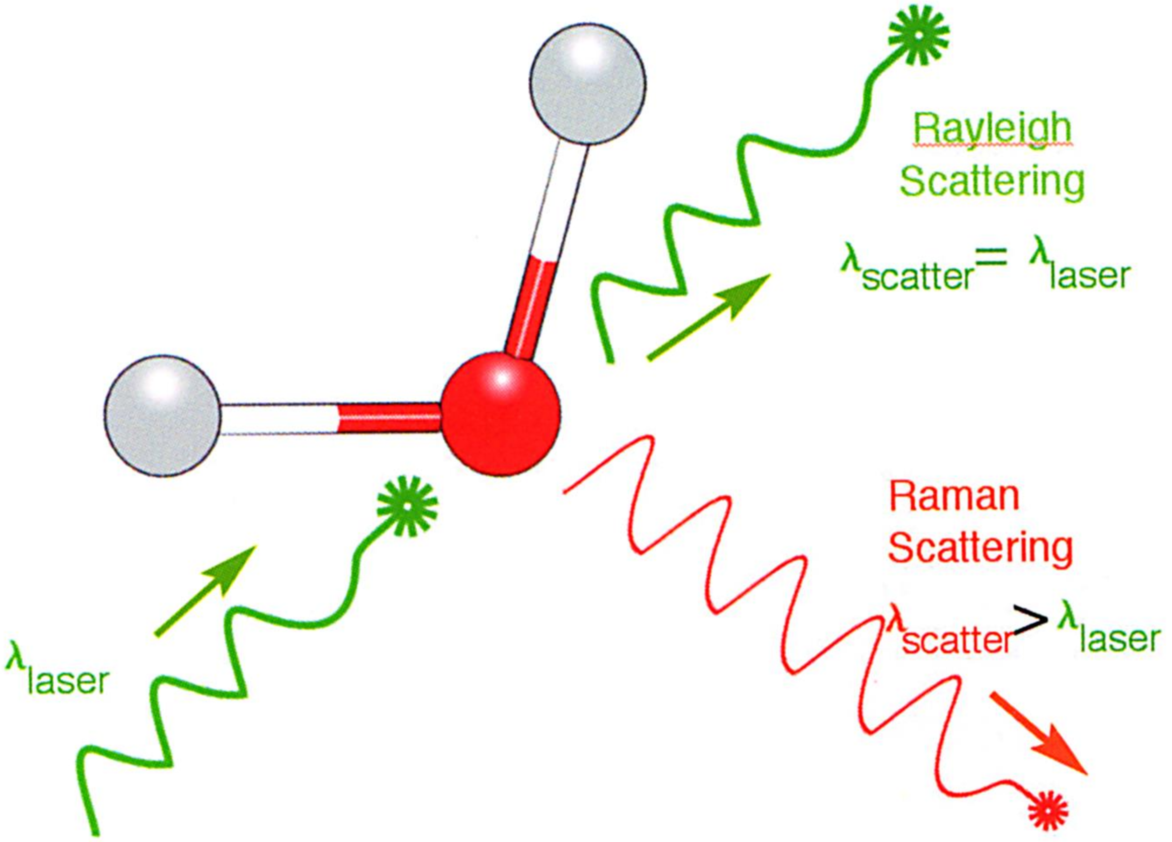
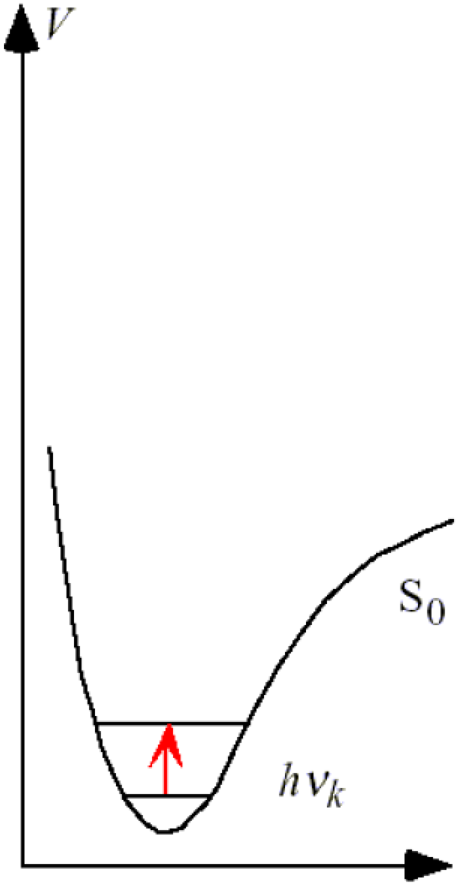
Lasers – Raman spectrometry

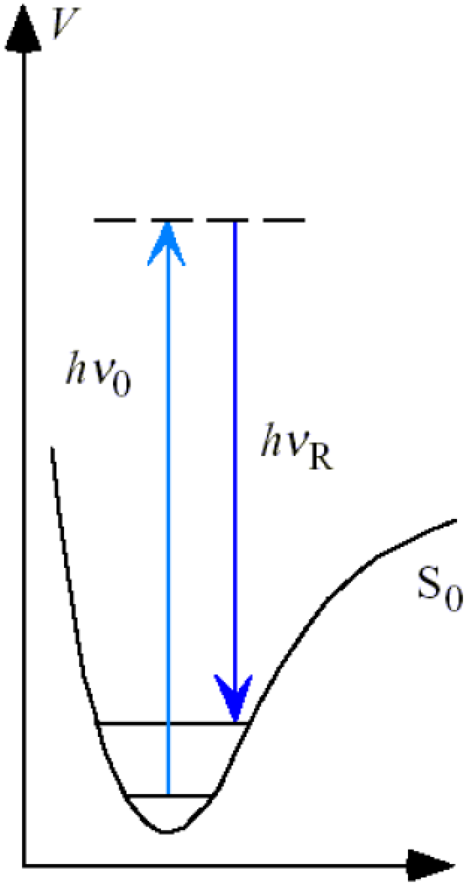
Vítězslav Otruba



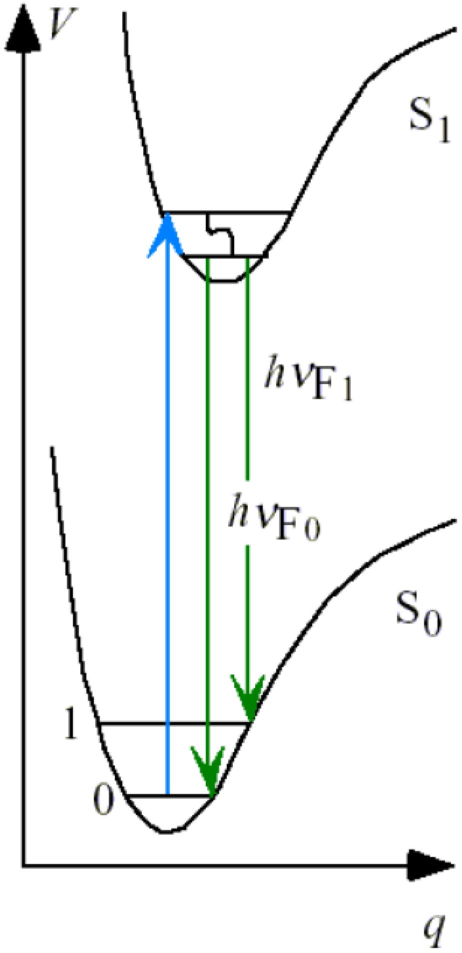
Raman scattering provides highly specific and non-destructive information on molecular vibrations, enabling analysis often not obtainable from other techniques such as fluorescence, chromatography and optical microscopy.



IR absorption



Raman scattering



Fluorescence

Principles of the Raman effect

SCATTERING of RADIATION

the scattered photon has a different energy than the incident photon

radiant two-photon transition between two stationary states of molecules whose vibration energies are E_1 a E_2,

caused by interaction with the photon of incident radiation with frequency

$$\nu_0 > (E_2 - E_1) / h,$$

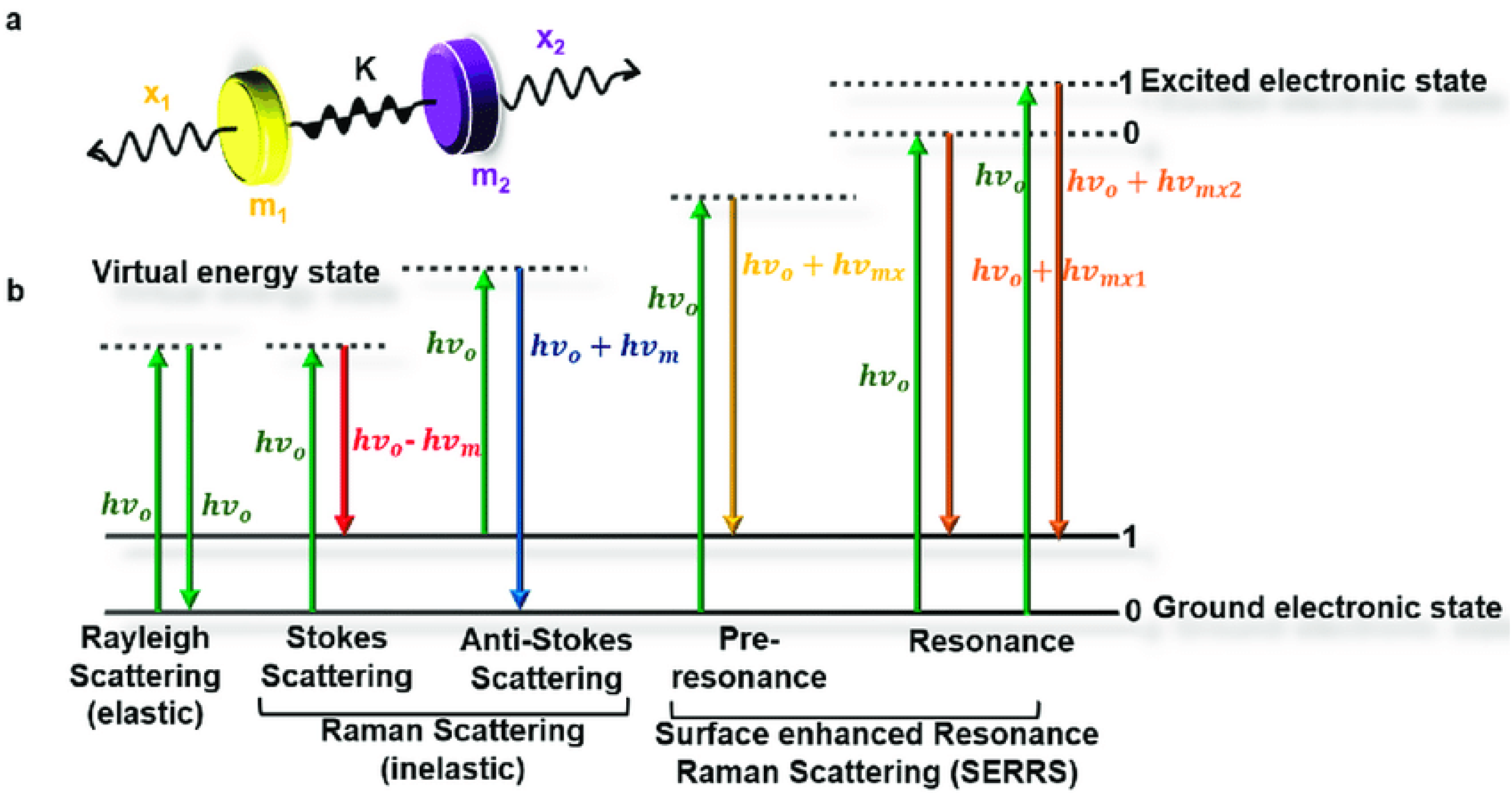
accompanied by radiation of scattered photon with energy

$$h\nu_R = h\nu_0 \pm (E_2 - E_1),$$

where $h\nu_{vib} = E_2 - E_1$

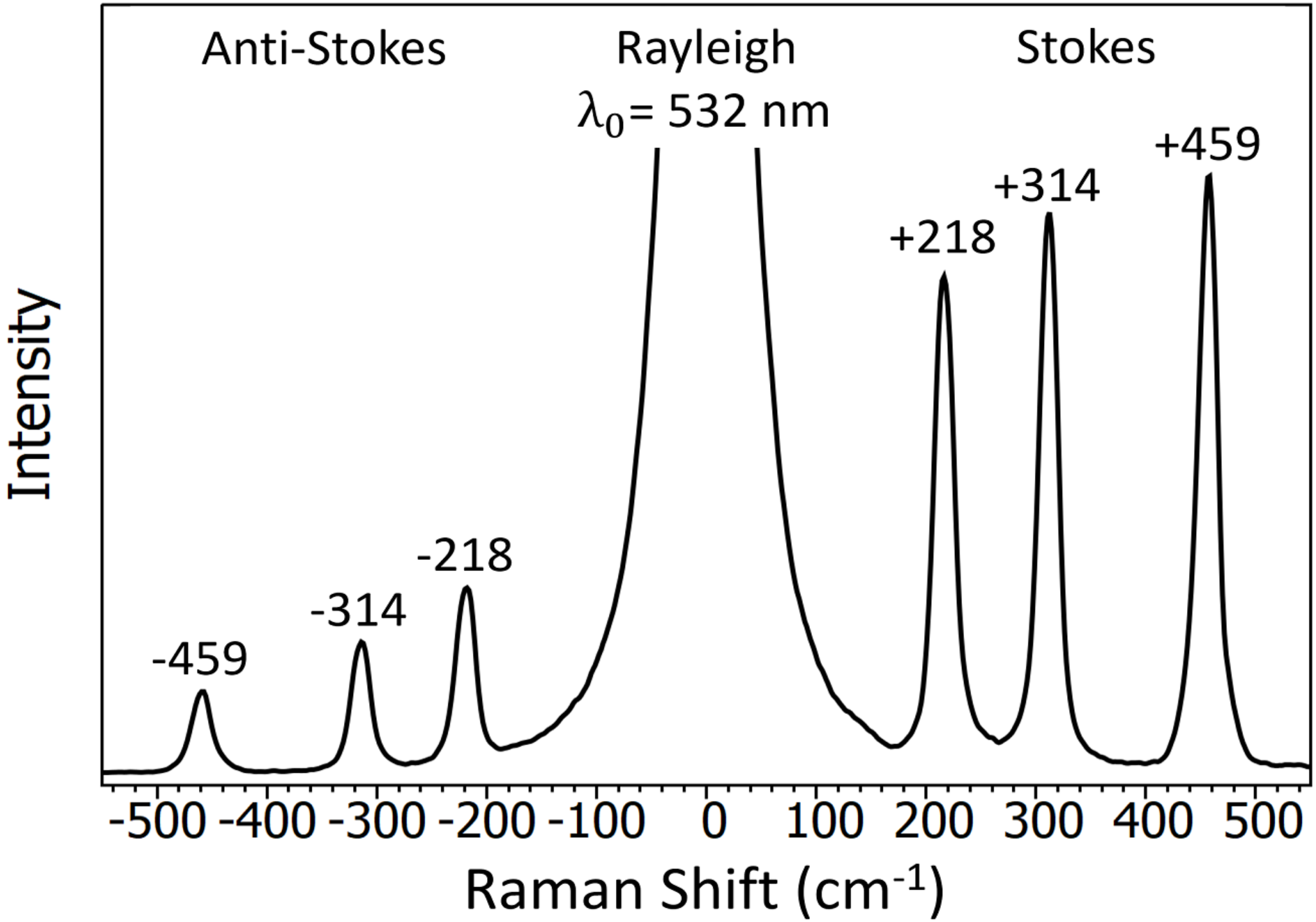
The scattered photons carry information about the energetic spectrum of the scattering center as well as the spatial orientation of a particular chemical bond, (it is like molecular "business card"). However, without special measures, only a single photon of hundreds of millions to hundreds of billions of incident photons is scattered in this way. The Raman scattering cross-section is about 10^{-30} cm^2.

Light scattering occurs in all directions around the scattering particle.



Rayleigh (molecular) scattering

Lord John Rayleigh rightly found that the intensity of the scattered radiation was directly proportional to the fourth power of the radiation frequency, or inversely proportional to the same power of its wavelength; light scattering occurs in all directions around the scattering particle and is even of three kinds: scalar scattering in which the intensity is uniform in all directions, scattering symmetrical and antisymmetric, the intensity of which varies in different directions, as well as the polarization of scattered radiation. At the same time, if a monochromatic light falls on a particle of frequency ν_0, then the diffuse radiation has the same frequency ν_0 ', but its intensity is much lower than the intensity of the incident radiation; in this case we say that the spectrum of scattered radiation consists of a single spectral line. All the experiments of Rayleigh's contemporaries confirmed this theory. Neither he nor any of his contemporaries could, however, suspect that there were at least ten types of light scattering, or that the blue sky was not caused by light scattering on individual molecules but by so-called atmospheric density fluctuations, ie random random clusters of molecules contained in an atmosphere that lasts an extremely short time, that is, only for the duration of the collision of at least three such molecules.


Anti-Stokes
Rayleigh
λ_0= 532 nm
Stokes
-459
-314
-218
+218
+314
+459
Intensity
-500
-400
-300
-200
-100
0
100
200
300
400
500
Raman Shift (cm^{-1})

Principle of Raman spectrometry 1

- The Raman scattering is based on the radiant two-photon transition between two stationary vibrational states of a molecule whose energies are E_1 and E_2, induced by interaction with the photon of incident radiation at the frequency
- $\nu_0 > | E_2 - E_1 | / h$,
- where h is Planck's constant, and is accompanied by the photon emission of scattered radiation at the frequency ν_R. This scattering effect can be simply imagined as the simultaneous absorption of the exciting photon by a molecule as the molecule moves to a virtual energy level and the emission of a secondary photon, subject to the condition of energy conservation:

$$h\nu_R = h\nu_0 \pm (E_2 - E_1) \quad (1)$$

- There are several possibilities of such a transition according to the position of the virtual energy level in relation to the actual states of the molecule (eg normal and resonant Raman effect).

Principle of Raman spectrometry 2

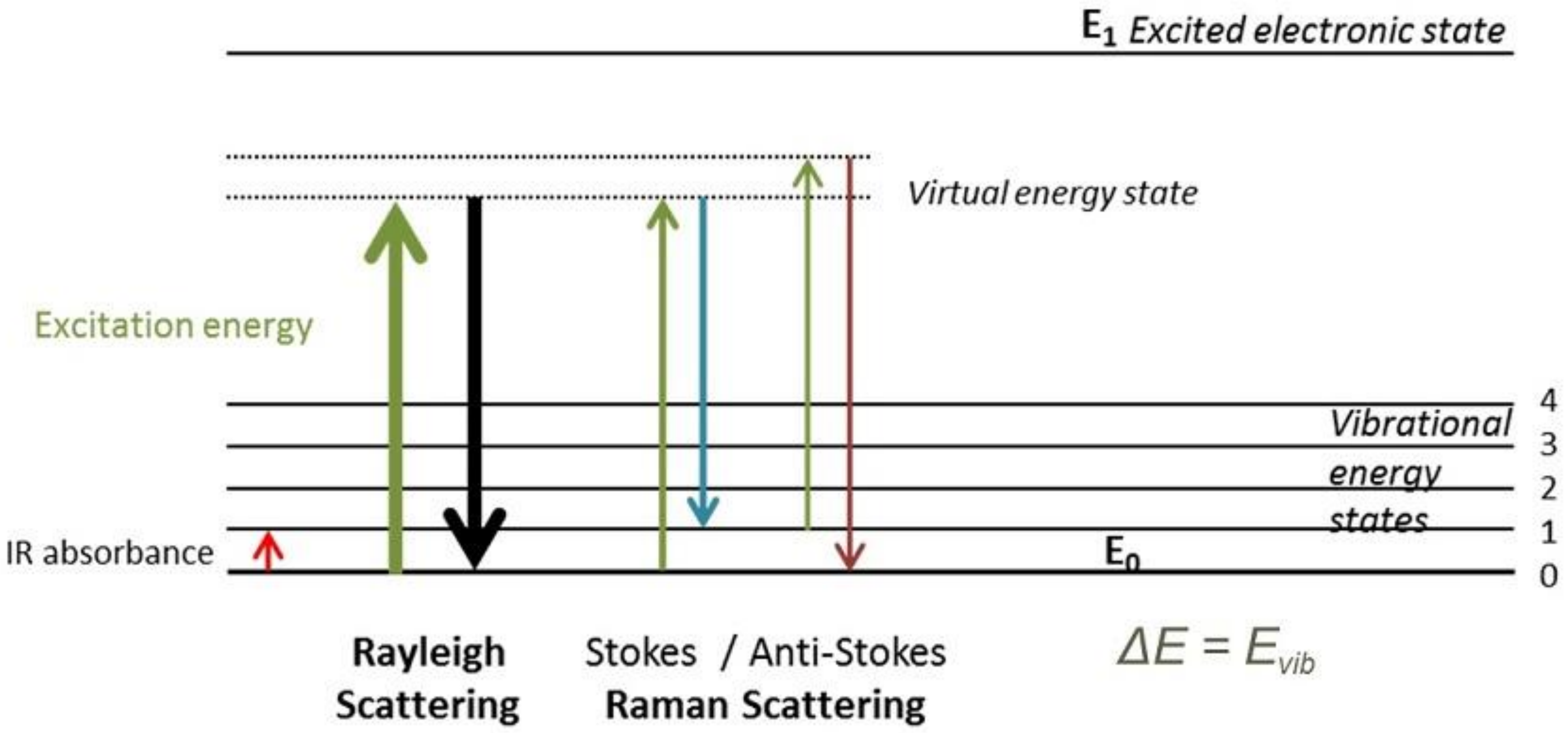
In the classical approximation, for a molecule interacting with radiation, the dipole moment ***p*** is induced in the molecule :

$$\boldsymbol{p} = \alpha \boldsymbol{E} \cos\left(\underline{2\pi \nu_0 t}\right) + \frac{1}{2}\frac{\partial \alpha}{\partial q} q \boldsymbol{E} \left\{\cos\left[2\pi\left(\underline{\nu_0 - \nu_{vib}}\right) t\right] + \cos\left[2\pi\left(\underline{\nu_0 + \nu_{vib}}\right) t\right]\right\}$$

where ν_0 is the frequency of excitation radiation, ν_{vib} is the vibration frequency, ***E*** is the vector of the electric field intensity of the incident radiation, *q* are the internal coordinates of the molecule and α is the polarizability of the molecule (polarisability is the degree of "difficulty" with which a negative charge is deflected by electric field). The equation shows that the molecule emits radiation at an unchanged frequency(ν_0 - Rayleigh scattering ▬) and radiation with frequencies($\nu_0 + \nu_{vib}$ ▬) a ($\nu_0 - \nu_{vib}$ ▬), which are collectively called Raman scattering, with the lower frequency ($\nu_0 - \nu_{vib}$) corresponding to Stokes scattering, while the higher frequency ($\nu_0 + \nu_{vib}$) belongs to anti-Stokes scattering. From the equation it is also evident that for the formation of the Raman lines is necessary that the vibratory motion cause change in polarizability, namely that:

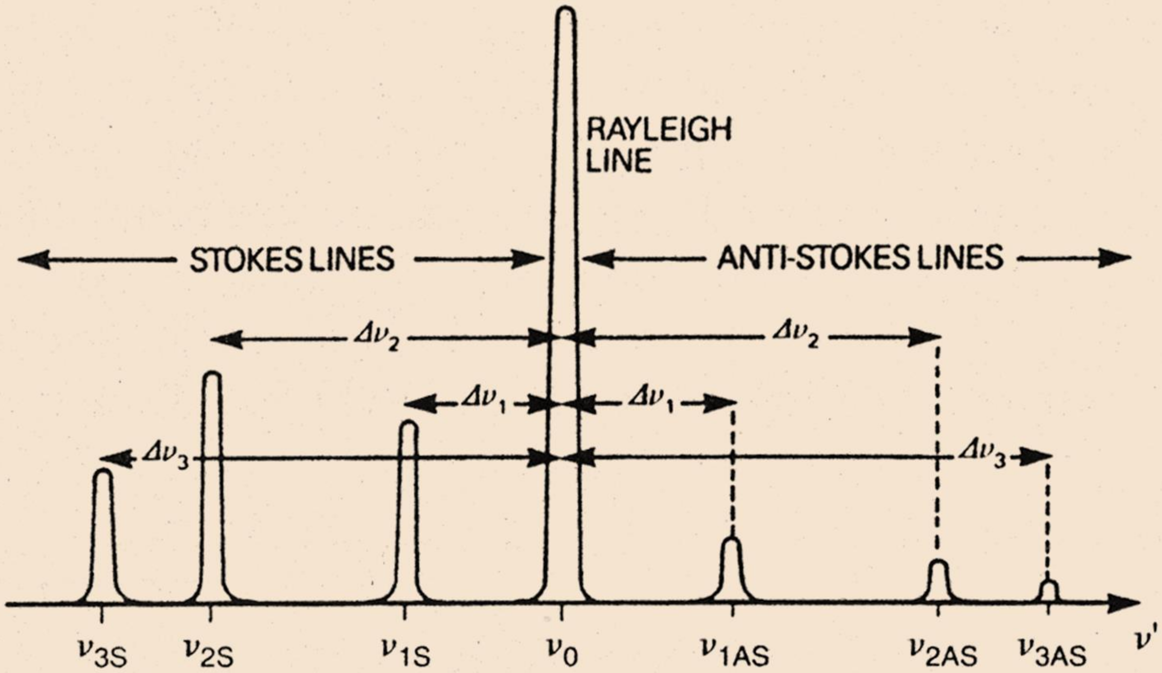
$$\frac{\partial \alpha}{\partial q} \neq 0$$

Non-resonance Raman spectra



The magnitude of the frequency shift ω_v does not depend on the frequency of the incident radiation. The probability of the Raman effect increases with the fourth power of the incident radiation frequency and is three orders of magnitude less than the Rayleigh scattering

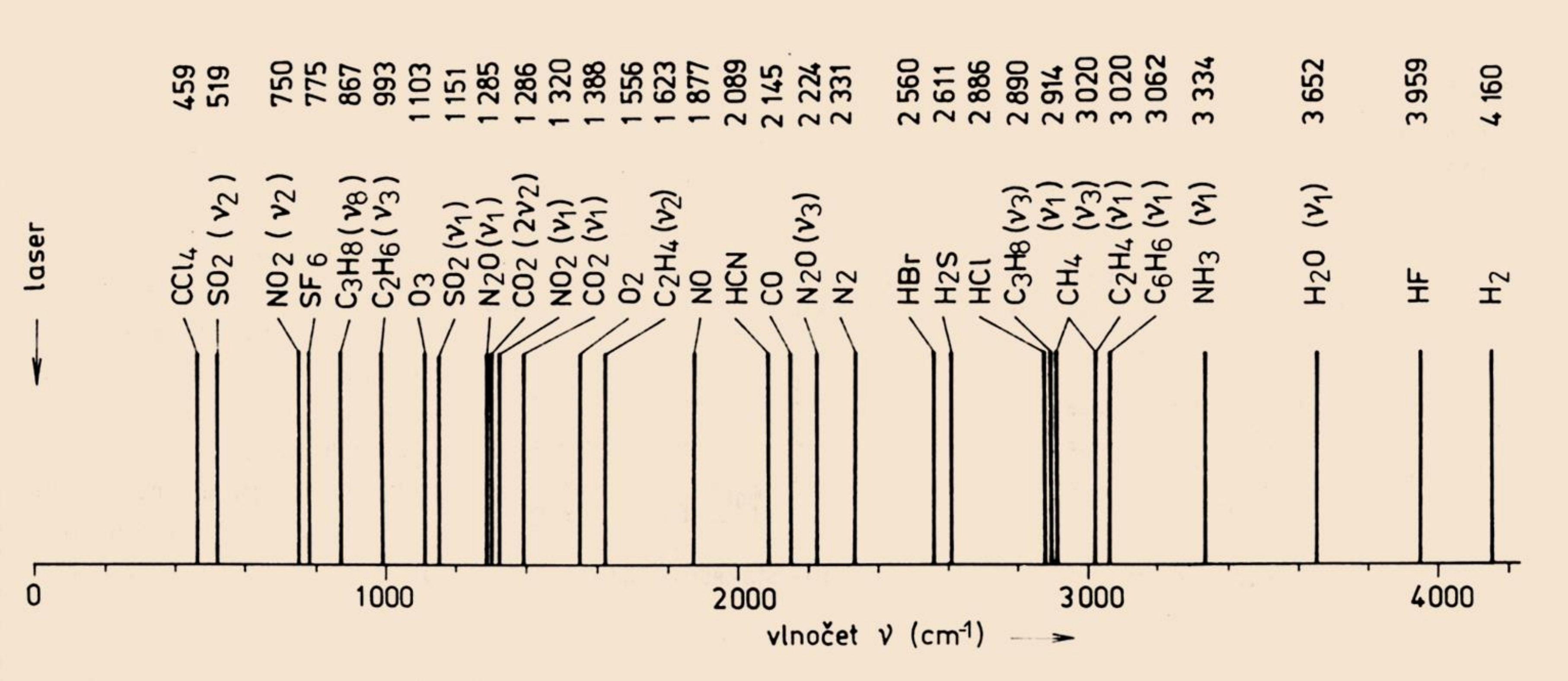
Raman spectrum



Model Raman spectrum showing the intensity of light scattered with frequency ν'

$$I_{AS}/I_S = \{(\nu_0 + \Delta\nu)/(\nu_0 - \Delta\nu)\}^4 (g_2/g_1) \exp(-h\Delta\nu/kT)$$

Raman spectrum of substances present in the atmosphere



Spectrum of Raman Lidar

Raman resonance spectra

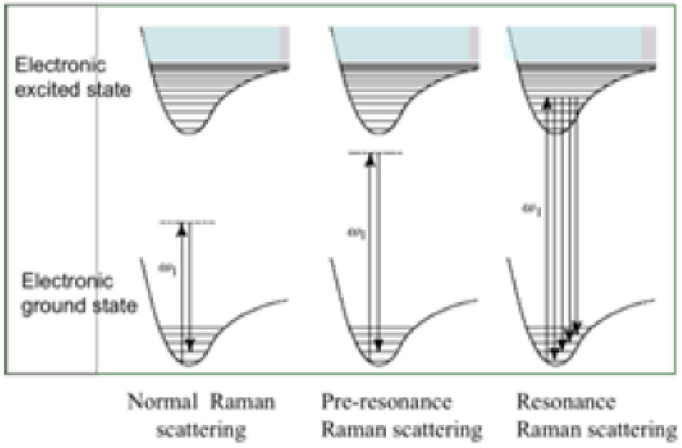
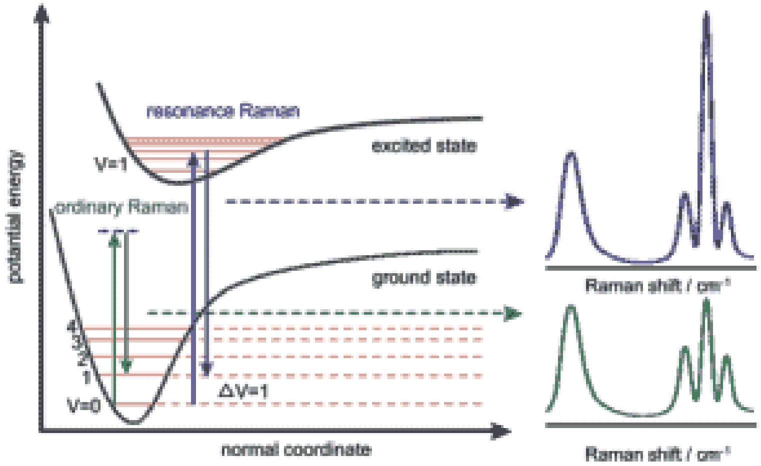


Figure 1: Comparison of normal, pre- resonance and resonance Raman scattering.



Resonance Raman scattering occurs when the frequency of radiation incident on the scattering particle matches or approaches the frequency of the particle's quantum transition. Compared to the radiation intensity of non-resonant Raman scattering, the intensity can be increased by 3 to 6 orders.

