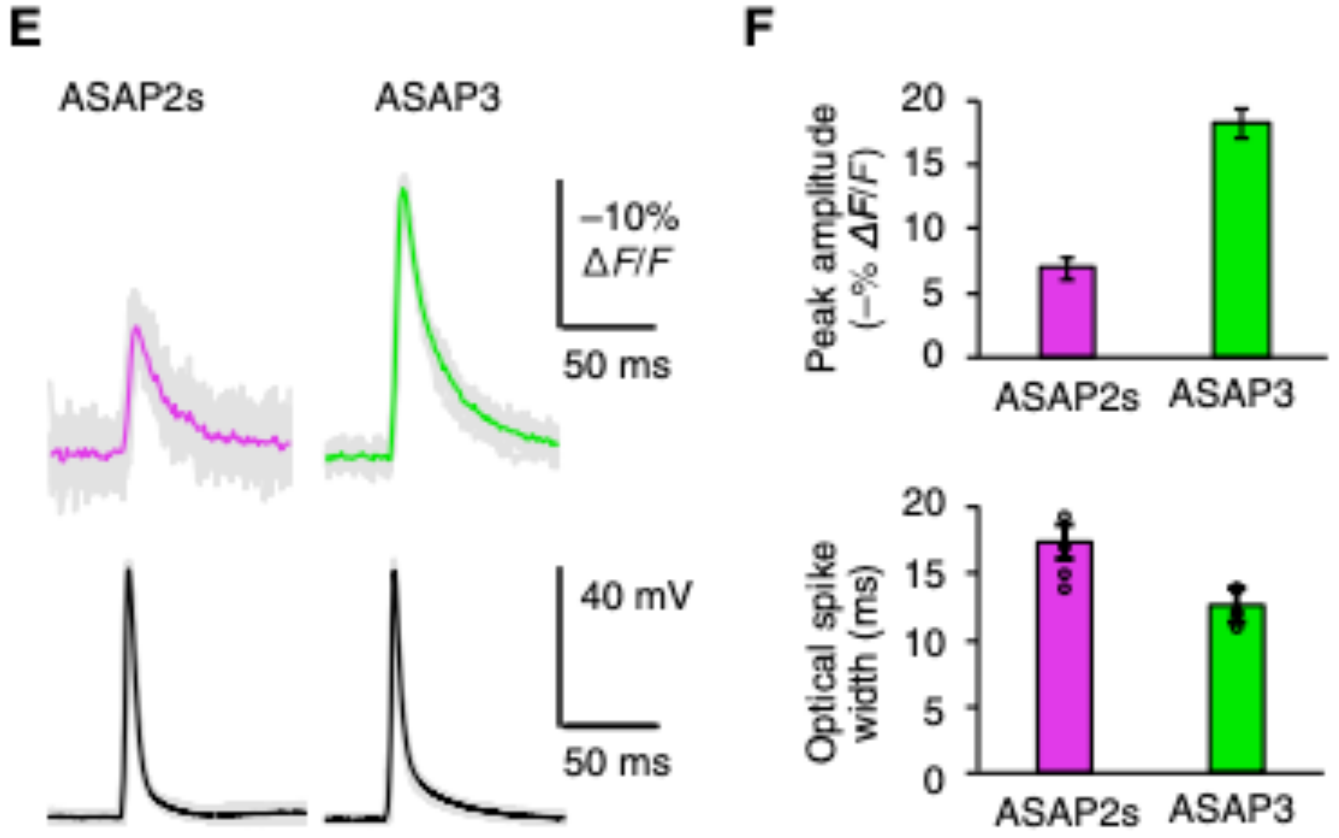
High Speed Imaging

‘Breaking the KHz barrier’

Jacques Carolan

Why

- Calcium dynamics ~100 ms vs APs ~ 1 ms
- Genetically Encoder Voltage Indicators:
  - ASAP3[1], QuasAr3[2], JEDI[3]

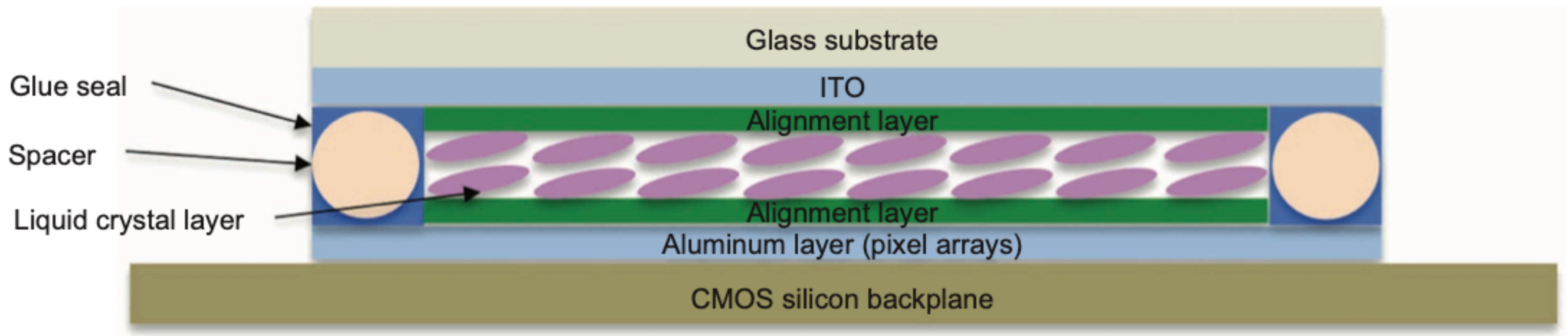


- Sub-threshold events
- All-optical electrophysiology[4] & connectomics

[1] Villette et al., (2019) [2P]
[2] Adam et al., (2019) [1P]
[3] St-Pierre Lab, Zoe + Junlang! [2P]
[4] Y. Adam (2021)

Challenges

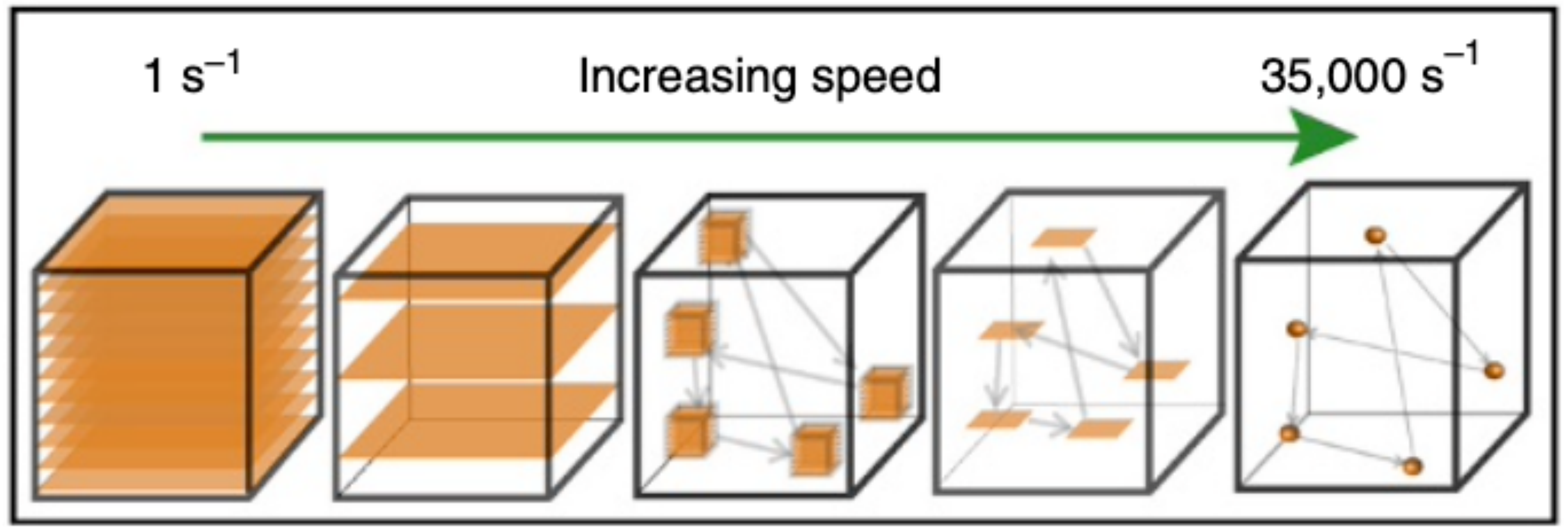
- Beam steering (e.g. Galvo):
  - Moving a mass (e.g. a mirror) has an inherent bandwidth → ~10 KHz = 0.1 ms
- Beam patterning (e.g. SLM):
  - Vicious liquid crystals → ~100 Hz =10 ms



Zhang et al,. Science & Applications (2014)

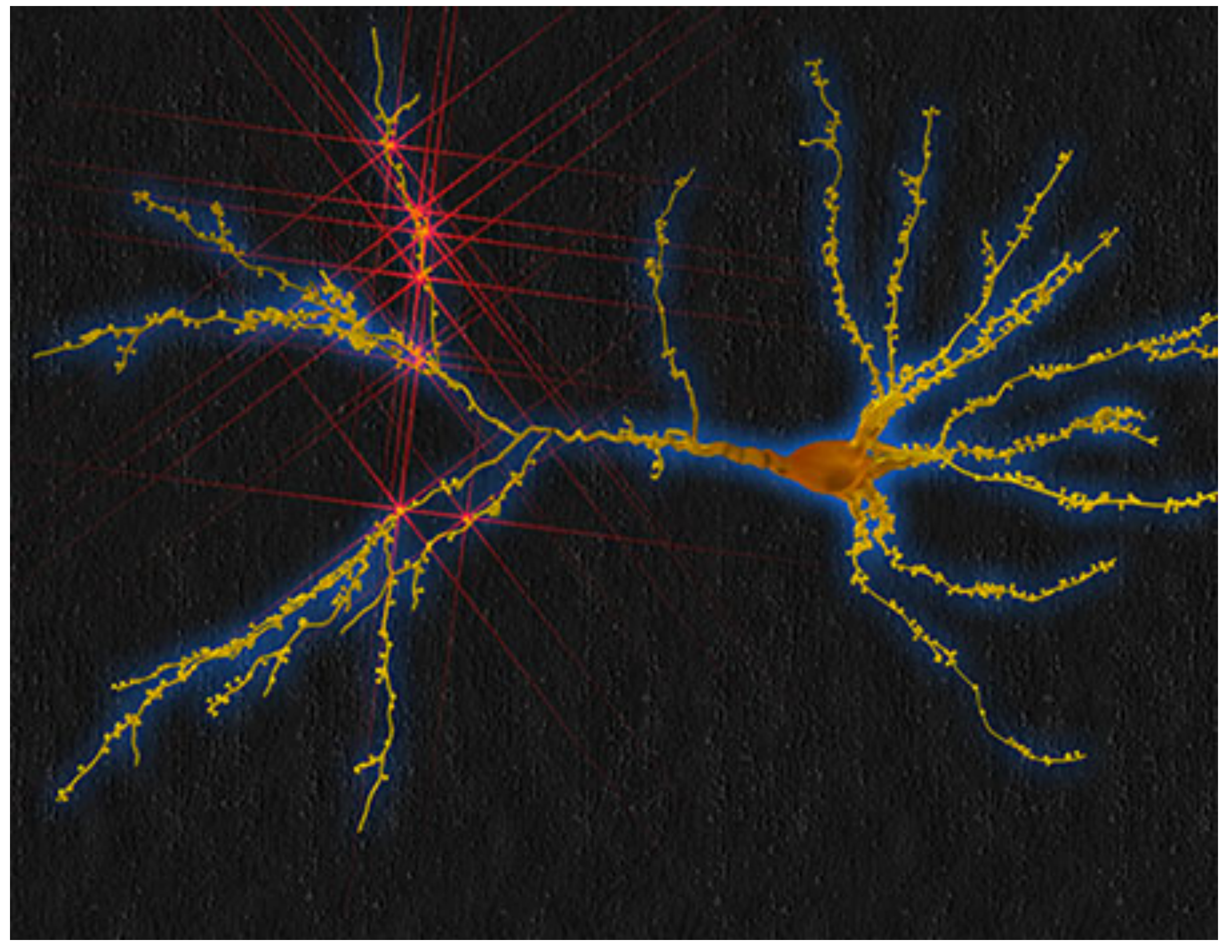
- Cameras (e.g. sCMOS):
  - 100 fps (2000 x 2000 px)

Random Access



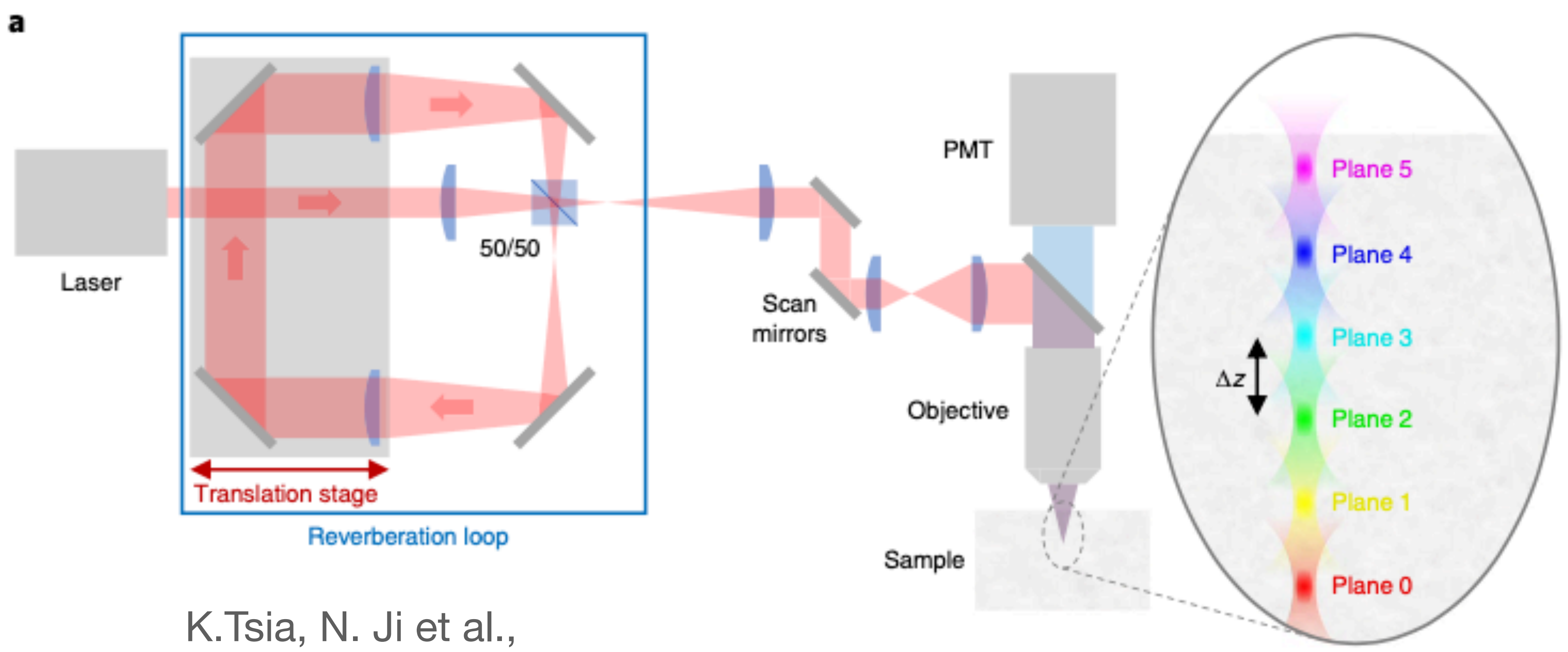
K. M. N. S. Nadella … P. A. Kirkby, and R. A. Silver, *Nat Meth* (2016).

Computational



K. Podgorski

Multiplexing



K.Tsia, N. Ji et al.,

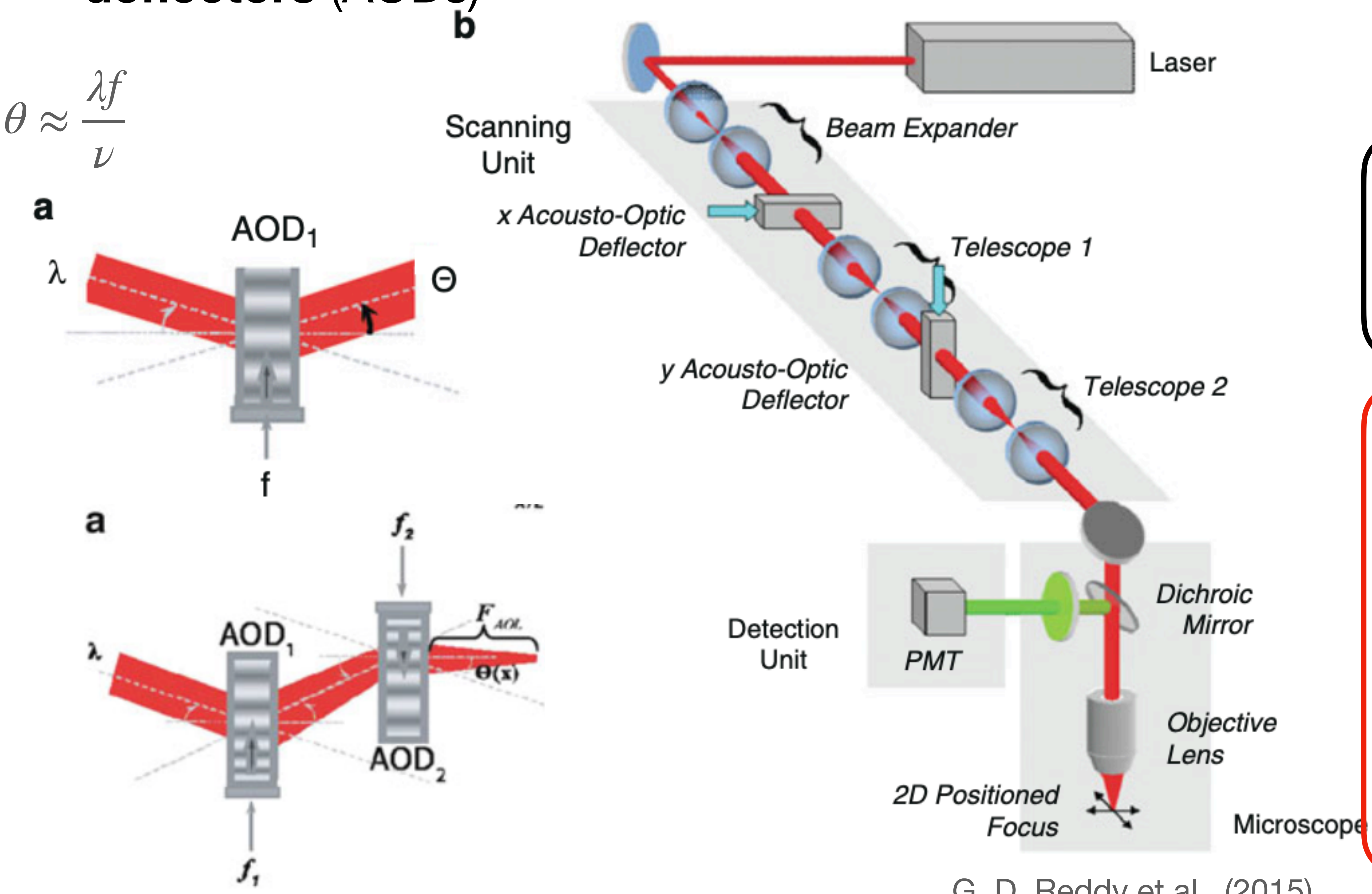
Aims:

- Broad overview of approaches
- Technological advances to keep an eye on
- Opportunities for us

Random Access Microscopy

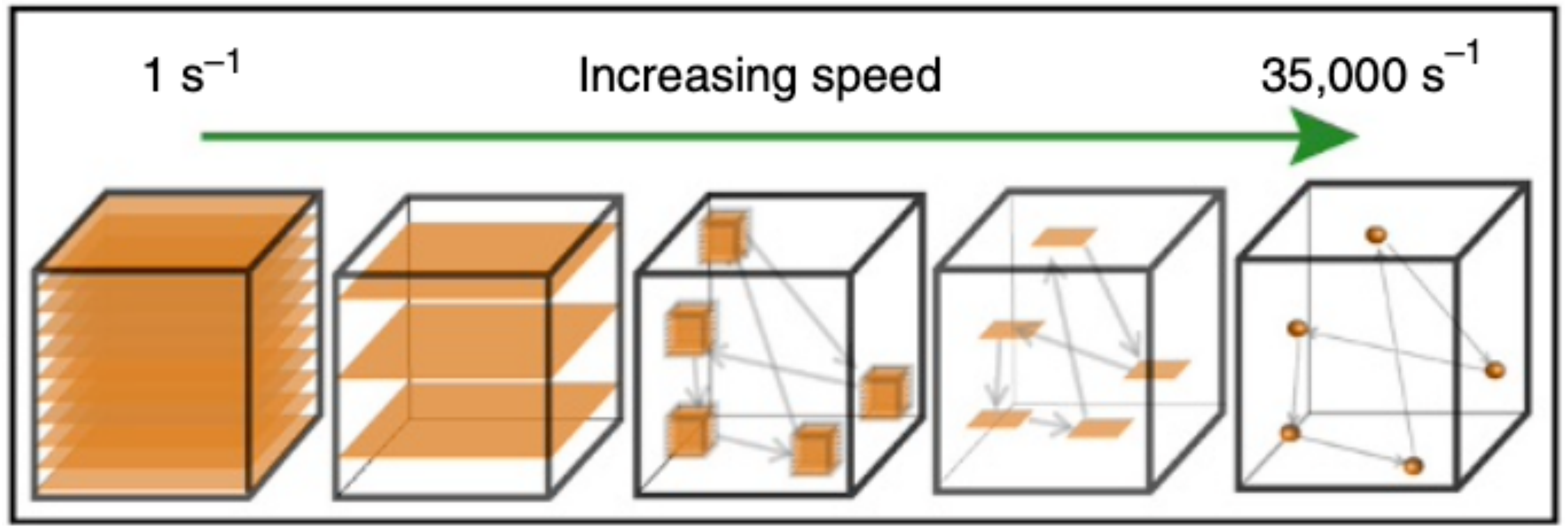
- Only access predetermined ROI's
- Programmable scanning enabled by **acoustic optic deflectors** (AODs)

$$\theta \approx \frac{\lambda f}{\nu}$$



Chirp f(t)

