

DIGITAL CAMERA

# ORCA<sup>®</sup>-Flash4.0 V2

## Versatile by design

A game changer from inception and a proven performer since its initial release, the ORCA-Flash4.0 V2 offers unrivaled flexibility across a wide range of imaging applications. Easily change from USB to Camera Link connectivity. Switch from a blazing fast scan to a virtually noiseless slow scan by a simple click in software. Use our Lightsheet Readout Mode for seamless integration with light sheet microscopy systems. Robust triggering allows the ORCA-Flash4.0 V2 to drive other devices or be driven by them. And then there's the highest QE of any sCMOS camera on the market.

Exceptional quantum efficiency

Over 70 %  
at 600 nm

Low noise

1.0 electrons median 1.6 electrons rms  
Standard scan at 100 frames/s

0.8 electrons median 1.4 electrons rms  
Slow scan at 30 frames/s

High-speed readout

100 frames/s  
Camera Link at 4.0 megapixels



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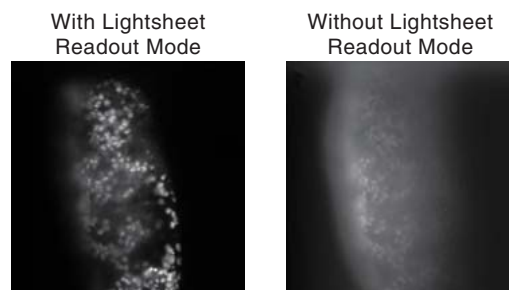
# What's New in the ORCA-Flash4.0 V2

## ● Two Scan Speeds

While the read noise at standard scan is only 1.6 electrons rms (1.0 electrons median), there are some experiments for which even lower noise is more important than raw speed. New in the ORCA-Flash4.0 V2 is an additional slow scan readout mode with read noise of just 1.4 electrons rms (0.8 electrons median). Both the USB and Camera Link configurations of the camera have this low noise capability.

## ● Lightsheet Readout Mode (Patent pending)

To enable the best speeds and synchronization for light sheet microscopy, the ORCA-Flash4.0 V2 configured with the Camera Link interface can be read out in one sweep across the sensor from top to bottom or bottom to top using our new Lightsheet Readout Mode.



Courtesy of Dr. Hufnagel and Dr. Krzic, EMBL Heidelberg, Germany

## ● Global Exposure Flexibility

By adding a Global Reset function to the ORCA-Flash4.0 V2, users can acquire global exposures and choose to have either an external source or the camera be master of the timing.

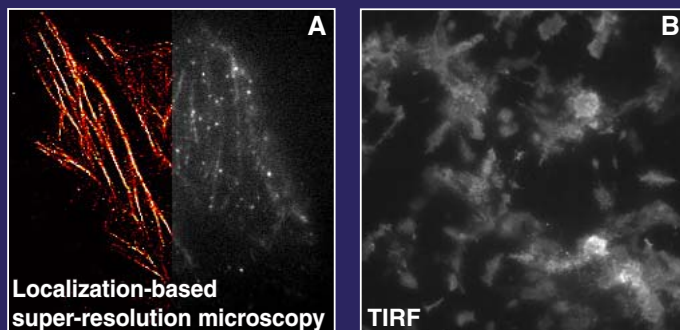
## ● Individualized Documentation

Knowing as much as possible about your camera helps increase confidence in the results it produces - especially under demanding experimental conditions. Every ORCA-Flash4.0 V2 is individually characterized at the factory before it ships, and the results of these tests are included with each camera. A measured noise histogram, photon transfer curve, rms noise value and conversion factor (electron/count) are provided along with simple formulas to make use of this information. Next time you're asked how many photons were detected you'll know the answer!

## → Applications

The ORCA-Flash4.0 V2 is ideally suited for fluorescence and other widefield microscopy applications.

- Super-resolution microscopy
- TIRF microscopy
- Ratio imaging
- FRET
- High-speed  $\text{Ca}^{2+}$  imaging
- Real-time confocal microscopy
- Light sheet microscopy



**A** HeLa cells labeled with d2EosFP. Left: reconstructed image. Right: single TIRF image from data used for reconstruction.

