Pokročilé spektroskopické metody analýzy léčiv

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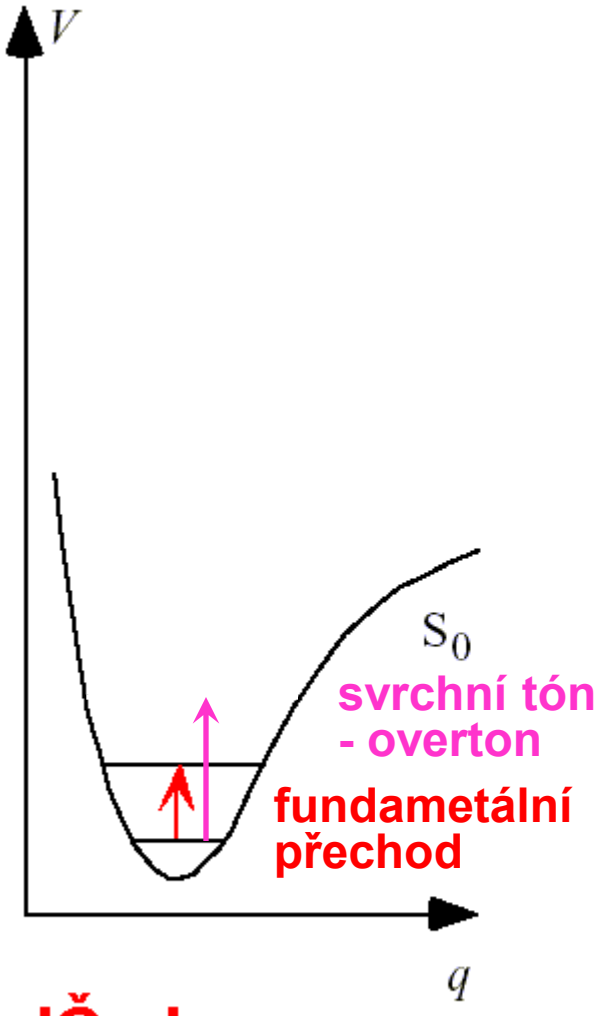
VŠCHT Praha

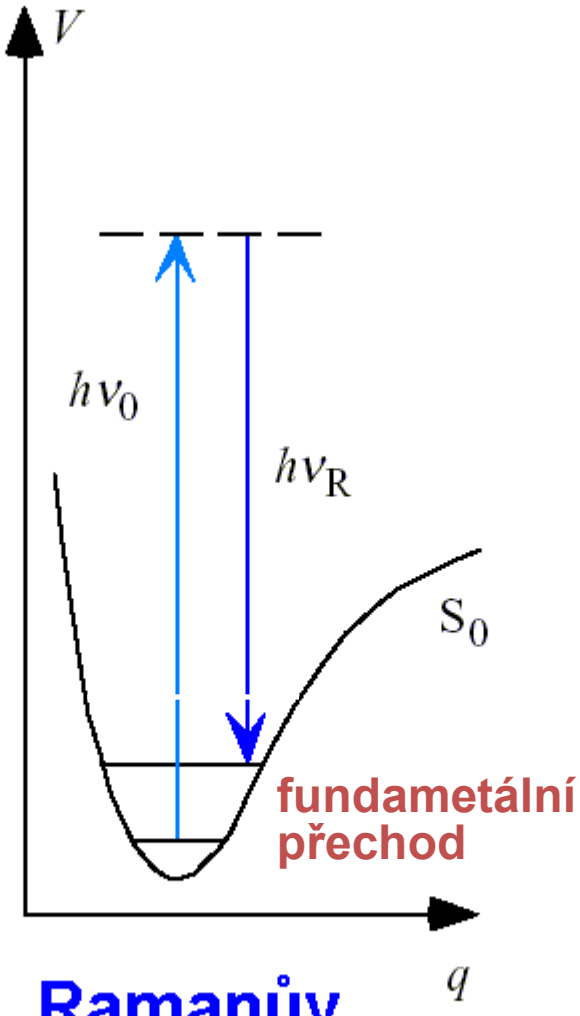
Sylabus předmětu - výchozí

1. Principy optické mikro- a nano- spektroskopie
2. Infračervená mikro- a nano- spektroskopie
3. Ramanova mikrospektroskopie a hrotem zesílená Ramanova spektroskopie
4. Povrchem zesílené vibrační spektroskopie a efekt plasmonové rezonance
5. Analýza vícesložkových léčiv metodami vibrační spektroskopie
6. Detekce aktivních složek a jejich metabolitů metodami optické spektroskopie
7. Tvorba knihoven spekter, elektronické databázové systémy
8. Využití multivariačních statistických metod při klasifikaci spektrálních dat
9. Využití multivariačních statistických metod při kvantitativní analýze spektrálních dat
10. Spektrometry s vláknovou optikou a in situ analýza
11. Mobilní a přenosné spektrometry
12. Analýza vícesložkových léčiv metodami NMR spektrometrie v roztoku
13. Analýza vícesložkových léčiv metodami NMR spektrometrie v pevné fázi
14. NMR analýza v pevné fázi v nehomogenním magnetickém poli

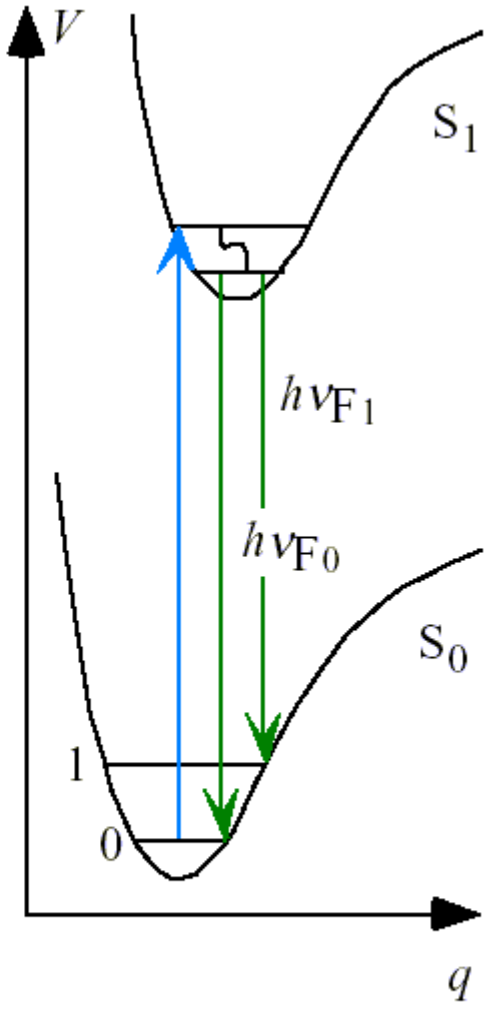
Schéma hladin



IČ absorpce



Ramanův rozptyl



Fluorescence

Infračervená spektrometrie

Podstata infračervené absorpce

jednofotonový přechod
mezi dvěma
vibračními (vibračně-rotačními) stavy molekuly,
jejichž energie jsou E_1 a E_2,
vyvolaný interakcí s fotonem dopadajícího záření

$$h\nu_{abs} = |E_2 - E_1|$$

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

pro fundamentální přechody

Infračervená spektrometrie

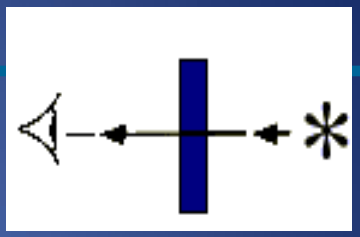
Podstata infračervené absorpce

přechody mezi

vibračními (vibračně-rotačními) stavy

- typy možných přechodů při absorpci IČ záření
  - v rámci jednoho vibračního modu
    - fundamentální (změna kvantového čísla o jednotku)
    - vyšší harmonické - svrchní tóny
  - zahrnuto více vibračních modů
    - kombinační

Infračervená spektrometrie



- **TRANSMISNÍ MĚŘENÍ**

- plyny - plynové kyvety - optická délka 1 cm - 10 m

- roztoky - kapalinové kyvety - 0,01 mm - 10 mm

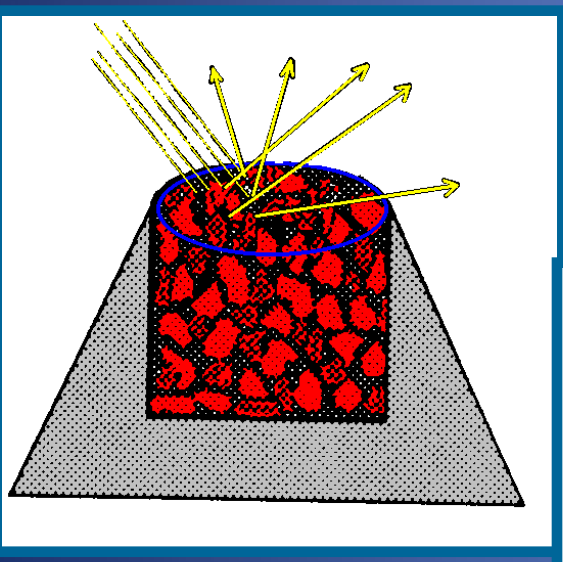
- kapaliny - kapalinové kyvety - 0,002 mm - 0,05 mm

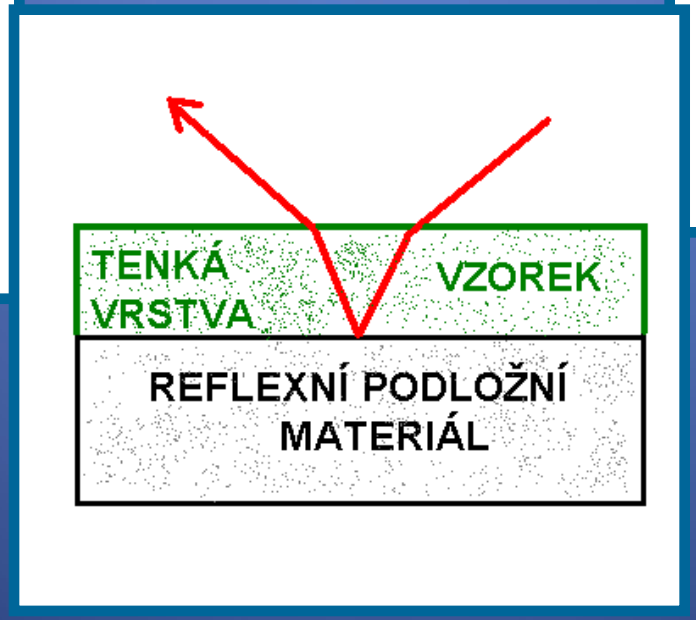
- pevné látky - suspenze s Nujolem, Fluorolube - kapalinové kyvety

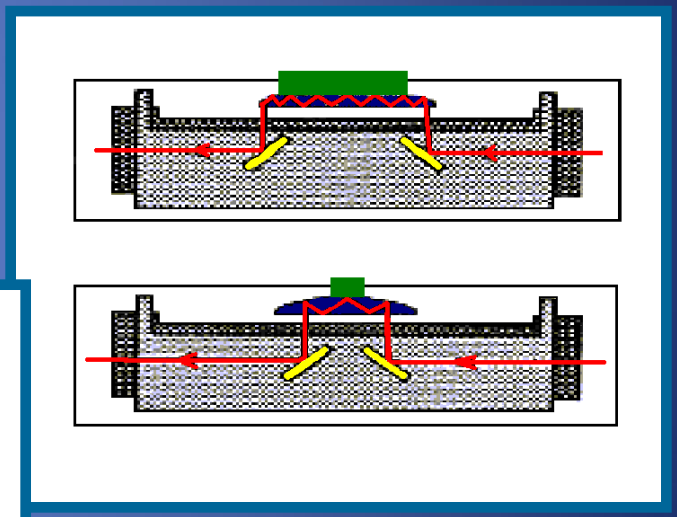
- tablety s KBr

Přehled technik – molekulová analýza

- **Reflexní techniky infračervené spektrometrie** – ATR, DRIFT, IRRAS
- Infračervená mikrospektroskopie





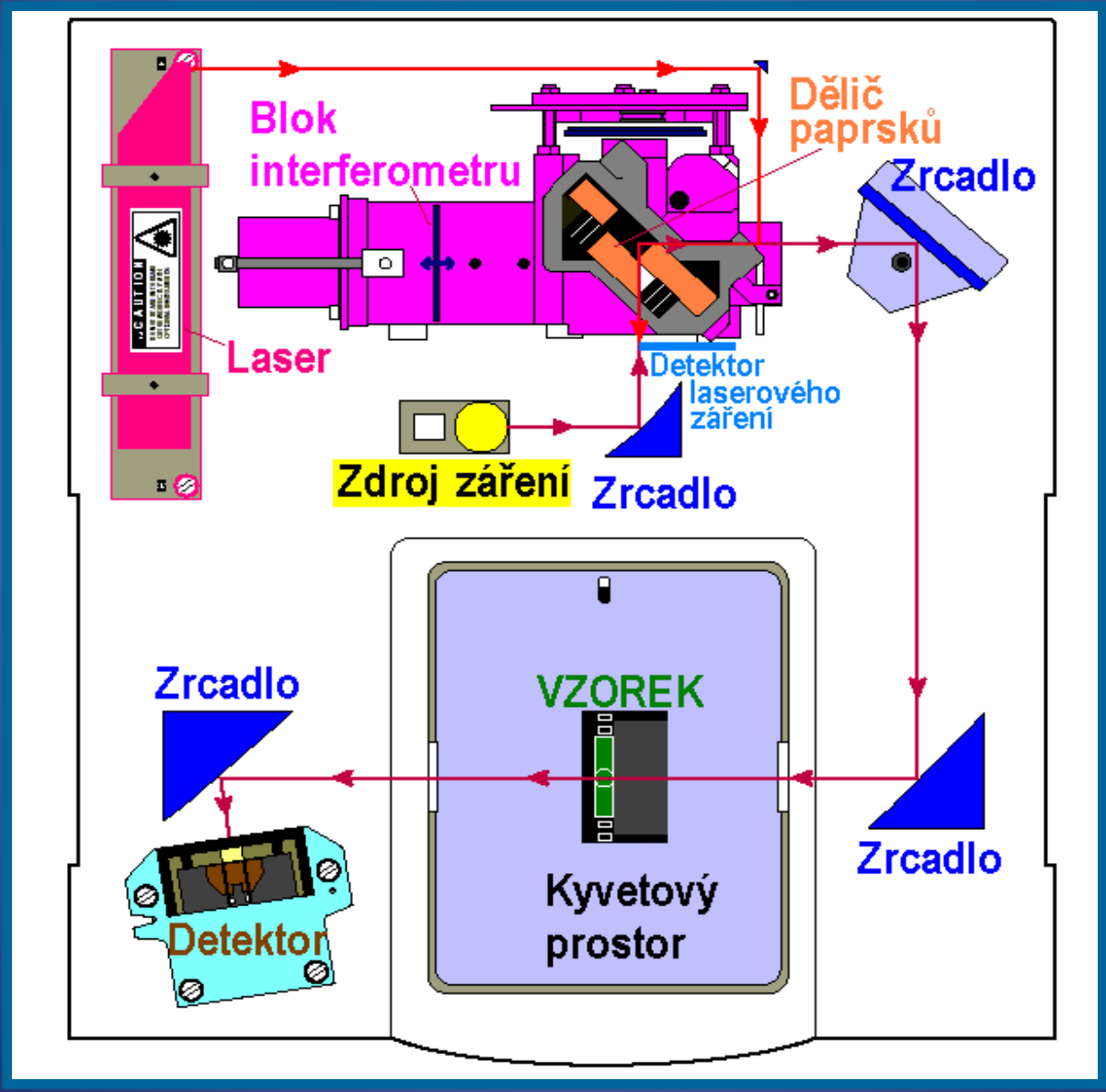


Infračervená spektrometrie

ANALYZOVANÉ TYPY MATERIÁLŮ

- plyny - analýza složení vzduchu v inkubátoru
  - monitoring vzdušných polutantů
- kapaliny, roztoky - analýza olejů
  - analýza vodných roztoků
  - analýza disperzí
- práškové vzorky - analýza léčiv, drog, trhavin
  - analýza rud, hnojiv
- fázové rozhraní – analýza kůže, dermatologie

Infračervená spektrometrie - instrumentace



Infračervená spektrometrie

Oscilující dipólový moment

pohyb molekuly spojený se změnou elektrického dipolového momentu
vede k absorpci (nebo k emisi) záření

$$p = p_0 + \left(\frac{\partial p}{\partial q}\right)_0 q$$

p - aktuální dipólový moment
p_0 - dipólový moment v rovnovážné poloze
q - normální souřadnice vibračního módu

Infračervená spektrometrie

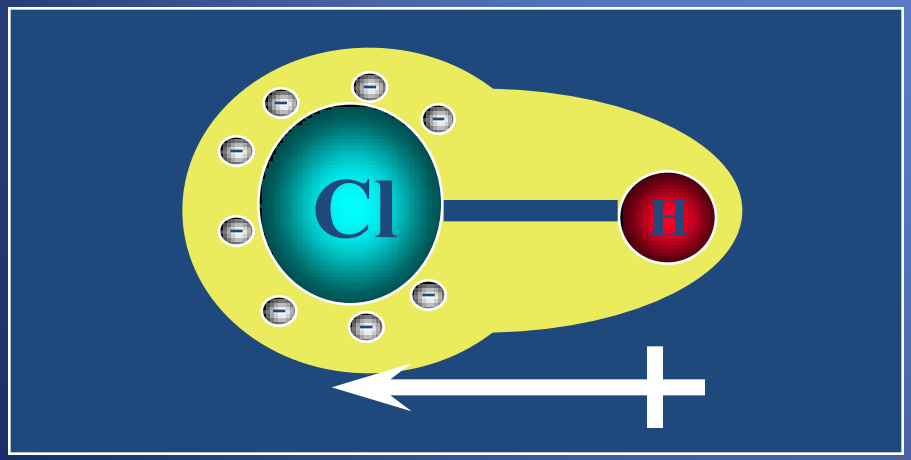
Základní výběrové pravidlo infračervené absorpce

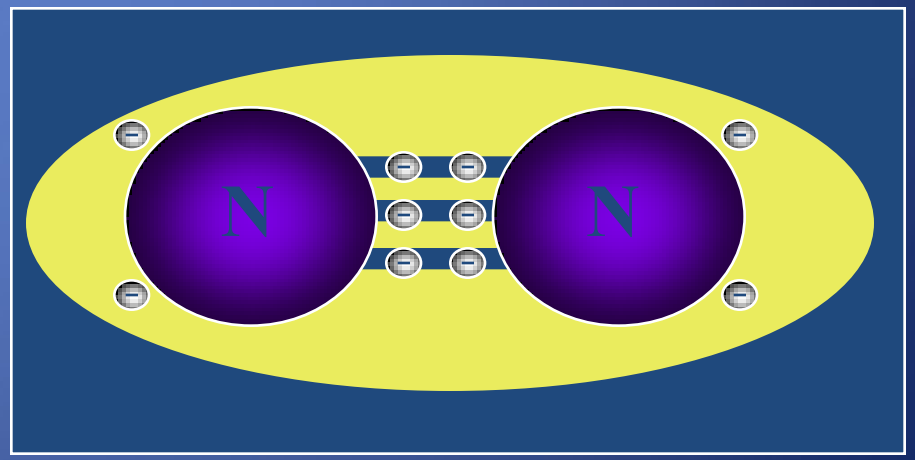
$$\frac{\partial p}{\partial q} \neq 0$$

INTENZITA PÁSŮ ÚMĚRNÁ ZMĚNĚ DIPOLOVÉHO MOMENTU BĚHEM VIBRAČNÍHO POHYBU

The Dipole Change...

- To absorb energy, the DIPOLE must change when the transition occurs
- The intensity of the absorption is proportional to the magnitude of the dipole CHANGE





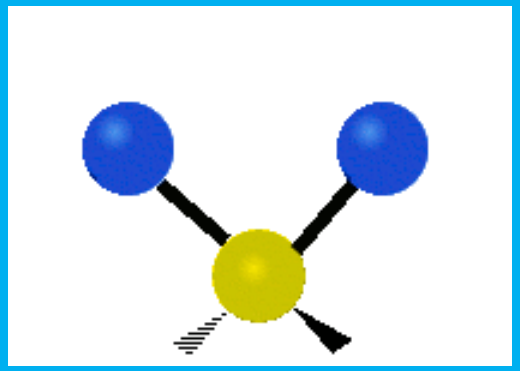
Infračervená spektrometrie

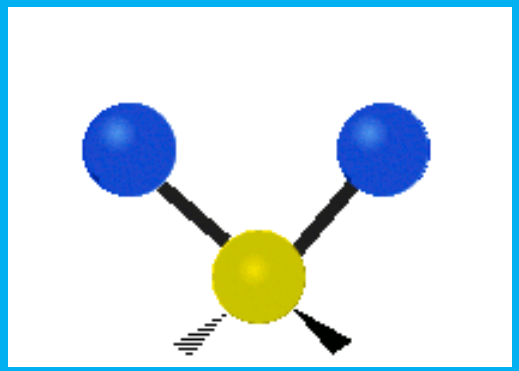
TYPY VIBRACÍ

- VALENČNÍ – ZMĚNA délky vazby/vazeb

» SYMETRICKÁ

» ANTISYMETRICKÁ

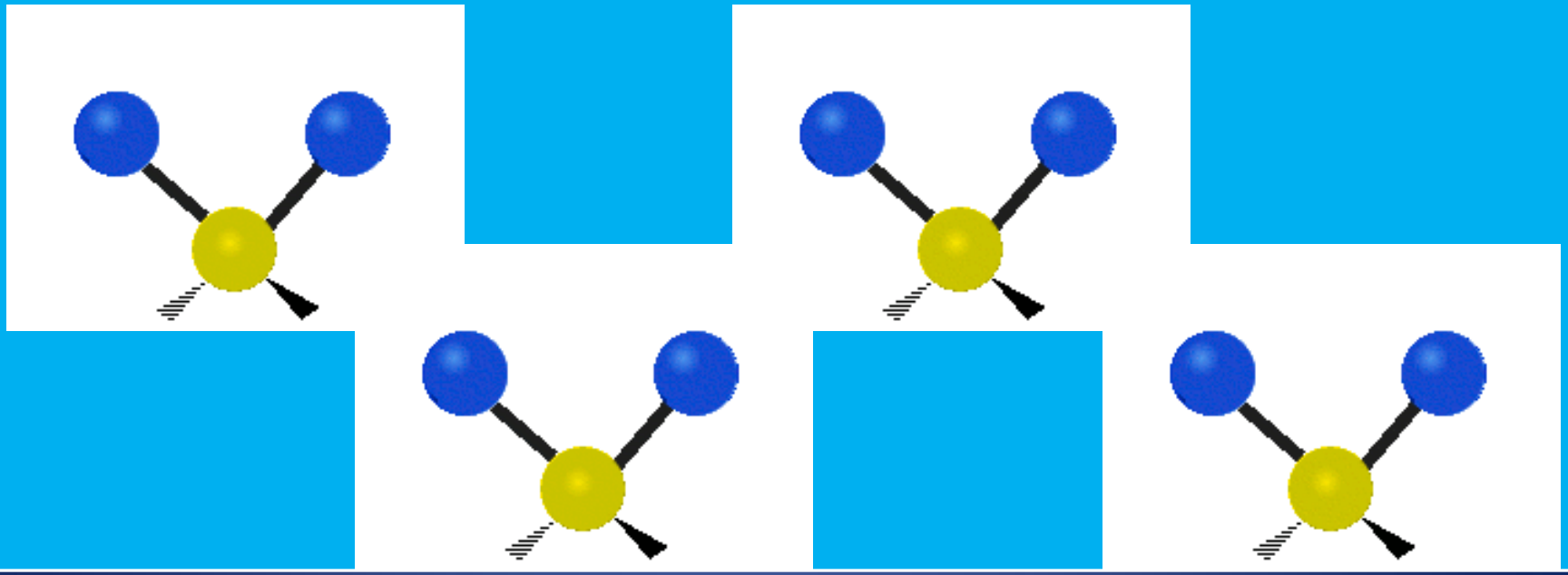




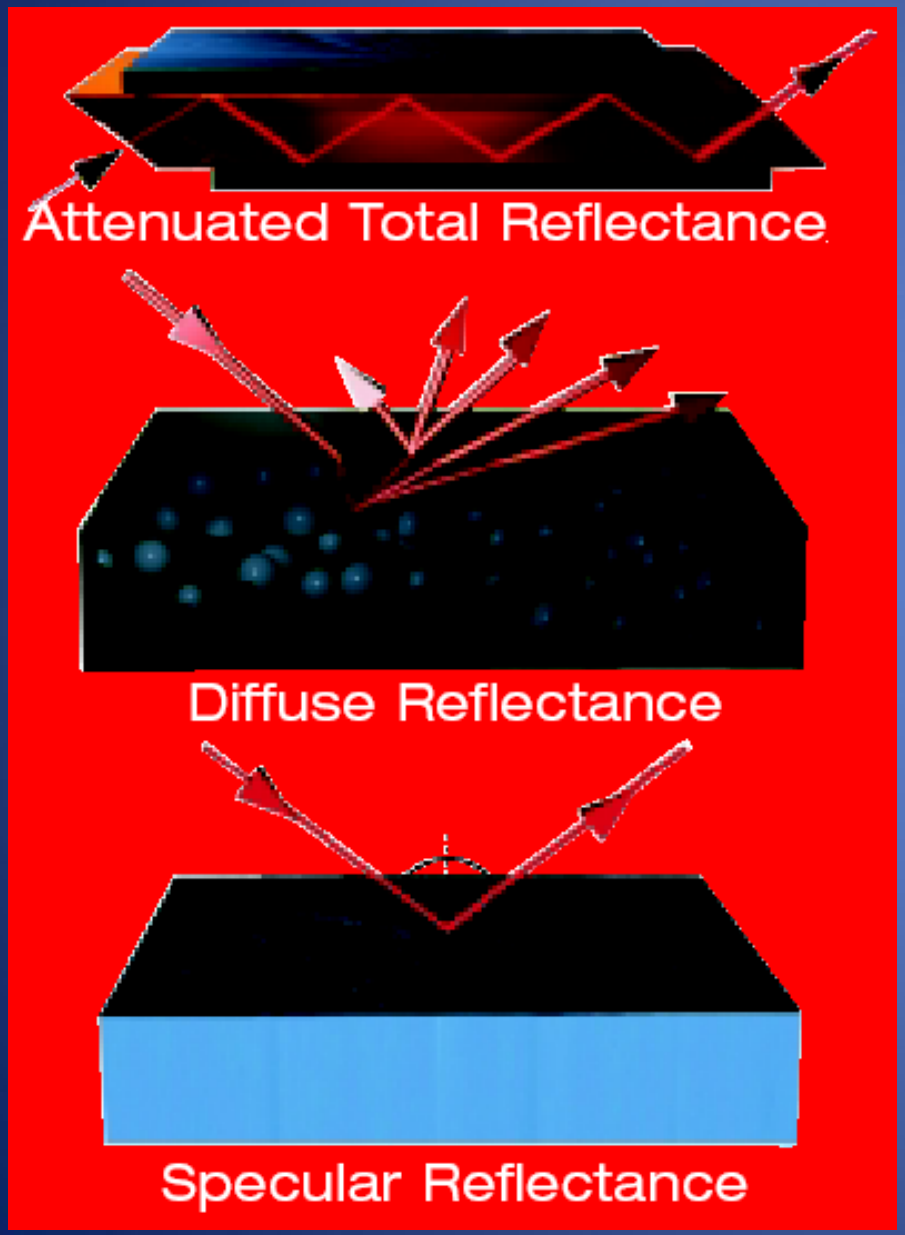
Infračervená spektrometrie

TYPY VIBRACÍ

- DEFORMAČNÍ - změny úhlů (vazebné úhly, torsní úhly)
- nůžková, kolébavá, kývavá, kroutivá



FT-IR reflexní techniky



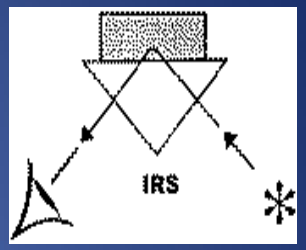
- Infračervený paprsek se odráží od fázového rozhraní úplným vnitřním odrazem
- Vzorek musí být v optickém kontaktu s krystalem
- Snímaná informace z **povrchové vrstvy** vzorku

- Pevné látky – prášková forma , případně naředěná IČ transparentní matricí - (KBr)
- Informace **z objemu vzorku**

- Reflektující vzorek či spíš tenká vrstva na zrcadlovém podkladu
- Informace **z tenké (povrchové) vrstvy**

