Life Science Cameras – Spring 2013

HAMAMATSU

PHOTON IS OUR BUSINESS

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Hamamatsu Life Science Cameras

Spring 2013

The most beautiful data are in your images.

It is a wonderful coincidence that it is possible to have meaningful scientific information embedded within images that we can experience as beautiful on a purely human level. The art of creating these data-rich images is not simple and requires a unique fusion of biology, physics and engineering. This is why achieving maximum camera performance while simultaneously ensuring quantitative data integrity is paramount at Hamamatsu. And, the fact is, we enjoy the beauty of the images too.

In previous editions of this catalog we've stated that we think the ORCA-Flash4.0 is, for the most part, making other cameras relevant for only specialized applications and that there has been a re–thinking of where sCMOS cameras fit into the world of scientific imaging. Now an emerging body of peer reviewed work is confirming that this is the case. The new ORCA-Flash4.0 V2, introduced in this catalog, should move this trend even further. With a blazing standard scan, a virtually noiseless slow scan and a readout mode designed specifically for Light Sheet Microscopy the ORCA-Flash4.0 has become even more versatile.

The ORCA-Flash4.0 V2 is at the core of Hamamatsu's camera offerings. But when experimental conditions require specialized capabilities, Hamamatsu has a complete product line affording scientists the opportunity to choose both the right technology and Hamamatsu quality. Our new ImagEM X2 is a perfect example of this. It is the fastest 512 x 512 EM-CCD on the market. With exceptionally stable gain and very low noise in the non-EM mode it promises to perform in situations where EM-CCD technology simply couldn't in the past.

The purpose of this catalog is to help you understand ways of determining which camera technology will work best for a given configuration of experimental conditions. Scientific CMOS, CCD, EM-CCD – they all have strengths and we are pleased to offer you cameras that have been designed for scientific discovery in each of these categories.

We hope you find this guide and interactions with Hamamatsu to be both a learning experience rooted in scientific principles as well as transparent in its nature. Thank you for taking the time to read it through.

Stephanie Fullerton, Ph.D.
Manager, Camera Products Group
Hamamatsu Corporation

Mark Hobson
Marketing Manager for Scientific Cameras
Hamamatsu Corporation

Changing the Game

ORCA-Flash4.0 V2

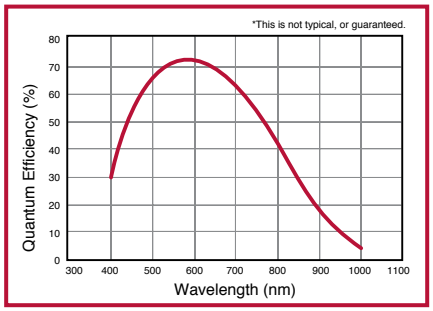
When it comes right down to it, every photon in a fluorescence experiment is valuable and hard earned. We loathe the idea that the most commonly used camera technologies waste photons by the bucket, either by not detecting them or rendering them indistinguishable from the noise. The unique combination of specifications in the new ORCA-Flash4.0 V2 enables the most efficient use of every photon, turning light into quantitative data.

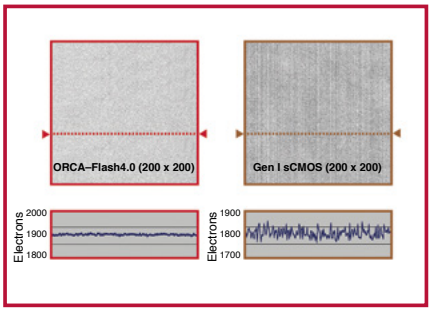
The performance of the ORCA-Flash4.0 V2 begins with a revolutionary new Gen II sCMOS detector designed to have superior quantum efficiency over existing Gen I sCMOS sensors while simultaneously capitalizing on CMOS high speed readout and large field of view. The second step is engineering a camera that nurtures the intrinsic qualities of the sensor by crafting a virtually noiseless environment even at the highest frame rates.

Specifications

- **Quantum Efficiency**
  72% at 580 nm
- **Read Noise**
  1.9 electrons rms (1.3 e- median) at 100 fps. standard scan
  1.5 electrons rms (0.9 e- median) at 30 fps. slow scan
- **Embedded FPGA**
  Hot-pixel correction, user switchable
- **Ideal Pixel Size**
  6.5 x 6. 5 µm
- **ImageConductor Connectivity™**
  30 fps with USB 3.0
  100 fps with CameraLink

Fluorescence Microscopy

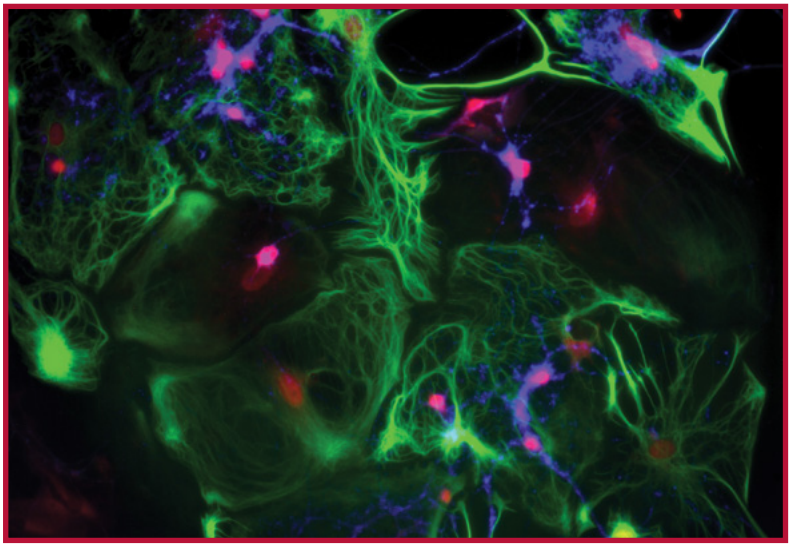




Outstanding Image Uniformity. At all input light levels the ORCA-Flash4.0 V2 shows exceptional image uniformity across the entire sensor as compared to cameras based on Gen I sCMOS technology.

The result is the ORCA-Flash4.0 V2: a camera that delivers unprecedented sensitivity (because of high QE and low noise), has minimal pixel gain variation (i.e., no stripes!); offers user–switchable, real–time, FPGA–embedded hot–pixel correction; and can sustain minutes of full–field streaming at 100 fps.

The ORCA-Flash4.0 V2 challenges the performance of all CCDs, EM-CCD*, and Gen I sCMOS and is poised to become the preferred camera for everything from routine fluorescence microscopy to advanced imaging applications.



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

Single Molecule Fluorescence

"turning light into quantitative data"

* *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 www.hamamatsucameras.com/flash4*

culture

"Hamamatsu has a culture of learning... learning from our customers and sharing our knowledge with them."

– Kate Pritchard
Camera Applications
and Support Engineer
Hamamatsu Corporation

New in the ORCA-Flash4.0 V2

"individually characterized"

Two Scan Speeds

While the read noise at standard scan is only 1.9 electrons rms 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 1.5 electrons rms. Both the USB and CameraLink configurations of the camera have this low noise capability.

Lightsheet Readout Mode™

To enable the best speeds and synchronization for light sheet microscopy the ORCA-Flash4.0 V2 configured with the CameraLink 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™.

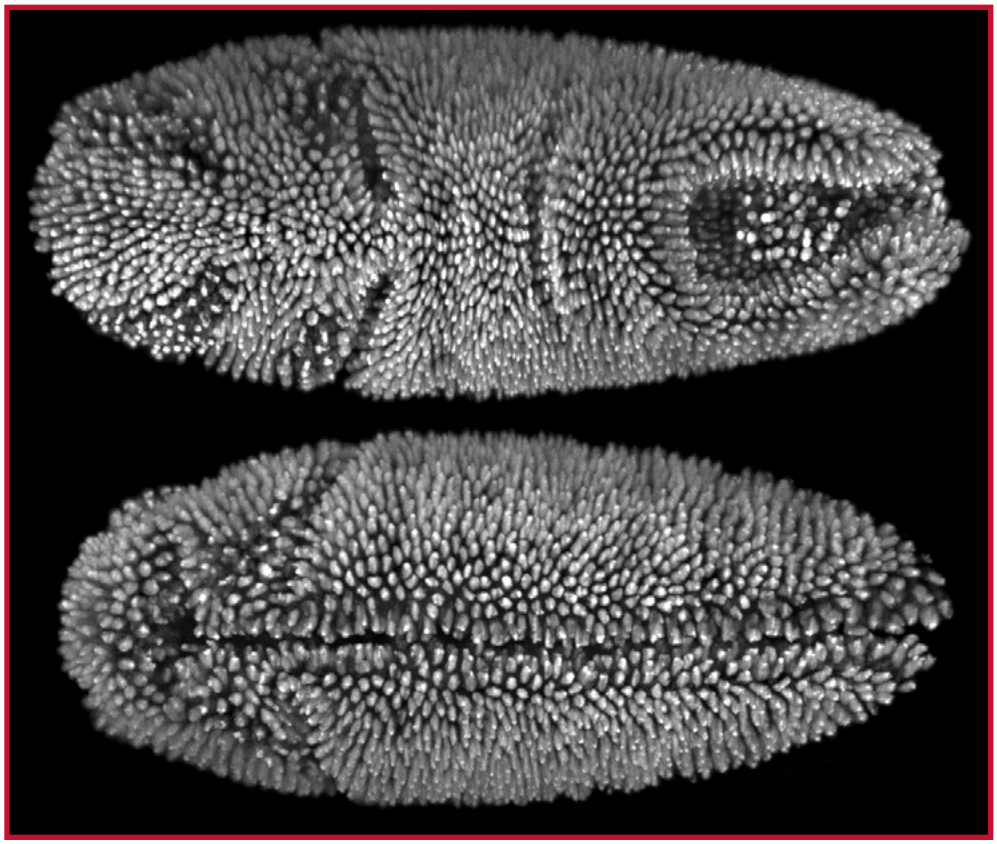
3D Structured Illumination

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 (e-/DN) 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!



A Drosophila embryo approximately 3 hours post fertilization (top: dorsal view, bottom: ventral view). The embryo, which expresses a genetically encoded marker labeling all cell nuclei, was recorded simultaneously from four different directions with a SiMView light-sheet microscope equipped with two Hamamatsu ORCA-Flash4.0 cameras. William Lemon and Philipp Keller, HHMI/Janelia Farm. http://www.janelia.org/lab/keller-lab

Read Noise (N_r)

There is always noise generated as acquired signal passes through the electronics of a sensor. Read noise is the uncertainty associated with shifting the electron charge in the sensor through amplifier(s) and resetting their base voltage to nominal zero. In the not too distant past, read noise was a considerable source of noise, as high as 10-15e- rms. We became conditioned to think of read noise as THE spec that defines camera sensitivity and this is true at low light. What's amazing is that by reducing read noise by an order of magnitude using sCMOS technology, we have redefined the meaning of low light. Low light is no longer 100s of photons per pixel. It is now possible to image, without any EM gain, signals of <10 photons per pixel. This advancement will allow scientists to push the boundaries of imaging by permitting shorter exposure times that contain meaningful image data.

Quantum Efficiency (QE)

If read noise is the queen of sensitivity at the very lowest light, QE is king at every other intensity. In sensor terms, the QE is the wavelength dependent probability that a photon is converted to a photoelectron. The QE of the ORCA-Flash4.0 V2 peaks at 72%. Functionally, having a high QE means that the detector is collecting photons more rapidly than a similar sensor with lower QE. With faster accumulation of photons, each pixel breaks out of the camera noise regime more quickly and rises to a shot noise only regime. At this point, with every additional photon the overall SNR increases and camera read noise becomes an inconsequential fraction of the total noise.

