Electro-Magnetic Infrared RAdiation

# Synchrotron Infrared Microspectroscopy In Environmental Science: Bioremediation

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# Outlines

**I. Heavy metals accumulation in plants**

**II. Phytoremediation**

**III. Basic principles of Infrared Microspectroscopy**

**IV. Application in Environmental Science**

*a) Nickel hyperaccumulating and non-hyperaccumulating genotypes of* ***Senecio coronatus*** *from South Africa*

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# Heavy metals accumulation in plants

- What are heavy metals?
- How are they bound to the soil
- Plant uptake of heavy metals
- Reducing heavy metal contamination

# What is a heavy metal?

- Loose definition- specific gravity
- Usually associated with toxicity in plants (but some micronutrients produce toxicity symptoms as well) or animals
- “trace metal” metals in ppm concentrations in earth’s crust

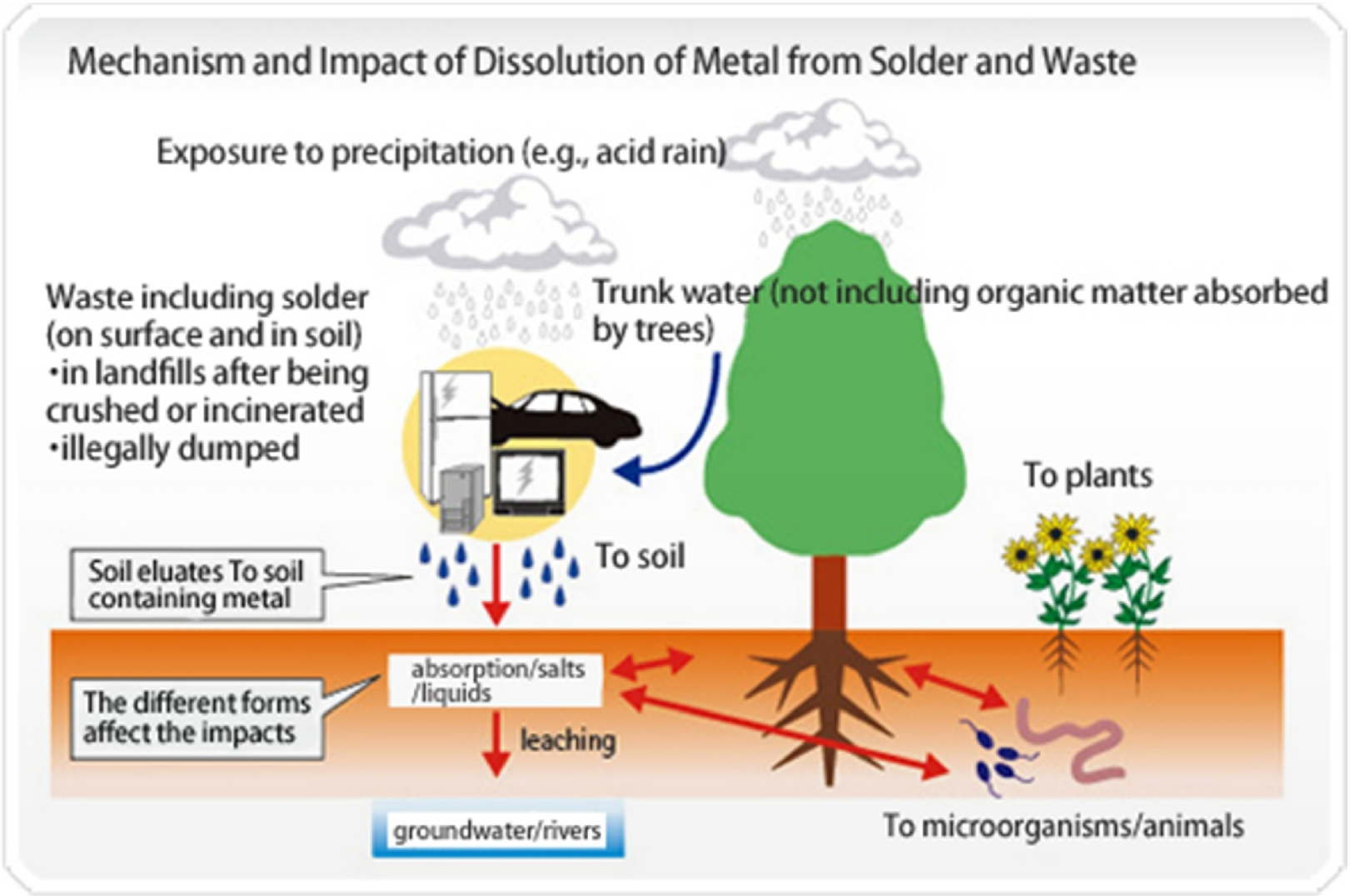
specific density > 4-7 $g/cm^3$

# What is a heavy metal?

The most common heavy metals are:
Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sn, Zn

Are often very toxic to living organisms over a certain concentration threshold

# Heavy metals in the environment



# Some heavy metals and their environmental and physiological effects

**TABLE 18.8 Sources of Selected Inorganic Soil Pollutants**

| *Chemical* | *Major uses and sources of soil contamination* | *Organisms principally harmed*[a] | *Human health effects* |
|---|---|---|---|
| Arsenic * | Pesticides, plant desiccants, animal feed additives, coal and petroleum, mine tailings, and detergents | H, A, F, B | Cumulative poison, possibly cancer |
| Cadmium | Electroplating, pigments for plastics and paints, plastic stabilizers, batteries, and phosphate fertilizers | H, A, F, B, P | Heart and kidney disease, bone embrittlement |
| Chromium | Stainless steel, chrome-plated metals, pigments, refractory brick manufacture, and leather tanning | H, A, F, B | Mutagenic; also essential nutrient |
| Copper * | Mine tailings, fly ash, fertilizers, windblown copper-containing dust, and water pipes | F, P | Rare; essential nutrient |
| Lead * | Combustion of oil, gasoline, and coal; iron and steel production; and solder on water-pipe joints | H, A, F, B | Brain damage, convulsions |
| Mercury | Pesticides, catalysts for synthetic polymers, metallurgy, and thermometers | H, A, F, B | Nerve damage |
| Nickel | Combustion of coal, gasoline, and oil; alloy manufacture; electroplating; batteries; and mining | F, P | Lung cancer |
| Selenium | High Se geological formations and irrigation wastewater in which Se is concentrated | H, A, F, B | Rare; loss of hair and nail deformities; essential nutrient |
| Zinc | Galvanized iron and steel, alloys, batteries, brass, rubber manufacture, mining, and old tires | F, P | Rare; essential nutrient |

[a] H = humans, A = animals, F = fish, B = birds, P = plants.
Data selected from Moore and Ramamoorthy (1984) and numerous other sources.

Brady and Weil, 1999

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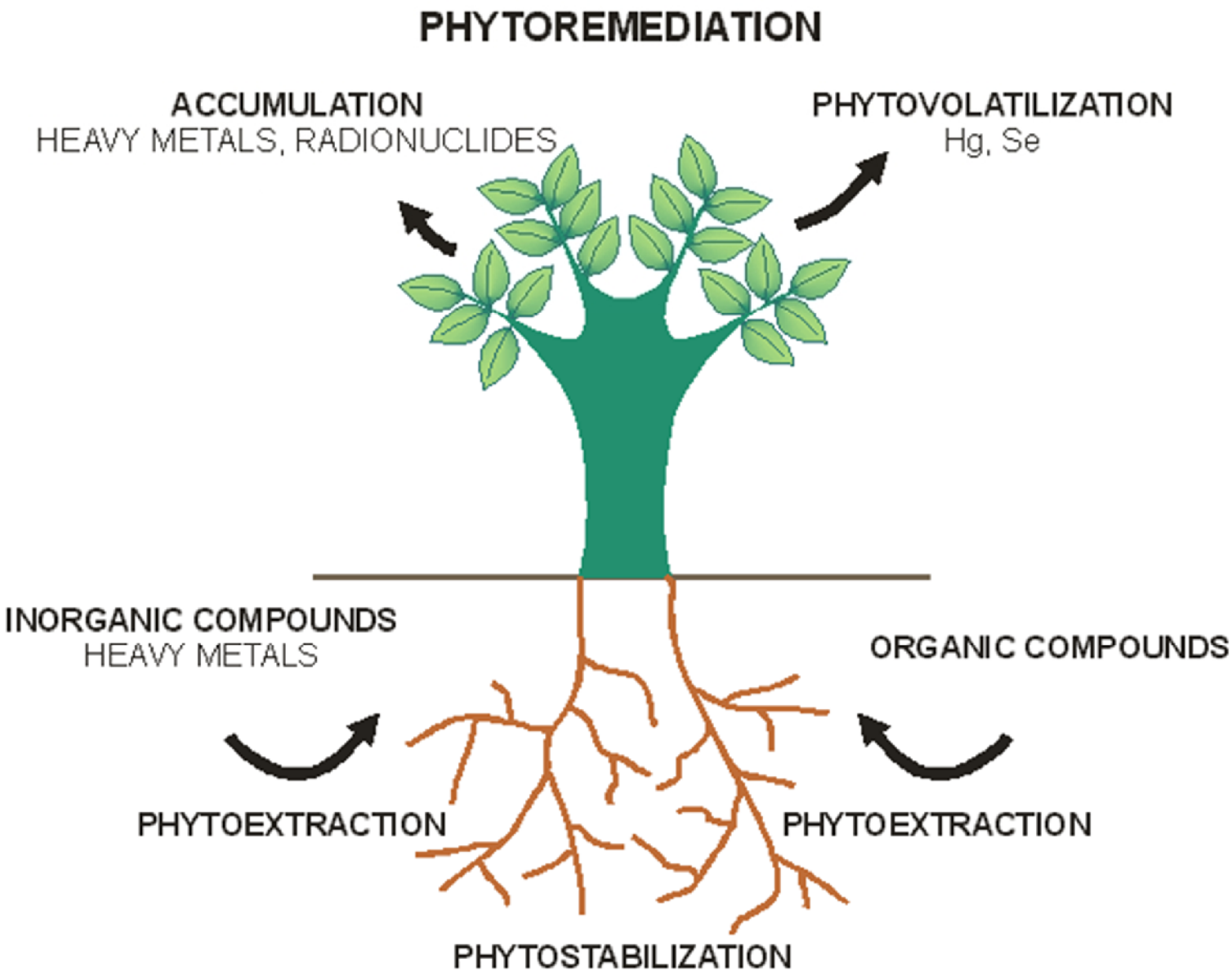
# Phytoremediation

**The use of plants to remove heavy metals and other pollutants from soils and waters**

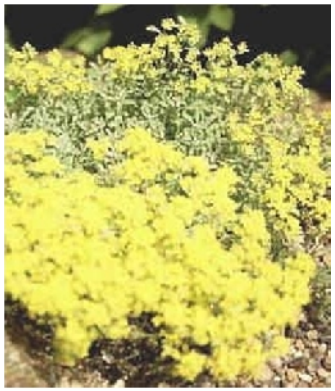

PHYTOREMEDIATION
ACCUMULATION
HEAVY METALS, RADIONUCLIDES
PHYTOVOLATILIZATION
Hg, Se
INORGANIC COMPOUNDS
HEAVY METALS
ORGANIC COMPOUNDS
PHYTOEXTRACTION
PHYTOEXTRACTION
PHYTOSTABILIZATION

# Phytoremediation of Heavy metals

Phytoextraction is a subprocess of phytoremediation in which plants remove dangerous elements or compounds from soil or water.

