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).

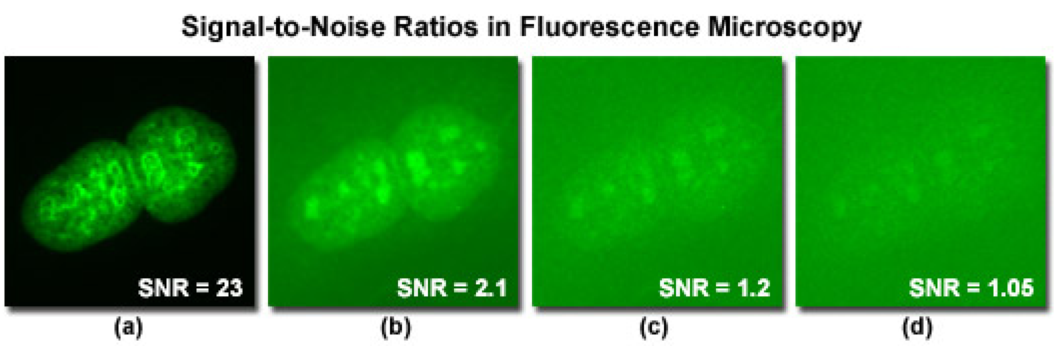

Figure 1: Signal to noise ratio comparison in fluorescence microscopy. Noise is clearly dominant at SNR=1-2 but improves slightly as it is increased to SNR=2. At SNR=23 there can be full measurement confidence with extremely minimal noise impact. Adapted from olympus-lifescience.com.

