Comparing the Performance of EM-CCD and CCD Cameras for Raman Microscope Applications

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Introduction

EM-CCD (electron multiplied charge-coupled device) and CCD (charge-coupled device) cameras are the most common choices for detectors for visible Raman spectrometers. While there is more to a Raman spectrometer than just the camera, a basic understanding of the differences between these technologies can be useful in selecting the appropriate instrument for a particular application. While both options can be used for most applications, there are some differences that make one or the other the optimal choice.

Background

In photometry, the intensity of light is referenced to the sensitivity of the human eye. With CCD and EM-CCD cameras, sensitivity depends on converting impinging photons into an electrical charge and measuring that charge in the presence of the noise associated with the measurement. The signal-to-noise ratio (S/N) is a measure of how well the signal can be distinguished from various sources of noise. This can be used as a key metric when assessing camera sensitivity. To achieve a high signal-to-noise ratio, it is desirable to have a camera with a high quantum efficiency (QE) to maximize the signal while keeping the various sources of noise to a minimum. The quantum efficiency is the measure of the ability of the camera to convert impinging photons to a measurable electronic signal. A high QE is especially important with samples exhibiting weak Raman scattering or when using measuring conditions that severely limit the number of photons reaching the detector. The signal generated by the camera (detector) is the product of the number of photons impinging on the camera and the quantum efficiency.

A discussion of the various technologies to enhance quantum efficiencies of CCDs and EM-CCDs is beyond the scope of this report and will not be covered here. For this report, it is sufficient to note that EM-CCD and CCD cameras can be either front or back illuminated and that back illuminated (back thinned) cameras typically have higher QEs than front illuminated cameras.

There are many sources of noise associated with camera measurements and as the technologies change the contributions from the different sources can become more or less significant. The major sources of noise to consider for CCD cameras are signal shot noise, dark noise, and readout noise. The signal shot noise is noise that is intrinsic to the measurement process and is proportional to the signal. The dark noise arises from thermally generated electrons and this noise is minimized by cooling the camera. The dark noise increases with exposure time so detector cooling is especially important when analyzing samples that require long exposure times. Long exposure time may be required for weak Raman samples or when experimental requirements, such as the use of low laser power to avoid damaging sensitive samples, limit the number of Raman photons produced. The readout noise is noise associated with reading out the registry of the camera. The more often the registry is read out, the more noise is generated. These various sources of noise affect the balance between exposure time and the number of exposures when trying to optimize results. The read noise is an important factor affecting the S/N of CCD cameras but it is not multiplied in the multiplication register of an EM-CCD, so the relative contribution of the readout noise compared to the resulting signal is significantly reduced in EM-CCD cameras. The readout speeds of EM-CCD cameras are much faster than CCD cameras so they excel at high-speed applications with multiple scans. The total noise of the camera is the square root of the sum of the squares of the various sources of noise.

EM-CCD cameras are different from CCD cameras in that they employ a multiplication register to amplify charges. The amplification process used is known as a clock-induced charge. A photon impinges on the camera and produces an electron. As the charge is being transferred for read out it passes through a multiplication registry. The registry consists of a large number of cells where high voltage is applied to impart additional energy to the electrons. When an electron has sufficient energy, impact ionization can occur and this creates an additional electron-hole pair. In this way additional charge is created, effectively amplifying the signal. The gain is controlled by adjusting the applied voltage which affects the probability that impact ionizations will occur. The multiplication registry amplifies any charge and thus it not only amplifies charges associated with the signal but also charges associated with different types of noise. However, since the read noise is not multiplied it becomes essentially insignificant when using an EM-CCD and thus is not a limiting factor of the S/N of the camera. The amplification process itself has a noise contribution (noise factor) associated with it which contributes to overall noise. A complete description of the various sources of noise imparted by the various technologies and their effects is beyond the scope of this report but what is important is an understanding of what situations favor the use of an EM-CCD or CCD camera.

