Hz
SLAP FOV
Cell 2
Cell 1
50 µm
[redacted]
SLAP
1016 Hz
50 V/cm stim
Cell 1
Cell 2
20% ΔF/F0
50 ms
Widefield
400 Hz
50 V/cm stim
Cell 1
Cell 2
10% ΔF/F0
50 ms