

Ultrafast Confocal Raman Imaging

Acquiring Spectra in a Few Milliseconds with Improved Sensitivity

In Confocal Raman imaging the acquisition time for one Raman spectrum is a crucial value, as it influences the acquisition time of the image which typically consists of tens of thousands of Raman spectra. This article describes how the use of a spectroscopic EMCCD as the detector can significantly reduce the acquisition time down to a few milliseconds per spectrum, as well as tremendously improve sensitivity.

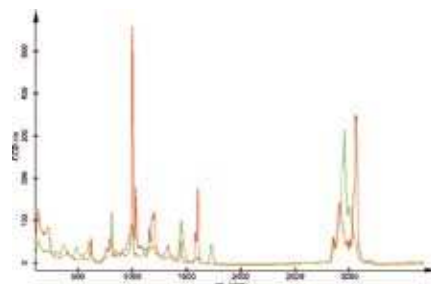
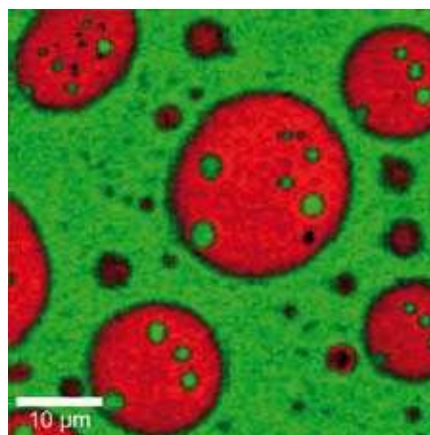


Fig. 1: a) Confocal Raman Image of a PS-PMMA blend acquired with the WITec Ultrafast Raman Imaging Option. 120 x 120 pixel = 14,400 spectra. Acquisition time for one spectrum: 2,3 milliseconds; total acquisition time for the image 67 seconds. Red: PS; green: PMMA. b) Corresponding Raman spectra

Introduction

In confocal Raman imaging, as in the WITec alpha300 R system which was used for the experiment described here, only light from the image focal plane can reach the detector, which strongly increases image contrast and slightly increases resolution. Special filters are used to suppress the reflected laser light while enabling the Raman scattered light to be detected with a spectrometer/CCD camera combination. To obtain an image, thousands of spectra are acquired in a very short time with typically less than 100 ms integration time per spectrum using a normal CCD camera. In order to further improve the overall sensitivity of the system a EM-CCD can be used.

Spectroscopic EMCCD

An electron multiplying CCD (EMCCD) is a normal CCD with an additional readout register which is driven with a much higher clock voltage than a normal CCD readout register. Due to this high clock voltage, an electron multiplication through impact ionization is achieved with an adjustable total amplification of the signal of up to 1000 times. With this setup, it is always possible to amplify the signal above the readout noise so that the S/N ratio is always limited by the Poisson noise of the signal, even if a very fast readout amplifier is used. As an example, a 1600 x 200 pixel EMCCD with a 2.5 MHz readout amplifier, as used for the experiments in this article, can be read out in only 1.7 ms.

The following calculations show the improvement in S/N that can be expected for different signals. It is assumed that the quantum efficiency (QE) of the CCD is 90% and that the amplification of the signal is set to a value at which one A/D count equals the number of electrons of the readout noise (1 A/D count = 30 electrons for a 2.5 MHz readout amplifier).

If 100 photons fall on a CCD pixel in a given integration time, 90 electrons will be generated and converted to 3 A/D counts. The readout noise will be 1 A/D count and the Poisson noise will be 9.5, which is approximately 0.3 A/D counts. With these numbers, the S/N ratio is about 2.6.

In an EMCCD, the signal will be multiplied by the electron gain factor which can be as high as 1,000. A smaller amplification factor would generally be used, but for the calculation it does not make a difference. 90 electrons will be amplified to 90,000 electrons resulting in 3,000 A/D counts. The Poisson noise is 9,500 electrons which translates to 317 counts, while the 1 count readout noise is completely negligible. S/N is 9.5, which is an improvement of a factor of 3.6.

If the signal is only 10 photons, this will result in a signal of only 0.3 counts for a normal CCD. Poisson noise can be neglected in this case. With 1 count readout noise, S/N is 0.3, hardly a detectable signal.

For an EMCCD, the signal is 333 counts and Poisson noise is 100 counts which gives a S/N of 3.3, an improvement of 11 times over a normal CCD.

In reality the electron multiplying process itself adds an additional, so called excess noise factor of about 1.4, so that the real improvements in S/N are reduced to 2.6 and 7.9 respectively for the above examples.

For higher signals, in which the signal intensity is no longer readout limited, the excess noise factor of the EM process reduces the S/N ratio of an EMCCD to below that of a normal CCD. In this case, the EM register can be switched off and then the "normal" readout register is used. Thus, the EMCCD behaves just as a normal back-illuminated CCD.