High Speed
Bright Field

PALM Ratio
Imaging

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 seems 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 fps in rolling shutter mode, the Gen II ORCA-Flash4.0 has 1.9 e- rms while Gen I has 2 e- 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 2/.54 = 3.7, while the Gen II is just 1.9/.72 = 2.6. So now, in photons, the Gen I sCMOS has 42% higher read noise than the ORCA-Flash4.0.

Finally, consider the outcome of this exercise when the ORCA-Flash4.0 V2 is running in slow scan mode with only 1.5 e- rms noise. The read noise is a mere 2.08 photons.

A Simplified Signal to Noise Equation

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

Signal & Background

S = signal, I_b = background. Photons falling on the sensor have an average photon flux. The fluctuations in this rate are governed by Poisson statistics and therefore have a standard deviation that is the square root of the number of photons (i.e. photon shot noise). In imaging, there are two sources of photons (and photon shot noise): the signal of interest (S) and the signal from the background (I_b). Limiting the amount of Ib and increasing S is critical to getting images with high SNR.

Quantum Efficiency

The QE of a camera is the wavelength dependent probability that photon is converted to a photoelectron. High QE is a fundamental attribute for obtaining high SNR, since QE is a predominant factor in the SNR equation.

EM-CCD only

M = EM gain, F_n = noise factor. EM gain occurs in a voltage dependent, step-wise manner and the total amount is a combination of the voltage applied and number of steps in EM register. EM gain has a statistical distribution and an associated variance, which is accounted for by F_n. At typical EM-CCD gains, $F_n = \sqrt{2} \cong 1.4$. All signal in an EM-CCD is subject to this additional noise. Since CCD and CMOS do not have EM gain, F_n = 1 in these cameras.

Camera Noise

N_r = read noise (e-). This is a statistical expression of the variability within the electronics that convert the charge of the photoelectrons in each pixel to a digital number expressing intensity.

N_d = dark noise (e-) (not shown above). This is camera noise that comes from thermally generated electrons and is time and sensor temperature dependent. N_d is not presented as a factor here because it is low and exposure times are short enough that it does not contribute significantly to the total noise.

Adding Noise Sources

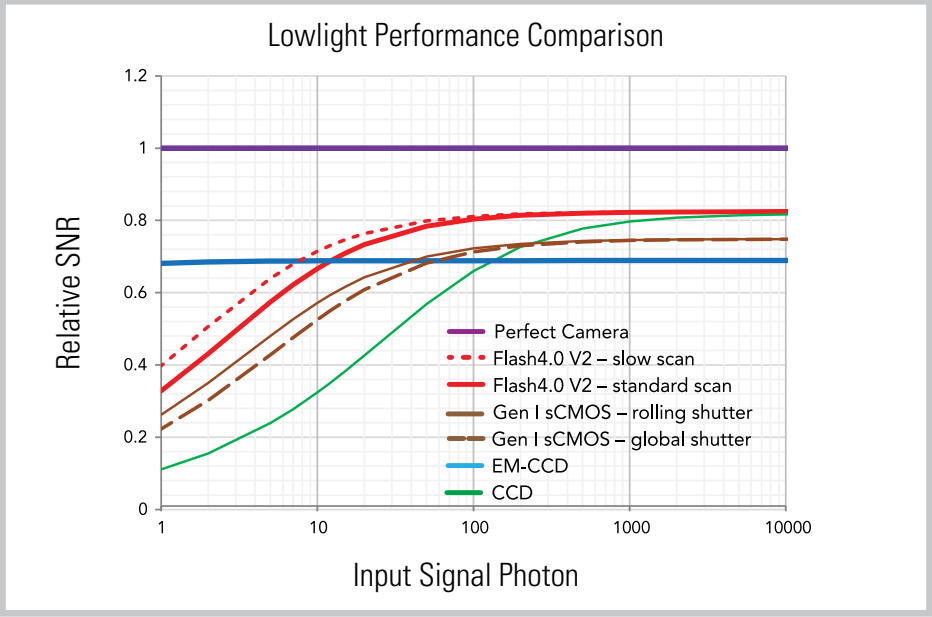
Uncorrelated noise is added in quadrature. This means that each noise term must first be squared, then added to other terms, before the total noise can be calculated by taking the square root. The effect is meaningful: a read noise of 2 e- contributes 4 e- of noise to the total noise, while a read noise of 4 e- contributes 16 e-.

ORCA-Flash4.0 V2

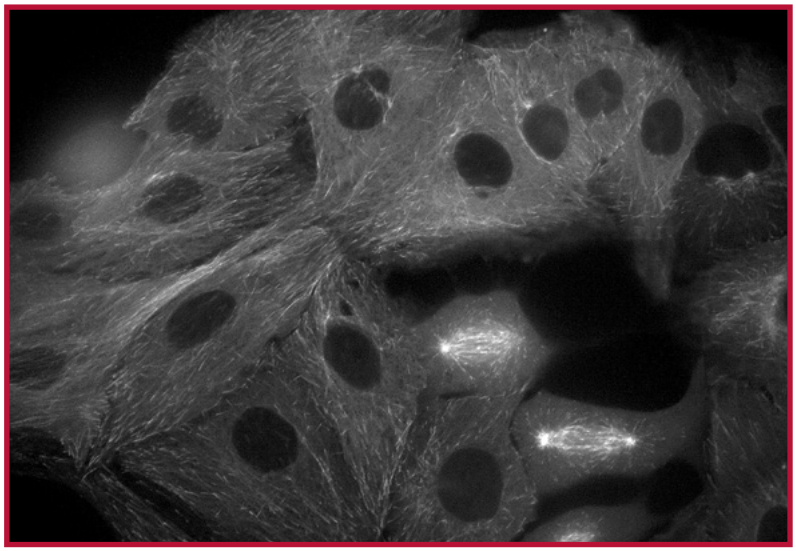
Allegro or Presto? You Are The Conductor.

When conducting imaging with a camera that has 4,194,304 pixels and 16-bit data depth, a single image is 8.39 megabytes, or the equivalent of two long mp3 songs. But capturing single frames is child's play. What really matters is sustained, sequential image capture. Hamamatsu's ImageConductor Connectivity™ 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 fps of full frame acquisition. If you choose, upgrade to our fully supported Firebird PCI Express x8 CameraLink card using that very same camera, without any additional modifications, can achieve 100 fps full resolution speed: >4 x 10^8 pixels per second or 839 megabytes of data per second…that is serious throughput. Combining the CameraLink version with our recommended solid state hard drive and high-speed computer keeps your data flowing, for up to 40 minutes of full speed, full resolution recording. 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 1.9 e- rms read noise for the ultimate in versatility and performance.

Bessel Beam Plane Illumination



Using relative SNR curves calculated from published values, it is possible to compare the low light performance of the ORCA Flash4.0 V2 in slow and standard scan to EM-CCD, CCD and Gen I sCMOS in both rolling and global shutter mode. At both speeds and at all light levels, the ORCA Flash4.0 V2 achieves higher signal noise than Gen I sCMOS. Due to the ORCA Flash4.0 V2's low rms read noise in slow scan, it exceeds the SNR performance of EM-CCD's at about 6-7 photons per pixel.



Spinning disc confocal image of LLC-PK1 cells showing EB3 fluorescence. Special thanks to Michael Davidson (Florida State University), Bruce Gonzaga (Molecular Devices) and the 2012 AQLM course at the MBL.

Calcium Imaging Blood Flow

Pixel Noise and Photon Conversion Fundamentals

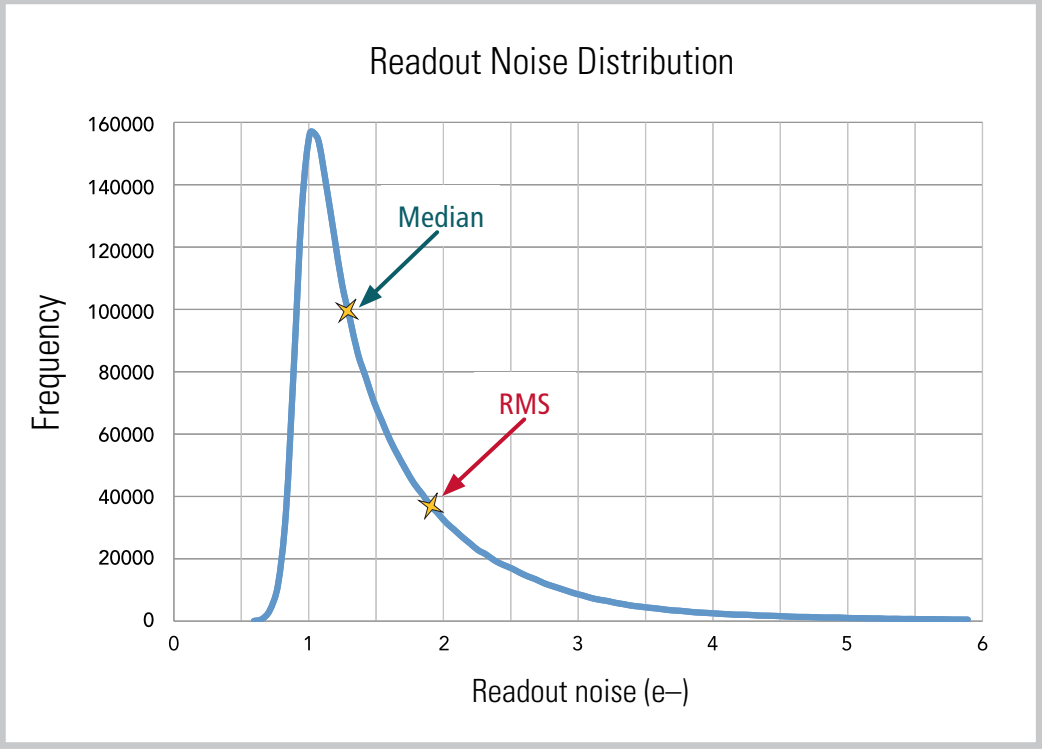
Read noise: Only rms is meaningful.

With any statistical parameter there are multiple models available to apply to the data. The classic electrical engineering method for calculating read noise is to define the root mean square (rms). This has always been the method used to calculate read noise for CCDs. Median and rms are both perfectly valid statistical models, but only rms noise accurately represents the experience that a user can expect from a camera. 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. So 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. For example, SNR measurements made empirically align with theory only when these simulations are done using rms noise values. Currently there is no industry standard in life science imaging for reporting noise specifications and it has become common practice for sCMOS to be specified by median read noise values. We include median noise data to facilitate superficial comparison with other sCMOS cameras, but we encourage users to be skeptical of median noise as a specification and to demand the more meaningful rms noise. The ORCA-Flash4.0 V2 Gen II sCMOS has 1.9 e- rms and 1.3 e- median typical read noise at standard scan.

"rms noise accurately represents the experience that a user can expect from a camera"

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.



The median value shown is simply the point at which half the pixels have more read noise and the other half have less. Given the nature of noise distribution in sCMOS cameras it is not particularly informative. RMS is the root mean square value of the read noise across all pixels and offers meaningful insight into image quality with pixel correction OFF. It is the value best used in image SNR calculations.

How Many Photons Do I Have?

With the ORCA-Flash4.0 V2, calculating the number of photons in a given pixel is straightforward. The gain conversion factor, i.e., the number of electrons represented by a single digital number (grey level), is 0.46 e-/DN. This value is critical for quantification of intensities including calculations of signal to noise and camera comparisons.

