What is Scientific Imaging Quality?

Dr Louis Keal – Version A3, 19/11/2020

Achieving the highest standards of image quality is an aim for all camera manufacturers – but in scientific imaging, Image Quality is defined by a distinct set of performance characteristics that make cameras more or less suitable for the rigorous demands of scientific imaging.

This document outlines the key factors that define the scientific image quality of a scientific camera and explores how this can be maximized.

Contents

Light Collection

The light collection ability of a camera is one of the most fundamental determining factors of resulting image quality. The more photons the camera is able to collect, the better the resulting image quality.

The following factors determine light collection efficiency.

Quantum Efficiency (QE)

Quantum efficiency (QE) is the measure of the effectiveness of an imaging device to convert incident photons into electrons. For example, if a sensor had a QE of 100% and was exposed to 100 photons, it would produce 100 electrons of signal.

The main factor determining quantum efficiency is Front vs Back Illumination, shown in Figure 1. For front-illuminated cameras, the electronics and wiring to manage the sensor is between the light-detecting silicon and the incoming photons, and microlenses to steer some photons past the wiring. Although simpler to manufacture, this sensor design typically loses at least 25% of the photons that reach it. A more efficient design is to back-illuminate, where the sensor is inverted and photons directly hit the light-sensitive silicon, with no microlenses necessary. This requires the silicon to be thinned down to the light-sensitive ~1.1 µm thick region, but leads to the best QE.

The highest-end scientific cameras can achieve up to 95% QE through back-illumination, but this is dependent on the wavelength of light being detected, as seen in Figure 2. In the near-red and violet regions of the visible spectrum the sensor has a lower QE, especially in front-illuminated cameras.

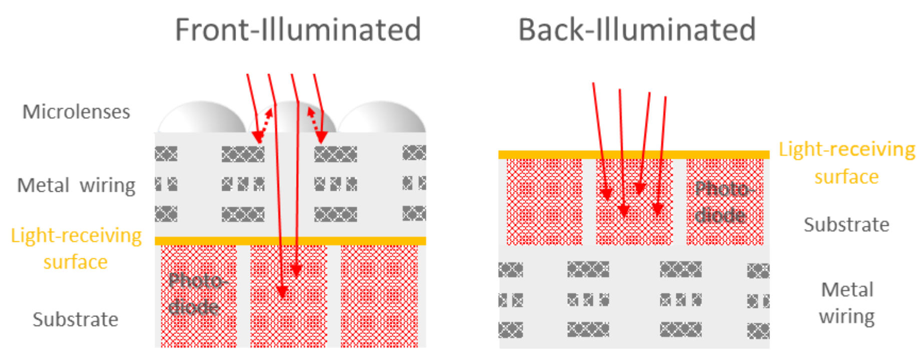


Figure 1: Schematic showing the difference between Front and Back-illuminated sensors. Front illuminated sensors include wiring and microlenses between the incoming photons and the light-sensitive silicon, blocking some photons from being detected. Back-illuminated sensors use thinned silicon, with photons directly striking the light-sensitive surface, for higher quantum efficiency.

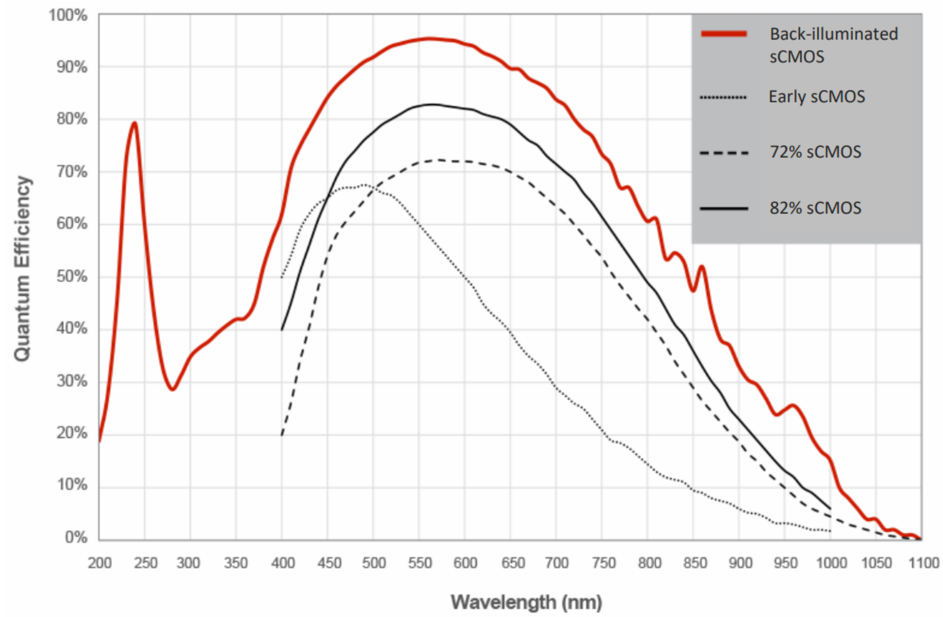


Figure 2: Spectral Response Curves showing Quantum Efficiency versus Wavelength of incident photon for a range of sCMOS camera sensors.

Ultimately, high quantum efficiency is vital for most applications, as the more photons we can collect, the better quantitative accuracy is possible in measurements, and the better image quality can be.