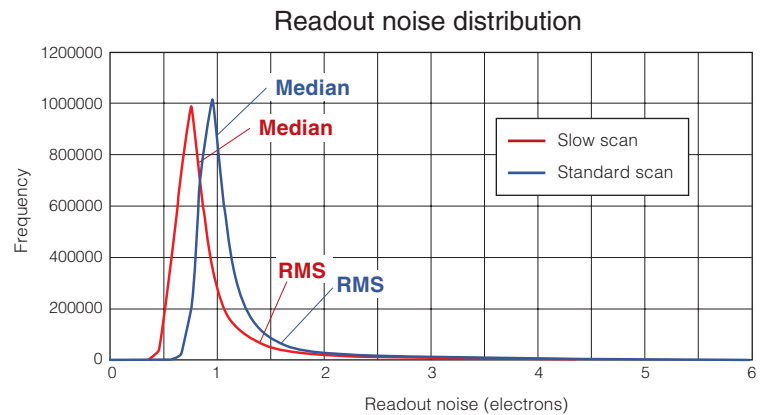
(Images courtesy of Prof. Zhen-li Huang, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology.)

**B** Ins-1 cell MARCS-DsRed (Image courtesy of Dr. Hideo Mogami, Hamamatsu University.)

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://www.hamamatsu.com/all/en/c11440-22CU.html>

## → Read noise: rms or median?

RMS and median are both valid statistical models for evaluating the central tendencies of data distributions, such as pixel noise. With CCDs there are never any issues regarding which model to use because the typical read noise for all pixels is very similar; thus rms and median are equivalent. With sCMOS, the structure of the sensor inherently has more pixel variation and the extreme low noise of the sensor makes variation more statistically significant. But when it comes to evaluating camera performance, the truly meaningful spec is rms noise. The rms noise value provides insight into image quality as well as being the appropriate noise variable in quantitative calculations. The ORCA-Flash4.0 V2's median noise data of 1.0 electrons (typical) is included only to facilitate apparent comparison with other sCMOS cameras. For truly quantitative imaging, rms noise must be known. The ORCA-Flash4.0 V2 Gen II sCMOS has 1.6 electrons rms typical read noise.



### All pixels or some pixels?

RMS or median noise values are valid only if all the pixels in the sensor are used or if the exclusion of outlier pixels is documented and explained. For the ORCA-Flash4.0 V2, we calculate both the rms and median read noise using every pixel in the sensor. This is done without any pixel correction functions or prequalification of the data. Since one goal of providing a spec is to enable accurate quantification of imaging results, this approach is consistent with our goal of providing the best quantitative scientific cameras.

## → Thinking in photons

There is a disconnect in imaging: we image photons but we talk about camera specs in electrons. This gap can be bridged easily, making camera comparisons more meaningful. Consider the difference between Gen I sCMOS and Gen II sCMOS. On the face of it, read noise specs seem rather equivalent (be careful to compare rms to rms, median to median under analogous modes and speeds). But if the read noise is considered first in electrons rms and then converted to photons, using QE at a particular wavelength, the differences are pronounced. At 100 frames/s in rolling shutter mode, the Gen II ORCA-Flash4.0 V2 has 1.6 electrons rms while Gen I has 1.8 electrons rms. The QEs at 550 nm for Gen I and Gen II are 54 % and 72 %, respectively. Using these numbers, the read noise in photons for Gen I is  $1.8/0.54 = 3.3$ , while the Gen II is just  $1.6/0.72 = 2.22$ . So now, in photons, the Gen I sCMOS has 50 % higher read noise than the ORCA-Flash4.0 V2.

Finally, consider the outcome of this exercise when the ORCA-Flash4.0 V2 is running in slow scan mode with only 1.4 electrons rms noise. The read noise is a mere 1.9 photons.

## High sensitivity means extreme versatility

The ORCA-Flash4.0 V2 is changing the game of scientific imaging. For years, cooled CCDs have been the go-to technology for fluorescence applications such as GFP or multi-channel imaging that require high signal to noise, high contrast images. EM-CCDs have been scientists' choice for low-light, often high speed applications such as TIRF or spinning disk confocal. For lack of a better choice, the same technology has been adopted for localization microscopy. The ORCA-Flash4.0 V2 offers such a multitude of benefits that it not only easily accomplishes each of these applications -- it may do them better.

