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SPRING 2016 SCIENTIFIC CAMERAS

2016 V2

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When the history of this decade is written, what will be the memorable scientific achievements?

Usually it's the big discoveries or culminating achievements that are celebrated. But every day, every scientist takes small steps toward a potential breakthrough. In this regard, scientific research is the embodiment of Kaizen, the Japanese philosophy of continuous, incremental improvement rooted in empirical investigation and relying on teamwork and best of practice methods. Within Hamamatsu engineering, Kaizen is never discussed, but it is always practiced. Since the launch of the ORCA-Flash4.0 sCMOS camera, we have stood side by side with researchers to learn what works and what can be done better. As of October 2015, every ORCA-Flash4.0 V2 has exactly what you asked for...increased quantum efficiency. We understand that by capturing more of the light emitted from you samples, you are now free to go a little faster, to resolve those extremely dim structures, to achieve greater localization precision in super-resolution experiments and to more efficiently image developing organisms with light sheets. We believe a small change can have a big impact.

ORCA-Flash4.0 V2

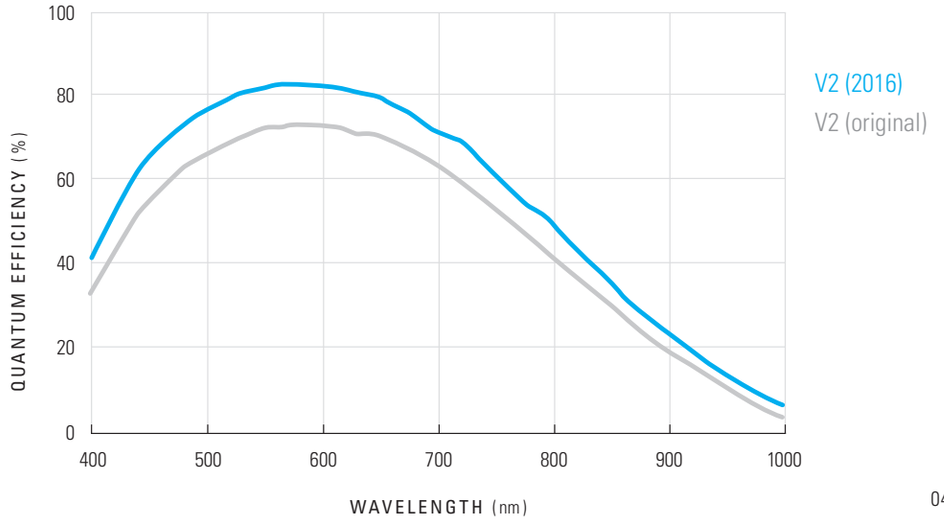
DISCOVER THE BREAKTHROUGH

From its introduction the Flash4.0 has challenged the status quo of imaging and undergone a series of useful enhancements. The most recent is perhaps the most exciting; a notable increase in the ability to detect photons. This enhanced QE means that you have a possibility of detecting the faintest of signal or for brighter samples that ideal image quality is achieved with shorter exposure times, perhaps saving your cells from phototoxicity or bleaching. With the ORCA-Flash4.0 V2 already delivering wide field of view, large dynamic range, and fast frame rates, this QE enhancement only makes it more versatile and powerful. If you have not yet discovered the performance of the ORCA-Flash4.0 V2 sCMOS now is the time. What biological breakthrough will you make with your extra photons?

FRAME RATE
100 FPS max @ full resolution

DYNAMIC RANGE
37,000:1

READ NOISE
1.4 electrons rms minimum



ORCA-Flash4.0 LT

EXPLORE MORE WITH CMOS

Ready to make the jump from a traditional CCD to the increased flexibility and sensitivity of a scientific CMOS (sCMOS) camera? With established performance and an affordable price, the ORCA-Flash4.0 LT fits into any experiment that needs simple connectivity, moderately fast frame rates, and great sensitivity. The ORCA-Flash4.0 LT is designed to bring all the advantages of sCMOS technology—wide field of view, low-light sensitivity, and large dynamic range—to every research lab. Easy connectivity and powerful performance help you explore your pressing biological questions. And when your green channel is screaming but your red is ho-hum horrible, the LT's "W-VIEW Mode" in combination with the W-View Gemini (p.12) ensures more balanced exposure for reliably quantitative dual wavelength imaging. Think of all that LT can help you discover.

