ORCA®-Flash4.0 V3

NEW

Digital CMOS Camera C13440-20CU

"Data reduction function" now available!

For Scientists Everywhere!

The next step in CMOS cameras rigorously supports cutting-edge research.

Hamamatsu Photonics, the leader in life science cameras, launched the ORCA-Flash4.0 digital CMOS camera for scientific quantitative imaging in 2011. Since then, we have achieved various advances through refinements and the development of new functions.

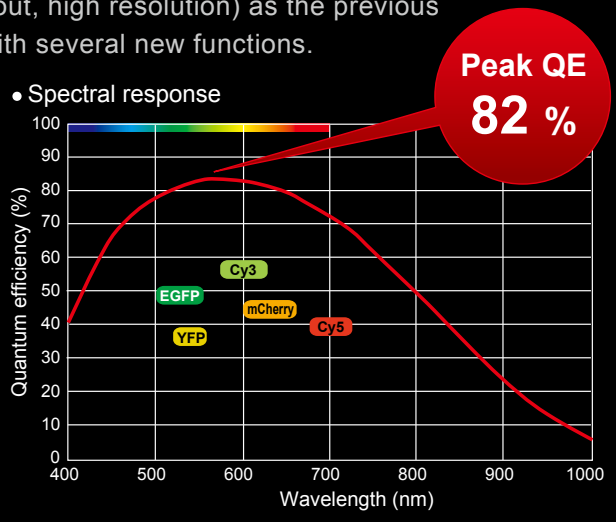
By focusing our expertise in circuit technology to develop the ORCA-Flash4.0 V3, the next step in the advancement of scientific cameras is achieved. This new camera will offer exceptional support to scientists everywhere.

Main Functions and Features

The ORCA-Flash4.0 V3 still has the same superior performance (high quantum efficiency, low noise, high speed readout, high resolution) as the previous ORCA-Flash4.0 V2, but now comes with several new functions.

- **High Quantum efficiency**
  - 82 % (Peak QE)
- **Low noise**
  - 0.8 electrons (Slow scan mode 30 frames/s)
  - 1.0 electrons (Standard scan mode 100 frames/s)
- **High speed readout**
  - 100 frames/s (Camera Link)
  - 80 frames/s New Feature (USB 3.0/8 bit)
  - 53 frames/s New Feature (USB 3.0/12 bit)
- **High resolution**
  - 4-megapixel resolution

• Spectral response



New Functions

- 8 bit/12 bit output
- Lightsheet Readout Mode (Patented)
- Dual Lightsheet Readout Mode (Patented)
- W-VIEW Mode
- Data Reduction Function

HAMAMATSU

PHOTON IS OUR BUSINESS

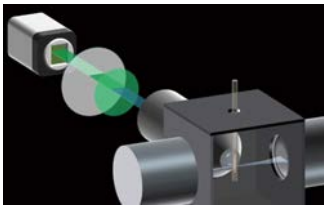
Hamamatsu introduces truly unique functions

Lightsheet microscopy and multi-wavelength imaging are rapidly becoming important tools for biological research since these techniques lower phototoxicity to the sample and reduce photo-bleaching of the fluorophores.
Hamamatsu Photonics deploys unique technology for the ORCA-Flash4.0 V3 to meet these leading edge research requirements.

Lightsheet Readout Mode **Patented**

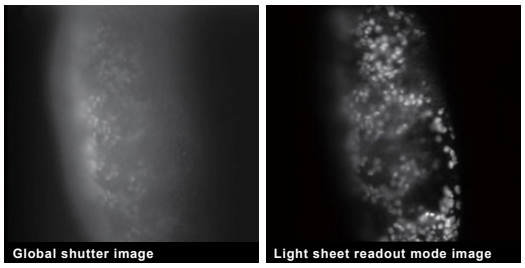
Lightsheet Readout Mode is one of the readout methods for an sCMOS camera used to enhance the S/N of lightsheet microscopy. Lightsheet microscopy is a type of fluorescence microscopy that can acquire high resolution optical sectioning images by using laser entering the sample perpendicular to the direction of observation.
In Lightsheet Readout Mode, the user can adjust the camera readout speed to synchronize with the scanning of their illumination light, thereby reducing the acquisition of scattered light and producing high S/N images.

● Conceptual diagram of lightsheet microscopy



Camera readout timing synchronized to the movement of the excitation light

● Result of light sheet readout function (sample image)



Data courtesy: Dr. Hufnagel, Dr. Krzic
(EMBL Heidelberg. Germany)

W-VIEW Mode

In W-VIEW Mode, each half of the sensor can be set an independent ROI, exposure time, and readout direction, enabling balanced dual color imaging with a single camera.
This function is optimized for simultaneous image acquisition of dual wavelength images with our W-VIEW GEMINI Image Splitting Optics, expanding the possibilities of low light imaging applications.

