Mikroskopické techniky v Ramanově spektroskopii

Vladimír Baumruk

Fyzikální ústav UK

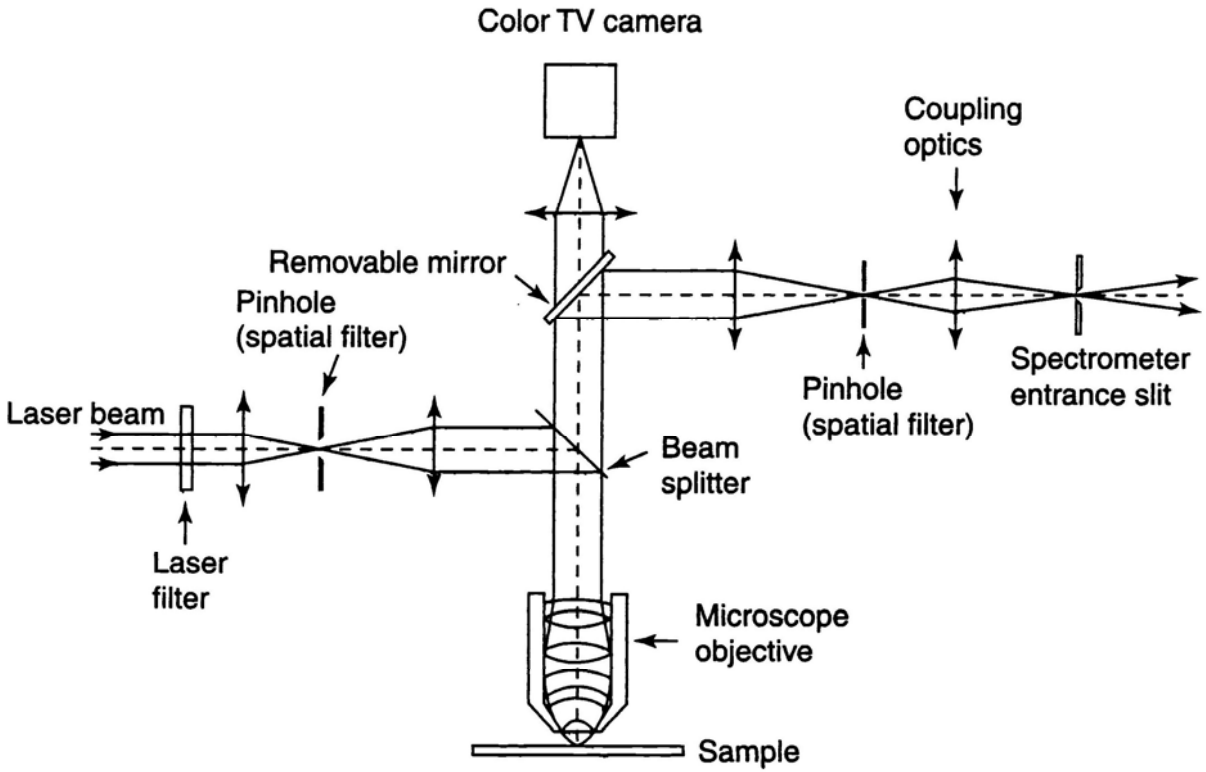
PROČ ?

- vysoké prostorové rozlišení v ploše (rovina XY) a v případě konfokálních systémů i v hloubce

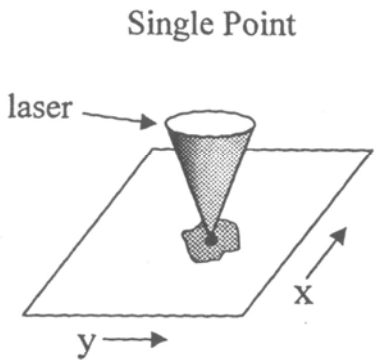
VÝHODY

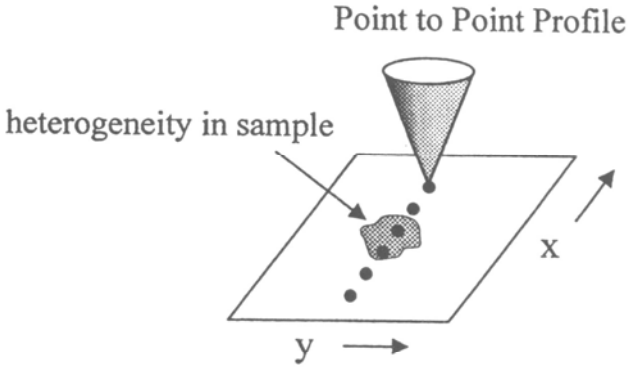
- při vysoké numerické apertuře v geometrii zpětného rozptylu (180°) je dodatečnou výhodou široký kónus sběru rozptýleného záření
- při zobrazení malé stopy budícího svazku na vstupní štěrbinu monochromátoru lze dosáhnout toho, že při vstupních šterbinách 100 μm a větších analyzujeme prakticky veškeré sebrané záření

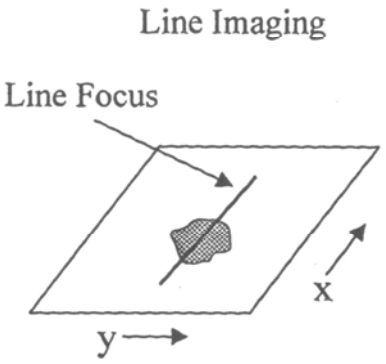
Optical scheme of the widely adopted illuminating and collecting optics for micro-Raman spectrometer