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

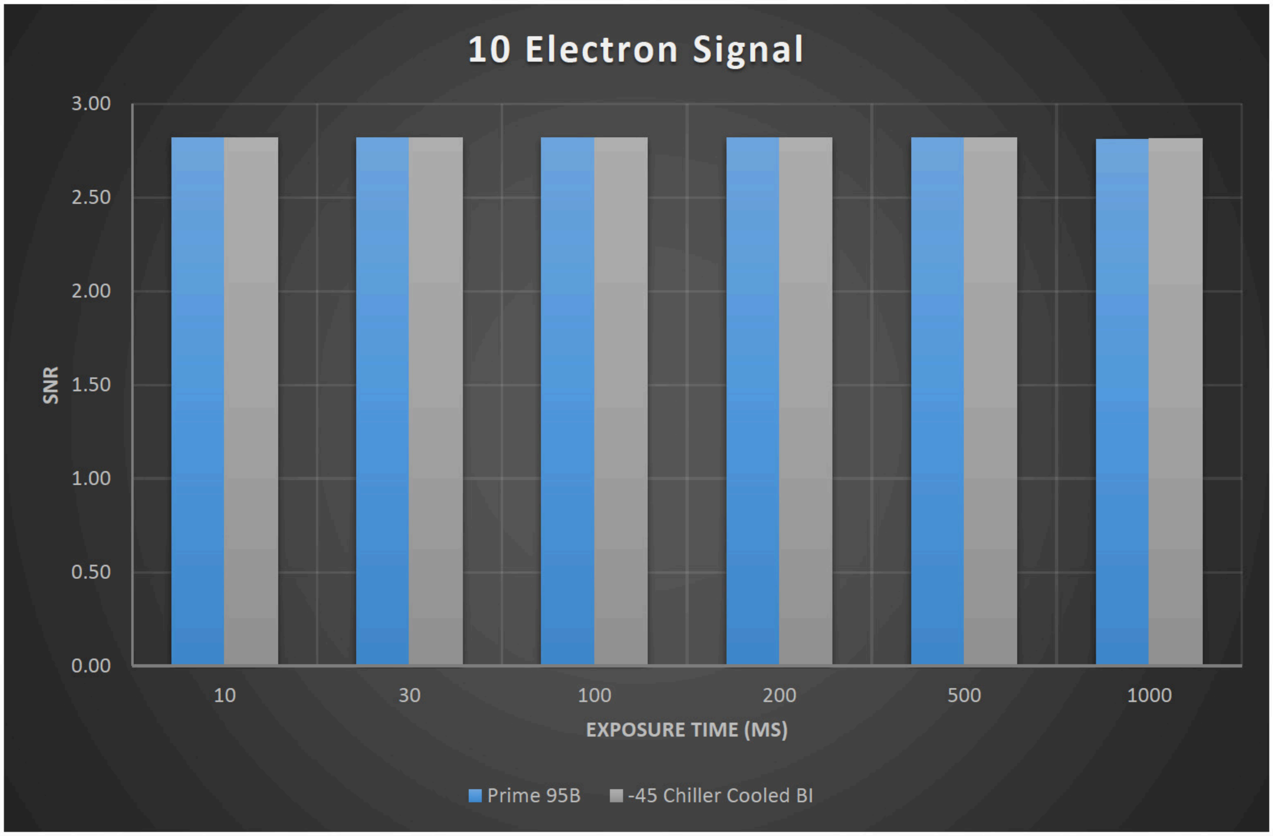

Figure 2: Signal to noise comparison of two back-illuminated CMOS cameras with 10 electrons of detected signal. Exposure times range from 10 milliseconds to 1000 milliseconds (1 second).

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

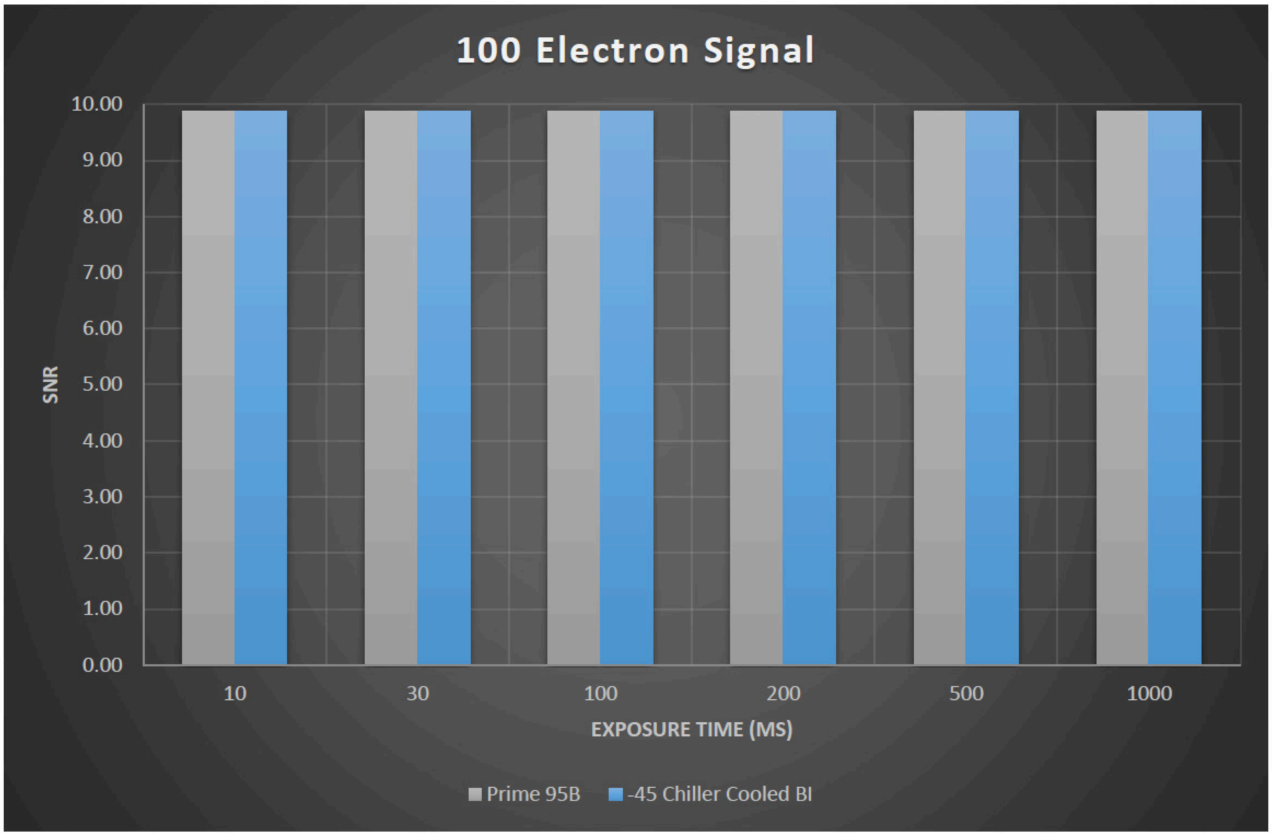

Figure 3: Signal to noise comparison of two back-illuminated CMOS cameras with 100 electrons of detected signal. Exposure times range from 10 milliseconds to 1000 milliseconds (1 second).

	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*