TELEDYNE PHOTOMETRICS
Everywhereyoulook™

PRIME 95B™

Customer Applications Handbook

TELEDYNE PHOTOMETRICS
Everywhereyoulook™
Part of the Teledyne Imaging Group

© Copyright 2019 Teledyne Photometrics
3440 East Britannia Drive
Tucson, Arizona 85706
Tel: +1 520.889.9933
Fax: +1 52 0.295.0299

Contents

Prime 95B Technical Notes

Camera Test Protocol

photometrics®

PRIME 95B™

Back Illuminated Scientific CMOS

Discovery depends on every photon

Prime 95B is the Scientific CMOS with extreme sensitivity using high Quantum Efficiency (QE) Backside Illumination (BSI), a first for Scientific CMOS cameras. The 95B's sensor converts up to 95% of incident photons into a measurable signal. Unlike microlens approaches to increasing QE, which lose effectiveness as objective magnification is increased, Prime 95B's BSI sensor brings light into the pixel photodiode from behind, avoiding structures that reflect or absorb light. When combined with large 11µm pixels, Prime 95B can deliver over 300% more signal than other sCMOS cameras at 100X magnification.

More importantly, Prime 95B outperforms EMCCD cameras—with no excess noise that negates the benefit of using a high QE sensor, and additional limitations from EM gain calibration, stability, expense, and sensor lifetime With a true 16-bit dynamic range, Prime 95B easily accomplishes what EMCCD can not—detect weak and bright signals within the same image with photon-noise limited performance.

The extreme sensitivity not only allows fainter signals to be detected, it provides the flexibility to increase frame rates, or turn down the excitation intensity to reduce cellular photo-damage. Yet Prime 95B maintains the same high frame rates, field-of-view and extremely low read noise that has made sCMOS so popular for live-cell imaging.

Primary applications:

Super-Resolution Microscopy

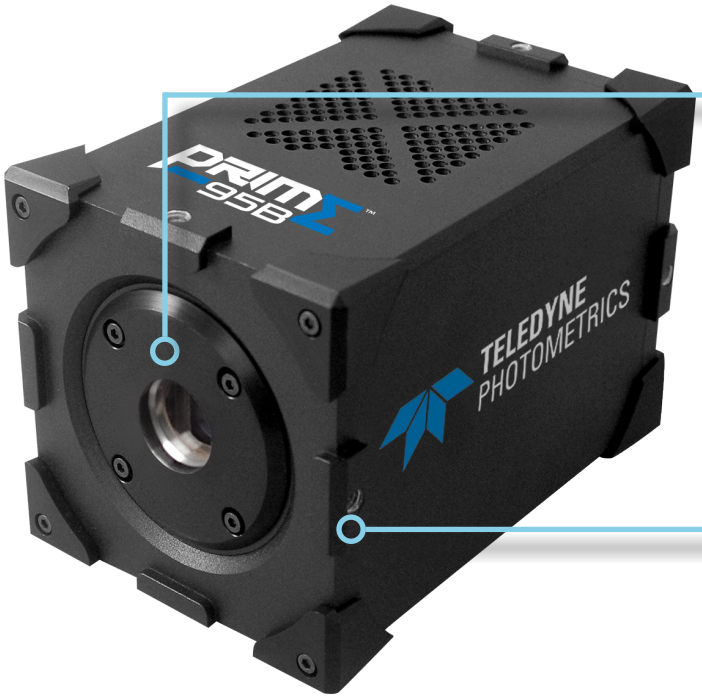
Confocal Microscopy

Single Molecule Fluorescence

Light Sheet Microscopy

- 95% Quantum Efficiency
- 11µm x 11µm Pixel Area
- 1.6e- Read Noise (median)
- 41fps @ 16-bit / 82fps @ 12-bit
- PrimeEnhance increases SNR 3-5X

Features	Advantages
High Quantum Efficiency 95% Peak QE	Maximizes ability to detect weak signals, enables short exposure times for high frame rates, minimizes phototoxicity across a wide range of wavelengths
Large 11µm Pixel Size	Maximize light collection while maintaining proper spatial sampling
Extremely Low Read Noise	Maximize your ability to detect faint fluorescence
Fast Frame Rates	Capture highly dynamic events with high temporal resolution
Large Field of View	Maximize the number of cells that can be tracked and monitored per frame
Prime Enhance™	Real-time quantitative denoising algorithm that improves image clarity by reducing photon-shot (Poisson) noise. Delivers an increase in Peak Signal to Noise Ratio of 3x to 5x
PrimeLocate™	Dynamically evaluates and acquires only the relevant data for localization based super-resolution applications
Enhanced Dynamic Range	Measure both bright and dim signal levels within the same image 50,000:1 Dynamic Range (94 dB)
Multiple Expose Out Triggering	Control up to four light sources for multi-wavelength acquisitions
SMART Streaming™	Faster acquisition rates with variable exposures, ideal for multi-probed live cell imaging Compatible with Multiple Expose Out Triggering



1.4 Megapixel BSI CMOS Sensor

Backside Illuminated Sensor
1.6e- Read Noise (Median)
>95% peak QE
80,000e- full well
11 x 11µm pixels
18.7mm diagonal

Easily Mounted and Secured

C-Mount
Two ¼"-20 mounting holes per side

Convenient Interfaces

16-bit Data
- 41 fps

12-bit Data
- 82 fps

Multiple Cooling Options

Forced Air Cooling
- -20°C Cooling
- Selectable Fan Speed

Liquid Cooling
- -25°C Cooling
- Leak-proof, quick-disconnect ports

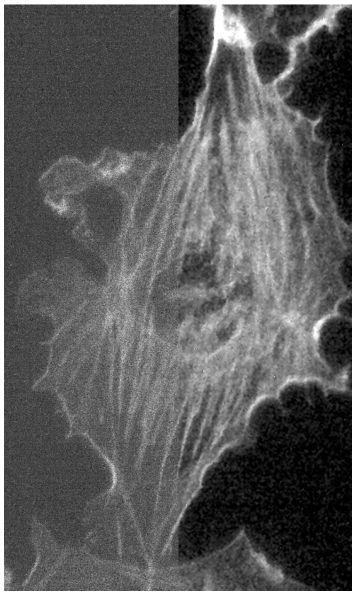
Advanced Application Triggers

Effective Global Shutter
Up to four selectable expose-out lines

TELEDYNE IMAGING
Everywhereyoulook™

Real-Time Application Optimization

PrimeEnhance™



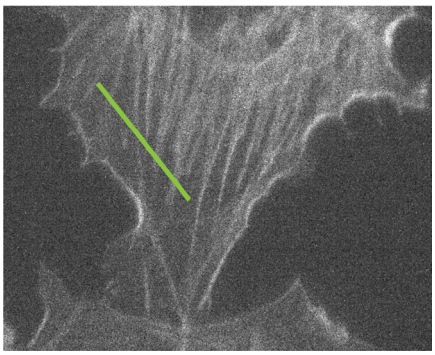
With the near-perfect sensitivity of Backside Illuminated Scientific CMOS sensors, the latest generation of scientific cameras have enabled imaging using only a few photons per pixel. Unfortunately, these minute signals are dominated by the natural Poisson variation in light levels preventing useful quantitation.

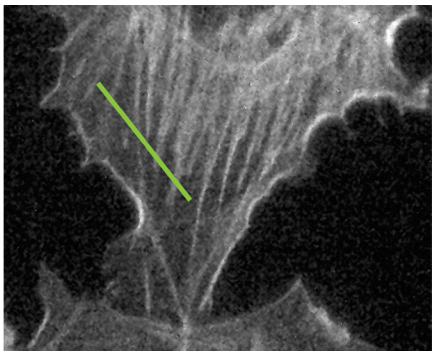
PrimeEnhance uses a quantitative SNR enhancement algorithm used in Life Science imaging to reduce the impact of photon shot-noise present in acquired images, leading to an increase in Signal to Noise Ratio (SNR) by 3x to 5x with equivalent exposure times.
With PrimeEnhance, the exposure times can be reduced by a factor of 8-10x while maintaining the Signal to Noise ratio. This reduces the effects of cellular photo-damage and extends cell lifetimes.

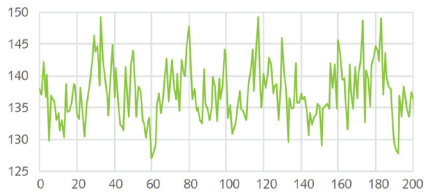
Invented at INRIA and further optimized for fluorescence microscopy at the Institut Curie, the denoising algorithm used in PrimeEnhance uses a patch based evaluation of image data and knowledge of the each individual camera's performance parameters to reduce the effects of photon shot-noise. The patches of image intensities and their noise characteristics are processed and evaluated with increasing neighborhood sizes during which weighted intensity averages are taken. This iterative process preserves not only the quantitative nature of the measured intensities, but also the maintains the finer features present in biological samples.

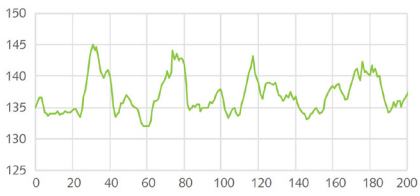
- Increase SNR 3x to 5x at low light levels by reducing photon shot-noise
- Preserve signal intensities ensuring quantitative measurements
- Extend cell lifetimes with reduced phototoxicity and photobleaching
- Extremely useful for low light imaging applications dominated by noise

Detailed performance and methodology of the algorithm is available in the following publication:
Patch-based nonlocal functional for denoising fluorescence microscopy image sequences.
Boulanger J, Kervrann C, Bouthemy P, Elbau P, Sibarita JB, Salamero J. IEEE Trans. *Med Imaging* 2010 Feb.









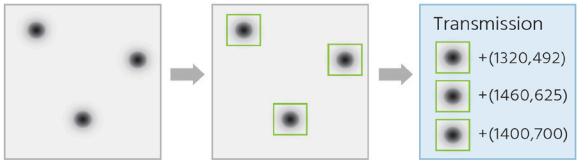
Real-Time Application Optimization

PrimeLocate

Localization based super-resolution microscopy requires a sparsity of data to ensure proper localization of emitting molecules. Even with this sparsity, the full image frame is transferred to the host computer to be analyzed, creating a large amount of data to be processed without adding useful information.

PrimeLocate dynamically evaluates image data and locates 500 regions per frame containing single molecule data relevant for super-resolution localization. Only these 500 regions are transferred to the host computer, drastically reducing the amount of data and time required for analysis.

By transferring only the relevant raw data, users have the freedom to use their preferred localization algorithm to generate super-resolution images.

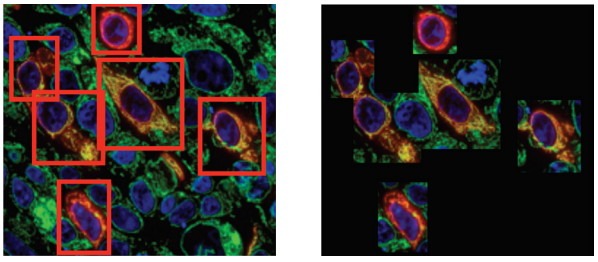


- Only the data within the patches is transferred to the host computer
- Processing time and storage requirements are easier to manage with the acquisition of only relevant data
- Ability to transfer 500 regions per frame
- Allows freedom to select preferred super-resolution localization algorithm

Multi-ROI

The surplus of data generated by sCMOS devices is challenging to acquire, analyze, and store, requiring special interfaces and expensive SSDs. While a large Field of View (FOV) is convenient for imaging, at times, only certain areas contain the desired information.

Multi-ROI allows users to select up to 15 unique ROIs within the FOV, and only these selected regions are transferred to the host computer. This allows for a large reduction in the amount of data acquired but ensures that the critical information is obtained.



- Only the data within the user-defined ROIs is transferred to the host computer
- Select up to 15 unique regions
- Significantly reduce the amount of data being acquired

Real-Time Application Optimization

Live Particle Tracking

Single molecule tracking is a technique often used to observe molecular interactions and behaviours at the single molecule level with high spatial and temporal resolution.

Teledyne Photometrics Live Particle Tracking performs this process live on the camera with live statistics.

The Live Particle Tracking algorithm works by identifying individual single molecule particles and tracking them across the field of view by adapting a published algorithm[1] tuned for two-dimensional tracking.

Firstly, the camera determines only the dynamic portions of the image and disqualifies anything static from detection. The data is then run through a restoration step (Figure 1) which reduces both the high frequency and low-frequency noise, and allows the correction of any noise variation on a pixel-to-pixel basis as well as any background intensity modulations due to uneven illumination.

The points are then processed to determine the local-maxima and go through a refinement process to ensure a high efficiency in particle detection based on a threshold to reduce the susceptibility to false positives. Any remaining artifacts are filtered out during the non-particle discrimination step, aimed at hot pixels and cosmic events.

The particles are tracked and linked through the acquired frame stack. The metadata included with all images is updated to include the particle data within each frame, providing particle IDs as well as the ability to display particle path traces as well as boxes to outline each detected particle.

Live Particle Tracking can be used to determine whether the particles are behaving as expected before time consuming data acquisition for post-processing tracking analysis.

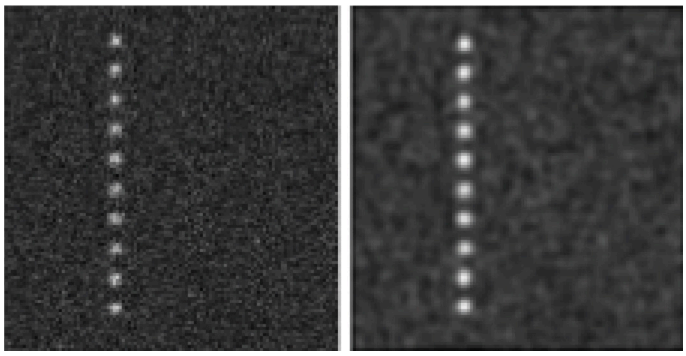


Figure 1: The input image of simulated single-particle data and the output of the image-restoration step to reduce image noise and pixel-to-pixel variation

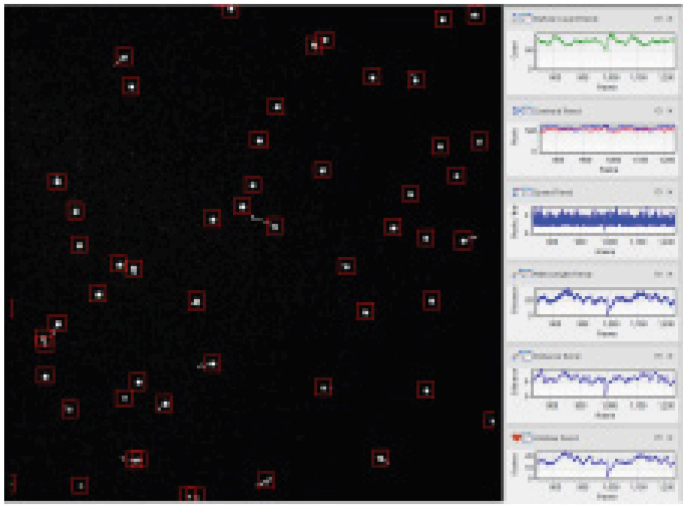


Figure 2: Live Particle Tracking running in Ocular software. Movement information of each particle is recorded, allowing tracking statistics to be displayed

[1] I.F.Sbalzarini & P. Koumoutsakos. (2005)
Feature point tracking and trajectory analysis for video imaging in cell biology. *J Struct Bio*. Aug;151(2):182-95.

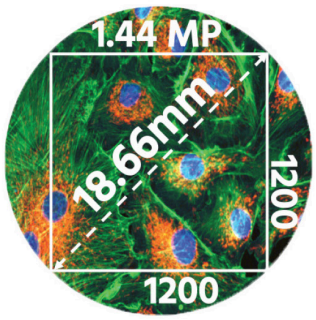
Three Field Of View Options

Most modern microscope camera ports have a maximum field of view of 19 mm, 22 mm or, more recently, 25 mm. The Prime 95B Series is uniquely positioned to match each of these ports to deliver the largest obtainable field of view for imaging.

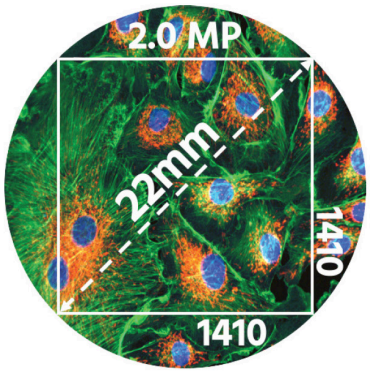
The Prime 95B and Prime 95B 22mm connect via the standard microscope C-mount and the Prime 95B 25mm connects via the larger format F-mount.

- Match the Prime 95B to the largest available microscope port
- Maximize field of view
- Increase throughput and maximize sample imaging area

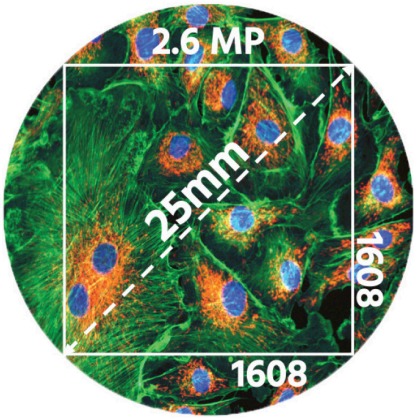
Prime 95B



Prime 95B 22mm

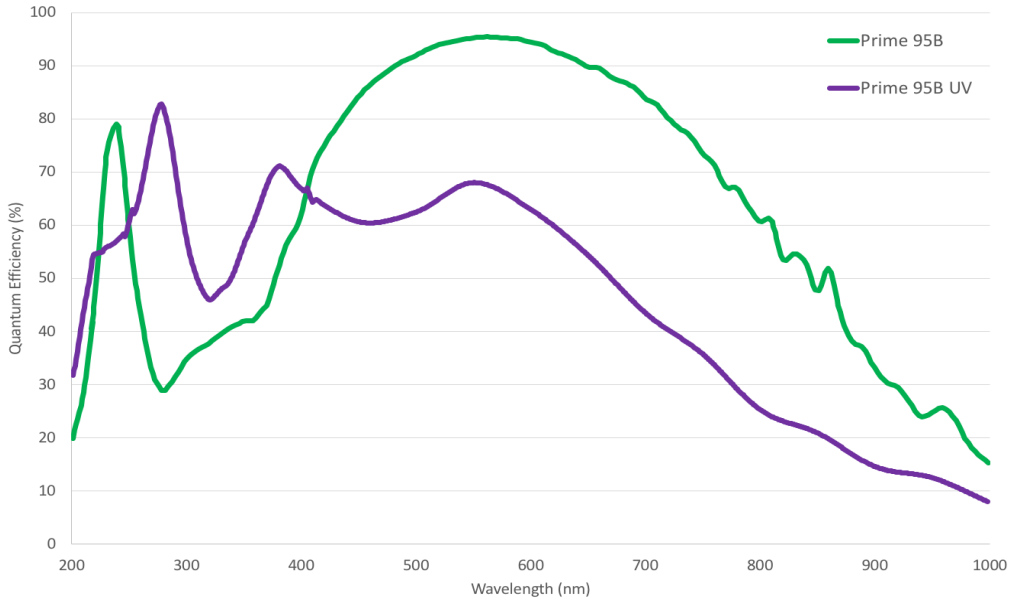


Prime 95B 25mm



Blue and UV sensor Variants

Maximize sensitivity in the Blue and UV with the Prime 95B sensor variants, Prime 95B Blue (310–450 nm) and Prime 95B UV (250-310 nm). Capture more photons than before at these difficult wavelengths to reduce exposure times and increase speeds.

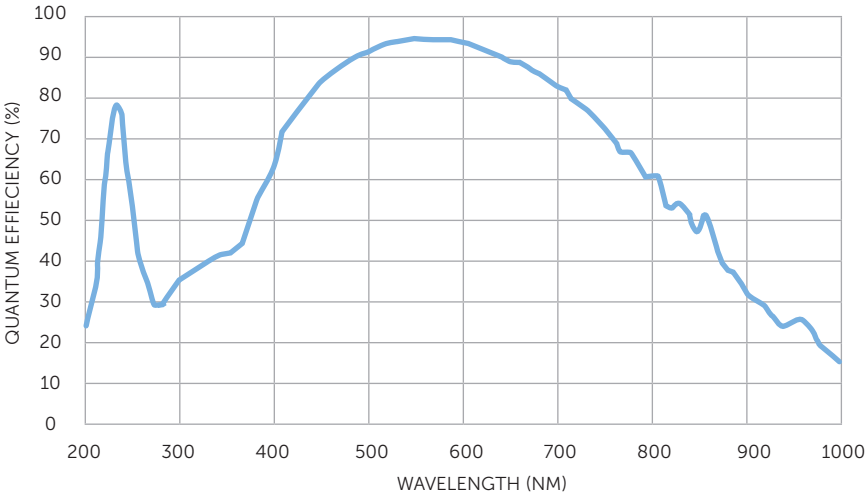


Specifications	Camera Performance
Sensor	GPixel GSense 144 BSI CMOS Gen IV, Grade 1 in imaging area
Active Array Size	1200 x 1200 pixels (1.44 Megapixel)
Pixel Area	11µm x 11µm (121µm^2)
Sensor Area	13.2mm x 13.2mm 18.7mm diagonal
Peak QE%	>95%
Read Noise	1.6e- (Median) 1.8e- (RMS)
Full-Well Capacity	80,000e- (Combined Gain) 10,000e- (High Gain)
Dynamic Range	50,000:1 (Combined Gain)
Bit Depth	16-bit (Combined Gain) 12-bit (High Gain)
Readout Mode	Rolling Shutter Effective Global Shutter
Binning	2x2 (on FPGA)
Linearity	>99.5%

Cooling Performance	Sensor Temperature	Dark Current
Air Cooled	-20°C @ 25°C Ambient	0.55e-/pixel/second
Liquid Cooled	-25°C @ 25°C Ambient	0.3e-/pixel/second

Specification	Camera Interface
Digital Interface	PCIe, USB 3.0
Lens Interface	C-Mount
Mounting Points	2x 1/4 "-20 mounting points per side to prevent rotation
Liquid Cooling	Quick Disconnect Ports

Triggering Mode	Function
Input Trigger Modes	Trigger First: Sequence triggered on first rising edge Edge: Each frame triggered on rising edge SMART Streaming: Fast iteration through multiple exposure times
Output Trigger Modes	First Row: Expose signal is high while first row is acquiring data Any Row: Expose signal is high while any row is acquiring data All Rows: Effective Global Shutter – Expose signal is high when all rows are acquiring data Signal is high for set Exposure time Rolling Shutter: Effective Global Shutter – Expose signal is high when all rows are acquiring data Signal is High for set Exposure time – Readout Time
Output Trigger Signals	Expose Out (up to four signals), Read Out, Trigger Ready



Frame Rate (PCIe interface)		
Array Size	16-bit	12-bit
1200 x 1200	41	82
1200 x 512	96	192
1200 x 256	192	384
1200 x 128	384	736

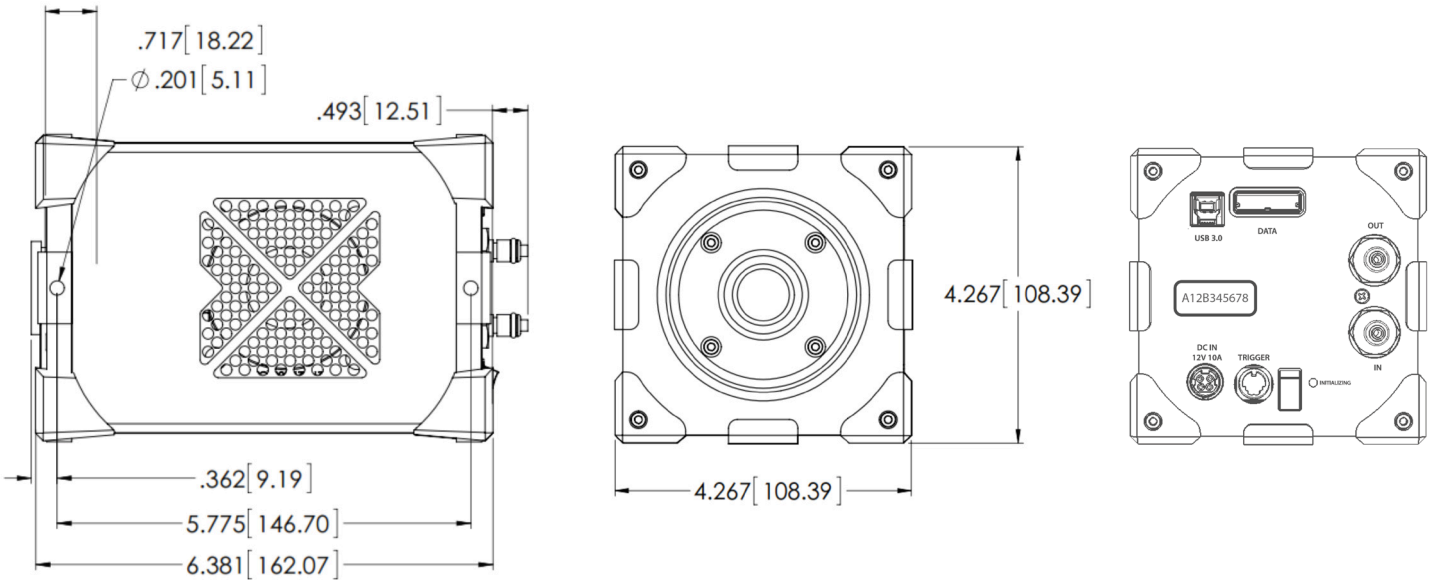
Accessories (Included)

- PCIe Card/Cable
- USB 3.0 Cable
- Trigger Cables
- Power Supply
- Manuals and QuickStart Guide
- Performance and Gain Calibration Test Data

Accessories (Additional)

- Liquid Circulator
- Liquid Cooling Tubes

Distance from C-mount to sensor



TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Photonics and Super-Resolution

Paul French, Professor

Photonics Group, Physics Department, Imperial College London

BACKGROUND

The Photonics Group in the Physics Department at Imperial College London develops instrumentation for multidimensional fluorescence imaging - spanning a wide range of applications, from super-resolved microscopy through automated fluorescence lifetime imaging for high content assays to endoscopy and optical tomography.

The availability of Scientific CMOS cameras has been transformative for their research because the technology provides unprecedented imaging performance with high resolution and high frame rates. The team particularly uses Scientific CMOS cameras for localization and light sheet microscopy.

> “The Prime 95B camera is specified to provide >95% quantum efficiency, giving us the advantages of Scientific CMOS with fantastic sensitivity.”

CHALLENGE

STORM[i, ii] for super-resolved microscopy is a particular interest and the team recently published an approach using low-cost diode lasers[iii]. “Our goal is to develop instruments that provide state-of-the-art performance while reducing the cost where possible so that more users are able to access such advanced imaging capabilities,” shared Professor Paul French.

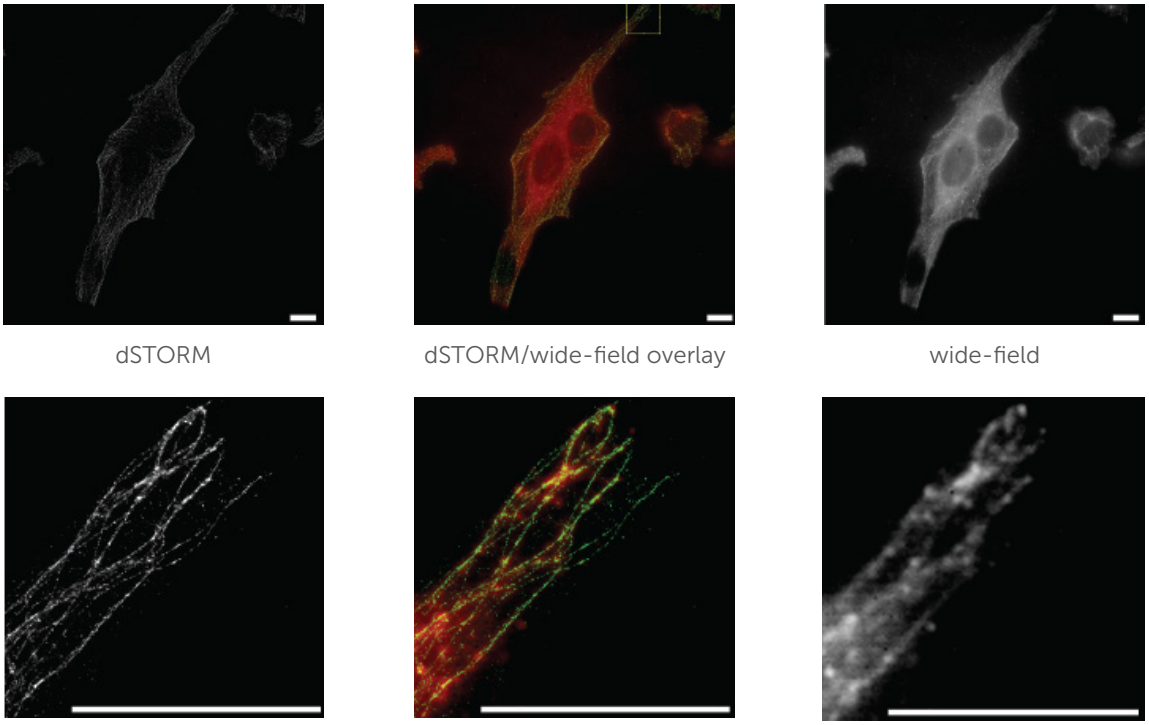
TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Prof. French explained, "Since the achievable resolution is a function of the number of photons detected, we were excited to learn about the back-illuminated Scientific CMOS camera from Teledyne Photometrics. The Prime 95B camera is specified to provide >95% quantum efficiency, giving us the advantages of Scientific CMOS with fantastic sensitivity."

When the group made a comparison of images taken with the Prime 95B and a standard Scientific CMOS camera, they were immediately impressed with the increase in signal to noise.

Prof. French concluded, "We look forward to implementing the Prime 95B camera in the new STORM microscopy platform that we are currently developing."



dSTORM | dSTORM/wide-field overlay | wide-field

Figure 1 NIH3T3 mouse embryonic fibroblast, starved overnight and treated with 1 µM Trichostatin A for 4h prior to fixation. Cell is stained by anti-acetylated tubulin with an Alexa Fluor 647 secondary antibody. Using a Cairn OptoTIRF system, 5000 image frames were taken with a 30ms exposure time which composed 2768763 individual localisations. Mean uncertainty is 11.54 nm. (Scale bars are all 1 µm).

REFERENCES

[i] M. J. Rust, M. Bates, and X. Zhuang, Nat. Methods 3, 793-796 (2006).

[ii] M. Heilemann, S. van de Linde, M. Schuttpelz, R. Kasper, B. Seefeldt, A. Mukherjee, P. Tinnefeld, and M. Sauer, Angew. Chem. Int. Ed. 47, 6172-6176 (2008).

[iii] K. Kwakwa, A. Savell, T. Davies, I. Munro1 S. Parrinello, M.A. Purbhoo, C. Dunsby, M.A.A. Neil and P.M.W. French, J. Biophotonics 9 (2016) 948-95, DOI 10.1002/jbio.201500324

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Super-Resolution Fluorescence Microscopy

Sang-Hee Shim, Principal Investigator and Assistant Professor of Chemistry

Shim Group, Center for Molecular Spectroscopy and Dynamics,

Institute for Basic Science, Korea University

BACKGROUND

The Shim Group at Korea University is an interdisciplinary lab covering physical chemistry, biophysics and cell biology.
Sang-Hee Shim, principal investigator and assistant professor of chemistry, leads a team composed of postdoctoral researchers and graduate students to develop new microscopic methods and apply them to answer complex biophysical questions.

Their core focus is to better understand life at the molecular scale by visualizing cell dynamics and the interactions of intracellular molecules. To do so, they explore the frontiers of optical microscopy with super-resolution fluorescence imaging.

> “For single-molecule images like in DNA-PAINT, the Prime 95B combined with PrimeEnhance allows us to conduct super-resolution imaging with higher spatial resolution than that of EMCCD technology.”

CHALLENGE

The Shim Group previously used EMCCD technology for localization-based super-resolution fluorescence microscopy. However, although EMCCD offers better sensitivity than sCMOS technology, it suffers from excess noise generated by the process of electron multiplication. The precision and resolution of their experiments are highly dependent on the sensitivity and noise level of the camera so this presents an issue.

The group investigated potential solutions and found the Prime 95B Scientific CMOS camera from Teledyne Photometrics - the first and only 95 percent quantum efficient CMOS device. The camera affords comparable sensitivity to EMCCD, yet offers far higher imaging speed and a larger field of view. After testing the camera, the group also found some cases in which it produced even better spatial resolution when compared to their existing EMCCD.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The team compared the Prime 95B to their EMCCD camera using single-molecule DNA-PAINT imaging and found that the camera gave improved localization precision. This is suggested to be because the Prime 95B does not rely on electron multiplication to increase sensitivity. By removing the excess noise factor generated by the electron multiplication process, the Prime 95B Scientific CMOS can achieve a higher signal to noise ratio than an EMCCD.

The team also investigated PrimeEnhance™, the active denoising algorithm that accompanies the Prime 95B camera. They found that PrimeEnhance can amplify some noise and produced false localizations. When the localization software was optimized for PrimeEnhance, the localization precision was further improved and gave the best results among all tested conditions.

Shim explains, "For single-molecule images like in DNA-PAINT, the Prime 95B combined with PrimeEnhance allows us to conduct super-resolution imaging with higher spatial resolution than that of EMCCD technology." Shim adds, "Plus, the Prime 95B offers the additional benefits of higher frame rate and a larger field of view."

Figure 1 Surface-immobilized DNA origami with 3 docking DNA strands with 80 nm gaps, imager DNA oligo transiently binding to the docking DNA strand, labelled with Atto 655

A. 1024x1024 EMCCD camera with a 130 nm effective pixel size 32x32 field. Acquired at 47 fps with 20 ms exposure, 30x EM gain. **B.** Prime 95B with a 110 nm effective pixel size. Acquired at 50 fps with 20 ms exposure time. **C.** Prime 95B plus PrimeEnhance with a 110 nm effective pixel size. Acquired at 28 fps with 20 ms exposure time.

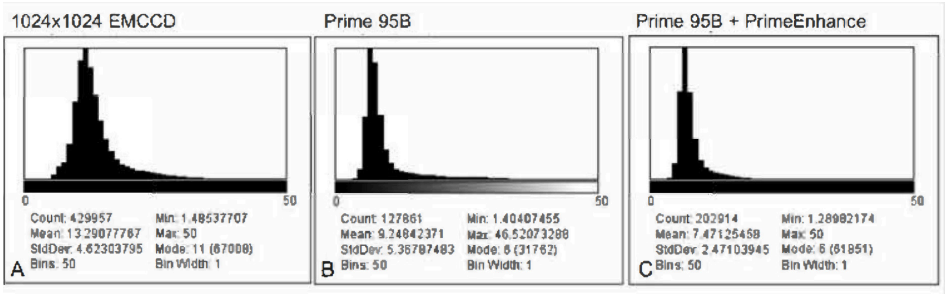


Figure 2 Localization accuracy analysis of the DNA origami

A. The 1024x1024 EMCCD camera shows a mean localization accuracy of 13.29 nm. **B.** The Prime 95B shows a mean localization accuracy of 9.25 nm. **C.** The Prime 95B plus PrimeEnhance shows a mean localization accuracy of 7.47 nm.