G. D. Reddy et al., (2015)

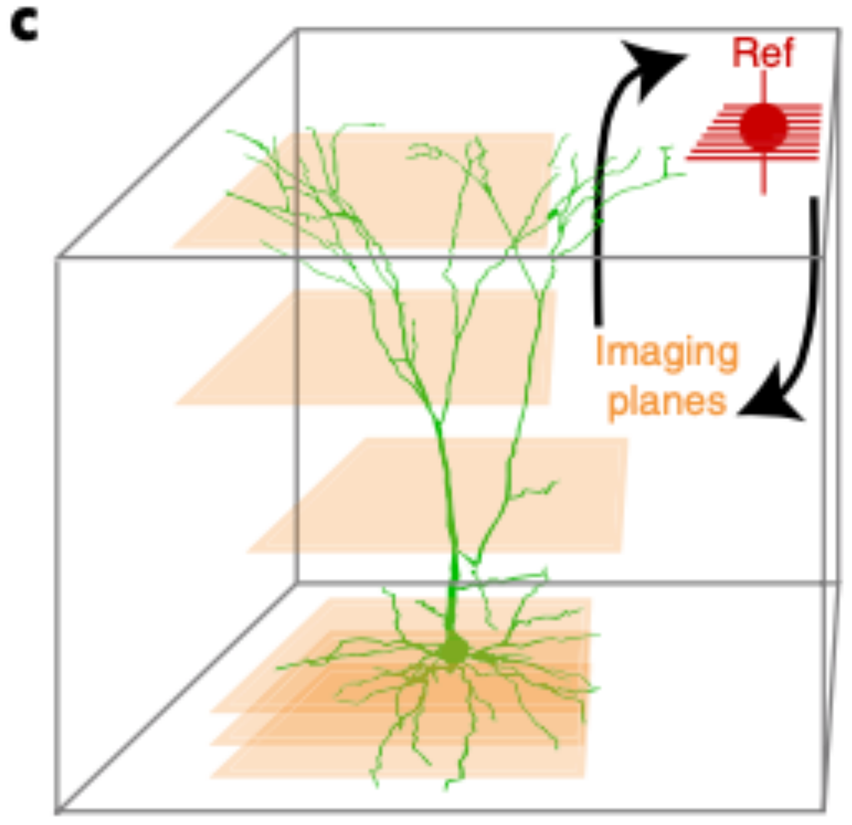


K. M. N. S. Nadella … P. A. Kirkby, and R. A. Silver, *Nat Meth* (2016).

Materials: Tellerium Dioxide / Quartz / **Glass**
Speed of sound = 4000 m/s
Switching speed = 20 mm / 4000 m/s = **5 μs**

+Fast
+3D
+Versatile
+Well developed tech

- Sparsity
- Dispersion comp.
- Movement correction

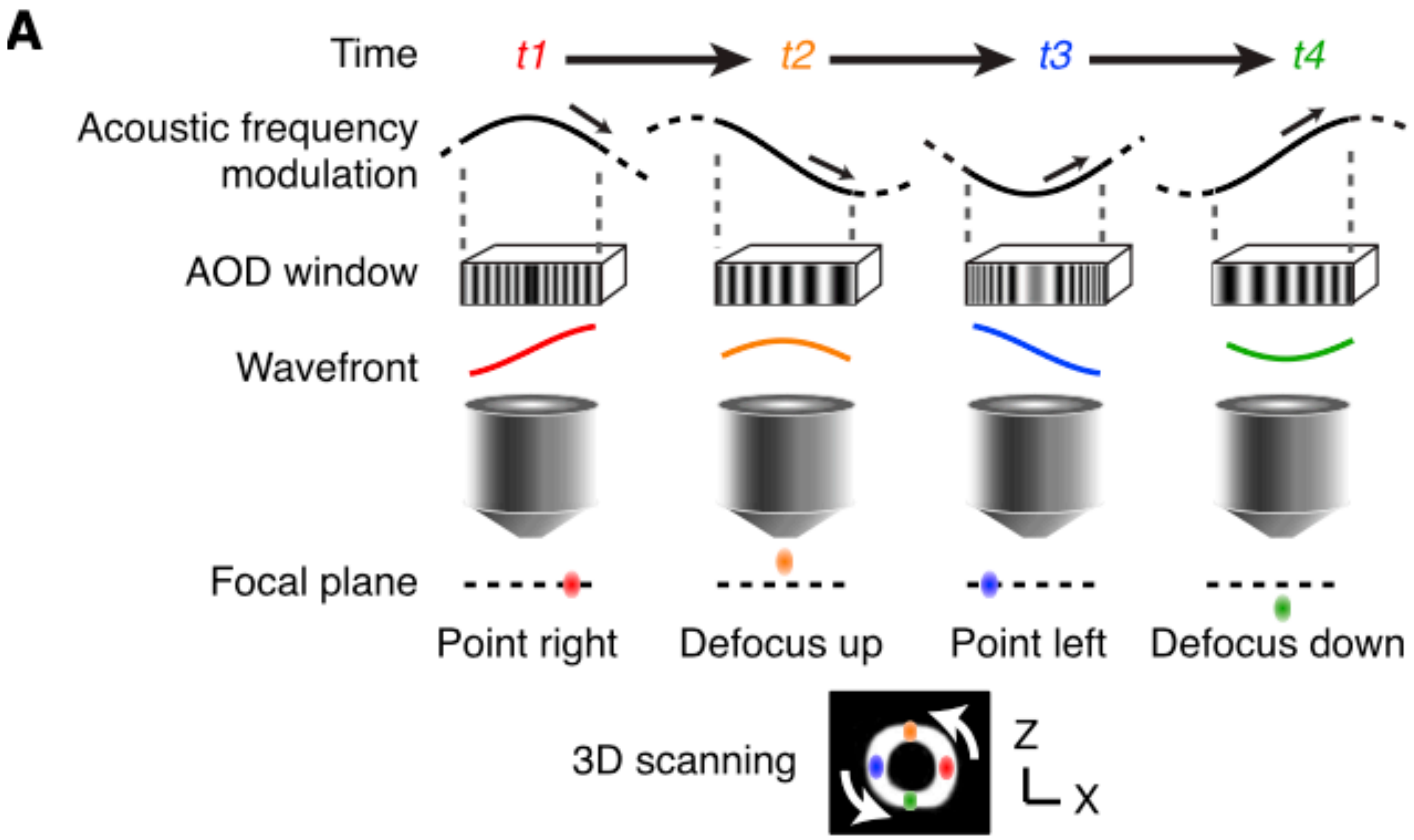


Griffiths et al., *Nat Methods* (2020)

RAMP for GEVI

- Challenges:
  - membrane confined molecules → diffraction limited spot gives low signal
  - Slow diffusion limits replacement rates and causes photobleaching

ULoVe



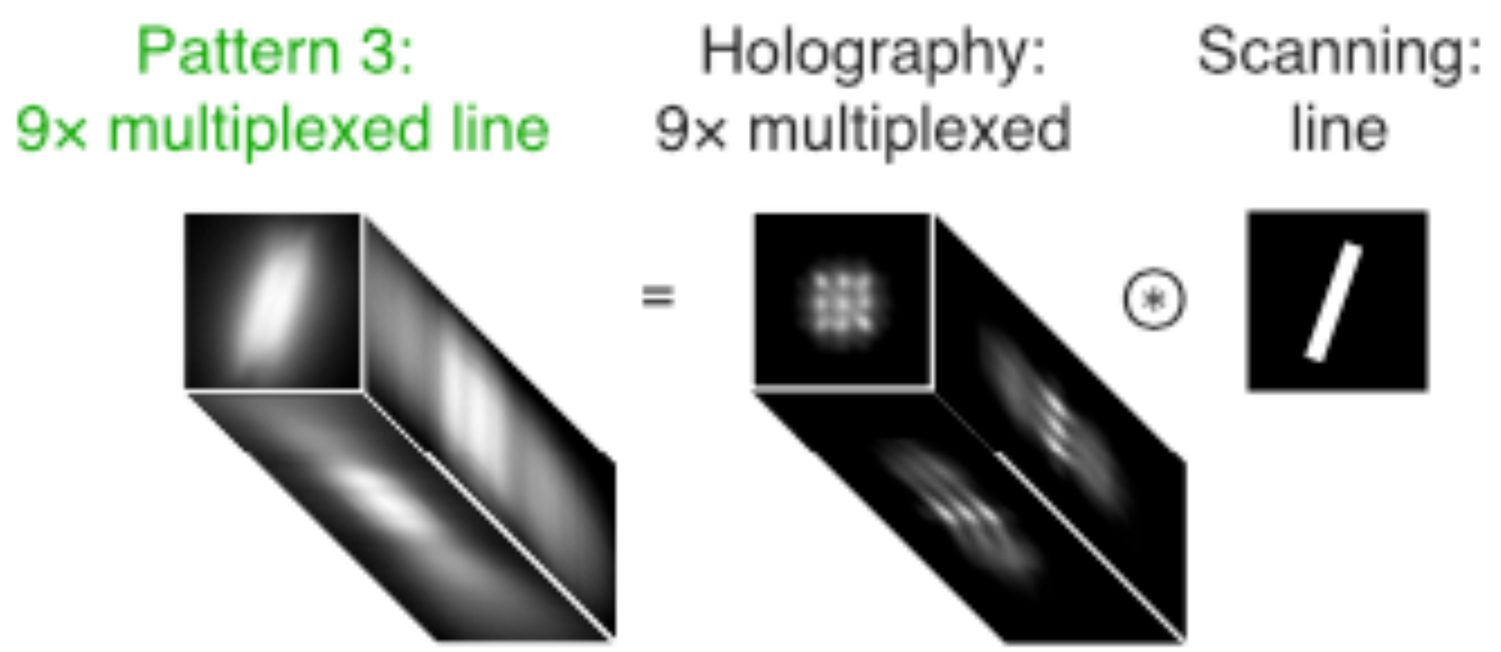
Cell

Resource

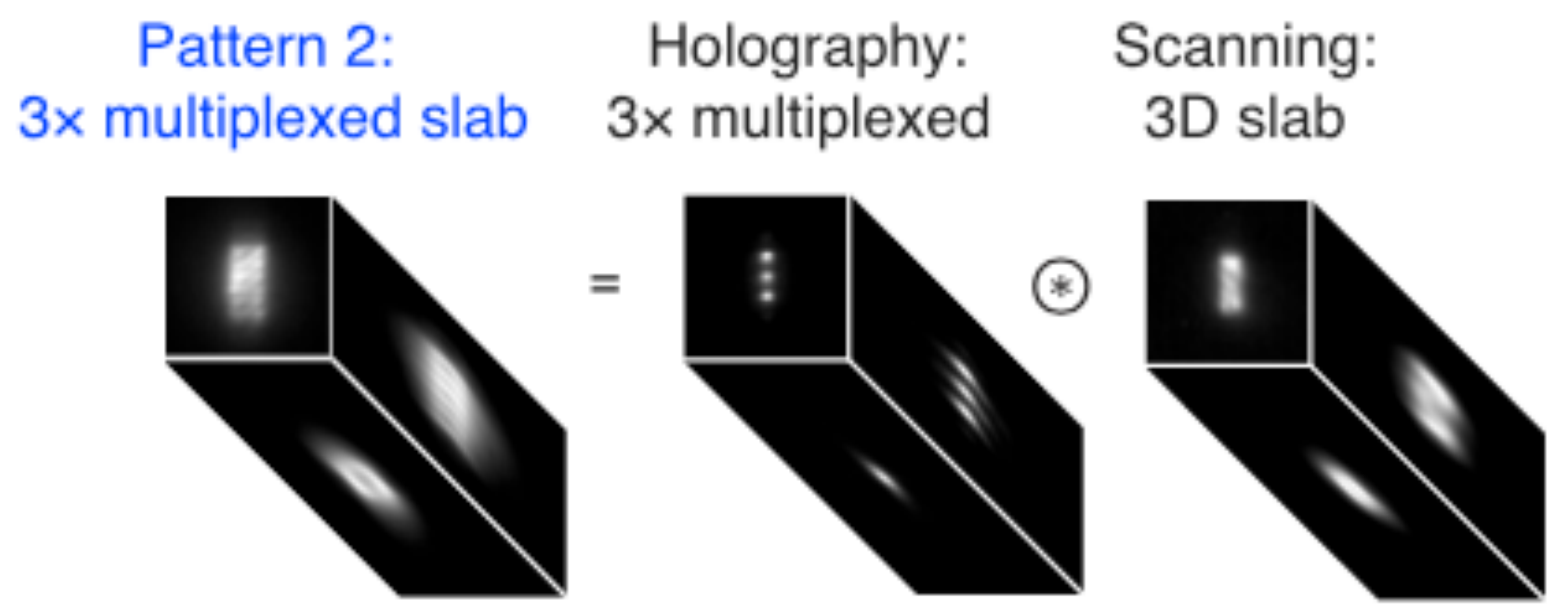
ASAP3

Ultrafast Two-Photon Imaging of a High-Gain Voltage Indicator in Awake Behaving Mice

Vincent Villette,[1,13] Mariya Chavarha,[2,3,13] Ivan K. Dimov,[2,4] Jonathan Bradley,[1] Lagnajeet Pradhan,[2,4] Benjamin Mathieu,[1] Stephen W. Evans,[2] Simon Chamberland,[7] Dongqing Shi,[2,5] Renzhi Yang,[6,11] Benjamin B. Kim,[3] Annick Ayon,[1] Abdelali Jalil,[9] François St-Pierre,[8] Mark J. Schnitzer,[4,12] Guoqiang Bi,[5,10] Katalin Toth,[7] Jun Ding,[6] Stéphane Dieudonné,[1,*] and Michael Z. Lin[2,3,14,*]

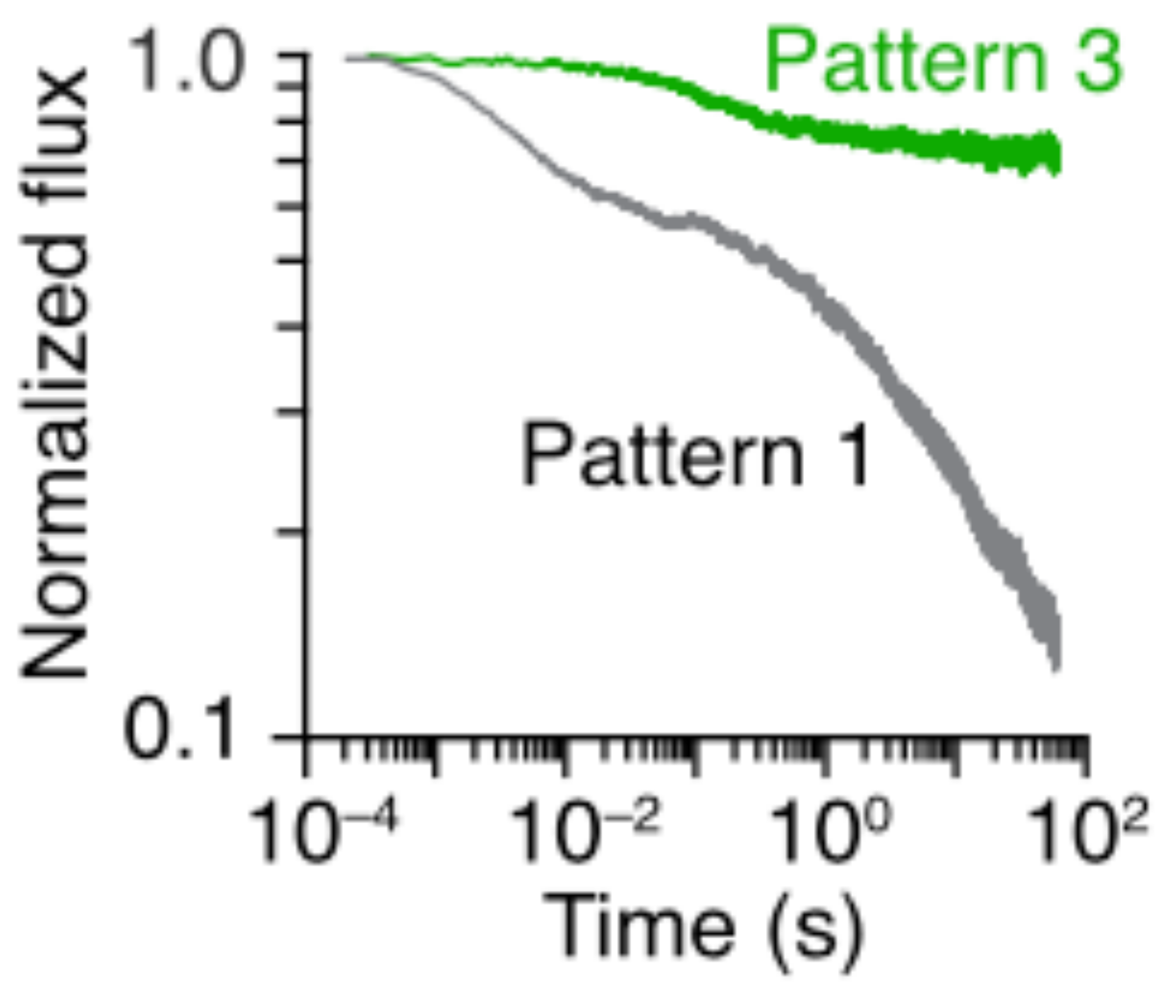


Akemann, W. Et al., Opt. Express 23, 28191–28205.

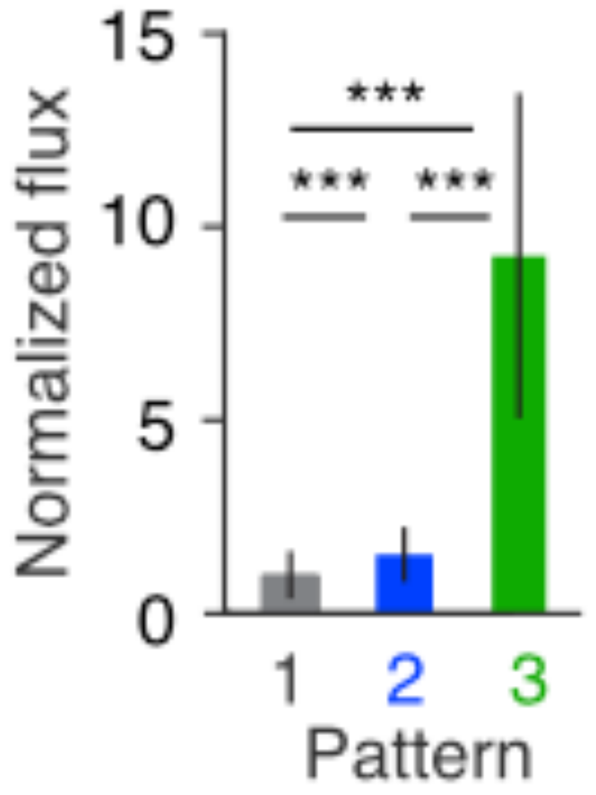


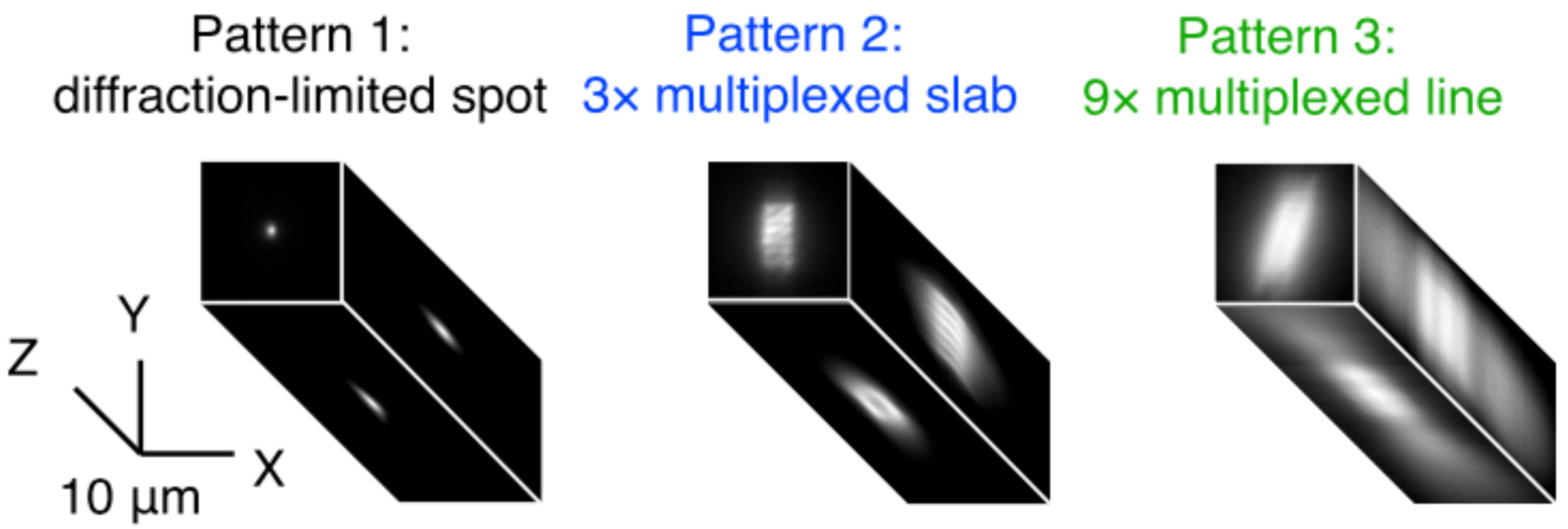
Villette et al., (2019)