Fan Long, Shaoqun Zeng, and Zhen-Li Huang. "Localization-based super-resolution microscopy with an sCMOS camera Part II: Experimental methodology for comparing sCMOS with EMCCD cameras," Optics Express, Vol. 20, Issue 16, pp. 17741-17759 (2012) <http://dx.doi.org/10.1364/OE.20.017741>

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

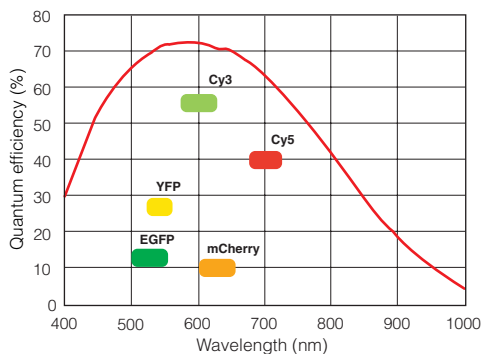
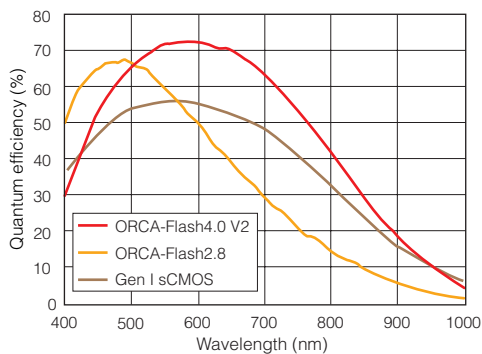
The ORCA-Flash4.0 V2 is engineered to outperform all other cameras for fluorescence microscopy. With carefully designed pixels and on-chip lens technology, its Gen II sCMOS sensor provides high QE across the range of wavelengths most commonly used in fluorescence microscopy.

### Low noise

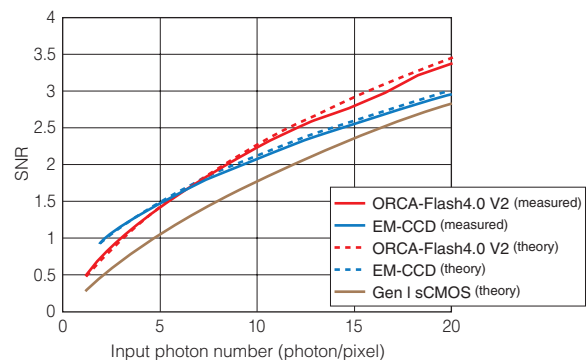
The ORCA-Flash4.0 V2 has the lowest read noise at 100 frames/s of any CCD or sCMOS camera. Even EM-CCDs trade off “relative” low read noise for multiplicative noise by using on-chip gain. But the ORCA-Flash4.0 V2 requires no tradeoffs. Our “quiet” electronics successfully lower the limit of detection, 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.

Spectral response

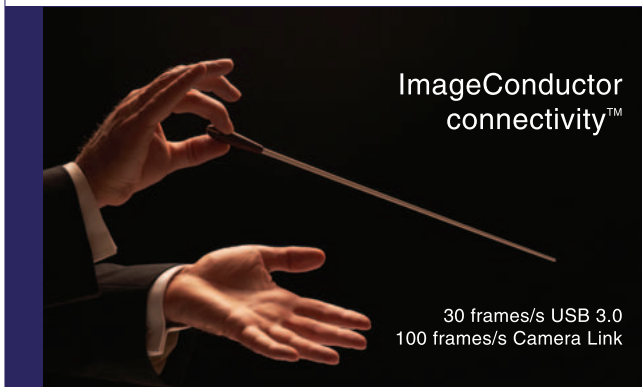


SNR Comparison of ORCA-Flash4.0 V2, Gen I sCMOS and EM-CCD



The ORCA-Flash4.0 V2 SNR exceeds that of EM-CCDs at about 6 photons/pixel. The solid lines show measured data at 533 nm. This measurement aligns well with predicted values (dotted line) for EM-CCD and ORCA-Flash4.0 V2. For comparison, the theoretical line for the Gen I type sensor is shown. Due to low QE and higher read noise, the Gen I camera does not compete with EM-CCD or Gen II ORCA-Flash4.0 V2 at these low light levels.  
 ORCA-Flash4.0 V2: QE = 70 %, Nr = 1.6 electrons rms as measured for this camera  
 EM-CCD: QE = 91 %, Nr = 0.2 electrons rms  
 Gen I sCMOS: QE = 52 %, Nr = 2 electrons rms as reported in literature