FRAME RATE
30 FPS max @ full resolution

DYNAMIC RANGE
33,000:1

READ NOISE
1.5 electrons rms minimum

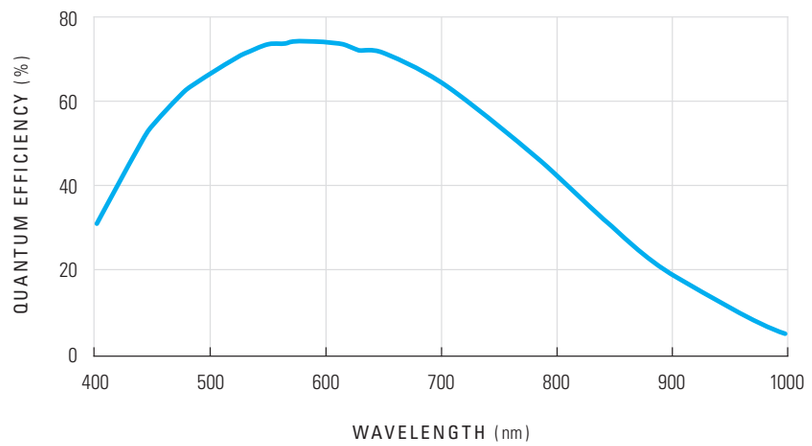


ImagEM X2 512

ImagEM X2-1K

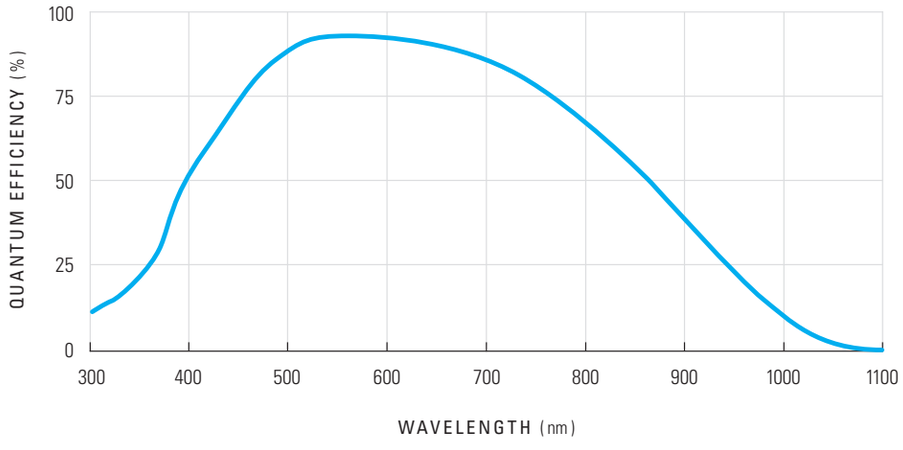
ImagEM X2 SERIES

PUSH THE LIMITS OF LOW

What do you do when your light levels are truly low? How low? As in the dimmest of dim, just a few photons-of-signal-per-pixel dim with very little background. In these demanding situations EM-CCDs still shine. Why? It's all in the EM-CCD's larger pixel size and specialized architecture, which multiplies the weak input signal before it reaches the amplifier and the unavoidable addition of readout noise. The ImagEM X2 (512 x 512 pixels) and the ImagEM X2-1K (1024 x 1024 pixels), are ideal for applications like luminescence that require long exposures, big pixels and/or ability to analog bin.

