Ultrafast Confocal Raman Imaging

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A thorough knowledge of structural and chemical properties is essential in the fields of nanotechnology, materials research, and life science, leading to a growing demand for characterization methods for heterogeneous systems on the nanometer scale. However, certain properties are difficult to study with conventional characterization techniques due to either limited resolution or the inability to differentiate materials chemically without inflicting damage or using invasive techniques such as staining. Confocal Raman Imaging can effectively overcome these fundamental obstacles. In Confocal Raman Imaging, the acquisition time for one Raman spectrum is a crucial value, as it determines 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 Electron Multiplying-CCD (EMCCD) as the detector can significantly reduce the acquisition time to a few milliseconds per spectrum, as well as tremendously improve sensitivity.

Confocal Raman Imaging

In Raman spectroscopy, a vibrational quantum state is excited or annihilated within a molecule, leading to an energy shift between the incident light and the scattered light. This energy shift is unique to each molecule and allows the chemical identification of compounds within a sample. By integrating a sensitive Raman spectrometer within a state-of-the-art confocal microscope setup, Raman imaging with a spatial resolution down to 200 nm laterally and 500 nm vertically can be achieved using visible light excitation. 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 a typical integration times per spectrum in the range of 20 to 100 ms using a normal CCD camera. In order to improve the overall sensitivity of the system further an EMCCD can be used.

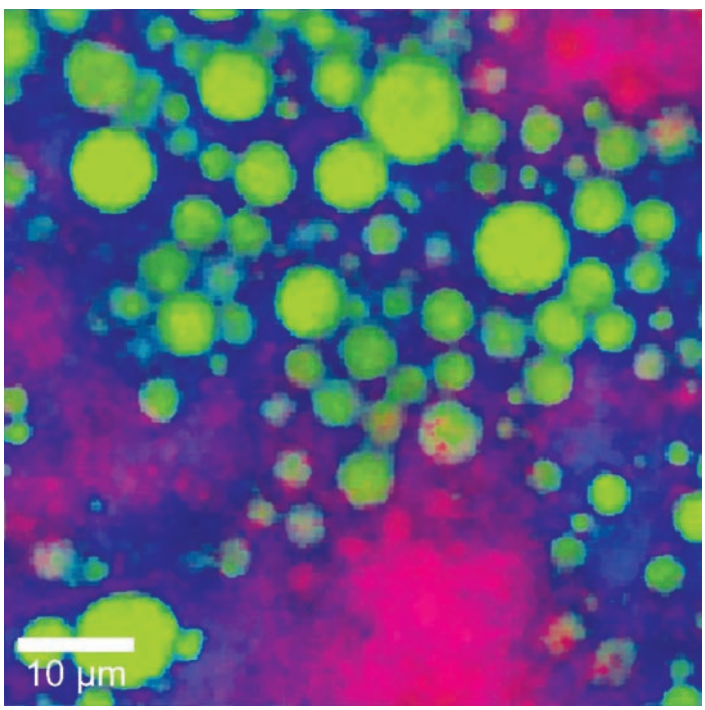


Fig. 1) Confocal Raman Image of an oil-alkane-water emulsion; scan range: 60×60 µm, 200×200 pixels, 40,000 pixel spectra, 760 µs/spectrum, 42 sec./image, excitation: 532 Nd:Yag; Green: oil, Red: alkane, Blue: water.