> **In summary: Higher quantum efficiency in your detected wavelength will lead to improved scientific image quality and quantifiability.**

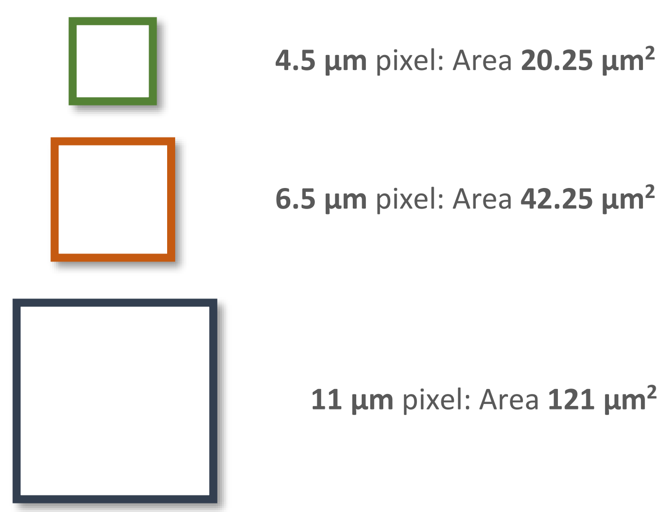
Pixel Size

The pixel size, also called 'Pixel Pitch' of a scientific camera refers to the xy width and height of the camera pixel on the sensor. The larger the camera pixel area, the more photons the pixel will be able to collect. Hence a doubling of pixel size leads to 4x the light collecting ability, so increasing pixel size is a powerful way to improve camera sensitivity.

The increase in pixel size however can come at the cost of imaging resolution, unless resolution is limited by the optical system.

Larger pixels are also able to hold more charge, giving them a higher full well capacity, and hence better dynamic range.

> **In summary: Larger pixels collect significantly more photons according to their area, potentially at the cost of resolution depending upon optics.**



Full Well Capacity

Full well capacity defines the amount of charge an individual pixel can hold before saturating. For scientific cameras, the term 'linear full well capacity' is more accurate, as the entire physical capacity of pixels to store photoelectrons is not used. This is because as pixels start to approach their maximum capacity, the presence of so many photoelectrons leads to charge exclusion effects that reduce the pixel's ability to collect additional photoelectrons, affecting linearity (see the section below). While not an issue for non-scientific applications, this loss of linearity is unacceptable for scientific cameras.

The available full well capacity depends also on camera mode, as it is determined by camera Bit Depth (the number of available grey levels) multiplied by camera Gain (the conversion factor from electrons to grey levels, in e-/grey), both of which can change based on camera settings.

A large full-well capacity can be important in imaging techniques that require high dynamic range, as in strong signals and weak signals in the same image. It is also useful for improving signal to noise in high light level techniques. However, for techniques that maintain a low light level such as typical fluorescence microscopy, full well capacity is not an important factor.

In summary: **Full Well Capacity influences image quality for imaging techniques that require a large dynamic range or use high light levels.**

The Role of Noise

Signal-to-noise ratio (SNR) describes the quality of a measurement. In scientific imaging, SNR refers to the relative magnitude of the signal compared to the uncertainty in that signal on a per-pixel basis. Specifically, it is the ratio of the measured signal to the overall measured noise (frame-to-frame) at that pixel. High SNR is particularly important in applications requiring precise light measurement.

Signal to Noise Ratio

Signal to noise ratio (SNR) for an intensity measurement is simply the signal divided by the sum of the three main noise sources: photon shot noise, dark current and read noise. It can be expressed by the following equation:

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

Where:
S = Signal (e-)
N_d = Dark current (e-/p/s)
N_r = Read noise (e-)
t = Exposure time (s)

A high SNR indicates images clear of 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. Additionally, many quantitative analysis techniques have a threshold SNR below which they will perform poorly.

There are no hard rules about what an ideal minimum signal to noise ratio is because this often depends on the sample, the analysis technique and the needs of the application. Figure 3 explores different signal to noise ratios and their corresponding image quality.

