https://youtu.be/Hz0VlUVjYfI

Science 20 Apr 2018: DOI: 10.1126/science.aaq1392

"Understanding biology and its complexity is probably one of the last human frontiers. It's the age of exploration, but instead of looking out at the galaxies, we're looking at the galaxies inside the cells."
-Gokul Upadhyayula, Scientific Director | UC Berkeley Advanced Bioimaging Center &Chan Zuckerberg Initiative

https://medium.com/@cziscience/a-picture-is-worth-a-thousand-words-bce6e17f289a

HAMAMATSU

PHOTON IS OUR BUSINESS

Do Dim Things.

Why low light camera capabilities are relevant to health and medicine.

Stephanie M. Fullerton, Ph.D.

Life Science Marketing Manager

Hamamatsu Corporation

07.23.2020

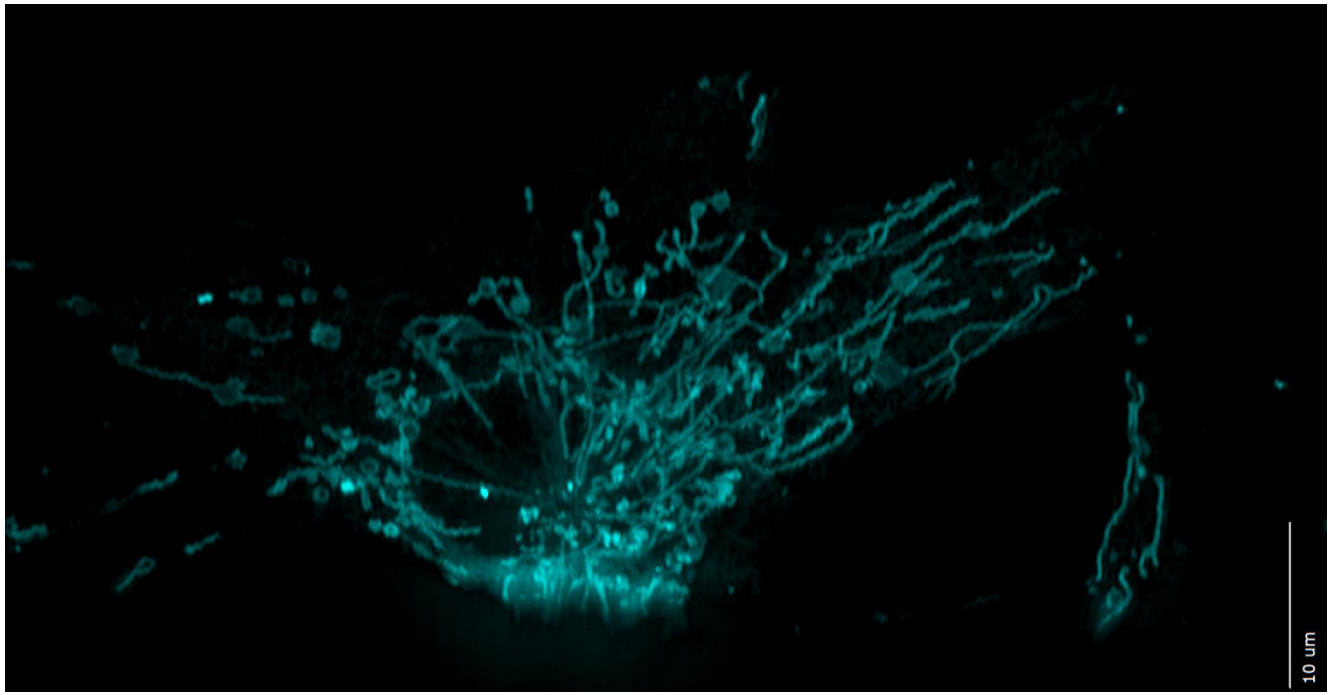
Do Dim Things

- Why does “low” light imaging matter?
- What makes a camera “good” in low light?
- Dim things done by bright people in Research
- Dim things leads to bright futures in Medicine

https://www.cell.com/pictureshow/2019-ki-image-awards

Why care about (low light) imaging?

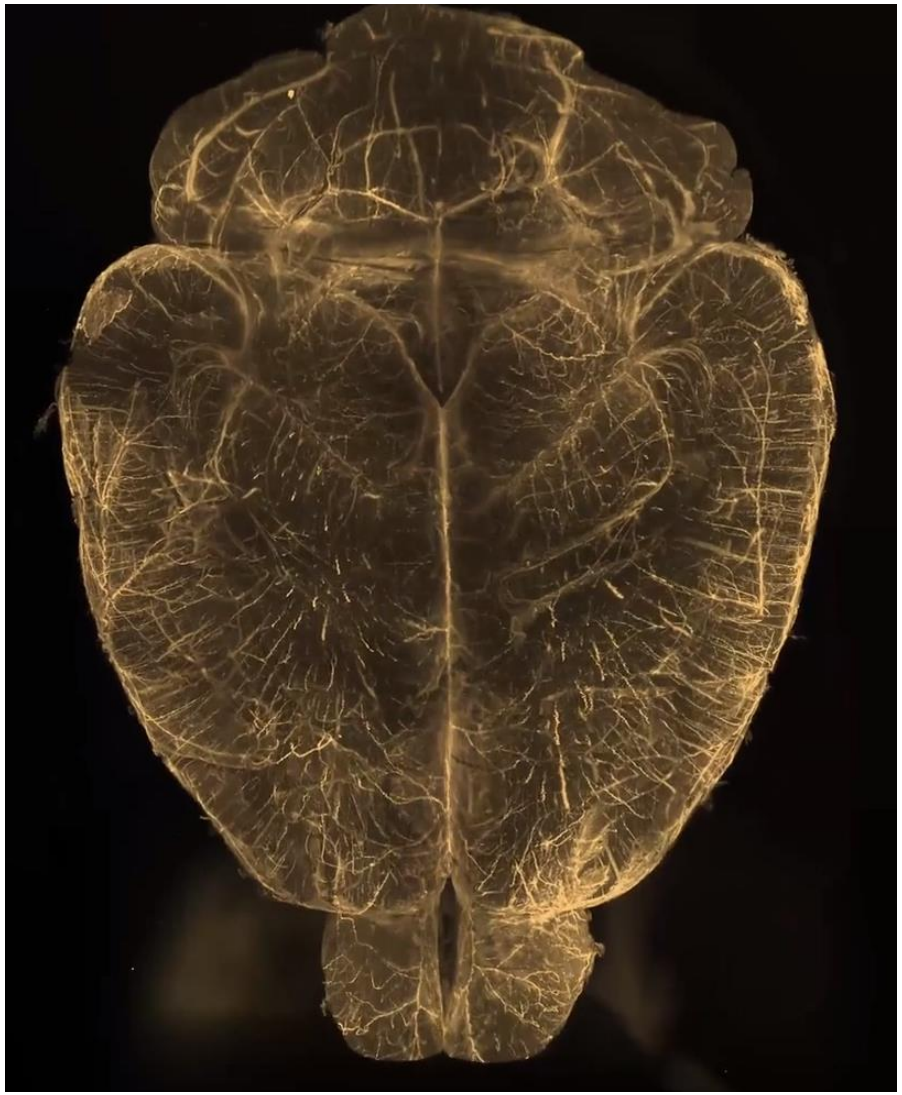
Imaging of Living Cells in 3D



PAE cells labeled with MitoTracker Red. Imaged on a 3i Lattice LightSheet in Structured Illumination (SIM) mode

Why care about (low light) imaging?