1. The metal needs to be dissolved in something the plant roots can absorb.
2. The plant roots need to absorb the heavy metal.
3. The plant needs to chelate the metal in order to both protect itself and make the metal more mobile(this can also happen before the metal is absorbed).

# HYPERACCUMULATOR SPECIES & PHYTOREMEDIATION PLANTS



*Alyssum serpyllifolium*

*Brassica juncea*

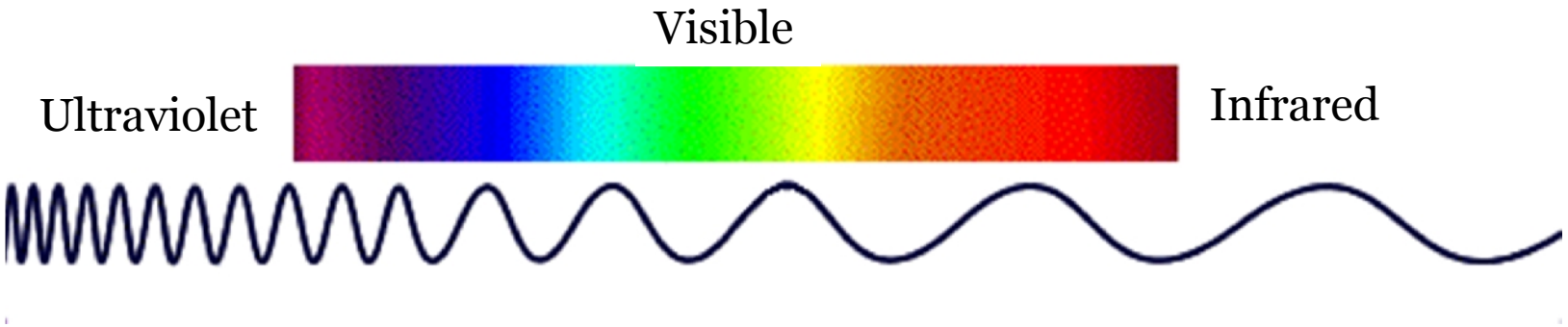
*Thlaspi caerulescens*

*Liriodendron tulipifera*

*Senecio Coronatus*

*Pteris vittata*

# FOURIER-TRANSFORM INFRARED SPECTROSCOPY AS A TOOL TO MONITOR CHANGES IN PLANT STRUCTURE IN RESPONSE TO SOIL CONTAMINANTS



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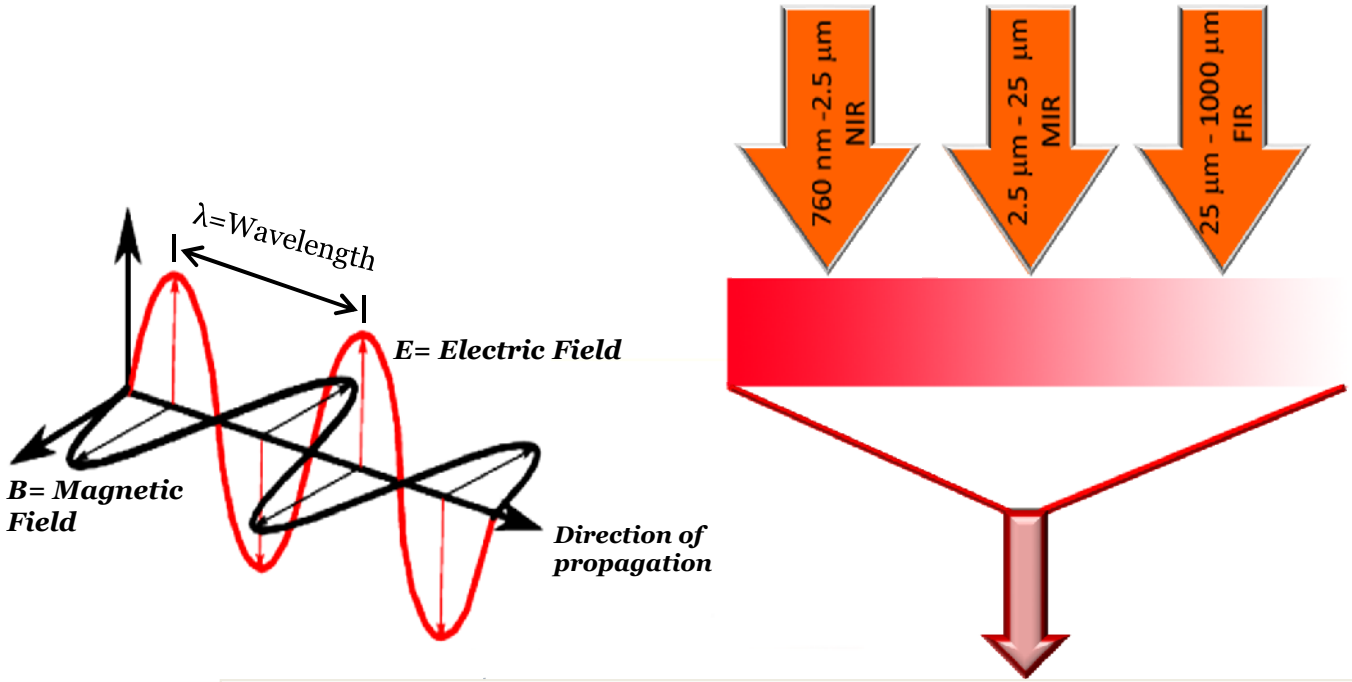
# The Infrared energy range




✓ Energy range: 1 to ~500 µm

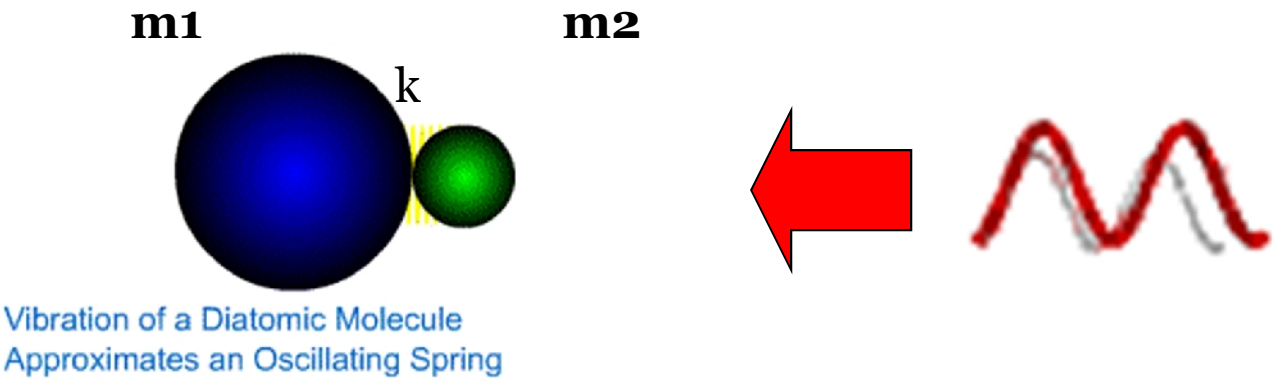
( 10000 to 20 cm-1 or 1.23 to 0.0025 eV)

- ✓ ~1 to ~2.5 µm ( 10000-4000 $cm^{-1}$) Near IR
- ✓ ~2.5 à 20 µm ( 4000-500 $cm^{-1}$) Mid- IR
- ✓ ~20 à ~2500 µm ( 500-50 $cm^{-1}$) Far IR

✓ They are long wavelengths, distributed in a wide range!

## The IR Spectroscopic Process

A varying electromagnetic field is absorbed - a covalent bond oscillates – due to the oscillation of the dipole of the molecule




Vibration of a Diatomic Molecule
Approximates an Oscillating Spring

$$\upsilon_{osc} = \frac{1}{2\pi}\sqrt{k\frac{m_1 + m_2}{m_1 m_2}}$$

**Frequency shift with:**

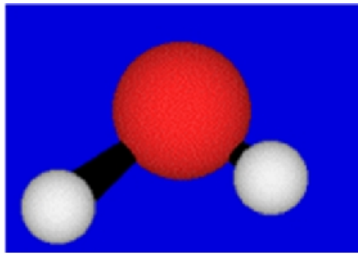
**- nature of atoms**

**- environment change**

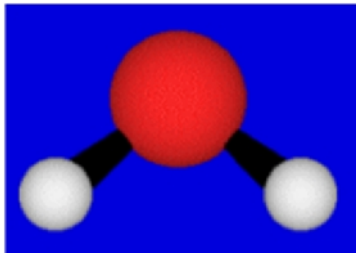
- ***IR Spectroscopy -*** study of the interaction of atoms and molecules with electromagnetic IR radiation

