Confotec series 3D scanning confocal microscopes: latest technologies and applications

S. Shashkov (SOL instruments LTD, Minsk, Belarus)
Confocal Raman Microscopy. Principles.

A typical Raman spectroscopy set-up is shown in the next Figure. A pinhole blocks the scattered light which is coming from the out-of-focus points. A suitable light source and efficient detection system are necessary for Raman measurements.

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3D Raman Confocal Microscope Confotec®

Layout of the Raman instrument – Confotec NR500 (SOL instruments)

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Instrumentation
3D Raman Confocal Microscope Confotec®

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Objective numerical aperture

\[ NA = n \sin \theta \approx n \frac{D}{2F} \]

Focused spot diameter

\[
\text{Spot diameter} = \left( \frac{4\lambda}{\pi} \right) \left( \frac{F}{D} \right) * M^2
\]

\[
\text{Spot diameter} = 0.61 \frac{\lambda}{\text{NA}} * M^2
\]

Laser focus depth

\[
\text{DOF} = \left( \frac{8\lambda}{\pi} \right) \left( \frac{F}{D} \right)^2 * M^2
\]

\[
\text{DOF} \approx \frac{4\lambda}{\text{NA}^2} * M^2
\]

Thus, the spatial resolution is determined by the wavelength of light (\( \lambda \)), medium (n) and the lens numerical aperture (NA).
Lateral (XY) spatial resolution

\[ R_{\text{Lateral}} = \frac{0.61 \cdot \lambda}{\text{NA}} \]

\[ R_{\text{Lateral}} = \frac{0.50 \cdot \lambda}{\text{NA}} \]

\[ R_{\text{Lateral}} = \frac{0.47 \cdot \lambda}{\text{NA}} \]
Lateral resolution is 360 nm with a 514.5 nm laser and NA=0.9 objective.
Objective types: air and immersion

Refractive index:
Air $n=1$
Oil $n=1.515$
Water $n=1.33$
Glycerin 1.45

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$$NA = n \sin \theta \approx n \frac{D}{2F}$$
SIL objective

Checkpoint technologies objective
NA3.3

Johnson Kasim, Yu Ting, You Yu Meng, Liu Jin Ping, Alex See, Li Lain Jong, Shen Ze Xiang, Near-field Raman imaging using optically trapped dielectric microsphere, Optics Express (2008), vol. 16, no. 11, 7976-7984
Lateral resolution with SIL objectives

<table>
<thead>
<tr>
<th>SIL Material</th>
<th>SIL Refractive Index</th>
<th>Resolution (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>2</td>
<td>~350</td>
</tr>
<tr>
<td>Glass</td>
<td>1.845</td>
<td>178</td>
</tr>
<tr>
<td>Silicon</td>
<td>3.48</td>
<td>230</td>
</tr>
<tr>
<td>Silicon</td>
<td>3.48</td>
<td>~100*</td>
</tr>
<tr>
<td>GaAs</td>
<td>3.48</td>
<td>350</td>
</tr>
<tr>
<td>Sapphire</td>
<td>1.76</td>
<td>243</td>
</tr>
</tbody>
</table>

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The dimensions of the nanojets are smaller than the optical diffraction limit. Such nanojets can enhance the backscattering of visible light by several orders of magnitude. This may provide a new near-field technique.
Structured sample illumination

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Lateral resolution with a doughnut-shaped illumination

NA=0.5 and λ=371 nm

1.18 times better

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TERS

TERS (tip-enhanced Raman scattering) involve the use of metal coated AFM/STM tips to boost the amount of Raman scattering from molecules very close to them. The enhancement can be significant.
AFM – Raman combined system

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Electromagnetic enhancement

Light field enhancement at metallic nanostructures.