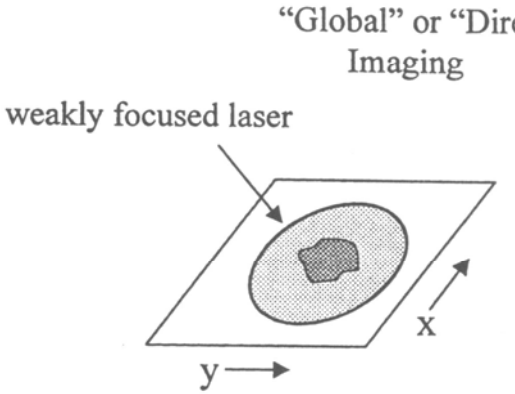


Imaging modes for Raman microscopy

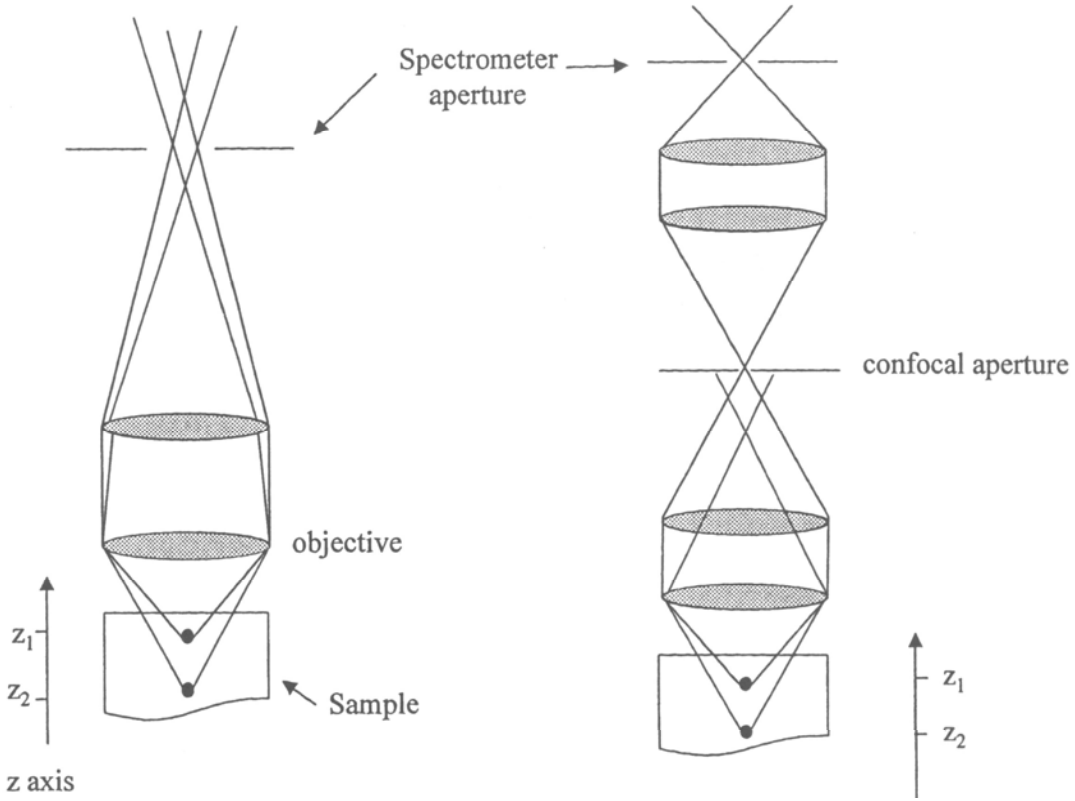






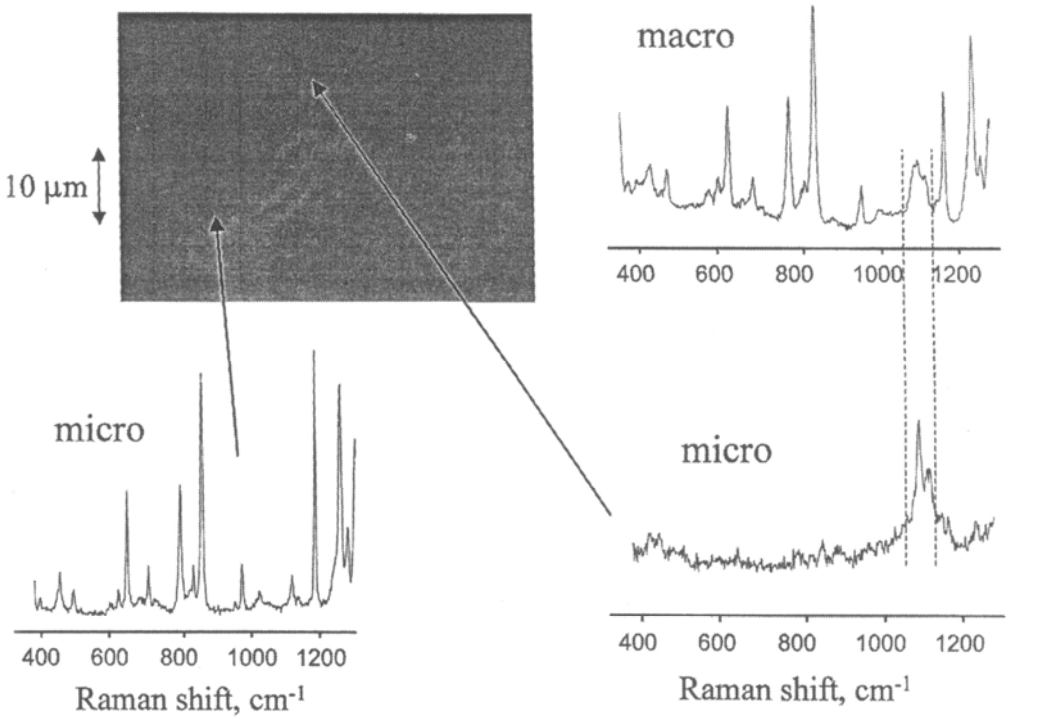


Schematic of confocal microscopy optics, showing addition of a „confocal aperture“ that restricts sampling depth