High throughput imaging of large number of fixed cells



https://www.intelligent-imaging.com/ctls Smooth muscle cells in the arteries, veins, and capillaries of mouse brain cleared with PEGASOS labeled by NG2BacDsRed. Sample courtesy of Dr. Woo-Ping Ge, University of Texas Southwestern Medical Center.

Why are these techniques low light imaging?

It’s all about

TIME

Why Time?

1. to collect enough photons
2. to resolve temporal events
3. to preserve biological samples
4. to increase throughput

Still missing one piece

INFORMATION

Fast imaging with maximum information content

"Information you don't have, cannot be extracted back"

-Na Ji

UC Berkeley

Advanced Imaging Workshop, January 2020

Dynamic super-resolution structured illumination imaging in the living brain

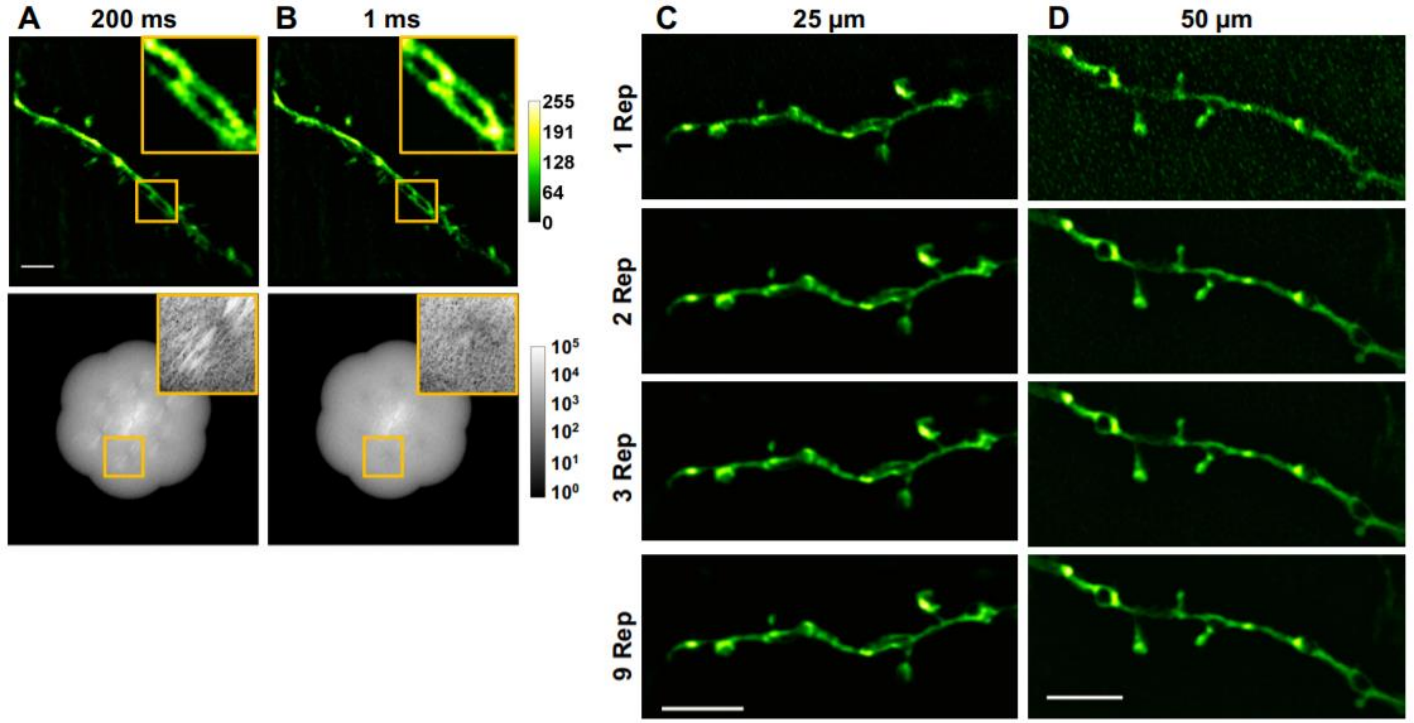


Fig. S6. Super-resolution SIM images of the *in vivo* mouse brain. (A,B) Short camera integration time reduces motion artifacts. (Top) SIM images and (bottom) their associated OTFs from raw data acquired with an integration time of (A) 200 and (B) 1 ms. Scale bar: 3 μm; inset width: 3.6 μm. (C,D) *in vivo* SIM images at depths of (C) 25 μm and (D) 50 μm for different numbers of repetitions: 1, 2, 3, and 9 repetitions (scale bar: 5 μm). Images were normalized independently. Most of the improvement in image quality is obtained when going from 1 to 2 repetitions. Occasionally artifacts are still present with 2 repetitions, thus 3 repetitions are therefore necessary.

Is this dim?

1. Enough to see it
2. Enough to quantitate it
3. Enough to compare it
4. Enough to computationally correct and analyze

What information do you seek?

