Vibrational spectroscopies: infrared and Raman

From theory to pharmaceutical and biomedical applications

2

- Middle infrared; near infrared: instrumentation and applications

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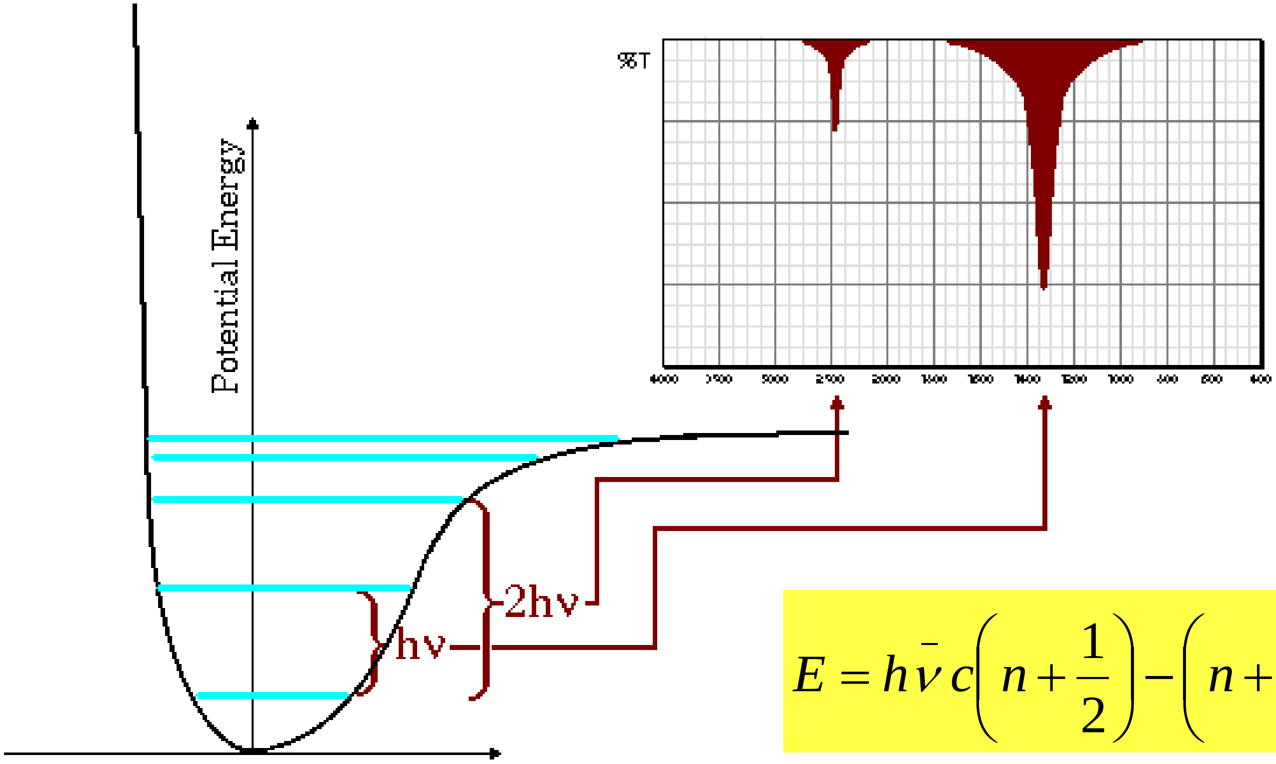
Lipides: systèmes analytiques et biologiques
Lip(Sys)²-EA7357

NEAR INFRARED SPECTROSCOPY

Theory of molecular vibrations



$$E = h\bar{\nu}c\left(n+\frac{1}{2}\right)-\left(n+\frac{1}{2}\right)^2 h\bar{\nu}cX_e$$

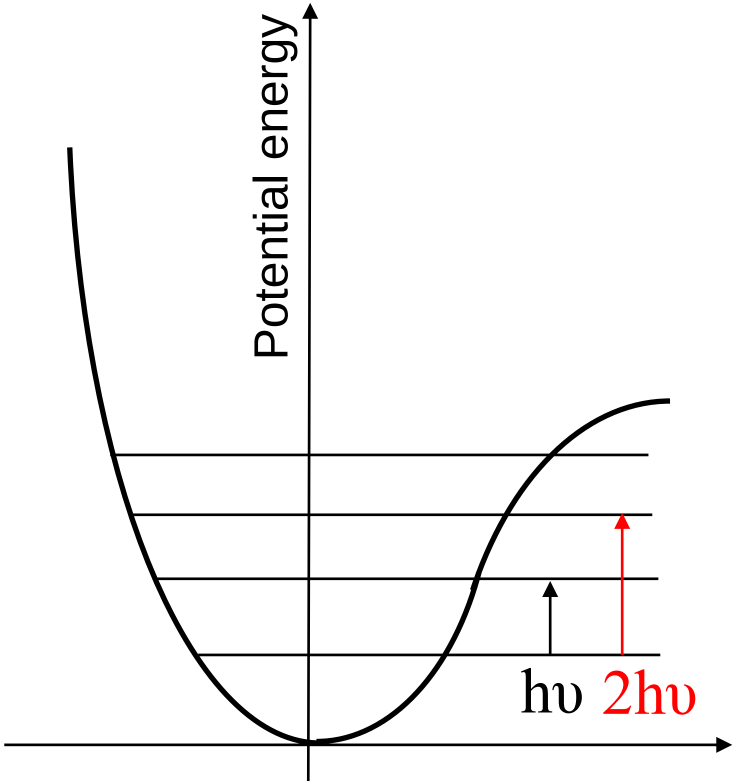
X_e= anharmonicity constant

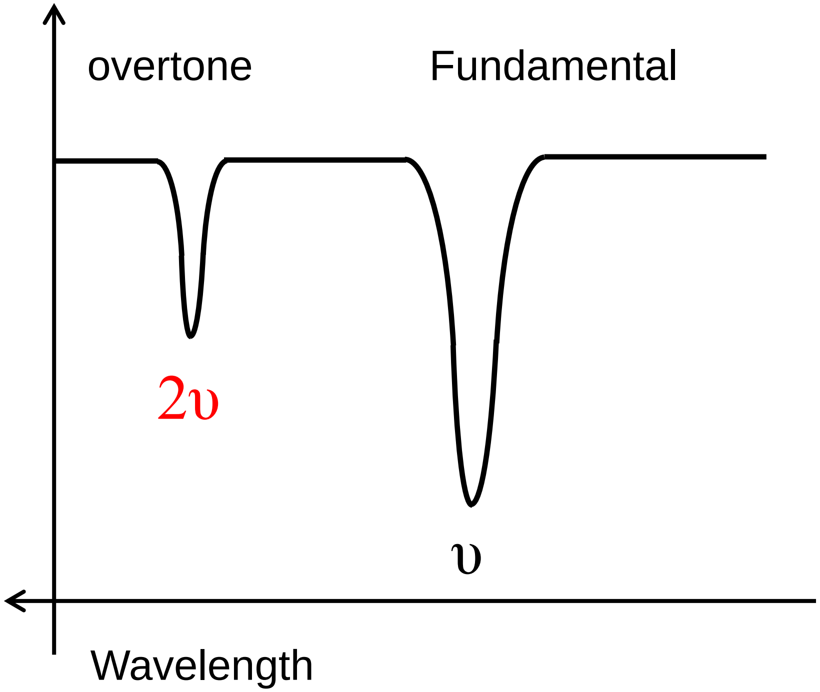
Quantum mechanics

The selection rule, which allowed transitions between one level and the next higher (or lower) is not rigorously true. A transition with Δn = +2, called an **overtone**, corresponds to ΔE approximately 2.h.ν

The overtone band appears at a little less than twice the frequency of the fundamental band, and is less intense. Often the overtone band is so small it cannot be found.

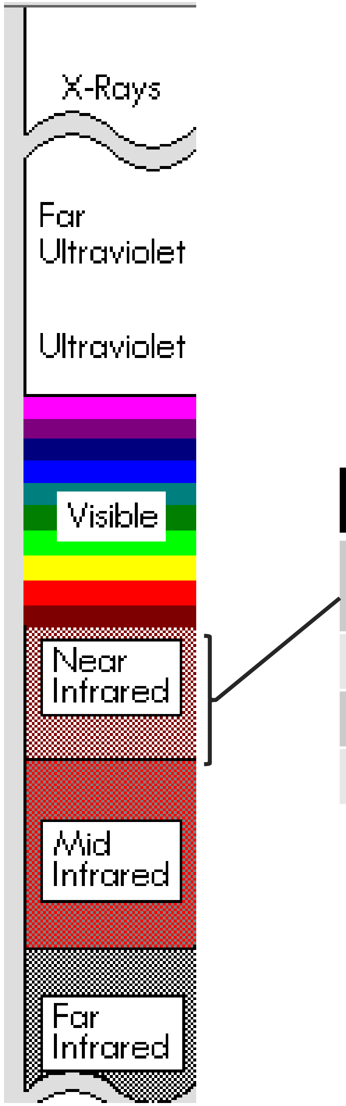
Theory of molecular vibrations





The molecule can absorb the energy corresponding to both transitions

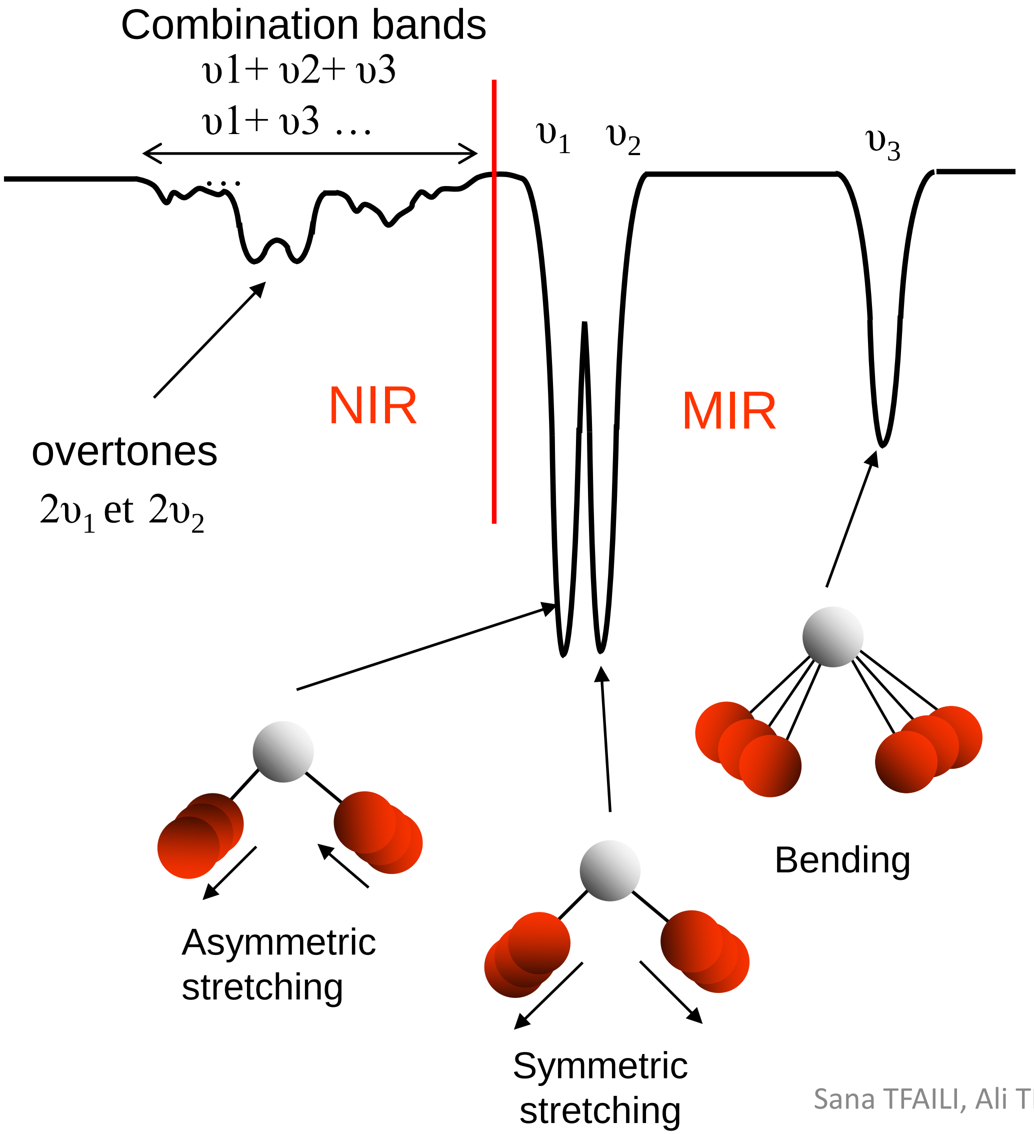
Near infrared



Infrared	λ (µm)	ω (cm^{-1})
Near	**0,78-2,5**	**12800-4000**
Mid	2,5-50	4000-200
Far	50-1000	200-10
Most common for mid	2,5-25	4000-400

Radiation in the near infrared region (12800-4000 cm^{-1}) corresponds to overtones and combinations of fundamental vibration modes.

Near infrared spectroscopy



NIR spectroscopy operates in the wavenumber range from 12500 to 4000 cm^{-1}, (800-2500nm):

It is located between visible and middle-infrared (MIR) spectral ranges.

In this domain, energy absorption is essentially caused by **vibration modes of C-H, N-H, S-H, O-H and C=O bonds**.

The NIR spectrum is divided into several domains corresponding to :

- **combinations of fundamental vibrations (5000-4000 cm^{-1}),**
- **first overtones (6500-5000 cm^{-1}),**
- **second overtones (9000-6000 cm^{-1})**
- **third overtones (12500-8500 cm^{-1}).**

Intensities are in between **10**, for combinations, up **to 1000**, for successive overtones, **times lower than** the absorption resulting from **fundamental vibrations**.

The most interesting range for quantification is the overtone region (10000-5000 cm^{-1}).

Near infrared spectroscopy

NIR spectroscopy operates in the wavenumber range from 12500 to 4000 cm^{-1}, (800-2500nm):

The spectral occurrences in the NIR region are **dominated by overtones and combination** absorption bands.

However, **some other effects**, associated with the higher order terms, **may be observed** and contribute to the complexity of the NIR spectrum.

Coupling or resonance between different vibrations of the same functional group can occur as a function of the third and fourth order terms.

Fermi resonance occurs between a fundamental and an overtone when their difference in energy is very low. This type of resonance results in a greater separation between the position of the two bands and in the intensification of the overtone band.