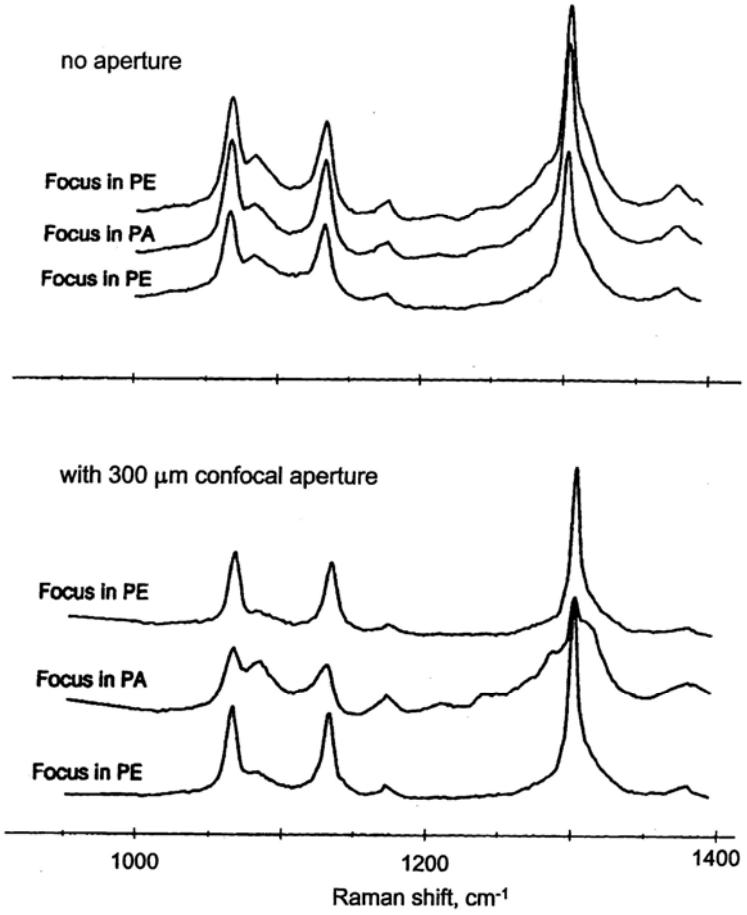
Conventional Confocal

Single point Raman microspectroscopy

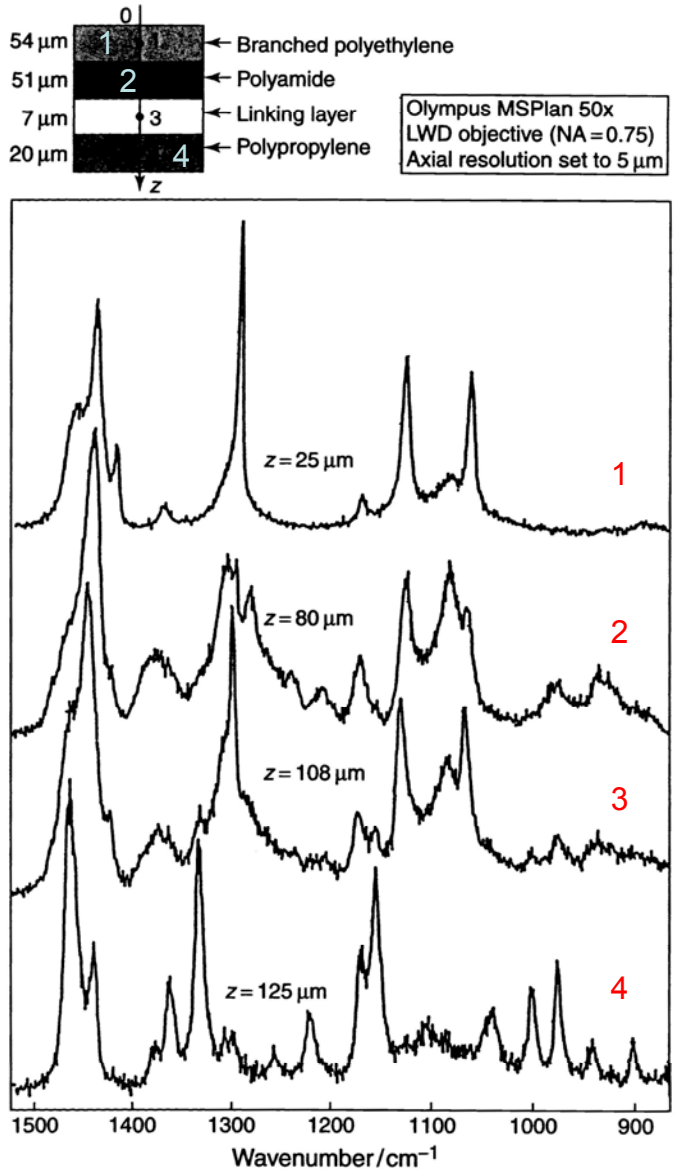


Single point Raman microspectroscopy of a pressed tablet containing acetamidophenol and microcrystalline cellulose. The macro was obtained with an ~100 µm-diameter sampling area, and the micro spectra were obtained at the locations indicated.

