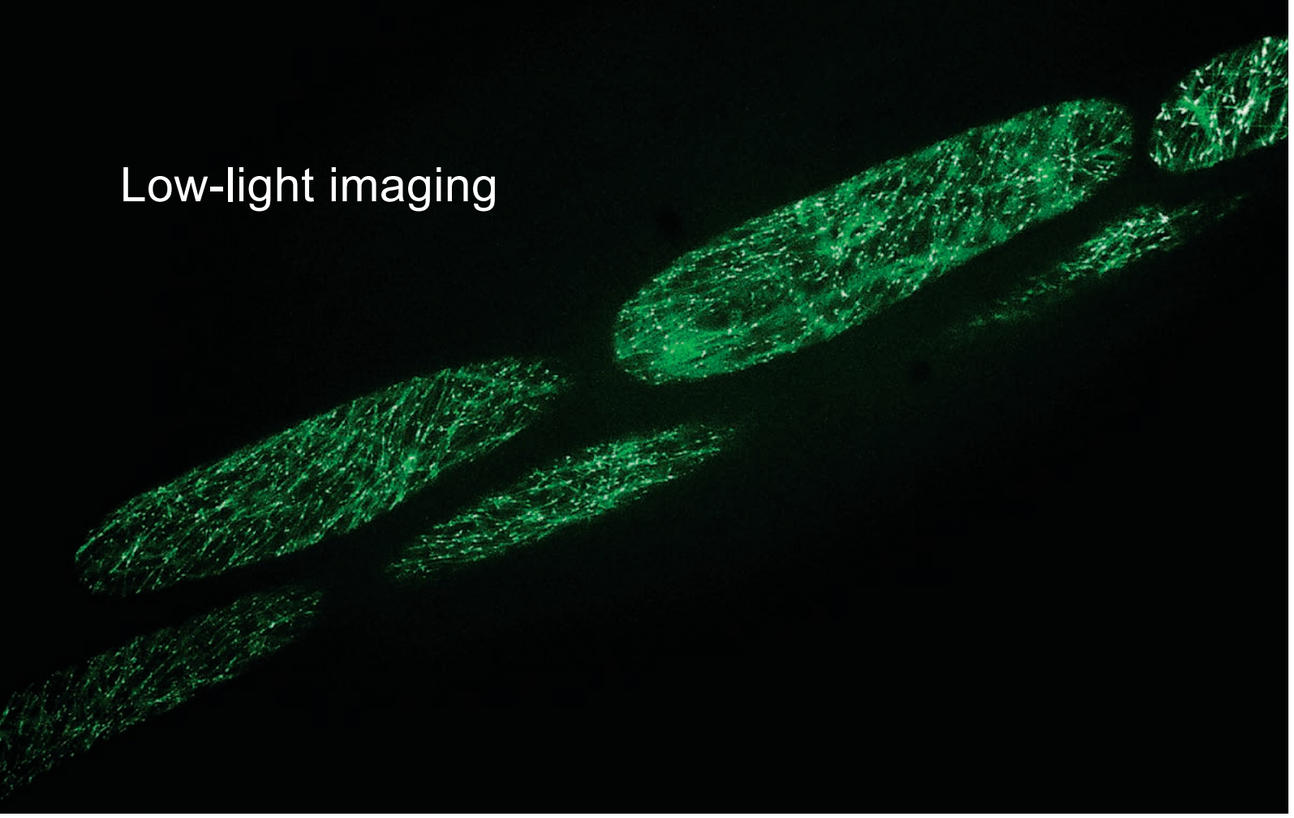
ORCA®-Fusion

HAMAMATSU

PHOTON IS OUR BUSINESS



The ORCA-Fusion, built from the sensor up, balances the complex nuances of camera features to provide beautiful images and robust data at all light levels, but especially in tough low-light conditions. The exceptionally low and highly uniform readout noise of the ORCA-Fusion means that when the sample emits even just a handful of photons, either by default or by experimental design, they are not lost in the noise, but detected and reliably quantified. After all, when you want to hear a whisper it's best to be in a quiet place.