EM-CCD cameras excel in situations that benefit from faster read out speeds (short exposures and multiple exposures) and are capable of achieving relatively high S/N even when operating at these faster rates. The advantage of the EM-CCD camera is lost as longer and longer exposure times are used. Longer exposure times might be required because the samples of interest are weak Raman samples or because the Raman photons produced are limited because of analysis requirements. Longer exposure times result in increased signal because the camera is exposed to impinging photons for a longer period of time. In those situations, the camera is read less often and so the relative effect on read noise is diminished while the noise factor associated with the amplification process still remains. It should be noted that an EM-CCD can be operated with the amplification turned off (gain =1) in which case it operates essentially like a standard CCD camera.

Experimental

While it might be possible to review the specifications of the cameras themselves to evaluate their respective differences, the real question is how they perform as part of Raman spectrometers. In order to investigate these differences, this report will compare results obtained using a Thermo Scientific™ DXR™2 Raman Microscope that uses a front illuminated CCD camera with those obtained from a Thermo Scientific™ DXR™2xi Raman Imaging Microscope that uses a back illuminated EM-CCD camera. These Raman microscopes have very similar optics for collecting and directing the photons to the cameras so that should not be a significant source of variation. They use the same lasers, filters, and gratings. However, these instruments were designed to satisfy different analytical needs and application requirements so there are some differences in hardware and software. The DXR2xi imaging microscope was designed for Raman imaging and utilizes a faster and more accurate stage as well as software that was designed to handle the collection of large amounts of spectral data very quickly. These components work well together with the EM-CCD to collect spectral data very quickly. The DXR2 microscope was designed for single-point analysis and Raman mapping, and it can use longer exposure times. These differences will not preclude a comparison of the two detectors but it is necessary to consider these differences when comparing things like acquisition times for imaging data or when considering the ceiling on achievable instrument S/N. Like any comparison of S/N measurements, there will be some variability with the experimental parameters used to collect the spectra.

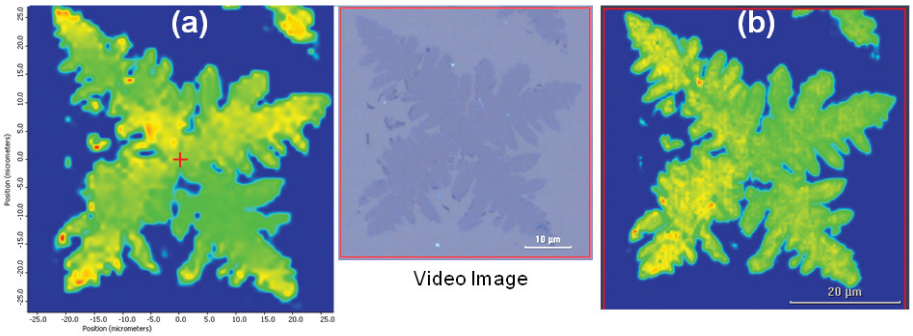
Three samples were chosen to illustrate a range of different types of Raman applications with varying analytical requirements. The first sample is an imaging (mapping) sample which consists of single layer of graphene on a silicon substrate. This represents applications where the sample is amenable to relatively fast Raman imaging. To eliminate any extraneous differences associated with stage movement during data collection, the second type of sample chosen involved single point measurements on a polystyrene sample, a moderately strong Raman sample. The third sample – a simple glass slide – was chosen to represent samples exhibiting weak Raman signals which require longer exposure times. While the choice of these three samples is somewhat arbitrary, they are not meant as suggestions for S/N standards they are only meant as examples of samples that have different experimental requirements. They are certainly not meant to cover all possible sample types but just serve as illustrations of the types of variations that can be observed when analyzing different types of samples.

Results

Both the DXR2 Raman microscope and the DXR2xi Raman imaging microscope can collect Raman spectra across a sample area to create Raman images.

The resulting images can be quite similar despite the fact that there are differences in how the data is collected. Figure 1 compares the Raman images of a monolayer graphene sample on silicon collected using the DXR2 microscope (a) and the DXR2xi imaging microscope (b). The Raman images are based on the intensity of the 2D peak of the graphene with the colors indicating differences in intensity. The samples areas were quite similar (54 x 54 µm and 55 x 55 µm respectively). The DXR2 microscope data was collected with 1 µm step sizes and the DXR2xi imaging microscope data was collected using an image pixel size of 1 µm. This means the images are made up of 3025 and 3136 spectra respectively. The spectral data was collected using an exposure time of 100 ms with the DXR2 microscope and 10 ms with the DXR2xi imaging microscope.

