



Ultrafast Confocal Raman Imaging

Harald Fischer and Olaf Hollricher, WITec GmbH

In confocal Raman imaging, as in the WITec alpha300 R system that 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, an EM-CCD can be used.

EM-CCD

An electron multiplying CCD (EM-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 is always limited by the Poisson noise of the signal, even if a very fast readout amplifier is used. As an example, a 1600×200 pixel EM-CCD with a 2.5 MHz readout can be read out in only 2.3 ms.

Results

Figure 1a shows the confocal Raman image of an extremely thin PMMA film, spin coated onto a glass substrate. The image was obtained by acquiring 200×200 Raman spectra in a $50 \times 50 \mu\text{m}$ scan range. Excitation power was 20 mW @532 nm using an oil immersion objective with NA = 1.4. Using an EM-CCD, the integration time was 7 ms/spectrum, resulting in a total acquisition time of 5.4 min (including 0.3 s/line for the back-scan). 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. 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 pres-

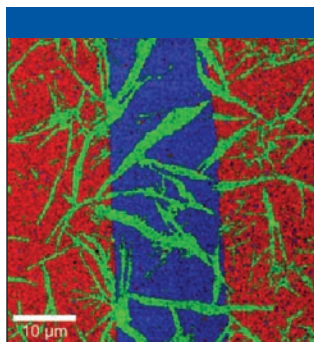


Figure 1: Color-coded confocal Raman image of a 7.1 nm PMMA layer (red) and a 4.2 nm contamination layer (green) on glass (blue). 200×200 spectra, 7 ms integration time/spectrum. Total acquisition time 5.4 min.

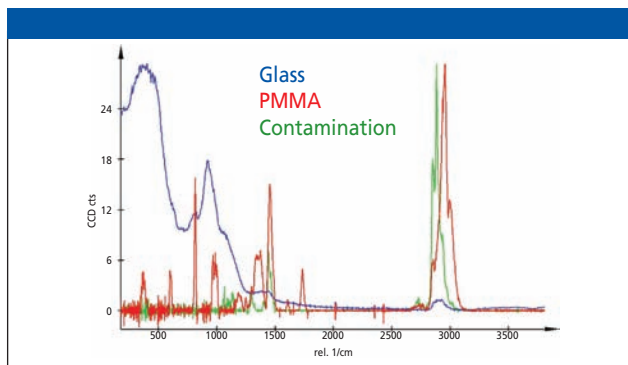


Figure 2: Raman spectra as calculated from the Raman measurement in Figure 1, displayed with identical maximum intensities.

ent, 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 contamination) could be obtained and were color-coded (blue = glass, red = PMMA, and green = contamination) to visualize their distribution. Figure 2 shows the spectra of the different components. For better comparison, they are displayed with identical maximum intensities. The PMMA spectrum is amplified about 20 times and the contamination spectrum about 15 times with respect to the glass spectrum. From this, the green spectrum (contamination) could be easily identified as an alkane.

Summary

The use of an EM-CCD camera can greatly increase detection efficiency and speed, especially for the short integration times necessary with a confocal Raman microscope. The distribution of a 7.1 nm PMMA as well as a 4.2 nm alkane layer on a glass substrate could be easily detected and identified with an integration time of only 7 ms per spectrum, which reduced the overall acquisition time to 5.4 minutes for a 200×200 (= 40,000) spectra confocal Raman image. For very small signals that are dominated by the CCDs readout noise, the use of an EM-CCD can improve the S/N by a factor of 5–10 compared to the best available standard CCDs.

WITec GmbH

Hörvelsinger Weg 6
89081 Ulm
Germany

Tel. +49 (0)731 140 700, Fax. +49 (0)731 140 7020
Harald.Fischer@WITec.de, www.WITec.de