Photobleaching:



Signal:

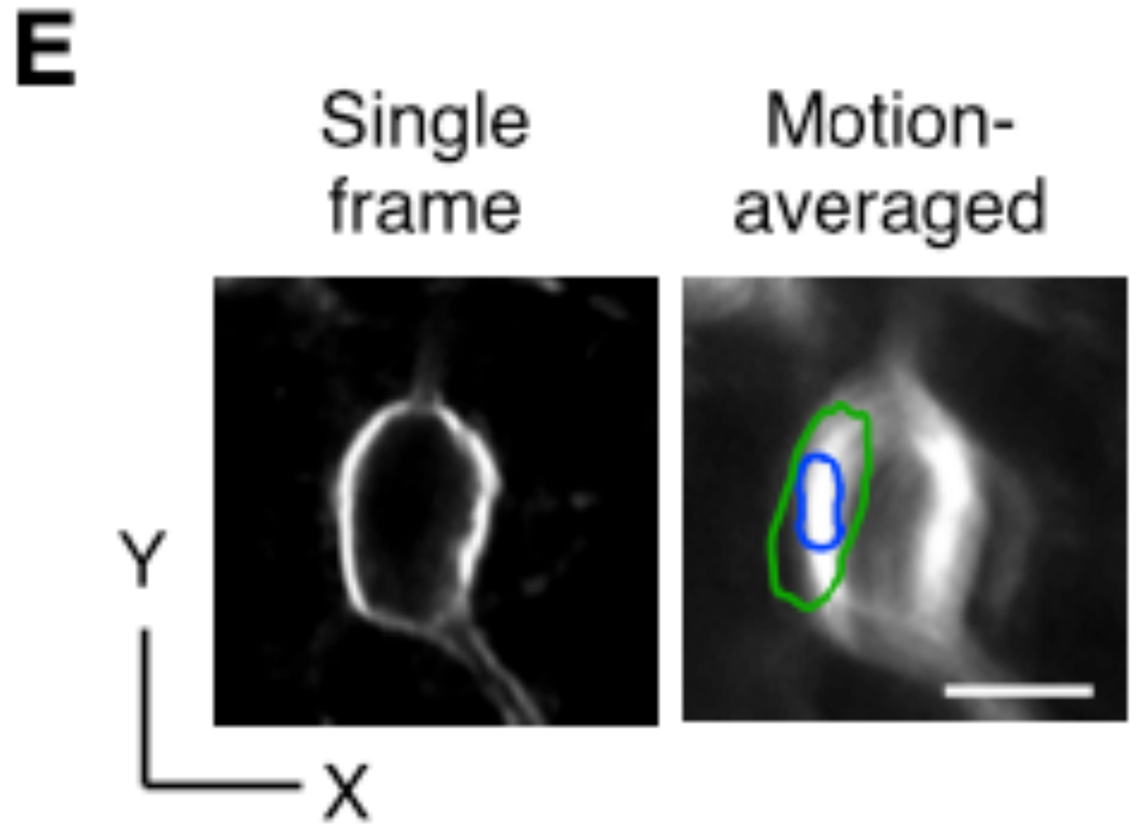


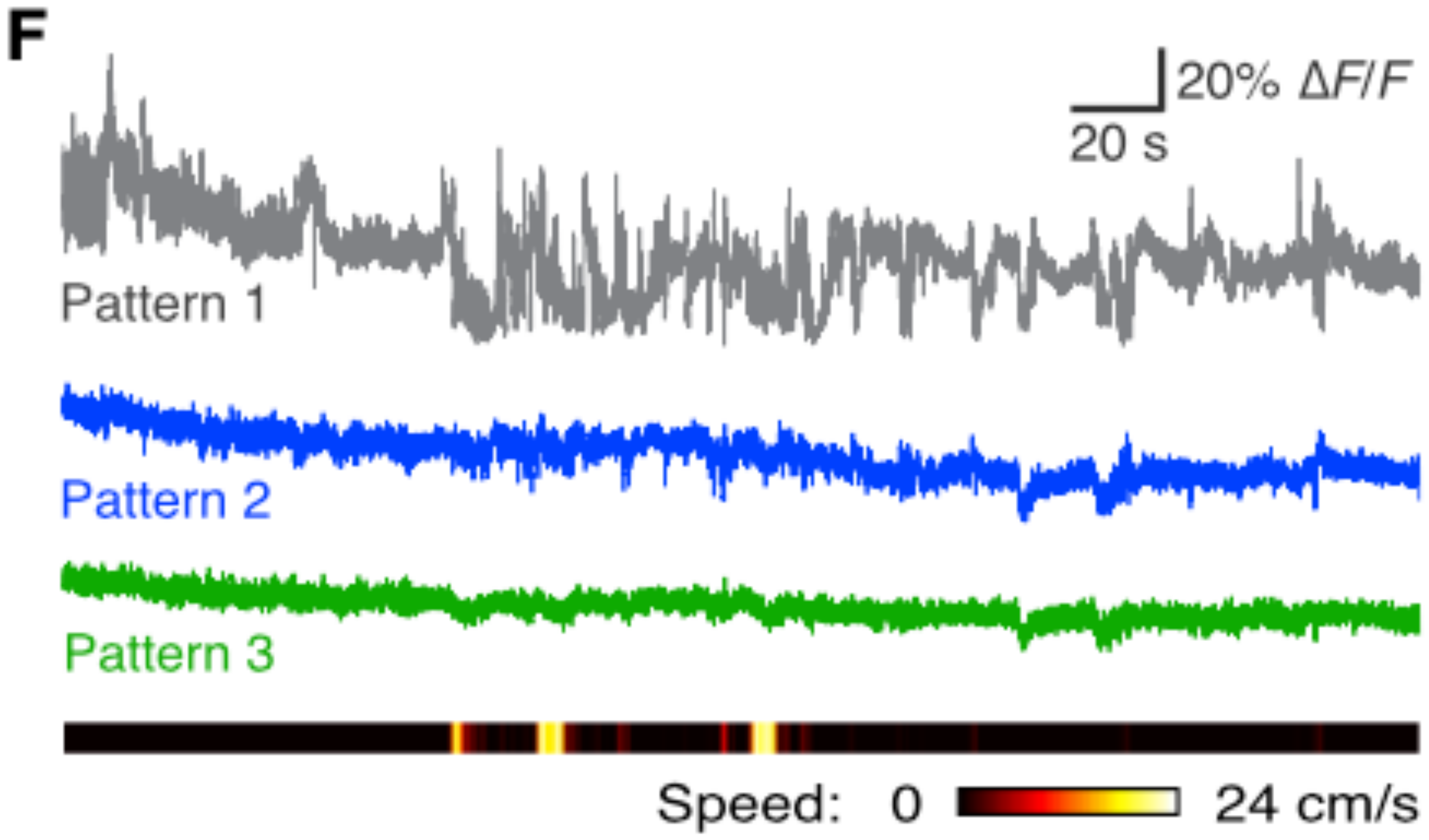


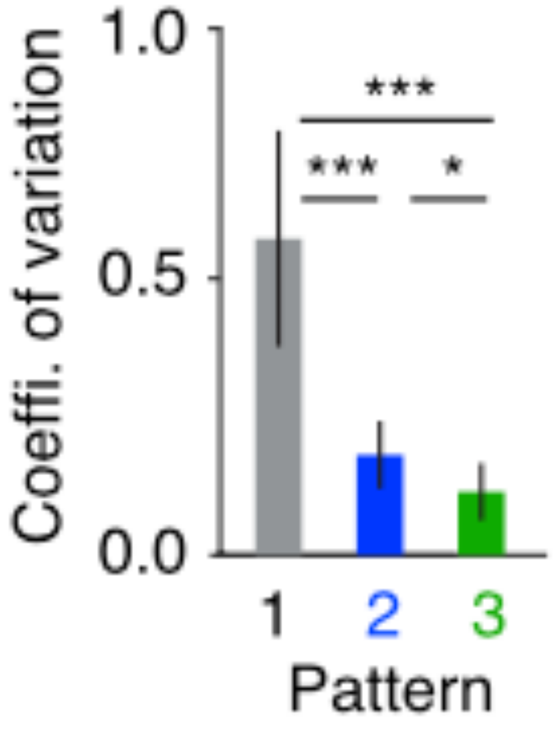
+ Increased signal
+ Reduced photobleaching
+ Movement correction

- 20 KHz per volume (cell)

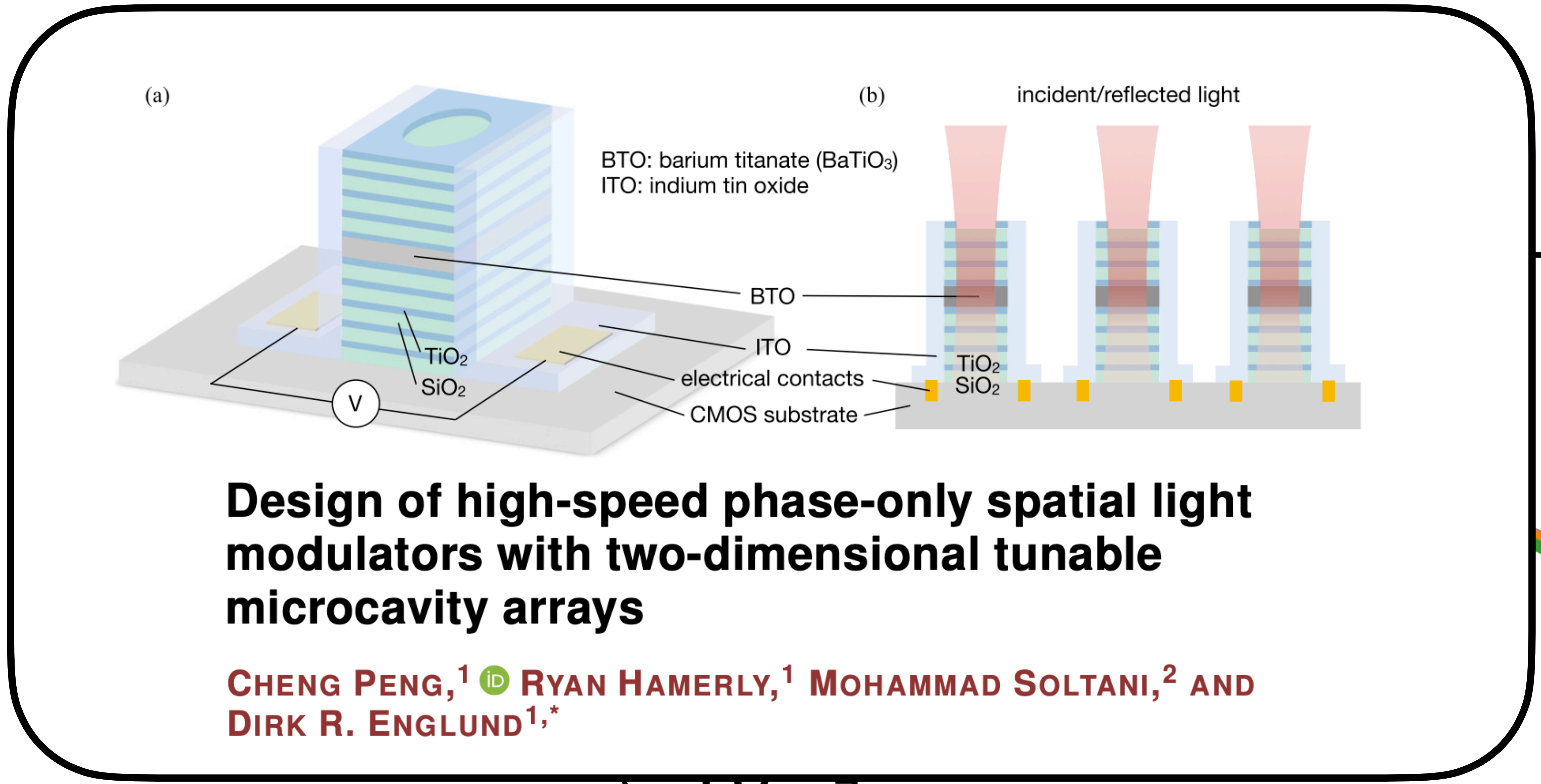
Movement:





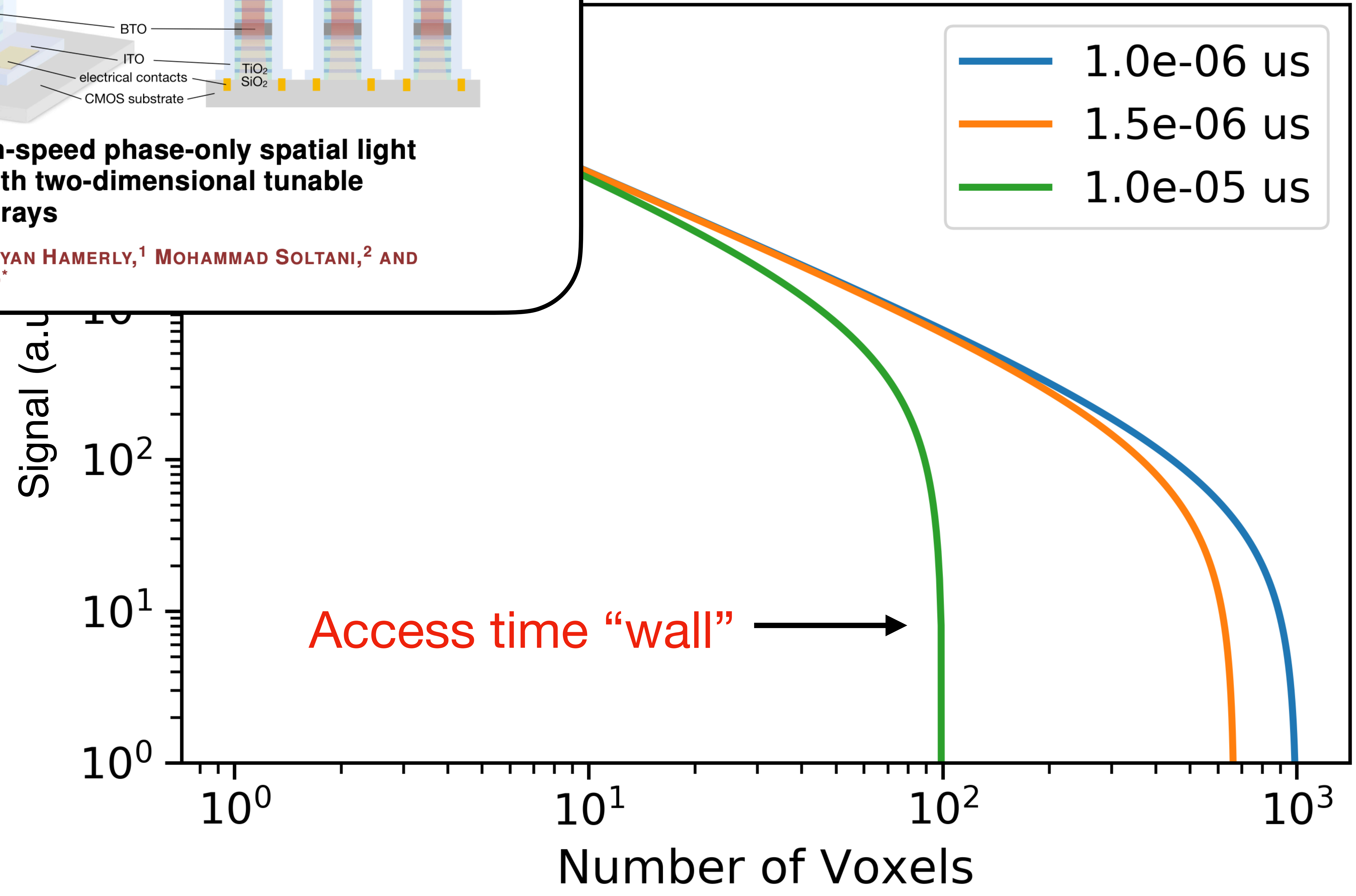


Villette et al., (2019)

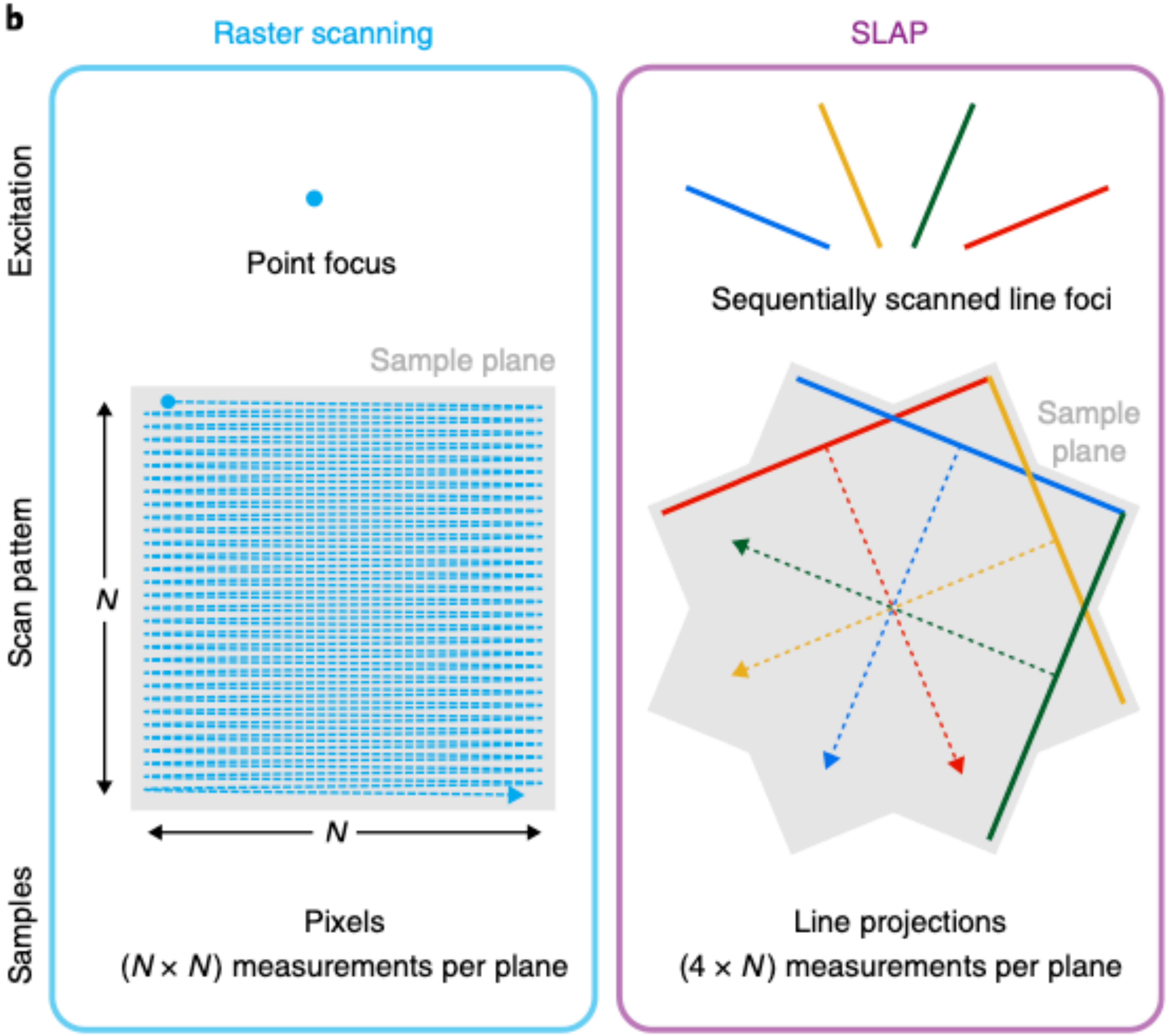


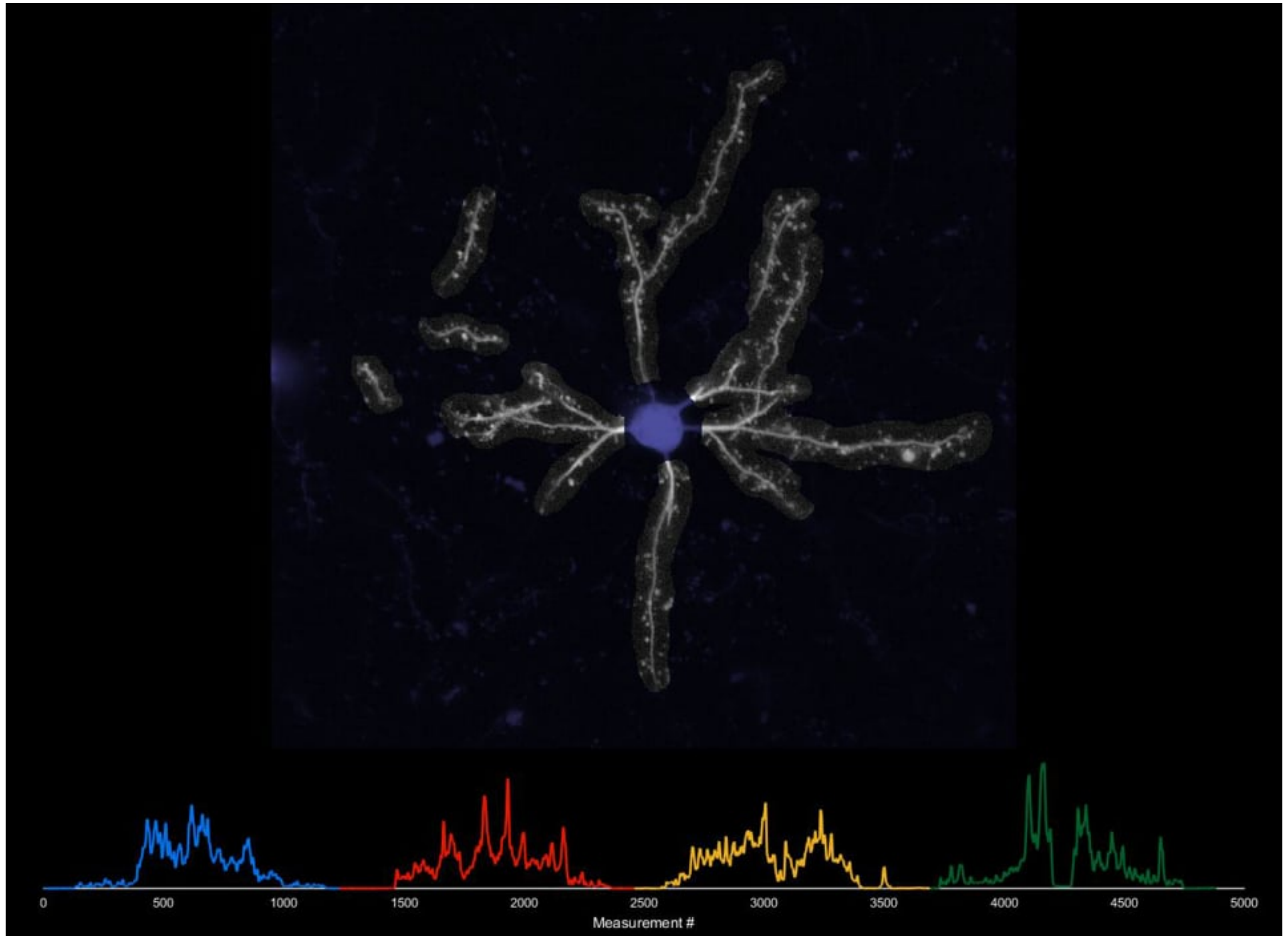
Design of high-speed phase-only spatial light modulators with two-dimensional tunable microcavity arrays