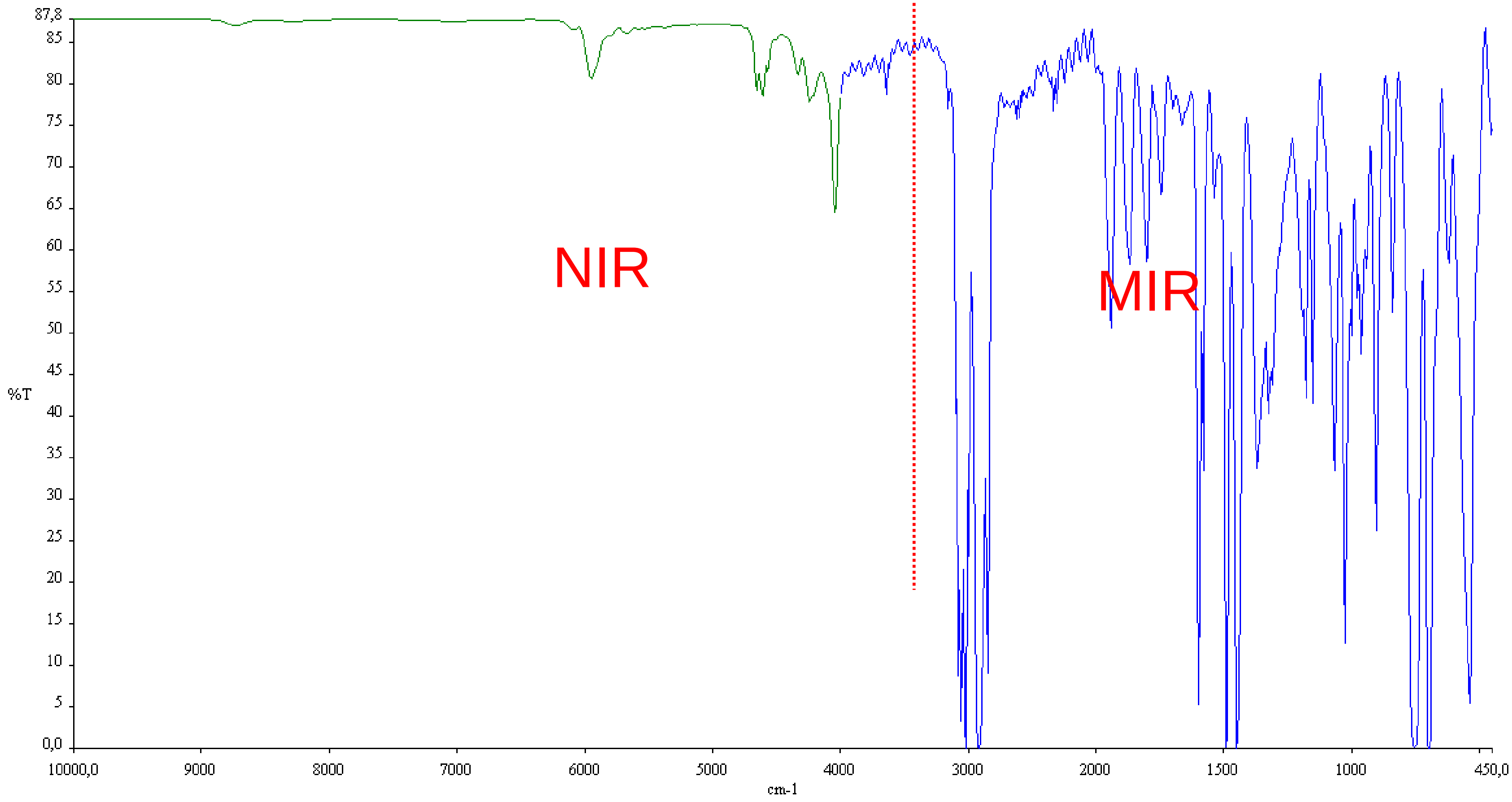
Darling-Dennison resonance may promote the interaction between two high level overtones of a molecule and a combination band, for example, and is particularly intense for bonds containing hydrogen atoms.

The complexity of the combination spectral region in the NIR spectrum of hydrocarbons is partly due the possibility of resonance between the combination bands and high order overtone for C-H bonds. The primary practical consequence of both types of resonance on a NIR spectrum is the possibility of the appearance of two instead of one band in the combination region (1600 – 2500 nm).

Near infrared spectroscopy

NIR spectroscopy operates in the wavenumber range from 12500 to 4000 cm^{-1}, (800-2500nm):

Polystyrene (38µm thick film)

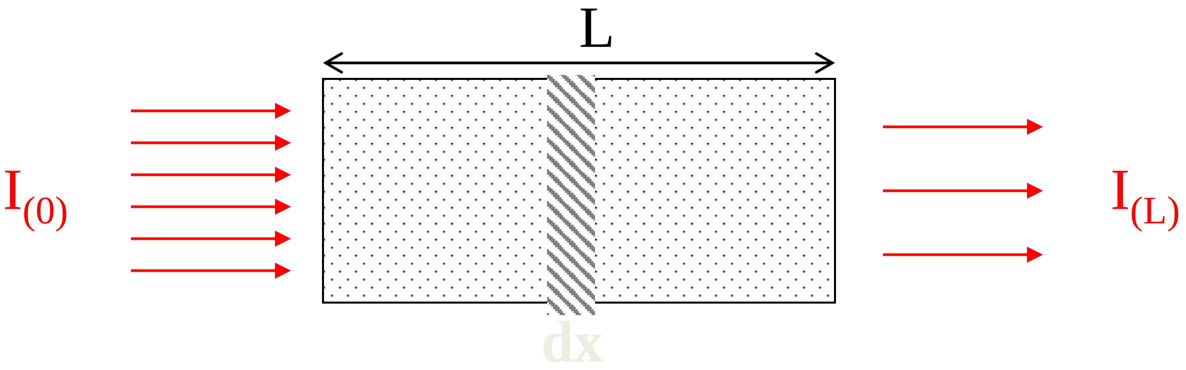


INFRARED

Measurment of the absorption

$$A = \log\left(\frac{I_{(0)}}{I_{(L)}}\right)$$

$I:/I_0$	Transmission in percentage	A	Absorbance in percentage
1	100%	0	0%
0,1	10%	1	90%
0,01	1%	2	99%
0,001	0,1%	3	99,9%



$$-dI_{(x)} = KI_{(x)}cdx$$

$$-\left[\ln I_{(x)}\right]_{I_{(o)}}^{I_{(L)}} = \left[Kcx\right]_{0}^{L}$$

$$\ln\left(\frac{I_{(0)}}{I_{(L)}}\right) = KcL$$

ou

$$\frac{I_{(L)}}{I_{(0)}} = e^{-KcL}$$

A , ABSORBANCE

$$A = \log\left(\frac{I_{(0)}}{I_{(L)}}\right)$$

$$\ln\left(\frac{I_{(0)}}{I_{(L)}}\right)=\ln 10\cdot\log_{10}\left(\frac{I_{(0)}}{I_{(L)}}\right)=kcL$$

$$A=\log\left(\frac{I_{(0)}}{I_{(L)}}\right)=\frac{k}{\ln 10}cL$$

$$A=\varepsilon cl$$

$1<\varepsilon<1000$ $\varepsilon>50$ **Is considered high**

BEER-LAMBERT law

- ABSORPTION is function of the quantity of the material crossed by the light

$$A = \varepsilon c l$$

A = ABSORBANCE

ε= Absorption coefficient

c = Concentration

l = Length of the light pathway

MIR and NIR instrumentation

MIR and NIR: instrumentation

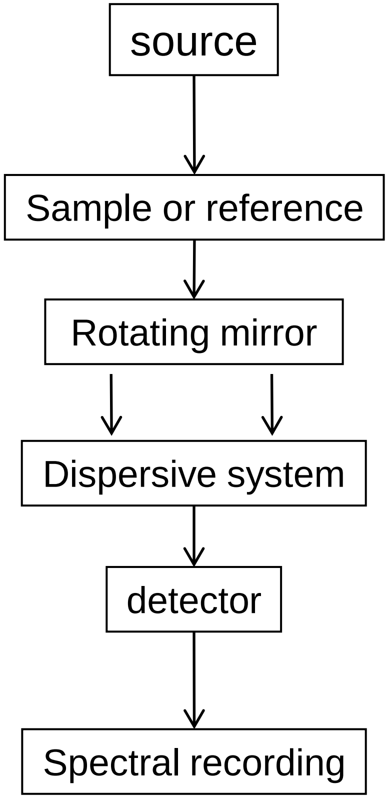
There are two major types of MIR spectrometers: **Dispersive and Fourier Transform FT**

Source:

- Nernst lamp: ceramic tube (zirconium oxide - yttrium oxide) eclectically heated to 1200°C -> infrared throughout (4000-200 cm^{-1})
- Tungstene filament
- Globar, a silicon carbide rod electrically heated up to 1000- 1650 °C-> infrared throughout (4-15 µm: 2500-667 cm^{-1})
- SYNCHROTRON radiation

Optical materials:

- Only mirrors (not lenses) are used. Glass and quartz absorb MIR light
- NaCl and KBr (water soluble)
- Cesium iodide
- Silver chloride
- KRS-5: Thallium bromo-iodide
- Tungstène filament
- AMTIR: glass compose of Germanium, Arsenic and Selenium



source

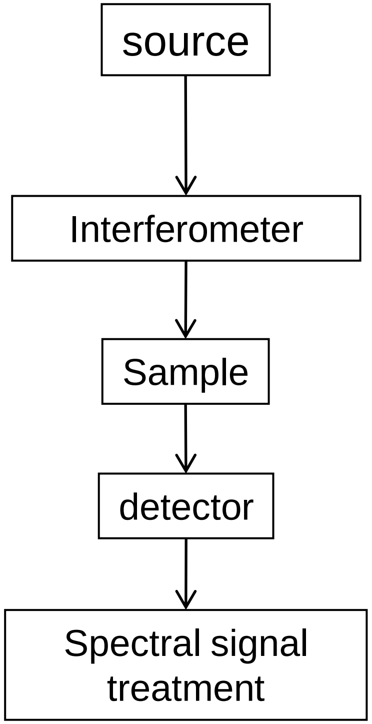
Sample or reference

Rotating mirror

Dispersive system

detector

Spectral recording



source

Interferometer

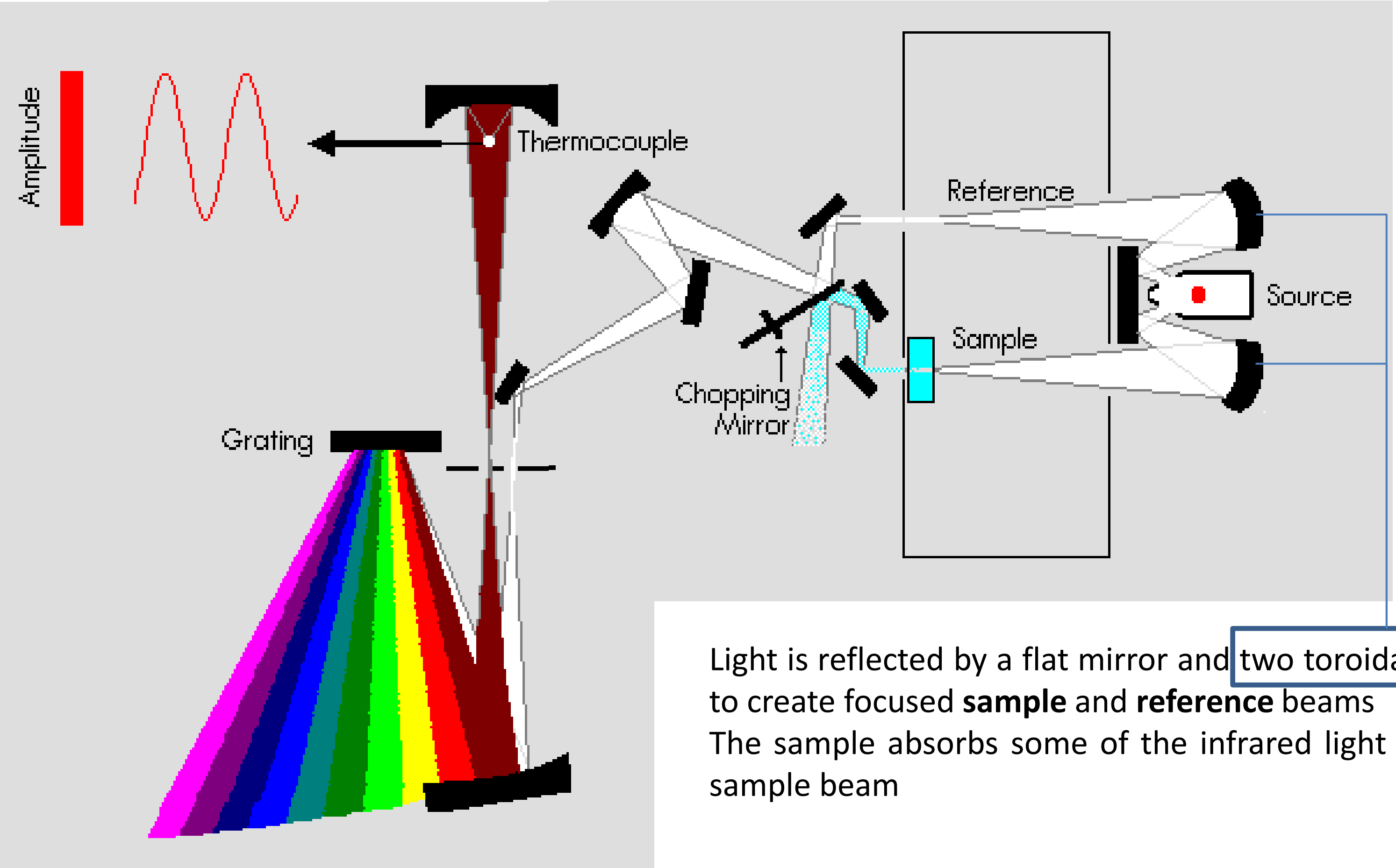
Sample

detector

Spectral signal treatment

MIR and NIR: instrumentation

Dispersive spectrometers:

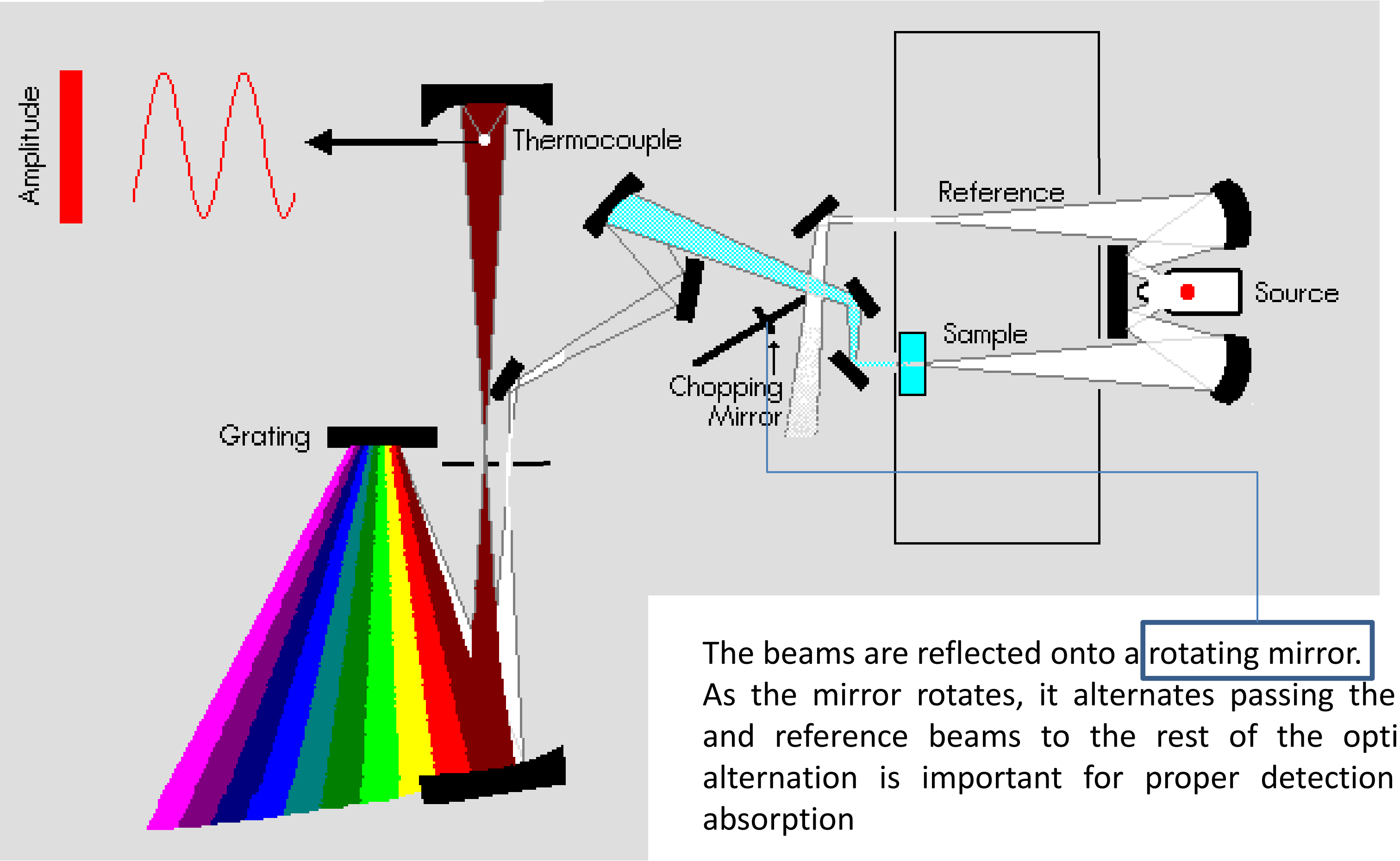


Light is reflected by a flat mirror and two toroidal mirrors to create focused **sample** and **reference** beams

