

Automated Raman Imaging

Raman imaging is a versatile technique for investigating the chemical and molecular composition of materials. Here we present the new automated Raman microscope *apyron*.

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With confocal Raman imaging, the properties of solids and liquids can be analyzed with diffraction-limited spatial resolution down to 200 nm. As WITec developed advanced confocal Raman imaging systems used by scientists all over the world to produce two- and three-dimensional, high-resolution images of their samples, the obvious question is: "Why bother automating Raman microscopy?"

Indeed, there are many reasons for automating even an extremely successful system such as streamlining the work process and enhancing reproducibility, minimizing errors and inefficiency. The automated Raman imaging system *apyron* not only stores the measurements' settings but allows each person working on the instrument to easily refer back to the previously used conditions. Operation of the hardware is consolidated in software and the accumulation and processing of data is guided by automated workflow tools. All together, automation simplifies and accelerates the user's collection of data. However, any new development must not be achieved at the expense

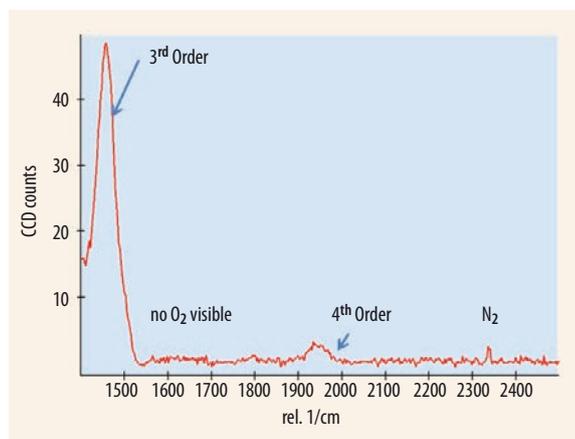


Fig. 1 The fourth order Raman band of silicon is a proof of sensitivity and optical efficiency for every Raman microscope. This spectrum was measured with the automated *apyron* system, using 532 nm laser wavelength, 3mW laser power, 20 minutes integration time, and a UHTS600 with 300 g/mm grating. Background data were subtracted.

of performance in resolution, speed or sensitivity of the microscope.

Confocal Raman imaging

The Raman effect, discovered by Nobel Laureate Chandrasekhara Venkata Raman, is based on light interacting with the chemical bonds of a sample. When photons strike a molecule they induce transitions between vibrational and rotational states of the molecule resulting in specific energy shifts of the scattered photons. The scattering molecule can either absorb energy (Stokes scattering) from the pho-

tons or transfer energy to them (anti-Stokes scattering). The shifts in energy (Raman lines) that are characteristic for individual molecular bonds can be recorded as a spectrum and thus they provide qualitative and quantitative information – a “fingerprint” – of the material. The method is non-invasive and non-destructive, and the samples don't require any preparation. For Raman imaging a complete Raman spectrum is recorded at every pixel of the measurement area. An appropriate software tool can, through integration of the data in certain spectral areas, generate a Raman image depicting the spatial

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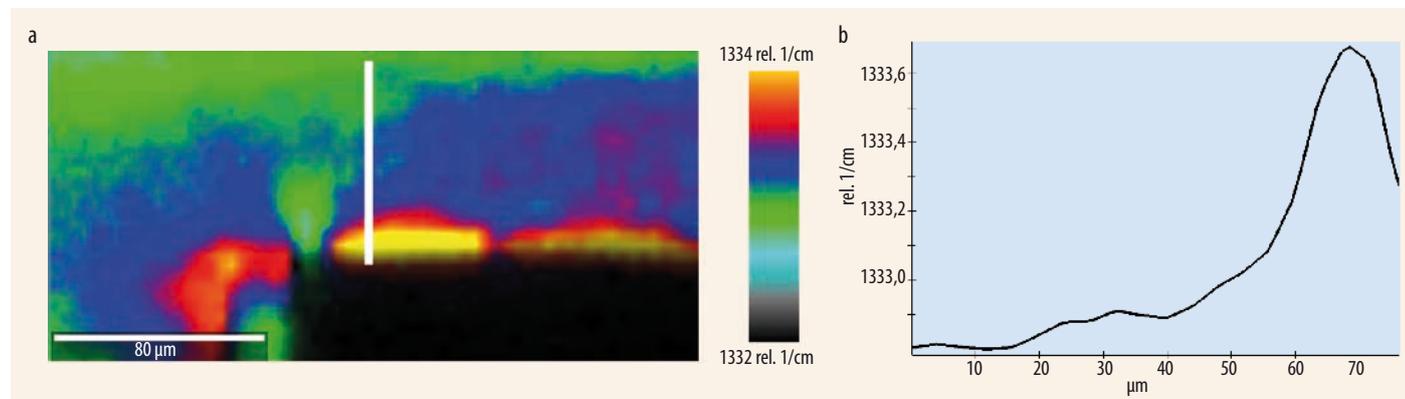