Depth discrimination in Raman microspectroscopy

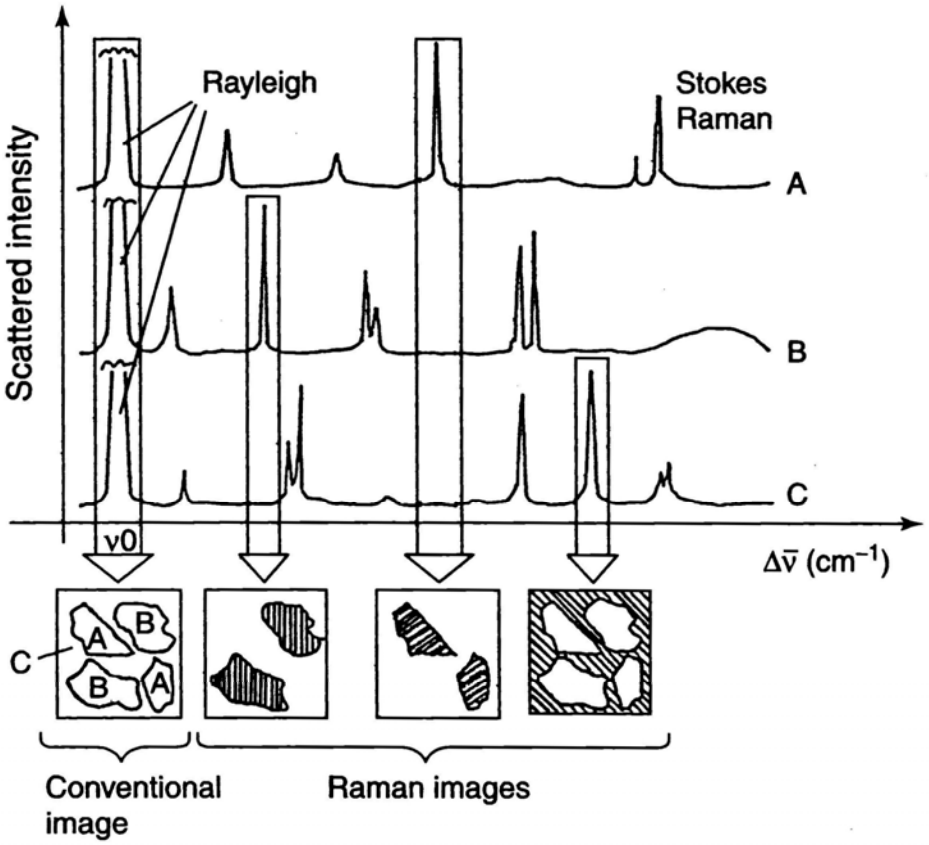


Raman spectra of a polyethylene (26 µm thick) / polyamide (26 µm) / polyethylene (26 µm) laminate with and without a confocal aperture. The improved depth resolution with the aperture permits discrimination of polyethylene and polyamide spectral features.



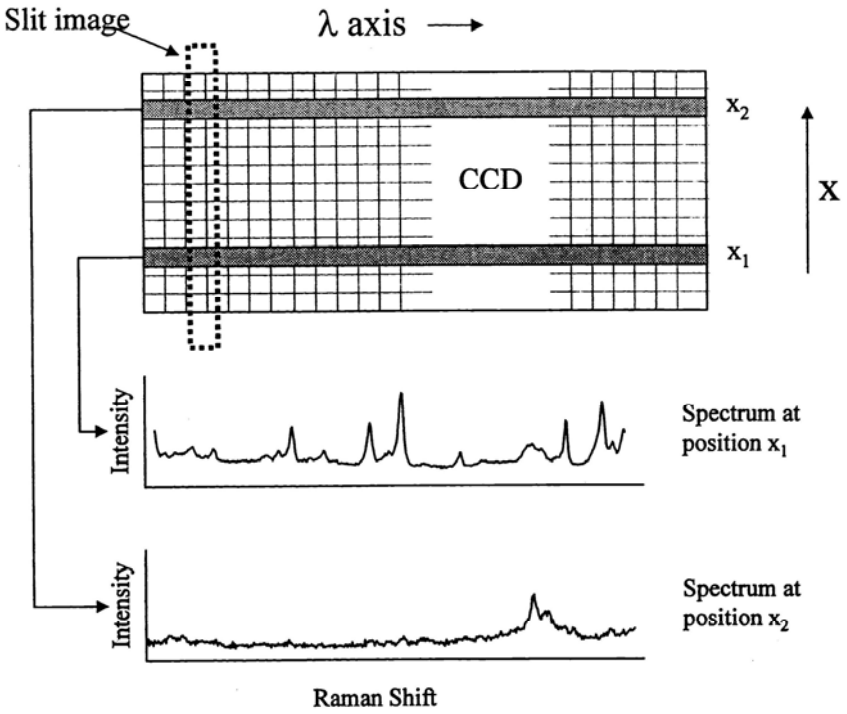
Depth analysis of a multilayered laminate polymer.