Camera noise and the limit of detectability

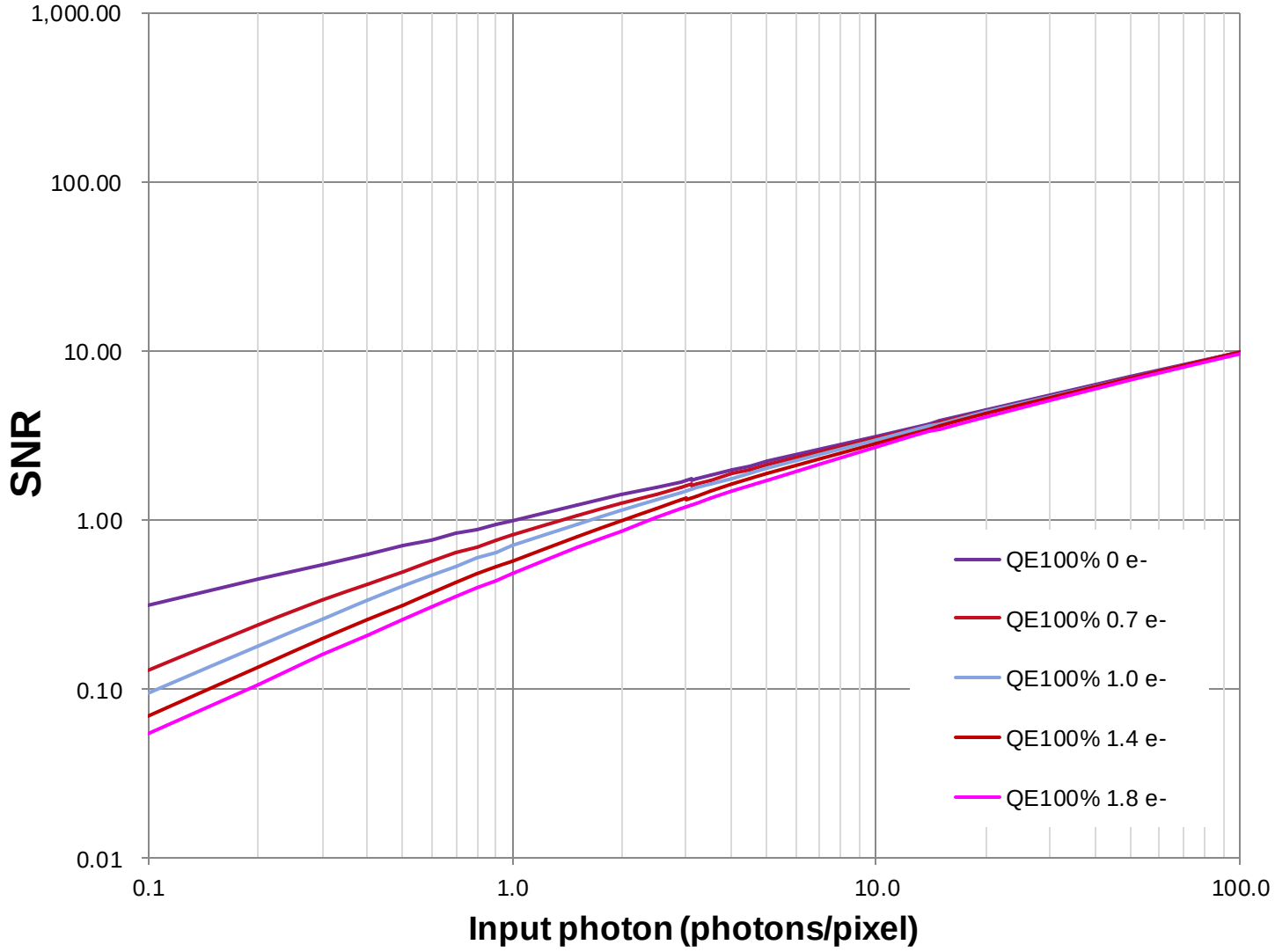
SNR as an indicator of image quality

For CCD and sCMOS

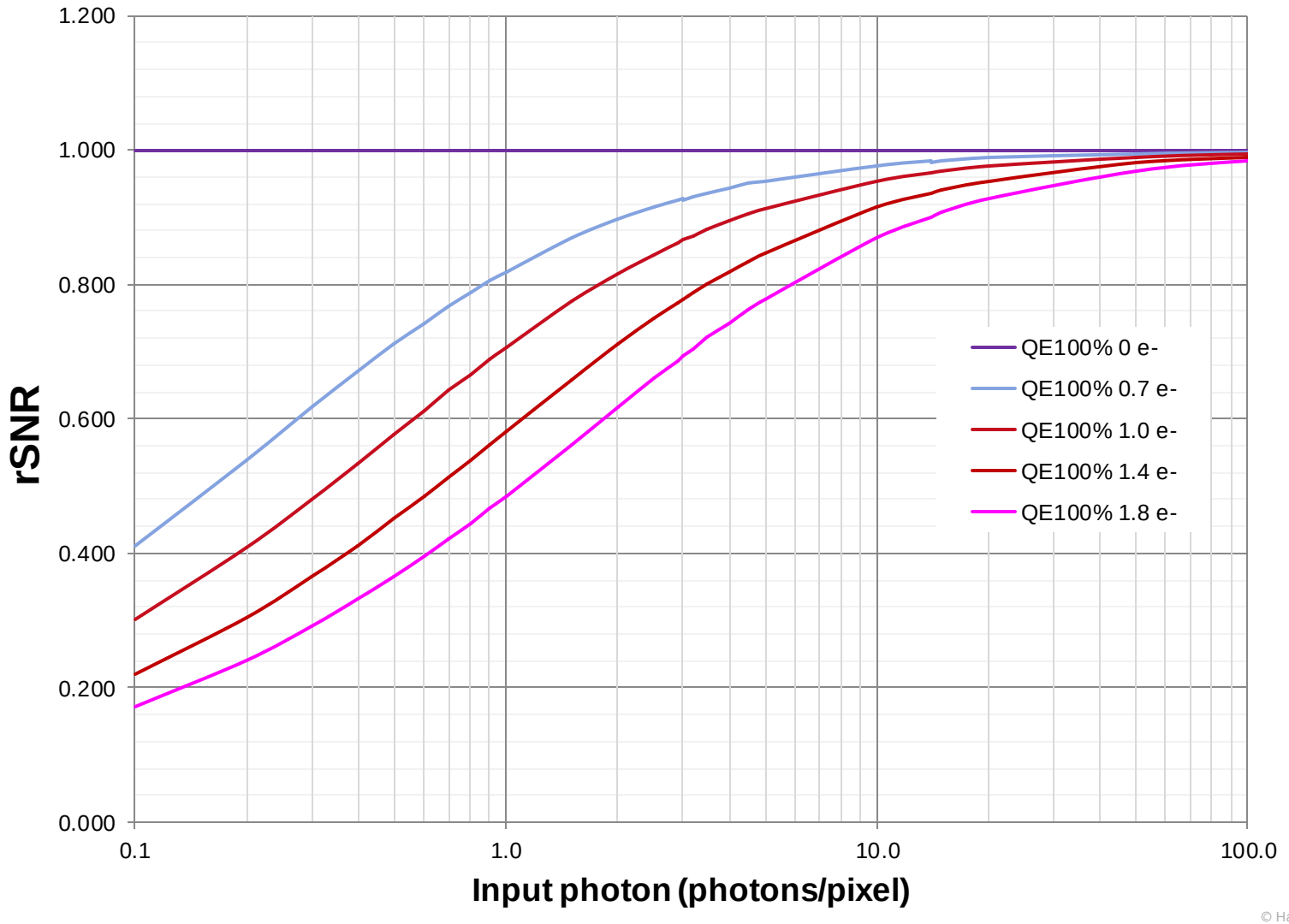
$$\mathrm{SNR} = \frac{\mathrm{QE} * S}{\sqrt{\underbrace{N_r{}^2}_{\text{Read Noise}} + \underbrace{(QE * S)}_{\text{Signal Noise}} + \underbrace{(D * t)}_{\text{Dark Noise}}}}$$

$S = \text{Signal } (photons)$
QE = Quantum Efficiency
D = dark current
t = exposure time
N_r = read noise