● Setting example of exposure time

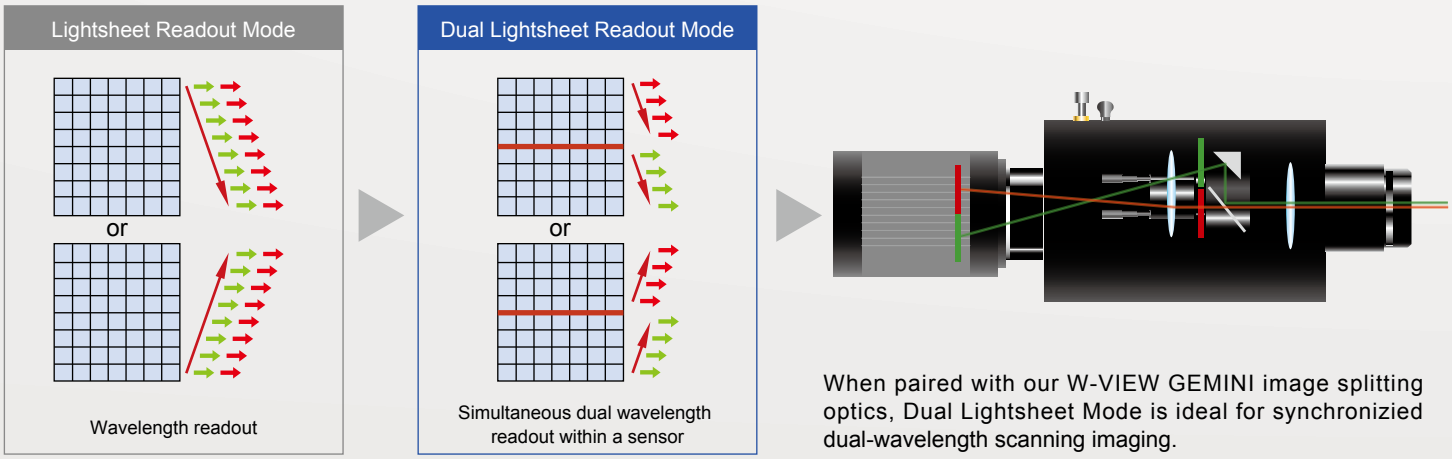
Unbalanced exposure image

×5 long exposure only for yellow

Balanced exposure image

Dual Lightsheet Readout Mode **Patented**

The Dual Lightsheet Mode scans each top and bottom halves of the sensor simultaneously and independently. By synchronizing two scanning light beams with the rolling exposure readout, it boosts each image acquisition time at 2 times as fast as the normal Lightsheet Mode. Furthermore, Dual Lightsheet Mode provides a capability to set an independent ROI in each half of the sensor allowing the user further flexibility.



When paired with our W-VIEW GEMINI image splitting optics, Dual Lightsheet Mode is ideal for synchronized dual-wavelength scanning imaging.

8 bit/12 bit mode for faster frame rates. (USB 3.0)

Today, handling an array of mass image data has become popular due to the recent scientific CMOS technology which delivers fast frame rates, wide field of view, and high resolution. On the other side of it, researchers are faced with the task of increasing analysis time and labor due to the large amount of data produced. It also requires mass storage and a proper archiving environment.
The ORCA-Flash4.0 V3's 8 bit/12 bit mode enables users to reduce their image data volume and analysis time while achieving faster frame rates.

8 bit/12 bit output

The ORCA-Flash4.0 V3 offers user-controllable Look Up Tables (LUT) for 8 bit or 12 bit data in order to record only the necessary range of digital output. With this capability, users can not only reduce image data volume but also improve the camera frame rates by eliminating the need to record unnecessary image data.

● Readout speed comparison when using USB 3.0

Effective number of pixels	ORCA-Flash4.0 V2		ORCA-Flash4.0 V3	
	Digital output	Frame rate	Digital output	Frame rate
2048×2048	16 bit	30 frames/s	16 bit	**40** frames/s
			12 bit	**53 frames/s**
			8 bit	**80 frames/s**
1920×1080	16 bit	60 frames/s	16 bit	**75** frames/s
			12 bit	**100 frames/s**
			8 bit	**151 frames/s**

High Quantitation and Image Quality for scientific research

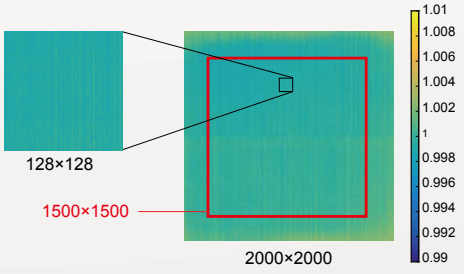
Quantitative accuracy is a requirement for scientific cameras. In order to achieve excellent quantitative performance, good linearity, reduced fixed pattern noise and minimal pixel differences are needed, allowing the user to acquire uniform background images. Especially, important for super resolution imaging, such as localization methods, since pixel differences generate a controversial impact on the precision of single molecule position. Hamamatsu builds in outstanding uniform image quality using of our many years of knowledge and experience with digital circuit technology.

Pursuit of Quantitation

Our attention to detail delivers outstanding linearity, especially at low light, and offers improved photon response non-uniformity (PRNU) and dark signal non-uniformity (DSNU) to minimize pixel differences and reduce fixed pattern noise.

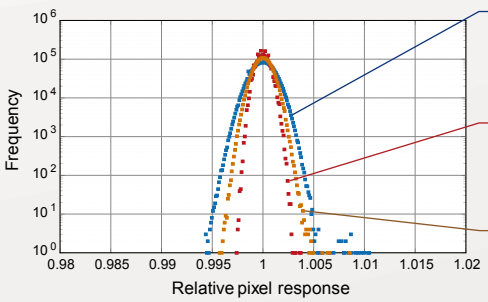
● Measurement example of photo-response non uniformity under uniform illumination

32686 DN= 15035.56 e⁻ mean illumination (1500×1500)



Normalized averaged image from 1000 frames
1500×1500 area used for statics

Log-Linear Histogram of pixel response under uniform illumination



9914.2 DN in 1500×1500 ROI
Photoelectron (e⁻) = 4560.5 e⁻
SD = 0.0011
PRNU = 0.11 %

32686 DN in 1500×1500 ROI
Photoelectron (e⁻) = 15035.56 e⁻
SD = 0.00062362
PRNU = 0.062 %

50050 DN in 1500×1500 ROI
Photoelectron (e⁻) = 23023 e⁻
SD = 0.00083367
PRNU = 0.083 %

Versatile Image Quality Improvement

Depending on your uses and purposes, user-selectable, multi-level hot pixel reduction can eliminate CMOS intrinsic noisy pixels, especially during long exposures.

Data reduction

Data Reduction Function

- **The ORCA-Flash4.0 V3 camera can deliver 4.2-megapixel, 16 bit images at 100 fps resulting in 800 MB of data output per second.**
- **For experiments lasting over long time courses the storage and archiving of data can be both expensive and cumbersome. With its new Data Reduction Function the Flash4.0 V3 offers users the flexibility to keep only the data that matters.**

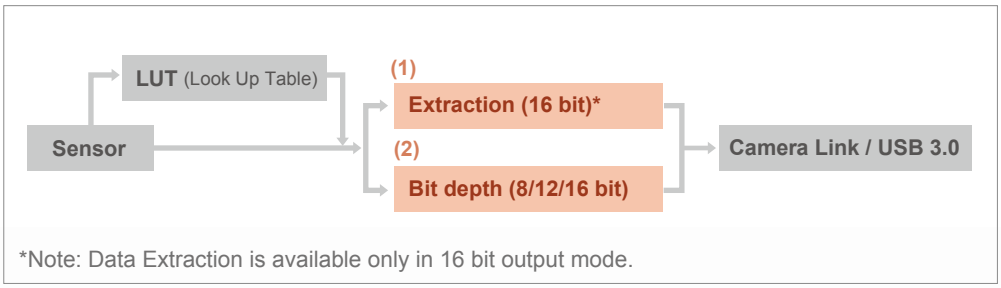
The Data Reduction Function is composed of Data Extraction and 8 bit/12 bit Digital Output

(1) Data Extraction

When this function is enabled, the ORCA-Flash4.0 V3 transfers only regions of interest.