Principle of Raman mapping

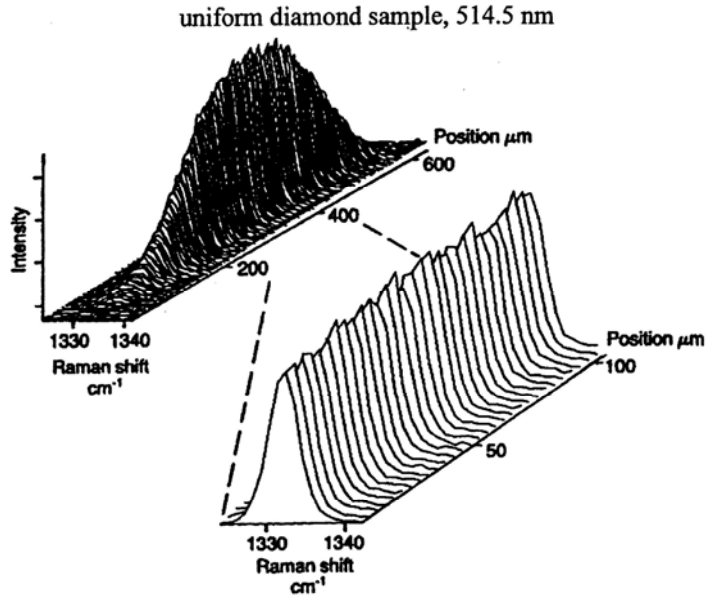


Selective maps of the sample depicting the distribution of a given molecular species can be obtained by passing the Raman scattered radiation through the spectrograph, acting as a narrow-band optical filter, which isolates a narrow spectral region centered on a characteristic Raman wavenumber of this species.

Line imaging



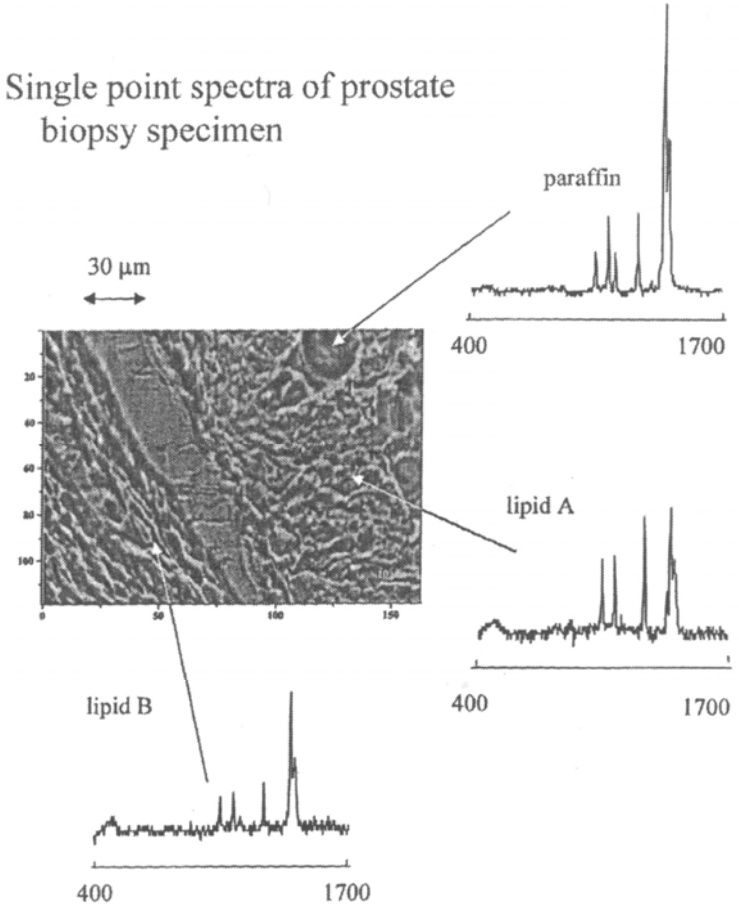
Schematic of line imaging onto a CCD. A line focus at the sample projected onto the slit of an imaging spectrograph generates a 2D image of intensity vs. x and $\Delta\tilde{\nu}$.



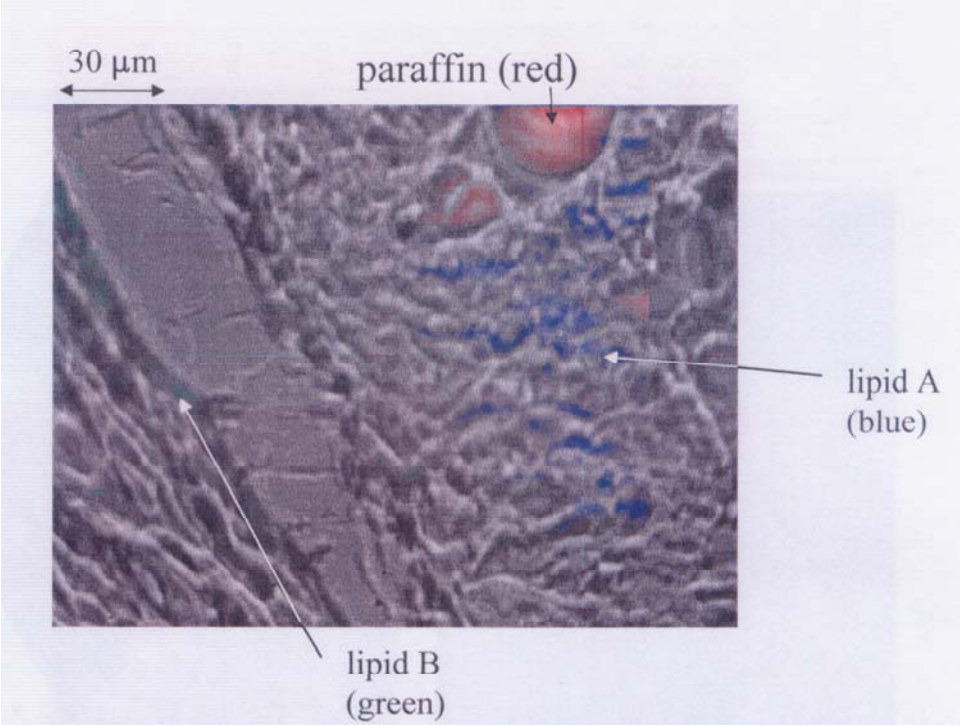
200 Raman spectra of the 1332 cm^{-1} feature of diamond obtained with a 600 μm long line focus. The expansion of the middle 100 μm of the line demonstrates relatively constant laser intensity.

