“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://youtu.be/Hz0VIUVjYfi

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

https://medium.com/@cziscience/a-picture-is-worth-a-thousand-words-bce6e171289a
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
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

\[
SNR = \frac{QE \times S}{\sqrt{N_r^2 + (QE \times S) + (D \times t)}}
\]

- \(S\): Signal (photons)
- \(QE\): Quantum Efficiency
- \(D\): dark current
- \(t\): exposure time
- \(N_r\): read noise

Reading Noise: Signal Noise: Dark Noise
At low light, read noise is significant

![Graph showing SNR (Signal-to-Noise Ratio) vs. Input photon (photons/pixel) with different QE (Quantum Efficiency) values. The graph indicates that as the input photon density increases, the SNR also increases, with higher QE values resulting in higher SNR.]
At low light, read noise is significant
At low light, read noise is significant

![Graph showing rSNR vs. Input photon (photons/pixel)]

**rSNR**

- **QE100% 0 e-**
- **QE95% 1.0 e-**
- **QE95% 0.7 e-**
- **QE95% 1.6 e-**
- **QE82% 1.0 e-**
- **QE82% 1.6 e-**
At low light, read noise matters

Read noise (e- rms)

80% QE

100% QE

5 photons/pixel average signal in each image
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
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\textsuperscript{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

Kiry D. Piatkevich1,2,11, Seth Bensussen1,11, Hua-an Tseng1,11, Sanaya N. Shroff, Violeta Gisselle Lopez-Huerta, Demian Park1,2, Erica E. Jung5, Or A. Shemesh1,2, Christoph Straub, Howard J. Gritton3, Michael F. Romano3, Emma Costa1, Bernardo L. Sabatini, Zhanyan Fu4, Edward S. Boyden1,2,7,8,9,10* & Xue Han*  

“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.”

https://doi.org/10.1038/s41586-019-1641-1
Dims things by bright scientists: Xue Han Lab, Boston University
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

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.”
Do Dim Things

- What is “low” light imaging?
- What makes a camera “good” in low light?
- Dim things done by bright people in Research
- Dim things leads to bright futures in Medicine
Where will advances in imaging impact health and medicine?

- Neuroscience
- Cancer
- Genetics
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:  
Do Dim Things

Thank you!

Stephanie Fullerton
sfullerton@hamamatsu.com