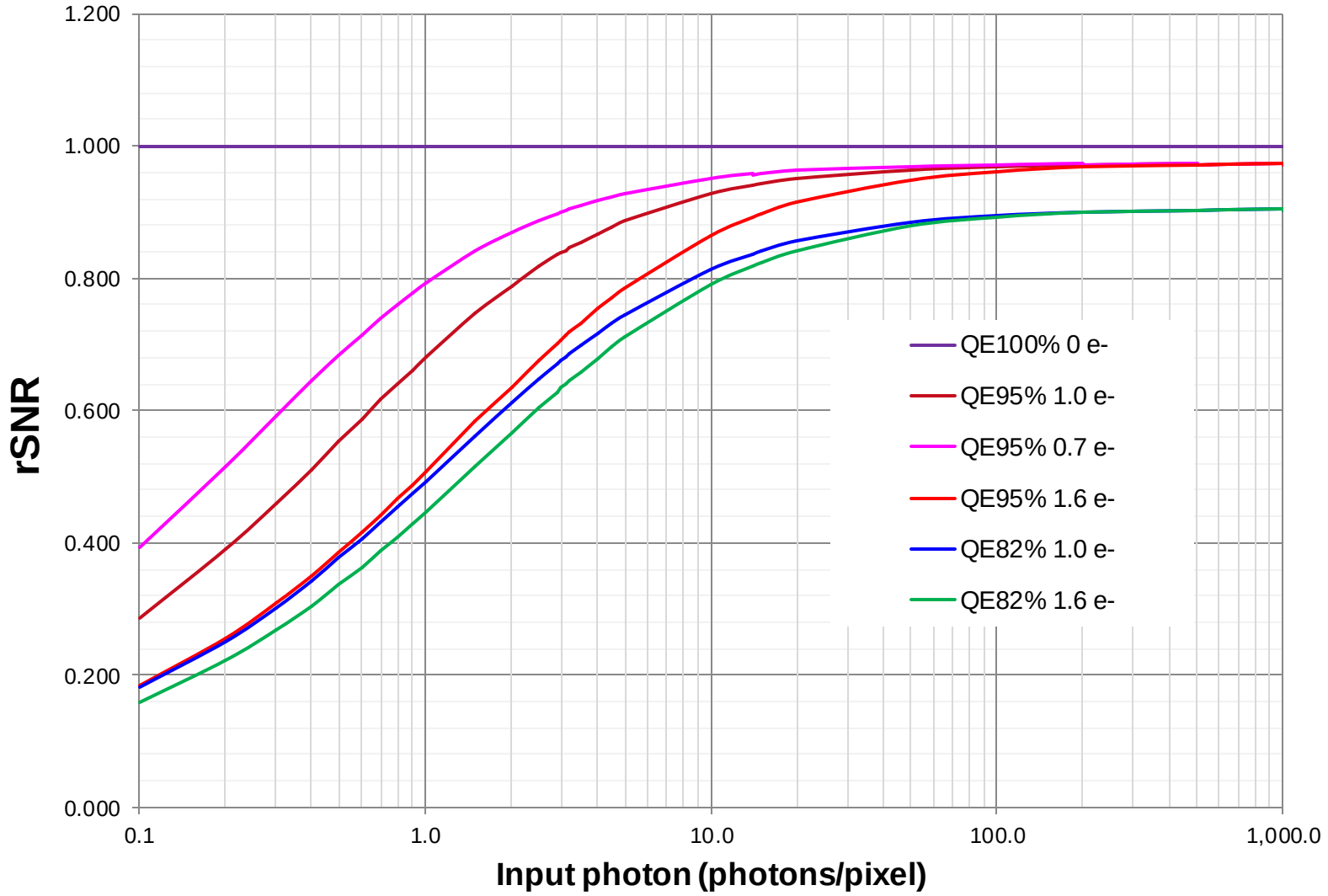
At low light, read noise is significant



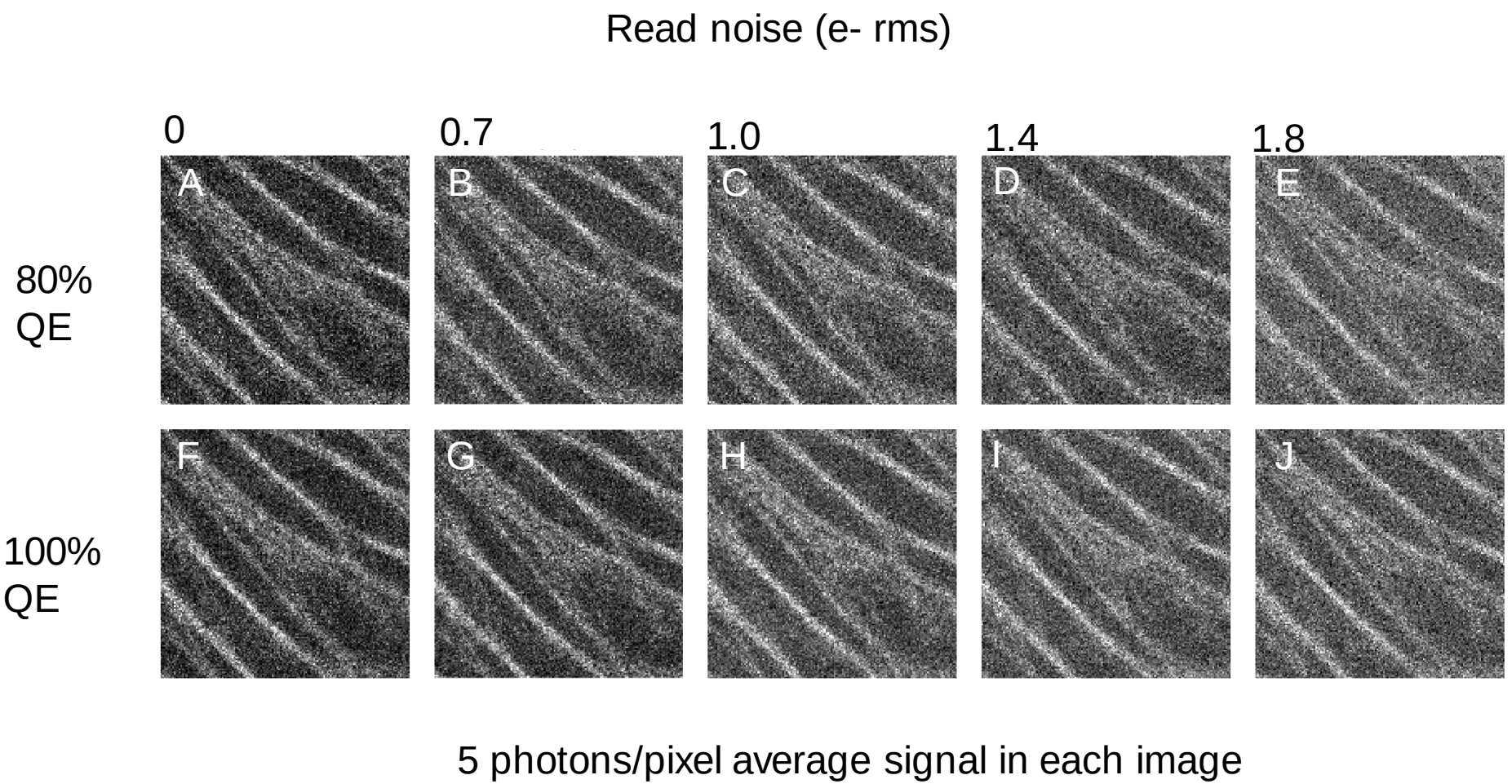
At low light, read noise is significant



At low light, read noise is significant



At low light, read noise matters



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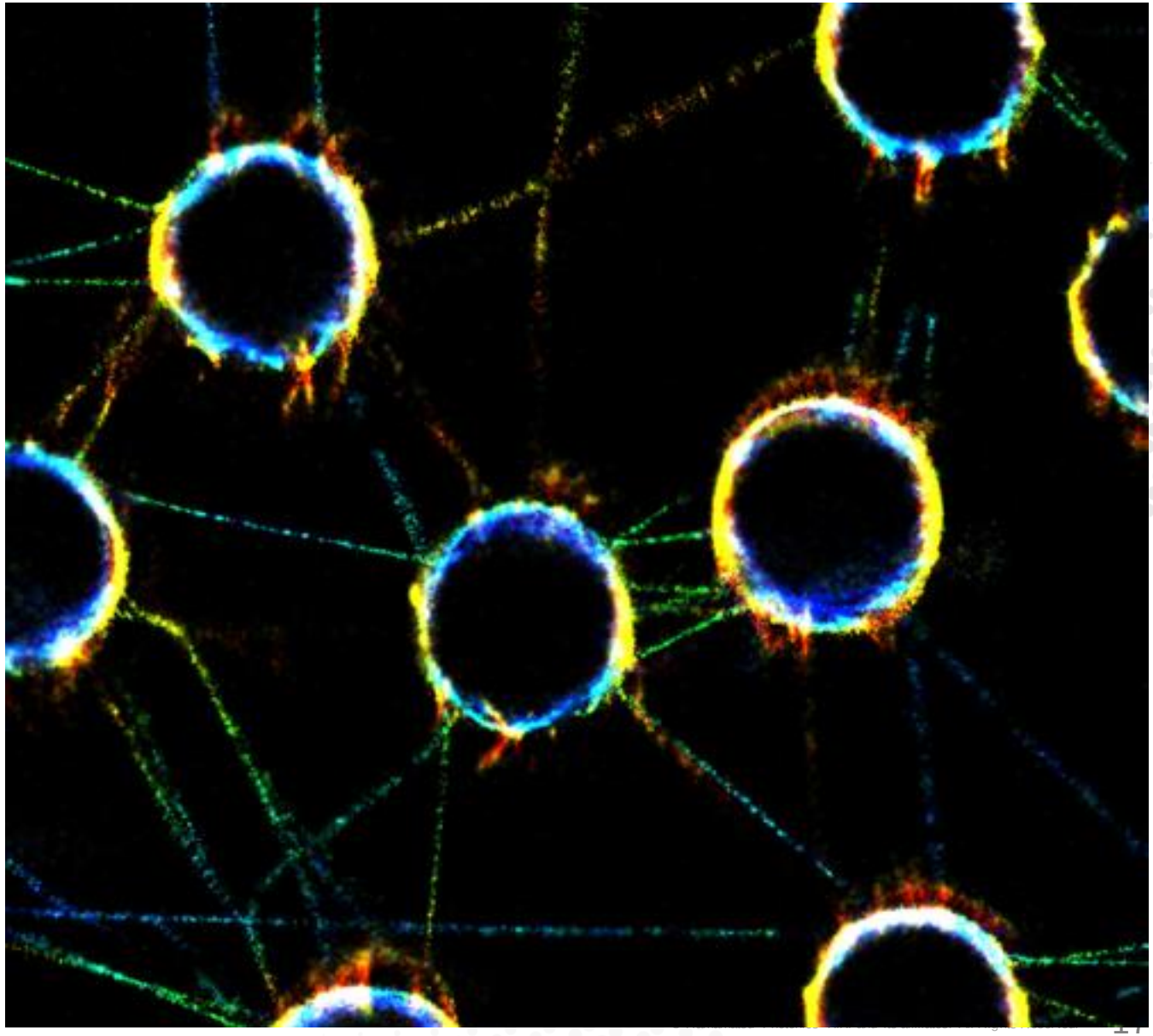


Visualizing Neuronal Activity

The Question:

Can we visualize membrane voltage changes in populations of cells in awake behaving animals?