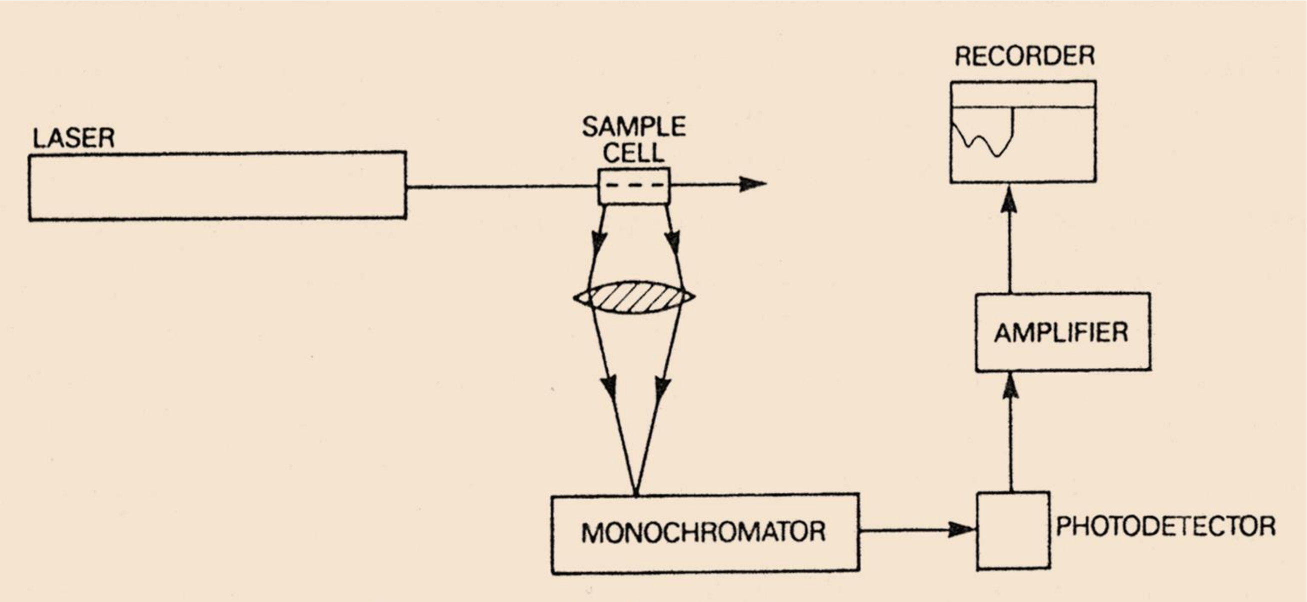
Resonance Raman scattering

- virtual level near the electron excited state
- UV resonance Raman spectroscopy - nucleic acids, proteins
- Visible range - coordination compounds, organic dyes, hemoproteins
- NIR - "pre-resonance"? - low energy electron transitions
- Excitation profiles - dependence of Raman spectra (selected bands) on excitation wavelength

Raman resonance spectrometry

- Enhancement factor 10^2–10^4
- Practical aspects
  - For solutions - the question of choice of concentration and position of the excitation beam
  - Self-absorption
  - Fluorescence
  - Choice of beam geometry, focus
  - Concentration profile

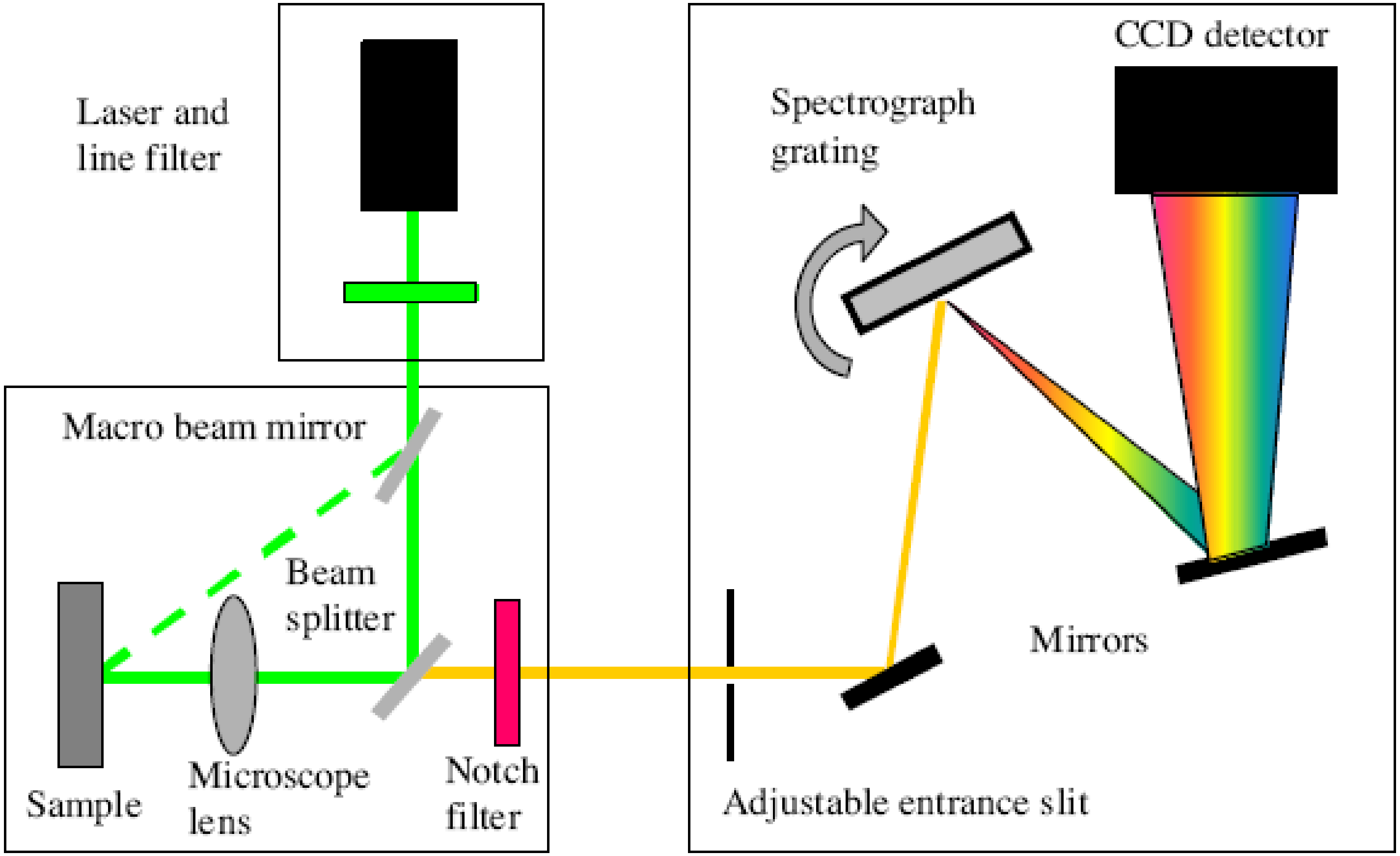
Instrumentation for Raman spectrometry



Instrumentation for laser Raman spectroscopy

Solid, respectively liquid sample is placed in front of the slot of the spectrometer. The sample is irradiated with a focused laser beam with an output power greater than 100mW, usually of an ion Ar (λ = 514.5 nm - green and 488 nm - blue) or a He-Ne laser (λ = 632.8 nm). The Nd:YAG laser with frequency multiplier and laser diodes is often used recently. The monochromator must have very low diffuse radiation (usually double). The detector is usually a photomultiplier, respectively. intensified CCD detector.

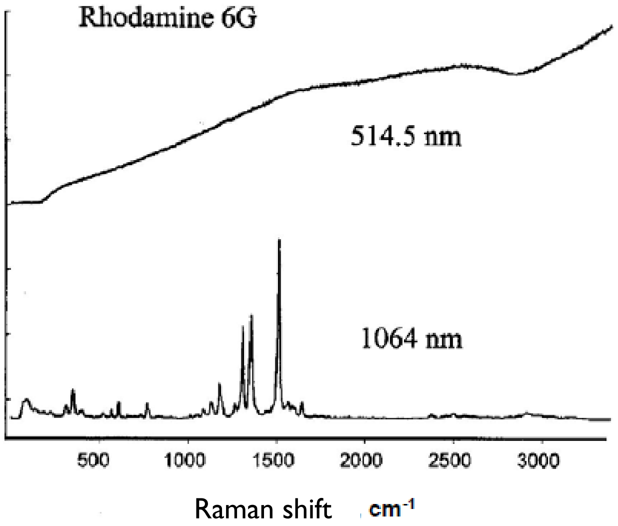
Diagram dispersive Raman instruments

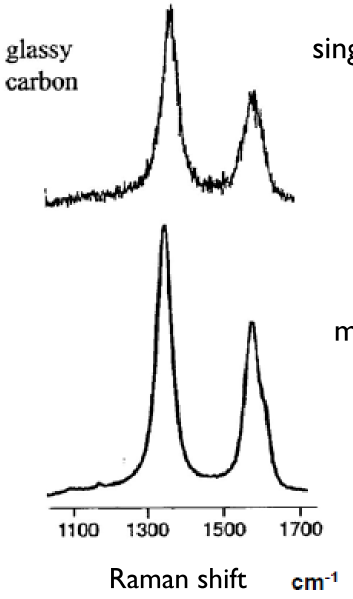


Technological advances in Raman spectroscopy in the past 20 years

1. FT Raman spectrometry

+ excitation in the NIR region - solves the problem with fluorescence
+ high accuracy of wavenumbers
- lower scattering intensity - more difficult to detect





single channel /PMT

Spex 1403

20 minut

SNR ~ 28

multi channel /CCD

Chromex 250

5 sekund

SNR ~ 280

2. Multichannel CCD detectors

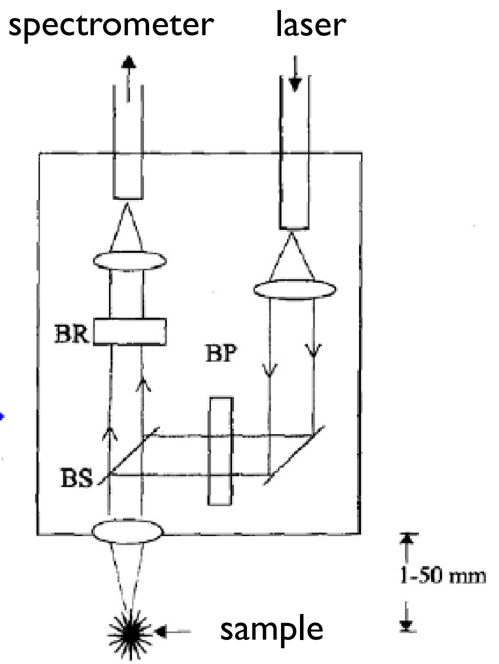
+ Significant improvement in signal to noise ratio (SNR)
+ Significant reduction in measurement time

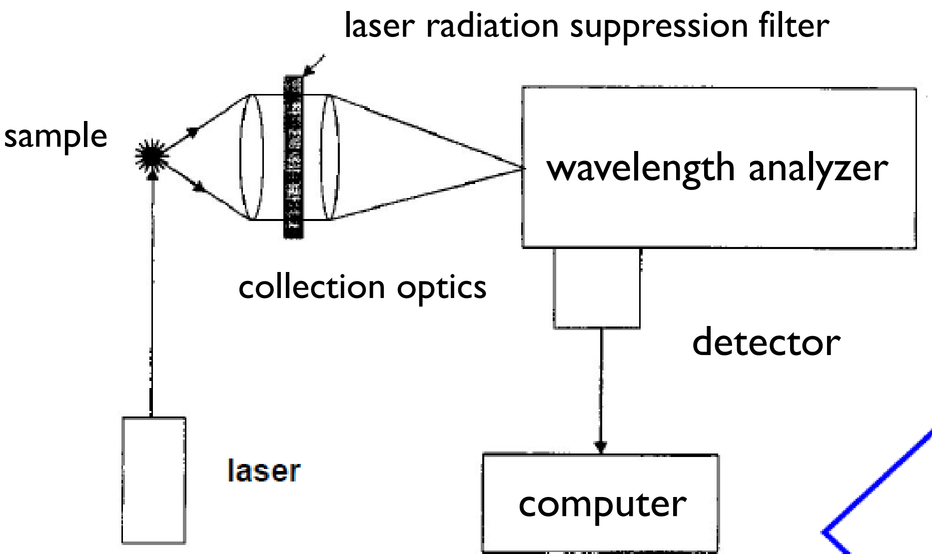
(in both cases excitation **514.5 nm / 50 mW)**

3. Fiber optics (integrated probes)

+ use for process monitoring and control
+ significant increase of application potential
+ "remote" spectrometer

integrated fiber probe containing the necessary filters (BP, BR) and focusing optics





4. Holographic filters

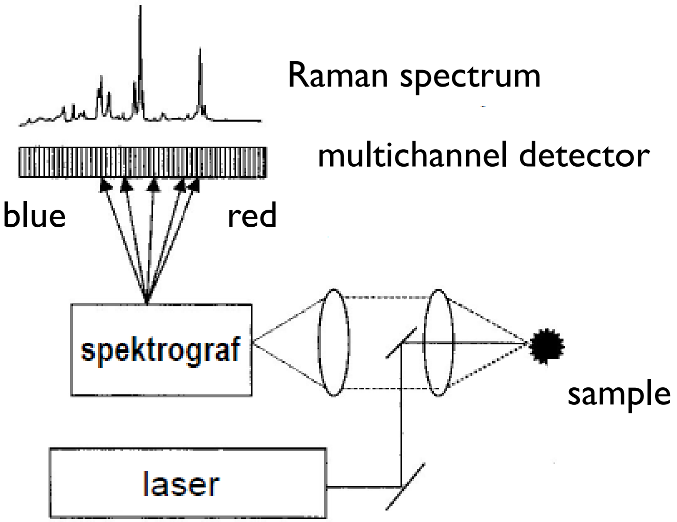
+ significant reduction in spectrometer dimensions and cost
+ more radiation on the detector
- an individual filter is required for each excitation wavelength

Scheme compact Raman spectrometer

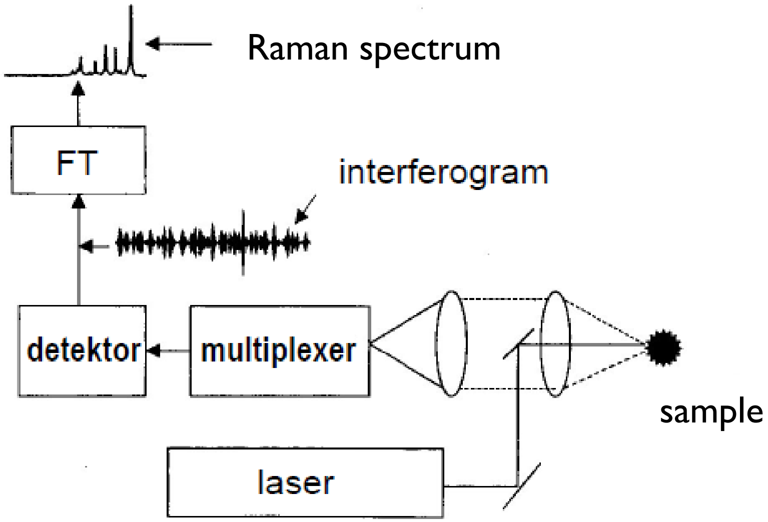
b

Dispersion and non-dispersive spectrometers

Dispersion multichannel Raman spectrometer



Non-dispersive FT Raman spectrometer



Advantages

- sensitivity
- higher signal to noise ratio
- λ_{exc} 200-800 nm
- (limited by CCD detector response)

- high frequency determination accuracy
- higher aperture
- always λ_{exc} greater than 1064 nm
- usually without fluorescence
- zpravidla bez fluorescence

Disadvantages

- a compromise between resolution and range
- more fluorescence
- changing spectral resolution

- worse signal to noise ratio
- often high excitation power of the laser

Lasers for Raman spectroscopy

$\lambda_{exc} < 900$ nm $\Rightarrow$ dispersive Raman spectrometers (limited by silicon CCD detectors)

$\lambda_{exc} = 1064$ nm $\Rightarrow$ non-dispersive FT Raman spectrometers

Continuous (CW) lasers

typ	λ (nm)	typical power
second harmonic Ar^+	224, 257, 229	15-200 mW
Ar^+ air cooled	488, 514.5	5-50 mW
Ar^+ water cooled	351.1, 454,5, 457.9, 465.8 476.5, **488**, 496.5, 501.7, **514.5**	0.1-10 W
He-Ne	632.8	5-100 mW
He-Cd	442 nm	5-50 mW
Kr^+	406.7, 413.1, **647.1**, 752.5	0.1-4 W
Nd:YAG	1064	0.1-10 W
second harmonic Nd:YAG	532	0.05-5 W
diodový (external cavity, fixed λ)	785	300 mW
diodový (external cavity)	780-1060	500 mW
dye, Ti: sapphire	continuously tunable	

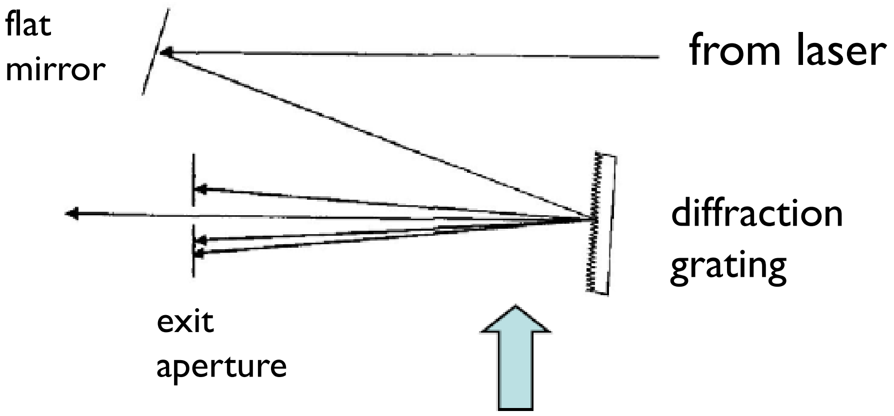
Filtration of the excitation light

Why?

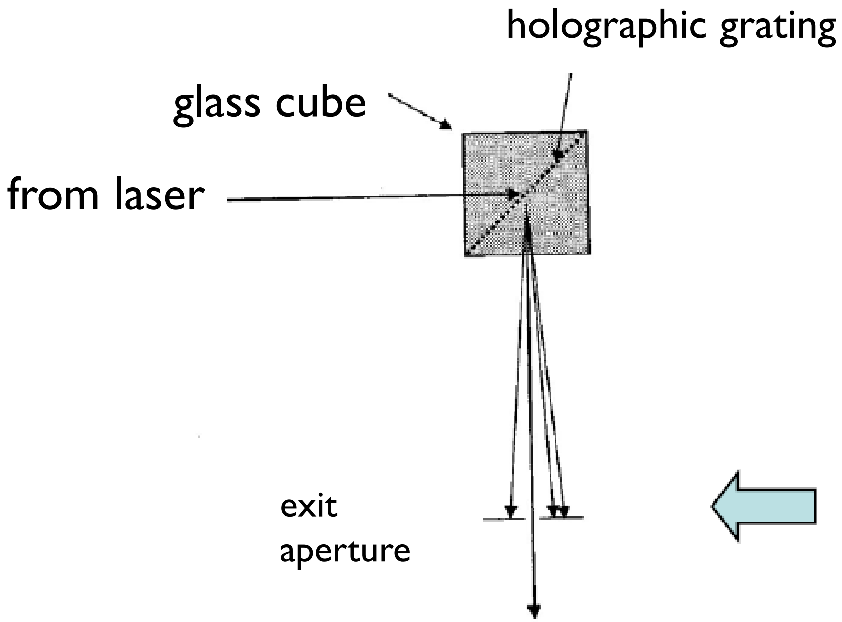
for suppressing unwanted spontaneous emission (eg. plasma lines in ion lasers, wide background from Nd:YAG and diode lasers)

How?

- interference filters
- pre-monochromator like filter



Grating premonochromator for filtering plasma lines from the collimated beam



high pass holographic bandpass filter, up to 80% (Kaiser Optical System)

Laser band-pass filters

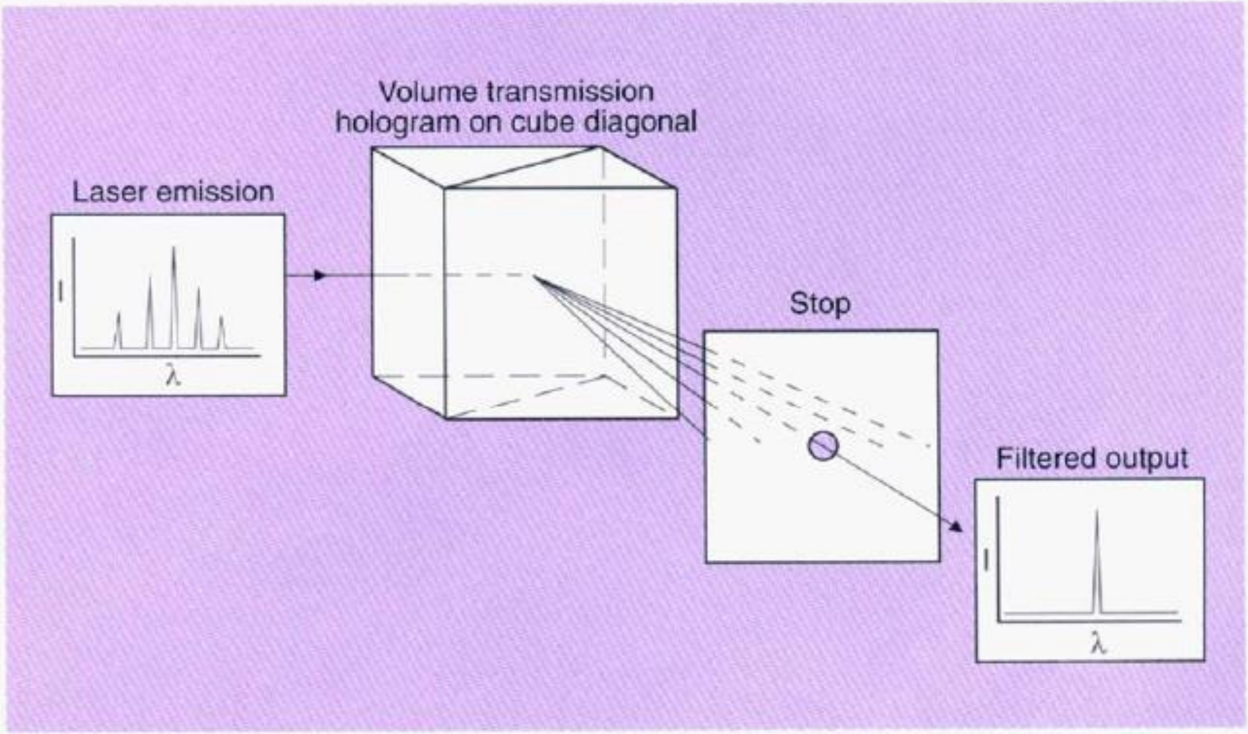
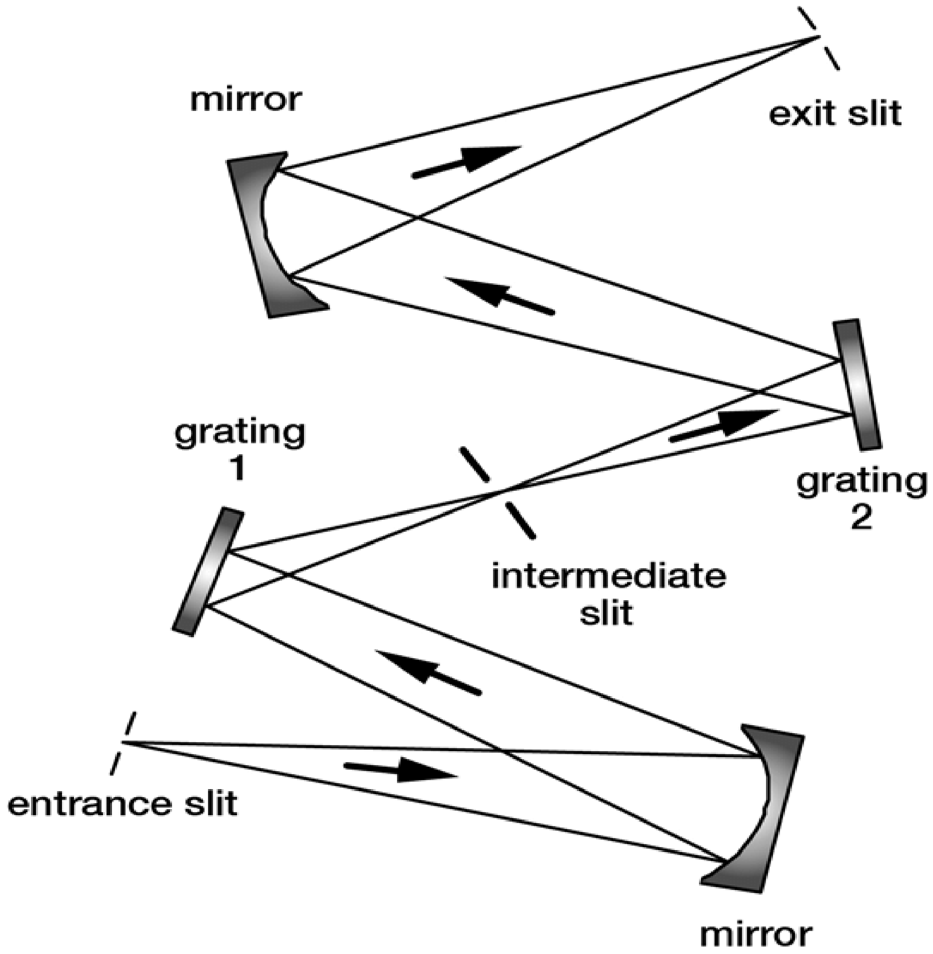


Figure 2. Laser bandpass filter configuration using volume transmission hologram embedded in a cube for maximum dispersion.

A very effective suppression of the plasma lines of the ion laser

Double monochromator



Double monochromator scheme
(eg. Spex 1403)
70s to 80s

Advantages:

high resolution

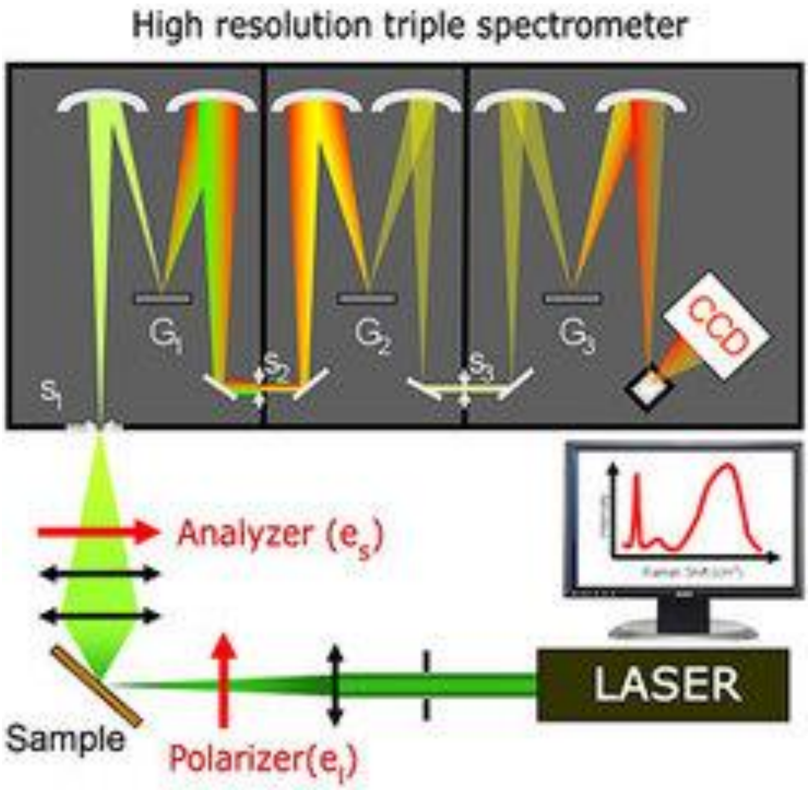
excellent background separation

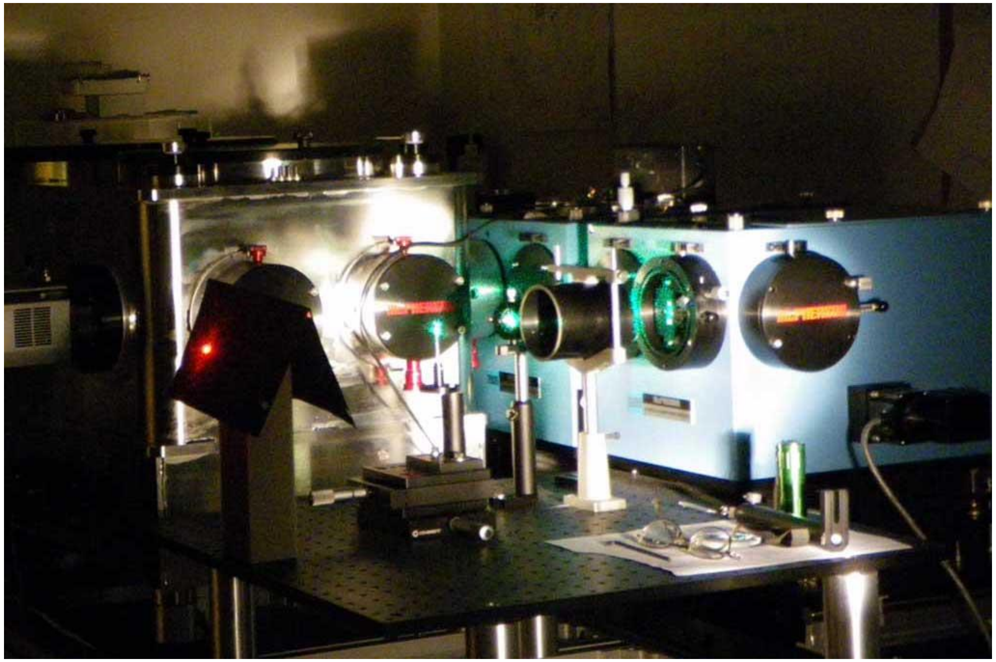
measurements near the excitation line

Disadvantages:

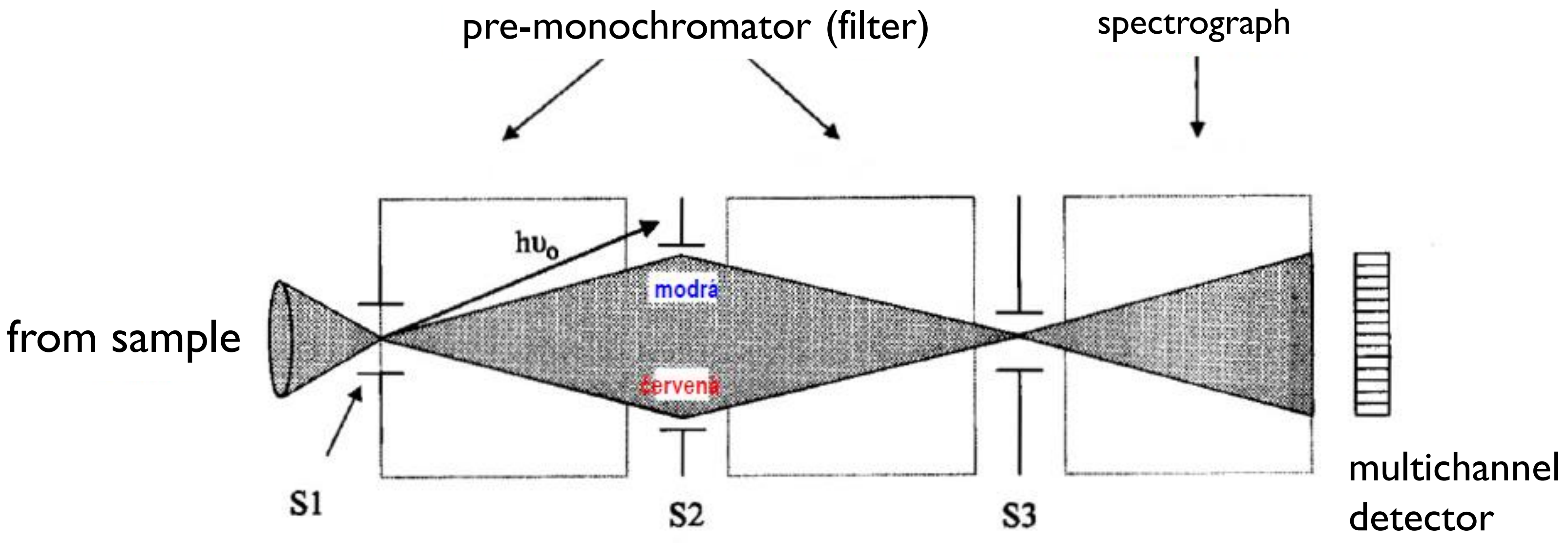
slow measurement (step by step)
too large dispersion for multi-channel detection

Triple spectrometer





Triple spectrometer



A diagram illustrating the operation of a triple spectrograph

Advantages:

excellent background separation

10^{-12} - 10^{-14}

measurements near the excitation line

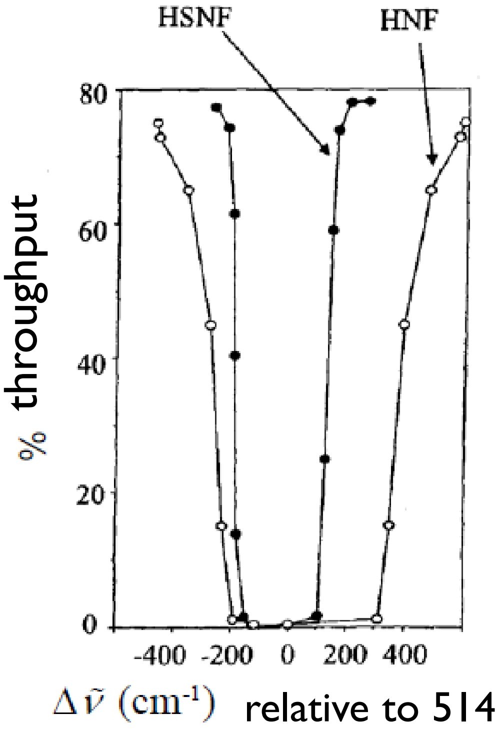
versatility

Disadvantages:

low aperture (low light on detector)

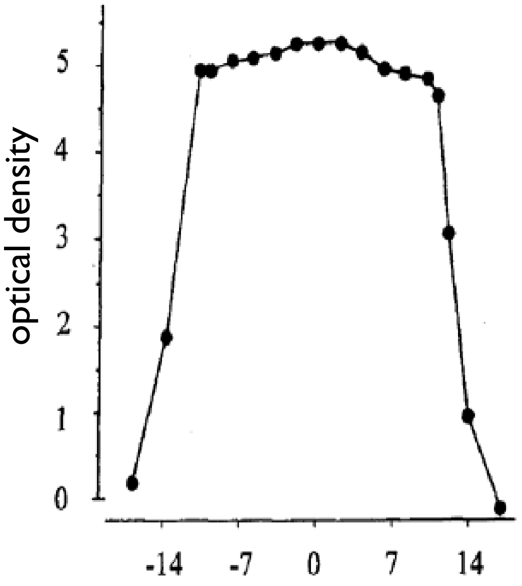
high price

Holographic notch filters



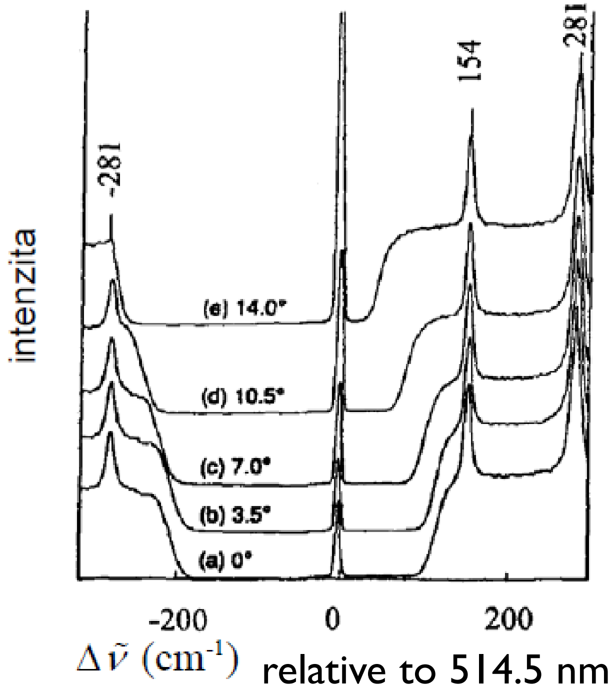
relative to 514.5 nm

Holographic Notch Filter (HNF) and Holographic Super Notch Filter (HSNF) Throughput



angle to the normal

Dependence of optical density HNF on the angle of rotation of the filter



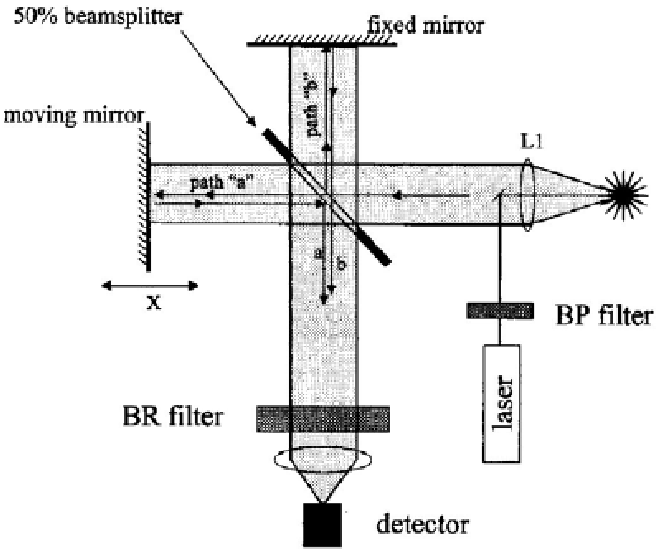
relative to 514.5 nm

Angular Tuning of HNF Filter (Calcite Spectrum)