Readout noise
0.7 electrons rms
Ultra quiet scan

DSNU
0.06 electrons

PRNU
0.06 %
At 7500 electrons

High speed
100 frames/s
At 2304 × 2048 ROI

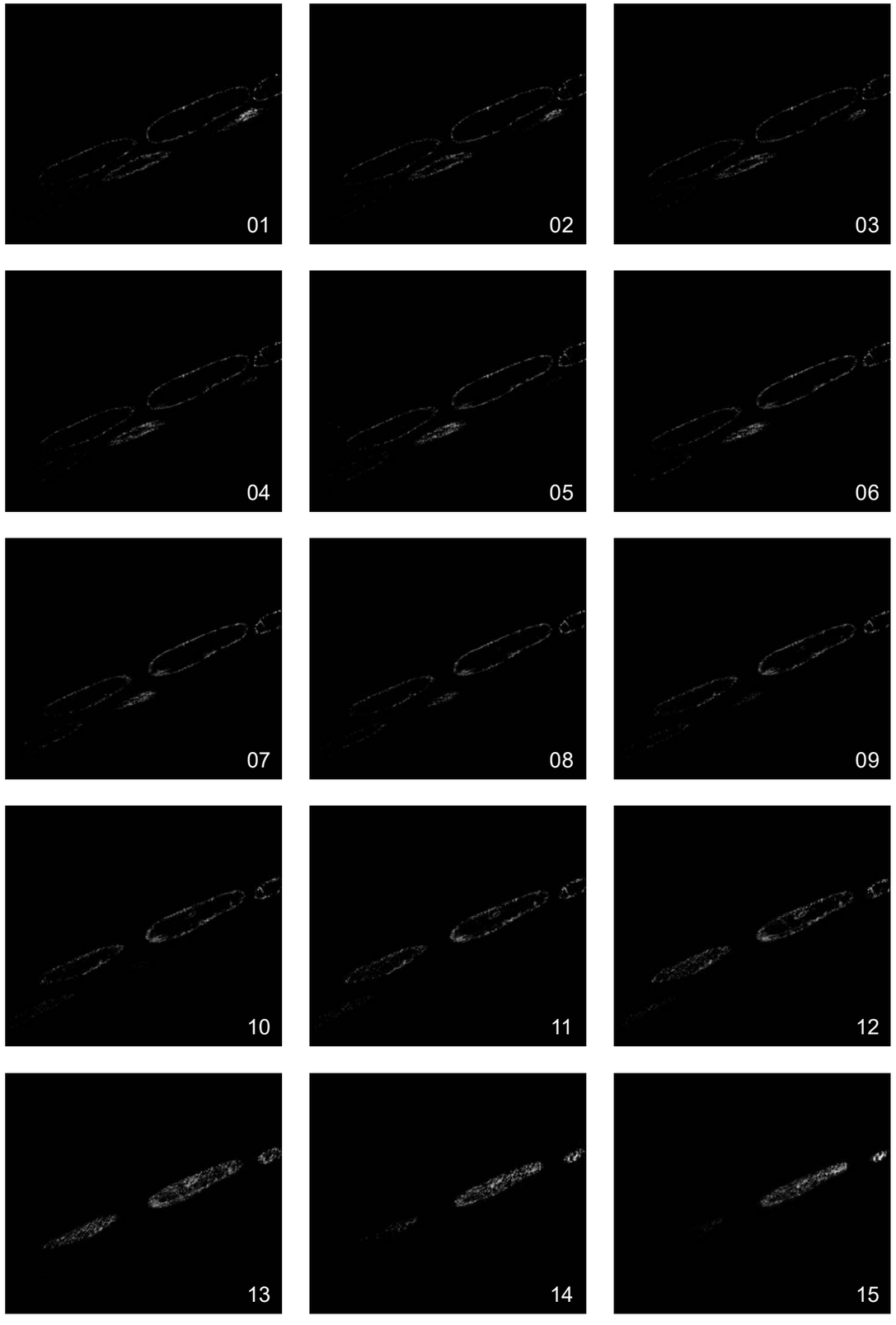
High resolution
2304 (H) × 2304 (V)
5.3 Megapixels