2D-Raman imaging

Data analysis techniques based on chemometrics and factor analysis may be applied to large spectral data sets to provide greater chemical selectivity. Instead of observing distribution of a particular Raman band, the distribution of an entire spectrum is determined.

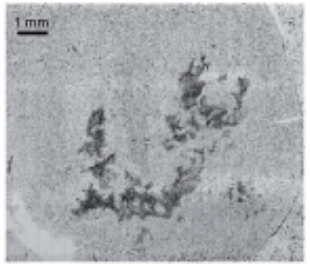


Raman microspectroscopy of a human tissue slice from a prostate cancer biopsy specimen mounted in parafin.

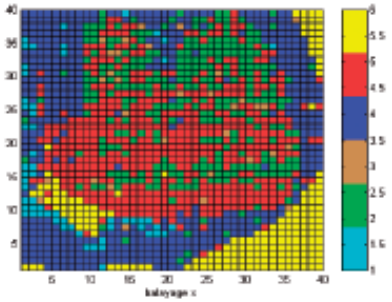


Distribution of components overlaid on the video image. Each color represents the contribution of a particular component to the Raman spectrum observed at each position of a 28 x 28 grid.

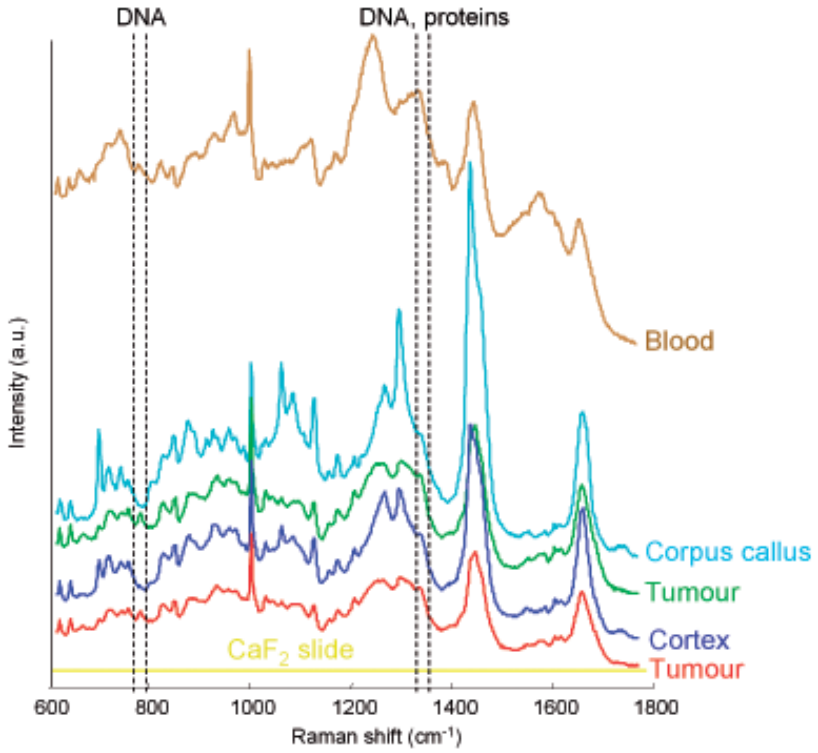
Results from mapped imaging of an unstained rat brain tissue section



