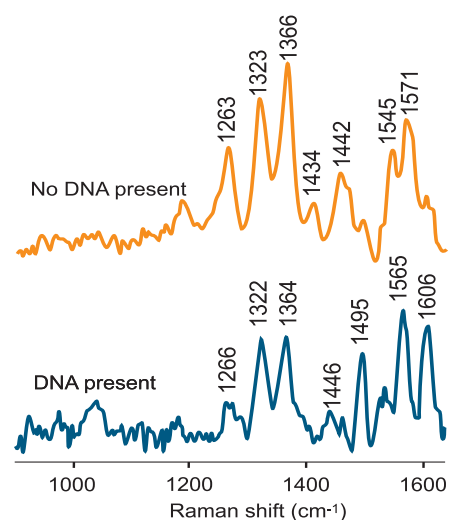
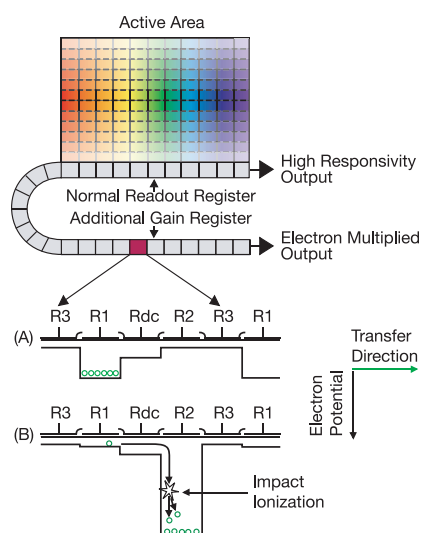


spectroscopy

Newton EMCCD

A New Approach to Spectroscopy



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World's First Electron Multiplying (EMCCD) Camera for Spectroscopy!



The Newton^{EM} delivers the highest sensitivities possible for any CCD camera, offering up to 95% QE and single photon detection. With multi-megahertz readout rates, deep thermoelectric cooling and negligible dark noise, it provides the fastest spectral readout rates for quantitative spectroscopy.

It is pushing back the frontier of conventional CCD spectroscopy and opening up a whole new range of application areas.

The Newton^{EM} is effectively two cameras combined into one package: an EMCCD and a conventional high speed CCD camera.

Sensitivity: single photon sensitive, with 1000X EM gain!

Noise: <1 electron, with minimal dark charge & spurious charge.

Cooling to -100°C

Ultra-Fast Spectral Rate: 600 sp/sec (1300 sp/sec in crop mode)

Digitization: 16 bits

Quantum Efficiency: >95%

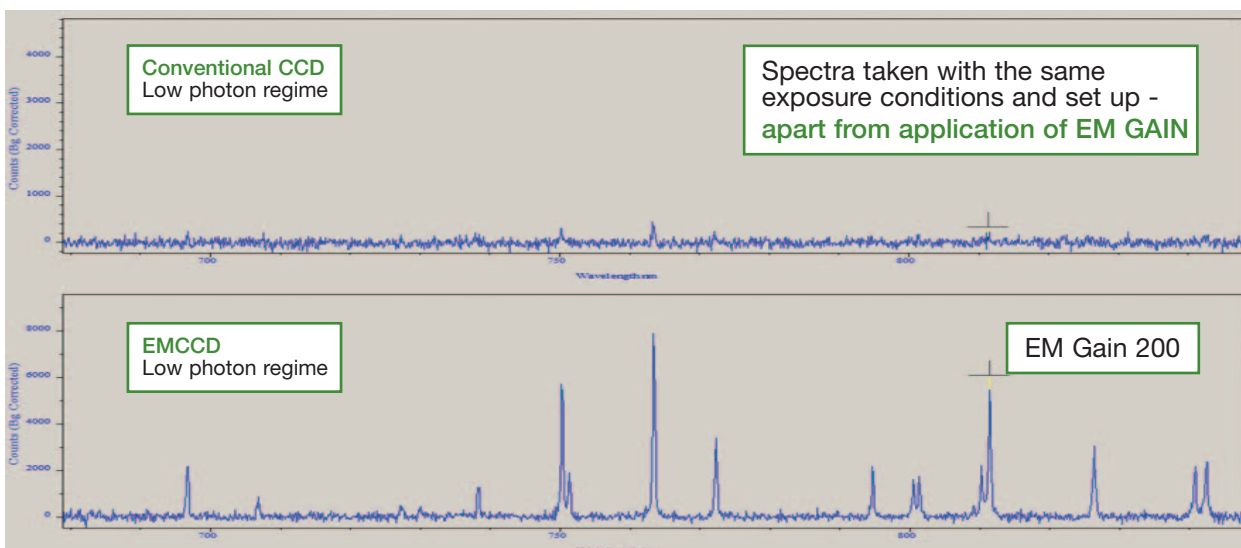
USB 2.0 Connectivity

Dual Output Amplifiers

Guaranteed Hermetic Vacuum Seal Technology

Versatility: Two Cameras in One

The Newton^{EM} camera comes with dual output amplifiers: conventional CCD register and EM gain register. Therefore the Newton^{EM} is really two cameras in one - a conventional CCD and an EMCCD combined and it is easy to switch between the two via the software.



Overview



- The Newton series of EMCCD cameras from Andor Technology offer best in class performance among high-end multi-channel spectroscopy detectors.
- The Newton^{EM} draws and builds upon the innovative EM technology introduced by Andor in 2001 and the significant system developments incorporated since then.
- It offers the ultimate performance in terms of sensitivity and quantitative measurement.
- Innovations include baseline clamping, a method for achieving the lowest clock-induced-charge, and a range of versatile triggering configurations to suit a wide and diverse range of experimental setups.
- These cameras are now being used all over the world in a multitude of applications, where the Newton^{EM} has extended traditional applications into another regime of detectivity and opened up the door to whole new areas of investigation previously inaccessible to conventional CCD technology.
- EMCCD technology is particularly suited to those applications requiring high sensitivity combined with high speed.

The Newton^{EM} Family

The Newton^{EM} comes in two EMCCD models depending on the size of sensor, the DU970N and DU971N. A range of different sensor types, including both front illuminated (FI) and back illuminated (BI), are available depending on the wavelength region of interest and where the highest quantum efficiency (QE) is desirable. The table below summarizes some details of the models available.