Notch	OD > 4.0	bandwidth	700 cm^{-1}	edge width	300 cm^{-1}
Notch Plus	OD > 6.0	bandwidth	700 cm^{-1}	edge width	300 cm^{-1}
Super Notch	OD > 4.0	bandwidth	350 cm^{-1}	edge width	150 cm^{-1}
Super Notch Plus	OD > 6.0	bandwidth	350 cm^{-1}	edge width	150 cm^{-1}

Non-dispersive spectrometers

FT Raman - a multiplex technique where many wavelengths are generated by an interferometer and which generates an interferogram recorded by one detector



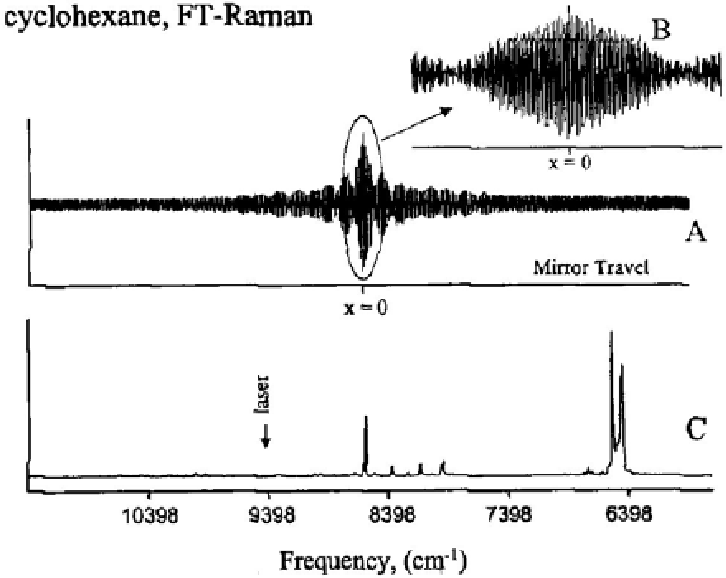


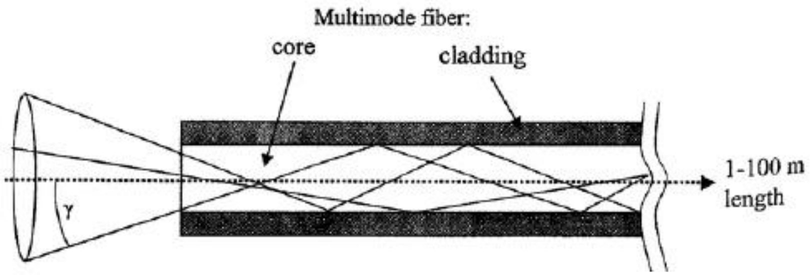
Figure of FT Raman spectrometer based on Michelson interferometer

Interferogram (A) for cyclohexane excited at 1064 nm and Raman spectrum (C)

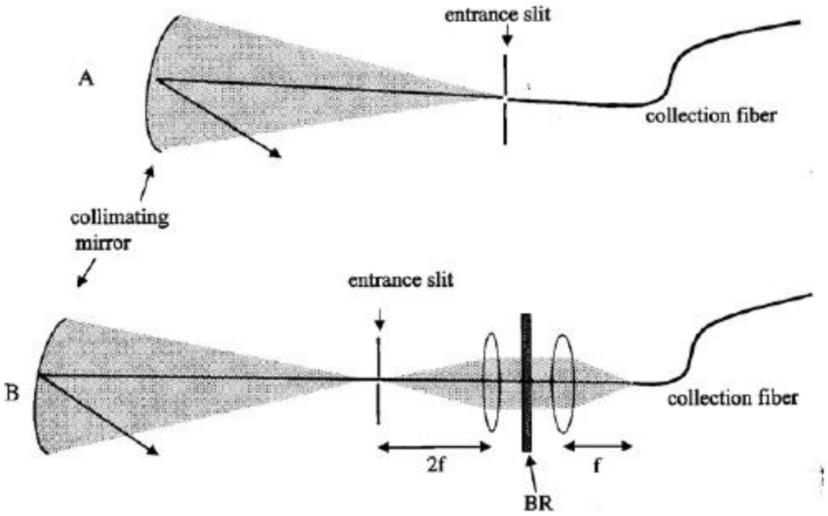
modulation - linear motion of the mirror, which generates the path difference a-b = 2x

resolution $\delta\tilde{\nu} = \dfrac{1}{\Delta x_{\max}}$ ⟸ maximum mirror path

Fiber probes



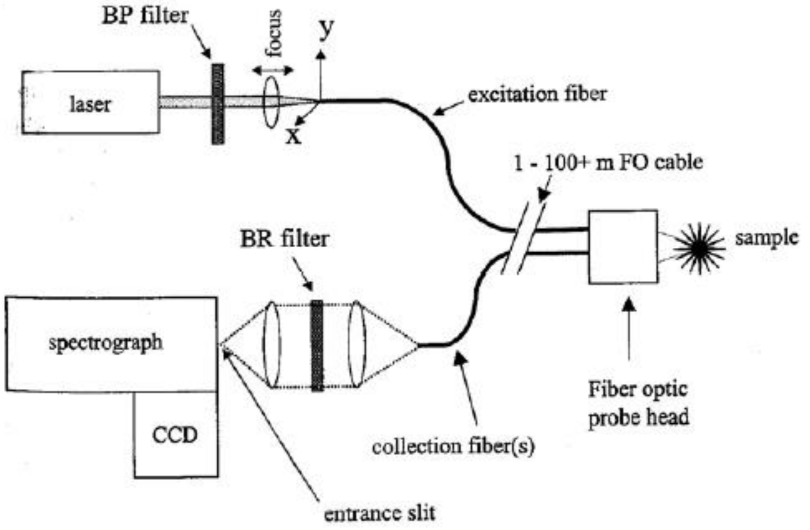
propagation of light in optical fiber



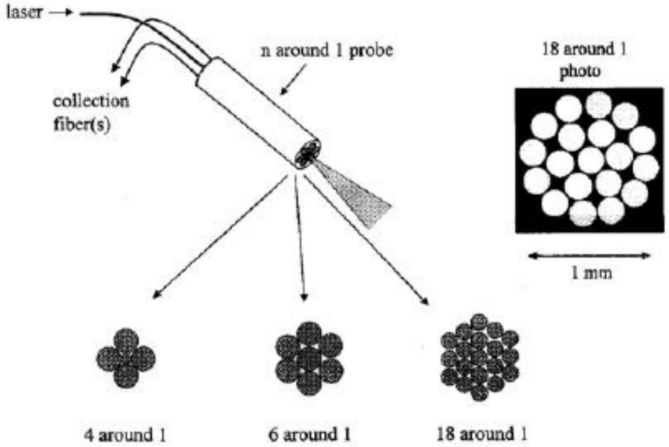
Two methods of connecting optical fibers to the spectrometer:

A) direct connection without possibility of f# spectrometer adaptation

B) a conventional method allowing even placing the BR filter in a collimated beam

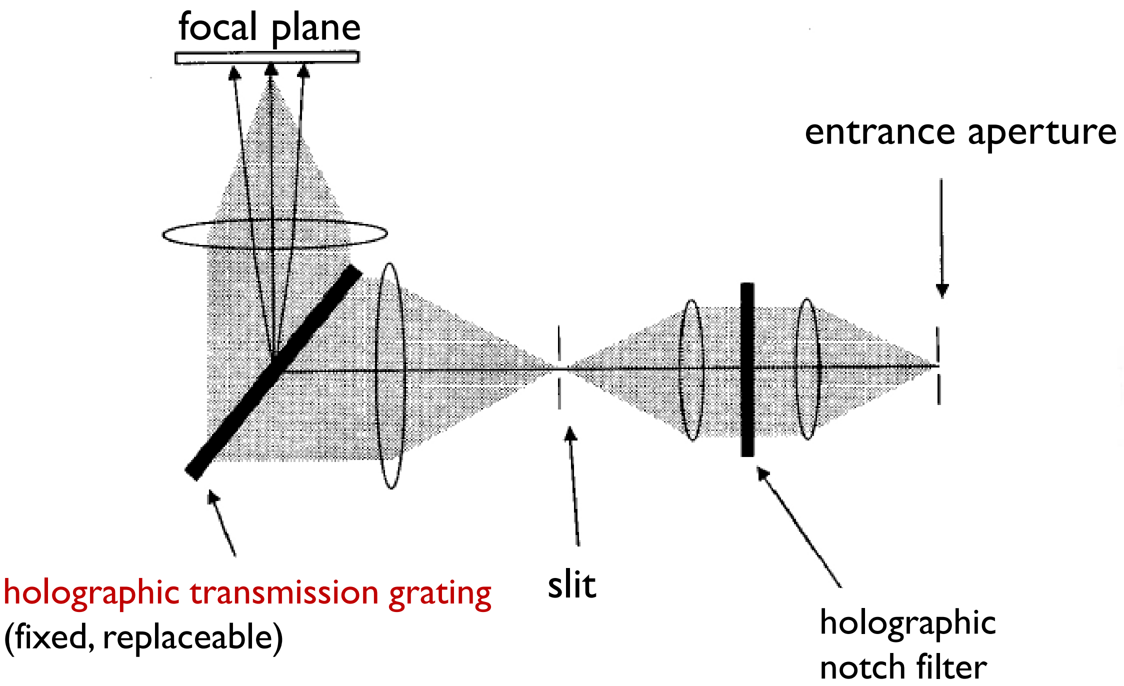


general scheme for the use of optical fibers



fiber probe arrangement (n around 1)

High aperture holographic imaging spectrograph



Scheme of transmission holographic spectrograph (e.g. Kaiser 1.8i)

Advantages

large aperture (transition from f / 4 to f / 1.4 represents almost an order increase of signal on detector (4 / 1.4) 2 = 8.2)
compactness (small size)
high dispersion

Disadvantage

different gratings for different excitation wavelengths

Nonlinear methods - SRS and ASRS

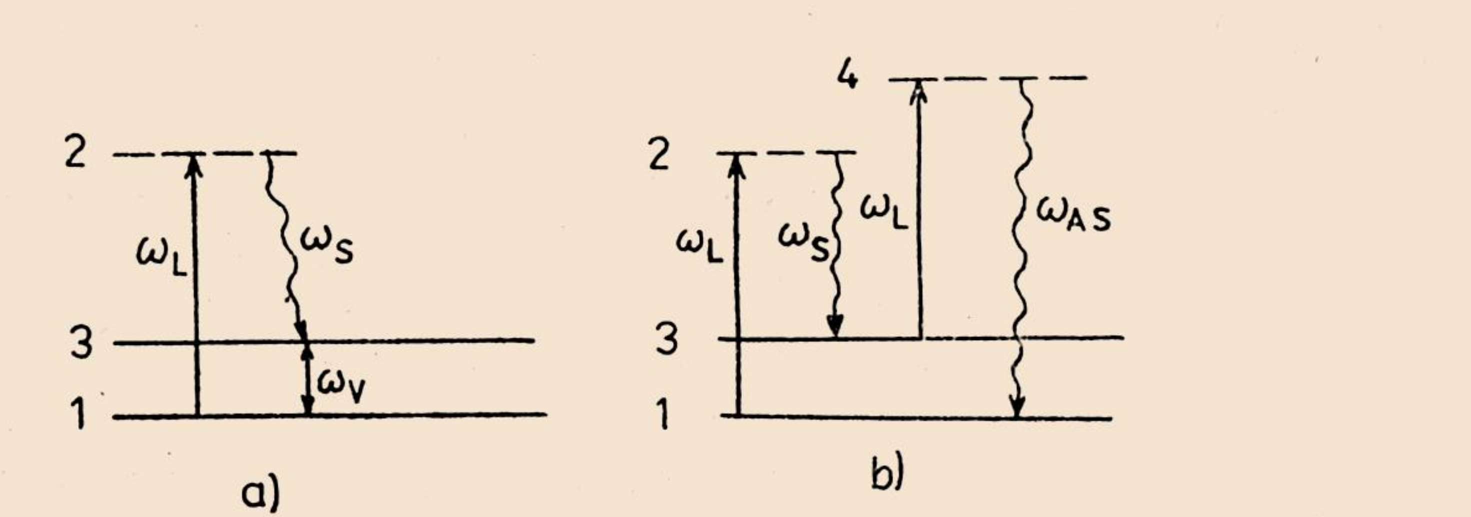
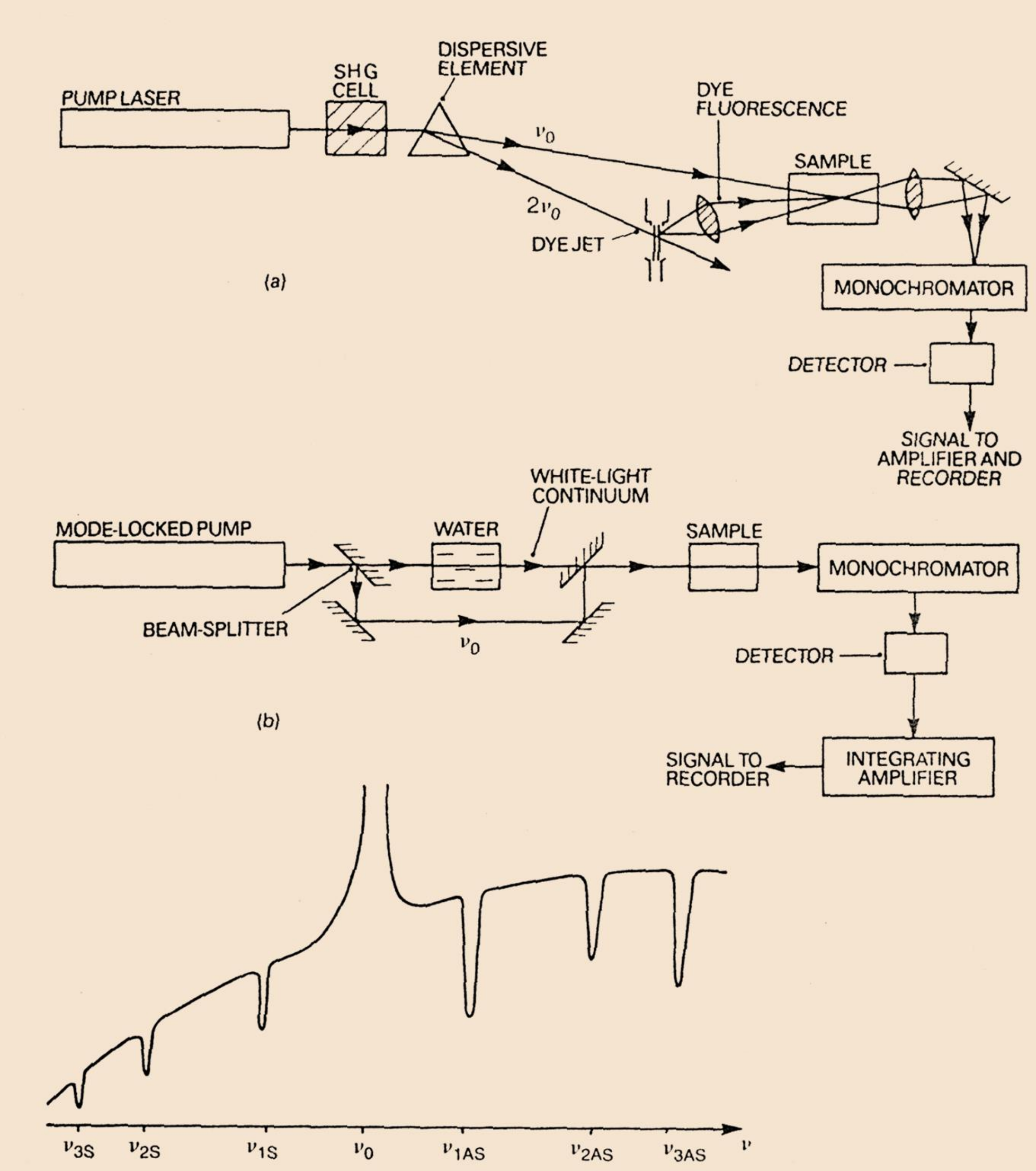


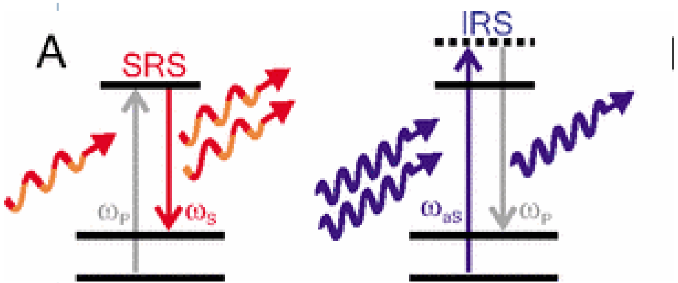
Diagram of energy levels and transitions for a) stimulated Raman scattering, b) anti-Stokes stimulated Raman scattering

SRS (Stimulated Raman Scattering) is a variant of the dual resonance (a) method in which the laser (frequency ω_L) level 2 is virtual. If ω_V is the frequency of the vibration transition, then $\omega_L - \omega_V = \omega_S$. If we insert an auxiliary beam into the cuvette with the frequency ω_S, ie with the Stokes frequency of the Raman spectrum, the amplification at this frequency occurs. In the case of ASRS (Antistokes SRS) two virtual levels 2 and 4 are excited and $\omega_L + \omega_V = \omega_{AS}$. The methods are used to measure high resolution up to 0.01 cm^{-1}. Tunable infrared (Raman) lasers are based also on this principle.



Inverse Raman spectrometry

- (a) Using a laser dye to generate a continuum
- (b) Using a white light continuum produced by slf-focusing of picosecond laser pulses in water
- (c) Illustratinon of the spectrum



Raman spectroscopy

- ***Each line of the Raman spectrum is dependent on its properties:***
  - ***on the number and mass of co-vibrating atoms of a molecule***
  - ***on their spatial arrangement***
  - ***on a molecular internal force field***
- ***Obviously, Raman spectra can be used analytically, especially in solving some differences in constitutions which are difficult to prove chemically***

Prof. Dr. Arnošt Okáč: Výklad k základním operacím v chemické analyse, JČMF 1948

Experimental advantages

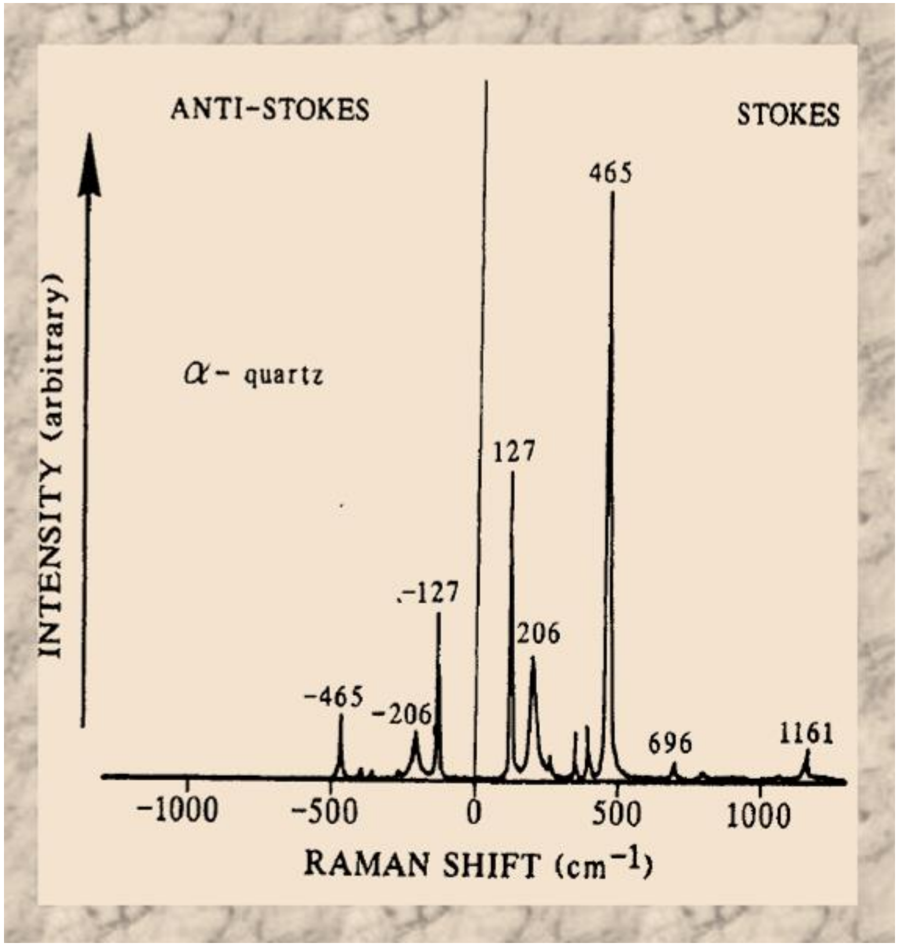
- possibility of measurement in aqueous environment
  - ➥ low intensity of Raman scattering for water
  - ➥ the optical materials used are not sensitive to moisture
- possibility of measurement in glass containers
  - ➥ measurement in closed ampoules – e.g. under vacuum
- easy to use glass fiber optics
- minimum treatment requirements for solid samples
- intense bands -C=C-, -N=N-, -S-S and other symmetric vibrations

Application of Raman s. in geology

- phase identification and analysis: in some cases, RS is the simplest or even the only one method available to identify a mineral, especially when encased in another transparent, non-fluorescent mineral. It makes it easier to distinguish members of isomorphic series more easily than X-ray diffraction.
- to identify gases in gas - liquid enclosures
- study of structure (especially bond of OH groups), structure order
- Phase transitions (temperature change of perovskite structure in the Earth mantle)
- Determination of thermodynamic properties of minerals

Other applications of RS in geology

- resonance Raman spectroscopy
- Electronic Raman spectroscopy
- hyper-Raman spectroscopy
- coherent antistokesian Raman scattering (CARS)



Geological materials - hematite

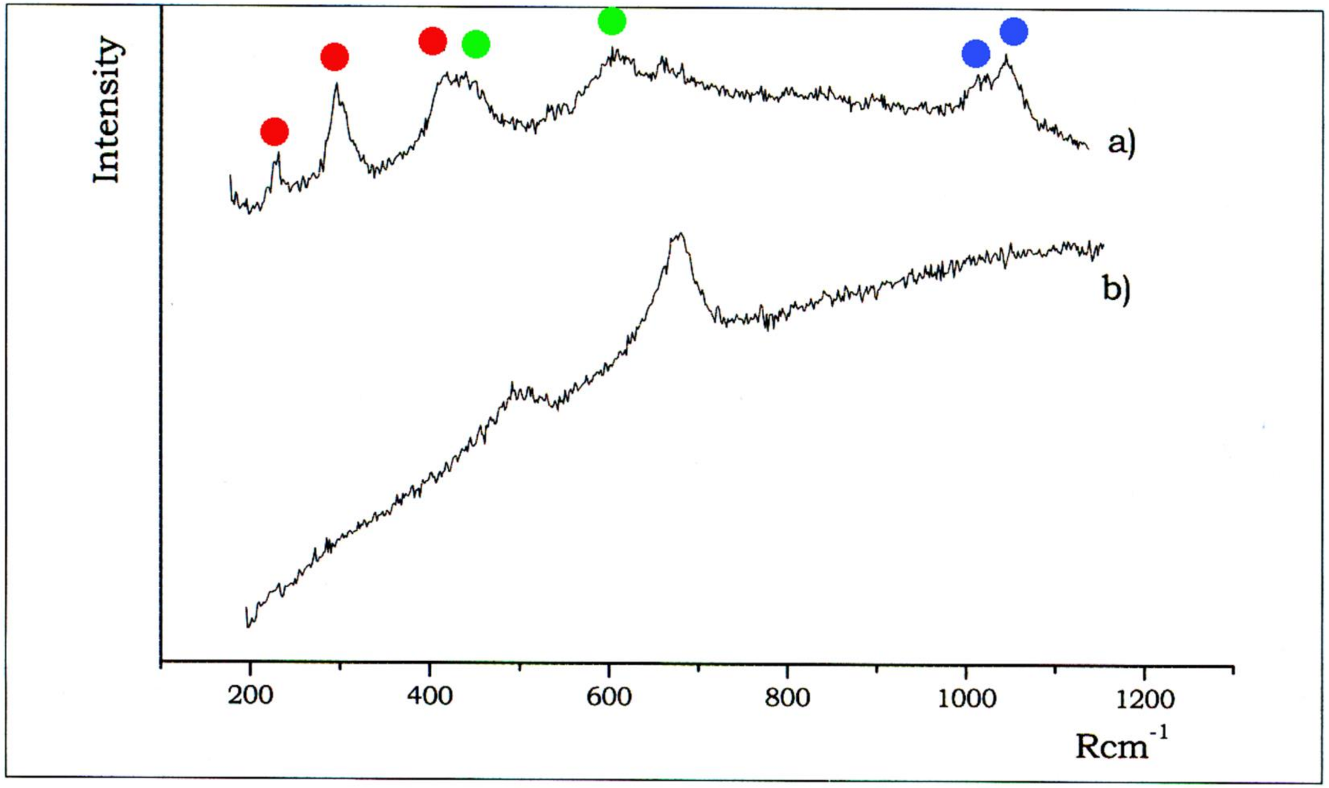
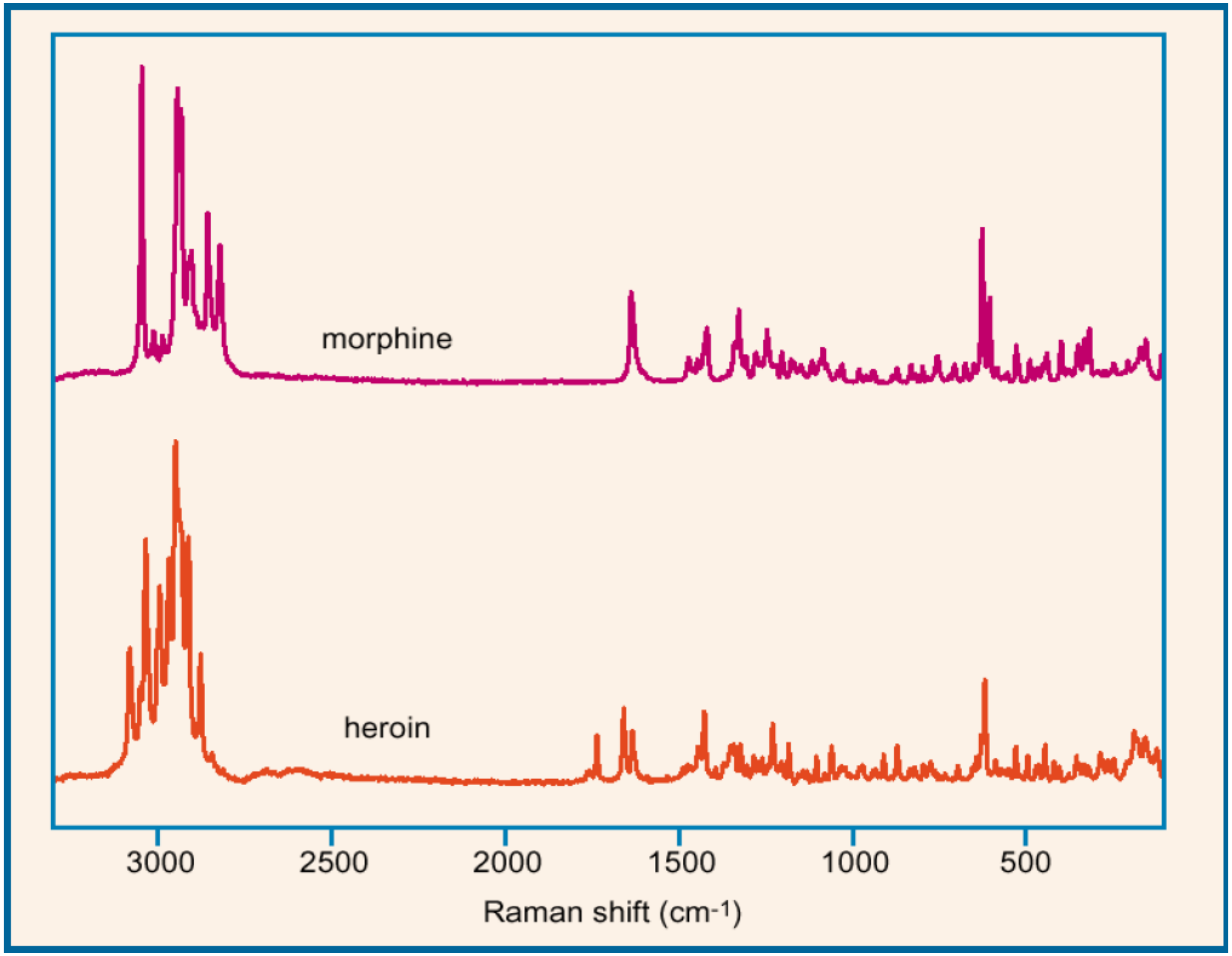
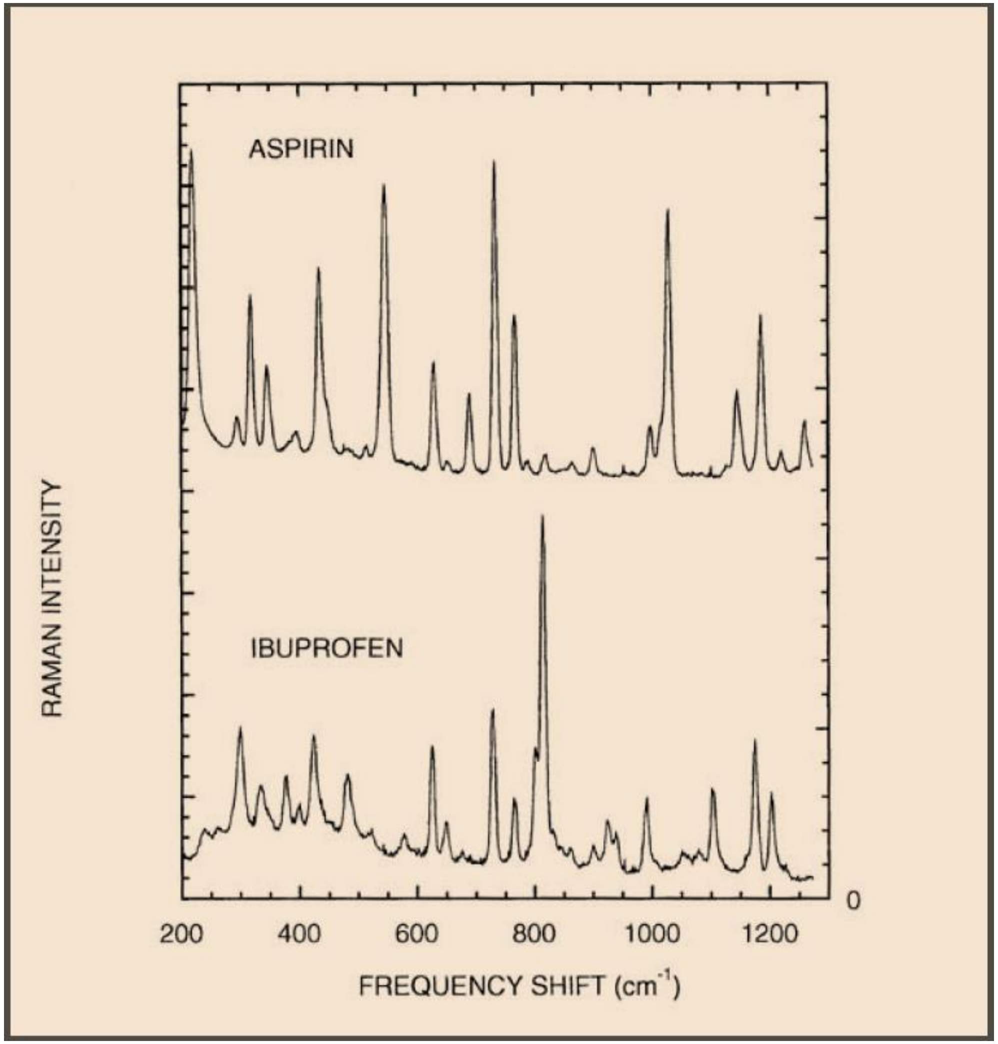


Figure 4. Raman spectra of (a) hematite and (b) magnetite, acquired on B and A sherds, respectively. In the (a) spectrum the bands of hematite are indicated by a 🔴, while those of rutile are labelled with a 🟢 symbol; the doublet marked with 🔵 is due to Cr(III) luminescence.