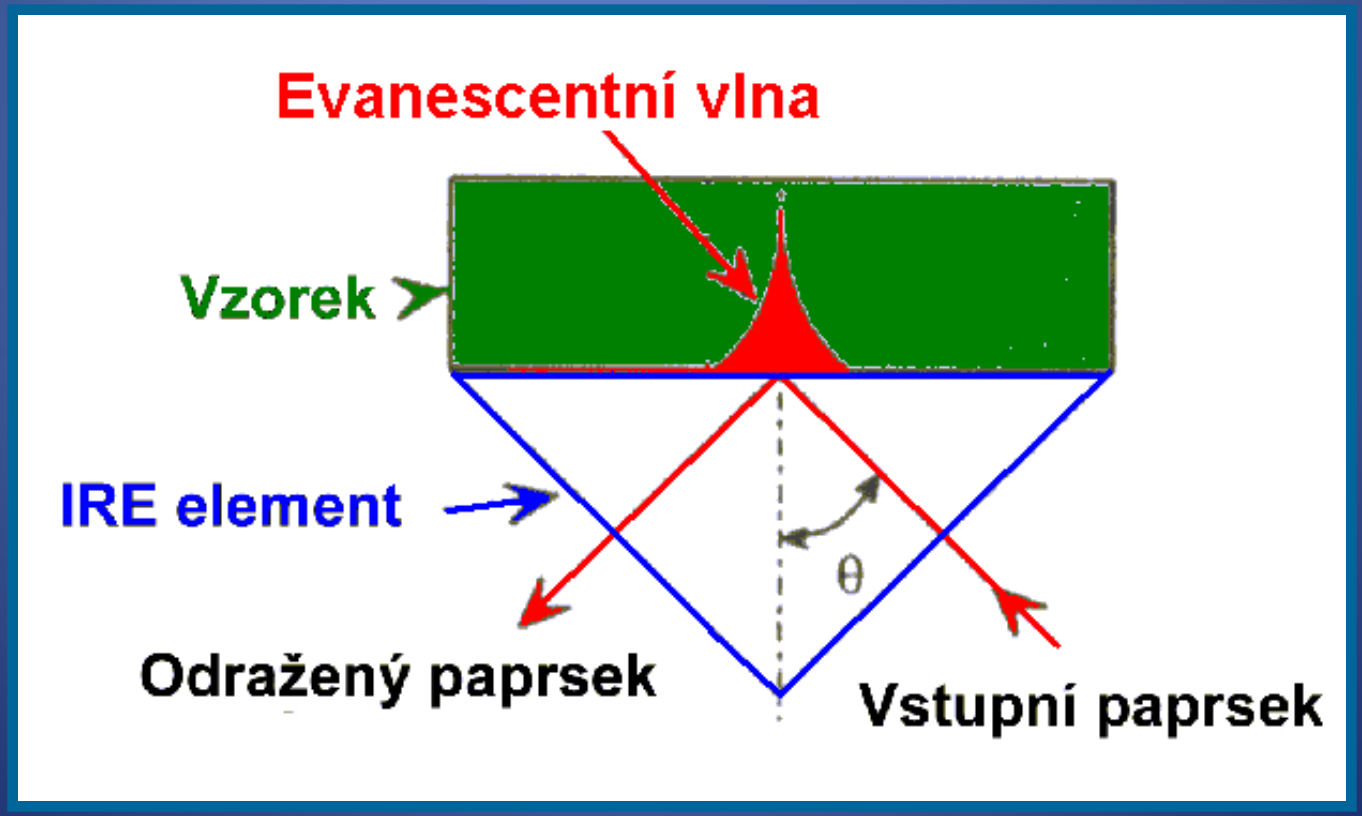
Infračervená spektrometrie

- **Reflexní techniky**

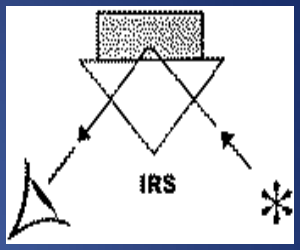


ATR - attenuated total reflection

- zeslabený úplný (vnitřní) odraz



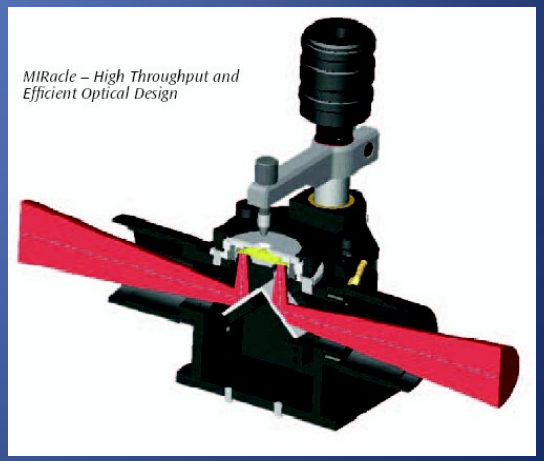
Infračervená spektrometrie



- Faktory, které ovlivňují ATR spektrální analýzu

POUZE ODRAZ - NIKOLI LOM !

- Vlnová délka infračerveného záření
- Index lomu IRE a vzorku
- Hloubka průniku
- Efektivní délka dráhy
- Úhel dopadu
- Účinnost kontaktu se vzorkem
- Materiál IRE (ATR krystalu)



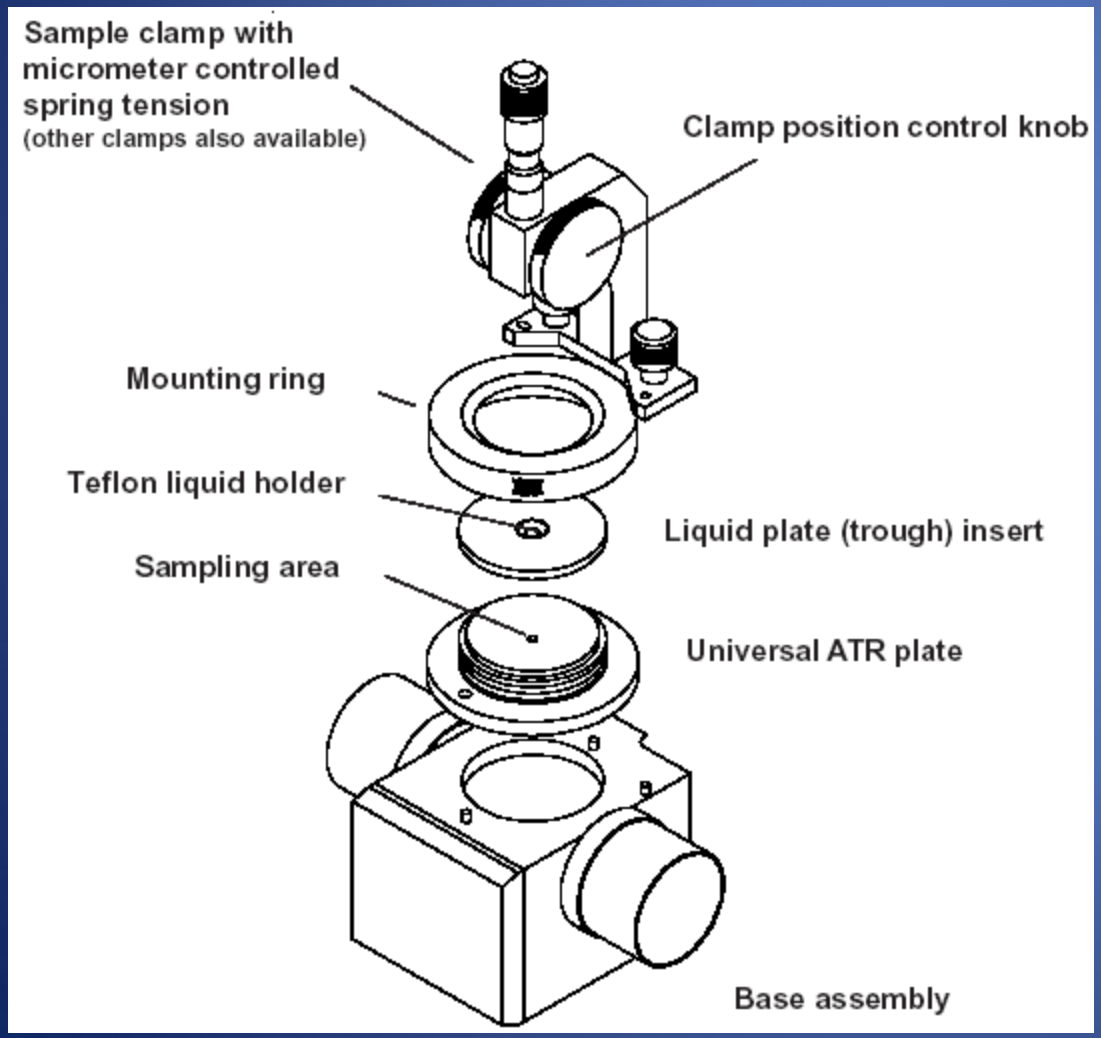
Zeslabený totální odraz (ATR)

Chart of Common Crystal Materials

Material	ATR Spectral Range (cm^{-1})	Refractive Index	Depth of Penetration (u) (at 45° & 1000 cm^{-1})	Uses
Germanium	5,500 - 675	4	0.66	Good for most samples. Strong absorbing samples, such as dark polymers.
Silicon	8,900 - 1,500 & 360-120	3.4	0.85	Resistant to basic solutions.
AMTIR	11,000 - 725	2.5	1.77	Very resistant to acidic solutions.
ZnSe	15,000 - 650	2.4	2.01	General use.
Diamond	25,000 - 100	2.4	2.01	Good for most samples. Extremely caustic or hard samples.

Infračervená spektrometrie

ATR - instrumentace



ATR - instrumentace

MIRacle Pressure Clamps are Pinned-in-Place and Easily Upgraded

MIRacle Digital Clamp *Ideal for Controlled Pressure*

MIRacle Rotating Clamp *Ideal for Cleaning Tip of Debris*

MIRacle Viewing Clamp *Ideal for Placing Fibers or Crystals*

MIRacle High-Pressure Clamp *– Ideal for Routine Sampling*

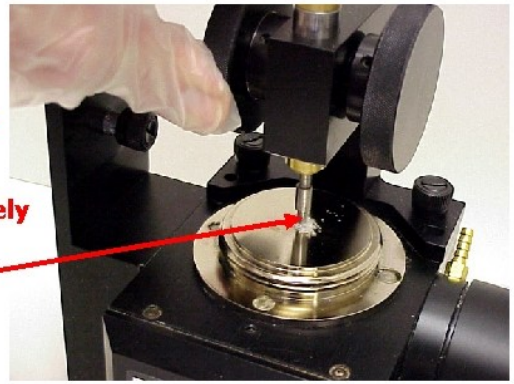
MIRacle Micrometer Clamp *– OK for Low Pressure Applications*

Slide 12

Sample should completely cover the ZnSe Crystal indicated with the arrow below.

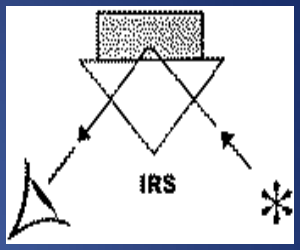
Slide 13

Be sure that the press is rotated completely to the lowest level.

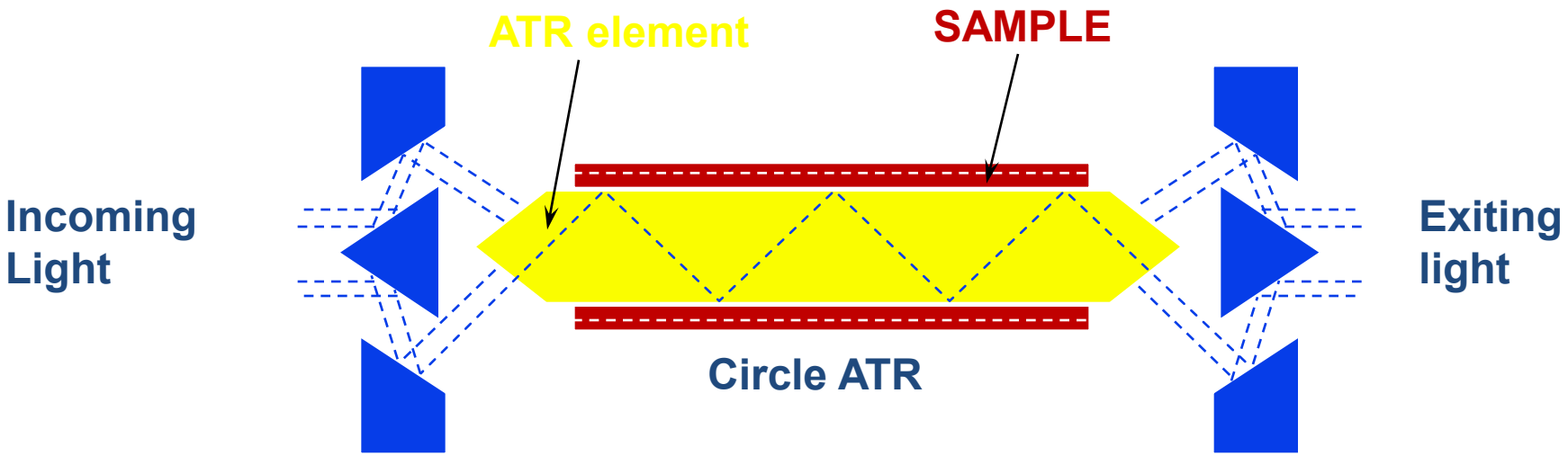


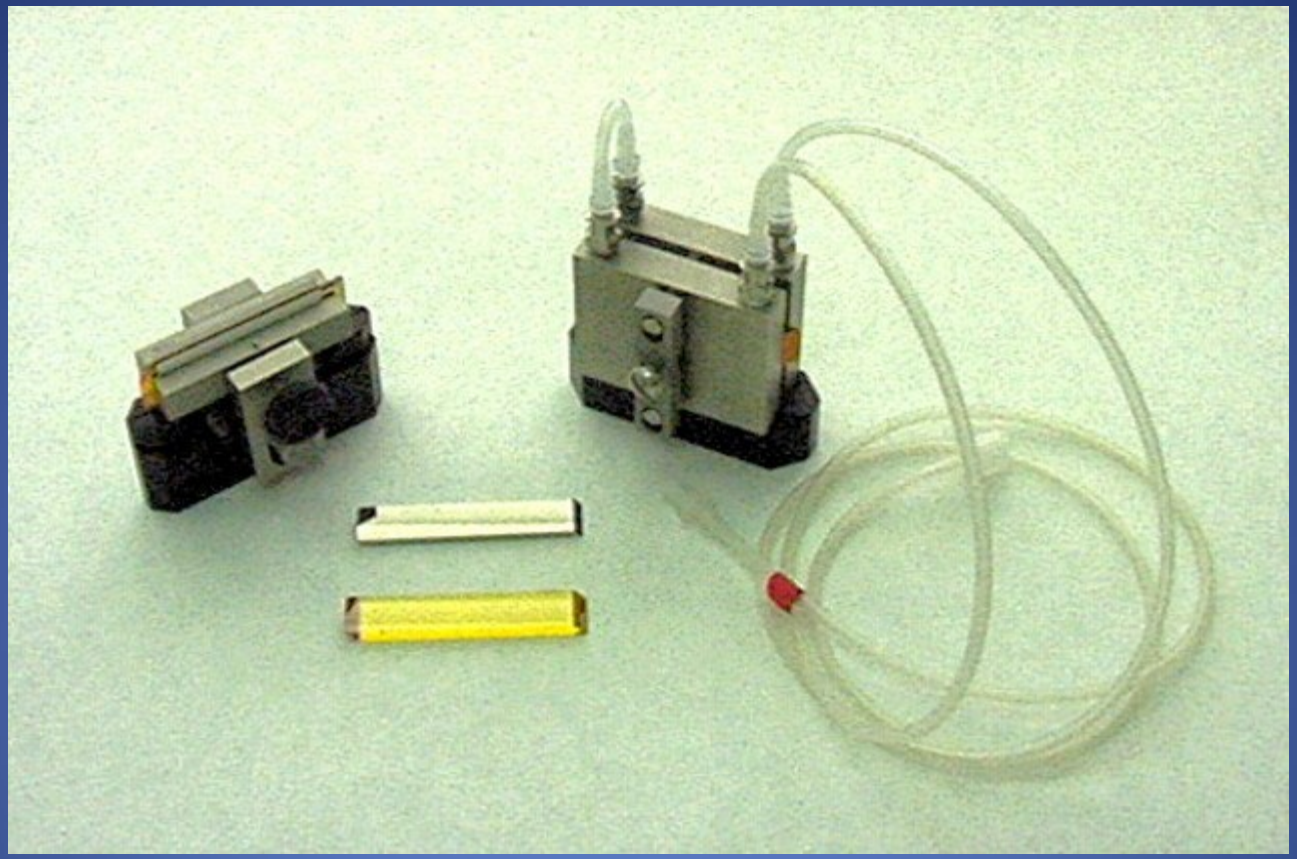
ATR - instrumentace

Zeslabený totální odraz (ATR)



Scheme of the Circle ATR Cell

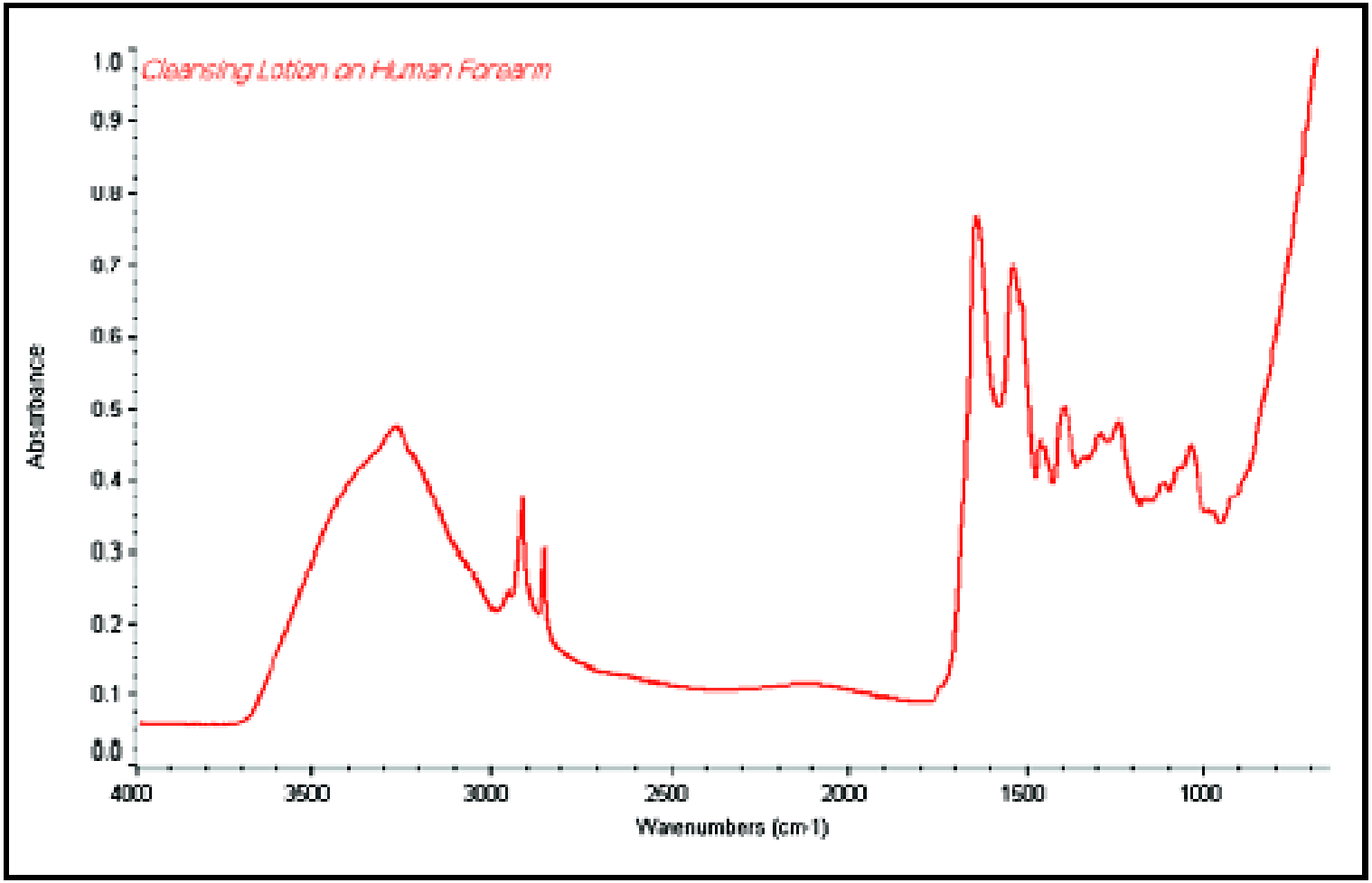




ATR/IR průtočná cela
germaniový a ZnSe krystal

Attenuated Total Reflection (ATR)

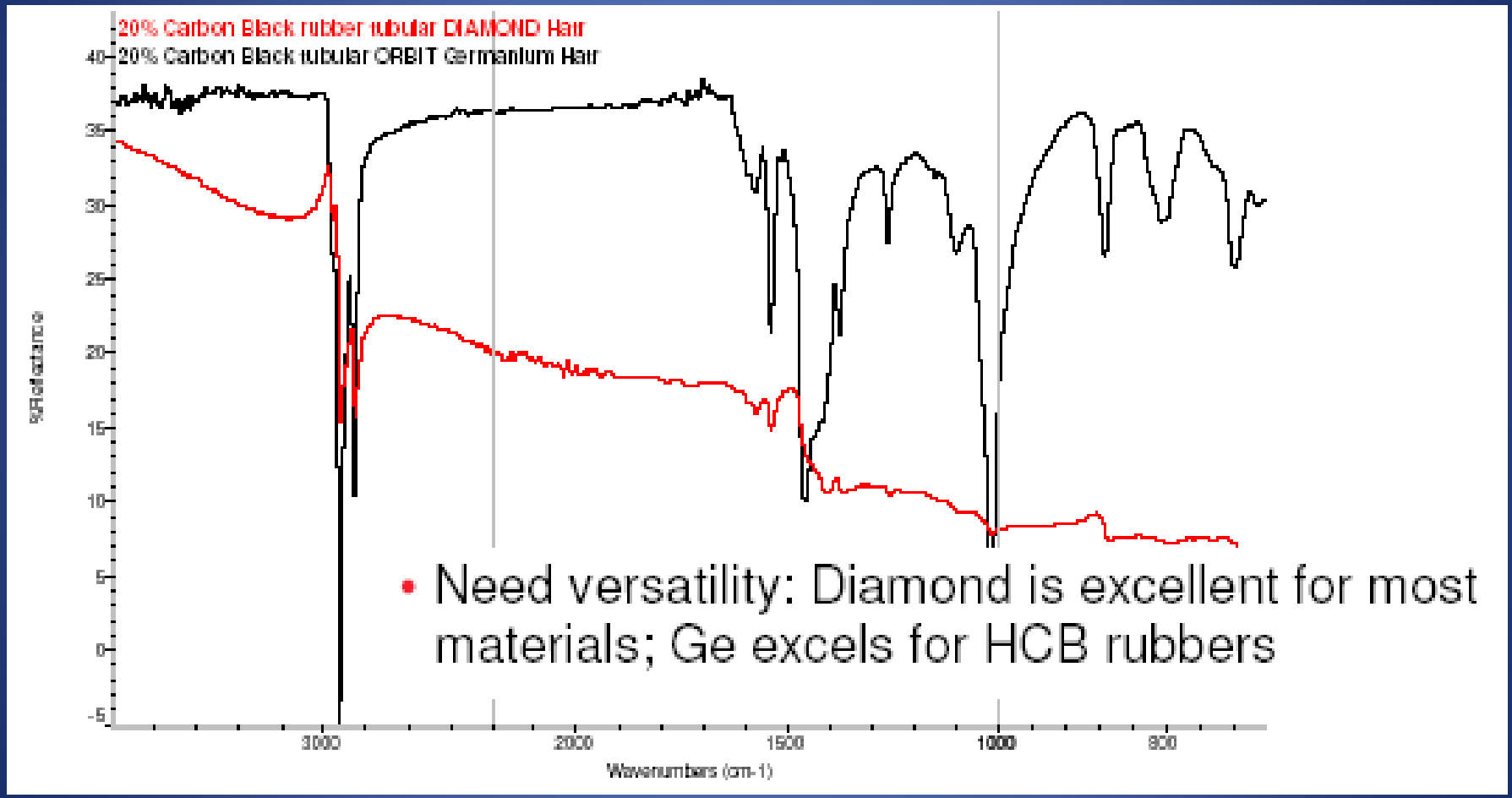
ATR Spectra



FTIR spectrum of cleansing lotion on human forearm – using the HATRPlus with flat plate Ge crystal.

Attenuated Total Reflection (ATR)

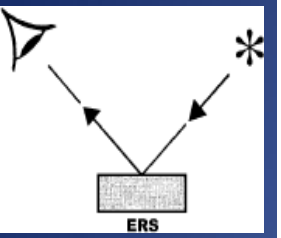
ATR Spectra of Rubber – Diamond vs Germanium IRE

