

Electron-Multiplying (EM) Gain

©2006, 2007 QImaging. All rights reserved.

In order to gain a clearer understanding of biological processes at the single-molecule level, a growing number of experiments are being conducted using small-volume samples. Both the lower fluorophore concentrations and the faster kinetics associated with these experiments establish key criteria for choosing an appropriate camera system.

This technical note endeavors to provide a comprehensive look at the advantages and limitations of electron-multiplying (EM) gain, a CCD technology designed for low-light, high-speed imaging.

The following topics are discussed:

- Low-light, high-speed challenges
- Applicable popular technologies
- Electron-multiplying gain

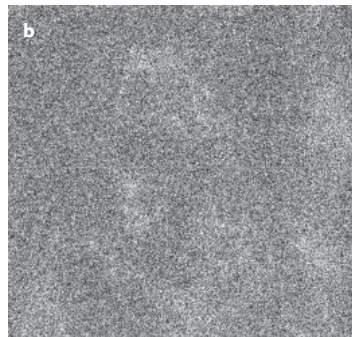
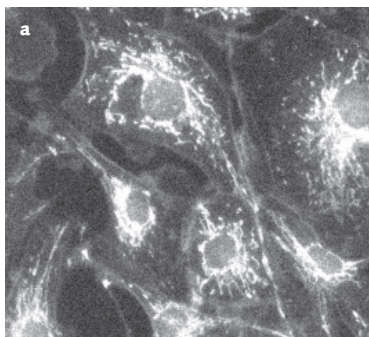


Figure 1. Low-light sensitivity (a) increases with low read noise and (b) decreases with high read noise.

Imaging at Low Light Levels

Requirements

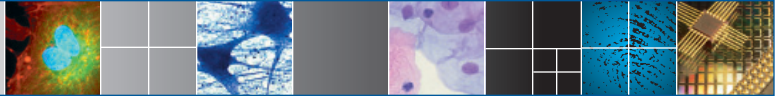
CCD performance has improved significantly through the years. Reductions in read noise and increases in quantum efficiency (QE) have served to lower the detection limits of leading-edge imaging systems. For example, QImaging® offers back-illuminated CCD cameras that boast QE greater than 90% and read noise as low as 2 e⁻ rms (see **Figure 1**).

However, the best read-noise performance is attainable only when readout speed is reduced considerably (i.e., into the range of “a fraction of a frame” to “a few frames” per second). Thus, traditional low-light-level imaging systems face a fundamental challenge when they are required to capture low-light events at video frame rates and faster.

FACT CCD read noise increases as readout speed increases.

Intensified CCDs

In order to overcome the limitation on sensitivity imposed by read noise at higher speeds, the signal itself is often amplified above the read noise. Photomultiplier tubes were among the first to implement this strategy.



FACT Amplifying the incoming signal effectively reduces the input-referenced read noise.

Today, image intensifiers are frequently employed for low-light-level imaging. In an intensified CCD (ICCD) camera system, incoming photons are multiplied by the image intensifier and subsequently detected by a traditional CCD.

ICCD camera systems offer a proven solution for applications such as single-molecule fluorescence (SMF), a type of live-cell imaging that demands very high detector sensitivity along with readout rates equal to and beyond those associated with video. However, while vast improvements have been made to these vacuum devices in terms of sensitivity and resolution over the years, they still suffer from a few disadvantages, including susceptibility to damage under high-light-level conditions as well as lower spatial resolution.

ICCD PROS Good low-light-level sensitivity and the ability to act as a fast shutter (psec or nsec gating)

ICCD CONS Susceptibility to damage, lower spatial resolution, high background noise

As with ICCDs, electron-bombardment CCD (EBCCD) camera systems use a photocathode to convert incoming photons to electrons; the charge is then amplified and detected by a CCD. The technology also carries similar lifetime, resolution, and background-noise limitations.