The Problem:

- Need indicator response to be fast. Current indicators are Ca^{2+} based & slow
- Need indicator to be bright photostable and membrane localized.
- Need ms resolution to resolve action potential. Typically not enough photons
- Need to visualize multiple cells. Too little field of view
- Want to make easy to do. Techniques are cumbersome or expensive

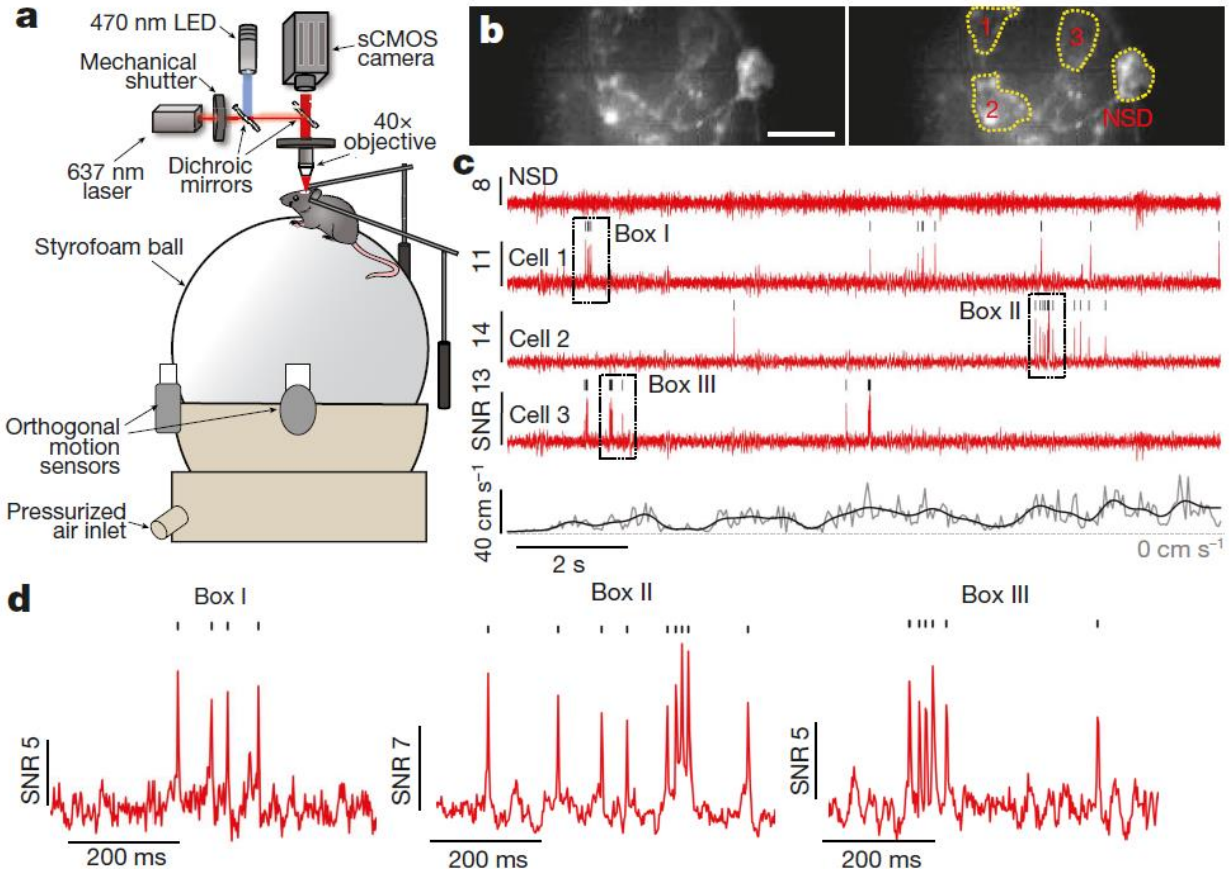
The Solution:

Population imaging of neural activity in awake behaving mice

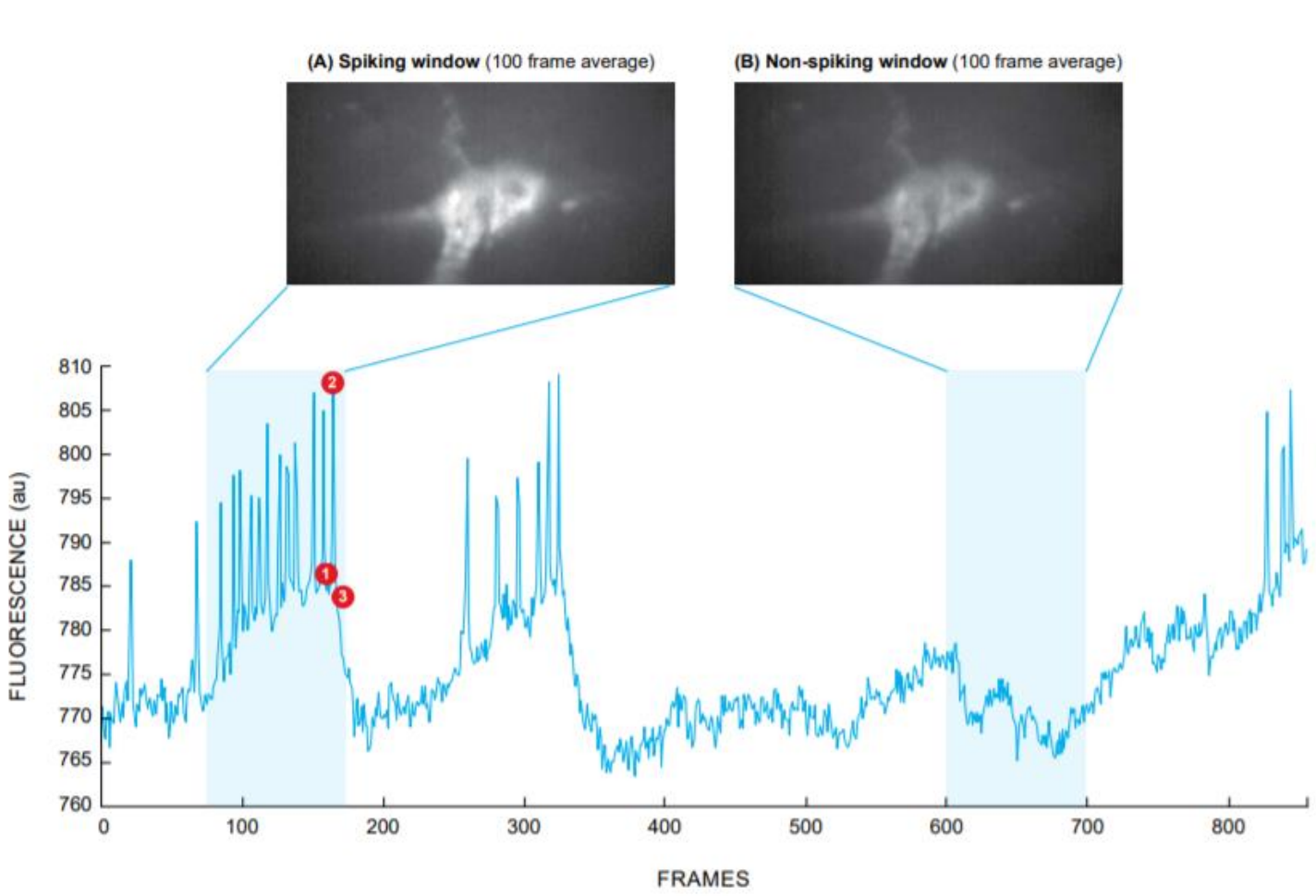
Kiryl D. Piatkevich[1,2,11], Seth Bensussen[3,11], Hua-an Tseng[3,11], Sanaya N. Shroff[3], Violeta Gisselle Lopez-Huerta[4], Demian Park[1,2], Erica E. Jung[1,5], Or A. Shemesh[1,2], Christoph Straub[6], Howard J. Gritton[3], Michael F. Romano[3], Emma Costa[1], Bernardo L. Sabatini[6], Zhanyan Fu[4], Edward S. Boyden[1,2,7,8,9,10]* & Xue Han[3]*

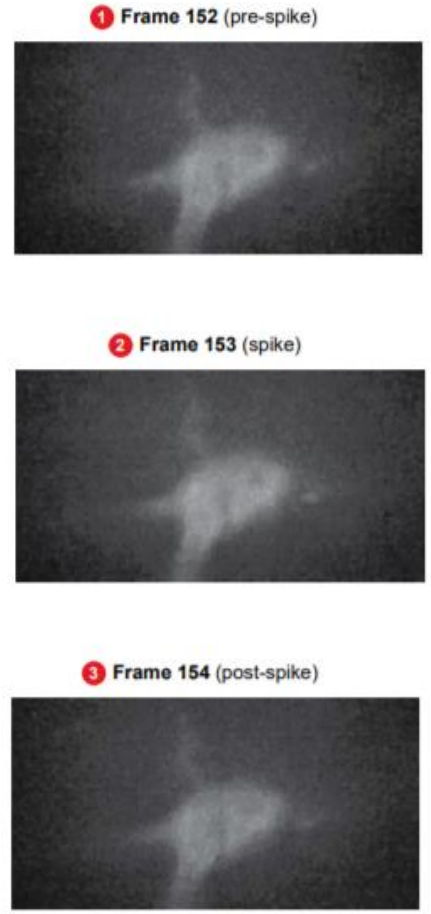
https://doi.org/10.1038/s41586-019-1641-1

"We could detect individual spikes in single cells in all four brain regions. The SNR per action potential ranged from about 7-16 across the brain regions examined. To our knowledge, no other paper has reported SNR values per action potential in living brain, so we cannot directly compare our molecules to others in this regard."



Dims things by bright scientists: Xue Han Lab, Boston University





Genetically Encoded Voltage Sensitive Indicators

The Future:

“As camera performance improves in years to come, and as further evolution of GEVI’s continues, we anticipate that it might be possible to image tens to hundreds of neurons using simple one-photon optics in the near future.”

How does functional neural system organization develop?

The Question:

How do developing neurons assemble into circuits that produce activity patterns capable of instructing behaviors?

The Problem:

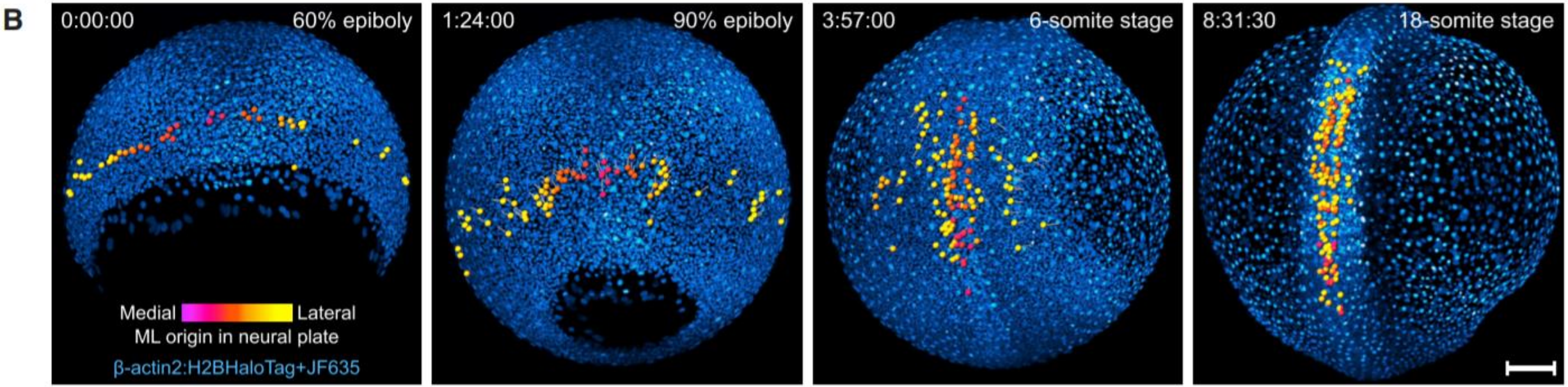
- Needs to be fast.
- Needs to have high info content including lineage, movement and molecular identity AND activity

The Solution:

Single-Cell Reconstruction of Emerging Population Activity in an Entire Developing Circuit

Authors

Yinan Wan, Ziqiang Wei, Loren L. Looger, Minoru Koyama, Shaul Druckmann, Philipp J. Keller



Wan et al., 2019, Cell 179, 355–372 October 3, 2019 https://doi.org/10.1016/j.cell.2019.08.039

The Future:

The methodology presented here for the first time provides access to the functional maturation of an entire circuit at the single-cell level, from neuronal birth to the emergence of patterned activity.”