Parameter	DU970N	DU971N
Sensor format / Active pixels	1600x200	1600x400
Sensor size, mm	25.6 x 3.2	25.6 x 6.4
Pixel size	16 μ m x 16 μ m	
Sensor types	BV, FI, UVB, UV	
Min. temp.	-100 °C	
Pixel readout rate	2.5MHz, 1.0MHz, 50kHz	
Vertical shift speeds (VSS)	4.95 μ s - 38.6 μ s	
PC interface	USB 2.0	
Digitization	16 bit	

Newton^{EM} Application Areas

- Raman spectroscopy
- Micro-spectroscopy
- Micro-Raman Spectroscopy
- Multi-spectral Imaging
- Hyper-spectral Imaging
- Fast Reaction Processes
- Fluorescence Resonance Energy Transfer
- Single Molecule Detection
- Transient (Pump-Probe) Spectroscopy
- Nano-dot Photoluminescent Spectroscopy
- Organic Luminescence
- Multi-track Spectroscopy
- Atomic Emission Spectroscopy
- NIR spectroscopy
- Absorption / Transmittance / Reflection Spectroscopy

The full benefit of having EM Gain is to be seen in the low photon signal regime...

A low signal may arise due to the following scenarios:

- The experiment gives intrinsically low signals - e.g. single molecule detection or FRET studies
- To facilitate the fastest spectral rates for fine time resolution in fast processes, where the shortest exposures and fastest spectral rates are desirable
- To facilitate low excitation powers of samples in order to reduce damage and prolong sample lifetime - such as studies of photolabile organic and inorganic samples
- To cope with multi-track spectroscopy where many tracks are needed such that few pixel rows are available to capture any given spectrum
- To facilitate spectral mapping of samples thus giving spectral images of samples - e.g. Raman spectral imaging of live-cells

- Provision of High Dynamic Range through accumulations
- Ensuring visibility of very weak signals during alignment and focusing optimization
- Excited state studies (pump-probe), where probe interactions need to be kept very low to minimize population of excited state

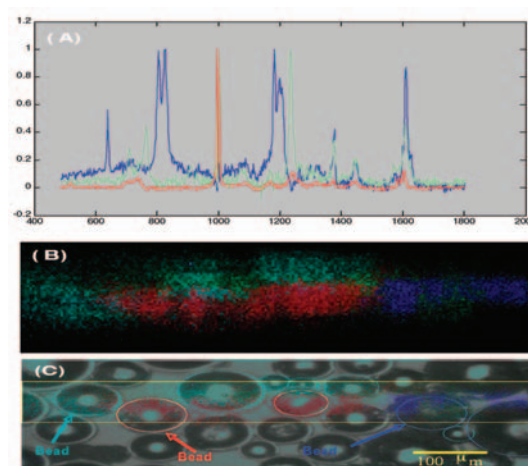


Figure 1 Raman spectral imaging of polystyrene micro-beads as used in bio-chemistry studies^[8]. The Newton^{EM} gave faster data acquisition and image scanning. Beads of three diameters were used. Top - Raman spectra, Middle - Raman spectral image, Bottom - image of sample showing individual beads.

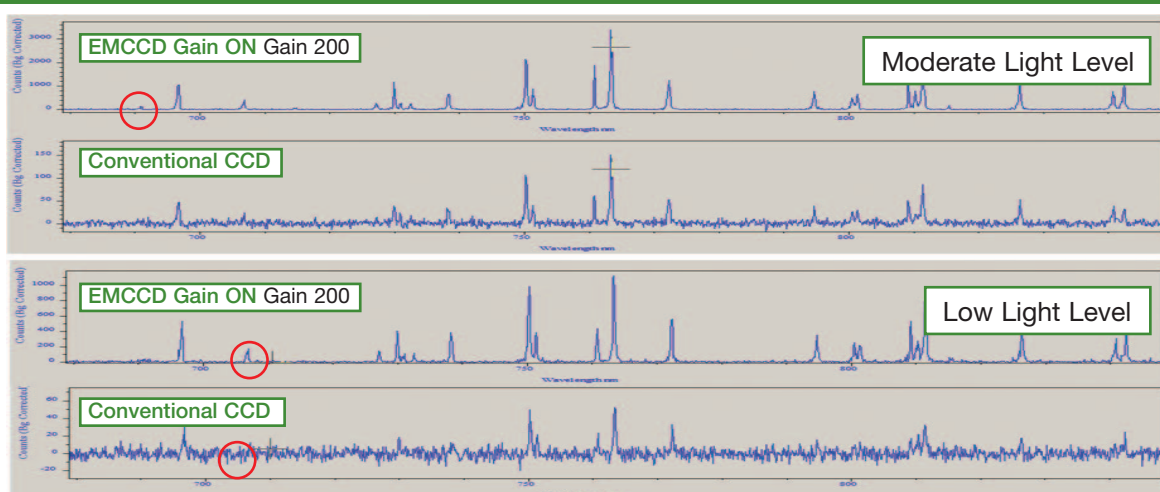
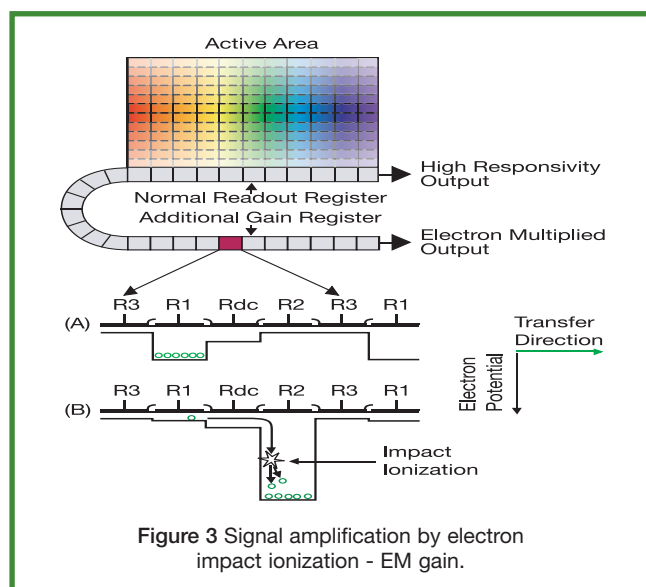


Figure 2 Comparison of EM Gain with conventional CCD for moderate and low light levels. Clearly even the weakest spectral features can be observed with the application of EM gain as shown for the red circles. Each data set, moderate and low, were taken under the same setup conditions apart from the amplifier used - EM or conventional. This data was captured for a Newton DU971N-BV camera on a Shamrock SR500 spectrograph using single scans with full vertical binning (FVB). (Low light conditions were achieved with an ND filter)

Key Performance Features

1. Sensitivity

The Newton EMCCD uses the latest EM technology to enhance the signal/noise for measurements in low signal level or photon starved experimental setups. The basic principles behind this technology are illustrated in the figure below.