Additional information about the Shim Research Lab and their work is available at: http://sodaus.wixsite.com/shimku

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

STORM Super-Resolution Microscopy

Yandong Yin, PhD, Postdoctoral Fellow
Eli Rothenberg, PhD, Associate Professor
New York University, School of Medicine

BACKGROUND

The laboratory of Dr. Rothenberg at the New York University School of Medicine focuses on new optical methods to study biological molecules and processes at real time and nanometer scale. The Rothenberg research team studies the mechanisms of enzymes and proteins that participate in repair of DNA damage leading to cancer, and develops new imaging methods that will enable them to visualize the behavior of individual biological molecules. STORM Microscopy is used to localize and track DNA as it replicates in the cell. "We try to look at the nucleus of a cancer cells as they are replicating the DNA. The DNA and proteins involved in DNA replication are labelled so we can understand what is going on when replication happens," Yandong Yin, PhD. Postdoctoral fellow states.

> "If you have a shorter exposure time, you can track faster kinetics. More sensitivity and shorter exposure times with the Prime 95B allow you to image faster and track kinetics better."

CHALLENGE

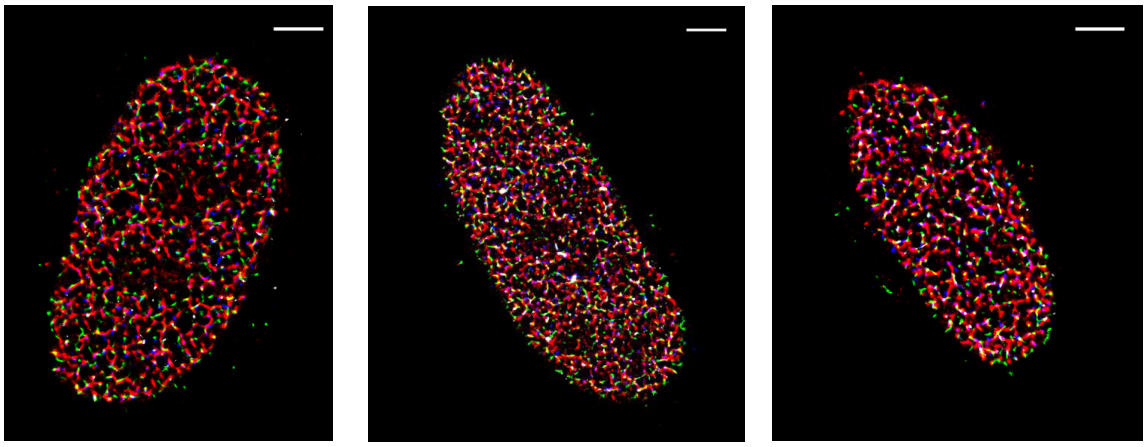
One of the challenges of imaging replicating DNA is that inside the nucleus of the cell there are many labeled components crowded together, as well as very small components that need to be clearly resolved. To determine how each component is organized spatially, the lab often performs STORM imaging using three or four colors sequentially, which makes resolution, sensitivity, and localization accuracy a great concern. "The DNA replication fork is very small. We can't image it without super resolution," says Yin. The laboratory calibrates their STORM post-processing conditions based on the variances of each pixel in the chip of the camera, correcting for any major variations, in order to better fit the point spread function of each fluorophore. Because of this, pixel to pixel variations, like those seen in patterned noise on CMOS cameras becomes a major problem.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The move away from EMCCD technology to the Prime 95B back-thinned CMOS was made easier because of the improved sensitivity in the detection of low-emission fluorophores, and the reduction in pattern noise when compared to other CMOS cameras. “For single-molecule localization imaging, the most important thing is the reconstruction process in how we fit each single-molecule point spread function into its centroid coordinate” Yin noted on his use of the Prime 95B. The post-processing of images collected with the Prime 95B are made significantly easier because of the reduction in pixel to pixel pattern noise, which makes the localization and reconstruction of fluorophores of multiple colors easier to do. Yin continued, “The variance for each pixel is much smaller than what is reported on other sCMOS cameras. We have found that more than 90% of the pixels fall within a very tight noise distribution.”

Additionally, the large field of view coupled with the improved sensitivity allows the team to get an image that contains useful information more often. Since they often work with samples that are dark at the start of acquisition, it was an issue that they couldn’t guarantee seeing something with the smaller field of view of an EMCCD. Yin says “Previously we used an EMCCD, but the EMCCD has a smaller chip. With a bigger chip we can see multiple cells simultaneously.”



Cell Type: U2OS cells
Exposure time: 30 ms
Magnification: 150 times (the configured pixel size is ~ 73 nm)
Reconstruction Algorithm Used: Maximum Likelihood Estimation (MLE) method for single PSF fitting

Learn more about the Rothenberg Group at New York University:
https://med.nyu.edu/biomolpharm/research/biochemistry-macromolecules/eli-rothenberg

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Super-Resolution Fluorescence Microscopy

Ke Xu, Principal Investigator and Assistant Professor of Chemistry

Ke Xu Group, University of California Berkeley, College of Chemistry

BACKGROUND

The Ke Xu Group at Berkeley is an interdisciplinary lab that combines biophysics, physical chemistry and cell biology. Their goal is to understand how orders emerge in biological systems at the nano-meter scale from the interaction between biomolecules. They achieve this goal experimentally through the development and synergistic application of innovative quantitative methods such as super-resolution fluorescence microscopy.

Ke Xu, principal investigator and assistant professor of chemistry, successfully opened his lab in 2013 and today, leads a team that includes post-doctoral researchers and graduate students. The team recognizes and respects how living systems achieve versatile structural organizations at the nanoscale. Their dedication to gaining a greater understanding of this phenomenon has led to their ability to consistently achieve publication of their research findings.

> “[Prime 95B] allows us to conduct our STORM experiments with higher frame rates... 95 percent quantum efficiency allows for super-resolution imaging that’s not achievable with conventional sCMOS cameras.”

CHALLENGE

Previously, EMCCD technology was the primary imaging solution in the Ke Xu Group. However, STORM experiments presented increasing demands on the existing imaging setup. The team decided to look at other technologies, specifically sCMOS solutions due to new advancements in CMOS sensors and more advanced capabilities becoming available.

Having reviewed available products, the team found the Prime 95B Scientific CMOS camera from Teledyne Photometrics. The company was touting the first and only 95 percent quantum efficiency sCMOS camera which piqued their interest. Having the opportunity to fully test the camera, they discovered it offered comparable, if not better, results when compared to their existing EMCCD.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

When comparing to the EMCCD camera that was used previously, the Prime 95B provides many more benefits; faster imaging, comparable spatial resolution and a larger field of view. “The Prime 95B Scientific CMOS camera allows us to conduct our STORM experiments with higher frame rates and a larger field of view than with EMCCD technology,” Xu shares. “Plus, the 95 percent quantum efficiency allows for super-resolution imaging that’s not achievable with conventional sCMOS cameras,” he adds.

Figure 1
Prime 95B
Scientific CMOS
Camera Test
ß13-tubulin-AF647
160 Hz
50k frames

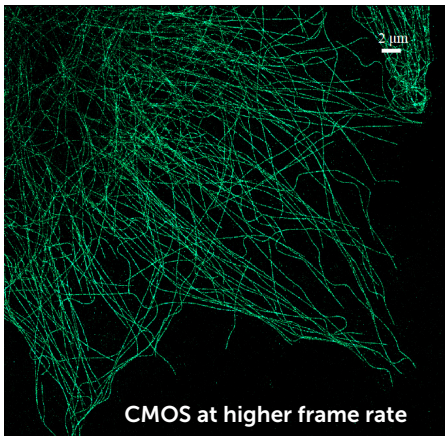
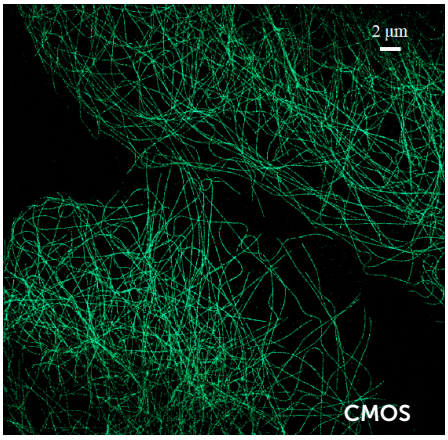
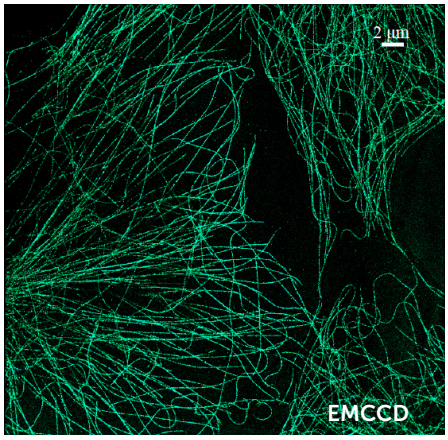


Figure 2
Prime 95B Scientific
CMOS vs EMCCD
Camera Test
ß 13-tubulin-AF647
110 Hz
50k frames





Learn more about the Ke Xu Group at the University of California Berkeley:
http://www.cchem.berkeley.edu/xuklab/

Images courtesy of Rui Yan and Ke Xu, UC Berkeley

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Super-Resolution Fluorescence Microscopy

Dr. Kyle M. Douglass, Post-Doctoral Researcher
The Laboratory of Experimental Biophysics EPFL
Suliana Manley Lab, Lausanne, Switzerland

BACKGROUND

Dr. Kyle M. Douglass, a research scientist at the EPFL, has spent the past several years developing high-throughput and automation methods for super-resolution fluorescence microscopy. The Laboratory of Experimental Biophysics, which is led by Prof. Suliana Manley, uses these techniques to study the structural biology of multi-protein complexes such as chromatin foci, the bacterial division machinery, and the centrosome.

From the perspective of the technology, these structures share a common theme in that they require large datasets of high quality images to computationally combine into a structural model which can possibly consist of one or more disordered components. It is therefore imperative to acquire as much data as possible and to ensure that it meets the exacting standards required by the computational reconstruction pipelines.

> “We can now more precisely locate each fluorescent dye that is targeting a protein within a complex. This has the effect of improving the resolution of our structural models, allowing us to see details inside these complexes that we could not before.”

CHALLENGE

These multiprotein complexes are well-suited to super-resolution approaches like STORM and PAINT because they are too small to see with traditional light microscopy. Furthermore, their rich and often heterogeneous composition precludes a complete study with electron microscopy but this problem is easily overcome with multi-color super-resolution. Unfortunately, not every available dye is bright and stable. The quality of the measurements depends critically on how many photons can be recorded from each dye molecule, which means that the protein maps that are reconstructed from weak dyes will suffer from a loss of resolution and quality.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Because every photon counts, Douglass and colleagues upgraded their cameras to the Teledyne Photometrics Prime 95B. The high sensitivity and large field of view allows the researchers to simultaneously image numerous structures at the same time while capturing even more photons than before. The increased throughput and quality of the data is paying dividends.

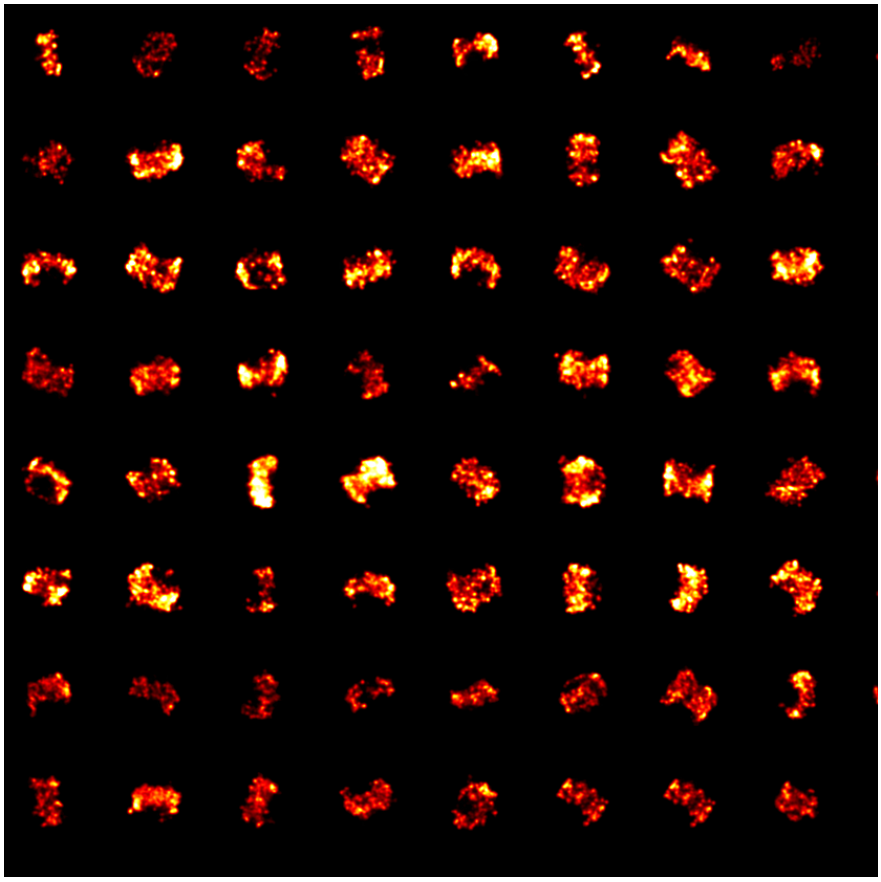


Figure 1 Image shows a montage taken from a particle library showing images of centriolar protein Cep152. Each particle is from a single centriole. The raw library typically consists of over 1 ,000 particles.

Learn more about the work of Dr. Douglass at EPFL:
http:/ /kmdouglass.github.io

Visit the Laboratory of Experimental Biophysics at EPFL:
http://leb.epfl.ch/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

SRRF and Super-Resolution Microscopy

Dr. Ricardo Henriques, LMCB Group Leader, UCL Senior Lecturer, Experimental Optics Leader

Medical Research Council Laboratory for Molecular Cell Biology (LMBC)

University College London (UCL)

BACKGROUND

The Henriques group use various super-resolution microscopy techniques to investigate cell signalling and host-pathogen interactions as well as creating and developing technology for cell biology research.

A big challenge in super-resolution microscopy is the requirement for intense illumination but this is usually phototoxic and incompatible with live-cell imaging. To tackle this problem the group developed a new approach – Super-Resolution Radial Fluctuations (SRRF) – which enables super-resolution imaging using any fluorophore with far lower illumination intensities than conventional super-resolution techniques.

> “The Prime 95B Scientific CMOS is an outstanding camera, particularly due to its low-noise, high-sensitivity and large field-of-view. ”

CHALLENGE

The Henriques group recently started using the Prime 95B Scientific CMOS camera for some of their work. Dr. Henriques told us, “We’ve been activeley using the Prime 95B as one of out main cameras for low-signal and super-resolution imaging at UCL. The Prime 95B Scientific CMOS is an outstanding camera, particularly due to its low-noise, high-sensitivity and large field-of-view.”

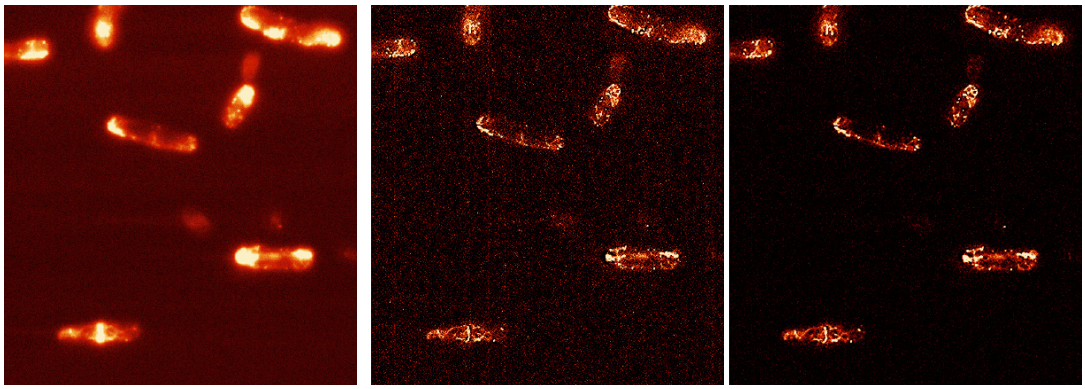
The group is also using the Prime 95B as they adapt the SRRF algorithm for use with CMOS cameras. Dr Henriques explains, “Super-resolution microscopy using our SRRF method was designed for EMCCD cameras but we are currently updating the SRRF algorithm for improved quality and performance when using data from modern CMOS devices to take advantage of the large field of view and higher speeds available.”

The accuracy of SRRF is directly related to the speed of acquisition so a faster camera would be advantageous.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The updated algorithm is expected to be released soon. Below are images obtained with the Prime 95B to show how SRRF is evolving to work with Scientific CMOS sensors:



Widefield | NanoJ-SRRF | SRRF4CMOS

Figure 1 LifeAct-GFP labelled fission yeast

Left: Widefield image
Middle: Image after applying the conventional NanoJ-SRRF algorithm
Right: Image after applying the in-development novel version NanoJ-SRRF algorithm optimized for CMOS devices (to be named SRRF4CMOS).

All images acquired with the Prime 95B scientific CMOS camera with a 10 ms exposure time (yielding 1 super-resolution frame per second).

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Confocal Microscopy and STORM

Uri Manor, Biophotonics Core Director

Salk Institute for Biological Studies, Biophotonics Core Facility

BACKGROUND

The Salk Institute is home to a highly collaborative cadre of scientists who delve into a broad range of research areas, from aging, cancer and immunology to diabetes, brain science and plant biology. The group is supported by on-campus research centers and core facilities that are equipped with cutting-edge technology.

The Institute embodies Jonas Salk's mission to dare to make dreams into reality by exploring the very foundations of life, seeking new realities in neuroscience, genetics, immunology and more. The team lives to discover, be it cancer or Alzheimer's, aging or diabetes, they understand that every cure has a starting point. Salk is where cures begin.

Uri Manor, biophotonics core director works with the Salk researchers to provide collaborative support for a wide variety of research projects that require scientific imaging. Manor also works with the faculty steering committee to incorporate new and advanced imaging technologies into the repertoire of resources offered through the Biophotonics Core Facility.

> "The Prime 95B provides the speed, field of view and resolution of a CMOS camera, with the added sensitivity of an EMCCD camera for our more demanding experiments."

CHALLENGE

The Facility provides technical and logistical access to Salk faculty, enabling the integration of imaging tools into a variety of biological research programs. To maintain its ability to advance science, the Facility must maintain the latest, cutting-edge commercial imaging and data analysis technologies available. This is especially important given that most projects involve sophisticated and complex research techniques.

The primary instrumentation that core researchers must have access to include technologies that support confocal microscopy (both fixed and live cell), TIRF microscopy, two-photon microscopy, electron microscopy and super-resolution microscopy as well as in-vivo imaging modalities.

TELEDYNE IMAGING
Everywhereyoulook™

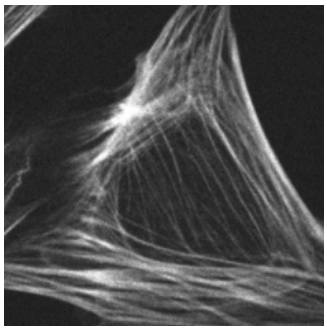
SOLUTION

To continue supporting the diverse work of the Salk researchers, Manor must stay abreast of the advancements being made in scientific imaging. It is important that the team always have access to the newest and most advanced solutions.

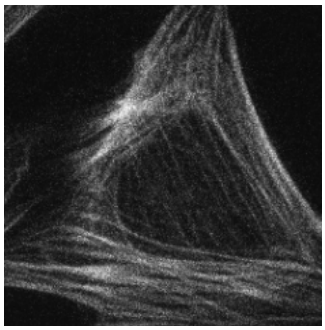
Manor learned of the Prime 95B Scientific CMOS camera from Teledyne Photometrics and was interested in learning more about the claim for 95 percent quantum efficiency. Of greater importance was how the camera was being touted as the most sensitive in the industry - sensitivity is always in high demand among Salk researchers.

After seeing the camera and testing it with his own samples, Manor realized the camera did stand up to the hype and would integrate well into the Facility's imaging tools. The back illuminated technology and high QE make the camera exceptionally versatile. Manor shares, "The Prime 95B provides the speed, field of view and resolution of a CMOS camera, with the added sensitivity of an EMCCD camera for our more demanding experiments."

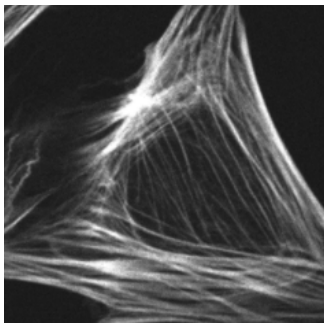
The Prime 95B easily supports multiple scientific applications, this flexibility also makes it a very good investment for the Salk Institute.



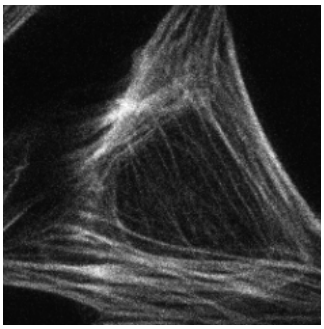
25 ms
Scientific CMOS



25ms, 400x EM
gain EMCCD



50 ms
Scientific CMOS



50ms, 400x EM
gain EMCCD

Figure 1 Images depict the actin cytoskeleton as stained by AlexaFluor488-Phalloidin. EMCCD used was the Teledyne Photometrics Evolve 512 and the Scientific CMOS was the Teledyne Photometrics Prime 95B. Images were captured using Micro-Manager on a Zeiss Confocal microscope with a Yokogawa spinning disk scan head. 25 ms and 50 ms exposures were acquired with laser power set to the minimum of 5 % with the EM Gain of the EMCCD set to 400.

Learn more about the Salk Institute for Biological Studies:
http:/ /umanor@salk.edu

View Uri Manor's professional profile at Salk Biophotonics Core:
http://www.salk.edu/science/core-facilities/advanced-biophotonics/faculty-staff/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Spinning Disk Confocal

Peter March, Senior Experimental Officer

University of Manchester, Bioimaging Facility

BACKGROUND

The research being performed at the University of Manchester has a real-world impact beyond the lab. The team is at the forefront of the search for solutions to some of the most pressing issues in biology, medicine and health. The Bioimaging Facility delivers a broad range of state-of-the-art imaging solutions to the University, Faculty of Biology, and Medicine and Health. A key technology used in biological imaging of live cells is Spinning Disk Confocal Microscopy. Spinning Disk allows for long-term, high-speed, three-dimensional imaging of live samples with multiple channels of illumination.

> "The Prime 95B is the perfect camera for Spinning Disk - the image quality is a big improvement over our EMCCDs, and the field of view makes samples much easier to find."

CHALLENGE

One of the primary reasons for using Spinning Disk Microscopy is to generate confocal images without photobleaching or damaging live samples. "Bright cells are not necessarily healthy cells," warns Peter March, senior experimental officer at the university. "Using less GFP in cells matches their natural behavior more closely," he adds.

Correspondingly, sensitivity is among the most important features of a camera. Until recently, and due to their ability to achieve >90% quantum efficiency, EMCCD cameras were the preferred imaging device for Spinning Disk Microscopy. March and his team typically use a 60x objective, resulting in the need to address the large pixels of an EMCCD, which require extensive optical adjustments to reach acceptable sampling levels. This severely limits field of view, making samples harder to find and capture. Additionally, EMCCD cameras cause excessive, visible noise across the sample, even at high exposures.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The Prime 95B back-illuminated Scientific CMOS is the perfect match for Spinning Disk Microscopy because it delivers much greater image quality at a higher resolution than possible with a EMCCD camera. In addition, its sensitivity is much higher than other CMOS cameras currently on the market. March is most impressed with the increase of field of view as he shares "With EMCCD cameras, finding the sample was often an issue. Field of view is all-important, and the Prime 95B is a big improvement here."

Without the excess noise factor of EMCCDs or the pattern noise seen in 2x2 binned front-illuminated sCMOS, March says, "The difference in image quality is huge". The Prime 95B provides the ability to capture more of a sample at equal exposure times compared to EMCCD cameras, and it produces more impressive images as a result.

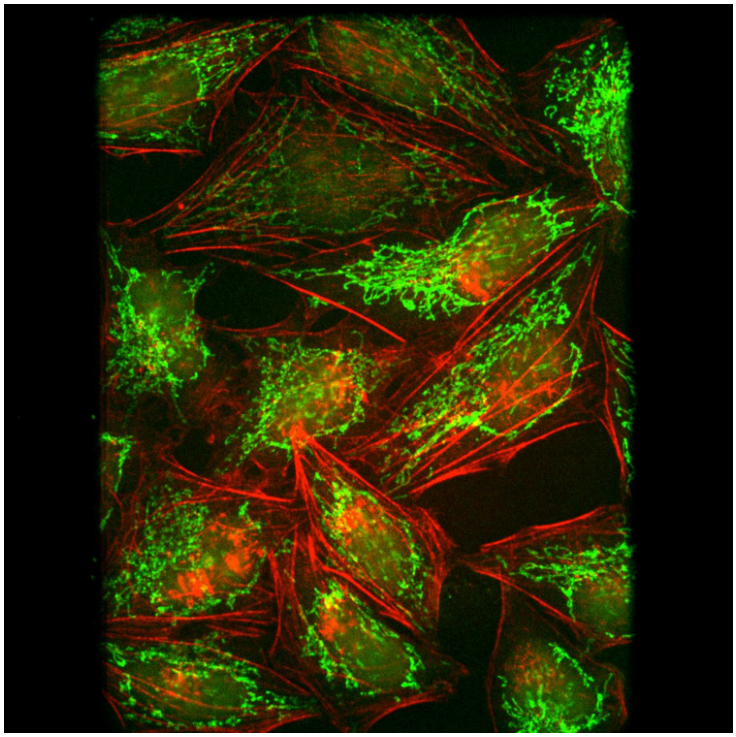


Figure 1 Mitochondria (green, 488nm) and Actin filaments (red, 566nm).

Sample prepared by Viki Allen, imaged using a Yokogawa CSU-X1 with a 63x oil, 1 .4NA objective.

Additional information about the research being performed in the Bioimaging Facility at the University of Manchester is available at
https:/ /www.bmh.manchester.ac.uk/research/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Structured Illumination Microscopy (SIM)

Guy Hagen, PhD, Research Associate

University of Colorado, Colorado Springs

BACKGROUND

Dr. Guy Hagen, Research Associate from the University of Colorado, Colorado Springs creates high performance image reconstruction methods and open-source software to process super-resolution microscopy data.

In 2014 Dr. Hagen released ThunderSTORM, an ImageJ plug-in for automated processing of photo-activated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) data[1]. In 2016, he introduced SIMToolbox, a MATLAB toolbox for processing SIM data. It has the flexibility to process 2D and 3D images, including both optical sectioning and super-resolution applications, and can be used on data acquired from commercial systems[2].

SIMToolbox also includes maximum a posteriori probability estimation (MAP-SIM), a super-resolution restoration method that suppresses out of focus light, improves spatial resolution, and reduces reconstruction artifacts[3].

Dr. Hagen is currently developing live cell imaging using SIM, and is continuing to develop data analysis methods for super-resolution microscopy.

> “Imaging twice as fast with the Prime 95B is a great advantage. The reduction of pixel-to-pixel variance and the reduction of visible column structure in the camera greatly improves our results.”

CHALLENGE

SIM is a widefield fluorescence technique that uses illumination patterns with high spatial frequency to illuminate samples. Algorithms applied to a combination of images taken with different phases and orientation of the illumination pattern are used to reconstruct a high-resolution image.

Reconstruction of images routinely requires many images per focal plane. Fluorescent signals need to withstand photobleaching and the acquisition rate must be fast enough to observe live cell dynamics.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Because multiple images are required for the reconstruction of SIM data, a sensitive camera will allow for shorter exposure times and faster acquisitions, reducing phototoxic and photobleaching effects on samples.

The back-illuminated Prime 95B Scientific CMOS camera has a near perfect 95% quantum efficiency and large 11 µm pixels, making it extremely sensitive. The Prime 95B allowed Dr. Hagen to reduce exposure times to acquire a set of SIM images in about half the time required with typical sCMOS cameras.

Pixel-to-pixel and column-to-column gain variations which are present in typical sCMOS cameras can degrade SIM images during the reconstruction process. The Prime 95B reduces image artifacts by implementing several pixel noise filters to detect and correct dynamic fluctuations, and the static variation in gain and offset is calibrated for every pixel. Because of this, raw SIM images have a reduction in noisy pixels and visible readout lines, resulting in higher quality processed SIM images.

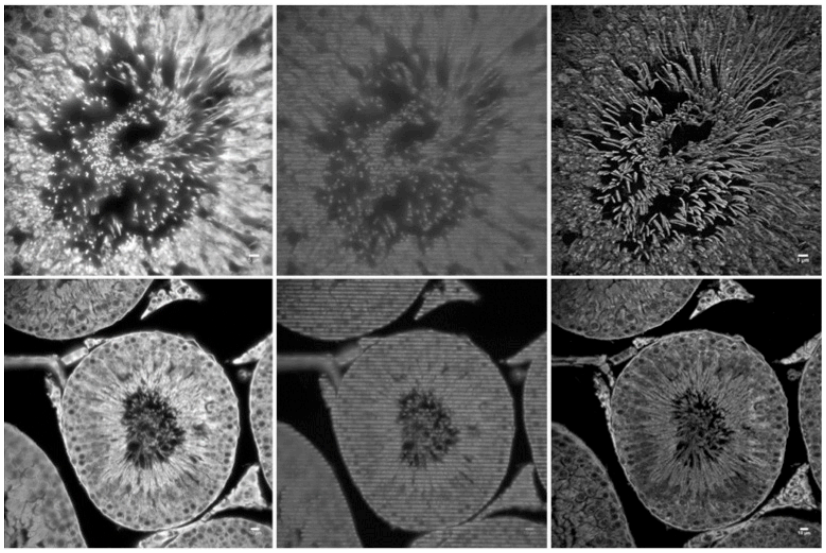


Figure 1 Cross section of a rabbit seminiferous tubule acquired using Dr. Hagen's structured illumination microscopy system and a Prime 95B camera with a 100X/1.47NA objective (top row) or 40x/1.3NA objective (bottom row). Images were reconstructed using SIMToolbox [2]. Conventional widefield fluorescence images (left) display out of focus light, raw SIM images (center) display the illumination pattern, and the 3D SIM reconstruction (maximum intensity projection, right) shows high quality, optically sectioned images.

References

1 Ovesny, M., Krfzek, P., Borkovec, J., Švindrych, Z., and Hagen, G. M. ThunderSTORM: a comprehensive ImageJ plugin for PALM and STORM data analysis and super-resolution imaging. Bioinformatics (2014), doi: 10.1093/bioinformatics/btu202

2 Krfzek P, Lukes T, Ovesny M, Fliegel K, Hagen GM. (2016). SIMToolbox: a MATLAB toolbox for structured illumination fluorescence microscopy. Bioinformatics (2016), doi: 10.1093/bioinformatics/btv576

3 Lukes T, Krfzek P, Švindrych Z, Benda J, Ovesny M, Fliegel K, Kif ma M, Hagen GM. Three-dimensional super resolution structured illumination microscopy with maximum a posteriori probability image estimation. Opt Express (2014), doi: 10.1364/0E.22.029805

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule Fluorescence Imaging

Anders Kyrsting, Post-doc

The Linke Group, University of Lund, Sweden

BACKGROUND

The Linke Group at the University of Lund, Sweden, creates artificial molecular motors and nanowires to better understand the role of biological motors in cellular processes such as cargo transportation, muscle contraction and cell division. The group tags the motors with quantum dots and images them using single molecule TIRF. They have further plans to expand their investigation with optical trapping and STORM super-resolution microscopy.

> “I don’t think I’d use an EMCCD again, I don’t know why I’d use it with the performance we get out of the Prime 95B.”

CHALLENGE

Anders Kyrsting, post-doc with the Linke Group, explained, “Molecular motors move very fast so to track them, we need a camera with a very high frame rate.” The group was previously using an EMCCD camera but the slower frame rate of its architecture meant that they had very limited temporal resolution.

Kyrsting continued, “Investigating single molecules means working with very low fluorescence signal. So, a camera with high sensitivity is equally as important as our need for a fast camera.” For this reason, the group couldn’t afford to sacrifice sensitivity for speed.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The near-perfect 95% quantum efficiency and high speed of the Prime 95B Scientific CMOS camera made it a clear fit for their work and the group was excited to implement the camera into their system.

Kyrsting told Teledyne Photometrics, "The Prime 95B enabled us to reach the 200 fps speed that we needed with a far larger field of view than would be possible on any EMCCD camera."

Additionally, with the large 11 µm pixel of the Prime 95B, the group could achieve perfect diffraction limited resolution with a 100x objective, without using any additional optics.

Kyrsting continued, "The speed and sensitivity were exactly what we were looking for and the bonus of having such a large field of view has really helped our data throughput." In conclusion, Kyrsting added, "I don't think I'd use an EMCCD again, I don't know why I'd use it with the performance we get out of the Prime 95B."

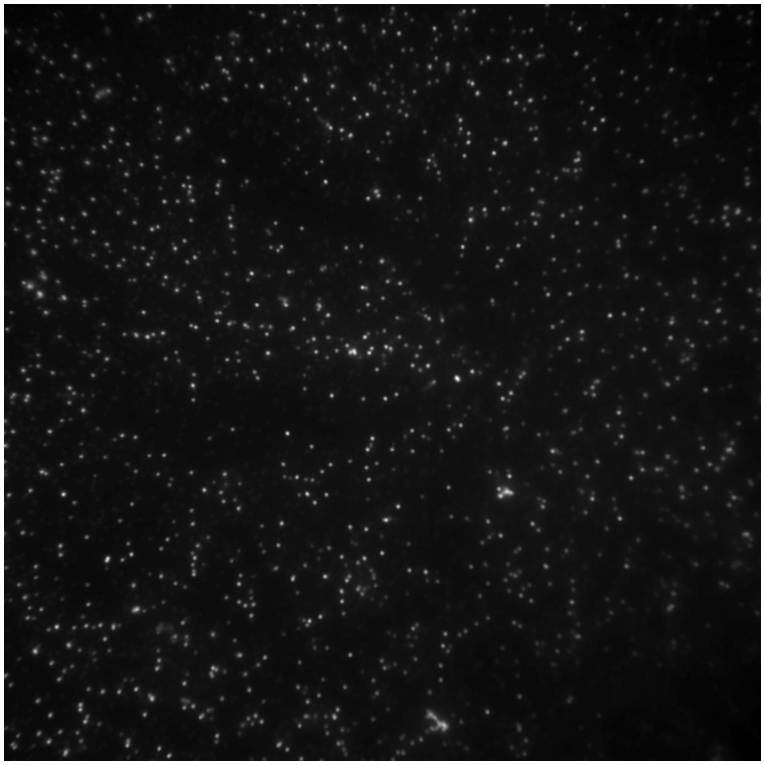


Figure 1 Alumina (Al2O3) coated gallium phosphide (GaP) nanowires functionalized with biotinylated BSA (bovine serum albumin). Streptavidin labelled with three fluorescent dyes (FITC, TRITC and Cy5), imaged under 100x magnification.