Fig. 2 A confocal Raman image of a diamond reveals stress levels around a garnet inclusion (a). High stress levels are displayed in yellow and red. Note that stress levels differ only by

two wavenumbers. The image was taken with the parameters: 100 × 30 pixels resolution, 0.1 s integration time per spectrum, 532 nm excitation wavelength, and 20 mW laser power.

The cross section along the white line in (a) shows the minimal differences in wavenumbers between 1332 and 1334 rel. 1/cm (b). The resolution of the peak shift is 0.005 rel. 1/cm.

distribution of different molecular species in the sample. Confocal Raman microscopy systems enable the layered representation of samples along the vertical z -axis without the need for sectioning. In this way, 3D images and depth profiles can be generated with high resolution [1].

Automated functions

Integrated measurement routines can be selected and initiated through the user interface, with all hardware settings centrally controlled and then stored along with the acquired data. The choice of the wavelength, the adjustment of the laser power with TruePower, the selection of the measurement area and the position of the sample as well as the automatic calibration and signal maximization with TrueCal and TrueScan are important features of the user interface.

A change between different excitation wavelengths can be an effective method to suppress potential fluorescence effects, to increase the intensity of the Raman signal, or to achieve a greater penetration depth. The excitation wavelength can be chosen through a simple drop-down menu. In the background, the appropriate optical components such as beam splitters and filters are then automatically brought into the beam path.

Laser power is an extremely important parameter, especially if

a measurement is to be repeated under the same conditions, a sample is extremely delicate, or both. The *apyron* Raman imaging system allows the absolute determination of laser power in 0.1 mW increments. Using TruePower, the actual laser power value is continuously measured and recorded enabling it to be matched precisely in subsequent measurements.

The integrated video camera with Koehler illumination allows the sample area of interest to be selected, then quickly and easily brought into focus while being displayed on the monitor. Switching between the white light view and Raman imaging is accomplished on-screen, as is the determination of imaging parameters such as scan range, number of pixels, and integration time per pixel.

The microscope includes a built-in calibration source so that an automatic calibration of the spectrometer may be performed (TrueCal). The necessary calibration modes for the optical and mechanical components are defined in the software and can therefore be retrieved at any time. The TrueCal function consists of a software-controlled routine that enables the optical signal to be directed under video control for maximum power into the entrance of the fiber that leads to the spectrometer. The result is a constantly-optimized optical throughput for high performance spectroscopy with Raman imaging.

A recent advance in system architecture that has been implemented in all WITec microscopes features a new beam path and fiber coupling that enables the integration of various spectrometers. The focal length of the spectrometer, its grating, and the CCD camera strongly influence the spectral resolution of the microscope. In general, the longer the spectrometer's focal length, the higher the groove density of the grating and the smaller the camera's pixels, the better the spectral resolution, as outlined in the following.

Automation in use

To demonstrate the *apyron's* performance we measured the fourth order Raman band of silicon. Measurements of higher order overtone Raman bands of the Si Raman peak at 520 rel. 1/cm are commonly considered an indicator of the sensitivity of a spectroscopic system because of their low intensities. Using the UHTS600 spectrometer with a confocal length of 600 mm, the corresponding Raman band could be resolved even at the extremely low laser power of 5 mW (Fig. 1).