(2) 8 bit/12 bit Digital Output

Using the 8 bit or 12 bit depth enables you to reduce image data volume.



*Note: Data Extraction is available only in 16 bit output mode.

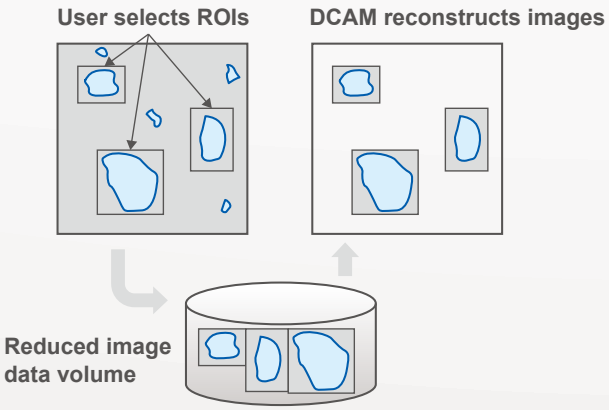
(1) What is Data extraction?

Data Extraction

When this function is enabled, the ORCA-Flash4.0 V3 transfers only the pixel data predetermined by ROI or Byte mask, reducing the image data volume recorded as DCIMG* files.

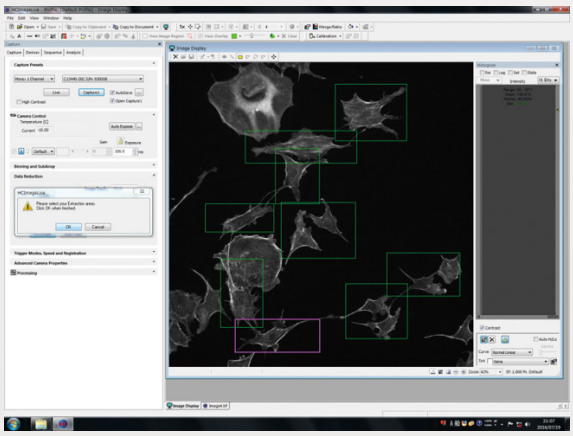
<Benefits>
Users can store only specified regions of pixel data, eliminating the need to manipulate massive amounts of pixel data containing no useful information.

Data Extraction can result in the following reduction;
- Data Size
- Cost of storage
- Analysis time and labor for data handling

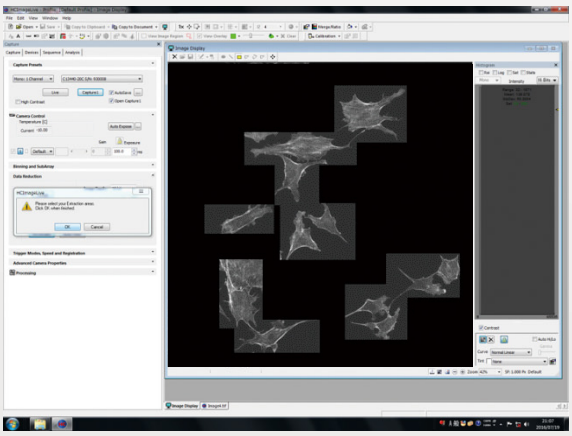


■ Example of Data Extraction in HCImage

● Select ROIs (or Byte Mask)

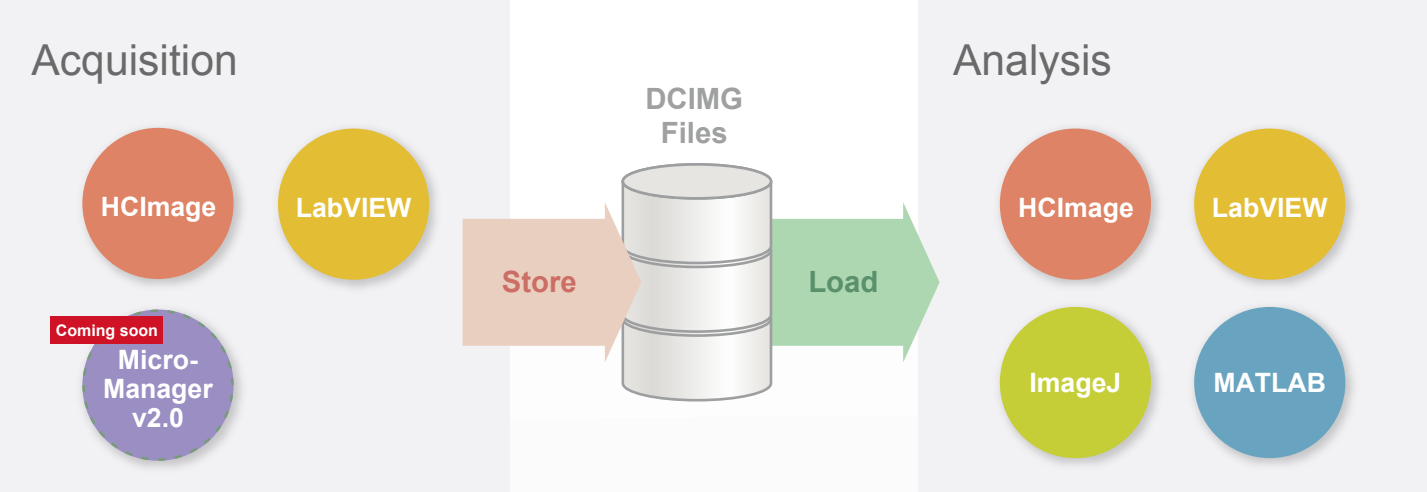


● Reconstructed images are displayed, only with predetermined pixels data



Software support

Data reduction function and reading DCIMG files are supported by major third-party softwares used for research application.



*** What is DCIMG file format?**
DCIMG is our unique file format generated by the DCAM-API recording feature, enabling high speed and mass data recording with Hamamatsu cameras.

< Advantage >
The DCAM-API recording feature is able to store the image data directly from the camera into the DCIMG file format.
Because there is no encoding or decoding of the data, optimal storage performance can be achieved with no data loss.
New camera features result in reduced data output from the camera.
The DCIMG file format takes advantage of this data reduction thereby minimizing data storage requirements.