Pixel size
6.5 µm × 6.5 µm

Dynamic range
21 400:1

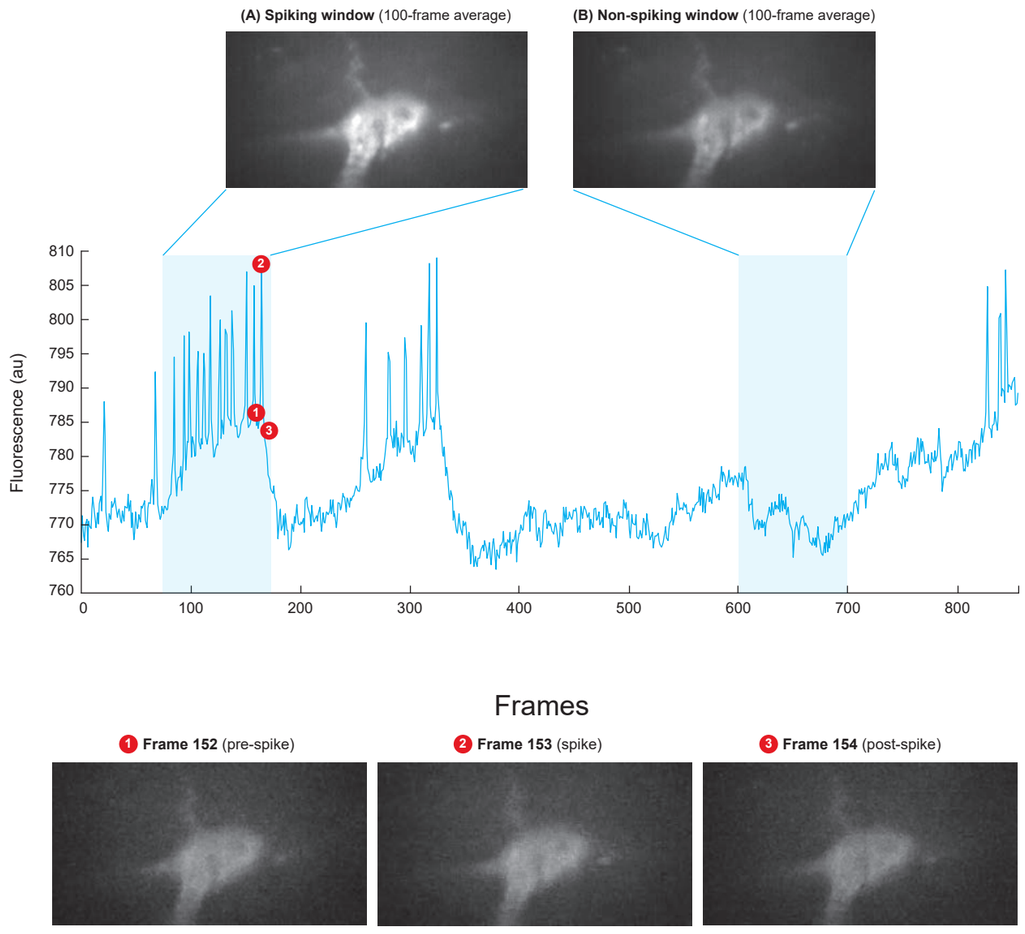
Peak QE
80 %

Individual focal planes (right) and a maximum projection of microtubules (above) from Arabidopsis thaliana captured on a spinning disc confocal microscope. An end-binding version of green fluorescent protein provides the fluorescence for these images. Images courtesy of 89 North.



Genetically encoded voltage imaging

Below: Spontaneous neural activity in a hippocampal neuron, captured at 828 Hz and reported by the fluorescent voltage indicator SomArchon, (Piatkevich et al., 2019, bioRxiv). These images represent the average intensity of 100 frames in spiking (A) and non-spiking (B) states.



Above: The same data showing individual frames at points before, during, and after a single spike.

Image courtesy of *Han Lab, Boston University. https://www.bu.edu/hanlab/*

The neural activity holy grail

Imaging neuronal activity using voltage-sensitive indicators is a scientific quest that challenges the limits of neuroscience and compels advances in chemistry and protein engineering, as well as live-animal, deep-tissue, high-speed and computational imaging. Scientists are making great progress in each of these areas and see a bright future for this burgeoning field (Please see the “References” below for a sampling of this work). A huge hurdle is finding imaging technology that can deliver the necessary speed, sensitivity and field of view, while also offering scientific quality, stability and tools for high speed data acquisition. Because this imaging is done with millisecond temporal resolution, the amount of light reaching any detector, even from a bright indicator is very limited. The ORCA-Fusion, with extremely low read noise combined with high QE and fast frame rates, makes it possible to capture neuronal spiking as shown in the figures to the left.

A camera is nothing without good software

But it’s not just the camera performance specs that enable this achievement. All Hamamatsu scientific cameras, regardless of what user interface software you use, run on our DCAM-API® platform. This underlying layer of software, invisible to camera users, but essential and easy to implement for software developers is the foundation of every successful cutting-edge experiment with a Hamamatsu camera. Our developers have created a robust, adaptable and stable library of functions that manages and optimizes interface communication, is backwards and forward compatible with all Hamamatsu cameras and is specifically designed for high speed, large volume data sets.

And sometimes it’s the little things that matter most. For the experiment shown here, we provided programming guidance on how to read our lossless, high-speed streaming DCIMG file format directly into the user’s custom Python code which corrects for animal movement - saving the lab hundreds of hours of processing time every time they analyze data. Our software team was able to listen to these scientists and deliver exactly what they needed. We value our relationships with our customers and know that our cameras are only as good as they are easy to use and relevant to the question at hand.

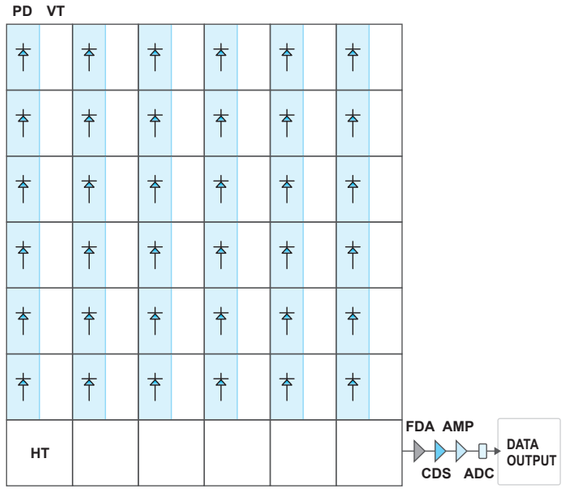
References: Highlights of voltage sensitive imaging publications