The sample absorbs some of the infrared light from the sample beam

MIR and NIR: instrumentation

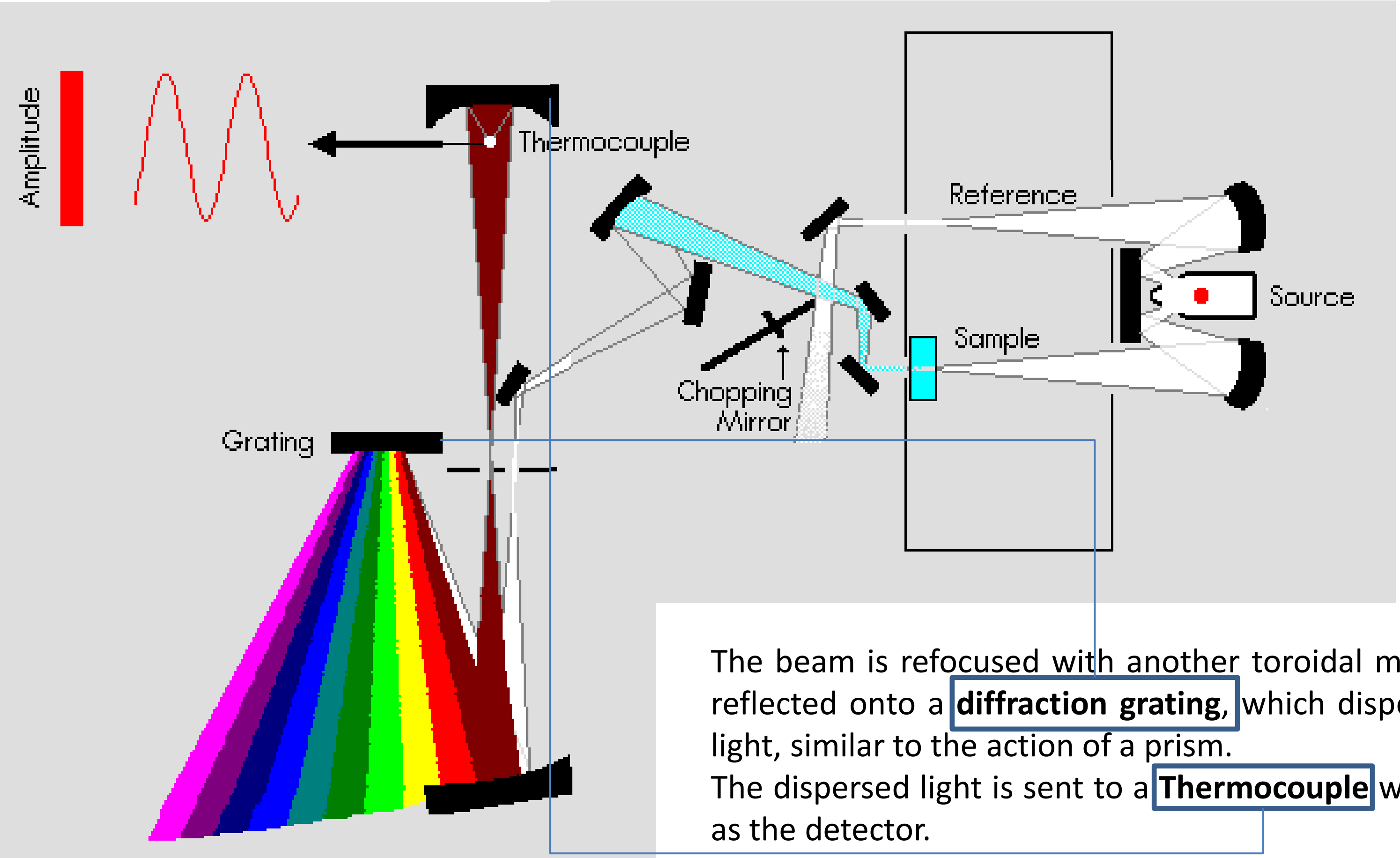
Dispersive spectrometers:



The beams are reflected onto a rotating mirror.
As the mirror rotates, it alternates passing the sample and reference beams to the rest of the optics. This alternation is important for proper detection of the absorption

MIR and NIR: instrumentation

Dispersive spectrometers:



The beam is refocused with another toroidal mirror and reflected onto a **diffraction grating,** which disperses the light, similar to the action of a prism.
The dispersed light is sent to a **Thermocouple** which acts as the detector.

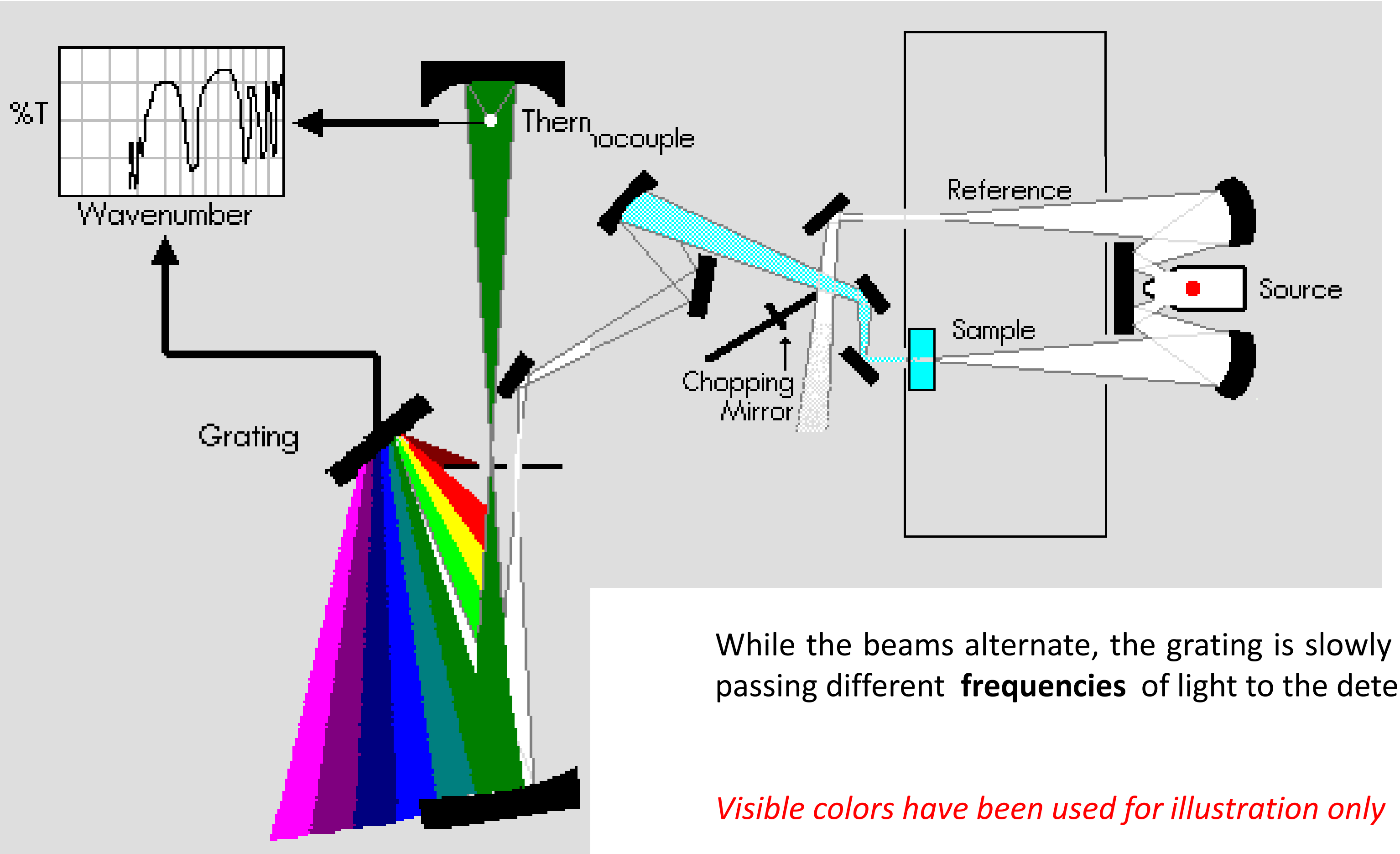
Visible colors have been used for illustration only

Detectors:

Thermocouple, thermopiles, thermistors

MIR and NIR: instrumentation

Dispersive spectrometers:

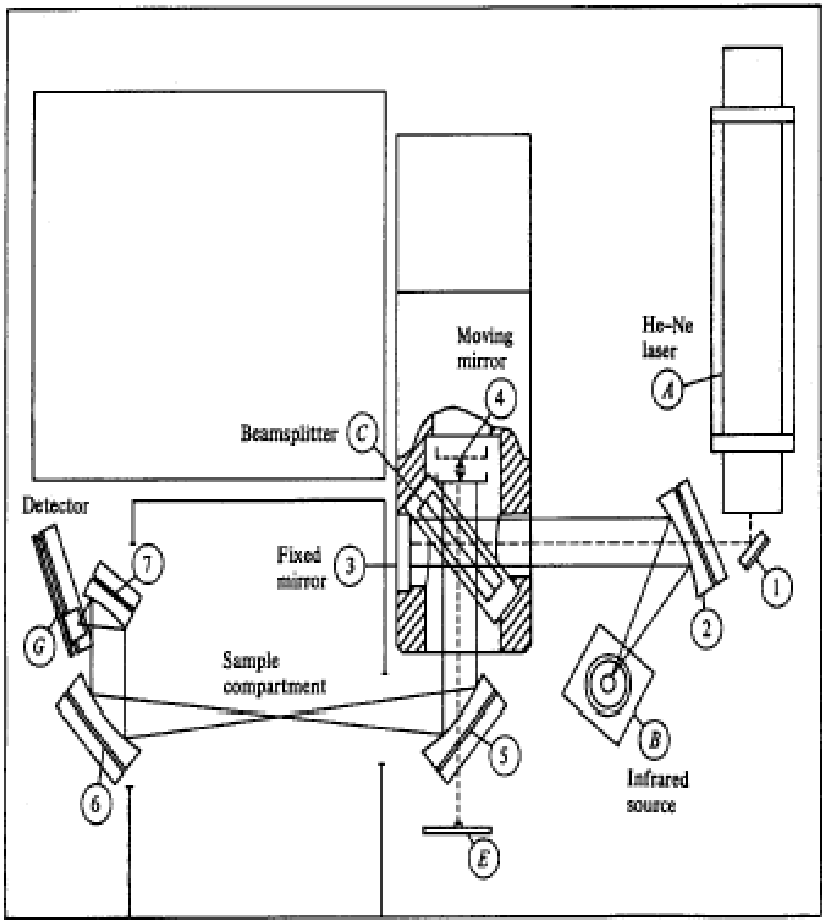


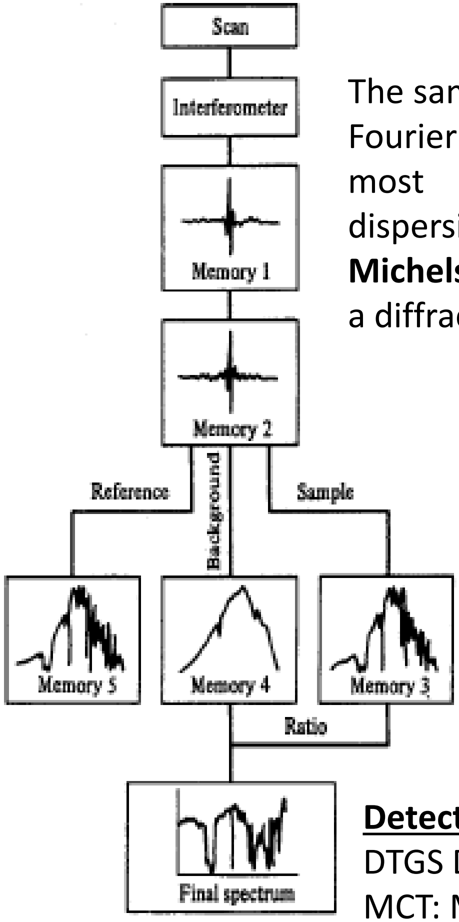
While the beams alternate, the grating is slowly rotated, passing different **frequencies** of light to the detector

Visible colors have been used for illustration only

MIR and NIR: instrumentation

Fourier Transform spectrometers:



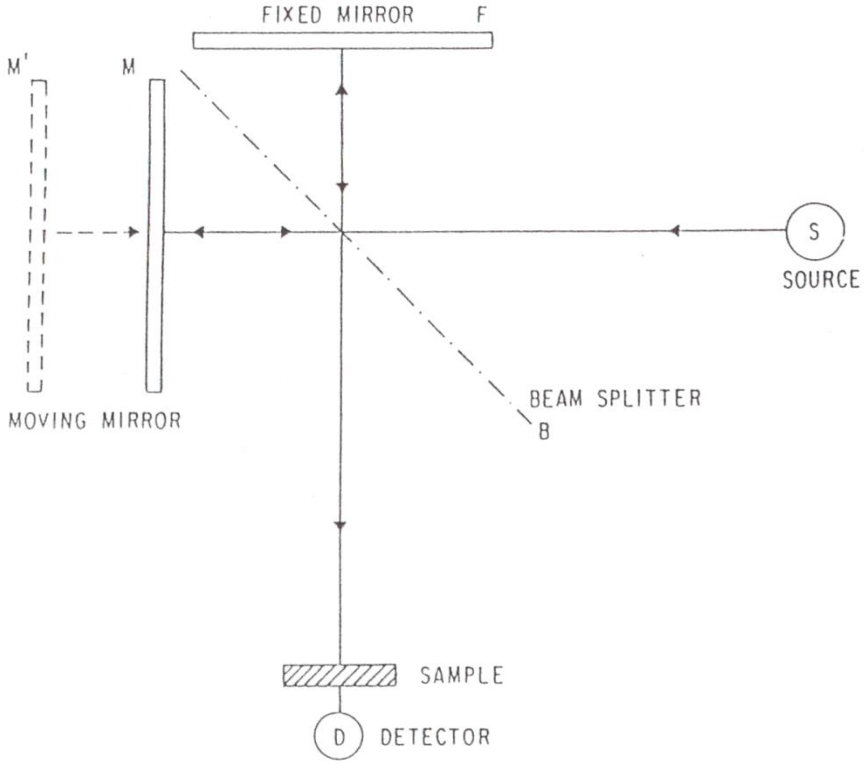


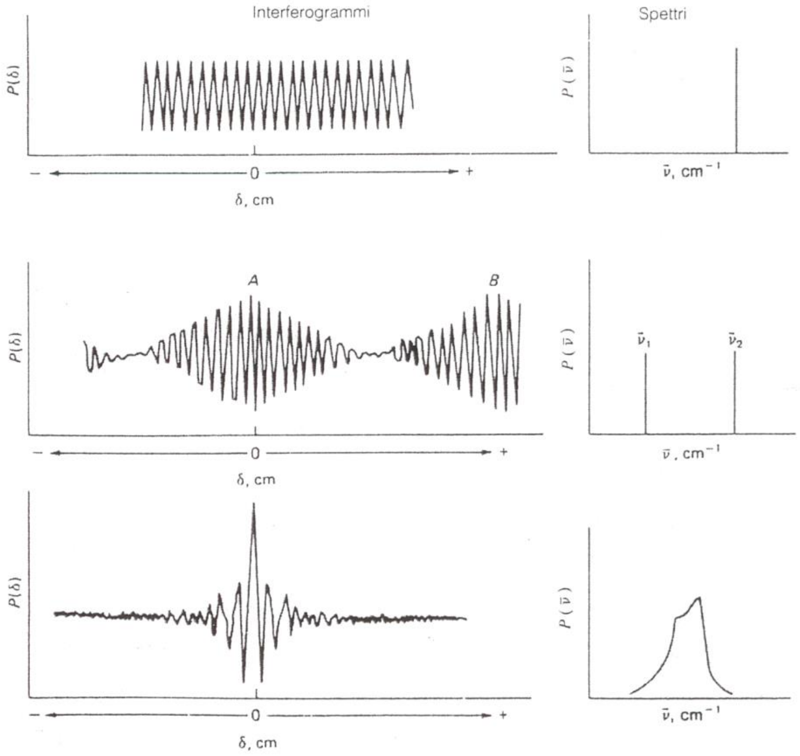
The same information can be obtained Fourier Transform spectrometers. The most important difference with dispersive systems is the use of a **Michelson interferometer** rather than a diffraction grating

Detectors:
DTGS Deuterated Triglycine Sulfate
MCT: Mercure Cadmium Telluride

MIR and NIR: instrumentation

Michelson interferometer:

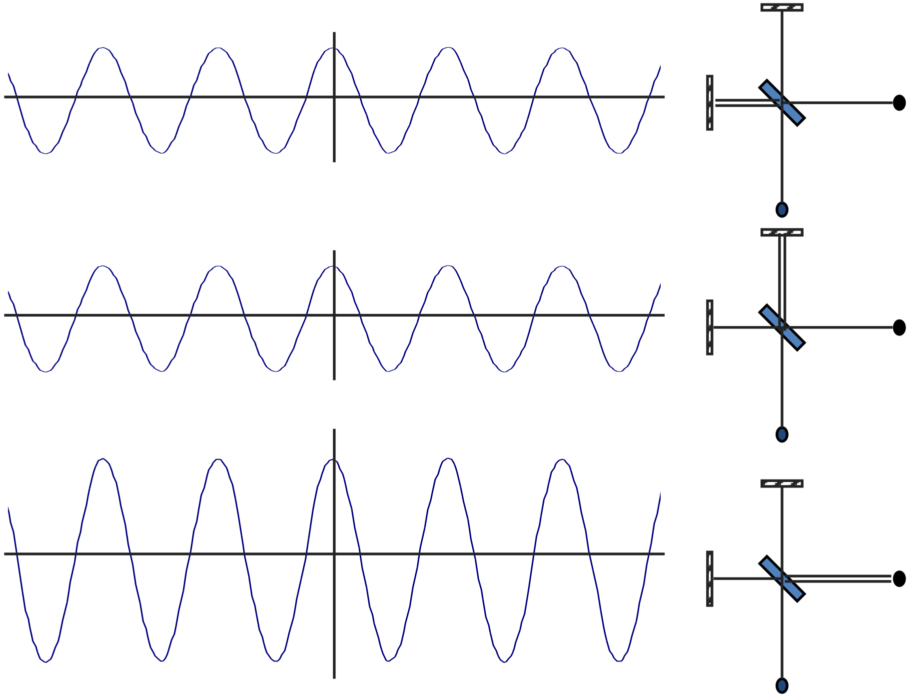




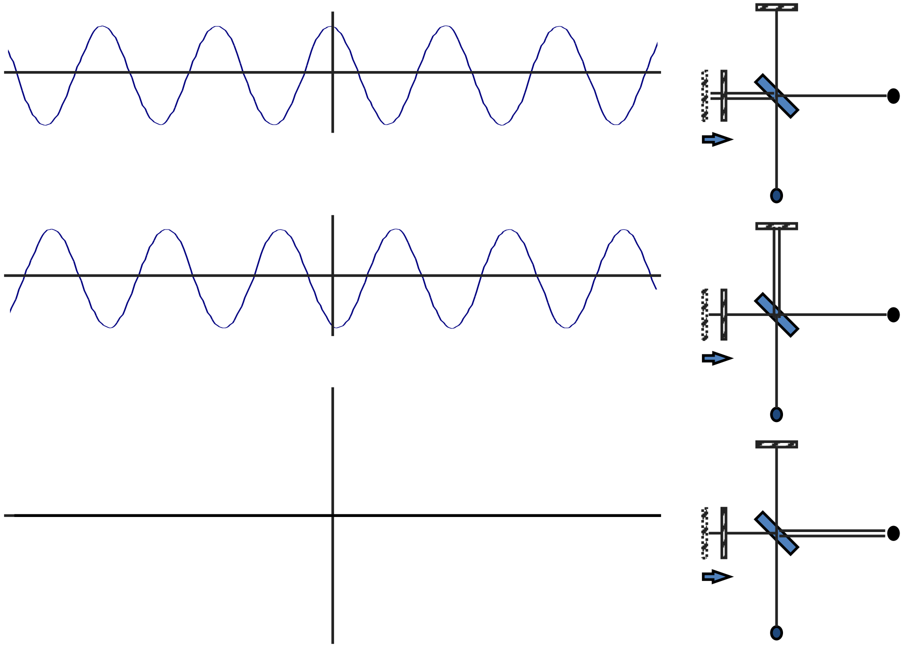
MIR and NIR: instrumentation

Michelson interferometer: Recombination of the beams

Identical light paths: constructive interference



Different light paths: destructive interference, minima at 180°



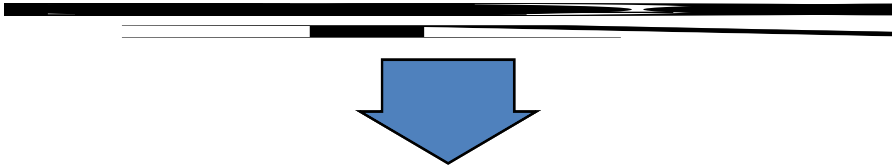
MIR and NIR: instrumentation

Michelson interferometer: Recombination of the beams

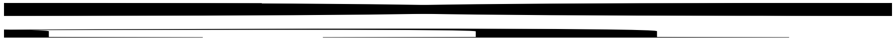
Signal with different wave lengths

Fourier Transform

Without sample



With sample

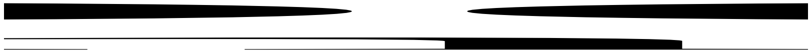


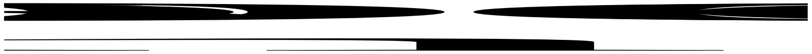
MIR and NIR: instrumentation

Michelson interferometer: Recombination of the beams

Ratio: sample/ background

Ratio



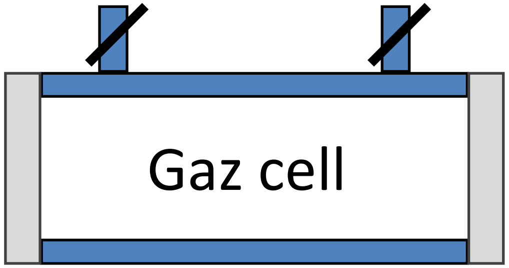


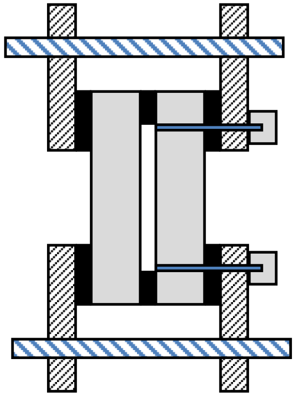
- Higher signal to noise ratio
- All wavenumbers are simultaneously obtained
- Higher spectral resolution

MIR and NIR: measurement modes

Transmission:

- **Gaz samples:** Optical pathway of few cm
- **Liquid samples:** a thin layer between NaCl disks
- **Solid samples:**
  - KBr pellets:
    - Smash the sample and KBr
    - Apply high pressure (10 tones) and vacuum
  - Micrometric sections (Microspectroscopy)
- **NIR** radiation are much less absorbed. Transmission spectra can be obtained across several mm of solid or liquid samples




Liquid cell

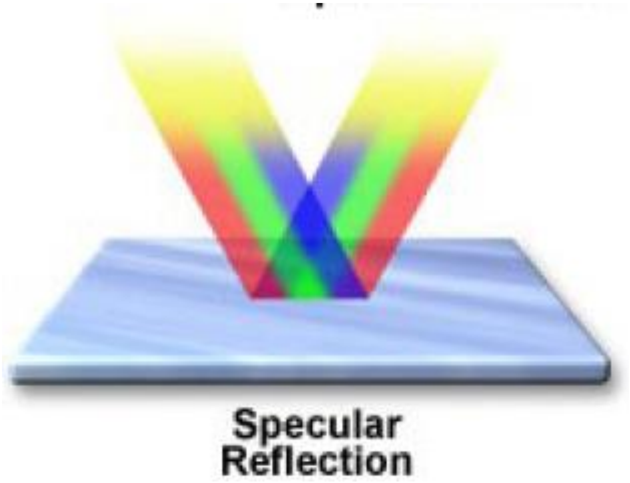
MIR and NIR: measurement modes

Reflection:

A- Specular reflection

defined as light reflected from a smooth surface (such as a mirror, any irregularities in the surface are small compared to λ) at a definite angle.

The reflectance of the sample (I) is compared to that of an Aluminum mirror (I_0)



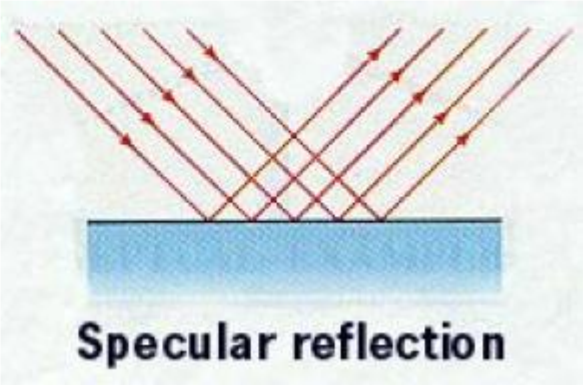
Reflectance spectrum $R=I/I_0=f(\lambda)$
(close to the derivative)

↓

Kramers-Kronig (K-K) transformation

↓

Pseudo-absorbance spectrum



https://www.perkinelmer.com/lab-solutions/resources/docs/TCH_reflection-Measurements.pdf

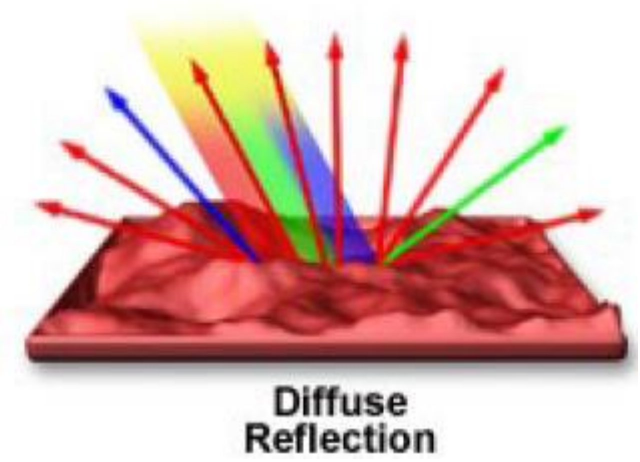
MIR and NIR: measurement modes

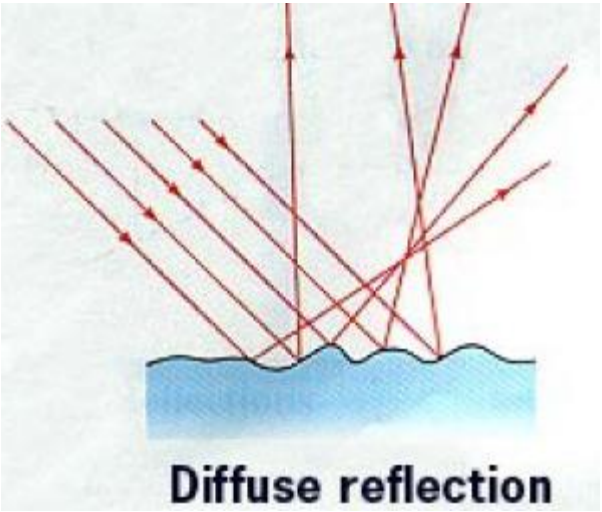
Reflection:

B- Diffuse reflection

Is produced by rough surfaces that tend to reflect light in all directions. There are far more occurrences of diffuse reflection than specular in ou everyday environment.

the reflection spectra do not come from the front surface alone. Radiation that penetrates into the material can reappear after scattering or reflection at a second surface. When this radiation emerges it will have experienced some absorption, depending on the path traversed. This component of the spectrum will have the general character of a transmission spectrum. Spectra of this type are called diffuse reflection spectra but it is important to remember that they always contain some amount of reflection from the surface of the sample.