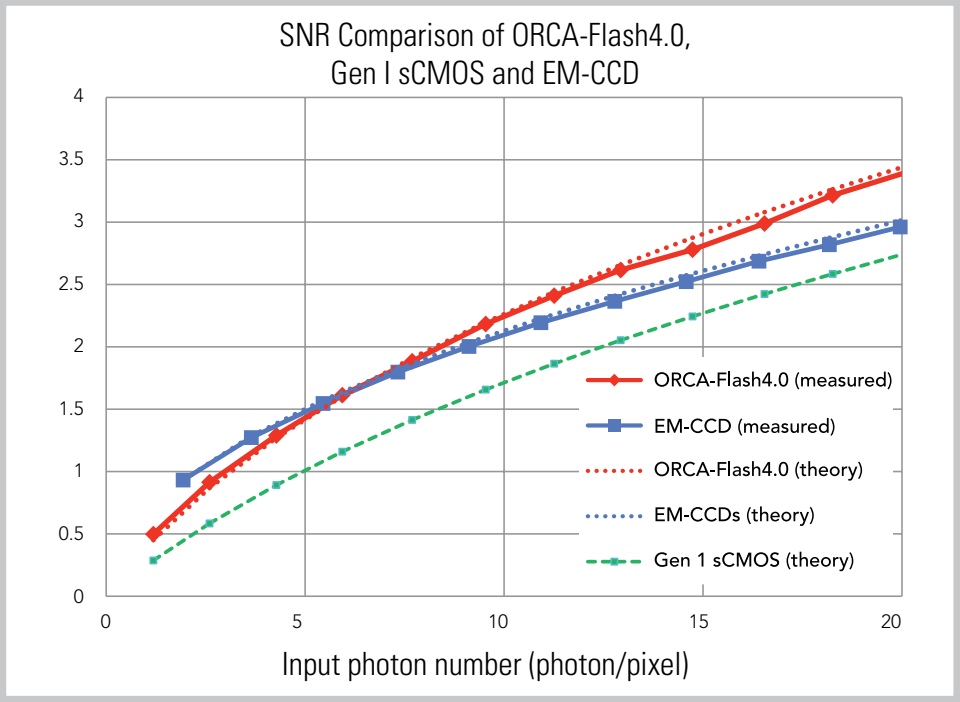
For example, if the camera output in a pixel is 10,100 grey levels, subtracting the offset of 100 grey levels and then multiplying by 0.46 electrons/grey level gives a signal of 4,600 electrons.

Furthermore, since the QE of the camera is 72% at the wavelength of interest of 550 nm, the number of photons represented by that pixel can be back-calculated: 4,600 e-/0.72 electrons/photon = 6,388 photons.

ORCA-Flash4.0 V2

The New Rules of the Game

- The ORCA–Flash4.0 V2, because of a combination of high QE and low read noise, without multiplicative noise, is capable of replacing traditional interline CCDs and EM-CCDs for most fluorescent imaging. In addition to having equal or greater sensitivity as EM-CCDs in demanding low light applications (>6 photons per pixel, measured at 533 nm), the ORCA–Flash4.0 V2 also offers larger field of view and faster frame rates than EM-CCDs.
- EM-CCDs are still the best choice for extremely low light applications (lower than approximately 6-12 photons per pixel, depending on wavelength) that have no background.
- The advantage of traditional interline cameras will continue to be their native low dark current and excellent pixel uniformity, making them the idea choice for long (minutes) exposure and slower speed experiments with moderate to high light levels.
- Background from the sample must be considered and may become the defining factor in application dependent camera selection.



The ORCA-Flash4.0 SNR exceeds that of EM-CCDs at about 6 photons per pixel. The solid lines show measured data at 533nm. This measurement aligns well with predicted values (dotted line) for EM-CCD and ORCA-Flash4.0. 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 at these low light levels. ORCA-Flash4.0: QE =70%, N_r = 1.6 e- rms as measured for this camera; EM-CCD: QE = 91%, N_r = 0.2 e- rms; Gen I sCMOS: QE = 52%, N_r = 2 e- rms as reported in literature.

Localization Microscopy

Super–Resolution

The measurement of data quality in super-resolution techniques like PALM and STORM is the precision of molecular positions. The precision of the data is limited by the noise in both the optical and detection systems. At the typical intensities observed in super-resolution, the ORCA-Flash4.0 V2 achieves better signal to noise ratios than EM-CCDs leading to increased precision (Long, et al. 2012 – see sidebar). This result is possible because of the ORCA-Flash4.0's combination of high quantum efficiency, low read noise and lack of multiplicative noise. Furthermore, because super-resolution experiments require hundreds to thousands of raw images to make a meaningful reconstruction, the fast frame rates of the ORCA-Flash4.0 V2 mean faster time to results, without sacrificing field of view.

TIRF

For the observation of fine structures and molecular tracking near the plasma membrane TIRF is a powerful technique. The ORCA-Flash4.0 V2 has the resolution required to take advantage of what TIRF can offer. With its small pixel size (6.5 µm) and great sensitivity the ORCA-Flash4.0 V2 enables high signal to noise images which are limited only by optical resolution. Rapid movements of single molecule dynamics are resolved both spatially and temporally.

In addition to the optimally sized pixels, the large area Gen II sCMOS sensor of the ORCA Flash4.0 V2 allows you to collect 2.6x the field of view and 16x the number of pixels possible with the standard 512 x 512 EM-CCDs. More useful data acquired in less time is an excellent combination.

Jiro Yamashita, Product Manager
Scientific Imaging and Imaging Application Group
HAMAMATSU PHOTONICS K.K.

STORM Light Sheet Microscopy

Independent Validation

"Localization–based super–resolution microscopy with an sCMOS camera Part II: Experimental methodology for comparing sCMOS with EMCCD cameras." Fan Long, Shaoqun Zeng, and Zhen–Li Huang. Optics Express, Vol. 20, Issue 16, pp. 17741–17759 (2012).

http://www.opticsinfobase.org/oe/abstract.cfm?uri=oe-20-16-17741

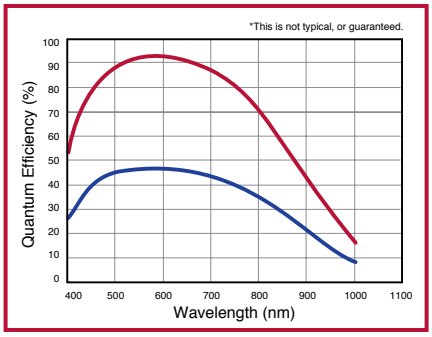
ImagEM X2 Series

Specifications

- Quantum Efficiency
  92% at 580 nm
- High Speed Readout
  70 fps at full resolution
  1076 fps with binning and subarray
- Large Collection Capability
  512 x 512 array with 16 x 16 µm pixels
- High Gain
  Exceptionally stable gain of up to 1200x

ImageEM X2 Multiply Faster

The ImagEM X2 is an extremely versatile camera that quietly delivers 70 fps at full frame and up to 1076 fps with analog binning and regions of interest. With very high signal to noise in the near dark conditions, extremely low dark current and global shutter, the ImagEM X2 enables quantitative ultra-low light imaging – both for long integration times and at high speed. With EM gain off, the extremely deep full well capacity can extract information from the lowest contrast bright images. Additional new features allow for optimized camera triggering, on-board shuttering for capture of truly dark reference images, streamlined connectivity through IEEE1394b, improved overall signal to noise and increased non-EM dynamic range. Hamamatsu has taken the beloved 512 x 512 EM-CCD sensor and created a masterfully redesigned camera that delivers maximum speed and precision performance.



Red line represents native Quantum Efficiency.
Blue line represents Effective Quantum Efficiency.

"visually pleasing and quantitatively meaningful images"

Widefield Fluorescence Luminescence

Hungry for Photons

With large pixels, high QE, and relatively zero read noise, EM-CCD technology performs in low light conditions. How low light? When you've got fewer than 10 photons per pixel (i.e. the dimmest of the dim samples) between the sample and background, EM-CCDs are the perfect tool for the job, delivering the best SNR of any camera technology (1). For high mag, biologically relevant applications with routine exposure times of 10-30ms, the sample is likely emitting 100s-1000s photons per pixel. But with faster speeds come shorter exposure times, risking the ability to capture more than 10s of photons per pixel in one shot and therefore pushing the application into the ultra-low light zone. The ImagEM X2 makes these super-fast exposures possible and has the sensitivity to provide visually pleasing and quantitatively meaningful images in a photon-starved environment.

When Photon Flux is Just Right (i.e. The Old Low Light Level)

When CCDs were our only imaging options, low light imaging meant 100s of photons per pixel. Visually this translates to very dim fluorescence… the kind that's hard but possible to see with our eyes and makes for grainy (noisy) images using CCDs. With EM-CCD we could now readily image samples at less than 100 photon per pixel level. Yes, the realization of the significance of the EM-CCD excess noise factor combined with the arrival of the high QE plus low noise sCMOS has shaken the position of EM-CCDs as the king of all low light scientific imaging. The rightful challenger to the throne, the ORCA Flash4.0 Gen II sCMOS has redefined low light imaging and is gaining acceptance in many very low to medium light quantitative, high speed applications including precision localization, TIRF, single molecule fluorescence and spinning disk (2). Yet, even for these applications, when the system is already optimized both optically and algorithmically for the 16um pixel size of an EM-CCD, the ImagEM X2 is an appropriate solution, delivering speed with high SNR.

High Light

An often overlooked benefit of EM-CCD technology is the ability to utilize the camera as a standard CCD. In non-EM mode, there is no effect of excess noise and the large full well capacity and high dynamic range are idea for bright light applications that have large intrascene dynamic range. The ImagEM X2 provides a low read noise non-EM mode that can be an ideal choice for such applications.

The Big Bucket Advantage

In most life science microscopy, the large 16um pixel size of EM-CCD is not advantageous and diminishes spatial resolution. Yet having a large pixel allows for collection of many photons per pixel quickly and permits the absolutely shortest time of illumination for non-photostable samples. The high speed of the ImagEM X2 combined with large pixels makes it ideal for such applications.

ImagEM X2 Series

New Features

Faster readout: By clocking pixel readout at 22MHz, the ImagEM X2 is able to achieve 70 fps with full frame resolution. That's more than 2x the original ImagEM and is faster than any commercially available camera using the sensor.

Lower read noise: In any image sensor, faster read out means increased read noise. Yet read noise is considered irrelevant for EM-CCDs because of the EM gain. Remarkably, the ImagEM X2, even before applying EM gain, has the fastest speed and lowest read noise of comparable cameras. But didn't we just say read noise in EM-CCD was irrelevant? Yes, in SNR equations this is true. However, if the primary purpose of EM-gain is to overcome read noise, then this will be accomplished with less gain in the ImagEM X2 and less voltage in the EM register translating into theoretically more stable EM gain calibrations and greater sensor longevity.

Mechanical Shutter: Many quantitative protocols require the capture of a dark reference frame. This image defines the no light parameter and is critical for accurate measurements and gain calibration. To ensure a completely dark reference image, the ImagEM X2 includes an integrated mechanical shutter that is software controlled.

IEEE 1394b connectivity: The data rates of the ImagEM X2 are well suited to the trusted and easy to use 1394b connectivity.

EM gain measurement and calibration: Gain aging is a known and expected process in EM technology. Even when every care is taken to minimize gain aging, use of the camera in EM mode, especially with high gains or high intensity light can degrade the gain. Since this is a use-dependent phenomenon, it's important to know when it's happened and to have the ability to easily recalibration. These two functions in the ImagEM X2 make this crucial maintenance of the camera software accessible and user friendly.

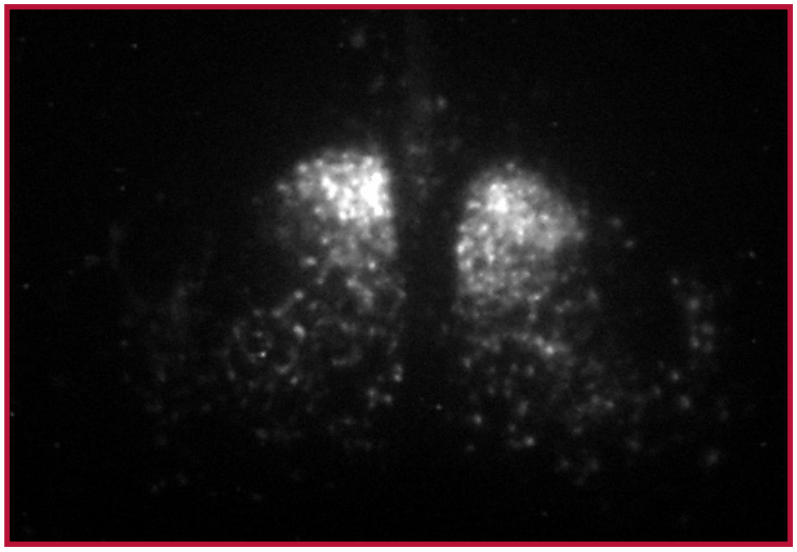
Corner Readout: By selectively imaging at the edge of the sensor, closest to the read register of the chip, it is possible to achieve even greater speeds of small ROIs.