The biggest difference when using these two Raman microscopes is the time required to collect the images. The collection time using the DXR2xi imaging microscope was just under 4 minutes where the DXR2 microscope took approximately 5 hours. A considerable part of this difference can be attributed to the differences in the stage movement and the way the data is collected. However, even leveling the field (using the same exposure time, number of exposures, and number of spectra) without any additional overhead it would take 25 minutes to collect this data set using the DXR2 microscope versus 2 minutes and 37 seconds with the DXR2xi imaging microscope. The images in Figure 1 are similar, but how do the underlying spectra compare qualitatively? Figure 2 shows a visual comparison of representative spectra from both of the images. Upon visual inspection, these spectra appear reasonably equivalent – but recall that the exposure time was ten times greater for the DXR2 Raman microscope spectrum.



DXR2 mapping image based on peak height of the graphene 2D Peak

DXR2xi image based on the peak height of the graphene 2D Peak

Figure 1: Raman images of monolayer dendritic growth graphene on silicon based on the peak intensity of the 2D peak of graphene. **(a)** Data collected using the DXR2 Raman microscope **(b)** Data collected using the DXR2xi Raman imaging microscope.

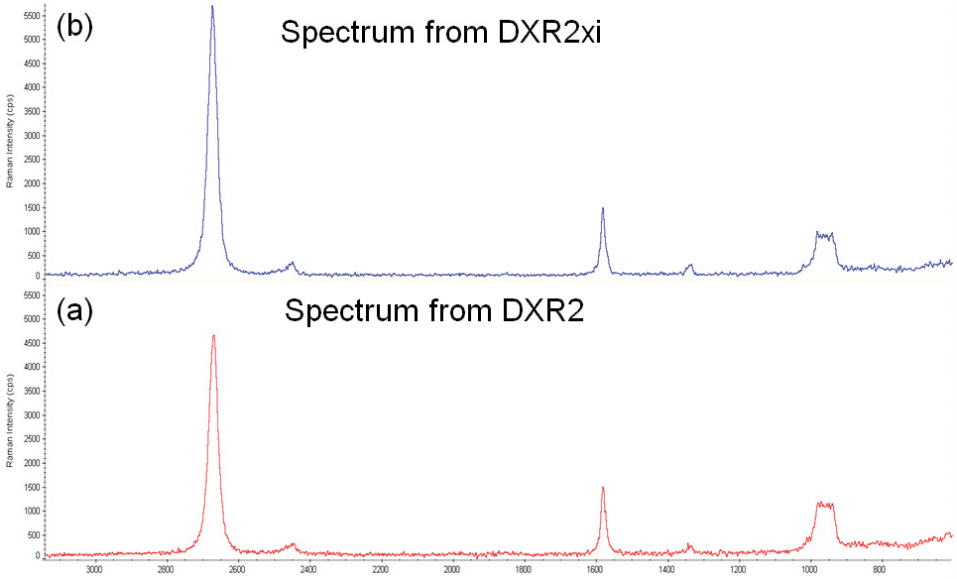


Figure 2: Representative Raman spectra of the graphene sample. **(a)** Collected using the DXR2 Raman microscope **(b)** Collected using the DXR2xi Raman imaging microscope.

The best comparison in this imaging application involves the signal-to-noise ratio calculated across the whole image. Figure 3 shows the results of using a signal-to-noise calculation as the basis of the Raman image. The values are calculated from the intensity of the graphene 2D peak and the root mean squared noise from the 2100-2000 cm^{-1} spectral region. The colors represent the range of signal-to-noise values and the two images have the same color scale. The fuchsia color represents higher signal-to-noise values – about 1.6 times greater than the regions colored yellow. The spectra collected using the DXR2xi imaging microscope had consistently higher signal-to-noise ratios. This illustrates the advantage of the EM-CCD camera, which allows for collection of the Raman spectra much faster with better spectral quality. The DXR2xi imaging microscope could collect this image even faster with exposure times up to six times shorter. These results are not surprising since the DXR2xi Raman imaging microscope was designed specifically for Raman imaging and the sample is suitable for fast Raman imaging. While this is an important type of application, it would be good to look at a situation where the stage movement is not a contributing factor.