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Total Internal Reflection Fluorescence (TIRF)

Shiaulou Yuan, Ph.D.

Postdoctoral Associate in Pediatrics (Cardiology)

Yale University

BACKGROUND

The Martina Brueckner lab at Yale University School of Medicine studies the genes of children with congenital heart disease. "We take a human genetics approach combined with animal models, such as mouse and zebrafish, and in vivo imaging. In particular, we do a lot of live imaging of cilia, hair-like structures on the cell surface that function as cellular antennas," Shiaulou Yuan, a postdoctoral researcher explained.

"The cilium is a tiny organelle that is packed with hundreds of distinct signaling molecules. We know these must be important because mutations in genes that are important for cilia biogenesis or signaling can cause congenital heart disease. The challenge is that for many of these genes, we simply don't know how they can cause congenital heart disease. To understand how these genes are functioning requires us to create a whole-animal, as well as apply high-resolution live imaging approaches, that are guided by human genetics. It's exciting because it's the type of science that can only be done nowadays rather than twenty years ago, because of the remarkable advances in genomic and imaging technologies. It all comes together to enable us to understand the problem from new angles."

> "The quantum efficiency of the camera is a really important factor for us. If we can use less excitation power, we can increase the length of our imaging and minimize photodamage to the animal. The sensitivity of the Prime 95B is truly transformative for our type of work."

TELEDYNE IMAGING
Everywhereyoulook™

CHALLENGE

Image collection for Dr. Yuan relies not only on sensitivity and resolution, but also fast speeds. "Much of work depends on genetically encoded biosensors or GFP-knock-ins, which are endogenously tagged with a single copy of GFP. They are not that bright, but at the same time, we are also doing live imaging of mouse or zebrafish embryos that require rapid image acquisitions over several hours as the animals happily develop. We have a limited photon budget, yet we must capture a lot of images very rapidly and over a long period of time. We also need high resolution because we're looking at tiny cilia, but also speed, because they move very fast. Finally, on top of all this, we must keep the laser excitation power low because the animal has to stay alive during all of this - the imaging needs to be gentle."

SOLUTION

"The quantum efficiency of the camera is a really important factor for us. If we can use less excitation power, we can increase the length of our imaging and minimize photodamage to the animal. The sensitivity of the Prime 958 is truly transformative for our type of work. Besides the sensitivity, the combination of speed and resolution of the Prime 958, which is superior to an EMCCD, makes it killer for our experiments. In fact, we have been limited in the past due to insufficient camera technologies. With the Prime 958 on hand, we are now able to attack these questions."

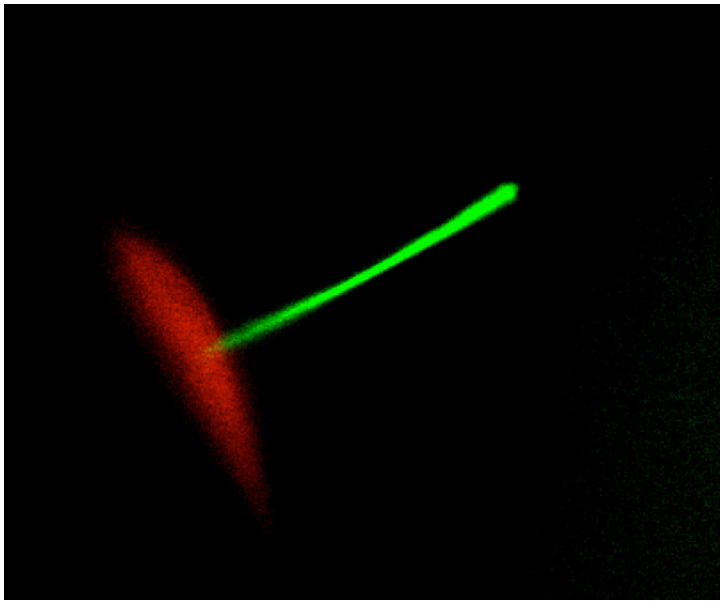


Figure 1 A live image of a cilium from a mouse cell expressing a genetically encoded calcium biosensor (green) and a membrane localized fluorophore (red).

Recording by Shiaulou Yuan and Mohammed Mahamdeh using a Zeiss 63X 1.2NA water immersion objective.

Learn more about the Martina Brueckner lab at Yale University School of Medicine: https://medicine.yale.edu/pediatrics/cardiology/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule TIRF Microscopy

Aleks Ponjavic, Postdoctoral Researcher

Klenerman Group, University of Cambridge

BACKGROUND

The Klenerman group investigates intracellular signalling in T-cells, a vital component of the human adaptive immune response. They are particularly interested in the kinetic-segregation model of T-cell signalling which proposes that signalling is only possible when CD45 molecules on the T-cell surface are sterically excluded from the T-cell receptor site.

The group observes these cell-surface molecules using single molecule TIRF microscopy to add further structural support for the kinetic-segregation theory.

> “I find the performance of the Prime 95B to be comparable to state-of-art EMCCDs.”

CHALLENGE

The group needs to ensure reliable detection of single molecules so high signal to noise is of great importance. To achieve this, they have been using the Teledyne Photometrics Evolve® 512 EMCCD camera but made the decision to purchase a Prime 95B because of its high sensitivity combined with the multiple benefits of CMOS architecture. Aleks Ponjavic, postdoctoral researcher with the Klenerman group, told us, “I was very interested to compare the high sensitivity of the Prime 95B to an EMCCD for single molecule imaging.”

CHALLENGE

When asked how the Prime 95B compared to an EMCCD camera, Aleks told us, “I find the performance of the Prime 95B to be comparable to state-of-the art EMCCDs but at lower cost and higher speed.” He went on to say, “I would definitely choose the Prime 95B over an EMCCD for any high sensitivity application that would benefit from the high speed offered by a CMOS camera.”

TELEDYNE IMAGING
Everywhereyoulook™

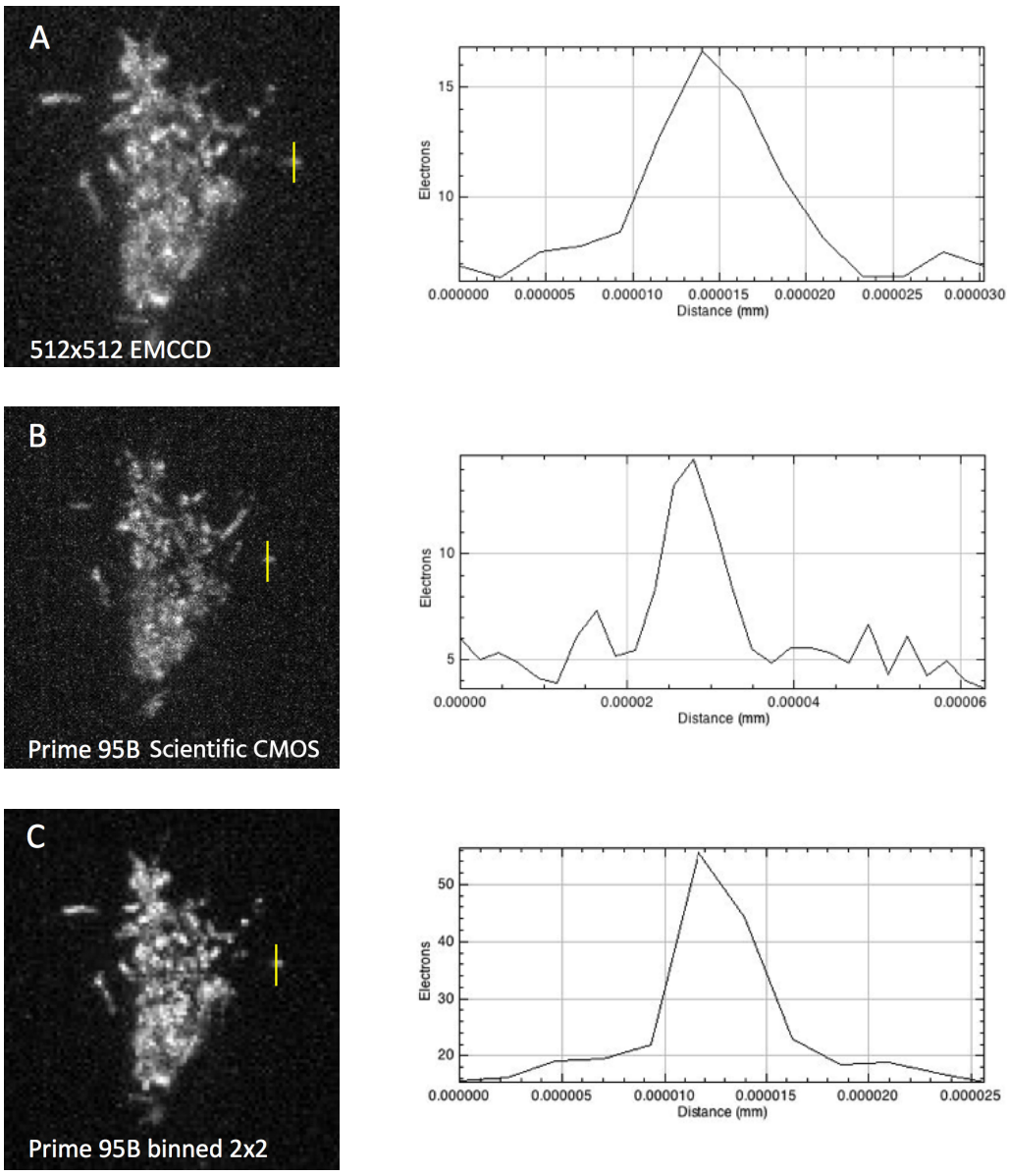


Figure 1: Jurkat T-cells bound to fibronectin. CD45 molecules on the cell surface labeled with Alexa 488.

A. *Left*: Maximum intensity projection of 500 frame 33 ms exposures taken with the Evolve Delta EMCCD camera using 250x EM gain. *Right*: Line profile taken through the marked area of the image (yellow line).

B. *Left*: Maximum intensity projection of 500 frame 33 ms exposures taken with the Prime 95B. *Right*: Line profile taken through the marked area of the image (yellow line).

C. *Left*: Maximum intensity projection of 500 frame 33 ms exposures taken with the Prime 95B binned 2x2. *Right*: Line profile taken through the marked area of the image (yellow line).

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule Microscopy

Prof. Mark Leake

Professor Anniversary Chair of Biological Physics

University of York

BACKGROUND

Prof. Leake founded and leads the Biological Physical Sciences Institute at the University of York, which brings together scientists researching the biomolecular interactions, biological modelling imaging and quantitation of complex data. His work targets a broad range of fundamental processes and open questions in biology, and has provided insight into topics such as the behavioural mechanics of the flagellar motor of bacteria, protein transport, DNA replication, repair and remodelling, signal transduction, gene regulation and oxidiative phosphorylation.

> “Altogether there is no better for cutting edge single molecule microscopy than the Prime 95B Scientific CMOS.”

CHALLENGE

Prof. Leake’s lab is at the forefront of developing new biophysical instrumentation to probe cells and biological specimens on a single-molecule level. Single-molecule imaging is a hugely powerful cutting-edge technique for examining fundamental processes and interactions in biological systems. However, the technique is also among the most demanding low-light imaging applications, as the fluorescent response from a single fluorophore is very low, and photobleaching must be avoided.

Determining the precise quantity and localization of single-molecule signals requires an excellent signal to noise ratio for the weakest of signals, while simultaneously delivering high spatial and temporal resolution.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The Prime 95B Scientific CMOS camera uses back illumination to reach near-perfect quantum efficiency, which, combined with low readout noise and large pixels, provides the sensitivity required for single-molecule fluorescence. “The Prime 95B combines the speed of a CMOS camera with sensitivity at or better than an EMCCD, allowing us to push single-molecule microscopy into larger specimens, imaging even faster phenomena than before,” Prof. Leake tells us.

“The camera is extremely easy to set up, with very few settings so you can get straight to imaging. It even includes its own programmable logic outputs which allow the 95B to control and synchronize with other components on the microscope. Altogether, there is no better camera for cutting-edge single-molecule microscopy than the Prime 95B Scientific CMOS.”

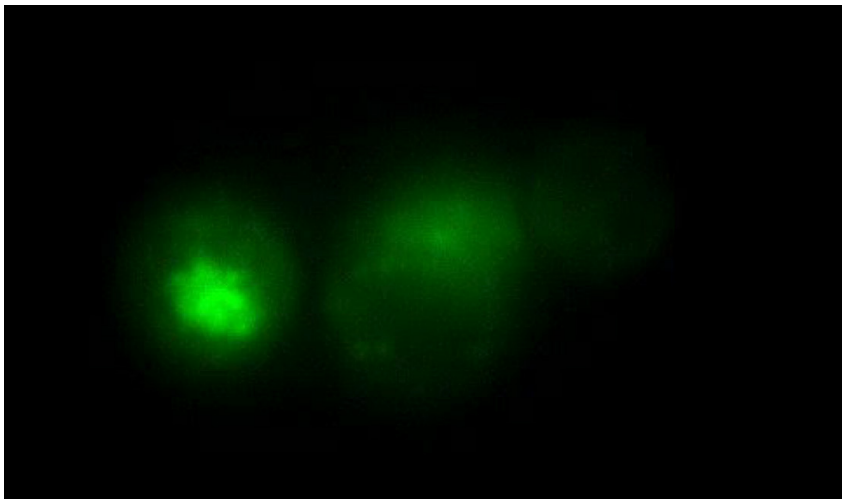


Figure 1 Live yeast cells displaying GFP labeled Mig1, an essential transcription factor, at millisecond sampling and single-molecule sensitivity.

Additional information is available at: http://single-molecule-biophysics.org/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule Biophysics and DNA Mechanics

Dr. Christoph Baumann

Lecturer in Molecular Biophysics

Department of Biology, University of York

BACKGROUND

Dr. Christoph Baumann, Lecturer at the University of York, Department of Biology and his group work with advanced imaging techniques to push forward our understanding of spatio-temporal dynamics in the bacterial cell envelope. Using a Teledyne Photometrics camera, the group was the first to observe that, contrary to expectations, proteins in the outer cell membrane don't diffuse significantly when tracked, and that new proteins are inserted predominantly at mid-cell during growth[1]. This means that bacteria can very quickly turnover their outer membrane proteins to adapt to new environmental challenges during growth, and this work initiated a new investigation of inter-membrane crosstalk in the Gram-negative bacterial cell envelope[2].

> "Better temporal and spatial resolution means better quality data. We'll definitely be using the Prime 95B Scientific CMOS camera for our upcoming experiments."

CHALLENGE

To pursue this research area, Dr. Baumann and his colleagues use TIRF microscopy alongside laser scanning confocal FRAP microscopy. When tracking single molecules, sensitivity and speed are all-important. "Better temporal and spatial resolution means better quality data," Dr. Baumann shares. Previously, the large pixels, slow speed, and the excess noise factor of their EMCCD camera limited both these aspects. Further, the small sensor size and the need to use a 1.6x magnification optic to match pixel size lead to a very small field of view, and using additional lenses lost them precious light.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The Teledyne Photometrics Prime 95B Scientific CMOS camera was the perfect fit for the team's research, with the 11 µm pixels giving optimal resolution paired with the 100x objectives used for TIRF microscopy. The faster speed and higher signal to noise ratio of the back-illuminated CMOS also provided better temporal resolution. "This increased resolution is of great benefit for all the research we do, not just this experiment," Dr. Baumann told us. "Since the camera is USB3.0, it's very easy to move the camera to other setups for short periods."

The huge field of view of the Prime 95B is not only useful for increasing throughput, but allows the team to use single-camera optical splitting technology. Dr. Baumann concluded, "We're going to use a polarization splitter in an upcoming experiment, and the Prime 95B's chip is large enough to still have a great field of view with both images side by side."

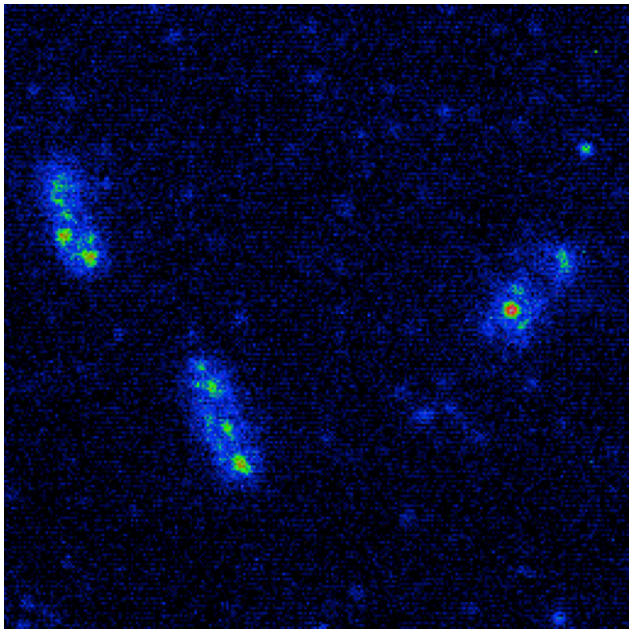


Figure 1: Alexa Fluor 488-labelled colicin E9 protein molecules bound to extracellular face of BtuB transmembrane receptors in the outer membrane of live Escherichia coli JM83 cells (256 x 256 pixels2, 16.9 x 16.9 µm2, 30 frame sum, video data collected with 33 ms exposure and global shuttering).

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Light Sheet and Single Molecule Tracking

Dr. Martin Lenz, Senior Research Associate
Cambridge Advanced Imaging Centre (CAIC)
University of Cambridge

BACKGROUND

The Cambridge Advanced Imaging Centre (CAIC) at the University of Cambridge develops modern imaging techniques to answer some of the most pressing and challenging biological questions. Keeping in mind the needs and demands of biologists, one of the current developments is a localization based 3D super-resolution microscope. One of its applications include investigation of Notch pathway transcription factor dynamics in Drosophila salivary gland cells and mapping out the arrangement of chromatin inside Drosophila spermatocytes. Working in close collaboration with biologists requires CAIC to adapt and apply technological advancements in biomedical imaging to answer some of the most challenging questions in the field.

> “The high QE of the Prime 95B will allow us to improve our investigation of protein dynamics and extend single molecule tracking to more challenging samples.”

CHALLENGE

The research team uses single molecule tracking (SMT) to explore protein dynamics in living tissues of Drosophila and Zebrafish. A complete picture of different diffusing populations require images with high signal to noise ratio (SNR) at low excitation laser powers and short exposure times. One of the key points for achieving this is an efficient collection and detection of emitted photons.

To investigate the architecture of chromatin in Drosophila spermatocytes the team uses single molecule detection in combination with double-helix point spread function (DHPSF). This can give high-resolution in all three spatial dimensions. Losses in generating DHPSF and splitting the number of photons into two lobes of the DHPSF, requires highly efficient collection of single molecule emissions for this technique to be successful.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Dr. Lenz, Senior Research Associate at CAIC shares, "The Teledyne Photometrics Prime 95B will help us to progress in both SMT as well as chromatin mapping projects. The high QE of the 95B compared to other sCMOS cameras currently used by us, will allow us to improve our investigation of protein dynamics and extend SMT to more challenging samples."

Single molecule light sheet microscopy is one of the key applications for future work that will benefit the most from the increased sensitivity. For this application, the larger than usual pixel size of 11 µm together high quantum efficiency will be highly advantageous.

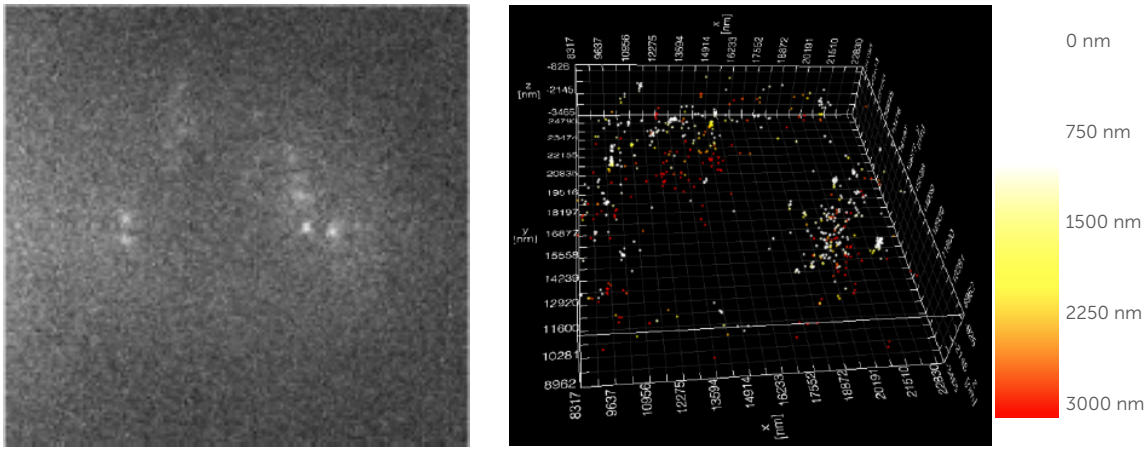


Figure 1: A) Raw image of spermatocytes using double-helix point spread function and **B)** 3D reconstruction from the raw data. Localizations in different colors represent their axial position.

Additional information is available at: https:/ /caic.bio.cam.ac.uk/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Mizar TILT Light Sheet Fluorescence Microscopy

Dr. Paul Maddox, Assistant Professor

The University of North Carolina at Chapel Hill, Biology Department

BACKGROUND

Conventional light sheet fluorescence microscopy (LSFM) is performed with two objectives oriented orthogonally to each other so that one objective introduces the light sheet and the other detects the fluorescence signal. However, this orientation requires the detection objective to be placed slightly away from the sample to prevent the two objectives colliding in space. Therefore, a long working distance detection objective is necessary which means that high NA, oil-immersion objectives are incompatible with the conventional LSFM design.

This presents a problem for the detection of cellular or subcellular structures which require a high NA detection objective and coverslip-based mounted samples for the superior resolution and light collection efficiency.

The Mizar TILT overcomes this problem by removing the illumination objective and introducing a tilted light sheet through a photomask and cylindrical lens which can be made to converge at the working distance of high NA objectives. In this way, high magnification and high NA (60x, 1.49), oil-immersion objectives can be used to image coverslip-based mounted samples.

> “The Prime 95B Scientific CMOS camera is, right now, the best solution we have found for TILT imaging. Coupling the Prime 95B to the TILT generates an extremely powerful imaging system!”

CHALLENGE

Like most light sheet systems, the Mizar TILT is designed to minimize photodamage and photobleaching to live samples by reducing the light source intensity and reducing exposure times. This allows for longer acquisitions to be made to monitor live processes over longer timescales.
One way to reduce exposure times is to use a more sensitive camera. CMOS devices are typically used in LSFM for the combination of a large field of view and fast speed but the sensitivity isn’t that high. EMCCD cameras are more sensitive than CMOS devices but suffer from small fields of view and slow speeds which makes them unappealing for LSFM applications.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The back-illuminated Prime 95B Scientific CMOS camera with an almost perfect 95% quantum efficiency and large 11 µm pixels is the perfect fit for the Mizar TILT.

The sensitivity of the Prime 95B is equivalent to an EMCCD but it retains the field of view and speed advantages of a CMOS camera. Furthermore, the larger pixels of the Prime 95B allow for high-resolution imaging with higher magnification objectives which the Mizar TILT was designed to use. This allows exposure times to be reduced and cells to be imaged for much longer with high detail.

Dr. Paul Maddox, assistant professor at the University of North Carolina at Chapel Hill is the creator of the Mizar TILT and founder of Mizar Imaging, shares with us, "The Prime 95B Scientific CMOS camera is, right now, the best solution we have found for TILT imaging. The outstanding quantum efficiency and pixel size allow imaging of a wide diversity of samples of varying brightness whilst enabling Nyquist sampling in space and time for even the most challenging samples. Coupling the Prime 95B to the TILT generates an extremely powerful imaging system!"

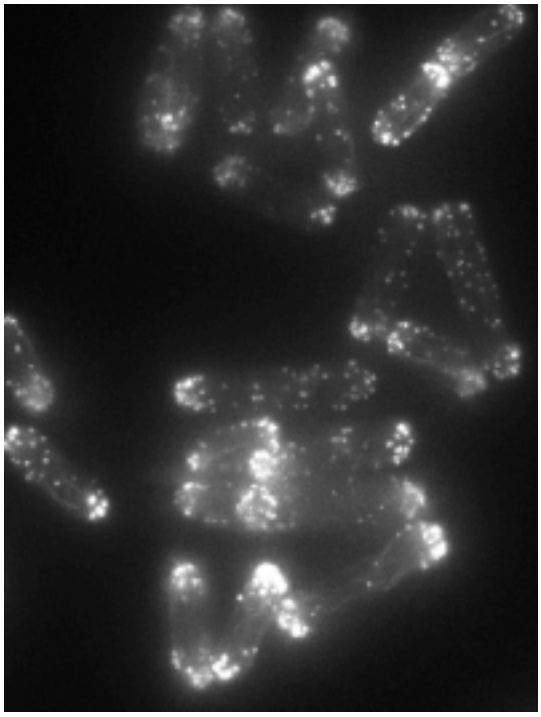


Figure 1 Maximum intensity projection of fission yeast expressing LifeAct-mCherry

Image acquired on the Mizar TILT under 150x magnification with a 1.49NA TIRF objective, 0.2 µm step size. 100 ms exposure using the Prime 95B scientific CMOS camera, cropped to 550x750 pixels.

Sample kindly provided by
Dr. Dan Mulvihill,
University of Kent
https://www.kent.ac.uk/bio/profiles/staff/mulvihill.html

Additional information is available at:
http:/ /bio.unc.edu/people/faculty/maddox-paul/
https://mizarimaging.com/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Microfluidics and Live Cell Imaging

Andrew deMello, Principal Investigator and Professor for Biochemical Engineering

Simon Berger, Doctoral Student

deMello Group, ETH Zurich (Switzerland) Department of Chemistry and Applied Biosciences

BACKGROUND

The deMello Group at ETH Zurich is engaged in a broad range of activities in the general area of microfluidics and nanoscale science. Primary specializations include the development of microfluidic devices for high-throughput biological and chemical analysis, ultra-sensitive optical detection techniques, nanofluidic reaction systems for chemical synthesis, novel methods for nanoparticle synthesis, the exploitation of semiconducting materials in diagnostic applications, the development of intelligent microfluidics and the processing of living organisms.

In recent years the deMello group has developed a range of microfluidic tools for the long-term imaging of living organisms, specifically the nematode *Caenorhabditis elegans*. Currently, work is focused on the creation of novel microfluidic devices for worm manipulation and the study of a wide range of developmental processes, previously inaccessible.

> “The Prime 95B allowed us to acquire high contrast fluorescence images using low excitation intensities, and subsequently allowed us to image over longer periods of time and at higher frequencies than previously possible.”

CHALLENGE

Live fluorescence imaging has seen a tremendous change over recent years. The development of sCMOS cameras has transformed image acquisition rates, fields of view and noise suppression, while also lowering unit costs. However, until recently the EMCCD has been the gold-standard for high sensitivity applications, significantly outperforming sCMOS devices with quantum efficiencies in excess of 95%, but lacking considerably with respect to sensor size and acquisition speed.

Simon Berger, doctoral student with the DeMello group, explains, “The primary challenge in live cell/organism imaging is the extraction of high quality images, both bright and with high contrast, while ensuring that phototoxicity and photobleaching are kept to a minimum. In this way, imaging does not affect sample viability and the biological processes under investigation.”

(continued...)

TELEDYNE IMAGING
Everywhereyoulook™

CHALLENGE

The need to ensure low photobleaching/phototoxicity often limits attainable image quality, as well as the frequency and detail with which images can be acquired. While the higher sensitivity associated with EMCCDs can remedy the effects of photobleaching/phototoxicity, the acquisition rates, the small fields of view and limited dynamic range, severely limit their usefulness for in vivo imaging.

SOLUTION

Compared to its peers, the Prime 95B combines the features of sCMOS cameras (high acquisition rates, large sensor size, low noise and high dynamic range) with the exceptional sensitivity previously only available through EMCCDs.

Simon explains, "The Prime 95B allowed us to acquire high contrast fluorescence images using low excitation intensities, and subsequently allowed us to image over longer periods of time and at higher frequencies than previously possible. This allowed for intrusion-free study of many sensitive developmental processes."

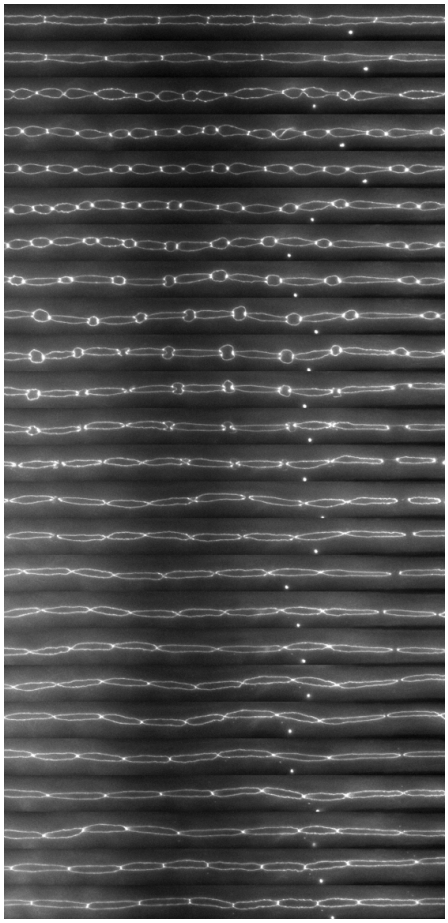


Figure 1 Expression of DLG-1 ::eGFP, apical junctional protein in the seam cell epithelium. Each image represents a 10 ms exposure taken every 20 minutes for a full sequence time of 4.5 hours.

C. elegans strain courtesy of Dr. H. Ribeiro Pires and Dr. M. Boxem, Utrecht University.

Additional information about the the deMello Group and their research is available at: https://demellogroup.ethz.ch/en/index

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Neuroscience and Calcium Imaging

Geoffrey G. Murphy, Ph.D., Research Professor
Molecular & Behavioral Neuroscience Institute
University of Michigan

BACKGROUND

Dr. Geoffrey Murphy, professor of physiology at the University of Michigan's Molecular & Behavioral Neuroscience Institute studies the how the mammalian brain encodes, stores and retrieves information. Dr. Murphy explains, "We do a lot of mouse molecular genetics, in vitro neurophysiology, and mouse behavior. We are interested in the dendritic architecture, and the calcium signaling within the dendritic structure versus the somatic structure."

> "The Prime 95B allowed us to not only increase the frame rate we were using to acquire images, but we also achieved higher resolution. For us, that meant being able to look at subcellular structures in real time."

CHALLENGE

For many neuroscience researchers, speed, sensitivity, and resolution are all critical to visualizing small changes in calcium signals in different regions of the neuron. Dr. Murphy told us, "Being able to image at 100 Hz is very important to us. That allows us to get high resolution, rapid calcium signals." Additionally, the samples to be imaged require a sensitive camera to visualize as the samples are often thick and can be troublesome to image through.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

For Dr. Murphy, the 11 µm pixel size and speed of the Prime 95B provided a solution to the challenges of calcium imaging in neurons. Dr. Murphy told us, "Previously, we had been using cameras that had a lower frame rate and worse resolution, so using the Prime 95B allowed us to not only enhance the frame rate that we were using to acquire images, but get higher resolution too. For us, that meant being able to look at subcellular structures in real time so the speed is certainly something that we like. Also, it's a really easy camera to use and it interfaces well with our preferred software application [for further analysis]."

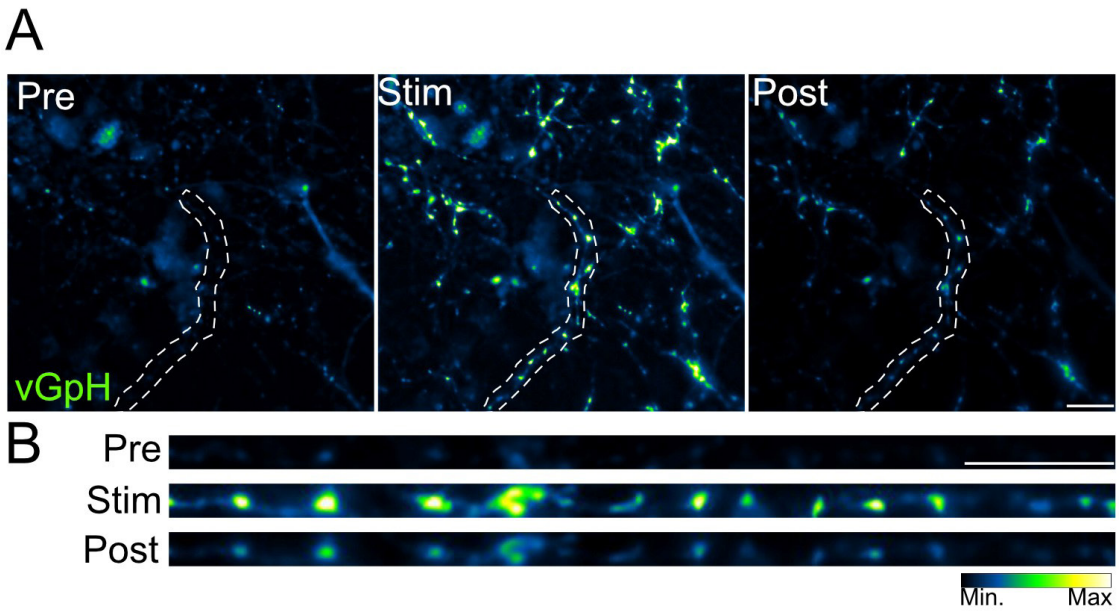


Figure 1 Fluorescence imaging of the fluorescent reporter for vesicle cycling (VGLUT1-pHluorin, vGpH) in hippocampal neurons.
A: Images of fluorescent intensity before (Pre), during (Stirn) and after electrical stimulation (100 pulses, 10 Hz) of neurons.
B: Enlarged view of an axonal segment (from outlined region in A).

Learn more about the Murphy Lab at the University of Michigan:
https:/ /sites.google.com/a/umich.edu/murphy-lab/home

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Fluorescence Correlation Spectroscopy

Prof. Enrico Gratton, Founder and Principle Investigator

Laboratory of Fluorescence Dynamics

University of California, Irvine

BACKGROUND

The lab of Prof. Enrico Gratton at the University of California, Irvine, is interested in the dynamics of the cell interior. The group investigates this using microscopy combined with mathematical approaches such as fluorescence correlation spectroscopy. As the founder and Principle Investigator of the Laboratory of Fluorescence Dynamics, Prof. Gratton's team has developed numerous fluorescence-based methods to measure diverse cell properties. This includes measurement of the absolute concentrations of molecules within cellular compartments, detection of aggregation of proteins and the detection of barriers to diffusion. Prof. Gratton also developed Globals for Images, a set of software packages to perform this analysis.