SMA triggering ports: In its new incarnation, the ImagEM X2 sports four shiny and compact SMA ports, one for input of an external trigger and three for output to other devices. These ports can be used to access an array of triggering options including three additional features: programmable trigger input/output, trigger delay and trigger ready.

There is no denying that EM-CCD technology offers the best SNR for the ultra-low light imaging and the ImagEM X2 offers the fastest speeds combined with multiple engineering enhancements to allow you to make the most of this technology.

Mind Your F_n's and eQE's

Through the use of electron multiplying gain, EM-CCDs amplify signal. Because EM gain (M) happens before pixels are read out, the read noise is not amplified. Rather the read noise becomes relative to the gain (N_r/M), effectively making it irrelevant even at low gain. The same process that achieves this signal amplification and relatively reduced read noise also introduces a new noise source, termed multiplicative noise or noise factor (F_n). EM gain occurs on chip in a voltage dependent, stepwise manner and the total amount is a combination of the voltage applied and number of steps in the EM register. EM gain has a statistical distribution and an associated variance, which is accounted for by F_n. At typical EM-CCD gains, $F_n = \sqrt{2} = 1.4$. All signals in an EM-CCD are subject to this additional noise. Since CCD and CMOS do not have multiplicative gain, $F_n = 1$ in these cameras. When F_n is properly included in signal to noise equations, the equation reduces to $QE/(F_n)^2$. Thus for EM-CCDs, but not sCMOS or CCDs, effective QE (eQE) is half of the nominal QE. We include this consideration because it is relevant to choosing between the ImagEM and ORCA-Flash4.0 V2. At first glance, EM-CCD seems the obvious choice for all low light applications. But check carefully, at extreme low light, lower than ~6-12 photons per pixel (depending on wavelength), EM-CCDs are best. This does not hold true at higher photon intensities or when the sample has any background photons.



Luminescence from suprachiasmatic nucleus of Per1-luc transgenic mouse slice. One hour exposure. Dr. Sato Honma, Hokkaido University

Circadian Rhythms

The ImagEM excels in applications where there is very low light and little background. Circadian rhythm experiments that capture gene expression by luminescence are good examples of this. With very weak emissions over a long period it sometimes can take weeks to capture meaningful data. The ImagEM has the ultimate in very low light detection sensitivity. Exposure times of up to two hours make observation of long-term phenomena without signal loss possible. Samples that could never have been seen by the human eye are captured brilliantly.

Jiro Yamashita,
Product Manager
Scientific Imaging and
Imaging Application Group
HAMAMATSU PHOTONICS K.K.

ORCA-R2

Specifications

- Quantum Efficiency
  72% at 550 nm
- Read Noise
  6 electrons
- Speed
  16.2 fps at full resolution

ORCA-R2

The ORCA-R2 is quite possibly the most widely used and well-respected cooled CCD used in laboratories today. For years it has been and remains a workhorse camera for researchers across the world in a variety of applications ranging from calcium imaging to spinning disc microscopy. Versatility and image quality define the core character of this camera.

At the heart of the ORCA-R2 is Hamamatsu's proprietary ER–150 CCD. With improved quantum efficiency in red shifted wavelengths compared to ICX–285 based cameras (56% vs. 32% at the Cy5 peak emission of 670 nm) and read noise of only 6 electrons, this camera can accommodate samples with dynamic range of up to 6000 to 1. Cooled by air (or by water if desired), dark current is minimized so as to be insignificant even after minutes of exposure. Images from the ORCA-R2 contain not only quantitatively relevant data but also possess an appealing visual quality that is simply not achieved by other cooled CCD cameras.

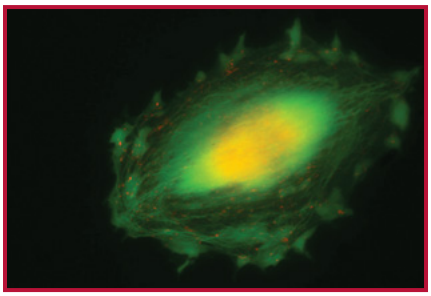
An array of one million pixels with full frame acquisition speeds of 16 frames per second and Hamamatsu's ability to enable extended red sensitivity, the ORCA-R2 can rapidly image fluorescence from GFP to mCherry and Cy5. As with all Hamamatsu cameras, it is supported by our bullet proof DCAM–API drivers (used by virtually every scientific imaging software package). The ORCA-R2 is simple to use and interacts seamlessly with automated microscopes and accessories.

A proven, reliable and multipurpose camera, the ORCA-R2 delivers exceptional images and data.

Fixed Cell Fluorescence TIRF

Dark Charge and Deep Cooling

Dark charge consists of thermally induced electrons which build up over time on a CCD or sCMOS sensor. For short exposures dark current is never a problem. But the longer the exposure becomes the more opportunity there is for dark charge to accumulate. Dark charge can be greatly reduced by cooling and the heat is typically dissipated by air flow. Very long exposures may require deeper cooling which is accomplished by using water circulation through the camera head. With water cooling the ORCA–R2 has dark current of only 0.0005 electrons per pixel per second. This adds up to just 2.1 electrons over an exposure of 70 minutes – which is less noise than the shot noise from just 3 photons of signal. In the spec wars of the camera business, cooling temperature is often used to outmaneuver competitors. We think this is a deceptive practice. If the dark charge of a camera is low enough so as not to be significant over the expected exposure time, then the camera is adequately cooled – no matter what the temperature specification.



An overlay of two wavelength fluorescence captured using the ORCA-R2.

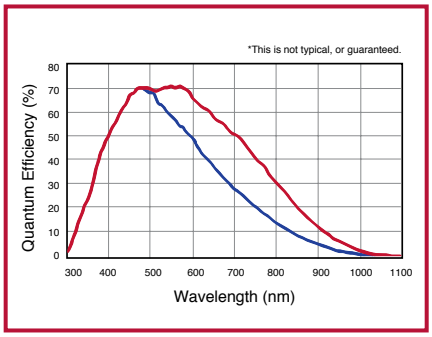
Widefield Fluorescence

BRET IRDIC

Spinning Disc Confocal

Ratio Imaging

Live Cell Fluorescence

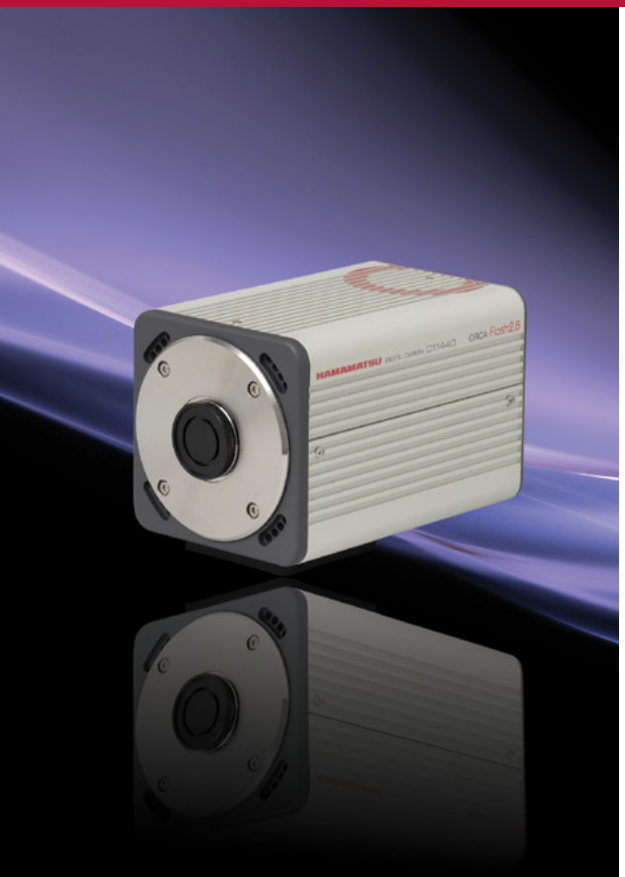


Red line: Low Light Mode
Blue line: High Light Mode

CCD or Gen I sCMOS?

When photons are relatively plentiful (>1000 per pixel) and read noise is much less than shot noise, the ORCA-R2 offers distinct advantages over Gen I sCMOS with similar size pixels. Due to the structure of CCDs and their serialized pixel read out through a single amplifier, CCDs intrinsically have excellent flat field uniformity and linearity. These properties of CCDs can simplify data analysis and provide excellent reproducibility over consecutive measurements in a range of medium to high light intensities.
In addition to the benefits of the native properties of CCD, the ORCA-R2 has the added advantage of Hamamatsu engineering, in both the sensor and camera.

ORCA-Flash2.8



Specifications

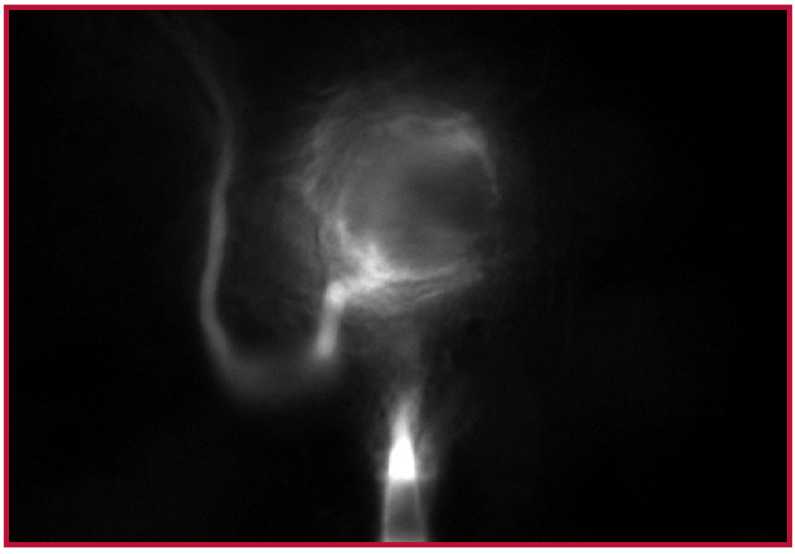
- Quantum Efficiency
  68% at 490 nm
- Read Noise
  3 electrons
- Speed
  45 fps at full resolution
- Dynamic Range
  4500:1

ORCA-Flash2.8

Don't forget the little guy…. Packed with 2.8 million 3.45 μm^2 pixels, this affordable high speed sCMOS camera delivers big performance. At 45 frames per second with full resolution, 3 e– read noise and peak QE of 70% at 550 nm, the ORCA-Flash2.8 holds its own in high speed fluorescent applications and excels when resolution is essential.

When coupled with a 0.5x c–mount adapter, the ORCA-Flash2.8 pixel size is comparable to classic cooled CCDs, while achieving faster frame rates, larger field of view and better SNR (at green wavelengths), at a fraction of the cost.

The ORCA-Flash2.8 is a great performer at an unbeatable price. When every research dollar matters, you might be surprised to find that this camera delivers exactly the performance that you need.