- ***Probe the world at molecular level***

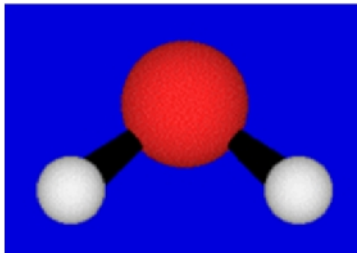
~3.3 μm



3756 cm-1

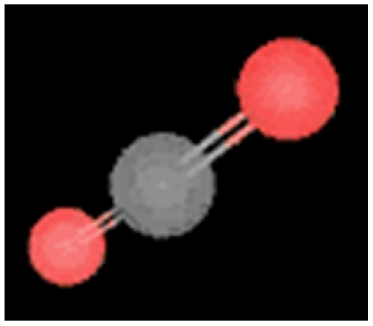


3652 cm-1

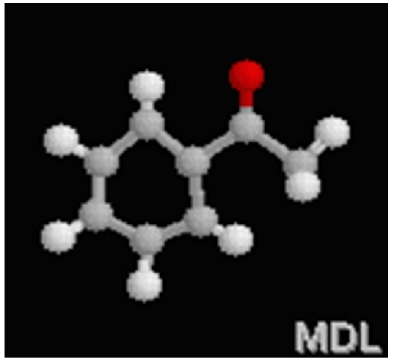


1595 cm-1

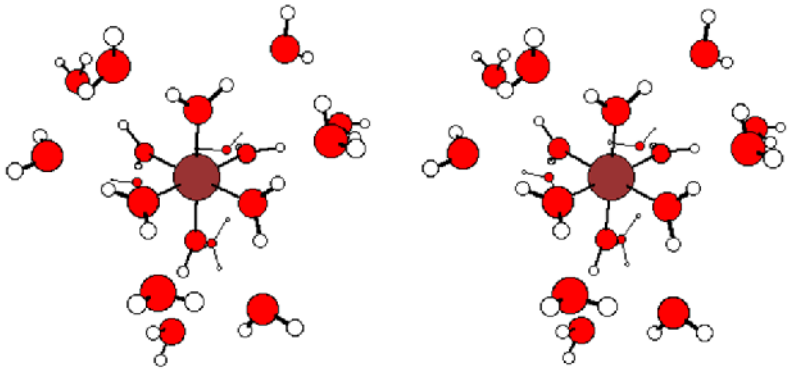
~6 μm



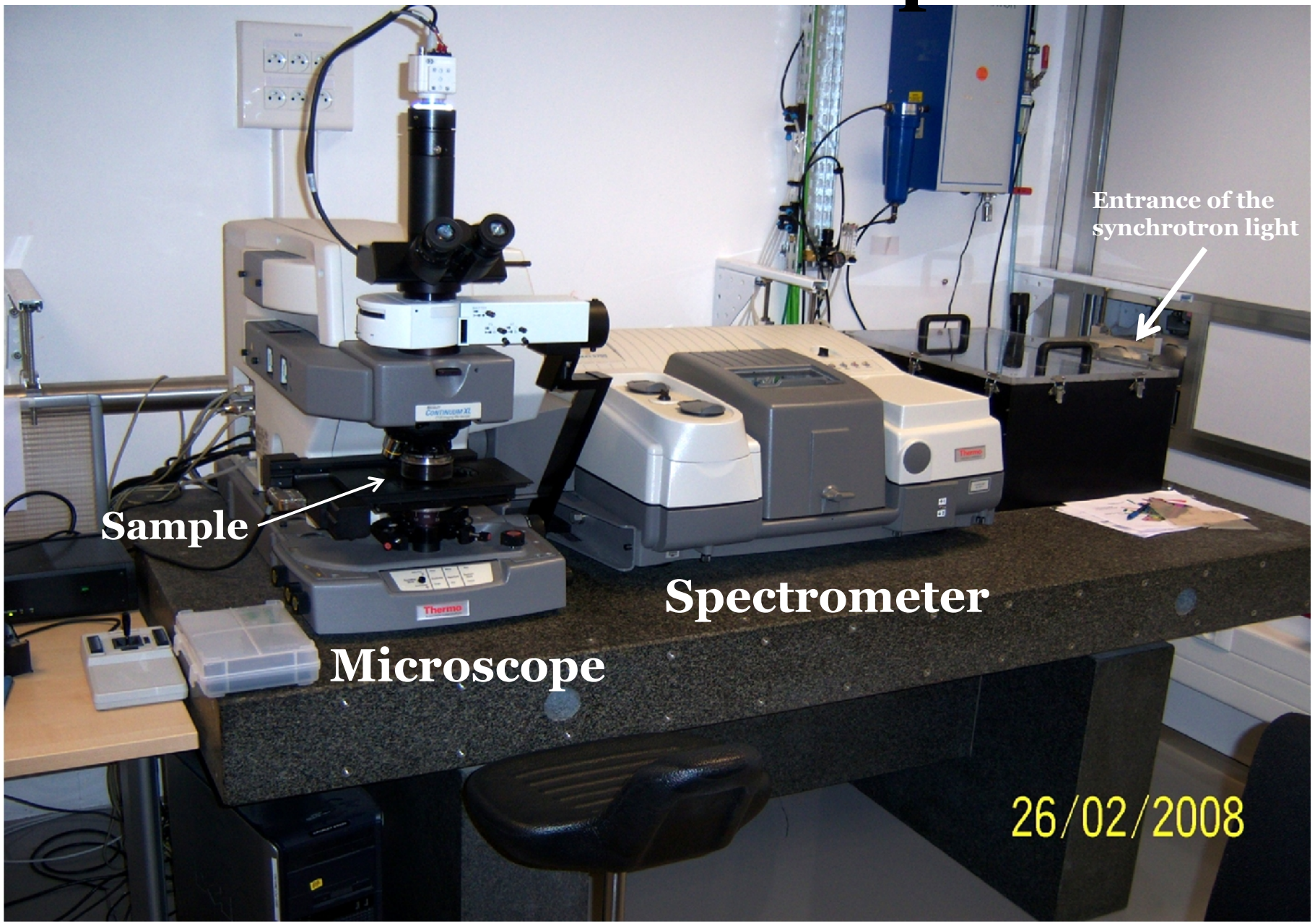
~10 μm




~30 μm



# Infrared Setup



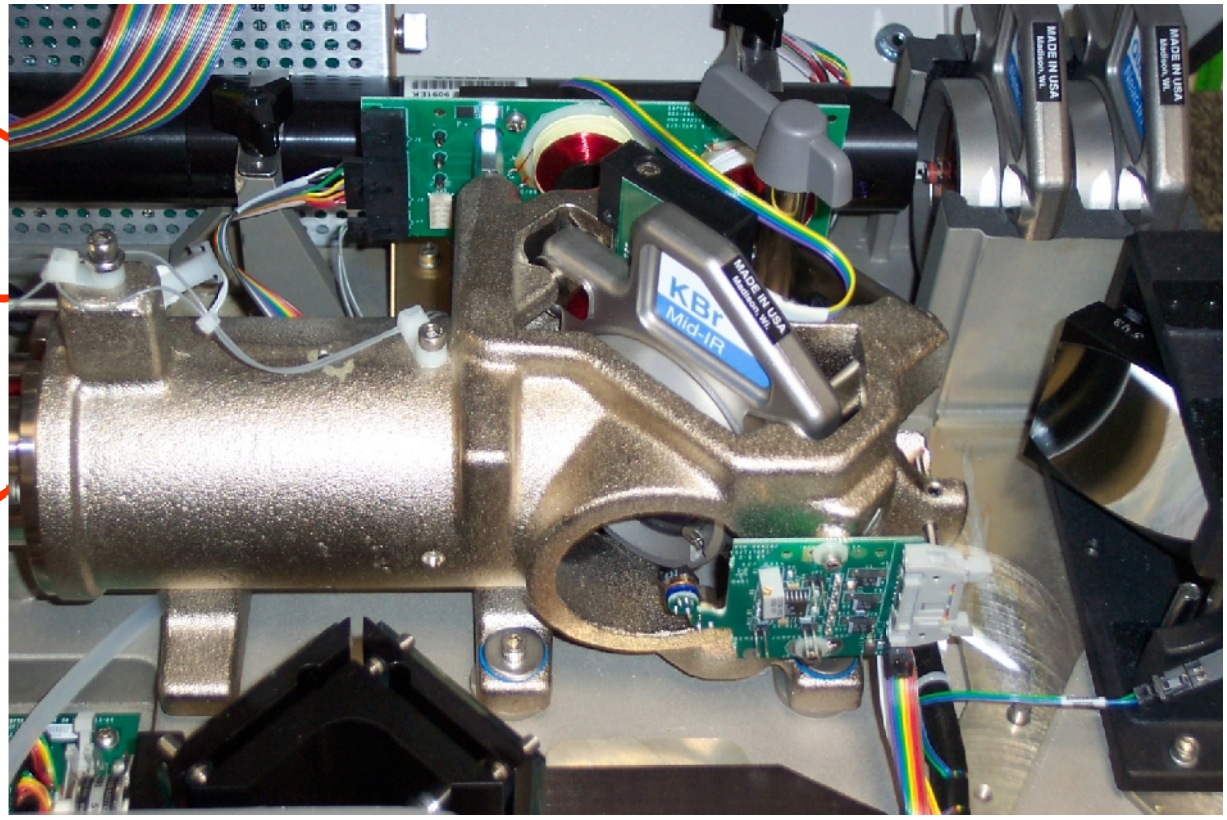
# Infrared spectroscopy today…

- ✓ Widely used in academic as well as in industry , primilarly for compound identification
- ✓ « Classical » infrared spectrometer is composed of three main components:

1- An IR source

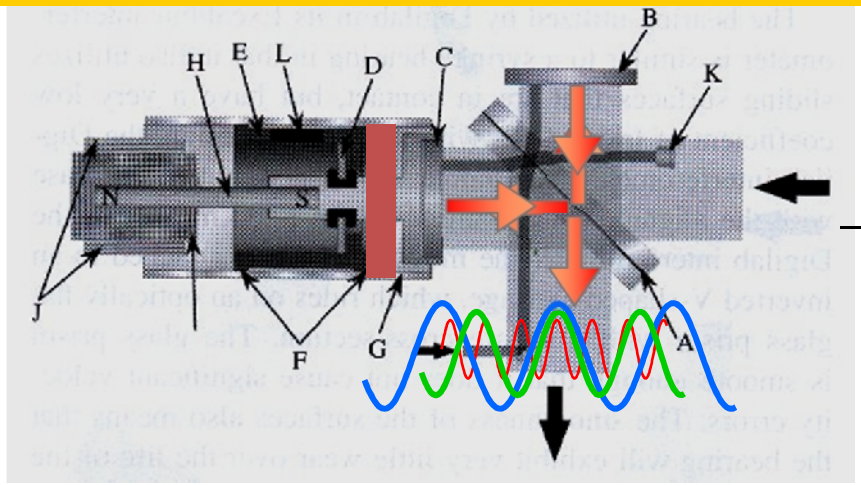
2- Interferometer

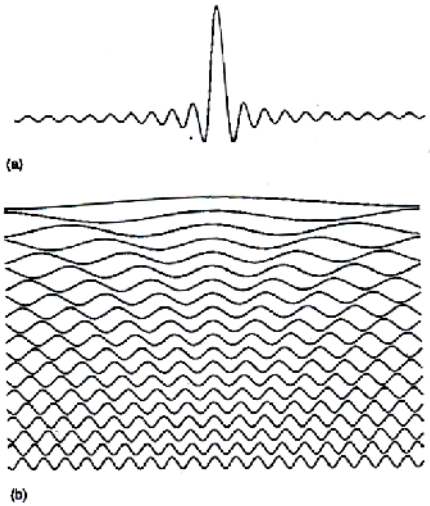
3- Detectors

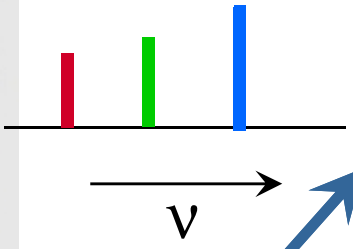


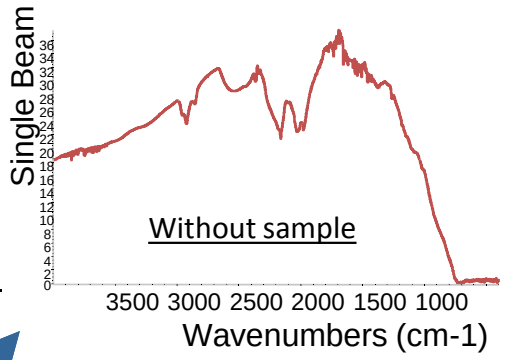
# Fourier Transform Infrared Spectroscopy

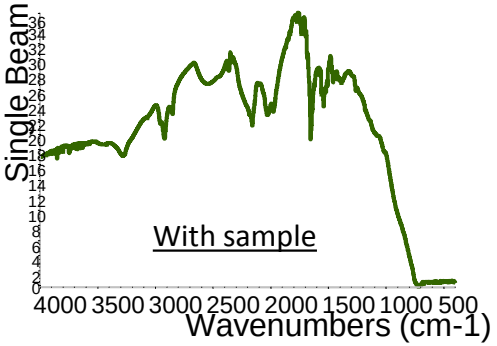
No monochromator, all wavelengths collected



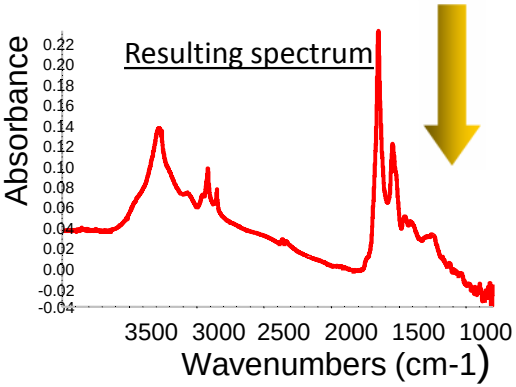



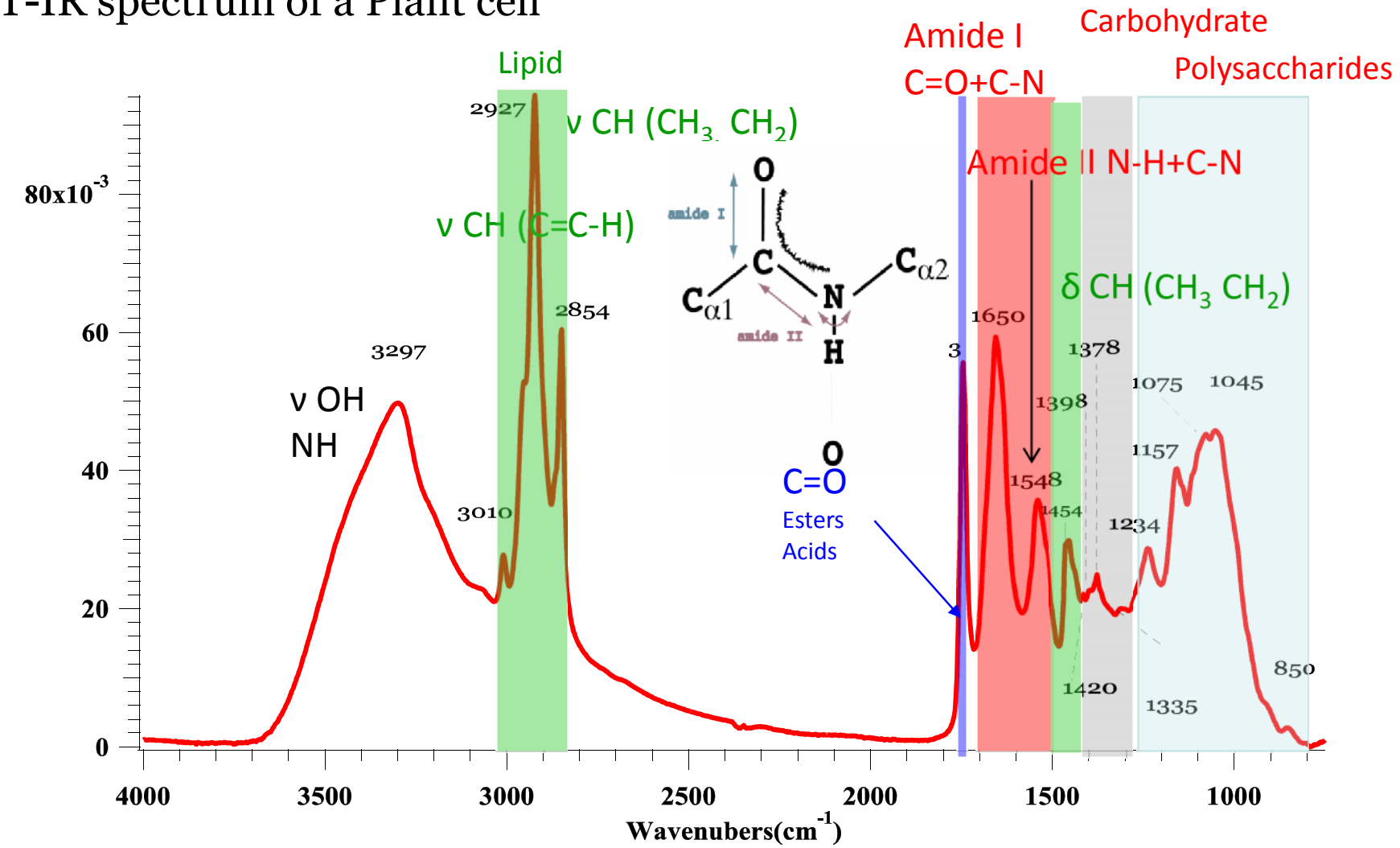




Fast Fourier Transform



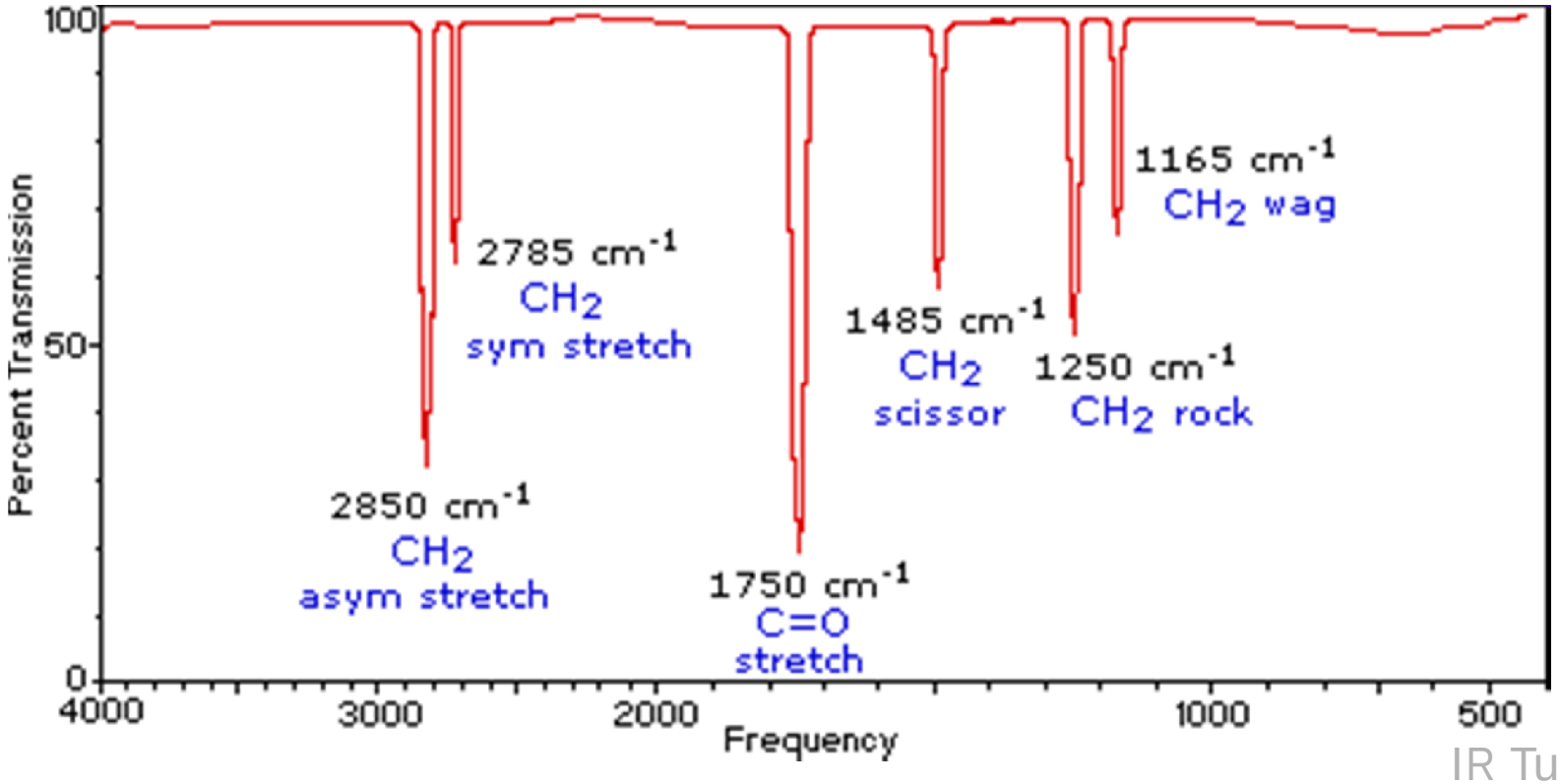
# Fourier transformed infrared spectroscopy and plant biology