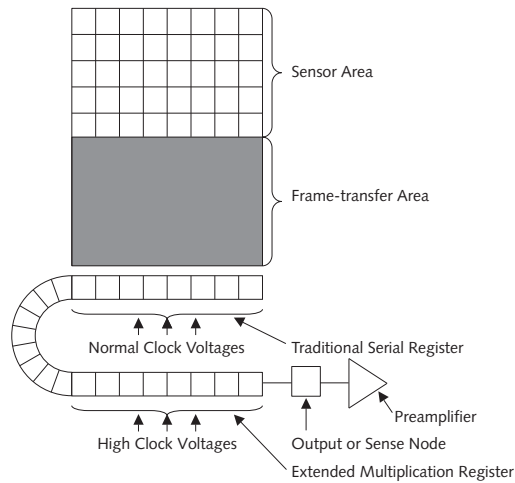


Figure 2. This example of an electron-multiplying CCD has a frame-transfer architecture.

Electron-Multiplying Gain

High Performance in Low Light

A few years ago, CCD manufacturers introduced novel, high-sensitivity CCDs engineered to address the challenges of ultra-low-light imaging applications — without the use of external image intensifiers. These detectors utilize *electron-multiplying gain* technology to raise photon-generated charge above the read noise, even at supraviduo frame rates.

This special, signal-boosting process occurs before the charge reaches the on-chip readout amplifier, effectively reducing the CCD read noise by the EM gain factor, which can be greater than 1000x. The main benefit of the technology, therefore, is a far better signal-to-noise ratio for signal levels below the CCD read-noise floor.

The principal difference between an electron-multiplying CCD (EMCCD) and a traditional CCD is the presence of a special extended

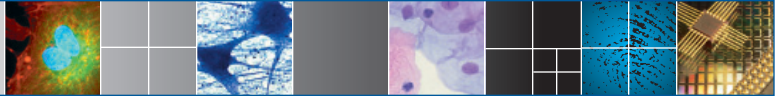
serial register, known as a *multiplication register*, in the EMCCD (see **Figure 2**). Note that the EM gain takes place after photons have been detected in the device's active area.

Electrons are accelerated from pixel to pixel in the multiplication register by applying higher-than-typical CCD clock voltages (up to 50 V). Secondary electrons are generated via an impact-ionization process that is initiated and sustained when these voltages are applied. The EM gain can be controlled by increasing or decreasing the clock voltages; the resultant gain is exponentially proportional to the voltage.

FACT EM gain is achieved by generating secondary electrons via impact ionization.

Technology Description

As mentioned earlier, the gain factor achieved via the impact-ionization process can be greater than 1000x. In fact, EM gain is actually a complex function of the probability of secondary-electron generation and the number of pixels in the multiplication register.



Mathematically, it is given by

$$G = (1+g)^N,$$

where N is the number of pixels in the multiplication register and g is the probability of generating a secondary electron. The probability of secondary-electron generation, which is dependent on the voltage levels of the serial clock and the temperature of the CCD, typically ranges from 0.01 to 0.016. Although this probability is low, the total gain can actually be quite high, owing to a large number of pixels in the multiplication register. For example, a CCD with pixels N equal to 400 and probability g equal to 0.012 produces EM gain G of 118.

FACT EM gain has an exponential relationship to the CCD's high-voltage serial clock.

Figure 3 clearly illustrates that the “last few volts” of the applied voltage result in a large increase in the EM gain. In practice, the level of voltage is commonly mapped to a high-resolution DAC (digital-to-analog converter) and controlled through software.

Effects of CCD Cooling

Another factor that influences EM gain is the CCD temperature. Simply put, the colder the temperature, the more likely it is for a primary electron to generate a secondary electron in the silicon, resulting in higher EM gain (see **Figure 4**). Studies show that greater than 1000x EM gain can be achieved by cooling the detector to -25°C or below. This strong performance dependency underscores the importance of selecting the optimum CCD temperature and preventing its fluctuation with the environment.