Application Examples

Figure 1 a) shows a confocal Raman image of a PS-PMMA polymer blend spin-coated onto a glass slide. It was imaged with the WITec Ultrafast Raman Imaging Option for the alpha300 R Confocal Raman Microscope using a spectroscopic EMCCD as the detector. The scan range was 50 x 50 μm with 120 x 120 image pixels = 14,400 spectra. The acquisition time per spectrum was only 2.3 milliseconds, resulting in a total acquisition time for the complete image of 67 seconds (including retrace at the end of the line). The image was obtained by evaluating the different peak characteristics of PS and PMMA as shown in figure 1b. with the WITec Project software package. This results in a color-coded image of the distribution of the two compounds (red: PS; green: PMMA).

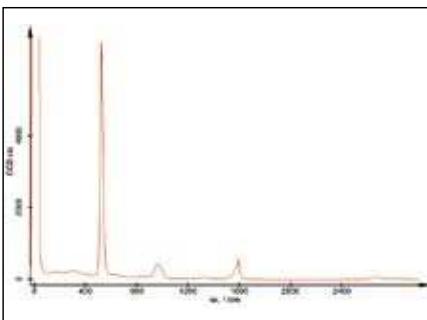
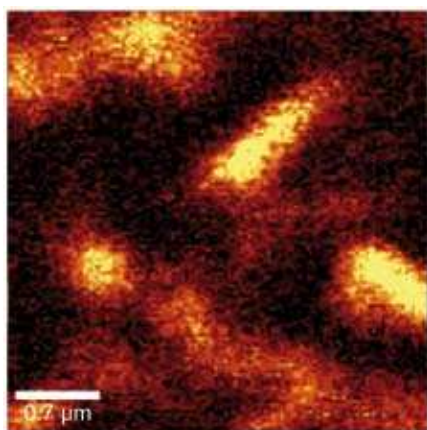
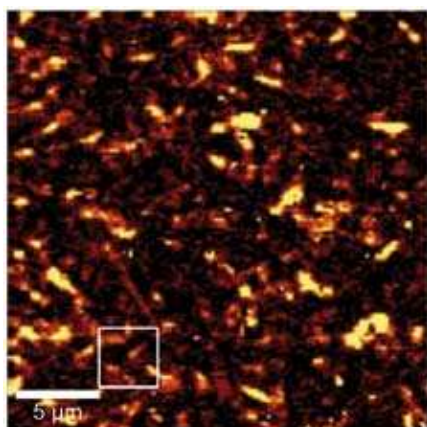


Fig 2: a) Overview scan of carbon nanotubes on a silicon substrate, $25 \times 25 \mu\text{m}$, 120×120 pixel = 14,400 spectra. b) Zoom-in of the marked area. Scan Range $3.5 \times 3.5 \mu\text{m}$, 120×120 pixel = 14,400 spectra. Total acquisition time of both images was 96 seconds. c) Typical measured spectrum obtained by averaging some of the 14,400 spectra which showed a clear signal from the CNTs.

In a second experiment, carbon nanotubes (CNTs) on a silicon substrate were imaged using the WITec Ultrafast Raman Imaging Option. As the first step, a large overview scan with a scan range of $25 \times 25 \mu\text{m}$ with 120×120 pixels = 14,400 spectra was performed. The acquisition time was 4 ms per spectrum, so the measurement required only 96 seconds to complete the image shown in figure 2 a). It clearly reveals the heterogeneous distribution of the CNTs on the substrate.

In a second step, a zoomed-in image of the marked area with a scan range of $3.5 \times 3.5 \mu\text{m}$ and 120×120 pixels was obtained as shown in figure 2 b). Due to the optimized readout of the EMCCD, the acquisition time per spectrum was again 4 ms and 96 seconds for the complete image (including retrace at the end of the line). Figure 2 c) shows a typical measured spectrum of the CNTs also showing peaks from the Si-Substrate. It was obtained by averaging some of the 14,400 spectra which showed a clear signal from the CNTs.

Summary

It was demonstrated that the use of an EMCCD camera can greatly increase detection efficiency and speed, especially for the short integration times necessary with a confocal Raman microscope. For very small signals that are dominated by the CCD's readout noise, the use of an EMCCD can improve the S/N ratio by a factor of 5 to 10 compared to the best available standard CCD's, while for larger signals the electron multiplying circuit can simply be switched off and all properties of a standard (back-illuminated) CCD are maintained.

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Highlights

- extremely fast image recording -1GB/s
- 636 fps at full resolution
- CMOS image sensor with 1280x1024 pixel resolution
- image memory in camera up to 4GB
- 10bit dynamic range
- standard interfaces (IEEE 1394, camera link)

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