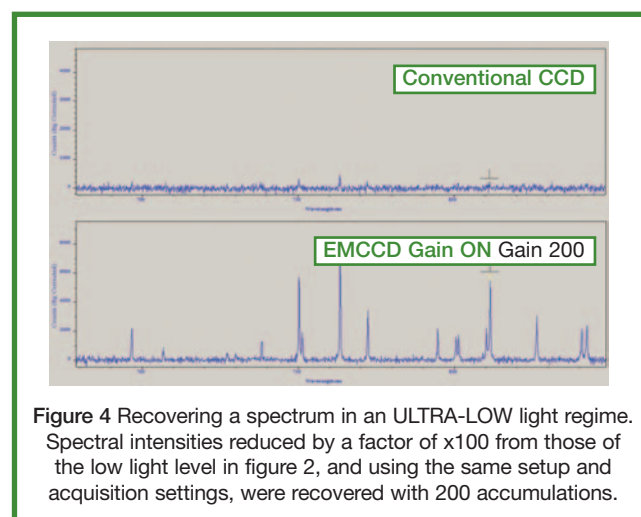
There are two readout registers on the chip - a conventional register and an EM register. The clocking voltages used on the EM register are higher than for conventional clocking. These higher voltages cause the electrons to acquire sufficient energy that impact ionization can occur where extra electrons are produced and stored in the next pixel. There is a small probability of electrons acquiring sufficient energy to create additional electrons but with many elements within the readout register significant gains of up to factors of ~1000x are possible - hence Electron Multiplication (EM).

The key benefit here is that the gain register amplifies signal before readout to ensure the signal is not readout noise limited i.e. the signal is raised well above the noise floor which is largely determined by the readout noise of the readout electronics (pre-amplifier and A/D converter).

The following examples illustrate the power of EM gain when measuring moderate to weak spectra. In figure 2, spectra taken at moderate and low signal levels are compared for a conventional CCD mode and an EM-CCD mode of acquisition using a Newton DU971N_BV camera. The same set up conditions were used with just a difference in exposure times to distinguish the moderate from low light regimes. For the EM acquisitions the EM gain was set to 200. Within each signal level regime, the only difference was whether a conventional mode or EM gain mode was used.

Clearly the improvement in S/N is striking in both cases with the use of EM gain. The weakest spectral features stand out clearly when EM gain is applied compared with the conventional CCD mode. In the low light case with the conventional CCD, only the strongest spectral features can be discerned; the weakest features are lost in the background noise.

As a further illustration of the power of the EM-CCD, an ND filter was introduced to reduce the low light signal level of that shown in figure 2 by a factor of x100. With the same exposure time and settings, no spectral features were clearly discernible in either the conventional or EM mode (with gain) for a single scan acquisition. However, using accumulations of multiple scans allows the recovery of the signal as illustrated in figure 4.

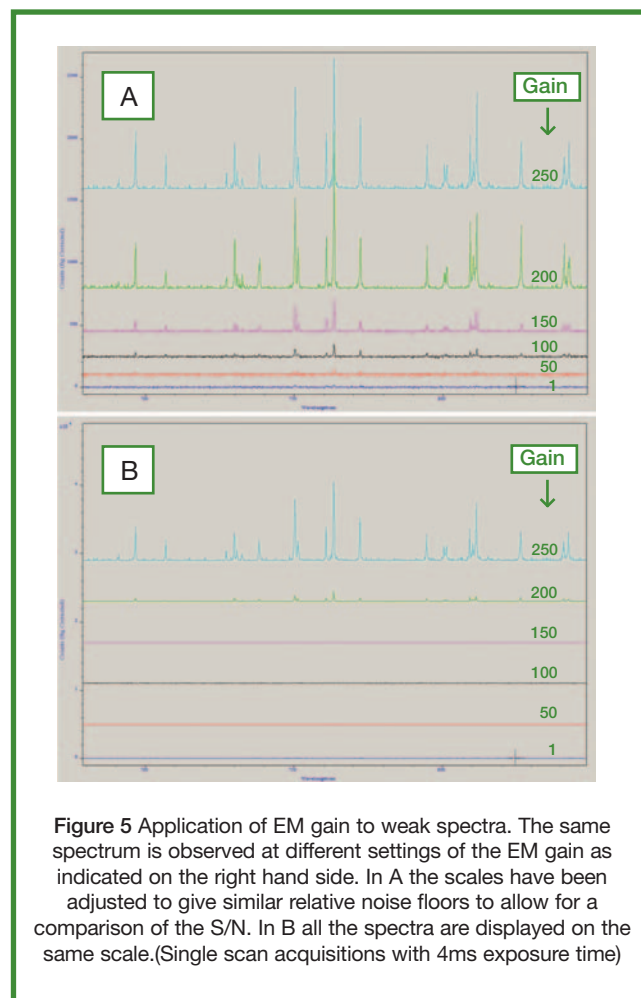


In this ultra-low light regime EM gain can recover successfully the spectral signal with a good S/N ratio, unlike the conventional CCD where only a few of the stronger features are barely observable.

The benefits of the EM gain are clearly evident, where the low spectral signal has been lifted above the read noise floor, resulting in a meaningful measurement, whilst for the conventional CCD the signal has by and large remained buried in the noise. Such performance is particularly relevant to those types of applications where:

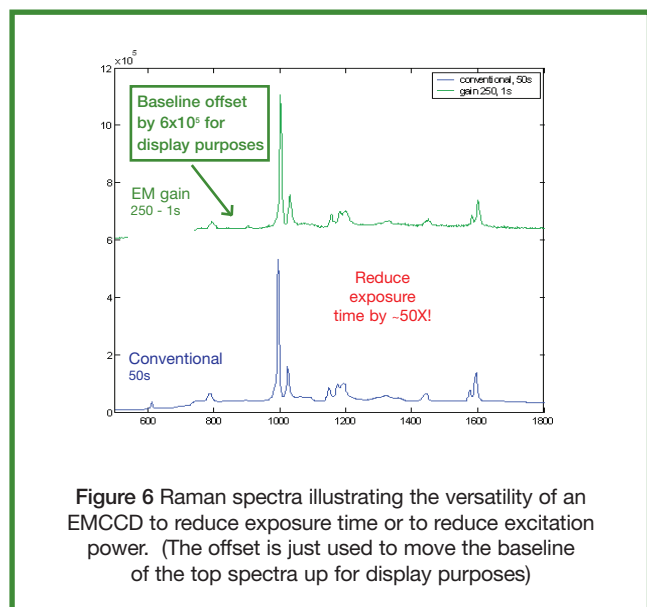
- Pulsed spectroscopy is used with low signal levels and multiple scans are needed to achieve a satisfactory S/N; data may be collected by accumulations or using a long exposure time and using integration-on-chip (IOC).
- Where long exposures are required to deal with very weak samples, but it is desirable to see the signal build up at intervals to ensure validity of data. With the EMCCD, this means this can be achieved without summing together multiple read noise floors.
- Dynamic range is critical and needs to cope with very strong and very weak spectral features simultaneously, therefore multiple accumulations are required.
- Low excitation energies are required to prolong sample life or avoid sample damage; in cases thermal damage can occur with high fluences and/or there is sufficient time for heat dissipation. Unlike conventional CCDs, the EM-CCD affords the versatility to make measurements when forced to operate in such a regime.