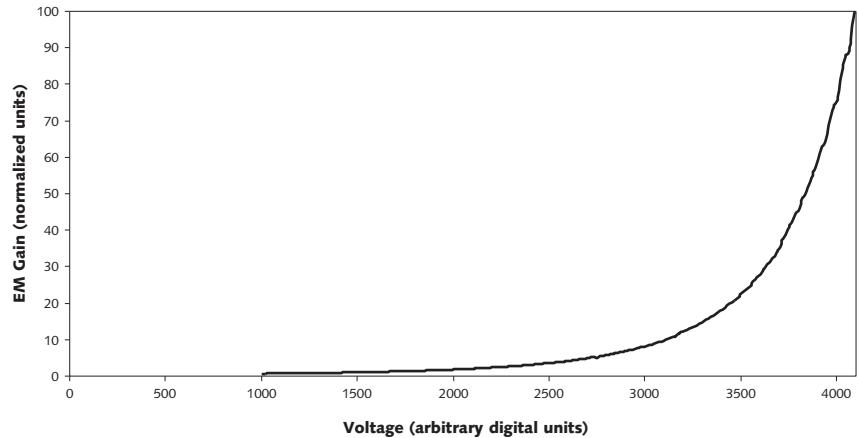


Figure 3. EM Gain vs. Voltage

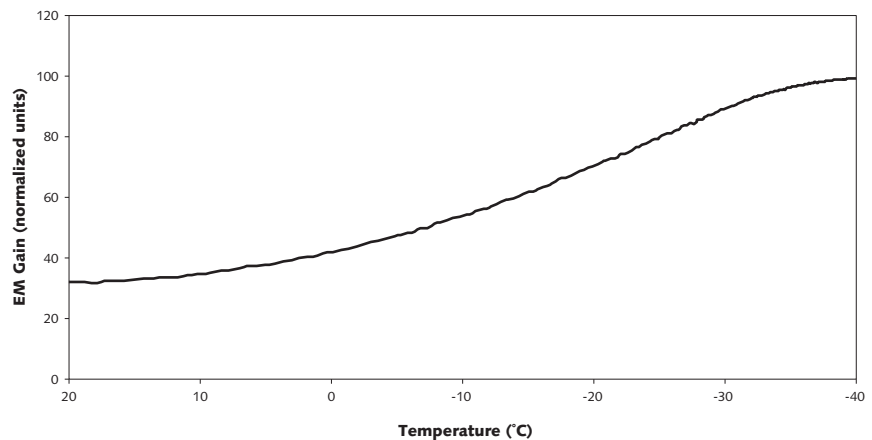


Figure 4. EM Gain vs. Temperature

As with traditional detectors, cooling a CCD that utilizes EM gain reduces the dark current generated in the pixels of the device. In EMCCDs, however, it is even more important that the dark current be minimized, since this unwanted contributor to system noise is multiplied in conjunction with the desirable, photon-generated signal via impact ionization.

Although cooling the CCD is often beneficial, it can also increase the occurrence of a lesser-known phenomenon called *spurious charge*.

FACT Cooling reduces dark current, increases EM gain, and increases spurious charge.

Spurious Charge

When electrons are clocked (moved) through the multiplication register's pixels, the sharp inflections in the clock waveform occasionally produce a secondary electron even if no primary electron is present. As noted previously, this phenomenon, called *spurious charge*, increases slightly as temperature decreases. Exposure time has no effect on spurious charge.

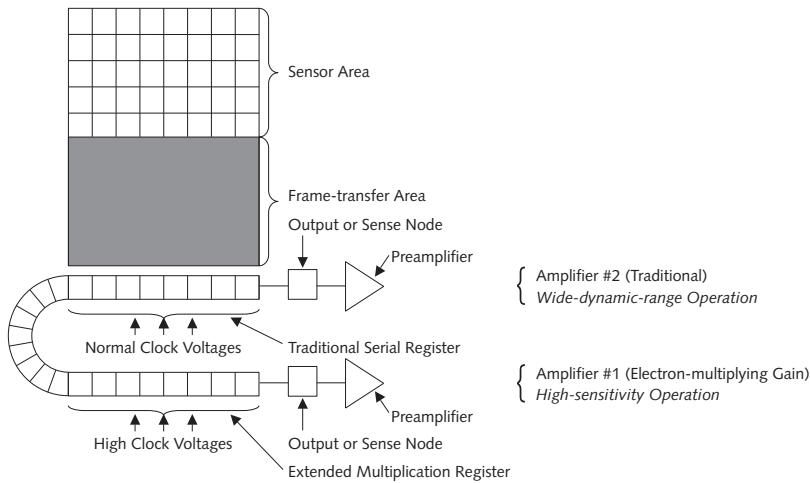
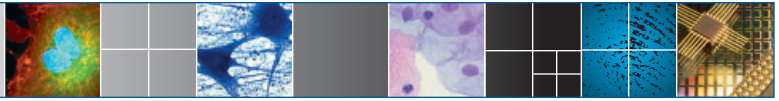


Figure 5. A second, “traditional” readout amplifier makes the Rolera-MGi more versatile by enabling the camera to be used for wide-dynamic-range applications.