> "The reliability and stability of the pixel noise makes the Prime 95B Scientific CMOS camera an ideal imaging solution for fluorescence correlation spectroscopy methods."

CHALLENGE

To reliably detect the incredibly small fluctuations in image signal required for fluorescence correlation spectroscopy – on the order of 10 electrons of signal - stable and predictable noise characteristics are necessary. Noise is an inherent feature of camera sensors and is corrected to reveal fluorescent fluctuations which are detected and measured.

Typical experiments using EMCCD cameras require the noise characteristics, including correlated noise and light-independent pixel variance, to be mapped before every experiment to account for instability in the camera noise over time. After fast, time series fluorescence imaging, the previously mapped noise is removed to detect the variance due to fluorescent fluctuations in each pixel, and allowed mathematical analysis of the signal.

With fluctuations in signal being less than 10 electrons in amplitude, high camera sensitivity and being able to accurately correct for the noise are crucial in providing the necessary signal to noise for this analysis.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Prof. Gratton now uses the Prime 95B back illuminated Scientific CMOS (sCMOS) camera, to take advantage of the stable noise and uncorrelated pixel noise properties. Light-independent pixel noise characteristics are stable over time which means the camera only requires calibration and mapping once, rather than before every experiment.

Prof. Gratton shared, "I didn't need to account for camera noise in the analyses, unlike when using EMCCDs and other CMOS cameras, which suffer from unstable noise. The reliability and stability of the pixel noise, makes the Prime 95B Scientific CMOS camera an ideal imaging solution for fluorescence correlation spectroscopy methods." Having predictable and easily modelled pixel to pixel variation produces the highest signal to noise ratio and results in detection of smaller fluctuations than possible when using an EMCCD.

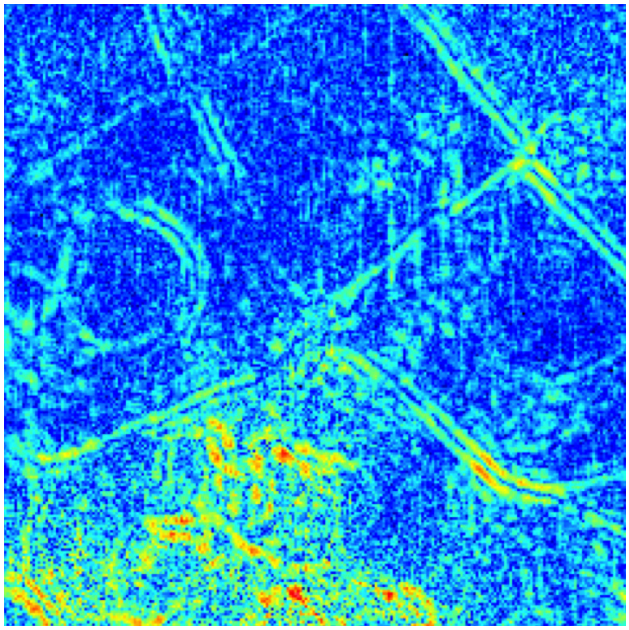


Figure 1: Arabidopsis in agar expressing GFP, imaged using SPIM with the Prime 95B uncorrected for camera noise. Pair correlation function showing obstacles to diffusion due to the cell walls in regions of strong anisotropic motion.

The parallel lines of signal clearly define the boundaries of diffusion at each side of the cell walls. Unlike super-resolution microscopy or other methods, fluctuation correlation spectroscopy images the diffusion itself, rather than the cellular structures which are often not visible.

Time series of 2100 frames over 10.5s, 256x256 pixels, 140 nm effective pixel size.

REFERENCE

http://users.df.uba.ar/mad/Workshop/Gratton%20FCS.pdf
http://www.rowland.harvard.edu/rjf/sahin/development/graphics/fcs_in_cells.pdf
http://www.globalssoftware.com/re.com/

Additional information about the Laboratory of Fluorescence Dynamics is available at:
https://www.lfd.uci.edu/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule Tracking PALM

Dr Thomas Etheridge

Antony Carr Group, Genome Damage and Stability Centre

University of Sussex

BACKGROUND

The Carr Lab at the Genome Damage and Stability Centre, University of Sussex, investigates DNA metabolism processes such as DNA replication and repair. They are interested in the challenges cells face during DNA replication and the cellular processes that help the cell overcome replication fork stalling or collapse. To study this, they focus on the behaviors and interactions of individual proteins involved in DNA replication in both fission yeast and human cells.

> “Compared to an EMCCD, the Prime 95B gives us a field of view large enough for our most demanding experiments as well as faster frame rates.”

CHALLENGE

One approach used to examine the DNA association of proteins is to break open the cell, extract the proteins and perform western blotting. However, this approach reveals nothing of the dynamics of association, and destroys cells in the process.

A more demanding alternative is to use an imaging technique capable of single protein resolution. To this end, Dr. Etheridge uses Single Molecule Tracking PALM to observe the association of proteins with the DNA in real time, non-invasively, in both yeast cells and human cells. Single fluorescent molecules move quickly and give off very little light so this technique requires high speed and the highest sensitivity.

The fluorescent proteins used in the yeast cells are very dim and, until now, Dr. Etheridge has been using an EMCCD camera to observe them. However, the field of view given by his EMCCD camera is quite small. For human cells, brighter synthetic dyes can be used so dynamics can be observed with better temporal resolution. Unfortunately, the lower speed of his EMCCD camera doesn’t allow for this.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The back illuminated Prime 95B scientific CMOS camera has the speed and field of view advantages expected of CMOS cameras, but with the sensitivity to rival or beat EMCCDs. This gives researchers using single molecule techniques unprecedented low-light detection and temporal resolution.

Dr. Etheridge shared, "To image the demanding yeast cells with our EMCCD, we were having to do multiple experiments on different areas of the sample to generate enough data for it to be worth processing. With the field of view of the Prime 95B, we can gather enough cells in the field of view to process in a single shot. In the human cell system, using the brighter dyes, we can image our favorite proteins at much faster speeds."

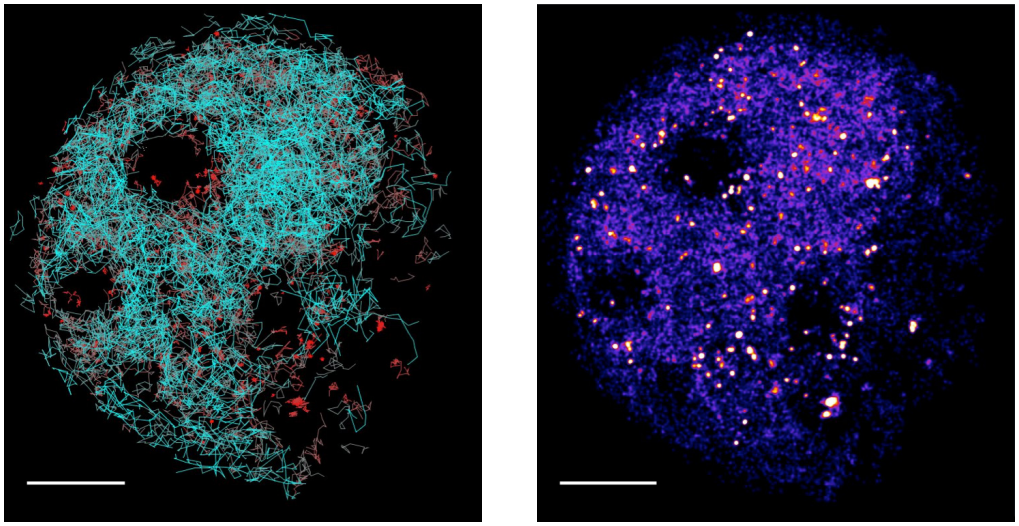


Figure 1: Human cell nucleus with DNA repair complex labelled with HaloTag-PA-JF549. *Left* Superimposed tracks of detected molecules. Red = DNA-bound, static molecule. Cyan= freely diffusing complexes. *Right* Localization map of complexes. Hot spots indicating locations of DNA bound molecules. Scale bar: 5µm.

Additional information about the Genome Damage and Stability Centre at the University of Sussex is available at: http://www.sussex.ac.uk/gdsc/

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Low-Light, High-Speed FRET Imaging

Professor Andrew Plested

Leibniz Research Institute for Molecular Pharmacology

BACKGROUND

The Plested lab investigates the characterization of receptors relevant to neuronal systems. They apply multiple techniques to image isolated neurons, including a combination of electrophysiology and fluorescence live-cell imaging.

One of their areas of study involves measuring Foerster Resonance Energy Transfer (FRET) upon electrical stimulation of the neurons. They label individual relevant structures and receptors with suitable fluorophores and observe the FRET signal in the dendritic region of cells of interest. This enables them to obtain information about the state, location, and behavior of channels, and particularly their colocalization in the nanometer range.

> “The Prime 95B lets us look at 5x the FOV at about twice the speed, but with the same sensitivity as our previous EMCCD system.”

CHALLENGE

The individual interactions the group is interested in observing only last for a very brief period of time (10-100 ms). Therefore, to capture the weak signal arising from the energy transfer from a donor fluorophore to an acceptor molecule, very fast imaging is required. This means the number of photons available for detection is very limited and an extremely sensitive imaging device is essential.

Their currently used EMCCD camera – although sensitive enough for detection – is only able to image a very small field of view which makes experimental measurements a time-consuming exercise. FRET measurements are also limited by the 30 ms read time per frame of the EMCCD camera, achieving just 30 fps (corresponding to ~15 fps FRET) at full field. Furthermore, binning had to be used to increase frame rate, reducing spatial resolution.

SOLUTION

The Prime 95B Scientific CMOS camera delivers three critical advantages to Dr. Plested's experiments: Sensitivity, field of view (FOV) and speed.

First, longer exposure times allowed by the Prime 95B mean the relevant power of excitation can be reduced by a factor of 5 – giving a more physiologically-relevant measurement and also reducing artefacts caused by bleaching.

Second, the larger sensor of the Prime 95B resulted in about five times the field of view, dramatically increasing the throughput of cellular regions per experiment.

And finally, along with the larger field of view, the frame rate can also be increased. By imaging at 50 fps (effective FRET measurement at 25 fps, with alternating excitation, see Figure 1) the data is much more relevant to synaptic transmission (timescale of interest 10-100 ms). This means that their measurements are now limited by probe properties, not the camera.

The Prime 95B gave the Plested lab an improved way to obtain their data, switching from essentially single object imaging to a multiple neurite view, yielding more detailed insight into synaptic transmission.

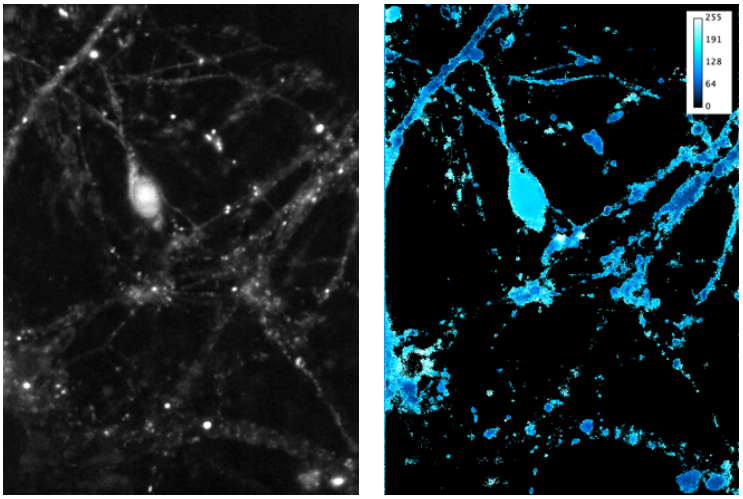


Figure 1. *Left* Hippocampal neuronal culture expressing receptor and scaffold protein tagged with GFP and mScarlet, respectively. Maximum intensity projection from both fluorophores combined. 488 and 561 nm excitation, Optosplit 550 nm dichroic, images aligned in Cairn Research ImageJ plugin.
Right Average FRET signal (8-bit, max 255) from 10 frames (100 ms total exposure). Maximum projection was thresholded and used to mask FRET calculation, restricting to cell processes. FRET calculation was done in IGOR Pro. Image manipulation in ImageJ.

Additional information available at http://www.leibniz-fmp.de/research/molecular-physiology-and-cell-biology/research-groups/plested/mnb0.html

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule Biophysics

Dr. Kevin Freedman, Assistant Professor, Bioengineering

University of California Riverside, Department of Bioengineering

BACKGROUND

The Freedman Lab studies single molecule biophysics using a variety of electrical and optical signal measurements. Most notably, the lab studies complex biological systems where molecular populations are heterogeneous and difficult to study using ensemble averaging. Whenever working with single molecules, sensitivity is a big challenge. As a lab which focuses on single molecule detection, they require high end imaging equipment. This is not only for high sensitivity but also for high temporal resolution (i.e. fast frame rates). In some cases, fluorescent nanoscale beads are used to characterize the devices custom-fabricated in the lab so the need for fast sampling is a limiting factor.

> “We’ve been using the Prime 95B as one of our main cameras for both single molecule sensing and nano-bead velocity measurements. The Prime 95B Scientific CMOS is excellent for low-noise, high sensitivity, and fast measurements.”

CHALLENGE

The Freedman Lab designs and characterizes nanofluidic devices that are used for biophysical or device physics (i.e. bead tracking) experiments. Often times, using the devices, nano-beads are transported at high velocities between two fabricated structures where speeds of up to 1000 fps are necessary to track them accurately.

To achieve this high framerate, the exposure time needs to be 1 ms. This means the camera must be capable of reaching this high frame rate as well as being sensitive enough to detect the nano-beads with such a low exposure time. This isn’t possible with the lower speed of an EMCCD camera, and front-illuminated sCMOS cameras lack the sensitivity.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

The Freedman Lab now uses the back-illuminated Prime 95B Scientific CMOS camera for their single molecule imaging experiments.

With 95% quantum efficiency, the Prime 95B has the sensitivity to detect particles with the 1 ms exposure time used in their challenging nano-bead velocimetry experiments. Also, with the increased speed afforded by CMOS technology, the Prime 95B can easily reach the desired 1000 fps framerate. This allows accurate bead tracking in a range of experimental parameters which were not accessible before.

Dr. Freedman told us, "We've been using the Prime 95B as one of our main cameras for both single molecule sensing and nano-bead velocity measurements. The Prime 95B Scientific CMOS is excellent for low-noise, high-sensitivity, and fast measurements."

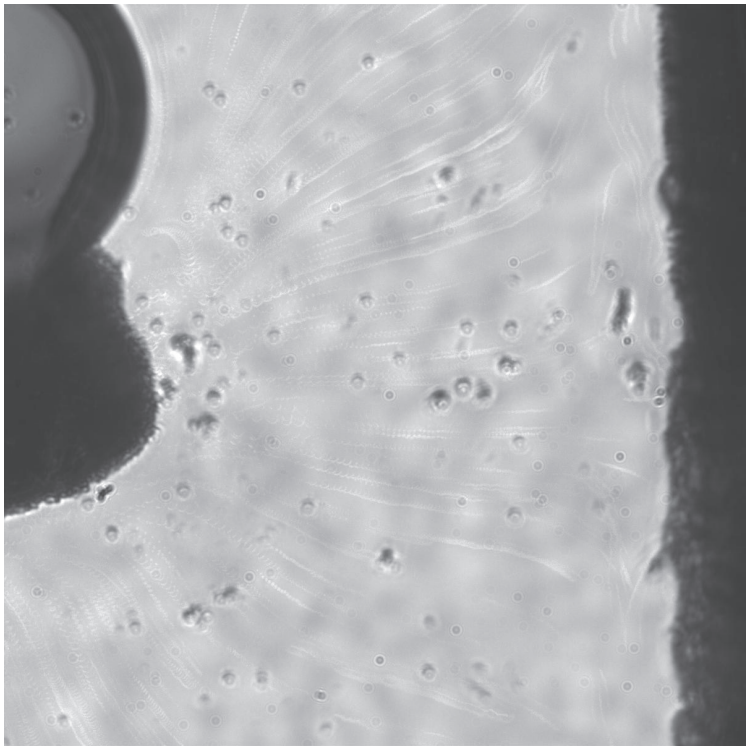


Figure 1 Maximum intensity projection of electrically charged beads moving through an electric field over time. Electrically charged ports on the left and right hand side of the image cause particles to move at high velocity through the pore. The tracks that can be seen show the movement of the beads.

Additional information about the Freedman Lab is available at:
https://www.engr.ucr.edu/people/kevinfreedman.html

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Calcium Imaging

Marc Freichel, PhD, Prof.

Volodymyr Tsvilovskyy, PhD.

Institute of Pharmacology, Heidelberg University, Germany.

BACKGROUND

Dr. Freichel's group are primarily interested in how ion channels regulate the influx of calcium ions (Ca^{2+}) as messengers for cellular and systemic functions.

The group is mainly interested in understanding transient receptor potential (TRP) and Ca^{2+} channels and their role in calcium signaling in cells of the cardiovascular system (endothelial cells, smooth muscle cells, cardiac fibroblasts, platelets), epididymal cells and mast cells. This gives the group important information about the corresponding integrative body functions such as vascular and cardiac contractility, transmitter secretion, blood pressure regulation, blood vessel formation, fertility and mast cell activation as well as their role during maladaptive cardiac remodelling and for the development of diabetes mellitus associated long term sequelae.

The laboratory uses transgenic approaches such as gene targeting in embryonic stem cells for the generation of disease models and for the identification and validation of new drug targets. Within Dr Freichel's lab, transgenic reporter lines (e.g. GCaMP6) are crossed with disease carrying lines to enable visualization of impaired signalling processes. In parallel, electrophysiological measurements via patch pipettes are also carried out.

> "The field of view of the Prime 95B is >2.5x bigger than our previous camera, allowing us to image more than twice the amount of data in a single imaging session and the high sensitivity of the Prime 95B dramatically reduced the exposure time needed to collect our images."

CHALLENGE

As with all reporters, individual light dose per image needs to be kept at a minimum to reduce potential side effects caused by phototoxic by-products. Moreover, the studied networks of cells exhibit spontaneous Ca^{2+} fluctuations which can be – dependent on cell type and disease model – very rare so many separate fields-of-view need to be imaged to reach a reliable statistical conclusion.

(continued...)

TELEDYNE IMAGING
Everywhereyoulook™

CHALLENGE

When transgenic reporters can´t be used, the lab applies indicator dyes such as AM-esters of Fura2 and Fluo-variants. Here, a further problem can occur when too high dye concentrations need to be used to reach a detectable signal level. This surplus of dye alters the kinetics of the physiological response, rendering it a non-physiological situation. This problem can only be overcome by reducing the concentration of dye, which means a scientific camera is needed that is sensitive enough to pick up very low signals.

Their current imaging solutions are either state-of-the-art CCD sensors or EMCCDs. Both require a very long exposure time and provide images with a suboptimal signal-to-noise ratio. The small field of view of CCD-based sensors also requires many imaging sessions to acquire statistically significant data.

SOLUTION

The Teledyne Photometrics Prime 95B back-illuminated sCMOS is the perfect match for the imaging demands of the Freichel lab.

The increased sensitivity achieved by the 11 µm pixel and the peak-quantum efficiency of 95% at a wavelength range which matters mostly for their reporters dramatically reduces the exposure time for individual images. This carries two benefits. Firstly, kinetics can be determined more accurately as more data points can be acquired in the same amount of time. Secondly, less light needs to be used to detect their cells which allows the group to obtain more physiologically relevant data.

The field of view of the Prime 95B is >2.5x bigger than their previous camera solution, allowing the group to image more than twice the amount of data in a single acquisition, further improving their statistics and their time spent at the microscope.

The Prime 95B is also fully supported in Zeiss´ ZEN software which is convenient as the group is already familiar with the interface.

In brief, the Prime 95B enhances the Freichel lab´s scientific output and opens up new ways for them to study their samples of interest, bringing them closer to finding answers to their scientific questions.

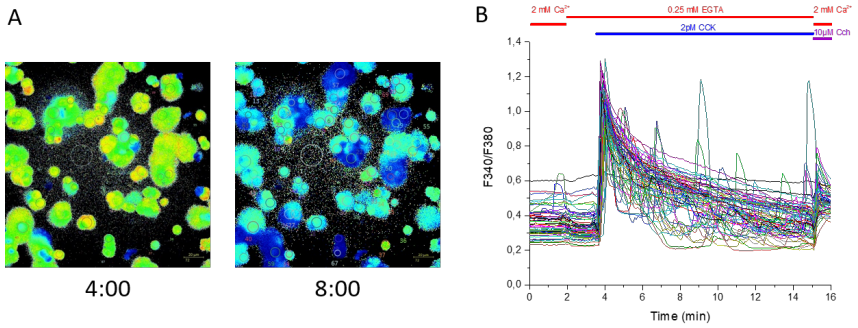


Figure 1. Time lapse of Fura-2 imaging in mouse pancreatic acinar cells. Cells were stimulated with 2pM cholecystokinin (CCK) in physiological salt solution (PSS) supplemented with 0.25mM EGTA following 10µM Carbachol (Cch) in PSS containing 2mM Calcium. Figure 1A visualizes intracellular calcium concentrations immediately after (time point 4:00 min) and 4 minutes after application of CCK (time point 8:00 min). Representative F340/F380 ratios of 65 cells in response to 2pM CCK and 10µM Cch can be observed in Figure 1B.

Further Information: http://www.medizinische-fakultaet-hd.uni-heidelberg.de/Research.107622.0.html?&L=en

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Nanophotonics

Dr. Mathieu Mivelle

Research Fellow, Sorbonne University

BACKGROUND

Dr. Mivelle's research lies within the field of nanophotonics and centers around investigations into the interactions between light and matter on the nanoscale to increase understanding of optical properties.

Dr. Mivelle explains, "Most current technologies make use of the electric field component of light, however, my research investigates the interaction of the magnetic optical field component with matter, which is much weaker".

Dr. Mivelle designs structures that are patterned at the nanoscale, and couples these to single quantum emitters, such as single fluorescent molecules or lanthanide doped nanocrystals to produce physical effects between the two that are not found in nature. This spatial coupling permits Dr. Mivelle to modify the quantum properties of the emitters which could provide new opportunities in photonics for technological applications.

> "We compared [the Prime 95B] with other EMCCD technologies and, for our application, the Prime 95B was the best."

CHALLENGE

Dr. Mivelle uses a custom set up that consists of an inverted microscope, on top of which is a near field scanning probe, not unlike those used in AFM, but with the nanopatterned structure attached.

The nanostructure is brought into close proximity (~10 nm) to the sample consisting of nanocrystals, 50 nm in diameter, doped with rare earth lanthanide ions, such as erbium and europium, which are used in many technologies. Lanthanides are known to be very dim emitters in terms of photon counts, Dr. Mivelle explained, "I require a very sensitive camera to be able to locate these single emitters during sample scanning at speed".

In order to couple the nanostructure to the single emitters, Dr. Mivelle must first locate them in the sample during scanning over tens of milliseconds, which provides a challenge in terms of speed and sensitivity.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Dr. Mivelle is using the Prime 95B in his custom-built setup to identify these single emitters during sample scanning at high speeds. He told us, "The combination of the high SNR and quantum efficiency [of the Prime 95B] allows me to see the very dim signals emitted from the lanthanide doped particles."
He continued, "We picked the Prime 95B based on the speed and the sensitivity, which was equivalent to EMCCDs but at a much better value for money. We compared with other EMCCD technologies and, for our application, the Prime 95B was the best."

Although field of view (FOV) is not currently the issue, Dr Mivelle anticipates that the increased FOV of the Prime 95B will provide benefits for future super-resolution localization (STORM) experiments.

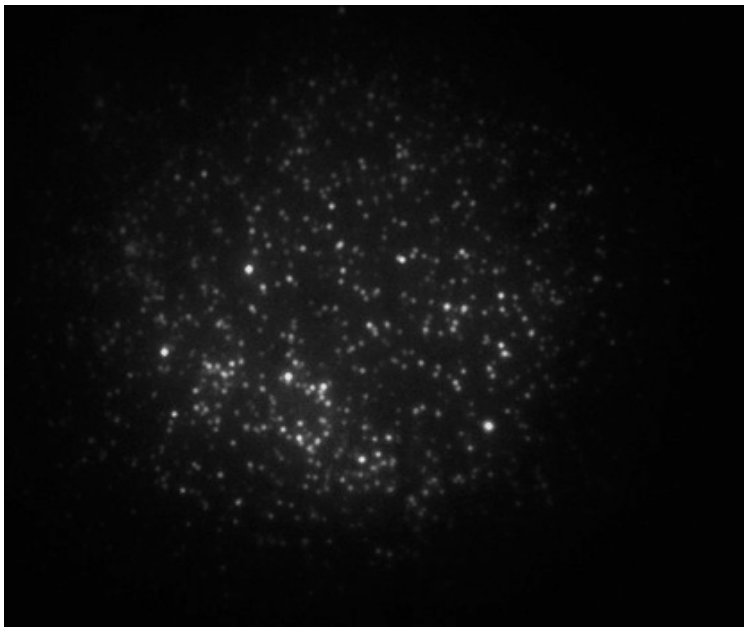


Figure 1. Quantum emitters imaged using the Prime 95B sCMOS camera on the custom-built microscope setup.

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Imaging Vesicular Neurotransmitter Release

Professor Kirill E Volynski

Institute of Neurology, University College London.

BACKGROUND

The Volynski group are primarily interested in understanding cellular regulation of synaptic release of neurotransmitters which forms the basis of communication among neurons in the brain.

The Volynski group uses fluorescent probes, such as vesicular release sensor synaptophysin-pHluorin (sypHy). They visualise synaptic transmission at the level of single synapses in order to understand the basic mechanisms of transmitter release and also the cellular mechanisms of synaptopathies – paroxysmal neurological disorders caused by mutations in synaptic proteins. These disorders include some forms of epilepsy, migraine and ataxias.

The sypHy probe consists of a pH sensitive GFP variant fused with the vesicular membrane protein synaptophysin. sypHy is widely used to image synaptic vesicle exo- and endocytosis. At rest, the sypHy fluorescence inside of a synaptic vesicle is quenched by the acidic vesicular pH. Upon synaptic vesicle exocytosis, caused by neuronal spiking, the sypHy probe becomes exposed to the extracellular medium with neutral pH, which leads to an increase in its fluorescence. Quantification of the fluorescence signal provides a means to monitor the response of individual synaptic boutons to different stimuli, thus providing a readout for the effects of disease-linked mutation effects on synaptic function.

> “We switched to the Teledyne Photometrics Prime 95B and now have very stable responses, the camera works faster, and the SNR is very comparable to EMCCDs.”

CHALLENGE

Professor Volynski was initially capturing images with a 512x512 EMCCD camera, however imaging routines often consisted of several seconds which led to problems with drift in the temperature of the EMCCD sensor accompanied by a drift in the camera gain and ultimately the signal. Although this can be corrected post-hoc it is non-trivial and complicates the analysis.

(continued...)

TELEDYNE IMAGING
Everywhereyoulook™

CHALLENGE

In addition to this, Prof. Volynski also wanted to image at higher speeds to capture the fluorescence associated with neurotransmitter release as well as synchronizing the camera with electrophysiological traces. However, when using the EMCCD, speed was limited to 30 fps and often frames were dropped randomly, which led to difficulties in the acquisitions and syncing.

SOLUTION

The Volynski group is now using the Teledyne Photometrics Prime 95B to image synaptic transmission. Professor Volynski told us that "Stability and speed were the key issues here, and once we started using the Prime 95B the problem of instability totally disappeared. We switched to the Teledyne Photometrics Prime 95B and now have very stable responses, the camera works faster, and the SNR is very comparable to EMCCDs" He shared that, "We can acquire the same region of interest faster with the Prime 95B compared to the EMCCD."

Professor Volynski went on to say that, "Now, we have a very robust protocol for detecting synaptic responses both at the level of single boutons and populations of synapses which provided us with a reliable and fast method for testing the synaptic effects of the disease linked mutations".

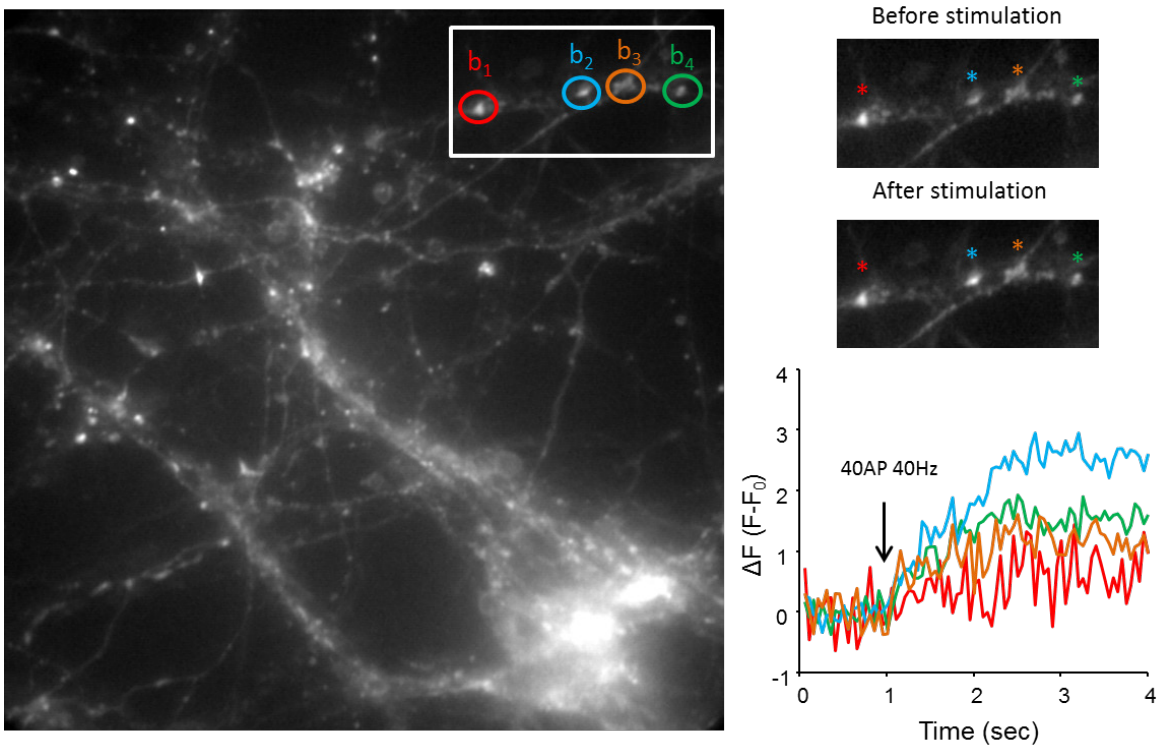


Figure 1. Representative image of mouse cortical neurons expressing the sypHy reporter. A small region of interest (right panels corresponding to the white box on the original image) containing several synaptic boutons is shown before and after stimulation with 40 action potentials at 40Hz. Action-potential-evoked fluorescence responses in the labelled boutons are shown on the graph bottom right.

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

CUSTOMER REFERENCE

Prime 95B™ Scientific CMOS Camera

Single Molecule FRET

Dr. Ehud Isacoff

Director, Helen Wills Neuroscience Institute, UC Berkeley

BACKGROUND

Prof. Ehud Isacoff's lab at the University of California, Berkeley, USA is interested in the mechanistic properties of metabotropic and ionotropic receptor subunits and the role the resultant protein complexes play in signal transduction. His group uses a combination of microscopy approaches including single molecule Föester resonance energy transfer (smFRET) in live cells, and in vivo imaging in a variety of species. They've developed a number of tools for the investigation of neuronal function. Having pioneered the manipulation of receptors with tethered ligands, more recently they've developed photoactivatable versions of sensors such as GCaMPs.

The effects of subunit composition on metabotropic receptor transduction can be subtle and impossible to separate in mixed populations of receptor subunits. In the brain of mammals, metabotropic glutamate receptor subunits have mixed and overlapping expression patterns, making it very difficult to understand the roles of particular subunits. Single molecule in vitro analysis of receptors with known subunit compositions is needed. This allows the full characterization of biophysical properties, such as conformation change kinetics, which can be measured by smFRET.

> "We have been using 10 ms exposure and getting 100 fps and I'm just as confident in the data at 100 fps with the Prime 95B as with 10 fps with the EMCCD."

CHALLENGE

To measure the conformation changes of single receptors, fluorescent receptor subunits expressed in bacteria are reconstituted into functional receptor types and immobilized on a surface for imaging. Specific manipulations like ligand applications can be performed to stimulate the receptors to change conformation, which is recorded in the FRET signal. The kinetics of the conformational change are accurately mapped by the temporal FRET response.

Single molecule FRET presents very specific problems for imaging. There are very few fluorophores present in each protein complex. Secondly, receptor kinetics can be extremely fast. High camera frame rates are needed to accurately sample fast events. This means exposure times are short and detecting the few photons being emitted is difficult. EMCCD cameras have traditionally been used due to their sensitivity, but they lack the speed to detect very fast events.

TELEDYNE IMAGING
Everywhereyoulook™

SOLUTION

Using the Prime 95B back-illuminated sCMOS camera, the Isacoff lab improved the speed at which they can record in smFRET experiments. Previously they achieved 10 fps with an EMCCD. They shared, "We have been using 10 ms exposure and getting 100 fps and I'm just as confident in the data at 100 fps with the Prime 95B as with 10 fps with the EMCCD." This increase in frame rate, whilst still maintaining the high signal-to-noise ratio needed to reliably record FRET changes, has allowed detection of events of 10 fold shorter duration than before.

This has allowed the group to confirm the kinetics of slower moving protein complexes, but also determine faster kinetics of other complexes for the first time. The combination of large 11um pixels with high 95% quantum efficiency and fast frame rates make the Prime 95B an excellent choice for smFRET where both sensitivity and speed are necessary to produce high-quality data.

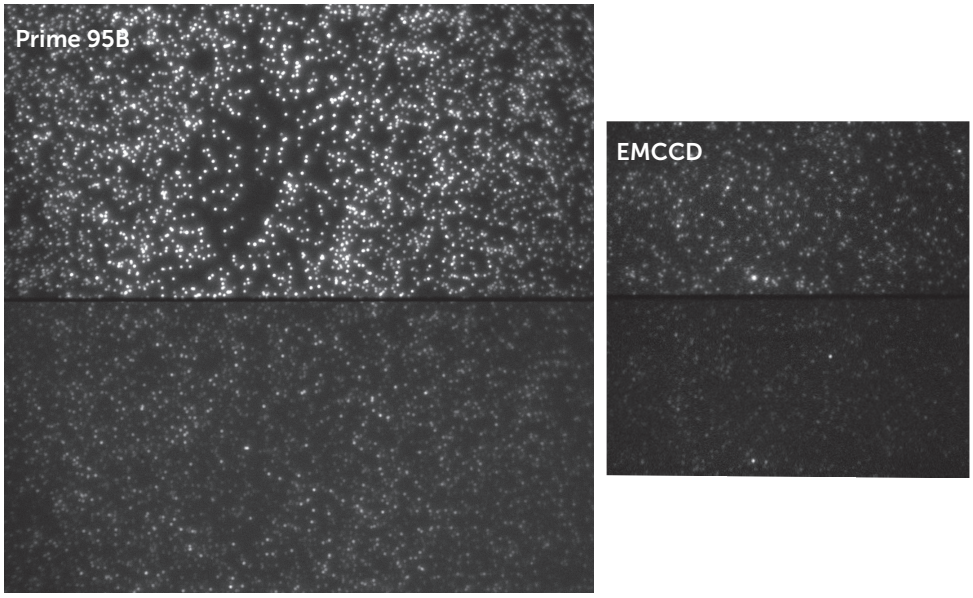


Figure 1: Donor and FRET signals separated to the top and bottom halves of the camera sensor. Comparison images were acquired on the Prime 95B sCMOS (left) and a 512x512 EMCCD (right) using the same exposure time. The increased field of view is obvious and subsequent image analysis revealed that the Prime 95B also had a higher signal-to-noise ratio. The Prime 95B is also capable of far higher speeds. This allows for higher quality data to be acquired and more protein complexes to be measured with faster sampling kinetics.