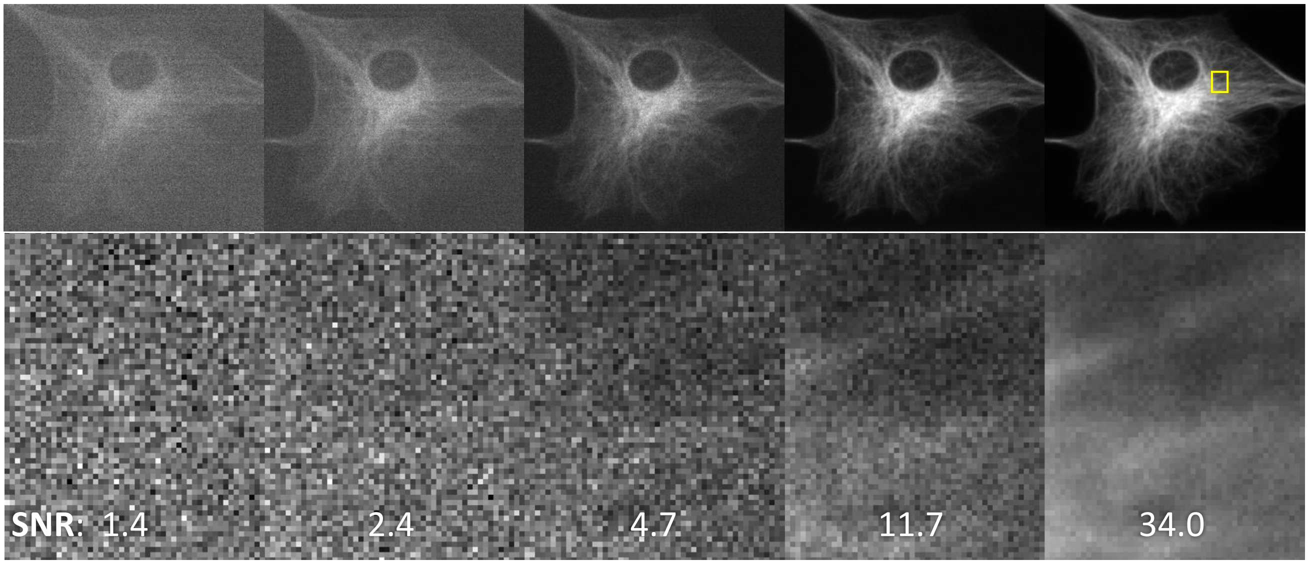


Figure 3: Signal to noise ratio comparison in fluorescence microscopy. Sample: Actin labelled BPAE fixed sample slide. Signal to noise ratios for images calculated based on average signal response from a 3x3 pixel region around the brightest point of the sample pictured. Yellow box on rightmost image shows the area used for inset below.

In summary: Signal to Noise Ratio (SNR) is a key quantitative indicator of image quality, encompassing both the signal collection and noise performance of the camera. However, patterns and artefacts are not accounted for in the SNR.

Photon Shot Noise

Photon shot noise is the randomness of emission of photons from practically every photon source, including fluorescent molecules. Due to this random emission, successive measurements of a constant signal will yield a distribution of photon counts with a known average – known as a Poisson distribution.

From measurement to measurement, a signal S will be expected to vary by $\sqrt{S}$. This is therefore a noise source that grows with signal, so at high signal levels it is Photon Shot Noise that will dominate.

Read Noise

Read noise is the uncertainty in measuring the detected signal in a camera, determined by speed of readout and quality of electronic design. CCD-based camera sensors have high read noise (6 to 10 electrons), as can industrial CMOS cameras with 4 to 10 or as much as 50 electrons of noise. Scientific CMOS cameras typically have around 1.0 to 1.6 electrons of read noise. High read noise can challenge low light imaging, and worsen image quality for dim signals.

The relative importance of Quantum Efficiency and Read Noise is a common question in scientific imaging, and is explored in Figure 4. Comparing a hypothetical camera with 0.7e- of read noise but only 80% QE to a camera with 1.0e- read noise and 95% QE, higher signal to noise ratio is attained for the higher QE camera at all signal levels above 1 photon per pixel, with typical signals in challenging low-light fluorescence microscopy for example typically ranging from 10 to 100 photoelectrons per pixel. Even increasing read noise to 1.6e- for the higher QE camera yields a better SNR for signals above 10 photoelectrons, a signal so low that most imaging applications would far exceed this value.

However, further reducing the read noise of a 95% QE camera from 1.0e- to 0.7e- has little effect on these signals, yielding a 2% increase in SNR at 10 photons and 0.2% at 100 photons.