CHENG PENG,[1] RYAN HAMERLY,[1] MOHAMMAD SOLTANI,[2] AND DIRK R. ENGLUND[1,*]

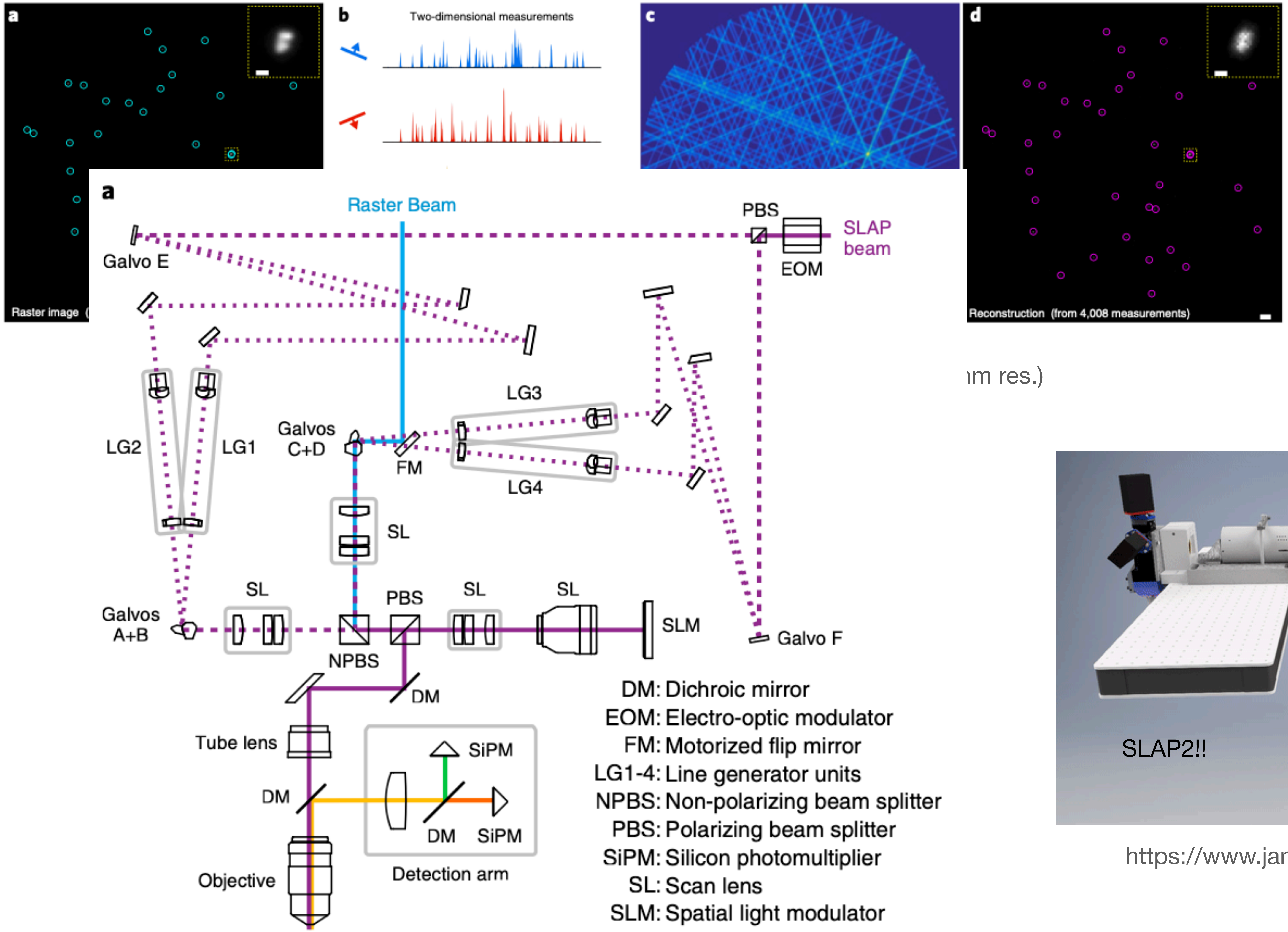


Computational Imaging (SLAP)



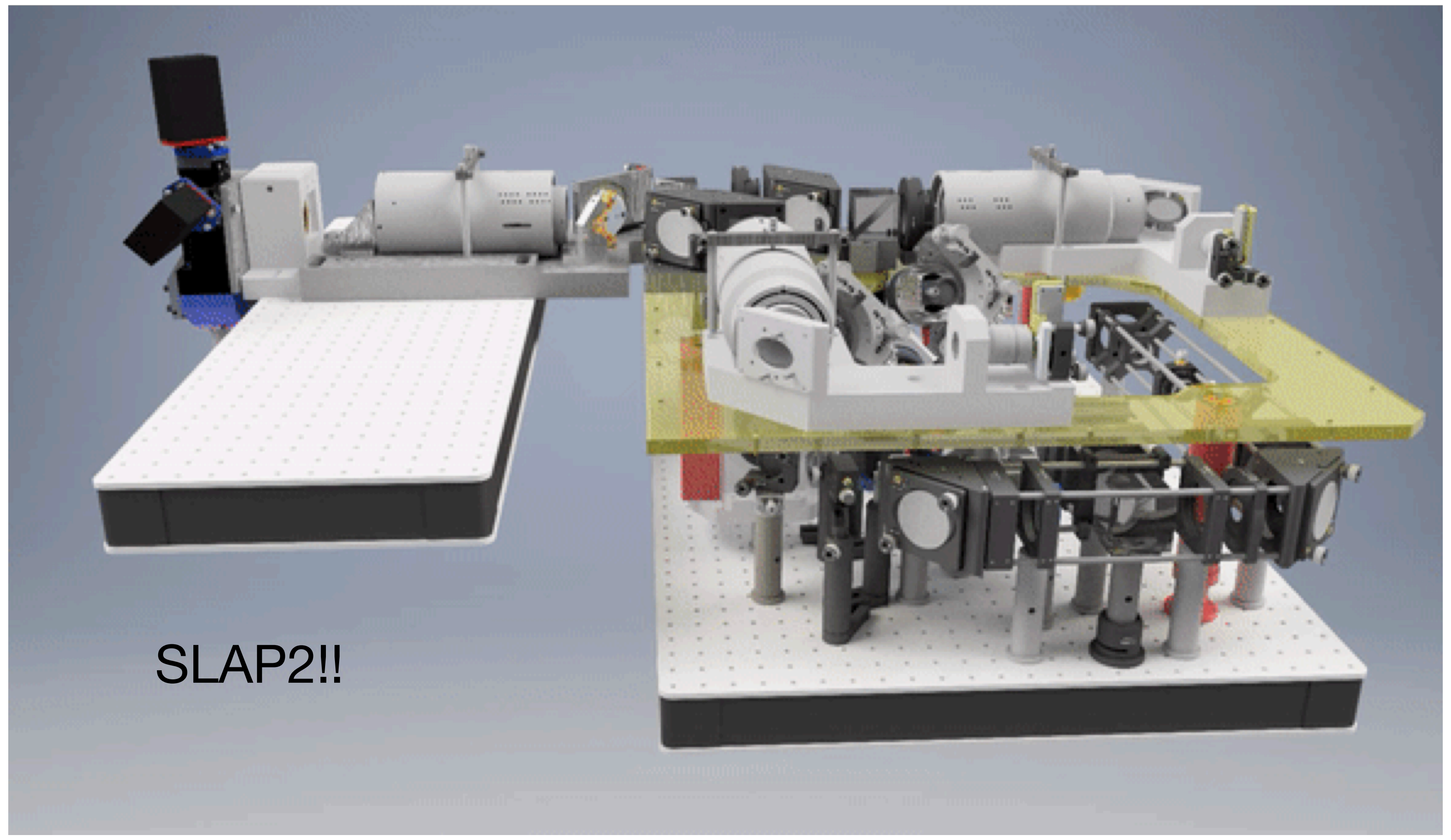


Kazemipour et al., (2019)



- \+ Fast
- \+ Dense or sparse
- \+ Robust to movement
- \+ Accurate reconstruction

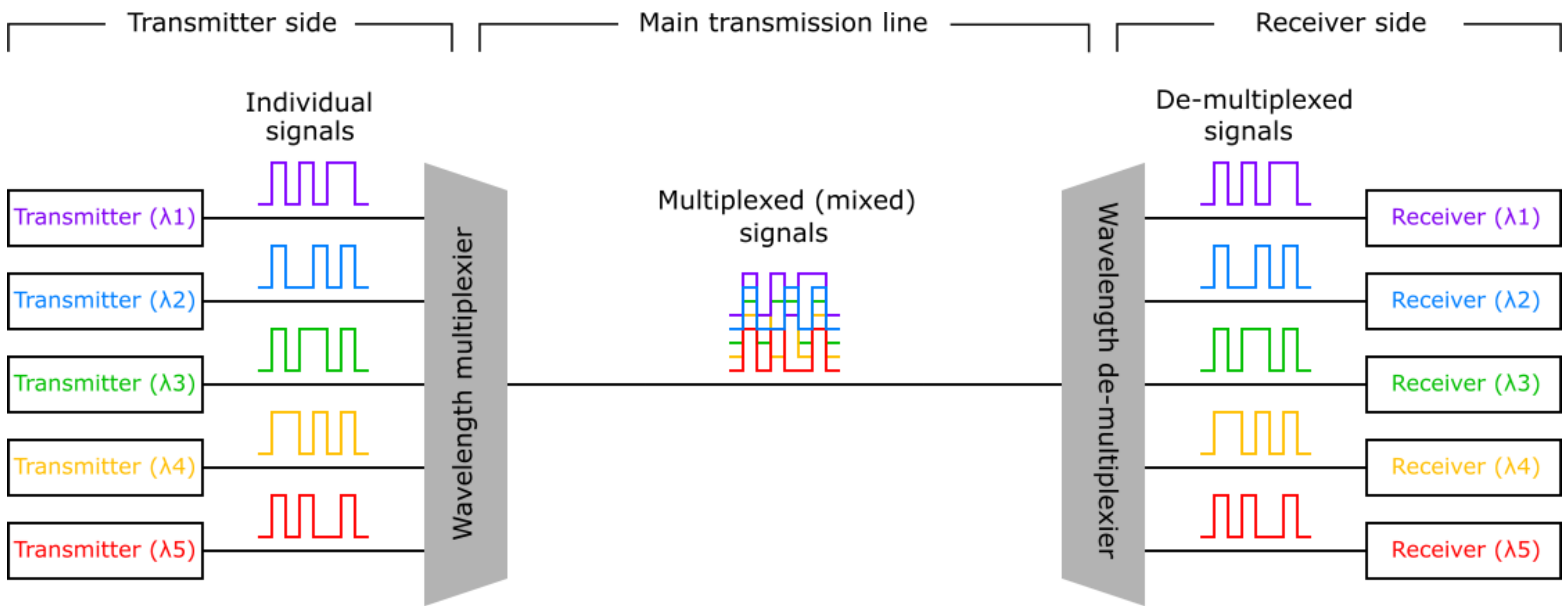
- \- Power hungry!
- \- Complex
- \- Limited FOV (~ 250 μm)
- \- Requires a prior



https://www.janelia.org/lab/podgorski-lab/slap2

Kazemipour et al., (2019)

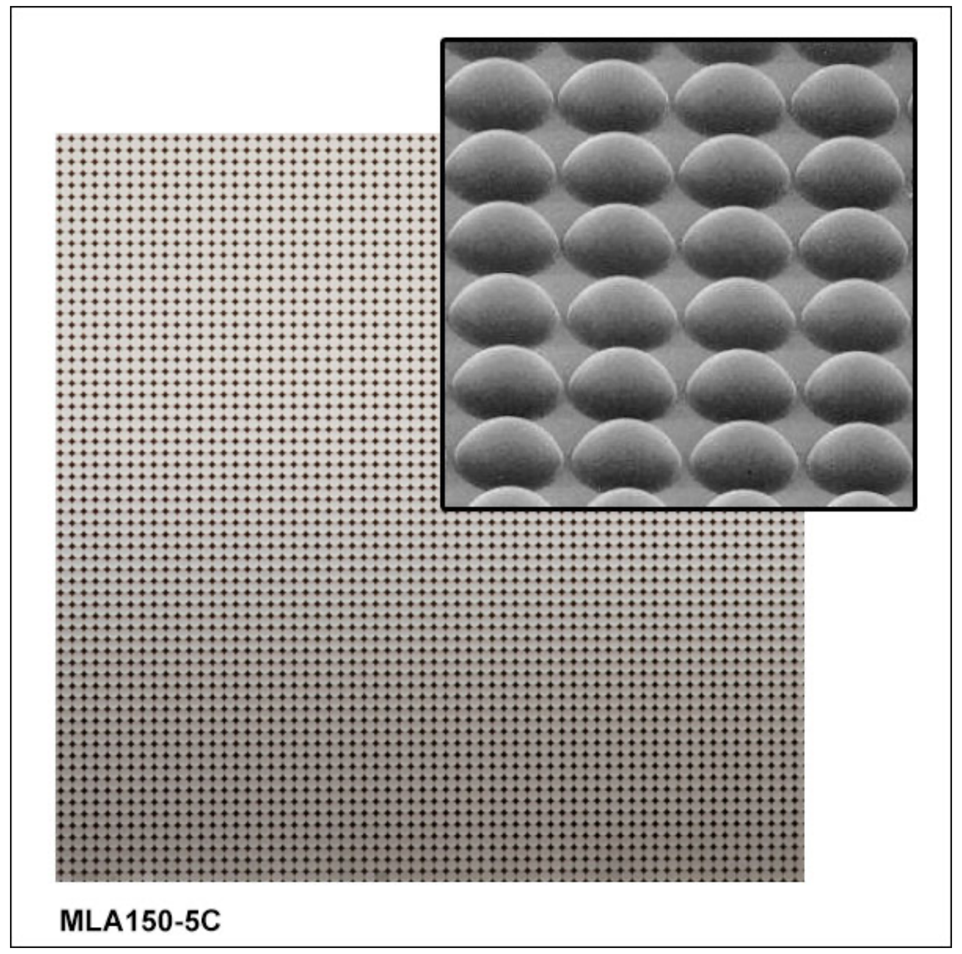
Multiplexing



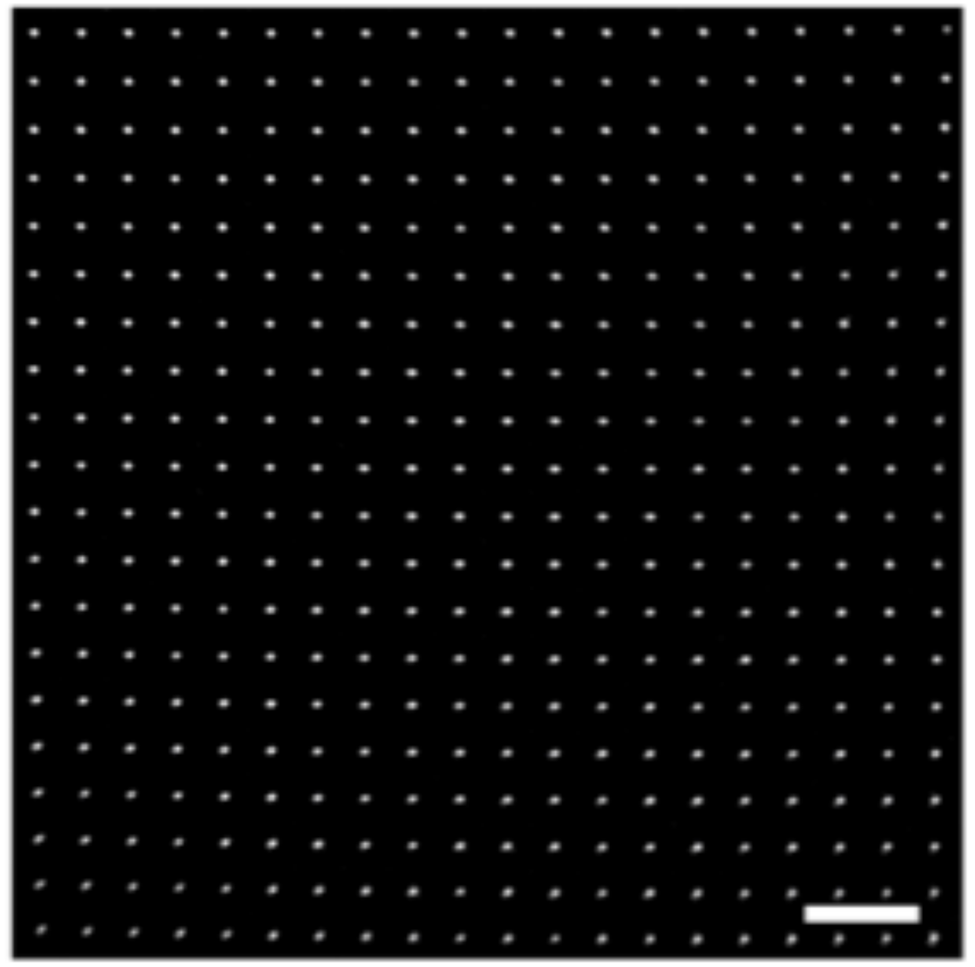
Generate many beams (x, y, z) → **Scan sample in parallel** → **Separate and detect fluorescent signals (time, space, freq)**

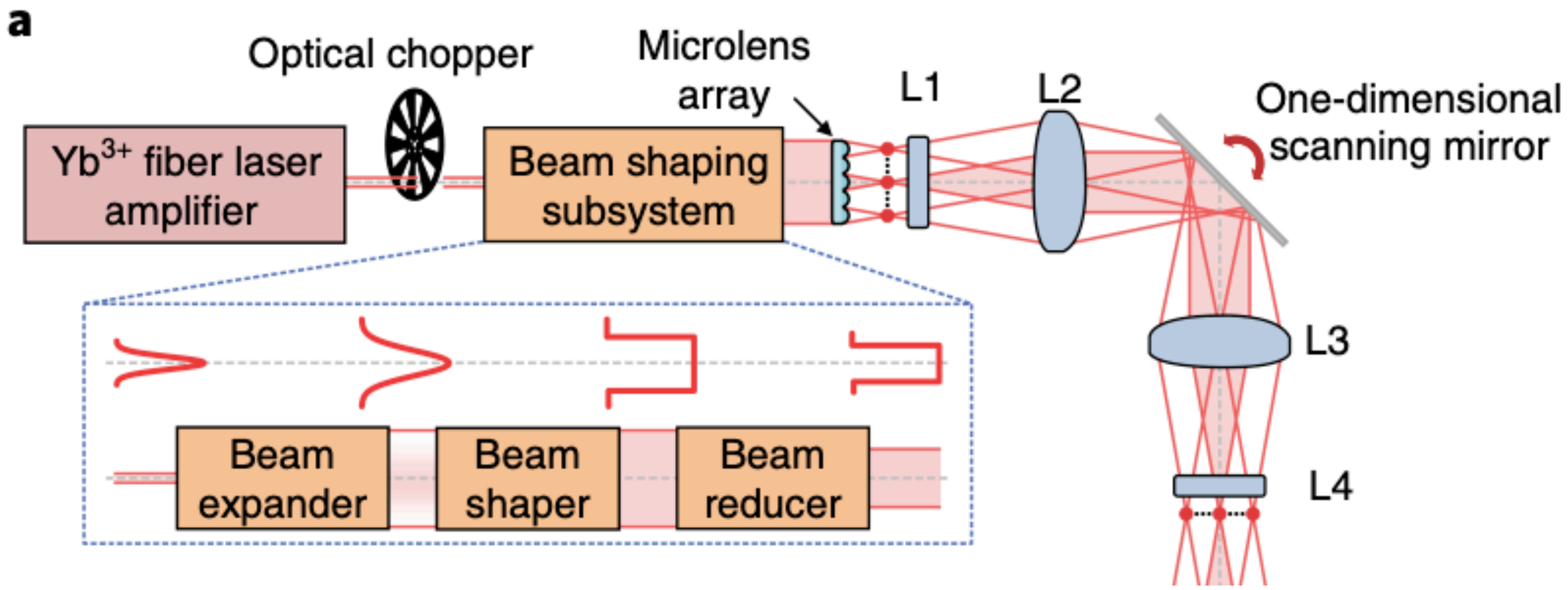
https://www.fiberlabs.com/glossary/about-wdm/