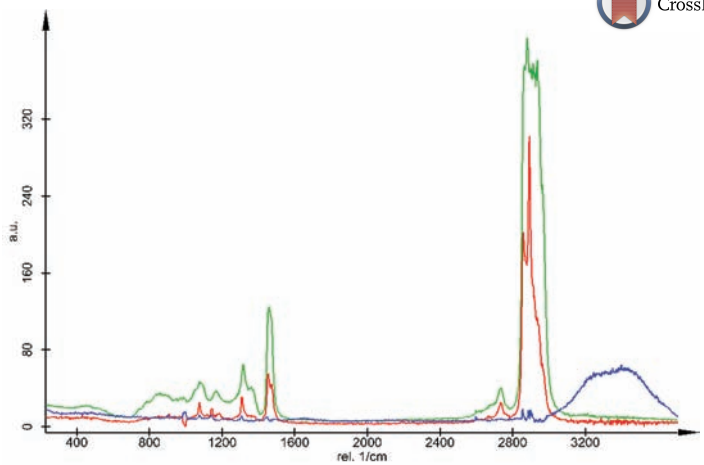


Fig 2) Corresponding Raman spectra of the compounds shown in Fig. 1. Green: oil, Red: alkane, Blue: water

Spectroscopic EMCCD

An electron multiplying CCD is a normal CCD with an additional readout register that 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 760 microseconds.

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 electrons, which is approximately 0.3 A/D counts. With these numbers, the S/N ratio is about 2.6.

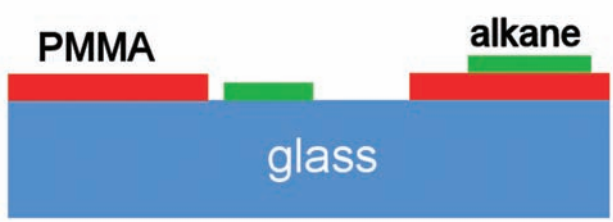
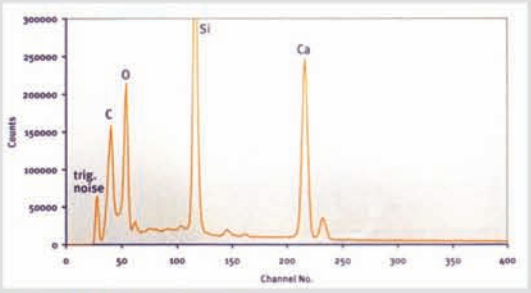


Fig. 3: Schematic of the glass-PMMA-alkane sample, 7.1 nm PMMA film and 4.2 nm alkane film on glass.

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.

SDD
Silicon Drift Detector

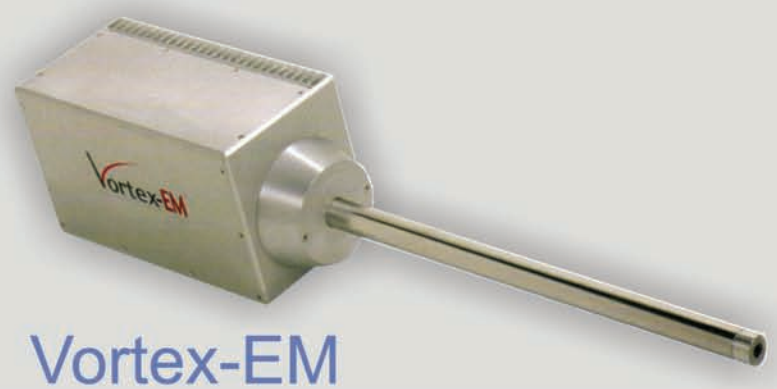


Typical light element spectrum

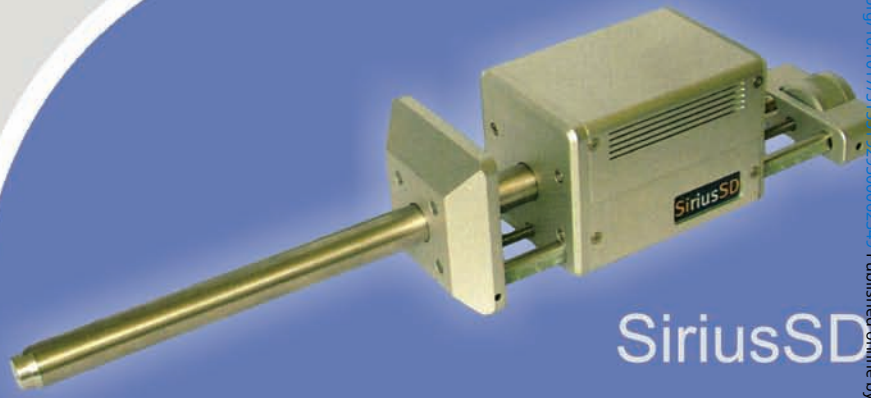
Energy Resolution (FWHM) @ 5.9 keV < 126eV
Crystal active area: 10-30 mm²
Crystal thickness: 450 µm
Window: Ultra Thin Window