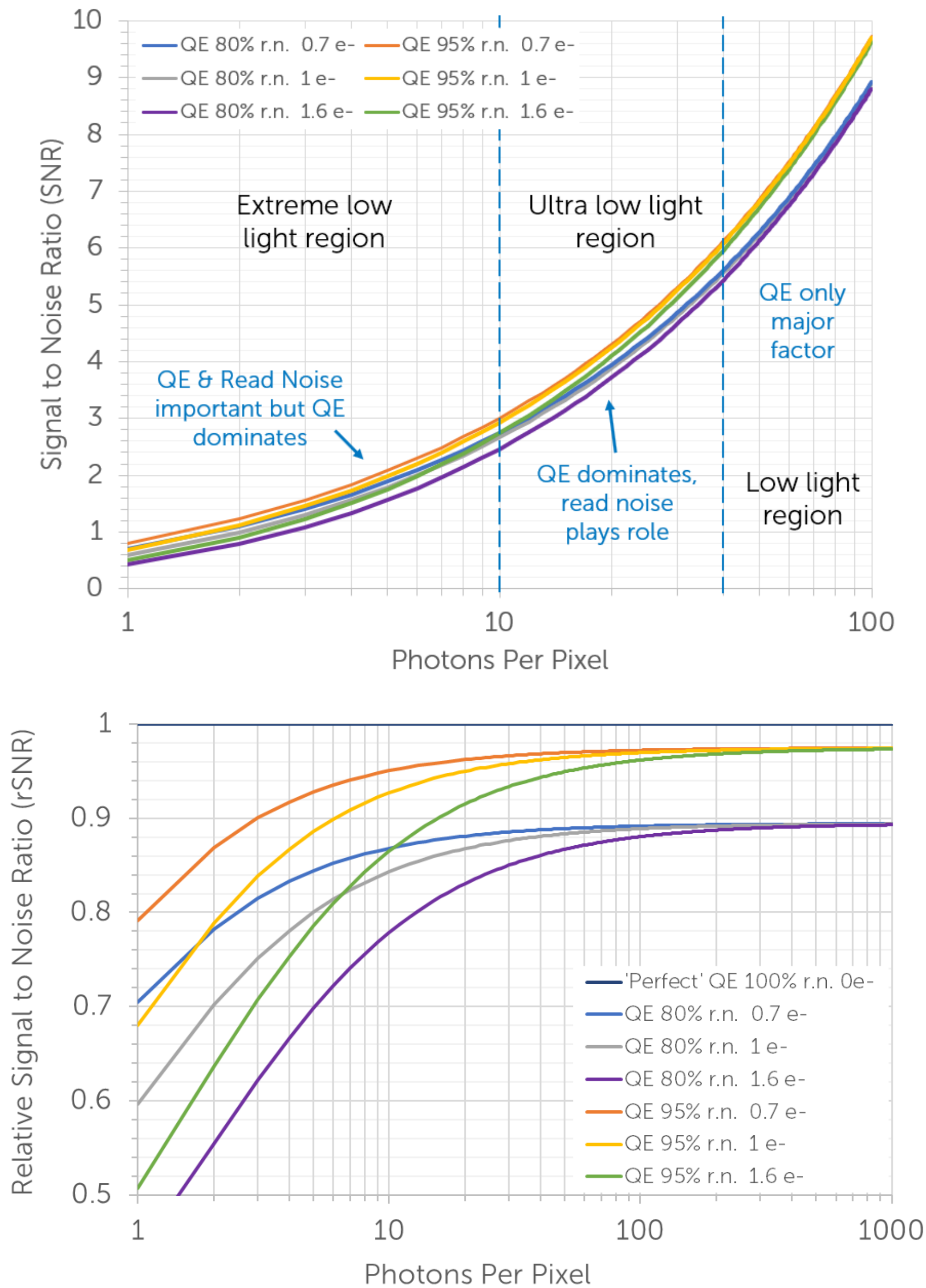


Figure 4: Signal to noise ratio versus incident signal comparison for 6 example hypothetical sensors with differing Quantum Efficiency (QE) and read noise (r.n.) values, calculated assuming dark current to be negligible. Top: Absolute signal to noise ratio. Bottom: Relative signal to noise ratio (rSNR) compared to a 'perfect' theoretical camera with 100% QE and no read noise.

In summary: Read noise is an important factor in low light imaging, but small differences only influence imaging at extreme low light levels. Quantum Efficiency plays a larger role.

Background Light

The role read noise plays in image quality also to some extent depends on the presence or absence of background light in the sample, from for e.g. out-of-focus fluorescence, autofluorescence, room light, and light leaks. As background light will also exhibit photon shot noise, unwanted signals of only 2-3 photoelectrons per pixel will already contribute more noise to measurements than the typical read noise for a CMOS camera, making differences in read noise between cameras far less significant for these setups.

EMCCDs and the Excess Noise Factor

The previous leading technology for low light imaging was the EMCCD camera, using electron multiplication before measurement to overcome the read noise of the camera. However, this multiplication is stochastic in nature producing a different multiplication for every pixel measured, and so introduces an additional noise factor called the Excess Noise Factor. When statistically modelled, this additional noise distribution has the effect of increasing all pre-multiplication noise sources by a factor of $\sqrt{2}$, or 41%. The most significant result of this is the multiplication of Photon Shot Noise discussed above, which grows with signal.

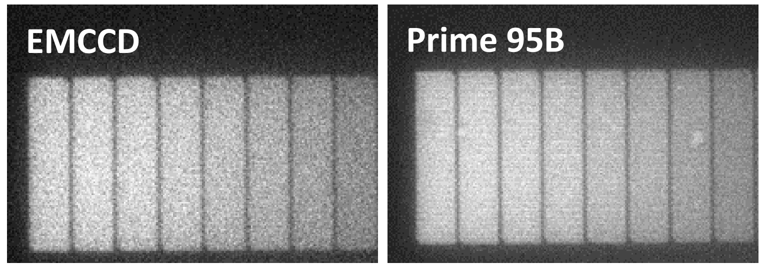


Figure 5: Comparison of calibration slide images for 13 µm pixel EMCCD (left) and 11 µm pixel back-illuminated CMOS (right), at the same exposure time and magnification level. The presence of the Excess Noise Factor is evident in the higher degree of 'grainy' noise for the EMCCD image, despite its 40% pixel area advantage.

As a result, a back-illuminated CMOS camera will yield an increasingly improved signal to noise ratio compared to an EMCCD for all signals above 2-3 photons per pixel, with the difference significant at the 10-100 photons range typical for challenging low light imaging. Additionally as the EMCCD's only advantage is in low read noise, the presence of any background light can nullify this advantage, as background light too suffers from additional photon shot noise due to the Excess Noise Factor.

However, for signals that are at maximum 1-2 photons per pixel such as in Single Photon Counting, the low read noise of the EMCCD still provides a better signal to noise ratio, so EMCCDs still have a niche within scientific imaging.

In summary: Though the Excess Noise Factor means EMCCD image quality is typically unable to compete with that modern back-illuminated CMOS cameras for biological imaging, applications for EMCCD cameras care still found.

Dark Current, and What Role Does Cooling Play?

Dark current is thermal noise that builds up during the duration of an exposure caused by electrons moving into the pixel well from surrounding silicon as if they were detected photoelectrons. The average quantity of dark current electrons in a pixel during readout is given by 'Specification Sheet' dark current (in electrons / pixel / second) D multiplied by t, the exposure time. As the collection of thermal electrons occurs at random times but with known average rate, this is another Poisson process like Photon Shot Noise, so the actual noise contribution of dark current is given by:

$$Dark\ Current\ Noise\ = \sqrt{D \times t}$$

Dark current is the primary reason for camera cooling, alongside reduction in hot pixels (individual pixels with unusually high dark current). However, the importance of dark current depends on the exposure time used. For short exposures of 10ms or less, even high dark current uncooled cameras can offer good signal to noise ratios – however, for 100ms exposures, dark current can come into play, and uncooled cameras can have much reduced SNR. By 1 second of exposure, depending on the dark current value, uncooled cameras can be effectively incapable of imaging, as seen in the table below.