ImagEM X2 512
Frame Rate: 1076 FPS MAX

ImagEM X2-1K
Frame Rate: 314 FPS MAX

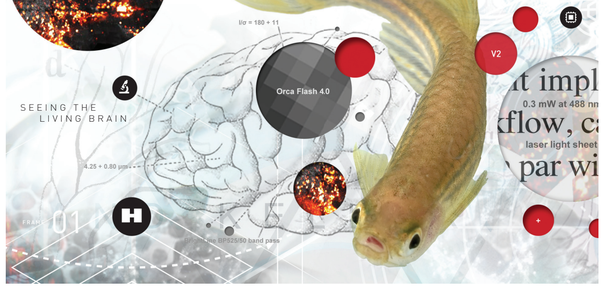


The Living Image

CELEBRATING THE LITTLE THINGS IN LIFE

Science is both beautiful and awe-inspiring. The images that are created in the process of scientific discovery astound us because of their significance and because of the raw beauty of life at the microscopic level. Although the images can and do stand on their own as art, there is a story behind every image. That story starts with a question. And, in the search for the solution, researchers encounter barriers at the bench that must be overcome. Eventually, the quest for answers is achieved leading to even greater possibilities.

The Living Image is an evolving collection of these Bench Stories from some of the best researchers in the world, on topics from light-sheet of whole embryos to implementing in vitro-like diagnostics in vivo. We invite you to read their stories and hope you can experience the same thrill that we do at seeing discovery in action. And through the library of articles and interactive tools in the Resources section explaining camera technology, we hope to make it easier to apply your camera as part of the solution. And, by all means, contact us if you'd like to see your work as a Bench Story—we'd love to help you share the story behind your work of art.



01 **THE QUESTION**

How does the brain work?

Scientists have been searching for answers to this question for decades. What's been missing is an actual image of the complete picture—the ability to directly view neurons firing in a whole, living brain in real time. This view is exactly what the Keller Lab delivers as they push the limits of light-sheet microscopy.

02 **THE BARRIERS**

Light-sheet technology

To acquire these groundbreaking images of the brain, Ahrens and Keller had to extend the capabilities of existing light-sheet technology to speed up volumetric acquisition time. Fast frame rates are important for imaging moving, living systems, whether you are looking at an entire brain or watching microtubules dynamics.

03 **THE SOLUTION**

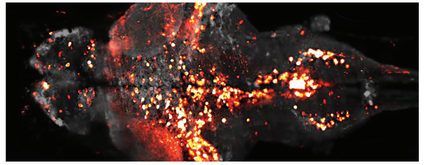
Redesigning the imaging strategy

Advancing light-sheet microscopy technology required a redesign of the existing imaging strategy. The team optimized the hardware components and configuration to streamline communications throughout the microscope control system.

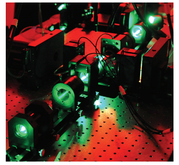
04 **THE POSSIBILITIES**

Next generation light-sheet microscopy

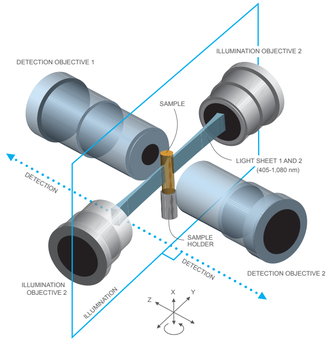
The promise of light-sheet is real-time imaging of neuronal ensembles, allowing scientists to probe functional patterns associated with sensory, motor and homeostatic behaviors. First demonstrated in 2013, the Keller lab radically advances the possibilities in 2015 with a multi-view hs-SiMView. In both systems, the speed of the ORCA-Flash4.0 V2 is essential.



Above: Ahrens et al. use wide field of view at single cell resolution, and imaging every 1.3 seconds to capture projections of whole-brain, neuron-level functional activity (reported by the genetically encoded calcium indicator GCaMP5G in an elavl3:GCaMP5G fish via changes in fluorescence intensity (ΔF/F), superimposed on the reference anatomy).

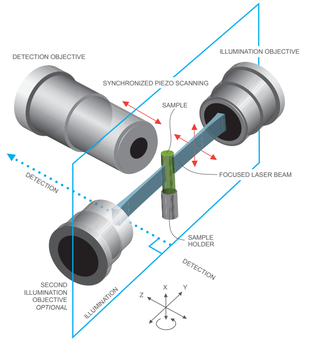


Above: The single view hs-SimView set-up.