1. Piatkevich, K. D., et al. “Population imaging of neural activity in awake behaving mice in multiple brain regions” *bioRxiv* (2019): 616094
2. Abdelfattah, Ahmed S., et al. “Bright and photostable chemigenetic indicators for extended in vivo voltage imaging.” *bioRxiv* (2018): 436840.
3. Piatkevich, Kiryl D., et al. “A robotic multidimensional directed evolution approach applied to fluorescent voltage reporters.” *Nature chemical biology* 14.4 (2018): 352.
4. Adam, Yoav, et al. “Voltage imaging and optogenetics reveal behaviour-dependent changes in hippocampal dynamics.” *Nature* 569.7756 (2019): 413.
5. Chavarha, Mariya, et al. “Fast two-photon volumetric imaging of an improved voltage indicator reveals electrical activity in deeply located neurons in the awake brain.” *bioRxiv* (2018): 445064.
6. Yang, Helen H., et al. “Subcellular imaging of voltage and calcium signals reveals neural processing in vivo.” *Cell* 166.1 (2016): 245-257.
7. Gong, Yiyang, et al. “High-speed recording of neural spikes in awake mice and flies with a fluorescent voltage sensor.” *Science* 350.6266 (2015): 1361-1366.

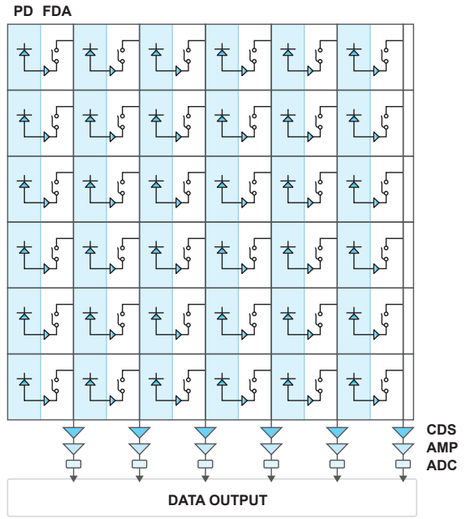
What is Read Noise?

To understand the significance of read noise in imaging, it's first necessary to review how digital images are formed in both CCD and CMOS. The basic principle is the same: incoming photons are converted to photoelectrons via the photoelectric effect in the sensitive layer of the silicon substrate. This electron charge is next converted to a voltage via floating diffusion node (FD), amplified via a source follower and then digitized through an analog to digital converter (ADC). CCD and CMOS differ in the on-chip location where each of these steps happen. Ultimately, an image is formed when the charge from each pixel is converted to a digital signal; this process is called read out.

CCD



CMOS

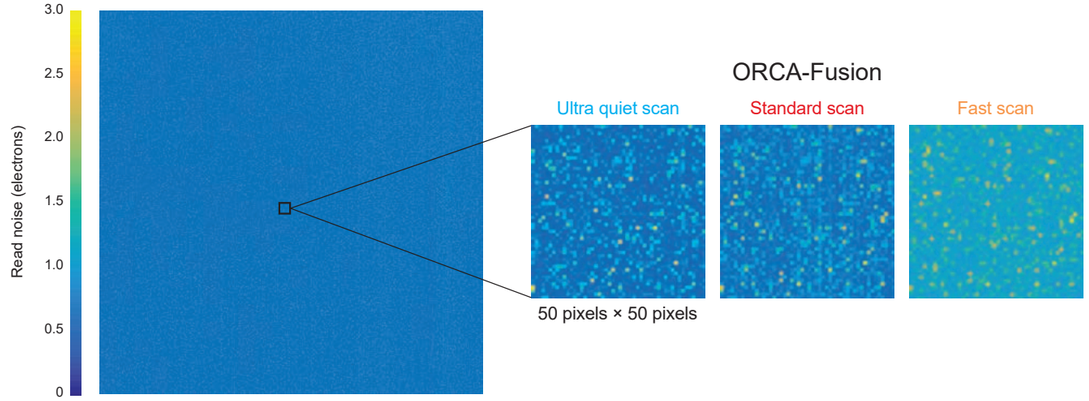


PD Photo Diode
HT Horizontal Transfer CCD
VT Vertical Transfer CCD
FDA Floating Diffusion Amplifier
CDS Correlated Double Sampling
AMP AMPlifier
ADC Analog to Digital Converter