FT-IR spectrum of a Plant cell



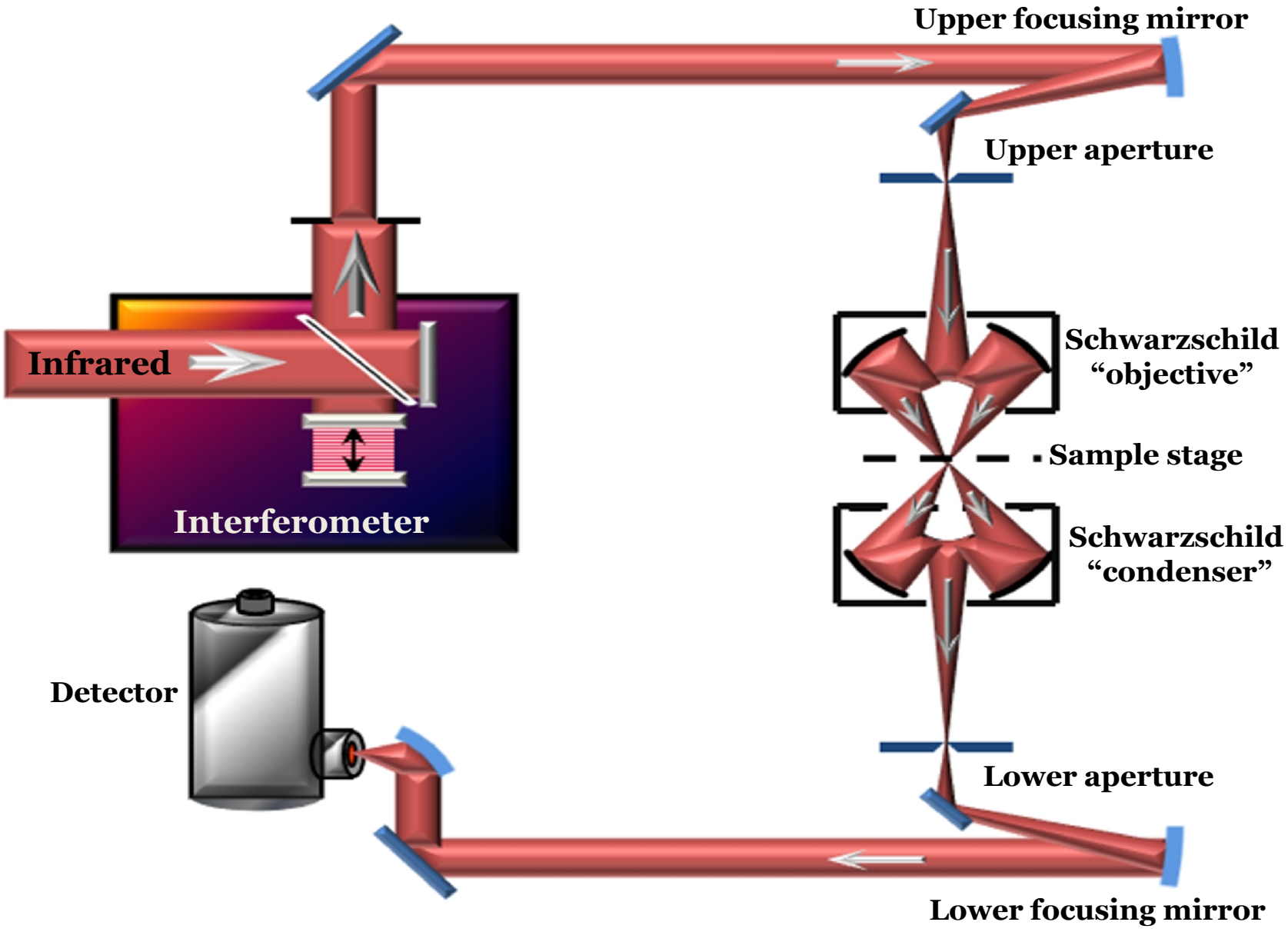
Infrared absorption phenomena: a kind of «  finger print » for each ensemble of molecular groups