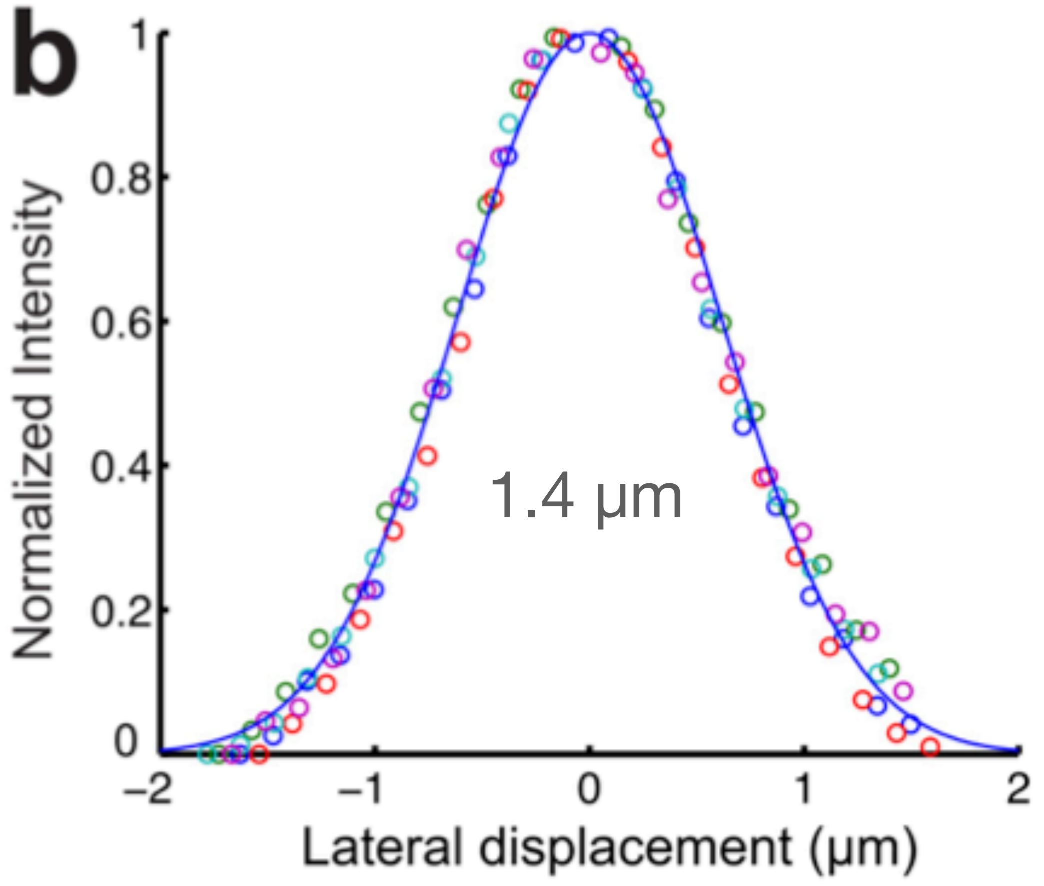
Spatial MUX

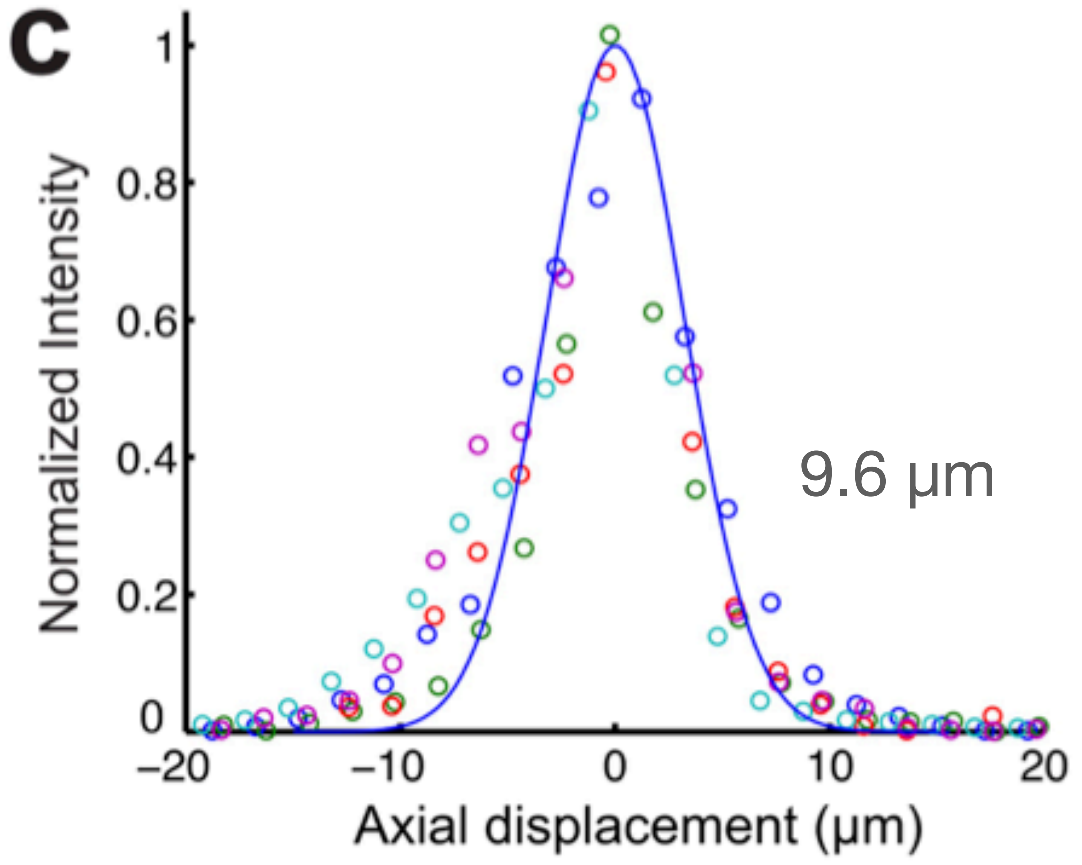


Laser intensity image of
20 x 20 laser foci



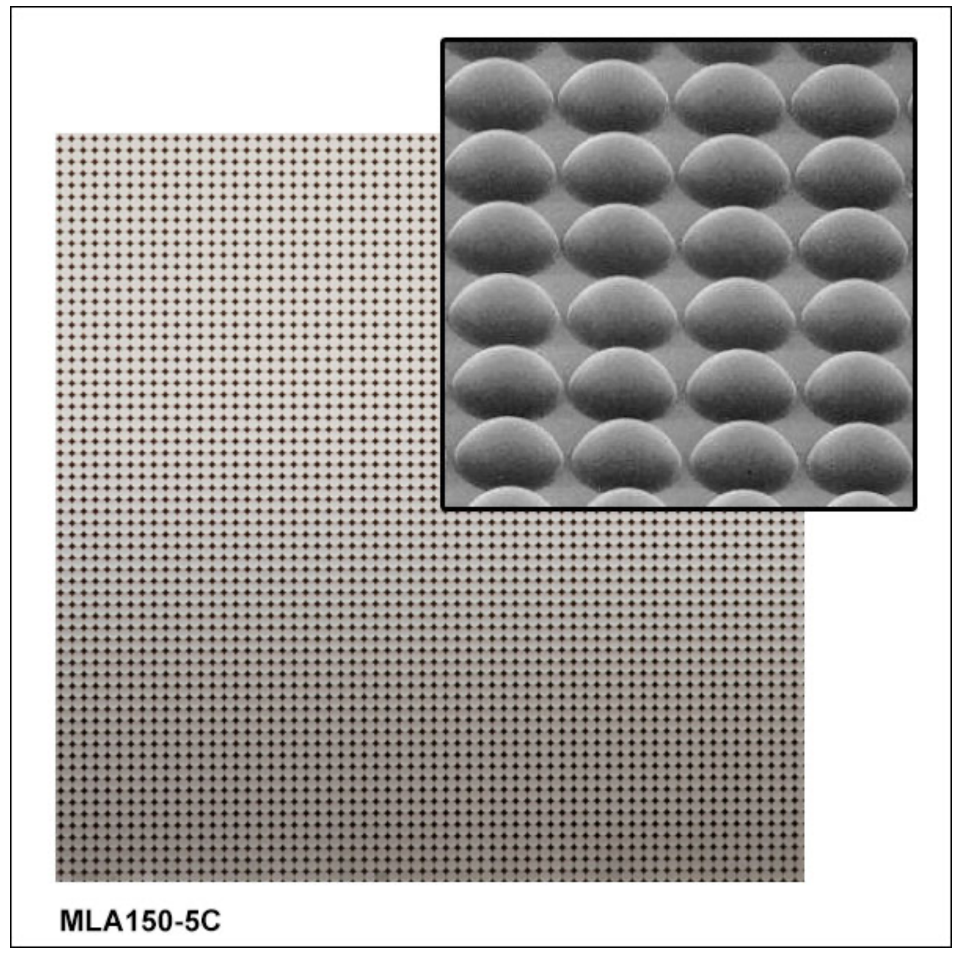






T. Zhang … M. J. Schnitzer, *Nat Meth* (2019).

Spatial MUX



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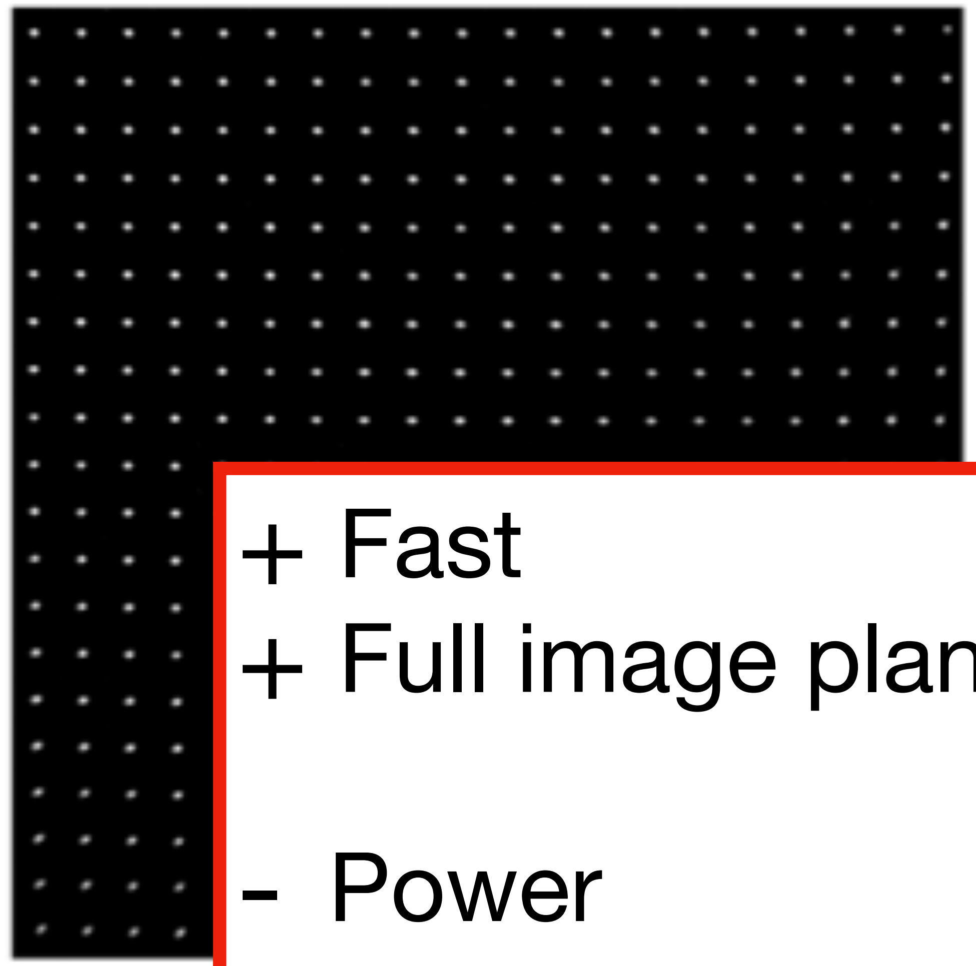
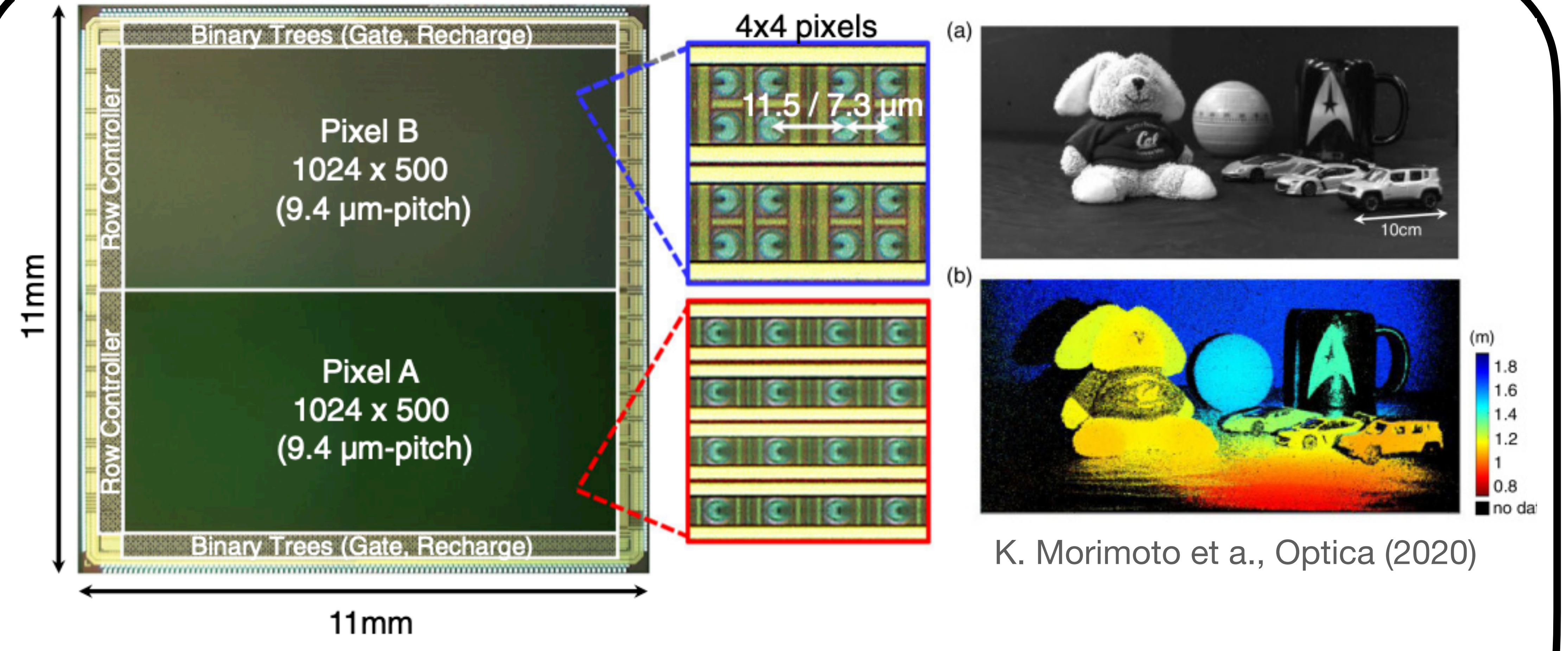


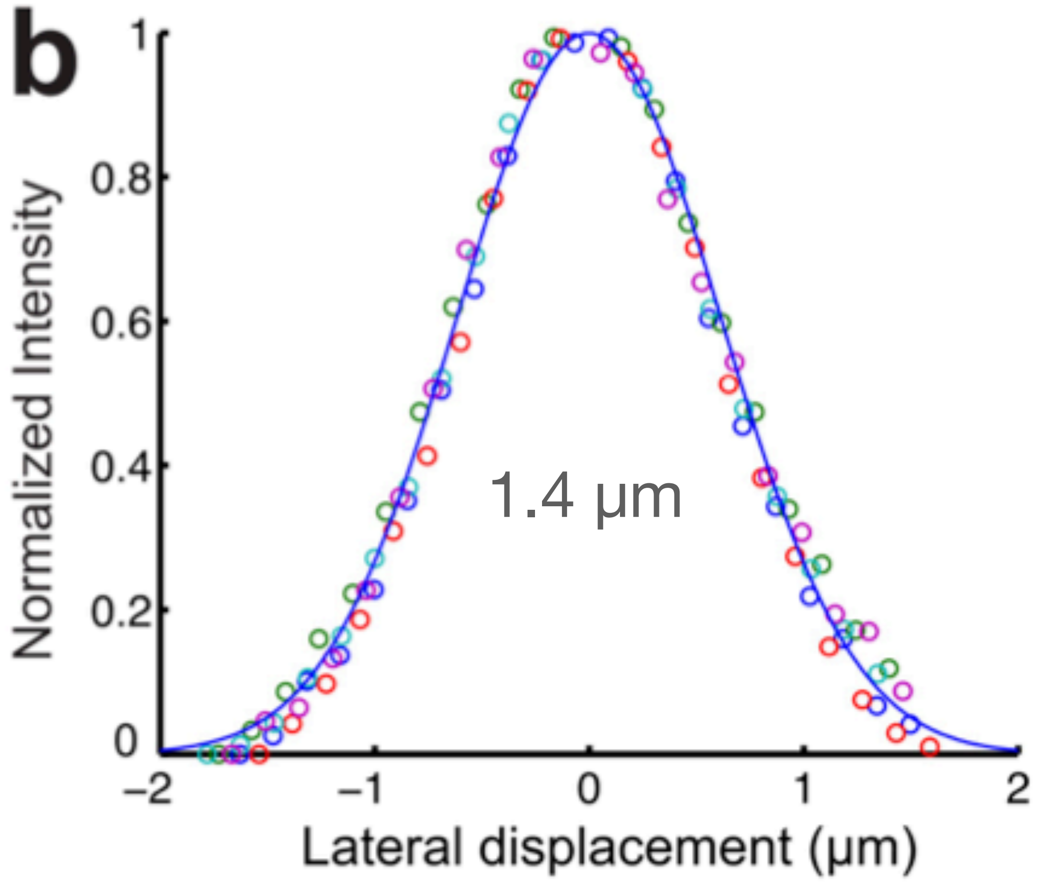


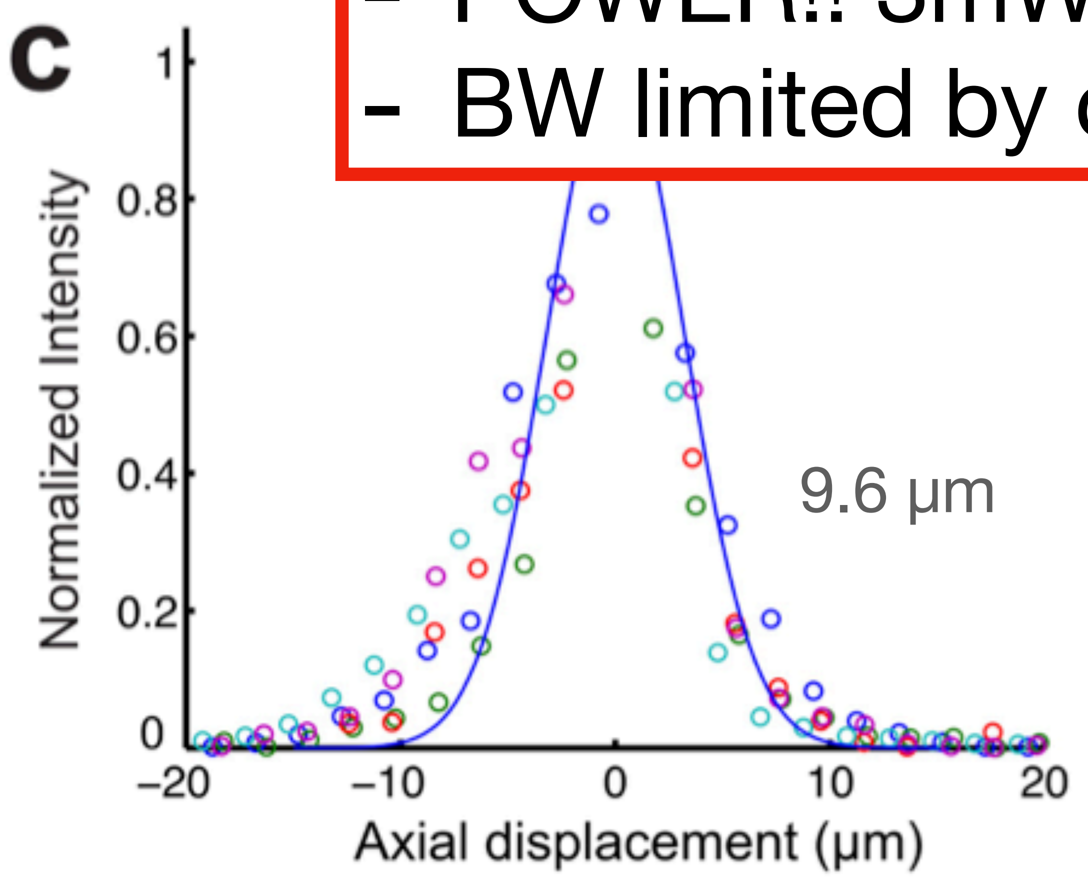
K. Morimoto et a., Optica (2020)

