# DIGITAL CAMERA ORCA-Flash4.0

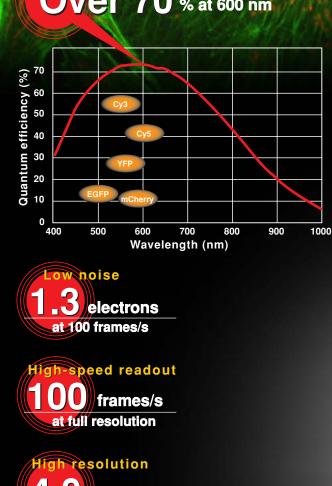
## Changing the Game

Hamamatsu's brilliantly designed ORCA-Flash4.0 is truly a game changer in the world of scientific imaging. Built on a revolutionary new Gen II sCMOS detector, the ORCA-Flash4.0 is the first sCMOS camera that challenges the performance of all CCD, EM-CCD, and Gen I sCMOS cameras. With its combination of low noise and high quantum efficiency, the ORCA-Flash4.0 delivers unprecedented sensitivity as well as high dynamic range, blazing fast speeds, large field of view, and excellent resolution—all at once. The new standard for sensitivity, speed, and resolution is here. We think you will enjoy the results.

HAMAMAT

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**- U/megapixels** at 6.5 μm × 6.5 μm pixels

Exceptional sensitivity

#### High sensitivity means extreme versatility

Cooled CCDs have been the go-to technology for fluorescence applications such as GFP or multi-channel imaging that require high contrast, high signal-to-noise images. The ORCA-Flash4.0 is changing the game.

And until now, scientists have used EM-CCDs for low-light, often high-speed imaging such as TIRF or spinning disk confocal. For lack of a better choice, the same technology has also been adopted for localization microscopy. Move over EM-CCD: the ORCA-Flash4.0 is changing this game, too.

The performance of the ORCA-Flash4.0 camera offers such a multitude of benefits that it not only easily accomplishes each of these applications—it does them better.

#### Quantum efficiency: higher than 70 % at 600 nm and 50 % at 750 nm

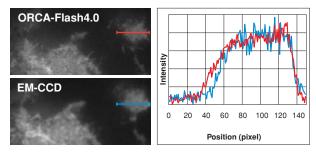
The ORCA-Flash4.0 is engineered to outperform all other cameras for fluorescence microscopy. With carefully designed pixels and on-chip lens technology, its Gen II sCMOS sensor provides high QE across the range of wavelengths most commonly used in fluorescence microscopy.

#### Low noise: 1.3 electrons

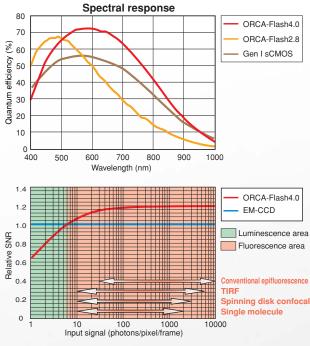
The ORCA-Flash4.0 has the lowest read noise at 100 frames/s of any CCD or sCMOS camera. Even EM-CCDs trade off "relative" low read noise for multiplicative noise by using on-chip gain. But the ORCA-Flash4.0 requires no tradeoffs. Our "quiet" electronics successfully lower the limit of detection, allowing you to take full advantage of high frame rates and see your signal with fewer photons.

The unique combination of high quantum efficiency and low noise, in the absence of EM-CCD multiplicative noise, means that your images are not limited by the camera. Detect signal at low light levels, compare small changes in intensity, and discriminate small signals amid large backgrounds—with ease. It's easy to see why the ORCA-Flash4.0 is changing the game.

For detailed information on the effects of multiplicative noise in EM-CCDs compared to Gen II sCMOS sensors please review our white paper "ORCA-Flash4.0: Changing the Game" at http://hamamatsucameras.com



For the same number of input photons the ORCA-Flash4.0 delivers consistently higher signal-to-noise ratios compared to EM-CCDs. The large intensity variation apparent in the data from the EM-CCD is a direct result of multiplicative noise.



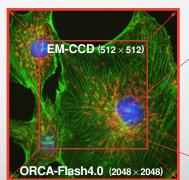
At just 5 photons per pixel, the ORCA-Flash4.0 exceeds the signal-to-noise performance of an EM-CCD camera.