However, the extent of cooling (i.e. the temperature achieved) is not the important factor, as different technologies and cameras can achieve massively different dark current performance with the same sensor temperature. Additionally, read noise will typically play a greater role for typical <2s exposures for cooled cameras. This is explored in the tables below.

Back-Illuminated 6.5um Pixel Cameras:	Prime BSI (-25°C Water Cooling)	Deep cooled (-45°C Chilled Liquid Cooling)	Uncooled Equivalent
Read Noise (Median, electrons)	1.0 e-	1.2 e-	1.8 e-
Dark Current (electron / pixel / second)	0.12 e-/p/s	0.10 e-/p/s	42 e-/p/s

Exposure Time (ms)	SNR at 10e- signal		
	Prime BSI	Deep cooled (-45°C)	Uncooled Equivalent
10 (100fps)	3.02	2.96	2.73
100 (10fps)	3.02	2.96	1.80
500 (2fps)	3.01	2.96	0.47
1000 (1fps)	3.01	2.96	0.24

Exposure Time (ms)	SNR at 100e- signal		
	Prime BSI	Deep cooled (-45°C)	Uncooled Equivalent
10 (100fps)	9.95	9.93	9.83
100 (10fps)	9.95	9.93	9.10
500 (2fps)	9.95	9.93	4.29
1000 (1fps)	9.95	9.93	2.31

Table 1: Top: Read noise and dark current comparisons for three cameras based on the same sensor from different camera manufacturers. Middle: Signal to noise ratios at 10 photoelectrons of detected signal for stated exposure time. Bottom: Signal to noise ratios at 100 photoelectrons of detected signal for stated exposure time. The low read noise and dark current of the Prime BSI means this camera leads in all cases. The difference between -25°C and -45°C cooling is negated even for 1 second exposure times by the difference in read noise between these two cameras. The uncooled camera yields only a fraction of the sensitivity for exposure times of 100ms and above.

In summary: For exposure times of 100ms-1s, cameras typically require cooling for low noise performance. However, sensor temperature is irrelevant; only the resulting dark current is important. For > 1 s exposures, low dark current can be essential.

Background & Signal Uniformity

One key aspect of scientific image quality that is not typically represented on camera specification sheets is the quality and uniformity of the background of the camera, on top of which signals appear. Structures and patterns in this background, both static and dynamic, can make the detection of weak signals more challenging, and the visual quality of images poorer. Removing these signals after acquisition, whether static or dynamic, is typically not possible as the contribution of the pattern to any given image can vary.

Background Quality

Static Patterns

In CMOS cameras, the measurement of signal happens independently for each column of the camera in analogue to digital converters (ADCs). Some cameras even split the sensor in two, with a row of ADCs at both the top and the bottom of the sensor to increase readout speed. However, this parallelized readout requires careful calibration and balancing of the respective outputs of each ADC and other factors, the quality of which can vary greatly between camera models and camera manufacturers, even when using the same underlying sensor. The underlying sensor patterns of two cameras are compared in Figure 6.

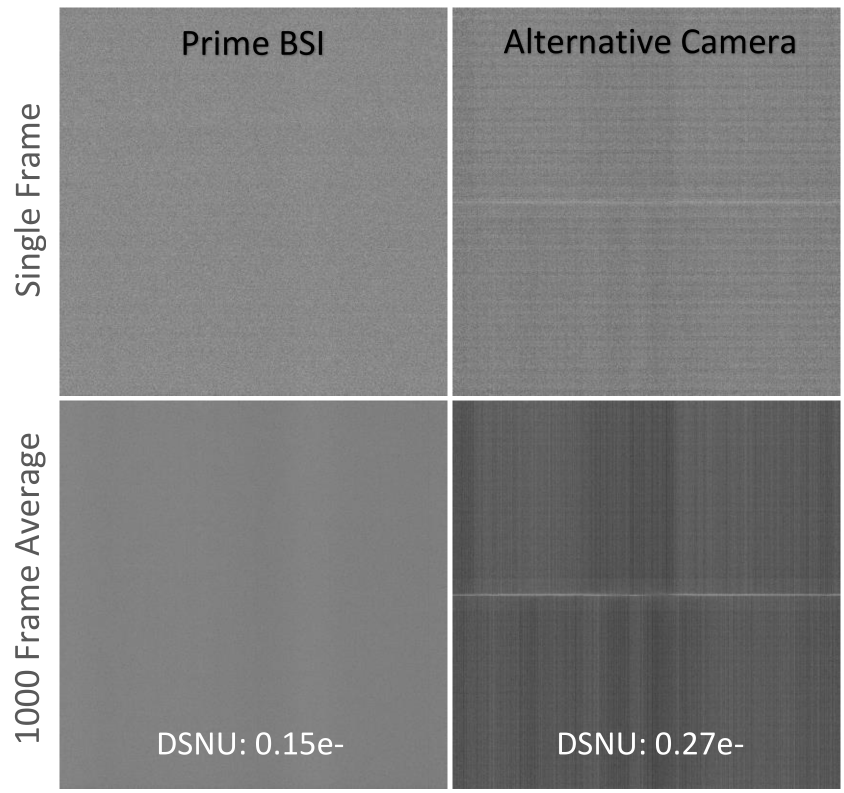


Figure 6: Background quality comparison for two cameras. The left column shows the Prime BSI, and the right column an 82% QE alternative camera. The top row shows single frames, image scaling for both images set to -4 e- black to 4e- white. Bottom row shows 1000 frame averages, with scaling as -1.5e- black to 1.5e- white. Neither frame for the Prime BSI shows significant pattern, however both for the alternative camera show massive levels of patterning. The static pattern in the single frame is somewhat obscured by the dynamic pattern discussed in the following section. DSNU numbers (discussed below) shown for averaged frames do not reflect extent of the difference in quality between these two images.