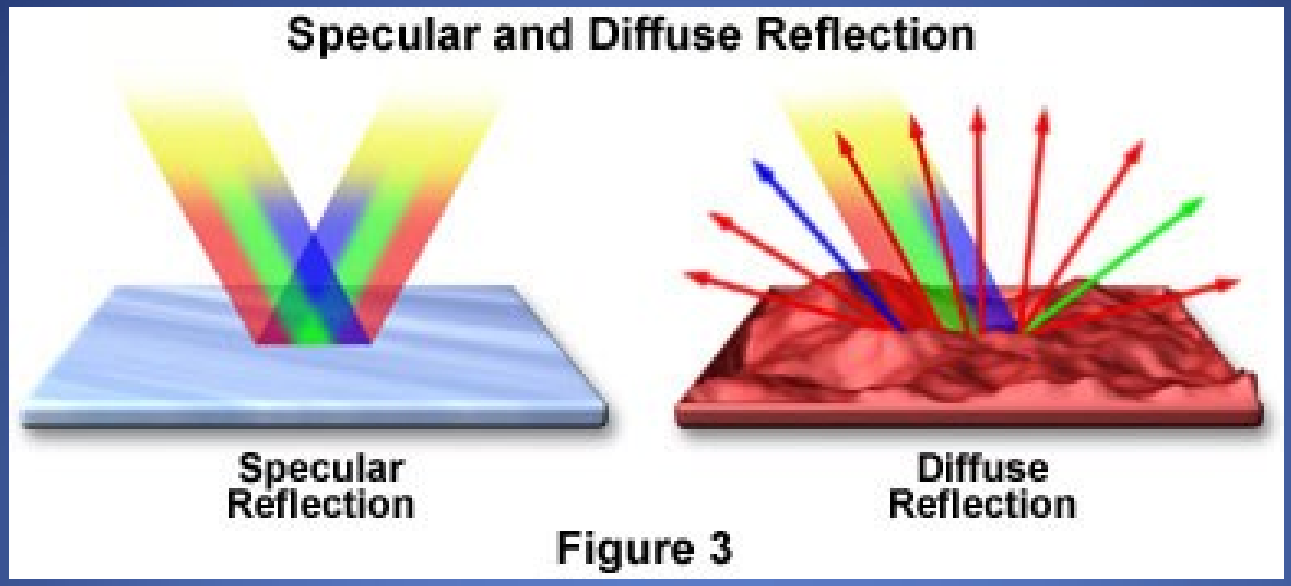


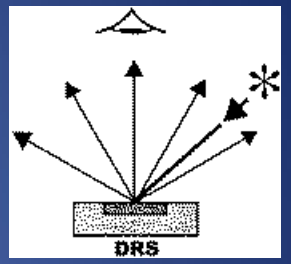
Refractive indexes: Ge=4

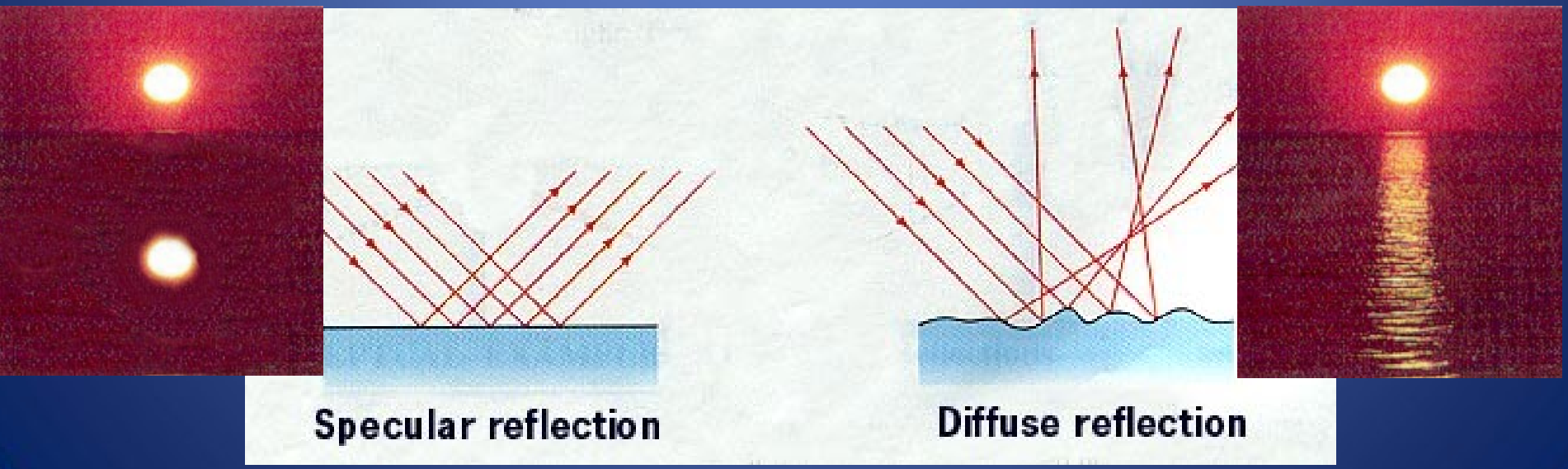
Diamond=2.4

Spekulární vs. Difusní Reflexe

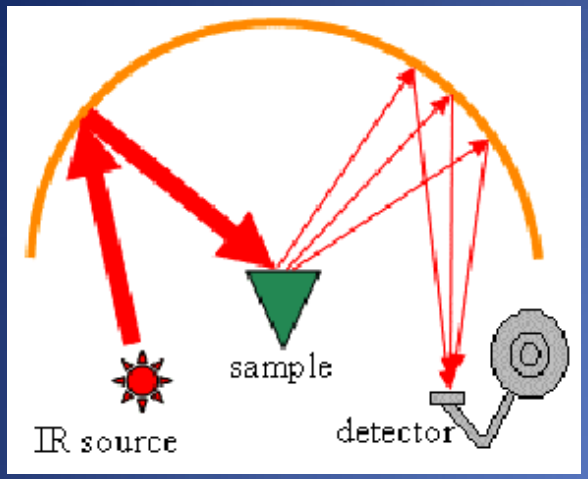


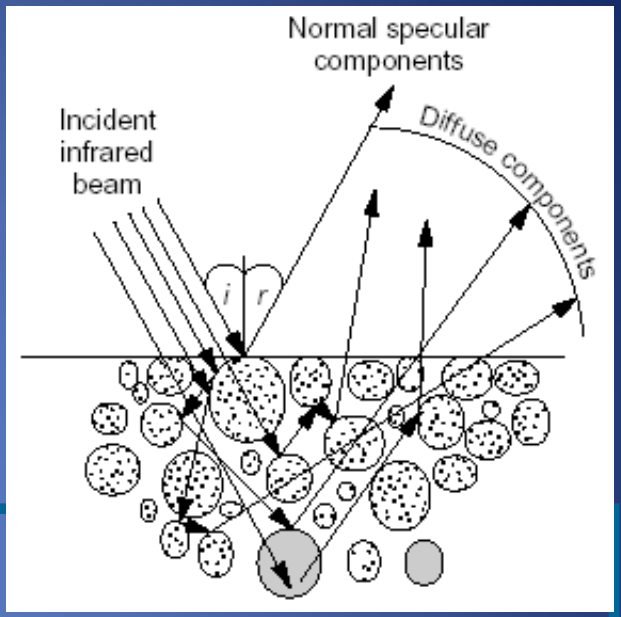






Reflexní techniky





-DRIFT

-rychlé měření práškových vzorků

-- nízká opakovatelnost dat

- složitý fyzikální popis jevu

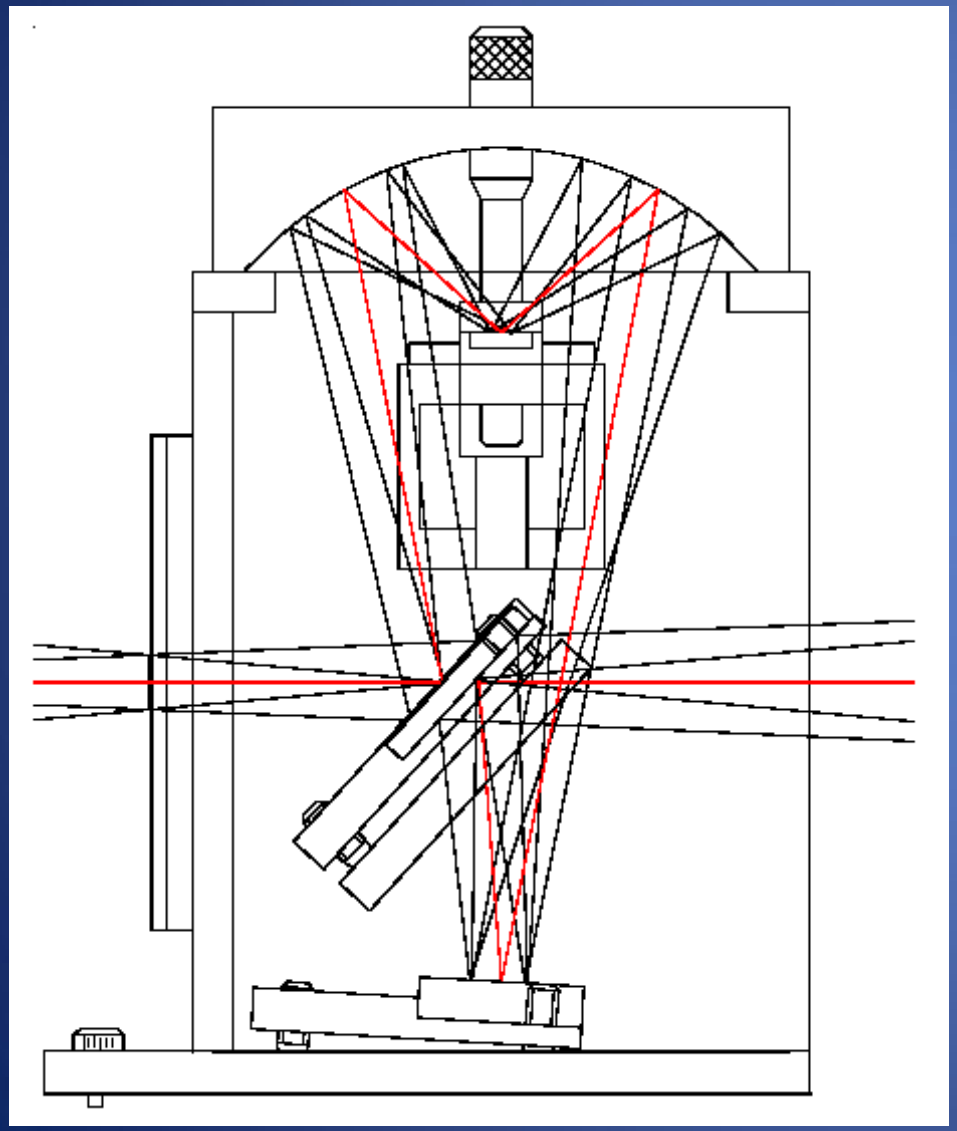
tvar částic, „zhutnění“ vzorku

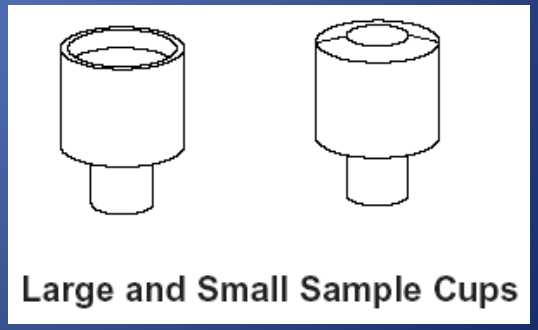
index lomu částic

reflektivita a absorpční vlastnosti částic

Diffuse Reflection

Experimental Setup

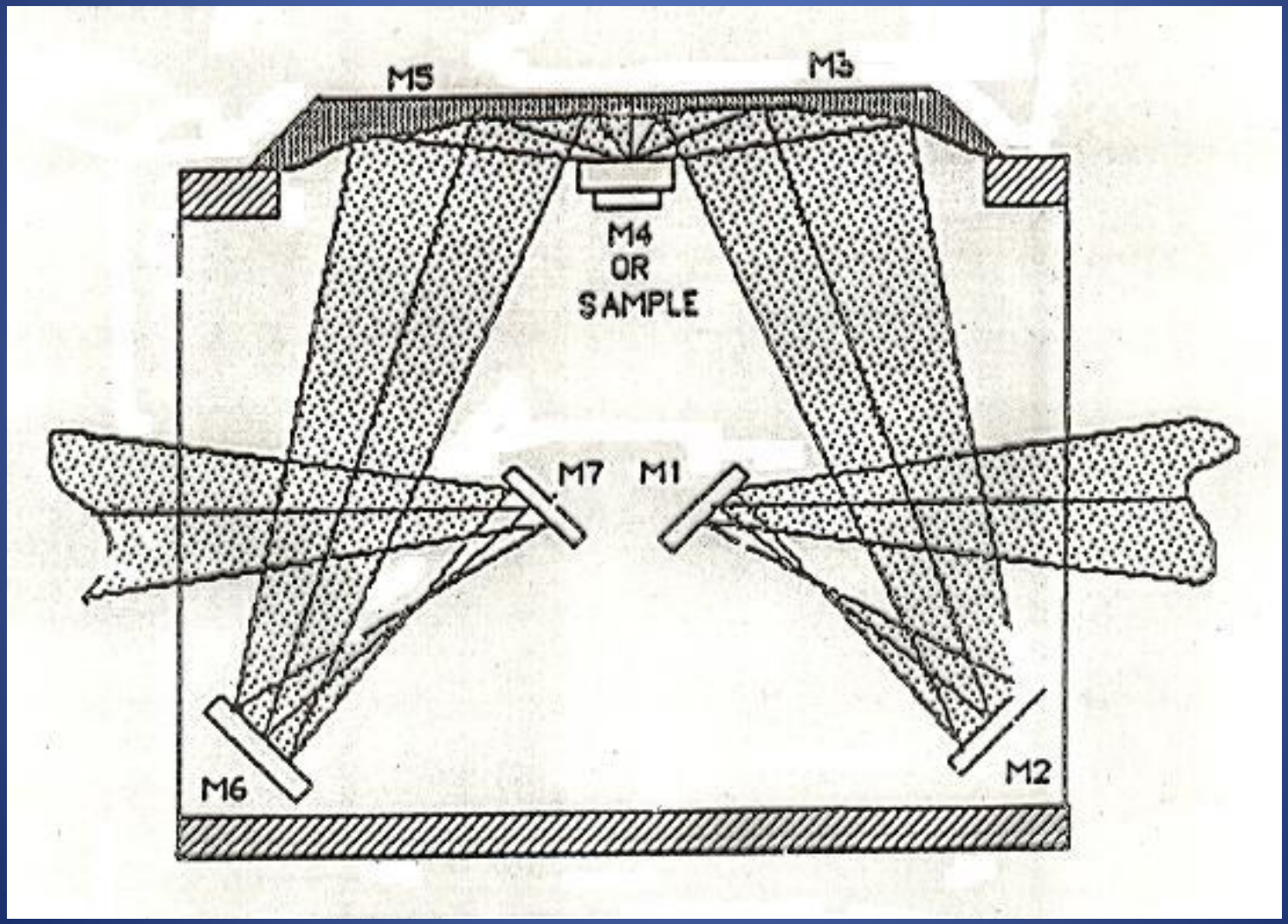




Large and Small Sample Cups

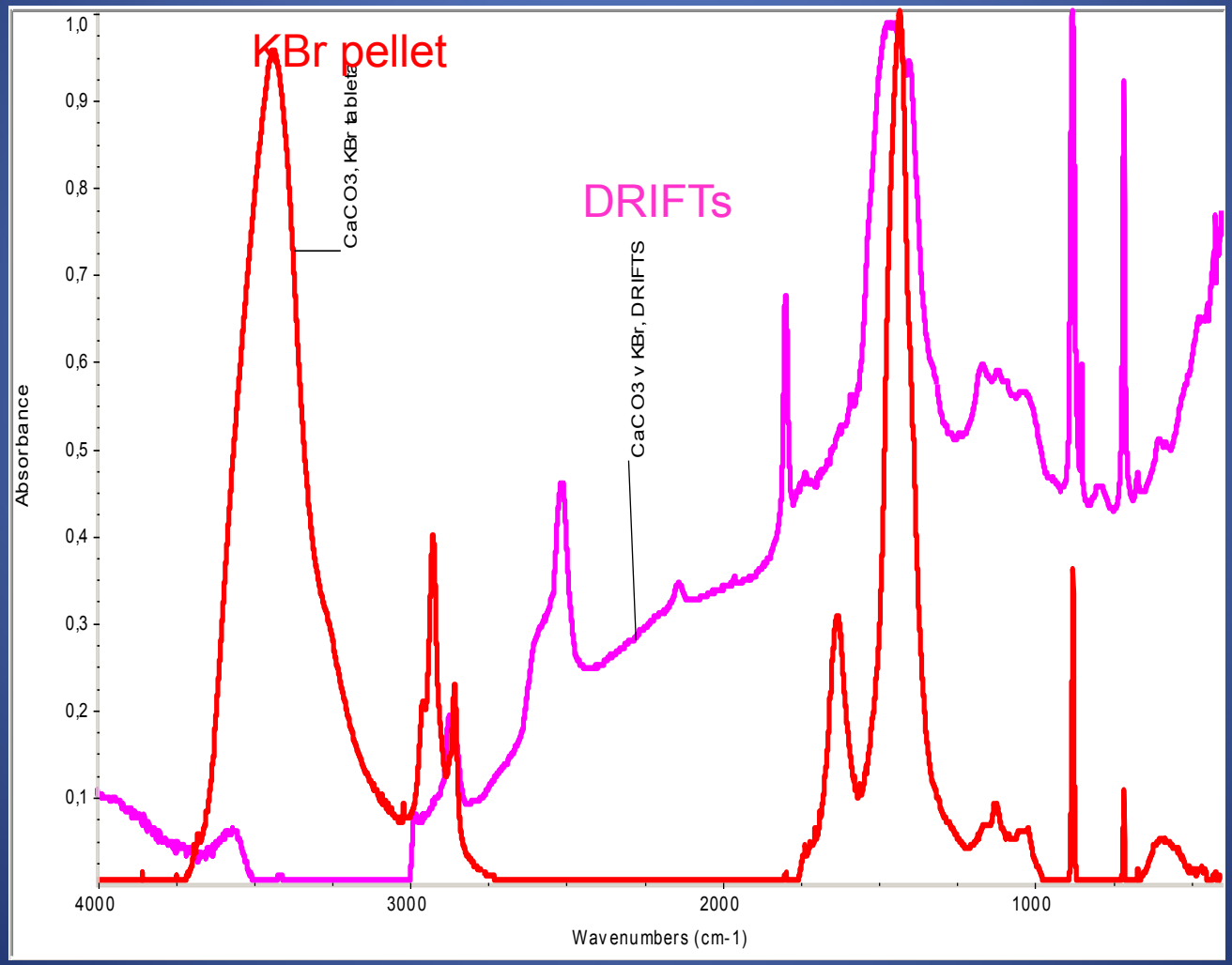
Diffuse Reflection

Experimental Setup – Different Geometry



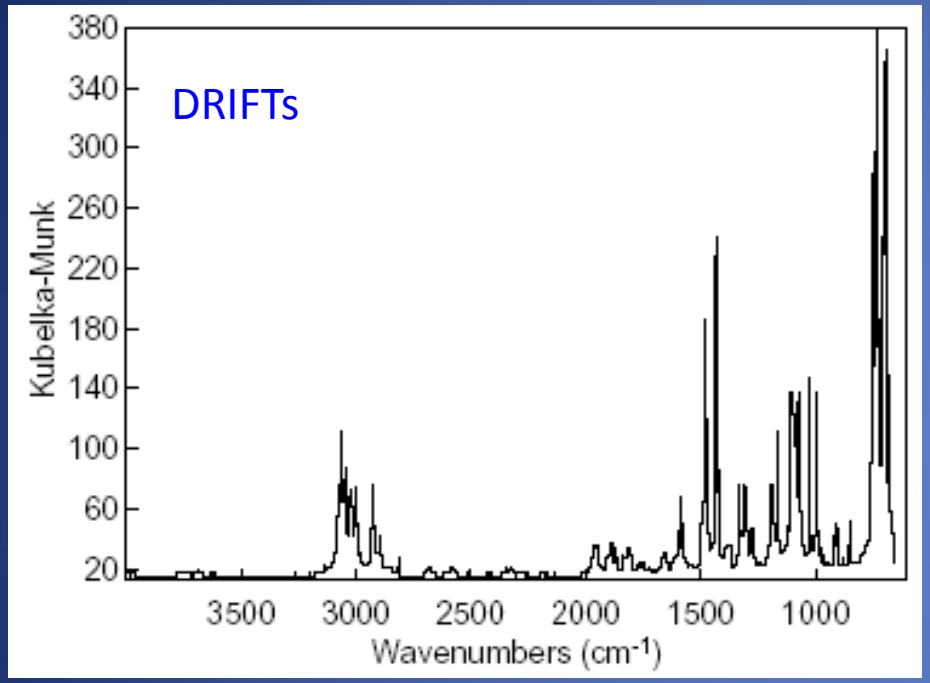
Diffuse Reflection

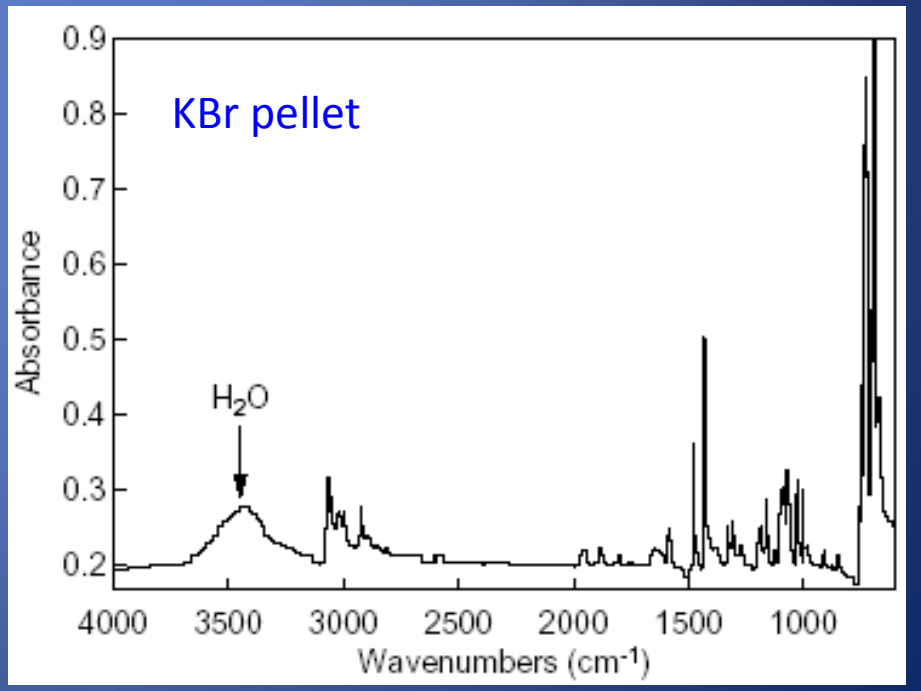
IR Spectra of $CaCO_3$



Diffuse Reflection

IR Spectra of 1,2-Bis(diphenyl phosphino)ethane





T Armaroli et al. Oil & Gas Science and Technology – Rev. IFP, 59 (2004), 215.

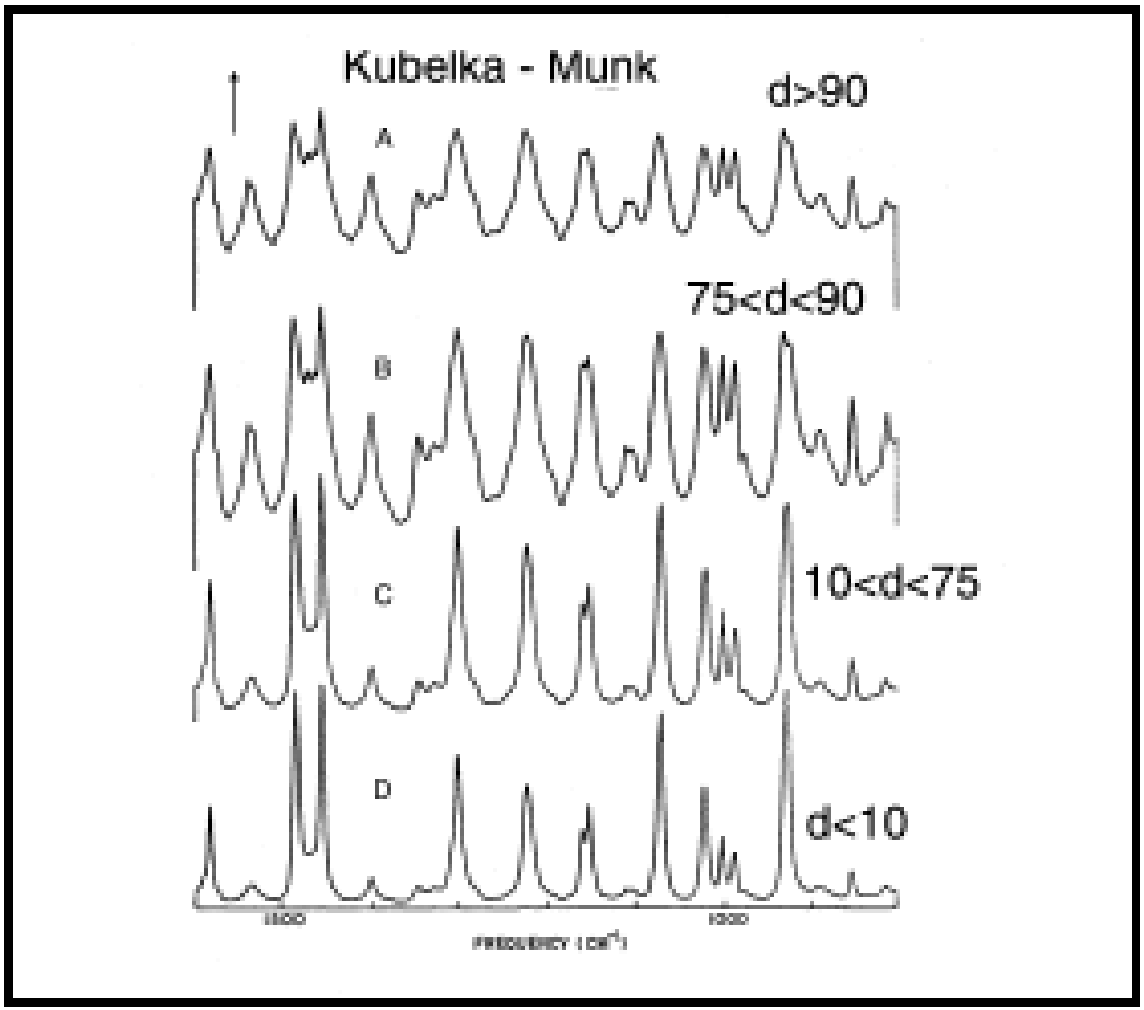


Figure 4: *Spectra of Azobenzene at varying particle sizes.*

Specular Reflection

If the surface is smooth like a mirror:

– reflection and the incidence angles are equal

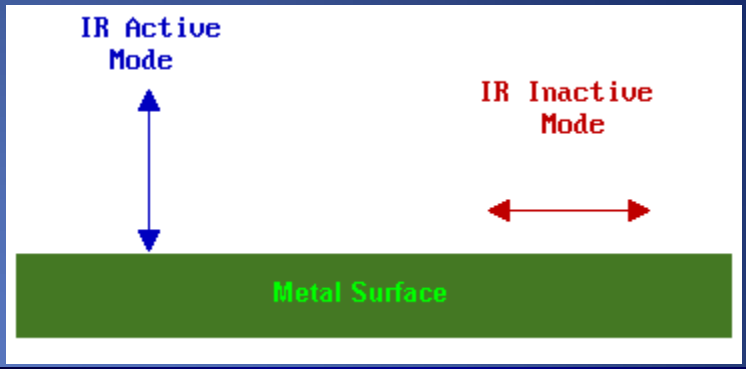
– reflected beam retains the polarization characteristics of the incidence beam

Thin layers: 0.5-20 mm $\Rightarrow$ angle ~20-60^{o} $\Rightarrow$ spectra similar to transmission ones

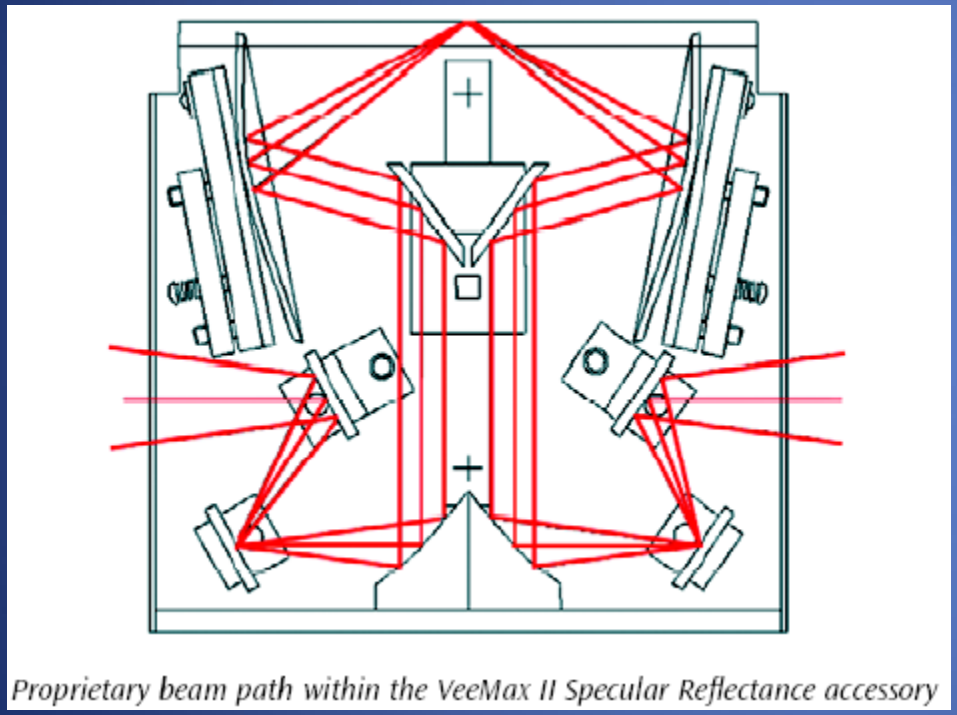
Monomolecular layers: angle ~60-85^{o} $\Rightarrow$ spectra predominatly a function of the **refractive index**

$\Rightarrow$ derivative shape of the bands arising from superposition of extinction coefficient and dispersion of refractive index