TECHNOLOGY BARRIERS

- *Communication speeds between existing hardware components of the laser illumination system limited image acquisition rate.*
- *Camera acquisition time of first gen sCMOS technology limited the rate of image capture.*
- *The imaging strategy of physically moving the sample extended the total acquisition time of each plane to allow for setting.*



REVISED IMAGING

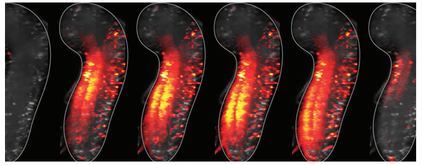
- *New camera technology was needed to keep up with the faster image acquisition times.*

Camera:
(2) Two Orca Flash 4.0 cameras were used

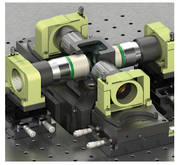
- *Faster image acquisition required the development of a more robust computational pipeline.*

Acquisition rate:
0.8 Hz (for a volume of the size 800 × 600 × 200 µm3)

- *Changing to an imaging strategy that keeps the sample immobile reduced the need for extended settle times.*



Above: Functional imaging of the entire, isolated central nervous system of a Drosophila larva.



Above: The multi-view hs-SiM view set-up.

By taking a multi-directional approach and revising the optical, mechanical and computational imaging strategy, the new hs-SiMView achieves a 25x speed improvement over the 2013 version. Because of these enhancements, it's now possible to perform functional imaging of non-transparent biological samples such as the Drosophila larva above.

All images courtesy of Phillip Keller, Lab Head, HHMI Janelia Research Campus

Find details of this 2013 work by Ahrens et al. at DOI:10.1038/NMETH.2434

For more information on this and other Bench Stories visit http://thelivingimage.hamamatsu.com/

Find details of this 2015 work by Lemon et al. at DOI:10.1038/ncomms8924

MATCHED TO THE PERFORMANCE OF GEN II SCMOS CAMERAS

CHROMATICALLY CORRECTED

USER-DEFINED FILTER COMBINATIONS

EASILY ALIGNED AND STABLE

HIGH TRANSMITTANCE

W-View Gemini

GET CLOSER TO THE BIOLOGY

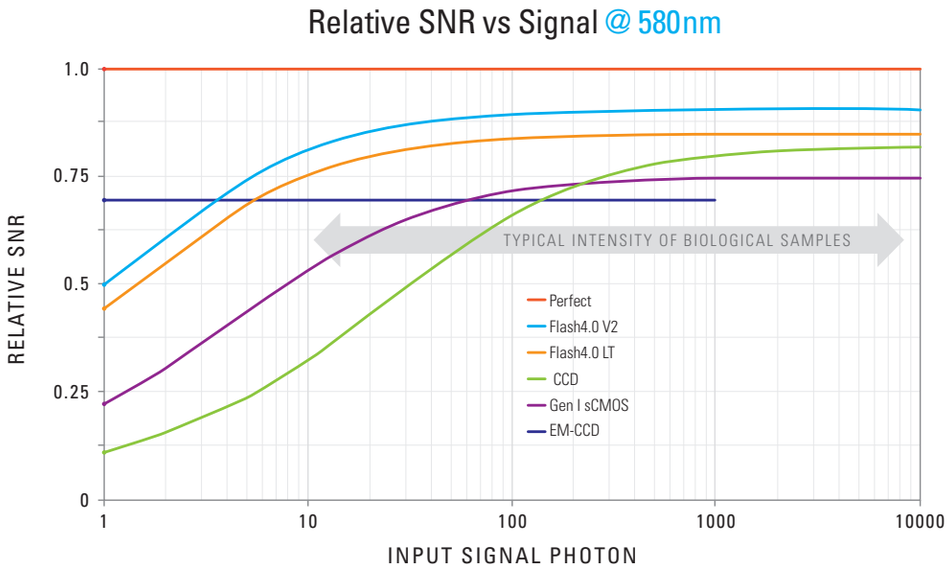
Have you ever thought, “Optical splitters—great idea but so hard to use!” They hold the promise of more efficient multi-wavelength imaging, but need careful alignment. The slightest bump of the microscope table or stomp in the neighboring lab can disrupt the setup. The team at Hamamatsu understands how important it is for tools to get out of your way and just work. They’ve taken on the optical splitter problem and crafted the W-VIEW GEMINI. Optically and mechanically stable, chromatically corrected, with simple software-assisted alignment and flexible configurations, the W-VIEW GEMINI lives up to the promise of what an optical splitter should be. And when you don’t need a splitter, just switch to “bypass mode”—it’s as though there’s nothing between your camera and your microscope. Simplifying multi-wavelength experiments like FRET, the W-VIEW GEMINI gets out of your way to bring you closer to the biology. What biological processes will the W-VIEW GEMINI bring closer to you?