It has been observed that a single spurious electron is generated for every 10 pixel transfers, thus yielding a value of 0.1 e-/pixel/frame. Typically, the spurious-charge component is added to the dark charge in order to determine the total dark-related signal. For example, a CCD camera cooled to -30°C with a dark current rate of 1.0 e-/pixel/sec (i.e., 0.033 e- per pixel per 30-msec frame) will have dark-related signal of 0.133 e-/pixel/frame.

FACT Total dark-related signal equals spurious charge plus dark charge.

Excess Noise Factor

Electron-multiplying gain is a probabilistic phenomenon, meaning there is a statistical variation in the gain (often, the reported EM gain is an ensemble average). The deviation or uncertainty in EM gain, which is related to the pulse-height distribution found in various scientific literature, introduces some amount of additional system noise, quantified by the *excess noise factor* (F).

Extensive investigations have been conducted in this subject area. Experimental results show that the excess noise factor is between 1.0 and 1.4 for levels of EM gain as high as 1000x. (When calculating total system noise, both the dark-related and photon-generated signals are multiplied by the factor *F* to account for excess noise.)

FACT The excess noise factor is between 1.0 and 1.4 for EM gain as high as 1000x.

Signal-to-Noise Ratio

A complete derivation of signal-to-noise ratio (SNR) is given in the Appendix. Simply expressed, the signal-to-noise ratio of a CCD with EM gain is given by

$$SNR_{Total} = (S * QE) / \sigma_{Total}$$

where

- S = total number of photons arriving at each pixel
- QE = fraction of photons detected
- σ_{Total} = total noise in system = $\sqrt{[(S * QE * F^2) + (D * F^2) + (\sigma_R / G)^2]}$

where

- D = total dark-related signal (including spurious charge)
- F = excess noise factor (typically between 1.0 and 1.4)
- σ_R = read noise of detector
- G = EM gain factor

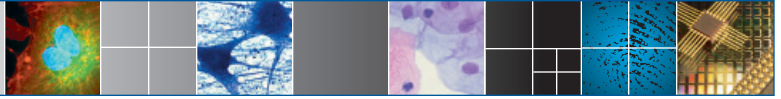
The first, second, and third terms of the denominator denote the effective photon (shot) noise, dark noise, and read noise, respectively, as a result of EM gain. Notice that the shot noise and dark noise are both increased by the excess noise factor, whereas the read noise is reduced by the EM gain factor.

Dual Amplifiers

Until now, one of the common limitations of cameras designed for low-light imaging was their inability to capture both bright and dim signals in the same frame (owing to a relatively narrow dynamic range).

Although low-light-level CCD cameras can be operated at unity gain for wide-dynamic-range applications, doing so does not match the dynamic-range capabilities of traditional CCDs. In CCDs with EM gain, this shortcoming stems from the fact that the readout amplifier (responsible for read noise) associated with the multiplication register is usually designed to run at higher speeds, resulting in higher read noise. While EM gain easily overcomes the elevated read noise, the dynamic range of the camera system suffers.

To preserve dynamic range, some CCD cameras with EM gain now feature a dual-amplifier design that incorporates a second, “traditional” amplifier for slower pixel readout. Thus, these high-performance EMCCD cameras can also be used for wide-dynamic-range applications like brightfield or fluorescence imaging (see **Figure 5**).



Back Illumination

Electron-multiplying gain is also being implemented in back-illuminated CCD architectures. As mentioned previously, back illumination offers greater than 90% QE, effectively compounding the sensitivity advantage provided by EMCCDs. This technology combination delivers the best available low-light-level sensitivity at fast frame rates. Some back-illuminated EMCCD cameras can be configured with dual amplifiers for broader application versatility.