Specular Reflection



Experimental Setup



Proprietary beam path within the VeeMax II Specular Reflectance accessory

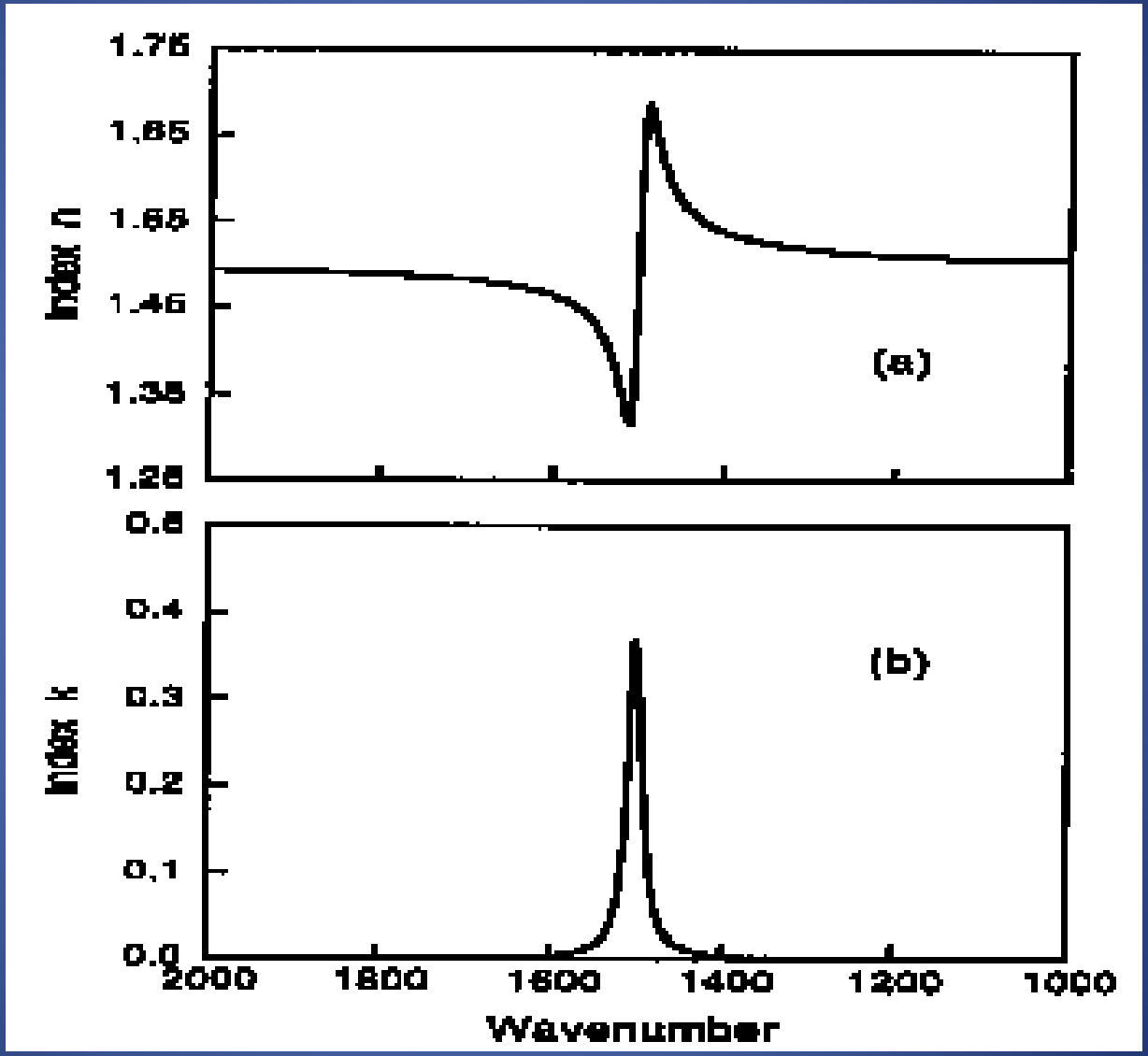
- **selection of incident angle**

Incident angle influences
- effective pathlength
- **polarized IR response**

Specular Reflection

Refractive index

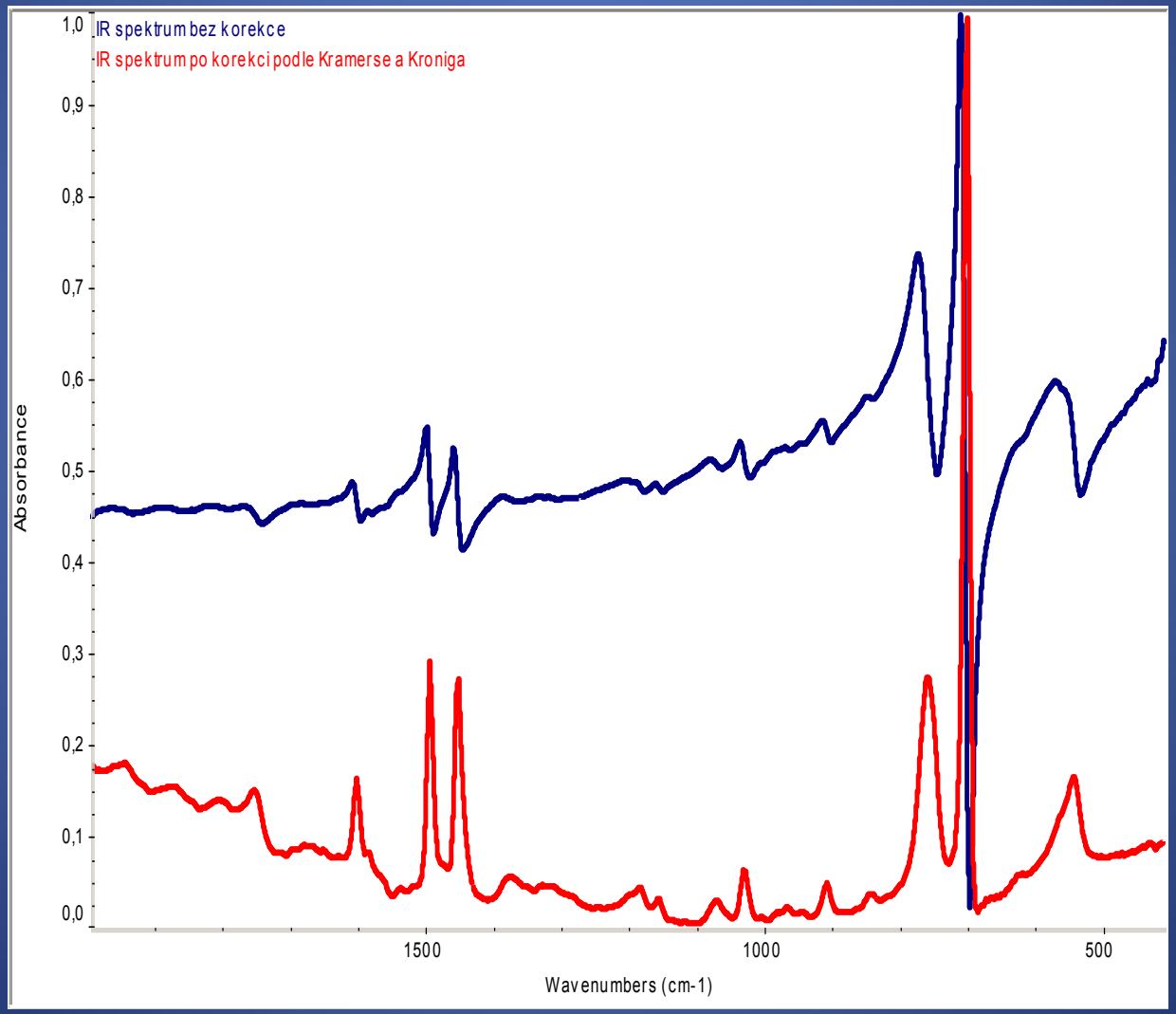
Absorbance index



K. Yamamoto and H. Ishida, Vibrational Spectrosc., 8, 1 (1994)

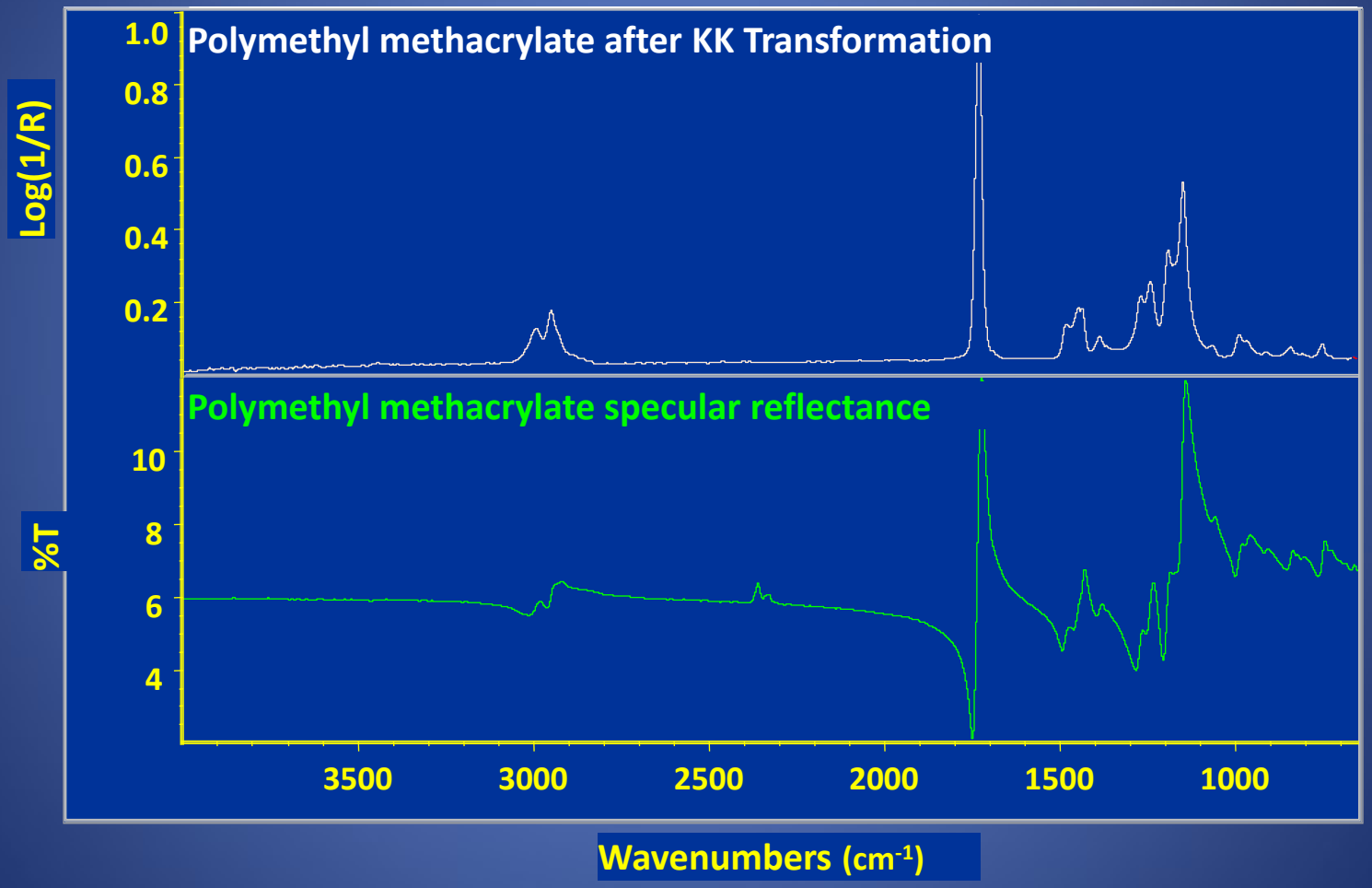
Specular Reflection

Correction of „Restrahlen „ bands



Specular Reflection

Correction Kramers-Kronig



Nanospectroscopy and microspectroscopy

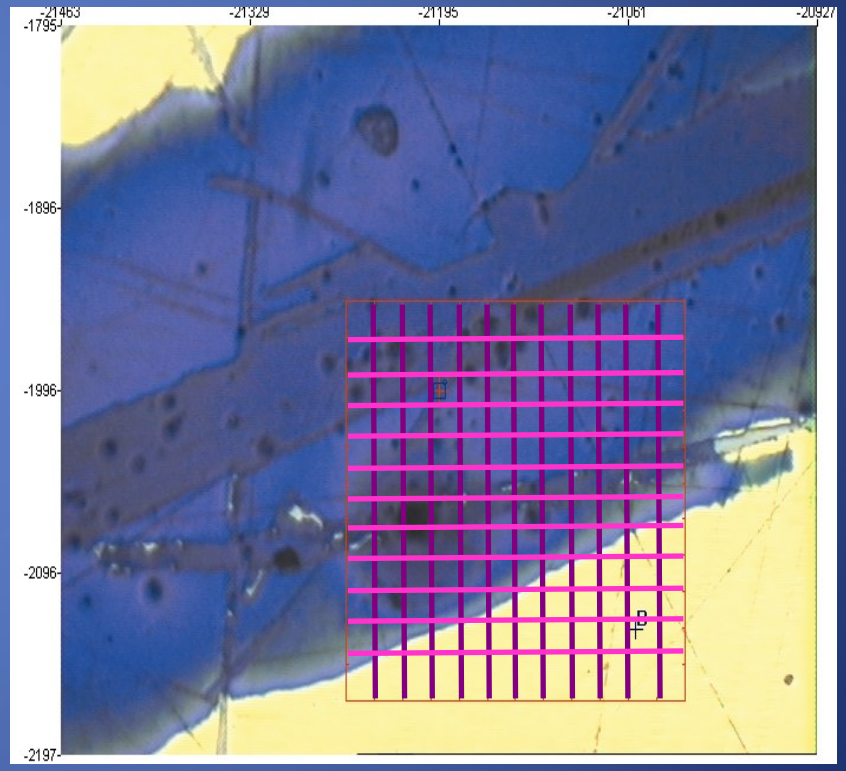
- **Chemical images of samples**
  - Generate from peak heights, areas, peak ratios, correlation, results of principal component analysis etc.
  - Useful for monitoring changes in chemical composition in a sample
    - inhomogeneities, defects, composite materials …

Nanospectroscopy and microspectroscopy

- **Chemical images of sample**
  - Group of points collected over the entire area of interest
  - Points can be collected in series (mapping – scanning the surface) or in parallel (imaging – multichannel detection)

Area Mapping and Imaging

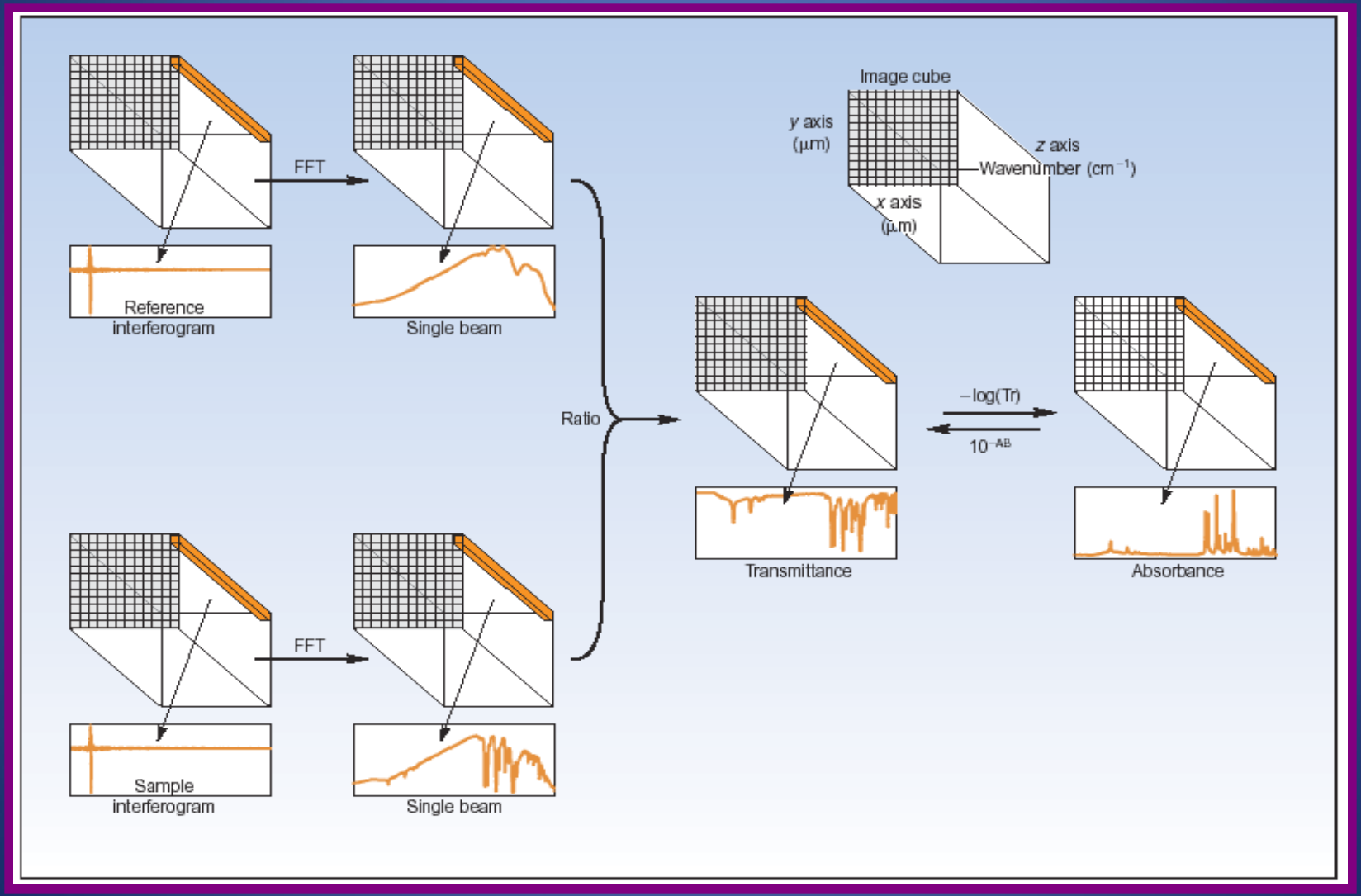
- Group of points collected over the entire area of interest
- Points can be collected in series (mapping) or in parallel (imaging)
- Ideal for analysis of highly heterogeneous samples



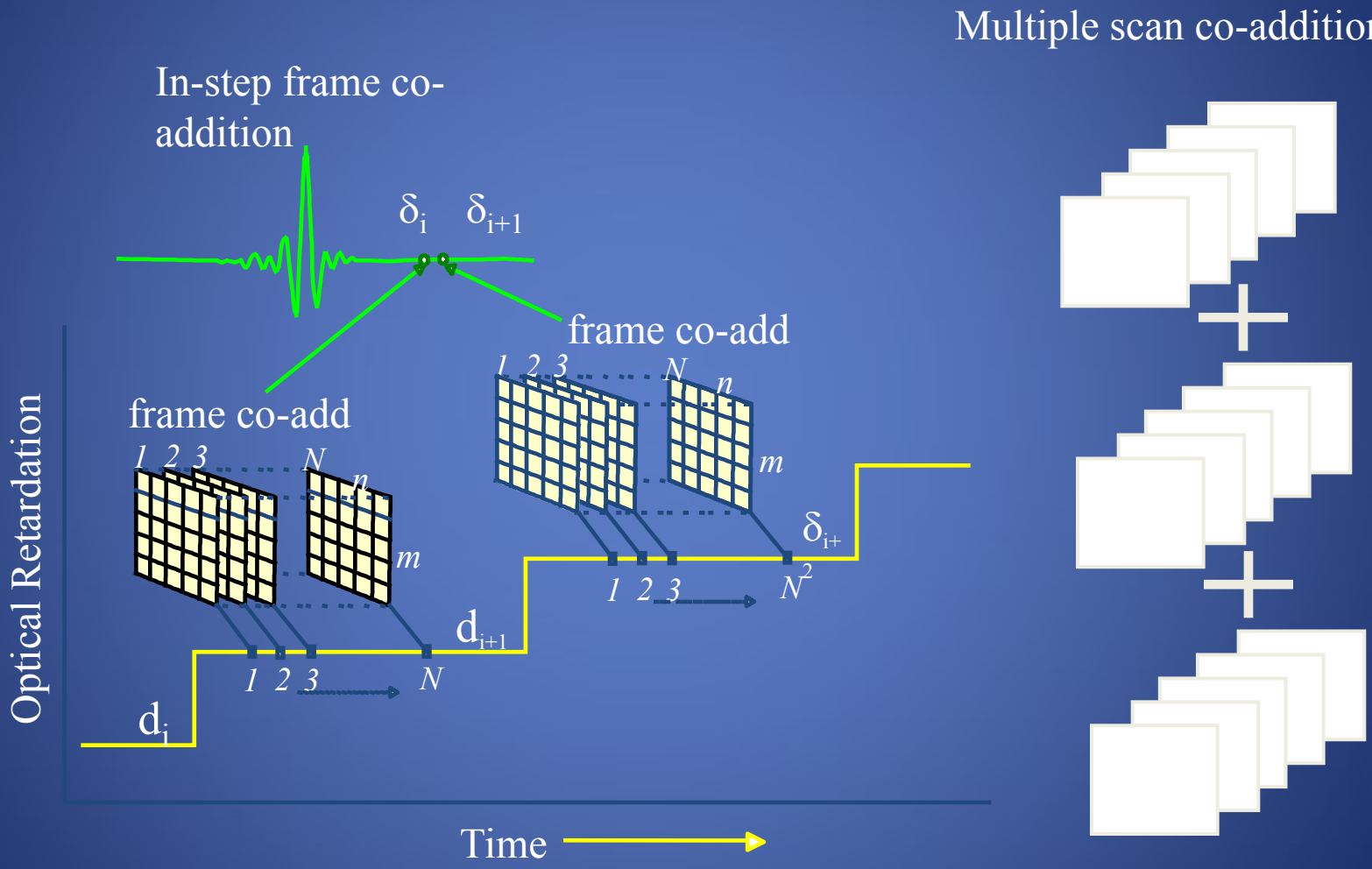
Infrared Microspectroscopy

- An exciting and versatile technology that combines light microscopy and FT-IR spectroscopy
- Visualization of the sample guarantees that you are analyzing the area of interest
- Understanding microscopy principals is the foundation of infrared microspectroscopy

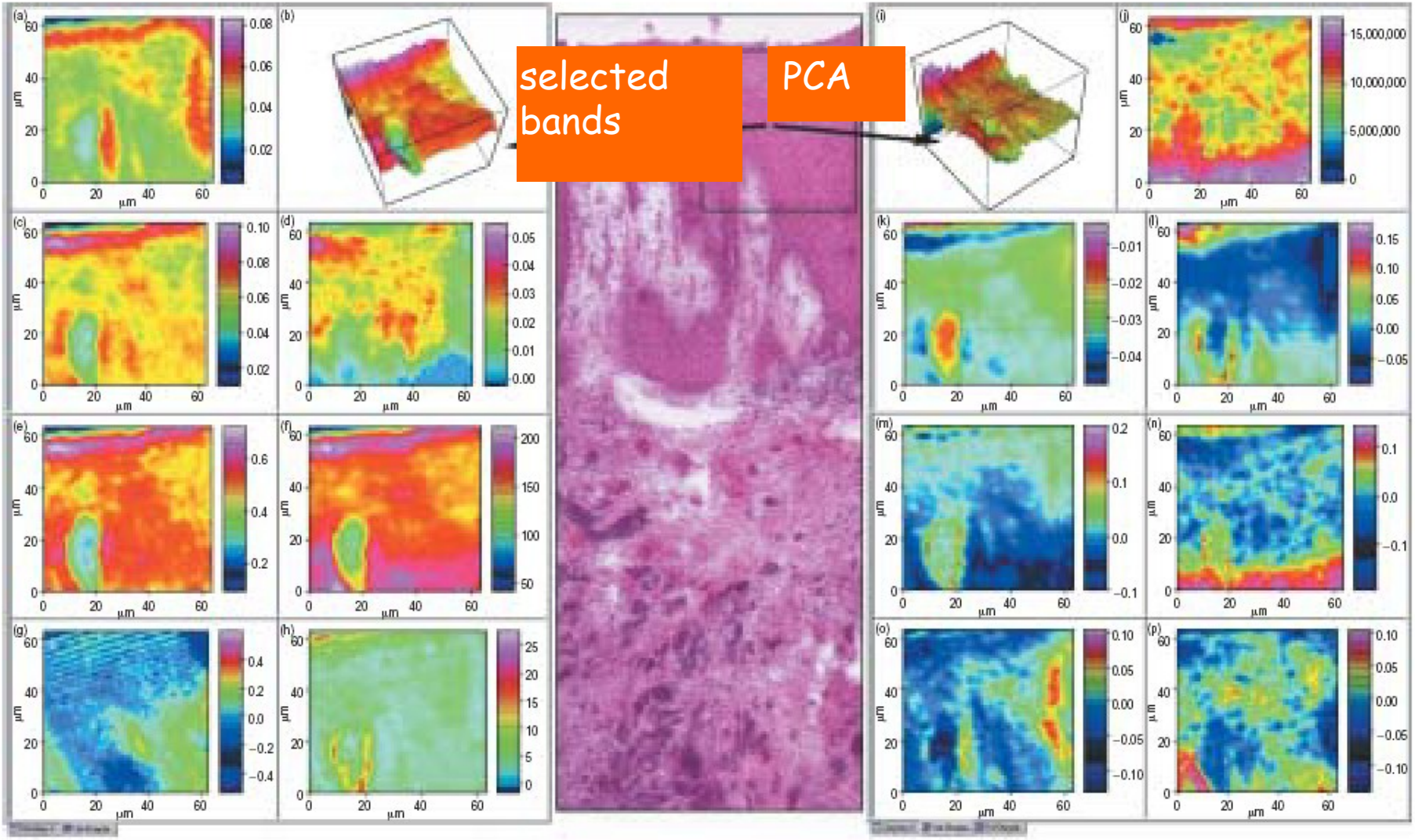
- **Chemical images of sample**
  - **IR imaging – multichannel detection**



Schematic Representation of Data Collection Protocol of FT-IR Spectral Imaging System

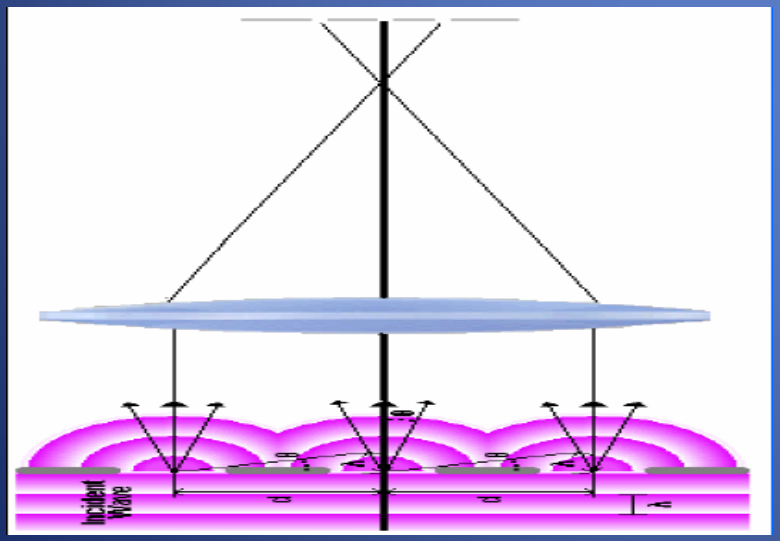


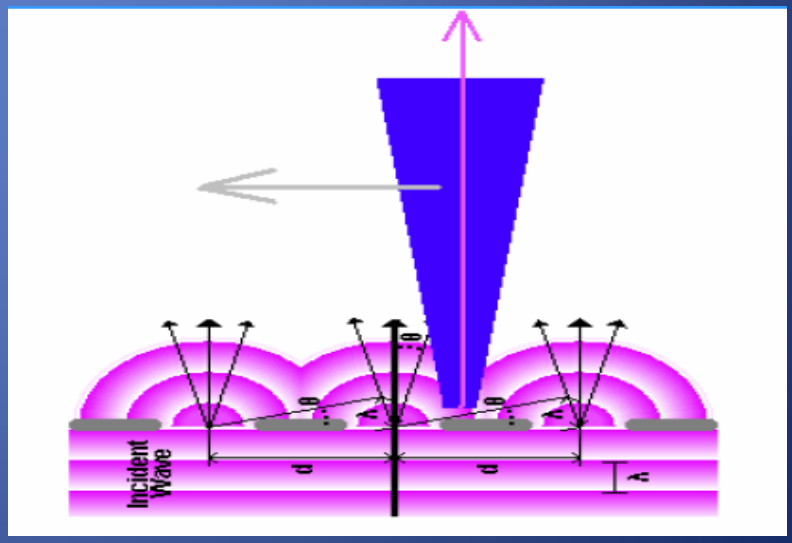
- Chemical images of sample
  - IR imaging – multichannel detection



Nanospectroscopy vs. microspectroscopy

- Microspectroscopy – techniques of far field
- Nanospectroscopy – techniques of near field
  - "coupling of a probe and surface"





Nanospectroscopy vs. microspectroscopy

- Microspectroscopy – techniques of far field
  - The maximum spatial resolution in a properly designed microscope is limited by the **diffraction** of light.
- Nanospectroscopy – techniques of near field
  - The maximum spatial resolution is under diffraction limit, it is limited mostly by **probe aperture** (probe diameter).