YOUR

50mm²



Vortex-EM

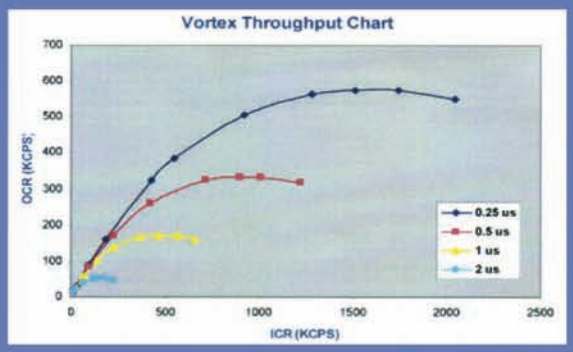


SiriusSD

126eV

CHOICE

Energy Resolution (FWHM) @ 5.9 keV < 133eV
Crystal active area: 50 mm2 (nominal)
Crystal thickness: 350 µm - 400 µm
Window: Ultra Thin Window



Throughput Chart

SDD
Silicon Drift Detector

IXRF Systems

IXRF Systems, IncHouston, TX77062 Ph: 281-286-6485 F: 281-286-2660

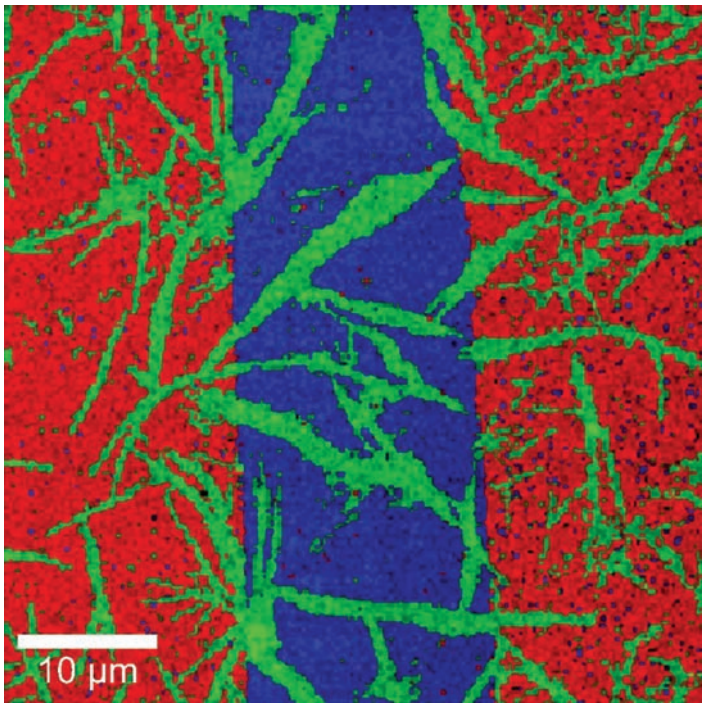


Fig. 4: Color-coded confocal Raman image of the 7.1 nm PMMA layer (red) and the 4.2 nm alkane layer (green) on glass (blue). 200×200 spectra, 7 ms integration time /spectrum. Total acquisition time: 5.4 min.

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 the "normal" readout register is used. Thus, the EMCCD behaves just as a normal back-illuminated CCD.