Kubelka-Munk correction

↓

Better fitting to transmission spectrum

https://www.perkinelmer.com/lab-solutions/resources/docs/TCH_reflection-Measurements.pdf

MIR: measurement modes

Reflection:

C- Attenuated total reflection

- introduced in the 1960s, now widely used
- light introduced into a suitable prism (high refraction index) at an angle exceeding the critical angle for internal reflection => an evanescent wave at the reflecting surface
  - sample in close contact with crystal
  - from the interaction of the evanescent wave (exponential decay) with the sample, a spectrum can be recorded with little or no sample preparation

Incident angle > limit angle

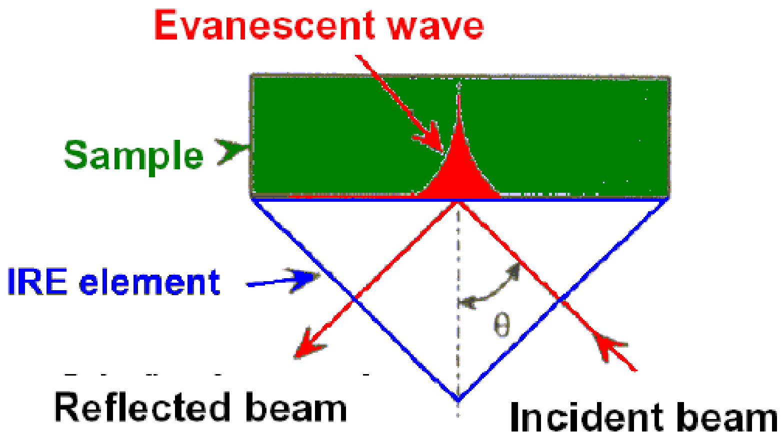
↓

Attenuated total reflections

↓

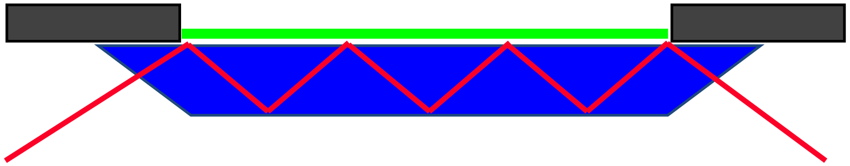
ATR spectral correction

Single Bounce ATR



IRE – internal reflection element = ATR crystal

Multiple reflection ATR



https://www.perkinelmer.com/lab-solutions/resources/docs/TCH_reflection-Measurements.pdf

MIR: measurement modes

Reflection:

C- Attenuated total reflection

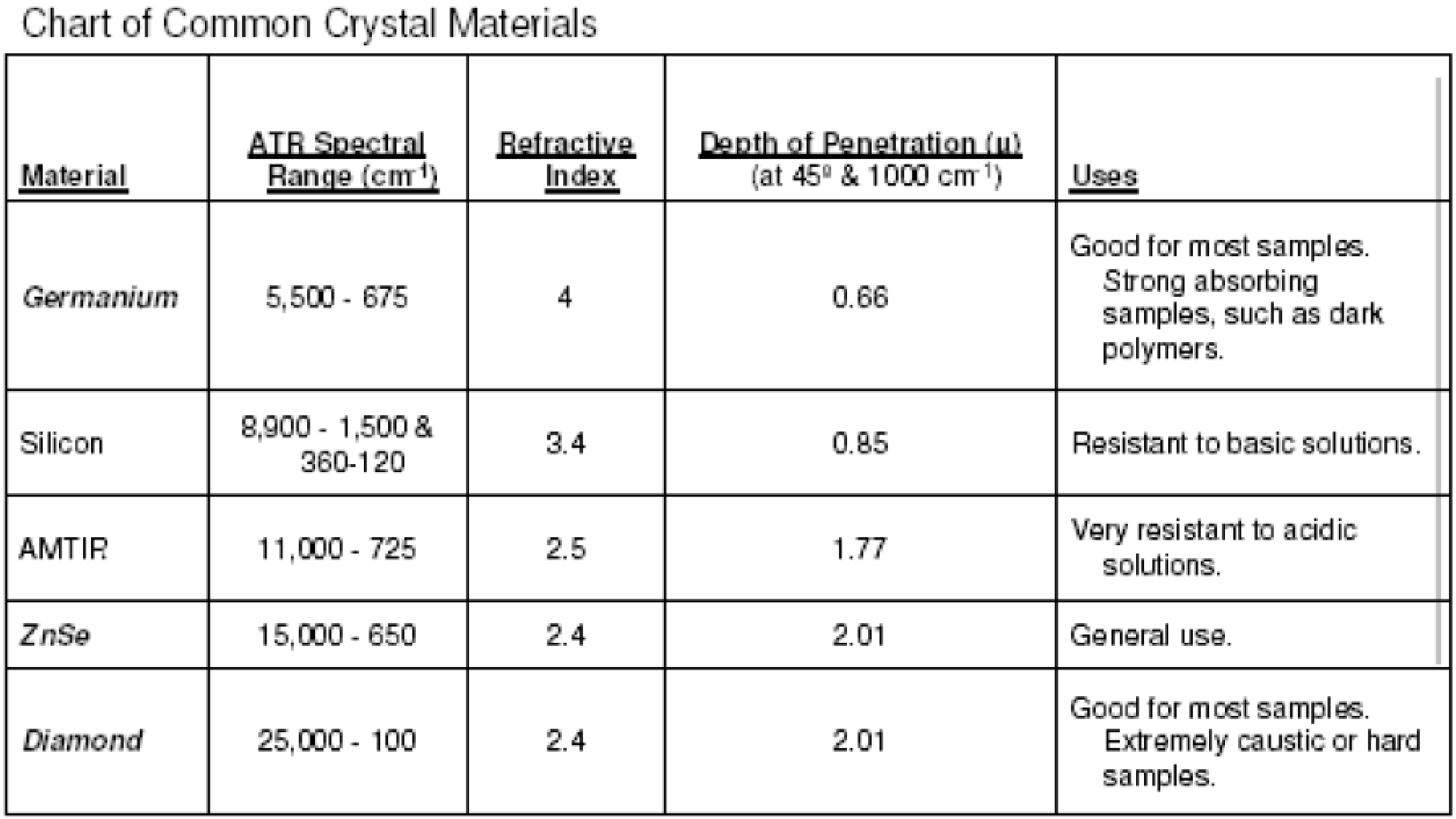
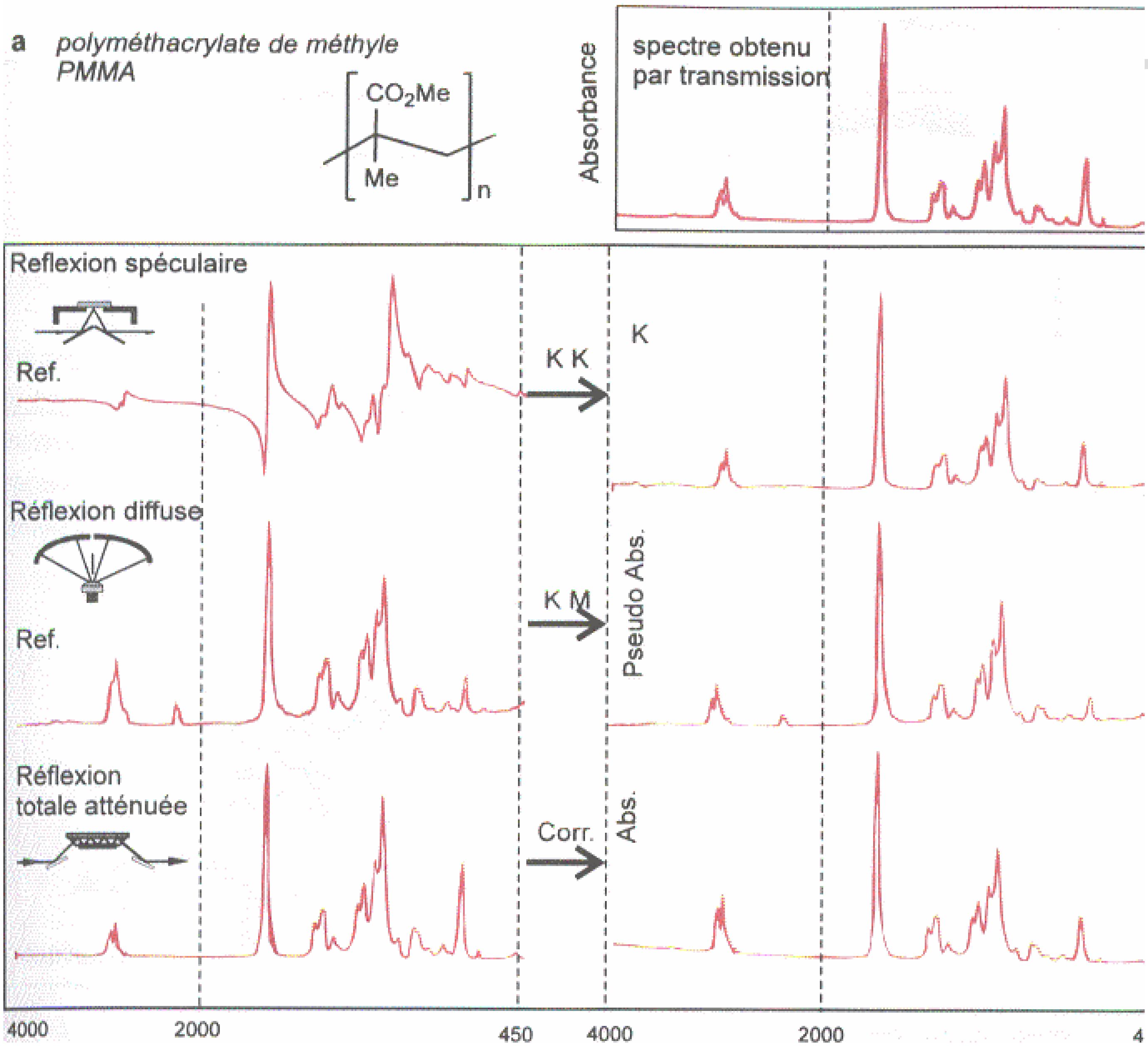


Chart of Common Crystal Materials

Material	ATR Spectral Range (cm^{-1})	Refractive Index	Depth of Penetration (µ) (at 45º & 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.

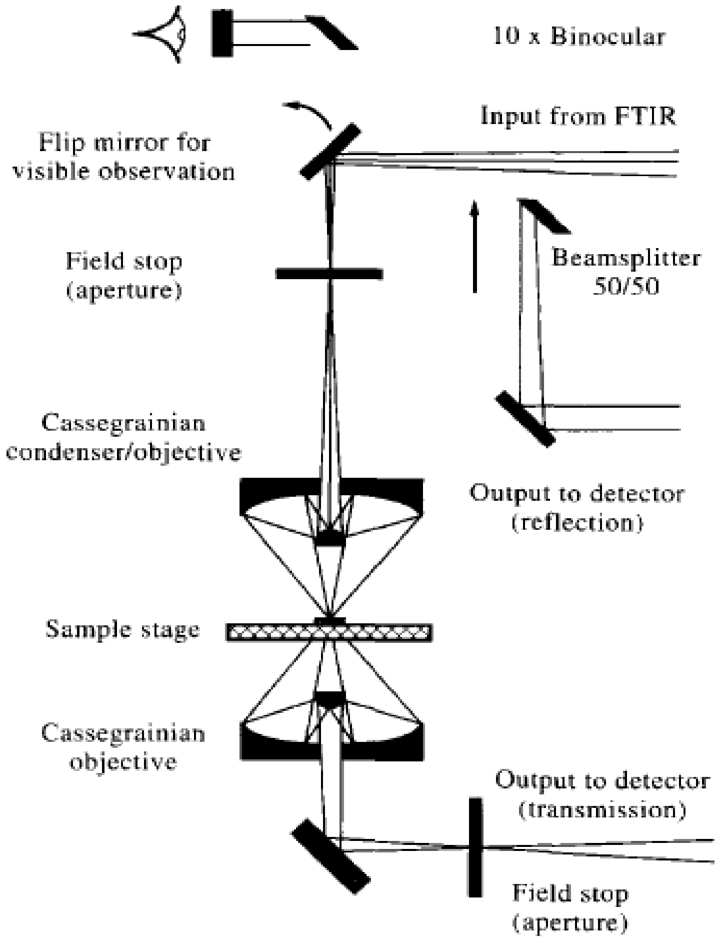
https://www.perkinelmer.com/lab-solutions/resources/docs/TCH_reflection-Measurements.pdf

Reflection:

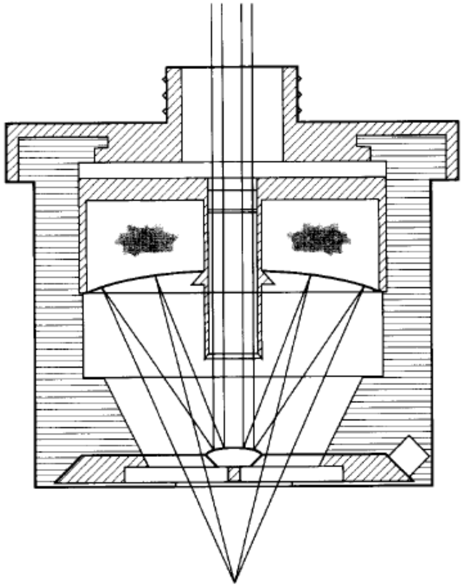

a polyméthacrylate de méthyle
PMMA
CO_2Me
Me
n
Absorbance
spectre obtenu
par transmission
Reflexion spéculaire
Ref.
K K
K
Réflexion diffuse
Ref.
K M
Pseudo Abs.
Réflexion
totale atténuée
Corr.
Abs.
4000
2000
450
4000
2000
4

MIR and NIR: measurement modes

IR microscopy



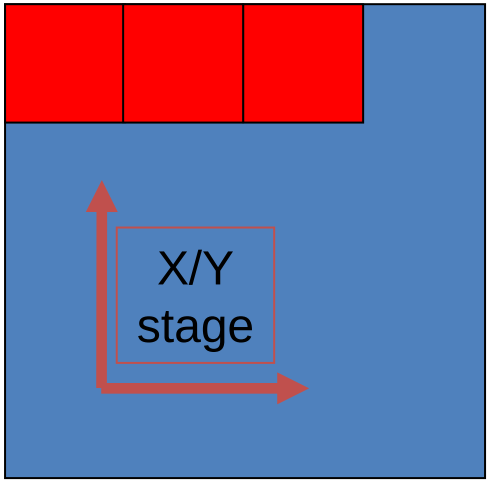
Optical diagram of an infrared microscope.
(from Katon, Micron, 27, 303-314, 1996).