Simultaneous Dual Wavelength Imaging

Relative SNR

How can I easily compare cameras?

No single technical specification can provide all the necessary information to match a camera to an application. But when the quantum efficiency and noise characteristics of a camera are considered in light of the signal and signal noise, we can understand the theoretical limits of a camera under the full range of light conditions. These signal to noise (SNR) curves provide tremendous value in predicting which camera performs best for certain applications, assuming the light levels for that application are known (more on this later). To make SNR data even more approachable, a useful variation is to look at relative SNR (rSNR), where all data is normalized to an imaginary "perfect" camera that has 100% QE and zero noise. With this transformation, it's easy to see that at the lowest light levels (less than 4 photons per pixel with 0 background), EM-CCDs achieve the highest possible SNR. And yet, above 4 photons per pixel, the 2016 ORCA-Flash4.0 V2 surpasses the SNR performance of the EM-CCD and exceeds all other technology, including CCD and previous generations of sCMOS. This SNR performance, combined with fast frame rates and large field of view make the ORCA-Flash4.0 V2 an excellent choice for most every biological application.



Relative SNR at 580nm. EM-CCD gain is 500x. Read noise: ORCA Flash4.0s = 1.4 e- rms, CCD = 6 e- rms, Gen I sCMOS = 2.4 e- rms. At 500x gain, EM-CCDs saturate around 1000 photons/pixel.

Calculating SNR

Calculating SNR is a simple ratio of the total signal to the total noise. For microscope cameras, the equation looks like this:

$$SNR = \frac{QE * S}{\sqrt{F_n^2 * QE * (S + I_b) + (N_r / M)^2}}$$

QE = Quantum efficiency

S = Signal (Photons/pixel/frame)

I_b = Background (Photons/pixel/frame)

N_r = Readout noise (e- rms)

M = EM gain

F_n = Excess noise factor
(1.4 for EM-CCD; 1 for CCD & sCMOS)

Thinking in Photons

How many photons do I have?

We capture images of light but rarely have any sense of the absolute amount of light that was incident on the camera. To get a useful estimate of your light levels, it's possible to make a quick calculation. Gather this information: the pixel full well capacity and bit depth of your current camera in the mode you normally use to collect images and without gain. Divide the full well by the bit depth (e.g. 30,000/65536 = 0.46). This number is the conversion from grey level (gl) to electrons. Then, from one of your typical images (collected without any EM or analog gain), identify a pixel or small region of pixels that represents typical brightness. Measure the intensity of this area in grey levels and subtract the camera offset (average grey level of a dark image; e.g. 1100 gl -100 gl = 1000 gl). Then convert the grey levels to electrons by multiplying the intensity in grey levels by the conversion factor (e. g. 1000 gl x 0.46 e-/gl = 460 e-). Now you have your intensity in electron and can convert to photons by dividing by the QE at the emitted wavelength (e.g. 460 e-/.82 = 560 photons). This is a rough but reasonable approximation of how many photons per pixel were captured from your sample in your imaging system.