Microspectroscopy

Spatial Resolution

- the ability to view two closely spaced points as distinct **objects**

Diffraction

- the bending (or “scattering”) of light/energy by an opening of an optical element (lens, aperture)

Microspectroscopy

Diffraction

- the bending of light by an opening of an optical element
- occurs when the wavelength of the light approaches the size of the opening
- for infrared spectroscopy ~ 10 µm
  - (1000 cm^{-1} is 10 µm)
- for Raman spectroscopy better than 1 µm
  - excitation in visible range

Microspectroscopy

Diffraction

$$d = \frac{1.22\,\lambda}{\text{NA obj.} + \text{NA cond.}}$$

For 1469 cm^{-1},

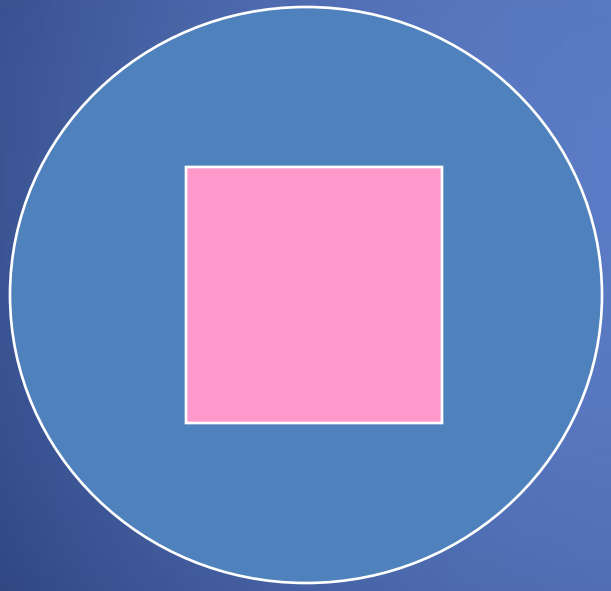
(1469 cm^{-1} = 6.8 μm)

$$d = \frac{1.22\ (6.8\ \mu m)}{0.58 + 0.71} = 6.4\ \mu m$$

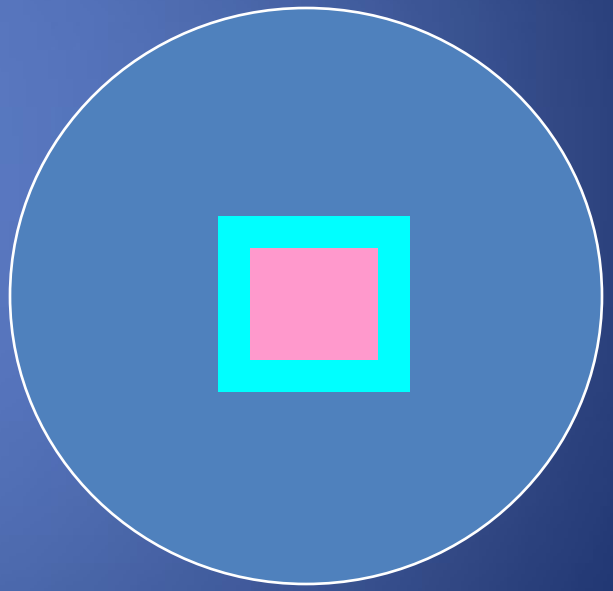
Microspectroscopy

Diffraction, resolution, sample size

Large sample (>100 μm)
No apparent diffraction



Small sample (<100 μm)
Diffraction Present



full field

sample area

Diffracted radiation

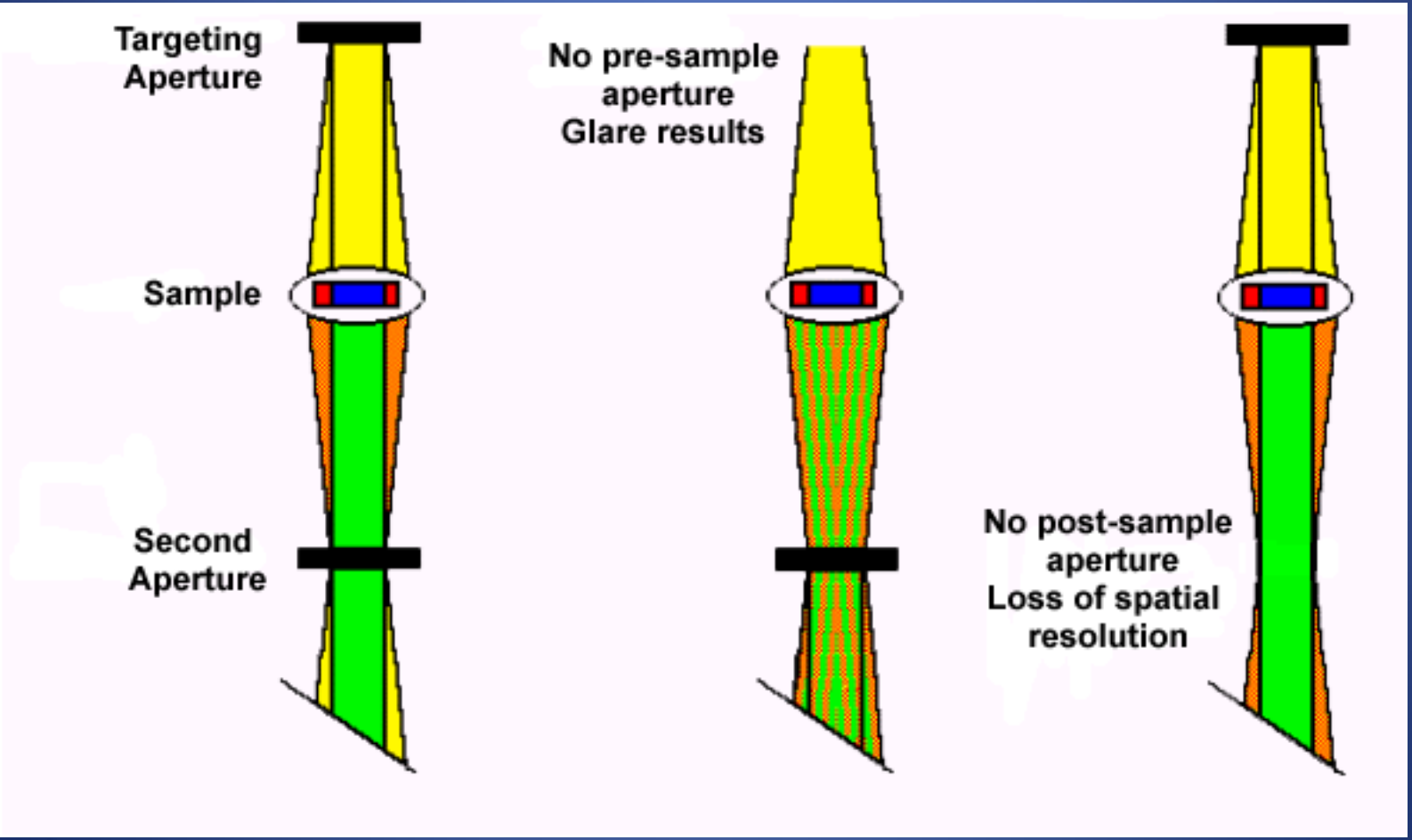
Microspectroscopy

Minimize Diffraction Effect - Dual Remote Aperture

- First aperture placed between infrared source and sample, limiting IR beam to desired sample area
- Second aperture placed between sample and detector to reduce amount of diffracted light detected

Microspectroscopy

Minimize Diffraction Effect - Dual Remote Aperture



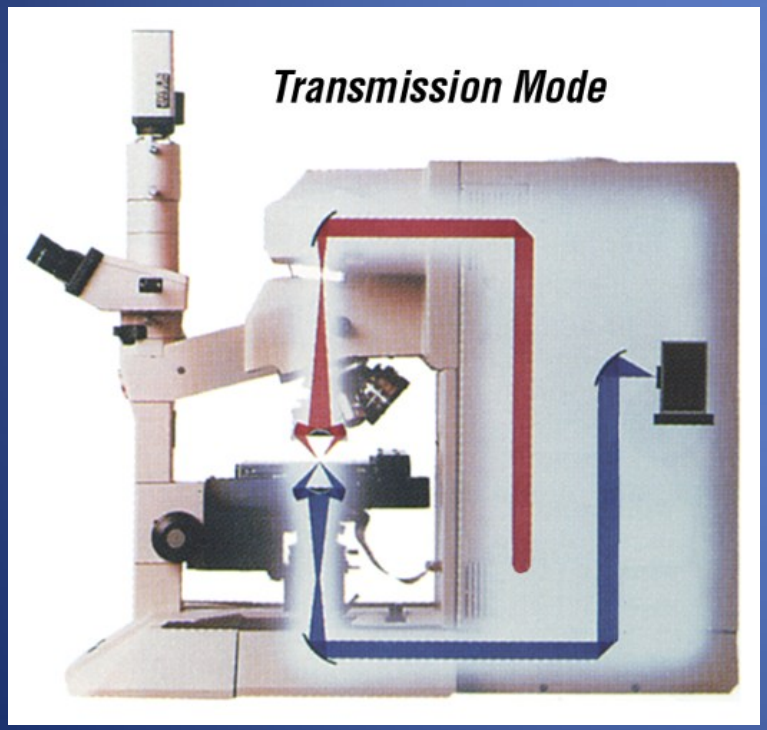
Design Considerations

- Contrast - without contrast, there is no resolution (illumination system)
- Aberrations - spherical or chromatic (minimized by proper design)
- Surface Defects - finish, shape and cleanliness (manufacturing)

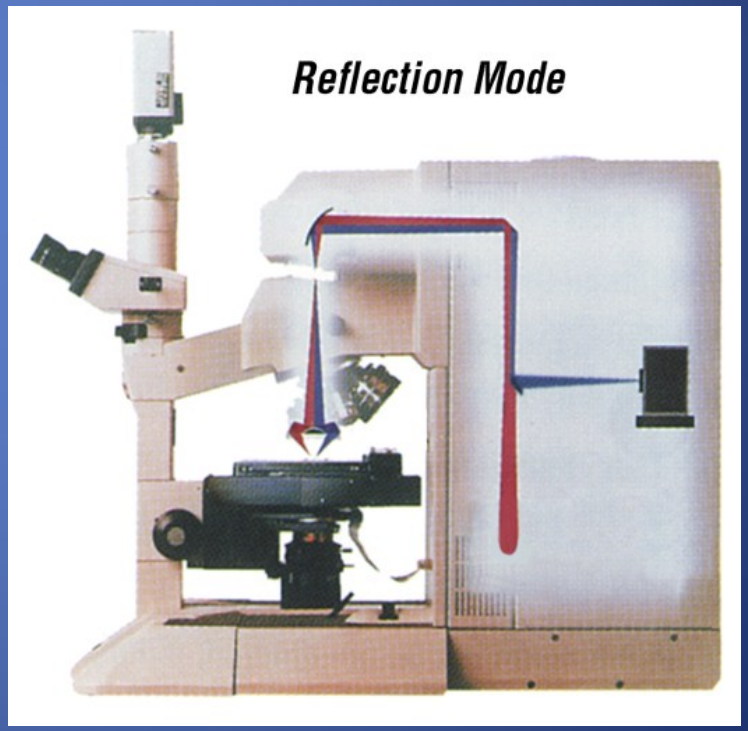
IR Microspectroscopy Sampling Modes

IR Microspectroscopy Sampling Modes

Transmission

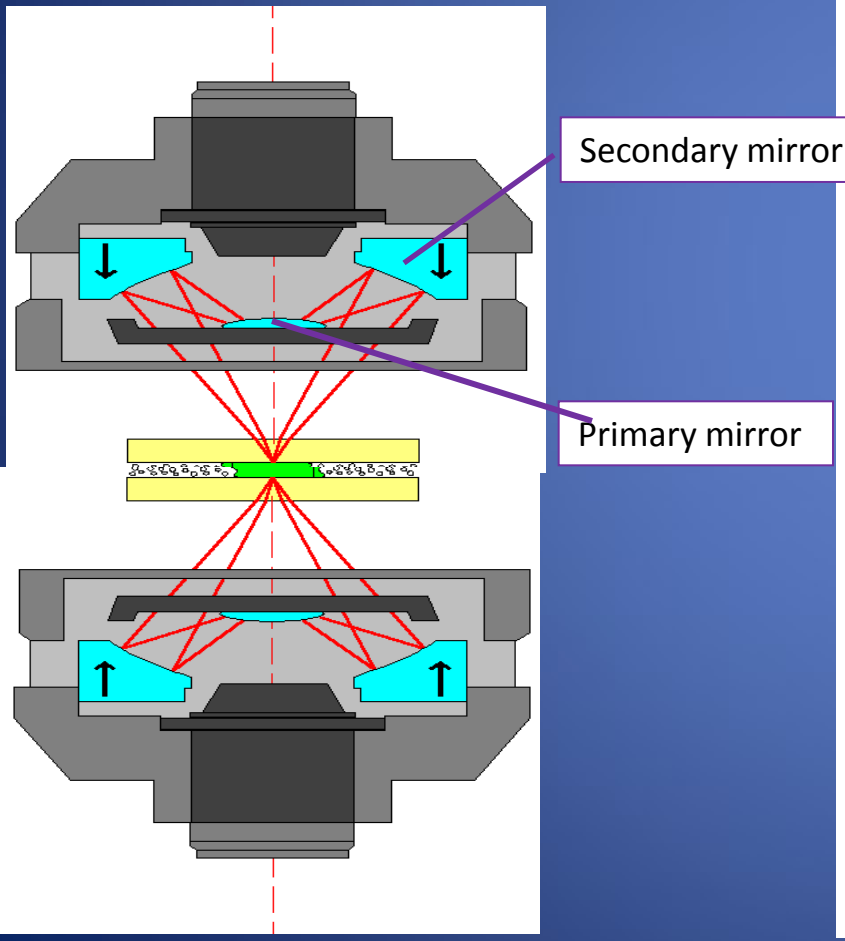


Reflection

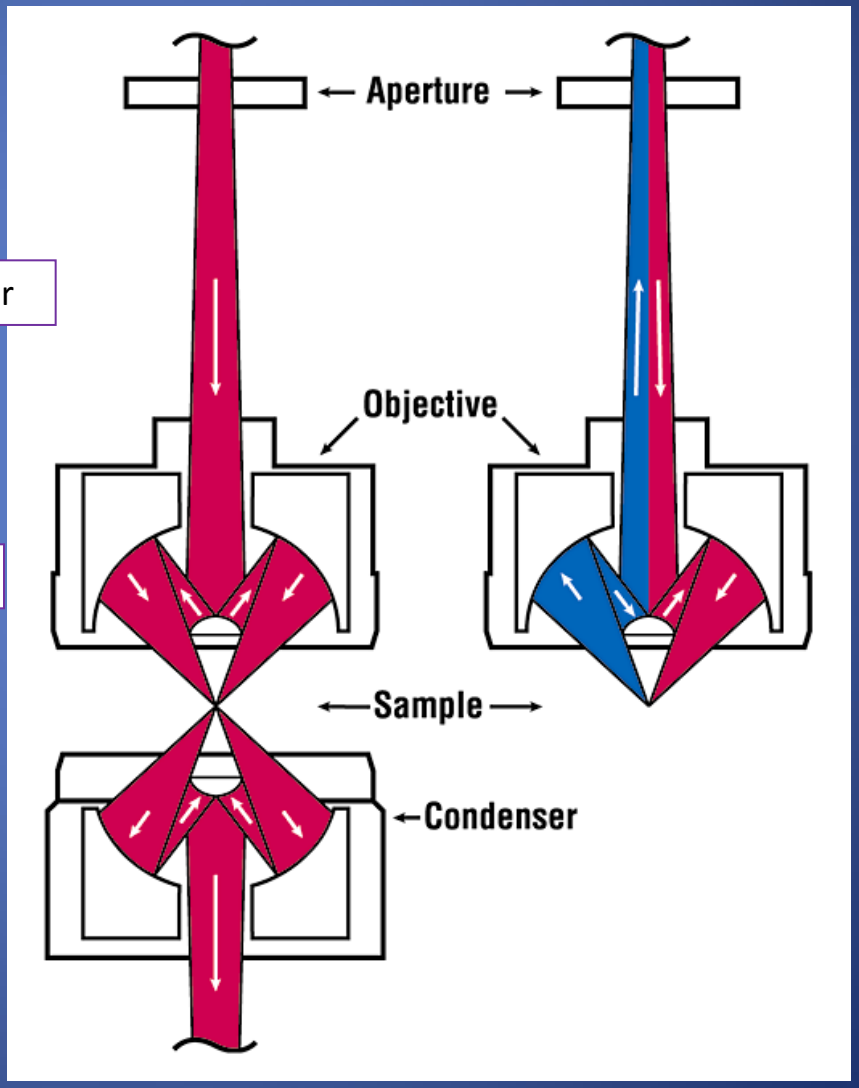


IR Microspectroscopy Sampling Modes

Transmission



Reflection



IR Microspectroscopy Sampling Modes

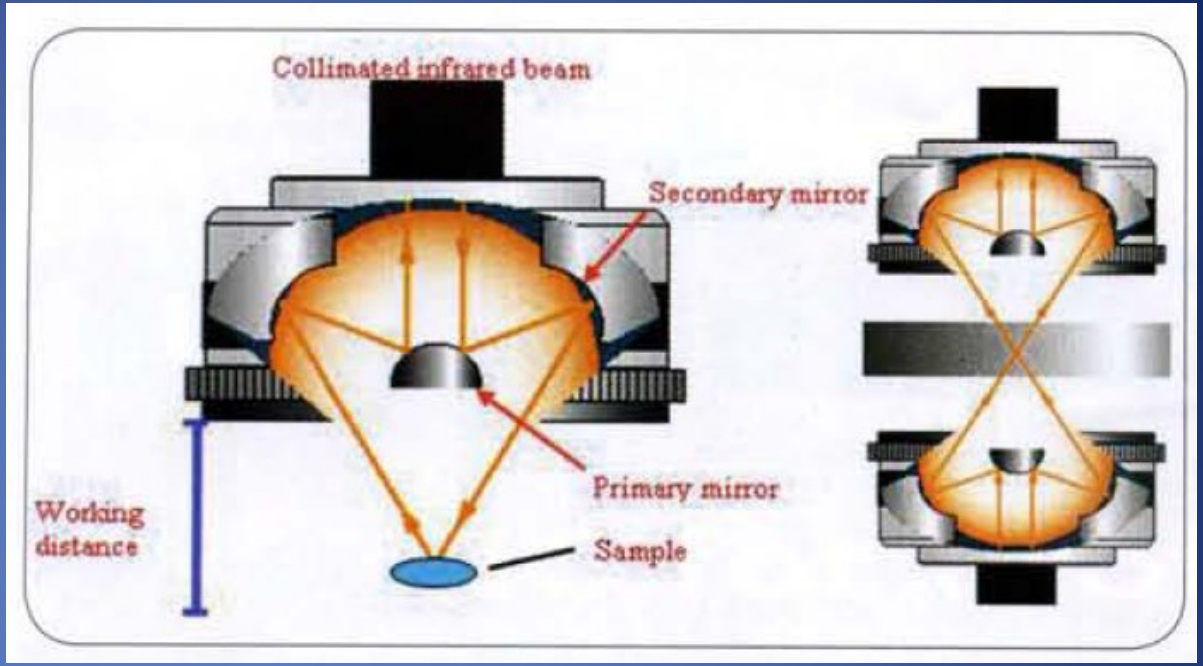
Transmission

- transparent samples, thin layers

Reflection

- ATR
- reflection - absorption,
  specular reflection,
  grazing angle

IR Microspectroscopy Sampling Modes



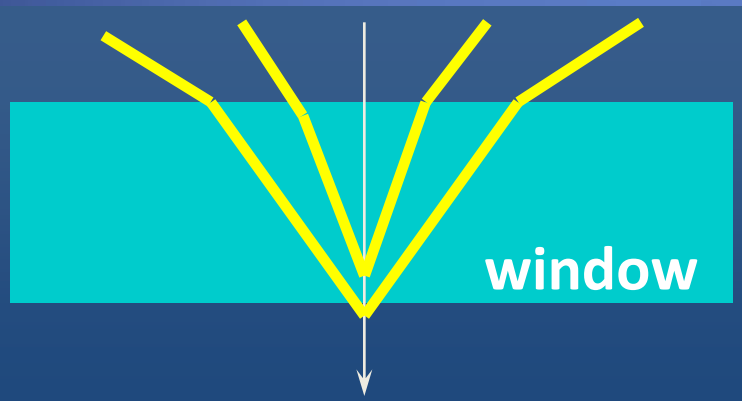
Transmission

- transparent samples, thin layers
  - 5 - 15 µm thickness
  - large and uniform surface

- mounting in compression cell between windows makes ideal transmission sample

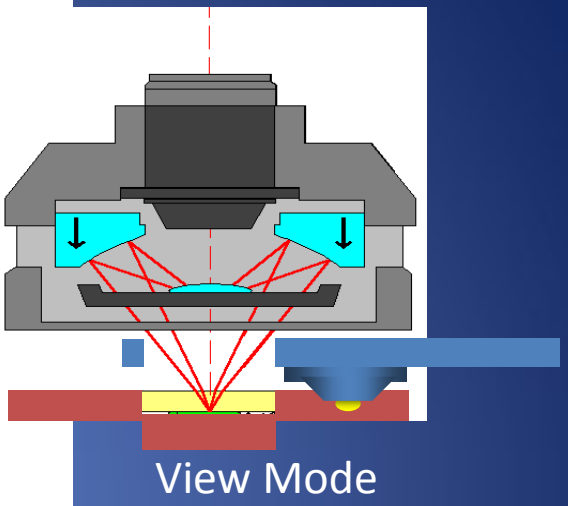
Window Sample Mounting

- Spherical aberrations are caused when the sample is placed on, or between, infrared transparent windows
- Spectra-Tech objectives and condensers correct these aberrations, resulting in improved resolution



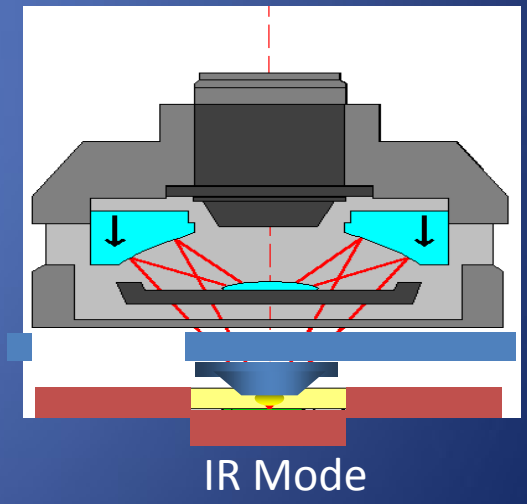
Spherical aberration caused by rays at high incident angles not coming to the same focal point as the low angle rays

IR Micr odes



Reflection - ATR

- simplifies sample preparation
- simplifies sample thickness problem
  (0.4 - 2.0 µm penetration depth)



- position sample on stage
- adjust for contact alert – contact sensor

IR Microspectroscopy Sampling Modes

Technical specifications

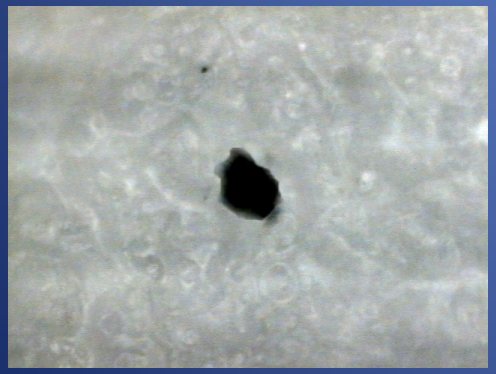
	Ge Hemispherical	Si Hemispherical	Ge Conical Tip
Material	Germanium	Silicon	Germanium
Refraction index	4	3.4	4
Depth of penetration @ 2000 cm^{-1}	0.4 micron	0.6 micron	0.8 micron
Medium angle of incidence	55 degrees	55 degrees	25 degrees
Contact area diameter	120 micron	120 micron	100 micron
Typical sample area	25 micron	25 micron	25 micron
Minimum sample area*	12 micron	12 micron	< 10 micron
Crystal probe length	2.5 mm	2.5 mm	6 mm
Survey ATR	Yes	Yes	Yes
Spectral range cut-off	650 cm^{-1}	650 cm^{-1}	650 cm^{-1}

** Note: Minimum sample area is typically achieved with a Continuum aperture size of 30 x 30 micron divided by the crystal refractive index.*

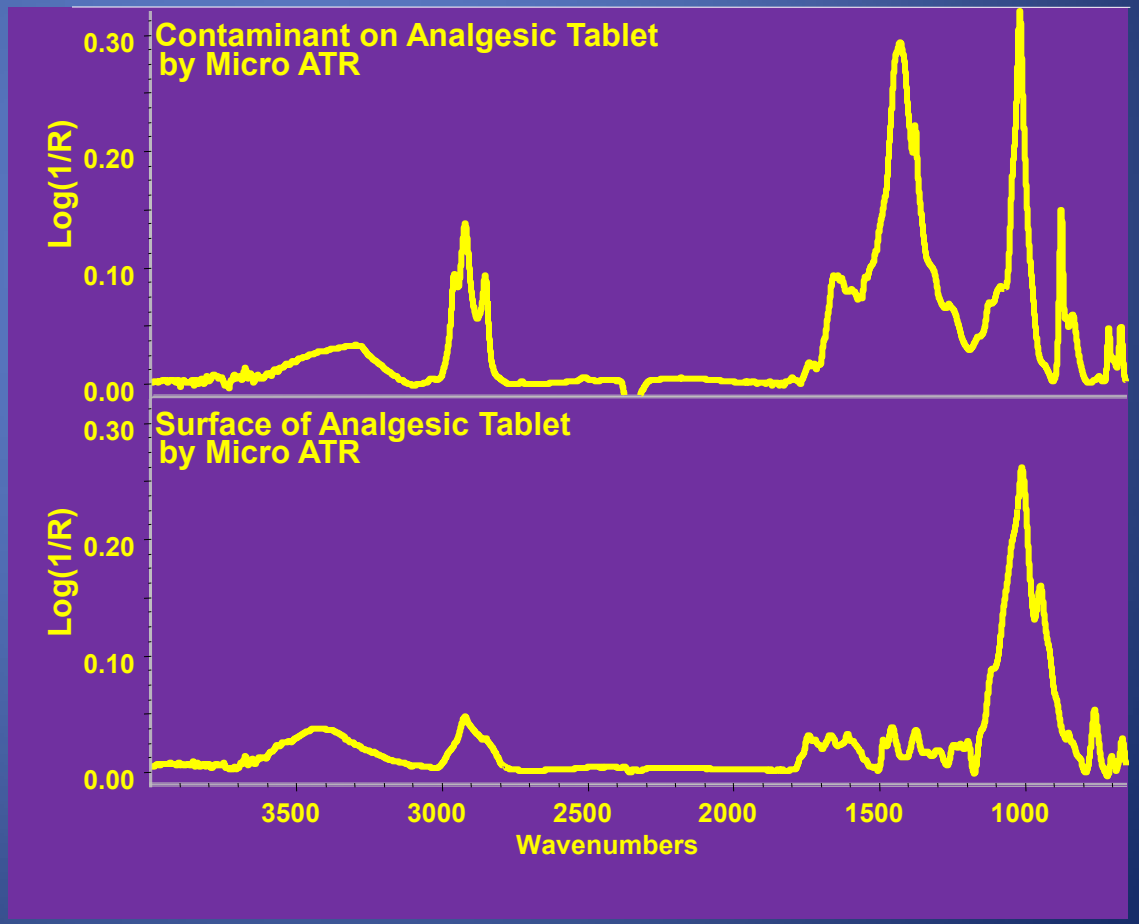
IR Microspectroscopy Sampling Modes

Reflection - ATR

- objective crystals - ZnSe, Ge, Si, Diamond



40 µm defect

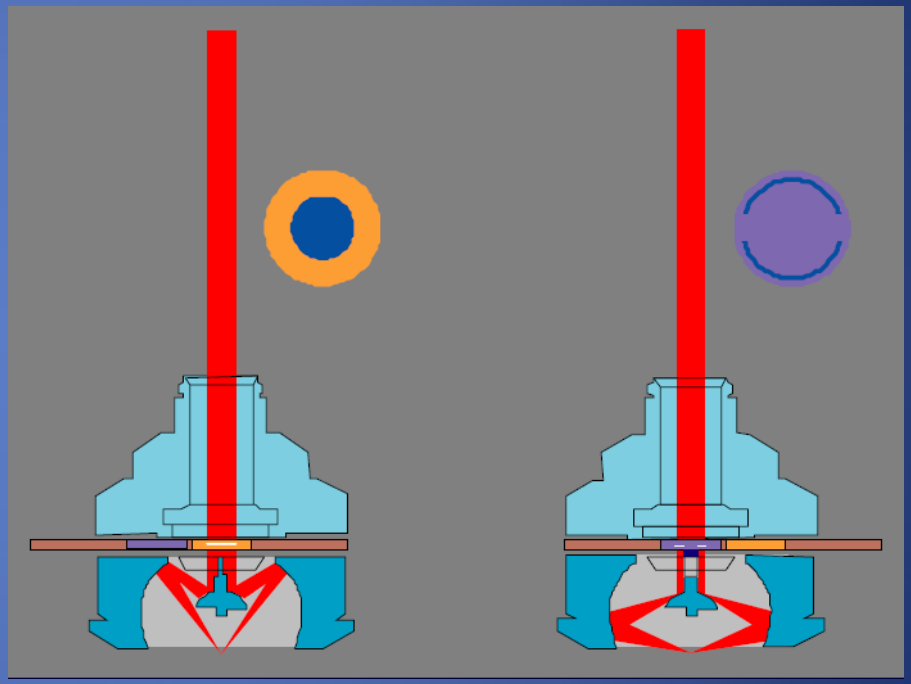


Grazing Angle Microsampling

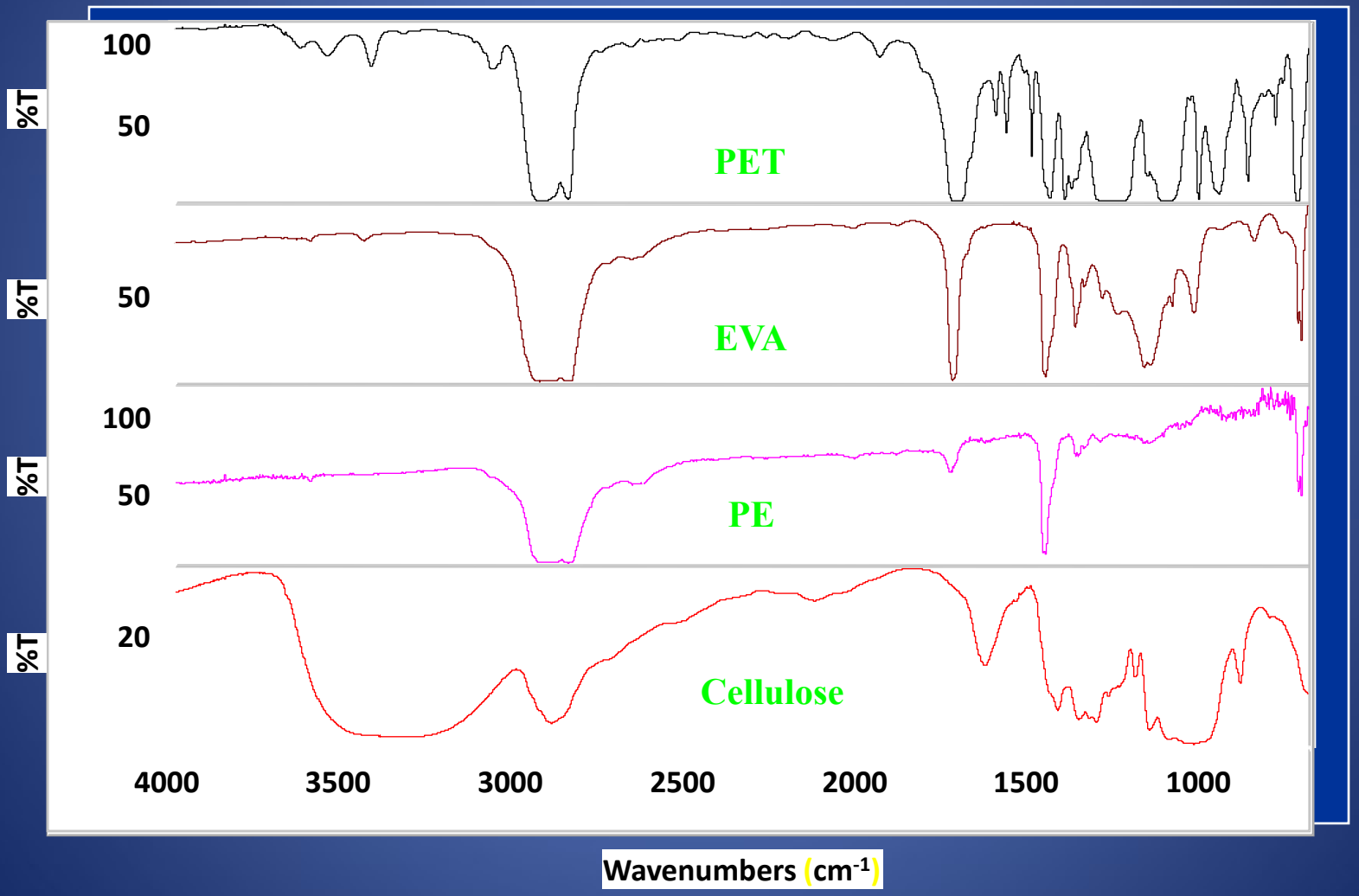
- Microscopic Analysis of Ultra-Thin (nanometer) Sample Deposits
- Operates in viewing and grazing mode