Photomicrograph

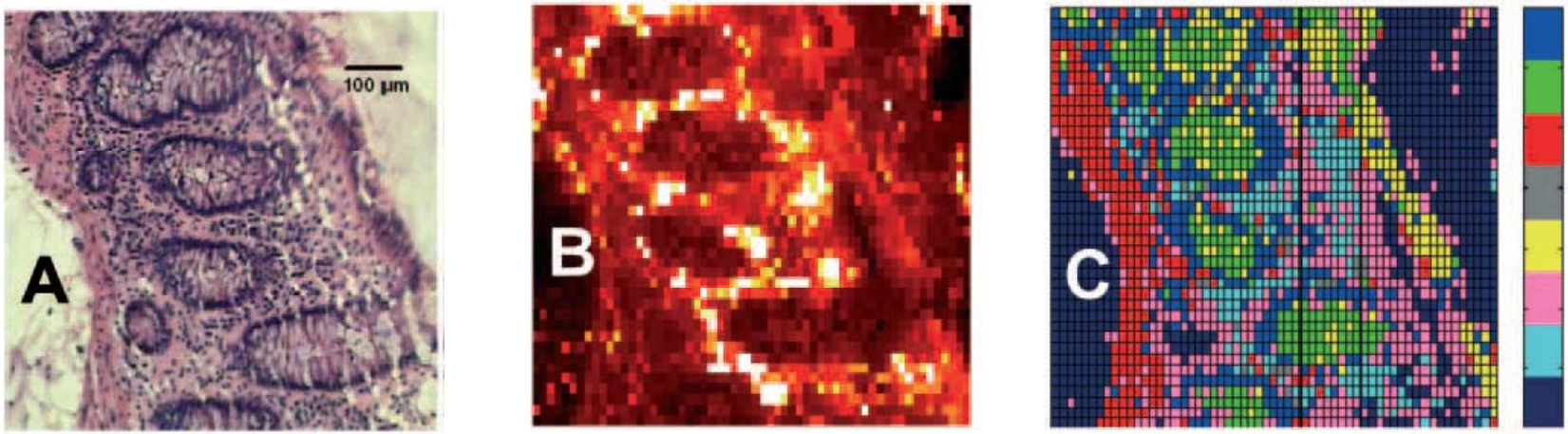


Raman mapped image



Photomicrograph and Raman mapped image of a rat brain tissue section, with coloured regions corresponding to the offset spectra shown on the right. Different tissue types are shown in the pseudo-colour Raman map, corresponding to both healthy (corpus callus, cortex, and blood) and diseased (tumoral) tissue. The red tumoral zone correlates directly to histopathological observations with H/E staining.

Raman (chemical) imaging



Analysis results for human colonic tissue section (A) optical image of histopathologic HE stained tissue, (B) Raman mapped image illustrating intensity of the Amide I band, and (C) pseudo-colour Raman maps created by using K-means cluster analysis in which each cluster (consisting of similar spectra) is assigned to one color.

It is immediately clear that the visible structural features of the tissue coincide with the chemical information provided by the Raman. Cluster analysis of the Raman data allows spectra of similar profile to be grouped and displayed as a single unit, allowing specific tissue types to be located. This technique makes use of the large information content present even in just one spectrum, which will give an indication of the presence of the various chemicals present within the tissue, such as DNA, RNA, protein, lipid and carbohydrate.

LabRam HR (Horiba Jobin Yvon)

verze VIS (400 - 1100 nm)
ohnisková vzdálenost 800 mm, flat field
2 mřížky 600 a 1800 vrypů/mm
LN2 chlazený CCD detektor 1024x256 pixelů
excitace He-Ne laser 633 nm (interní)
vzduchem chlazený Ar^+ 514.5, 488 nm (externí)
laserová dioda 785 nm (externí)

konfokální mikroskop Olympus BX41 (open)
motorizovaný XY stolek, krok 0.1 µm
hloubkové profilování rozsah 80 µm, krok 0.1 µm
optika pro pozorování v polarizovaném světle
měření polarizovaných Ramanových spekter

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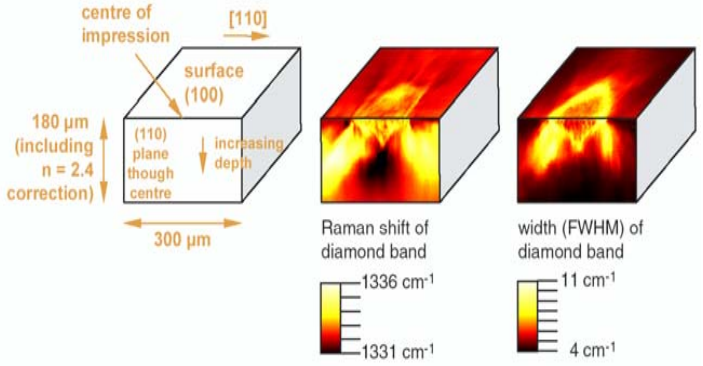
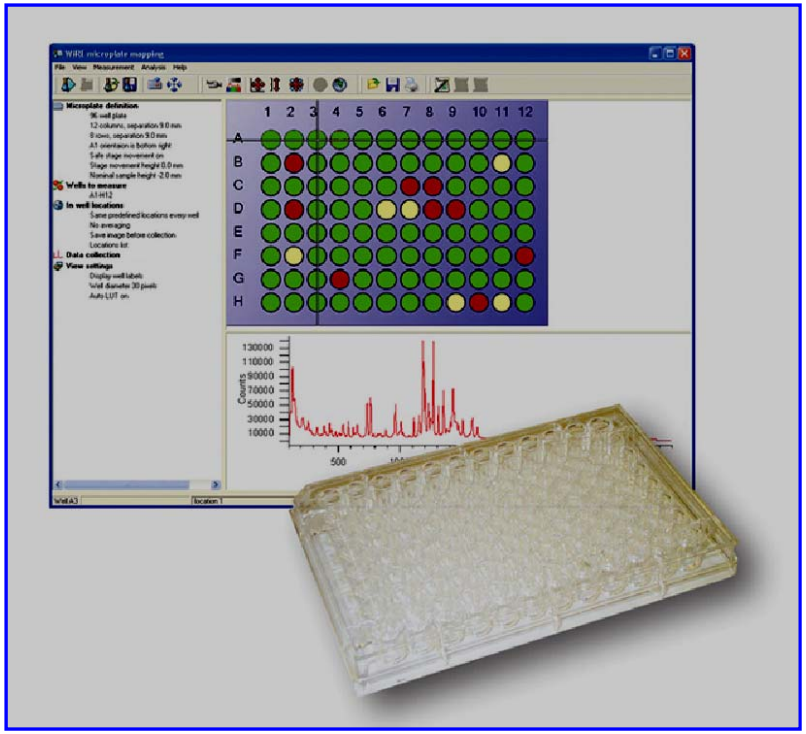
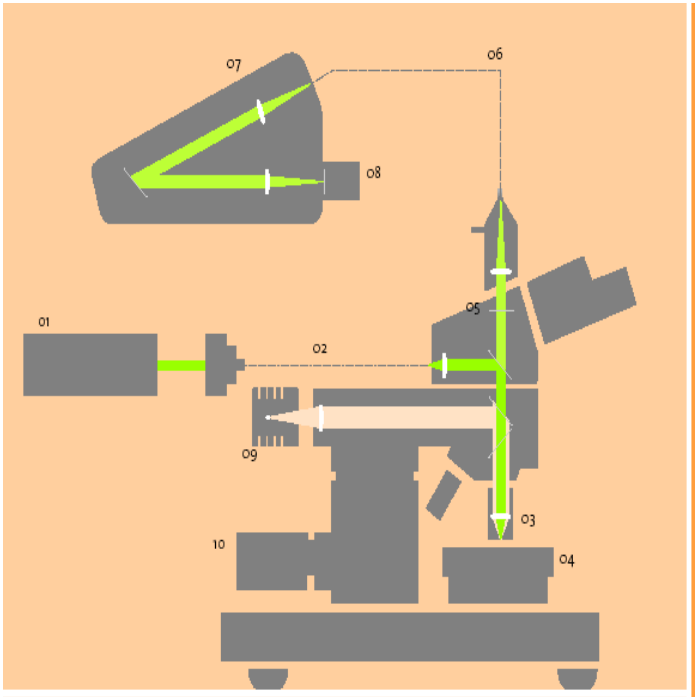


