



The Advantages of a Compact, Thermoelectrically-Cooled Fiber Optic Spectrometer for Raman and Fluorescence Spectroscopy

Compact spectrometers using array detectors and fiber optic light inputs are being utilized for a wide variety of spectroscopic applications. Historically, one of the limitations of this design has been poor detection limits, particularly for low light level applications such as fluorescence and Raman spectroscopy. The main reason for this performance degradation has been the higher noise characteristics associated with non-cooled detectors. However, breakthroughs in thermoelectrically (TE) cooled spectrometers has led to significantly lower noise specifications, which allow for longer signal integration times, resulting in lower detection limits. In this application note we demonstrate the benefits of a TE-cooled spectrometer by comparing the technology with a non-cooled device, configured for both fluorescence and Raman spectroscopy.

Detector Noise

To better understand the advantages of the thermoelectric (TE) cooling, it is important to first have a basic understanding of noise associated with any type of photon detector. Theory tells us that the total noise of any detector is calculated by taking the square root of the sum of all of the noise components. The four main sources of noise found in an array detector include: readout noise, shot noise, dark noise and fixed pattern noise.

- **Readout noise** is caused by electronic noise in the detector output stage and related circuitry, which largely dictates the detection limit of the spectrometer.
- **Shot noise** is associated with the statistical variation in the number of photons reaching the detector, which follows a Poisson distribution and, as a result, is proportional to the square root of the incident photon flux.
- **Dark noise** is associated with the statistical changes in the number of electrons generated in a dark state and is impacted by temperature.
- **Fixed pattern noise** is the variation in photo-response between neighboring pixels, which are caused by slight differences in the area and thickness of the detector during the fabrication process.

Thermoelectric Cooling

Cooling an array detector with a built-in thermoelectric cooler (TEC) is an effective way to reduce dark current noise, therefore enhancing the dynamic range and lowering the detection limit. Studies have shown that with silicon-based CCD (charged-coupled device) array detectors, the dark current goes up



by a factor of two when the temperature increases by approximately 5-7°C and goes down by a factor of two when the temperature decreases by the same amount. At room temperature (~22 °C) for example, dark noise is a significant contribution to the total signal with a non-cooled detector. This is exemplified in Figure 1, which shows the dark current noise for a non-cooled CCD spectrometer at room temperature and a TE cooled spectrometer, operated at 14°C, using an integration time of 30 seconds. The calculated RMS (root mean square) noise level of the TE-cooled spectrometer on the right is approximately five-fold lower than the non-cooled unit.

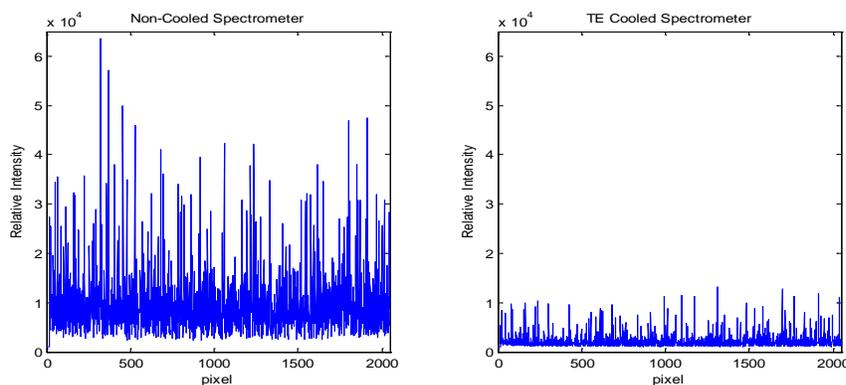


Figure 1: Dark current noise for a non-cooled CCD spectrometer at room temperature (left) and a TE-cooled CCD spectrometer at 14°C (right), using an integration time of 30 seconds

It should be emphasized that when a CCD based spectrometer is used for less demanding, high light level applications such as LED measurements, the dark noise reduction due to TE cooling is not so advantageous. The major reason being that readout noise is the dominant noise component when relatively short integration times are used (~ 200 ms). For that reason, there is no significant noise reduction due to the TE cooling, although the internal temperature control will be beneficial for long term baseline stability.

Let's now take a more detailed look at two low-light applications: fluorescence and Raman spectroscopy. All data in this study was generated using the Glacier® X, TE-cooled spectrometer (B&W Tek, Newark, DE). This system has been described in the open literature, and basically utilizes a compact high performance TE-cooled 2048 pixel CCD detector at 14°C. The optical bench utilizes a crossed Czerny-Turner spectrograph design with a resolution of < 0.2 nm (1).

Fluorescence Spectroscopy

In fluorescence spectroscopy, a molecule is typically studied by exciting it with a high energy photon which causes the electrons to transition from a ground state to a higher energy, excited state. When the electron falls back to the ground state, it emits a photon at a characteristic wavelength of light equal to the difference in the energy levels. Therefore, by using the fundamental relationship between the energy and frequency of the emitted light, the different electronic and vibrational states of a molecule can be studied.



This is exemplified in Figure 2, which shows a comparison of fluorescence spectra of a quantum dot made up of a cadmium selenide (CdSe) core and a zinc sulfide (ZnS) shell, engineered to fluoresce at 584 nm. These measurements are taken using a UV excitation source and are collected using a collimating lens and a fiber optic patch cord connected to a non-cooled spectrometer (blue) and TE-cooled spectrometer (red). Both units are configured with a spectral range of 350nm -1050nm and a spectral resolution of 4nm. The integration time of both spectrometers is set at 120 seconds. By analyzing the raw data plots on the left, it becomes clear that the data collected with the non-cooled unit has significantly higher noise than the TE-cooled unit. As a result of this noise signal, the 584nm fluorescence peak on the non-cooled unit is almost indistinguishable from the noise, but is clearly visible in the spectrum collected using the TE-cooled system. This difference is shown more clearly in the plot on the right where the dark current noise has been removed.