### Conduct your research

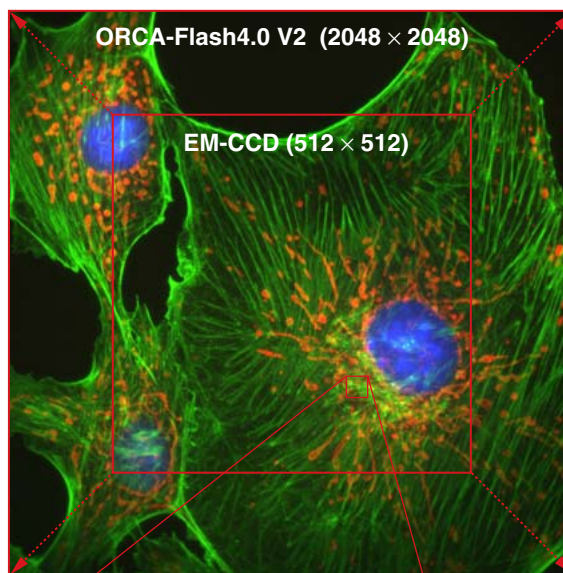
Every ORCA-Flash4.0 V2 includes ImageConductor connectivity™ so that it's enabled for both USB 3.0 (default) and high speed Camera Link. If your imaging tempo is 30 frames/s, then the default configuration with USB 3.0 is right for you. If you need something a little more lively... presto, just add a Camera Link board now or later to achieve 100 frames/s of full 4-megapixel images. Both options deliver the same low noise, high quantum efficiency imaging for unprecedented sensitivity. With Hamamatsu's versatile ImageConductor connectivity™ you direct the show.

## Wide field of view & high resolution

With 4.0 megapixels at  $6.5 \mu\text{m} \times 6.5 \mu\text{m}$  each, the ORCA-Flash4.0 V2 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 V2 delivers beautiful images.

### Comparison of field of view

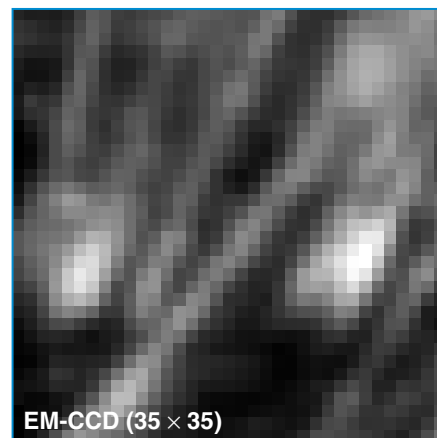
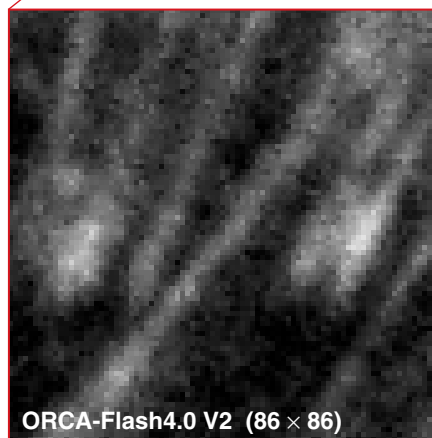
Field of view is 2.5x larger than that of a standard EM-CCD camera.



Sample: FluoCells Prepared Slide #1  
Objective lens: S Plan Fluor 100x

### Comparison of resolution

The  $6.5 \mu\text{m} \times 6.5 \mu\text{m}$  pixels of the ORCA-Flash4.0 V2 enable much finer detail to be resolved when compared to the  $16 \mu\text{m} \times 16 \mu\text{m}$  pixels of an EM-CCD camera.