<Available Software>
Images can be extracted from the DCIMG file with a variety of popular software packages, including LabVIEW, ImageJ, and MATLAB.
In addition, software developers can easily extract decoded images directly from DCIMG files using our DCIMG-SDK.

(2) 8 bit/12 bit Digital Output

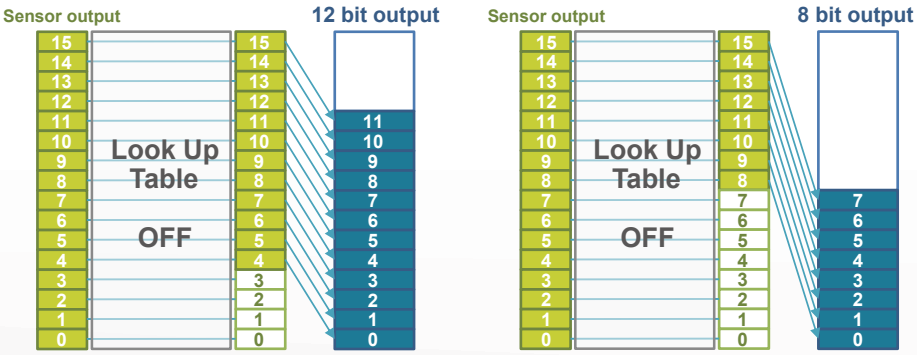
Selectable Pixel Bit Depth

Using the 8 bit (256 gray levels) or 12 bit (4096 gray levels) depth is a method to reduce the image data volume to a user significant resolution.

- 12 bit digital output: The data is reduced to 3/4, of the 16 bit output.
- 8 bit digital output: The data is reduced to 1/2, of the 16 bit output.

Thus 8 bit or 12 bit digital output can also boost the USB 3.0 interface frame rates, while reducing your image data volume.

● LUT OFF

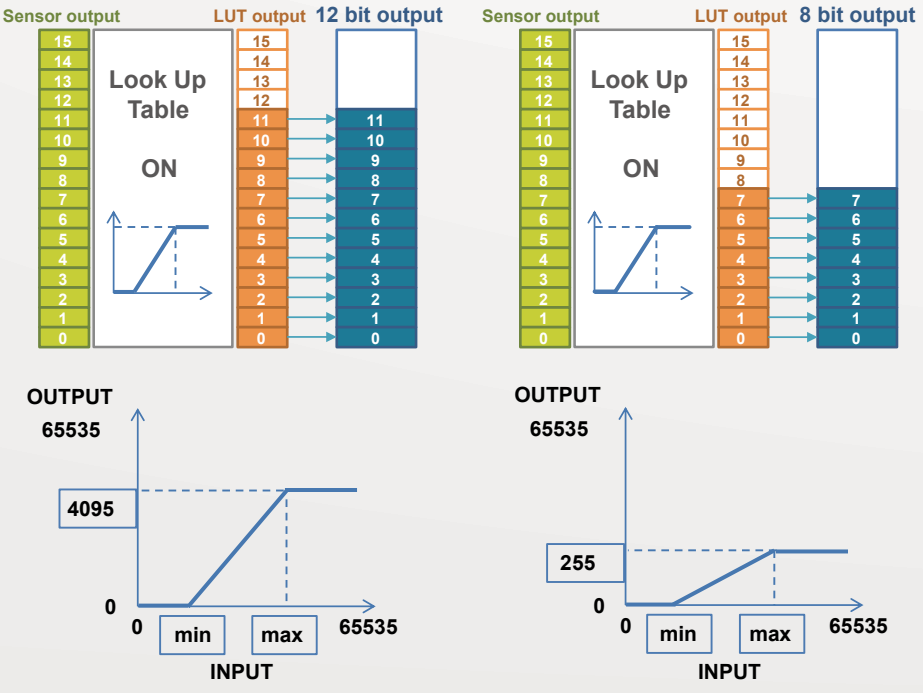


User-Controllable Look Up Table

The reduced 8 or 12 bit-depth acquisition can result in the compression of pixel intensity data thereby reducing intensity resolution.
The User-Controllable Look Up Table (LUT) can be used to regain intensity resolution by allowing a selectable, reduced range, of intensities to mapped into the reduced bit-depth.

Selectable LUT is adjustable up to the 16 bit-depth resolution.

● LUT ON

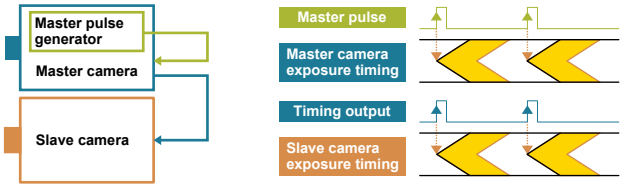


More Flexibility, More Simple

Master Pulse

Conventional systems have required an additional external pulse generator in order to synchronize multiple cameras and devices.
Our new "Master Pulse" allows the ORCA-Flash4.0 V3 to truly run the trigger timing with its simple, built-in timing generator.

● Example of exposure timing control

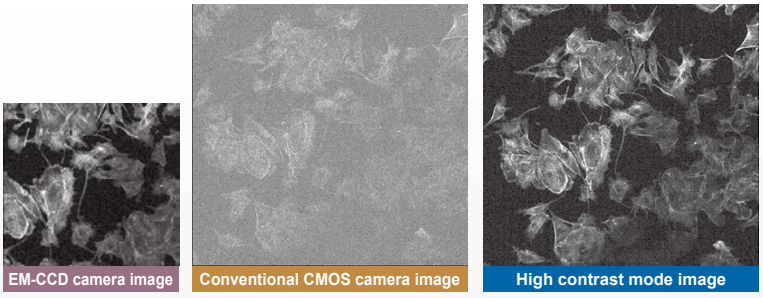


Exposure timing of the master and slave camera can be controlled easily with 2 cameras operated in synchronized state.

Enhanced Visualization Mode

CMOS cameras have the ability to detect low signals over background, resulting in images with the appearance of low contrast and less sharp backgrounds. Better visualization of these low light images are desired, especially during the setup of many low light experiments, such as those that were previously imaged using EM-CCDs. The ORCA-Flash4.0 V3's Enhanced Visualization Mode enriches the contrast of the displayed image, while saving the sCMOS raw image data to disk.