The calyx of Held terminal and axon in rat auditory brainstem slice, filled with Alexa 568. Jun Hee Kim, Ph.D. UTHSCSA
http://physiology.uthscsa.edu/new/research/faculty_view.asp?id=88

Fixed
Fluorescence

When Small Pixels Shine

There are numerous online resources available that carefully explain resolution in terms of pixel size and number (for example http://learn.hamamatsu.com/tutorials/java/airymag/). Experienced imagers know that the general rule of thumb, after considering all the theorems, is to use 3 pixels per minimum object dimension. For those working with 60-100x objectives with high NAs, the current pixel size range of 6-16 μm^2 for standard scientific cameras fits the rule fairly well. But for those working at 40x with high NA or at even lower mag, there are few ideal options. And this is where the ORCA-Flash2.8 shines. With 2.8 million pixels at 3.63 μm^2, the ORCA-Flash2.8 is an excellent choice for low mag, high resolution imaging and also delivers on signal to noise and speed.

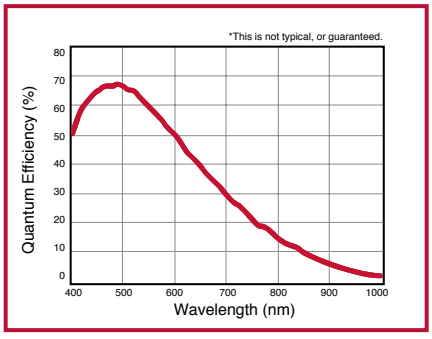
Brightfield

IR DIC

LiveCell Fluorescence

Light Sheet Microscopy

GFP Fluorescence



High Speed DIC

For rapidly moving samples imaged with brightfield, DIC or IR-DIC, the ORCA-Flash2.8 has remarkable performance. For these experiments pixel size (spatial resolution possible), dynamic range (range of sample contrast that can be accommodated) and frame rate are the fundamental characteristics to consider. The ORCA-Flash2.8 is well positioned for this work with 3.6 µm pixels, 4500:1 dynamic range and 45 frames per second at full resolution. It even outperforms many analog cameras conventionally used for these applications. We think that digital and analog camera users will be pleased with the great cost performance, resolution, speed and image quality of the ORCA-Flash2.8.

Jiro Yamashita,
Product Manager
Scientific Imaging and
Imaging Application Group
HAMAMATSU PHOTONICS K.K.

ORCA-D2

Specifications

- Quantum Efficiency
  Over 70% at 500 nm
- 2x Full Resolution
  Dual wavelength imaging with two sensors
- Versatility
  Interchangeable filter blocks
- Reproducible Results
  One–time setup

ORCA-D2

The simultaneous acquisition of two perfectly aligned, focused and properly exposed images has conventionally required multiple cameras, repeated complex optical alignments and virtual gymnastics in imaging software to make sense of the results.

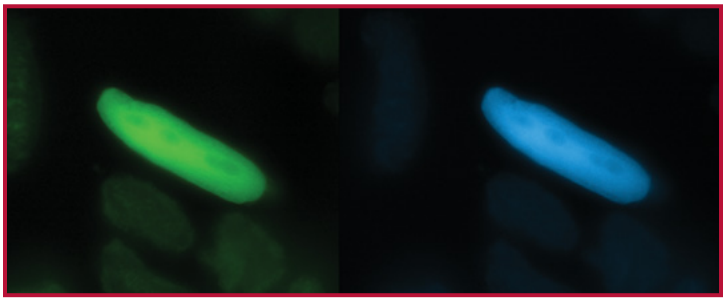
Enter the ORCA-D2. A unique camera based on two of Hamamatsu's proprietary ER–150 CCDs – the same CCD used in our venerable ORCA-R2. With one million pixels on each sensor, great sensitivity from the green through the far red (peak QE over 70%) and low read noise of only 8 electrons, it is perfectly suited for capturing multiple wavelength fluorescence at the same, precise moment in time. A simple, one time alignment (software assisted) which is memorized by the camera facilitates the generation of image pairs that are aligned to single pixel registration in the x, y and z. Because different fluorophores often produce signal of very different brightness the ORCA-D2 allows for independent exposure of each of the sensors maximizing signal to noise in both channels.

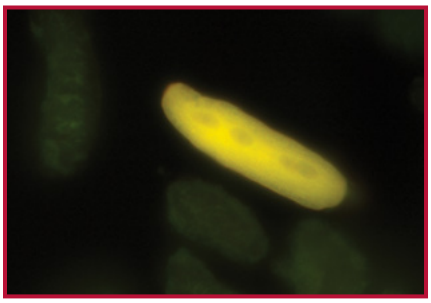
For experiments that require fast acquisition of multiple focal planes a 50/50 optical block is available. This, combined with the ORCA-D2's ability to adjust in the z-axis, now makes it possible to collect two image planes for every stop of the microscope stage. No–hassle acquisition of multiple wavelengths simultaneously or a z–series acquisition in half the time – beautiful images which are perfectly aligned are effortlessly captured with the ORCA-D2.

FRET Ratio Imaging

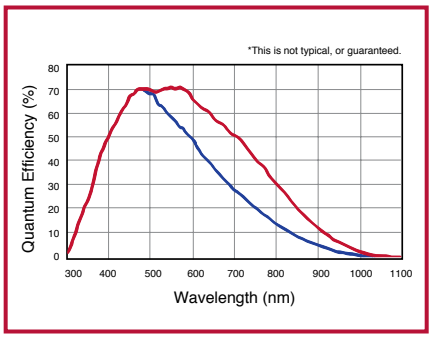
FRET

If you've ever done FRET with two cameras, you may have seen a color fringe on the edge of some cells. This is due to chromatic aberration caused by different wavelengths or a shift in position related to the optics. In the ORCA-D2 each color channel is aligned to within one pixel. With a single camera you can do an experiment without having to worry about aberration or alignment.





An acutely isolated adult rat ventricular myocyte expressing a FRET based cAMP biosensor. In the dual image figure, ECFP (left panel) and EYFP or FRET (right panel) images were obtained simultaneously using the ORCA-D2. Robert Harvey, Ph.D., University of Nevada School of Medicine
http://www.medicine.nevada.edu/dept/pharmacology/faculty/harvey.html



Red line: Low Light Mode
Blue line: High Light Mode

Co-Localization

The positional relationship between two fluorescently labeled objects is a common measurement. But if the sample is moving, this is not easy. The ORCA-D2 can acquire perfectly simultaneous two color images at high speeds avoiding the possibility of positional deviation. Even revealing the changes in distance between moving flagella and a labeled basal body is within the capabilities of the ORCA-D2.

Jiro Yamashita,
Product Manager
Scientific Imaging and
Imaging Application Group
HAMAMATSU PHOTONICS K.K.

Widefield Fluorescence Electrophysiology

Rolling and Global Shutter

Some Basics about Rolling and Global Shutter

CCD and CMOS sensors differ in the way they read out an image. Understanding these read methods and how they are triggered facilitates understanding how a particular camera will fit into an experimental protocol.

For a CCD sensor, this readout process is achieved serially. The data from each pixel is passed through a single readout amplifier and circuit and transferred digitally to the computer. CCDs transfer all pixels simultaneously to a storage region for readout. This simultaneous transfer means that the exposure time for all pixels will start and end concurrently.

For a CMOS sensor, the readout process is achieved by a combination of parallel and serial readout circuits. Each pixel has its own readout amplifier and with multiplexing it is possible to maintain the pixel map for each line while reading out the complete line simultaneously. The readout of each line is then done sequentially. This “rolling shutter” speeds data rates enormously and has a temporal effect on the image. Rolling shutter means that each pixel has an equal amount of exposure time, but the start and end of each sequential line has a small shift in time relative to the line before. For the rates of motion presented by almost all biological samples this effect will not be measurable or significant. This is the most common way scientists use our ORCA-Flash4.0 sCMOS camera.

How We Accomplish Global Exposure

While the vast majority of experiments work perfectly with rolling shutter, there are a handful of protocols in which the signal from all pixels must be captured at exactly the same time. Of the two ways to accomplish this timing in CMOS cameras, Hamamatsu has implemented the solution which offers the fastest acquisition and best image quality possible.

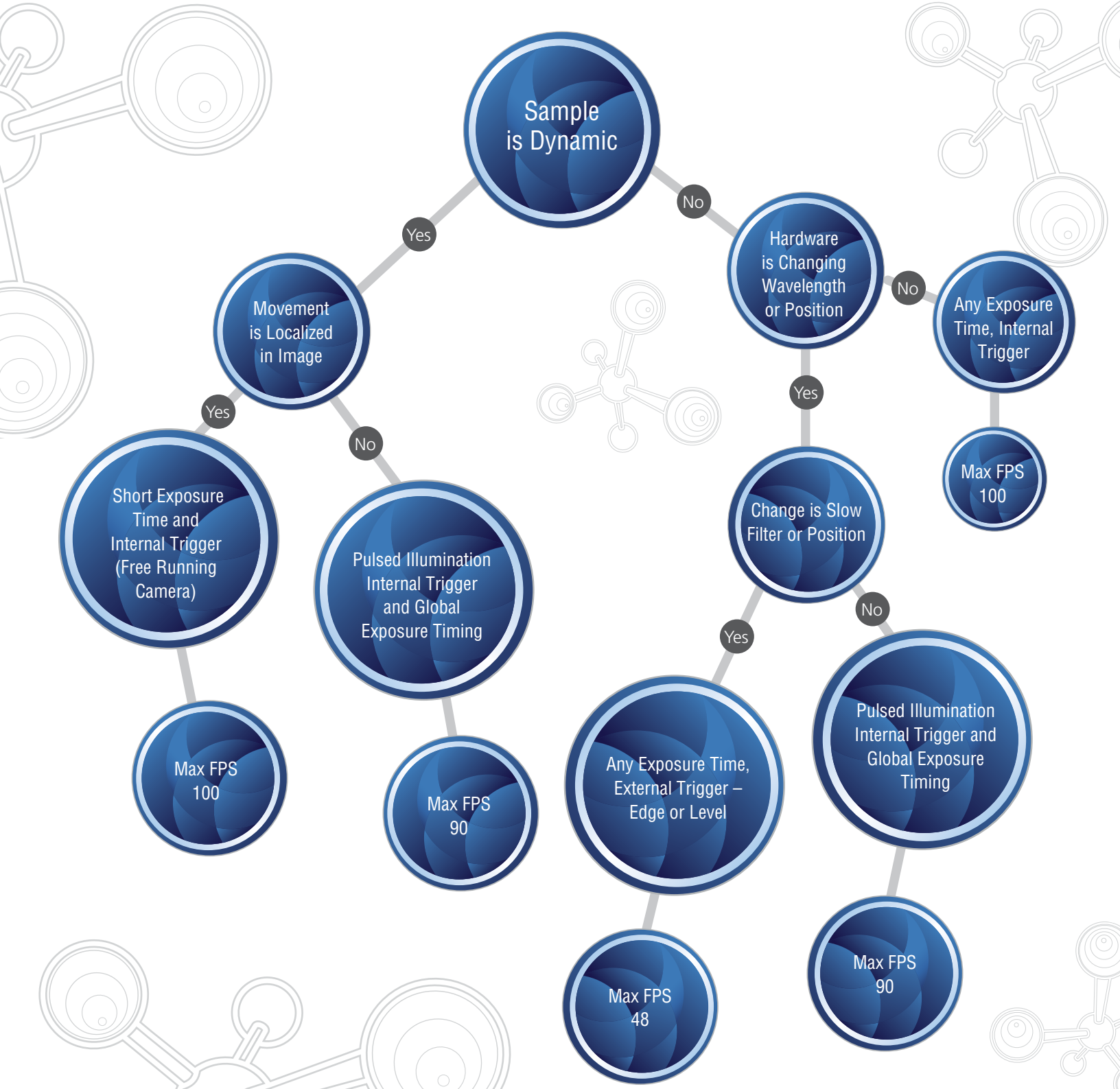
Global shutter read out mode requires each pixel to have an additional transistor. The complexity of this pixel design and its operation lowers the QE and increases dark current. Furthermore, for quantitative data, a reference frame must be acquired with each image, halving the effective frame rate and increasing the read noise by 1.4x. Because of these tradeoffs, Hamamatsu sCMOS cameras are designed without global shutter type sensors.