Increasing CMOS Camera Sensitivity Through Back-Illumination

Introduction

Scientific camera sensitivity is determined by three main factors; quantum efficiency (QE), pixel size and noise characteristics. Quantum efficiency is the measure of the effectiveness of the camera to produce electronic charge (electrons) from incident photons, where a higher QE results in the conversion of more photons to electrons of signal. Electrons go on to be converted into a digital signal that can be read by a computer and visualized. Pixel size relates to the physical area of the pixel, where a larger pixel is able to collect more photons and therefore deliver more electrons of signal. Noise characteristics, particularly read noise at low-light levels, determine how much the electron signal can fluctuate per pixel. The higher the signal over the noise, the higher the signal-to-noise ratio and therefore image quality. There will be no sample detection if noise exceeds signal.

This technical note will focus on quantum efficiency and how it was made possible to increase sensitivity on CMOS cameras by increasing QE to an almost perfect, 95% through the process of back-illumination. An easily repeatable experiment is also outlined to evaluate camera sensitivity.

Quantum Efficiency

Quantum efficiency can be pretty well defined as the percentage of electrons produced from the number of incident photons. For example, if 100 photons hit a 95% quantum efficient sensor, 95 electrons would be theoretically generated. Likewise, if 100 photons hit a 65% quantum efficient sensor, 65 electrons would be theoretically generated. This process is a property of the photovoltaic effect, where light energy (photons) incident on the silicon substrate of a pixel creates electron-hole pairs. These electrons are then read out by the device and converted into a digital signal that can be interpreted by a computer.

There are many conditions that affect the photovoltaic effect and thereby determine the number of electrons generated by a single photon. Of these, the two most important conditions are the absorption coefficient and the chemical and physical properties of the material on the sensor surface. As these conditions determine the number of electrons that can be generated by a single photon, they directly influence the quantum efficiency of the camera.

Camera-Related Noise

One of the main differences between standard CCD/EMCCD cameras and scientific CMOS cameras is the signal readout structure. A CCD/EMCCD camera converts charge (electron signal) to a voltage at the preamplifier, the voltage is then digitized by the Analogue to Digital Converter (ADC) to produce a digital signal that can be read by a computer (figure 1). CCD/EMCCDs, therefore, have a single preamplifier and ADC which digitize every single pixel one by one.

Absorption coefficient

The absorption of photons into the silicon substrate of the pixel is wavelength dependent. This is the reason why quantum efficiency is shown on camera datasheets as a curve, such as the curves shown in Figure 1.

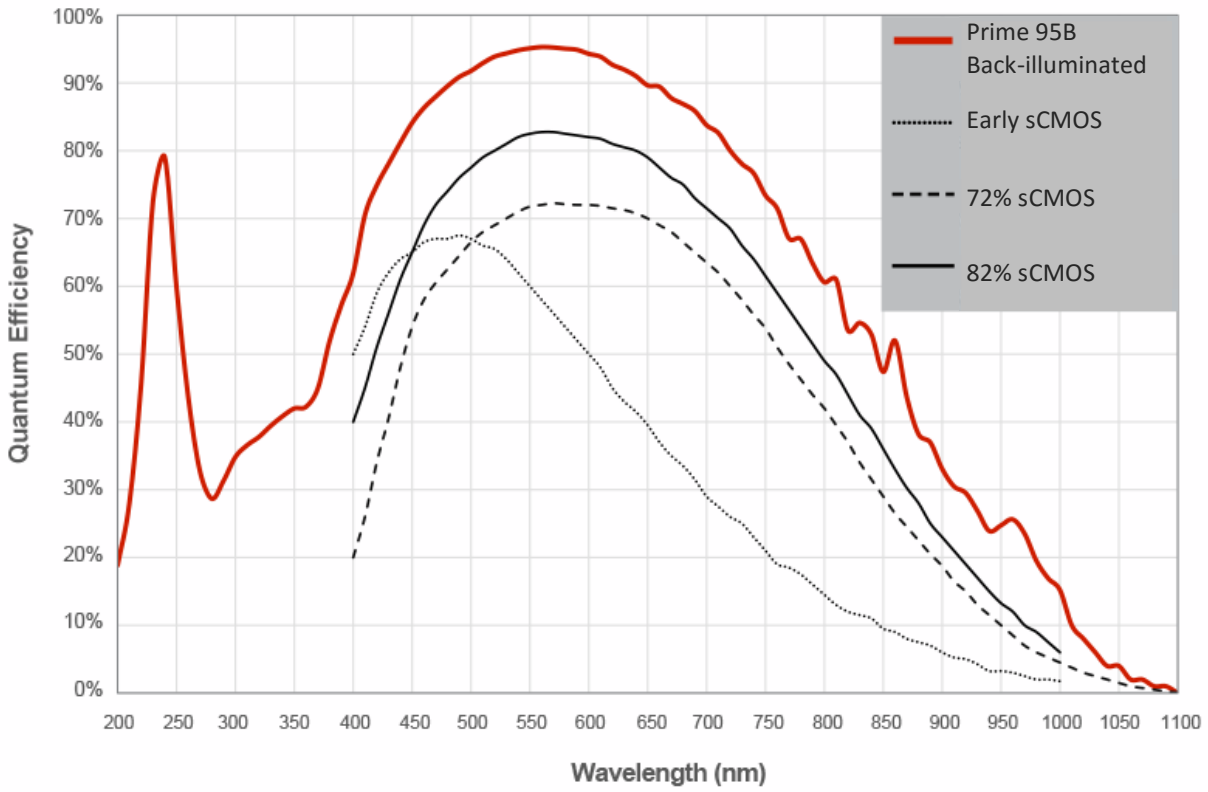


Figure 1: Comparison of quantum efficiency curves of some typical CMOS cameras
Adapted from Princeton Instruments, Kuro sCMOS.

Quantum efficiency is higher in the green and yellow region (500 nm – 600 nm) because these wavelengths penetrate well into the region of the silicon substrate of the pixel where the photovoltaic effect takes place (Figure 2).

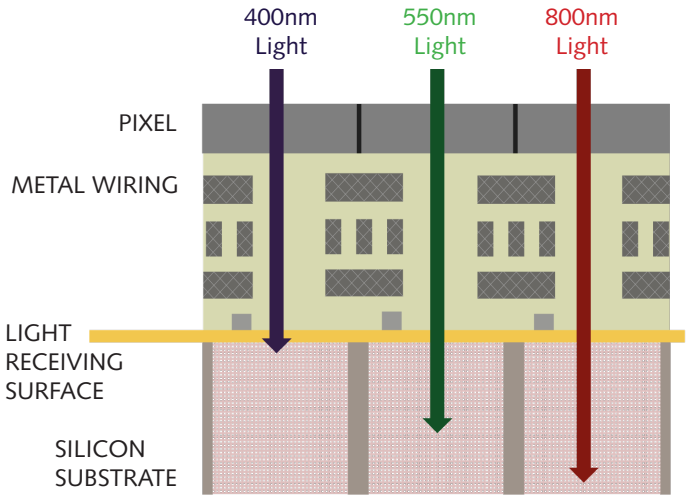


Figure 2: Light wavelength and silicon penetration.
Adapted from Sony, back-illuminated CMOS image sensor

Shorter wavelengths do not penetrate deep enough so many photons are lost before reaching the silicon substrate. At the other end of the spectrum, longer wavelengths penetrate too far so photons pass straight through the silicon substrate.

There is usually a quantum efficiency cut-off at around 400 nm where the majority of the photons are lost before they can reach the silicon substrate.

There is also a critical wavelength, usually at around 1100 nm, where incident photons have insufficient energy to produce an electron-hole pair so no signal can be generated.

The sensor surface

On CMOS sensors, a certain fraction of the pixel surface is covered in the metal tracks, wiring and transistors (the circuitry) necessary to collect and transport charge (Figure 3). This has the unfortunate side effect of making that area completely light insensitive.

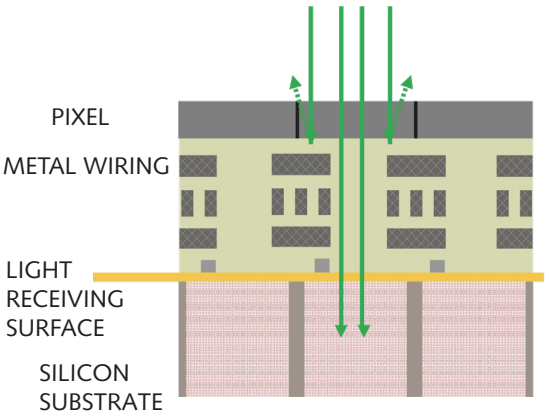


Figure 3: Photons unable to pass the metal tracks, wiring and transistors present in front of the CMOS sensor.

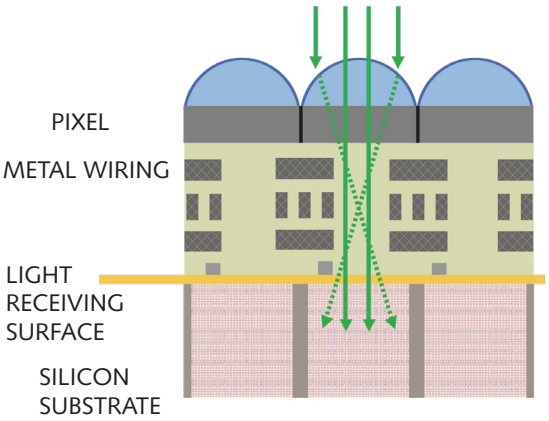


Figure 4: The use of microlenses to increase quantum efficiency of CMOS sensors.

The photons landing on this area can't reach the silicon substrate because they are physically impeded. These photons, therefore, won't be converted into electrons and so the quantum efficiency will be negatively affected. CMOS sensors using this architecture typically have a peak QE of 82%, so almost a fifth of the photons arriving at the pixel never make it to the silicon substrate.

The highest quantum efficiency sensors using this architecture was made possible through the addition of microlenses on the sensor surface (Figure 4). The microlenses are designed to focus the incident light away from the circuitry and onto the silicon substrate. This effectively increases the number of photons reaching the silicon substrate and therefore increases QE. CMOS Sensors using this architecture claim a QE of up to 82%.

A downside of microlenses, however, is that they are most effective when the incident angle of light is normal to the sensor surface. When light enters the sensor from any other angle, the effectiveness of the microlenses can become severely reduced. This means that the reported QE increase of a CMOS camera with microlenses may not accurately reflect the real QE increase.

Regardless of the issues with microlenses, the real problem to overcome is clearly the position of the circuitry. To address this, sensor manufacturers have recently started creating back-illuminated CMOS sensors. By inverting the sensor and bringing light in from the back, the circuitry can be avoided completely.

Back-Illumination

A back-illuminated sensor is one that has essentially been flipped over so light enters directly into the silicon substrate rather than having to pass through the circuitry (Figure 5). Any light loss due to objects on the sensor surface is thereby eliminated.

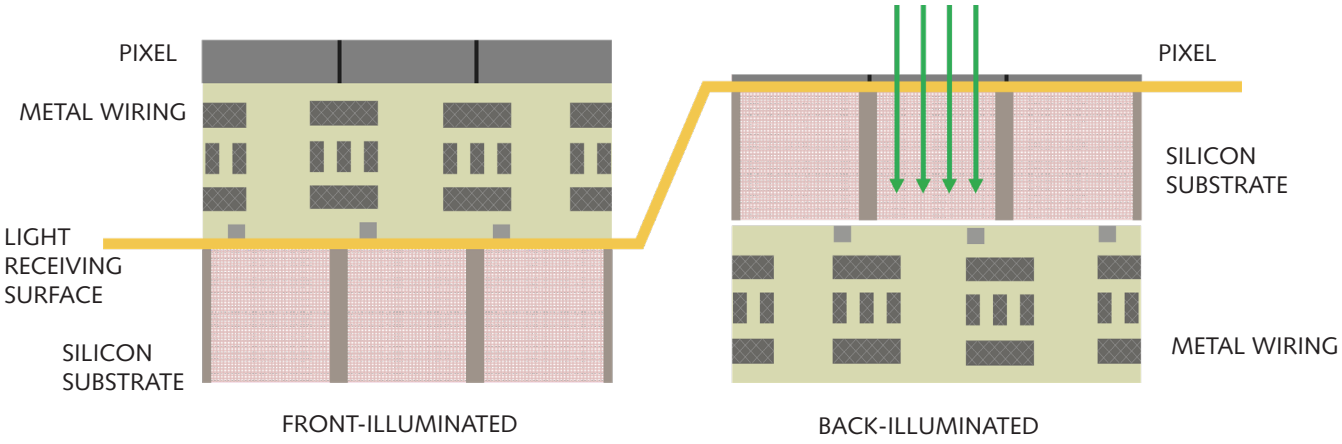


Figure 5: Comparison of front and back illuminated sensors.

To allow the photons to penetrate deep enough into the silicon substrate to be converted into electrons, the silicon must also be thinned at the back. For this reason, a back-illuminated sensor may also be referred to as a back-thinned sensor.

The result is a sensor with an almost perfect, 95% quantum efficiency at its optimum wavelength. This can be seen in Figure 1, which shows the QE curve of the Prime 95B back-illuminated CMOS compared to front-illuminated CMOS devices, such as the 82% CMOS camera with microlenses.

Another advantage of back-illumination, highlighted in Figure 1, is the ability to achieve a high QE with shorter, UV wavelengths of light. It's possible to achieve UV light detection on other CMOS devices but, as stated earlier, the fill-factor becomes limiting. This problem is completely overcome with a back-illuminated sensor. Moving the circuitry below the silicon substrate allows the fill-factor to reach 100%, granting both a high UV response and a high QE over a wide spectral range.

Summary

Camera sensitivity is determined by quantum efficiency, pixel size and noise characteristics. Increasing pixel size comes with the disadvantage of reducing resolution so increasing quantum efficiency is a more attractive method of increasing camera sensitivity. Recently, back-illuminated CMOS sensors have been developed which allow quantum efficiency to reach up to 95%.

Control of Noise and Background in Scientific CMOS Technology

Introduction

Scientific CMOS (Complementary metal–oxide–semiconductor) camera technology has enabled advancement in many areas of microscopy imaging. However, this technology also poses problems that camera manufacturers need to solve to produce a device capable of accurate quantitative imaging. To achieve this, several features of CMOS sensors have to be understood and then corrected for. In this technical note, we'll briefly discuss the most important points to consider when producing a scientific CMOS camera.

The main consideration is correction of noise, which will always occur during the acquisition of an image. Noise is the uncertainty which accompanies the acquired signal and can be divided into two major groups: camera-related noise and sample-related noise. As a camera manufacturer, there's little we can do to improve the sample-related noise but our goal is to minimize all camera-related noise.

Sample-Related Noise

Photon shot noise

Photon shot noise is the inherent natural variation of the incident photon flux - there is always uncertainty associated with the process of emission of photons from a fluorescent structure upon excitation (quantum nature). Photon shot noise follows a Poisson distribution and has a square root relationship between signal and noise where:

$$\text{Photon shot noise} = \sqrt{\text{Signal}}$$

All values are displayed in electrons (e^-).

This noise cannot be improved by advances in camera design as it is a physical phenomenon that can't be removed.

Camera-Related Noise

One of the main differences between standard CCD/EMCCD cameras and scientific CMOS cameras is the signal readout structure. A CCD/EMCCD camera converts charge (electron signal) to a voltage at the preamplifier, the voltage is then digitized by the Analogue to Digital Converter (ADC) to produce a digital signal that can be read by a computer (figure 1). CCD/EMCCDs, therefore, have a single preamplifier and ADC which digitize every single pixel one by one.

CMOS sensors, on the other hand, have a capacitor and amplifier on every pixel to convert charge into a voltage. This voltage is then digitized by an ADC at the end of every column (figure 2). This greatly increases the readout speed of the sensor but introduces other complications.

Read noise

Read noise is the noise introduced as the signal is read out i.e. passed through the preamplifier and ADC.

The architecture of CCD/EMCCD sensors (Figure 1) typically consists of a single preamplifier to convert charge into voltage. This means that every single pixel is treated the same way and so read noise will follow a Gaussian distribution. All pixels will be affected and fluctuate by a similar degree.

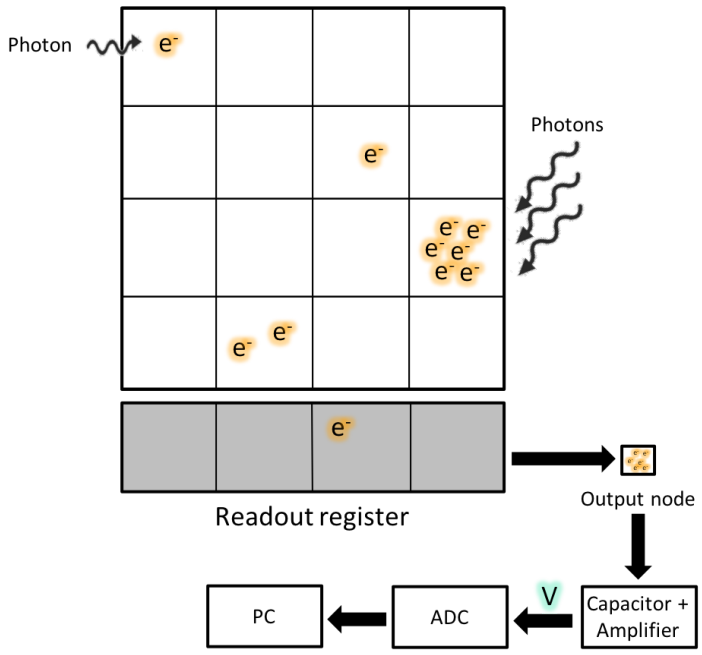


Figure 1: CCD architecture.
The number of electrons created is directly proportional to the number of photons hitting the pixel. After exposure, electrons are moved down, row by row, until they reach the readout register. The readout register shuttles the row of electrons one at a time into the output node which is connected to a capacitor and amplifier.

On CMOS sensors, each pixel has its own individual readout structure to convert charge into voltage. Furthermore, each column has its own ADC. Some scientific CMOS cameras also use a split sensor design where there are two ADCs instead, one for the top half of the chip and one for the bottom half. Although this results in a great increase in readout speed, a consequence of having multiple readout structures is that the read noise is now a distribution

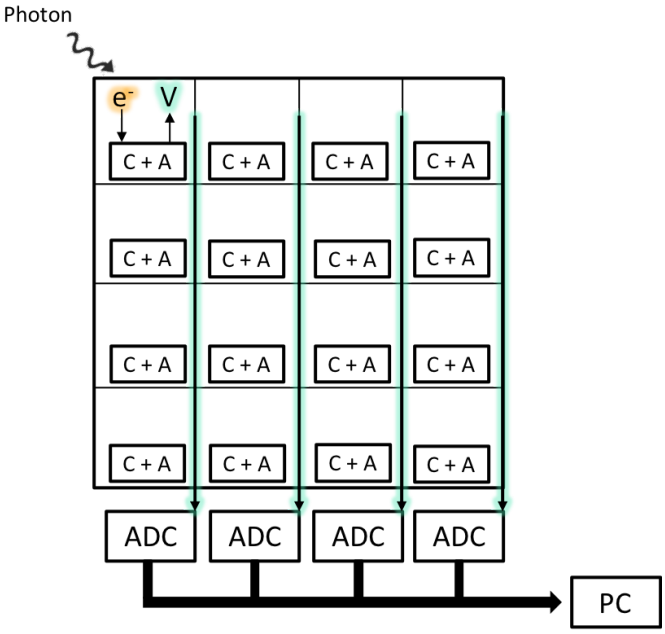


Figure 2: Scientific CMOS architecture. Photons hit the pixels and create electrons, individual capacitors and amplifiers are on every pixel. The generated voltages are sent down the whole column to the analogue to digital convertor (ADC) and the digital signal is read by a computer. This makes CMOS cameras much faster because they have one amplifier per pixel.

The true distribution of read noise which can be measured on CMOS sensors is not Gaussian but more of a skewed histogram. For this reason, read noise will be quoted on datasheets both as root mean square (RMS) and median. If a datasheet reports a median read noise of 1 electron, this means half of the pixels will report less than 1 electron read noise and half will report more. However, within this group, some pixels may report very high read noises such as 3 electrons or more. For this reason, the datasheet will also report an RMS value which is a far more meaningful description of read noise. This value will represent a true mean read noise and will, therefore, be higher than the median.

Pattern noise

Pattern noise (figure 3) is a noticeable pattern of 'hot' (bright) and 'cold' (dark) pixels in the background of the image and is produced regardless of illumination conditions. It goes hand-in-hand with CMOS read noise variation and a difference in the background offset (bias) value of individual columns, it's caused by small differences in the responsivity of individual pixels on the sensor.

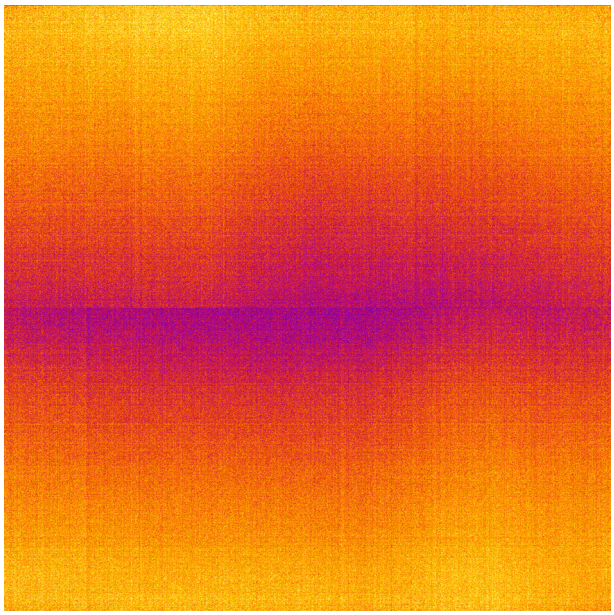


Figure 3: Fixed pattern noise. Fixed pattern noise on a typical scientific CMOS camera with a split sensor. Variation across the background is around 6 e^-.

Roll off

Roll off (figure 4A) is a phenomenon which can often be observed on CMOS sensors making use of split sensor technology. The roll off highlights the seam between the two sensors and displays a bias dip across it. This variation in the bias and the consequent lack of linearization makes quantitative imaging very difficult.

Newer, single-read and back-illuminated sensors such as those used in newer Teledyne Photometrics scientific CMOS cameras (Figure 4B) prevents this problem from occurring and provides a better environment for quantitative imaging.

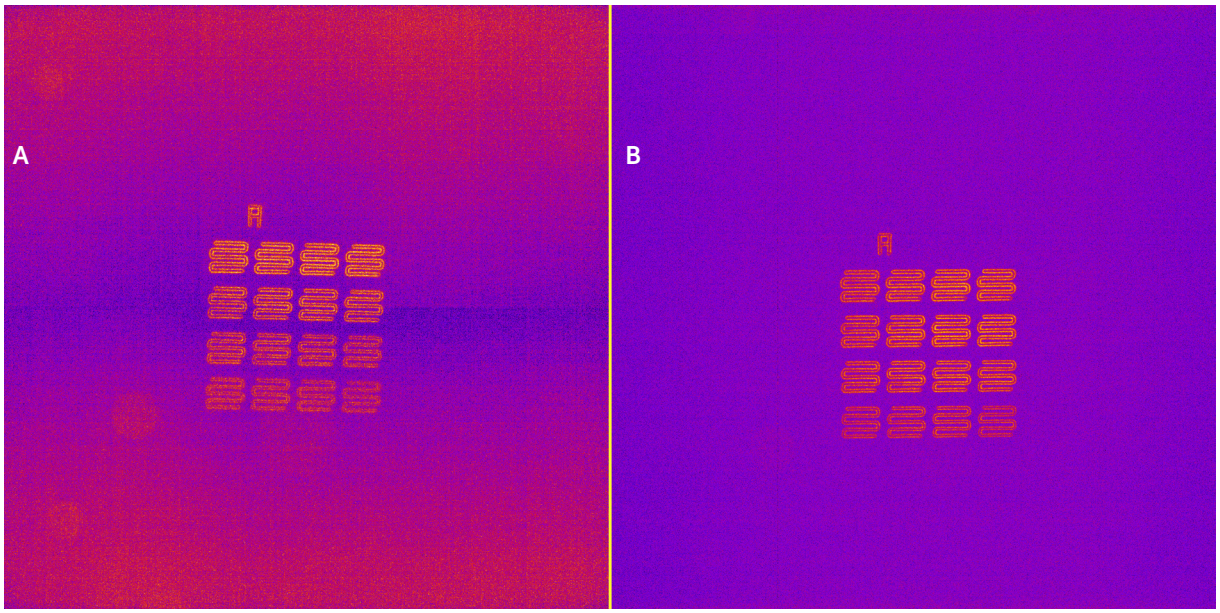


Figure 4: Roll off comparison
A) Current, standard, split sensor scientific CMOS camera and B) the GPixel GSENSE2020BSI sensor used in the Teledyne Photometrics Prime BSI.

Dark current

Dark current arises from charge building up on the sensor caused by thermal energy. Crucially, this noise is light independent. Because dark current is a thermal effect, cooling (e.g. with a Peltier element) is used as a measure to counteract this problem. Typically, dark current can be halved for every 7°C of cooling.

All high-performance scientific CMOS cameras will come with a dark current specification on the data sheet. For instance, a Teledyne Photometrics Prime BSI has a dark current specification of 0.5 e^-/p/s, resulting in 1 electron/pixel generated upon a 2 s exposure time.

Clock induced noise & random telegraph noise

It's possible to run EMCCD cameras at a very high clocking speed to achieve high frame rates but this introduces additional noise which can add up to 5 e^- of noise per pixel. This is called clock induced charge (CIC) and occurs when an electron is inadvertently generated without being induced by an incident photon. CIC has to be corrected for by using a spurious noise filter to identify the affected pixels and replace the measured signal by taking a mean value from its neighbours (nearest-neighbor correction).

On a CMOS sensor, the increased speed reduces the need to run the pixel clock of the chip at or over the maximum. As a result, the effect of clock induced noise is also greatly reduced.

However, CMOS sensor architecture suffers instead from random telegraph noise (RTN) or 'salt'n'pepper' noise (Figure 5). This noise is caused by charge moving in and out of pixel defects. The output from a single pixel thereby fluctuates between bright, average, and dark states many times over the course of an acquisition. Similar correction as that used for CIC applies to RTN and it is corrected on the sensor in engineering.

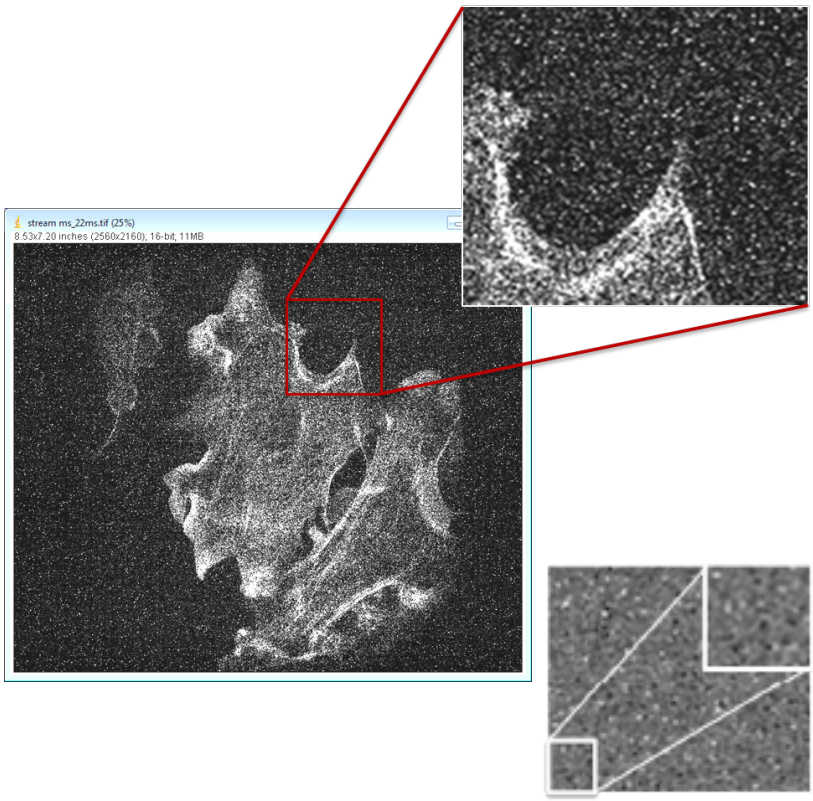


Figure 5: Random Telegraph Noise.
Example of random telegraph noise displaying the fluctuation between bright, average and dark pixel states.

Correlated noise

Correlated noise occurs due to capacitive coupling on the sensor. This is where energy is transferred between pixels by means of displacement current which causes neighboring pixels to share charge, effectively correlating the measured pixel signal. This results in an overestimation of the camera system gain which, by extension, overestimates the read noise, dark current and quantum efficiency of the camera.

This is also corrected in engineering until it is certain that all charge originates on the pixel and isn't being influenced by the charge of any surrounding pixel.

Further considerations

Binning is a common method to increase speed and sensitivity on CCD/EMCCD cameras. When binning, the signal of a 2x2 or 4x4 square of neighbouring pixels is combined. This increases the signal by summing the charge of all these pixels and as a result, fewer effective pixels require digitization which decreases the speed bottleneck at the ADC. On CCD/EMCCD cameras, summing the charge of neighboring pixels occurs before the ADC so read noise is only applied once.

In contrast, the parallelization of the read out on scientific CMOS cameras will apply read noise to each column. The combination of neighbouring pixels' signal will occur afterwards. Pixels from the same column will combine their information before read noise is applied but combining the information from pixels originating from neighbouring columns will sum their according read noise. Effectively, binning on scientific CMOS technology is partially performed in software.

As a result, binning does not have the same speed and noise benefits on scientific CMOS as it does on CCD/EMCCD cameras. Furthermore, scientific CMOS fixed pattern noise will be substantially amplified by binning. On early scientific CMOS cameras, this was a problem which couldn't be solved. However, improved correction algorithms for CMOS sensors have minimized this effect at the current state of this technology.

Teledyne Photometrics Solution for Minimizing Camera Noise

Our solution to the problems of camera noise is to carefully characterise each individual sensor and fine-tune for all eventualities. Scientific CMOS technology is in this respect very challenging as pixel-to-pixel variations, but also column-to-column variations need to be taken into consideration. Equalling bias value, linearizing the response and gain of each individual pixel across the chip is key to achieve those targets. The aim is for all columns to be perfectly balanced.

Other levers to unify the behaviour across the chip is tuning the use of facilitated correlated double sampling. Where two signals per pixel are effectively measured during acquisition: (a) bias for the offset and (b) amount of light. The difference between the two will effectively give the real signal.

Background Event Reduction Technology (BERT) enables researchers to identify the pixels that are likely to contain spurious event data and then correct the data if desired. Originally designed for EMCCD cameras, this technology has also been optimized for and applied to Teledyne Photometrics scientific CMOS cameras.
BERT removes spurious events which could not have been generated via incident photons in real-time. It allows the image captured to more accurately represent what the sample is and remove artefacts.
Spuriously large pixel values which could not have come from the actual sample being imaged are replaced with a best approximation by taking the mean of the surrounding pixels and absolutely minimizes the influence of thermally-induced amplified events on acquired data.

Maximizing Microscope Field of View

Introduction

Microscope field of view (FOV) is the maximum area visible when looking through the microscope eyepiece (eyepiece FOV) or scientific camera (camera FOV), usually quoted as a diameter measurement (figure 1). Maximizing FOV is desirable for many applications because the increased throughput results in more data collected which gives a better statistical measurement for detecting subtle effects and also decreases time needed at the microscope.

The FOV of a microscope is ultimately limited by a number of factors, such as the objective lens, the tube-diameter of the microscope's internal optical-system, the eyepieces, the scientific camera sensor size and the camera mounting adaptor.

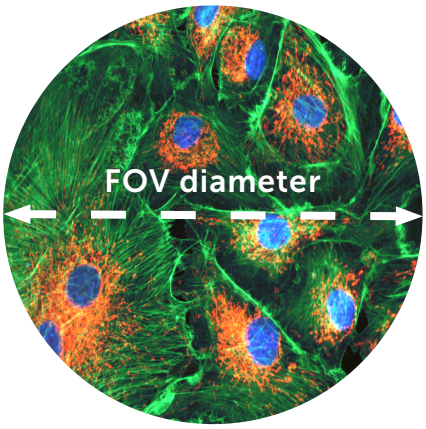
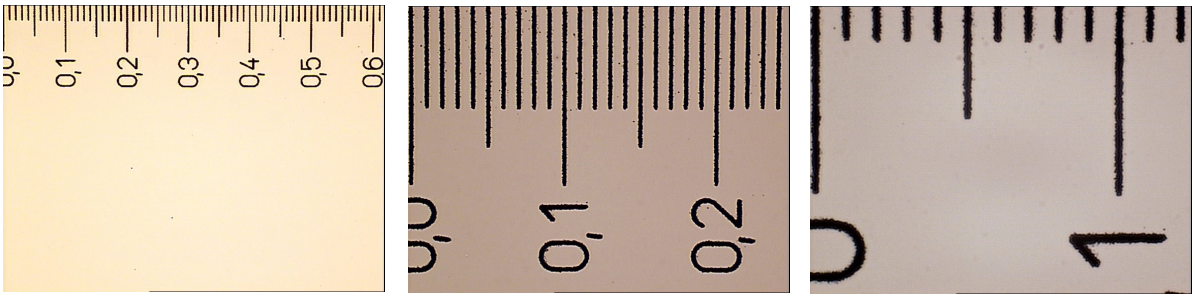


Figure 1: Microscope field of view is measured as the diameter.

It's usually possible to find the maximum FOV of the microscope by referring to the field number (FN) displayed on the eyepieces and on Figure 1: Microscope field of view some objective lenses. The field number is simply the maximum FOV of measured as a diameter the objective or eyepiece in millimeters, so an objective lens with a field number of 18 would have a maximum FOV of 18 mm. However, the field number always assumes no magnification so to calculate the actual FOV, the field number should be divided by the objective magnification:

$$\text{FOV} = \frac{\text{Field Number}}{\text{Objective Magnification}}$$

A 20x objective with a field number of 18 would actually have a FOV of 0.9 mm. Likewise, a 100x objective with a field number of 18 would have a FOV of 0.18 mm. The more an object is magnified, the smaller the field of view will be. Therefore, when looking to increase FOV, one of the first considerations should always be whether it's possible to decrease magnification (figure 2).