The application of EM gain can pull the weak spectra out of the read noise floor as illustrated in the figure below where the value of the EM gain is increased in steps.

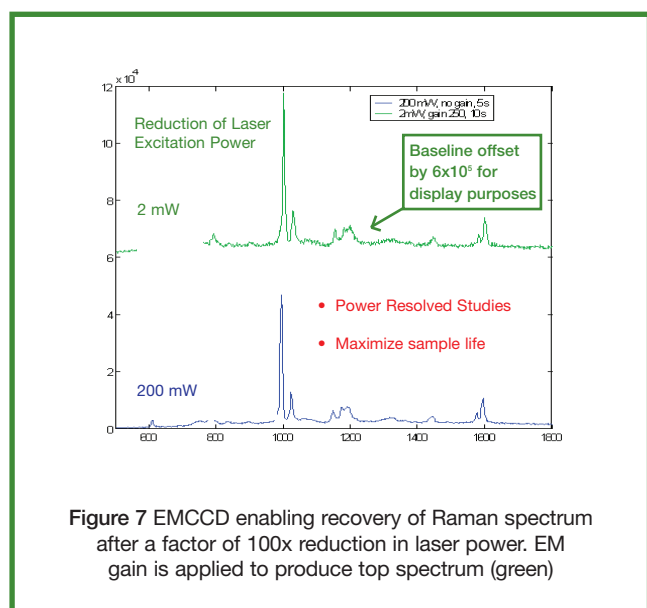


It is clear from figure 5(A) that there is steady improvement in the S/N with increased gain until a gain around 200 is reached. Increasing the gain beyond this shows no significant improvement as evidenced for the gain of 250. This points to one characteristic when using EM gain in practice - the gain should be increased to an optimum operating point (corresponding to when the effective readout noise <1 electron) and beyond which no further advantage is to be achieved. Operating this way will maximize the available dynamic range per scan.

Raman spectra taken from micro-beads, as used in biochemistry investigations, are shown in figure 6 below illustrating the potential of EM gain to facilitate reduced exposure times or reduce laser power.



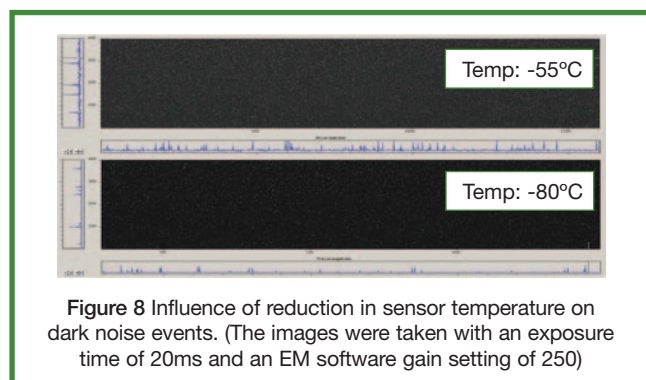
The potential to use lower excitation powers is illustrated in figure 7 below where Raman spectra are compared with different excitation powers and EM gain is used to recover the signal.



2. Deep Cooling: Minimizing dark current

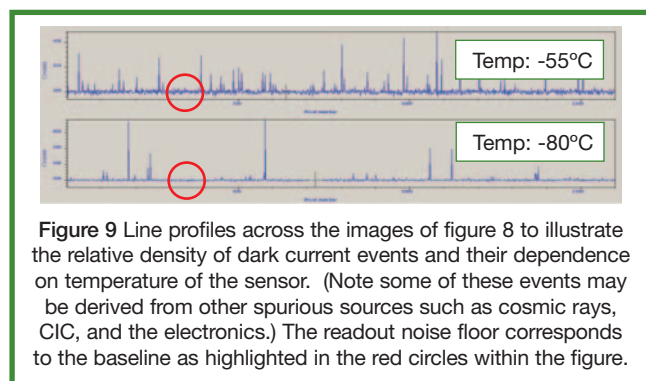
To access the full potential that EM technology can provide in terms of detectivity and dynamic range, it is of extreme importance to eliminate or minimize the various contributions to the overall noise captured within any acquisition of a signal. The main contributing sources of noise are the shot noise of the signal, the dark current of the sensor, spurious noise such as clock induced charge (CIC), and the readout noise from the output electronics. Shot noise within the photon signal is an intrinsic contribution related to fundamental quantum physics, and will always be part of any signal. It represents a fundamental limit for any noise reduction. The other sources of noise can be reduced and Andor uses a range of innovative techniques to minimize or eliminate their contributions.

Deep cooling is provided with Andor's innovative hermetically sealed permanent vacuum housing and 4-stage TE cooler. Temperatures as low as -100°C are possible. With deep cooling, the contribution from dark current can be reduced to insignificant levels relative to the readout noise. It becomes even more critical and beneficial to be able to deep cool the sensor when operating an EMCCD compared with a standard CCD, the reason being that the dark current events are amplified along with the main photon generated signal in the readout process. The reduction of this source of noise is particularly pertinent when long exposure times are needed. The images in figure 8 were taken with an EM software gain setting of 250.



This ensures that dark current events will be exposed as sharp spikes particularly at the lower temperatures where EM gain is highest. They were also taken with settings that minimized the possibility of CIC events (discussed later).