IR Microspectroscopy Sampling Modes

Reflection – grazing angle

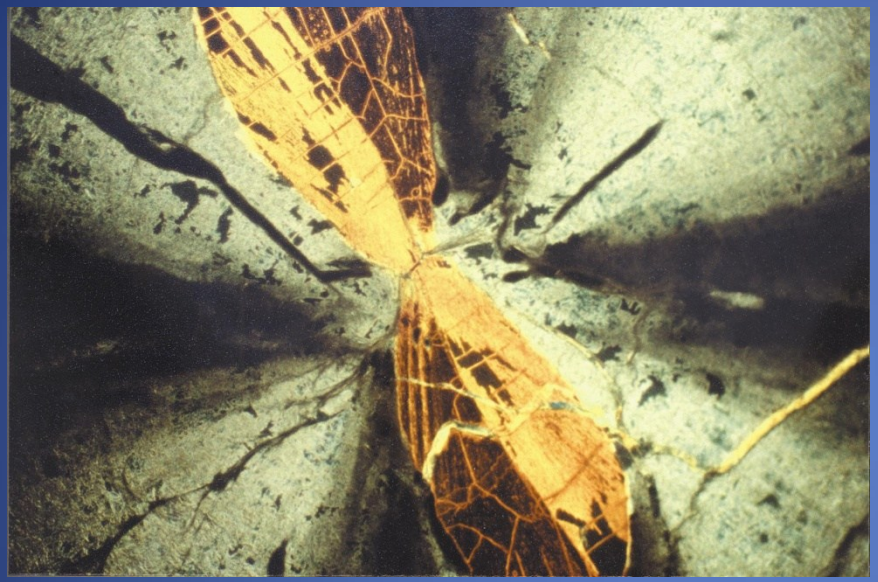


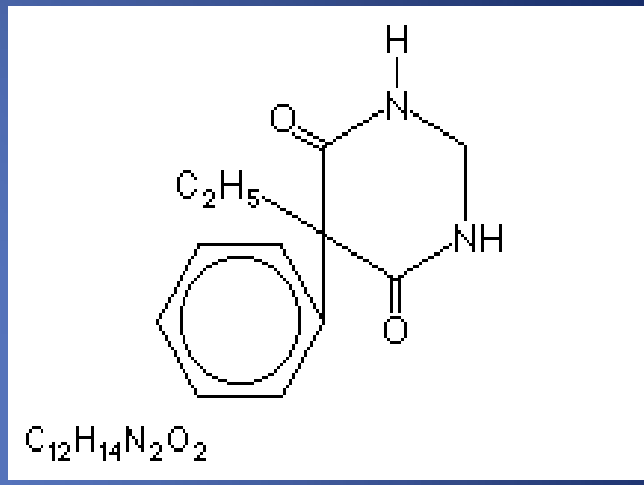
Polymer Laminate Spectra



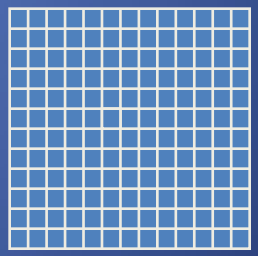
Primidone

5-ethyl-5-phenyl-hexahydropyrimidine-4,6-dione

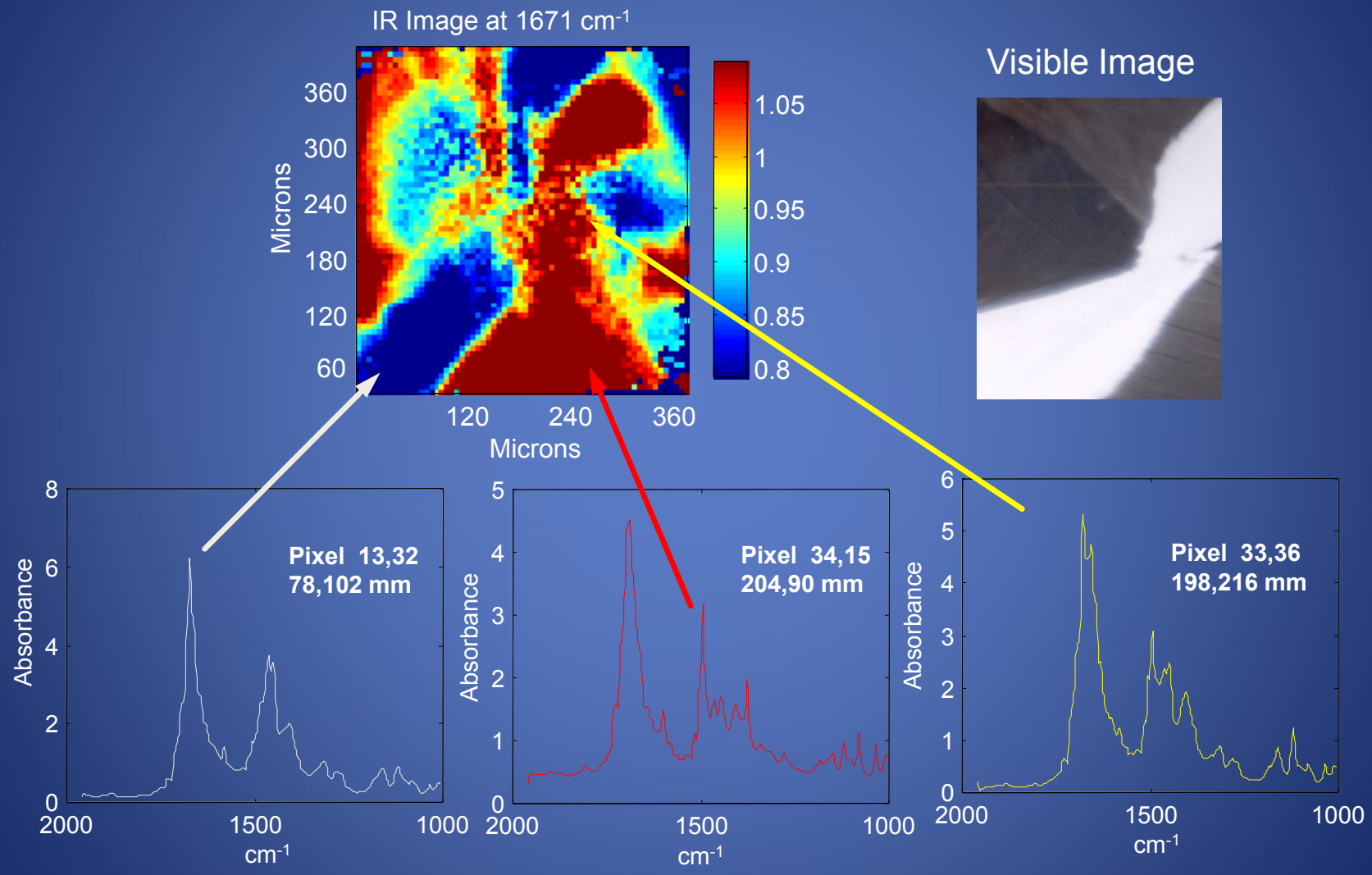




- Frequently used anti-convulsant
- Effective in the treatment of grand mal psychomotor epilepsy and Jacksonian seizures

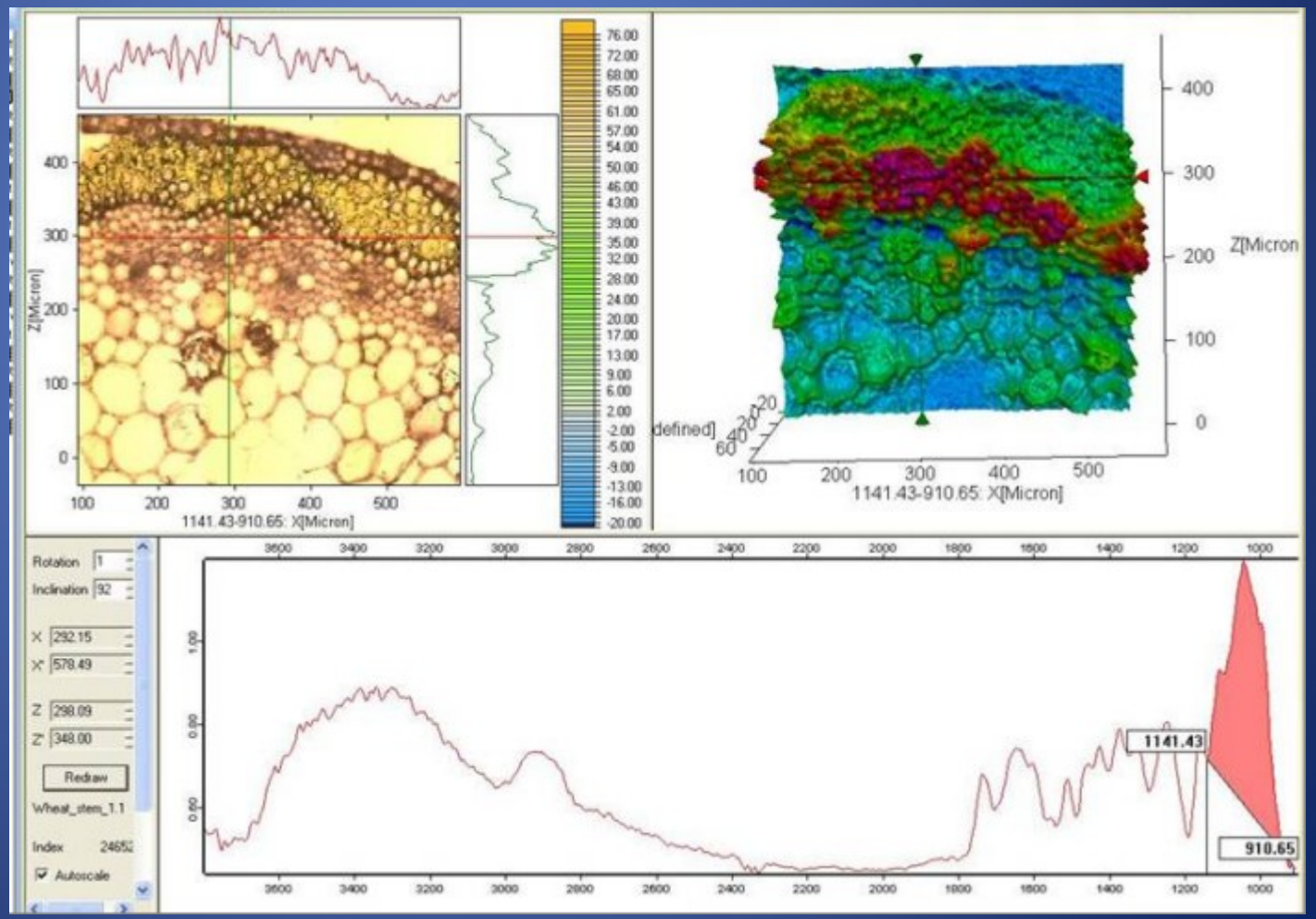


Primidone Polycrystalline Film



FTIR mikrospektroskopie – příklad

FTIR imaging microscope for chemical composition and spatial info at the cellular scale. Gives info like **lignin**, **starch**, **protein** and **oil** distribution.



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Research Article

The Use of FTIR and Micro-FTIR Spectroscopy: An Example of Application to Cultural Heritage

Mauro Francesco La Russa,[1] Silvestro Antonio Ruffolo,[1] Germana Barone,[2] Gino Mirocle Crisci,[1] Paolo Mazzoleni,[2] and Antonino Pezzino[2]

Samples	Sampling points and typology of degradation products
Sar1	Central *clipeo*, right side, black crust
Sar 2	Sixth *strigile*, at right-high side of *clipeo* black crust
Sar 4	Higher left side, orange patinas
Sar 5a	Inside left area, black crust
Sar 5b	Inside left area, orange patinas

(a) (b)

FIGURE 1: (a) The Roman sarcophagus; (b) particular of the facade of St. Giuseppe Church.

;radation

products

SG1	Calcarenite with black crust localized on the base of column right
SG2	Calcarenite with black crust localized on the base of column left
SG3	Calcarenite with black crust localized on the portal
SG4	Brown-orange patinas present on the facade
SG5	Brown-orange patinas present on the facade
SG6	Brown-orange patinas present on the facade

Fourier transform-infrared spectroscopy (FTIR) was performed for a mineralogical characterization of the powdered samples by means of comparison to a data base [10]. The equipment used was a Nicolet 380 with a Smart Orbit accessory used in the following arrangement: a K-Br beam-splitter, an HP-DTGS-KBr detector, and an Ever-Glow lamp used as source. In this configuration, the resolution was 4 cm^{-1}. The great advantage of this spectroscopic technique is the high sensibility which allows the detection of many components, even at very low amounts.

The qualitative distribution maps of mineralogical phases, performed on thin sections, have been obtained by using a micro-Fourier transform-infrared spectrometer (μ-FTIR) Spotlight 200 (Perkin Elmer) microscope, equipped with an MCT detector cooled by liquid nitrogen, a germanium μATR crystal, and a computer-controlled mapping stage programmable in the x and y directions. The spectra were recorded at 4 cm^{-1} resolution, mode with a spot of $100 \times 100\,\mu m^2$.

Point-by-point spectral mapping of the thin section was carried out in a grid pattern with the computer-controlled microscope stage with a spot of $100\,\mu m \times 100\,\mu m$. Each spectrum was collected between 4000 and 700 cm^{-1} at a spectral resolution of 4 cm^{-1}. The map spectra were collected with 4 scans for each spectrum. Maps have a resolution of 625 dots and a spatial resolution of 100 μm. They are based on the compare correlation value (calculated by Spotlight 200 software) of the recorded spectra with references ones. A compare correlation map indicates the areas of a map, where the spectra are most similar to a reference spectrum.

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FIGURE 1: (a) The Roman sarcophagus; (b) particular of the facade of St. Giuseppe Church.

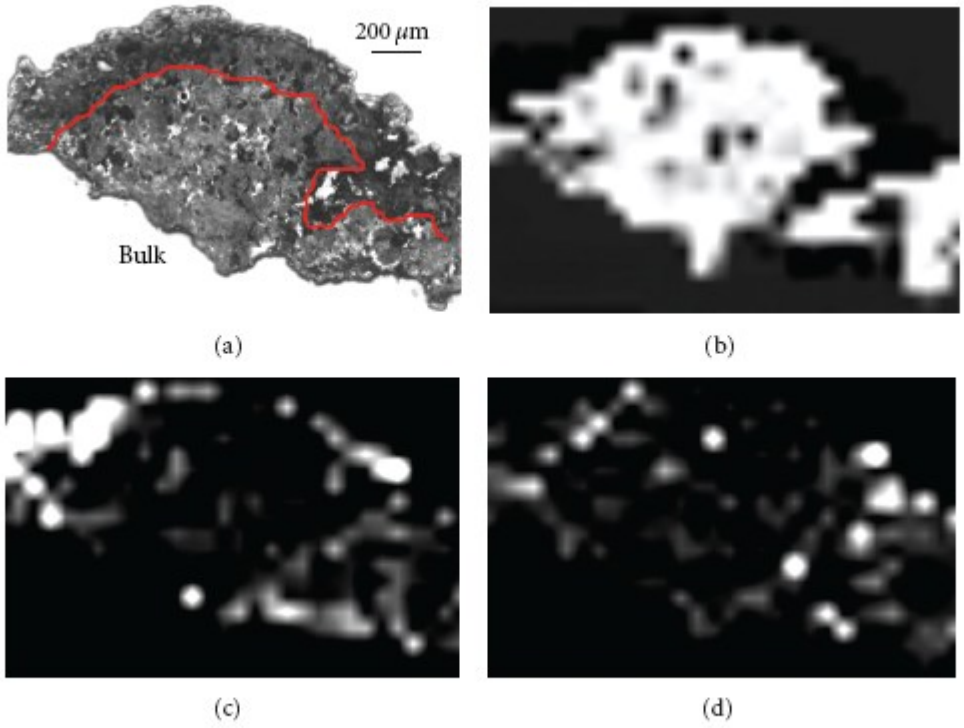

FIGURE 3: (a) Ultrathin section analyzed, (b) distribution maps of calcite, (c) gypsum, and (d) whewellite obtained by μ-FTIR-ATR spectroscopy. The bright areas indicate regions of high concentration, while dark areas indicate a low concentration.

FTIR mikrospektroskopie – synchrotronové záření

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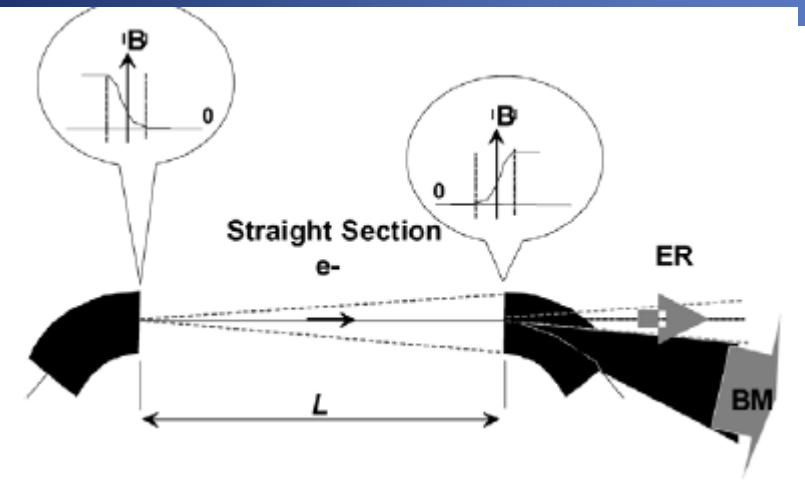
Physics and heritage / Physique et patrimoine

Recent applications and current trends in Cultural Heritage Science using synchrotron-based Fourier transform infrared micro-spectroscopy

Marine Cotte [a,b,*], Paul Dumas [c], Yoko Taniguchi [d], Emilie Checroun [e], Philippe Walter [a], Jean Susini [b]



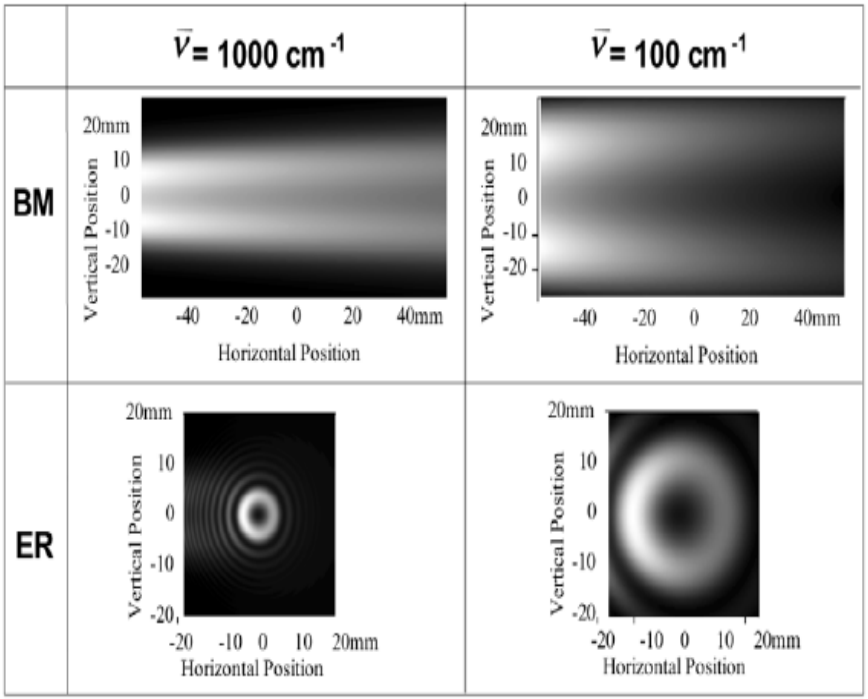


Fig. 2. Intensity distribution of edge radiation, for two wavelengths: λ = 10 μm (left column) and λ = 100 μm (right column). The distribution has been calculated for a bending magnet (BM) emission, and an edge radiation (ER) emission. This calculation has been carried out for the case of a storage ring of 3 GeV, and a vertical aperture of 20 mrad.

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FTIR mikrospektroskopie – synchrotronové záření

Flux and brightness for the two types of IR emission are almost equivalent, but the opening angle of the ER is narrower than that of the SR from constant field of a BM. The intensity profiles of the two types of emission are also different, and depend upon wavelength. In Fig. 2, the distribution profiles at two wavelengths (10 μm and 100 μm) have been calculated for a medium energy storage ring (3.0 GeV) for a prototypical opening angle of the dipole chamber of 20 × 40 mrad (vertical by horizontal) for the collection of BM infrared emission, and 20 × 20 mrad for the collection of the ER infrared emission. These profiles illustrate that, for BM emission, the angle of emission is larger than that of the ER. However, flux and brightness are equivalent for the two types of emission sources, and either one can be used for synchrotron infrared experiments.

This has some advantages for engineering purposes, since the dipole chamber exit port in a synchrotron storage ring has to be enlarged for BM radiation. Thus today, both sources are used for extraction, depending upon availability of a straight section and/or BM. The most recent synchrotron facilities are collecting both sources, and split them after the propagation to the instrument in order to have two spectroscopic branches with the same extraction port (Australian Synchrotron, SOLEIL, Diamond).

A marked difference between the two sources is their polarization properties. BM emission is strictly horizontally polarized in the plane of the electron trajectory, while ER has a radial polarization.

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Marine Cotte [a,b,*], Paul Dumas [c], Yoko Taniguchi [d], Emilie Checroun [e], Philippe Walter [a], Jean Susini [b]

FTIR mikrospektroskopie – synchrotronové záření

The IR light is then focused through an IR-transparent window (usually diamond, but in a few cases IR-transparent windows such as KBr, ZnSe and KRS5 have been used), which separates the ultra-high vacuum (UHV) conditions of the storage ring (10^{-9}–10^{-10} mbar) and the low vacuum of the SR-FTIR beamline (10^{-3}–10^{-4} mbar). This diamond window is usually a slight wedge shape to avoid interferences, and relatively small in diameter (10 to 40 mm clear aperture). Some sophisticated wheels containing windows of several materials (e.g. calcium fluoride, diamond, cesium iodide) have been recently implemented at the Swiss Light Source for the same purpose.

The low vacuum section of a SR-FTIR beamline is generally terminated with an IR-transparent window (KBr, CsI, polyethylene) either after a vacuum-based FTIR spectrometer or prior to a purged instrument. This window isolates the beamline vacuum from the ambient pressure of the spectrometer and/or microscope. To date, all commercial FTIR microscopes operate at ambient pressure and are typically purged with dry N_2 or dry air to remove any water vapor and carbon dioxide in the FTIR spectrum.

FTIR mikrospektroskopie – synchrotronové záření

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COMPTES RENDUS PHYSIQUE

Physics and heritage / Physique et patrimoine

Recent applications and current trends in Cultural Heritage Science using synchrotron-based Fourier transform infrared micro-spectroscopy

Marine

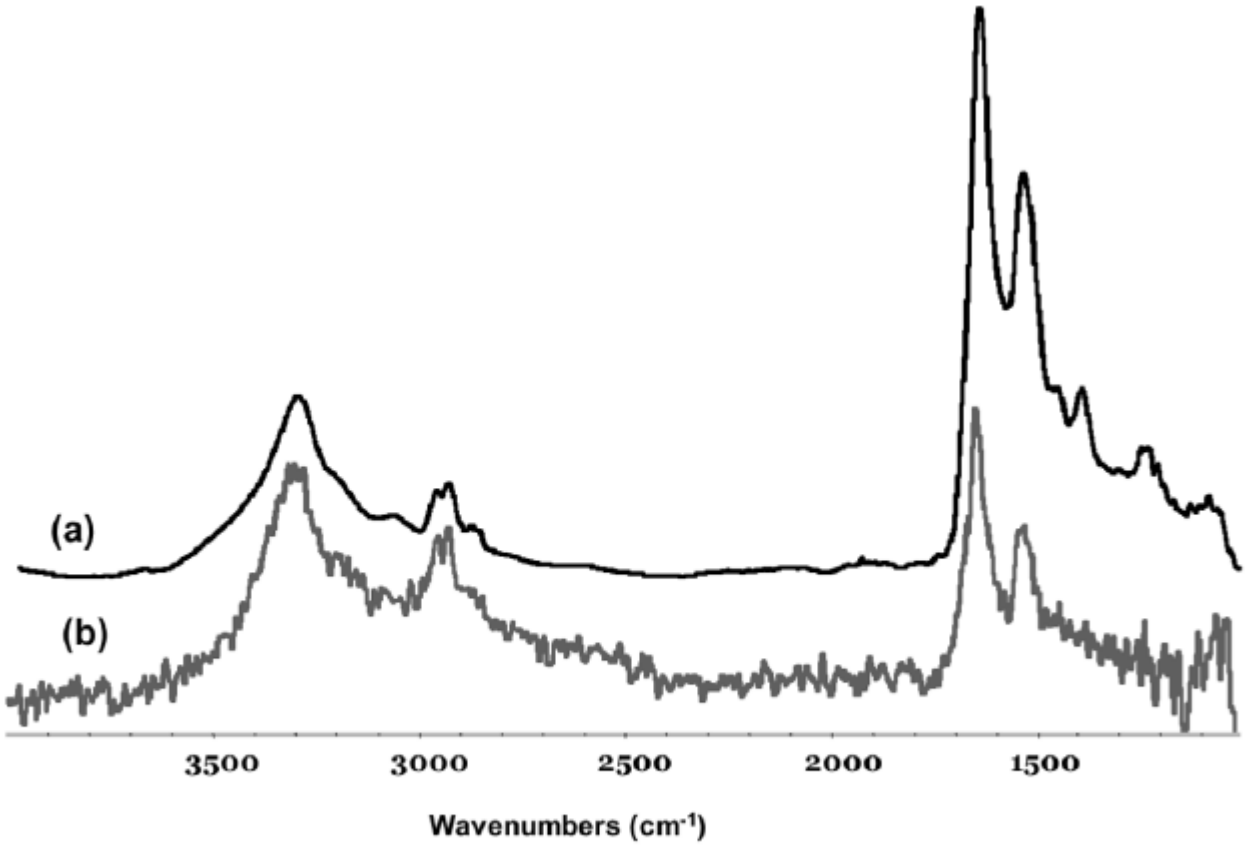

Fig. 4. FTIR spectra recorded with a dual aperture of $6 \times 6\ \mu m^2$, at $4\ cm^{-1}$ resolution, derived from a sum of 128 scans with (a) the synchrotron source and (b) with the internal (globar) source.