20x Objective (~0.6 mm horizontal FOV) | **50x Objective (~0.25 mm horizontal FOV)** | **100x Objective (~0.12 mm horizontal FOV)**

Figure 2: Reduction in field of view with increasing magnification. The visible length of the graticule measures ~0.6 mm under 20x magnification but ~0.25 mm at 50x and only ~0.12 mm at 100x.

Matching Scientific Camera FOV to Microscope FOV

Using the field number to calculate microscope FOV works well when imaging using the eyepieces but not when imaging using a scientific camera. Like most digital cameras, scientific cameras use square or rectangular sensors. This means that a scientific camera cannot capture the whole, circular FOV that the microscope is capable of. Instead, the camera FOV must fit inside the microscope FOV (figure 3).

Camera specification sheets will display the camera FOV as a diagonal measurement (usually in millimeters). Ideally, the diagonal camera FOV should match the diameter of the microscope FOV to capture as much of the available image as possible. However, this does mean that the horizontal and vertical FOV of the camera will be less than the microscope diameter.

It's possible to use a camera with a larger diagonal FOV than the microscope to capture the entire microscope FOV (figure 4). However, this is not optimal as there will be substantial vignetting at the corners of the image. Ideally, when choosing a scientific camera, it should have a diagonal FOV that matches the specifications of the microscope it will be used with.

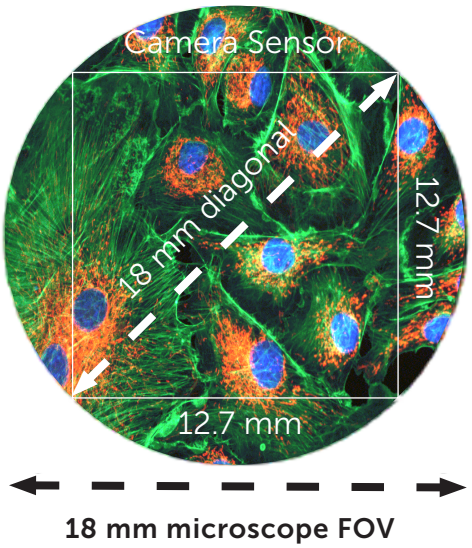


Figure 3: A scientific camera with an 18 mm diagonal FOV ideally fits an 18 mm microscope FOV. The x and y sides of the camera sensor measure 12.7 mm to allow for this diagonal FOV.

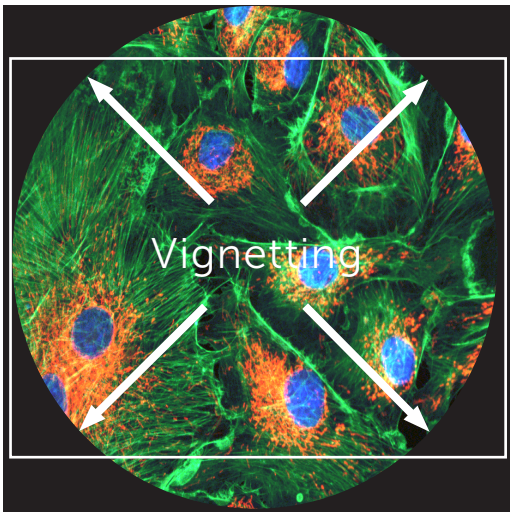


Figure 4: A camera sensor with a larger diagonal FOV than the microscope FOV would show considerable vignetting in the corners of the image.

Matching Adaptor FOV to Camera and Microscope FOV

A microscope C-mount or F-mount adaptor is needed to connect a scientific camera to the microscope camera port. The mount threading is standardized which means that a C-mount adaptor will connect to all scientific cameras that connect via C-mount. However, the adaptors are microscope specific which means that although any C-mount camera will connect to a C-mount adaptor, the adaptor will only fit microscopes of the matching brand.

Adapters can have lenses in them to magnify or demagnify the image before it reaches the camera. This can be used to better match the camera FOV to the microscope FOV. For example, if the camera has an 11 mm diagonal FOV but the microscope is capable of an 18 mm FOV, a 0.67x adaptor would demagnify the image and allow it to be displayed on the 11 mm camera. However, this increase in FOV comes at the cost of reduced resolution.

If the goal is simply to attach the camera to the microscope, a 1x adaptor contains no additional lenses and provides no additional magnification or demagnification. This is often the preferred method as it introduces no additional lenses into the system. Every extra lens reduces the number of photons reaching the camera by 3-4% so many researchers will try to avoid this.

Adaptors can also affect the microscope and camera FOV depending on the type of adaptor used. A C-mount adaptor is the most popular microscope camera adaptor and is restricted to a maximum 22 mm FOV. The F-mount adaptor is a larger format adaptor capable of reaching >30 mm FOV.

The development of larger FOV microscopes and scientific cameras that can take advantage of the F-mount is relatively recent - at the time of writing only one commercially available 25 mm microscope exists. Most modern microscopes have a 19 mm or 22 mm FOV and are therefore still able to use the C-mount. The largest format spinning disk confocal systems are also limited to a 22 mm FOV.

Choosing a Camera to Maximize Microscope FOV

At Teledyne Photometrics, we aim to create cameras that can optimally match the FOV of all modern microscopes (table 1). For this reason, the Prime 95B Series comprises a 19 mm camera, a 22 mm camera and a 25 mm camera. Additionally, the Prime BSI and Iris 9 both fit a 19 mm microscope FOV and the Iris 15 fits a 25 mm microscope FOV.

Camera	Microscope FOV 19 mm	22 mm	25 mm
Prime 95B™	✓		
Prime 95B 22mm™		✓	
Prime 95B 25mm™			✓
Prime BSI™	✓		
Iris 9™	✓		
Iris 15™			✓

Table 1: Teledyne Photometrics cameras are optimized to match any microscope with a FOV of 19 mm, 22 mm or 25 mm.

By recognizing that FOV requirements can be highly variable, we are able to better serve the needs of our customers and offer a broad range of camera FOV options.

Summary

The maximum field of view of the microscope is affected by the objective lens, the tube-diameter of the microscope's internal optical-system, the eyepieces, the scientific camera sensor size, and the camera mounting adaptor. For optimal imaging performance, it's best to match the microscope FOV to the scientific camera FOV to capture as much information as possible and avoid vignetting. Typical microscopes have a 19 mm, 22 mm or 25 mm FOV which is why Teledyne Photometrics cameras are designed to match these specifications to offer the maximum field of view possible.

The Effect of Camera Cooling on Signal to Noise Ratio

Introduction

Signal to noise ratio describes the relationship between the detected signal originating from the sample and the uncertainty involved in the measurement of that signal on a per-pixel basis. It is essentially the ratio of the measured signal to the overall measured noise. Most low-light microscopy applications look to maximize signal and minimize noise.

Noise, in this case, does not refer to background fluorescence or what may be referred to as 'sample noise', this is noise generated by the scientific camera. It is important to note that all scientific cameras generate noise, the three main sources of which are read noise, photon shot noise and dark current. One of the primary functions of camera cooling is to reduce dark current. Considerable effort is invested into minimizing and controlling these noise characteristics to ensure that scientific cameras perform as true quantitative measurement devices.

Noise values should be displayed on all scientific camera data sheets and they are always displayed in electrons. Therefore, when calculating signal to noise ratio, it is important to compare signal in electrons to noise in electrons. Determining signal in electrons from a biological sample is a relatively simple process, explained thoroughly in our camera testing protocol. This document will focus primarily on how cooling and dark current impacts signal to noise ratio.

Scientific Camera Noise Sources

Read noise

Read noise is the amount of noise generated by electronics as the charge collected on the pixels is transferred to the camera. It is a combination of all the noise generated by system components which convert the charge of each pixel into a signal for conversion into a digital unit (ADU or gray-level value) that can be displayed by the computer.

A lower read noise is always desirable. It allows for the detection of very weak signals that would have otherwise been hidden below the noise floor. It also allows for a higher dynamic range, enabling more accurate detection of the difference in signal levels.

Photon shot noise

Photons incident on the pixels of a scientific camera are converted to photoelectrons within the device's silicon layer. These photoelectrons constitute the signal but also carry statistical variation in the photon arrival rate at a given point. Photon noise, also known as photon shot noise, refers to the inherent natural variation of the incident photon flux. The number of photoelectrons collected by the pixel exhibits a Poisson distribution and has a square root relationship between signal and noise. Photon noise cannot be reduced via camera design.

Dark current

Dark current is caused by thermal energy within the silicon lattice comprising the scientific camera sensor. This thermal energy, or heat, can generate electrons. Typically, electrons are only generated from incident photons but electrons generated from dark current are independent of light falling on the sensor. The difficulty is that these electrons are still captured by the pixels and counted as signal.

Dark current builds up over time, therefore the number of electrons contributed by dark current is directly proportional to the exposure time. For this reason, dark current specifications on scientific camera data sheets are expressed in electrons per pixel per second (e^-/p/s). As an example, a scientific camera with a dark current specification of 1 e^-/p/s would generate 1 electron of dark current with a 1 second exposure time. However, if the exposure time was 10-fold lower, 100 ms, only 0.1 electrons of dark current would build up. This is an important factor to consider when considering the typical exposure time needed for the application.

Cooling is a necessary feature on scientific CMOS cameras. Cooling directly reduces dark current, lowering the noise floor, as well as minimizing the occurrence of hot pixels. An uncooled scientific camera would not only struggle with low-light detection but, due to hot pixels, would also not perform as a true quantitative measurement device. On an uncooled camera, hot pixels would otherwise need to be controlled by interpolation filters which can be problematic for some applications requiring quantitative pixel uniformity such as in super-resolution localization microscopy.

The most common method of cooling used by scientific cameras is thermoelectric cooling, or Peltier cooling, where heat is transferred away from the sensor and onto a heat sink which dissipates the heat. If increased cooling is needed, liquid cooling is often the preferred choice.

A common misunderstanding when comparing data sheets is to emphasize the temperature the camera is cooled to over the dark current specification. The primary goal of camera cooling is to reduce dark current so dark current is what should be compared. Ideally, dark current should be reduced to a point where its contribution is negligible for a typical exposure time.

Calculating Signal to Noise Ratio

Signal to noise ratio (SNR) is simply the signal divided by the sum of the three main noise sources. It can be expressed by the following equation:

$$\mathrm{SNR} = \frac{S}{\sqrt{S + [N_d * t] + N_r^2}}$$

Where:

S = Signal (e^-)

Nd = Dark current ($e^-/p/s$)

Nr = Read noise (e^-)

t = Exposure time (s)

A high SNR guarantees clear images with low distortions and artifacts caused by noise. The higher the SNR, the better the signal stands out, the better the quality of the images, and the ability to see the desired results is improved.

There are no hard rules about what an ideal signal to noise ratio is because this often depends on the sample and the needs of the application. However, generally speaking, an SNR greater than 1 is required for detection, greater than 5 allows structures to be segmented, greater than 10 allows noise to be mostly overcome and greater than 15 allows for full measurement confidence (Figure 1).

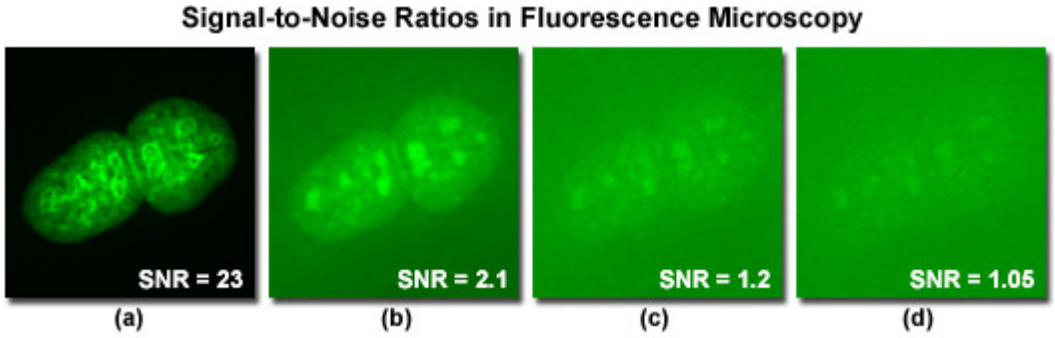


Figure 1: Signal to noise ratio comparison in fluorescence microscopy. Noise is clearly dominant at SNR=1-2 but improves slightly as it is increased to SNR=2. At SNR=23 there can be full measurement confidence with extremely minimal noise impact. Adapted from olympus-lifescience.com.

Increasing Signal to Noise Ratio

To increase image quality, SNR can be increased in a variety of different ways. Unfortunately, most of which have tradeoffs that also need to be considered:

Increase the exposure time

Collecting signal for a longer time allows for a higher signal level to be reached, potentially lifting the signal above the noise floor. However, this would sacrifice the ability to image at a desired, lower frame rate. It also exposes cells to more light, worsening the effects of phototoxicity and photobleaching. If the exposure time is long enough, the noise from dark current may also start to become a larger portion of the signal.

Frame averaging

Frame averaging reduces total image noise by the square root of the number of frames averaged. However, the ability to image at desired frame rates will again be sacrificed and this method is generally less effective than increasing exposure time.

Increase the excitation intensity

This allows for a higher signal level without trading off temporal resolution. However, the rate at which phototoxicity and photobleaching occur is also increased, reducing cell viability.

Denoising algorithms

Denoising algorithms increase signal to noise ratio by reducing the effects of photon shot noise at low light levels, improving the quality of images and data. However, there are many challenges when processing data to reduce noise such as preserving the quantitative nature of the recorded pixel intensities, as well as preserving key features like edges, textures, and details with low contrast. Further, processing has to be accomplished without introducing new image artifacts like ringing, aliasing or blurring. Some algorithms can be inflexible with different image types, resulting in these intrusive artifacts. Additionally, because noise tends to vary with the level of signal, it is also difficult for some denoising algorithms to distinguish signal from noise, and as a consequence, small details may be removed. It is very important when using denoising algorithms that the user knows what they are doing.

The best denoising algorithm we've found is the safir algorithm created at INRIA and optimized for fluorescence microscopy in collaboration with the Institute Curie1. This algorithm can be used live on the camera or offline using the free ImageJ plugin.

Improved camera specifications

No matter which additional techniques are used for increasing signal to noise ratio, using a scientific camera with high sensitivity and low noise characteristics will always be of benefit. A back-illuminated device with 95% quantum efficiency will collect as many incoming photons as possible and having a low read noise floor and dark current allows for the detection of the lowest signal levels.

It is also important to consider factors such as background quality, field uniformity and hot-pixel correction – all of which contribute to the scientific camera being a true quantitative measurement device.

In the following investigation, we look at the impact of the main types of camera noise on SNR and determine which specifications result in the highest SNR.

Analysis of Dark Current Impact on SNR

To investigate the impact of scientific camera noise on SNR we set up an analysis of two back-illuminated (BI) CMOS cameras with differing cooling and dark current specifications. The goal of this analysis is to show how dark current impacts SNR as exposure times get longer and to see at which point dark current becomes a significant enough noise source to significantly affect SNR. The names and noise specifications of the cameras are described in Table 1.

Camera	Read noise (e^-) (median)	Dark current (e^-/p/s)
Prime 95B	1.6	0.3
-45°C Chiller Cooled BI	1.6	0.2

Table 1: Comparison of the noise specifications of two back-illuminated (BI) CMOS cameras: The Prime 95B liquid cooled to -25°C and a -45°C chiller cooled back-illuminated CMOS.
All reported read noise values are median values and dark current specifications are taken directly from the camera specification sheets.

The cameras are compared over two conditions; 10 electrons of signal and 100 electrons of signal, representing low-light and moderate-light conditions, respectively. Both cameras are back-illuminated with 95% quantum efficiency and have the same pixel size, so the conditions assume that both cameras detect an equal number of electrons of signal. Signal to noise ratio is calculated using the SNR equation described above using the read noise and dark current specifications reported in Table 1. The photon shot noise value is given by the square root of the signal condition ie. In the 10 electron signal condition, the photon shot noise is $\sqrt{10}$.

10 Electron Signal

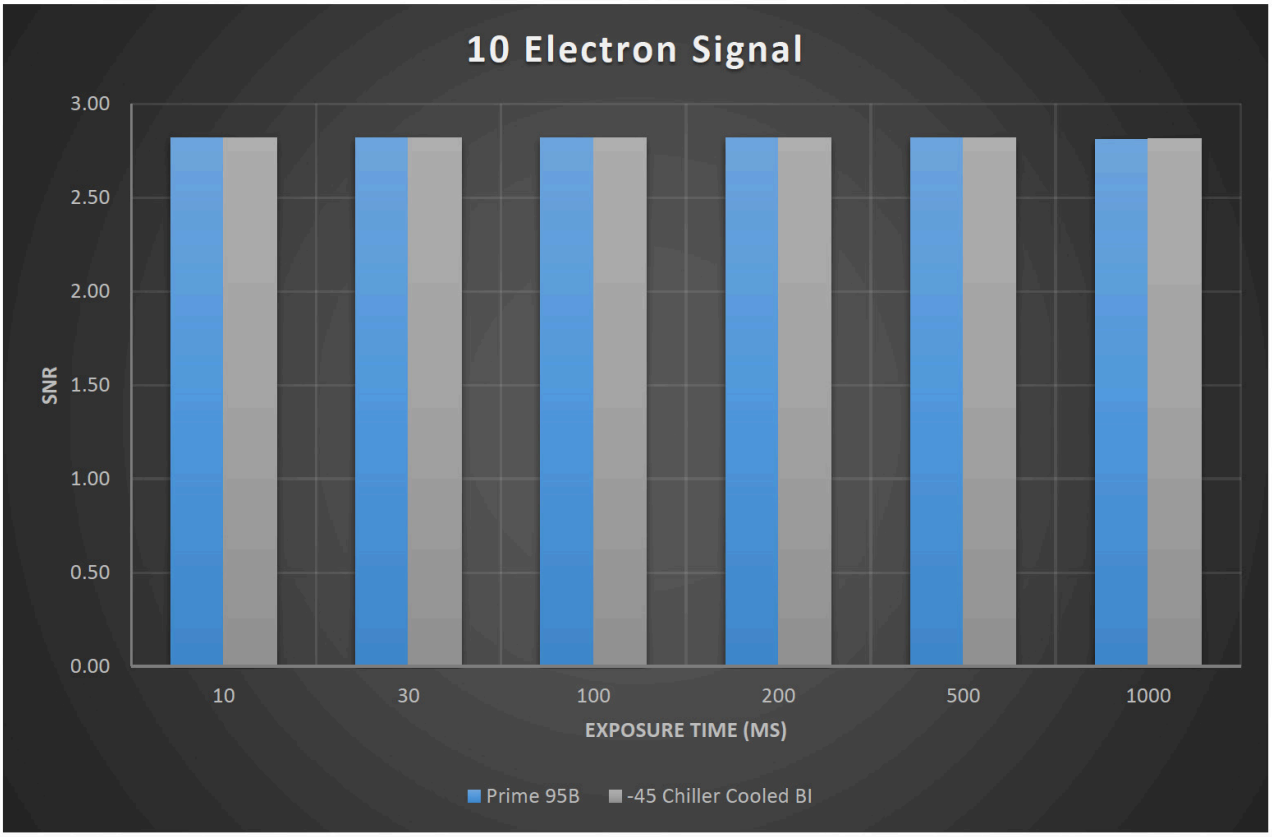


Figure 2: Signal to noise comparison of two back-illuminated CMOS cameras with 10 electrons of detected signal. Exposure times range from 10 milliseconds to 1000 milliseconds (1 second).

Exposure time (ms)	SNR at 10 e^-	
	Prime 95B	-45°C Chiller Cooled BI
10 (100 fps)	2.82	2.82
30 (30 fps)	2.82	2.82
100 (10 fps)	2.82	2.82
200 (5 fps)	2.82	2.82
500 (2 fps)	2.82	2.82
1000 (1 fps)	2.81	2.82

Table 2: Signal to noise comparison values from Figure 2 at 10 ms, 30 ms, 100 ms, 200 ms and 1000 ms exposure times representing 100 fps, 30 fps, 10 fps, 5 fps and 1 fps respectively.

Figure 2 and Table 2 show that the Prime 95B and the -45° Chiller Cooled BI both start with an SNR of 2.82 at 10 ms and stay the same across all exposure times until 1 second where they only differ by an SNR of 0.01.

10 Electron Signal

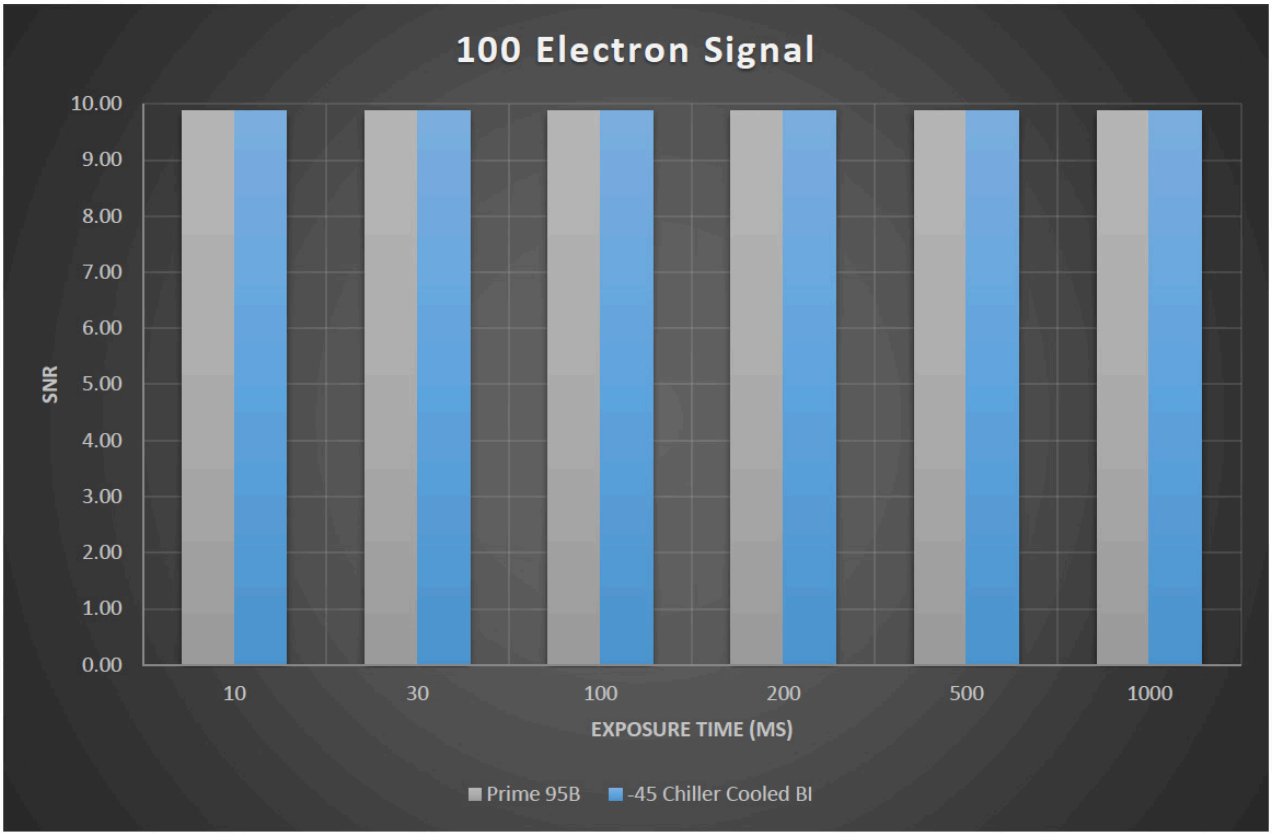


Figure 3: Signal to noise comparison of two back-illuminated CMOS cameras with 100 electrons of detected signal. Exposure times range from 10 milliseconds to 1000 milliseconds (1 second).

	SNR at 100 e^-	
Exposure time (ms)	Prime 95B	-45°C Chiller Cooled BI
10 (100 fps)	9.87	9.87
30 (30 fps)	9.87	9.87
100 (10 fps)	9.87	9.87
200 (5 fps)	9.87	9.87
500 (2 fps)	9.87	9.87
1000 (1 fps)	9.87	9.87

Table 3: Signal to noise comparison values from Figure 3 at 10 ms, 30 ms, 100 ms, 200 ms, 500 ms and 1000 ms exposure times representing 100 fps, 30 fps, 10 fps, 5 fps, 2 fps and 1 fps respectively.

Figure 3 and Table 3 show that the Prime 95B and the -45°C Chiller Cooled BI both start with an SNR of 9.87 and stay the same across all exposure times, even with a 1 second exposure time they are no different.

Noise Contribution

Signal to noise values give a strong indication of the image quality but it can also be useful to know the raw noise contribution from the main noise sources. The noise contribution in electrons of the two cameras at 10 e^- and 100 e^- of signal is summarized in Tables 4 and 5.

	Noise Contribution (e^-) at 10 e^- signal	
Exposure time (ms)	**Prime 95B**	**-45°C Chiller Cooled BI**
10 (100 fps)	3.54	3.54
30 (30 fps)	3.54	3.54
100 (10 fps)	3.54	3.54
200 (5 fps)	3.54	3.54
500 (2 fps)	3.55	3.55
1000 (1 fps)	3.56	3.55

Table 4: Noise contribution in electrons of the two back-illuminated cameras with 10 e- signal at 10 ms, 30 ms, 100 ms, 200 ms, 500 ms and 1000 ms exposure times representing 100 fps, 30 fps, 10 fps, 5 fps, 2 fps and 1 fps respectively.

	Noise Contribution (e^-) at 100 e^- signal	
Exposure time (ms)	**Prime 95B**	**-45°C Chiller Cooled BI**
10 (100 fps)	10.13	10.13
30 (30 fps)	10.13	10.13
100 (10 fps)	10.13	10.13
200 (5 fps)	10.13	10.13
500 (2 fps)	10.13	10.13
1000 (1 fps)	10.13	10.13

Table 5: Noise contribution of the three back-illuminated cameras with 100 e^- signal at 10 ms, 30 ms, 100 ms, 200 ms, 500 ms and 1000 ms exposure times representing 100 fps, 30 fps, 10 fps, 5 fps, 2 fps and 1 fps respectively.

The data displayed in Tables 4 and 5 make it clear that the relative noise contribution difference between the Prime 95B and -45°C chiller cooled BI cameras is extremely minimal. The increased cooling doesn't result in a noise difference until a 1 second exposure time where the difference is just 0.01 e^-, far too low to make a detectable difference in signal.

Summary

We can conclude from our SNR investigation of the Prime 95B and -45°C chiller cooled back-illuminated CMOS cameras that, in low-light conditions (10 electrons of signal), the advantage of increased cooling on dark current is so minimal that even with a one second exposure time, there is no detectable increase in SNR.

In moderate-light conditions (100 electrons of signal), camera noise characteristics have a much lower impact on SNR and there is no significant decrease in SNR on any camera due to dark current.

The results of this study show that when comparing cameras for low-light imaging, drilling down into the numbers and performing a signal to noise ratio comparison using typical exposure times and signal levels in electrons enables any user to determine how camera specifications affect the quality of their data.

References

[1] *Boulanger, J., Kervrann, C., Bouthemy, P., Elbau, P., Sibarita, J. B. & Salamero, J. (2010) Patch-based nonlocal functional for denoising fluorescence microscopy image sequences. IEEE Trans. Med Imaging*

Using Additional Optics to Adjust Pixel Size

Introduction

Additional optics such as magnification couplers (also known as camera mount adaptors) can be used to optically adjust the effective pixel size and field of view of scientific cameras to better match the microscope resolution and field of view, respectively. Couplers are placed just before the camera on the camera port of the microscope and are available in several magnification or demagnification values, the most common of which being 0.5x, 0.66x, 1x, 1.5x and 2x.

The way couplers function can be described quite simply; demagnification increases the effective pixel size and magnification decreases the effective pixel size at the sample. As Table 1 shows, an 11µm pixel demagnified with a 0.5x coupler would have an effective pixel size of 22 µm but when magnified with a 2x coupler it would have an effective pixel size of 5.5 µm. These two couplers therefore represent a doubling or halving of the initial pixel size.

11 µm Pixel	Magnification Coupler				
	0.5x	0.66x	1x	1.5x	2x
Effective Pixel size	22.0	16.7	11.0	7.3	5.5

Table 1: The effect of demagnifying and magnifying couplers on the effective pixel size of an 11 µm pixel camera with a 1.4NA objective.

Changing pixel size with magnification couplers allows the user to trade off sampling at the appropriate resolution for field of view or vice-versa. Using a demagnification coupler increases field of view at the expense of a larger effective pixel whereas using a magnification coupler decreases effective pixel size at the expense of a smaller field of view.

The ideal magnification coupler is almost always 1x, which contains no lens and allows light to travel from the microscope to the camera without any additional correction. This avoids the other issues inherent with magnification couplers such as a reduction in the number of photons (each additional lens in the light path reduces the number of photons by 3-4%), reduced image quality (couplers can contain lower quality lenses than other microscope optics) and uneven illumination of the camera sensor.

Magnification Means Pixels Cover a Smaller Sample Area

One of the most common uses of magnification couplers is to map the pixel across a smaller sample area. If the pixel is too large, the image cannot be sampled at the microscopes limiting resolution. Nyquist sampling is often desired in microscopy applications because it ensures the finest sample features possible are captured in the image while maximizing the sensors field of view.

A full explanation of Nyquist sampling and diffraction limited resolution can be found in the Teledyne Photometrics technical note on resolution.

As Table 2 shows, to achieve Nyquist sampling at 100x/1.4NA the largest effective pixel size that can be used is 11 µm. However, to achieve Nyquist sampling at 60x/1.4NA, a pixel size of 6.6 µm is required so an 11 µm pixel camera will not achieve Nyquist sampling. By cross-referencing with Table 1, the 11 µm pixel camera can be made to reach a minimum effective pixel size of 6.6 µm with a 2x magnification coupler to give an effective 5.5 µm pixel. A 1.5x coupler only reaches 7.3 µm which unfortunately isn't enough – using this would give equivalent resolution to a 60x/1.2NA objective.

Magnification	Pixel size for Nyquist (µm)
40x 1.3 NA	4.8
60x 1.4 NA	6.6
100x 1.4 NA	11

Table 2: Pixel size required for Nyquist at 40x, 60x and 100x assuming an emission wavelength of 510 nm (GFP)

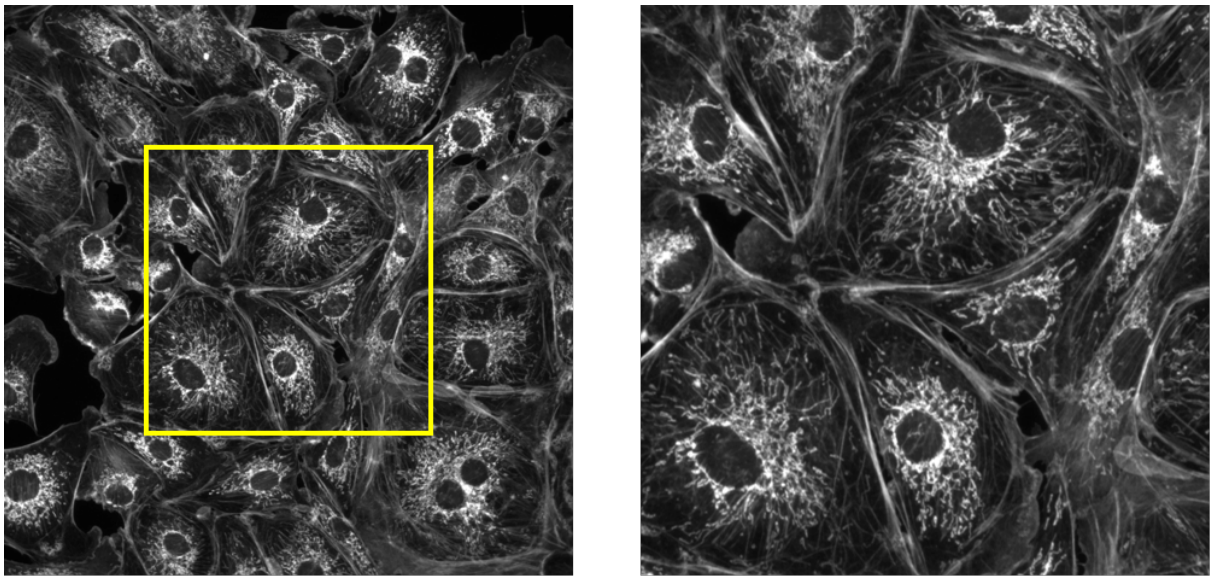
However, using a magnification coupler with an 11 µm pixel camera to reach the required pixel size for Nyquist sampling will have a significant effect on field of view. As Table 3 shows, using a 60x objective with a 2x magnification coupler means the final magnification will be 120x which results in a 50% reduction in field of view. The more the sample is magnified, the less sample area can be seen.

Magnification	Coupler Magnification	Actual Magnification	Field of View
40x	1x	40x	100%
	1.5x	60x	75%
	2x	80x	50%
60x	1x	60x	100%
	1.5x	90x	75%
	2x	120x	50%
100x	1x	100x	100%
	1.5x	150x	75%
	2x	200x	50%

Table 3: The difference in objective magnification and actual magnification when applying 1x, 1.5x and 2x magnification couplers.

The difference in field of view when comparing 60x magnification with 60x magnification with a 2x coupler is illustrated in Figure 1. The sacrifice in field of view when using an 11 µm pixel camera to match Nyquist at 60x is quite considerable.

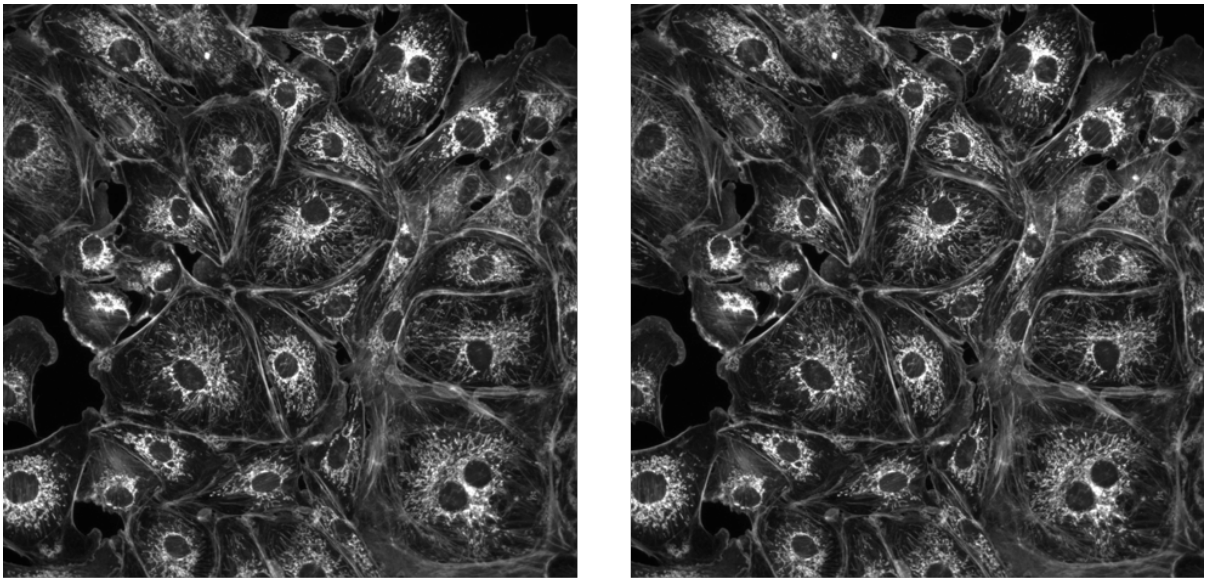
A more favorable solution that would allow for Nyquist sampling at 60x whilst maintaining the field of view would be to use a scientific camera with a 6.5 µm pixel size. This would also result in a more sensitive camera because a larger pixel has a larger area for photon collection. A 6.5 µm pixel has a 42.25 µm2 pixel area whereas a 5.5 µm pixel has a 30.25 µm2 pixel area, which gives ~30% reduction in sensitivity. The field of view comparison between an 11 µm pixel camera and a 6.5 µm pixel camera at 60x magnification is shown in Figure 2.