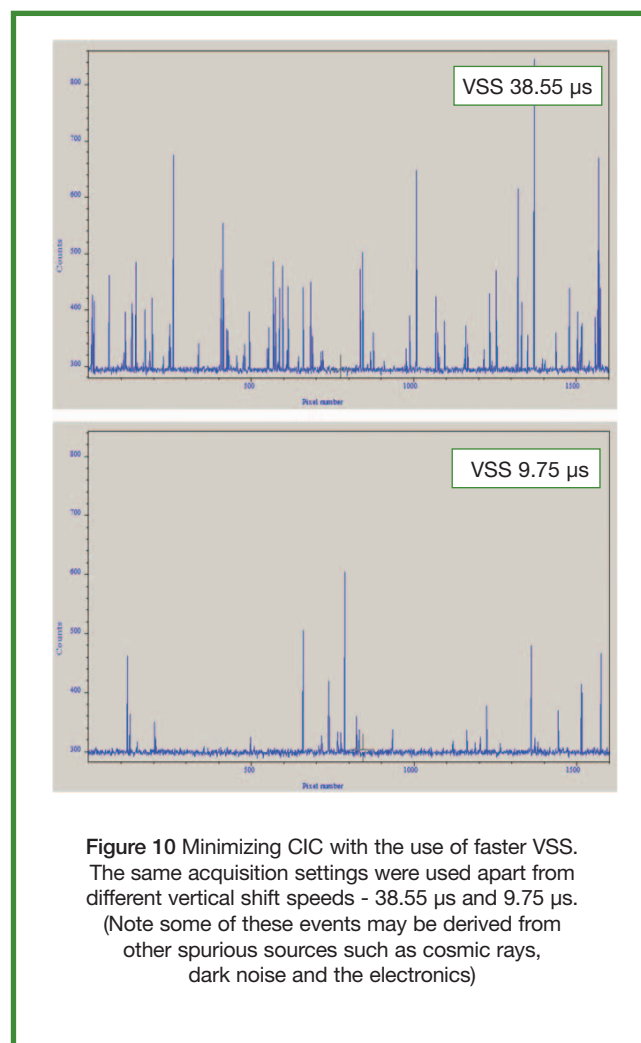
Clearly there is a striking reduction in dark current events with reduction in temperature. Cooling deeper to below -95°C reduces the dark current contribution to be insignificant compared with CIC and readout noise. Line profiles taken across the images are shown in figure 9 below.



In the deep cooling regime as evident in the bottom trace, the detection limit of the system is set by these background events - showing up as spikes here - and not the readout noise floor. This means the EM gain can amplify even the weakest signal to well above the read noise floor. Clearly if one electron events can be amplified to form these spikes, it is easy to see how one photon producing a 'one-electron' event will result in a clearly visible spike. So the key to the optimization for such ultra-sensitivity is to minimize or eliminate these background events. Deep cooling is one part of the answer but the other is to reduce CIC events.

3. Minimizing Clock Induced Charge (CIC)

Andor has addressed this spurious noise source by developing fine tuned and very well controlled clocking voltages. In particular fine control over clocking edges down to nanosecond resolutions have been implemented. There is a close relationship between vertical clock speeds and the probability of CIC events. Possibly contrary to expectations, the faster the vertical shift speed the lower the occurrence of CIC spurious events. Figure 9 below shows data for line profiles taken through two dark images with the shortest of exposure times to minimize the contribution of dark current thermal events. The EM gain was set at 250. So the only significant parameter of difference was the vertical shift speed (VSS). The difference can be clearly seen and the reduction of CIC events with increase in VSS.



4. Speed of spectral acquisitions

The speed at which the data can be read from the sensor depends on two key parameters of the sensor functions, a) the pixel readout rate (readout register) and b) the vertical shift speed (VSS), and whether binning is being used or not. Sensors for spectroscopy are designed to be long and narrow. More often than not full vertical binning (FVB) mode is used, to ensure the capture of individual spectra at the highest possible rates. The pixel readout rate is the speed at which charge can be shunted out through the EM gain register and the following readout electronics (preamp, A/D). The Newton offers three readout speeds (Table 1), the fastest being 2.5MHz. The VSS is the rate at which charge is shifted vertically down the rows of the sensor. It is given here as the time in μs to make one shift from row to row: shift speeds of $4.95\mu\text{s}$ to $38.6\mu\text{s}$ are available. Binning is available in a range of custom options which are readily selectable through the software, as well as the classic spectroscopy mode of full vertical binning. FVB is where all the rows of 'pixel charge packets' are shifted down into the readout register where their individual charges are integrated or summed before being read out through the gain register. Table 2 below summarizes the fastest spectral speeds available, including that for full frame readout (imaging mode) where no binning occurs. They have been taken with the fastest VSS ($4.95\mu\text{s}$) at 2.5MHz readout rate and with the shortest exposure times. The table also gives spectral acquisition rates using crop mode; crop mode allows for very high spectral acquisition rates.

The Newton has the capability to allow the sensor to be effectively cropped in size to a narrow sensor requiring fewer vertical row shifts to acquire a given spectrum. Figure 11 below gives an example of the use of crop mode. A spectrum has been aligned along the bottom rows of the sensor so that all the signal falls in the bottom twenty rows and it is ensured that NO signal falls on the rest of the sensor. In the acquisition process, the sensor is treated as one of just 20 rows in height. These are read out in FVB mode, i.e. the 20 rows are integrated in the readout register before reading out. This is a very powerful feature for applications requiring high speeds of acquisition but it is of critical importance that no signal falls on the rest of the sensor as this would corrupt successive spectra. In the bottom of figure 11(C), a 3D view of the line profiles across the sensor show that light only falls on the bottom rows of the sensor.

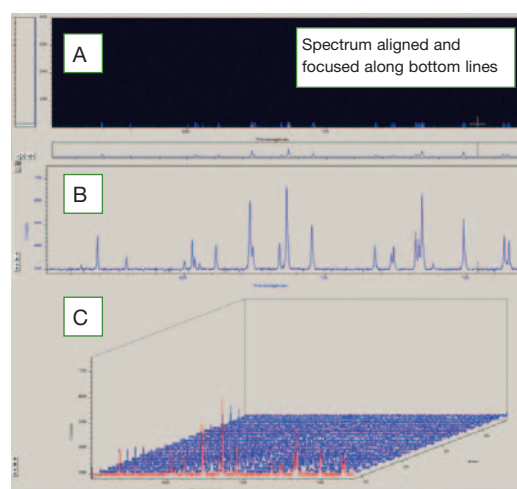
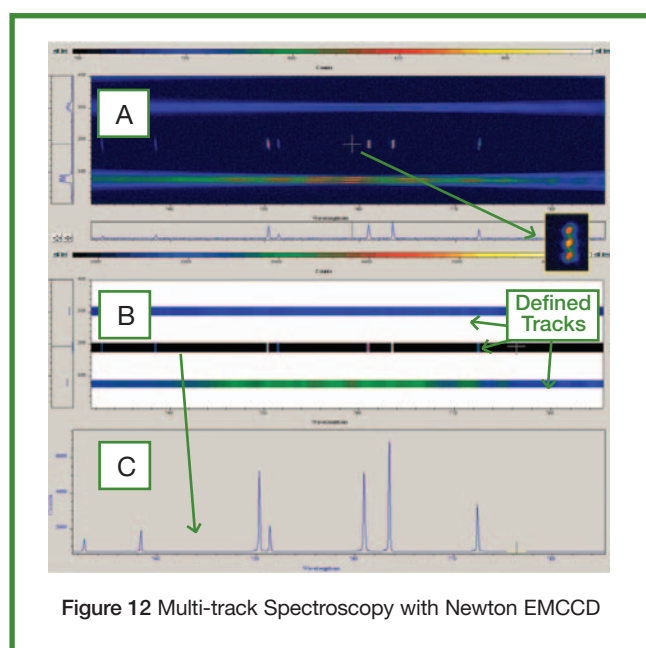


Figure 11 Illustration of the use of crop mode with 20 rows to achieve the fastest spectral acquisition rates. The spectral profile is shown centre and the 3D image at the bottom (C) shows that no light falls on the rest of the sensor.