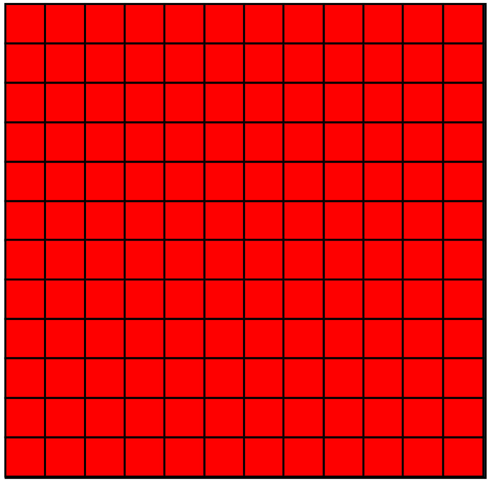
Optical design of a Cassegrain lens used in infrared microscopes
(from Katon, Micron, 27, 303-314, 1996)

MIR and NIR: measurement modes

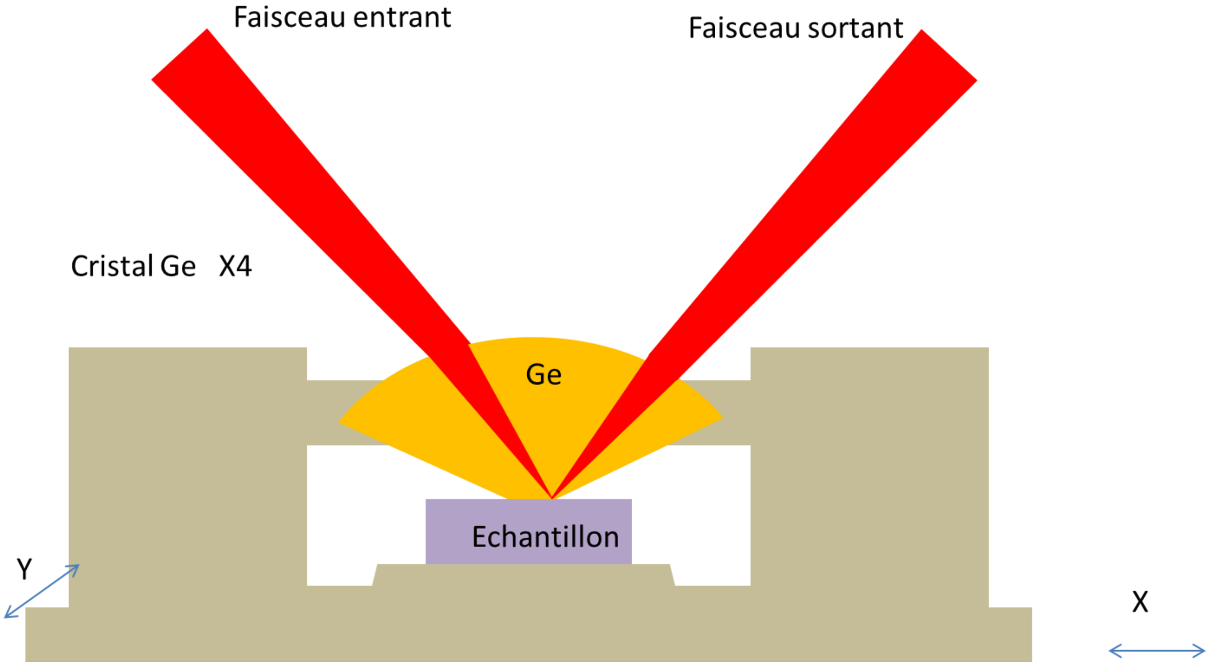
IR imaging*:



Point by point



Simultaneous



ATR imaging

*** hyperspectral imaging**

Quizz

- NIR : 1
- MIR: 2

https://orgchemboulder.com/Technique/Procedures/IR/IRliquid.shtml

Quizz

- NIR : 1
- MIR: 2

https://www.azom.com/article.aspx?ArticleID=12539
25/01/2021

Quizz

- NIR : 1
- MIR: 2

https://www.metrohm.com/en/products/spectroscopy/nirs-lab-analyzers/
25/01/2021

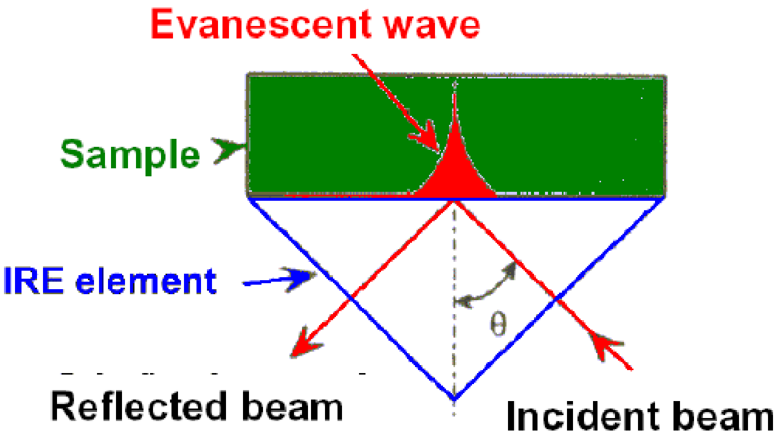
Quizz

- NIR : 1
- MIR: 2

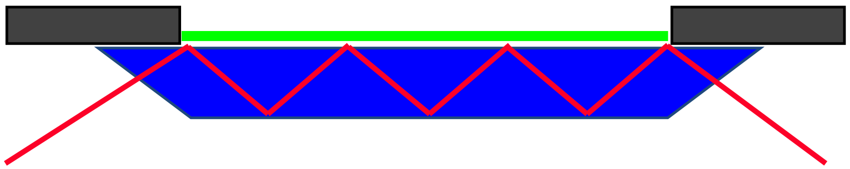
1- https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Book%3A_Physical_Methods_in_Chemistry_and_Nano_Science_(Barron)/04%3A_Chemical_Speciation/4.02%3A_IR_Spectroscopy
2- https://lab-training.com/2015/05/20/comparison-of-different-solid-sampling-techniques-for-ft-ir-spectroscopy/
3- https://qd-europe.com/at/en/products/spectroscopy/ftir-sample-preparation-presses-dies/
4- https://www.reddit.com/r/chemistry/comments/2qs5dt/after_four_hours_of_trial_and_error_the_perfect/

Quizz

- NIR : 1
- MIR: 2



Single Bounce ATR



Multiple reflection ATR

Quizz

- NIR : 1
- MIR: 2

https://www.topsante.com/medecine/votre-sante-vous/sante-pratique/medicaments-il-ne-faut-pas-ecraser-ses-comprimes-248371

25/01/2021

Quizz

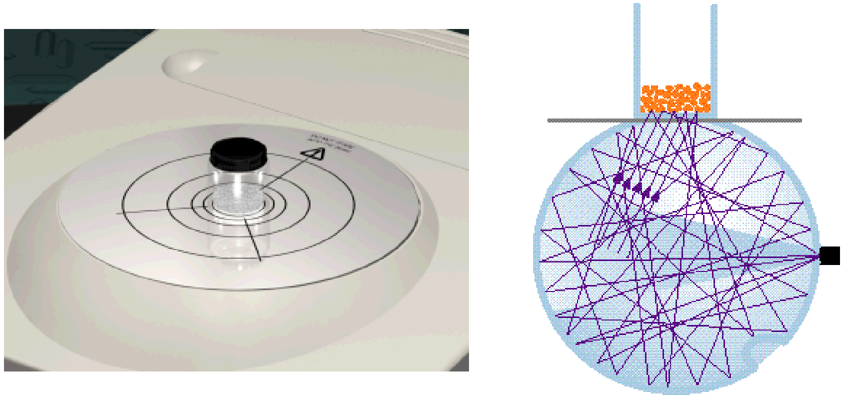
- Transmission : 1
- Diffuse reflection : 2

https://www.topsante.com/medecine/votre-sante-vous/sante-pratique/medicaments-il-ne-faut-pas-ecraser-ses-comprimes-248371

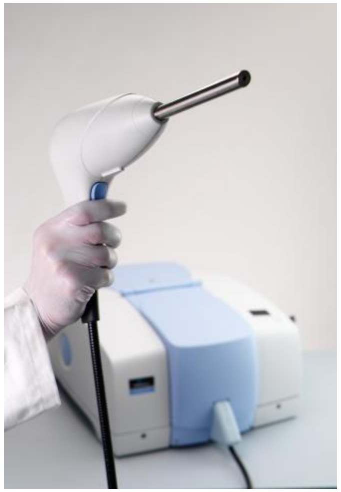
MIR and NIR: measurement modes

NIR measurement accessories:

liquid probe



Vial on a diffuse reflectance accessory



Probe for powder analyses

MIR and NIR applications

MIR and NIR applications

The signal obtained for several applications may be influenced by parameters not related to the chemical nature of the sample

Preprocessing and processing: a key step in spectral data analysis

- Atmospheric correction
- Mie-scattering
- Baseline correction
- MSC- EMSC
- Normalization
- …
- Chemometric approaches

MIR and NIR pharmaceutical applications

Qualitative analyses:

- Identification of raw materials at reception
- Process Analytical technology (PAT)
- Conformity check
- Identification of unknown products:

    - Use of reference spectra or spectral databases: **search, interactive interpretation, knowtitall …**

- Expertise, faults analyses, criminology, counterfeits …
- Final products control
- Homogeneity distribution
- …

Quantitative analyses:

- Quantification of drugs

MIR and NIR pharmaceutical applications

Identification of raw materials at reception:

In order to obtain a reliable method, some key steps must be followed during the method construction and validation, the method design should considerate the following points:

- Definition of the different sample collections
- Acquisition of spectra
- Defining acquisition parameters influencing the quality of spectra
- Choice of possible spectrum processing and identification algorithm
- Validation of the method

Identification is possible if:

Intra-class variability < inter-class variability;
The variability might originate from both the sample and/or the device itself

??

Differences are due to chemical structure or physical properties
Same chemical class
Number of lots / family of samples
Time required for method development and the frequency of the tests

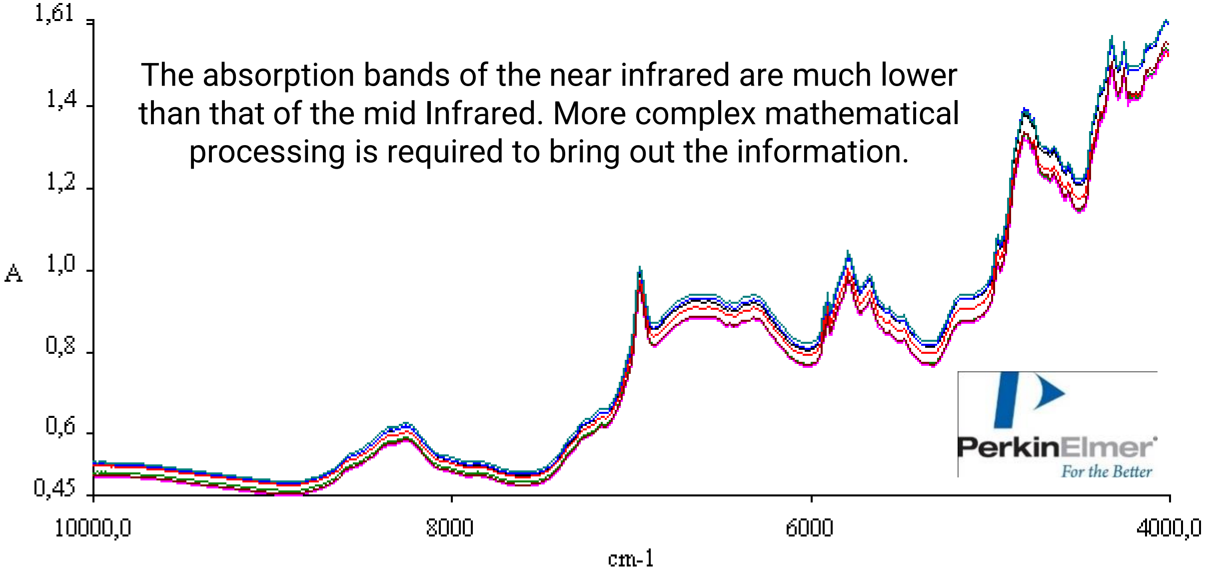
MIR and NIR pharmaceutical applications

General variation origin

Evolution of the samples in time
Different Suppliers
Temperature (especially liquid)
Operator
Sampling method
Instrumental variations
Other…

Variation within the sample

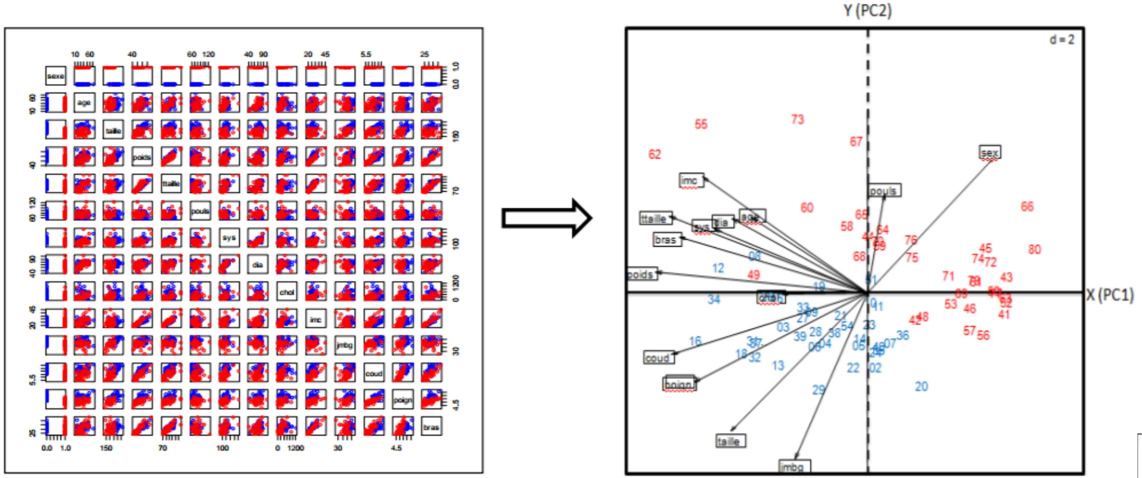
Humidity
Particle size
Residual solvents
Product degradation
Change in Formulation
Other physical or chemical properties


The absorption bands of the near infrared are much lower than that of the mid Infrared. More complex mathematical processing is required to bring out the information.

Chemometrics applied to NIR

Chemometrics uses multivariate statistics, applied mathematics, and computer science, in order to address problems and **extract information** in **chemistry**, biochemistry, medicine, biology and chemical engineering.

Principal Component Analysis [Jackson, 1991; Wold, et al., 1984; Wold, et al., 1987] is the simplest method and is commonly used in first place in multivariate data analysis. PCA is a descriptive method. It consists on reducing the dimensionality of data by retaining the information with the greatest variation within the data.



MIR and NIR pharmaceutical applications

Counterfeit detection

Within the drugs industry, the market for counterfeits is constantly expanding. Due to the health problems that could potentially be caused, searching for and identifying counterfeits is a major challenge for actors in the pharmaceuticals sectors and for health authorities.