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Spectral resolution

The spectral resolution of a spectrograph can be defined as the Full Width at Half Maximum (FWHM) of its response to a perfectly monochromatic radiation:

Two spectral lines of equal intensity can be resolved, if the distance between two lines is more than their Full Width at Half Maximum.
Echelle grating

An echelle grating is a special type of diffraction grating characterised by a relatively low groove density, but a groove shape which is optimized for use at a high incidence angle and therefore in high diffraction orders.
Some Raman system producers are using the special definitions of spectral resolution:

**Pixel resolution (nm/pixel)**
Pixel resolution indicates a quantity of Raman light, which is scattered by a diffraction grating and delivered to a CCD (nm per pixel).

**Wavelength repeatability (accuracy)**
It is a precision of the fitted peak position.
Spectral resolution. Raman spectra of polypropylene.
Comparison of stress measurements (Raman peak position shift) using different laser excitation.

The sample consists 1, 1.5, 2 and 4 µm wide Si stripes separated by 4 µm shallow trenches.
Confotec™ NR500 with 5 lasers

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Input port of Confotec® NR500

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Detectors
Accessories

Cryostat (2.2 - 500 K)

TS1000 (up to 1000°C)

THMS350V (-196°C до 350 °C)

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Ultra-Low Frequency (Low Wavenumber) Raman

Typical transmission of Raman filters: Semrock "notch" NF01-633E-25 (curve 1), Semrock "edge" LP02-633RE (curve 2), OptiGrate BragGrate BNF-633 (curve 3)
RazorEdge® ultrasteep long-pass edge

LP03-532RE-25

186 cm\(^{-1}\)

LP03-532RU-25

90 cm\(^{-1}\)

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Edge Filter tilt

![Graph showing Raman intensity vs. Raman shift with a tilt in the filter.](www.solinstruments.com)
White light transmission

\[ \lambda(\theta) = \lambda_0 \sqrt{1 - (\sin\theta/n_{\text{eff}})^2} \]

\( n_{\text{eff}} = 2.08 \) for s-polarized light, \( n_{\text{eff}} = 1.62 \) for p-polarized light.

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Bragg notch filters

Volume Bragg gratings (VBG) is a volume hologram formed by photo-induced modulation of the refractive index of the recording media (photo-thermo-refractive glass).

The thickness of glass is 1-5 mm with modulation in the range of 5000 - 10000 planes/mm.

A large reflectivity of VBG may be reached in a narrow spectral range around a certain wavelength which fulfills the Bragg condition:

\[ \Lambda = \frac{\lambda}{2n} \]

where \( \Lambda \) is the grating period, \( \lambda \) is wavelength, \( n \) is refractive index.

The wavelength selectivity \( \Delta \lambda \) can be approximately estimated as

\[ \frac{\Delta \lambda}{\lambda} \approx \frac{\Lambda}{L} \]

where \( L \) is the grating thickness.

\[ \frac{\Delta \lambda}{\lambda} \approx 10^{-5} \]
Low frequency Raman spectroscopy provides valuable information on a wide variety of materials, e.g.

- **Polymers** (LA modes),
- **Crystals** (lattice modes),
- **Carbon nanotubes** (Radial breathing modes),
- **Graphene layers** (C-bands),
- **Semiconductors** (folded acoustic modes),
- **Gases** (rotation modes),
- **Pharmaceutical tablets** (low frequency signatures), etc.
Advantages of VBG

- VBG allow measurements down to 10 cm$^{-1}$
- Operating range: 400 – 2500nm
- BNF’s enable simultaneous measurements of Stokes and anti-Stokes Raman bands
- Raman signal transmittance: up to 95%
- No polarization dependence
- No degradation in high power laser

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Confocal Raman microscope Confotec for ultra-low frequency Raman measurements

1 – laser
2 – laser line cleaning filter (BragGrate-Bandpass filter)
3 – aperture diaphragm
4 – beam expander
5 – BragGrate-Notch filters (bandwidth < 10cm⁻¹)
6 – XY scanning mirrors
7 – optical microscope
8 – aperture diaphragm
9 – monochromator-spectrograph
10 – spectral CCD

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Amplified Spontaneous Emission (ASE):