11 µm pixel camera at 60x

11 µm pixel camera at 120x (60x objective with 2x magnification coupler)

Figure 1: Field of view comparison of an 11 µm pixel camera at 60x magnification (left) with an 11 µm pixel camera at 60x magnification with a 2x magnification coupler resulting in an actual magnification of 120x (right). The yellow box highlights the field of view from the image right.



11 µm pixel camera at 60x

6.5 µm pixel camera at 60x

Figure 2: Field of view comparison of an 11 µm pixel camera at 60x magnification (left) with a 6.5 µm pixel camera at 60x magnification (right).

Image Quality is Only as Good as The Worst Lens

One of the major issues when using magnification couplers is that it introduces another lens into the system. This may be undesirable for two reasons; firstly, every additional lens in the light path loses 3-4% of the photons through reflection, and secondly, the quality of the image is only as good as the worst lens in the system.

The quality of the lenses in a microscope determine how well the system performs. Objective lenses are among the most expensive part of the microscope and for a good reason, they are extremely high-quality lenses with multiple corrections to ensure high quality light transmission.

Apochromatic objectives, for example, are chromatically corrected for red, blue, and yellow. They also provide spherical aberration correction for two to three wavelengths and generally have a high numerical aperture (NA) and long working distance. Plan, also known as planar, semi-plan, semi-planar, or microplan, objectives correct for field curvature. Field curvature is a type of aberration present when the off-axis image cannot be brought to focus in a flat image plane, resulting in a blurred image as it deviates from the optical axis. Plan objectives display better than 90% of the field flat and in focus.

Magnification couplers designed by the microscope manufacturers should use the same high-quality lenses expected of their objective lenses however third-party magnification couplers may use cheaper lenses which don't have the same correction quality as the rest of the lenses in the system.

Maximizing Optical Efficiency

To best way to maximize optical efficiency is to use the largest pixel camera possible that matches Nyquist with the objective being used. This allows for sensitivity to be maximized by using the largest pixel area possible and resolution to be maximized by matching Nyquist sampling with the objective. This also avoids using extra, unnecessary lenses and losing light through reflection.

When choosing between cameras that satisfy these conditions, the next step is to match the camera field of view (via pixel count) to the maximum field of view possible with the microscope. This is usually done by choosing a camera with a 19 mm, 22 mm or 25 mm field of view depending on the microscope used.

Summary

Magnification couplers are often used to change the pixel size or field of view of a scientific camera, either to match Nyquist sampling or to match the field of view of the camera sensor to the microscope. However, there are trade-offs when using magnification couplers such as incorporating a lower quality lens into the system and having uneven illumination of the sensor due to using a sensor larger than the microscope was designed for.

Teledyne Photometrics does not recommend using a magnification coupler if it can be avoided for low light imaging. When choosing a camera for a microscope system, it is far better to select a camera with the right pixel size that matches the objective used for the application and a field of view that matches the maximum output of the microscope.

For this reason, Teledyne Photometrics offers cameras with a range of pixel sizes; 4.25 µm to match 40x, 6.5 µm to match 60x and 11 µm to match 100x, as well as cameras with multiple field of view options to optimally fit 19 mm, 22 mm and 25 mm microscopes.

PrimeEnhance™

2D Active Image Denoising

There are several sources of noise when imaging faint signal levels which can affect the Signal-to-Noise Ratio (SNR) of your measurement, the main types being dark noise, read noise and shot noise. Camera manufacturers make design choices to minimize the presence of noise in the image and to maximize the quality and SNR of the collected images. Dark Noise is reduced by cooling the sensor, and read noise is minimized through sensor performance and electronic design.

Photon shot noise however, is an inherent property of light. There is always a statistical variation in the number of photons (or photoelectrons) detected in a given time period. This uncertainty is dependent on the amount of signal photoelectrons being measured and has the statistical property of a Poisson distribution. This relationship is expressed as:

$$\text{Shot Noise} = \sqrt{\text{Signal}}$$

While shot noise increases with signal, it increases more slowly (as the square root). This results in SNR improving with light levels. At low light levels, SNR is low even with a perfectly acquired image.

Signal Level (e^-)	Shot Noise (e^- RMS)	Percent of Signal
5	2.23	44.8%
10	3.16	31.6%
50	7.07	14.1%
100	10	10%
500	22.36	4.5%
1000	31.62	3.2%

Table 1

At these lower signal levels, there have been only a few ways to improve SNR, each with a tradeoff.

- **Increase the exposure time and collect signal for a longer time**
  This allows for a higher signal level, reducing the impact of shot noise. The ability to image at a desired frame rate may be sacrificed, and the cell is illuminated for a longer time, increasing phototoxicity and photobleaching. Finally, if the exposure time is long enough, the noise from dark current can become a larger portion of the signal.

- **Average frames to reduce noise**
  This allows for a reduction in total image noise as a square root of the number of frames averaged. The ability to image at adequate frame rates will again be sacrificed, and is generally less productive than simply increasing exposure time.

- **Increase the excitation intensity**
  This allows for a higher signal level without trading off temporal resolution. The rate at which phototoxicity and photobleaching occurs is also increased, reducing cell viability.

A remaining technique for the reduction of noise is the use of a “denoising algorithm” that dynamically examines the image collected in order to separate and remove noise. The Prime™ family of cameras from Teledyne Photometrics introduces a new real-time method for dynamic noise reduction called PrimeEnhance.

PrimeEnhance

There are many challenges when processing data to reduce noise such as preserving the quantitative nature of the recorded pixel intensities, as well as preserving key features like edges, textures and details with low contrast. Further, processing has to be accomplished without introducing new image artifacts like ringing, aliasing or blurring. Many algorithms are inflexible with different image types, resulting in these intrusive artifacts. Additionally, because noise tends to vary with the level of signal, it is difficult for many denoising algorithms to distinguish signal from noise, and as a consequence, small details tend to be removed.

Using an algorithm invented at INRIA and optimzed for fluorescence microscopy in collaboration with the Institute Curie, PrimeEnhance implements a 2D denoising process which evaluates and processes incoming images to reduce the effects of photon shot noise at low signal levels. The algorithm also preserves the finer details and features of biological samples, and does not introduce image artifacts. One key facet of PrimeEnhance is the quantitative nature of the algorithm, ensuring that intensity values remain unchanged.

PrimeEnhance works by being aware of each camera's characteristics and specifications. It uses this knowledge to first evaluate the image data and perform a variance stabilization transform, which removes the dependency between the mean intensities and their noise characteristics. Then a small patch of pixels is compared to similar sized patches in iteratively increasing areas of surrounding pixels (neighborhoods). The pixels within the neighborhood are selectively weighted based on their similarity to the intensity values of the original patch, and using these weighted corrections, the original patch is updated. This process is repeated through the entire image, updating each patch and reducing the impact of shot noise. Once this process has been completed, the inverse variance stabilizing transform is applied to ensure that the quantitative nature of the pixel values is maintained.

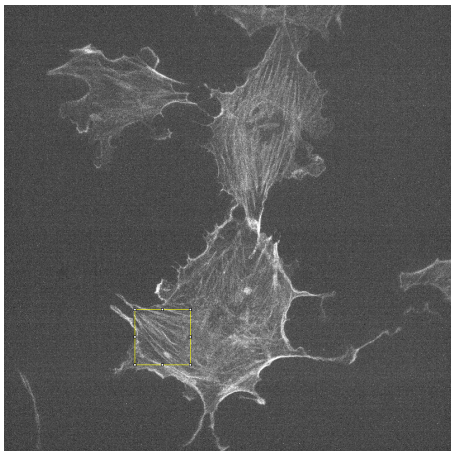


Figure 1a
Original Image

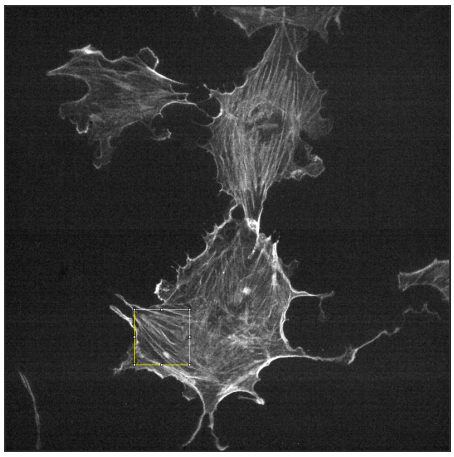


Figure 1b
PrimeEnhance

		Original Image	PrimeEnhance
Full Image	Average Intensity	131.7	131.1
	St Dev	6.68	4.78
	Min Value	72	71
	Max Value	363	362
Region of Interest	Average Intensity	138.8	138.2
	St Dev	7.98	5.02
	Min Value	87	88
	Max Value	241	241

Table 2

PrimeEnhance Evaluation

Fluorescence images were acquired with (Fig 1a) and without (Fig 1 b) PrimeEnhance enabled, to demonstrate its functionality and give a proper comparison. The following image was acquired with a 100ms exposure time. The image statistics are avaiable in Table 2.

As shown by the intensities, the mean intensity values, minimum intensity value, and maximum intensity value remain essentially unchanged between the original noisy image and the denoised image - ensuring that all measurements made remain quantitative and are relatable to each other. The standard deviation has been reduced, indicating the removal of noise.

A difference image (Fig 1c) between the original and PrimeEnhance image shows that only noise has been removed by PrimeEnhance, with the brighter regions showing higher noise levels in keeping with the relationship discussed in the introduction.

Figure 2a and 2b provide an increased zoom level on the structures within the cell, and show that features are preserved while no artifacts have been generated. The line profiles demonstrate PrimeEnhance's ability to reduce the shot noise present in the image, extracting features that were previously undistinguishable from the noise.

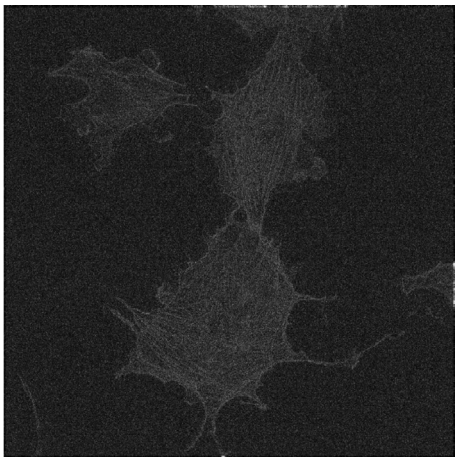


Figure 1c. Difference Image

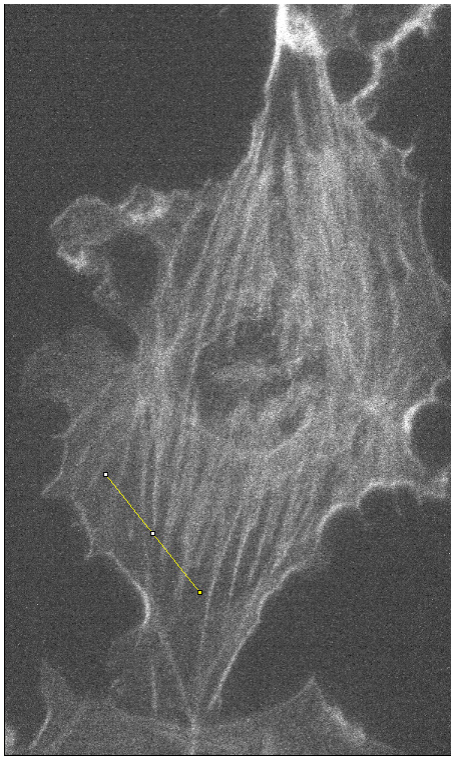


Figure 2a. Original Image

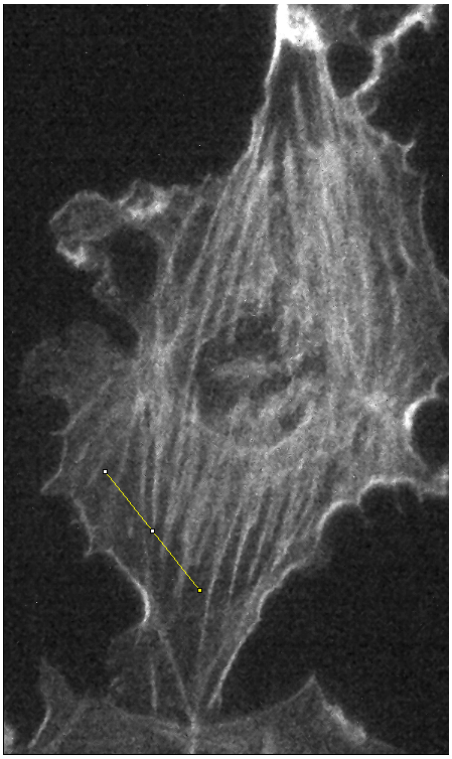


Figure 2b. PrimeEnhance

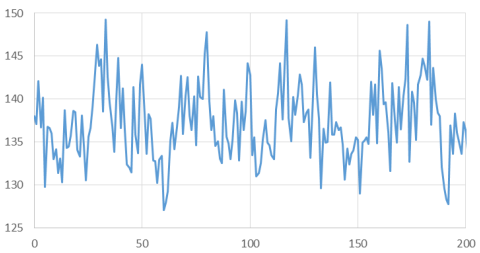


Figure 2c. Line Profile for Original Image

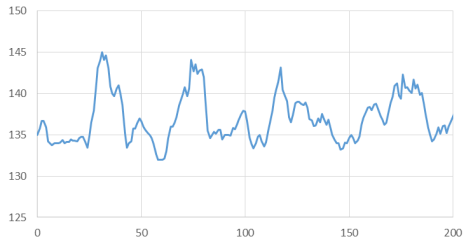


Figure 2d. Line Profile for PrimeEnhance

PrimeEnhance Experimental Impact

By increasing the effective signal to noise in each frame, it is possible to acquire high quality images at lower exposure times, reducing the effects of phototoxicity and photobleaching on samples. The following are images acquired of a faint samples with a 100ms exposure compared to images acquired with an 800ms exposure.

The comparison between the 800ms exposure and the 100ms PrimeEnhance exposure, as evidenced by the line-profiles, demonstrates the increase of image and data quality possible with PrimeEnhance at 8X lower exposure times.

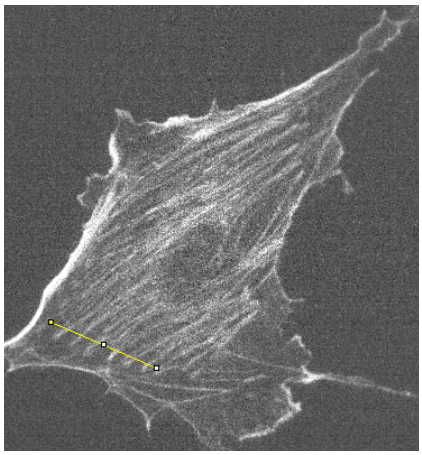


Figure 3a. Raw Image at 100ms exposure

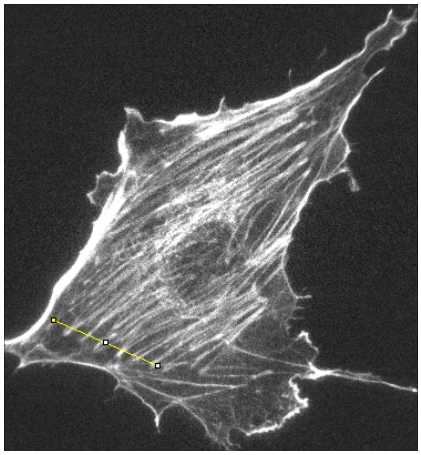


Figure 3b. PrimeEnhance at 100ms exposure

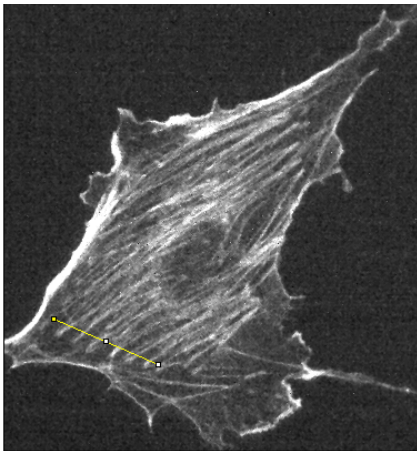


Figure 3c. Raw Image at 800ms exposure

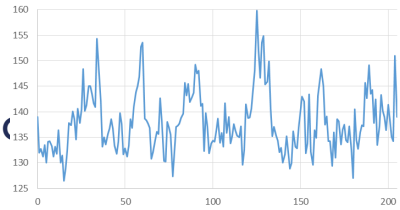


Figure 3d. Line Profile of Raw Image at 100ms

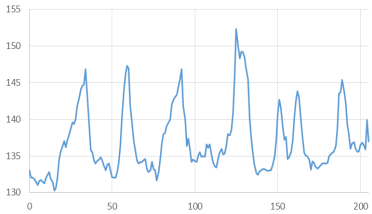


Figure 3e. Line Profile of PrimeEnhance Image at 100ms

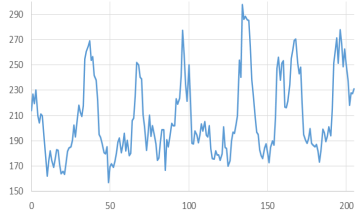


Figure 3f. Line Profile of Raw Image at 800ms

Conclusion

PrimeEnhance provides a real-time quantitative increase in signal to noise ratio by reducing the effects of photon shot noise at low light levels, which improves the quality of images and data. The finer features within images are preserved and no unwanted processing artifacts are generated. A comparison between a 100ms denoised image and a 800ms standard fluorescence image shows equivalent results in image quality, indicating the ability to significantly reduce exposure times while maintaining the quality of captured data.

Vision Systems Design 2016 Innovators Awards

PrimeLocate™

An excess of image data may seem like an abundance of riches, except when the data does not contain useful information. Then it becomes data that must be stored, processed and ultimately discarded. The Prime™ family of cameras from Teledyne Photometrics solves this problem at its source with PrimeLocate, one of its advanced, real-time processing features. When enabled, PrimeLocate automatically finds and transfers regions of interest to the host computer, either over PCIe or USB3. In this way massive amounts of data containing no useful information is eliminated from consideration. *PrimeLocate can identify up to to 512 of the brightest 3x3 patches within each image and transmit only that portion of the image, reducing the data and processing requirements from 6.7Gbps down to 100Mbps.*

The PrimeLocate algorithm is most appropriate for localization microscopy methods such as STORM and PALM. The hallmark feature of localization microscopy is that sparse images of individual point emitters blink at random times during an image sequence. By finding the centroid of each emitter's diffraction limited spot in a given frame and combining the localization results from each frame, a super-resolution image of the original fluorescence can be reconstructed.

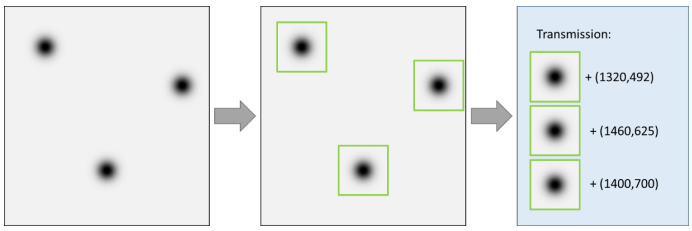


Figure 1. The PrimeLocate process in the camera. From left, an image containing sparse events is analyzed. The regions containing these events are identified, and only those regions are transmitted. Coordinates of the regions are also transmitted as metadata attached to each region.

The PrimeLocate algorithm scans each full image (or single ROI, if enabled) for the brightest local maxima within a 3x3 window. Each identified bright local maxima forms the center of a transmitted patch. The transmitted patch size is under user control and can vary from a radius of "1" to transmit a patch of 3x3 pixels, to a radius of "15" for transmitting a patch of 31x31 pixels. The number of transmitted patches can vary from one to a maximum of 512 patches per frame.

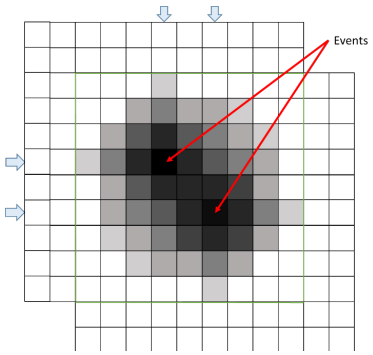


Figure 2. Example of PrimeLocate for two partially overlapping fluorescence events. The patch maxima are separated by more than two pixels, so two patches are transmitted with partially redundant pixel data. This ensures all data needed for correct localization is included. The blue arrows mark the center rows and columns for the patches.

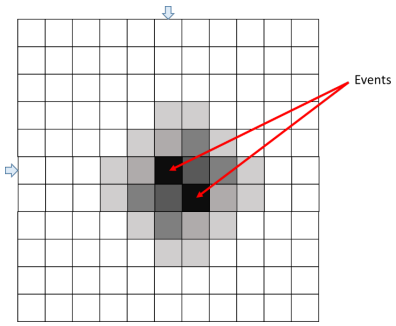


Figure 3. Example of 11x11 patch data for two small, overlapping fluorescence events. The event maxima are close enough together that a single 11x11 patch captures both. The blue arrays mark the center row and column for the patch.

Using a small 3x3 search window ensures that when two fluorescence events happen in close proximity, two or more patches are transmitted to capture both events (Figure 2). The 3x3 exclusion region discounts two or more events occurring within a radius of two pixels as being independent, as events this close together can be captured within a single, transmitted patch (Figure 3).

PrimeLocate always transmits the predetermined number of patches per frame, regardless of the number of fluorescence events present. For example, when using the maximum 512 regions, after patches containing true fluorescence events are transmitted, remaining patches will simply contain the brightest noise peaks from the

image. In this way, a consistent amount of data is transmitted per frame. These patches will be low-intensity compared to the brightest events and can be ignored. Should more than 512 events occur within a single frame, those events will be lost.

PrimeLocate does not attempt to localize events to precision better than a pixel, as this step is expected to be performed on the host. Leaving this step to the host allows choice of localization algorithm and configuration. The data transmitted to the host includes the patch data itself as well as the patch's (X,Y) location, so that the sparse image can be reconstructed at the host.

PrimeLocate and µManager

µManager is an excellent open-source microscope control application that provides image acquisition and device control (www.openimaging.com). µManager supports PrimeLocate along with several new innovative features of the Prime family of cameras. Settings for PrimeLocate are found in the "Device Property Browser…", or as shown below, the "Configuration settings" dialog when using the Hardware Configuration provided with the Prime and Prime 95B cameras.

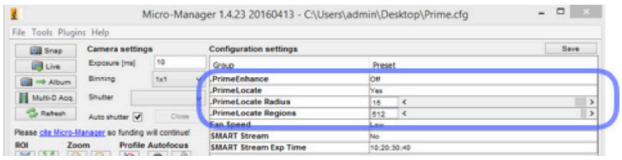


Figure 4. PrimeLocate settings in µManager

There are three highlighted controls:

Name	Description	Selection
PrimeLocate	Controls if PrimeLocate is enabled	Off/On
PrimeLocate Radius	Controls the size of the region transferred around each local maxima	1 to 15
PrimeLocate Regions	Controls the number of maxima transferred	1 to 512

Table 1. Highlighted Controls.

In the following example, a 512x512 portion of the sensor was used to acquire 1000 frames at 300 FPS. In each frame, 16 regions were selected for transfer using PrimeLocate with a size of 31x31 pixels (radius=15).

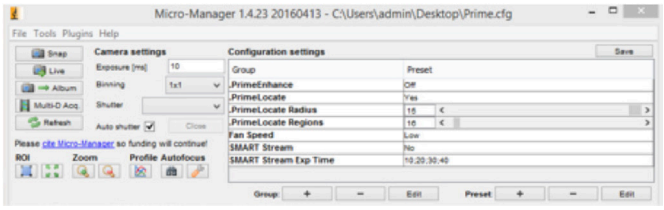


Figure 5a. PrimeLocate settings in µManager.

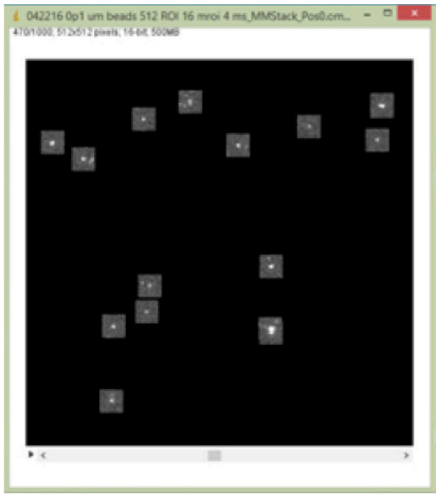


Figure 5b. Example of a 512x512 region, with PrimeLocate configured to find the 16 brightest regions, and return them as 31x31 pixels patches.

PrimeLocate and Data Reduction

Windows provides a convenient method for taking advantage of compression by saving images to a "compressed" folder on the hard disk. Windows will dynamically compress the image data as the images are written.

An uncompressed TIFF format image stack consisting of 1000 frames of 512x512, 16-bit image data without PrimeLocate enabled is 504MB uncompressed, and 346MB when saved to a compressed folder.

However, when using PrimeLocate configured for 16 regions of 31x31 pixels, the same file consumes only 54MB in the compressed folder as the blank-zero valued area will now efficiently compress using Windows compression algorithm.

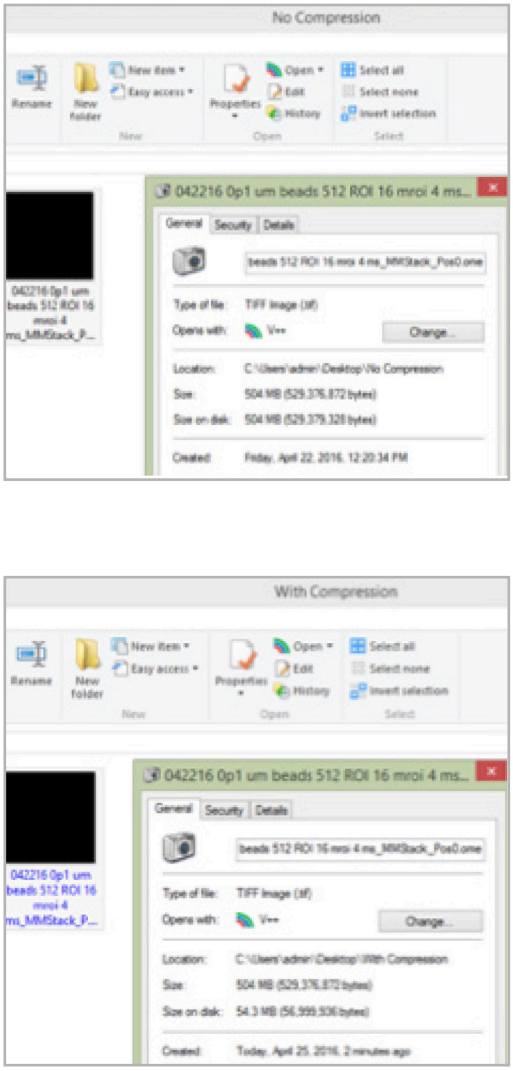


Figure 6. PrimeLocate enables effective compression using Windows compressed folder mechanism, resulting in a 10x reduction in storage requirements in the 1000 frame example shown.

When using the maximum 512 regions, more data from the original image is transferred, and this same file is reduced to 257MB. These results are typical, the exact amount of compression depends on the image data and the number and size of stored regions.

Conclusion

This brief introduction is designed to provide background on how PrimeLocate works. The next White Paper for PrimeLocate will focus on how it can be adapted for live particle tracking.

Live Particle Tracking

Introduction

The Prime 95B™ Scientific CMOS camera is great for imaging situations requiring both extreme sensitivity as well as a high acquisition rate, making it ideal for techniques such as super-resolution localization microscopy and single-molecule tracking. The Prime camera platform incorporates a powerful FPGA and performs real-time image processing optimized for specific applications to provide more useful experimental data.

Live Particle Tracking is the latest addition to the real-time processing capabilities of the Prime 95B camera, and is designed to increase the efficacy of single molecule particle tracking experiments.

How It Works

Live Particle Tracking, as its name suggests, is designed to identify individual single molecule particles and track them across the field of view by adapting a published algorithm tuned for two-dimensional tracking. The algorithm processes the images in the following order:

1. Image detection
2. Image restoration
3. Estimation and refinement of point locations
4. Non-particle discrimination
5. Trajectory linking
6. Image output with tracking meta-data

The image detection step requires the camera to determine only the dynamic portions of the image and disqualify anything static from detection. The data is then run through a restoration step which behaves as a bandpass filter, reducing both the high frequency and low-frequency noise, and allows the correction of any noise variation on a pixel-to-pixel basis as well as any background intensity modulations due to uneven illumination.

The points are then processed to determine the local-maxima within the radius of the evaluation kernel, and go through a refinement process to ensure a high efficiency in particle detection based on a threshold to reduce the susceptibility to false

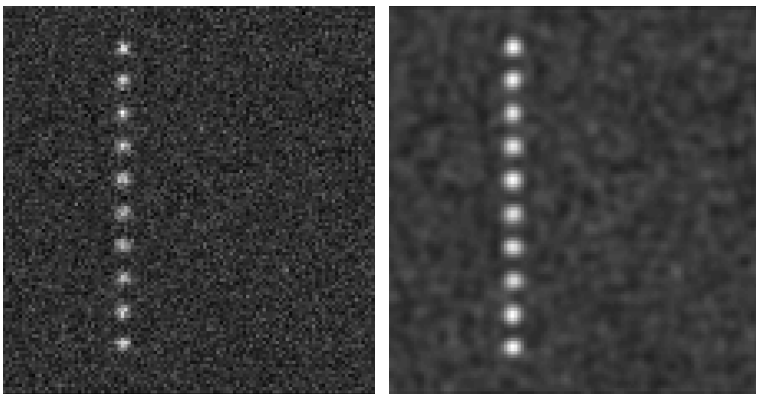


Figure 1. The input image of simulated single-particle data and the output of the image-restoration step to reduce image noise and pixel-to-pixel variation.

positives. Any remaining artifacts are filtered out during the non-particle discrimination step, aimed at hot pixels and cosmic events.

Finally, the particles are tracked and linked through the acquired frame stack. The metadata included with all images is updated to include the particle data within each frame, providing particle IDs as well as the ability to display particle path traces as well as boxes to outline each detected particle.

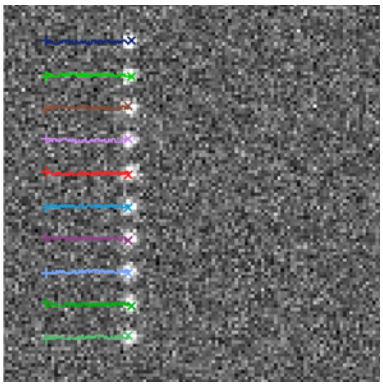


Figure 2: The live particle tracking feature adds meta-data to each image providing the movement information of each particle.

Conclusion

The Live Particle Tracking feature of the Prime 95B Scientific CMOS camera provides the ability to evaluate the behaviour of single-molecules during the acquisition, ensuring that expected behaviour can be determined early in the experimental process. By reducing the number of experiments that do not meet the required or expected rates of movement by the particles, the efficacy of experiments can be increased significantly.

References

I.F. Sbalzarini, P. Koumoutsakos. 2005. Feature point tracking and trajectory analysis for video imaging in cell biology

Camera Test Protocol

Introduction

The detector is one of the most important components of any microscope system. Accurate detector readings are vital for collecting reliable biological data to process for publication.

To ensure your camera is performing as well as it should be, Teledyne Photometrics has designed a range of tests that can be performed on any microscope.
The results of these tests will give you quantifiable information about the state of your current camera as well as providing a method to compare cameras, which may be valuable if you're in the process of making a decision for a new purchase.

This document will first take you through how to convert measured signal into the actual number of detected electrons and then use these electron numbers to perform the tests. The tests in this document make use of ImageJ and Micro-Manager software as both are powerful and available free of charge.

Table of Contents

Part 1.
Working With Photoelectrons

Measuring Photoelectrons

Background

A fluorescence signal is detected when photons incident on the detector are converted into electrons. It's this electron signal that's converted by the analog-to-digital converter (ADC) in the camera to the Grey Levels (ADUs) reported by the computer.

Although grey levels are proportional to signal intensity, not every camera converts electrons to the same number of grey levels which makes grey levels impractical for quantifying signal for publication.

Instead, signal should be quantified in photoelectrons as these are real world values for intensity measurement that allow for consistent signal representation across all cameras. This signal can then be compared against noise to assess the quality of images by signal to noise.

Method

To convert signal in grey levels to signal in electrons:

1. Load an image into ImageJ, pick a fluorescent spot and draw a line across it.
2. Select Plot Profile from the Analyse menu (Figure 1) to get a peak representing the signal across the line in Grey Levels. Find the value at the top of the peak.

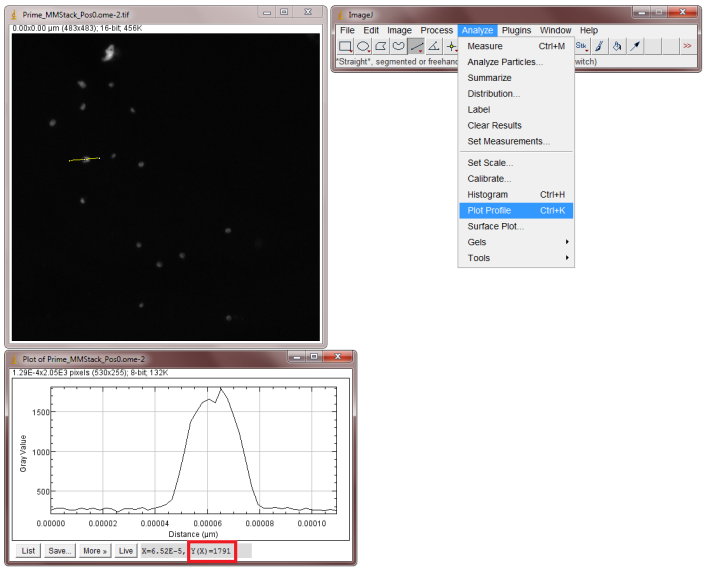


Figure 1

3. Subtract the camera bias from this Grey Level signal.
4. Multiply the result by the camera system gain.

The full equation is:

Signal in Electrons= (Signal in Grey Levels - Bias)*Gain

The camera bias and camera system gain can be found on the Certificate of Performance (COP) or other information provided with the camera or they can be calculated by tests explained below.