Identification of drugs



Identification of drugs



Study of complexes

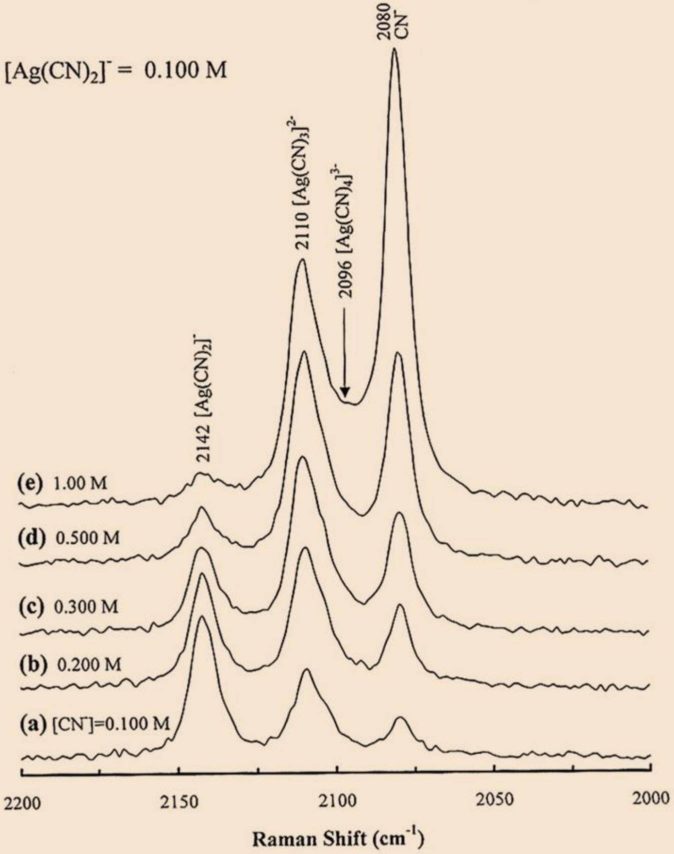


FIG. 3. Raman spectra at different ratios of CN^- with respect to $[Ag(CN)_2]^-$. The concentrations of CN^- ranged from 0.00 to 1.00 M at the constant $[Ag(CN)_2]^-$ concentration of 0.100 M before mixing the two solutions. (*a*) 0.100 M, (*b*) 0.200 M, (*c*) 0.300 M, (*d*) 0.500 M, and (*e*) 1.00 M of the CN^- concentration.

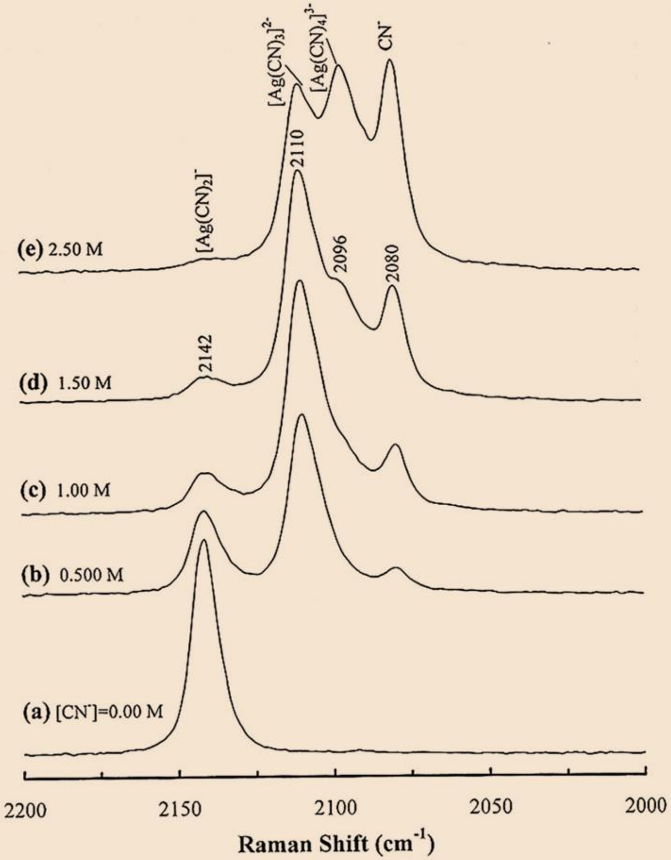


FIG. 4. Raman spectra at different ratios of CN^- with respect to $[Ag(CN)_2]^-$. The concentrations of CN^- ranged from 0.00 to 2.50 M at the constant $[Ag(CN)_2]^-$ concentration of 0.500 M before mixing the two solutions. (*a*) 0.00 M, (*b*) 0.500 M, (*c*) 1.00 M, (*d*) 1.50 M, and (*e*) 2.50 M of the CN^- concentration.

Archeology - ceramics

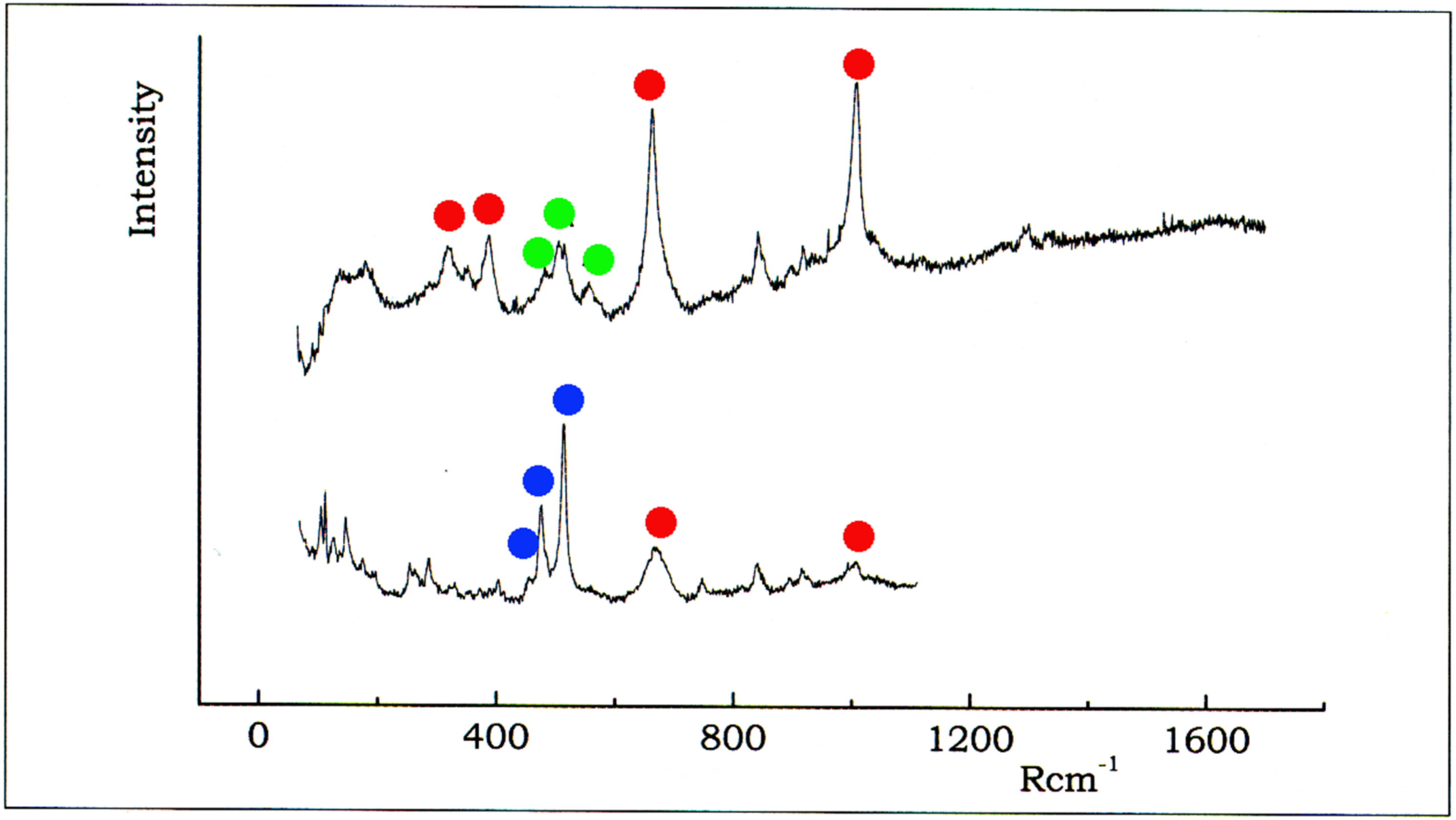
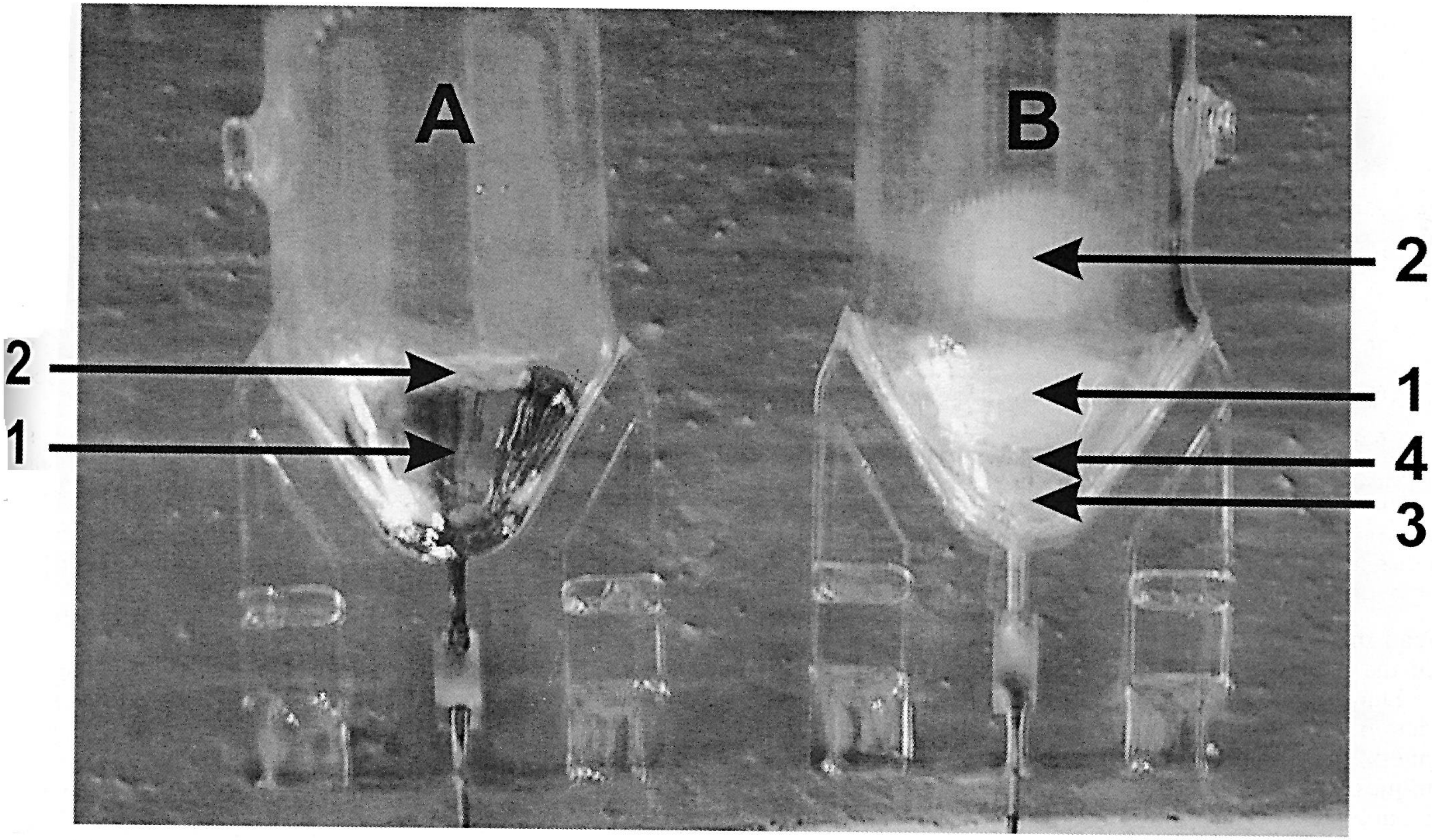


Figure 3. Spectra recorded on A wares containing characteristic peaks of diopside (🔴), K-feldspars (🔵) and plagioclases (🟢).

Analysis of compounds in the discharge lamp



Photographs of lower ends of arc tube A immediately after seasoning (i.e., 7 min vertical burning) and of arc tube B after burning vertically for 125 h.

Analysis of compounds in the discharge lamp

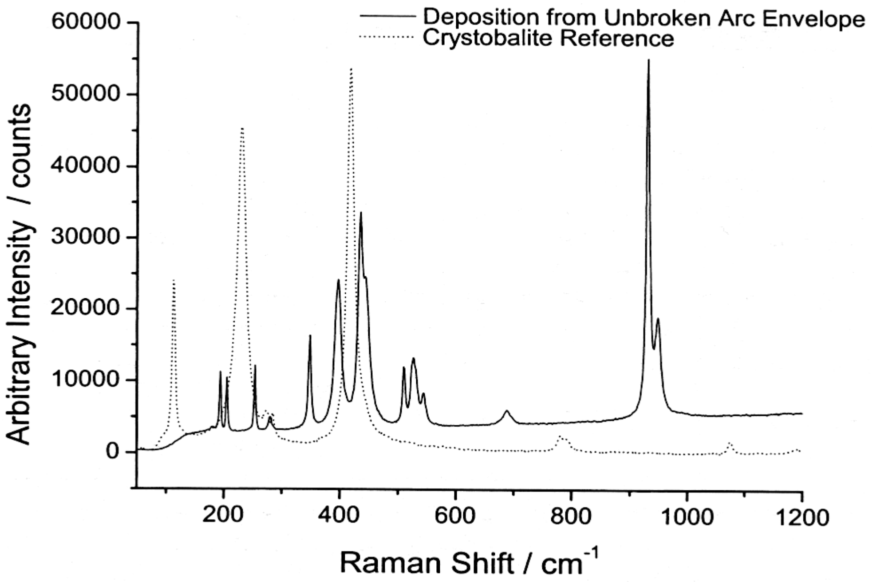


FIG. 9. Raman spectrum of white lamp envelope deposit (Positions 1 and 2 on arc tube B shown in Fig. 2) and an α-crystobalite reference spectrum from the HTSL Raman Database.

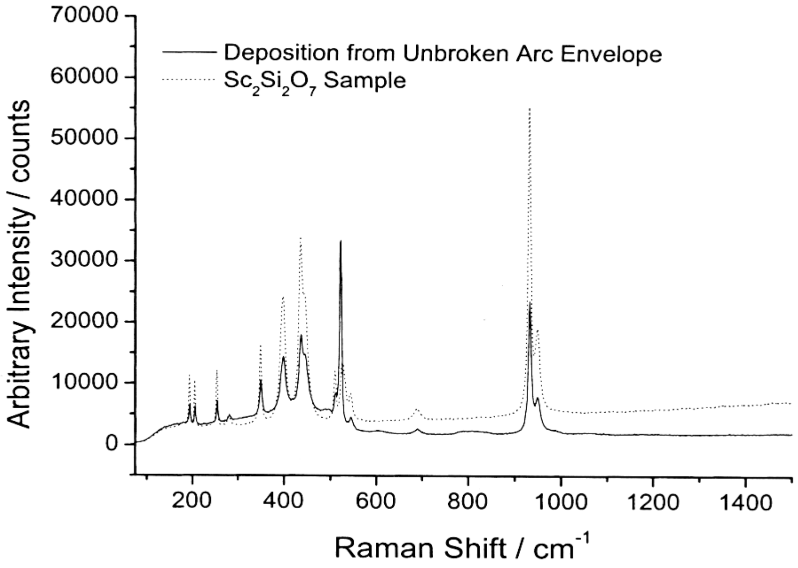
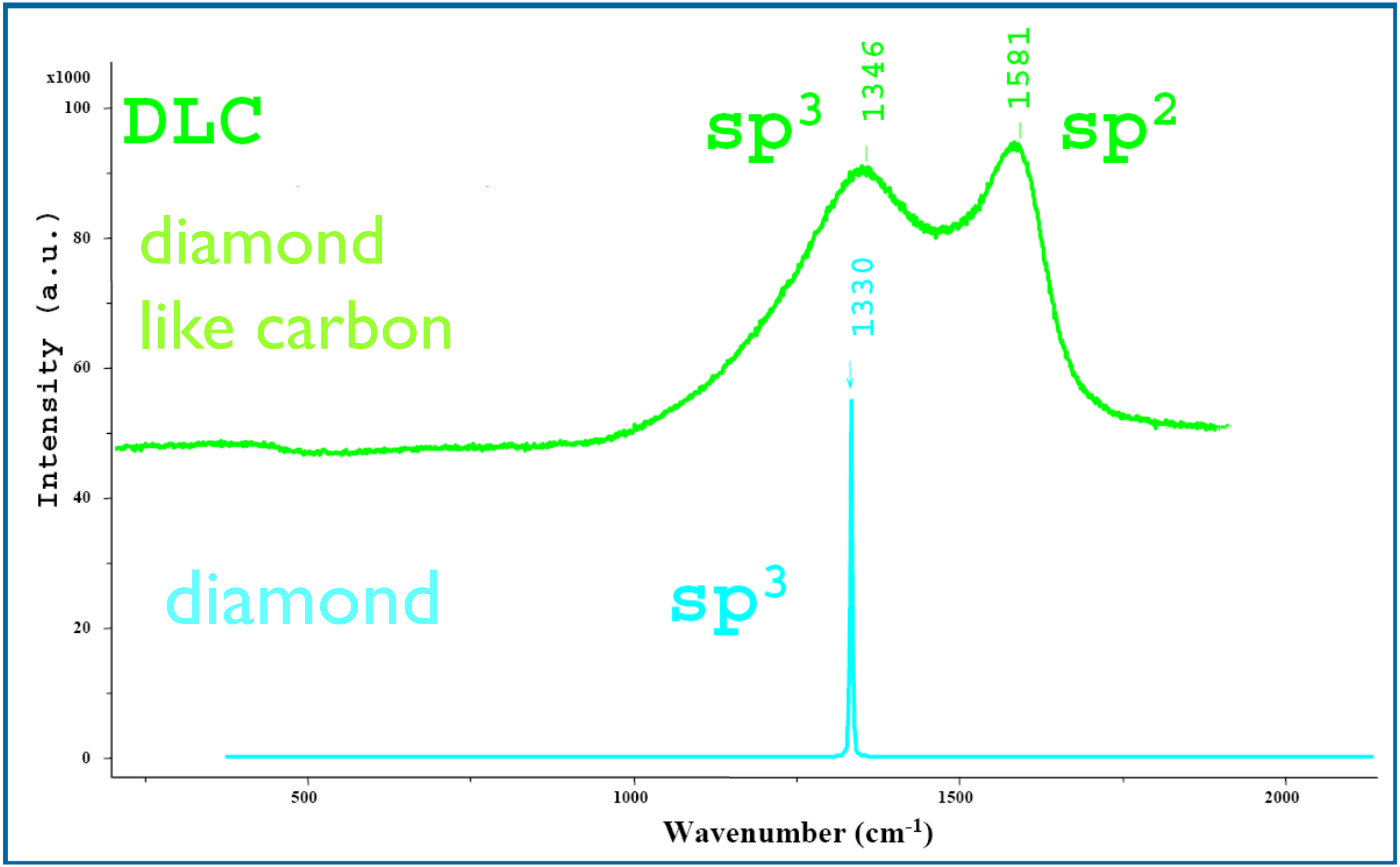
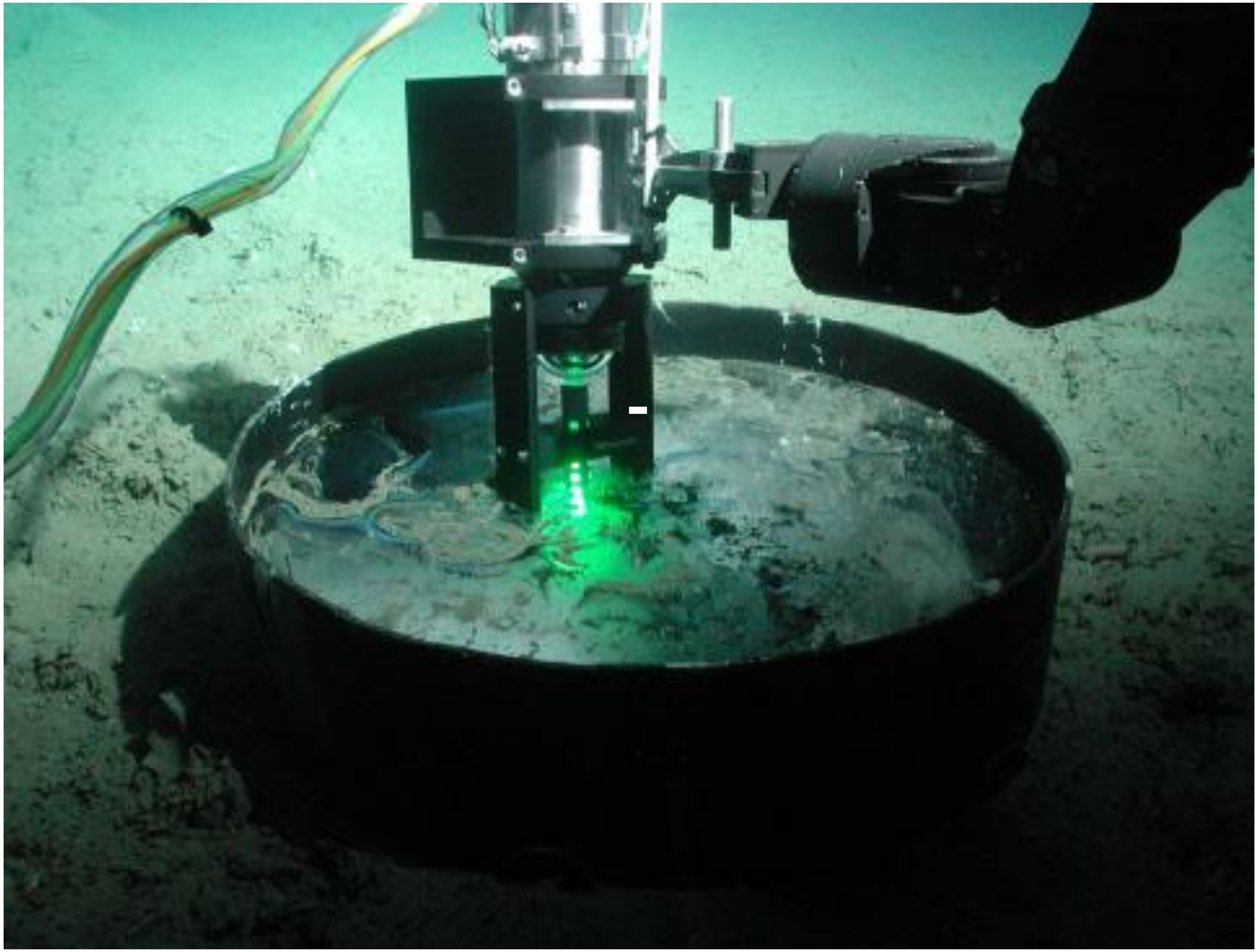


FIG. 11. Raman spectrum of white envelope deposit (Positions 1 and 2 on arc tube B shown in Fig. 2) and a $Sc_2Si_2O_7$ reference spectrum from the HTSL Raman Database.

Analysis of carbon materials



DORISS system (depth of 3607 meters in Monterey Bay)



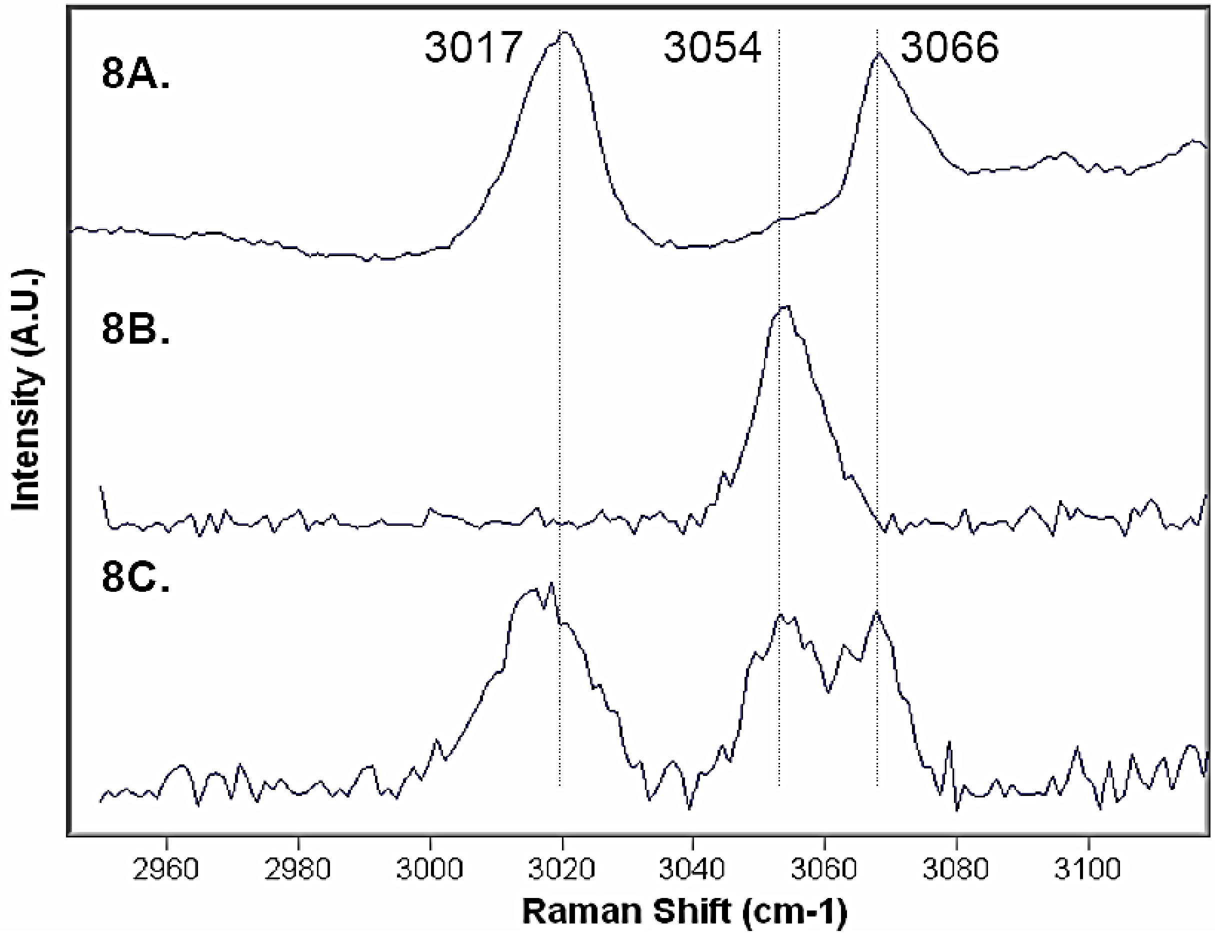


Figure 8. Methane Raman spectra. Spectrum 8A (5 MPa, 278 K, 150 sec (15 sec X 10)) shows two peaks (3017, 3066 cm^{-1}) assigned to methane gas. Spectrum 8B (7 MPa, 276 K, 200 sec (20 sec X 10)) shows one peak (3054 cm^{-1}) for pure synthetic methane hydrate. Spectra 8C (7.7 MPa, 275.4 K, 25 sec (5 sec X 5)) shows three peaks (3017, 3054, and 3066 cm^{-1}) for a natural hydrate sample measured *in situ*. Of the three peaks in 8C, two peaks (3017 and 3066 cm^{-1}) can be assigned to gas phase methane and 3054 cm^{-1} can be assigned to methane in the hydrate phase.

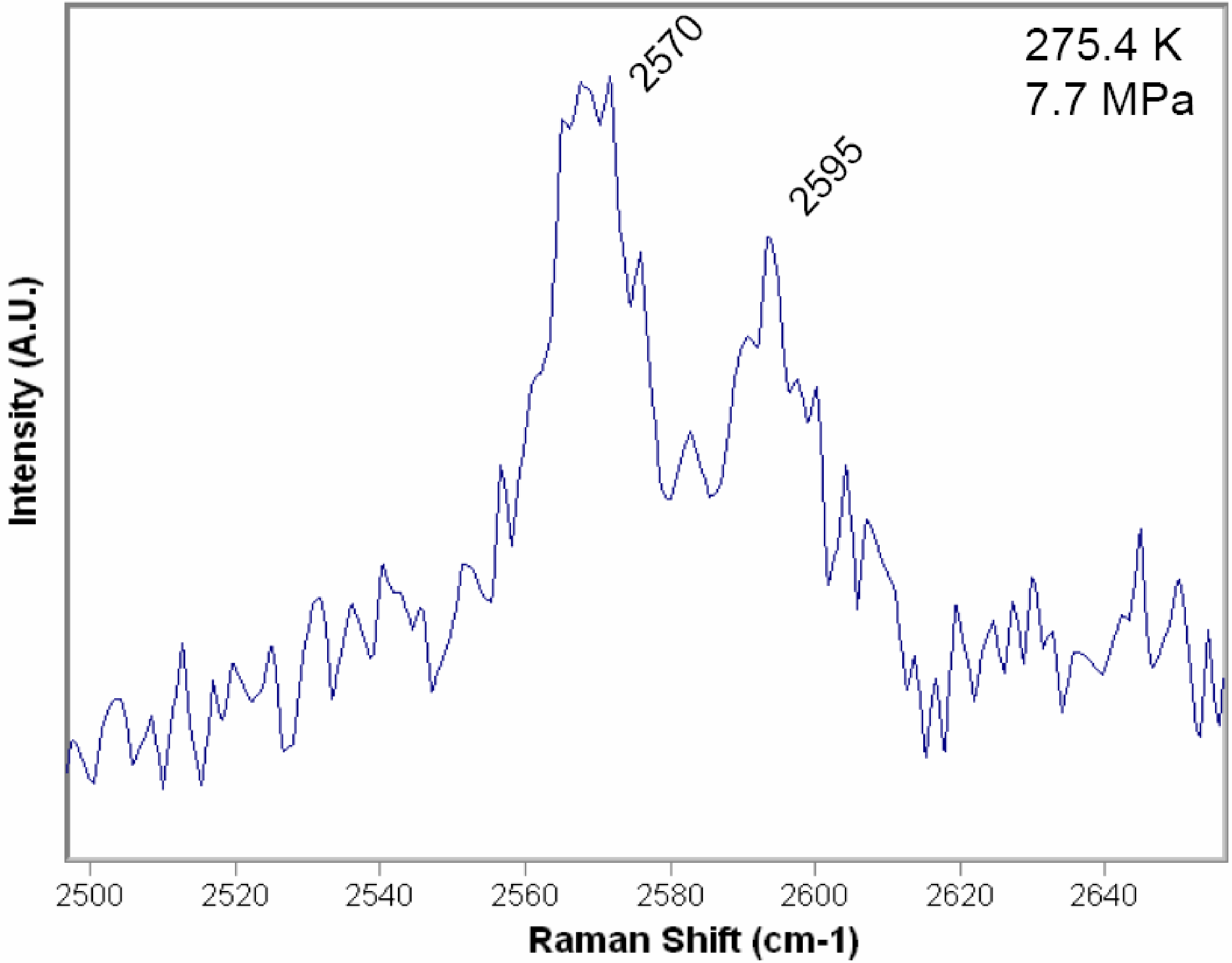


Fig. 9. Raman spectra showing H_2S in the $5^{12}6^2$ cage (The peak at 2570 cm^{-1} is a vibrational mode from methane in the hydrate), (400 sec, 20 sec X 20)