SPECS

	ORCA-Flash4.0 V2 USB 3.0	ORCA-Flash4.0 V2 With Camera Link Option	ORCA-Flash4.0 LT USB 3.0	ImagEM X2 512	ImagEM X2 1K
Product Number	C11440-22CU	C11440-22CU	C11440-42U	C9100-23B	C9100-24B
Imaging Device	sCMOS	sCMOS	sCMOS	Back-Thinned EM-CCD	Back-Thinned EM-CCD
Cell (pixel) **Size** (µm)	6.5	6.5	6.5	16	13
Pixel Array (horizontal by vertical)	2048 x 2048	2048 x 2048	2048 x 2048	512 x 512	1024 x 1024
Effective Area (horizontal by vertical in mm)	13.312 x 13.312	13.312 x 13.312	13.312 x 13.312	8.19 x 8.19	13.3 x 13.3
Dark Current (electrons/pixel/sec.) – Air Cooled	0.06	0.06	0.6	0.005	0.01
Dark Current (electrons/pixel/sec.) – Water Cooled	0.006	0.006	N/A	0.0005	0.001
Full Well Capacity in electrons (typ.)	30,000	30,000	30,000	370,000[7]	400,000[8]
Readout Noise (N_r) median in electrons (typ.) slow scan	0.8 @ 30 fps	0.8 @ 30 fps	0.9 @ 30 fps	-	-
Readout Noise (N_r) **rms in electrons** (typ.) **slow scan**	**1.4 @ 30 fps**	**1.4 @ 30 fps**	**1.5 @ 30 fps**	**8 @ 4x gain**	**3 @ 10x gain**
Readout Noise (N_r) median in electrons (typ.) standard scan	1.0 @ 30 fps	1.0 @ 100 fps	1.3 @ 30 fps	-	-
Readout Noise (N_r) **rms in electrons** (typ.) **standard scan**[1]	**1.6 @ 30 fps**	**1.6 @ 100 fps**	**1.9 @ 30 fps**	**<1 @ 1200x gain**	**<1 @ 1200x gain**
Dynamic Range (typ.)	37,000:1	37,000:1	33,000:1	Gain Dependent	Gain Dependent
Peak Quantum Efficiency (QE)	(QE) **82%** @ 560 nm	(QE) **82%** @ 560 nm	(QE) **73%** @ 580 nm	(QE) **92%** @ 580 nm	(QE) **92%** @ 580 nm
Quantum Efficiency (QE) @ 500 nm	(QE) **77%** @ 500 nm	(QE) **77%** @ 500 nm	(QE) **67%** @ 500 nm	(QE) **91%** @ 500 nm	(QE) **91%** @ 500 nm
Quantum Efficiency (QE) @ 670 nm	(QE) **76%** @ 670 nm	(QE) **76%** @ 670 nm	(QE) **68%** @ 670 nm	(QE) **83%** @ 670 nm	(QE) **83%** @ 670 nm
Quantum Efficiency (QE) @ 750 nm	(QE) **61%** @ 750 nm	(QE) **61%** @ 750 nm	(QE) **53%** @ 750 nm	(QE) **66%** @ 750 nm	(QE) **66%** @ 750 nm
Noise Factor (F_n)[2]	1	1	1	1.4	1.4
Minimum Exposure Time	1 ms[4]	1 ms[4]	1 ms[4]	13.85 ms[5]	52.7 ms[5]
Maximum Exposure Time	10 s	10 s	10 s	2 hours	2 hours
In-Camera Binning	2 x 2, 4 x 4 (digital)	2 x 2, 4 x 4 (digital)	2 x 2, 4 x 4 (digital)	2 x 2, 4 x 4[6]	2 x 2, 4 x 4
Subarray	Yes	Yes	Yes	Yes	Yes
Maximum Full Resolution Frame Rate (fps)	30	100	30	70.4	18.5
Absolute Maximum Frame Rate (fps)[3]	25,655	25,655	25,000	1076	314
Electron Multiplying Gain	N/A	N/A	N/A	4 - 1200x	10 - 1200x
Analog Gain	No	No	No	Yes	Yes
A/D Converter	16 bit	16 bit	16 bit	16 bit	16 bit
Interface Type	USB 3.0	CameraLink	USB 3.0	IEEE 1394b	IEEE 1394b
Lens Mount	C-mount	C-mount	C-mount	C-mount	C-mount

[1] 2.0 electrons rms, guaranteed.

[2] If this value is greater than 1, multiplicative noise is present.

[3] Using maximum binning and/or smallest subarray.