Ultrafast Image Acquisition

To demonstrate the benefit of this technique, an emulsion was analyzed with a confocal Raman Microscope attached to a Raman spectrometer with an EMCCD detector unit (WITec alpha300 R, Ultrafast Confocal Raman Imaging Option). Emulsions play an important role in various production processes e.g. in the food, pharmaceutical and cosmetics industries. As an example of an emulsion, a mixture of oil, alkane and water was imaged. The scan range was 60x60 µm and 200×200 pixels, resulting in a total of 40,000 Raman spectra. The acquisition time was 760 microseconds per spectrum and 42 seconds per image. The resulting image is shown in Fig. 1 and the corresponding spectra with the same color-coding are shown in Fig. 2. Using a standard CCD camera, the fastest acquisition time achievable is 13 milliseconds, thus the acquisition of the image would have taken approx. 9 minutes. With this sample, the enhanced setup including the EMCCD results in a 13-fold reduction in acquisition time.

Sensitivity

In this study an extremely thin PMMA film, spin-coated onto a glass substrate was investigated. In the center of the images, a vertical scratch was made with a metal needle to remove the PMMA layer. The film thickness, as measured with an AFM across this scratch was 7.1 nm. It was observed that there was an additional contamination layer of 4.2 nm thickness consisting of alkane. Fig. 3 shows a sketch of the sample. The images were obtained by acquiring 200 x 200 Raman spectra in a 50 x 50 µm scan range. Excitation power was 20 mW @532 nm using a 100×, NA=1.4 oil-immersion objective. Integration time was 7ms/spectrum, resulting in a total acquisition time of 5.4 min. (including 0.3s/line for the back-scan). Due to the limited confocal depth resolution, none of the spectra obtained was a pure PMMA spectrum or a pure spectrum of the contamination layer. However, by averaging all spectra acquired in the area of the scratch with no contamination present, a pure glass spectrum was obtained. By subsequent subtraction of the glass spectrum from the PMMA spectrum as well as from the spectrum of the contamination, pure PMMA and contamination spectra could be calculated. These spectra were taken for a basis analysis, in which each measured spectrum is fitted as a linear combination of basic spectra. Using this technique, three images with the distribution of the three components (glass, PMMA and alkane) could be obtained which were color-coded in one image (blue=glass, red=PMMA and green=alkane) to visualize their distribution (Fig. 4). Fig. 5 shows the three spectra of glass, alkane and PMMA with an identical intensity scale. It is obvious that the confocality of the Raman system is crucial for the delectability of thin layers. Even with the best confocal setup, the information depth is at least 500 nm which means that 500 nm of glass contribute to the Raman signal. As the Raman signal is proportional to the amount of material, a standard (non-confocal) setup would have collected a glass signal more than 300 times higher (170 µm cover glass thickness), making the detection of the thin coating layers impossible, even with much longer integration times.

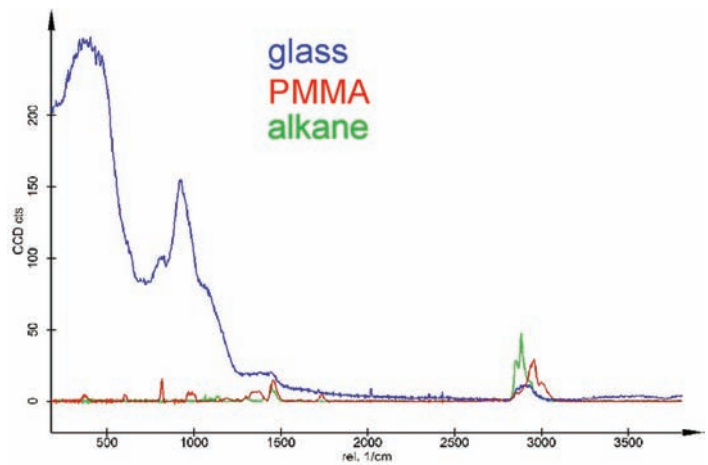


Fig. 5: Corresponding Raman spectra of PMMA (red), alkane (green) and glass (blue)

Summary

It was demonstrated that the use of an EMCCD camera could greatly increase detection efficiency and speed, especially with the short integration times necessary for a confocal Raman imaging 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 - 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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