Raman micro probe

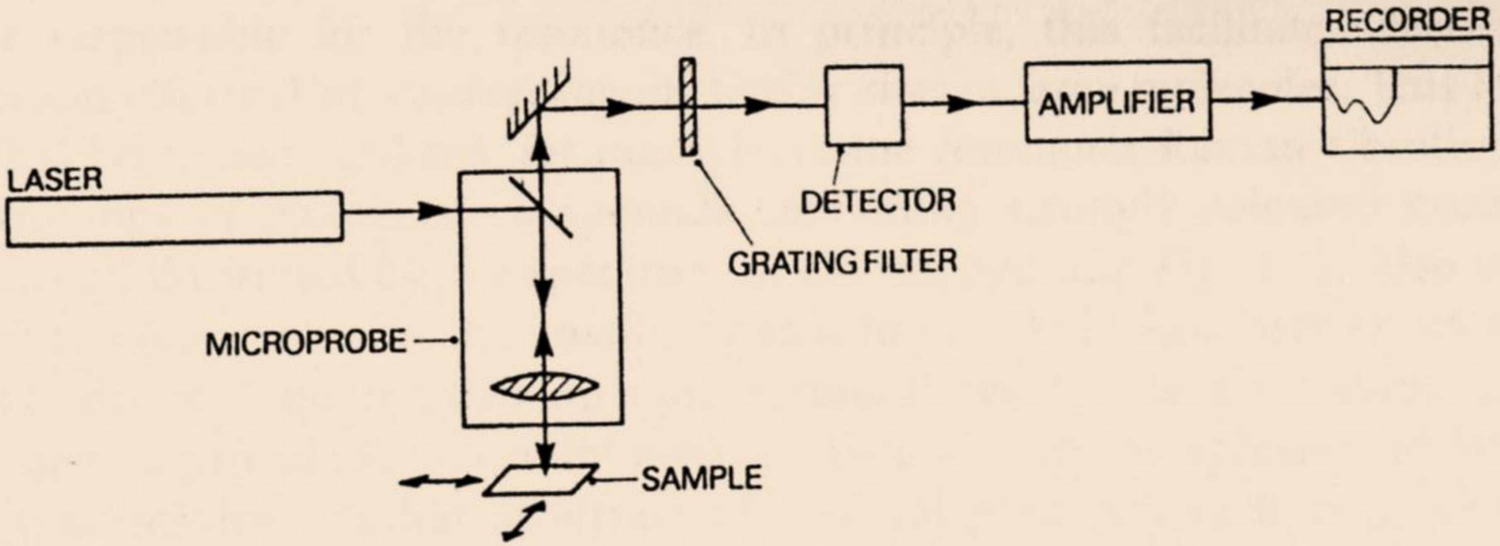
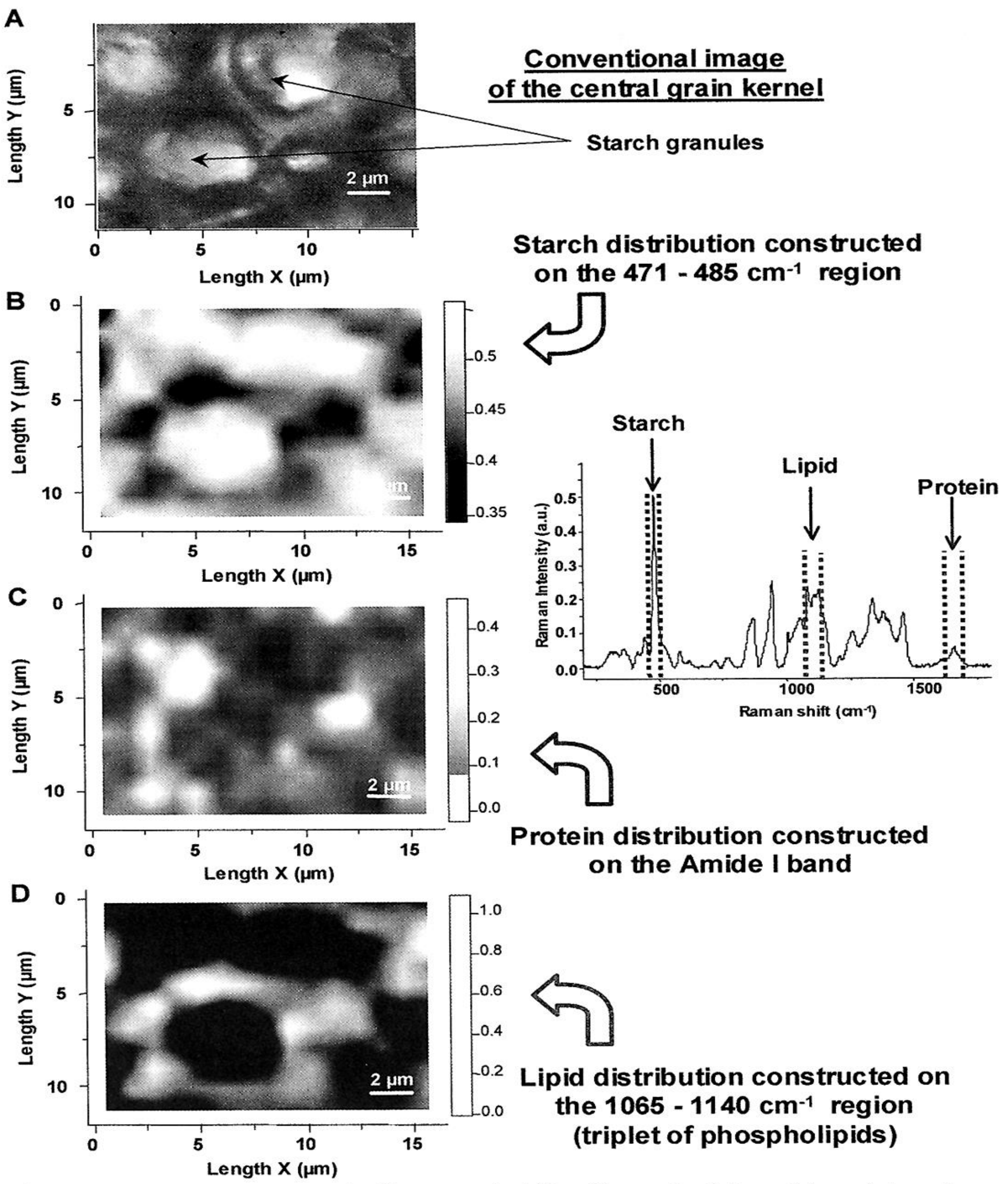


Fig. 4.25 Raman microprobe (point illumination) instrumentation. In the alternative global illumination configuration, a larger surface area is illuminated, and the detection equipment is replaced with an image intensifier phototube and camera

Analysis of cereal grains



Spectral images were generated from a set of 15 x 11 spectra taken at 1 µm interval

Paper surface mapping

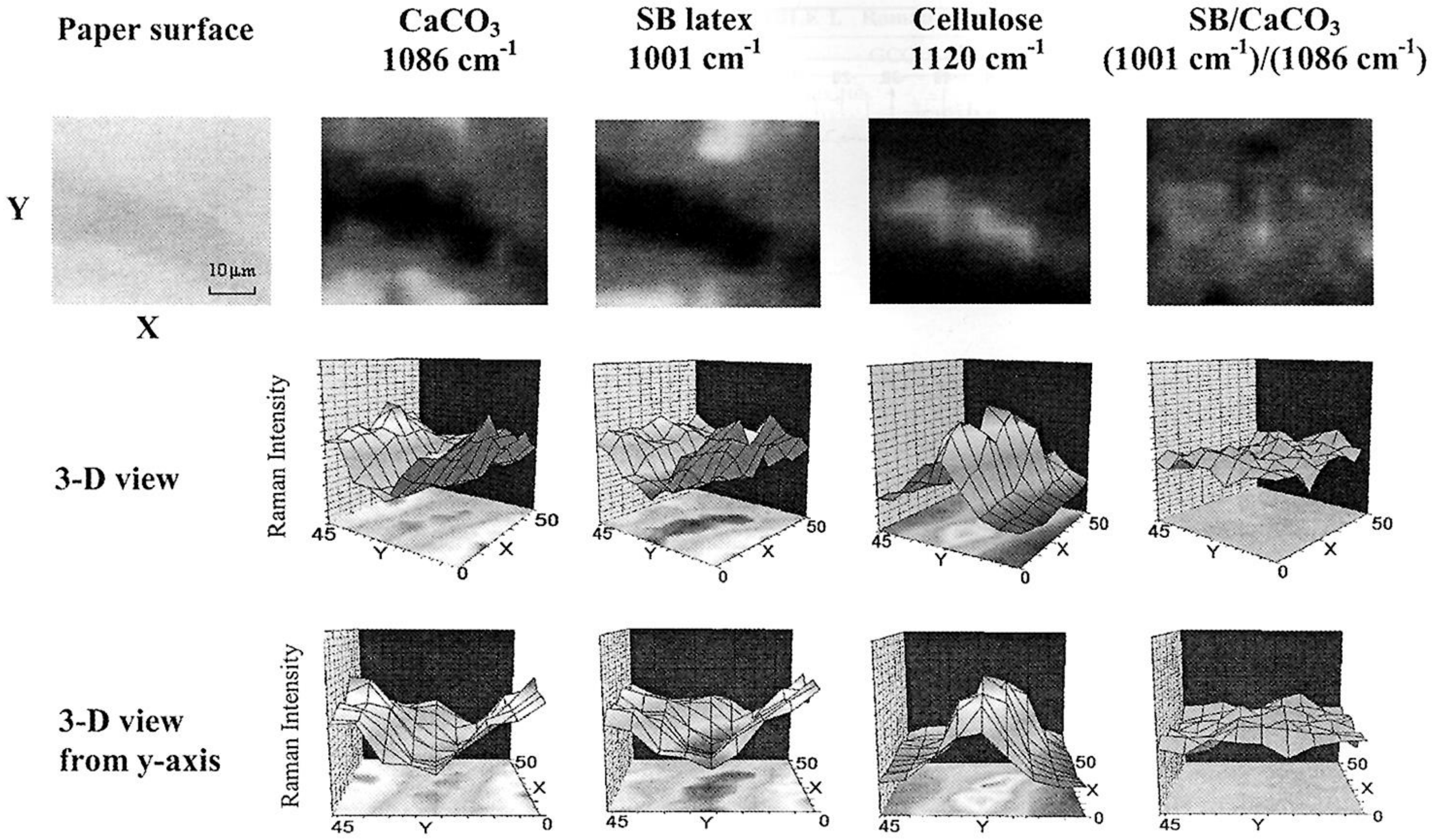
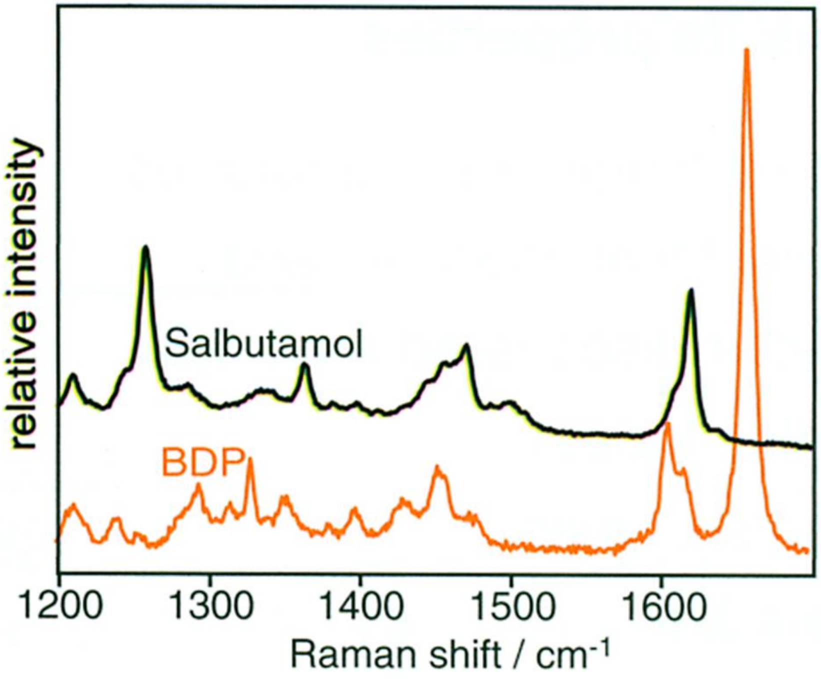
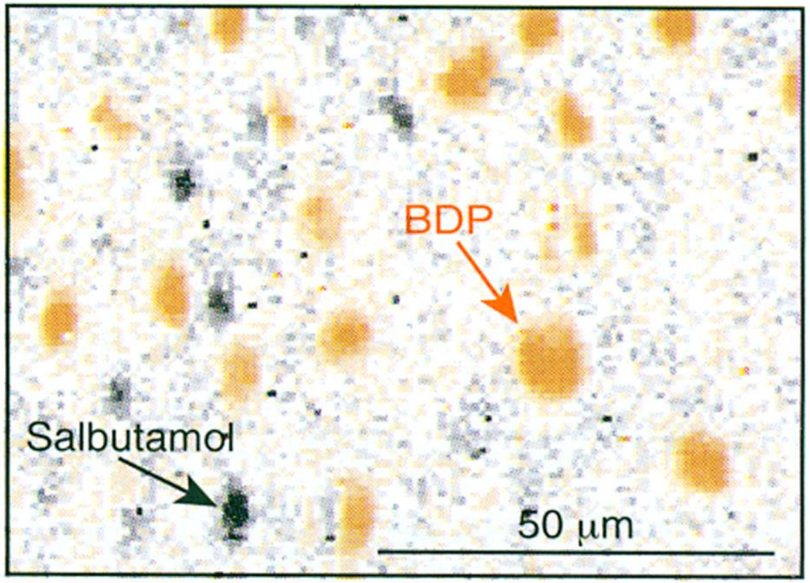


FIG. 6. Surface mapping of SB/$CaCO_3$ coated paper.

Spatial distribution of aerosol



Raman spectra of pure samples of BDP and salbutamol



Raman map of BDP and salbutamol distribution

BDP - **beclomethasone**

Detection of infected erythrocytes

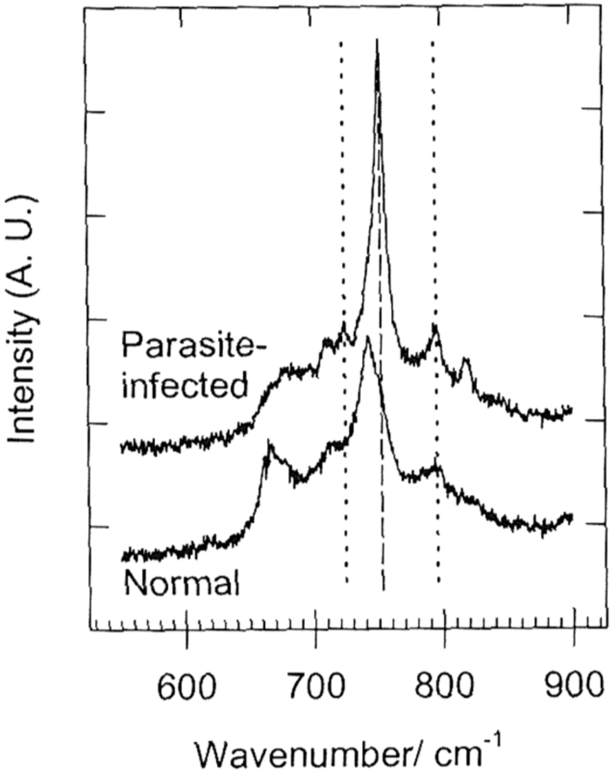


FIG. 4.
the atom

FIG. 6. Raman spectra comparing the differences between the normal and parasite-infected erythrocytes in the 550–900 cm^{-1} region with the 632.8-nm HeNe laser. The band due to the vibrational mode C_aNC_a occurs at 743 cm^{-1} for normal erythrocytes and 754 cm^{-1} for parasite-infected erythrocytes, as indicated by the dashed line. More differences can be identified by the weaker bands, at 724 and 795 cm^{-1}, indicated by the dotted lines.

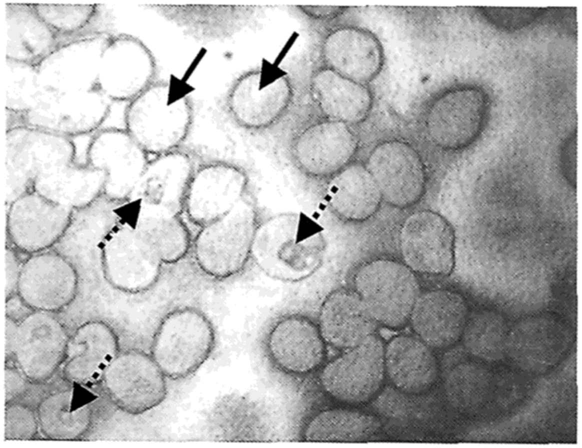


FIG. 2. Optical images of uninfected and *P. berghei*-infected erythrocytes. The uninfected mouse erythrocytes are indicated by solid arrows and the *P. berghei*-infected erythrocytes by dashed arrows. Mag: 1000×.

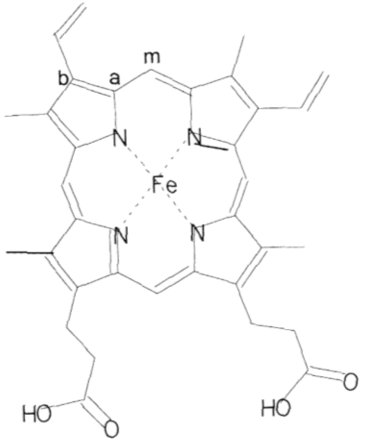
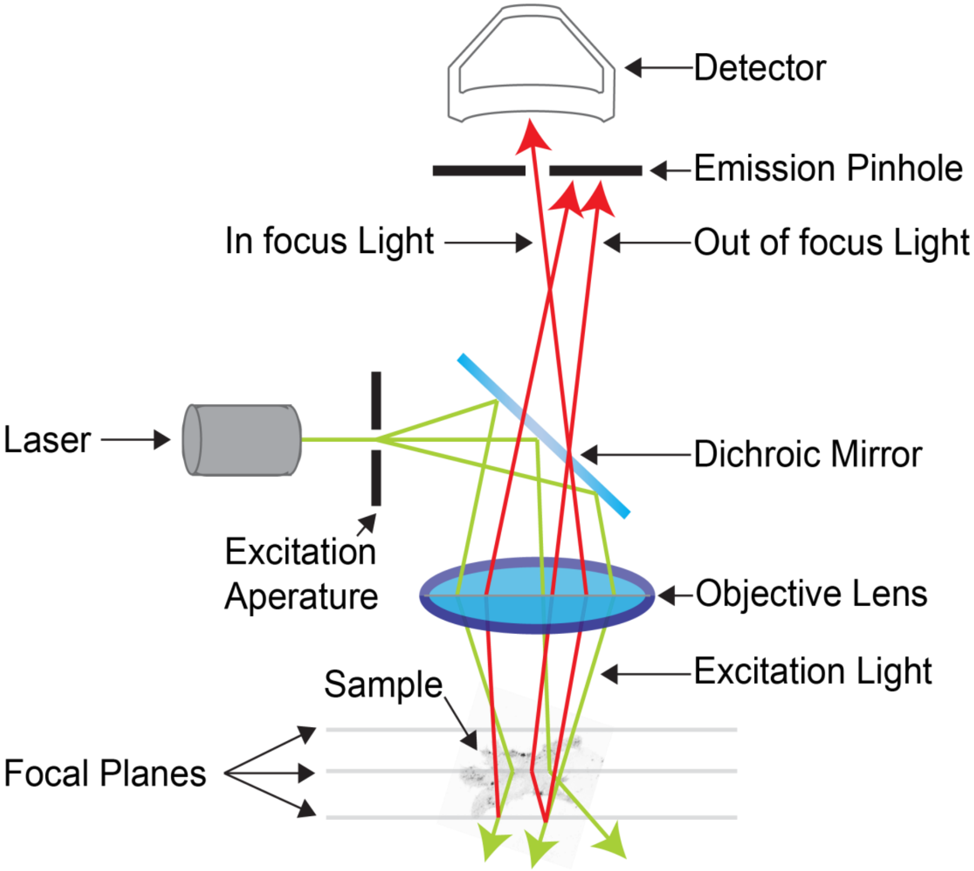


FIG. 4. Schematic representation of the iron protoporphyrin showing the atoms referred to in the band assignments.

Confocal microscope

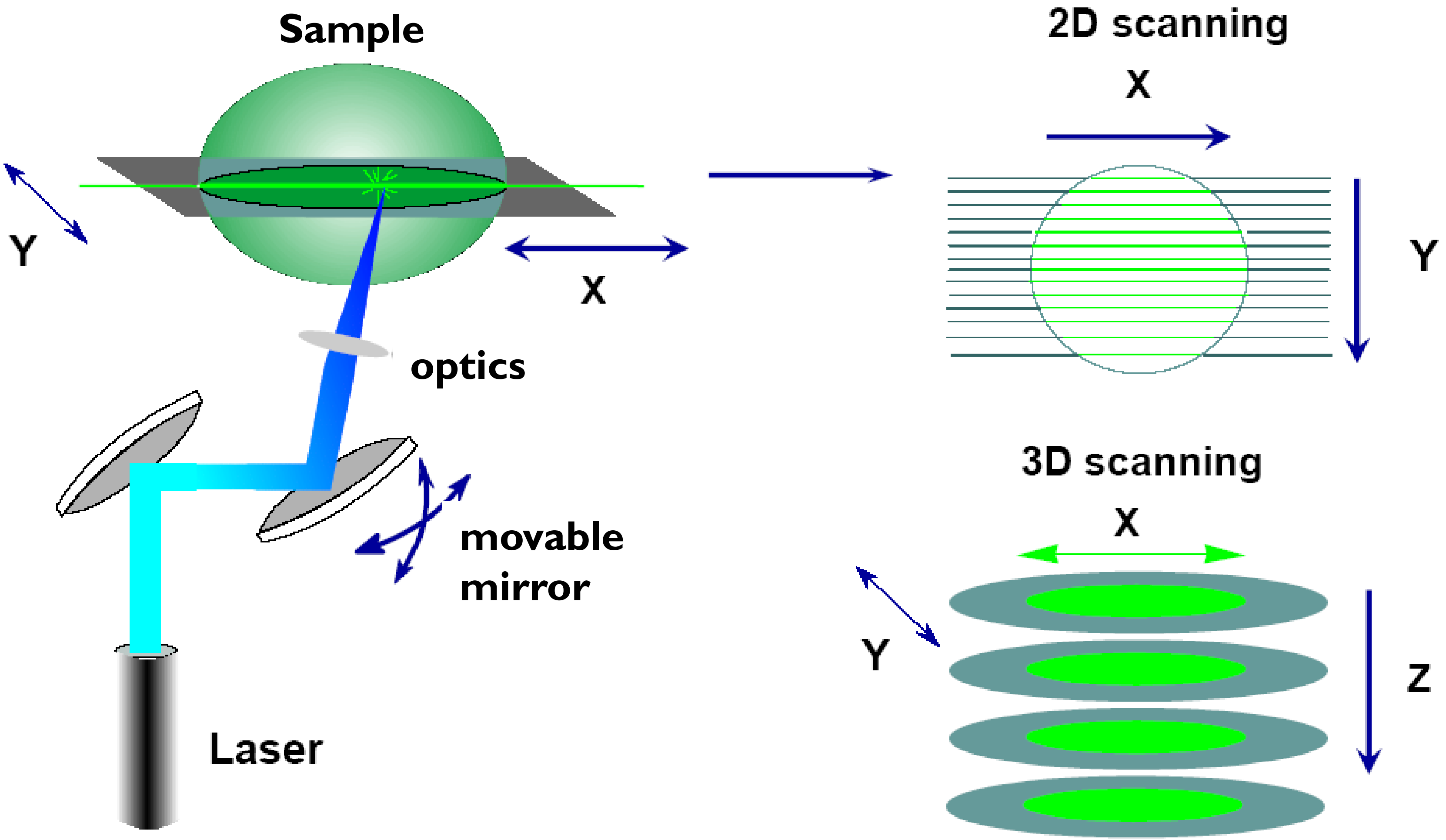


Focused beams of laser light are scanned across the sample (Laser Scanning Microscopy, LSM)

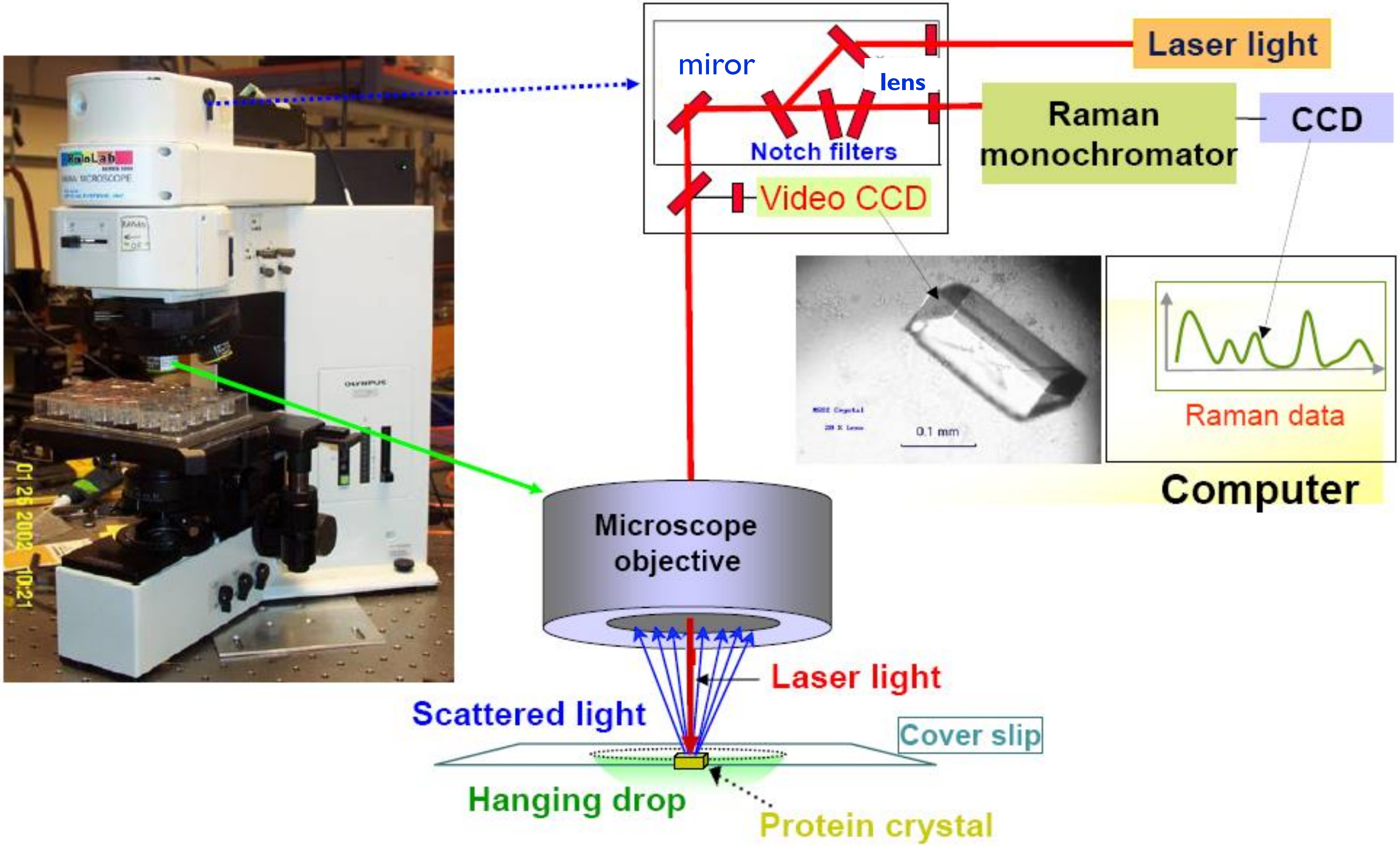
and light only from the desired focal plane is allowed to enter the detector.

These microscopes provide excellent axial resolution, and very good signal to noise sampling, however this often comes with a sacrifice in temporal resolution due to the slow nature of scanning pixel-by-pixel across the image during capture. Some systems overcome this speed issue with either faster scanners.

Scanning mirrors



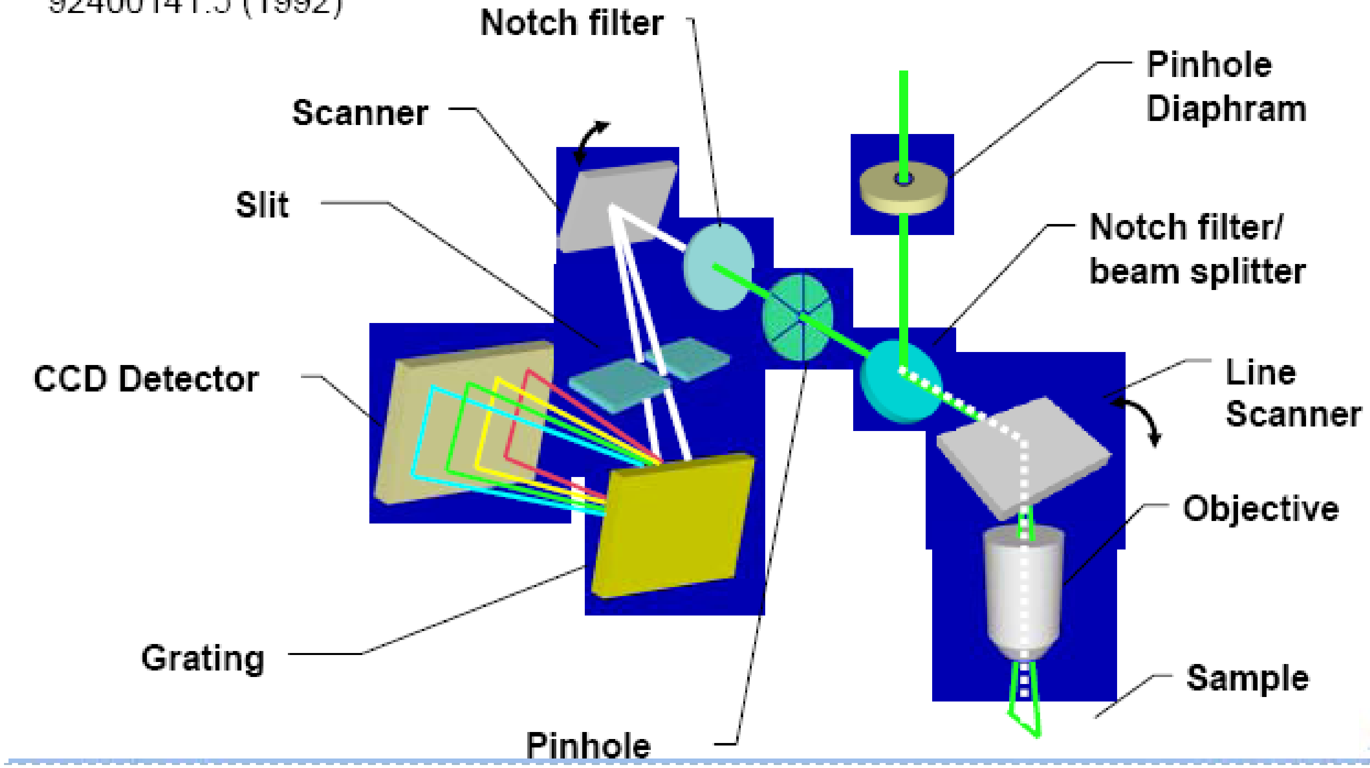
Confocal Raman microscope



Raman microspectroscopy

Confocal Line Scanning

Patented unique method to scan laser through objective to avoid aberrations and maintain diffraction-limited spot. Then the spectrum of each spot on sample is displayed on a different row of the CCD, **assuming that the spectrograph is stigmatic.** European patent # 92400141.5 (1992)

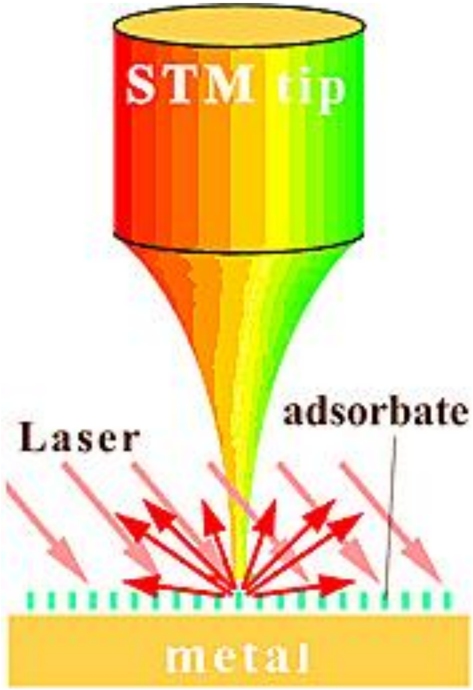


Raman nanospectroscopy

- Near field techniques
  - probe near surface („near field“)
- Near-field spectroscopy

- Near field microscopy
  - SNOM – scanning near-field optical microscopy
  - UV-vis, IR (IR-SNOM), Raman spectroscopy

    \+ TERS
  - photoluminescence, fluorescence
    - ☐ resolution better than 50 nm
    - ☐ spectroscopy of single molecule

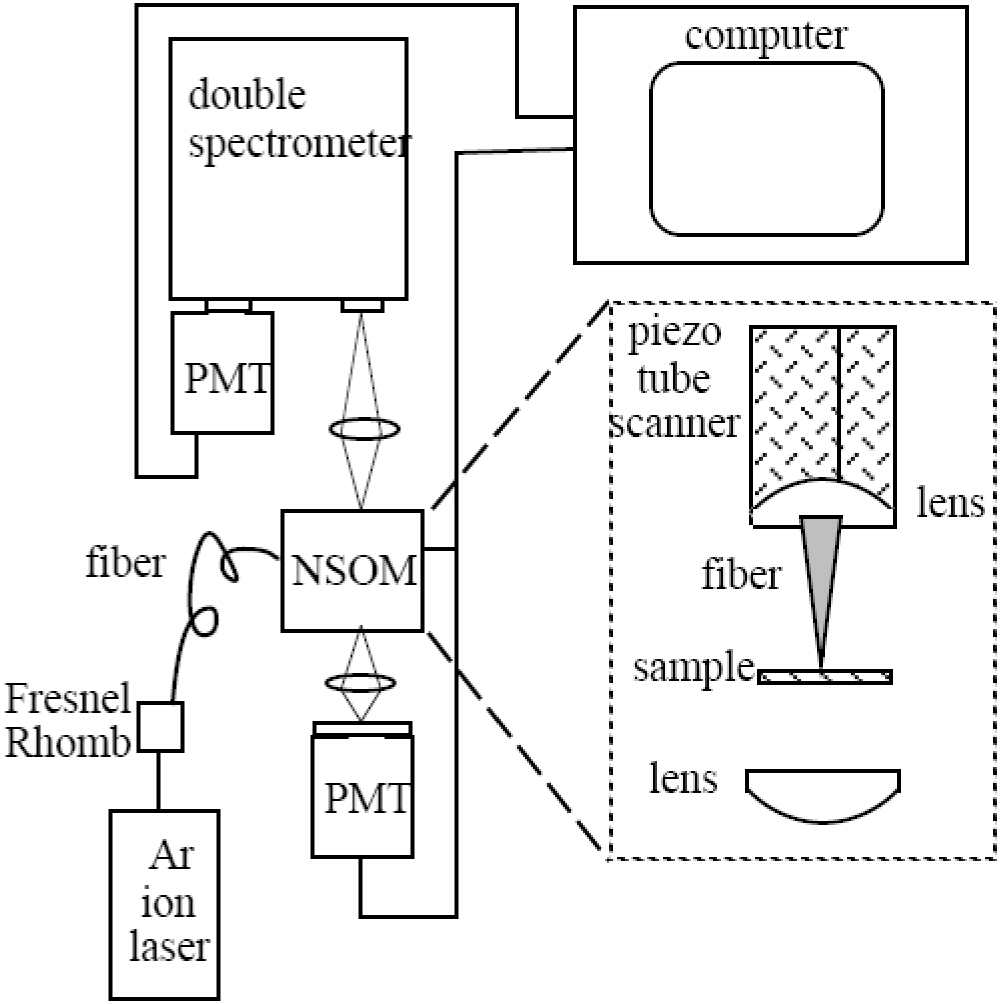


Application of SNOM

- **Single Molecule Detection**J.K.Trautmanet al. Nature 369,40, (1994)
- **Raman Scattering**C.L. Jahnckeet al. Appl. Phy. Lett. 67 (17), 2483 (1995)
- **Polarization and Orientation**B. McDaniel et al. , Appl. Opt. 37, 84 (1998)
- **Magnetic-Imaging**U. Hartman, J. Magn.& Magn. Mater. (1996)
- **Data Storage**H.J. Mamin, IBM J. Res. Develop. (1995)
- **Biological Imaging**. VanHulstet al. J. Struct. Bio., 119,222 (1997)
- **Quantum Dots, Quantum Wires** H.F. Hess et al. Science 264, 1740 (1994)
- **Lithography** S. Madsen et al. J.App. Phy.82 (1) 49(1997).
- **Photonic Device Characterization** S.K. Burrattoet al. App. Phy. Lett.65, 2654 (1994)
- **Semiconductor/ Defect Characterization** LaRosaet al. Mater. Res. Soc. Symp. Proc. 406,189-194 (1996)