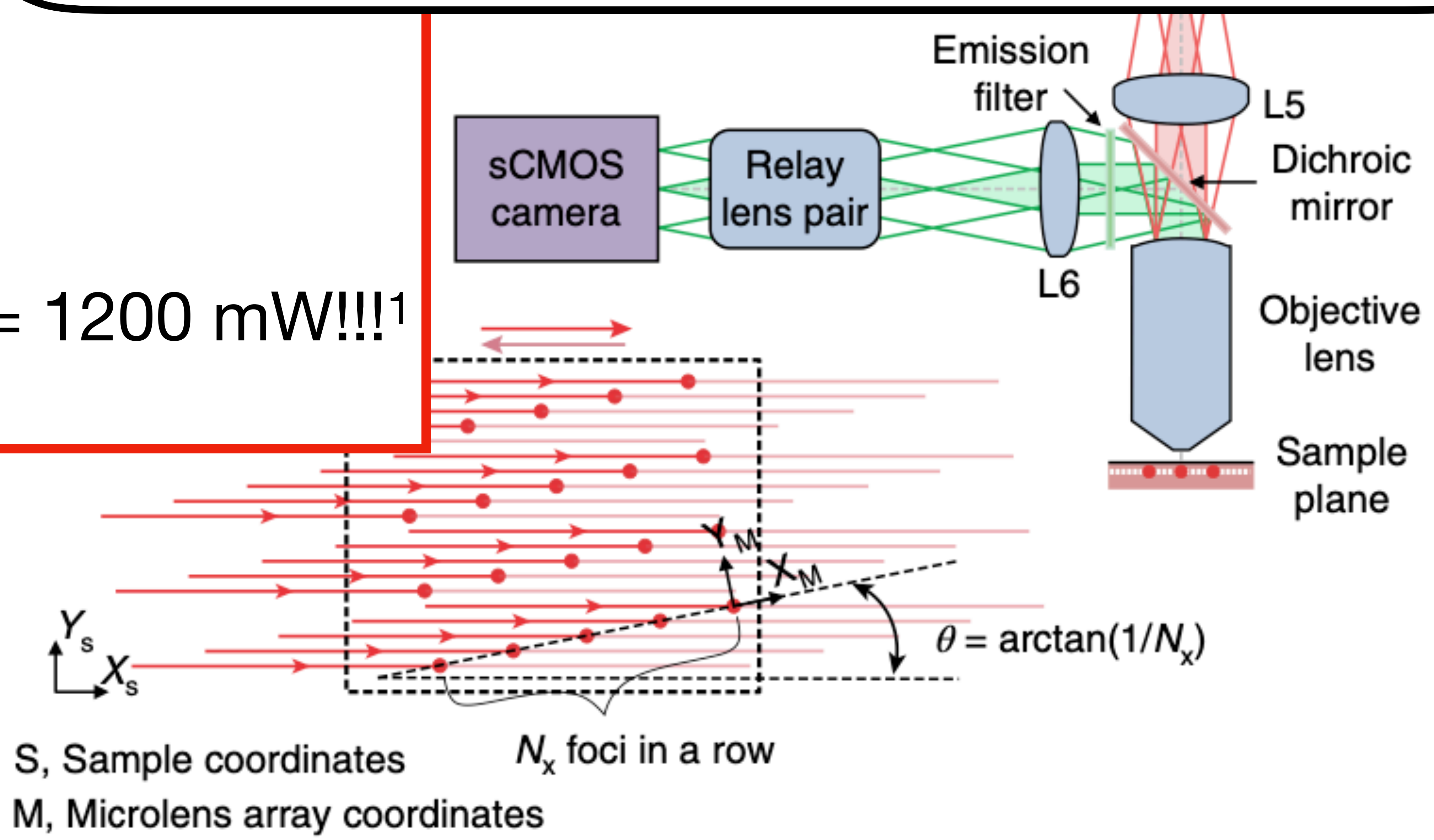
1 MP - 27% QE - 24 KHz - 4 ns resolution

+ Fast
+ Full image plane

- Power
- Power!
- POWER!! 3mW x 400 = 1200 mW!!![1]
- BW limited by camera







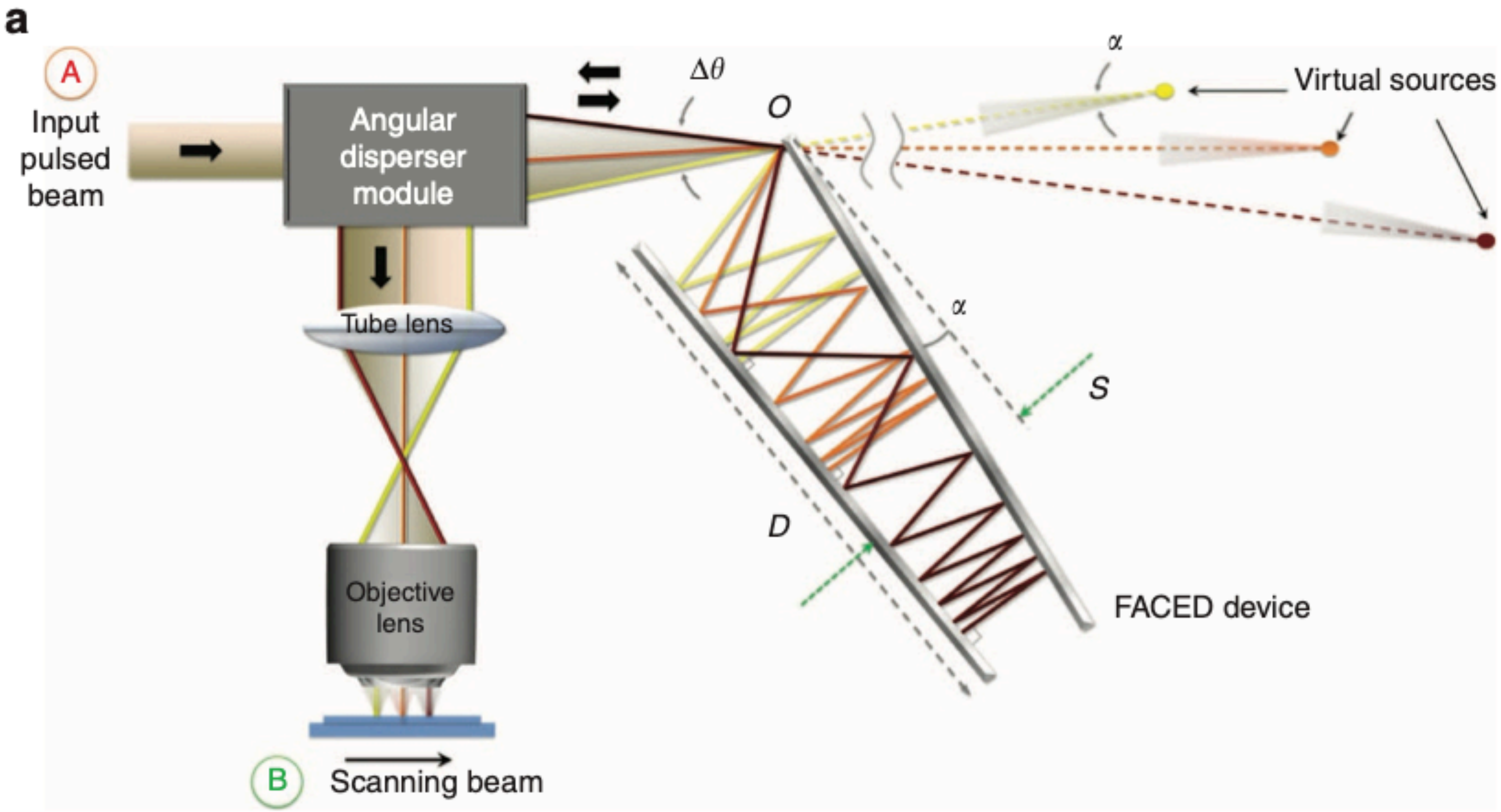
T. Zhang … M. J. Schnitzer, *Nat Meth* (2019).

[1]Podgorski and Ranganathan (2016)
[Max 250 mW]

Spatiotemporal MUX in X

FACED Microscopy

Line scanning at the rep-rate of the laser!! ~ MHz



$N = \Delta\theta / \alpha$

J. Wu … M. Z. Lin, K. K. Tsia, and N. Ji, *Nat Meth* (2020).
J.-L. Wu … K. K. Tsia, Light Sci Appl 6, e16196 (2017).

Spatiotemporal MUX in X

FACED Microscopy

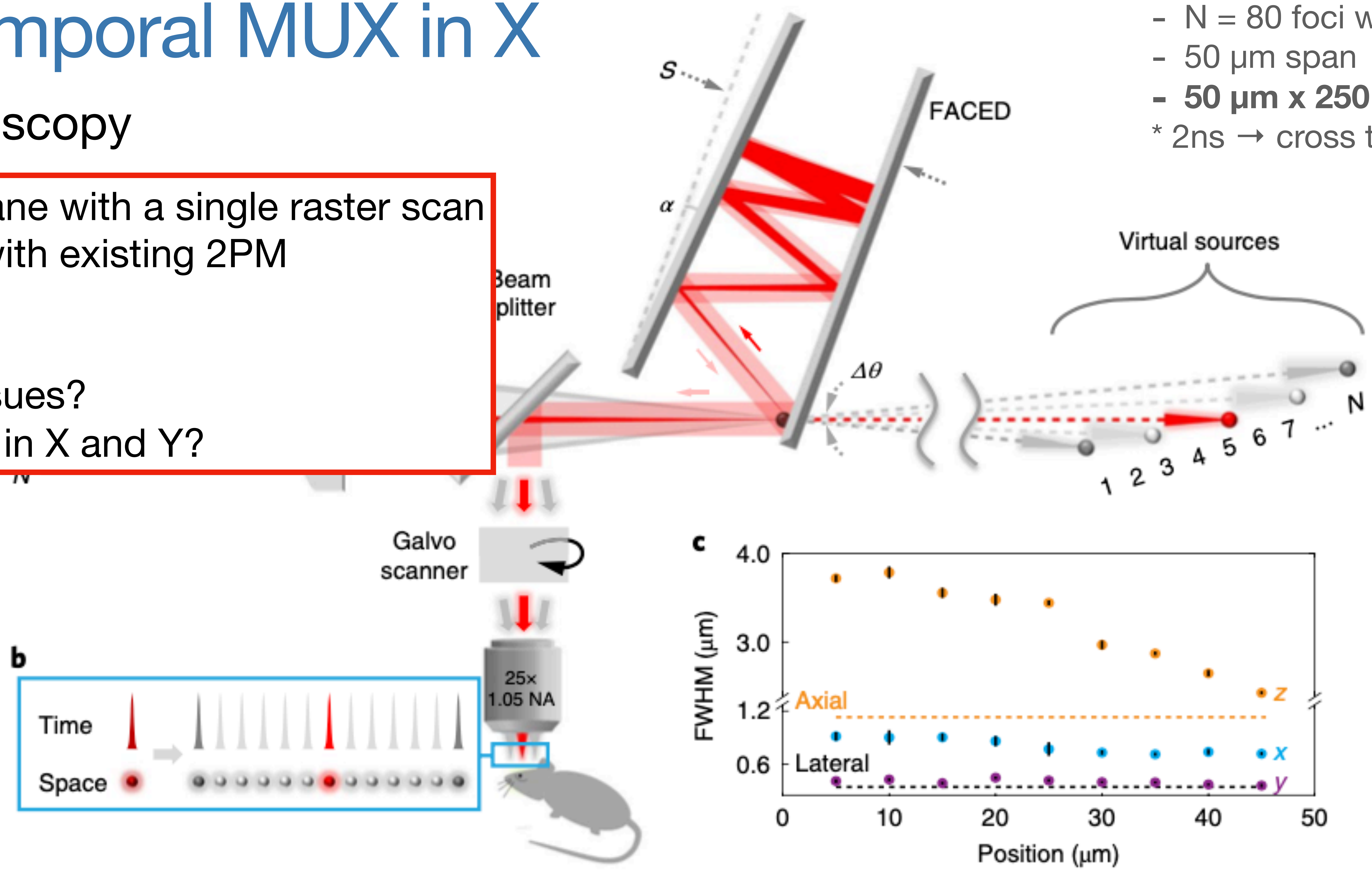
+ Full image plane with a single raster scan
+ Compatible with existing 2PM

- Limited FOV
- Dispersion issues?

→ two together in X and Y?

- 1 MHz laser
- N = 80 foci with 2 ns delay*
- 50 µm span
- **50 µm x 250 µm at 1 KHz**

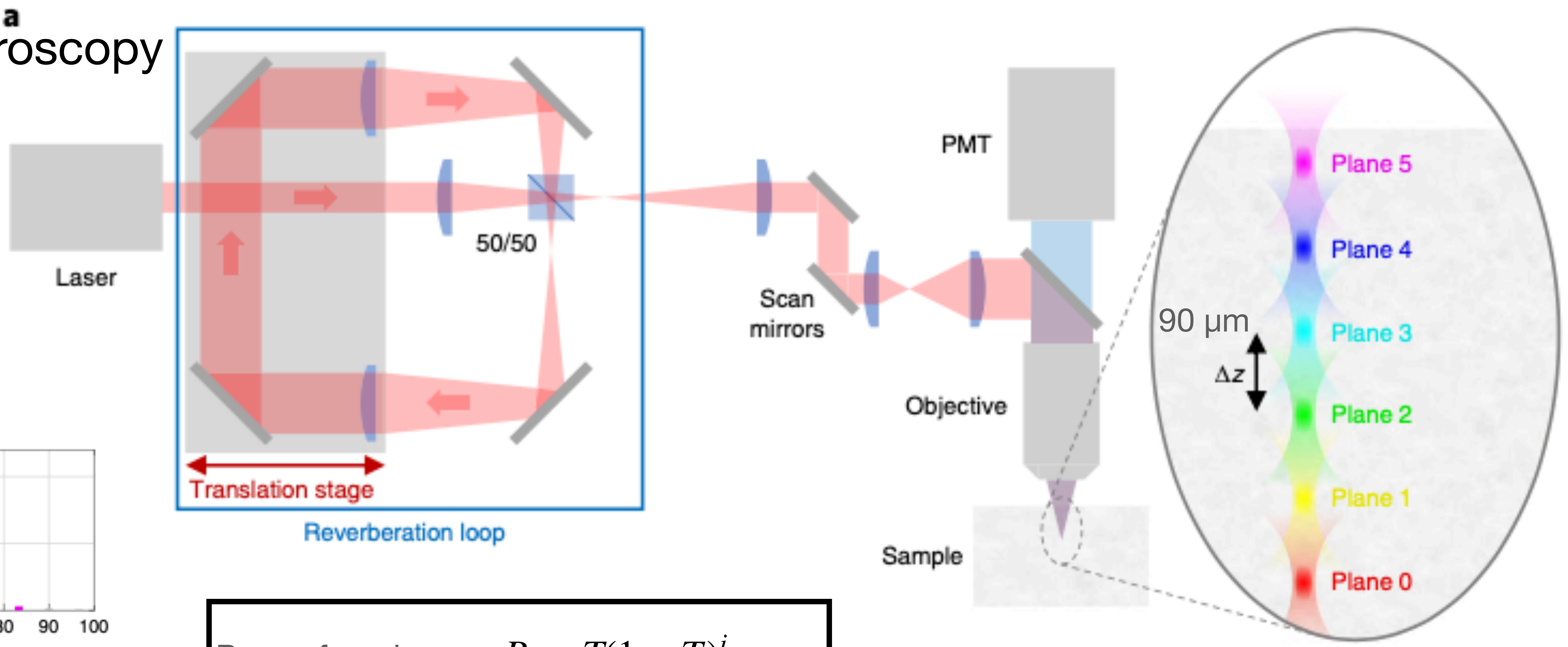
* 2ns → cross talk?! GCaMP6f/s



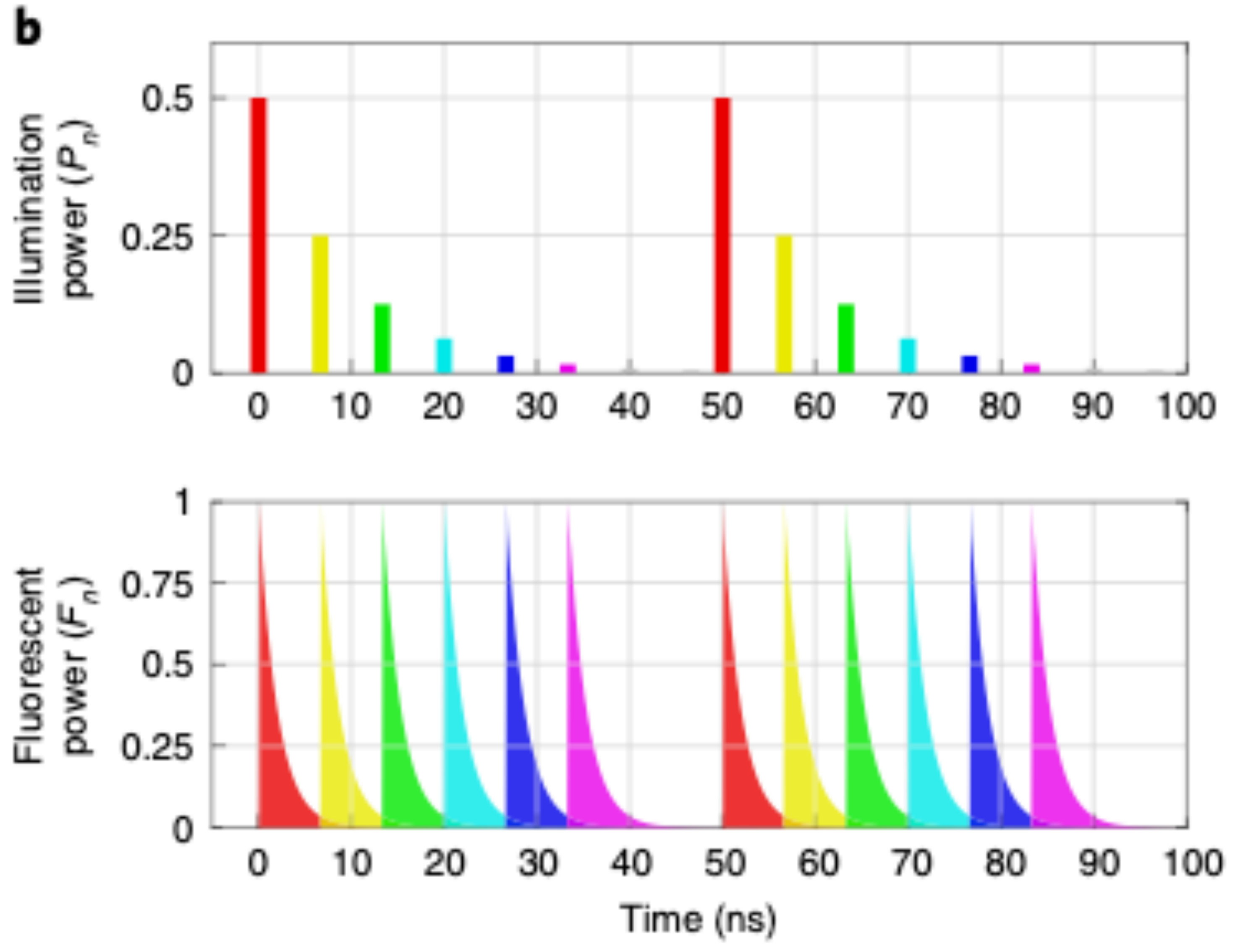
J. Wu … M. Z. Lin, K. K. Tsia, and N. Ji, *Nat Meth* (2020).
J.-L. Wu … K. K. Tsia, Light Sci Appl 6, e16196 (2017).