“The general design of our methodological approach should enable the systematic interrogation of developmental processes and functional roles of neurons in a variety of neuronal systems.”

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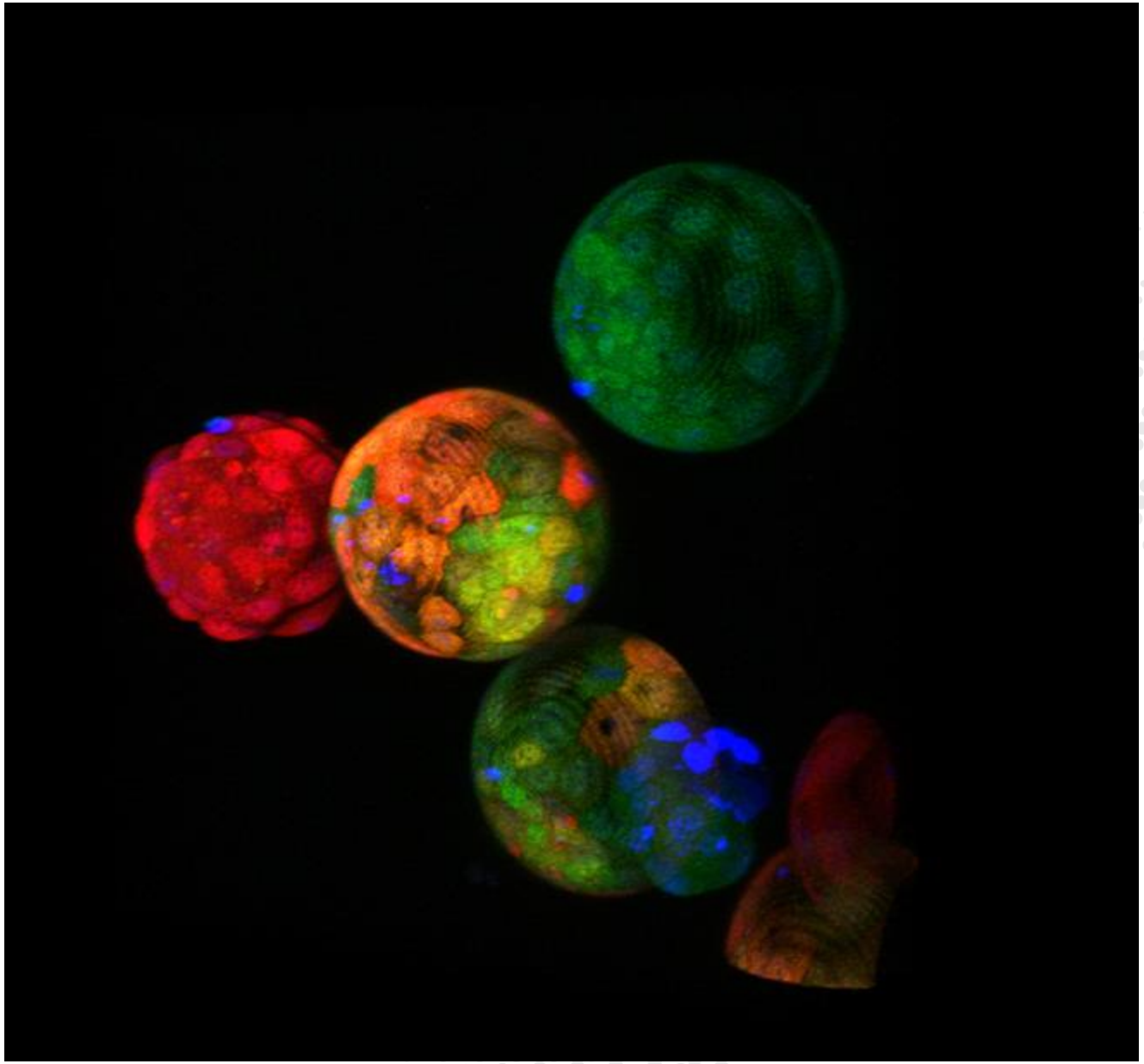
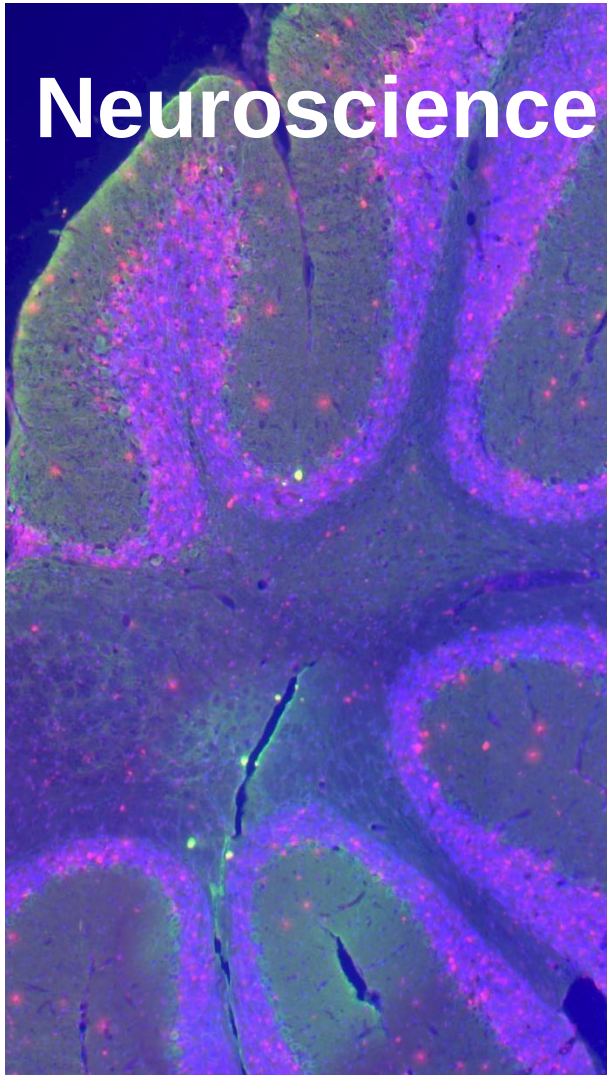
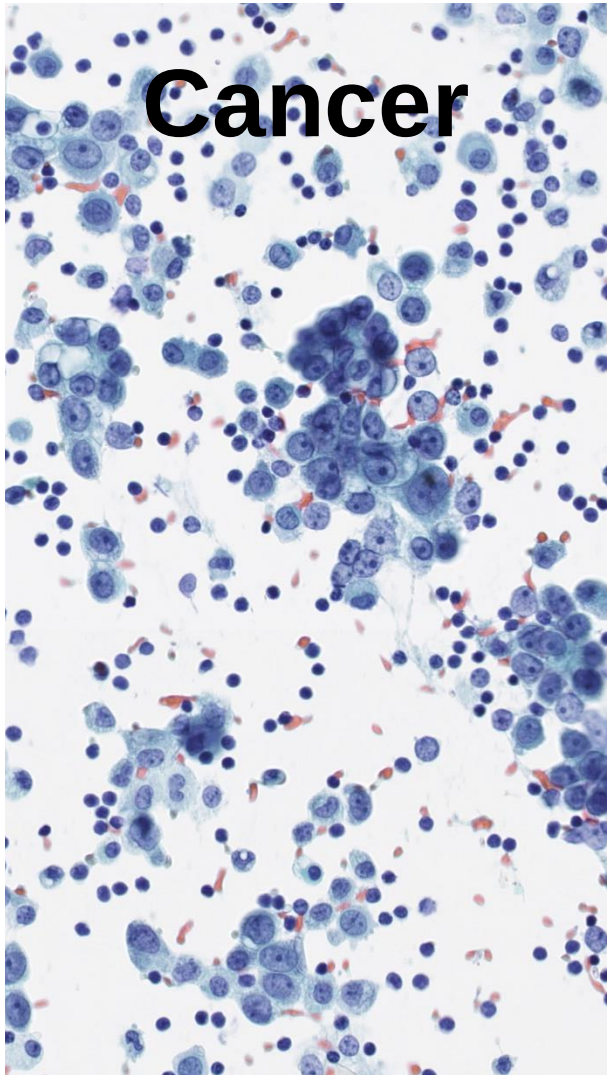
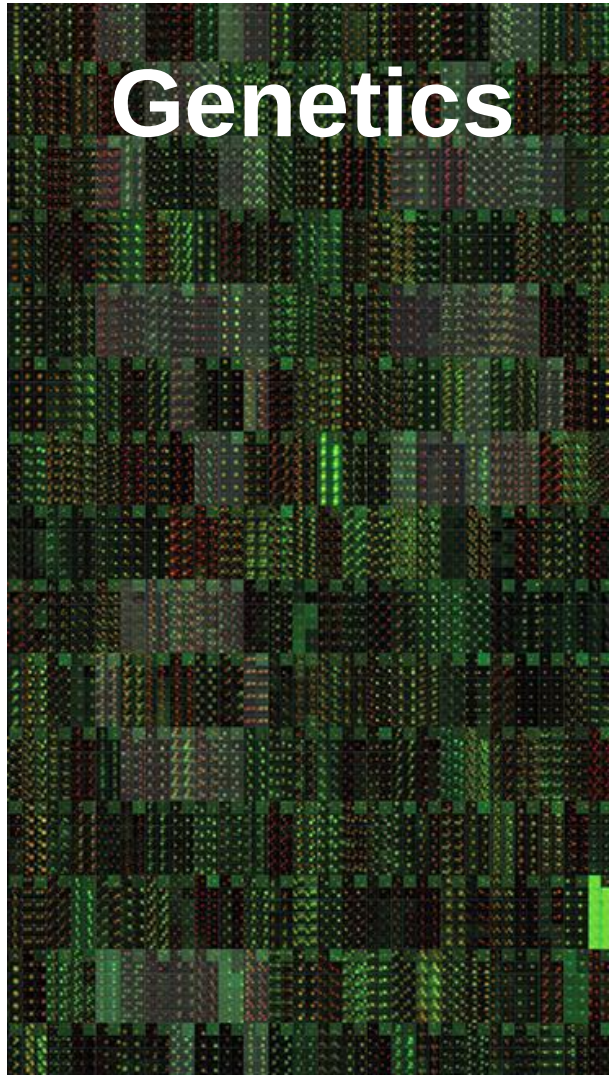