Raman - SNOM

- probe distance - up to 10 nm
- probe aperture
- scanning modes
  - transmission (transparent samples only)
  - reflective - sharp probe - transmitter, receiver, both
  - scattering - transmitter, receiver, both

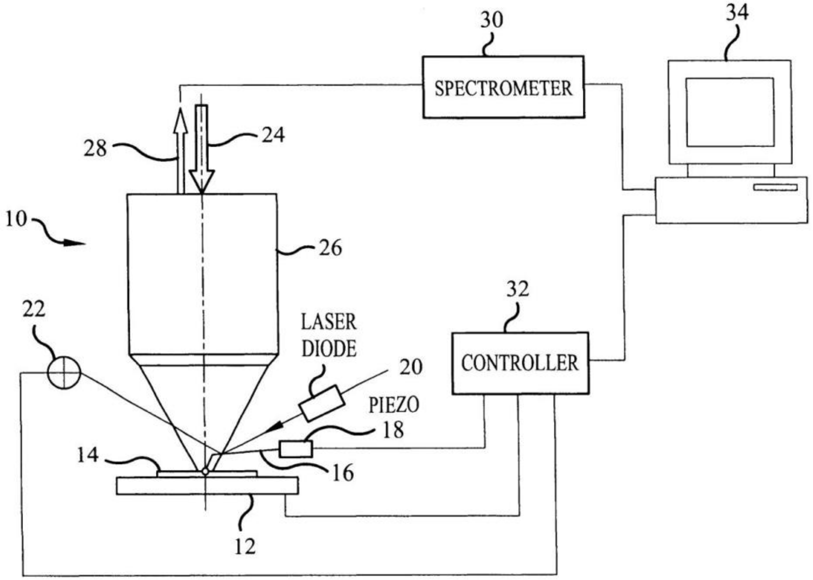


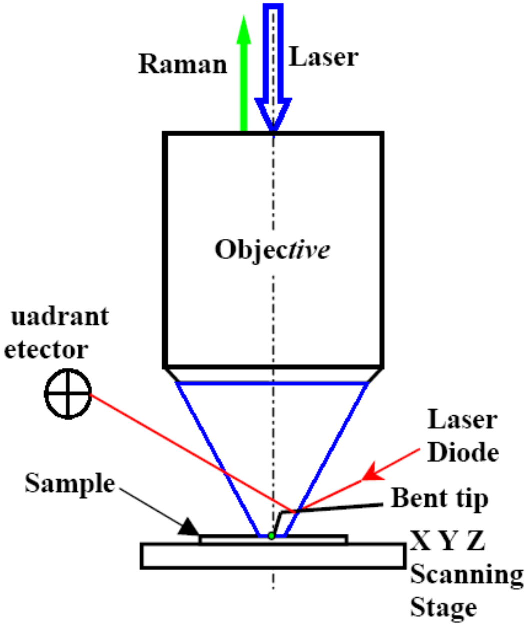
Raman-SNOM

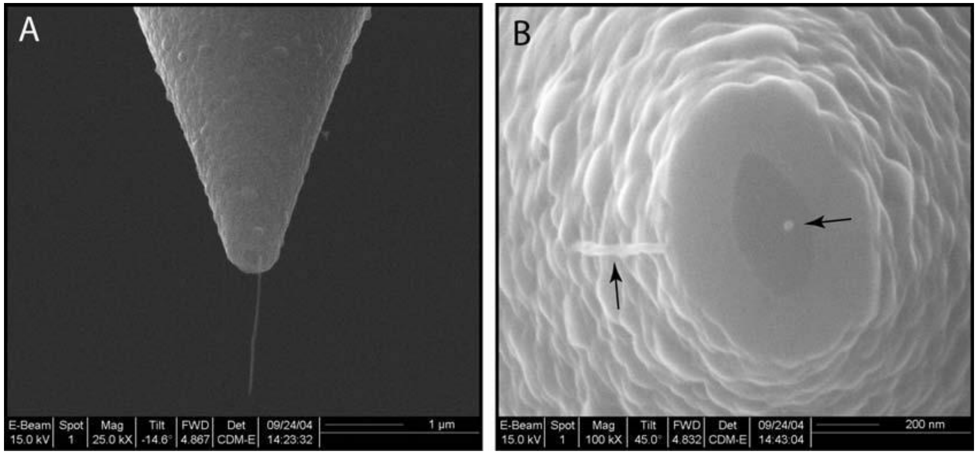
probe distance –to 10 nm

- probe aperture
- scanning modes

  perpendicular or oblique laser excitation







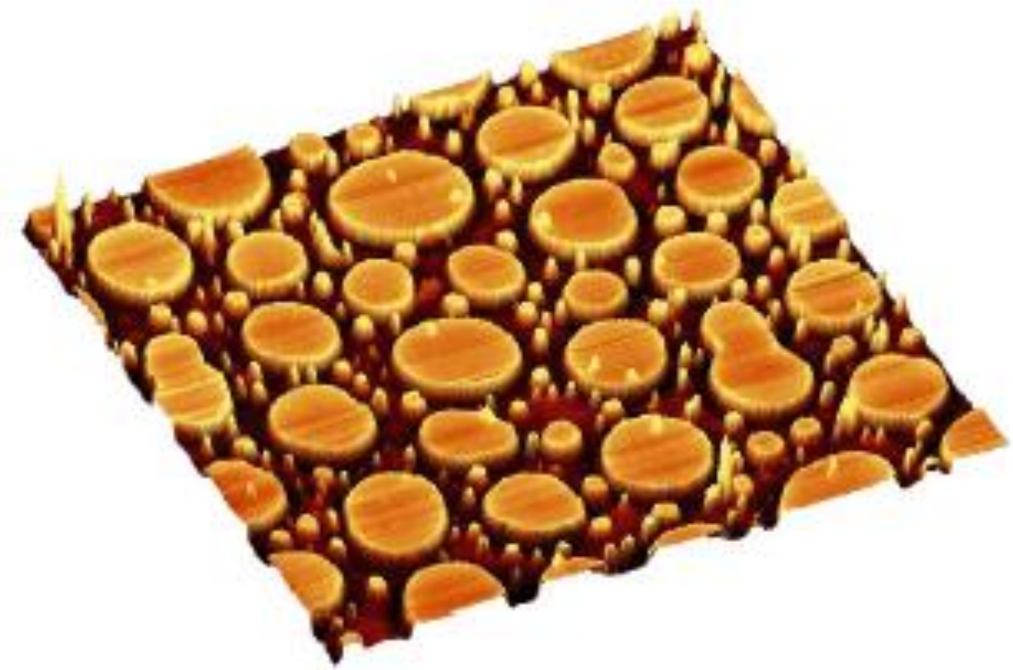
Phase separation

AFM image of PMMA-SBR polymer blend, centrifugally

(polymethymethacrylate - styrene-butadiene-rubber)

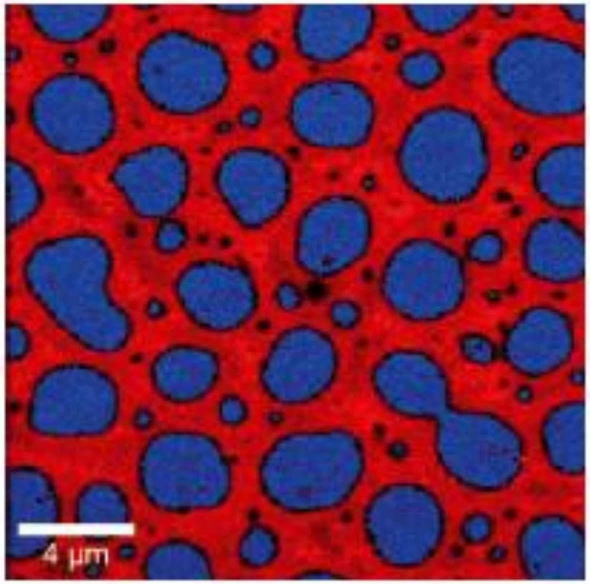
applied to a glass substrate. Scan 20x20 µm, topographic

30 nm scale



Raman image of PMMA-SBR polymer blend.

PMMA surfaces are color coded in blue, SBR surfaces are displayed in red. views: 200x200 spectra



Measurement of stress in material

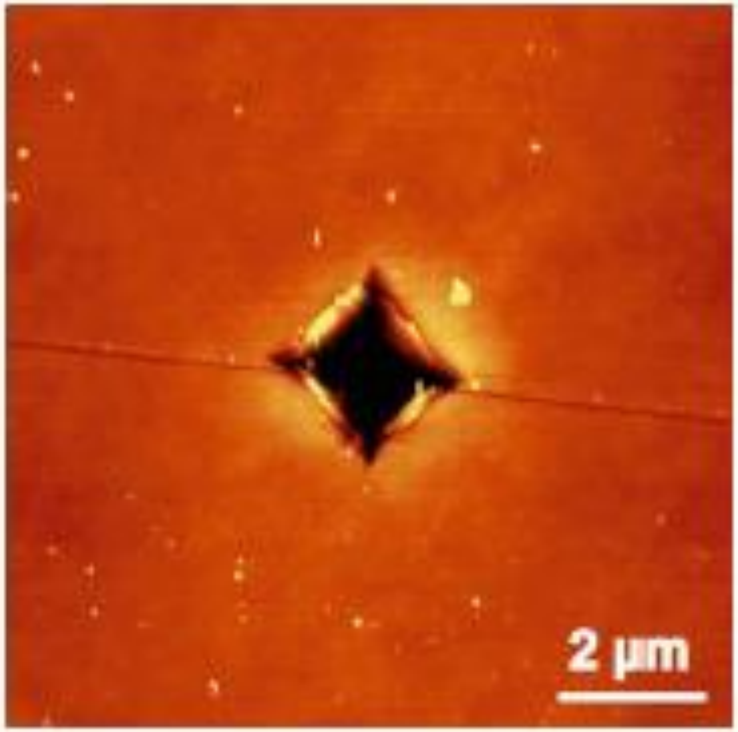
AFM measurement of indentation from Vickers hardness test Si.

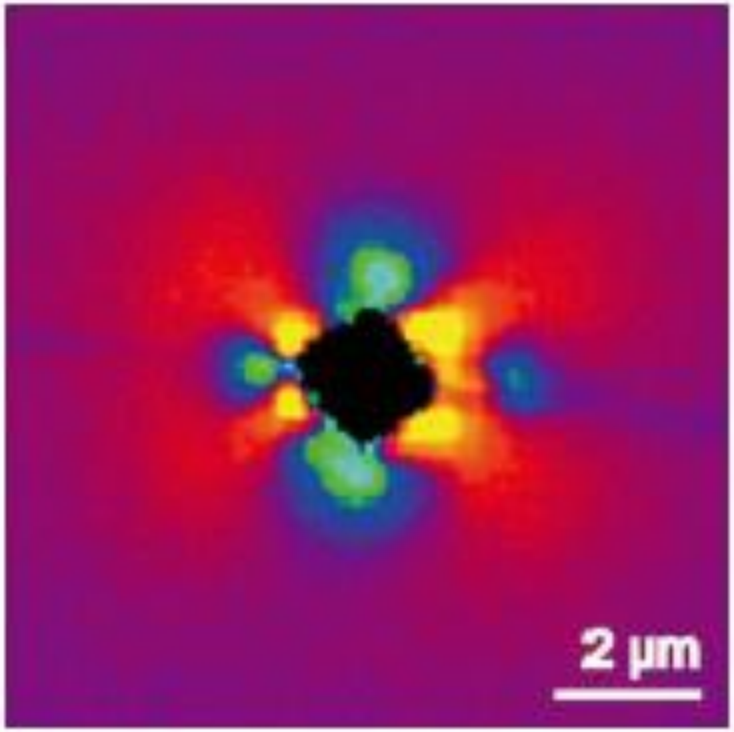
A 2.75 μm diagonal and 210 nm depth imprint was created by force

50 mN. Scan area: 10x10 μm.

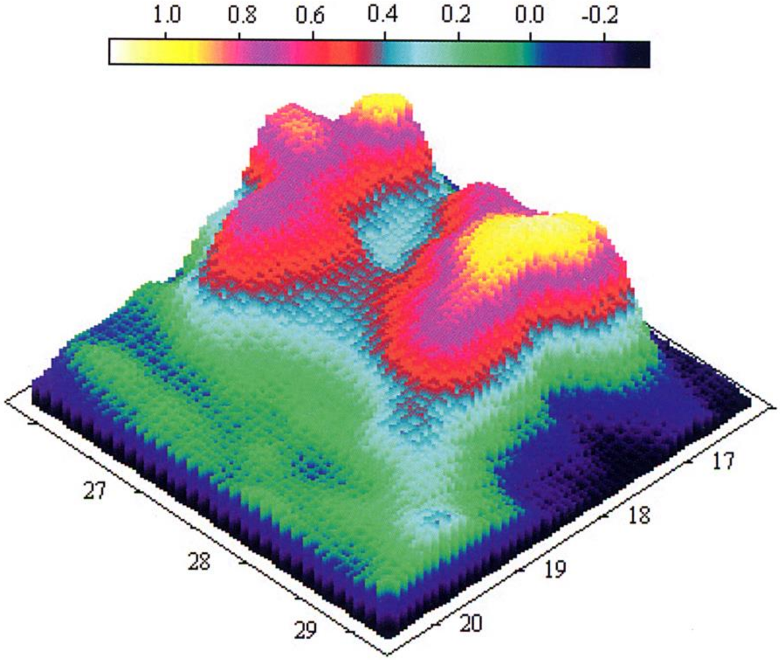
Raman display the same areas as in the adjacent figure.

The image was calculated from the position of the parabolic approximation peak





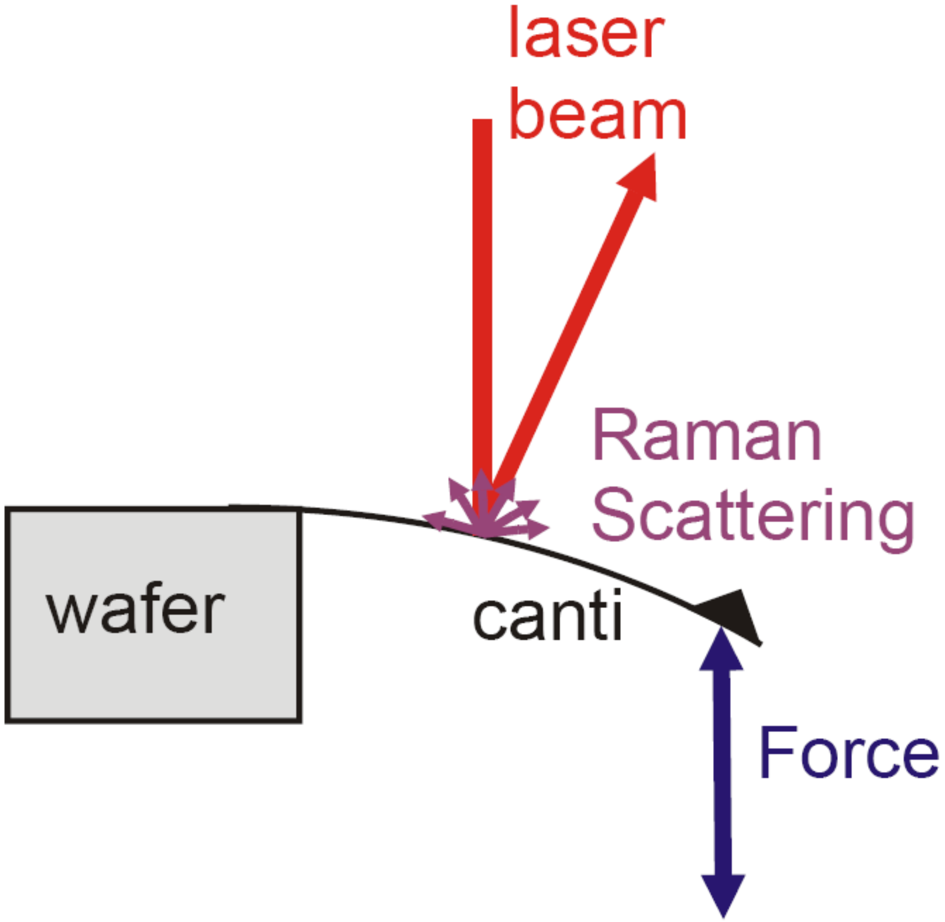
Measurement of stress in material _ phonons



High resolution analysis can provide very detailed information on intrinsic stress and strain in a sample. Raman map of a sub-micron structure (0,6 x 0,6 µm²) showing the Si-phonon shift used for stress analysis.

- **Fonon -** quasi-particles of crystal lattice vibrations, vibrational quantum spreading through the crystal lattice. Phonons can be used to describe the propagation of sound waves in solids. The name phonon originated as an analogy to a photon. A photon is a particle of an electromagnetic field, a phonon is a quasi-particle of an undamped sound field in a solid. It ranks among the **bosons**.

Mechanical stress in nanostructures



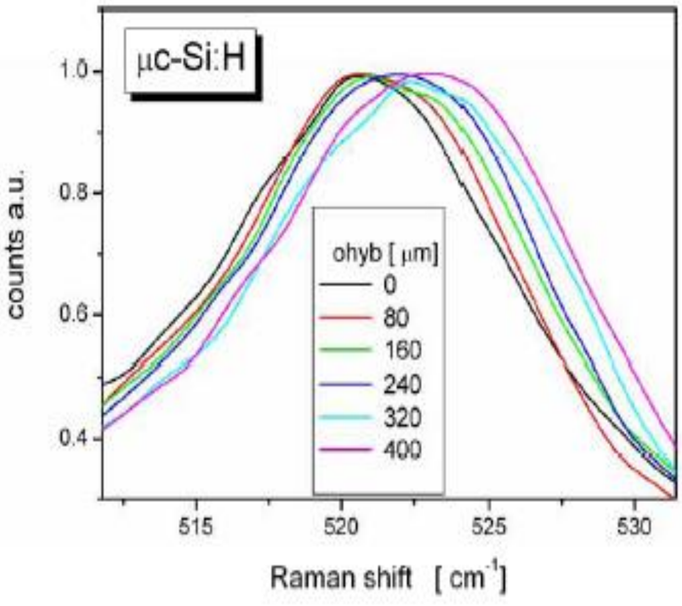
laser beam

Raman Scattering

wafer

canti

Force

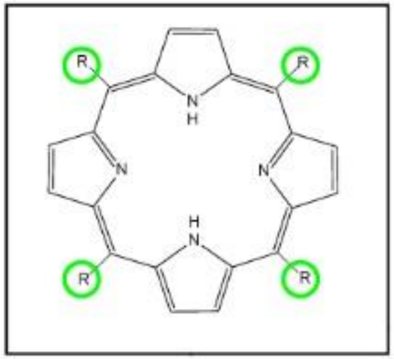


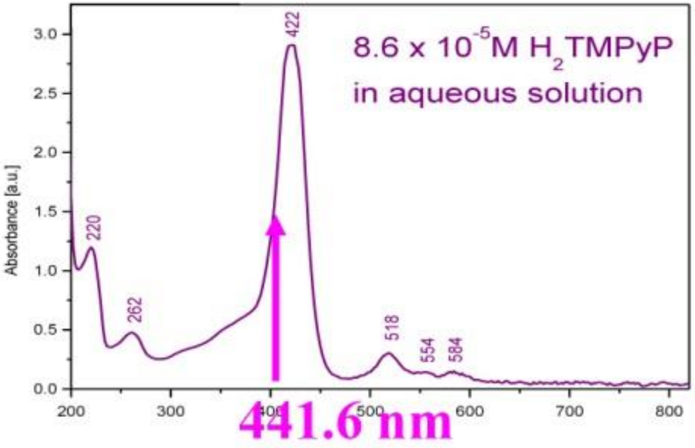
Special techniques of RS

- resonance - RR
- surface-enhanced - SERS
- surface enhanced resonance - SERRS
- photoacoustic– PARS (non-linear, pulsed)
- hyperRaman (two photon pumping)
- coherent anti-Stokes - CARS
- coherent Stokes - CSRS

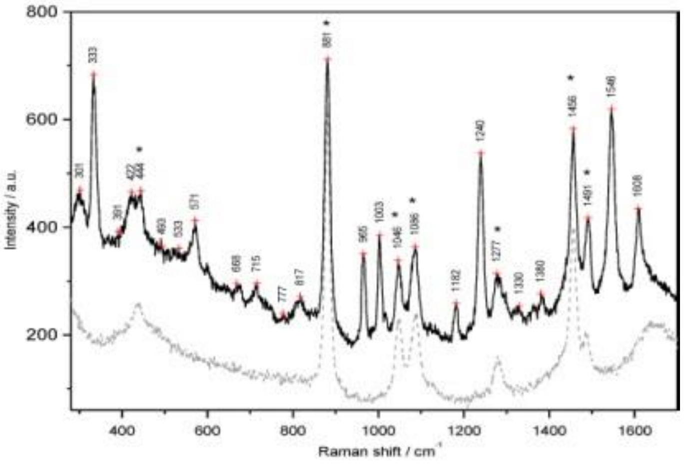
RRS (Resonance Raman Scattering

- Excitation into the absorption band of the molecule, but there is a risk of photodegradation and interference with the fluorescence

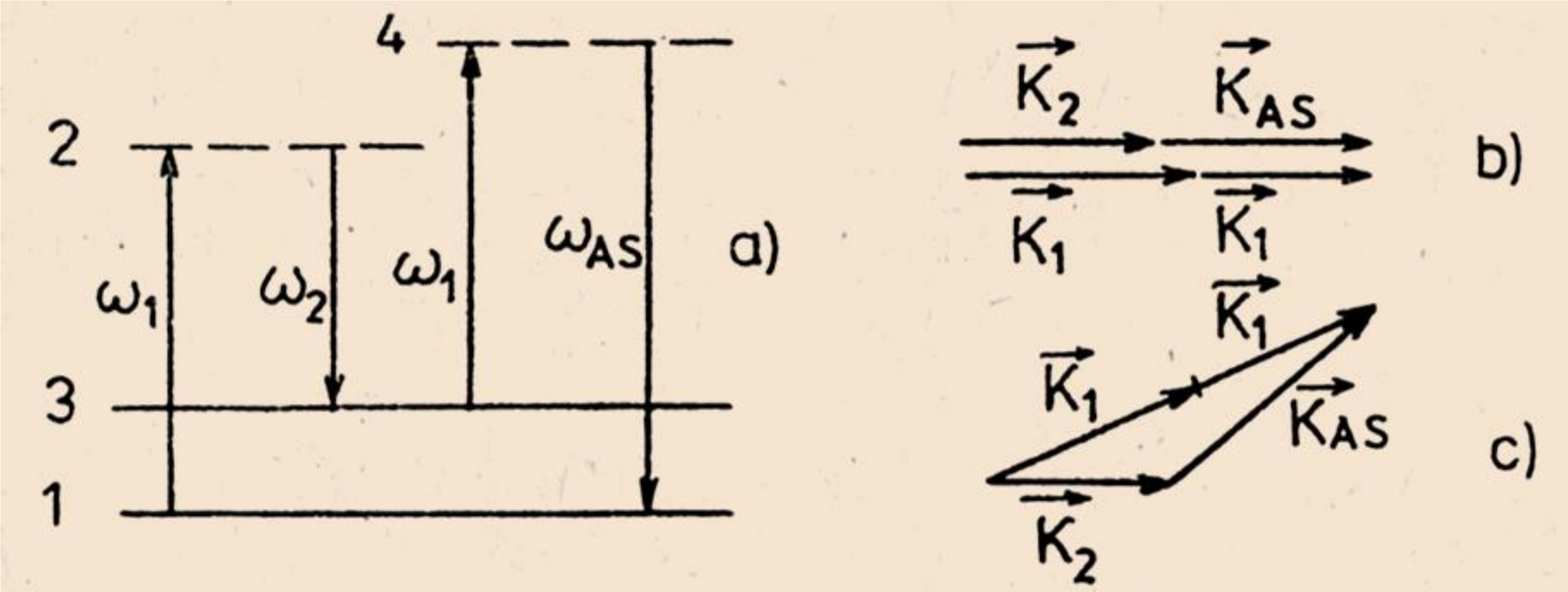




RRS
10^{-6} M porphyrin



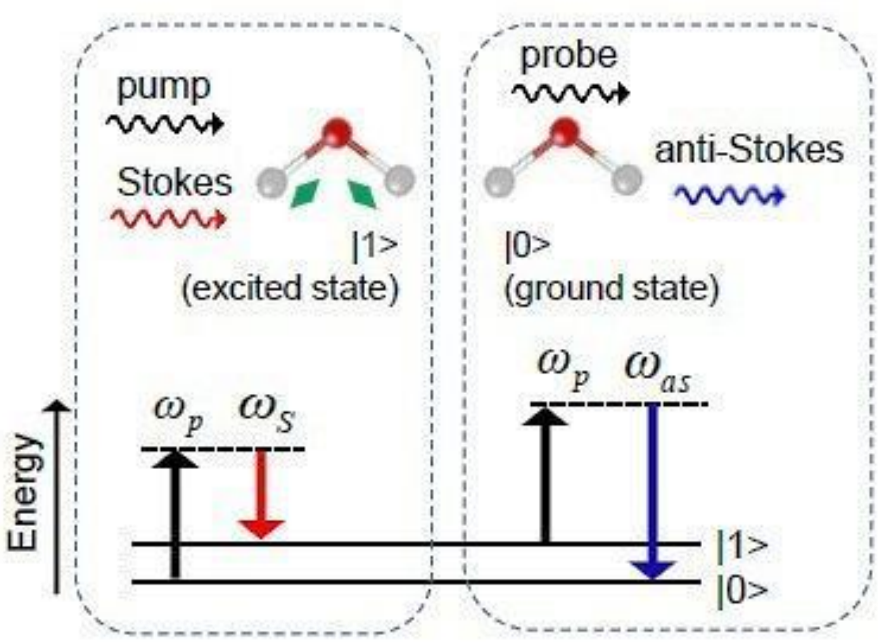
Nonlinear methods - CARS



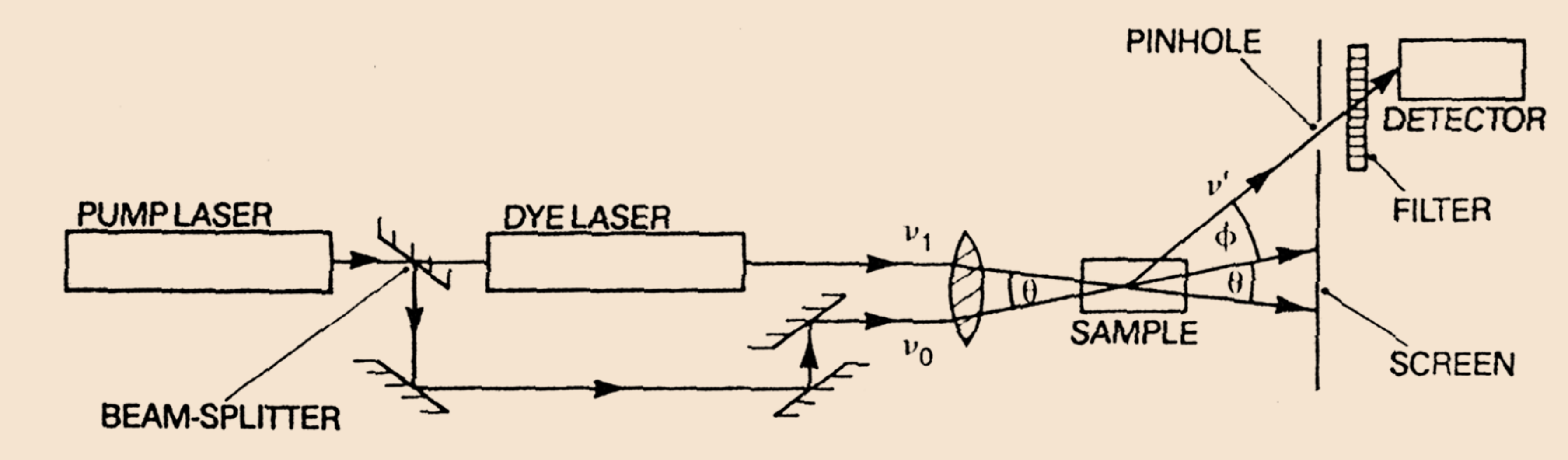
In the CARS ("four-frequency mixing") method, unlike ASRS, relaxation from the first virtual level 2 is forced by radiation from the second laser at ω_2, which causes an increase in the population of level 3 and the antistokes transition is stimulated emission of coherent and directed radiation. The ω_{AS} emission is based on a narrow cone. In dispersion samples (liquids), the wave vectors are summed in vector (c), so that no dispersion element is required to separate the detected radiation, unlike gases (b).

Coherent Anti-Stokes Raman Scattering

- CARS can stimulate the production of a significantly larger amount of signal than spontaneous Raman microscopy. Like spontaneous Raman, CARS probes vibrational modes in molecules and does not require the introduction of exogenous dyes or markers, which is advantageous in imaging small molecules, such as metabolites, for which labeling may significantly affect their molecular properties.
- CARS is a process that involves four photons that interact with the third order nonlinear susceptibility of the sample, which is a function of the vibrational frequencies . To understand a CARS event, consider two photons: a pump, of energy , and a Stokes, of lower energy . Consider also a molecule with a single resonance, represented by a third order susceptibility. A CARS event can be understood in two steps. Upon the illumination of the molecule with the pump and Stokes photons, the first step is initiated if the condition is met; that is, if the diference in energy between the pump and Stokes photons matches the energy of the excited vibrational state of the molecule, so that the molecule is excited. Once this happens, the second step is the result of the interaction of this excited state with a third photon, known as the probe, of energy . This photon gains the energy of excitation of the molecule, and an anti-Stokes photon is emitted with an energy that has a higher frequency than any of the incident photons.

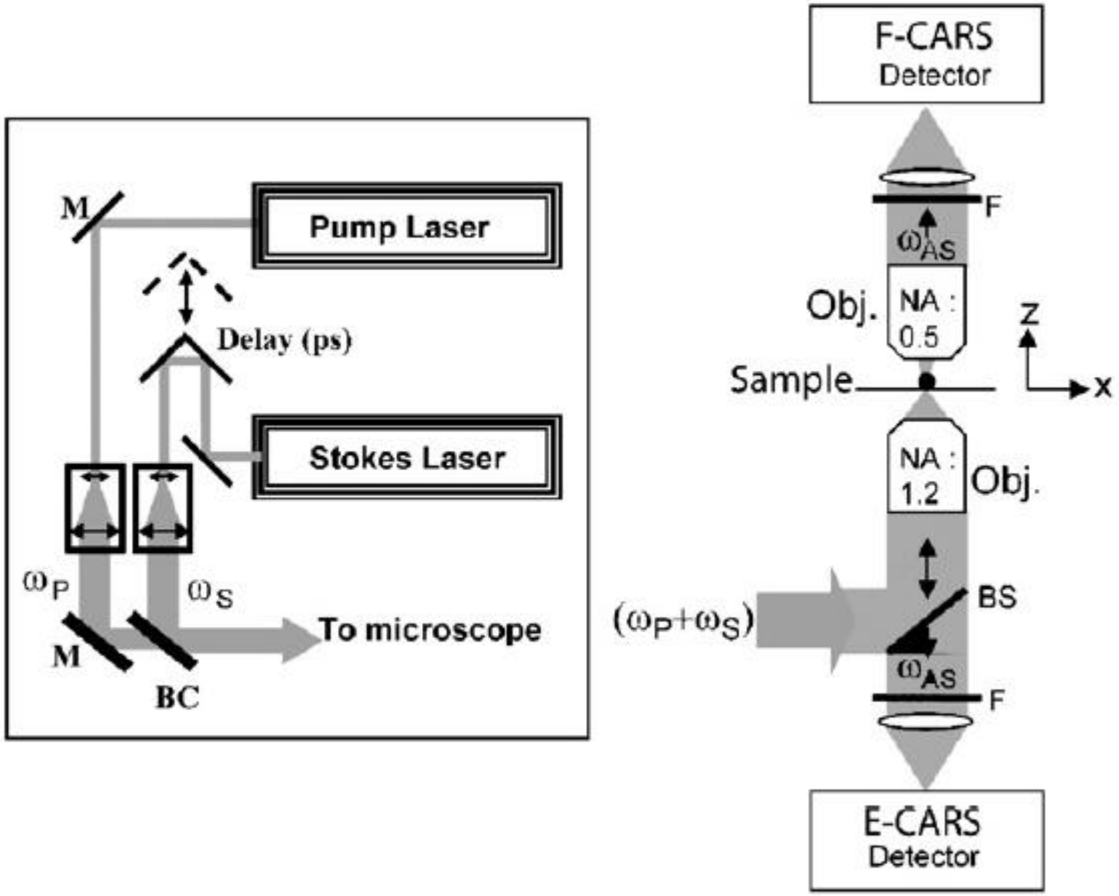


CARS instrumentation



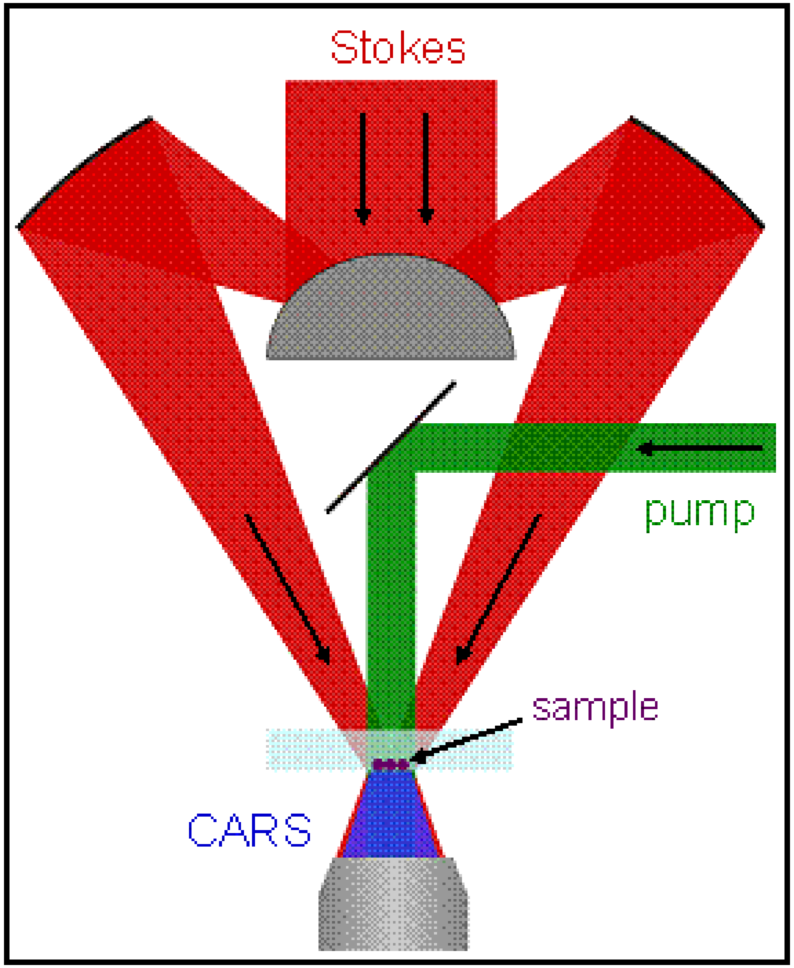
CARS requires first power tunable lasers that are technically and economically challenging. Unlike conventional Raman spectrometry, the radiant fluxes in CARS are very intense, so the signal detection requirements are minimal. E.g. when monitoring the benzene vibration transition at 992 cm^{-1} (totally symmetrical vibration of the benzene ring), excitation to virtual levels at 513 nm, P = 100 kW / 6μs, stimulation at 540 nm, P = 30 kW / 6μs, coherent radiation power from Interactive space 300W! Efficiency can be achieved up to 10%, for spontaneous Raman scattering it is 10^{-5} to 10^{-8}%.