Figure 2
Raman maps of type IIb diamond



ÚMCH , FZÚ, PřF UK, …



CRM 200 Beam Path

01. Laser
02. Single mode fiber
03. Objective
04. Scan table
05. Filter
06. Multi mode fiber
07. Lanse based spectroscopy system UHTS 300
08. CCD Detektor
09. White light illumination
10. Z-stage for focusing

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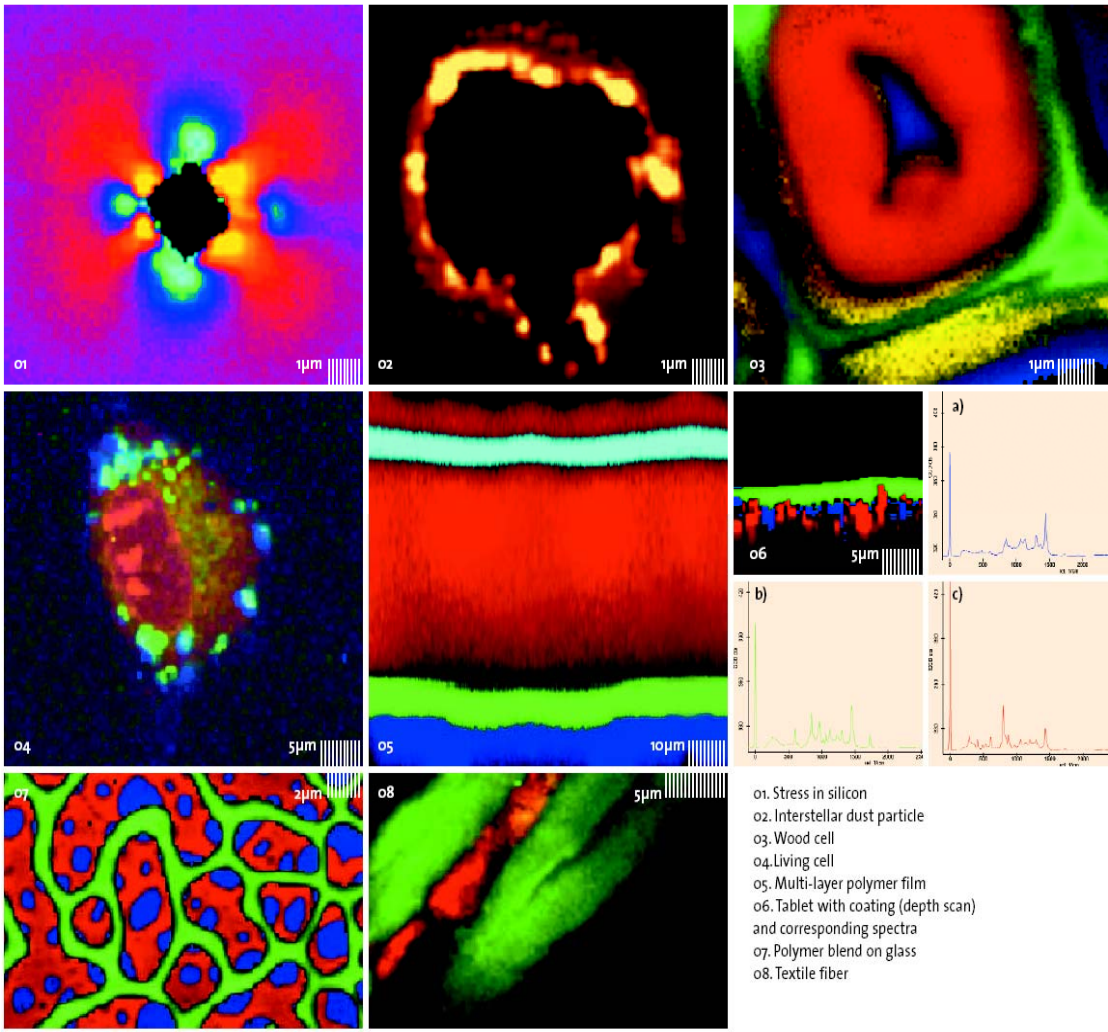
focus innovations



01. Stress in silicon
02. Interstellar dust particle
03. Wood cell
04. Living cell
05. Multi-layer polymer film
06. Tablet with coating (depth scan) and corresponding spectra
07. Polymer blend on glass
08. Textile fiber

Kombinace s AFM