Where will advances in imaging impact health and medicine?



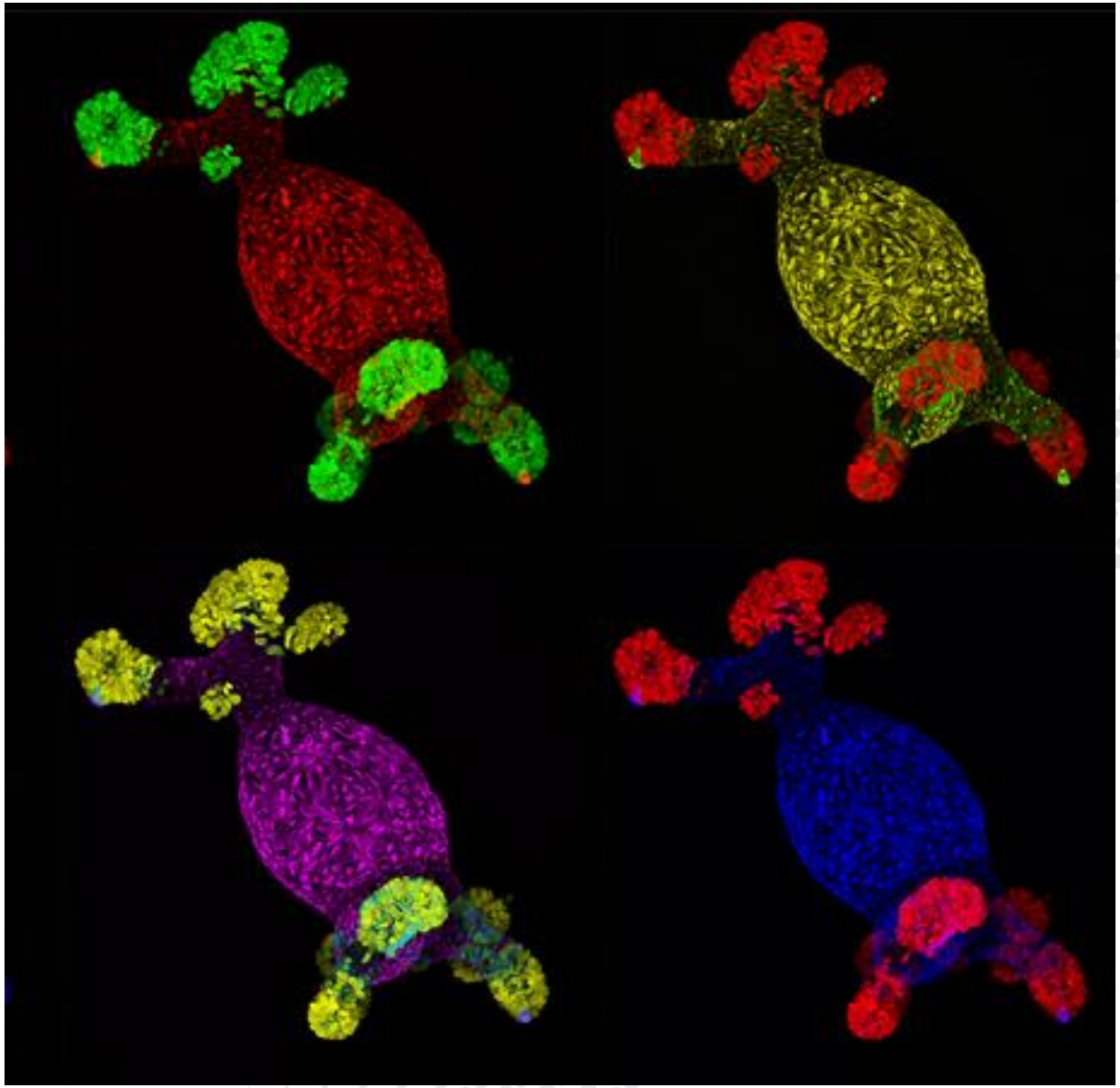




Do Dim Things

- Live cell and high throughput imaging eventually become low light imaging due to time
- Read noise is critical for detectability
- Bench top advances in imaging are already appearing in medicine

https://www.cell.com/pictureshow



Upcoming Hamamatsu Photonics Seminars

Mid-Infrared (MIR) Technologies & Applications	July 28 and July 30
Photon Counting Detectors – SiPM and SPAD	August 11
Using SNR Simulation to Select a Photodetector	August 18

To register for other webinars or hear previous webinar recordings, please visit link below:
https://www.hamamatsu.com/us/en/news/event/2020/20200526220000.html

Do Dim Things

Thank you!

Stephanie Fullerton

sfullerton@hamamatsu.com