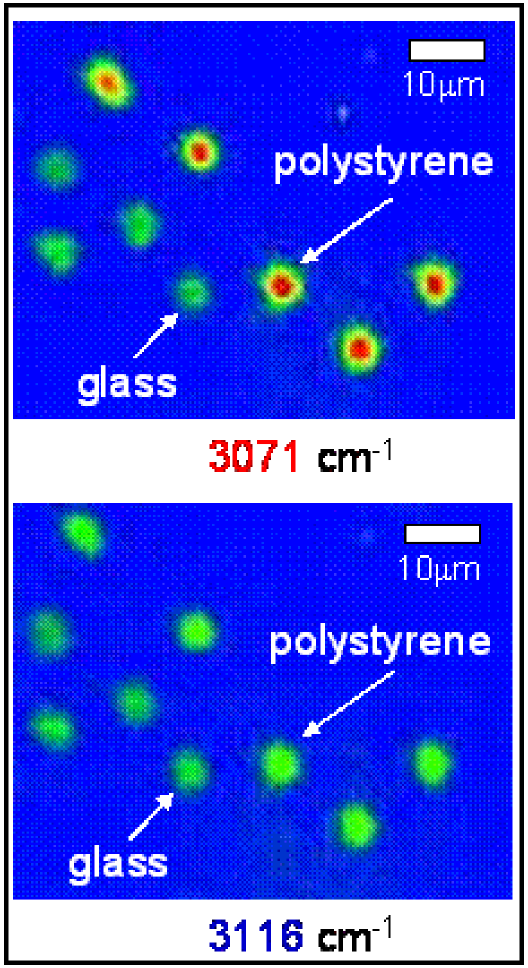
Coherent anti-Stokes Raman scattering microscopy (CARS):



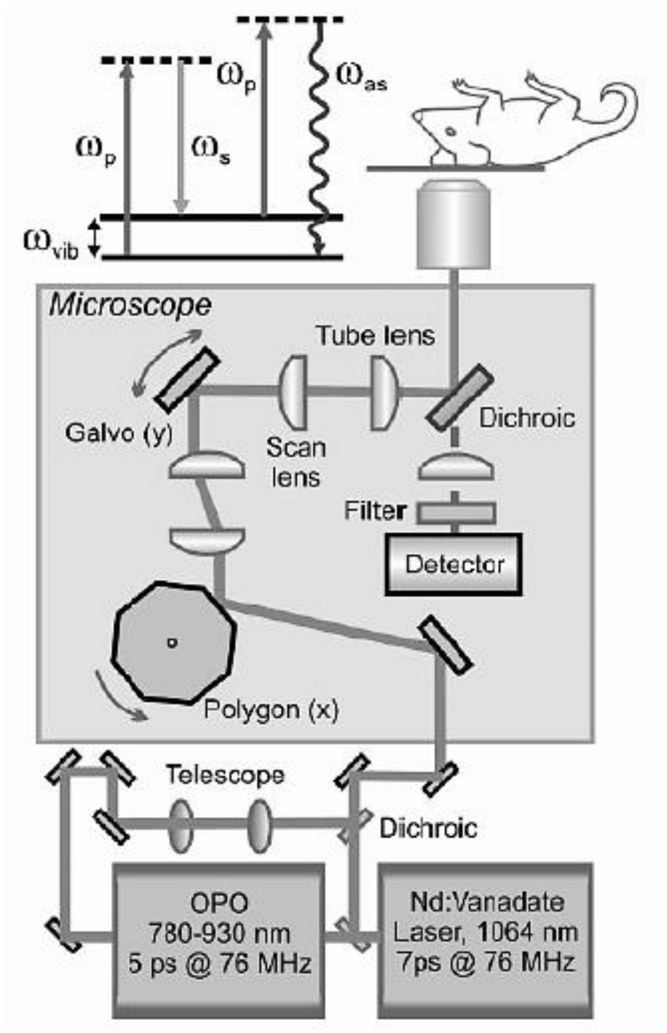
- F-CARS and E-CARS microscopy with co-propagating incident beams, forward and backward signal collection, respectively
- Obj., objective lens; F., filter; BC., beam combiner; L., lens; M., mirror

CARS microscopy





Broadband Coherent anti-Stokes Raman Microscope for Materials Research – CARS



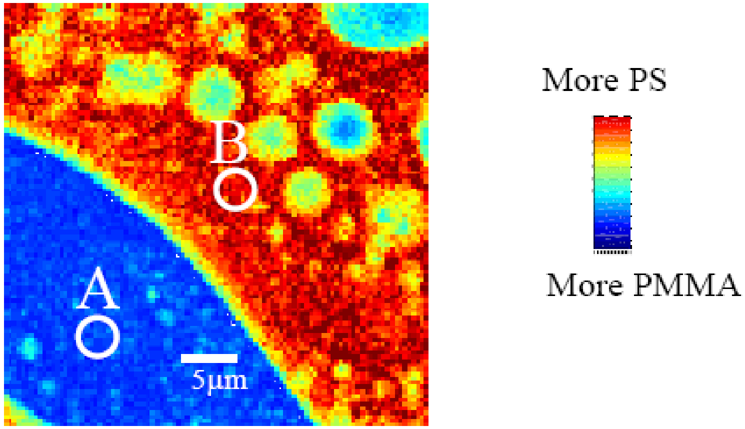
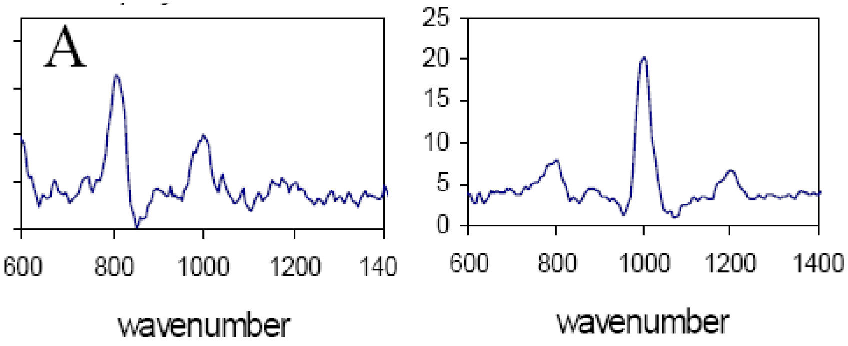
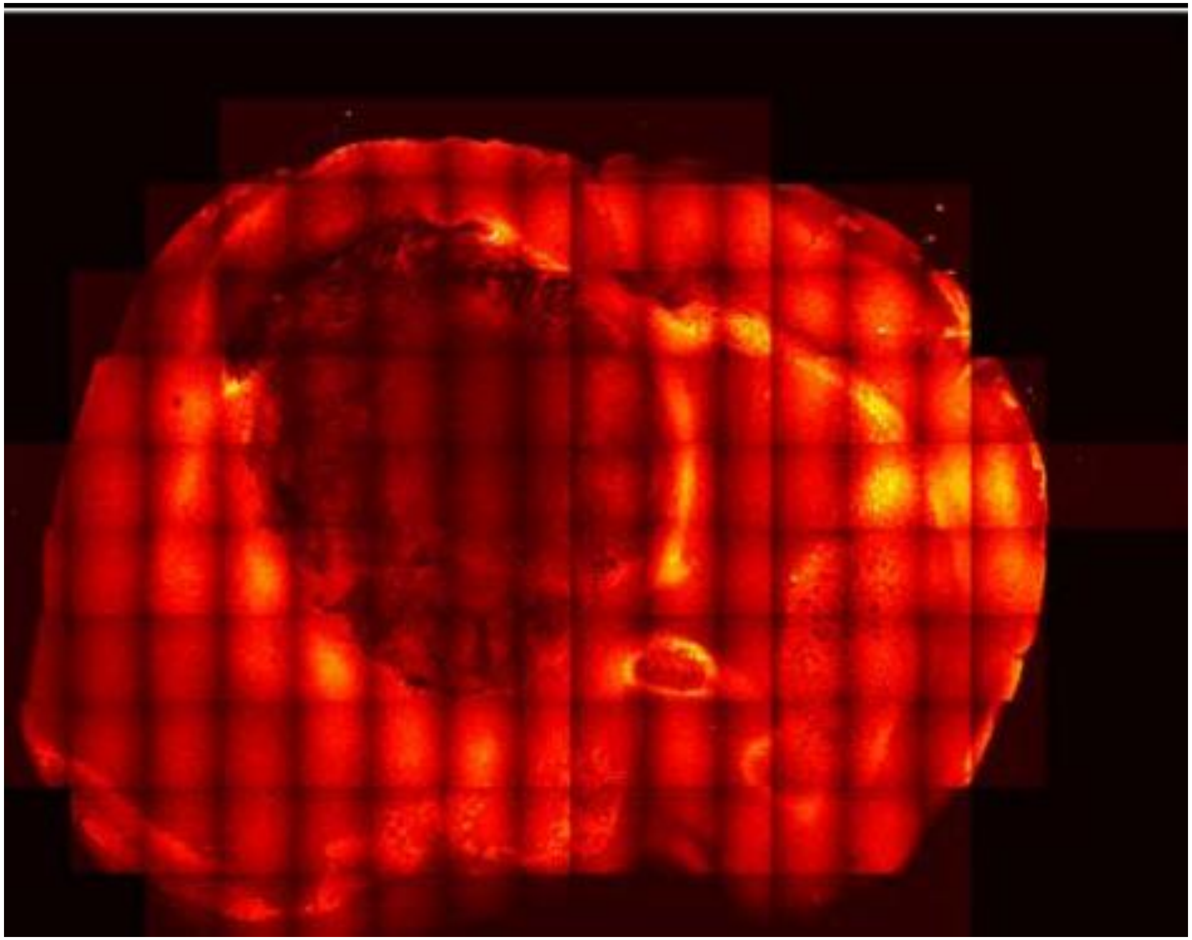


Fig. Shows the distribution of individual components in a mixture of polystyrene (PS) and polymethyl methacrylate (PMMA). The image is generated based on the relative ratio between peaks of 800 and 1000 cm^{-1}.



epi-CARS microscopy



We used epi-CARS microscopy to image *ex vivo* mouse brain tissues. Epi-CARS microscopy suppresses the nonresonant background from the aqueous medium.

Mosaic picture of an epi CARS mouse brain image. The brain tumor is on the left side and extends across the center line and distorts the symmetry of thebrain. The magnification was 20x. Image is displayed in pseudo-color.

Surface Plasmon Resonance(SPR)

Plasmonics

Surface Enhanced Raman Spectroscopy

SERS

Tip Enhanced Raman Spectroscopy/Microscopy

TERS

Aplication of SPR

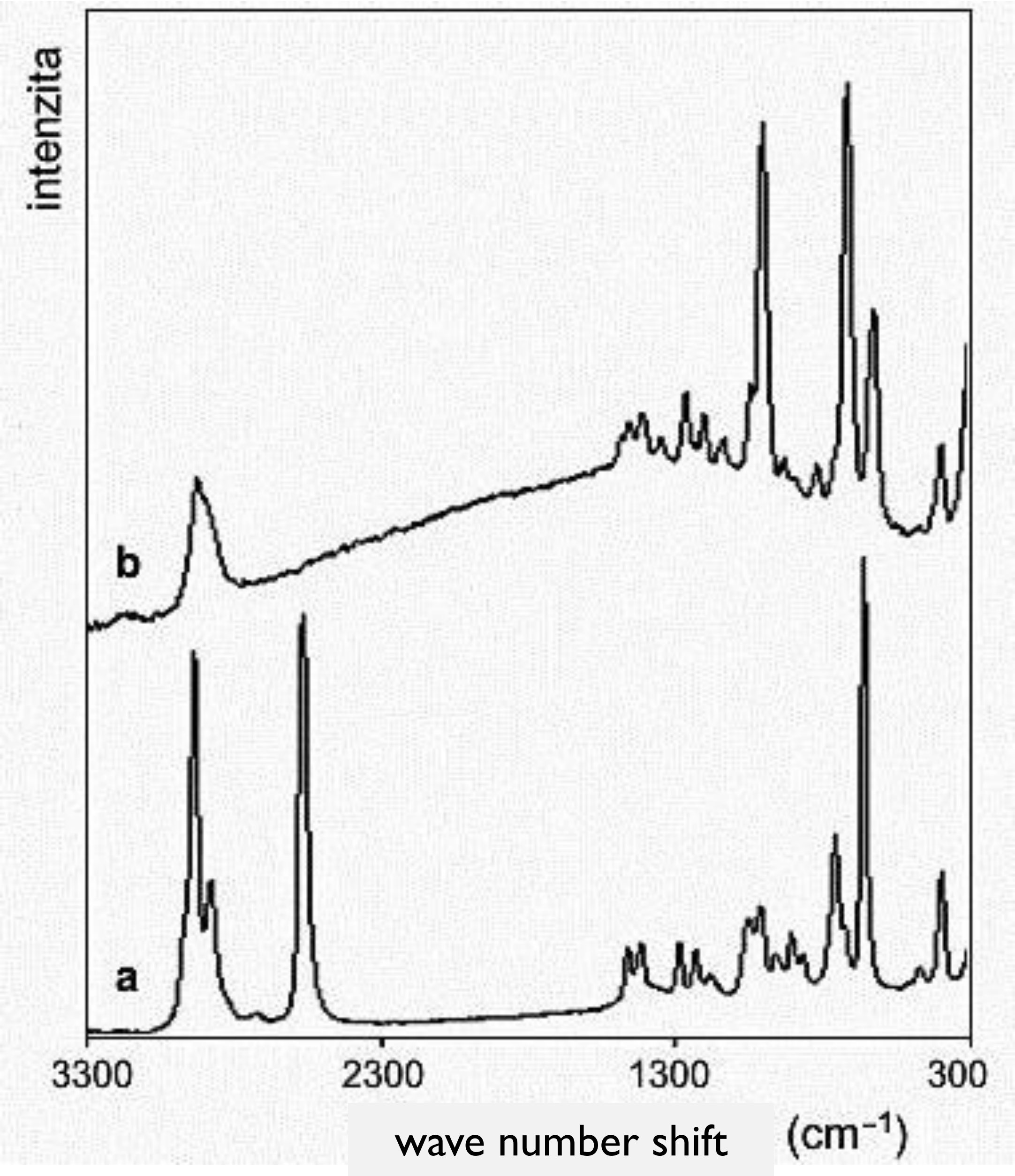
- **sensitivity enhancement of spectroscopy techniques** including fluorescence, Raman spectroscopy … (surface enhanced Raman spectroscopy ~ $10^{14} – 10^{15}$x allows identification of a single molecule)
- change of refractive index by adsorption of molecules on metal-dielectric interphase
- shift of resonance due to adsorption of molecules on the interphase
- noble metal nanoparticles exhibit strong UV-Vis absorption bands (not present in "macro")
- measurement of thickness adsorbed layers, binding constants of ligands ...

Basic nomenclature

- **SERS** – Surface Enhanced Raman Scattering. Raman scattering on molecules bound to the surface of a precious metal (gold, silver) can increase both scattered and incident radiation due to the resonance interaction of photons with the quanta of electron gas oscillations in the field of crystal lattice ions bound to the surface.
- **Plazmon** – quasi-particles (quantum) of longitudinal electron gas oscillations in solids (in crystal lattice of metals, in non-metals, in plastics). In metals, for example, it is possible to excite plasma oscillations as a collective excitation of conductive electron gas against the background of crystal lattice cations. Reflected or transmitted electrons or photons interacting with plasmons exhibit energy losses equal to integral multiples of the plasmon's energy. The generation of plasmon (in most materials with an energy of 10 ÷ 20 eV) leads to energy losses, which are manifested in the form of so-called Ferrel radiation (discovered in 1960) in the UV or visual field.
- **Surface plasmons** – plasmons occurring at the interface of vacuum or material with positive relative permittivity and environment with negative relative permittivity (usually metals or doped semiconductors). They interact strongly with photons to form another quasi-particle - the polariton.

SERS

- Surface Enhanced Raman Scattering – the method brings a great improvement in MS by applying suitable metal molecules or nanoparticles (eg Ag) to the investigated surface.
- The amplification of Raman signals is of the order of 10^4 - 10^6, in some systems it may be even higher.
- The improved sensitivity of the method is related to the fact that molecules near Ag or Au nanoparticles exhibit surface plasmon resonance. However, this explanation is not the only one.
- *Surface plasmon is a quasi-particle. It is a collective excitation of free electrons on the conductor-insulator interface.*

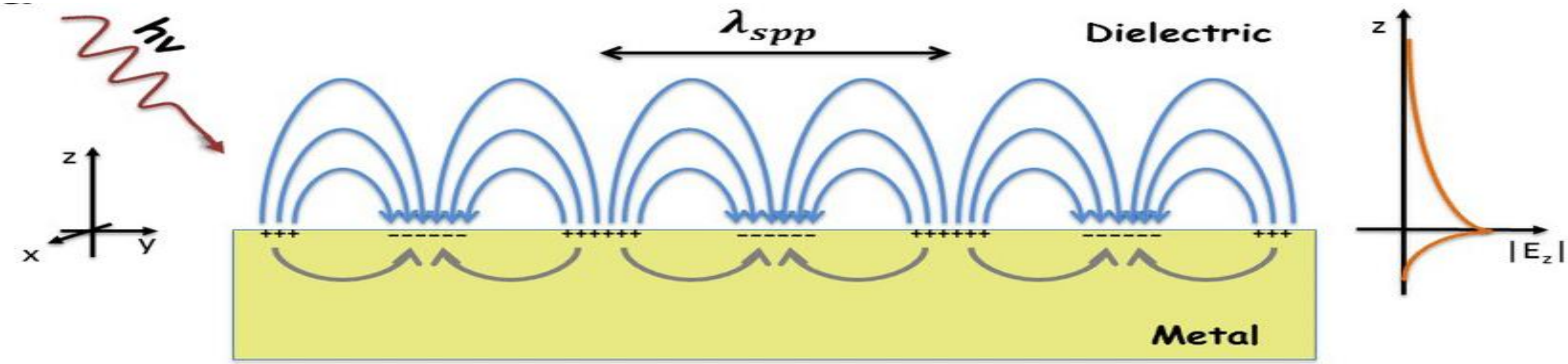


Surface-enhanced Raman scattering, SERS

The difference between the SERS spectrum of the 2-mercaptoethanol monomolecular layer on the surface of the roughened silver (a) and the spectrum of liquid 2-mercaptoethanol (b). Due to the attractive surface forces that modify the structure of the electron sheath, the two spectra differ. (For clarity, the spectra are offset and displayed at different scales.) Source: Wikipedia.

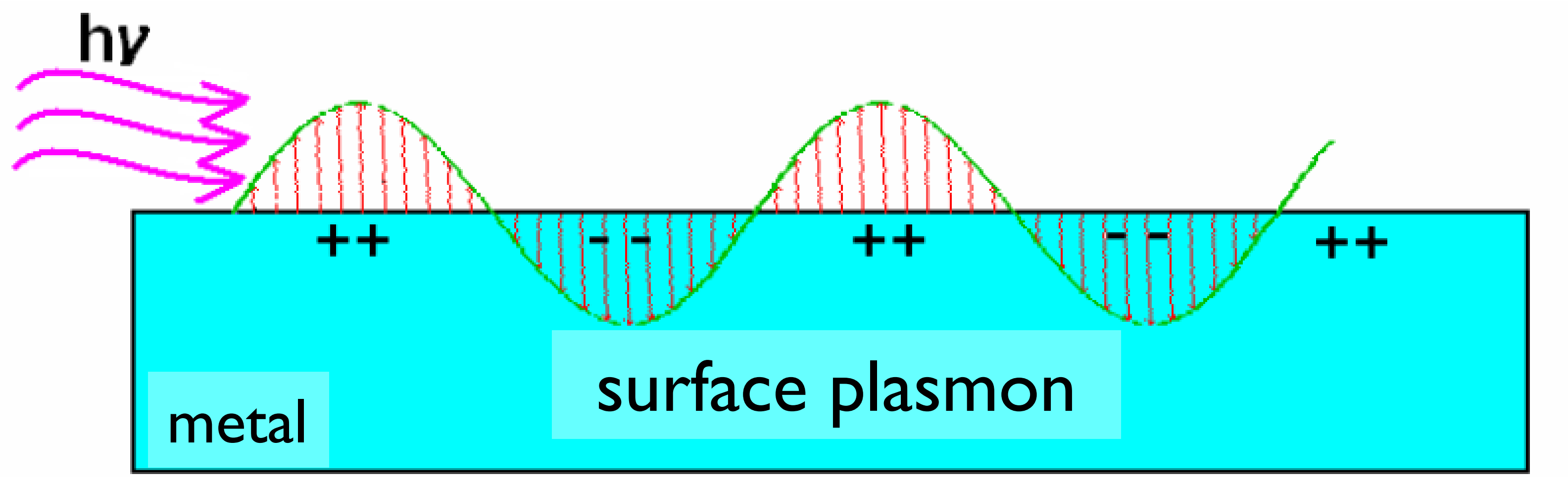
Surface plasmons

- *Surface Plasmon - polariton* = coherent "collective" oscillations of electrons in the conductive band
- Its electromagnetic states are coupled to the metal / dielectric interface
- formed by charge in metal (e-) and elmag. field in both phases
  - \- resulted in associated oscillations e-density and elmag. field
  - (= "Levels" of electron density oscillations)
  - \- field intensity exponentially decreases with distance from the surface of the metal phase
  - (=> localization in the interphase) **propagates as a longitudinal wave at the interface**
- *The properties of the plasmon* depend on**- composition of interphases**(ϵ, $\boldsymbol{R_a}$)
  - **- refractive index of dielectrics** (light guide, detection of chemical bonds, nanostructures)



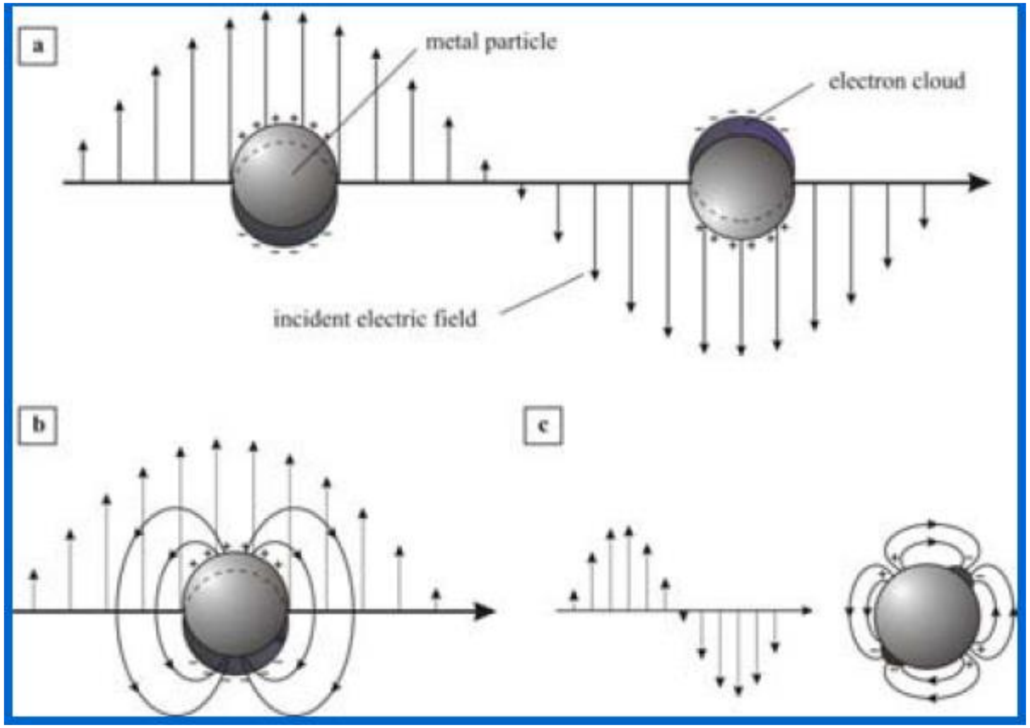
Mechanism of surface plasmon resonance(Surface Plasmon Resonance)

- The incident light **λ (hν)** excites the electron cloud oscillations of the conductive band with subsequent amplification of the elmg. field at the surface interface
- ⇒ in light absorption resonance, λ_{SPEC} increases by several orders of magnitude (= surface plasmon resonance)
- Metallic nanostructure acts as an antenna.



Nanoparticle plasmons

- **Nanoparticle plasmon -** no longer exists localized energy levels (forming a band / cloud). Min. particle size:> 2 nm.
- **Interaction with light** => excitation of e-cloud oscillations => polariton (el. Polarization)
  - ***Small nanoparticle*** interaction with light => dipole radiation (E-field) (a, b)
  - larger nanoparticles => quadrupole radiation (c)

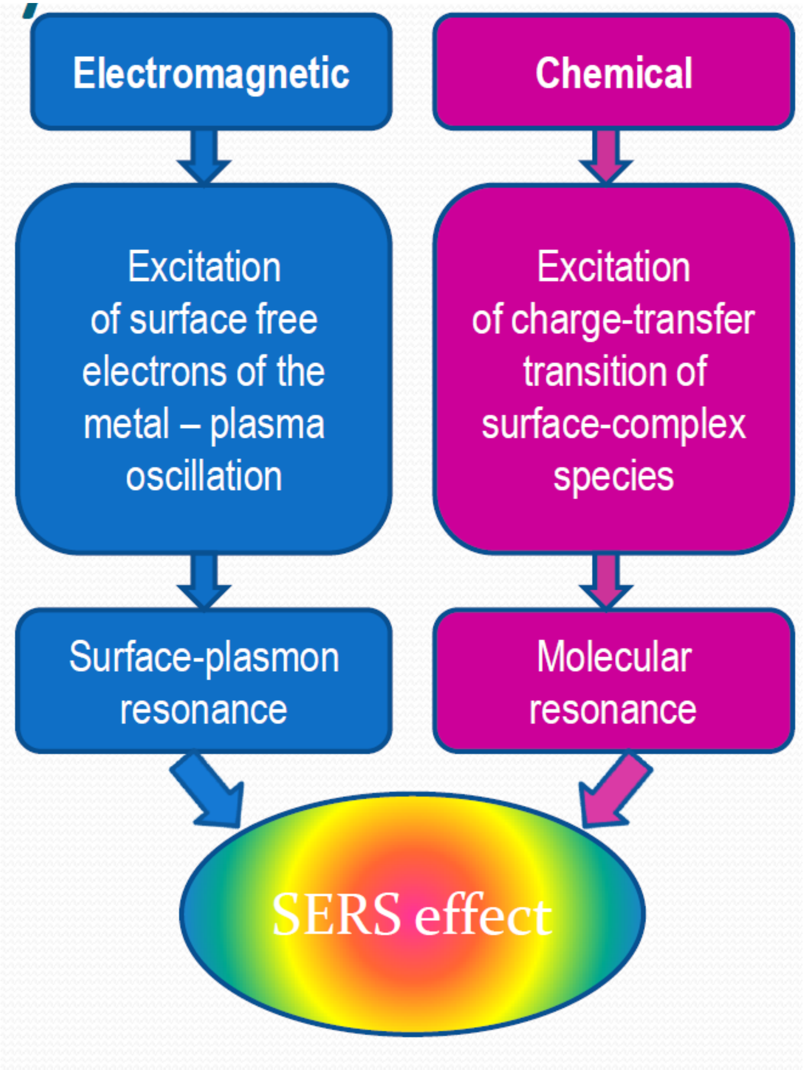


Surface Enhanced Raman Spectroscopy

- **Conditions :**
  - Max. enhancement (incident and scattered light (Raman) is amplified by plasmon resonance) for frequencies with minimal shift Δλ (both can not be very shifted in resonance => less gain)
  - Plasmon oscillations must be perpendicular to the surface
  - use of Au, Ag, Cu (NIR-Vis) nanostructures
  - „Hot-Spots“ (the signal is not representative to the surface)
- **combination of advantages**
  - fluorescence - high light gain
  - Raman spectroscopy - structural information
- Theory:
  - **binding** - charge transfer, formation of bonds
  - **excitation** of surface plasmon
  - **?**

SERS Spectroscopy

- giant enhancement of Raman signal
- two mechanisms involved
  - **electromagnetic**-long range, depends on metal-substrate properties (surface plasmonsare involved)–coinmetals –Au, Ag, Cu
  - **chemical**-local, molecular structure plays an important role (formation of surface complex)



SERS - history

- An important milestone in the use of combination scattering was the discovery of surface-enhanced Raman scattering in 1977 by two groups of researchers independently of each other. Historically, the first SERS - Surface Enhanced Raman Scattering - of pyridine adsorbed onto the surface of electrochemically roughened silver was measured in 1974, but was not correctly interpreted. At the same time, both groups proposed two primary SERS theories, recognized to this day: electromagnetic, based on excitation of surface bound plasmon, while chemical theory is based on charge transfer complexes.
- The most commonly used materials for SERS are gold and silver with a surface with irregularities at least one order of magnitude less than the wavelength of incident light. The resonant frequencies of these materials fall within the range of visible light and near infrared radiation. The amplification of the combination scattering for a flat substrate is in the range of $10^3 \div 10^6$.

SERS on nanostars

- Calculated enhance and cross-section values for a hypothetical nano-star with two-pronged.
- Source: P. S. Kumar et al; Nanotechnology **19** (2008).