2 - BragGrate-Bandpass filter (ASE filter)
This spectrum demonstrates the measurement capability (as well as the Stokes and anti-Stokes measurements)
Several folded modes have been observed. A narrow peak at 15.8 cm$^{-1}$ is the $E_2$ mode of the 4H polytype (space group $C_6^{4v}$). Two further bands correspond to $E_g$ mode (45.1 cm$^{-1}$) and to $A_g$ mode (111.3 cm$^{-1}$) of the 2H polytype with $D^{3}_{3d}$ symmetry.
The interlayer shear Raman mode of few layer graphene (C-band) can be observed from $43 \text{ cm}^{-1}$ (in bulk graphene) to $32 \text{ cm}^{-1}$ (in bilayer graphene).
C-band

P.H. Tan, The shear mode of multi-layer graphene
Further to previous data, various pharmaceutical tablets exhibiting low frequency Raman features were analyzed. All observed Raman peaks have frequencies very close to the excitation line.
Raman spectrum of Acetylsalicylic acid
Raman spectrum of Acetaminophen
Raman spectrum of Citramon
Raman polarization studies of highly oriented samples

The capability of polarized Raman spectroscopy to investigate the structure of single crystals and orientation of functional groups is discussed below. Suppose a crystal is irradiated with polarized radiation from the Y-direction with the electric vector of the incident radiation vibrating parallel to the Z-axis. The Raman scattering is observed in the X-direction with its polarization in the z-direction. According to Porto’s notation, this scattering geometry is \( y(zz)x \). The letters in the parenthesis give the components of the derived polarizability tensor that are measured \( \alpha_{zz} \).

Some scattering geometries for Raman spectra of crystals:

- \( y(zz)x \)
- \( y(xz)x \)
- \( y(zy)x \)
- \( y(xy)x \)

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Polarized Raman measurements with a Confotec® system

The analyser and the half-wave plate ($\pi/2$) may be installed in the next orientations:

<table>
<thead>
<tr>
<th></th>
<th>Laser light polarization</th>
<th>Raman detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Perpendicularly</td>
<td>—</td>
<td>$z(\text{xy})z$</td>
</tr>
<tr>
<td>Parallel</td>
<td>—</td>
<td>$z(\text{yx})z$</td>
</tr>
<tr>
<td>Perpendicularly</td>
<td>—</td>
<td>$z(\text{yy})z$</td>
</tr>
</tbody>
</table>

To analyze (zx), (xz), (zy), (yz) and (zz) components of the polarizability tensor, orientation of the crystal should be changed.

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Polarizer

Glan-Taylor prism

www.solinstruments.com
A half-wave plate allows control the light polarization
Half-wave plate

\( \lambda / 2 \)

- **Input polarization plane**
- **Linearly polarized input**
- **Crystalline optic-axis direction**
- **Output polarization direction**
Symmetry of vibration mode $\nu$ at polarized Raman spectroscopic measurements can be determined on base of experimental Raman tensors $\alpha_{\nu}$, compiled from the scattering intensities $I_{kl}$ ($k, l = x, y, z$) at the frequency $\nu$:

$$\alpha_{\nu} \sim \begin{pmatrix} I_{xx} & I_{xy} & I_{xz} \\ I_{yx} & I_{yy} & I_{yz} \\ I_{zx} & I_{zy} & I_{zz} \end{pmatrix}.$$  

$(\text{NH}_4)_2\text{Mg(SO}_4)_2\cdot6\text{H}_2\text{O}$ is monoclinic (space group P21/a or $C_{2h}^5$) with two units per crystal cell ($Z=2$). A transparent single crystal sample was prepared in the form of a cube with the facet length of 8 mm oriented according to the crystallographic axes $a$, $b$, $c \cdot \sin \beta$.

Site symmetry of ammonium ions and water molecules is C1.

Factor-group analysis for $(\text{NH}_4)_2\text{Mg(SO}_4)_2\cdot6\text{H}_2\text{O}$ predicts 36 Raman active vibrational modes ($18A_g+18B_g$).
Raman tensors of the $A_g$ and $B_g$ modes have the following overall form

$$A_g \begin{pmatrix} 0 & 0 & e \\ 0 & 0 & f \\ e & f & 0 \end{pmatrix}$$

$$B_g \begin{pmatrix} a & d & 0 \\ d & b & 0 \\ 0 & 0 & c \end{pmatrix}$$

$A_g$ modes should be active in XX, XY, YX, YY and ZZ geometries (XY and YX are identical).