The second example involves a single point analysis of a moderately strong Raman sample, a polystyrene film. While polystyrene is a relatively good Raman scatterer, this analysis was done at low laser power using a 532 nm laser at 0.5 mW, not to avoid damaging the sample but, to prevent saturating the EM-CCD camera at long exposure times. The analysis was done this way so that the results from the two cameras could be compared using longer exposure times. If the goal was simply the analysis of the sample, the experimental parameters would be optimized for each of the different types of Raman instruments. Figure 4 shows the polystyrene spectra collected from the sample at exposure times of 7 s and 0.1 s using both the DXR2 microscope and DXR2xi imaging microscope. The spectra at 7 s exposure times appear to have similar quality but there is a real visual difference in the spectra collected at 0.1 s exposure – the EM-CCD data quality is far superior to that from the standard CCD. Again it is instructive to compare the signal-to-noise ratios.

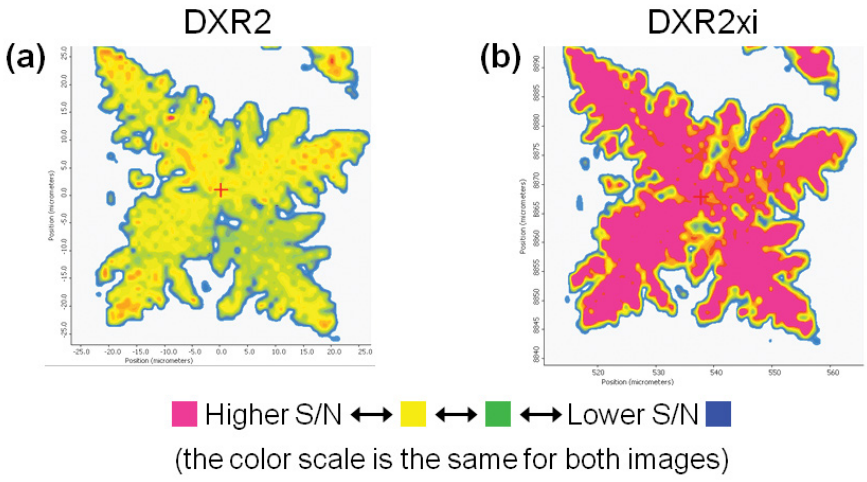


Figure 3: Raman images of monolayer dentritic growth graphene on silicon based on signal-to-noise calculations. The signal is defined as the peak intensity of the graphene 2D peak and the noise is the RMS noise from the 2100-2000 cm^{-1} region of the spectra. Colors indicate S/N values and the two images are on the same color scale. (a) DXR2 data (b) DXR2xi data.

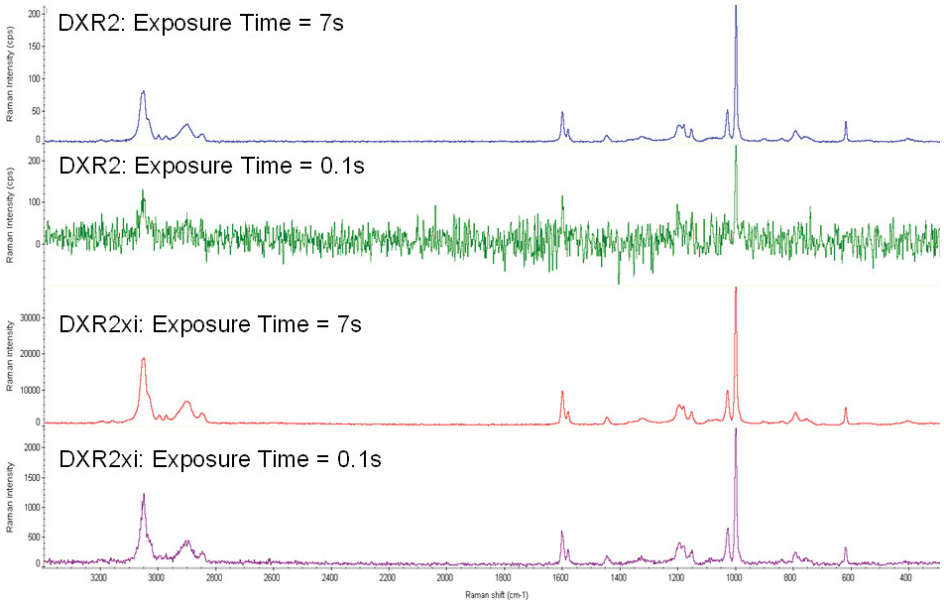


Figure 4: Raman spectra (532 nm laser, 0.5 mW) from the polystyrene sample collected using exposure times of 0.1 and 7 s.