Camera Model	DU970N		DU971N	
Mode of Operation	Conv.	EM	Conv.	EM
Max. spectra per sec (FVB)	602	606	377	379
Max. spectra per sec with Crop mode (20 rows)	1316	1316	1316	1316
Max. full frames per sec	7.3	7.3	3.6	3.6

5. Multi-track Spectroscopy

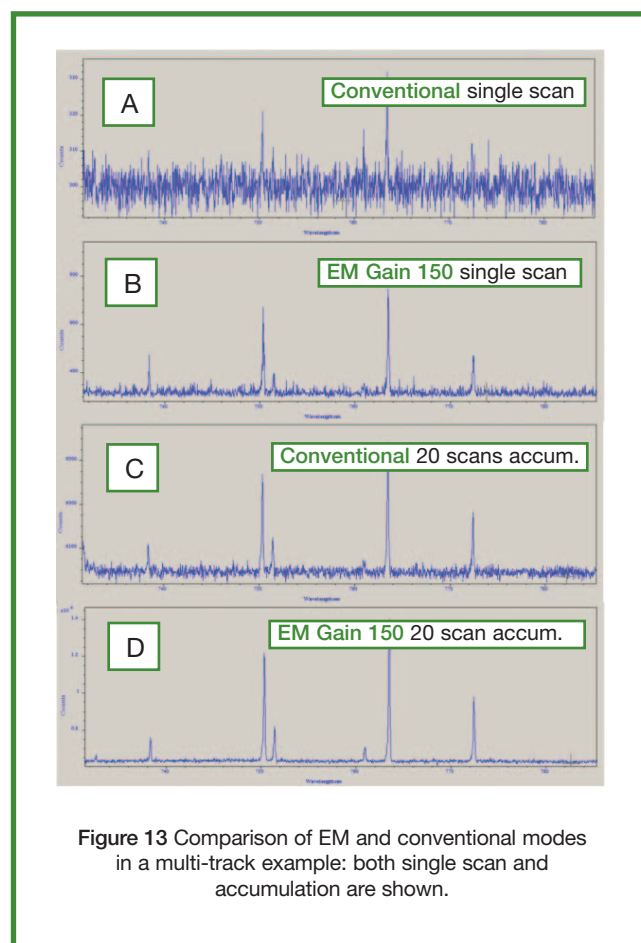
In some applications it is desirable to capture several independent spectra at the same time on the same sensor. Typically the spectra are captured as tracks on the sensor in the spatial/vertical dimension and the signals are coupled into the spectrograph via a multi-channel fibre. Many tracks can be catered for on the DU970N and DU971N models of the Newton with their sensor heights of 3.2 and 6.4mm respectively.



Multi-track spectroscopy is usually carried out on imaging spectrographs such as the Shamrock 303i. Figure 12 illustrates the use of three spectral tracks across the DU971N. The top and bottom spectra were illuminated from a broad band source and the middle was illuminated from a standard HgAr lamp. Each channel was formed with three 100 μ m core fibres as can be seen in the inset on right hand side of the figure. It can be seen, depending on the design of the multi-channel fibre, that it would be possible to have as many as twenty channels on this sensor. The middle part of the figure shows how the sensor is defined into individual tracks to enhance the speed of spectral acquisitions and optimized binning. The lower part is just the spectral profile resulting from the HgAr track in the centre.

The problem ultimately with using narrower tracks and more of them is the sensitivity required for the resultant weaker signals. This is where EM technology comes to the aid of multi-track spectroscopy. The EM gain allows for the weaker signals to be captured with reasonable S/N, and the fast speed of the Newton maintains fast spectral rates. Dynamic range may also be an issue with multiple spectra with some being strong and some weak: the larger capacity within the EM readout register pixels offers an advantage here as well.

Figure 13 gives a comparison of conventional CCD versus EM-CCD modes. The middle track spectrum of figure 11 was set up in a low photon regime using an ND filter to reduce signal level by a factor of $\sim 150\times$.



It is clear that EM can offer significant advantage in those multi-track applications where the signals are too weak for a conventional CCD.

6. Stability and Gain Characteristics

EMCCD cameras are susceptible to a number of factors which can impact on data acquisition stability and consequently on the accuracy of quantitative measurements. It is of critical importance that these are addressed to ensure reliable quantitative capture and analysis of data. The two main tools in the Newton platform to ensure stability are baseline clamping and optimized temperature stability.

Baseline Clamping

A baseline or bias level is an applied DC offset added to the output signal from the EMCCD sensor when it is being readout through the readout electronics (pre-amp and A/D) to ensure the displayed signal is always a positive number of counts and within a stable regime of the output amplifier. It is important to note that this is a DC offset and does not add any noise to the final measured signal. Therefore it does not affect sensitivity. If the data acquisition type is selected with background correction, then this offset can be automatically removed in the final display of the data. It must also be remembered that the baseline needs subtracted from a raw signal in counts (not background corrected) when performing signal to noise calculations. In practice small changes may occur in the temperature of the read out electronics and sensor which can lead to drift in the baseline level. This would be particularly significant if long exposure times or long kinetic series were being used and would lead to variation in the apparent signal being collected even for an ideal uniform source. Baseline clamping corrects each individual acquisition for drift. It monitors for each acquisition an average baseline which it uses to correct for drift, thereby ensuring a uniform effective baseline is used for all acquisitions. The baseline clamp feature can be selected in the software as ON or OFF. Figure 14 illustrates the importance of baseline clamping, showing the drift when it is off and how it is held steady with it on.

It is worth noting that the baseline is susceptible to variation when EM gain is

used. The Newton takes care of this in any case, ensuring the bias level is clamped irrespective of the gain being used.