**IR spectra of  formaldehyde , $H_2C=O$**

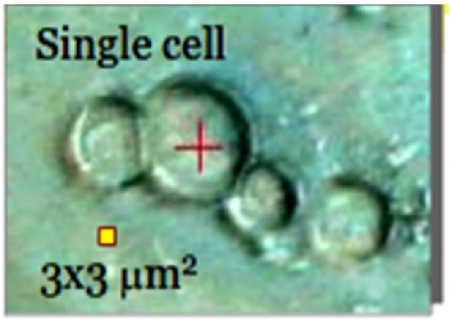


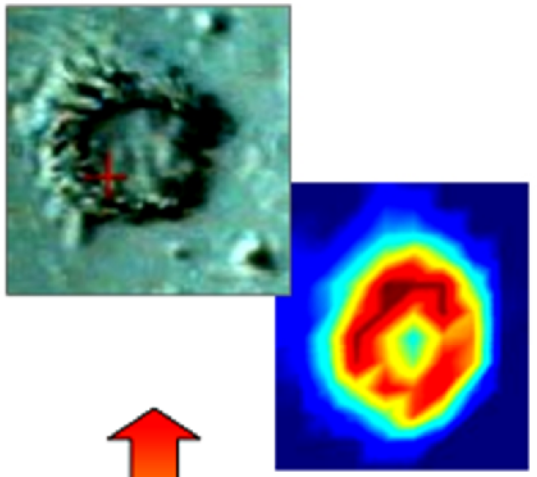

IR Tutorial

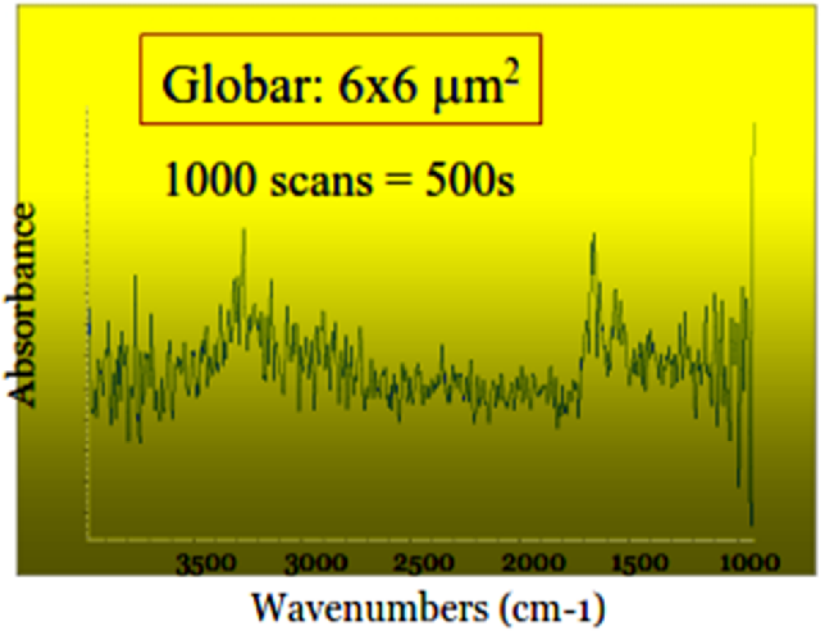
# IR Microspectroscopy

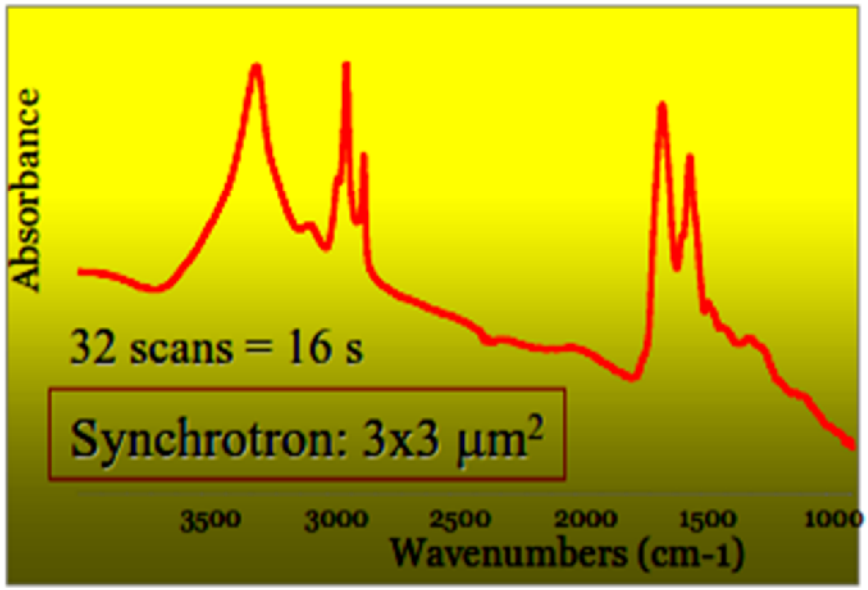


# Synchrotron radiation as infrared source in Microspectroscopy

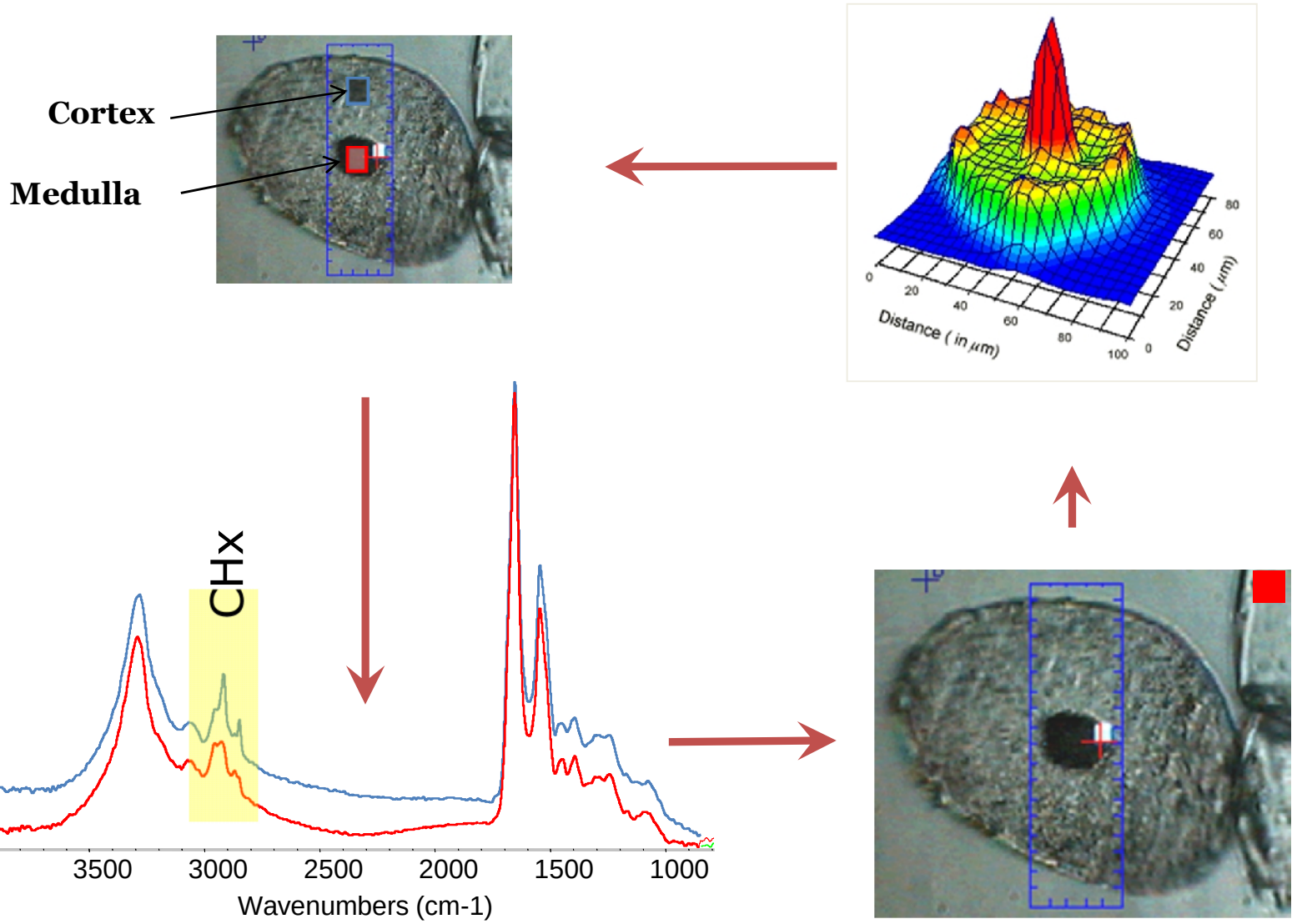




# From microscopy to chemical imaging



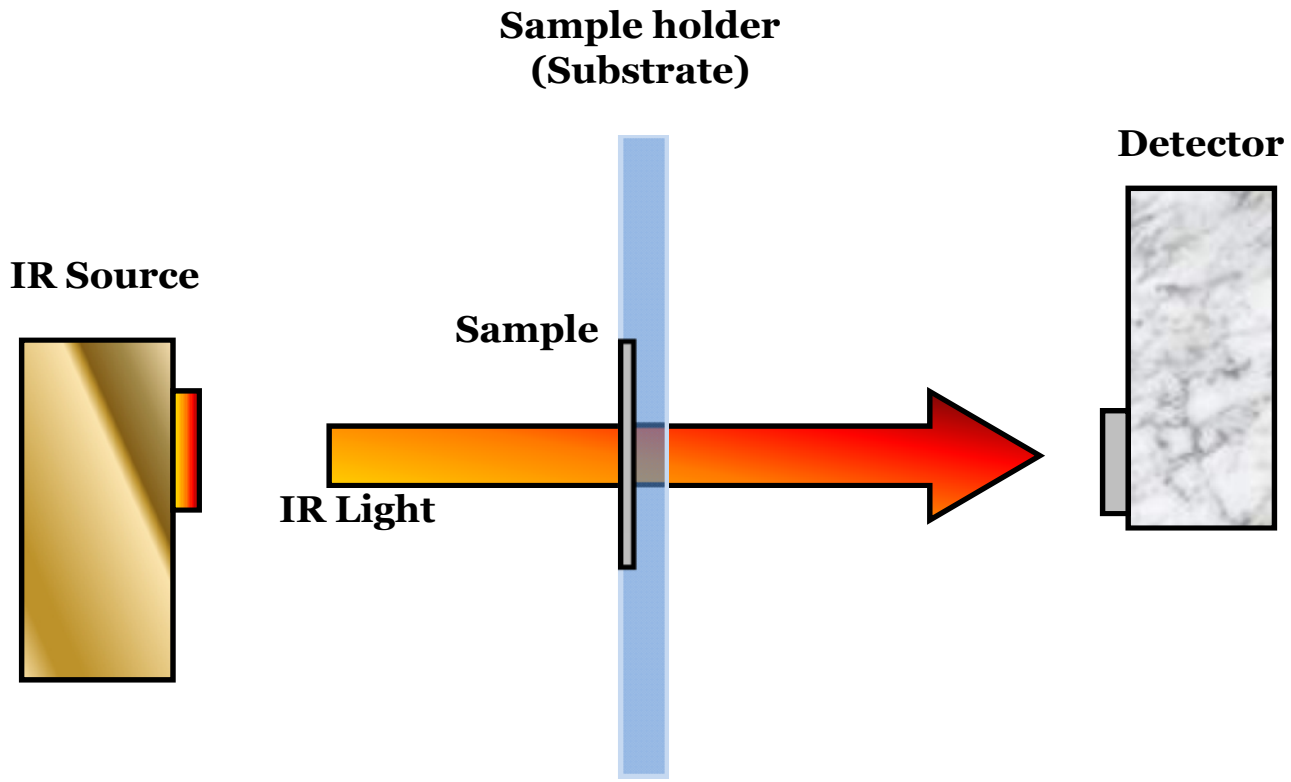
# Which type of samples can be studied Using infrared spectroscopy ?

- Any sample in practically any state may be studied:

1. Solids:
2. Liquids:
3. Gases:

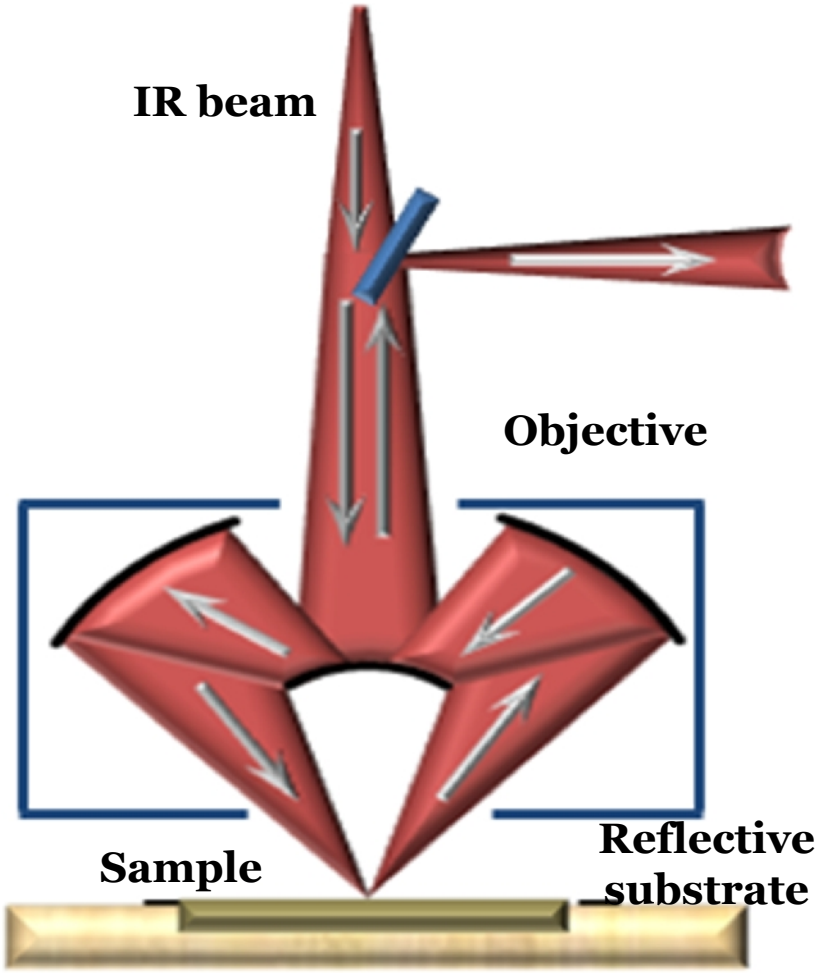
# Sampling techniques

- **Transmission**



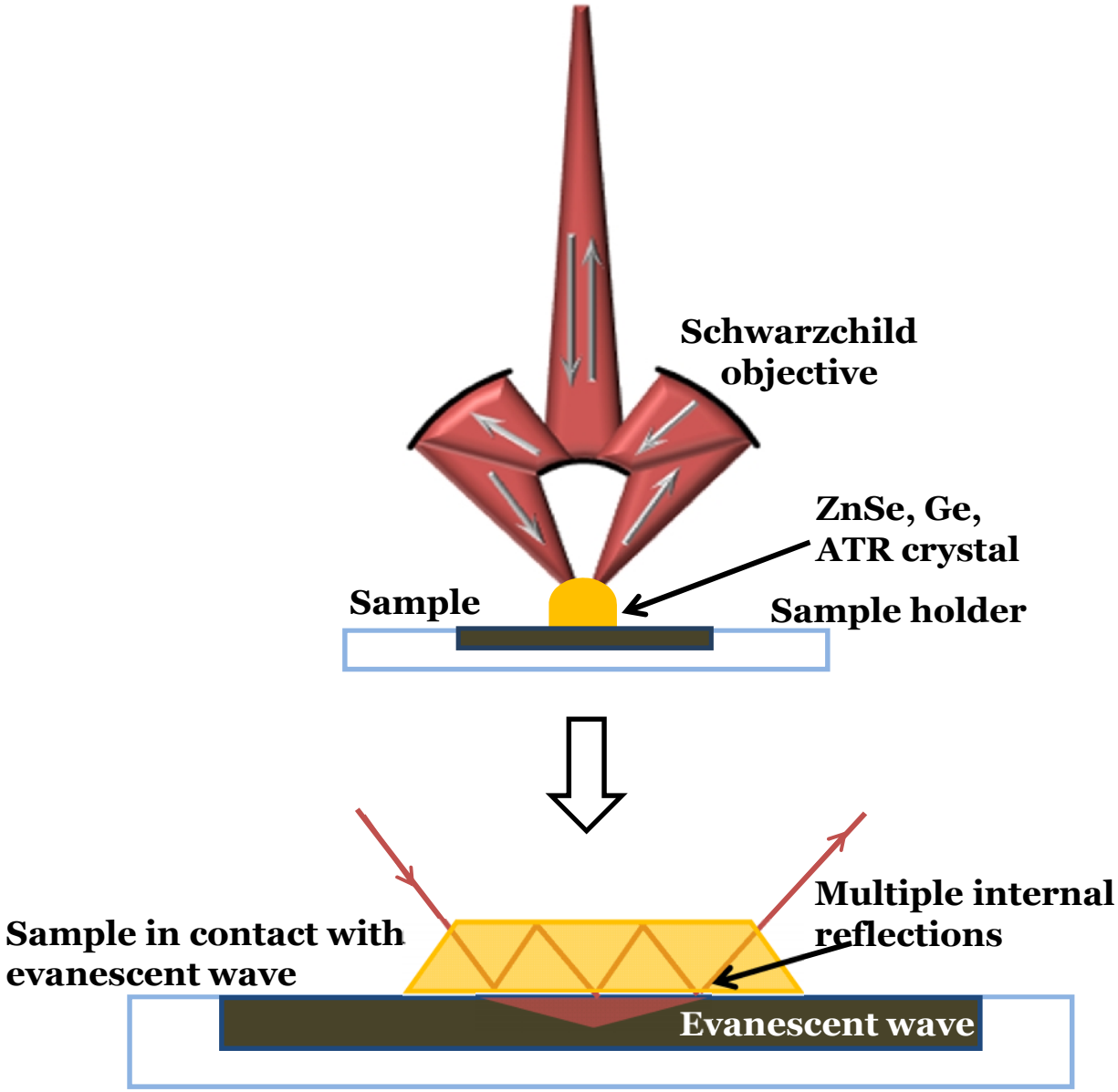

- An infrared transparent material like (KBr, $CaF_2$, $BaF_2$, ZnSe, ZnS and diamond)

# Reflection




**Samples which cannot be cut by microtome to a thin section**

•**Attenuated total reflection (ATR)**



# An Example of Synchrotron-based infrared Source..

**The infrared beamline at SEMAME Synchrotron**

## I. Simulation and Design of an Infrared Beamline for SESAME

***a)*** *The Infrared Source Characteristics for SESAME*

***b)*** *Optical Design Simulation*

***c)*** *Beamline Design*

***d)*** *Experimental Station*

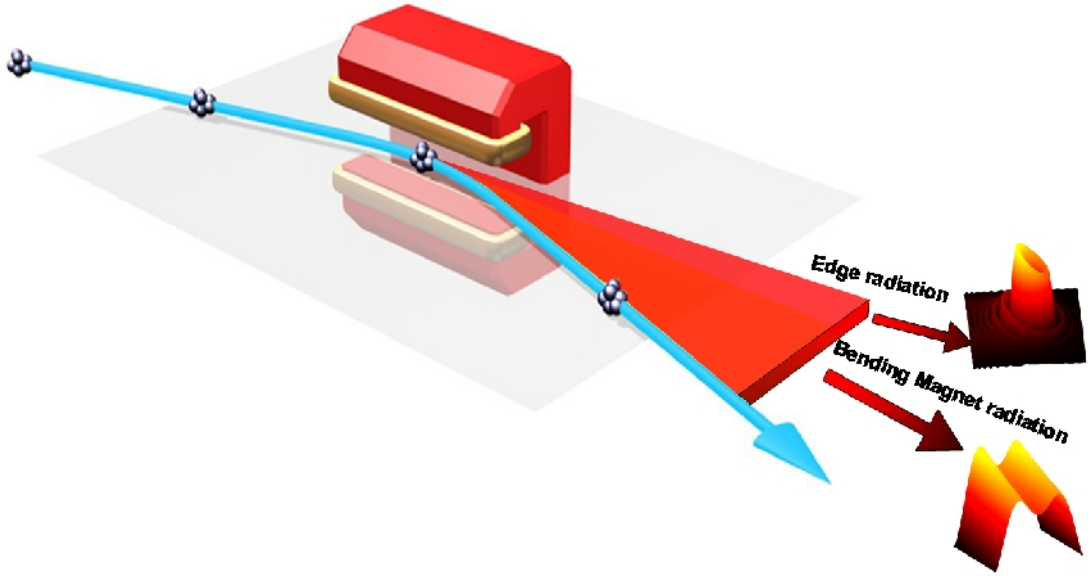
# SESAME Storage Ring Parameters

| Parameter | Description | Value |
|---|---|---|
| **E** | **Electron energy** | **2.5 GeV** |
| **B** | **Magnetic field** | **1.4554 T** |
| **R** | **Bending radius** | **5.726 m** |
| **I** | **Electron current** | **400 mA** |
| **L** | **Straight section length** | **4.4 m** |

- IR beamline will be one of the three Day-1 beamline at SESAME
- IR beamline will be the first one  fully designed at SESAME

# Collection and Design Details

- **Infrared light will be collected from (ER +BM sources)**

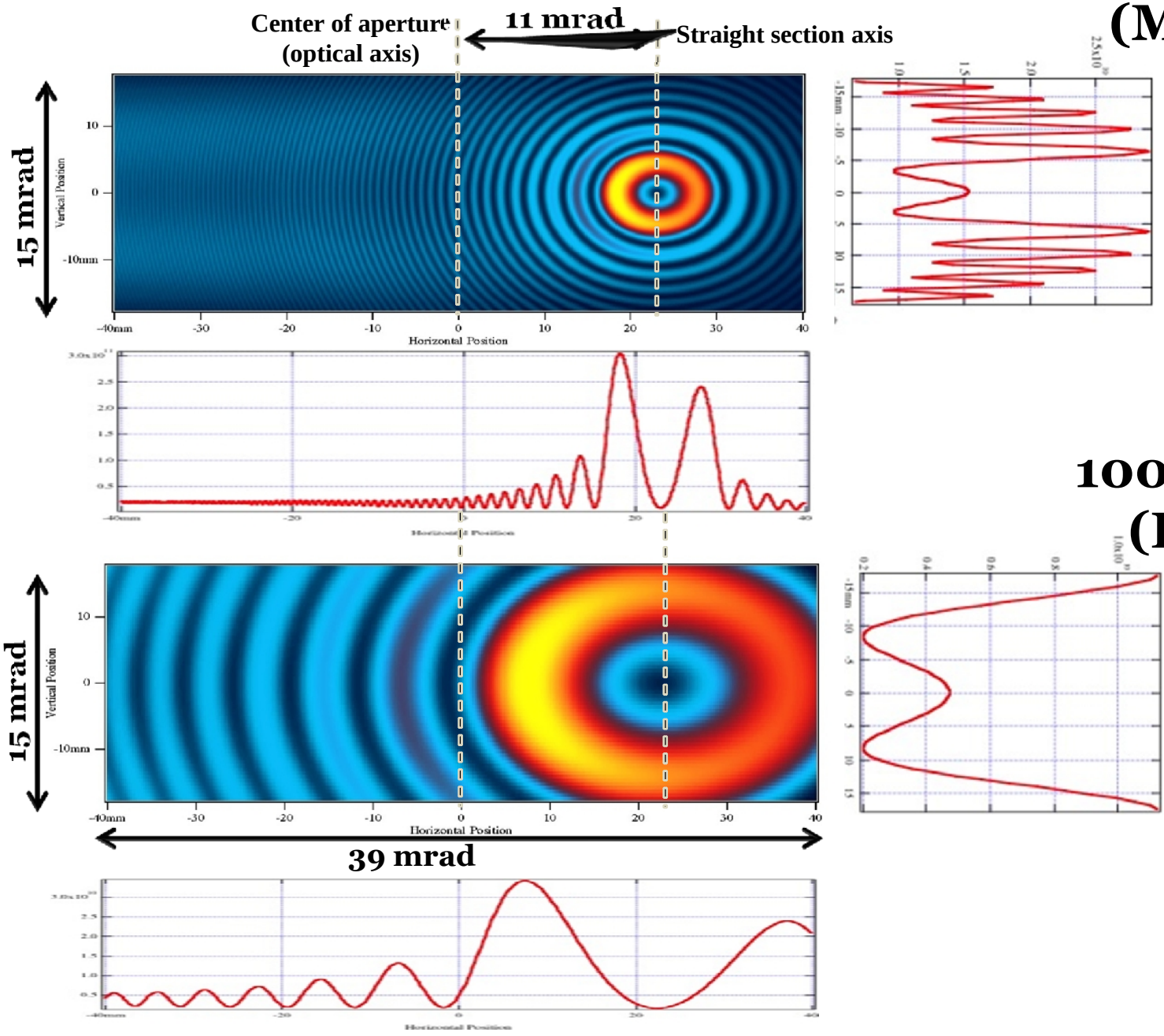



- **Vertical collection angle = 15 mrad**
- **Horizontal collection angle = 39 mrad**