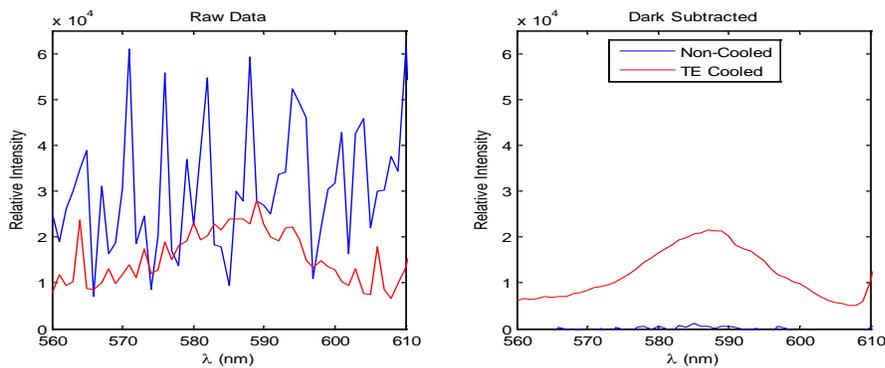


Figure 2: A comparison between a non-cooled (blue) and TE-cooled (red) spectrometer showing the fluorescence spectra of a quantum dot made up of a cadmium selenide (CdSe) core and a zinc sulfide (ZnS) shell, engineered to fluoresce at 584nm. The plot on the left shows the raw data (noise included), while the plot on the right shows the spectra with the dark noise subtracted out.

Raman Spectroscopy

Raman spectroscopy measures vibrational, rotational, and other low frequency interactions between wavelengths of light and a molecule of interest. The Raman Effect occurs when the incident photon is absorbed by the molecule, exciting the electrons into a virtual state equal to the photon energy. While the vast majority of electrons return to the ground state and emit a photon of energy equal to the incident photon, a small number of the excited electrons will decay to an intermediate energy state before returning to the ground state. When this happens, the molecule emits a photon of lower energy and the difference in energy is equal to the vibrational energy of the covalent bond. This process of the Raman Effect is known as Stokes scattering, and only occurs in 1 out of every 10^6 incident photons. There is also a strong probability that the photon will gain energy equal to the vibrational energy. This process is known as anti-Stokes scattering and is usually much weaker than Stokes scattering.

The energy of a photon determines the wavelength; so by measuring the change in wavelength from a monochromatic excitation source (typically a laser), Raman spectroscopy allows for the interrogation and identification of vibrational states of molecular bonds. However, since the Raman Effect has such a low



probability of occurring as previously mentioned, it is essential to integrate the signal for long periods of time when measuring the scattered light. This makes it extremely challenging to use a traditional, non-cooled spectrometer for carrying out Raman studies.

Figure 3 demonstrates the advantages of a TE-cooled spectrometer over a non-cooled unit for evaluating the Raman spectrum of acetaminophen. For the acetaminophen measurement, the integration time for both spectrometers was set at 7 seconds. It is clear from the spectral data on the left that the dark current noise for the non-cooled system is at such a high level that it is difficult to differentiate all the Raman peaks in the spectrum. Whereas the spectrum collected with the TE-cooled system on the right shows well-defined Raman peaks, with very low baseline noise.

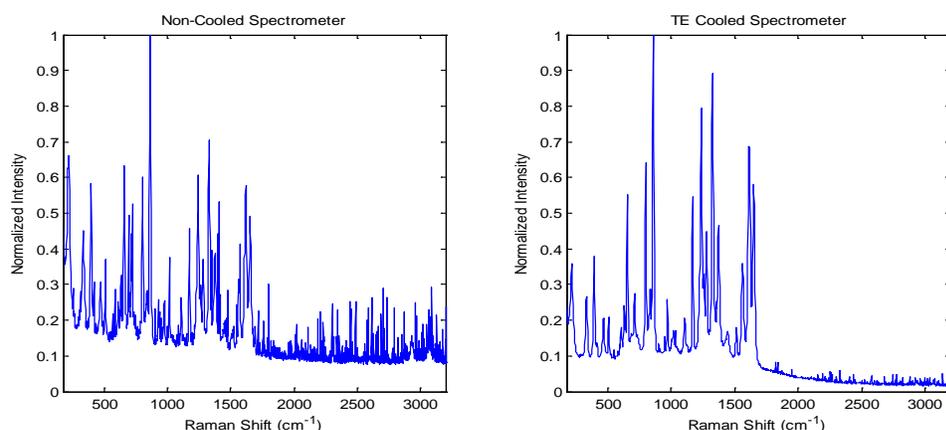


Figure 3: Raman spectrum of acetaminophen collected using a non-cooled spectrometer (left) and a TE-cooled system (right)

Conclusion

The study has shown that by lowering the temperature of a spectrometer's CCD detector using thermoelectric cooling, dark current noise can be reduced quite significantly. For low-light applications where the analyte signal is often very difficult to differentiate from the background noise, such as in fluorescence and Raman spectroscopy, this allows for longer signal integration times, which translates into less noise, lower detection limits, wider dynamic range and better long-term stability.

Further Reading

1. Glacier X® compact high performance TE-cooled CCD spectrometer:
<http://bwtek.com/products/glacier-x/>
2. Exemplar® Plus high performance smart spectrometer:
<http://bwtek.com/products/exemplar-plus/>