Raman imaging has been widely used to evaluate internal stresses around impurities and grain interfaces in diamond. A shift of the diamond band from approximately 1332 rel. 1/cm to higher wavenumbers corresponds to in-

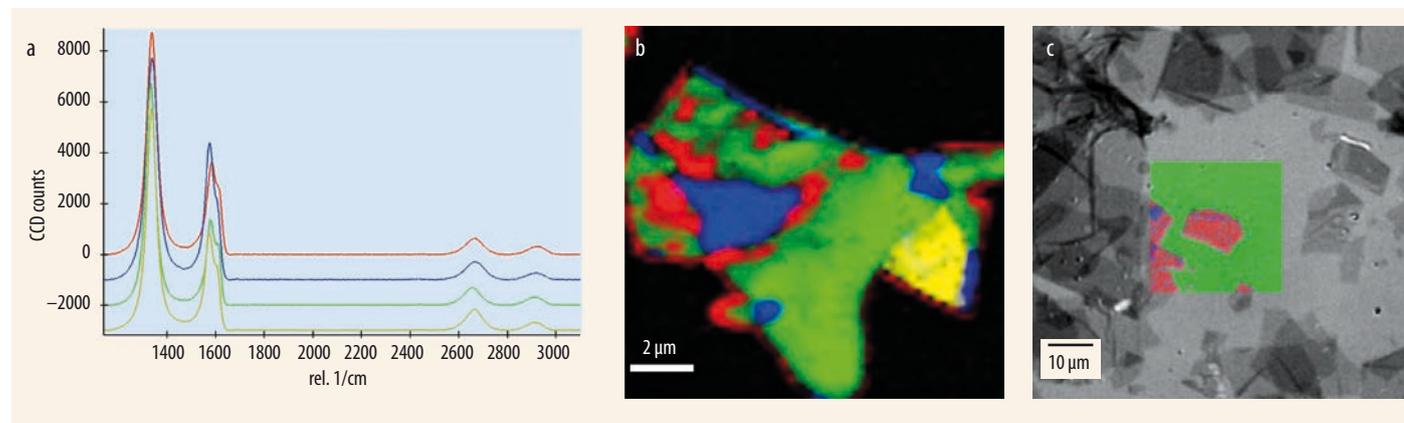


Fig. 3 Four averaged Raman spectra of graphene oxide clearly show the typical characteristics such as the D-band and the G-band (a). The background is corrected for

and an offset is applied to distinguish the spectra. A color-coded Raman image (b) is depicted from the spectra with the image parameters: $15 \times 15 \mu\text{m}^2$ size, 60×60 pixels resolution,

0.0016 s integration time per spectrum, 532 nm excitation wavelength, and 47 mW laser power. A video image of the sample (c) shows the color-coded Raman image overlaid onto the scan area.

creasing stress. Here, such an analysis reveals the sensitivity, speed and spectral resolution of the automated *apyron* system (Fig. 2). The stress levels around the inclusion could be differentiated on a scale of only two wavenumbers (rel. 1/cm) at a peak shift resolution of 0.005 rel. 1/cm.

To illustrate the speed of an *apyron* system equipped with the most sensitive accessories, a graphene oxide flake was measured (Fig. 3). Raman spectroscopy can differentiate graphite, fullerenes, carbon nanotubes, and graphene by their typical Raman bands [2]. For graphene the prominent features are denoted as the D-band at approximately 1345, the G-band at 1582, D'-band at 1620 and 2D-band at 2680 rel. 1/cm. The ratio of intensity of D/G-bands is a measure of defect density present in the structure [3]. The examined sample shows a narrow, sharp D-band and a much smaller G-band. Two spectra were detected with slightly different

relations of the D- to the G-band intensities. This can be interpreted as resulting from local variations in the functionalization density. The spectral data were recorded extremely quickly at 1.6 milliseconds per spectrum using a camera in EMCCD (Electron Multiplying Charge Coupled Device) mode and a 600 mm focal length spectrometer.

Summary

The automation of Raman imaging realized by the *apyron* drastically reduces the time and effort required to become familiar with the operation of the instrument. This accelerates the initiation of measurements and increases the rate of sample turnover compared to conventional systems. With the *apyron's* straightforward interface, the user can concentrate on the experiment without being distracted by com-

plex operating procedures. Such a system is well suited for multi-user laboratories with changing staff, for beginners in Raman imaging, or for specialists who rely on high performance. It simplifies and expedites the workflow for every user.

- [1] T. Dieing, O. Hollricher, and J. Toporski (Eds.), *Confocal Raman Microscopy*, Springer (2011)
- [2] Y. Stubrov et al., *Nanoscale Research Letters* **11**, 2 (2016)
- [3] L. G. Cancado et al., *Nano Letters* **11**, 3190 (2011)



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