Dynamic Patterns

Dynamic patterns can reflect uncorrected issues with the readout process of a camera. These patterns are a common issue in CMOS cameras due to the expense and challenge of engineering cameras without them. Additionally these patterns are even more challenging than static patterns to remove after acquisition, and potentially more damaging to the quality and quantifiability of low light imaging data.

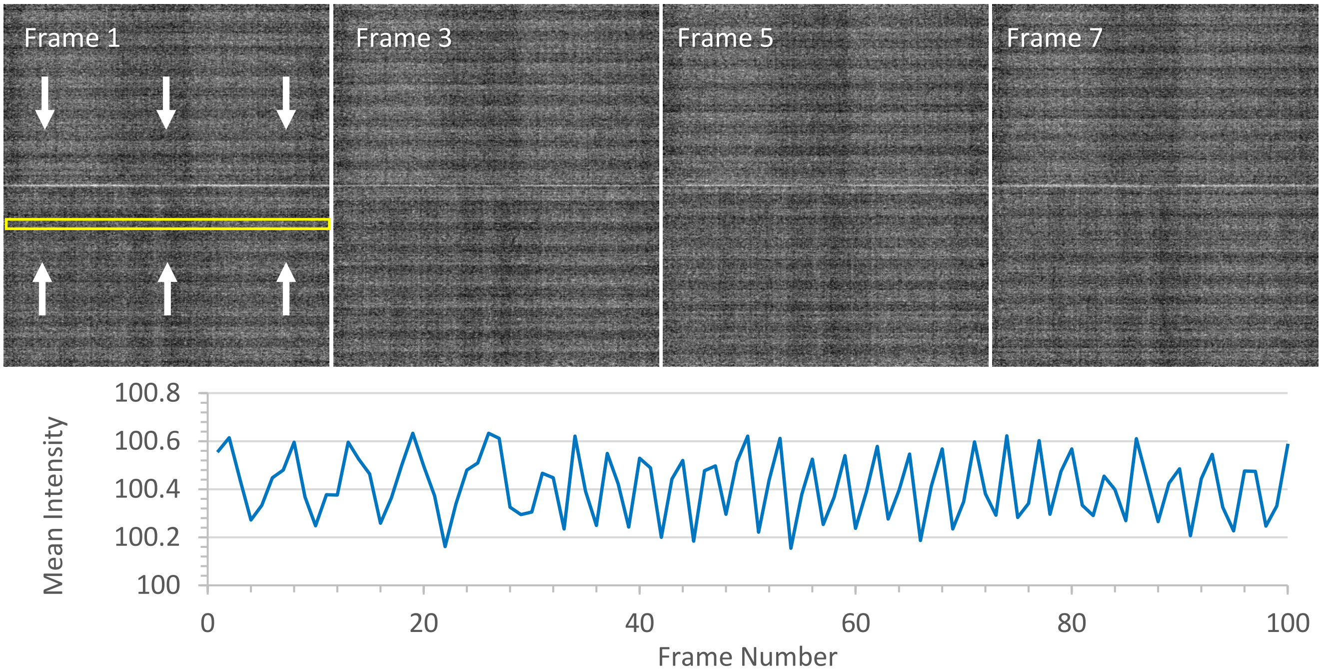


Figure 7: 82% QE alternative CMOS camera dynamic background artefacts. Top: Image sequence shows scrolling pattern of horizontal bands, converging on the center of the sensor. Image scaling is fixed at 98 to 101 grey levels. Yellow rectangle shows the 2048x10 pixel region the average value of which is plotted below the images for a 100 frame image sequence.

In summary: Some cameras can exhibit static and dynamic patterns in their backgrounds, one of the most challenging factors for image quality when imaging weak signals.

Binning & Background Quality

For challenging low-light imaging, binning can provide a method to increase signal to noise ratio at the cost of resolution. However, pattern issues such as those discussed above are worsened by binning.

Additionally, binning on CMOS cameras increases read noise, with 2x2 binning leading to 2x read noise. It is therefore important that read noise and patterns are both minimal if binning cameras.

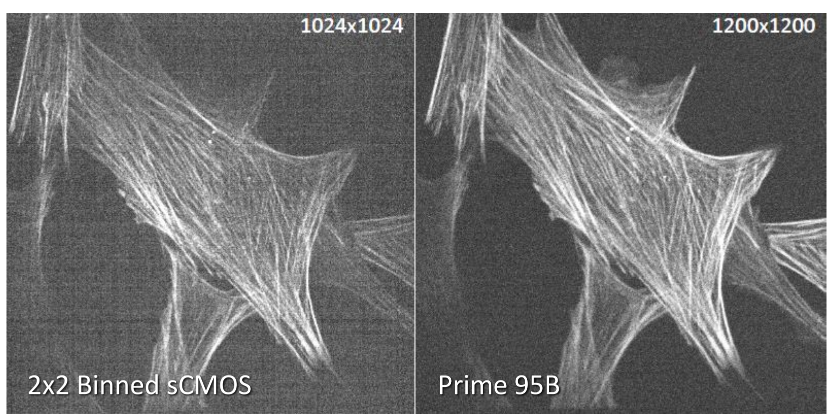


Figure 8: Left: 82% QE 6.5 µm pixel alternative CMOS camera with 2x2 binning. Right: 95% QE 11 µm pixel Prime 95B, unbinned. Images acquired with identical exposure times and light level. The left image shows a clear ‘checkerboard’ artefact.