Global exposure synchronization is a method of driving a rolling shutter CMOS so that there is a time when all the lines are exposed simultaneously, thus emulating a global shutter CMOS. This acquisition option is built into our ORCA-Flash series of cameras. To enable global exposure the camera is synchronized with a pulsed light source or fast external shutter. In the camera, the exposure window is expanded to be slightly longer than time the light source is on. This makes possible a slice of time in which all lines are receiving light simultaneously. The precise hardware triggering is handled in software, allowing the user to easily achieve the same temporal synchronization as global shutter while still offering low noise, high QE and fast frame rates.

Shelley Ziemski Brankner
Application Engineer, Technology Group
Hamamatsu Corporation

The Time Sensitive Experiment

Decision Tree to Visualize Five Types of Time Sensitive Experiments Using the ORCA-Flash4.0



Maximum frame rates are shown for full resolution (2048 x 2048) images for an ORCA-Flash4.0.

Software

The DCAM-API is a digital camera Application Programming Interface. This software module performs the communication between the application program and the hardware acting like a translator between the different camera types and the user's chosen application program. The software installation includes drivers for the various approved frame grabbers, interface ports and Hamamatsu cameras. Updates to the DCAM-API are available free of charge at **www.dcamapi.com**.

The DCAM-SDK is the DCAM-API Software Developer Kit. It is a documentation library licensed to commercial developers by Hamamatsu which includes the necessary input/output commands utilized in the application program for the purpose of setting parameters and acquiring image data from Hamamatsu cameras. The documentation also includes sample programming for commonly used subroutines such as camera initialization, image acquisition, and error checking. If you are a developer wishing to support Hamamatsu cameras, please contact us directly.

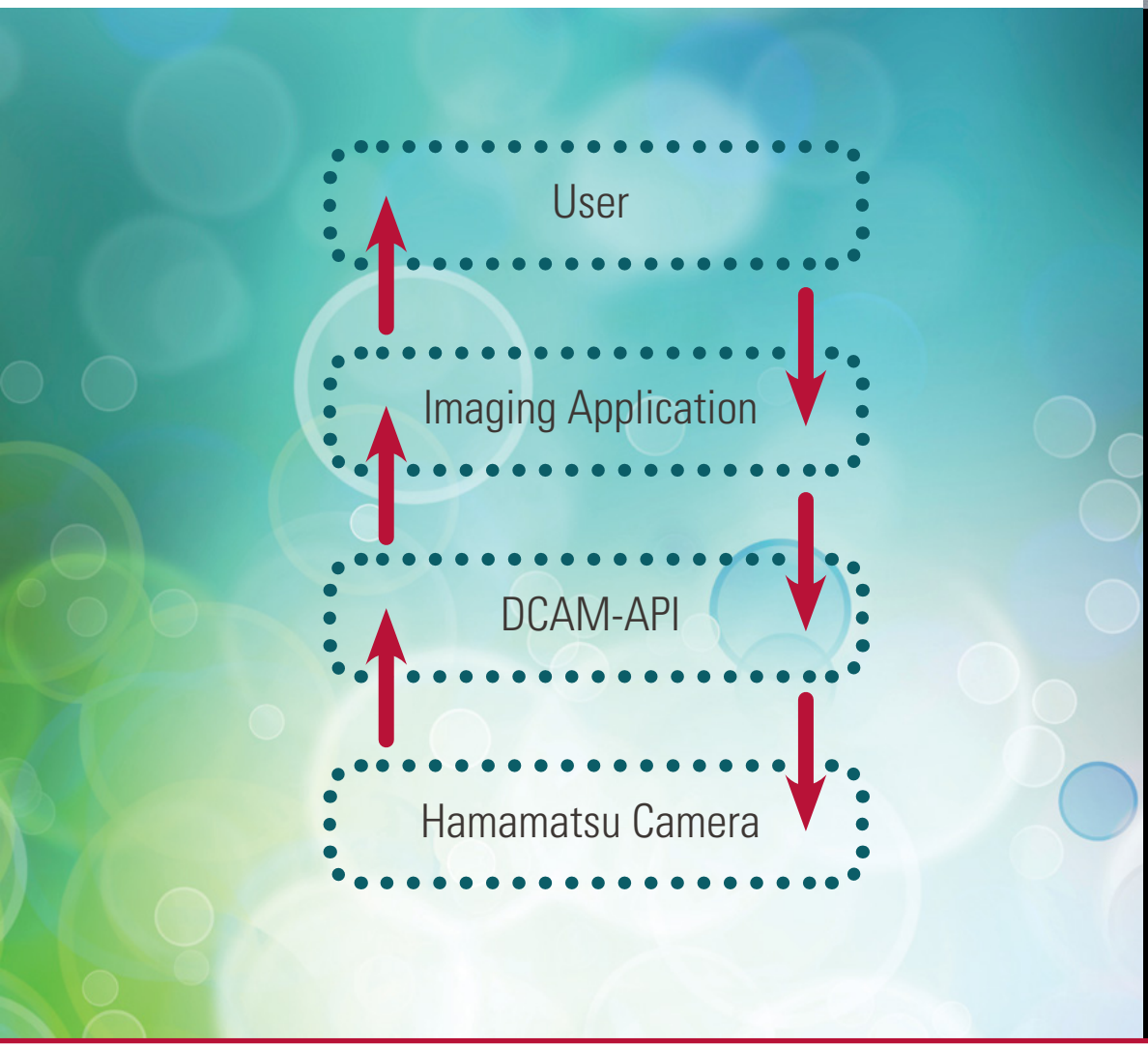
DCAM-API

To augment the characteristics of a great sensor, you need a superbly designed camera. But the process is not yet complete. The control mechanism required to have a truly useful digital device is software. Just like the OS on your computer is the essential interface between the hardware and an "Application," our most important software is the underlying code that allows for seamless integration of our cameras with the imaging application of your choice. For the end-user our DCAM-API is a transparent layer enabling a wide range of imaging software to run our cameras. For the developer our DCAM-SDK is a comprehensive and easily implemented library.

"a comprehensive and easily implemented library"

**While most of the imaging software packages listed above run all of Hamamatsu's cameras, please check with your software provider to confirm that their current version offers full support of the camera you have chosen.*

Software packages that support Hamamatsu cameras using the DCAM-API include: AxioVision, Olympus cellSens, Image-Pro Plus, HCImage, Imaging Workbench, iVision-Mac, LabVIEW, Leica Application Suite, MATLAB, MCID, MetaMorph, Micro-Manager, Neurolucida, NIS-Elements, OpenLab, SlideBook, Stereo Investigator, StreamanalySIS, StreamPix, Volocity and Zeiss ZEN.*



innovation

"Each Hamamatsu camera engineer brings a singular perspective and unique skills to a project. Through collaboration the best of these ideas are fused into a finely crafted tool for scientific research."

– Tadashi Maruno
General Manager
Scientific Imaging and
Imaging Application Group,
System Division
Hamamatsu Photonics K.K.

Out of the Labs of Academia, Into the World of Biotech. . .

Look around your lab; it's likely that at least one piece of equipment uses Hamamatsu technology inside. The products that make up our academic imaging portfolio are just a snapshot of our capabilities. Our OEM (Original Equipment Manufacturing) cameras deliver Hamamatsu quality and performance customized to suit a specific need. If you have an idea to commercialize a project and need imaging, we invite you to consult with us so that you can:

Experience our Experience

The photon is our business… Ranging from photomultiplier tube craftsmanship, to solid state sensor and optical assembly design to advanced electronics to maximize camera performance, Hamamatsu has experience in every facet of photon detection. Tap into our experience and benefit from the collective knowledge our engineers have amassed in the 55 years we've been in the photonics industry. Can you afford not to talk to us?

Rely on our Reliability

Unlike consumer electronics which seemed designed to fail within years, scientific instruments must be robust. Every instrument down means additional support costs and possibly lost data and irretrievable experimental sample. Hamamatsu Photonics is renowned for the most reliable products in the photonic performance, Hamamatsu OEM cameras are no exception. With over 25,000 OEM cameras in the field and a return/repair rate of <0.5%, our experienced design and quality manufacturing gives our customers peace of mind and lower overall cost of ownership. How do you want to sleep at night?

Create with our Technical Ingenuity

Experience is not often associated with innovation. Innovation is deemed the forte of young, nimble organizations that craft sleek and appealing marketing material. But at the core of innovation for OEM products is the experience to find just the right solution. Whether it's modification of an off the shelf device or designing a completely new camera from the sensor up, Hamamatsu engineers seek to understand your needs and apply our expertise with elegant simplicity. Our OEM camera design team understands the tradeoffs that come with every parameter (speed, resolution, sensitivity, etc.) and strive to deliver a solution with the performance you demand, at the cost that you need. We think design is the fun part, don't you?

Partner with our Team

Our best OEM customers understand that what we can deliver tomorrow is just the tip of the iceberg. By becoming true partners and sharing roadmaps for future development, we can exceed the boundaries of current technology and deliver the most advanced solutions, helping you capture your market and stay at the leading edge. Our team of hardware, software, sales and application engineers collaborates with you and for you throughout design, implementation and production. For an OEM camera to truly be an integrated part of your instrument now as well as 5 years from now and beyond, we need to share a vision. Can you envision the possibilities?

"exceed the boundaries of current technology"

Challenge Your Assumptions

At the beginning of this brochure we stated that the Flash4.0 is likely the best camera for almost every application. Yet we offer other cameras for those customers who have specific imaging priorities that are not best served by the Flash4.0. If you've read through the options and are now looking at the camera spec table on the preceding pages and still haven't decided, consider the following points; they may challenge your assumptions about camera selection leading to a more informed decision process.

1. Camera Selection is Not Only an Application Specific Decision

Our jobs would be infinitely simplified if we could provide recommendations for cameras purely based on the end-user application. Yes, application is important, but only because it offers some rough boundaries of sample parameters such as brightness, speeds, background levels and resolution requirements. In reality every application has such a wide range of each of these elements that it is not possible to say for certain that any one particular camera is the best choice for all flavors of an application.

When it comes right down to it, what really matters for most camera choices is the intensity of sample signal in the regions that hold the answers to your questions. All camera parameters are irrelevant unless you consider them in the context of your sample's brightness and your expected analysis.

This principle holds true whether you're imaging single molecules or whole embryos. Once these variables are defined, then it's possible to know what range of single pixel SNRs are possible throughout your image and whether camera noise will dominate your regions of interest or you'll be in that coveted place where shot noise in the sample is your primary noise source.

2. Camera "Sensitivity" is More Than a Single Parameter

"Which camera is most sensitive?" is an often used approach to camera selection. It seems straightforward and this question is often a proxy for "Which camera has the lowest read noise?" or "Which camera has the highest QE?" It turns out that both parameters are key to sensitivity and that the weight of each factor in determining sensitivity is highly dependent on the input light levels (i.e., back to point #1). At the low light levels, a pixel's SNR is dominated by read noise. In this light range and if the primary objective is photon detection, i.e., distinguishing some photons from no photons, QE/N_r offers the best thumbnail of sensitivity and an EM-CCD will demonstrate the best performance. Above the intensity at which the read noise becomes equivalent to the shot noise, then sensitivity as defined by SNR is a combination of read noise, shot noise and noise factor (F_n). In this phase, N_r/eQE offers a meaningful metric of sensitivity and the Flash4.0 is the best performer. At higher intensities, only eQE matters so sCMOS or CCD is the camera of choice. Again, to truly answer which camera is most sensitive, we must frame the question in the context of sample intensity and image analysis.

