

Molecular and Microanalysis Newsletter

Winter 2009

This newsletter is produced by HORIBA Scientific's Molecular & Microanalysis Team, to provide our customers, colleagues & friends with up-to-date information in the fields of Raman, fluorescence, SPRI & XRF Instrumentation and Applications.

What's inside:

Page 2: Raman in the Photovoltaic World

The photovoltaic industry, more commonly called the 'solar' industry, has been developing rapidly in the past several years. Raman spectroscopy is a very useful tool to analyze many materials used in the photovoltaic industry, especially silicon-based materials and the somewhat newer materials such as GaAs, CIS (CuInSe) and CIGS (CuInGaSe).

Page 3: New Nanosizer® software yields more power for analyzing Single-Wall Carbon Nanotube (SWCNT) photoluminescence data.

The new Nanosizer® software facilitates simulation of Excitation-Emission Maps from SWCNT species using a double-convolution (Patent Pending) algorithm to quantify model peak parameters that can be assigned to individual SWCNTs.

Page 4: The discovery of Van Gogh's black cat in "Le Jardin de Daubigny"

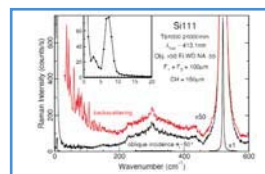
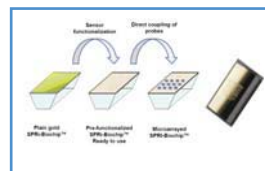
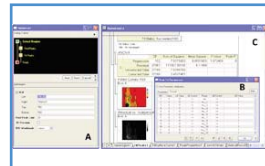
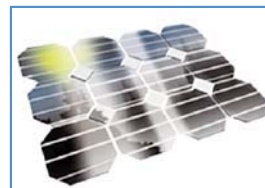
An XGT system with extra large sample chamber was used for the analysis of the famous painting. **Micro-XRF** imaging has been used to determine that a "black cat" was originally painted by Vincent van Gogh in his Le Jardin de Daubigny currently owned by the Art Museum of Hiroshima.

Page 5: Ready-to-use functionalized biochip for high throughput multiplex probes immobilization.

Microarrays are usually prepared with robotic spotters which deposit nanoliter droplets of probes solutions on a solid surface and in a pre-defined pattern. SAM surface chemistries developed on gold sensor and commercially available allow the immobilization of spots of native or conjugated probes directly on the surface.

Page 6: Off-axis illumination device for low frequency measurements

For exploring the very low frequency range of the Raman spectrum, the main challenge to overcome has to do with an appropriate rejection of the elastic Rayleigh scattering, which tends to mask the vibrational information of interest.



Ishai Nir, Marketing Manager, Horiba Scientific

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Raman in the Photovoltaic World

By Emmanuel Leroy, HORIBA Scientific

The photovoltaic industry, more commonly called the 'solar' industry, has been developing rapidly in the past several years, partly because oil prices seem to be rising dramatically, but also because many large players in the semiconductor industry saw photovoltaics as a normal transition out of the very competitive and high risk field of microchip manufacturing.

Silicon-based materials

The vast majority of photovoltaic substrates currently are based on silicon, a material that microchip makers understand well and have spent years developing processing tools for, while yield improvement and cost reductions are also very common to both semiconductor and photovoltaic industries. Even the metrology required for process monitoring is somewhat similar between the industries.

The one major difference is that the technology required to produce photovoltaics is more concerned about reducing materials costs and increasing its conversion efficiency than racing to shrink devices to the smallest possible area, making the process a lot less demanding in terms of cleanliness or accuracy.

Raman spectroscopy has been used in basic research for years to qualify stress in microchips but is just coming to maturity as far as being integrated into low level clean-rooms with robotics and low particle-emission. Raman has become a technique of choice easily integrated into this less stringent process environment for a much simpler application yet critical for the photovoltaic industry: measuring the ratio of amorphous to crystalline silicon.