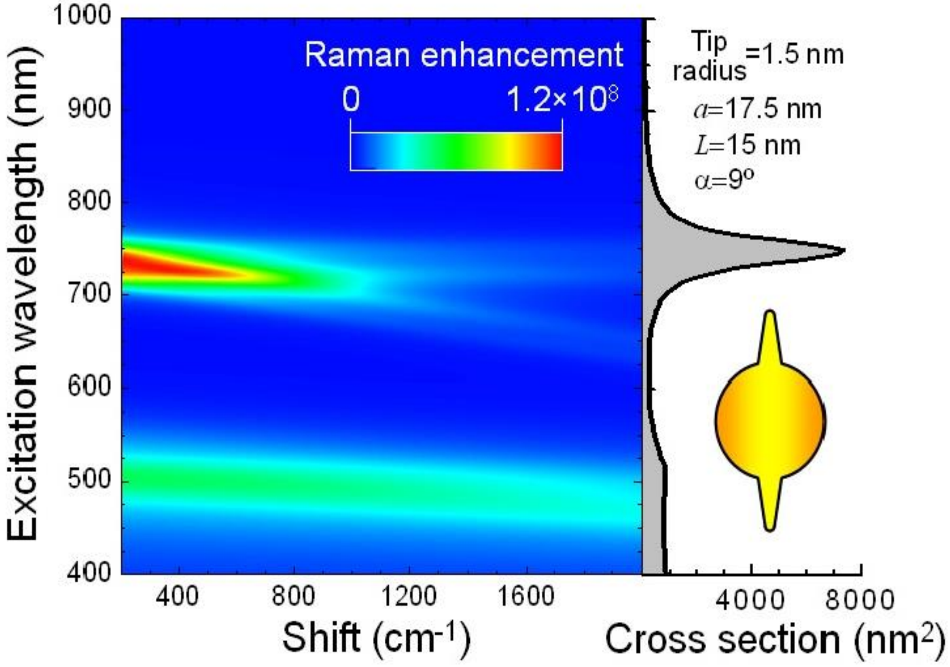
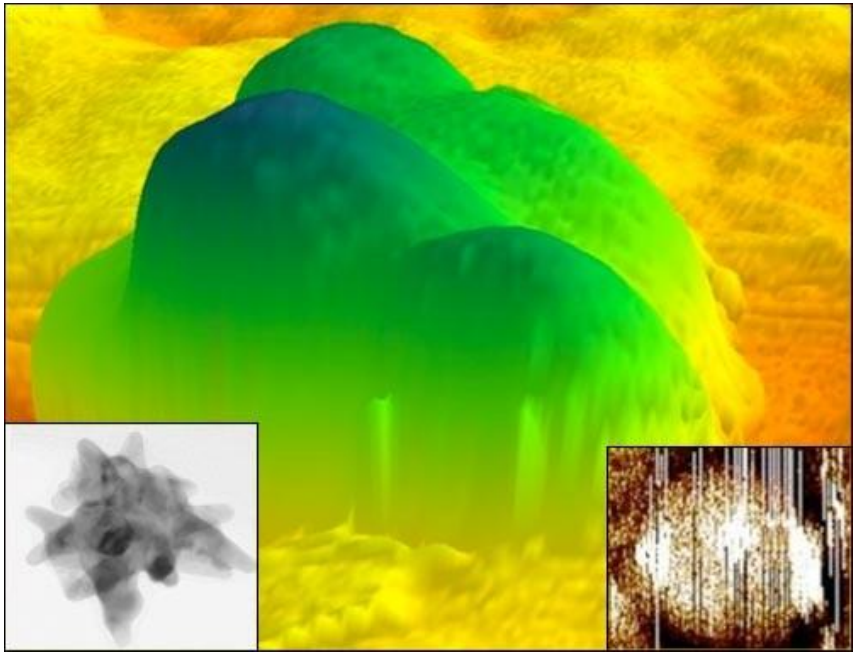


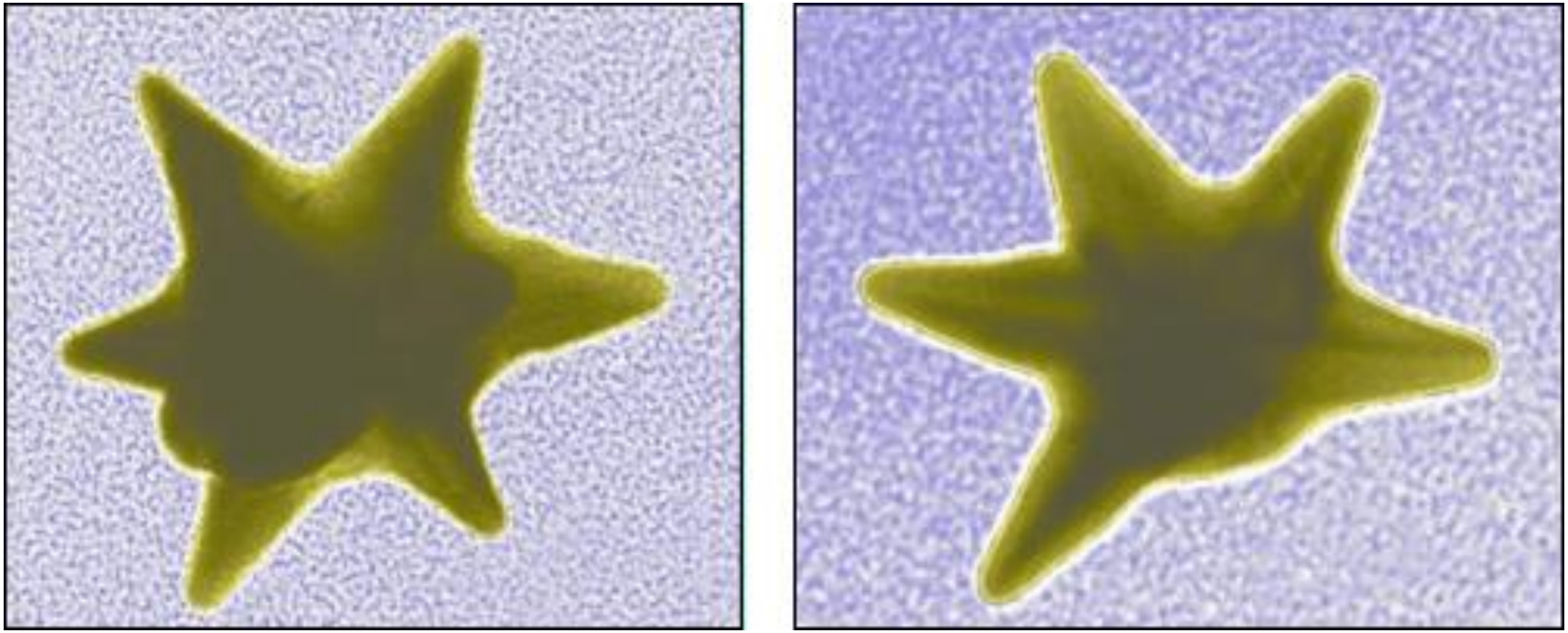
Figure 5. Left: calculated Raman enhancement in a two-tip gold particle in air. The transitions involved in the Raman process are assumed to occur 2 nm away from the gold surface. Right: scattering cross section of the same particle. The particle shape and geometrical parameters are shown in the insets.

Nanostars

Image of a nano-star in a transmission scanning electron microscope**TEM** - Transmission Electron Microscopy, creating an image of a thin object by passing energy electrons. The image formed by the passed electrons is then enlarged and focused by electron optics and converted to visible radiation on the screen, which is usually further recorded by a CCD camera. Another technique is **SEM**, in which the image is produced from reflected electrons. It is complemented by a single gold nanostar image using Atomic Force Microscope (AFM), an atomic force microscope. The machine scans the surface of the material using a tip suspended on a flexible swing arm. The tip is attracted by electrostatic and van der Waals forces. Movements of the arm above the surface are monitored by a laser. The microscope is so sensitive that it can track the electron orbitals of material molecules. The **AFM** microscope was invented in 1986 by G. Binnig, C. Quat and C. Gerber. (bottom left) and a phase portrait of a nano-star using electrostatic microscopy (bottom right, white areas show areas with charge accumulation at the sharp peaks of the star. Source: Nanotechweb).



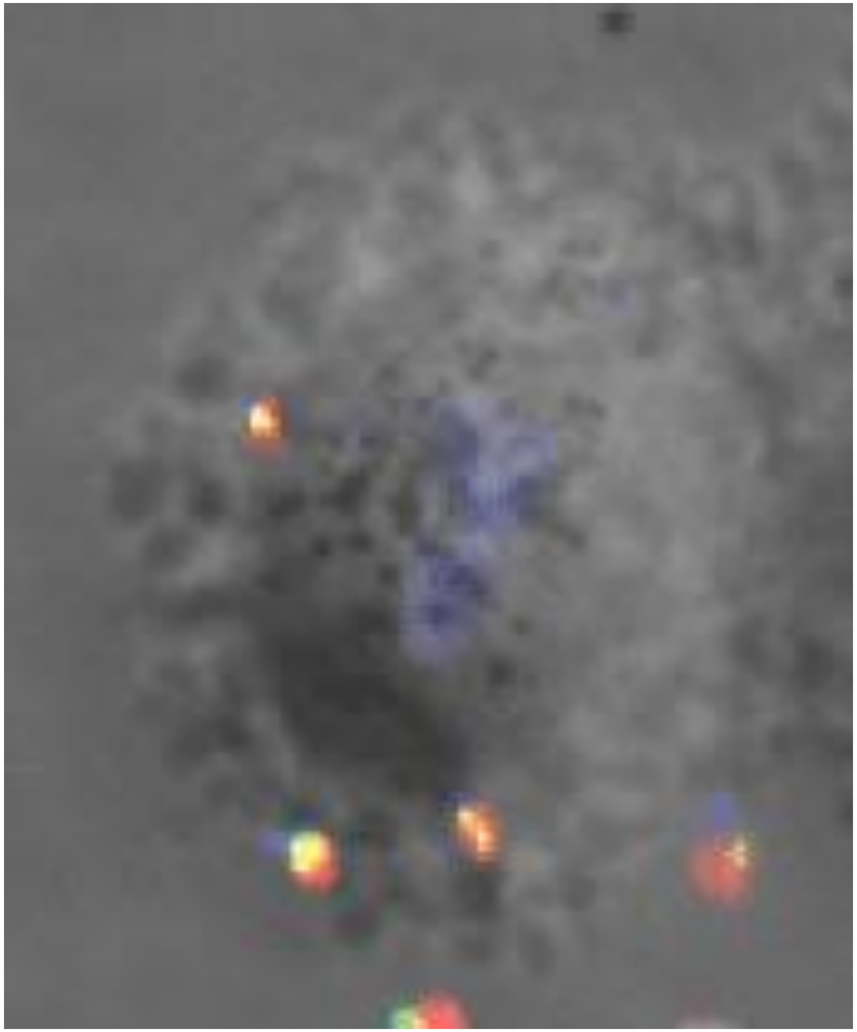
Individual nanostars



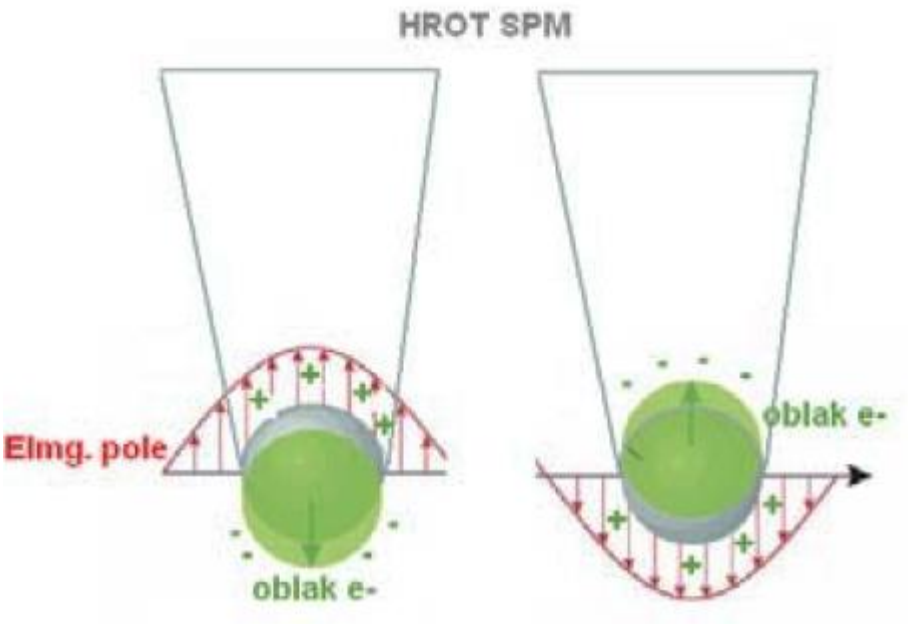
Source: NIST

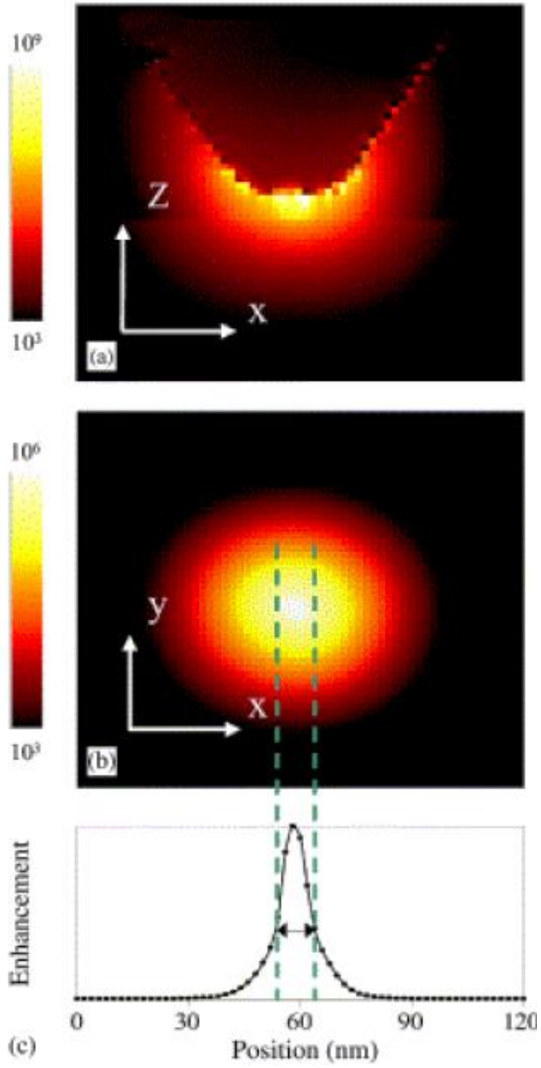
Nanostars penetrate to the living cell

- The video shows the movement of individual nanostars bound to EGFR (Epidermal Growth Factor Receptor) protein molecules in a living human cell grown from a cervical cancer. Note the unbound nanoparticles moving rapidly across the field of view. The bound nanoparticles move slowly, towards the cell nucleus.
- Source: Aaron et al.: Opt. Express 16/3 (2008).



Tip Enhanced Raman Spectroscopy

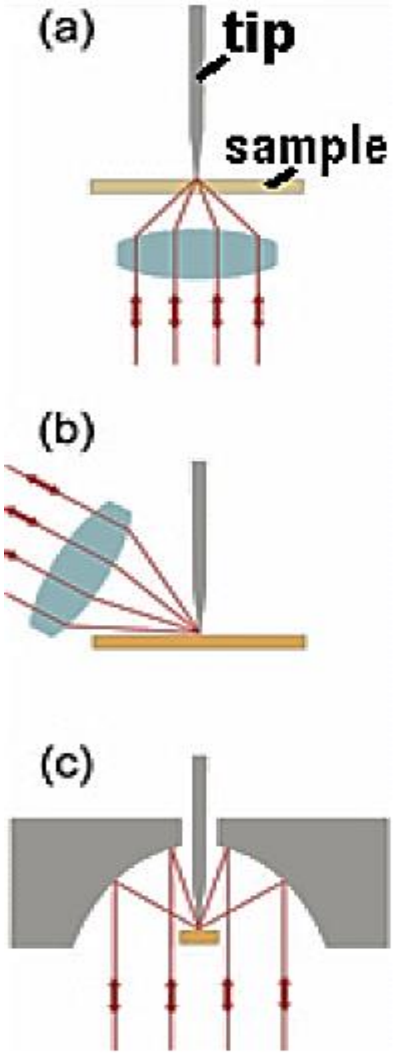


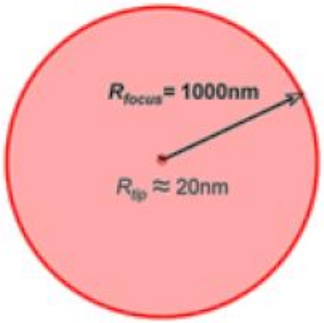


Section cut TER(S)
($A = I_{RT}/I_{R0}$)
λ = 541 nm,
$d_{T\text{-}S}$ = 4 nm

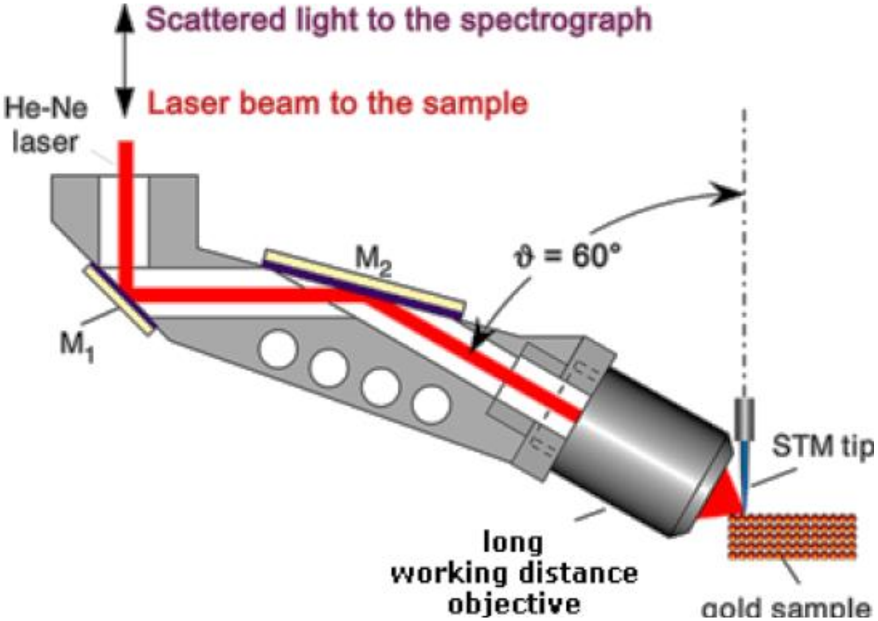
From nanoparticle plasmon resonance (SE) to tip enhancement (TE)
P. Hewageegana, M. I. Stockman: Plasmonics enhancing nanoantennas
Infrared Physics & Technology 50 (2007) 177–181

TERS instrumentation



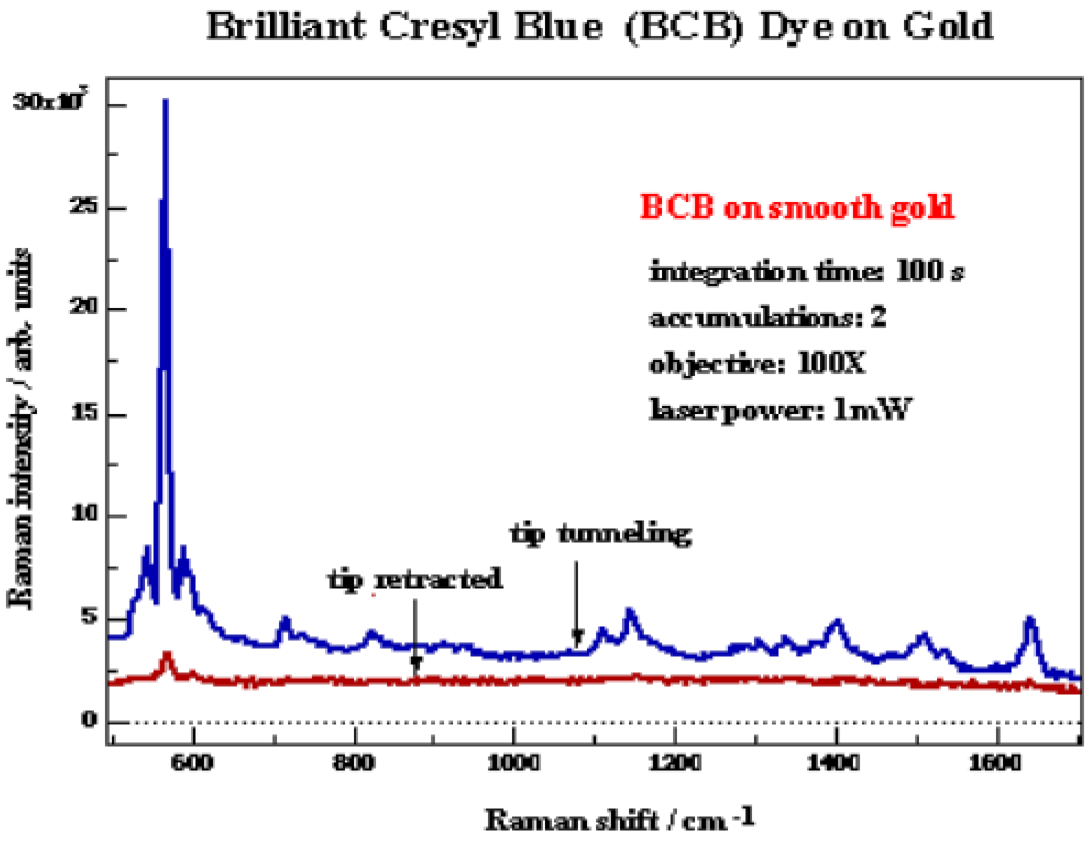


comparison of focus with tip apex area



Source: He-Ne laser (632.8 nm) ~0.3 mW on sample

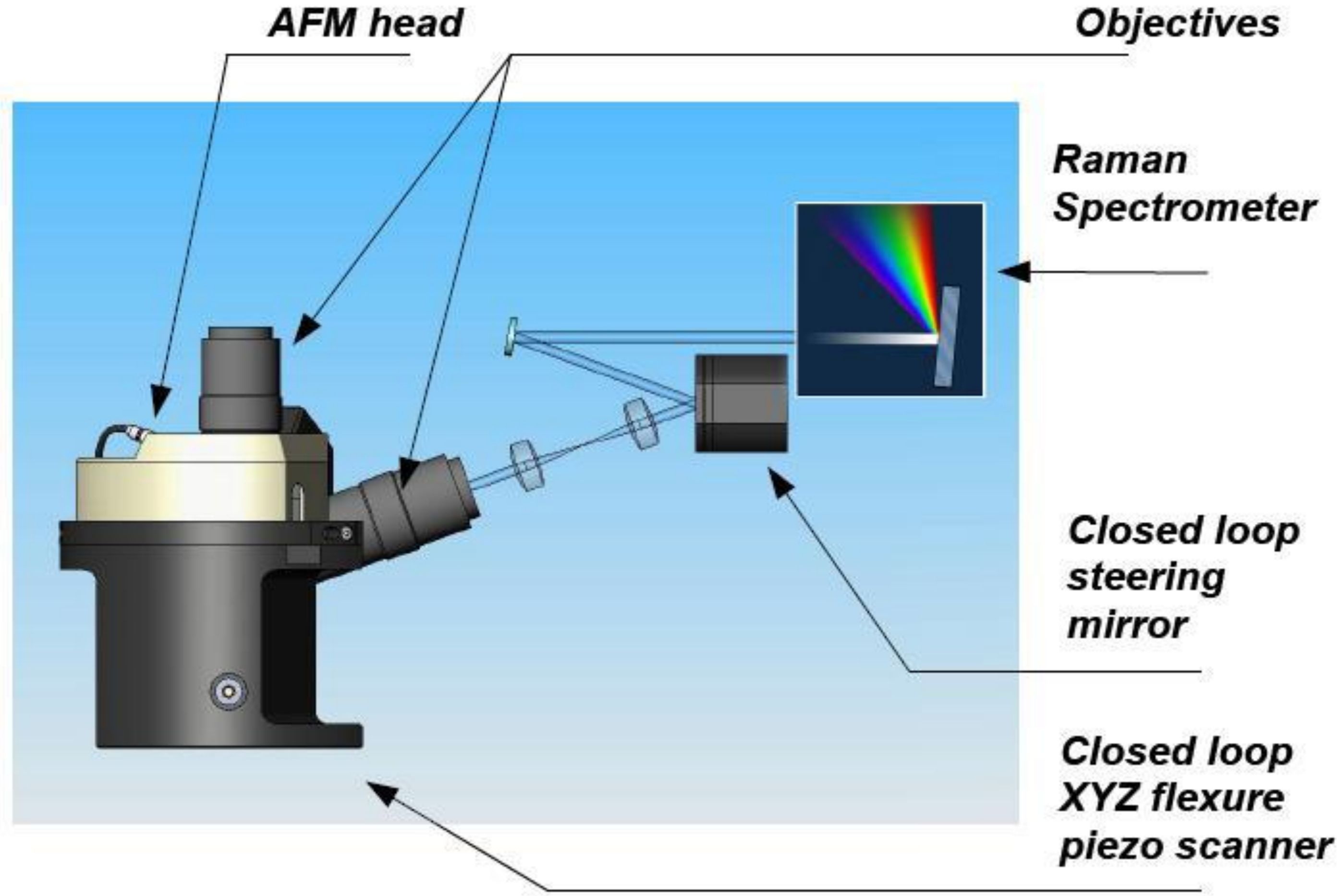
Example of TERS application



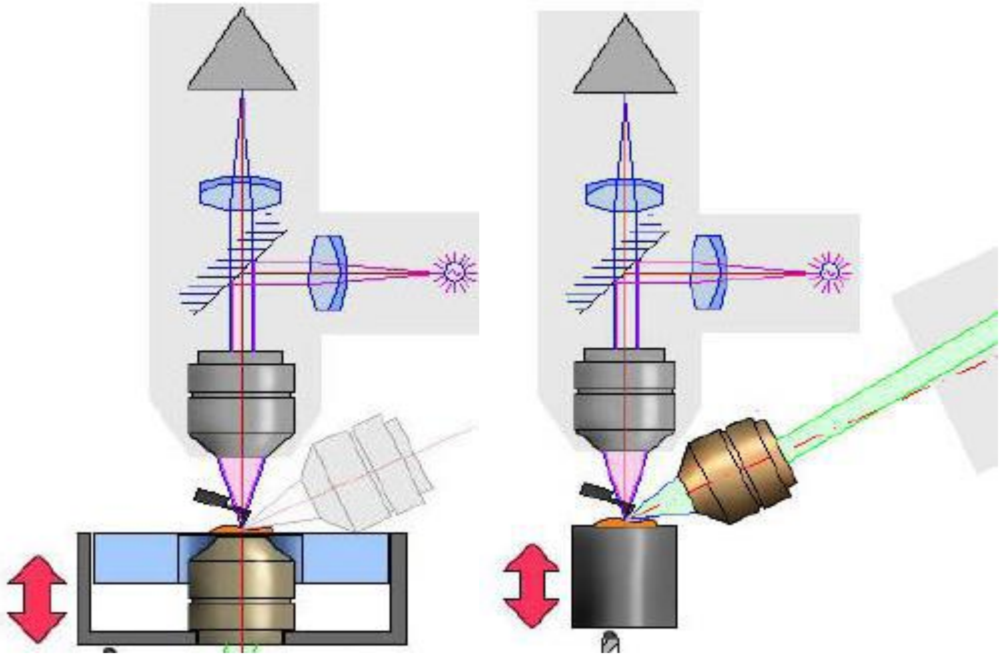
Monolayer of dye adsorbed on Au film, STM Ag-tip

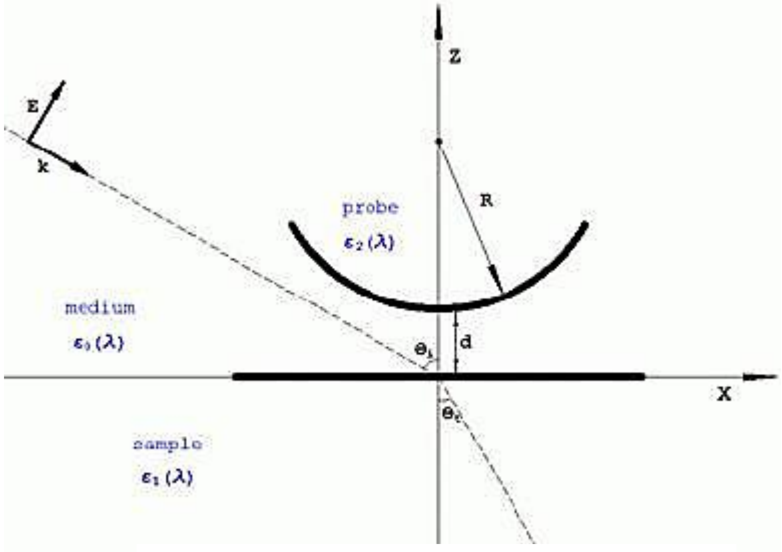
G. Picardi, K. Domke, D.Zhang, B. Ren, J. Steidtner B. Pettinger,
Fritz-Haber-Institut der Max-Planck-Gesellschaft

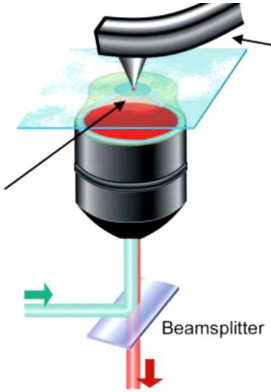
Instrumentation-integrated AFM + TERS



Instrumentation - integrated AFM + TERS two optical ports

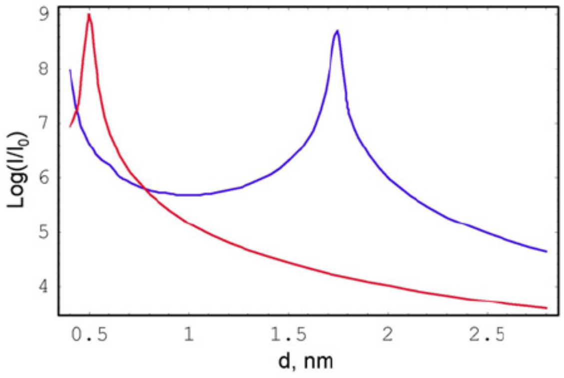






Modified AFM tip: Ag coating, colloid..

Only the area of the sample in contact with the tip experiences enhancement



Local field intensity dependence (logarithmic scale) on the probe-sample distance d.
Here R=100 nm, X=0 nm, Y=0 nm, Z=1 nm; light wavelength: 653 nm and 775 nm.

Combination - AFM, Raman nanomapping

Parallel images of silicon semiconductor

AFM image –9 x 7 µm

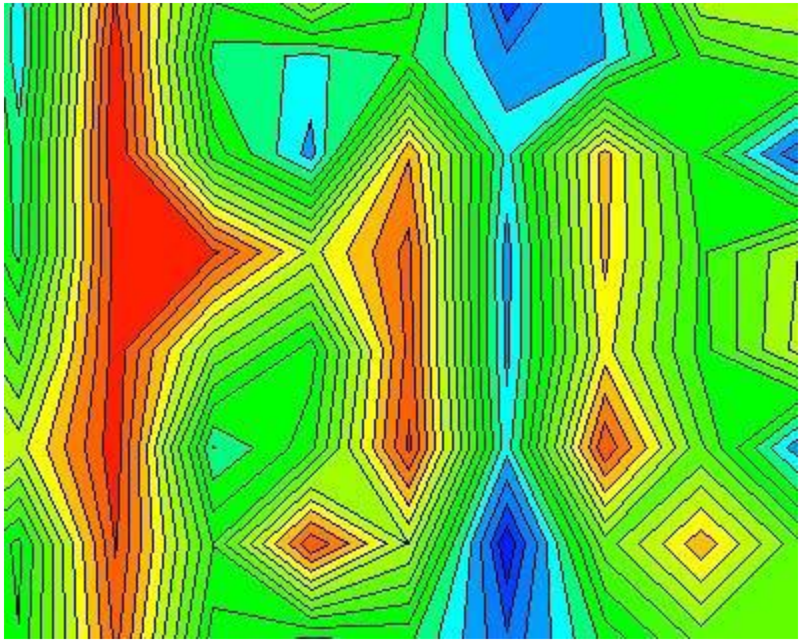


Image of Ramanovy intensity–520 cm-1, *the same area*

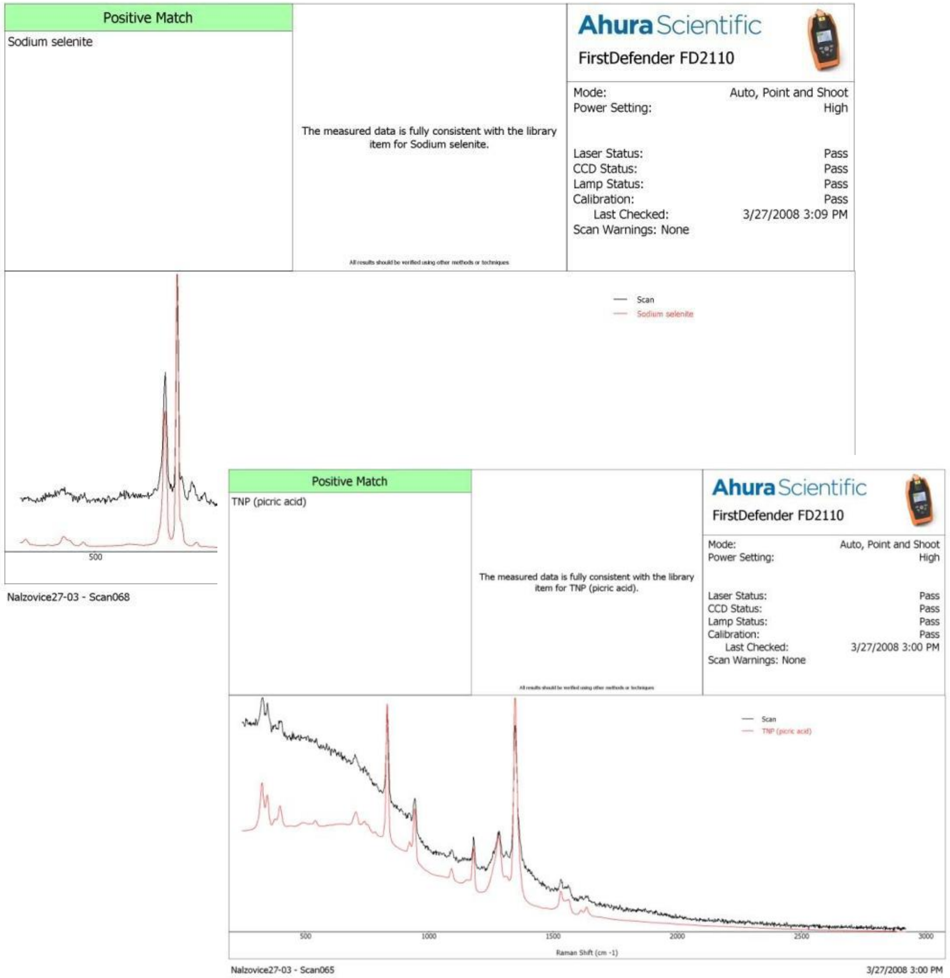
Application

- according to the type of investigated material
  - anorganic
  - organic
  - geological
  - biological …
- by instrumentation
  - dispersion vs. FT
  - macro x micro x nano
- according to the data evaluation method
  - spectra libraries, „ puzzles solving ", chemometrics …

Studied materials

- SAMPLES **– solids, liquids, phase interfaces**
- **examples**
  - **anorganic –***corrosion layeres, surfaces of hard disc*, silicon, *amorphous carbon, diamonds*
  - **organic -***supramolecular systems, contaminants in enviroment*
  - **polymers -***photolabile materials*
  - **biological-***in vitro, in vivo*
  - **geological -***minerals, rocks*
  - **archaeological -** *from the Paleolithic to the Modern Age*

Field measurements



Positive Match

Sodium selenite

The measured data is fully consistent with the library item for Sodium selenite.

All results should be verified using other methods or techniques

Ahura Scientific

FirstDefender FD2110

Mode: Auto, Point and Shoot
Power Setting: High

Laser Status: Pass
CCD Status: Pass
Lamp Status: Pass
Calibration: Pass
Last Checked: 3/27/2008 3:09 PM
Scan Warnings: None

Nalzovice27-03 - Scan068

Positive Match

TNP (picric acid)

The measured data is fully consistent with the library item for TNP (picric acid).

All results should be verified using other methods or techniques

Ahura Scientific

FirstDefender FD2110

Mode: Auto, Point and Shoot
Power Setting: High

Laser Status: Pass
CCD Status: Pass
Lamp Status: Pass
Calibration: Pass
Last Checked: 3/27/2008 3:00 PM
Scan Warnings: None

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3/27/2008 3:00 PM

Field measurements

Hanheld Raman spectrometer – AHURA