When properly integrated in a high-performance camera platform, EMCCDs provide researchers an excellent choice for nongated, low-light-level applications that require video (or supravideo) frame rates and excellent spatial resolution. Examples of such applications are intracellular ion imaging, biological fluid flow measurements, and SMF imaging. When EMCCDs are deeply cooled, with EM gain sufficiently higher than the read noise and a low photon-arrival rate, even photon counting should be possible without image-intensifier hardware.

Technology Summary

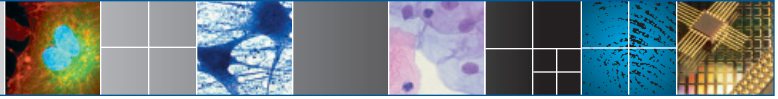
Making an Informed Choice

Much of the sensitivity advantage offered by traditional, cooled CCD cameras comes from their ability to integrate signal on the chip prior to readout and thereby only incur read noise once during measurement. Hence, for the long exposures required in many low-light-level applications, frame rates for these cameras are low.

However, because EM gain overcomes read noise, images can be acquired at faster frame rates with devices that feature this on-chip technology. Their electron-multiplying capability greatly improves the utility of EMCCDs for low-light-level work.

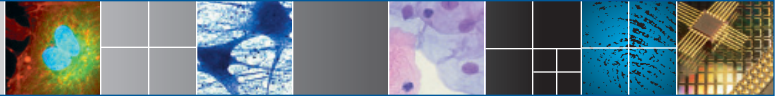
The net result is that devices with EM gain boast the sensitivity of intensified and electron-bombardment CCDs, but don't carry the risk of potential damage to external image-intensifier hardware. And because no photocathode or phosphor is involved, the spatial resolution provided is as high as that offered by traditional CCD imagers with the same array and pixel size.

The latest back-illuminated EMCCD cameras from QImaging feature dual amplifiers in order to ensure the highest level of performance not only for ultra-low-light imaging, but for wide-dynamic-range applications. Now, a single camera can be used for SMF and brightfield / fluorescence imaging.



Electron-Multiplying Gain			
Appendix			
<i>Derivation of Signal-to-Noise Ratio (for CCDs utilizing EM gain)</i>			
<i>Signal Calculation</i>			
1.	Number of incident photons at each pixel	S	
2.	Number of electrons generated at each pixel	S*QE	QE is the quantum efficiency at the wavelength of the photons.
3.	Number of electrons after the EM gain (S_{Total})	S*QE*G	G is the EM gain factor.
<i>Noise Calculation</i>			
4.	Photon (shot) noise	$G * F * \sqrt{S * QE}$	Incoming photons follow Poisson statistics and have an inherent noise called photon (shot) noise, which is given by the square root of the signal. In CCDs featuring EM gain, both the signal and the noise are multiplied by the gain factor (G). In addition, the shot noise is multiplied by the excess noise factor (F).
5.	Dark noise	$G * F * \sqrt{D}$	Total dark-related signal (D) includes dark charge and spurious charge. Similar to shot noise, dark noise is given by the square root of total dark-related signal (D). Since dark charge also goes through the multiplication process, both the EM gain and excess noise factors are applied.
6.	Read noise	σ_R	Since read noise occurs after EM gain, it is not affected by EM gain.
7.	Total system noise (σ_{Total})	$\sqrt{[(G^2 * F^2 * S * QE) + (G^2 * F^2 * D) + \sigma_R^2]}$	To derive the total system noise (σ_{Total}), the individual noise components in (4) , (5) , and (6) are added in quadrature (i.e., square the individual components, add, and take a square root of the total).
<i>Signal-to-Noise Ratio</i>			
	$SNR (S_{Total} / \sigma_{Total})$	$S * QE * G / \sqrt{[(G^2 * F^2 * S * QE) + (G^2 * F^2 * D) + \sigma_R^2]}$ $= (S * QE) / \sqrt{[(S * QE * F^2) + (D * F^2) + (\sigma_R / G)^2]}$	(3) / (7) Divide the numerator and denominator by G.