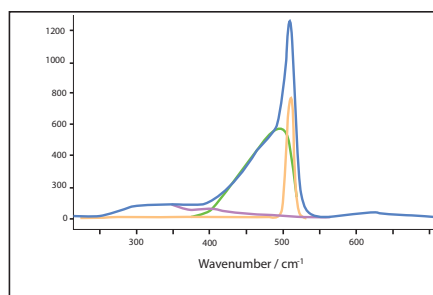


Fig1. Spectrum of a mixed amorphous/crystalline silicon glass

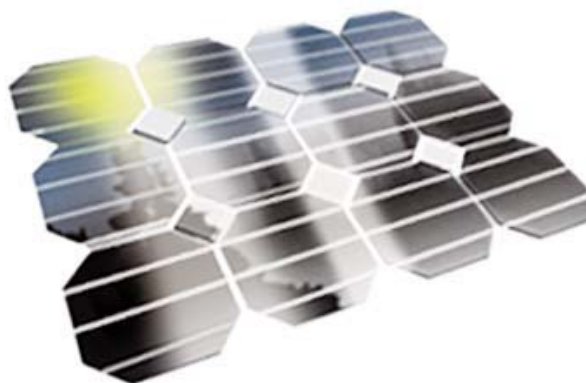
The right ratio is an optimum combination of high-efficiency but expensive crystalline silicon, and cheaper-to-produce but less-efficient amorphous silicon. Layers of only the necessary thickness are deposited on inexpensive substrates like glass to minimize cost.

Raman spectroscopy gives a clear answer to quantification of crystallinity in a few seconds, and can easily be fiber-optically coupled to small Raman probes, making integration into process lines simple, even for measurements over very large panels.

Other photovoltaic materials...

Raman spectroscopy is also a very useful tool to analyze many other materials used in the photovoltaic industry, especially for the somewhat newer materials such as GaAs, CIS (CuInSe) and most commonly CIGS (CuInGaSe). These compound semiconductors have been known for decades to be efficient photovoltaic materials based on laboratory experiments. Manufacturing formerly required slow, low throughput equipment until the semiconductor industry brought its expertise in growing complex films with more cost-effective methods.

The manufacturing process can be cost-effective only if it is possible to improve yields during the substrate growth phase by characterizing the films before building the substrate up into a device. Raman spectroscopy is an effective tool here as well, giving valuable and precise quantitative information on the stoichiometry of the film.



Raman spectroscopy is also an effective method for examining polymer-based photovoltaic materials like organic solar cells (PET, PEDOT:PSS, and P3HT), as well as many other materials used as transparent conductive oxides like ZnO.

Raman spectroscopy equipment can easily be configured to perform photoluminescence measurements, and may be combined with other techniques such as fluorescence lifetime measurements using TCSPC or FT-IR, making Raman spectroscopy a very versatile tool for the photovoltaic industry.

The 'OneCheck™' function for Auto-calibration

Dr Arnaud Zoubir, HORIBA Scientific

Analytical instruments used in regulated industries are increasingly required to follow strict protocols. The OneCheck package provides hassle-free validation of the most reliable results. A typical Raman spectrum consists of many data points, characteristic of the chemical nature of the sample. Proper interpretation of these results. The AutoCalibration and AutoValidation routines can even be automated.

The AutoCal function of the XploRA automatically compensates for drift. Not only calibrated in frequency, it also calibrated in intensity, so that the results are consistent. This makes it easier to compare spectra and perform spectral analysis.

The AutoVal function goes a step further by automatically checking if the signal intensity is above a pre-defined minimum and that the results obtained during the routine are stored in a log file accessible by the user. This helps in the detection and preventing possible failures.

New Nanosizer® software wields more power for analyzing Single-Wall Carbon Nanotube (SWCNT) photoluminescence data.

Adam M. Gilmore, Fluorolog Product Manager, HORIBA Scientific

Arguably the most important characteristic of SWCNTs is their bandgap semiconducting behavior which has the potential to revolutionize many electronic, biological, medical and even combined applications. The bandgap energy is primarily determined by the respective SWCNT diameter, as dictated by the 'quantum confinement effect', however, the helical twisting of the component graphene sheet can differentiate metallic from bandgap semiconductor species (1). Subsequent to the seminal observations of bandgap photoluminescence (PL) from SWCNTs (2), HORIBA Scientific developed the Nanolog® which rapidly measures the relationship between the absorbance and emission PL bands of semiconducting SWCNTs. The 3-dimensional PL excitation-emission maps (EEMs) obtained with the Nanolog® contain several key pieces of structural information regarding SWCNT samples including, among others, the diameter, helix (twisting) angle, concentration (2) and length (3) distributions in addition to information about energy transfer processes among SWCNTs in bundles (4).