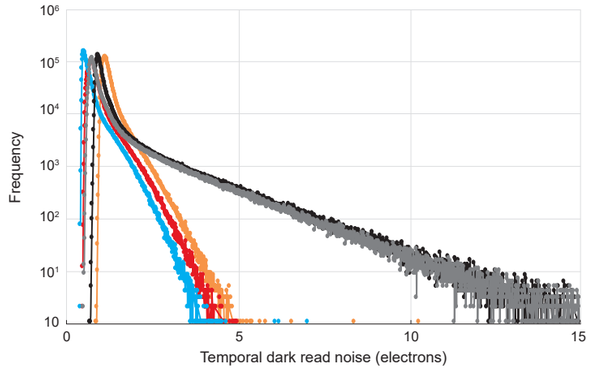
In a CCD, read out happens serially. Charge in each pixel is transferred from pixel to pixel in columns and then to a horizontal register. Every pixel is then read out through a single charge conversion, amplification and digitization circuit. In CMOS, each pixel has a floating diffusion amplifier (FDA) and source follower, so the charge is converted into a voltage and amplified in each pixel and then analog to digital conversion is achieved in column ADCs. In both CCD and CMOS, there is a process called correlated double sampling (CDS) that happens just before digital conversion that helps reduce noise and compensate for pixel offsets and drift.

Temporal Dark Read Noise

Read noise map comparison



Temporal dark read noise distribution



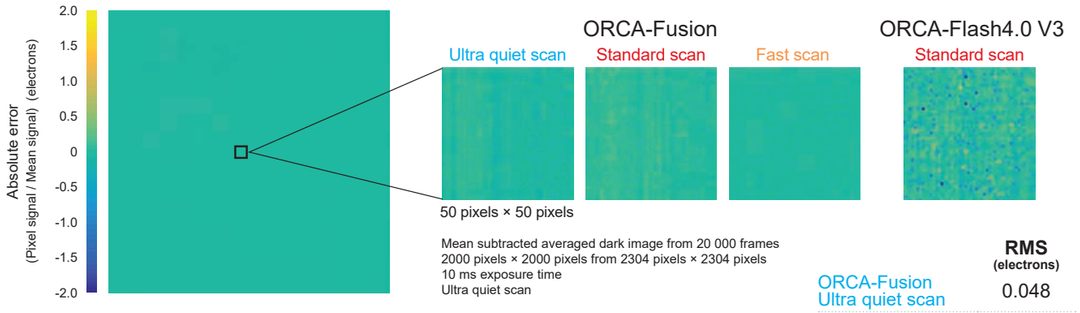
	RMS (electrons)	Median (electrons)	Mean (electrons)
ORCA-Fusion Ultra quiet scan	0.73	0.57	0.67
ORCA-Fusion Standard scan	0.91	0.77	0.85
ORCA-Fusion Fast scan	1.28	1.16	1.25
ORCA-Flash4.0 V3 Standard scan	1.63	0.99	1.30
ORCA-Flash4.0 V3 Slow scan	1.22	0.82	1.13

The entire readout process of detecting, converting, moving and digitizing the photoelectrons introduces temporal noise, termed "read noise" into the amplification and digitization process. Since in a CCD this process happens serially through a single read out chain, the read noise of each pixel is very nearly identical, and CCD cameras are adequately characterized by a single value for the read noise. In other words, a single pixel read noise value will have a Gaussian distribution around the mean in time and this distribution is the same if the measurement is done spatially by averaging many pixels in frame. However, in CMOS, since each pixel has its own charge to digitization chain, the pixel to pixel read noise can vary significantly, both spatially and temporally. Therefore, in CMOS the read noise distribution of pixels is not Gaussian and CMOS cameras are not adequately characterized by a single value for the read noise. A read noise histogram is necessary to fully characterize CMOS read noise. In particular, the "tail" of the read noise distribution, that is those pixels with high read noise can significantly affect image quality and create complications with computational algorithms.

Dark Signal Non-Uniformity (DSNU), Dark Offset

A camera is a scientific instrument because it is designed for quantitative measurements of intensity. To accurately measure image intensity there are three key camera parameters that must be calibrated by the manufacturer: Dark Signal Non-Uniformity (DSNU), Photo-Response Non-Uniformity (PRNU) and linearity.

Dark offset image

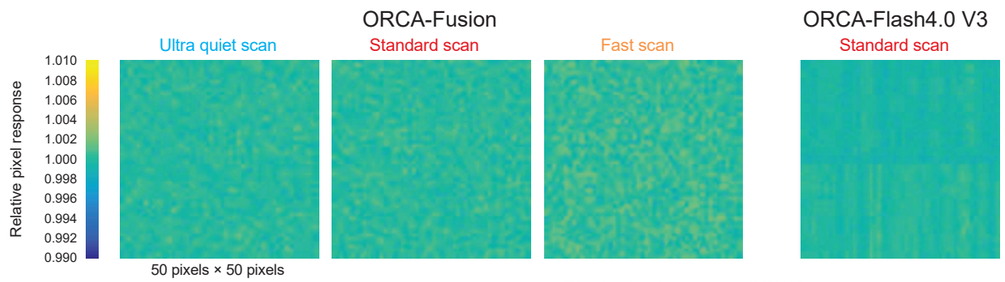


Mean subtracted averaged dark image from 20 000 frames
2000 pixels × 2000 pixels from 2304 pixels × 2304 pixels
10 ms exposure time
Ultra quiet scan

DSNU calibration uses masked pixels along the edge of the sensor to set the dark reference point (offset) for all pixels. By having a uniform offset, any increases in intensity can be attributed to signal. DSNU is measured in e- rms, should be less than a camera's read noise and is most noticeable in low light conditions. For the ORCA-Fusion, DSNU is 0.06 electrons.