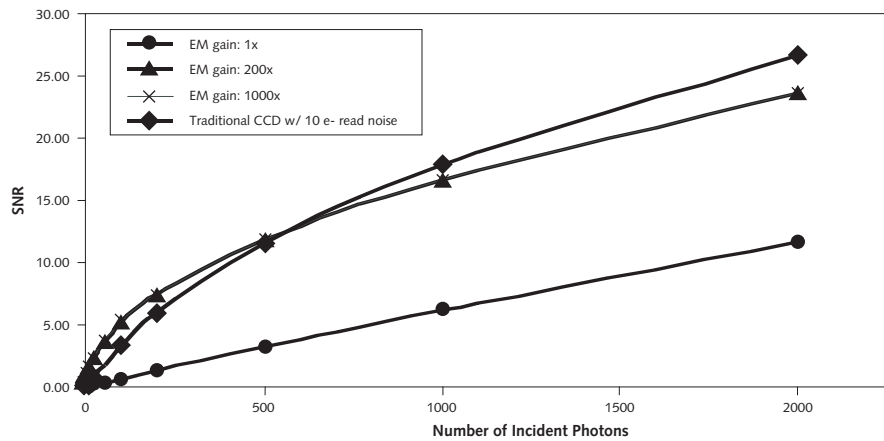
The first and second terms in the denominator of the final equation show that the shot noise and the dark noise are increased due to the excess noise of the electron-multiplying process, whereas the third term (read noise) is effectively reduced by the EM gain factor.



SNR Calculation

The following example illustrates the effect of EM gain on the overall system SNR for various incident-signal levels (i.e., for various numbers of incident photons).

By changing the QE in this example to 90% (or greater), it's easy to see that a back-illuminated version of an electron-multiplying CCD would yield even higher SNR.



Camera parameters used for this calculation:

Quantum efficiency

@ 600 nm (QE) = 40%

Read noise (σ_R) = 60 e- rms

Exposure time = 33 msec
(30 frames/sec)

Dark charge

(dependent on exposure time) = 1 e-/pixel/sec @ -30°C
(0.033 e-/pixel/frame)

Spurious charge = 0.1 e-/pixel/frame

Total dark-related

signal (D) = 0.133 e-/pixel/frame

Excess noise

factor (F) = 1.2

The signal-to-noise ratio at each signal level has been computed based on the equation derived earlier and then plotted in the graph. For comparison purposes, the SNR obtained with a similar — but traditional — slow-scan CCD is also presented.

The data indicates:

- CCDs with EM gain offer the greatest advantage at low light levels where the read noise of the CCD is the dominant factor (i.e., in the read-noise-dominant regime).
- EM gain is useful only up to the point of overcoming the read noise. In this particular example, there is very little difference between SNR performance at 200x and 1000x.
- Traditional slow-scan CCDs with sufficiently low read noise achieve better SNR in the shot-noise-dominant regime (i.e., at higher light levels). Thus, there is a distinct advantage in having a single camera with two readout amplifiers — one (EM gain) designed for ultra-low-light imaging and another (traditional) that offers better support for wide-dynamic-range applications.

References

Conference Proceedings

J. Hyneczek and T. Nishiwaki, "Excess noise and other important characteristics of low light level imaging using charge multiplying CCDs," IEEE Trans. Electron Devices, vol. 50, no. 1, pp. 239-245, Jan. 2003.

M. S. Robbins and B. J. Hadwen, "The noise performance of electron multiplying charge coupled devices," IEEE Trans. Electron Devices, T-ED Manuscript #1488R, received Dec. 2002.

Corporate Publications

The Use of Multiplication Gain in L3Vision™ CCD Sensors (Sep. 2002). A1A-Low-Light Technical Note 2, Issue 2, E2V Technologies Limited, 106 Waterhouse Lane, Chelmsford, Essex CM1 2QU, England.

Introduction to Image Intensifiers for Scientific Imaging (2000, 2002). Technical Note #11, Roper Scientific, Inc., 3440 East Britannia Drive, Tucson, AZ 85706.

Keep the Noise Down! Low Noise: An Integral Part of High-Performance CCD (HCCD) Camera Systems (1999). Technical Note #4, Roper Scientific, Inc., 3440 East Britannia Drive, Tucson, AZ 85706.