The new Nanosizer® software facilitates simulation of EEMs from SWCNT species using a double-convolution (Patent Pending) algorithm to quantify model peak parameters that can be assigned to individual SWCNTs. The new, enhanced software interface is contained within Origin®Pro8 and the essential elements are shown in Figure 1. Key features include the ability to globalize peak parameters (fixing, sharing, constraining), use of energetic (eV or cm^{-1}) or wavelength units, a Voigt function for deconvolving the instrument response from the emission line, complete workbook-style reports and themes for rapid, comparative fitting of related EEMs. The general flow of the program includes the following 5 steps: 1) select a region of interest from an EEM, 2) scan the EEM to generate the initial model parameter set, 3) adjust the fitting engine, global linking and report settings, 4) fit and evaluate the report and 5) assign and plot the SWCNTs based on the user-editable library. Figure 2 shows key example graphical report features from the new Nanosizer® from an analysis of an EEM of CoMoCAT (5) SWCNTs suspended in D2O with NaDDBS as a surfactant.

Notably, the new Nanosizer® is also capable of simulating 3D EEMs from other types of materials for purposes of component identification and quantification. Importantly, the Nanosizer® can be used to simulate 3-dimensional maps of Raman analysis signals corresponding to the SWCNT Radial Breathing Modes (RBMs) when the latter are measured as a function of excitation wavelength with a tunable Raman laser source (6). The new Nanosizer® has thus clearly evolved into a highly powerful tool for simulation and analysis of SWCNT data and is now applicable to both PL and Raman spectroscopies.

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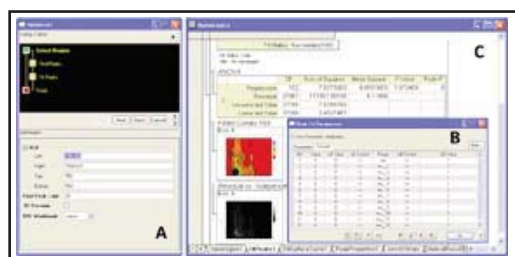


Fig1 shows the main Nanosizer® operating menu (A), the Fit Control menu (B) and the final report work-book (C).

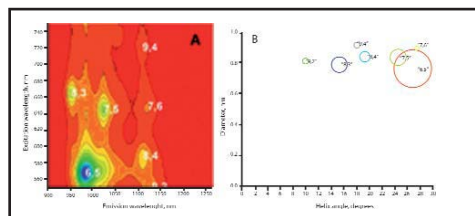


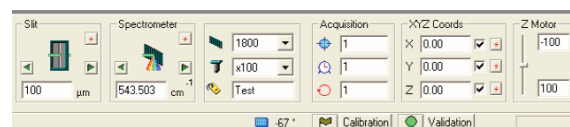
Fig2 shows the Excitation-Emission map for a typical CoMoCAT sample (A) and the corresponding plot of the Diameter and Helix angle for the Nanosizer analysis (B). The size of the circles in B is proportional to the peak intensity in A.

Automation and Auto-validation of Raman data

Increasingly required to comply with validation processes through the use of IQ/OQ/PQ proven methods for the XploRA system, to comply with these requirements and to ensure a number of peaks assigned to specific frequencies and having different intensities, calibration and validation of the system are a guarantee of precise and reliable data. Calibration can be scheduled periodically during the system's downtime to optimize working time.

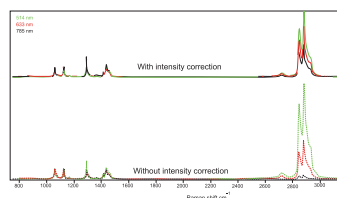
Ensuring any potential variations to ensure correct frequency labeling. The system is not just a data collection tool, it also ensures that spectra of the same sample have the same peak ratios with the different lasers. This is achieved through the use of the system's auto-validation feature.

Monitoring the performance of the instrument. It systematically checks that the reference requirements are met for a true confocal measurement. The reference spectra recorded by the system operator, in order to help trouble-shooting. It also allows monitoring the system evolution over time.