The variation of the signal-to-noise ratio with exposure time can be seen in the plot in Figure 5. The signal-to-noise ratios were calculated using the peak height of the 1001 cm^{-1} polystyrene peak and the root mean squared (RMS) noise from the spectral region between 2400 and 2300 cm^{-1}. The plot shows that the spectra obtained using the DXR2xi imaging microscope (the EM-CCD) consistently had higher signal-to-noise ratios even at exposure times out to 7 seconds. The relative differences are larger at shorter exposure times.

The final scenario involves samples exhibiting weak Raman scattering, where long exposure times are required to obtain reasonable Raman spectra. Weak Raman scattering is not uncommon, and there are also cases where the signal is purposefully limited such as when low laser power is required to avoid damaging a sample. Glass has a weak Raman signal, so a glass microscope slide was an attractive sample because it is very homogeneous and not prone to damage. This analysis involved just single point collections like the polystyrene example because that eliminates any variation related to differences in stage movement. The spectra were collected using a 532 nm laser. Even though the signal from glass is weak, it was possible to saturate the EM-CCD detector at very long exposure times, so a laser power of 2 mW was used.

Samples that require long exposure times to obtain reasonable Raman spectra reduce the advantages realized by the DXR2xi imaging microscope because the time per point becomes the overwhelming determinant of the collection time and read out noise is less of an issue. As mentioned previously it is possible to turn the amplification of the EM-CCD off (gain =1) and in that case the EM-CCD acts like a CCD camera. For this example, spectra were collected using the DXR2xi microscope with the amplification on and off.