	RMS (electrons)
ORCA-Fusion Ultra quiet scan	0.048
ORCA-Fusion Standard scan	0.16
ORCA-Fusion Fast scan	0.13
ORCA-Flash4.0 V3 Standard scan	0.28

Photo Response Non-Uniformity (PRNU)



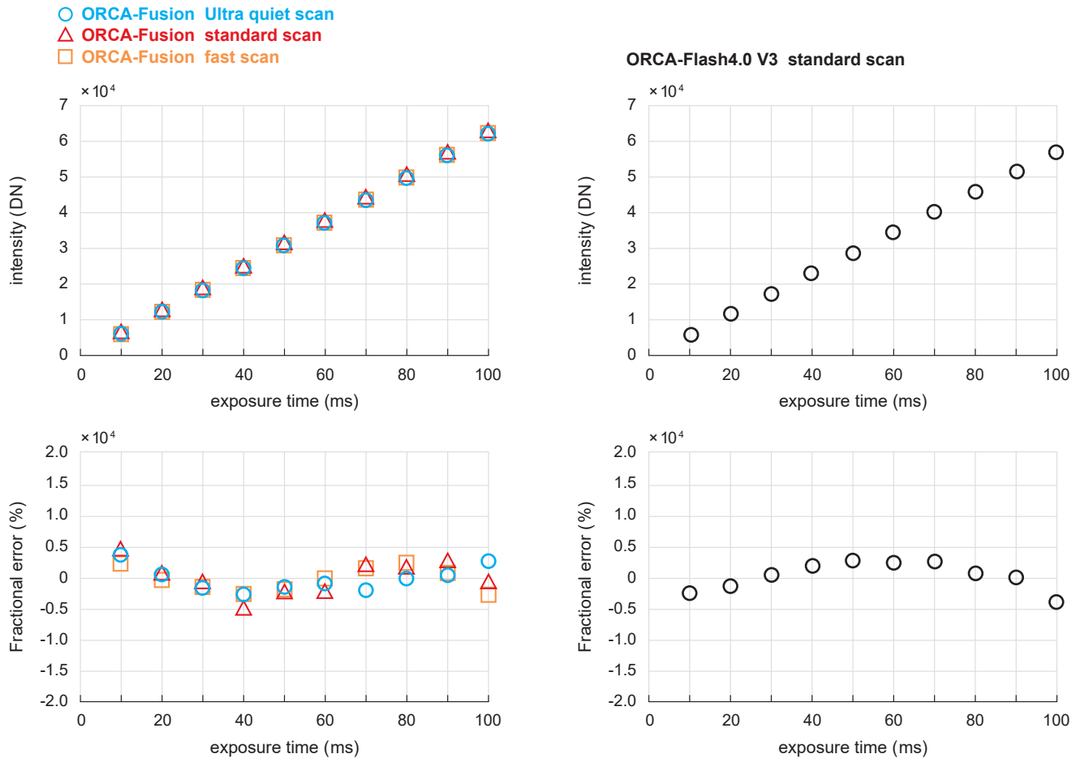
	Photoelectron (electrons)	PRNU (%)
ORCA-Fusion Ultra quiet scan	7199	0.062
ORCA-Fusion standard scan	7155	0.063
ORCA-Fusion fast scan	7398	0.079
ORCA-Flash4.0 V3 standard scan	15 036	0.062

PRNU calibration requires a very stable and uniform light source to detect pixel level differences that are the results of pixel variation in gain and QE. If left uncorrected, the variations would make quantification unreliable since the small difference would result in different output intensity levels in pixels receiving the same input light level. By comparing individual pixel intensity to the mean pixel intensity from a perfectly uniform image of known intensity, pixel variation can be mapped and then stored in the on-board camera FPGA to correct all images. PRNU specs are expressed as the % maximum from average and the ORCA-Fusion PRNU is 0.06 %.

Linearity

A linearly responsive camera will output a digital intensity value that is linearly proportional to the amount of input photons. In all current front and back illuminated sCMOS Gen II sensors, the analog signal generated by the incoming photons is converted to digital units by two separate digitizers, one for low light and one for high light. This pixel design requires matching the slopes of the two analog-to-digital converters (ADCs) to output linear 16 bit images. Design engineers must pay careful attention to the point of overlap of the two ADCs to make this low and high light merge seamless. The ORCA-Fusion Gen III pixel design utilizes only one ADC for 16 bit imaging, thus simplifying the linearity calibration. Linearity is measured according to EMVA 1288 standards (https://www.emva.org/standards-technology/emva-1288/).