3. A Camera That is Best for Extreme Low Light is Not Necessarily Best for Brighter Samples

From the mathematics of the SNR equation as discussed above, the camera with the best SNR at extreme low light (i.e., fewer than 20 photons per pixel across visible wavelengths) is the EM-CCD. What is not intuitive is that this advantage evaporates with increasing light. With more photons, the EM-CCD low read noise advantage is overcome by the effects of the EM excess noise making way for Gen II sCMOS to garner the top spot in performance.

The specification chart that follows is designed to help you compare the performance among our cameras — including those built with differing technologies.

Simply knowing the individual values for quantum efficiency, read noise or frame rate cannot provide an adequate basis for accurate comparisons of cameras. It turns out that relationships between the specifications, sample conditions and the type of measurement desired that make a camera truly more (or less) suitable for a given experiment.

To keep the comparison process straight forward some of the more specialized functions and specifications of the cameras are omitted. The complete individual datasheets for each camera are available on our website at **www.hamamatsucameras.com**. There you will find information on features like triggering, pixel clocks, scan modes and many other topics.

	ORCA-Flash4.0 V2 USB 3.0	ORCA-Flash4.0 V2 With CameraLink Option	ORCA-Flash2.8	ORCA-R2	ImagEMX2	ORCA-D2
Product Number	C11440-22CU	C11440-22CU	C11440-10C	C10600-10B	C9100-23B	C11254-10B
Imaging Device	FL-400	FL-400	FL-280	ER-150	CCD-97	ER-150 (x2)
Cell (pixel) Size (μm²)	6.5	6.5	3.63	6.45	16	6.45
Pixel Array (horizontal by vertical)	2048 x 2048	2048 x 2048	1920 x 1440	1344 x 1024	512 x 512	1280 x 960
Effective Area (horizontal by vertical in mm)	13.312 x 13.312	13.312 x 13.312	6.97 x 5.23	8.67 x 6.6	8.19 x 8.19	8.26 x 6.19
Dark Current (electrons/pixel/sec.) – Air Cooled	0.5	0.5	0.6	<0.001	0.005	0.01
Dark Current (electrons/pixel/sec.) – Water Cooled	0.015	0.015	N/A	0.0005	0.0005	N/A
Full Well Capacity in electrons (typ.)	30,000	30,000	18,000	18,000 or 36,000	370,000	18,000
Readout Noise (N_r) median in electrons (typ.) slow scan	0.9 @ 30 fps	0.9 @ 30 fps	–	–	–	–
Readout Noise (N_r) rms in electrons (typ.) slow scan	1.5 @ 30fps	1.5 @ 30 fps	–	6	4@ 4x gain	–
Readout Noise (N_r) median in electrons (typ.) standard scan	1.3 @30 fps	1.3 @ 100 fps	3	–	–	–
Readout Noise (N_r) rms in electrons (typ.) standard scan[4]	1.9 @30 fps	1.9 @100 fps	3	10	<1 @ 1200x gain	8
Dynamic Range (typ.)	33,000:1	33,000:1	4500:1	3000:1	Gain Dependent	2250:1
Peak Quantum Efficiency (QE)	72% @ 580nm	72% @ 580 nm	68% @ 490 nm	72% @ 550 nm	92% @580 nm	72% @ 550 nm
Quantum Efficiency (QE) @ 500 nm	71%	71%	67%	70%	90%	70%
Quantum Efficiency (QE) @ 670 nm	68%	68%	33%	58%	89%	58%
Quantum Efficiency (QE) @ 750 nm	52%	52%	20%	40%	80%	40%
Noise Factor (F_n)[1]	1	1	1	1	1.4	1
Minimum Exposure Time	1 ms	1 ms	20 μs	10 μs	13.85 ms	117 μs
Maximum Exposure Time	10 s	10 s	10 s	4200 s	7200 s	60 s
In-Camera Binning	2 x 2, 4 x 4 (digital)	2 x 2, 4 x 4 (digital)	2 x 2 (digital)	2 x 2, 4 x 4, 8 x 8	2 x 2, 4 x 4 [3]	2 x 2
Sub-array	Yes	Yes	Yes	Yes	Yes	Yes
Maximum Full Resolution Frame Rate (fps)	30	100	45.4	16.2	70	11.2
Absolute Maximum Frame Rate (fps)[2]	25,655	25,655	1273	115	1076	50.5
Electron Multiplying Gain	N/A	N/A	N/A	N/A	4-1200x	N/A
Analog Gain	No	No	Yes	Yes	Yes	Yes
A/D Converter	16 bit	16 bit	12 bit	12 or 16 bit	16 bit	12 bit
Interface Type	USB 3.0	CameraLink	CameraLink	IEEE 1394b	IEEE 1394b	IEEE 1394b
Lens Mount	C-mount	C-mount	C-mount	C-mount	C-mount	2/3 inch bayonet

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

[2] Using maximum binning and/or smallest sub-array

[3] 8 x 8, 16 x 16 binning optional

[4] 2.0 electrons rms, guaranteed

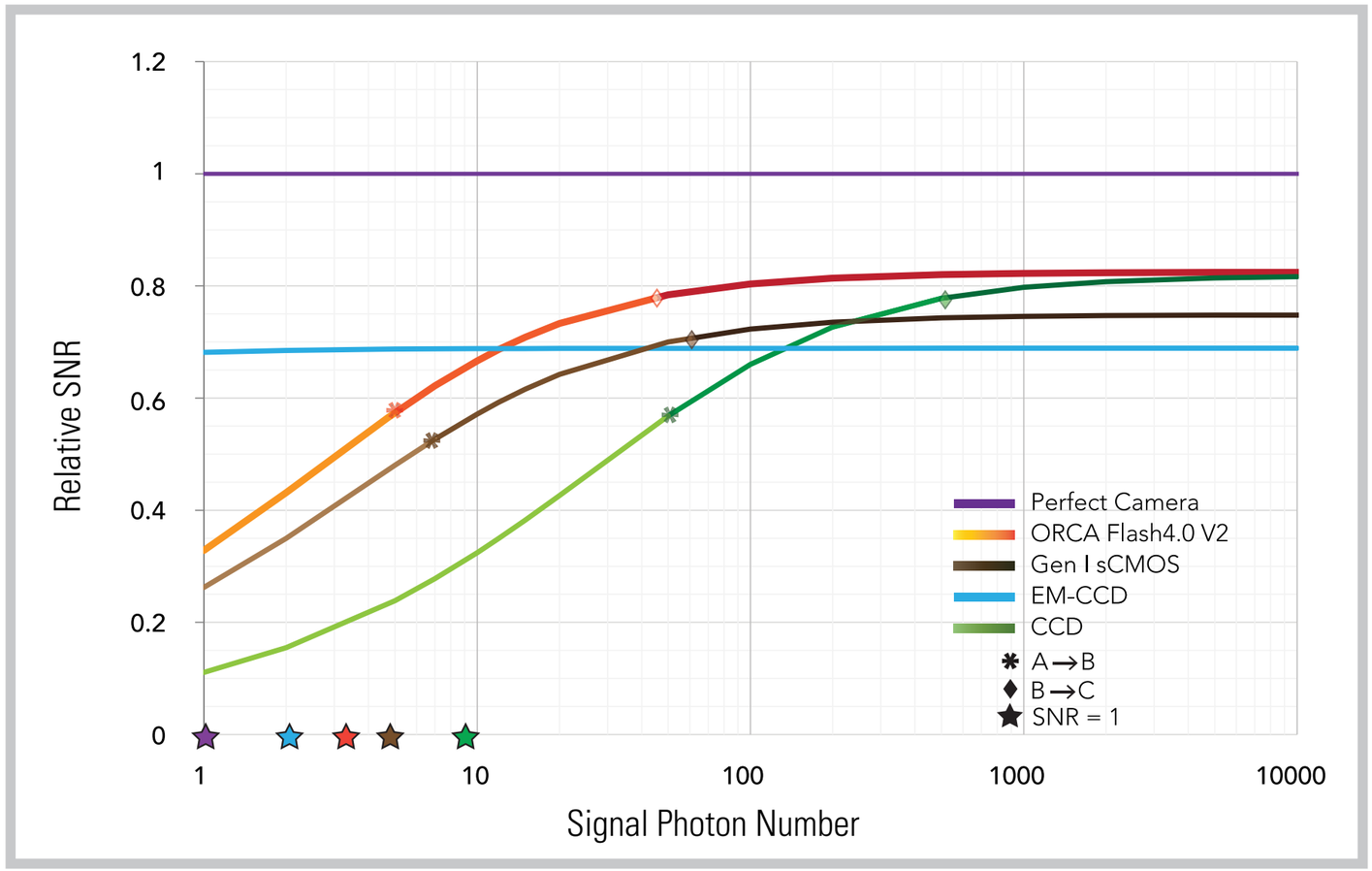
The Effects of Camera Specifications on Relative Signal to Noise Ratios (SNR)

Historically, rms read noise has been the primary camera spec used to define sensitivity. The performance characteristics of the ORCA-Flash4.0 have given us a great reason to think about sensitivity anew. To have a complete understanding of the topic it is imperative that we consider how quantum efficiency (QE), read noise (N_r), signal photon number (S) and, when present, multiplicative noise (F_n) fundamentally affect SNR – individually and especially in relationship to each other.

In the graph below, the purple line represents the relative SNR for a perfect camera. This is a theoretical line… every photon is detected and measured because there is 100% QE and zero N_r. While a perfect camera has no noise, signals do still have shot noise and therefore absolute SNR values using a perfect camera are the square root of the signal.

The perfect camera cannot exist, but the concept of the perfect camera provides context for the quality of the technology that we do have. Real cameras have limitations caused by the actual QE of the sensor, the effective lowering of QE by F_n (referred to as “effective QE” or eQE) and the effects of N_r. The calculated values for each of the four cameras shown on the graph are color coded to represent regions of significance.

At the lowest input light levels, region (A), N_r dominates relative SNR calculations and the crossover into shot noise dominated regions is the upper boundary of this region (asterisk, 5 photons for the ORCA-Flash4.0 V2, 7.4 photons for Gen I sCMOS and 50 photons for the CCD).



The **(B)** region is the intermediate zone, where N_r, eQE and F_n all contribute to the relative SNR. We have defined the upper boundary of this region as the point at which the curve is 95% of the maximum relative SNR for that camera (diamond, 45 photons for the ORCA-Flash4.0 V2, 60 photons for Gen I sCMOS, 550 photons for the CCD).

The **(C)** region is the high light region where eQE is the only camera parameter that matters. Because EM gain effectively removes N_r from SNR equations, the entire SNR curve for the EM-CCD reside in region C. But unlike CCDs and sCMOS, EM-CCD also has F_n. (sCMOS and CCD F_n =1; EM-CCD F_n =1.4) The EM-CCD curve mirrors the shape of the perfect camera almost exactly, except that EM-CCD relative SNR maxes out at 0.7. This is a direct result of F_n and makes it is clear that in spite of low N_r, and high apparent QE it is the eQE that tells the full story. The SNR of EM-CCDs is greatly affected by F_n and no matter what the input light level, eQE always dominates.

Finally, on the X-axis, stars indicate the input photon number where SNR = 1 for each camera. This benchmark is defined as a camera standard by the European Machine Vision Association (www.emva.org). Termed “Absolute Sensitivity” this is an informative value because it inherently considers multiple camera parameters, not a single spec, to assess low light performance.

Please note that the original version of this graph appeared in our white paper “ORCA-Flash4.0: Changing the Game” at www.hamamatsucameras.com/flash4. This version has been updated to reflect our thinking that SNR calculations for all cameras, including sCMOS, must use rms values for N_r. As noted on page 9, median values do not reflect the true performance of the camera. In this graph, rms N_r values were 1.9 e-, 2.0 e- and 6 e- for Gen II, Gen I and CCD respectively.