Spatiotemporal MUX in Z (axial)

Reverberation Microscopy



t = 6.7 ns (~9% cross talk)



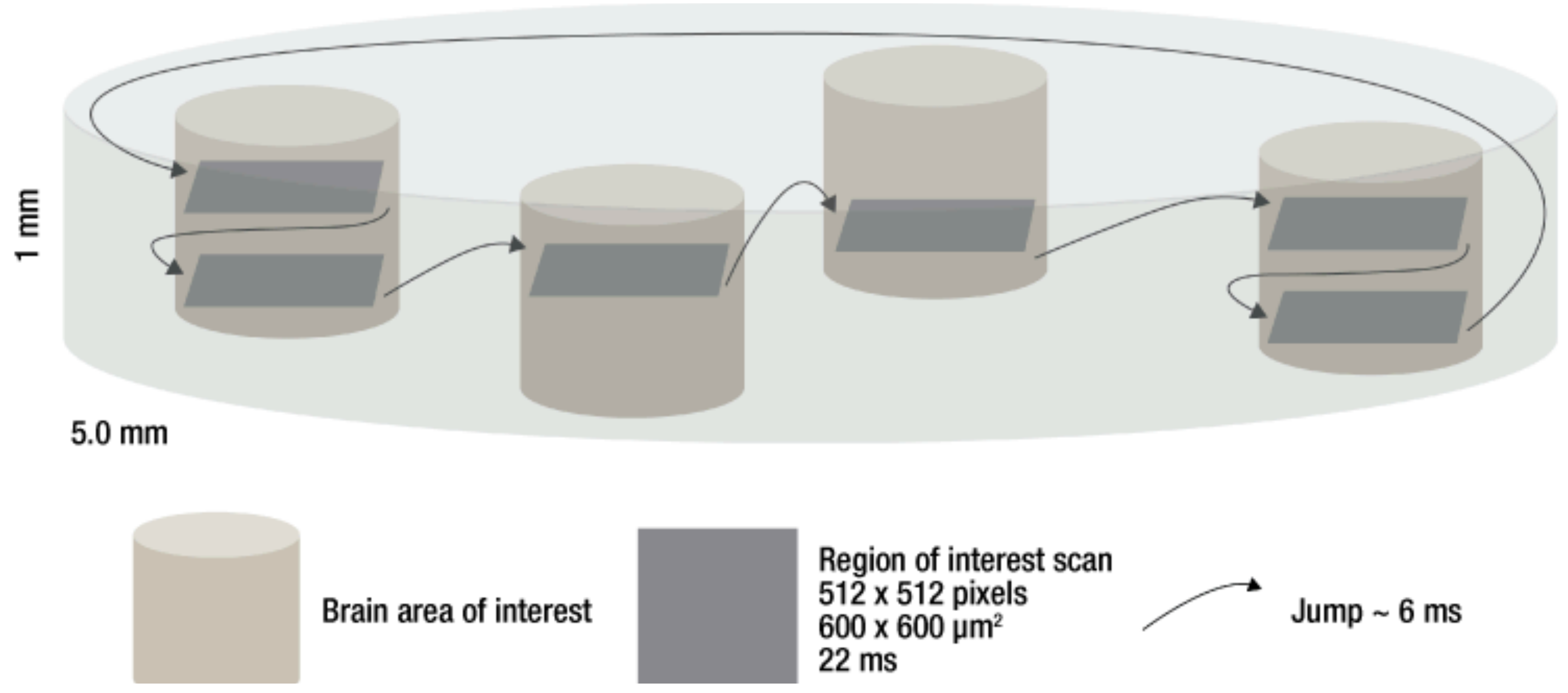
Power from loop: $P_i = T(1-T)^i$

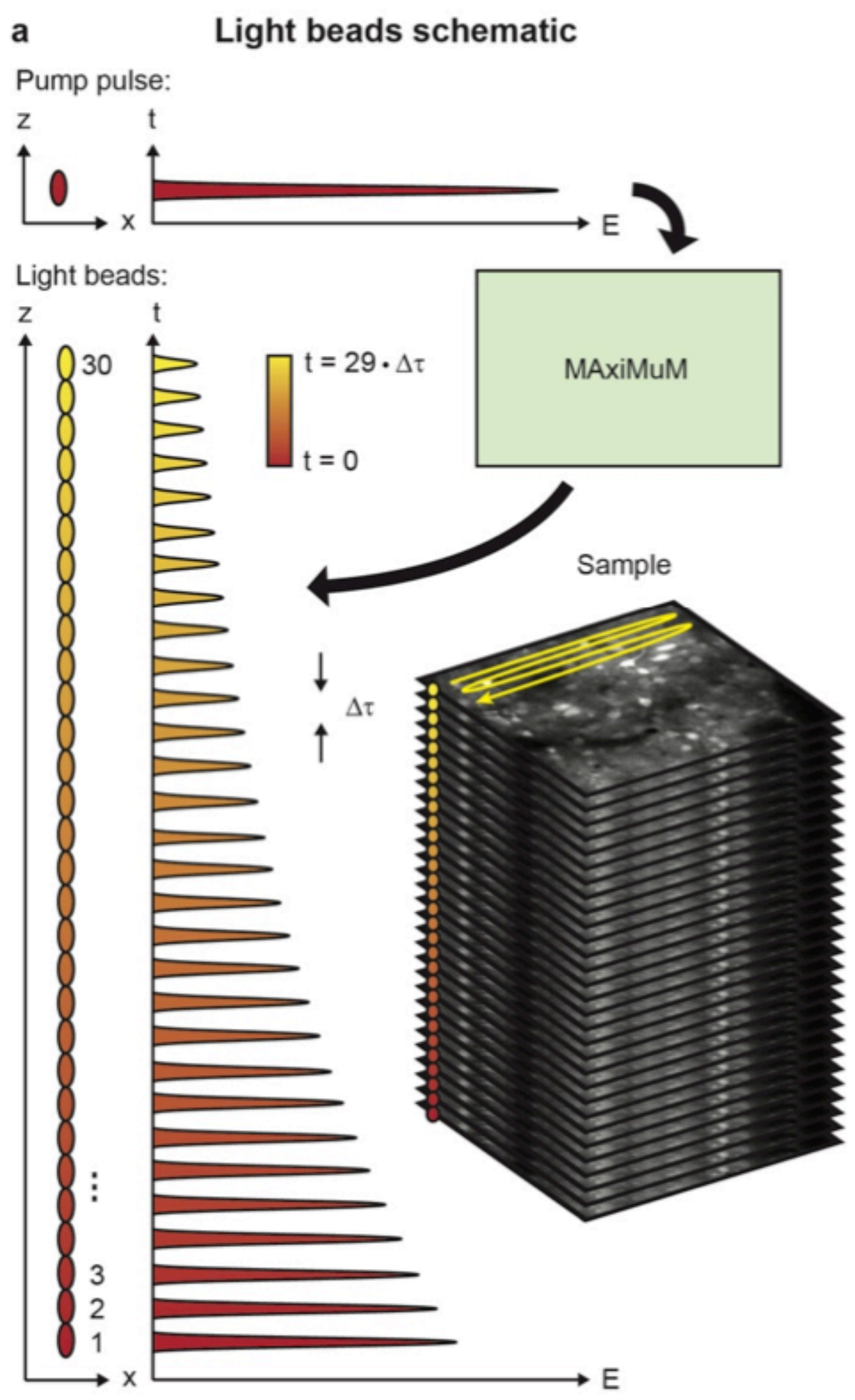
Fluorescence: $F \propto F_0 \exp(-D/l_s)$

D. R. Beaulieu, I. G. Davison, K. Kılıç, T. G. Bifano, and J. Mertz, Nat Meth 1 (2020).

Light Bead Microscopy

- ‘Optimal excitation’:
  - Temporal separation is ~7ns
  - $\delta z = 16$ µm (30 beads = 500 µm)
- Laterally:
  - Laser rep rate to deliver 1 pulse every 5 µm for 600 µm
- Coupled with the Thorlabs Mesoscope (Svoboda Lab) get to 5 mm^2 FOV

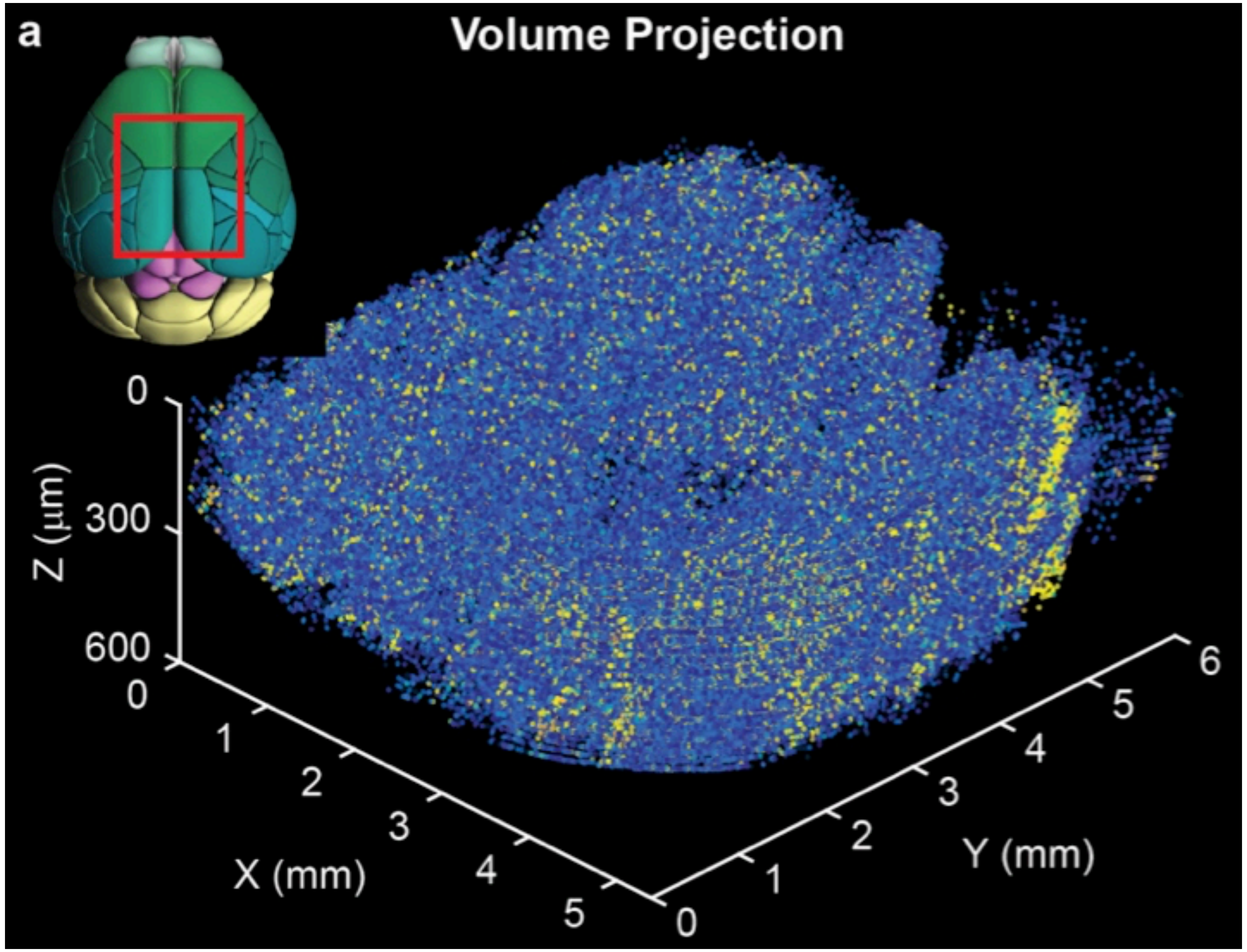




J. Demas et al., bioRxiv (2021)

~1M Neurons!!

- 807,000 neurons in ~5.4 x 6 x 0.5 mm^3 volume at 2.2 Hz
- 1 pulse / voxel
- Recording bilaterally from primary visual, primary somatosensory, posterior parietal and retrosplenial



"Stevensons Law"

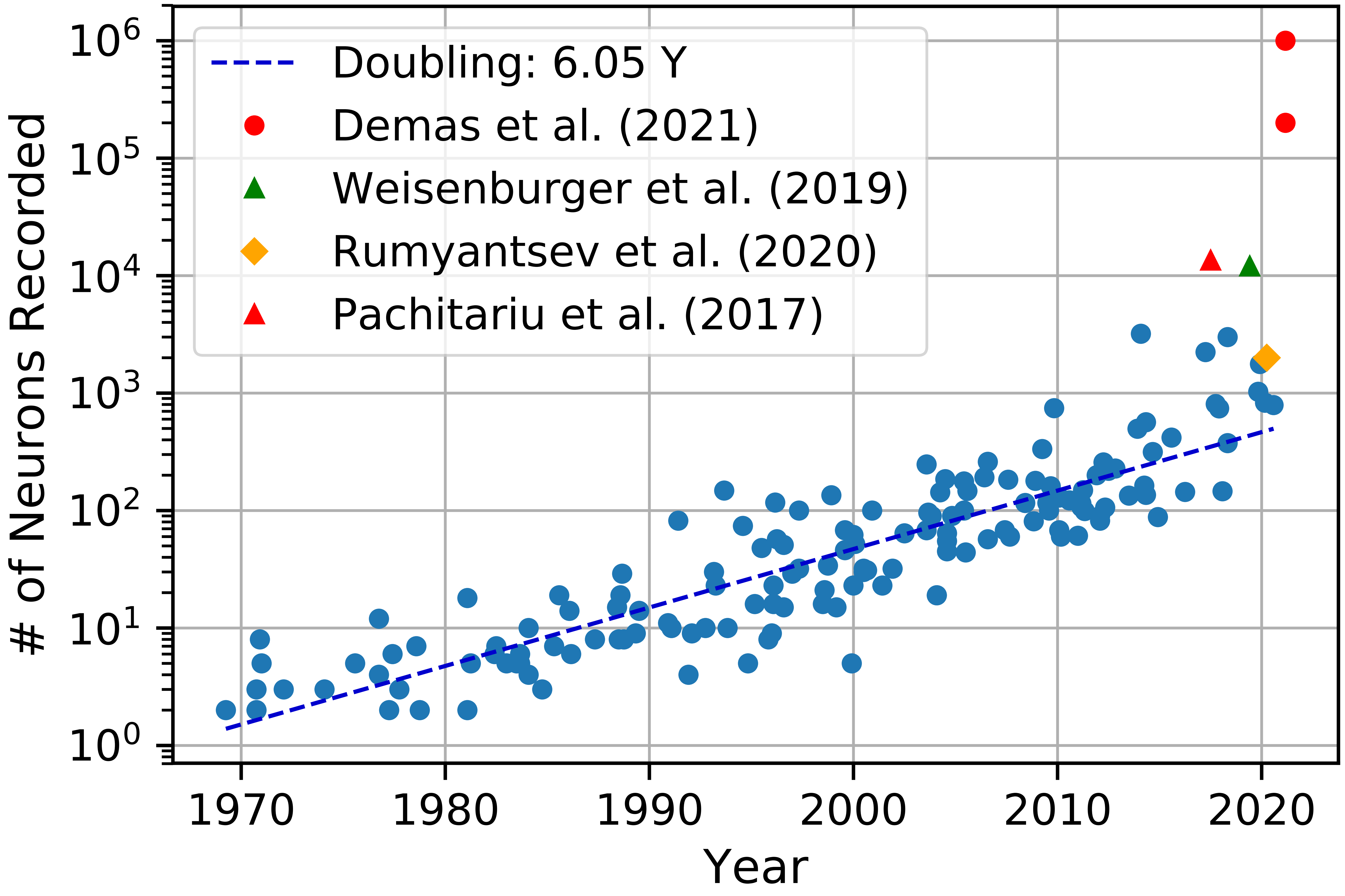
How advances in neural recording affect data analysis

Ian H Stevenson[1] & Konrad P Kording[1–3]

frontiers in
COMPUTATIONAL NEUROSCIENCE
HYPOTHESIS AND THEORY ARTICLE
published: 21 October 2013
doi: 10.3389/fncom.2013.00137

Physical principles for scalable neural recording

Adam H. Marblestone[1,2†], Bradley M. Zamft[3†], Yael G. Maguire[3,4], Mikhail G. Shapiro[5], Thaddeus R. Cybulski[6], Joshua I. Glaser[6], Dario Amodei[7], P. Benjamin Stranges[3], Reza Kalhor[3], David A. Dalrymple[1,8,9], Dongjin Seo[10], Elad Alon[10], Michel M. Maharbiz[10], Jose M. Carmena[10,11], Jan M. Rabaey[10], Edward S. Boyden[9,12‡], George M. Church[1,2,3‡] and Konrad P. Kording[13,14‡]*



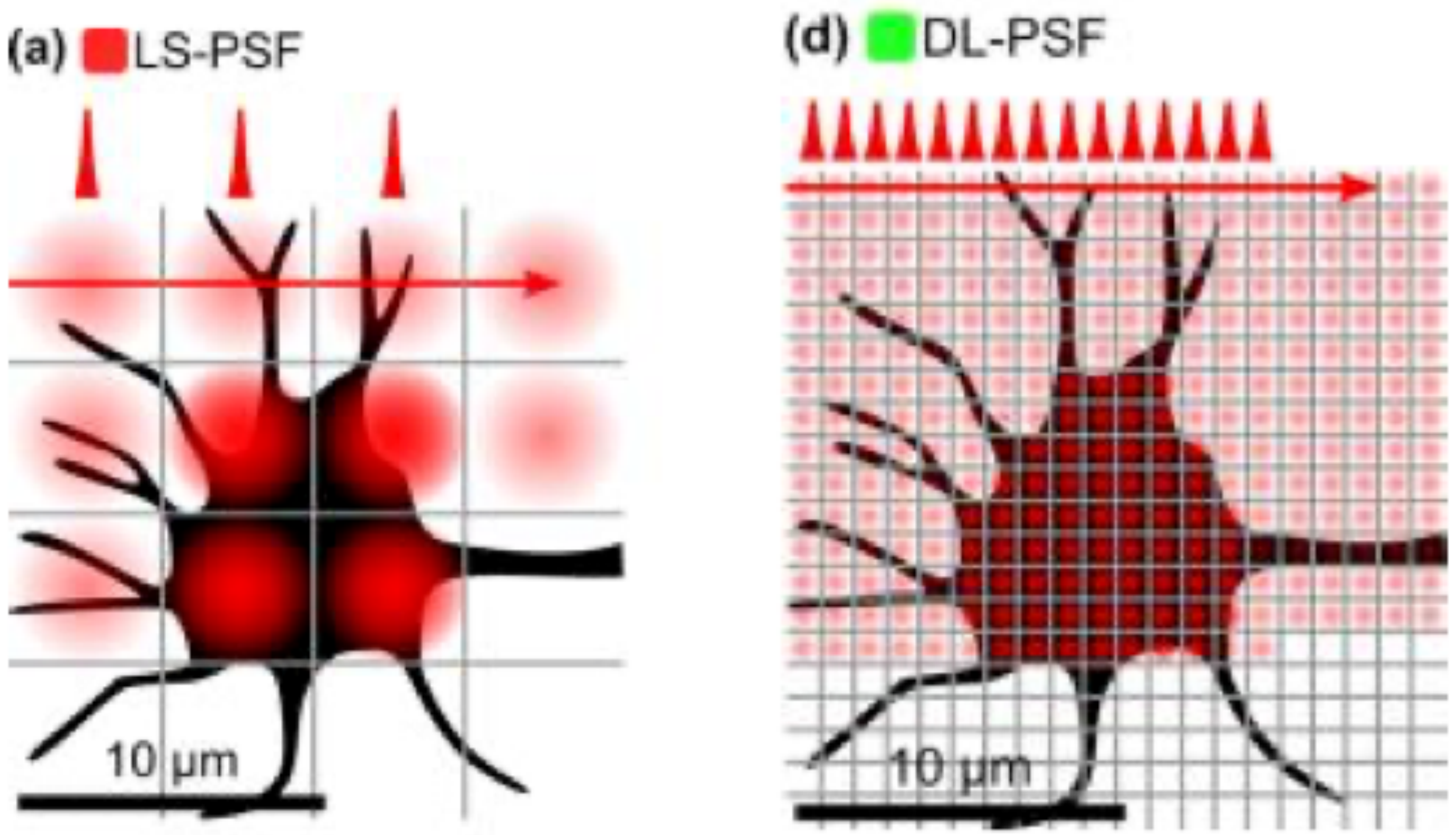
https://stevenson.lab.uconn.edu/scaling/

[1]S Herculano-Houzel et al., (2013)

Trade-offs

Quantitative evaluation of two-photon calcium imaging modalities for high-speed volumetric calcium imaging in scattering brain tissue

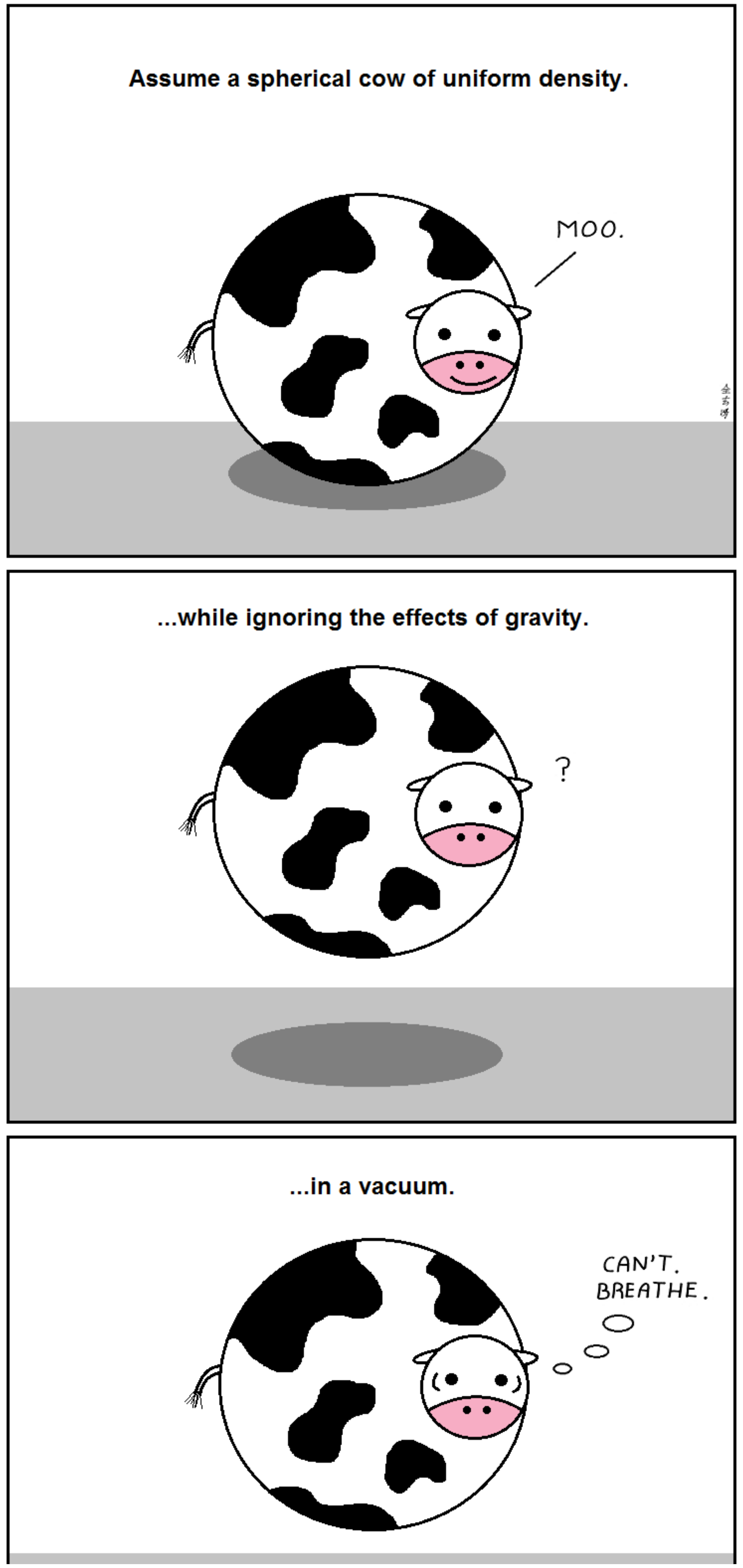
Siegfried Weisenburger[1,2*], Robert Prevedel[2,3*] and Alipasha Vaziri[1,2,†]