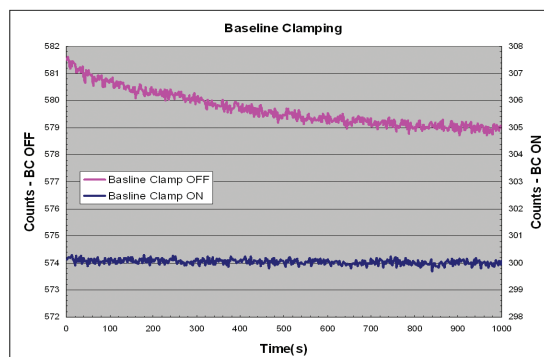


Figure 14 Comparison of Baseline clamping (BC) when on and off.

Gain stability

An interesting characteristic of EM gain is its sensitivity to temperature. The gain increases as the temperature of the sensor decreases, so little or no gain may be observed at -20°C and yet real gains around 1000 may be observed around -80°C . Figure 15 below shows typical characteristics for an EMCCD camera. The significant sensitivity and dependence of gain on both temperature and the EM clocking voltage is evident.

Clearly, if gain is temperature sensitive, it is of the utmost importance that it remains stable across data acquisitions particularly if data is being collected over relative long time scales. Andor provides optimized temperature stability regulation on the Newton platform, which maintains temperature variation within a fraction of one degree.

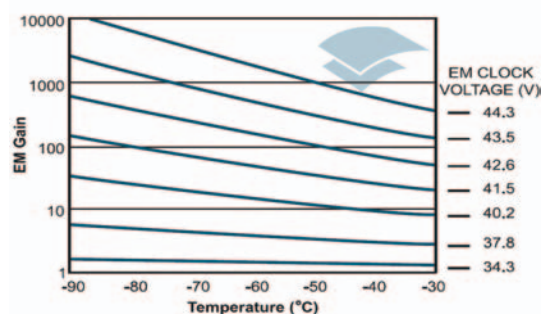
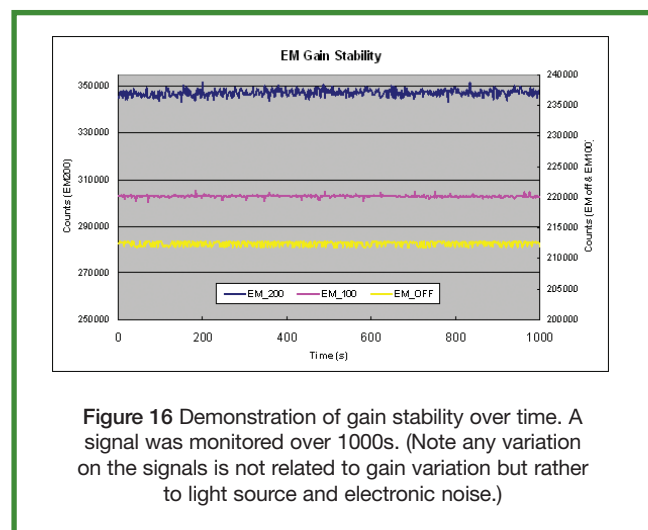


Figure 15 EM gain dependence on clocking voltage and temperature of the sensor.

The stability of the gain over a long period of time is illustrated in figure 16 below where time series are shown for a number of gains over an extensive period of time. The temperature was stabilised at -70°C and the baseline was clamped.



Ageing Effects

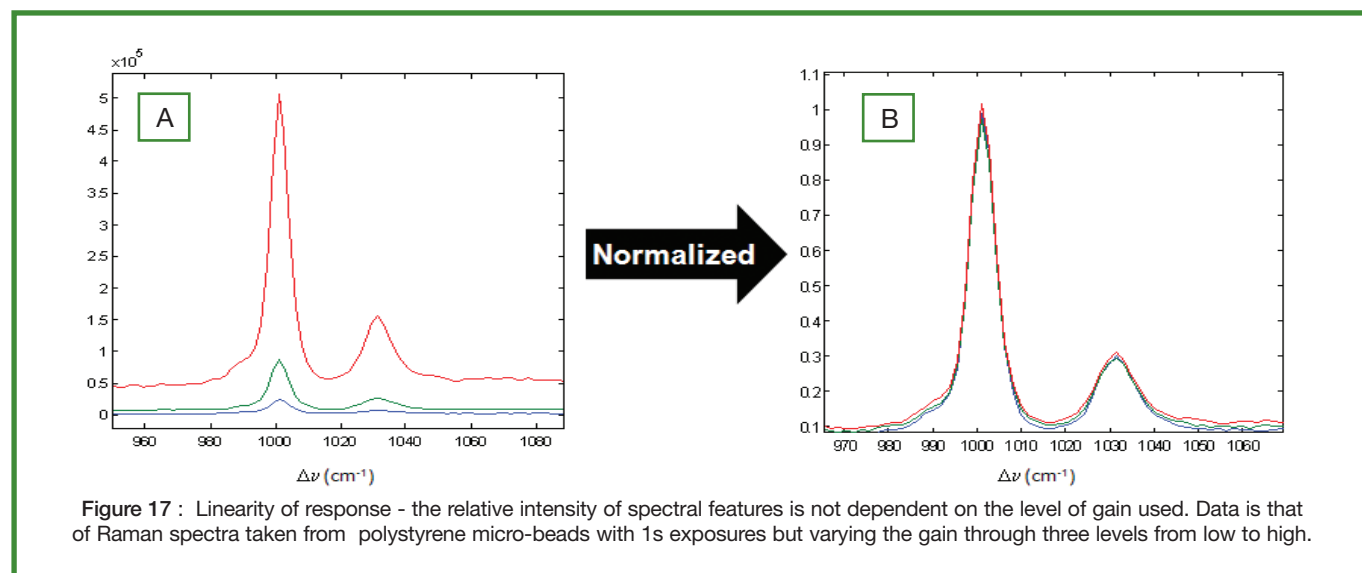
EMCCD cameras that incorporate L3VisionTM from E2V (Chelmsford, UK), tend to suffer from ageing effects over a prolonged period of time, where the effective gain tends to fall off. Ageing is related to the amount of charge run through the gain register. This fall-off in gain can be recovered by small adjustments in the EM clocking voltage. In most spectroscopy applications absolute calibrations of the actual gain are not needed, but if one is desired a calibration can be readily carried out with a stable source.