● High contrast mode image and comparison of field of view
(All images based on LUT)



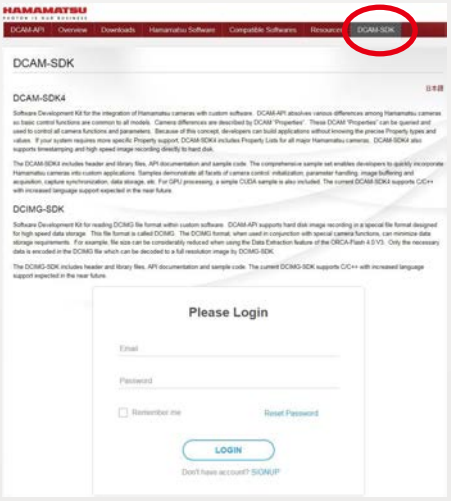
V2 Compatible Mode

"V2 Compatible Mode" is intended to ensure that users of established software, currently supporting our traditional ORCA-Flash4.0 V2, can continue working together with the ORCA-Flash4.0 V3 hardware.

Software Developer's Kit

DCAM-SDK & DCIMG-SDK

The SDK is a Software Development Kit for the integration of Hamamatsu cameras with the customer software. You may develop own application software depends on the needs, for camera control with the DCAM-SDK and for recording DCIMG files with the DCIMG-SDK.
You are available to download our DCAM-SDK and DCIMG-SDK from the link below with the user registration completed and please refer to Hamamatsu software information as well from the same page.
https://dcam-api.com/



Sample code for GPU

Various kinds of sample codes for application software Development are included in the DCAM-SDK, one of which is the use for incorporating GPU processing power into your image acquisition workflow. Through the combination of DCAM-API and CUDA, offering simple with powerful and native function groups, Hamamatsu is open for the free software development by the SDK users.

Enhanced Quantum Efficiency

High sensitivity means extreme versatility

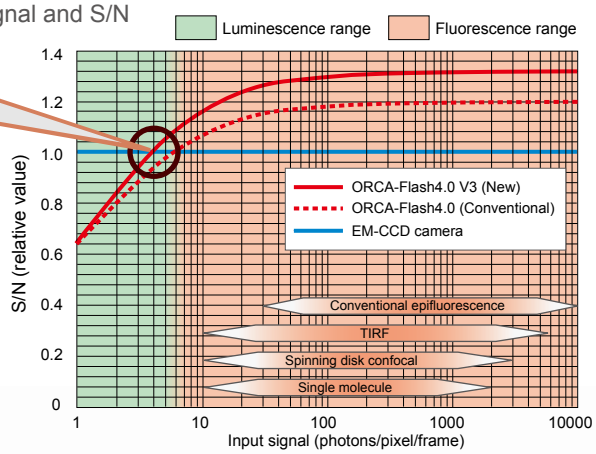
EM-CCDs have often been the scientists' choice for low-light, high speed applications such as TIRF or spinning disk confocal. The ORCA-Flash4.0 V3 is engineered to outperform all other cameras for fluorescence applications that require high contrast images.

Quantum efficiency: 82 % (Peak QE)

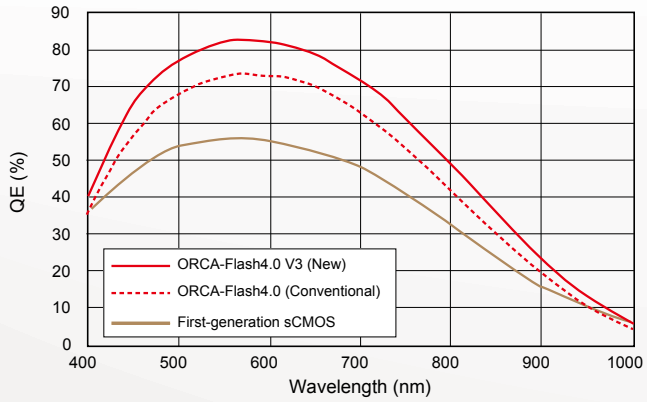
With carefully designed pixels and on-chip lens technology, its Gen II sCMOS sensor provides high QE across the range of wavelengths and offers greater sensitivity than competing conventional sCMOS cameras, even EM-CCDs. In low-light fluorescent imaging, the ORCA-Flash4.0 V3 enables not only excellent sensitivity but also stable imaging acquisition capabilities compared to the EMCCDs which have an unavoidable effect of low S/N due to the multiplicative noise introduced in the on-chip gain.

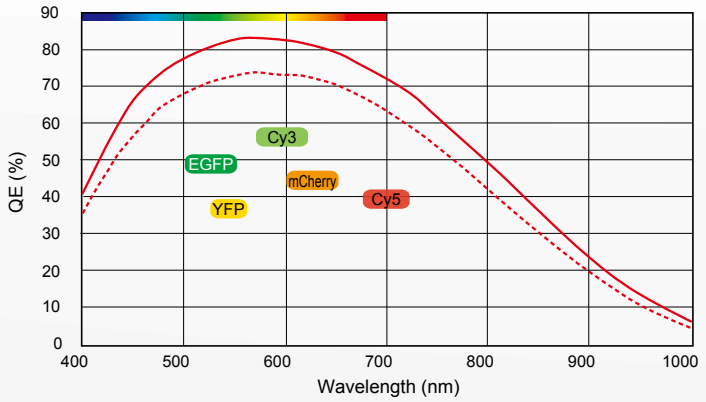
● Input signal and S/N

Showing higher performance than EM-CCD camera in the fluorescence range where only a small number of photons detected as the input signal