$B_g$ modes should be active in XZ, YZ, ZX and ZY geometries (XZ and ZX are identical, YZ and ZY are equivalent too).
Raman active modes at the spectral region between 2800 and 3600 cm⁻¹:

**A₉ symmetry:**
- 3 modes of $\nu_1$ ($\text{H}_2\text{O}$) (symmetrical stretching)
- 3 modes of $\nu_3$ ($\text{H}_2\text{O}$) (asymmetrical stretching)
- 1 mode of $\nu_1$ ($\text{NH}_4$) (symmetrical stretching)
- 3 modes of $\nu_3$ ($\text{NH}_4$) (symmetrical stretching)

**B₉ symmetry:**
- 3 modes of $\nu_1$ ($\text{H}_2\text{O}$) (symmetrical stretching)
- 3 modes of $\nu_3$ ($\text{H}_2\text{O}$) (asymmetrical stretching)
- 1 mode of $\nu_1$ ($\text{NH}_4$) (symmetrical stretching)
- 3 modes of $\nu_3$ ($\text{NH}_4$) (symmetrical stretching)
Vibrations with wavenumbers more than 3173 cm\(^{-1}\) assigned to water molecules.

The rest bands respect to vibrations of ammonium ions

### Wavenumbers (cm\(^{-1}\)) of stretching vibrations of isolated ammonium ion and water molecule

<table>
<thead>
<tr>
<th>Vibration</th>
<th>NH(_4^+)</th>
<th>H(_2)O</th>
<th>Symmetry of vibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>v1</td>
<td>3040</td>
<td>3657</td>
<td>A(_1)</td>
</tr>
<tr>
<td>v3</td>
<td>3145</td>
<td>3756</td>
<td>F(_2)</td>
</tr>
</tbody>
</table>

### Assignment of Raman spectra peaks

<table>
<thead>
<tr>
<th>xx</th>
<th>yy</th>
<th>zz</th>
<th>xy</th>
<th>xz</th>
<th>yz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>Ag</td>
<td>Ag</td>
<td>Ag</td>
<td>Bg</td>
<td>Bg</td>
</tr>
<tr>
<td>3353</td>
<td>3353</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3350</td>
<td>3344</td>
<td>3345</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3273</td>
<td>3276</td>
<td>3265</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3203</td>
<td>3173</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3139</td>
<td>3136</td>
<td>3137</td>
<td>3133</td>
<td></td>
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</tr>
<tr>
<td>3106</td>
<td>3100</td>
<td></td>
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<tr>
<td>3041</td>
<td>3079</td>
<td></td>
<td></td>
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<tr>
<td>2930</td>
<td>2928</td>
<td>2910</td>
<td>2910</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2857</td>
<td>2849</td>
<td>2857</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vibrations with wavenumbers more than 3173 cm\(^{-1}\) assigned to water molecules. The rest bands respect to vibrations of ammonium ions.
Carbon (graphite) fiber is a new material consisting of fibers about 5–10 μm in diameter and composed mostly of carbon atoms.
The **specific strength** is a material's strength (force per unit area at failure) divided by its density. It is also known as the **strength-to-weight ratio**.

<table>
<thead>
<tr>
<th></th>
<th>Tensile strength (MPa)</th>
<th>Density (g/cm³)</th>
<th>Specific strength (kN·m/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon fibre (AS4)</td>
<td>4300</td>
<td>1.75</td>
<td>2457</td>
</tr>
<tr>
<td>Stainless steel (304)</td>
<td>505</td>
<td>8.00</td>
<td>63.1</td>
</tr>
</tbody>
</table>

Specific tensile strength of various materials. Comparison of carbon fiber properties with other engineering materials.