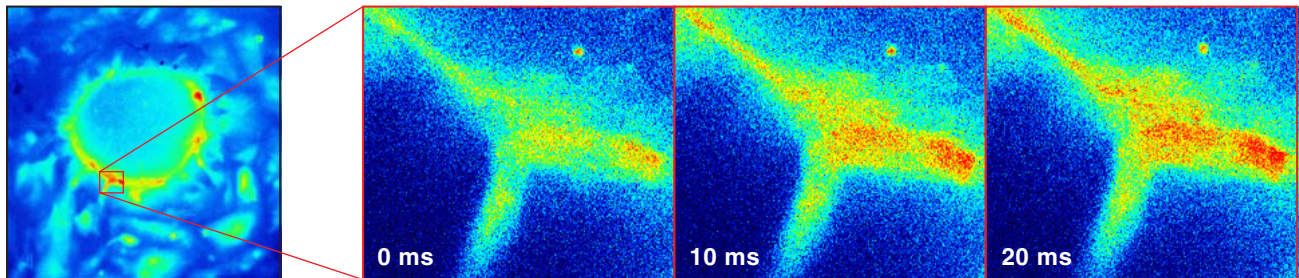
## High speed: allegro or presto? You be the Conductor.

When conducting imaging with a camera that has 4 194 304 pixels with 16-bit data depth, a single image is 8 megabytes. But capturing a single frame is child's play. What really matters is sustained, sequential image capture. Hamamatsu's ImageConductor gives you control over which speed works for you. In the default configuration, the ORCA-Flash4.0 V2 comes with a USB 3.0 card and cable and will deliver 30 frames/s of full frame acquisition. If you choose, upgrade to our fully supported FireBird PCI Express Gen II 8x Camera Link card, and that very same camera, without any additional modifications, can achieve 100 frames/s full resolution speed. Both camera configurations facilitate fine tuning of frame rates by allowing flexible region of interest, letting you select the area that matters. At all speeds, in every configuration, the ORCA-Flash4.0 V2 has just 1.6 electrons rms (1.0 electrons median) read noise for the ultimate in versatility and performance.

### Low noise and fast readout time simultaneously

Readout speed		Camera Link		USB 3.0		
		Horizontal pixel	Binning 2 × 2, 4 × 4	Horizontal pixel		Binning 2 × 2, 4 × 4
		2048 / 1536 / 1024 / 512		2048 / 1536 / 1024	512	
Vertical line	2048	100	100	30	100	100
	1024	200	200	60	200	200
	512	400	400	120	400	400
	256	801	801	240	801	801
	128	1603	1603	481	1603	1603
	64	3206	3206	968	3206	3206
	8	25 655	25 655	7894	25 655	25 655

Readout speed at center position (frames/s, typ.)



High-speed  $\text{Ca}^{2+}$  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 V2 image. Right: magnified images show rapid and finely localized changes in intracellular  $\text{Ca}^{2+}$  concentration associated with cardiomyocyte contractions.

 Specifications

<b>Product number</b>		C11440-22CU (ORCA-Flash4.0 V2)
Imaging device		Scientific CMOS sensor FL-400
Effective number of pixels		2048(H) × 2048(V)
Cell size		6.5 μm × 6.5 μm
Effective area		13.312 mm × 13.312 mm
Full well capacity (typ.)		30 000 electrons
Readout time	Standard scan (at 100 frames/s)	10 ms
	Slow scan (at 30 frames/s)	33 ms
Readout noise	Standard scan (at 100 frames/s, typ.)	1.6 electrons rms (1.0 electrons median)
	Slow scan (at 30 frames/s, typ.)	1.4 electrons rms (0.8 electrons median)
Dynamic range (typ.)*2		37 000:1
Quantum efficiency		Over 70 % at 600 nm and 50 % at 750 nm

Cooling method	Dark current (typ.)	Sensor temperature (nominal)
Forced air (Ambient at +20 °C)	0.06 electrons/pixel/s	-10 °C
Water (+20 °C)	0.02 electrons/pixel/s	-20 °C
Water (+15 °C)	0.006 electrons/pixel/s	-30 °C

Frame rate	Camera Link	USB 3.0
Full resolution	100 frames/s	30 frames/s
2048 × 1024 (at center position)	200 frames/s	60 frames/s
2048 × 8 (at center position)	25 655 frames/s	7894 frames/s
512 × 8 (at center position)	-	25 655 frames/s

Lightsheet Readout Mode (Camera Link only)	
Readout format	Seamless readout
Readout direction	Top to bottom / Bottom to top
Readout time	20 ms to 204.8 s (at full area readout)
Scan mode	Full area, Sub-array