Spectral response





Fluorescent Live Cell Imaging

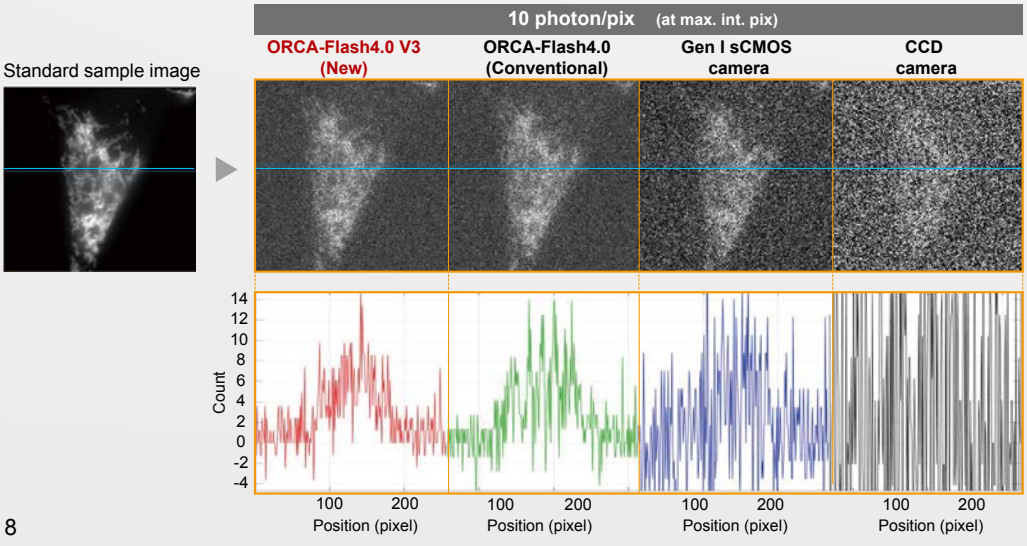
Improved S/N

Simulation condition

The below images represent the simulation results of measuring the same number of photons with the ORCA-Flash4.0 V3, Gen I sCMOS, and CCD. Each image is visually adjusted with a normalized LUT.

Results

The ORCA-Flash4.0 V3's high S/N outperforms other conventional cameras based on the simulation comparison of the same number of photons.
The unique combination of high quantum efficiency and low noise means that you have a possibility of long term time-lapses with low excitation light, saving your cells from photobleaching and phototoxicity.



Super Resolution Microscopy (Localization method)

Enhanced localization accuracy

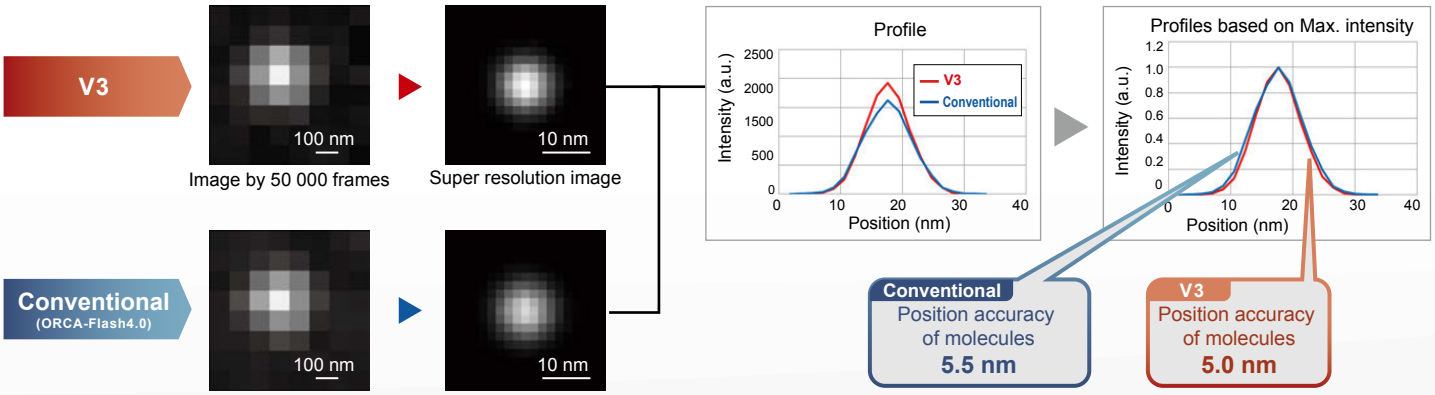
The enhanced QE of the ORCA-Flash4.0 V3 delivers precise location detect capability for super resolution localization imaging.

Simulation condition

The below images represent the simulation results in super resolution imaging.

Results

Position accuracy improved.



Large Field of View & High Resolution

2.5× larger field of view than that of EM-CCD cameras

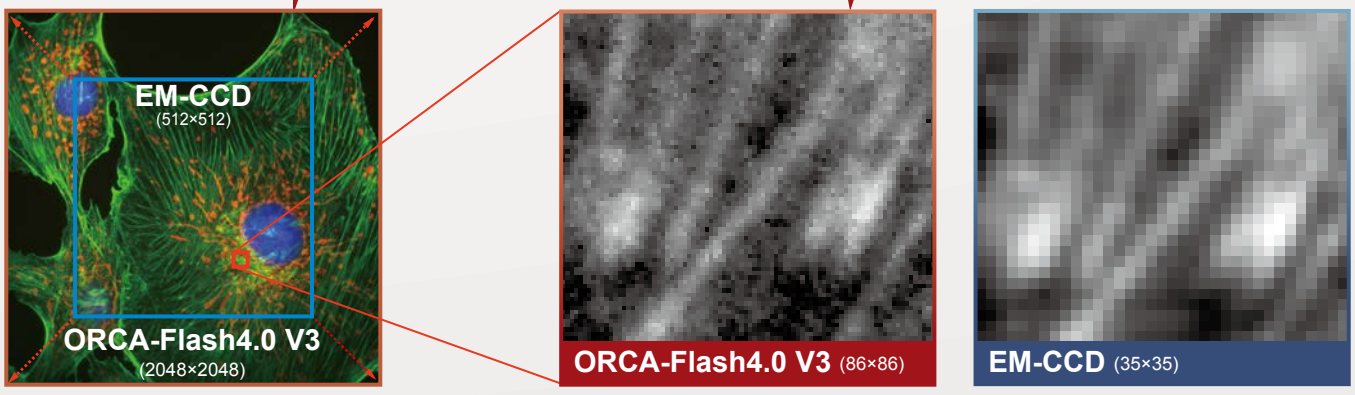
With 4.0 megapixels at 6.5 µm×6.5 µm each, the ORCA-Flash4.0 V3 is the ideal format for demanding microscopy applications, aiming to capture and resolve images of many cells, the ORCA-Flash4.0 V3 offers quality images.

Comparison of field of view

The field of view is 2.64×larger than that of a standard EM-CCD camera.
(4.0 megapixels at 6.5 µm×6.5 µm each)

Comparison of resolution

The 6.5 µm×6.5 µm pixels of the ORCA-Flash4.0 V3 enables much finer detail to be resolved when compared to the 16 µm×16 µm pixels of an EM-CCD camera.