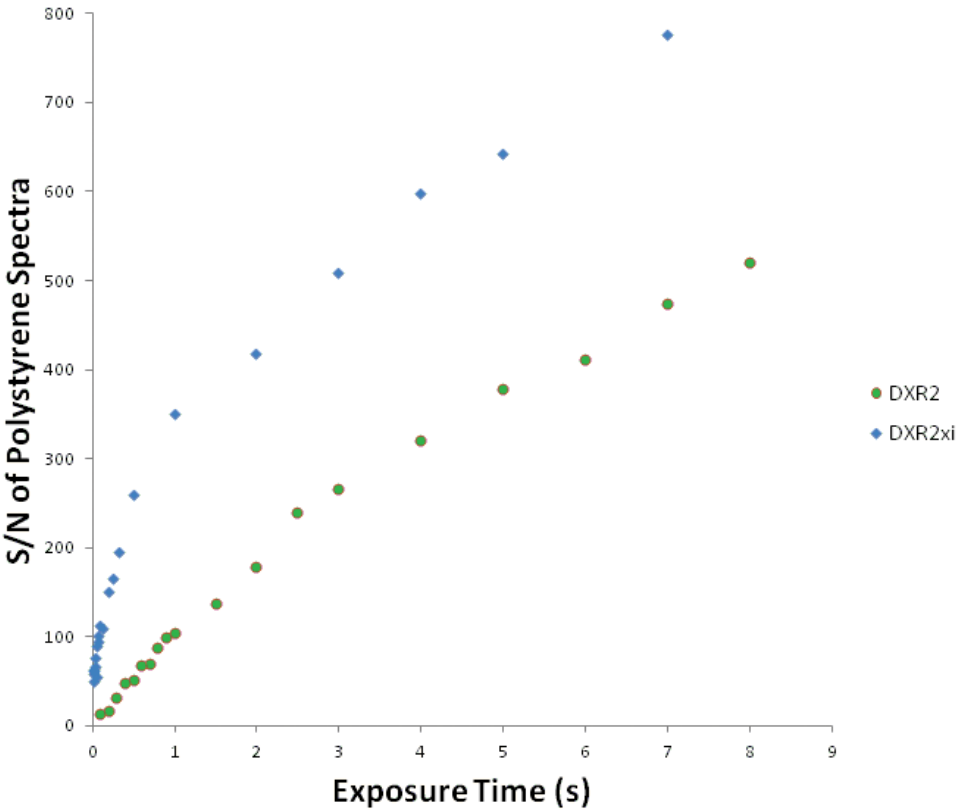
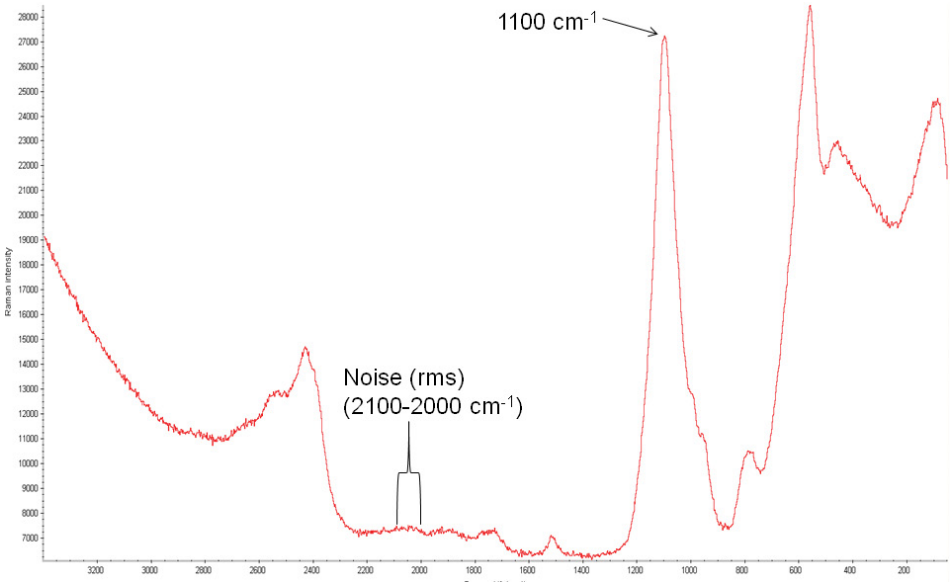


Figure 5: Plot of signal-to-noise ratios from the polystyrene sample (532 nm laser, 0.5 mW) as a function of exposure time. The signal was defined as the intensity of the 1001 cm^{-1} peak of polystyrene and the noise as the RMS noise in the 2400-2300 cm^{-1} region of the spectra. (●) Data from the DXR2 Raman microscope (♦) Data from the DXR2xi Raman imaging microscope

Figure 6 shows a representative Raman spectrum obtained from the glass slide collected at a long exposure time. As previously, the spectra are compared by calculating the signal-to-noise ratios. In this case, the peak height at 1100 cm^{-1} was used as the signal and the RMS noise in the region from 2100-2000 cm^{-1} was used as the noise. Figure 7 shows a plot of the signal-to-noise values for the spectra collected with the DXR2xi imaging microscope with the amplification on, the DXR2xi imaging microscope with the amplification off, and the DXR2 microscope. The insert is just an expanded view of the shorter exposure time region. This plot shows data out to much longer exposure times (out to 30 s).

At the shorter exposure time (<2 s), the DXR2xi imaging microscope with the amplification on gave the best results. At exposure times beyond 2 s, the DXR2xi imaging microscope with the amplification off gave the best results. This is not unexpected because with the amplification off the EM-CCD operates like a back-illuminated CCD camera and has a higher quantum efficiency than the front illuminated CCD in the DXR2 microscope. It should be noted that at exposure times above about 6 seconds the DXR2 microscope gave better results than the DXR2xi imaging microscope with the amplification on. Even with the lower laser power, the spectra started to saturate at the really long exposure times when using the DXR2xi imaging microscope with the amplification on (see the plot at exposure times >20 s). This shows a drawback of using the EM-CCD with the amplification on at long exposure times. Similar to cases where fluorescence contributes to the baseline, the exposure time cannot be increased to get better signal-to-noise because the camera saturates. With both microscopes having the amplification off, the exposure time could be increased or the laser power could be increased to get better signal-to-noise because there is still dynamic range available.