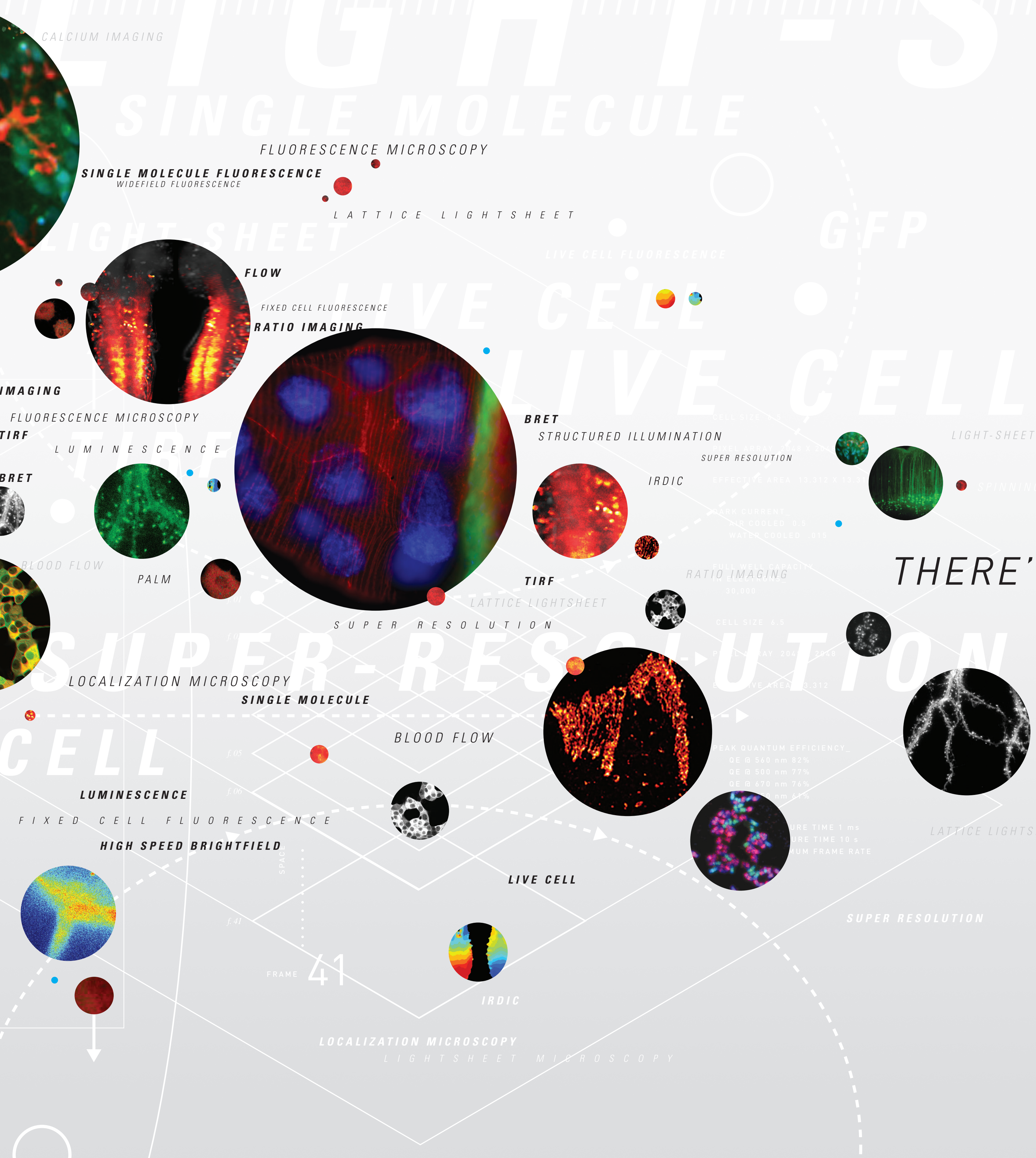
[4] 40 µs using internal trigger and subarray.

[5] 10 µs using external trigger.

[6] 8 x 8, 16 x 16 binning optional.

[7] FWC in EM-CCD Mode. FWC for normal CCD mode is 140,000 e-

[8] FWC in EM-CCD Mode. FWC for normal CCD mode is 50,000 e-



THERE'S A STORY
BEHIND EACH OF THESE IMAGES

WHAT'S YOUR STORY?