References

1. **"ORCA-Flash4.0: Changing the Game."** Stephanie Fullerton, Ph.D., Keith Bennett, Ph.D. (Hamamatsu Corporation), Eiji Toda, Teruo Takahashi (Hamamatsu Photonics K.K.)
http://sales.hamamatsu.com/assets/pdf/hpspdf/Flash4-ChangingTheGame.pdf

2. **"Localization-based super-resolution microscopy with an sCMOS camera Part II: Experimental methodology for comparing sCMOS with EMCCD cameras."** Fan Long, Shaoqun Zeng, and Zhen-Li Huang. Optics Express, Vol. 20, Issue 16, pp. 17741-17759 (2012)
http://www.opticsinfobase.org/oe/abstract.cfm?uri=oe-20-16-17741

3. **"Camera Simulation Engine Enables Efficient System Optimization for Super-Resolution Imaging."** Stephanie Fullerton, Ph.D., Keith Bennett, Ph.D. (Hamamatsu Corporation), Eiji Toda, Teruo Takahashi (Hamamatsu Photonics K.K.)
http://hamamatsucameras.com/assets/pdfs/camera-simulation-engine.pdf

4. **"Optimization of Precision Localization Microscopy using CMOS Camera Technology."** Stephanie Fullerton, Ph.D., Keith Bennett, Ph.D. (Hamamatsu Corporation), Eiji Toda, Teruo Takahashi (Hamamatsu Photonics K.K.)
http://hamamatsucameras.com/assets/pdfs/precision-localization-microscopy.pdf

5. **"Algorithm Specific Comparison of Gen II sCMOS and EMCCD Cameras for Precision Localization Microscopy using a Camera Simulation Engine."** Eiji Toda, Teruo Takahas hi (Hamamatsu Photonics K.K.), Stephanie Fullerton, Ph.D., Keith Bennett, Ph.D. (Hamamatsu Corporation)
http://hamamatsucameras.com/assets/pdfs/FOM2012_Algorithm_Abstract.pdf

6. **"Localization of High-Density Fluorophores using Wedged Template Matching."** Shigeo Watanabe, Teruo Takahashi, Tomochika Takeshima (Hamamatsu Photonics K.K.)
http://hamamatsucameras.com/assets/pdfs/FOM2012_CSE_Abstract.pdf

Glossary

- **Amplifier —** An electrical device that is used to increase (by gain) or transform the signal from one form to another (for example from current to voltage). There is a noise associated with the amplifier circuits which contributes to the overall system noise.

- **CCD —** Charge-coupled device (CCD) is a device for the movement of electrical charge, usually from within the device to an area where the charge can be manipulated, for example conversion into a digital value. This is achieved by "shifting" the signals between stages within the device one at a time. CCDs move charge between capacitive bins in the device, with the shift allowing for the transfer of charge between bins.

- **CNR —** Contrast to noise ratio, demonstrates the ability to see the signal over the background. The background, especially at low light levels, is usually limited by the noise floor of the camera. It is a demonstration on how we perceive the quality of an image.

- **Column Amplifier —** An amplifier that is specific to a column of pixels in the image sensor. Use of the column amplifiers in the sCMOS allows for each column to be read in parallel increasing the frame rate while keeping the noise low.

- **Dark Current —** Currents that are thermally generated when the imager is in the dark. These currents are normally generated in the bulk silicon of the detector. The dark current scales with operating temperature and exposure time. The dark current is reduced by half for each 7°C of cooling. Normally, the camera noise and dark noise are mixed together when reading the camera output in the dark. Therefore, you must calculate back to dark current by using the overall camera noise and the read noise values.

- **Dark Noise —** Shot noise associated with the dark current.

- **DCAM-API —** Digital Camera Application Programming Interface, it connects the hardware functions with the software commands. Hamamatsu standardizes the control for all of cameras and bridges different communication protocols (IEEE-1394, CameraLink, etc.) with a defined set of API functions. It is a simple yet powerful set of functions to control all the necessary features and data acquisition control functions of all Hamamatsu cameras.

- **Dynamic Range —** In a charge-coupled device (CCD) or complementary metal oxide semiconductor (CMOS) image sensor, dynamic range is typically specified as the maximum achievable signal divided by the camera noise, where the signal strength is determined by the full-well capacity, and noise is the sum of dark and read noises. As the dynamic range of a device is increased, the ability to quantitatively measure the dimmest intensities in an image (intrascene performance) is improved. The interscene dynamic range represents the spectrum of intensities that can be accommodated when detector gain, integration time, lens aperture, and other variables are adjusted for differing fields of view.

- **Electron Multiplier CCD —** By incorporating on-chip multiplication gain, the electron multiplying CCD achieves, in an all solid-state sensor, the single-photon detection sensitivity typical of intensified or electron-bombarded CCDs at much lower cost and without compromising the quantum efficiency and resolution characteristics of the conventional CCD structure.

- **eQE —** Effective QE, it is the quotient of the QE and the multiplicative noise as it reduces in the SNR equation. Because of noise factor, the QE is effectively reduced by QE/Fn^2 where F_n is the noise factor.

- **Excess Noise/Multiplicative Noise —** Noise introduced in the gain mechanism of electron multiplier CCD (EM-CCD) and intensified CCD (ICCD) cameras. It is a variation in the number of gain electrons from shot to shot. It increases the noise floor and background of your camera and must be included in SNR calculations. Normal CCDs and sCMOS cameras do not have internal chip gain and therefore do not have excess noise.

- **Gain** — Amplification of signal which usually has a noise associated with it.
- **Gain Conversion Factor** — Otherwise known as conversion efficiency, it is the number of electrons that correspond to 1 bit of grey level in the analog to digital converter of the camera system. Normally represented as electrons/grey level, this allows you to calculate back to the number of signal photoelectrons.
- **Nyquist** — Sampling frequency to ensure adequate resolution. Can be in the spatial, digital or temporal domain.
- **OEM** — Original equipment manufacturer, a company that purchases components and manufactures a complete system which is then retailed under the OEM's brand name.
- **Optical Background** — Light which arrives at your sensor which is not desired. Considered noise in your system, it can be from your environment or out of focus and misdirected light from your sample.
- **Photon** — Elementary particle which is the basic building block of light and other forms of electromagnetic radiation and the force carrier of the electromagnetic force. The photon has the dual nature of both particle and wave.
- **Photon Transfer Curve** — Testing methodology employed in the design, operation, characterization, optimization, calibration, specification and application of solid state imagers and camera systems. Generating a photon transfer curve (PTC) gives the user an understanding of and values for key performance parameters of their imaging system.
- **Pixel** — Size of the individual sensing elements in your image sensor array. The size directly affects the amount of signal which can be stored in each pixel as well as your resolution and field of view.
- **QE** — Quantum efficiency, the ratio of incoming photons converted into photoelectrons inside of the detector. It is usually represented in percentage and varies with wavelength.
- **Read Noise** — Noise induced by the readout electronics, typically dominated by the noise on the floating diffusion amplifier or the analog to digital converter, it typically increases with clocking speed or frame readout speed.
- **Read Register Amplifier** — Also known as the floating diffusion amplifier (FDA), it is the amplifier after the horizontal register on CCD chips. The signal charge in the CCD chip is transferred vertically through the CCD chip to the horizontal register. The horizontal register then shifts the charge horizontally to the floating diffusion amplifier which converts it from current (charge) to voltage. The FDA is usually responsible for the read noise in the CCD.
- **sCMOS** — Scientific complementary metal oxide semiconductor (sCMOS) image sensor. Differs from standard CMOS by parallel row A/D converters. The structure results in high speed, low noise operation of CMOS sensor. CMOS detectors are characterized by on pixel signal processing by amplifier circuit. A series of switches addresses the CMOS pixels to read out the signal, as opposed to CCD sensors.
- **SDK** — Software development kit, it is the access to the DCAM for third party software development.
- **Shot Noise** — Photons falling on the sensor have an average photon flux. The fluctuations in this rate (i.e., noise) are governed by Poisson statistics and have a standard deviation equal to the square root of the number of photons (or photoelectrons). Shot noise cannot be eliminated but it can be reduced by frame averaging. A pixel is said to be "shot noise limited" when the total noise is dominated by shot noise, not camera noise.
- **SNR** — Signal to noise ratio of the signal in your system to your noise. It describes the accuracy of your system. For applications such as super-resolution, position resolution is directly related to SNR value.

Notes:

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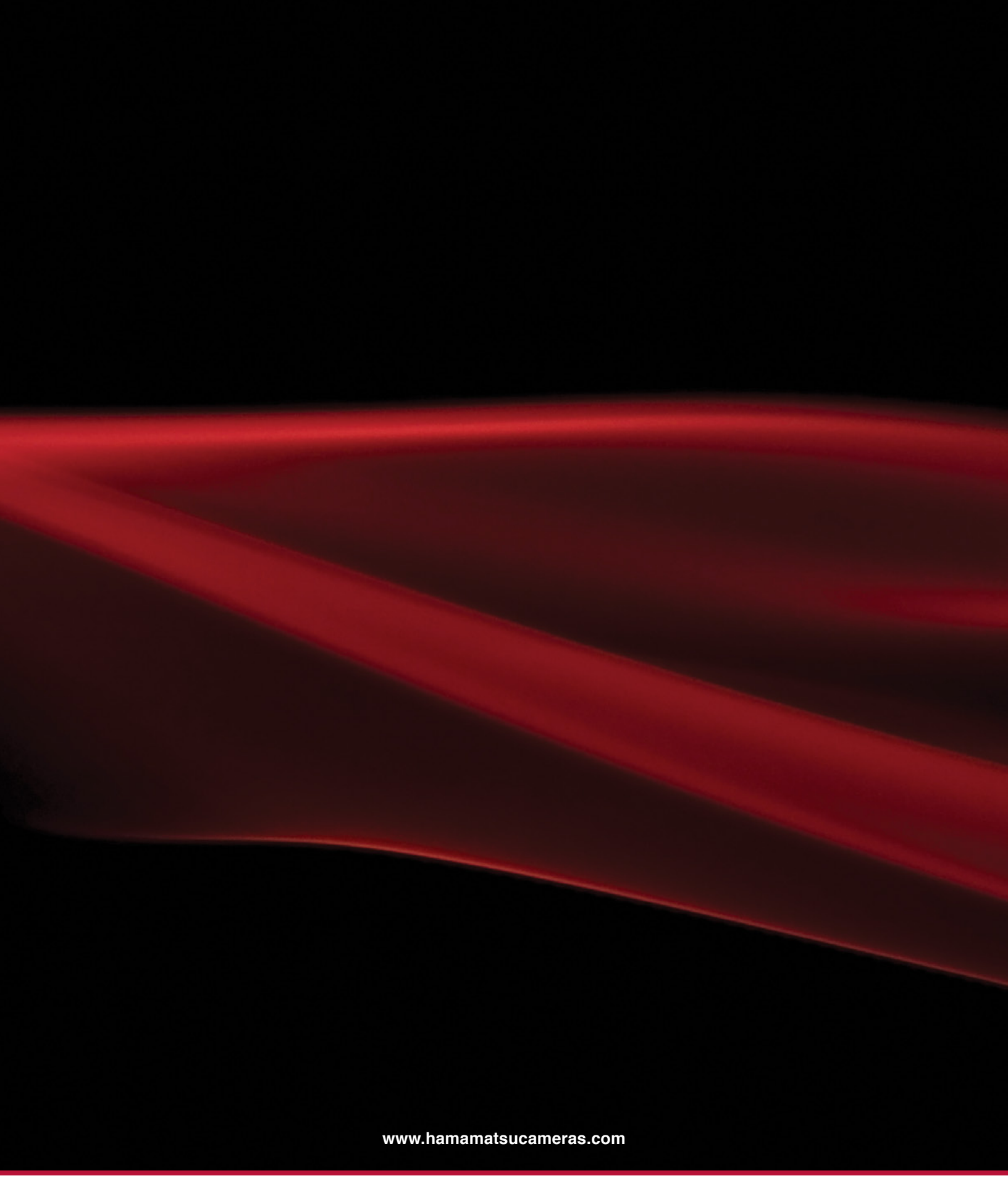

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