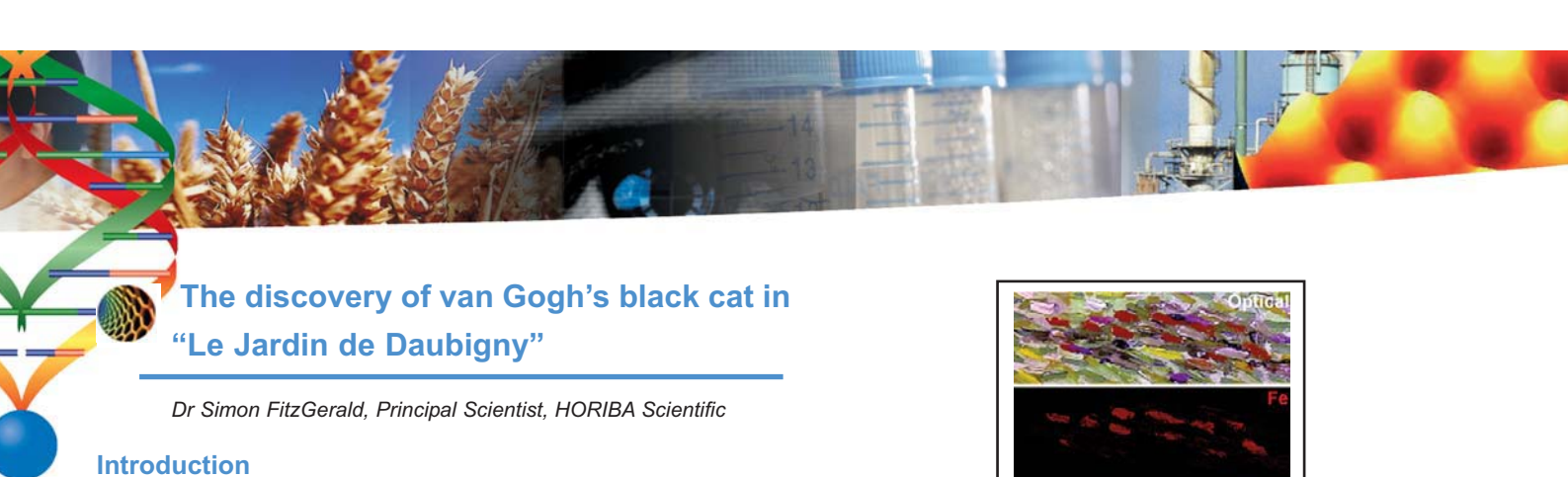


Color of the icons indicates the status of the system
Green = OK; Red = Not OK

- Self check-up for quick start-up
- Foolproof calibration for reliable results
- Traceability and easy trouble-shooting



The above figure shows spectra of LDPE polymer obtained at three different wavelengths with and without intensity correction. Without the intensity correction, the relative intensity of the peaks close to 3000cm^{-1} decreases dramatically with the longer wavelength due to the lower sensitivity of the CCD detector in this spectral region. The intensity correction gets rid of this effect.



The discovery of van Gogh's black cat in "Le Jardin de Daubigny"

Dr Simon FitzGerald, Principal Scientist, HORIBA Scientific

Introduction

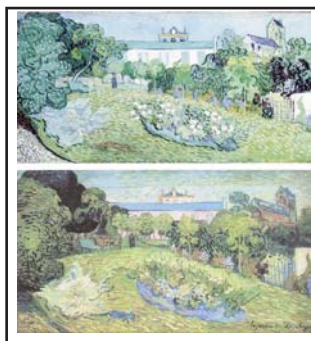


Fig1 Vincent van Gogh's *Le Jardin de Daubigny*. Top – Hiroshima version. Bottom – Basel version.

For three years the Hiroshima Museum of Art (Japan) has been conducting major research into an important work in its collection, Vincent van Gogh's *Le Jardin de Daubigny* (Daubigny's Garden). A work of the same name and composition and of similar size to this Hiroshima piece (Figure 1, top) can be found in the Kunstmuseum Basel (Figure 1, bottom). In one of his letters (No. 651), van Gogh attached a sketch of *Le Jardin de Daubigny* with the description of "a black cat in the foreground".

Comparing the two works, the Basel painting has a blue-colored 'black cat'. This cat is absent from the Hiroshima piece, and in its place is a dark brown touch-up. Hiroshima Museum of Art had analysed their *Le Jardin de Daubigny* using X-ray, ultraviolet ray, and infrared photography, but the existence of the black cat could not be confirmed. Recent work by Professor Susumu Shimoyama (KIBI International University, Japan) has used the XGT X-ray fluorescence microscope to analyse the 'black cat' region of the Hiroshima painting. The penetrating nature of the primary X-rays allows characterisation of pigments both at and beneath the surface of the painting.

Experimental Results

An XGT system with extra large sample chamber and a heavy duty stage (Fig2) was used for the analysis of the painting, allowing the entire painting (approximately 100cm x 50cm) to be accommodated within the safety enclosure.



Fig2: XGT-5000 with extra large sample chamber, showing the van Gogh painting set up for analysis.