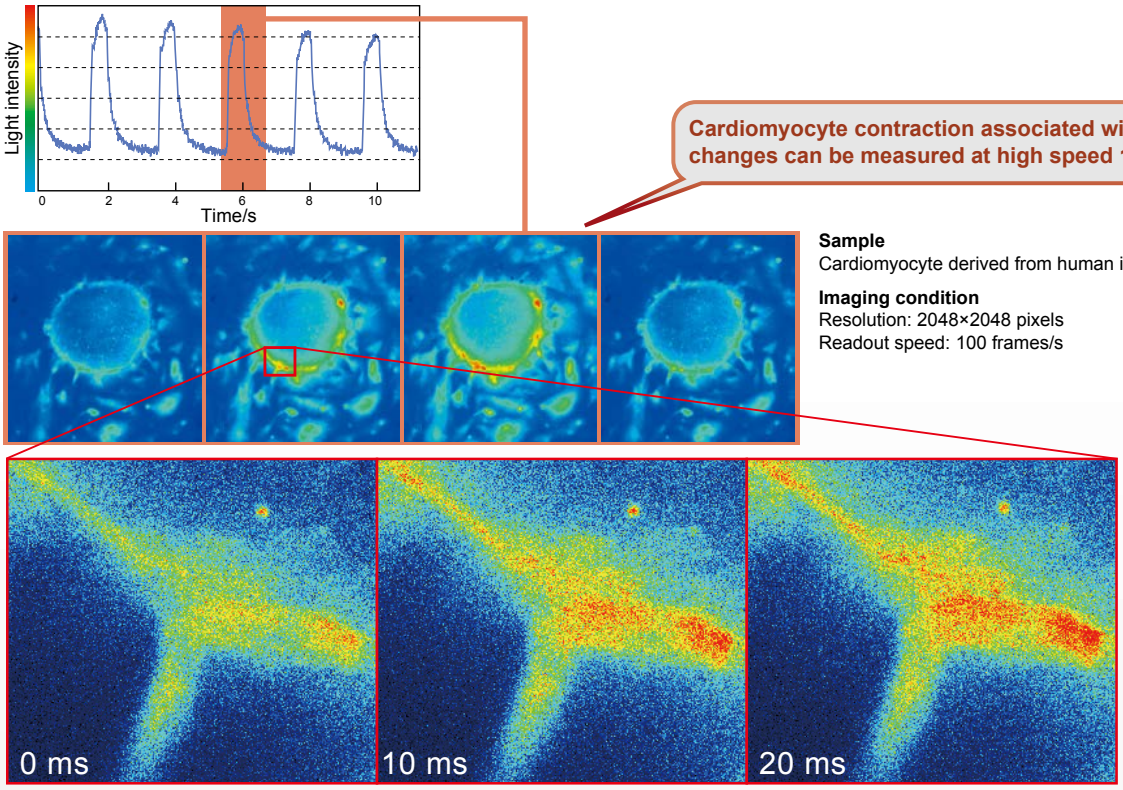


▲ Sample: Fluo Cells Prepared Slide #1
Objective lens: S Plan Fluor 100×

High Speed: Allegro or Presto? You be the Conductor

100 frames/s High Speed Imaging for instantaneous phenomenon

The ORCA-Flash4.0 V3 provides 100 frames/s high speed imaging with a 2048×2048 pixel wide field of view. This finely tuned data transfer efficiency allows users to follow faster signals, passing the performance of common CCD cameras.



Sample
Cardiomyocyte derived from human iPS cell stained with Fluo8-AM

Imaging condition
Resolution: 2048×2048 pixels
Readout speed: 100 frames/s

• Readout speed*

		Camera Link		USB 3.0				
		16 bit/12 bit/8 bit		16 bit	12 bit	8 bit	16 bit/12 bit/8 bit	
		Horizontal pixel	Binning	Horizontal pixel				Binning
		128 to 2048	2×2/4×4	640 to 2048			128 to 512	2×2/4×4
Vertical line	2048	100	100	40	53	80	100	100
	1024	200	200	80	106	160	200	200
	512	400	400	160	212	320	400	400
	256	801	801	320	424	641	801	801
	128	1603	1603	641	848	1282	1603	1603
	64	3206	3206	1282	1710	2565	3206	3206
	8	25 655	25 655	9329	12 827	17 103	25 655	25 655

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

Low read noise & Short time snap shot

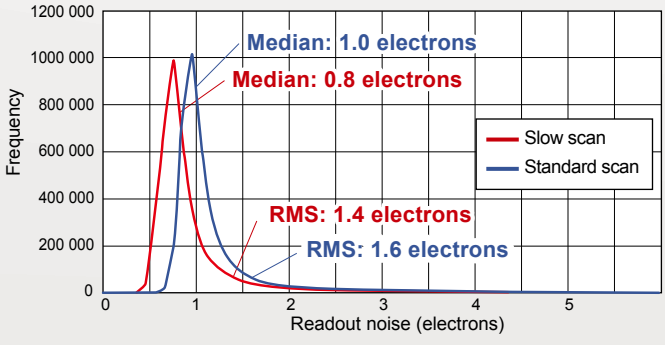
Slow Scan Mode

While the read noise at standard scan is only 1.0 electrons median, the ORCA-Flash4.0 V3 is an additional slow scan readout mode with read noise of just 0.8 electrons median. (at 30 frames/s) Both the USB and Camera link configurations of the camera have this low noise capability, especially for weak light detection.

Global Exposure Flexibility

By adding a Global Reset Mode function to the ORCA-Flash4.0 V3, users can acquire global exposures and choose to have either an external source or the camera be master of the timing even for short time snap shot.