#### Wide field of view & high resolution

With 4.0 megapixels at 6.5  $\mu$ m × 6.5  $\mu$ m each, the ORCA-Flash4.0 is the ideal format for demanding microscopy applications. Whether imaging at high magnification, requiring finely detailed images of an individual cell, or low magnification, aiming to capture and resolve images of many cells, the ORCA-Flash4.0 delivers beautiful images.

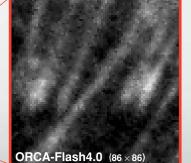
#### Comparison of field of view

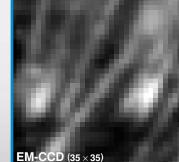
Field of view is  $2.5 \times$  larger than that of a standard EM-CCD camera.



#### Comparison of resolution

The 6.5  $\mu m \times 6.5$   $\mu m$  pixels of the ORCA-Flash4.0 enable much finer detail to be resolved when compared to the 16  $\mu m \times$  16  $\mu m$  pixels of an EM-CCD camera.



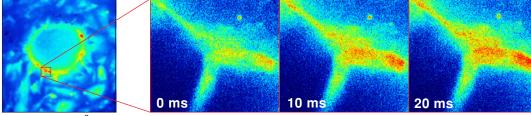


▲ Sample: FluoCells Prepared Slide #1, Object lens: S Plan Fluor 100×

#### **High speed** Up to 15 min. of continuous full speed, full resolution acquisition\*

4.0 megapixels, one hundred times per second-that is serious pixel throughput! Low noise and fast readout time simultaneously Building on our experience with high-speed scanning cameras, Hamamatsu engineers designed the ORCA-Flash4.0 with dual CameraLink cables connected to a single, state of the art CameraLink board. When combined with a recommended solid state hard drive and high-speed computer, this configuration keeps your full resolution data flowing. For those experiments that need the most temporal resolution, the ORCA-Flash4.0 features flexible region of interest to dial up your speed—up to 25 656 frames/s.

Readout method	Number of pixels	Readout speed
		at center position (frames/s)
Full resolution	$2048 \times 2048$	100
Sub-array readout	2048 × 1024	200
(typical examples)	2048 × 512	401
	$2048 \times 256$	802
	2048 × 128	1603
	2048 × 64	3207
	2048 × 8	25 656



▲ High-speed Ca<sup>2+</sup> imaging of cardiomyocyte derived from human iPS cell stained with Fluo8-AM. Sequential images were obtained every 10 ms. Left: whole FOV of the ORCA-Flash4.0 image. Right: magnified images show rapid and finely localized changes in intracellular Ca<sup>2+</sup> concentration associated with cardiomyocyte contractions.

#### Behind-the-scene quality & innovations

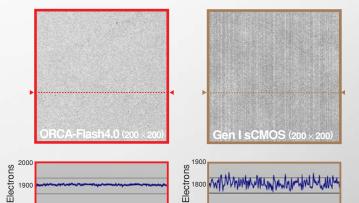
The ORCA-Flash4.0 is a solid piece of camera engineering. Our small package bundles many years of Hamamatsu experience with an array of important and innovative performance enhancements.

#### Performance optimized cooling

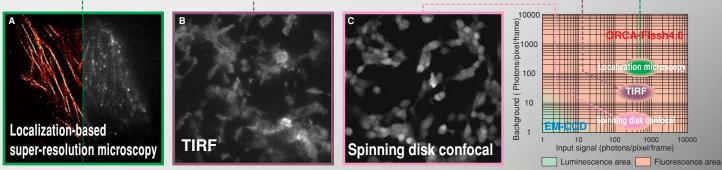
For 25 years, Hamamatsu has been an industry leader in the cooling of scientific cameras. The ORCA-Flash4.0 is no exception. Air-cooled to -10 °C, the ORCA-Flash4.0 finds the balance between meeting performance needs versus satisfying sleek marketing specs.

#### Outstanding image uniformity

At all input light levels the ORCA-Flash4.0 shows exceptional image uniformity across the entire sensor as compared to cameras based on Gen I sCMOS technology.



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HeLa cells labeled with d2EosFP. Left: reconstructed image. Right: single TIRF image from data used for reconstruction. А (Images courtesy of Prof. Zhen-li Huang, Britton Chance Center for BiomedicalPhotonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology.)