By careful and optimized operation of an EMCCD Newton, its lifetime can be prolonged for many years of quantitative use. For example running at excessive gain with moderate to strong light signals does speed the rate of degradation in gain. However, this is often to no advantage in terms of the optimized operating point for the best S/N measurements. As a rule, EM gain should be only used to recover weak photon signals and the level of gain used should be just sufficient to reduce the 'effective noise floor' to its lower limit (this usually corresponds to the reduction of the 'effective readout noise' to a level of 1 electron). Increasing the gain beyond this point offers no advantage.

Linearity of Response

This is a key question as it is important that, for a given gain setting, the response is not dependent on the level of signal captured - i.e. there is a linear response in amplification irrespective of signal level for a given gain setting. The linearity of the Newton EM for several different gain settings from low to high is illustrated in figure 17. The ratioed intensity of the spectral features remains constant irrespective of the gain used as can be seen from the normalized data in 17B.

This capability is of extreme importance, in those experiments where the same features may be getting analysed but the level of signals vary drastically between measurements requiring optimisation of data acquisition by adjustment of EM gain.



7. Dynamic Range

Dynamic range (DR) is related to the ability to measure very weak and very strong signals simultaneously, and for CCD detectors is usually taken as

$$DR = \frac{\text{Full Well Capacity}}{\text{Detection Limit}}$$

In optimized operation conditions, the detection limit is essentially determined by the readout noise. However, it is more complicated when estimating the dynamic range for EMCCDs, where the EM gain and various means of saturation have to be taken into account. Saturation can occur in three places, a) the pixel itself when the well capacity becomes full, b) the readout register pixels, and c) the A/D electronics. To facilitate high DR, the Newton EM sensors are designed with readout register pixels/elements with much higher capacities than for the conventional registers in order to cope with the amplification in electron charge packets. Figure 18 illustrates the dynamic range characteristics for the Newton DU970N camera.

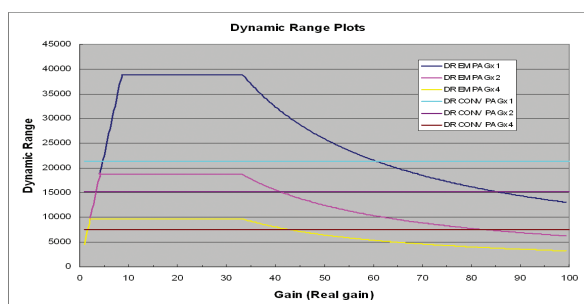


Figure 18 Dynamic range plots for Newton DU970N for single pixel configurations at 1MHz readout rate. Plots are included for different settings of the PAG.

As can be seen the dynamic range available when using EM gain depends on the operating point chosen. It also worth noting here, that the Newton offers analogue amplification at the pre-amplifier of the readout electronics. Pre-amplifier gains (PAG) of x1, x2 and x4 are available which can also be adjusted to switch between higher sensitivity and higher DR operation points.

Detection Limit and EM gain

The main function of EM gain is to eliminate the read noise detection limit. When EM gain is applied, the detection limit can be reduced to an 'Effective Read Noise' equal to the read noise divided by the gain. This can be reduced to the ultimate limit of one electron which corresponds to a signal discrimination of one photon i.e. you either have a photon or you don't but cannot have a fraction of a photon. The detection limit can not or should not be taken as less than one electron. Thus the DR is observed to increase as gain is increased until a limiting plateau corresponding to saturation occurs - either in the pixel elements or A/D electronics. As the gain is increased the detection limit eventually reaches its ultimate point limit.

At higher gains beyond this point, DR falls off as the effective capacity of the pixel is reduced with the continued amplification of the signal. What is clear is that, with the versatility of the EM Newton, one can switch between various configurations and maintain a good DR whilst still being able to measure the weakest signals.

8. Signal to Noise

The signal to noise ratio (S/N) is the key measurement factor in determining when EM gain will offer its advantages over the conventional CCD. There are four types of noise to be considered when considering the optimum S/N performance of any camera, those associated with the camera - dark current (N_{DN}), spurious or CIC (N_{CIC}) and readout noise (N_{RN}), and that associated with the signal itself - shot noise (N_{SN}). Figure 19 below shows noise characteristics for the Newton^{EM}.

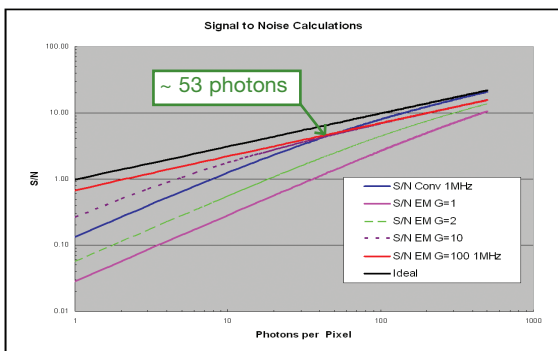


Figure 19 : S/N ratios for a DU970N Newton^{EM} operating at 1MHz readout rate. The conventional, ideal and curves for several values of gain are included.

The ideal curve corresponds to the ultimate noise limit of the signal shot noise. Several curves for different gains are included which show that at the lower photon signals increasing gain is beneficial whereas at higher signal levels there is a limit reached even with moderate gains. The characteristic for the conventional crosses over the EM curve - gain 100 - at a signal of ~53 photons per pixel. This means that weaker signals below this transition point will benefit from EM gain, whereas above there is better S/N with the conventional amplifier.

If this system were operated at the fastest readout speed of 2.5MHz, the readout noise would be greater and the benefits of EM gain would be seen for even stronger photon signals as shown in figure 20.

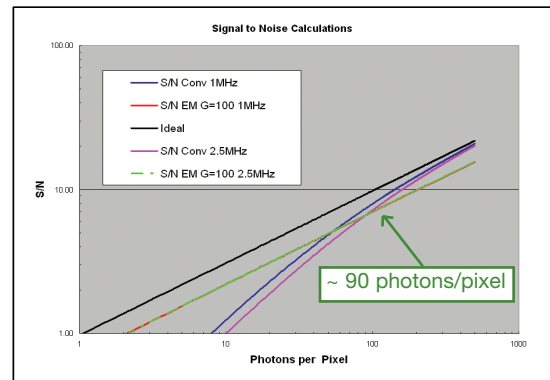


Figure 20 Comparison of S/N curves for different readout rates, 1 MHz and 2.5MHz. EM gain provides benefits at higher photon signals. Transition point at ~90 photons/pixel.

It will be noted that as the photon signal increases, the S/N ratio for the conventional CCD approaches that of the ideal curve i.e. the noise becomes limited by the intrinsic shot noise of the signal. In the case of the EM CCD the S/N ratio approaches the ideal but with an offset associated with the Noise Factor (NF) of the gain register. A noise factor is characteristic any amplification process and in the case of the EMCCD is ~1.4.

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