As an example, the data in the image above was taken with the Prime 95B™ which has a bias of ~100 and a gain of ~1.18. By inserting these values into the equation, we get the following result:

Signal in Electrons= (1791 - 100)*1.18

Signal = 1995 e^-

Measuring Camera Bias

Background

When visualizing a fluorescence image, we would expect the intensity value of a pixel to correspond only to the intensity of fluorescence in the sample. However, every camera has a background offset that gives every pixel a non-zero value even in the absence of light. We call this the camera bias.

The bias value is necessary to counteract fluctuating read noise values which might otherwise go below zero. The value of the bias therefore should be above zero and equal across all pixels. The bias value doesn't contain any detected signal so it's important to subtract it from an image before attempting to calculate the signal in photoelectrons.

Method

To calculate the camera bias:

1. Set your camera to a zero millisecond exposure time.
2. Prevent any light entering the camera by closing the camera aperture or attaching a lens cap.
3. Take 100 frames with these settings (Figure 2).

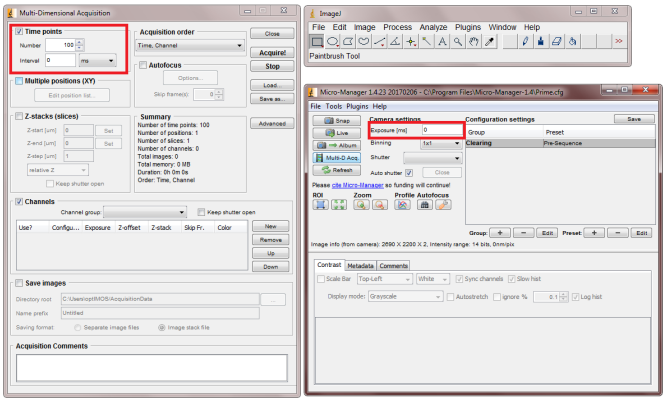


Figure 2

4. Calculate the mean of every frame by selecting Stacks from the Image menu and then clicking on Plot Z-axis profile (Figure 3). This should give you the mean values of every frame in the Results window.

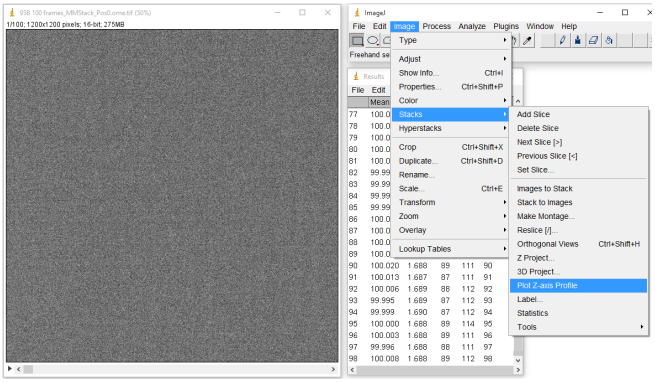


Figure 3

5. Calculate the mean of the 100 frame means by selecting Summarize in the Results menu (Figure 4).

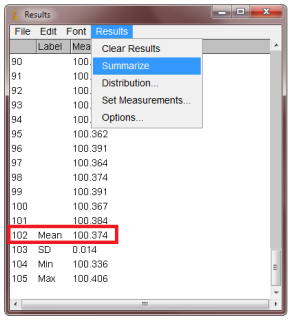


Figure 4

The bias is the mean of a single frame so by plotting the mean values of all 100 frames we calculate a more accurate bias.

Calculating Camera Gain

Background

When the amount of light entering a camera is linearly increased, the response of the camera in grey levels should also linearly increase.
The gain represents the quantization process as light incident on the detector is processed and quantified. It varies from camera to camera depending on electronics and individual properties but it can be calculated experimentally. If a number of measurements are made and plotted against each other the slope of the line should represent the linearity of the gain.

Method

Camera system gain is calculated by a single point mean variance test which calculates the linear relationship between the light entering the camera and the cameras response to it. To perform this test:

1. Take a 100-frame bias stack with your camera like in the previous section and calculate the mean bias.
2. Take 2 frames of any image using the same light level with a 5 ms exposure time.
3. In ImageJ, Measure the means of both images and average them. We'll call this $Mean_{Image1,\ Image2}$ (Figure 5).

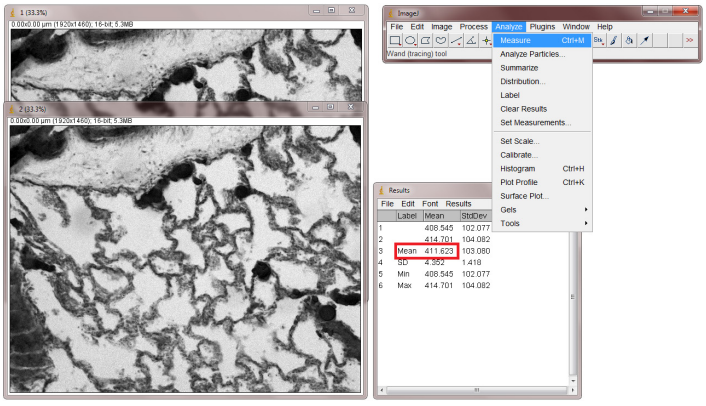


Figure 5

4. Calculate the difference between the two images by selecting Image Calculator from the Analyze menu. Select the two frames and Subtract, you will need to float the result. Press OK to generate the diff image (Figure 6).

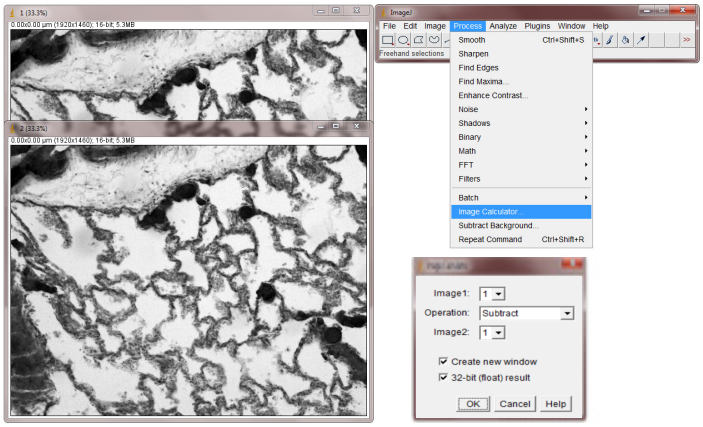


Figure 6

5. Measure the Standard Deviation of the diff image, we'll call this Standard deviation$_{\text{Diff image}}$ (Figure 7).

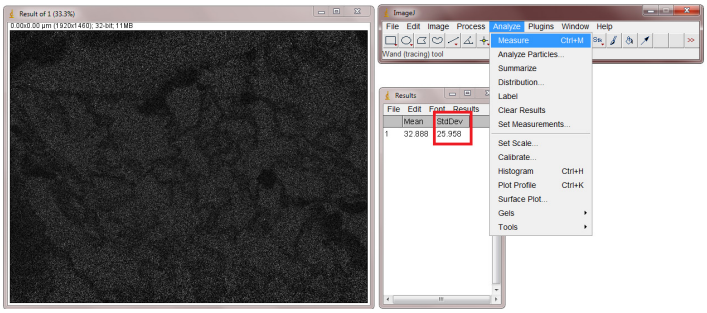


Figure 7

6. Calculate the variance of the two images with the following equation:

$$\text{Variance}_{\text{Image 1,Image 2}} = \frac{\text{Standard deviation}_{\text{Diff image}}^{2}}{2}$$

7. Calculate the gain from the variance using the following equation, remember to remove the previously calculated bias:

$$\text{Gain} = \frac{(\text{Mean}_{\text{Image 1,Image 2}}) - \text{bias}}{\text{Variance}_{\text{Image 1,Image 2}}}$$

Gain is represented as e^{-}/grey level.

8. Repeat this process with 10 ms, 20 ms and 40 ms exposure times to check that the gain is consistent across varying light levels.
9. You can also use the single-point mean variance (gain) calculator provided by Teledyne Photometrics on the website:
https://www.photometrics.com/learn/calculators

Calculating Signal to Noise Ratio (SNR)

Background

The signal to noise ratio describes the relationship between measured signal and the uncertainty of that signal on a per-pixel basis. It is essentially the ratio of the measured signal to the overall measured noise on a pixel. Most microscopy applications look to maximise signal and minimize noise.

All cameras generate electron noise with the main sources being read noise, photon shot noise and dark current. These noise values are displayed on the camera data sheet and are always displayed in electrons. This means that the most accurate way to calculate the signal to noise ratio is by comparing signal in electrons to noise in electrons.

Method

The signal to noise ratio can be calculated using the following equation:

$$SNR = \frac{S}{\sqrt{S + [Nd * t] + Nr^2}}$$

Where:
S = Signal in electrons.

The best way to calculate an electron signal for use in the equation is to use a line profile across an area of high fluorescence as described at the beginning of this document.

Nd = Dark current in electrons/pixel/second
Nr = Read noise in electrons
t = Exposure time in seconds

You can also use the signal to noise calculator provided by Teledyne Photometrics on the website:
https://www.photometrics.com/learn/calculators

Calculating Signal to Noise Ratio (SNR) of an EMCCD Camera

Background

EMCCD cameras are designed for very low light applications and function in the same way as a CCD but have additional electronics to multiply the captured electrons. This process occurs after the electron signal has been captured but before it's been read out.

The multiplication process means that the camera read noise is effectively reduced to less than 1 electron, allowing the detection of very low signal. However, this is not free in terms of signal to noise. The multiplication process is not a definitive event – there is a probability associated with gaining extra electrons and this uncertainty adds an extra noise source to the SNR calculation, Excess Noise Factor. Excess noise factor has a value of $\sqrt{2}$ and effectively cuts the sensors quantum efficiency in half. When calculating the SNR of an EMCCD camera, this must be added to the equation.

Method

The signal to noise ratio of an EMCCD can be calculated using the following equation:

$$\text{EMCCD SNR} = \frac{S}{\sqrt{[S * F^2] + [Nd * t * F^2] + [\frac{Nr}{E}]^2}}$$

Where:
S = Signal in electrons
Nd = Dark current in electrons/pixel/second
Nr = Read noise in electrons
t = Exposure time in seconds
F = Excess noise factor
E = EM gain

To get accurate electron counts from EMCCD data we recommend you use the QuantView™ function of the Teledyne Photometrics Evolve® Delta. QuantView converts Grey Level intensities into the number of electrons measured at the sensor so there are no calculations necessary to convert Grey Levels into electrons. To activate QuantView:

1. In Micro-Manager, open the Device Property Browser.
2. Scroll down to QuantView and change it from off to on (Figure 8).

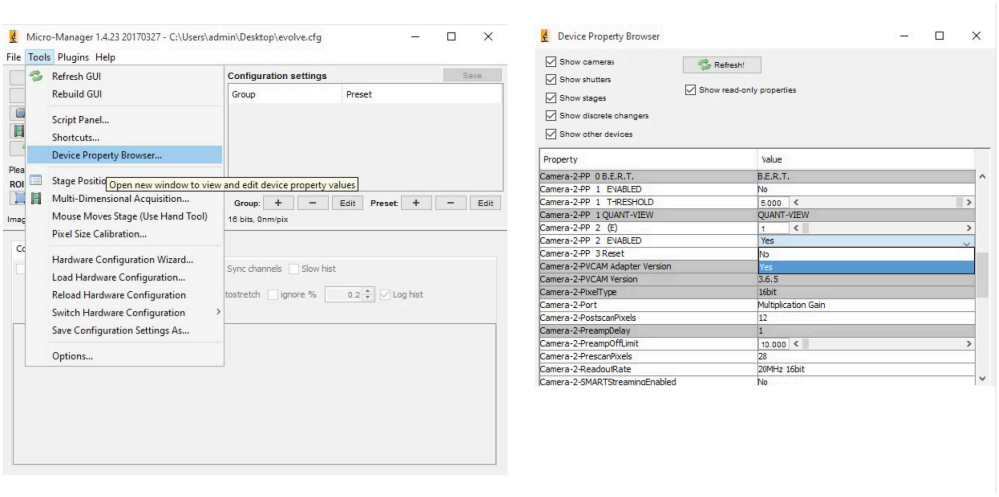


Figure 8

Alternatively, locate the gain value of the camera on the Certificate of Performance (CoP) or other information provided with the camera and perform the calculation given at the beginning of this document to convert Grey Levels to electrons.

To convert Grey Levels to electrons on non-linear gain EMCCDs such as the Teledyne Photometrics Cascade series, please see the following tech note:
https://www.photometrics.com/learn/calculators

Part 2.
Testing Camera Quality

Evaluating Bias Quality

Background

There are two important things to look for in a bias, the stability and the fixed pattern noise.

The stability is simply a factor of how much the bias deviates from its set value over time. A bias that fluctuates by a large amount will not give reliable intensity values.

Fixed pattern noise is typically visible in the background with longer exposure times and it occurs when particular pixels give brighter intensities above the background noise. Because it's always the same pixels, it results in a noticeable pattern seen in the background. This can affect the accurate reporting of pixel intensities but also the aesthetic quality of the image for publication.

Method

To evaluate the bias stability:

1. Plot the mean values of all 100 bias frames taken in the previous section.
2. Fit a straight line and observe the linearity.

Our goal at Teledyne Photometrics is to produce a stable bias that doesn't deviate by more than one electron, which is shown here using the Prime 95B™ Scientific CMOS data (Figure 9).

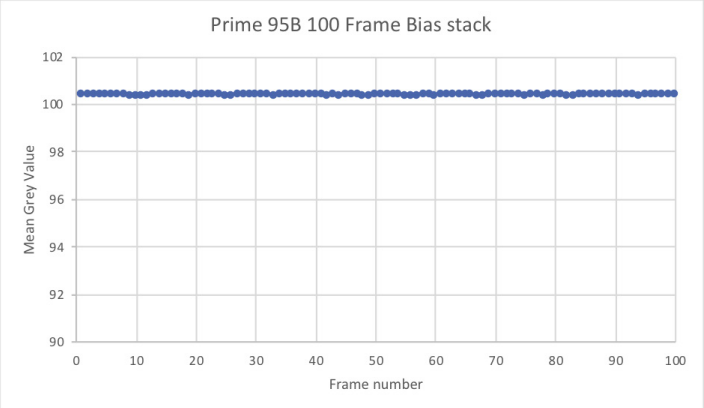


Figure 9

To evaluate fixed pattern noise:

1. Mount a bright sample on the microscope and illuminate it with a high light level
2. Set the exposure time to 100 ms
3. Snap an image
4. Repeat this experiment with longer exposure times if necessary

A 'clean' bias such as that demonstrated in figure 10 on the Prime 95B will give more accurate intensity data and produce higher quality images.

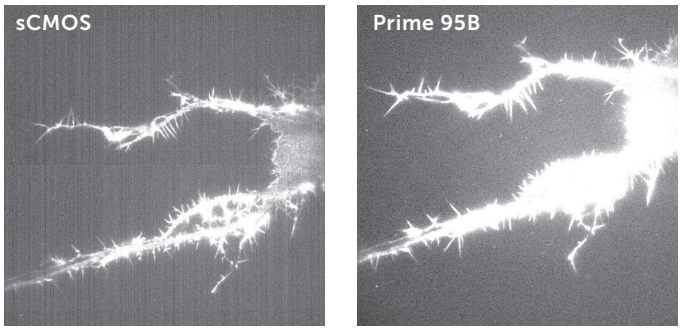


Figure 10

Evaluating Gain Quality

Background

Gain linearity is very important as the gain directly influences how the electron signal is converted into the digital signal read by the computer. Any deviation from a straight line represents inaccurate digitization.

Method

To evaluate the gain linearity:

1. Plot the $Mean_{Image1 - Image2}$ against the $Variance_{Image1 - Image2}$ data collected in the 'Calibrating your camera for photoelectron measurement' section
2. Fit a straight line and observe the linearity

Teledyne Photometrics recommends that any deviation from the line be no more than 1%, as shown in figure 11 using the CoolSNAP™ DYNO data:

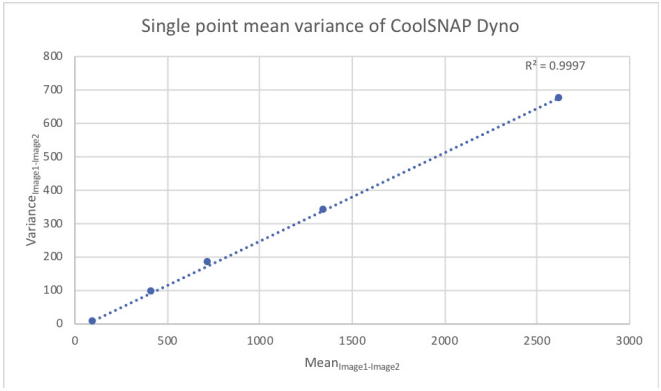


Figure 11

Evaluating EM Gain Quality

Background

All EMCCD cameras suffer from EM gain fall-off over time. This means that the EM gain multiplication of any EMCCD camera will be reduced with usage. Most modern EMCCD cameras have ways to recalibrate the EM gain multiplication so there will not be any noticeable change but eventually there will come a point when no more can be done.

This becomes a problem when, for example, 300x EM gain was used to overcome read noise but due to EM gain fall-off the camera can no longer reach this gain level. At this point the camera has lost it's EM gain functionality and the only option is to buy a new camera.

Method

To test the EM gain multiplication of your camera:

1. Take a 100-frame bias stack with your EMCCD camera and calculate the mean bias.
2. Take a long exposure (~1s) image of a dim sample without EM gain.
3. Without changing anything about the sample, take a short exposure (~10ms) with EM gain.

Note - It's necessary to lower the exposure time for point 3. to avoid saturating the pixels when using EM gain. We'll correct for time in point 4.

4. Subtract the bias value from both images and divide both by their respective exposure time in milliseconds to equalize them.
5. The factor difference in signal per time unit should be the EM gain multiplication factor If you're worried about EM gain fall-off, you can reduce its impact by following these guidelines:

If you're worried about EM gain fall-off, you can reduce its impact by following these guidelines:

1. Only use the EM gain necessary to overcome read noise. An EM gain of 4 or 5 times the root-mean-square (rms) read noise should be enough. It should almost never be necessary to go above an EM gain of 300 to achieve this.
2. If EM gain isn't necessary for your work, don't use it. Most EMCCD cameras have non-EM ports to read out the signal without using the EM register.
3. Avoid over-saturating the EMCCD detector.

Calculating Read Noise

Background

Read noise is present in all cameras and will negatively contribute to the signal to noise ratio. It's caused by the conversation of electrons into the digital value necessary for interpreting the image on a computer. This process is inherently noisy but can be mitigated by the quality of the camera electronics. A good quality camera will add considerably less noise.

Read noise will be stated on the camera data sheet, certificate of performance or other information provided with the camera. It can also be calculated as explained below.

Method

Read noise can be calculated with the following method:

1. Take two bias images with your camera
2. In ImageJ, calculate the difference between the two images by selecting Image Calculator from the Analyze menu. Select the two frames and Difference, you will need to float the result. Press OK to generate the diff image (Figure 12).

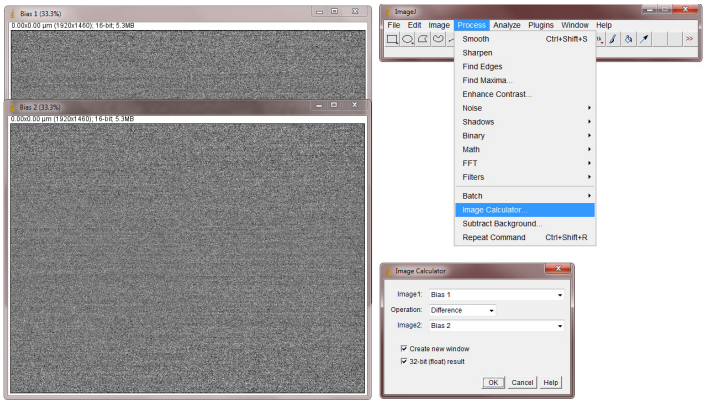


Figure 12

3. Measure the Standard Deviation of the diff image, we'll call this Standard deviation$_{\text{Diff image}}$ (Figure 13).

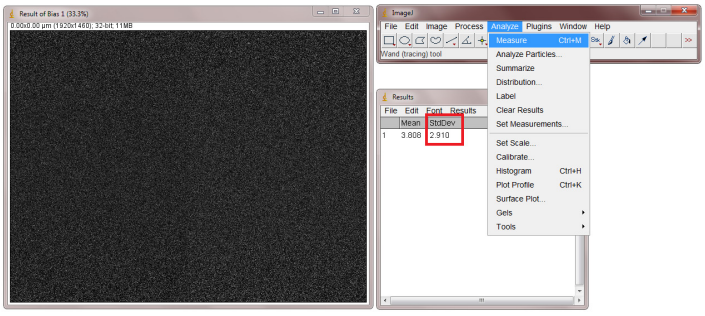


Figure 13

4. Use the following equation to calculate system read noise, you'll need the previously calculated gain value or you can use the gain value given in the information provided with the camera:

$$\text{Read Noise} = \frac{\text{Standard deviation}_{\text{Diff image}} * \text{Gain}}{\sqrt{2}}$$

You can also use the read noise calculator provided by Teledyne Photometrics on the website:
https://www.photometrics.com/learn/calculators

Calculating Dark Current

Background

Dark current is caused by thermally generated electrons which build up on the pixels even when not exposed to light. Given long enough, dark current will accumulate until every pixel is filled. Typically, pixels will be cleared before an acquisition but dark current will still build up until the pixels are cleared again. To solve this issue, dark current is drastically reduced by cooling the camera. You can calculate how quickly dark current builds up on your camera with the method below.

Method

To calculate how much dark current is accumulating over differing exposure times, you need to create a dark frame. A dark frame is a frame taken in the dark or with the shutter closed. By creating multiple dark frames with varying exposure times or acquisition times, you can allow more or less dark current to build up.
To do this:

1. Prevent any light entering the camera and take images at exposure times or acquisition times you're interested in. For example, you may use a 10ms exposure time but intend to image for 30 seconds continuously. In this case, you should prepare a 30 second dark frame.
2. Take two dark frames per time condition.
3. In ImageJ, calculate the difference between the two dark frames by selecting Image Calculator from the Analyze menu. Select the two frames and Difference, you will need to float the result. Press OK to generate the diff image.
4. Measure the Standard Deviation of the diff image, we'll call this Standard deviation$_{\text{Diff image}}$

5. Use the following equation to calculate system read noise and dark current:

$$\text{Read Noise + Dark current} = \frac{\text{Standard deviation}_{\text{Diff image}} * \text{Gain}}{\sqrt{2}}$$

Note - the equation remains the same as in the previous section but because we've allowed the camera to expose for a certain amount of time, dark current has now built up on top of the read noise.

6. Subtract the number of electrons contributed by read noise - calculated in the previous section - to be left with the noise contributed by dark current.
7. Compare the calculated dark current value to the acquisition time to determine how much dark current built up per unit time.
8. This experiment can be repeated at differing exposure times and temperatures to determine the effect of cooling on dark current build-up.

Counting Hot Pixels

Background

Hot pixels are pixels that look brighter than they should. They are caused by electrical charge leaking into the sensor wells which increases the voltage at the well. They are an aspect of dark current so the charge builds up over time but they are unable to be separated from other forms of dark current.

Method

To identify hot pixels:

1. Take a bias frame with your camera.
2. Prevent any light entering the camera and take a 10-frame stack with a long (~5 sec) exposure.
3. In ImageJ, subtract the bias frame from one of the long exposure frames by selecting Image Calculator from the Analyze menu. Select the two frames and Subtract, you will need to float the result. Press OK to generate the image (Figure 14).

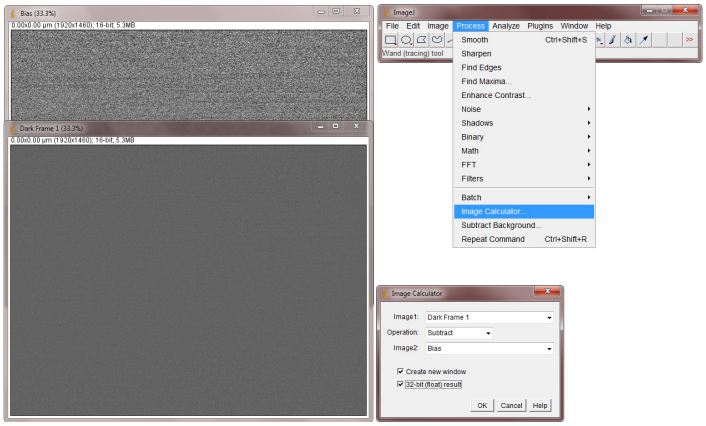


Figure 14

4. Hot pixels should immediately be visible as bright white spots on the dark background. Draw line profiles over individual hot pixels to measure the intensity (Figure).

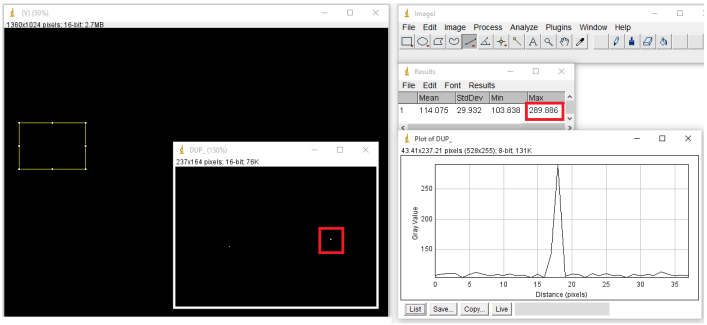


Figure 15

5. Compare hot pixels between all 10 long exposure frames.

The advantage of hot pixels is that they always stay in the same place so once they are identified these pixels can be ignored for data processing.

Like normal dark current, camera cooling drastically reduces hot pixel counts. If you are still having issues with hot pixels you may be able to adjust the fan speed of the camera to provide more cooling or even switch to a liquid cooled system.

Part 3.
Other Factors to Consider

Saturation and Blooming

Saturation

Saturation and blooming occur in all cameras and can affect both their quantitative and qualitative imaging characteristics.

Saturation occurs when pixel wells become filled with electrons. However, as the pixel well approaches saturation there is less probability of capturing an electron within the well. This means that as the well approaches saturation the normally linear relationship between light intensity and signal degrades into a curve. This affects our ability to accurately quantify signal near saturation.

To control for saturation, we call the full well capacity before it starts to curve off the linear full well capacity. A high-quality camera will be designed so that the linear full well capacity fills the full 12-, 14- or 16-bit dynamic range so no signal is lost. At Teledyne Photometrics, we always restrict the full well capacity to the linear full well so you'll never experience saturation effects.

Blooming

An additional saturation problem is that when the pixel reaches saturation, the extra charge can spread to neighbouring pixels. This spread is known as blooming and causes the neighbouring pixels to report false signal values.

To control for blooming Teledyne Photometrics cameras feature the anti-blooming technology, clocked anti-blooming. In this technique, during an exposure, two of the three clock-voltage phases used to transfer electrons between neighbouring pixels are alternately switched. This means that when a pixel approaches saturation, excess electrons are forced into the barrier between the Si and SiO_2 layers where they recombine with holes. As the phases are switched, excess electrons in pixels approaching saturation are lost, while the electrons in non-saturated pixels are preserved. As long as the switching period is fast enough to keep up with overflowing signal, electrons will not spread into neighbouring pixels. This technique is very effective for low-light applications.

Speed

Types of Speed

Biological processes occur over a wide range of time scales, from dynamic intracellular signalling processes to the growth of large organisms. To determine whether the speed of your camera can meet the needs of your research, you need to know which aspects of the camera govern its speed. These aspects can be broken down to readout speed, readout rate, readout time and how much of the sensor is used for imaging.

Readout speed tells you how fast the camera is able to capture an image in frames per second (fps). For a camera with a readout speed of 100 fps for example, you know that a single frame can be acquired in 10 ms. All latest model Teledyne Photometrics cameras are able to show hardware generated timestamps that give much more reliable readout speed information than the timestamps generated by imaging software. This can be shown in PVCAMTest provided with the Teledyne Photometrics drivers or turned on in Micro-Manager by enabling metadata. The .tiff header will then show the hardware generated timestamps.

Readout rate tells you how fast the camera can process the image from the pixels. This is particularly important for CCD and EMCCD cameras which have slow readout rates because they convert electrons into a voltage slowly, one at a time, through the same amplifier.

CMOS cameras have amplifiers on every pixel and so are able to convert electrons into a voltage on the pixel itself. This means that all pixels convert electrons to voltage at the same time. This is how CMOS devices are able to achieve far higher speeds than CCD and EMCCD devices, they have far higher readout rates.

Readout rate is typically given in MHz and by calculating 1/readout rate you can find out how much time the camera needs to read a pixel.

Readout time is only relevant for sCMOS devices and tells you the readout rate of the entire pixel array. This can be calculated as 1/readout speed, so if the readout speed of the camera is 100 fps, the readout time is 10 ms.

Binning and Regions of Interest (ROI)

When speed is more important than resolution pixels can be binned or a region of interest (ROI) can be set to capture only a subset of the entire sensor area.

Binning involves grouping the pixels on a sensor to provide a larger imaging area. A 2x2 bin will group pixels into 2x2 squares to produce larger pixels made up of 4 pixels. Likewise, a 4x4 bin will group pixels into 4x4 squares to produce larger pixels made up of 16 pixels, and so on (Figure 16).

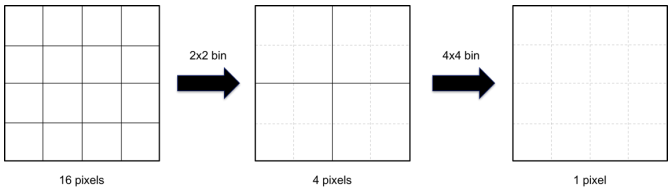


Figure 16

On a CCD or EMCCD, binning increases sensitivity by providing a larger area to collect incident photons as well as increasing readout speed by reducing the number of overall pixels that need to be sent through the amplifier.

Binning on an sCMOS also increases sensitivity but cannot increase readout speed because electrons are still converted to voltage on the pixel. Binning is therefore only useful to increase sensitivity and reduce file size.

Both devices can benefit from setting an ROI as this limits the number of pixels that need to be read out. The less pixels to read out, the faster the camera can read the entire array.

Camera Sensitivity

Quantum Efficiency

Sensitivity is a function of both quantum efficiency and pixel size.

Quantum efficiency (QE) tells you what percentage of photons incident on the sensor will be converted to electrons. For example, if 100 photons hit a 95% QE sensor, 95 photons will be converted into electrons.

72% QE sCMOS was made 82% quantum efficient with higher quality microlenses. By positioning microlenses over the pixels, light from wider angles was able to be directed into the active silicon. However, it's important to make a photoelectron detection comparison with both types of sCMOS as most light used in biological applications is collimated which gives limited light collection advantage to the microlenses.

Pixel Size

Pixel size on the other hand tells you how large an area the pixel has for collecting photons. For example, a 6.5x6.5 µm pixel has an area of 42.25 μm^2 and an 11x11 µm pixel has an area of 121 μm^2 which makes the 11x11 µm pixel ~2.86x larger than the 6.5x6.5 µm pixel. So, if the 11x11 µm pixel collects 100 photons, the 6.5x6.5 µm pixel only collects ~35 photons.

This means that, as far as sensitivity is concerned, a high QE and a large pixel are preferred. However, larger pixels can be disadvantageous for resolution.

Pixel Size and Resolution

The optical resolution of a camera is a function of the number of pixels and their size relative to the image projected onto the pixel array by the microscope lens system.

A smaller pixel produces a higher resolution image but reduces the area available for photon collection so a delicate balance has to be found between resolution and sensitivity. A camera for high light imaging, such as CCD cameras for brightfield microscopy, can afford to have pixel sizes as small as 4.5x4.5 µm because light is plentiful. But for extreme low light applications requiring an EMCCD or scientific CMOS camera, pixel sizes can be as large as 16x16 µm.

However, a 16x16 µm pixel has significant resolution issues because it can't achieve Nyquist sampling without the use of additional optics to further magnify the pixel.

In light microscopy, the Abbe limit of optical resolution using a 550 nm light source and a 1.4 NA objective is 0.20 µm. This means that 0.20 µm is the smallest object we can resolve, anything smaller is physically impossible due to the diffraction limit of light. Therefore, to resolve two physically distinct fluorophores, the effective pixel size needs to be half of this value, so 0.10 µm. Achieving this value is known as Nyquist sampling.

Using a 100x objective lens, a pixel size of 16x16 µm couldn't achieve Nyquist sampling as the effective pixel size would by 0.16 µm. The only way to reach 0.10 µm resolution would be to use 150x magnification by introducing additional optics into the system.

This makes it very important to choose the camera to match your resolution and sensitivity requirements. The table below outlines which Teledyne Photometrics cameras achieve Nyquist under which magnification:

Magnification	NA of objective	Wavelength of light	Required Pixel Size for Nyquist	Ideal camera (pixel size)
40X	1.3	509nm (GFP)	4.8 µm	CoolSNAP DYNO (4.54 µm)
60X	1.4		6.7 µm	Prime BSI (6.5 µm)
100X	1.4		11.1 µm	Prime 95B (11 µm)
150X	1.4		16.6 µm	Evolve 512 Delta 16 µm)

Table 1

Note – It's often the case that sensitivity is more important than resolution. In this case, choosing the Prime 95B for use with a 60x objective is far superior to choosing the Prime even though the Prime matches Nyquist. This is where the researcher will need to balance the demands of their application with the best available camera. Additional optics can always be used to reduce the effective pixel size without changing the objective.

Prime 95B Software Support

Supported Software

- ✓ Nikon NIS-Elements
- ✓ Zeiss Zen
- ✓ Olympus cellSens
- ✓ Leica LASX
- ✓ Molecular Devices MetaMorph
- ✓ Visitron VisiView
- ✓ Intelligent Imaging Innovations (3i) SlideBook 6
- ✓ Quorum Technologies Volocity
- ✓ National Instruments LabVIEW
- ✓ MathWorks MATLAB
- ✓ WaveMetrics Igor Pro 8
- ✓ Python 3.6
- ✓ Open CV
- ✓ Micro-Manager
- ✓ Teledyne Photometrics Ocular
- ✓ Camera driver SDK available for developers

Supported Operating Systems

- ✓ Windows 7, 8, 10 64-bit
- ✓ Linux

Notes

We recommend contacting your local third-party software office for minimum version numbers, support and relevant information.

TELEDYNE PHOTOMETRICS

Everywhereyoulook™

WWW.PHOTOMETRICS.COM