- B Ins-1 cell MARCS-DsRed (Image courtesy of Dr. Hideo Mogami, Hamamatsu University.)
- C HEK293 cells stained with Fluo8-AM, Yokogawa CSU Spinning Disk Confocal

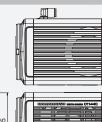


#### Specifications

Product number	C11440-22C (OR	,		
Imaging device	Scientific CMOS sensor FL-400			
Effective number of pixels	$2048(H) \times 2048(V)$			
Cell size	$6.5 \mu\text{m}  imes 6.5 \mu\text{m}$			
Effective area	$13.312 \text{ mm} \times 13.312 \text{ mm}$			
Full well capacity (typ.)	30 000 electrons			
Readout noise (at 100 frames/s, typ.)	1.3 electrons			
Dynamic range (typ.)	23 000:1			
Quantum efficency	Higher than 70 % at 600 nm and 50 % at 750 nm			
Cooling method	Sensor temp.	Dark current (typ.)		
Forced air (Ambient at +20 °C)	–10 ℃	0.5 electrons/pixel/s		
Water (+20 °C)	–20 ℃	0.15 electrons/pixel/s		
Water (+15 °C)	–30 ℃	0.05 electrons/pixel/s		
Readout speed				
Full resolution	100 frames/s			
1024 lines at center position	200 frames/s			
8 lines at center position	25 656 frames/s			
A/D conversion	16 bit output			
Readout modes	Digital binning $2 \times 2 / 4 \times 4$			
	Sub-array readout mode			
Exposure time	9.7 μs to 10 s			
Digital interface	CameraLink full configuration Deca mode			
Lens mount	C-mount	0		
Power requirement	AC 100 V to AC 240 V, 50 Hz/60 Hz			
Power consumption	Approx. 90 VA			
Trigger in External trigger mode	Edge, Level, Synchronous readout and Start trigger			
External trigger signal routing	SMA connector or CameraLink I/F			
External trigger delay function	0 to 10 s in 10 μs steps			
Trigger out				
External signal output	3 programmable timing outputs			
Fotomal simulation of a day days	Global exposure timing and Trigger ready output			
External signal output routing	SMA connector			
Software				
Software interface	PC-based acquisition package included			
	DCAM-SDK, commercially available software			

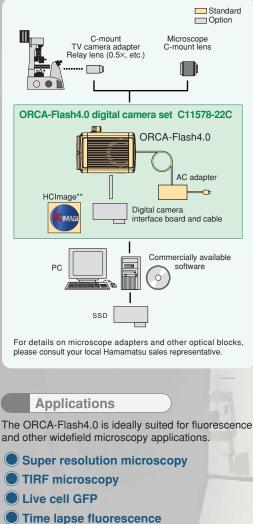
#### **Dimensional outlines**

Camera head (approx. 2.0 kg) Unit: mm





#### System configuration



- Ratio imaging
- FRET
- High-speed Ca<sup>2+</sup> imaging
- Real-time confocal microscopy
- Light sheet microscopy
- Morphology
- Fluorescence in situ hybridization (FISH)

This was tested with Dell T5500 (E5640.2.66GHz) + RAID 0 (LSI MegaRAID SAS 9260-8i) and 4 pcs SATA SSD drives (Crucial m4 SSD. CT256M4SSD2) as of Jan 2012.
"HCImage software provides standard image measurements functions. Upgrades to more feature-rich versions are available.

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#### HAMAMATSU PHOTONICS K.K., Systems Division

812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan, Telephone U.S.A.: Hamamatsu Corporation: 360 Foothill Road, P. O. Box 6910, Bridgewater, N.J. 08807-0910, U. Germany: Hamamatsu Photonics Deutschland GmbH: Arzbergerstr. 10, D-82211 Herrsching am Amm France: Hamamatsu Photonics France S.A.R.L.: 19, Rue du Saule Trapu, Parc du Moulin de Massy, 9 United Kingdom: Hamamatsu Photonics UK Limited: 2 Howard Court, 10 Tewin Road Welwyn Garden City I North Europe: Hamamatsu Photonics Norden AB: Thorshamnsgatan 35 16440 kista, Sweden, Teleph Italy: Hamamatsu Photonics Italia: S.R.L.: Strada della Moia, 1/E, 20020 Arese, (Milano), Italy, Telepho China: HAMAMATSU PHOTONICS (CHINA) Co., Ltd.: 1201 Tower B, Jiaming Center, No.27 Dongsanhuan Be

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