As can be seen, carbon fibre has a tensile strength almost 8.5 times greater than that of steel, yet is 4.5 times less dense.
A Raman spectrum of a carbon fiber is shown below

The disorder-induced band, or D-band, occurs at about 1372 cm\(^{-1}\). The Raman band at \(~1595\text{ cm}\(^{-1}\) (G-band) can be related to C–C vibrations in Graphite and is present in all carbon fibers.

The ratio of the integrated intensities of the D-band to the G-band is commonly used to characterize different kinds of disordered sp\(^{2}\) carbon.
A relationship between band intensities should be proportional to the degree of structural order with respect to graphite structure. Indeed, this relationship has been largely used in the literature in different forms:

1) total integral intensity ratio $I_D/(I_D+I_G)$,
2) integral intensity ratio $I_D/I_G$,
3) Raman peak intensity ratio $R = I_D/I_G$
1. Total integral intensity ratio: \( \frac{I_D}{I_D + I_G} = 61.3\% \)

The width of D band correlates well with the degree of disorder.

D-band width as a function of the degree of disorder [A. Cuesta, P. Dhamelincourt, Raman microprobe studies on Carbon materials, Carbon, Vol.32, No.8, pp.1523-1532]
2. The degree of structural disorder in the fibers may be characterized by the integral intensity \((I_D/I_G)\) ratio.

The respective surface crystalline size \((L_a)\) of the carbon fibers may be obtained by the following equation

\[
L_a = \frac{C}{(I_D/I_G)},
\]

where \(L_a\) is the surface crystalline size and \(C\) is equal to 44 Å.

Because the \(I_D/I_G\) ratio is 3.254, the surface crystalline size is 13.52 Å.

The slight distortion in graphitic structure of carbon fibers occur through the Breaking of aromatic bonds and reduction of surface crystallinity.
3. The Raman peak intensity ratio $R = I_D / I_G = 0.95$
Carbon fibers. 3D imaging

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MIPOS100
Carbon nanotubes

Carbon nanotubes (CNTs) have drawn much attention due to their unique structural, mechanical, thermal, and electrical properties.

Applications for nanotubes:

- Semiconductor devices
- STM / AFM tips
- New materials
- Battery addivities
- Polymer composites

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(Semiconducting) Single walled carbon nanotubes (SWCNs). Overall Raman spectrum.

The D (defect-activated)-mode peak is associated with the defects in the nanotube structure. The greater the relative intensity of this peak, the more defects in the structure appears.

The strong Raman peak at \(~1582 \text{ cm}^{-1}\) is related to the tangential C-C stretching vibrations (G-band).

The comparison of the ratios of these two peaks intensities gives a measure of the quality of the sample.

The RBM peak correspond to the radial breathing mode.
SWNT on Si substrate

Single walled carbon nanotubes have been deposited on a Si substrate by spin casting of their solution in ethyl alcohol.

Raman image of a CN
532 nm laser, 100 x NA0.95 objective, scanning step is 40 nm
According to atomic force microscopy characterization, the carbon nanotube diameters is 1.7 nm
Raman spectroscopy of a bent SWCN (RBM mode)

The radial breathing mode (RBM) is very sensitive to the diameter of SWCN.

The diameter of the nanotube is 1.75 nm (approximately) according to the relation

$$\nu_{RBM} (\text{cm}^{-1}) = \frac{223.5}{d_t \text{ (nm)}} + 12.5$$
Raman microscopy of a bent SWCN (G and D modes)

The tangential stretching mode is highly sensitive to strain and the local structural changes in CNs. The local structural changes and strain may occur due to curvature effects.

In the place of the greatest curvature, a decrease in the G-band frequency is clearly observed. The G-mode shifts downward upon nanotube bending, and it is attributed to the tensile stress.

Additionally, an increase of the D-band relative intensity at the given location of the carbon nanotube is also observed.

(The brighter the color corresponds to the higher frequency)
Polarization analysis of G band modes in CNs

SWCN emits and absorbs light polarized along the nanotube axis.
Cellulose analysis by Raman spectroscopy

- non-destructive testing method
- it can be used for depth profiling
- qualitative analysis of films
- quantitative analysis (degree of crystallinity, etc.)