Figure 3 shows the mapped images for chromium and iron, together with the optical image of the analysis region. The shapes of the cat's head, neck, forelegs and tail are discernible in the chromium element mapping image. Also, parts of the cat's head, forelegs and tail can be visualised in the lead image (not shown). This indicates that the black cat was indeed included in this painting, and that chrome yellow (lead chromate) was the chosen pigment.

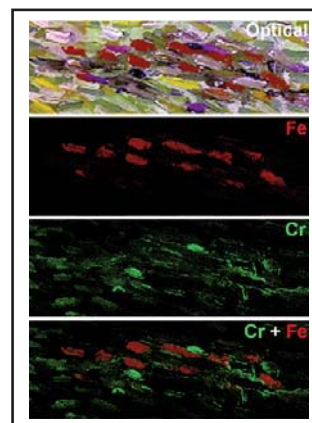


Fig3: optical (top) and chromium and iron element images from the 'black cat' region of the Hiroshima painting.

The iron image (Figure 3) shows correlation of this element with the dark brown paint used in the touch up, which is presumed to be ferric oxide. However, closer observation shows that there is low intensity iron overlapping with the chromium. Since no single paint colour contains both chromium and iron elements, it can be presumed that the faint overlapping iron pigment is derived from Prussian blue paint (ferric ferrocyanide). Thus it has been determined that the cat was painted using both chrome yellow and Prussian blue paints.

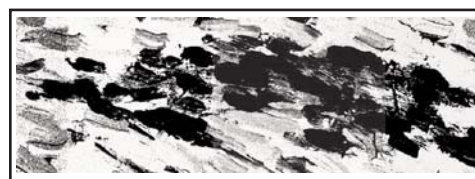


Fig4: zinc element image from the 'black cat' region of the Hiroshima painting.

The zinc mapping image (Figure 4) shows that this element is not present where the cat is found. Since the surrounding grass is painted with green paint mixed into a base of zinc white (zinc oxide) this indicates that by design the grass was not painted in the region where the cat was to be painted. It can be presumed that the cat was directly painted onto the canvas. Later on, the cat was covered up with a white paint (lead white) and brown touches.

Summary

Micro-XRF imaging has been used to determine that a "black cat" was originally painted by Vincent van Gogh in his *Le Jardin de Daubigny* currently owned by the Art Museum of Hiroshima. The combination of elemental specificity and analysis below the paint surface has finally solved the mystery which has surrounded this painting for many years.

Acknowledgments

Professor Susumu Shimoyama of the Graduate School of Conservation and Restoration for Cultural Properties Studies at KIBI International University, Japan, is thanked for kindly allowing his research data to be used for this application note.

Additional thanks are offered to the Hiroshima Museum of Art curator Mr Yoshiyuki Furutani, to painter and painting materials researcher Mr Hiroshi Yoshida, to Mr Hideyuki Oh-hara, who studied the work through the eyes of a restorer, and to Kibi International University assistant professor Mr Hideaki Takagi and research associate Mr Koji Ohsita.



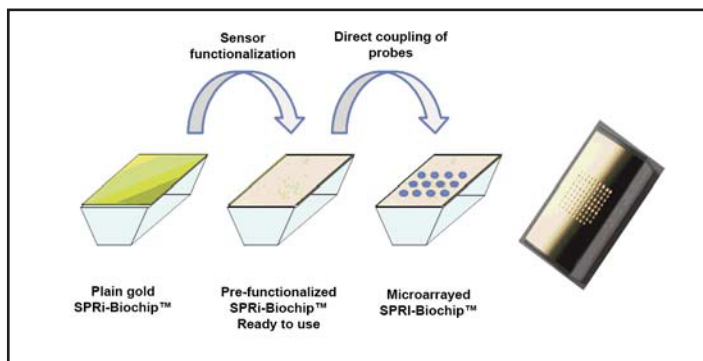
Ready-to-use functionalized biochip for high throughput multiplex probes deletes immobilization.

Sophie Bellon, Karen MercierGenOptics
Chiraz Frydman, HORIBA Scientific

Surface Plasmon Resonance is an emerging tool in the bio and life-sciences markets. It offers a new generation in the multiplex label-free interaction analysis of bio-molecules, providing information on kinetic processes (association and dissociation), binding affinity and real-time target molecule detection. The SPR imaging technology takes SPR analysis a step further. It is a sensitive label-free method of visualizing the whole surface of the biochip via a video CCD camera. This design enables the biochips to be prepared in a microarray format providing SPR interaction information simultaneously.