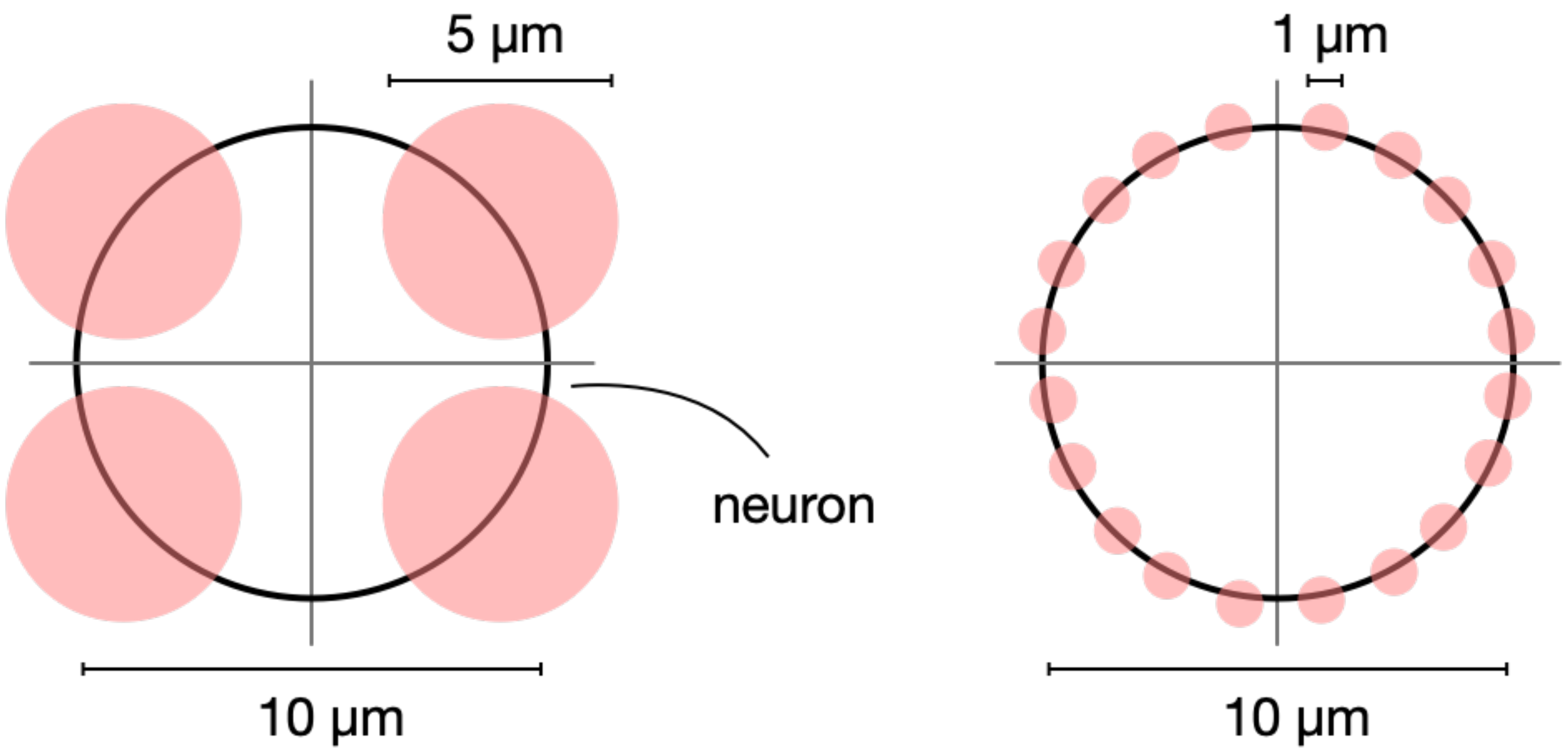
1. Less voxels → faster frame rate

2. For a given frame rate, less voxels → lower rep rate ∴ higher pulse energy

3. For a given intensity, more fluorophores are excited ∴ greater SNR (similarly for a fixed signal you can decrease the intensity)



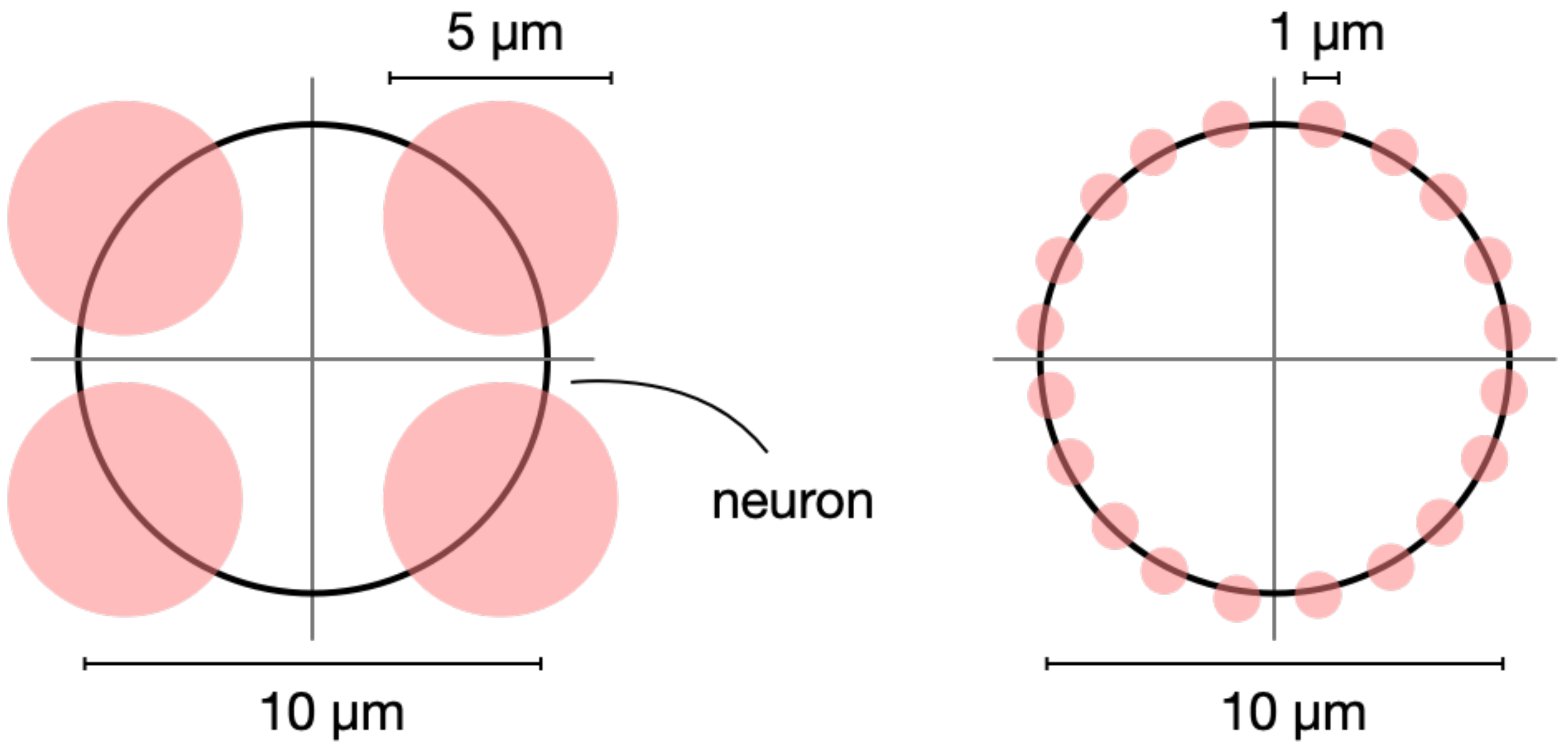
Abstruse Goose



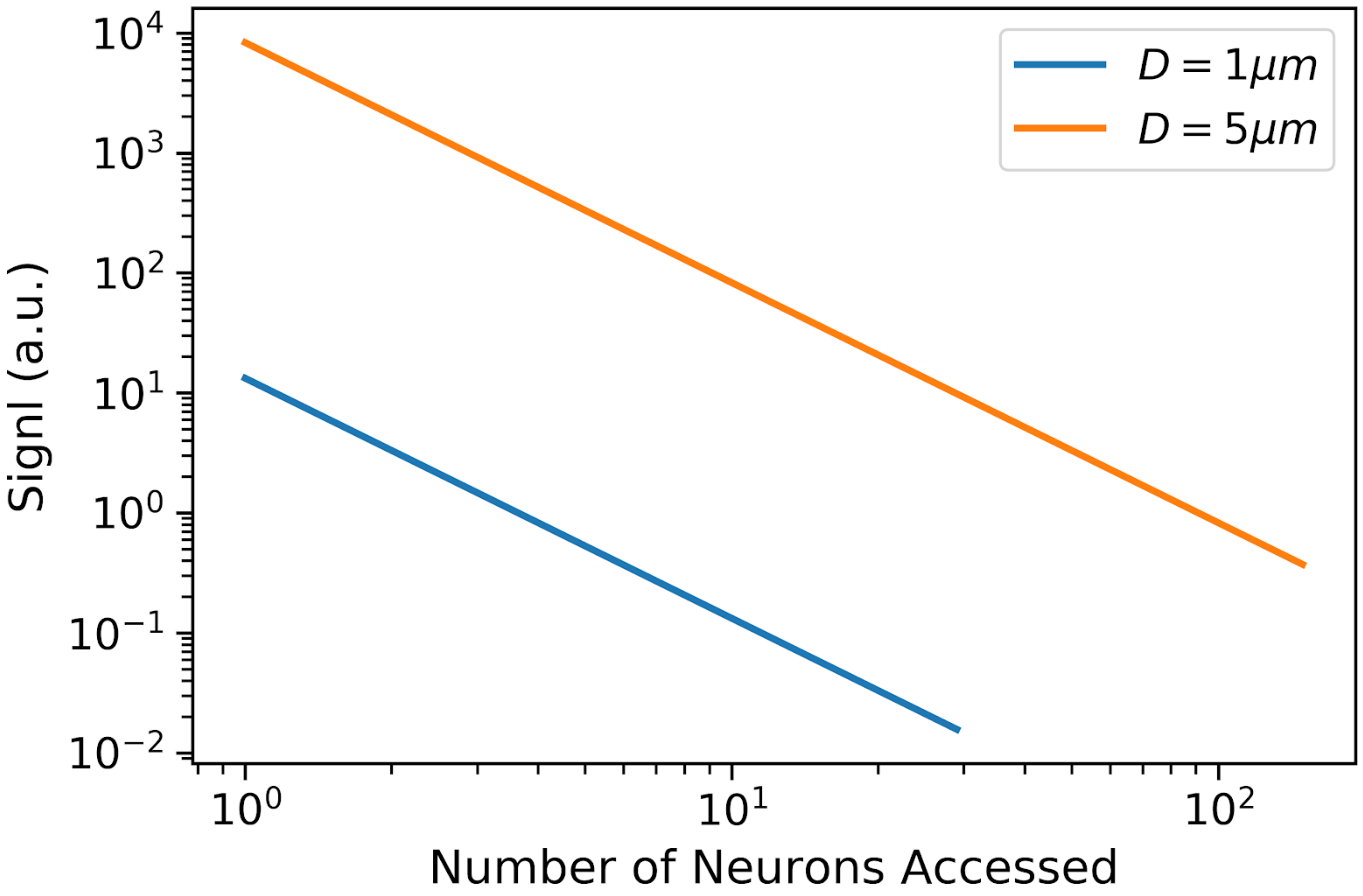
$$N_a \sim C \times \frac{P_0^2}{f^2 \tau} \left(\frac{\lambda}{A} \right)^2 V$$

C = constant
P_0 = average power
f = laser rep rate
τ = pulse width
A = illumination area
V = illuminated volume containing fluorophores

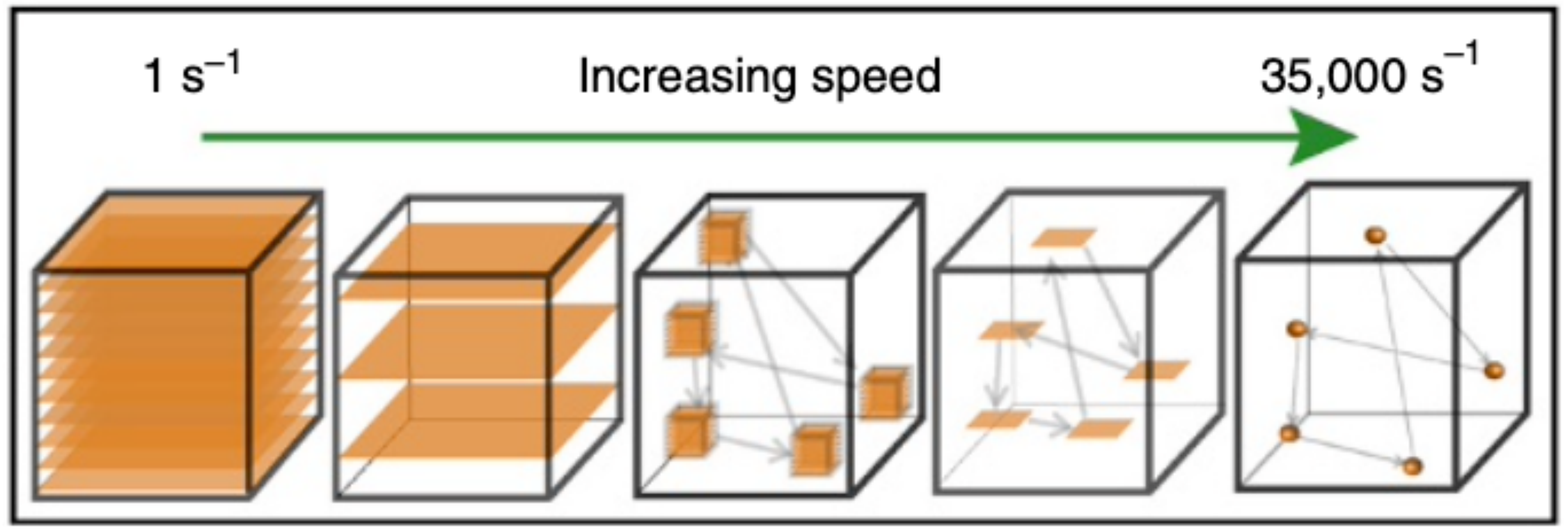
Weisenberger et al. (2017)



Assuming:
- Fixed energy density (E/A)
- Acquisition rate of 1 KHz
- 1 pulse / voxel
- Switching time of 1.5 µs

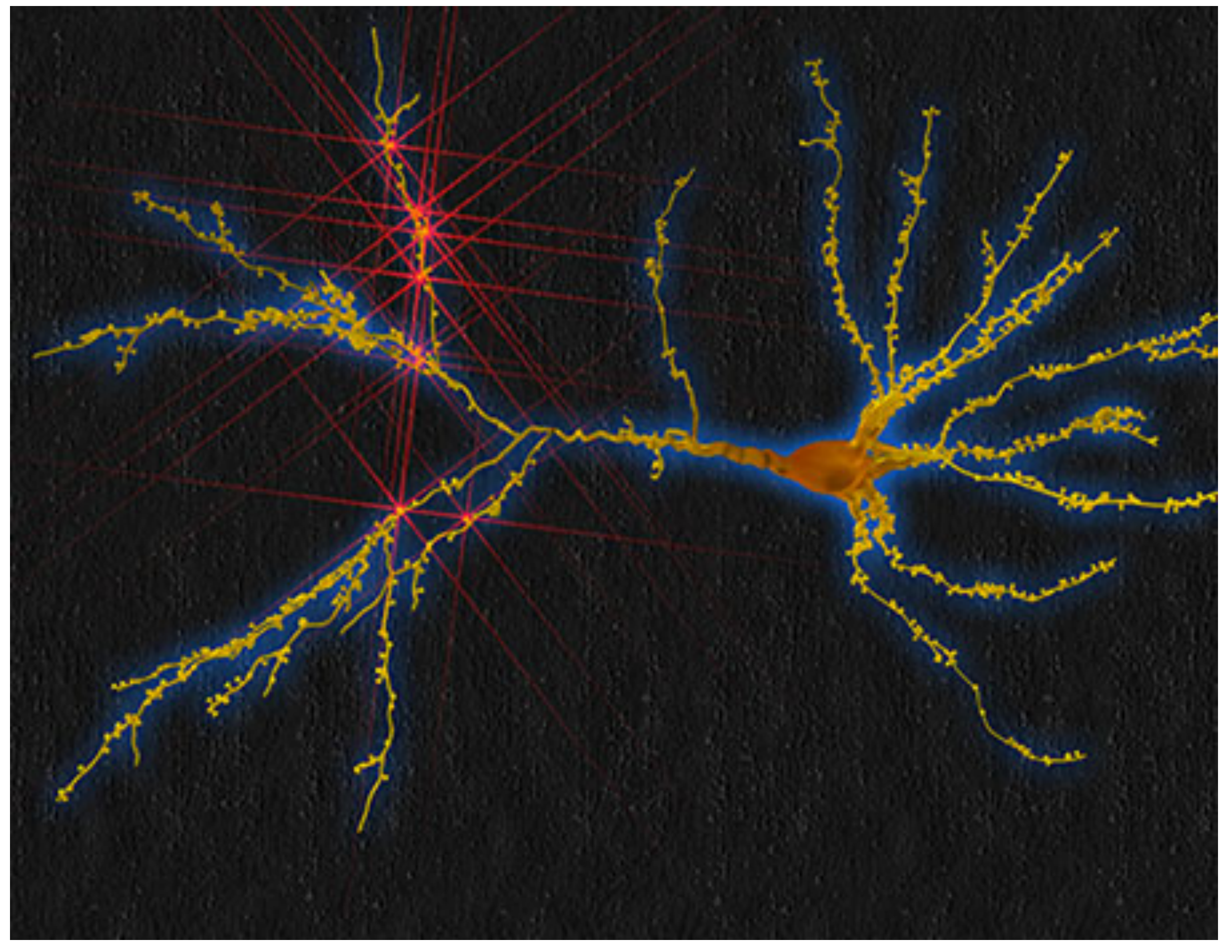


Random Access



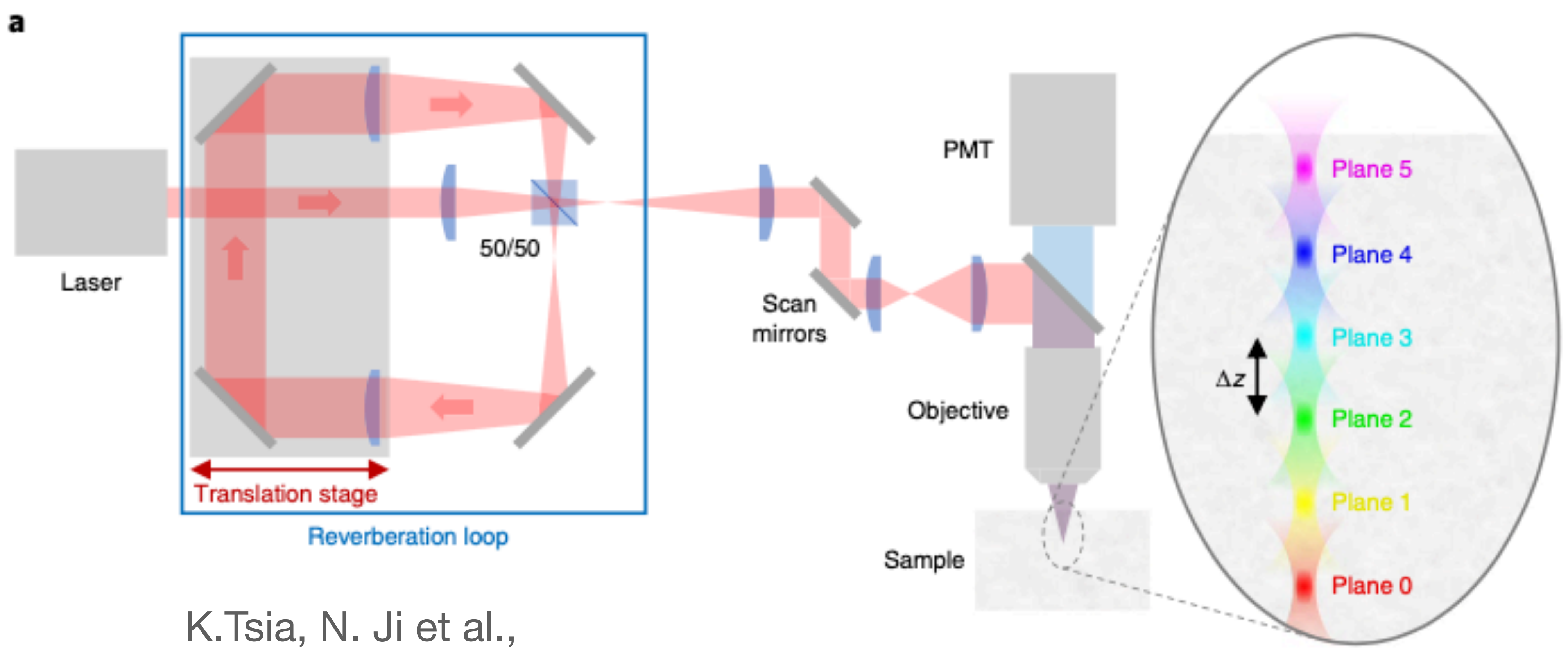
K. M. N. S. Nadella … P. A. Kirkby, and R. A. Silver, *Nat Meth* (2016).

Computational



K. Podgorski

Multiplexing



K.Tsia, N. Ji et al.,

Thanks!

- New technologies
- Where we could do something
- Would love to chat about these ideas or any others