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Raman spectrum of a film containing cellulose
532 nm, 2 sec exposure time, UPanFLN 40x NA 0.75

Reference spectrum of Microcrystalline cellulose
Raman spectrum interpretation

\[ \delta(\text{COH}) \ (\text{CCH}) \ (\text{OCH}) \]

\[ \nu_s(\text{COC}) \]

\[ \nu_{as}(\text{COC}) \]

\[ \delta(\text{CH}/\text{CH}_2) \text{ and } \delta(\text{OH}) \]
An empirical Method for estimating the degree of crystallinity of cellulose

The intensity of two spectral features present at 380 and 1096 cm\(^{-1}\) is significantly affected by crystallinity modification. The correlation between the degree of crystallinity and 380/1096 Raman intensity ratio is shown below.


Intensity ratio \(I_{380}/I_{1096}\) is 0.2
Degree of crystallinity is 26.3%
The increase in the degree of crystallinity of cellulose leads to the:

- higher mechanical strength
- higher density of the material
- lower chemical reactivity
- lower swelling
Micromechanical properties of cellulose

Cellulose Raman peak positions, as well as their shapes provide information about the stresses in the sample.

Mechanical strain ($\varepsilon$) affects the frequency shift ($\Delta v$) of the Raman band at 1096 cm$^{-1}$.

$$\frac{d\Delta v}{d\varepsilon} \propto E$$

$$E = \frac{\sigma}{\varepsilon} = \frac{F \times L_0}{A \times \Delta L}$$

- $E$: Young's Modulus/ GPa
- $\sigma$: Stress /MPa
- $\varepsilon$: Strain /%
- $F$: Force /mN
- $A$: cross sectional area /$\mu$m$^2$
- $L_0$: initial length /$\mu$m
- $\Delta L$: difference between initial and final length
XZ Raman profile of a multilayer polymer film. Different polymer layers are clearly observed.

Polypropylene

Acrylic layer

Polyethylene terephthalate

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Ge QD on a Silicon substrate

Self-assembled Ge dots grown on Si substrates are attracting attention because they have a potential to be simply integrated with the existing Si-based technology. The electronic properties of the Ge dots depend on many parameters, including shape, size and distribution of strain.

Raman scattering spectroscopy is absolutely necessary method for the characterization of QDs.

Raman spectroscopy allows to obtain information on the composition, strain in the structures, distribution of dots on the surfaces.
AFM Topography
Height of Ge dots are 150 nm approximately
Ge QD on a Silicon Substrate

Forming of three-dimensional Ge islands on Si substrate is illustrated below.

Growing of Ge dots on Si substrate (schematic diagram)
Optical image, 100x objective

Rayleigh scattering, 21x21um, 488 nm

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Samples exhibit two strong bands at 504.9 cm\(^{-1}\) and 521 cm\(^{-1}\), and many weak features. Ge–Ge, Si–Ge and Si–Si optical modes can be found at around 301, 405 and 432 cm\(^{-1}\), respectively.
There are 20 different alpha-amino acids that form proteins:

- Glycine
- Alanine
- Valine
- Proline
- Leucine
- Isoleucine
- Methionine
- Phenylalanine
- Glutamic acid
- Aspartic acid
- Lysine
- Arginine
- Glutamine
- Asparagine
- Histidine
- Tyrosine
- Serine
- Threonine
- Cysteine
- Tryptophan
Peptides are biologically occurring short chains of amino acid monomers linked by peptide (C-N) bonds.

A tetrapeptide (for example, \textbf{Val-Gly-Ser-Ala}) formation:

If the chain length is short (say less than 30 amino acids) it is called \textbf{a peptide}; longer chains are called \textbf{polypeptides} or \textbf{proteins}. 