Microarrays are usually prepared with robotic spotters which deposit nanoliter droplets of probes solutions on a solid surface and in a pre-defined pattern. SAM (Self Assembled Monolayer) surface chemistries developed on gold sensor and commercially available allow the immobilization of spots of native or conjugated probes directly on the surface.

Receptors of interest can be proteins (antibody, antigen, peptides),

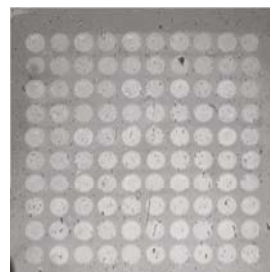


nucleic acids (DNA, RNA) in their native state or conjugated with biotin or GST-tag.

SAM consists on self assembled mono or multi-layers presenting a free chemical function or group on the upper layer for direct covalent binding with probes:

- Succinimidyl ester (NHS) or aldehyde (CHO) terminated chains (namely CS and CO respectively) are amine-reactive so proteins compounds presenting accessible primary amine (from Lysine amino-acid or N-terminal extremity) are directly grafted on the chains extremity.
- Receptors (protein or nucleic-acid) derivatized with biotin group can be also immobilized on surfaces coated with an extravidin extra-layer (CSe or COe biochips). Extravidin is covalently attached on the whole SAM and biotin-tagged proteins or DNA can be immobilized through extravidin-biotin interaction. Such an immobilization method is expected to better retain protein or DNA functionality than direct covalent binding.
- Fusion protein such as those with glutathione S-transferase (GST) obtained from recombinant technology can be used for the

immobilization of oriented protein of interest using a ready to use surface totally covered with an anti-GST antibody (CTg biochip).



SPRi image of 100 spotted receptors on SAM ready-to-use biochip

After receptors spotting, incubation time, washing of excess solution and a potential blocking step, microarray is ready for SPRi probe-target interactions study.

	DIRECT COUPLING				
	CS	CO	CSe	COe	CTg
	NHS	CHO	NHS/ext	CHO/ext	CHO/GST
Proteins	X	X	X	X	
Nucleic Acids			X	X	
Tagged Proteins					X

SAM SPRi biochip proposed

Immobilization by direct coupling of probes on functionalized "ready-to-use" surface is available:

- via probes amino group on CS, CO biochips
- via biotinylated probes on extravidin surface CSe, COe
- via GST-tagged probes on anti-GST antibody derivatized surface CTg

Functionalized SPRi Biochips enable rapid and efficient probes immobilization for biomolecular interaction study, within multiplex format, in real-time and without labelling.

Note: CS functionalized biochip' are compatible with MS (Mass spectrometry) coupling.

Off-axis illumination device for low frequency measurements

Sophie Morel, Sales Manager, HORIBA Scientific

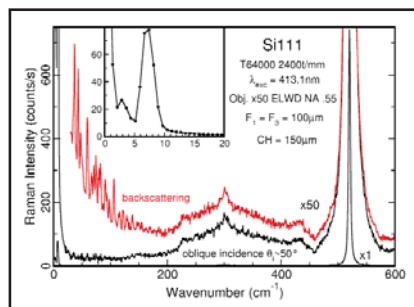
For exploring the very low frequency range of the Raman spectrum, the main challenge to overcome has to do with an appropriate rejection of the elastic Rayleigh scattering, which tends to mask the vibrational information of interest. In addition to using an optimal filtering device such as a double subtractive fore-monochromator, an off-axis illumination can allow a significant gain in terms of low-frequency cut-off and access to spectral features located in the direct vicinity of the laser peak.

This device relies on a separate side-arm with steering optics enabling laser light injection with an oblique or grazing incidence, thereby eliminating most of the Rayleigh scattering which is reflected out of the acceptance cone of the collection optics.

This option not only enlarges the field of low frequency Raman measurements, but also allows to explore new routes for specific polarisation analysis, measurements within Diamond Anvil Cells (DAC) or waveguides characterisation.



Data courtesy from Antoine Zwick, CEMES, Toulouse, France



Comparison between 2 silicon Si111 spectra recorded on a T64000 Raman system under a standard 180° backscattering configuration (blue) and an oblique incidence (red). The reduction of the Rayleigh scattering signal when using the off-axis configuration is clearly demonstrated. The Brillouin peak shown on the insert has been measured closing the intermediate slit of the T64000 fore-monochromator.

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