Pixel response linearity

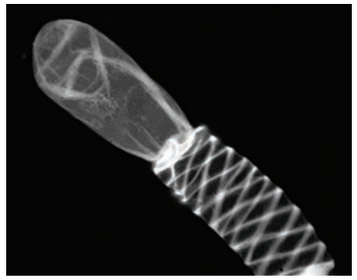


*Fractional error (%) based on EMVA 1288 standards

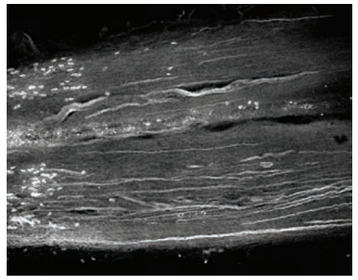
	Linearity error (%)	Linearity (%)
ORCA-Fusion Ultra quiet scan	0.33	99.67
ORCA-Fusion standard scan	0.47	99.53
ORCA-Fusion fast scan	0.26	99.74
ORCA-Flash4.0 V3 standard scan	0.33	99.67

Image examples

A. Regenerated lamprey spinal cord.
(E. Guadarrama and J. Morgan, MBL).



B. Plant tissue culture induced to make xylem.
(CT. Baskin, UMass Amherst, R. Oldenbourg, MBL).



C. Regenerated lamprey spinal cord.
(E. Guadarrama and J. Morgan, MBL)

Typical frame rates (frames/s)

Readout mode		Area readout mode				Lightsheet Readout Mode	
Scan mode		Fast scan		Standard scan	Ultra quiet scan	Fast scan	
X (pixels)	Y (pixels)	CoaXPress (16 bit)	USB 3.0 *1 (16 bit)	CoaXPress and USB 3.0 (16 bit)	CoaXPress and USB 3.0 (16 bit)	CoaXPress (16 bit)	USB 3.0 *1 (16 bit)
2304	2304	89.1	31.6	23.2	5.4	88.9	31.6
2304	2048	100	35.5	26.1	6.1	100	35.5
2304	1024	200	71.1	52.3	12.1	199	71.1
2304	512	400	142	104	24.3	397	142
2304	256	799	284	208	48.6	787	284
2304	128	1590	569	415	96.8	1540	569
2304	8	22 800	9330	5950	1380	15 800	9330
2304	4	41 000	18 600	10 700	2500	22 800	18 600

*1 The faster frame rates are available at 8 bit and 12 bit

Typical frame rates at 2×2 binning (frames/s)

Readout mode		Area readout mode			Lightsheet Readout Mode
Scan mode		Fast scan	Standard scan	Ultra quiet scan	Fast scan
X (pixels)	Y (pixels)	CoaXPress and USB 3.0 (16 bit)	CoaXPress and USB 3.0 (16 bit)	CoaXPress and USB 3.0 (16 bit)	CoaXPress and USB 3.0 (16 bit)
1152	1152	89.1	23.2	5.4	N/A
1152	1024	100	26.1	6.1	N/A
1152	512	200	52.3	12.1	N/A
1152	256	400	104	24.3	N/A
1152	128	799	208	48.6	N/A
1152	64	1590	415	96.8	N/A
1152	4	22 800	5950	1380	N/A
1152	2	41 000	10 700	2500	N/A

ORCA-Fusion

Camera
ORCA-Fusion
Digital CMOS camera

Product number
C14440-20UP

Pixel size
6.5 µm (H) × 6.5 µm (V)

Effective number of pixels
2304 (H) × 2304 (V)

Effective area
14.976 mm (H) × 14.976 mm (V)

*1 Typical value

*2 In Ultra quiet scan

*3 Calculated from the ratio of the full well capacity to the readout noise in Ultra quiet scan mode.

*4 The water temperature is +20 ˚C and the ambient temperature is +20 ˚C

*5 Dark current depends on cooling temperature

*6 Valid to 4 digits and rounded up to 5th digit

*7 USB 3.1 Gen 1 compatible

*8 The value with AC 240 V. (Approx. 70 VA with AC 100 V)