Dark Signal Non-Uniformity (DSNU)

Dark Signal Non-Uniformity is the general term given to all variations in a camera's offset, including visual images of patterns in a camera's background as shown above. However, it can also be used to describe a single quantity, that being the standard deviation of a frame formed from an average of 1000 or more dark frames, measured in electrons. This measure is intended to capture the level of patterns and artefacts in a camera's background that can obscure weak signals.

As shown in Figure 6 above, enormous differences in background quality can correspond to relatively minor differences in DSNU, indicating that this is not a useful representation of background quality and the presence of patterns.

Additionally, a newer model from the camera manufacturer for the alternative camera used in Figure 6 claims an improvement in pattern-free backgrounds compared to the camera pictured, however the DSNU figure claimed for this new camera is 0.3 electrons, compared to 0.27 measured for the camera above. This is further indication that DSNU is not a useful measurement of camera background performance.

In summary: DSNU does not accurately capture the background quality of a camera, as patterns that can be highly problematic for low light imaging are not represented well by this measurement.

Photo-response Non-Uniformity (PRNU)

PRNU is defined as the Standard Deviation of the pixel values of a camera under even illumination, often given as a percentage. This is intended to present a quantitative measure for sensor uniformity at high light levels, as a quantitative representation of patterns present on the sensor. However, it is highly light level dependent, and there is no meaningful industry standard for which light level should be used.

Typically in camera manufacturers' technical literature, PRNU is measured at half full well capacity for the sensor. This is highly unhelpful for comparison purposes - not only does full well capacity vary greatly for different sensors, it can vary between implementations of the same camera sensor by different manufacturers. Additionally, half full well capacity is far too high a light level to provide meaningful measurements for low light imaging techniques.

However, it can become even more clear on inspection of images acquired in measuring PRNU that this measurement is unsuitable for representing the presence or absence of patterns and non-uniformity. Figure 9 shows images and data from an alternative camera data sheet comparing PRNU measurements for two cameras, where one camera shows significant patterning on the sensor and the other does not, yet PRNU measurements are precisely equal.

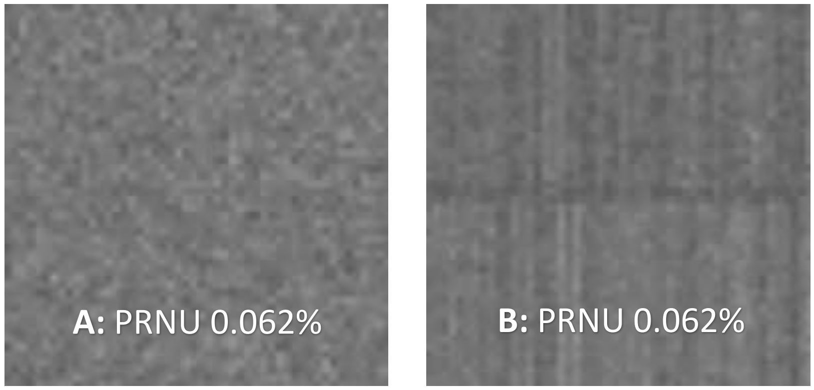


Figure 9: Central 50x50 pixels of two alternative camera sensors under even illumination at half full well capacity, with measured PRNU values. The images show significant patterns for Camera B, and not for Camera A. However, PRNU values are the same, showing PRNU is not a useful indicator of image quality. Values and images from Hamamatsu ORCA-Fusion Specification Sheet[1]

In summary: PRNU is not an appropriate comparison measurement for sensor uniformity as there is no industry standard light level used. PRNU also fails to represent the presence of sensor patterns at high light levels. Additionally, it is not relevant for low light imaging.

Linearity

When using a camera scientifically, i.e. as a quantitative device for measuring photons, a linear response to light is necessary, meaning that our digital signal out should be directly and constantly proportional to our detected signal in. However in reality this represents a considerable engineering challenge for camera sensors, with the majority of 'consumer' digital cameras being non-linear in their response. In scientific imaging, linearity is typically represented as a percentage, given by:

$$Linearity\ (\%) = 100 - \frac{(\text{Max Positive Deviation} + \text{Max Negative Deviation})}{\text{Max Detectable Signal}} \times 100$$

calculated based on successive measurements of a constant illumination source for increasing exposure time, up to the camera full well capacity. The Max Positive and Negative Deviations in the equation refer to amount of deviation from the ideal straight-line response.

For scientific cameras, linearity of 99% or above is typically enough to minimize linearity error below other sources of camera error such as the noise sources discussed above. For cameras with poor or no linearity correction, the linearity is typically not given on camera specification sheets, so the quantitative response of the camera is unknown.

One common issue with CMOS cameras with '16 bit high dynamic range' modes is linearity issues around 3-4000 grey levels. 16 bit images are typically acquired through combining the output of two 12 bit analogue to digital converters (ADCs), which unless precisely engineered, can lead to jumps in grey levels around the crossover point.