Figure 6: Representative Raman spectrum obtained from a glass microscope slide. The peak intensity at 1100 cm^{-1} and the RMS noise in the 2100-2000 cm^{-1} region were used to calculate signal-to-noise ratios.



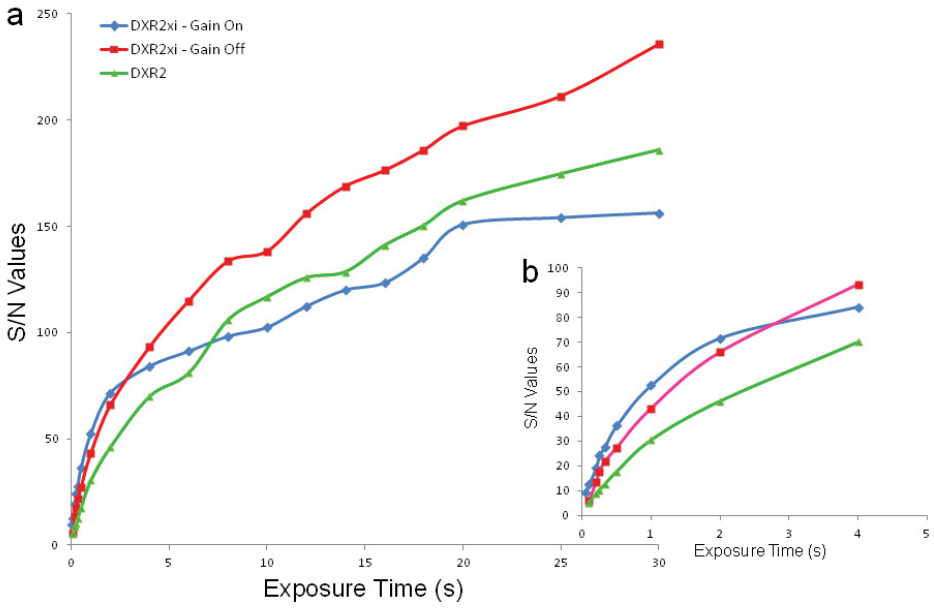


Figure 7: Plot of signal-to-noise ratios of Raman spectra obtained from the glass microscope slide as a function of exposure time. Data obtained from the DXR2xi Raman imaging microscope with the amplification of the EM-CCD turned on is shown in blue. Data from the DXR2xi Raman imaging microscope with the amplification of the EM-CCD turned off (gain =1) is shown in red. Data obtained from the DXR2 Raman microscope is shown in green. (b) is an inset that shows an expanded view of the lower exposure time region of the plot.

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Conclusions

The report illustrates the differences between EM-CCD and standard CCD cameras for different Raman applications. These were represented by the performance differences of the DXR2xi Raman imaging microscope (back-illuminated EM-CCD) and the DXR2 Raman microscope (front-illuminated CCD). The samples were chosen to represent a range of applications. Clearly the EM-CCD excels at collecting Raman spectra very quickly and with high signal-to-noise ratios. This shows that while the EM-CCD is uniquely suited to Raman imaging it is not necessarily the best solution for all types of applications. The other advantages of the DXR2xi imaging microscope, not related to the EM-CCD, in terms of the minimization of overhead in stage movement and data collection are also less significant when the exposure times are the dominate factor determining the acquisition time. The advantages of the EM-CCD and the DXR2xi imaging microscope over the DXR2 microscope become less forceful with applications requiring long exposure times.

The goal of any experimenter is to select the instrumental solution that best matches the application requirements. The examples shown covered a range of sample types, but there is undoubtedly going to be variation with different types of applications. For instance, none of the applications here addressed samples with significant fluorescence contributions. The issue of etaloning with the use of long wavelength lasers (785 nm) with a back illuminated EM-CCD or CCD camera will be addressed in a future white paper. Even so, the general trends outlined here are expected to hold for a wide range of samples, so this information can be used to understand when either an EM-CCD or a standard CCD might be the best choice.

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