\textbf{Protein Structures:}

\begin{itemize}
  \item Primary structure
  \item Secondary structure
  \item Tertiary structure
\end{itemize}
Keratin is a protein in human nails

Keratin is a family of fibrous structural proteins. Keratins have large amounts of the sulfur-containing amino acid cysteine, required for the disulfide bridges (S-S) that confer additional strength and rigidity.
Optical Image of the Human Nail
A typical Raman spectrum of the human nail from 300 cm\(^{-1}\) - 4000 cm\(^{-1}\)

Nail1

508, \(\nu\) (S-S)
621, \(\nu\) (C-S)
643, \(\nu\) (C-S)
1004, \(\nu\) (C-C)
1247, \(\delta\) (NH)
1318, \(\delta\) (CH\(_2\))
1442, \(\delta\) (CH\(_2\))
1618, \(\nu\) (C=C)
1663, \(\nu\) (C=O)
1663, \(\nu\) (C=O)
2735, \(\nu\) (C-H)
2871, \(\nu_s\) (CH\(_2\))
2932, \(\nu_s\) (CH\(_3\))
3059, \(\nu\) (C-H)

Intensity, a.u.
Raman spectra provide a method of non-invasive measurement of the degree of sulphation of proteins (the disulphide content of fingernails) that may be indicative of bone health.

**The T-Score**

<table>
<thead>
<tr>
<th>World Health Organization Definitions Based on Bone Density Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Level</strong></td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Low bone mass</td>
</tr>
<tr>
<td>Osteoporosis</td>
</tr>
<tr>
<td>Severe (established) osteoporosis</td>
</tr>
</tbody>
</table>

A plot of a normal distribution where each band has a width of 1 standard deviation:

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The S-S peak parameters are indicative of the presence of osteoporosis.

<table>
<thead>
<tr>
<th>Raman spectroscopy results for osteoporotic versus non-osteoporotic nail.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Width at half maxima for the S-S peak</strong> (cm(^{-1}))</td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Non-osteoporotic</td>
<td>27.68</td>
</tr>
<tr>
<td>Osteoporotic</td>
<td>39.20</td>
</tr>
</tbody>
</table>

\(v\) (S-S):

**Nail1**
- Intensity: 4281.5 counts
- Position: 508.5272 cm\(^{-1}\)
- FWHM: 45.1507 cm\(^{-1}\)

**Nail2**
- Intensity: 268.3 counts
- Position: 516.5 cm\(^{-1}\)
- FWHM: 37.3 cm\(^{-1}\)
If one text overlaps with the other one on the document, the fabrication may be investigated by checking the areas of intersections. In this case Raman spectroscopy technique may be used to determine the order of crossing lines.
To recognize different blue pigments of gel pens, Raman spectra has been used.

Raman spectra from two different blue inks

Raman image of crossing lines

The last written line on document is vertical
Geological application

Raman spectroscopy is a powerful tool to study a variety of geological samples.

The Raman spectral analysis method is used for:

- Mineralogy
- Geoarchaeology
- Palentology
- and so forth

Raman spectroscopy is an express method for the structural characterization of minerals (chemical composition, distribution of components).
Raman spectra of minerals
Raman spectrum of Anatase

Anatase (TiO$_2$) distribution in Gneis (India)

Rayleigh scattering

20um
Plagioclase is a series of tectosilicate minerals (NaAlSi$_3$O$_8$ — CaAl$_2$Si$_2$O$_8$ compositions)  
Olivine (Mg,Fe)$_2$[SiO$_4$]$_4$  
Carbon (C)
Plagioclase
Analysis of a Li-ion battery cathode

The electrode material can degrade after repeating charge and discharge process multiply. Some parts of the cathode turn into $\text{Co}_3\text{O}_4$ which can’t contribute to charge.

$3\text{LiCoO}_2 + \text{Li}^+ + e^- = \text{Co}_3\text{O}_4 + 4\text{Li}_2\text{O}$

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Raman spectra of a Li-ion battery cathode

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3D Raman image of a Li-ion battery positive electrode

Distribution of normal electrode material (LiCoO$_2$) and degradation material (Co$_3$O$_4$)

Raman intensity image of LiCoO$_2$ (595cm$^{-1}$)  Raman intensity image of Co$_3$O$_4$ (695cm$^{-1}$)
Thank you for attention!

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