A/D conversion*3	16 bit output	
Readout modes	Digital binning 2 × 2 / 4 × 4	
	Sub-array readout mode	
Exposure time*4	Internal trigger mode (at full resolution)	1 ms to 10 s
	Internal trigger mode with sub-array readout	38.96 μs to 10 s
	External trigger mode with sub-array readout	1 ms to 10 s
Digital interface	Camera Link *5 / USB 3.0	
Lens mount	C-mount	
Power requirement	AC 100 V to AC 240 V, 50 Hz/60 Hz	
Power consumption	Approx. 70 VA	

Trigger in	
External trigger mode	Edge, Level, Synchronous readout, Start trigger, Global reset edge and Global reset level
External trigger signal routing	SMA connector or Camera Link I/F
External trigger delay function	0 to 10 s in 10 μs steps

Trigger out	
External signal output	3 programmable timing outputs
	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

\*2 Full well capacity / Readout noise median in slow scan

\*3 The true 16 bit image data is achieved through seamless merging of the output from two 11 bit A/D converters.

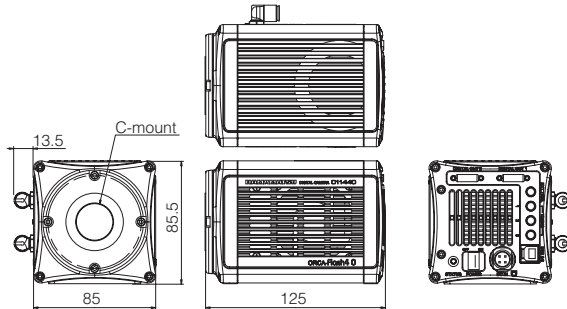
\*4 Minimum exposure time in internal trigger mode varies depending on sub-array setting. Minimum exposure time is in standard scan.

\*5 Proprietary mode equivalent of Camera Link 80-bit configuration

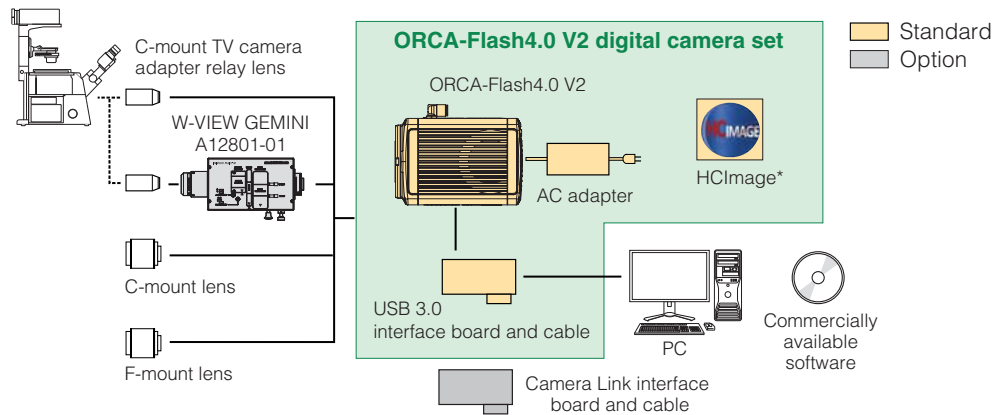
## Dimensional outlines

Camera (Approx. 2.0 kg)

Unit: mm




## Configuration example



\* HClmage software provides standard image measurement functions. Upgrades to more feature-rich versions are available.

Please contact your local Hamamatsu representative or distributor regarding actual configuration.



**Image splitting optics**  
**W-VIEW GEMINI A12801-01 (Option)**

The W-VIEW GEMINI is an image splitting optics which provides one pair of dual wavelength images separated by a dichroic mirror onto a single camera. Simultaneous image acquisition of dual wavelength images allows you high speed ratio metric imaging and other multiple fluorescence applications.

Cover image: Rat hippocampal neurons and glial fixed and immunostained with antibodies against HDAC6, GFAP and Synapsin1&2. Courtesy of Qi Zhang, Ph.D., Vanderbilt University  
<http://www.mc.vanderbilt.edu/labs/nano-neurosci/>

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Cat. No. SCAS0081E11

DEC/2014 HPK Created in Japan