In summary: Linearity of 99% or above is recommended though hard to engineer. Linearity values are often left unspecified outside of high-quality low light imaging scientific cameras.

[1] https://www.hamamatsu.com/resources/pdf/sys/SCAS0136E_C14440-20UP.pdf

Artefacts

Hot Pixels

Another common issue requiring correction for scientific imaging is the presence of individual pixels with an unusually high dark current. Cooling can reduce the occurrence of these pixels, but some sensors can still be prone to hot pixels despite cooling, especially at longer exposure times.

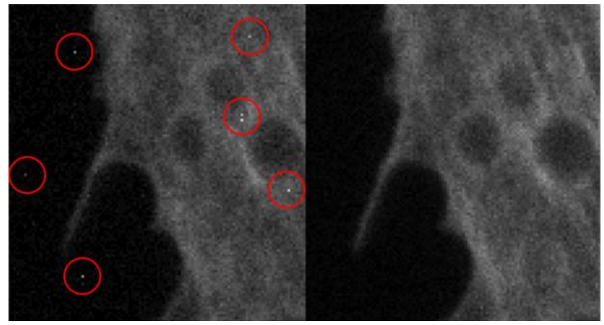


Image Corrections

Despeckle Correction Filters

The sensor design of CMOS cameras can introduce a minor additional noise source called Random Telegraph Noise, caused by the random movement of electrons into and out from defects in the silicon of the sensor. This leads to a very small percentage of pixels in an image exhibiting values much higher or much lower than the true detected signal, with which pixels affected changing dynamically from image to image. As these individual pixels can affect the max and min image values significantly, they can distort image scaling settings as well as worsening visual image quality.

To account for these dynamic spurious value events, the industry standard approach is to apply a conditional median filter to spurious pixels. The camera actively searches for individual pixels that have values much higher or much lower than their neighbors in a 3x3 area, and if the difference is greater than a factory-calibrated threshold, the pixel value is replaced with the median value of the 3x3 area. Typically in any given image only a few hundred to a few thousand pixels of a 1 MP camera require replacement in this way, but through this mechanism, known as ‘despeckle filtering’, a more accurate representation of the sample is attained.

The chances of false recognition of a genuine high value pixel as having occurred due to a spurious event is negligible for the majority of imaging applications, as optical lens-based or microscope-based systems are typically diffraction-limited, meaning that photons from a point source would be spread over multiple pixels, not concentrated on an individual pixel.

However, there are some applications such as single-molecule imaging where imaging subjects are frequently single point sources, and this likelihood is increased. Additionally applications using wide-angle lenses where light collection is no longer diffraction-limited, and large changes of contrast can occur in small distances. For these applications it is recommended camera despeckle filters are turned off.

In summary: Despeckle filters are important for maintaining CMOS camera image quality, however for applications exclusively imaging point sources such as single molecule imaging, it is recommended they be switched off.

Summary

To bring all of these factors together, here is a summary of what to look for in a scientific camera to achieve high image quality and quantitative accuracy:

Quantum Efficiency (QE)	**High** quantum efficiency (such as found in Back-illuminated sensors) increases signal detection, which directly improves image quality and quantitative accuracy.
Pixel Size	**Large** pixel sizes also increase signal detection, but must be balanced with need for resolution
Full Well Capacity (FWC)	Full Well Capacity influences image quality for imaging techniques that require a **large dynamic range** or use **high light levels**, but is not typically relevant for low light imaging.
Signal to Noise Ratio (SNR)	For a given photon signal and exposure time, the Signal to Noise Ratio can be calculated and compared between cameras. This is our **primary means of judging scientific camera image quality**. However, patterns and artefacts are not accounted for in the SNR.
Read Noise, and Read Noise vs QE	Read noise is an important noise characteristic for low light imaging – however the relative importance of read noise and QE will be signal dependent and must be checked through calculating SNR. For typical imaging applications, **high QE will be more beneficial than low read noise**.
Dark Current and Cooling	For exposure times of 100ms-1s, cameras typically require cooling for low noise performance. However, **sensor temperature is irrelevant**; only the resulting dark current is important. For > 1 s exposures, low dark current can be essential.
Background Light	Due to the effect of Photon Shot Noise, the presence of background light in an image can overwhelm other noise sources and should be avoided.
EMCCD Excess Noise Factor	This additional noise source for EMCCDs is the most significant reason for the performance improvement of back-illuminated CMOS over EMCCD.
Camera Background Quality	Some cameras can exhibit **static and dynamic patterns** in their backgrounds, one of the **most challenging factors for image quality** when imaging weak signals. These patterns can be worsened by the use of binning.
Dark Signal Non-Uniformity (DSNU)	DSNU does not accurately capture the background quality of a camera, as patterns that can be highly problematic for low light imaging are not represented well by this measurement.
Photon-Response Non-Uniformity (PRNU)	PRNU is not relevant for low light imaging. Further it is not an appropriate comparison measurement for sensor uniformity as there is no industry standard light level used. PRNU also fails to represent the presence of sensor patterns at high light levels.
Linearity	Linearity of **99% or above** is recommended though hard to engineer. Linearity values are often left unspecified outside of high-quality low light imaging scientific cameras.
Despeckle Filters	Despeckle filters are important for maintaining CMOS camera image quality, however for applications exclusively imaging point sources such as single molecule imaging, it is recommended they be switched off.