FTIR mikrospektroskopie – synchrotronové záření

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Recent applications and current trends in Cultural Heritage Science using synchrotron-based Fourier transform infrared micro-spectroscopy

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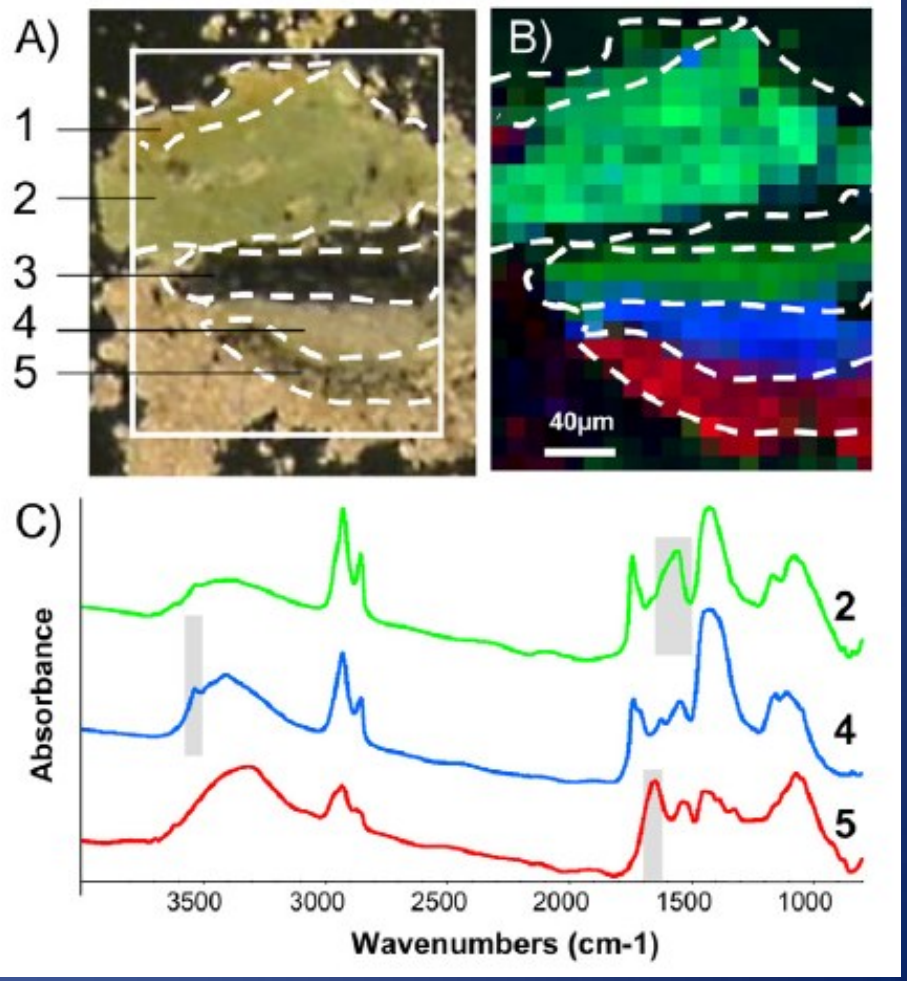

Fig. 5. A) Photomicrograph of pressed fragment of sample BMM035 from Cave N(a), Bamiyan, showing a multi-layered structure: 1 = yellowish transparent layer, 2 = green layer, 3 = black layer, 4 = white ground, 5 = transparent brownish layer, and earthen rendering. B) Chemical mappings obtained by SR-µFTIR, showing the distribution of three particular ingredients: in red, proteins; in green, carboxylates; in blue, hydrocerussite. Map size: 190 × 170 µm^2; step size: 10 × 10 µm^2. C) Average FTIR spectra obtained in the green layer (#2), the white ground layer (#4) and the transparent brownish layer (#5). The gray rectangles highlight the vibrational bands used to generate chemical mappings displayed in B).

FTIR mikrospektroskopie – srovnání s dalšími metodami

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Recent applications and current trends in Cultural Heritage Science using synchrotron-based Fourier transform infrared micro-spectroscopy

Marine Cotte [a,b,*], Paul Dumas [c], Yoko Taniguchi [d], Emilie Checroun [e], Philippe Walter [a], Jean Susini [b]

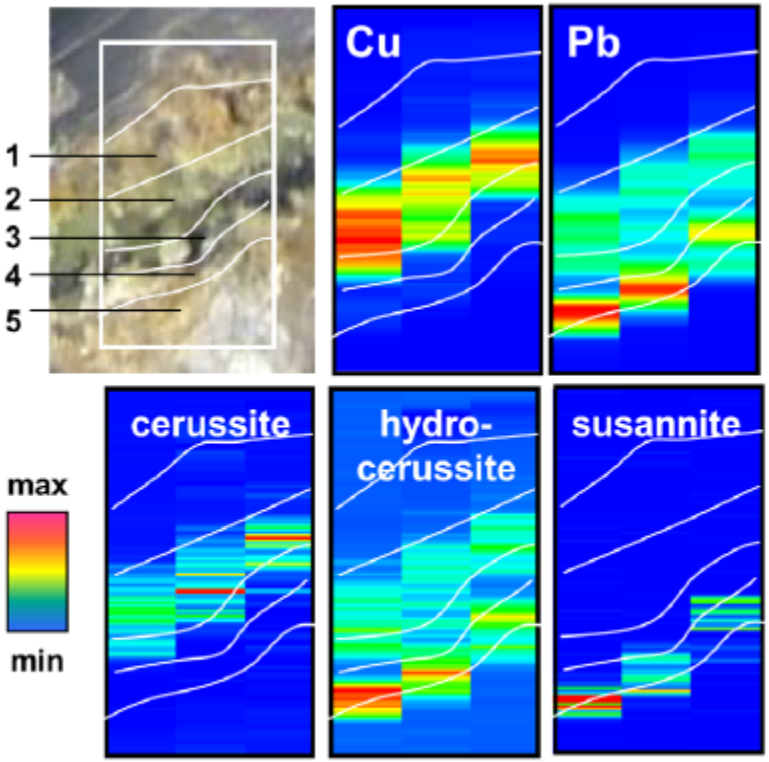


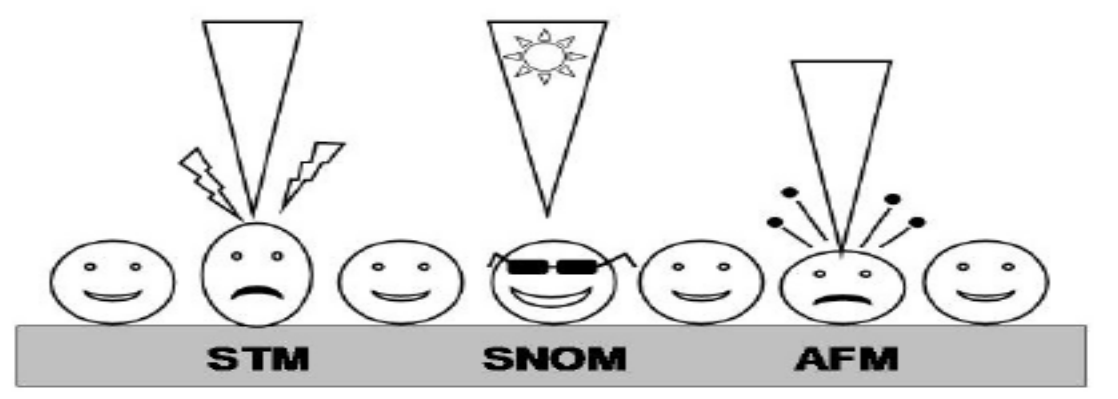
Fig. 6. Photomicrograph of sliced cross-section of sample BMM035 from Cave N(a), Bamiyan, showing a multi-layered structure: 1 = yellowish transparent layer, 2 = green layer, 3 = black layer, 4 = white ground, 5 = transparent brownish layer, and earthen rendering. Elemental and phase mappings obtained by μXRF and μXRD, respectively. Map size: 80 × 140 μm^2; beam and step size: 15 × 1 μm^2.

IR Microspectroscopy

Microscopy Applications

- Small samples
- Large Samples
- Plastics
- Packaging materials
- Pharmaceuticals
- Fibers
- Trace evidence
- Contaminants
- Failure analysis
- Coatings & inks
- Electronic materials
- Migration, diffusion and aging studies
- Reverse engineering
- Art conservation
- And much more

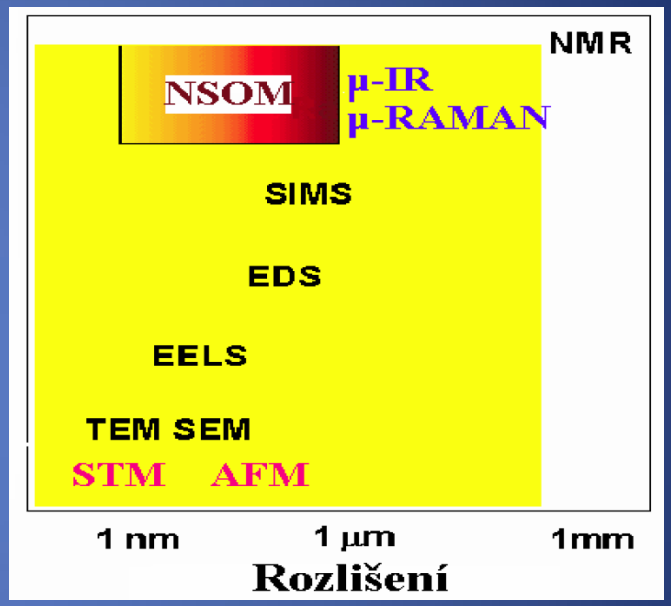
Infračervená nanospektroskopie vs. nanomikroskopie



- Nanospektroskopie
  - možnost nedestruktivního přístupu
  - nevyžaduje složitou přípravu vzorku
  - nevyžaduje vakuum

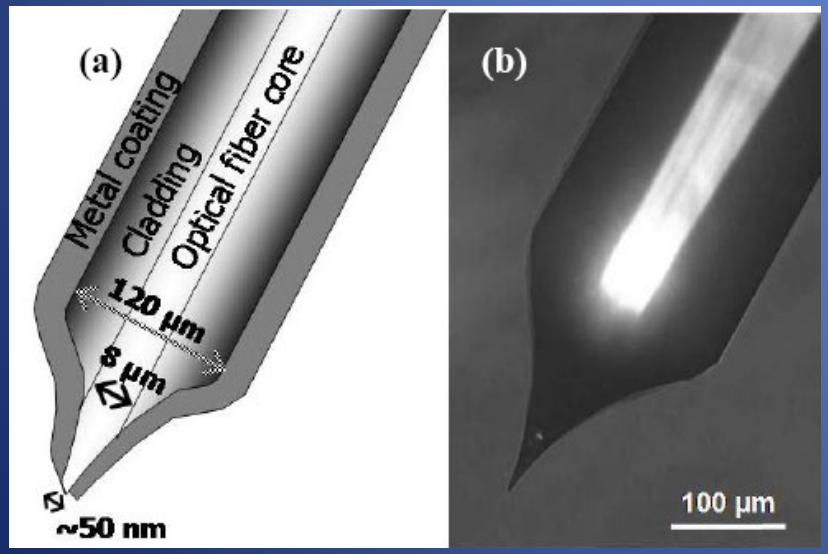
Infračervená nanospektroskopie

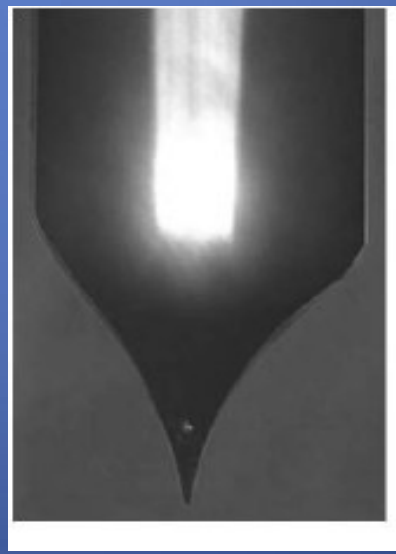
- Techniky blízkého pole
  - sonda v blízkosti povrchu („blízké pole“)
  - Spektroskopie blízkého pole
    - (near-field spectroscopy)
  - Mikroskopie blízkého pole
    - SNOM – scanning near-field optical microscopy
    - UV-vis, IR (IR-SNOM), Ramanova spektroskopie (vs. TERS)
    - fotoluminiscence, fluorescence
      - rozlišení lepší než 50 nm
      - spektroskopie jedné molekuly

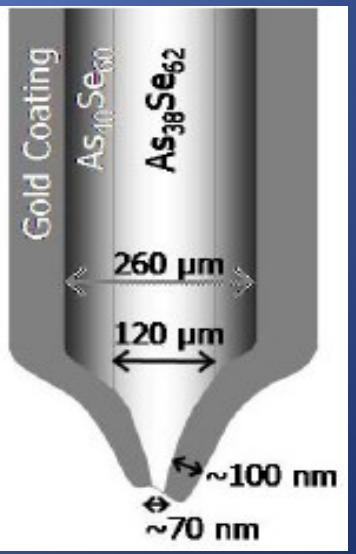


Infračervená nanospektroskopie

- Techniky blízkého pole
  - konstrukce spektroskopického obrazu rastrováním
  - sonda skenuje povrch – bod po bodu
  - kritická je apertura sondy a její vzdálenost od povrchu

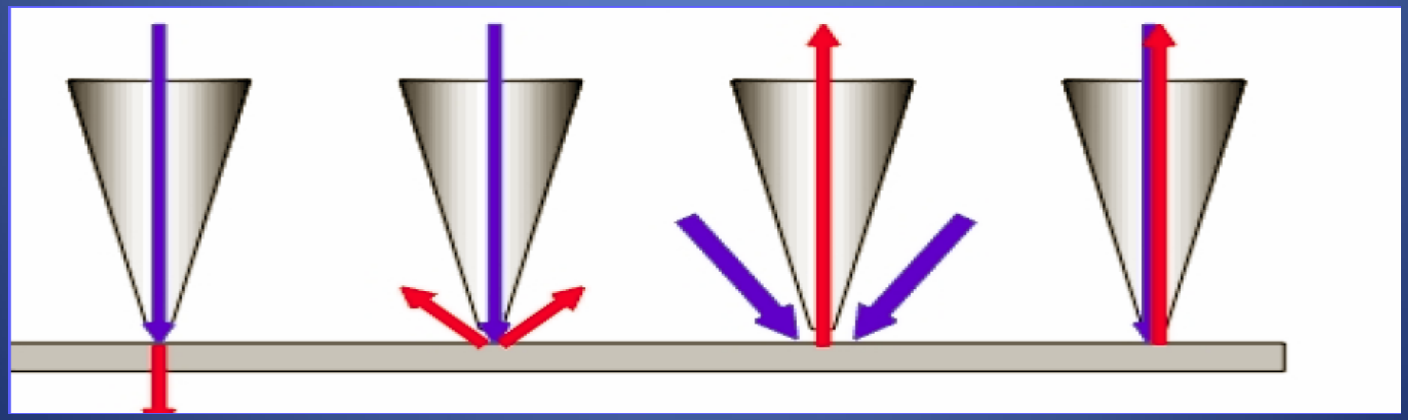






Infračervená nanospektroskopie

- vzdálenost sondy – ≈ 10 nm
- apertura sondy
- režimy snímání
  - transmisní (jen transparentní vzorky)
  - reflexní – ostrá sonda – vysílač, přijímač

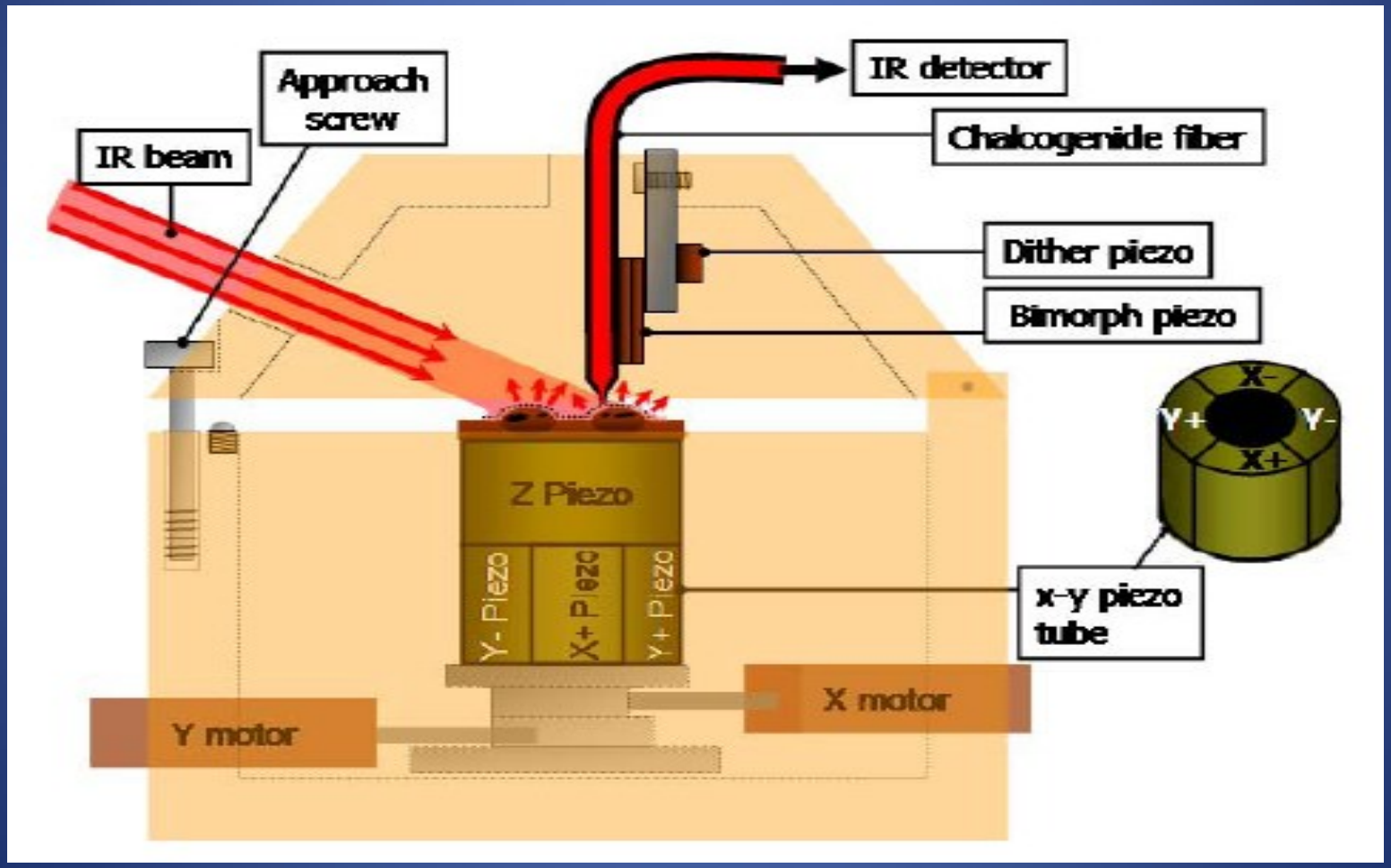


Infračervená nanospektroskopie

- vzdálenost sondy – ≈ 10 nm
- apertura sondy

- optické spřažení mezi špičkou sondy a vzorkem
- sonda reaguje na změny dielektrické funkce v jejím okolí

Infračervená nanospektroskopie



Výhody a problémy SNOM

- VÝHODY
  - překonání difrakční limity – „nanorozlišení“
  - nedestruktivní metoda
  - flexibilní režimy snímání
- PROBLÉMY
  - technologické nároky na konstrukci SNOM sondy
  - nízká intenzita detekovaného záření
  - nároky na citlivost detektoru

Výhody IR SNOM

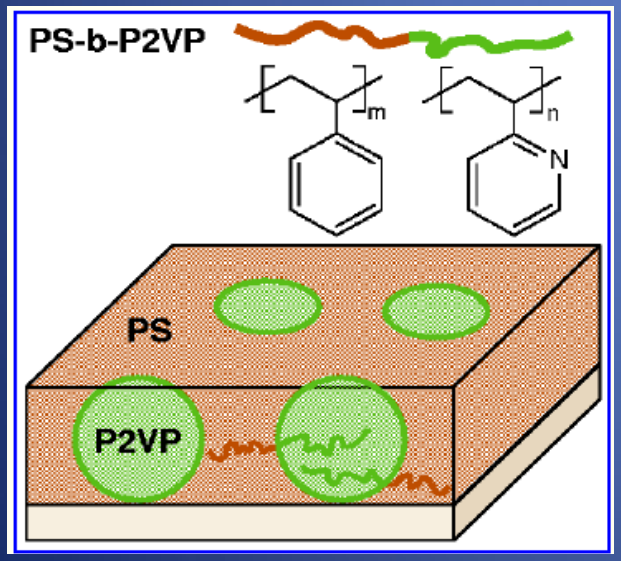
- kombinace SNOM a IR záření
- ➢ prostorové rozlišení SNOM – jednotky nanometrů
- ➢ chemické rozlišení - chemická specificita IR spekter

  - ➢ chemická charakterizace nanomateriálů
    - ➢ nanodomény

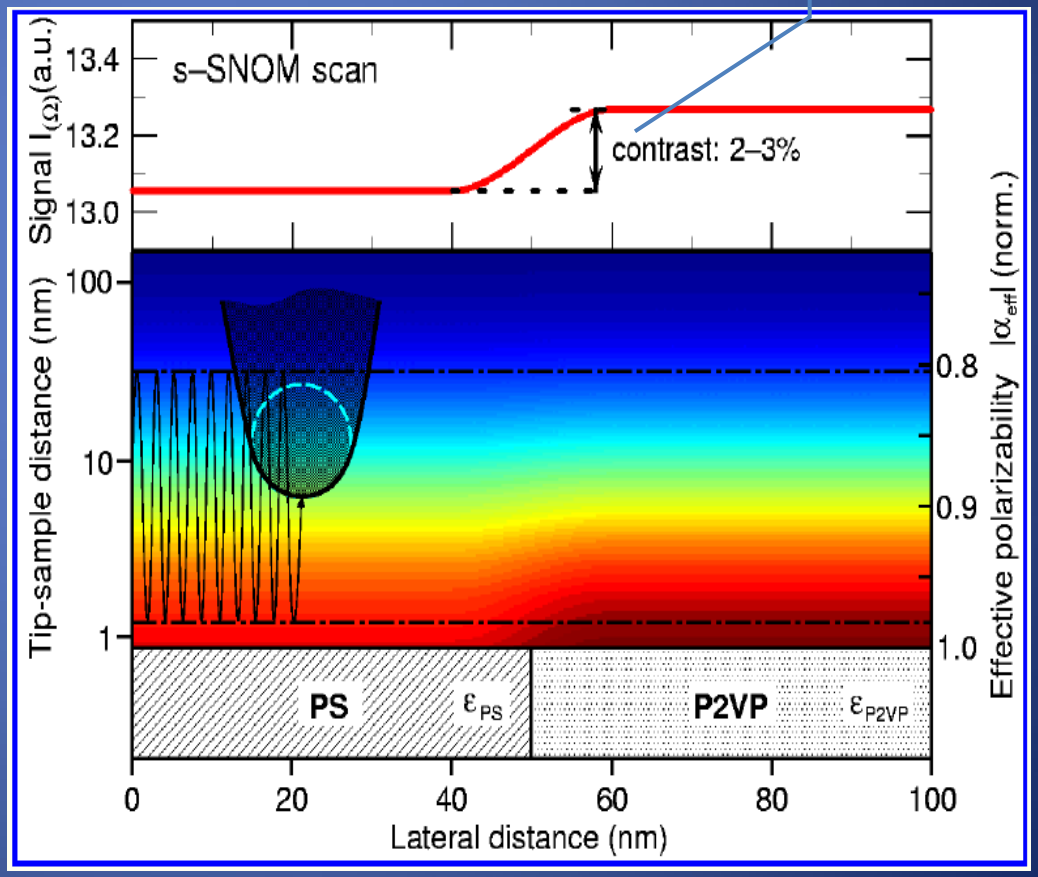
Příklady použití

– organické nanokompozitní materiály

- domény
  - polystyren
  - poly-2-vinylpyridin



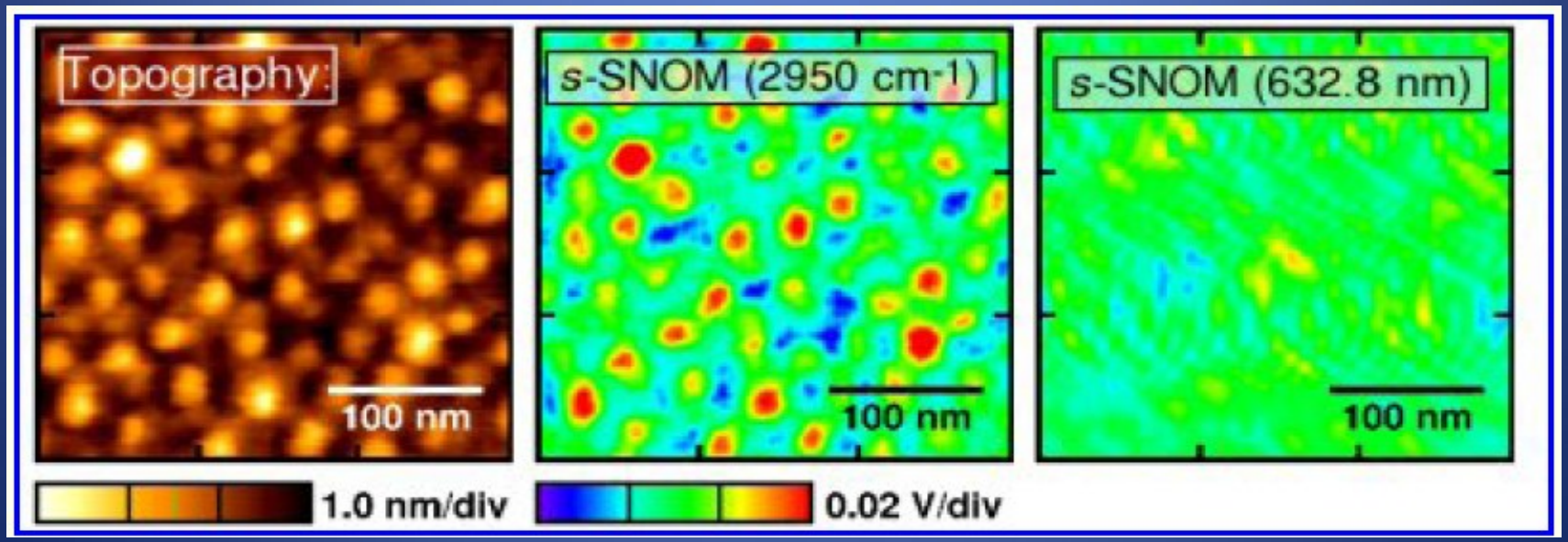
kontrast při 2950 cm^{-1}



Příklady použití

– organické nanokompozitní materiály

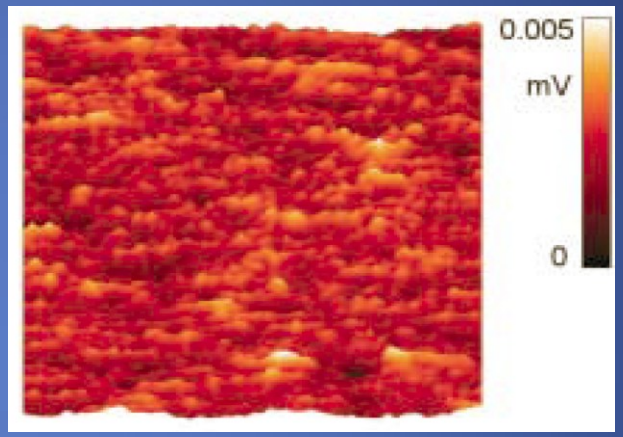
- domény
  - polystyren
  - poly-2-vinylpyridin