Counterfeits are in general products in which:

- **the active ingredient is absent, incorrectly dosed, incorrect, or in a polymorphous form different to that of the original drug,**
- **the active ingredient is correct but the excipient(s) is(are) incorrect,**
- **the formulation is identical to the original drug, but out-of-date and/or reconditioned.**

MIR and NIR:

Characterization and identification of suspected counterfeit miltefosine capsules

Thomas P. C. Dorlo, Teunis A. Eggelte, Peter J. de Vries and Jos H. Beijnen

Active: Miltefosine: treatment of visceral leishmaniasis

Problematic: suspected counterfeit products

Risk: risk to the individual health of patients but also severely threaten the control efforts for tropical infectious diseases and may even contribute to emerging drug resistance

Suspected products: ‘Miltefos’ produced in Bangkok, Thailand. Distributed in Bangladesh

Objective: Characterization et identification de la Miltefosine

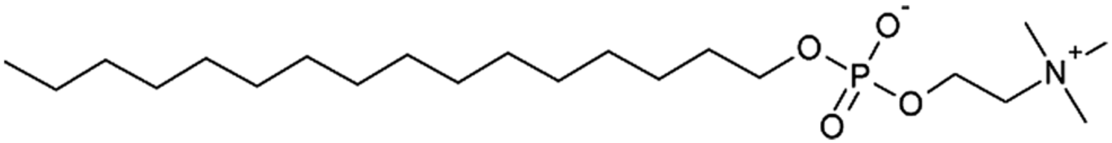
Methods: LC-MS/MS, MIR, NIR and colorimetric (low cost and quick method

MIR:

Characterization and identification of suspected counterfeit miltefosine capsules

Thomas P. C. Dorlo, Teunis A. Eggelte, Peter J. de Vries and Jos H. Beijnen

Miltefosine:



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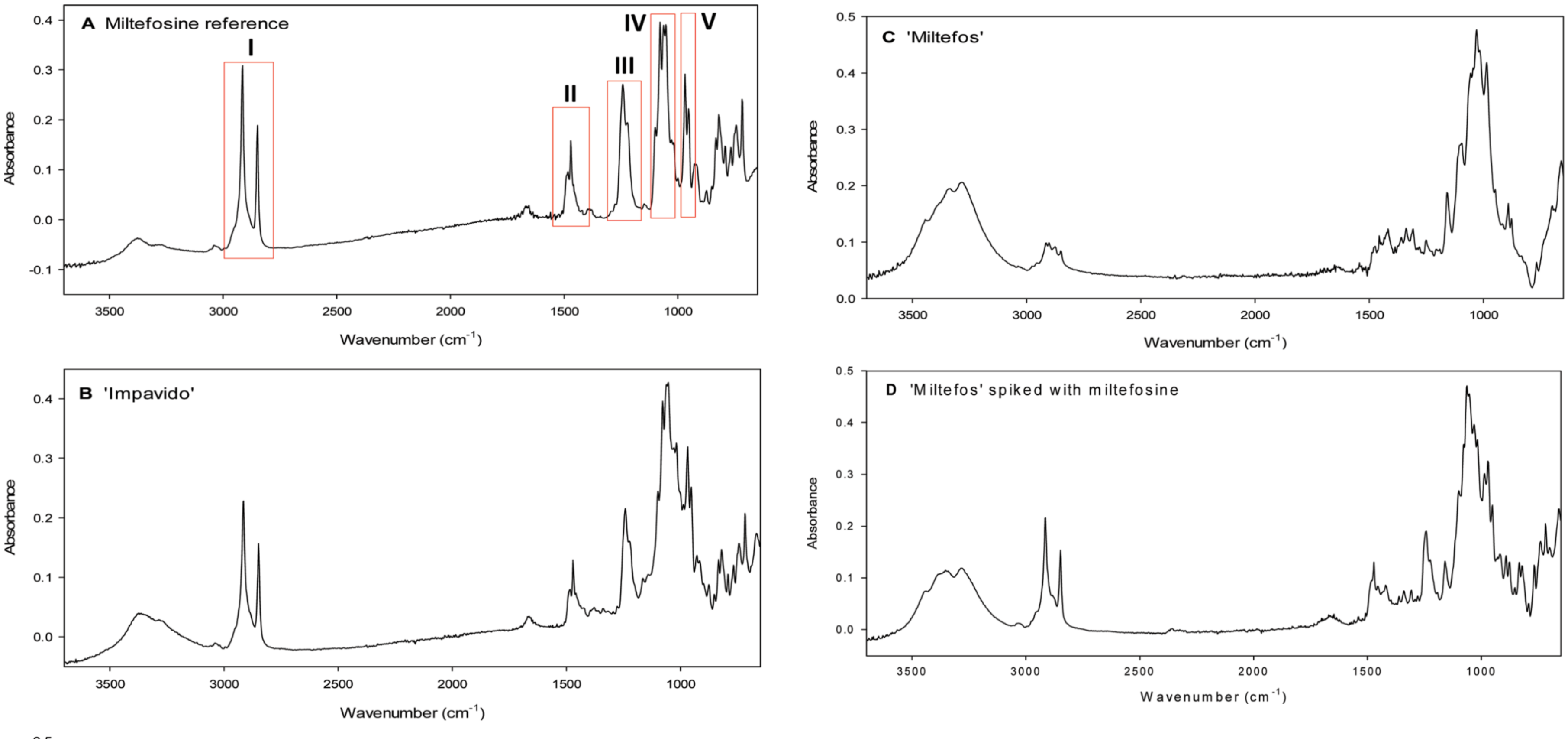
MIR: direct spectral information

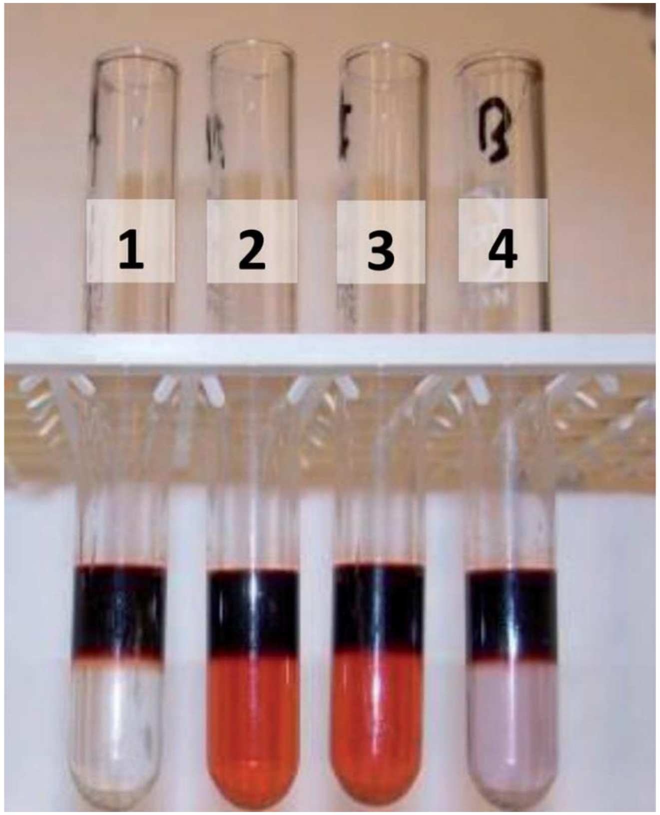
Dissolution in chloroform/water (1:1 / V:V)



Characterization and identification of suspected counterfeit miltefosine capsules

Thomas P. C. Dorlo, Teunis A. Eggelte, Peter J. de Vries and Jos H. Beijnen

Colorimetric test results for miltefosine utilizing the complex formation with ammonium ferrithiocyanate.
Vial 1 contains a blank solution (**wate**r),
vial 2 contains 2 mg mL1 **miltefosine in water**,
vial 3 contains 8 mg ‘**Impavido**’ capsule contents in water
vial 4 contains 8 mg ‘**Miltefos**’ capsule contents.



NIR and Process Analytical Technology (PAT)

The measurements can be carried out:

"on line": the material is diverted then reintegrated or not into the process

"at line": samples are taken and analyzed near to the process within a limited time interval

"in line": the measurements are carried out directly on the material, without modification of the process by in contact measurements or through a window.

NIR infrared spectroscopy may be used for the monitoring, the optimization and the determination of the end of the process. It is suited to the following formulation steps: mixing, granulation, Drying, coating/film coating, Lyophilization, Extrusion, encapsulation, etc.

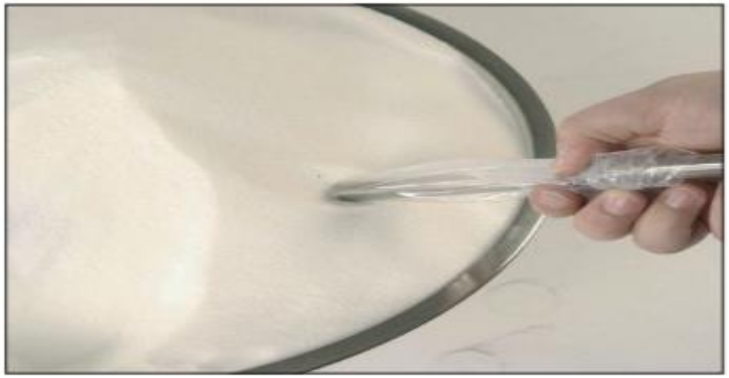


Image 1

Analyse de conformité de matières premières à réception. L'utilisation d'un sachet jetable de polyéthylène autour de la sonde en réflexion diffuse permet d'éliminer les risques de contamination croisée entre analyses. Image © ABB

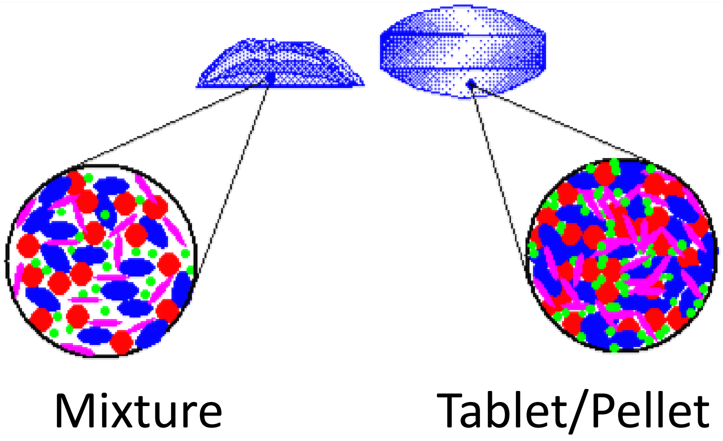
Image 2

Mélangeur ouvert, la sonde Proche Infrarouge est plongée au dessus du mélange (mesure en réflexion) et des pales du mélangeur. Image © Bruker Optics.

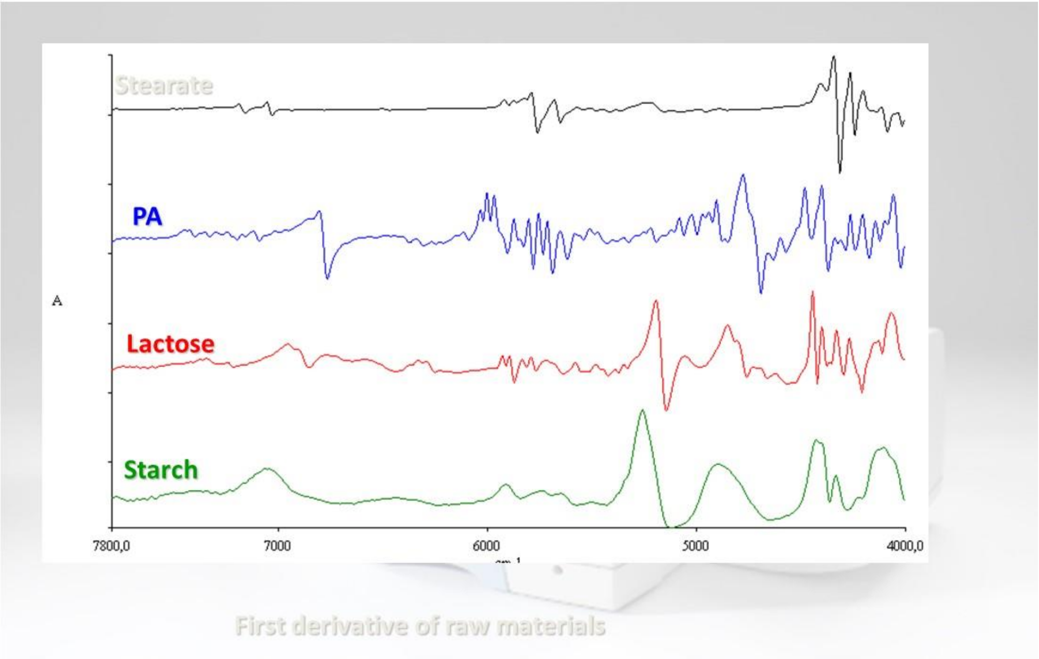
http://www.ondalys.fr/sites/pages/medias/pdf/presse/fchauchard-et-cfontange-spectra-analyse.pdf

NIR imaging at the scale of a pellet

- A pharmaceutical tablet contains various constituents whose content and distribution will influence many parameters:
  - stability, hardness, dissolution, bioavailability, ...
- The distribution depends on several factors:
  - granulometry, time and mixing processes, compression parameters, ...



NIR imaging

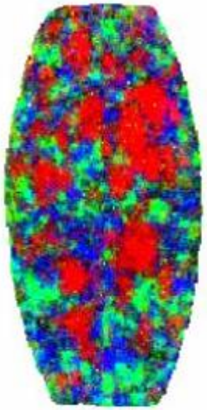


First derivative of raw materials

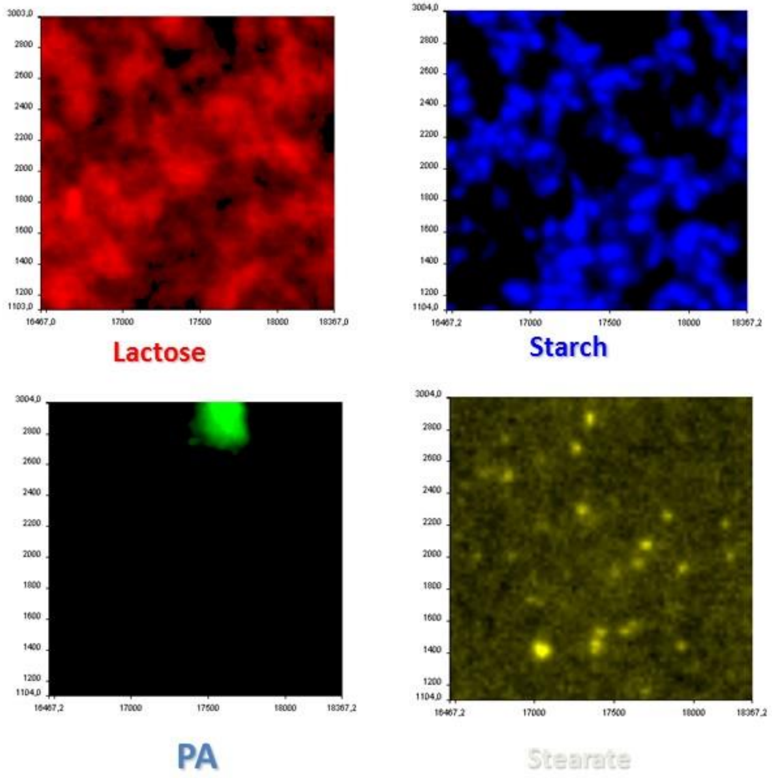
NIR imaging at the scale of a pellet

Information about this repartition within the pellet permits to:

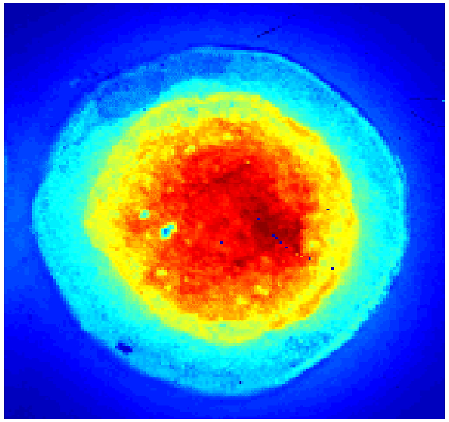
- Optimize the process parameters
- Identify the causes of defaults
- Verify the origin of the tablets
- Estimate the active ingredient release rate



Distribution of different raw components within the image, calculated by least square method



NIR imaging at the scale of a pellet



Encapsulation of the active ingredient into micro-spherical granules allows sustained release over extended time periods.