Readout noise *1	
Fast scan	1.4 electrons, rms / 1.3 electrons, median
Standard scan	1.0 electrons, rms / 0.9 electrons, median
Ultra quiet scan	0.7 electrons, rms / 0.6 electrons, median
Quantum efficiency *1	
@ 400 nm	65 %
@ 550 nm	80 %
@ 700 nm	70 %
@ 800 nm	50 %
Full well capacity *1	15 000 electrons
Dynamic range *3	21 400 : 1, typ. / 25 000 : 1, median
Conversion factor *1	0.24 electrons / count
Cooling Temperature	
Forced-air cooled	-5 ˚C (Ambient temperature: +25 ˚C)
Water cooled	-5 ˚C (Water temperature: +25 ˚C)
Water cooled (Max cooling)	Less than -15 ˚C *1, *4
Dark current *1, *5	
Cooling temperature: -5 ˚C	0.5 electrons/pixel/s
Cooling temperature: -15 ˚C	0.2 electrons/pixel/s
Dark offset	100 counts
Dark signal non-uniformity (DSNU) *1, *2	0.06 electrons
Photo response non-uniformity (PRNU) *1	0.06 %
At 7500 electrons	
Linearity error *1 (EMVA 1288 standard)	0.5 %
Readout modes	Full resolution, Digital binning (2×2, 4×4), Sub-array, Lightsheet
Readout times at full resolution *6	
Fast scan	11.22 ms (89.1 frames/s with CoaXPress or 31.6 frames/s with USB 3.0)
Standard scan	42.99 ms (23.2 frames/s with CoaXPress or USB 3.0)
Ultra quiet scan	184.4 ms (5.4 frames/s with CoaXPress or USB 3.0)
Lightsheet Readout Mode (Fast scan)	
Row interval time	4.868 µs to 963.8 µs *6
Readout time at full resolution	11.22 ms to 2.221 s *6
Readout modes	Full resolution, Sub-array
Readout directions	Top to bottom readout / Bottom to top readout
Exposure times	
Fast scan	17 µs to 10 s (4.87 µs step)
Standard scan	65 µs to 10 s (18.65 µs step)
Ultra quiet scan	280 µs to 10 s (80.00 µs step)
Trigger modes	Edge, Level, Sync readout, Start, Global reset edge, Global reset level
Trigger delay function	0 s to 10 s in 1 µs steps
Trigger output	3 programmable timing, Global exposure timing, trigger ready, low, high
Trigger input connector	SMA
Trigger output connectors	SMA
Master pulse mode	Free running / start trigger / burst
Digital output	16 bit / 12 bit / 8 bit
Interface	CoaXPress (Dual CXP-6) and USB 3.0 *7
Lens mount	C-mount
Power consumption	Approx. 150 VA *8
Ambient operating temperature	0 ˚C to + 40 ˚C
Ambient operating humidity	30 % to 80 %, with no condensation

ORCA-Fusion

Camera specs

Low noise and exceptional readout noise uniformity

Readout noise
0.7 electrons rms
Ultra quiet scan

DSNU
0.06 electrons

PRNU
0.06 %
At 7500 electrons

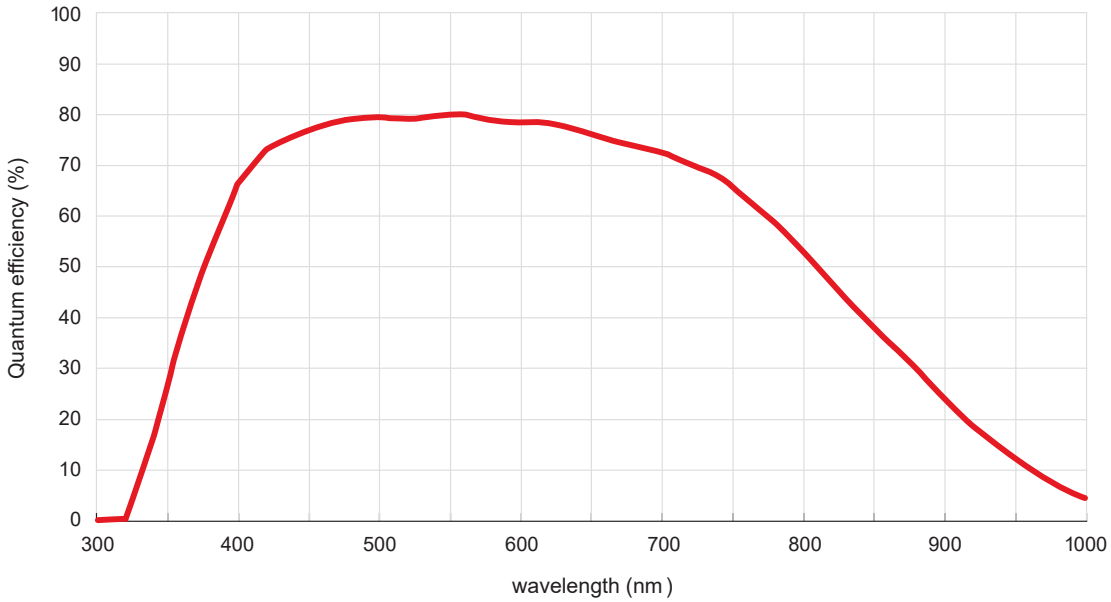
High speed
100 frames/s
At 2304 × 2048 ROI

High resolution
2304 (H) × 2304 (V)
5.3 Megapixels

Pixel size
6.5 µm × 6.5 µm

Dynamic range
21 400:1

Peak QE
80 %



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