● Readout noise distribution

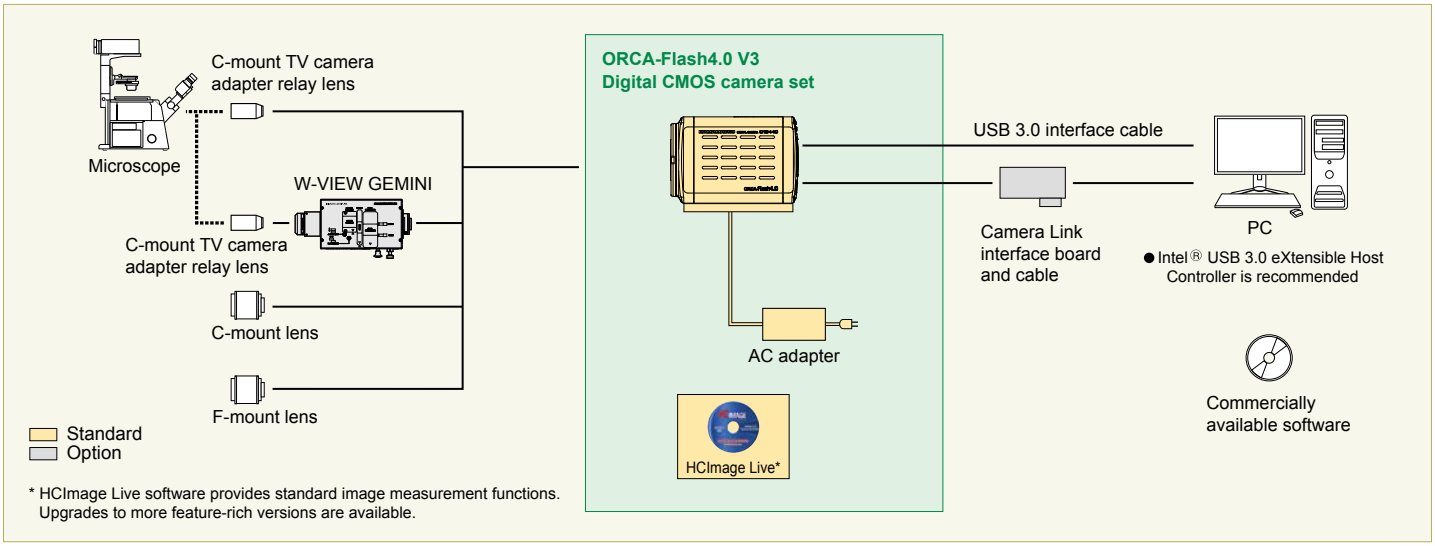


Specifications

Product number		C13440-20CU
Imaging device		Scientific CMOS image sensor
Effective number of pixels		2048 (H)×2048 (V)
Cell size		6.5 µm (H)×6.5 µm (V)
Effective area		13.312 mm (H)×13.312 mm (V)
Full well capacity		30 000
Dynamic range (Typ.)		37 000 : 1
Cooling method		Forced-air cooled
		Water cooled
Cooling temperature	Forced-air cooled (Ambient temperature: +20 °C)	-10 °C
	Water cooled (Water temperature: +20 °C)	-10 °C
Frame rate	Standard scan (Full resolution, Camera Link)	100 frames/s
Readout noise (rms) (Typ.)	Standard scan (100 frames/s)	1.6 electrons
	Slow scan (30 frames/s)	1.4 electrons
Readout noise (median) (Typ.)	Standard scan (100 frames/s)	1.0 electrons
	Slow scan (30 frames/s)	0.8 electrons
Dark current (Typ.)	Air cooled (Cooling temperature -10 °C)	0.06 electrons/pixel/s
Digital output		16 bit
Exposure time (Standard scan)	Internal trigger mode with 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
External trigger input mode		Edge trigger
		Level trigger
		Synchronous readout trigger
		Start trigger
Trigger delay function		0 s to 10 s in 10 µs steps
Trigger input connector		SMA
		Camera Link I/F
Trigger output		3 programmable timing outputs
		Global exposure timing output
		Trigger ready output
Trigger output connector		SMA
Binning		2×2
		4×4
Sub-array		Yes
Interface		Camera Link full configuration Deca mode
		USB 3.0
External control interface		Camera Link
		USB 3.0
Software interface		DCAM-SDK
		PC-based acquisition package included
		Commercilally available software
Lens mount		C-mount
Power supply		AC 100 V to AC 240 V, 50 Hz/60 Hz
Power consumption		Approx. 70 VA
Ambient operating temperature		0 °C to +40 °C
Ambient operating humidity	With no condensation	+30 % to +80 %
Ambient storage temperature		-10 °C to +50 °C
Ambient storage humidity	With no condensation	90 % Max.

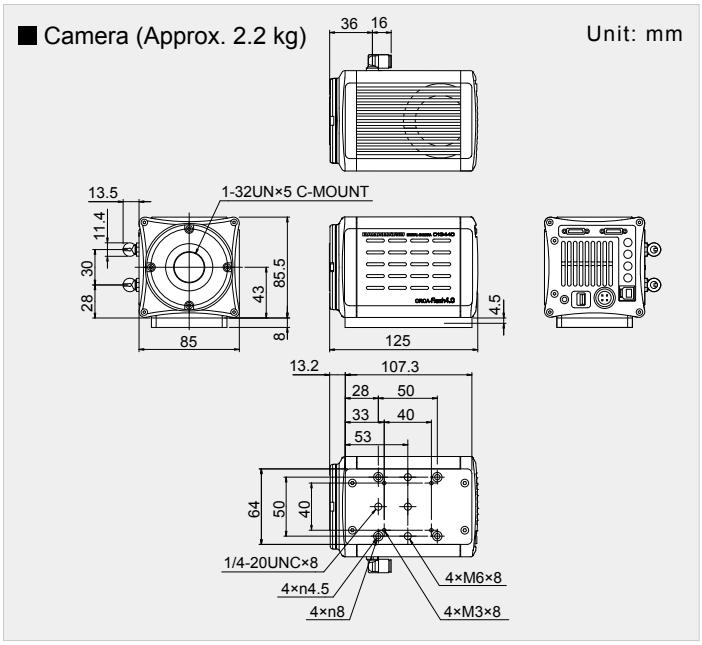
System configuration, Dimensional outlines and Options

System configuration example



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

Dimensional outlines



Options

Model Name	Product Name
A10788-04	Hose set w/no seams
A12106-05	SMA-BNC external trigger cable, 5 m
A12107-05	SMA-SMA external trigger cable, 5 m
A11185-01	Adjuster pole for C13440-20CU
A13261-02	Mounting bracket (C13440-20CU); for cables
A12801-01	W-VIEW GEMINI Image Splitting Optics
A12801-10	W-VIEW GEMINI-2C Image Splitting Optics

● W-VIEW GEMINI

Simultaneous dual wavelength imaging by a single camera

● W-VIEW GEMINI-2C

Simultaneous dual wavelength imaging with super resolution quality in wide field of view. (Dual wavelength imaging by dual cameras)

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