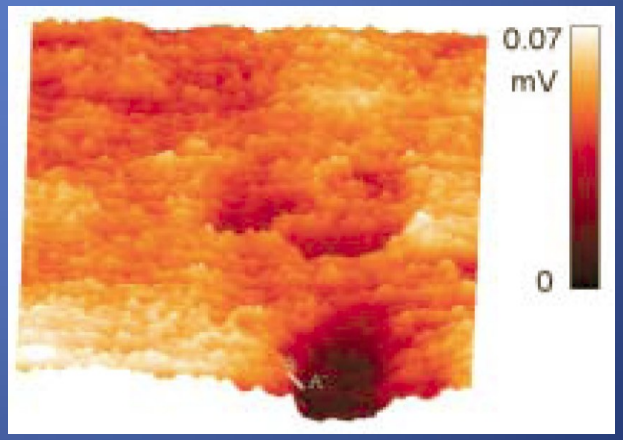
Příklady použití

– buněčné kultury

- 20 x 20 μm IR SNOM obrazy
  - 1515 cm^{-1}

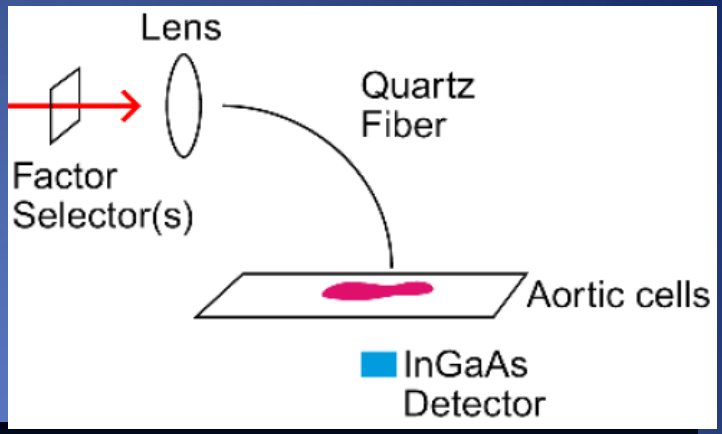


  - 1440 cm^{-1}

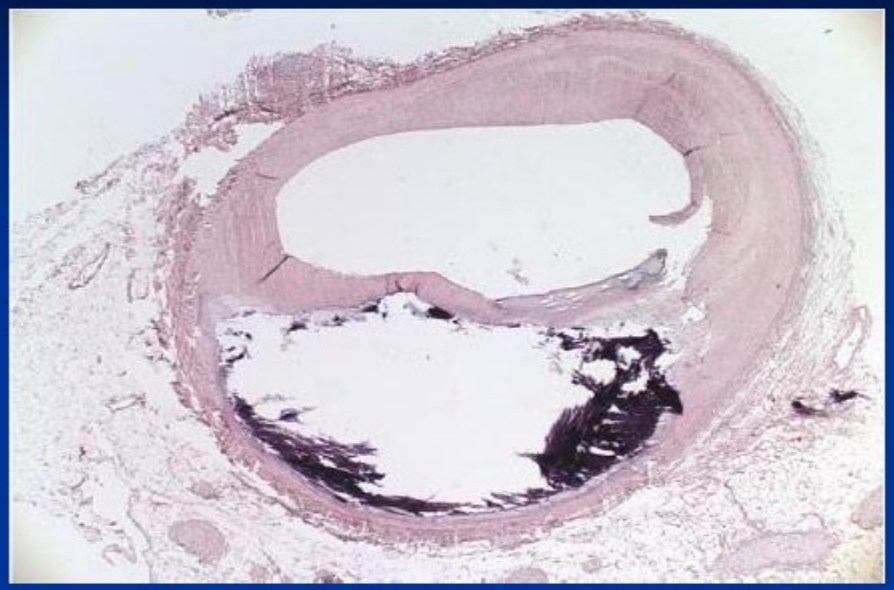


Příklady použití

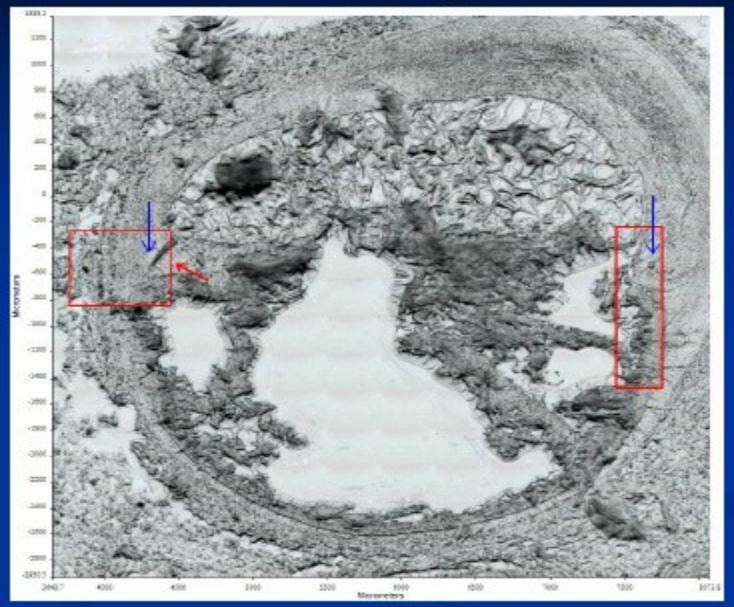
– NIR SNOM



Vulnerable Atherosclerotic Plaque



stained section



adjacent IR mapped section

Příklady použití

– NIR SNOM

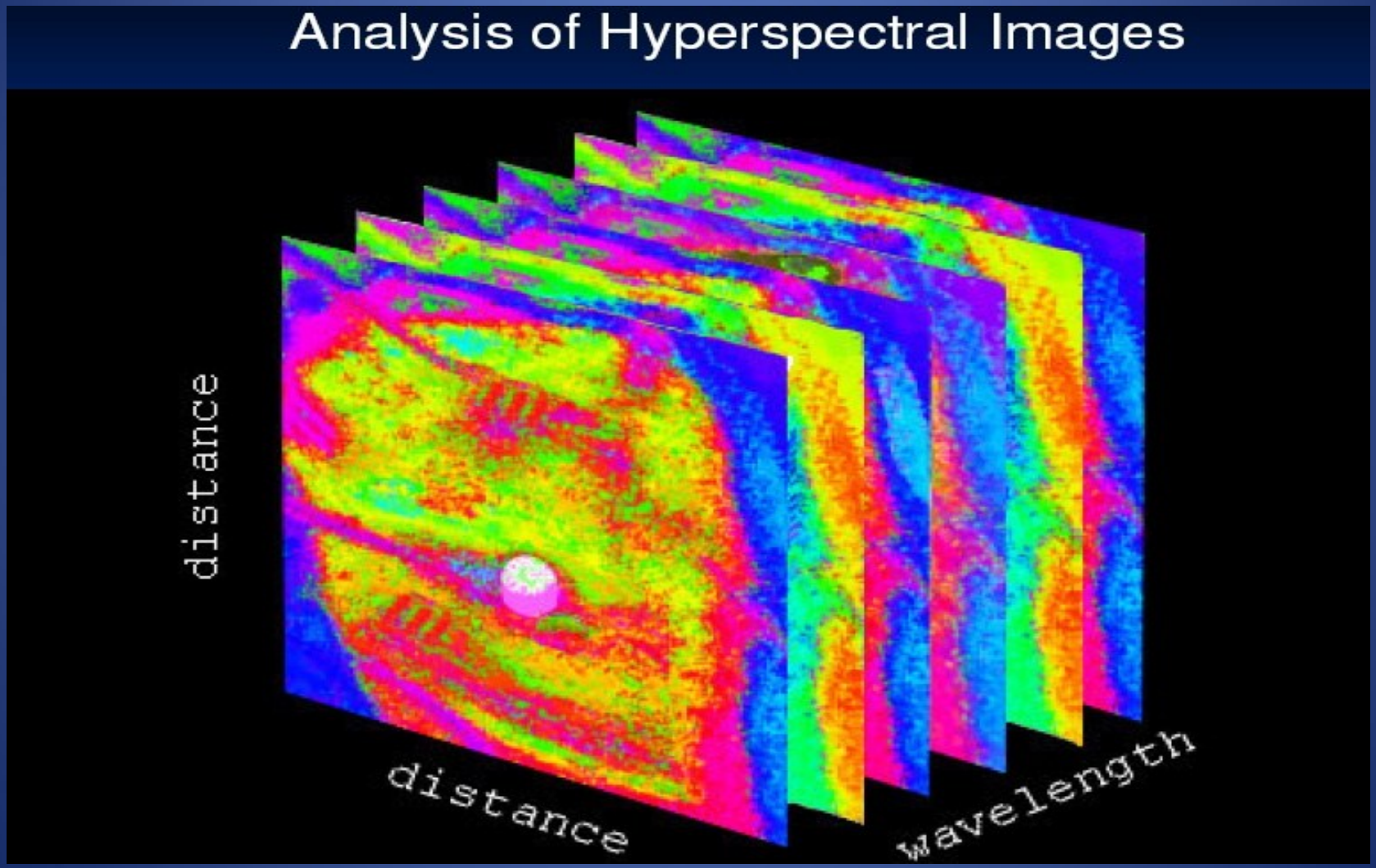
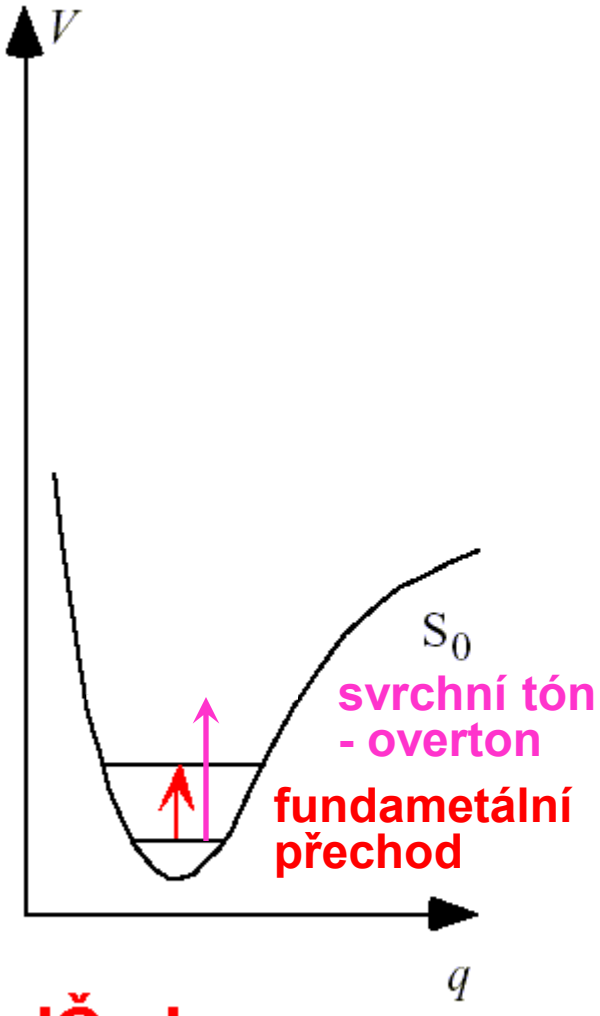
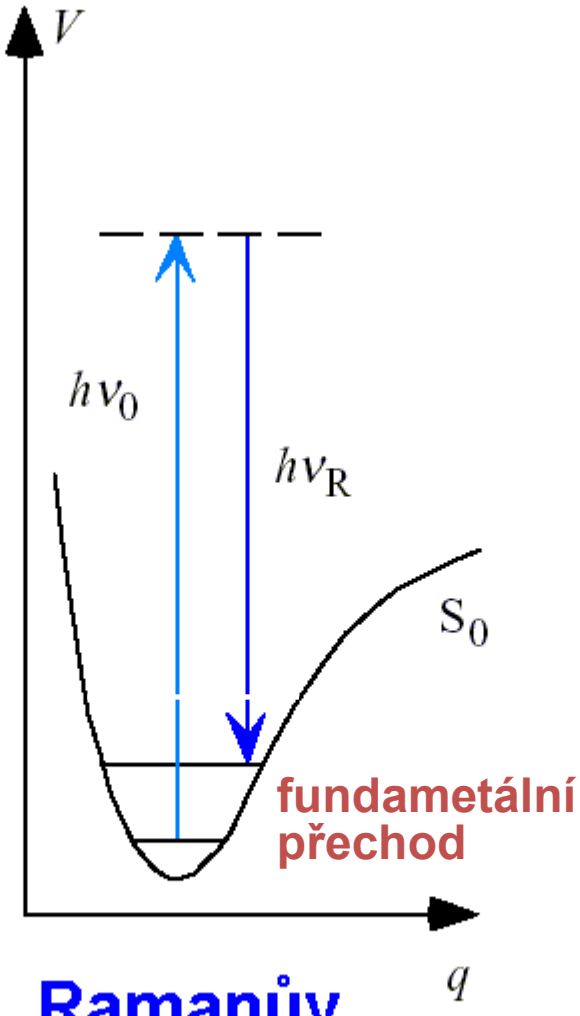


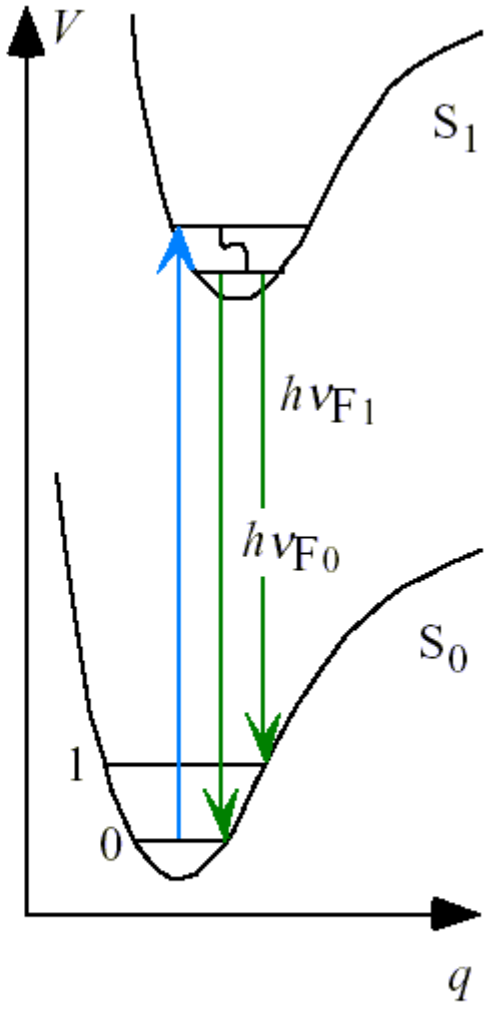
Schéma hladin



IČ absorpce



Ramanův rozptyl



Fluorescence

Ramanova spektrometrie

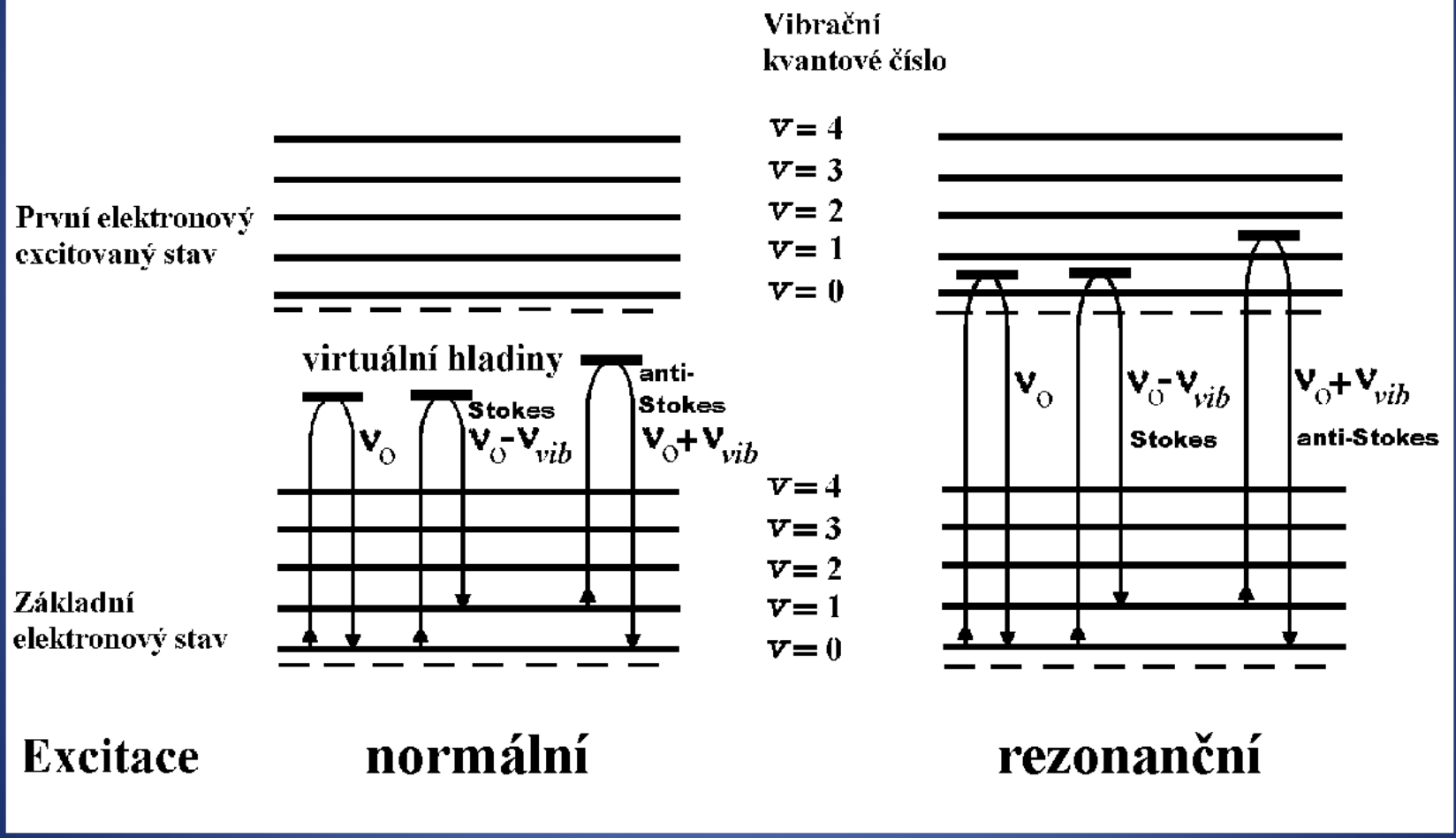
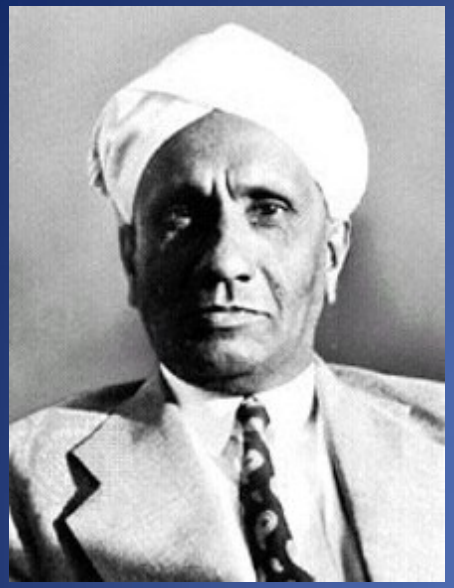


Schéma dvoufotonových jevů

Ramanův a Rayleighův rozptyl při excitaci normální a rezonanční

Ramanova spektrometrie



Sir Chandrasekhara Venkata Raman
1888 – 1970

Nobelova cena za fyziku 1930

A New Type of Secondary Radiation

C. V. Raman and K. S. Krishnan, Nature, *121(3048)*, 501, March 31, 1928

The experiments we have made have confirmed this anticipation, and shown that in every case in which light is scattered by the molecules in dust-free liquids or gases, the diffuse radiation of the ordinary kind, having the same wave-length as the incident beam, is accompanied by a modified scattered radiation of degraded frequency.

Principy Ramanovy a FT Ramanovy spektroskopie

Podstata Ramanova jevu

ROZPTYL ZÁŘENÍ

- rozptýlený foton má odlišnou energii oproti dopadajícímu

zářivý dvoufotonový proces
mezi dvěma stacionárními vibračními stavy molekuly,
jejichž energie jsou E_1 a E_2,

vyvolaný interakcí s fotonem dopadajícího záření
o frekvenci $\nu_0 > |E_2 - E_1| / h$,

provázený vyzářením rozptýleného fotonu
o energii $h\nu_R = h\nu_0 \pm (E_2 - E_1)$,

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

Rozdíly IČ a Ramanovy spektrometrie

Vibrační frekvence jednotlivých módů molekul jsou nezávislé na tom, zda je studujeme infračervenou nebo Ramanovou spektroskopií, avšak intenzity spektrálních linií budou pro obě spektroskopické techniky zřetelně odlišné.

Ramanův rozptyl – navíc informace z polarizace/depolarizace rozptýleného záření, z excitačních profilů (rezonanční efekt).

Ramanova spektroskopie

$$\vec{P} = \alpha \vec{E}$$

Klasické přiblížení

- Indukovaný dipólový moment úměrný intenzitě elektrického pole

$$p_x = \alpha_{xx} E_x + \alpha_{xy} E_y + \alpha_{xz} E_z$$
$$p_y = \alpha_{yx} E_x + \alpha_{yy} E_y + \alpha_{yz} E_z$$
$$p_z = \alpha_{zx} E_x + \alpha_{zy} E_y + \alpha_{zz} E_z$$

$$p_k = \alpha_0 E_0 \cos(2\pi \nu_0 t) + \frac{1}{2}\left(\frac{\partial \alpha}{\partial q_k}\right)_0 q_k^0 E_0 \left[\cos(2\pi(\nu_0 - \nu_k)t) + \cos(2\pi(\nu_0 + \nu_k)t)\right]$$

Rayleigh — Stokes — anti-Stokes

Základní výběrové pravidlo Ramanova rozptylu

změna polarizovatelnosti během vibračního pohybu

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

Principy Ramanovy a FT Ramanovy spektroskopie

Vztah intenzity pásů

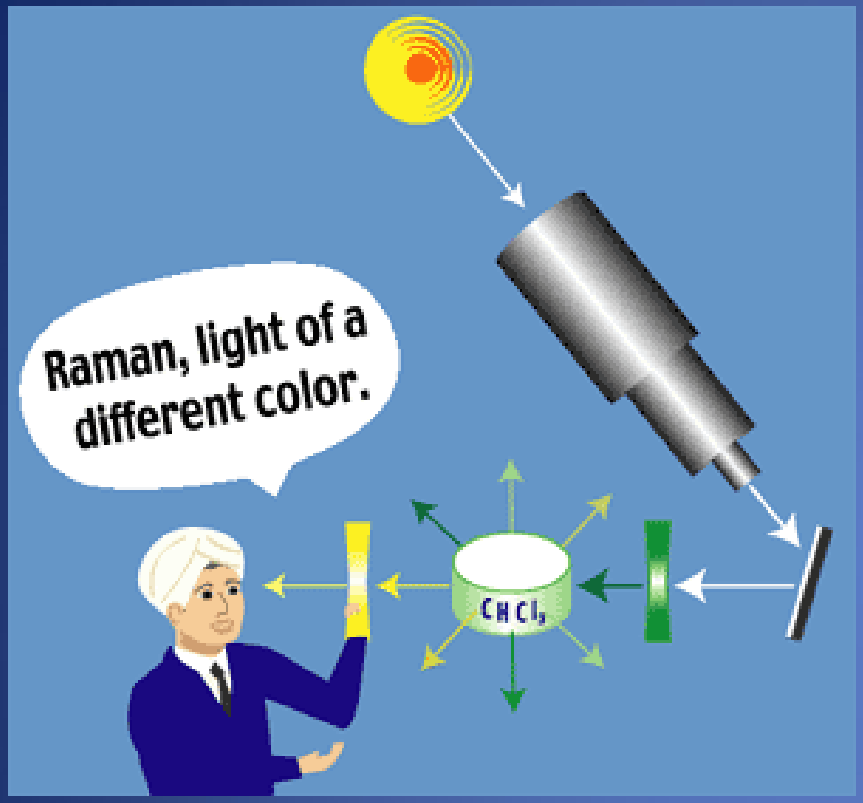
- možnost měření teploty vzorku

$$\frac{I_{\text{anti-Stokes}}}{I_{\text{Stokes}}} = \left(\frac{\nu_0 + \nu_{vib}}{\nu_0 - \nu_{vib}} \right)^4 \mathrm{e}^{-\frac{\mathrm{h}\,\nu_{vib}}{\mathrm{k}\,T}}$$

Ramanova spektrometrie

- možnost měření ve vodném prostředí
  - ↳ nízká intenzita Ramanova rozptylu pro vodu
  - ↳ používané optické materiály nejsou citlivé na vlhkost
- možnost měření ve skleněných nádobách
  - ↳ měření v uzavřených ampulích - např. pod vakuuem
- snadné využití skelné vláknové optiky
- minimální požadavky na úpravu pevných vzorků
- intenzivní pásy -C=C-, -N=N-, -S-S- a dalších symetrických vibrací

Ramanova spektrometrie



Zdroj záření

- Slunce a filtry
- rtuťová výbojka
- LASERY
  - monochromatické
  - koherentní

Detekce světla

- oči
- fotografické desky
- fotonásobiče
- CCD čipy

Instrumentace

The following experiment seems to us to be decisive: between the scattering quartz crystal and the spectrograph slit we placed a quartz vessel which was filled with mercury vapors and totally absorbed light with a wavelength of 2536 A. We did not obtain this line in the spectrogram, but obtained only the satellites.

G.S. Landsberg, L.I. Mandelstam, 1928

- zdroj excitujícího záření
- excitační optika
- vzorkový prostor
- sběrná optika
- „odlišení“ záření o různé energii
- detekce záření
- akviziční elektronika
- ukládání a zpracování dat

Instrumentace

- přenosné přístroje – „ruční“, mobilní
- stolní kompaktní spektrometry
- stolní spektrometry s volbou excitační vlnové délky
- stolní mikrospektrometry
- vědecké systémy

- průmyslové univerzální systémy
- aplikačně přizpůsobené (jednoúčelové) systémy

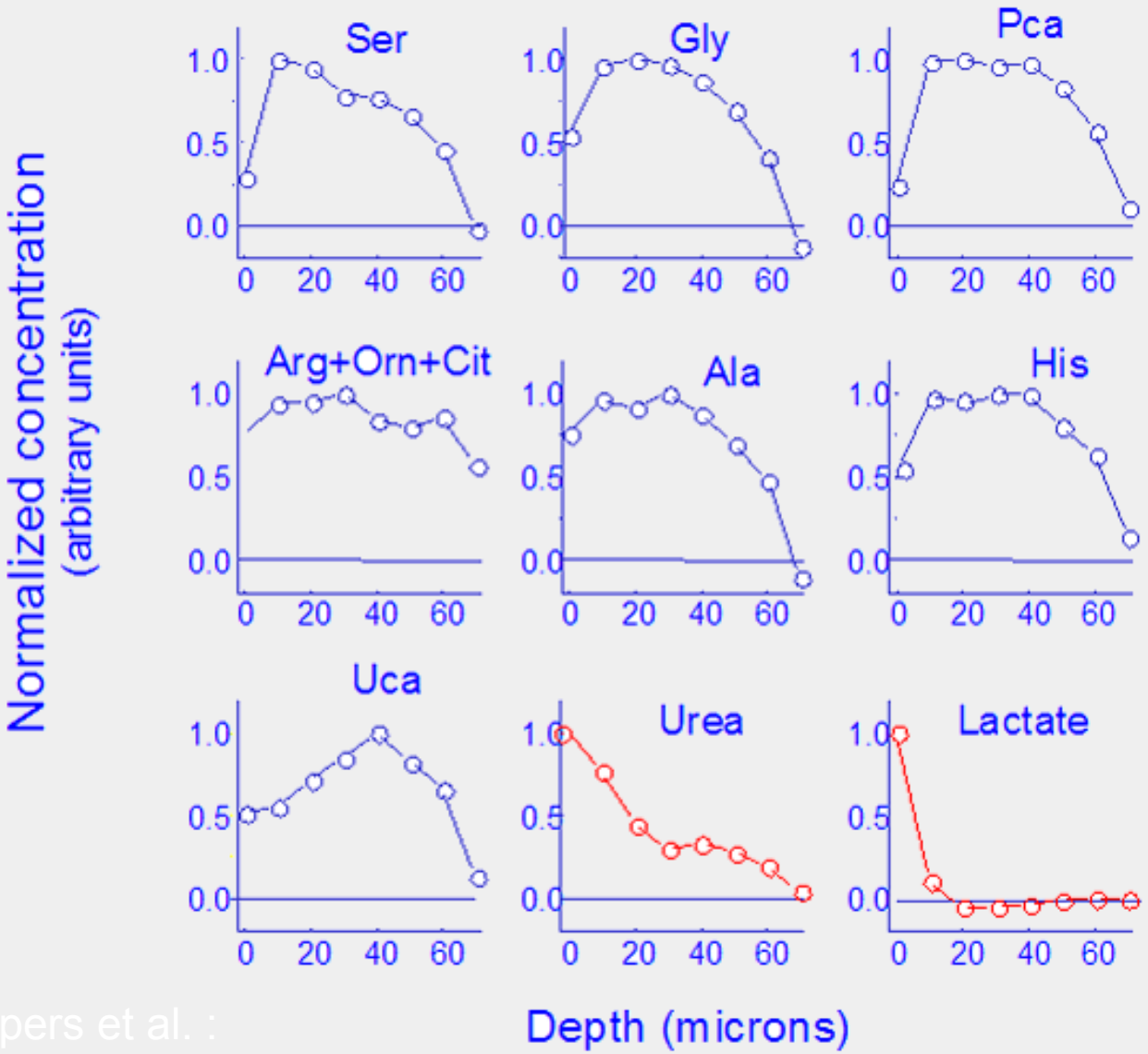
ANALÝZA kůže – jednoúčelové zařízení

River Diagnostics Model 3510 Skin Analyzer

-

http://www.riverd.com/instrumentation.htm

ANALÝZA kůže – jednoúčelové zařízení



Caspers et al. :
Journal of Investigative Dermatology 116(3):434-442 (2001)

Externí sondy – vláknová optika