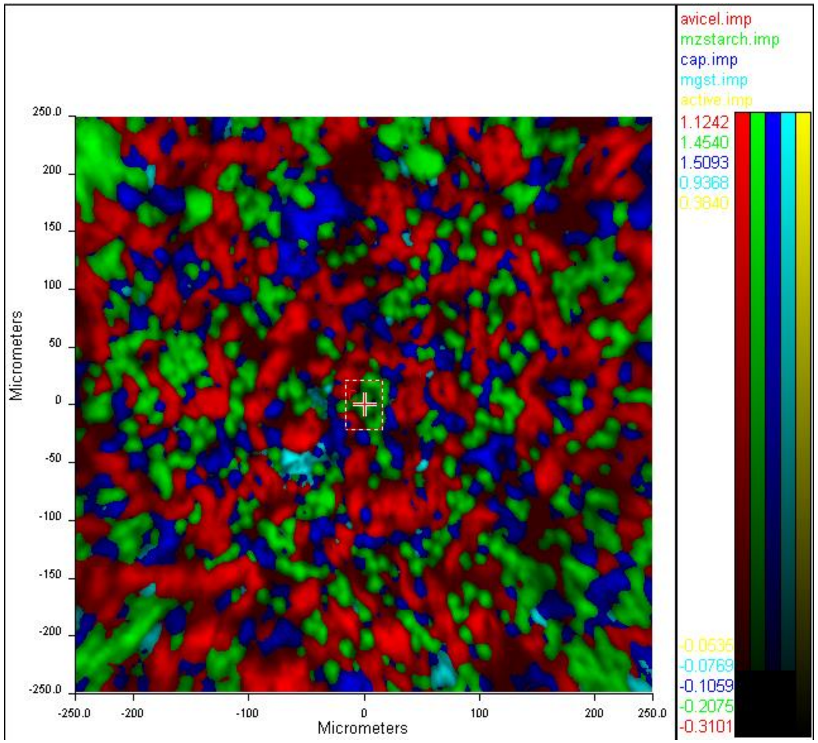
Granules of differing solubility are packaged together into a single capsule that staggers the release of the active ingredient in a complex pattern.

The distribution of different granules within a capsule may be determined by spreading the granules out and imaging the separated particles. By choosing the appropriate wavelength, different granules are chemically differentiated.

This makes use of the MatrixNIR’s ability to image a relatively large area (9x12 mm) in macro mode and conduct massively parallel spectroscopic measurements.

On closer examination, using the system’s micro mode, each granule can be seen to have an internal structure that determines its active release characteristics.
The image obtained, at 1520nm, from a selection of time release granules within a single capsule.
A typical, cold medicine, time release capsule The image above shows the chemical structure of a single, time-release granule (less than 1 mm in diameter) from the same cold medicine capsule shown at above right.

MIR ATR imaging



Avicel	🟥
Amidon	🟩
Calcium phosphate	🟦
Magnesium stearate	■

MIR and NIR: quantitative analysis

Quantitative analysis:

Chemical dosage; ppm to percentage:

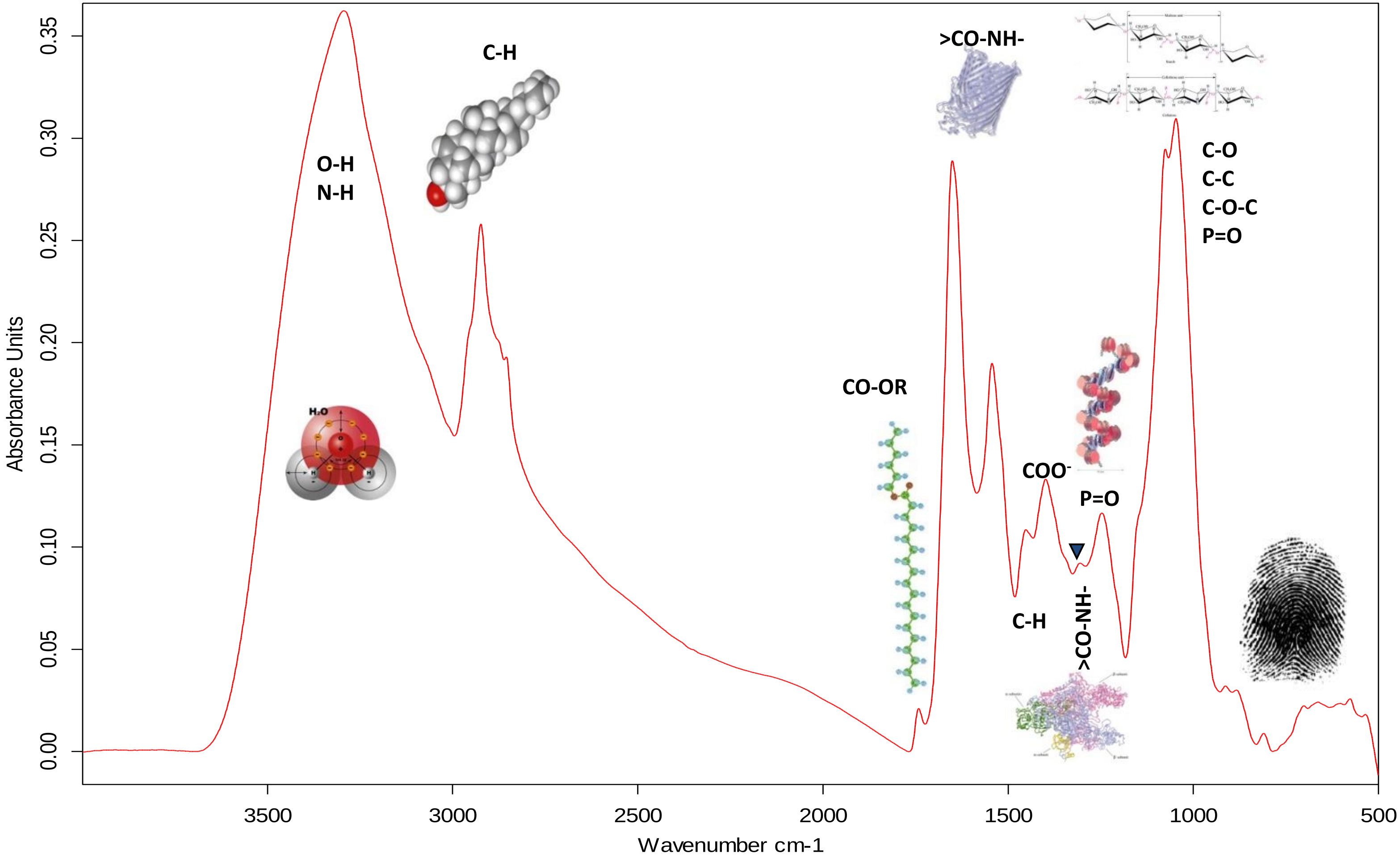
Ex: - active ingredient in tablets (NIR)

-hydrocarbon in water (MIR), benzene in gasoline (MIR), ester in gas oil (MIR)

Protein, moisture, fat, dry extract, in food matrices,

MIR and NIR biomedical applications

IR spectra of biological samples



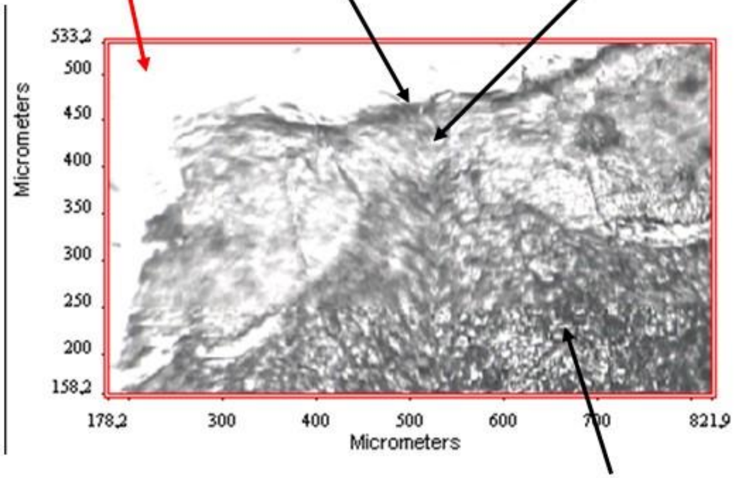
MIR biomedical applications

Treatment of spectral images – Elimination of outliers

paraffin

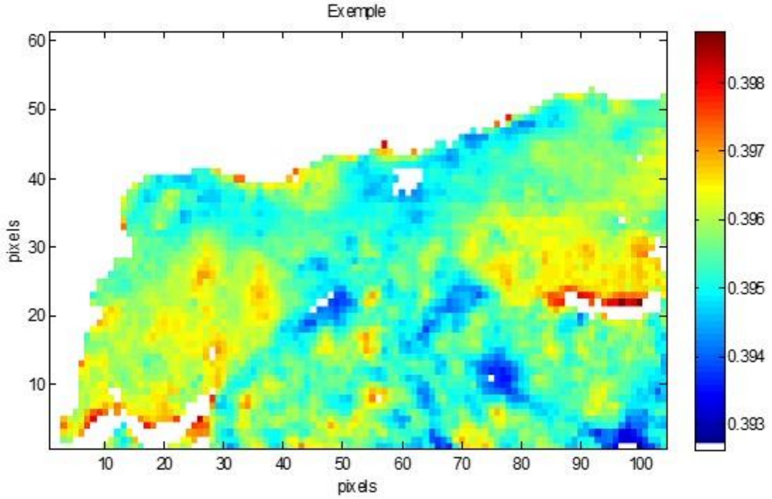
stratum corneum

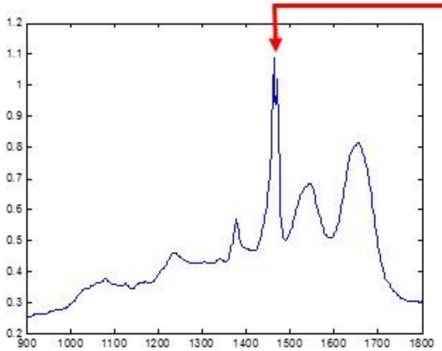
epidermis



dermis

Reconstructed spectral image (total AUC)



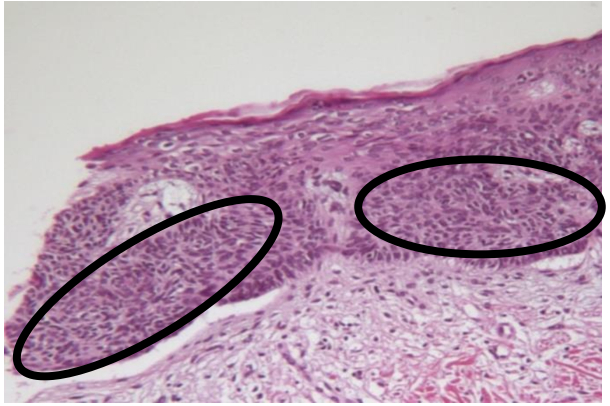


Contribution of wax

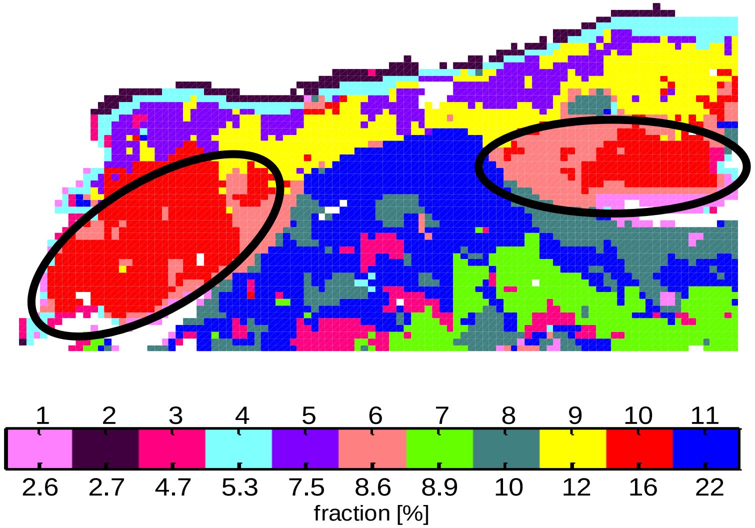
MIR biomedical applications

Multivariate data analysis

- Spectra classification, K-means



Standard histological image (HE staining)

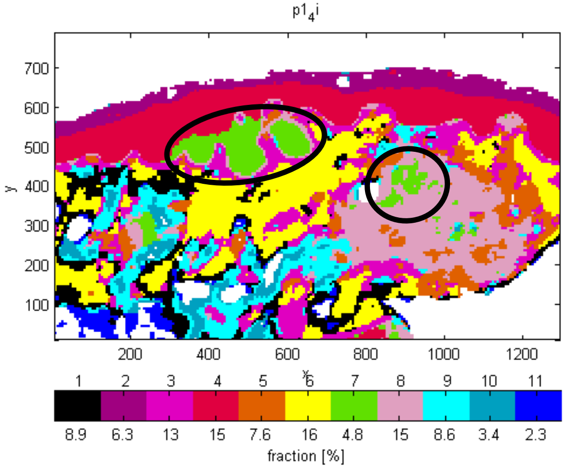


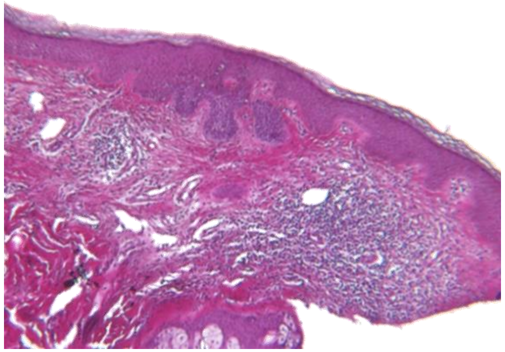
pseudo-color image based on 11 clusters

Superficial BCC

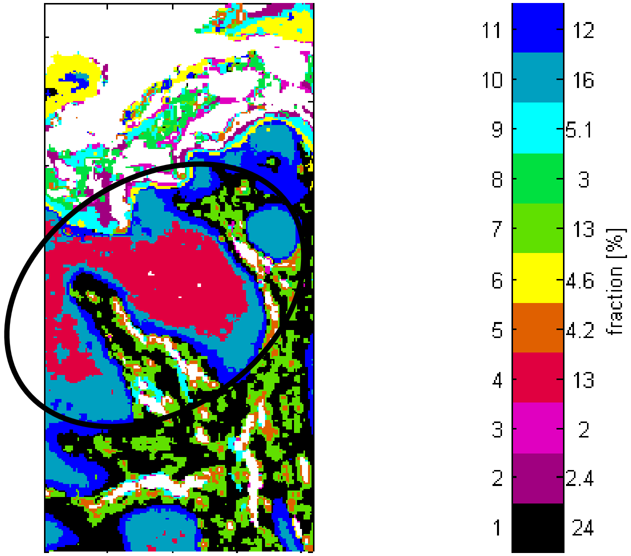
MIR biomedical applications

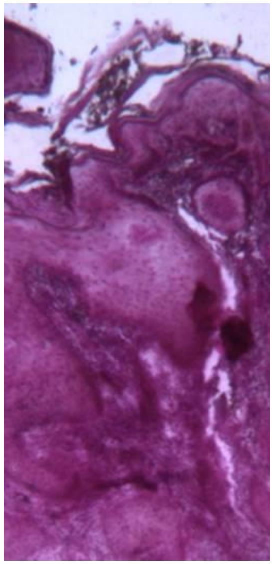
Pseudo-color images (K-means classification– 11 clusters)





BCC superficial





SCC