- **Collection geometry optimized using SRW and Rays Tracing**

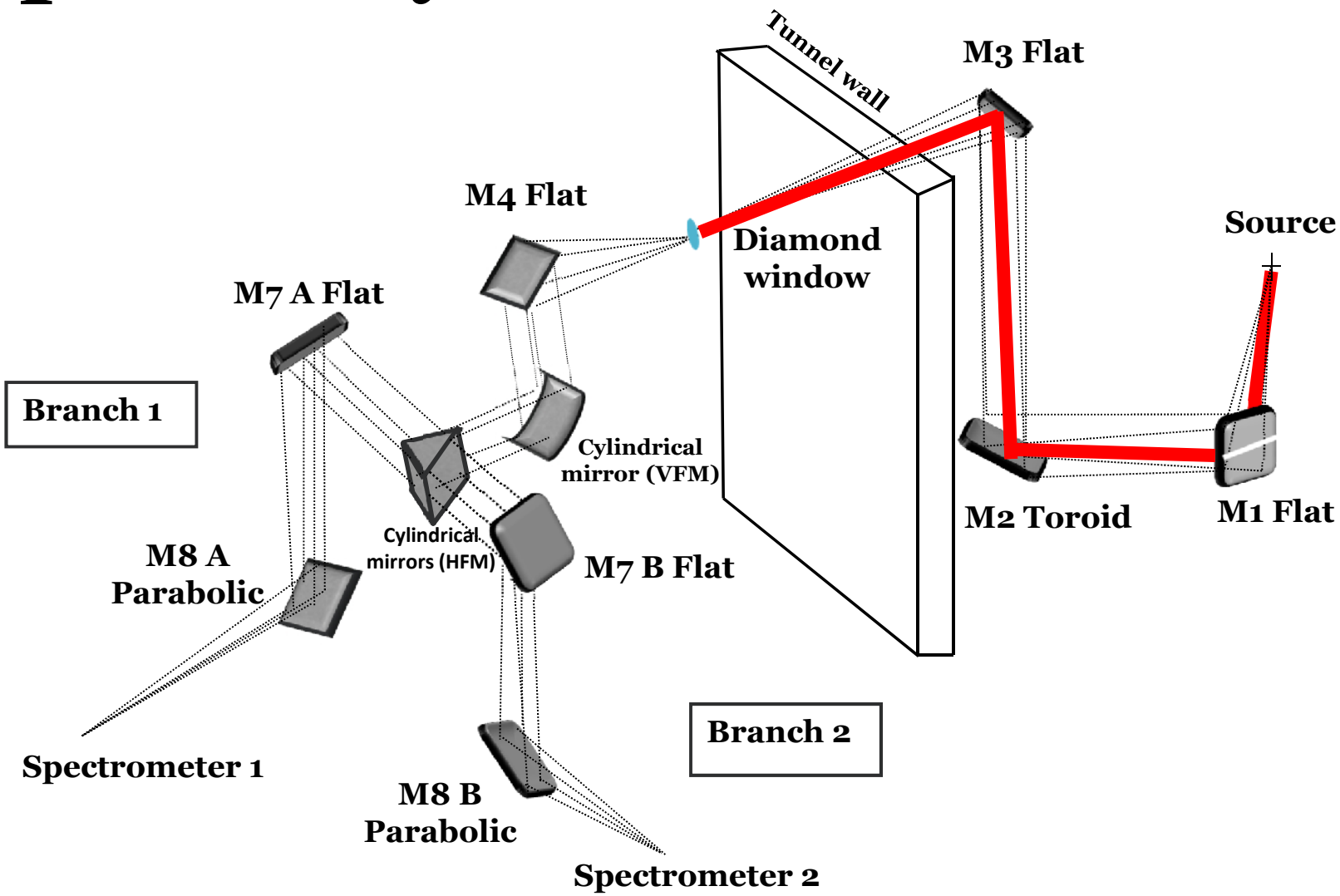
# Initial Wavefront Profile

**10 microns (Mid-IR )**



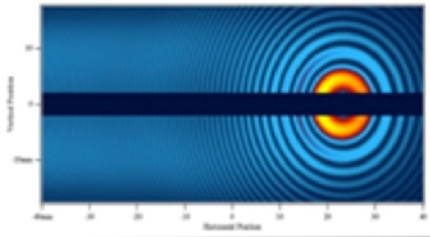
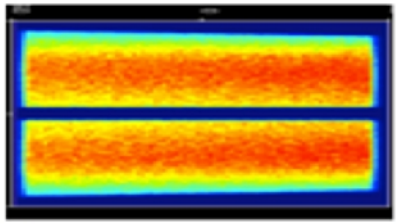
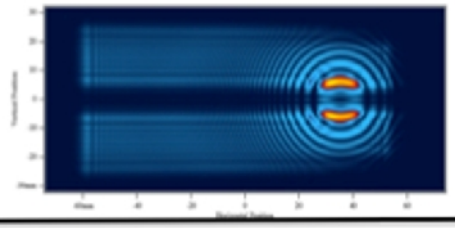
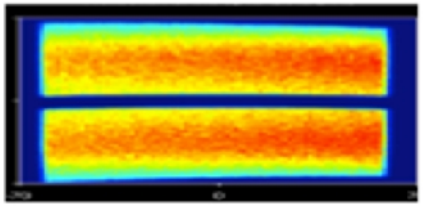
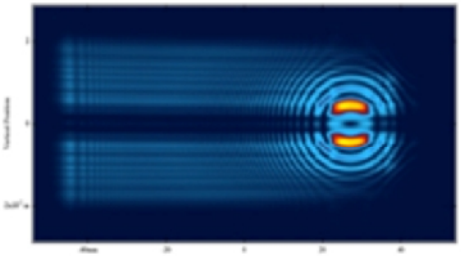
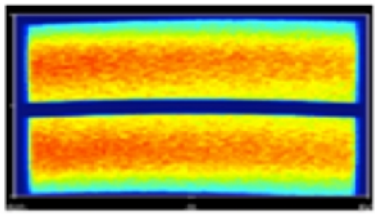
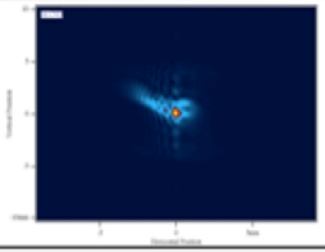
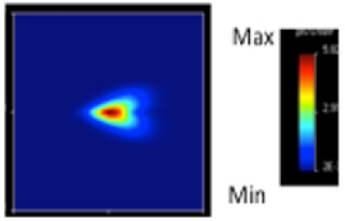

**100 microns (Far-IR)**

# Optical Layout

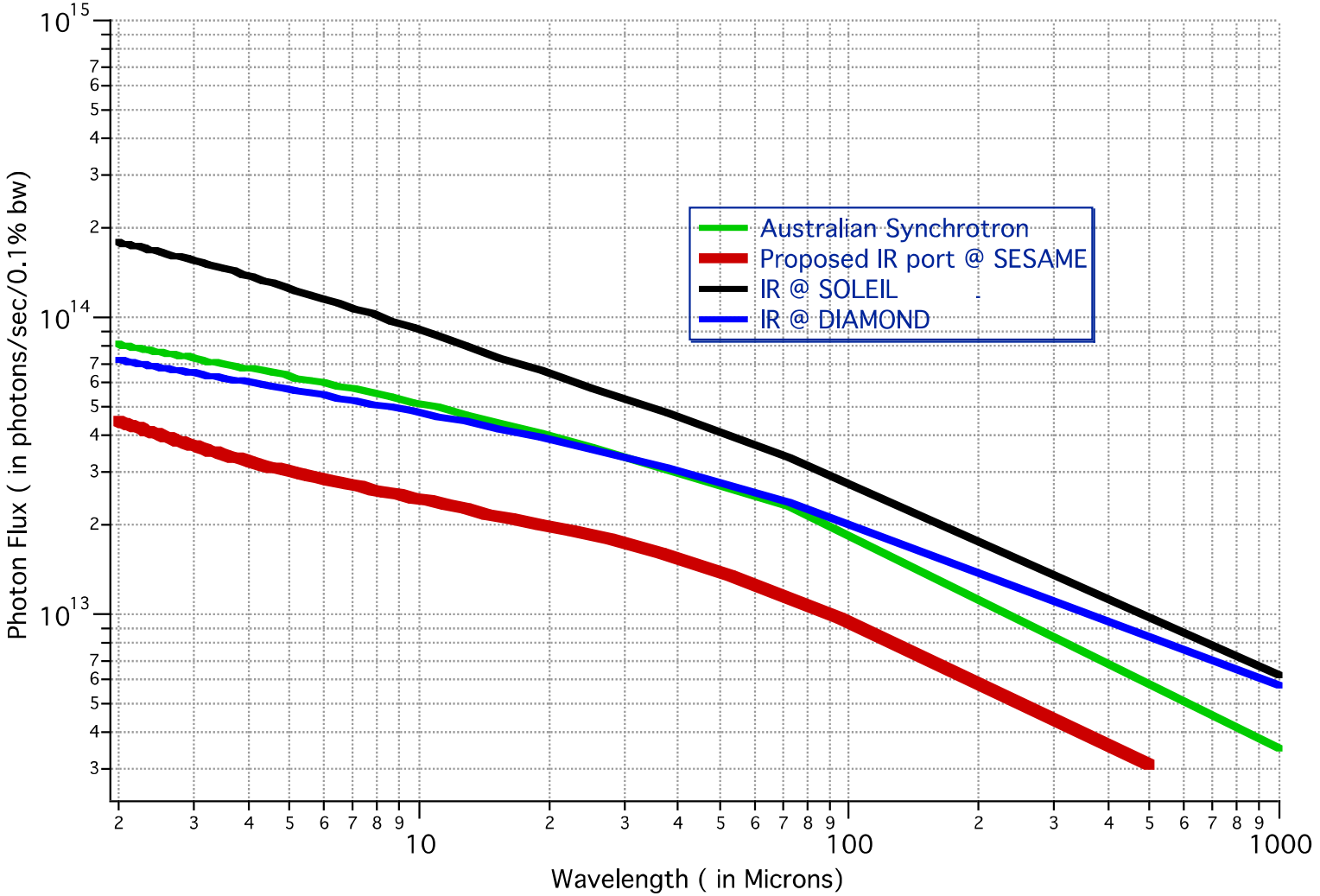



***Schematic layout of optical path from the source inside the tunnel wall until the instruments***

# Wavefront simulation using SRW and SpotX: 10 µm

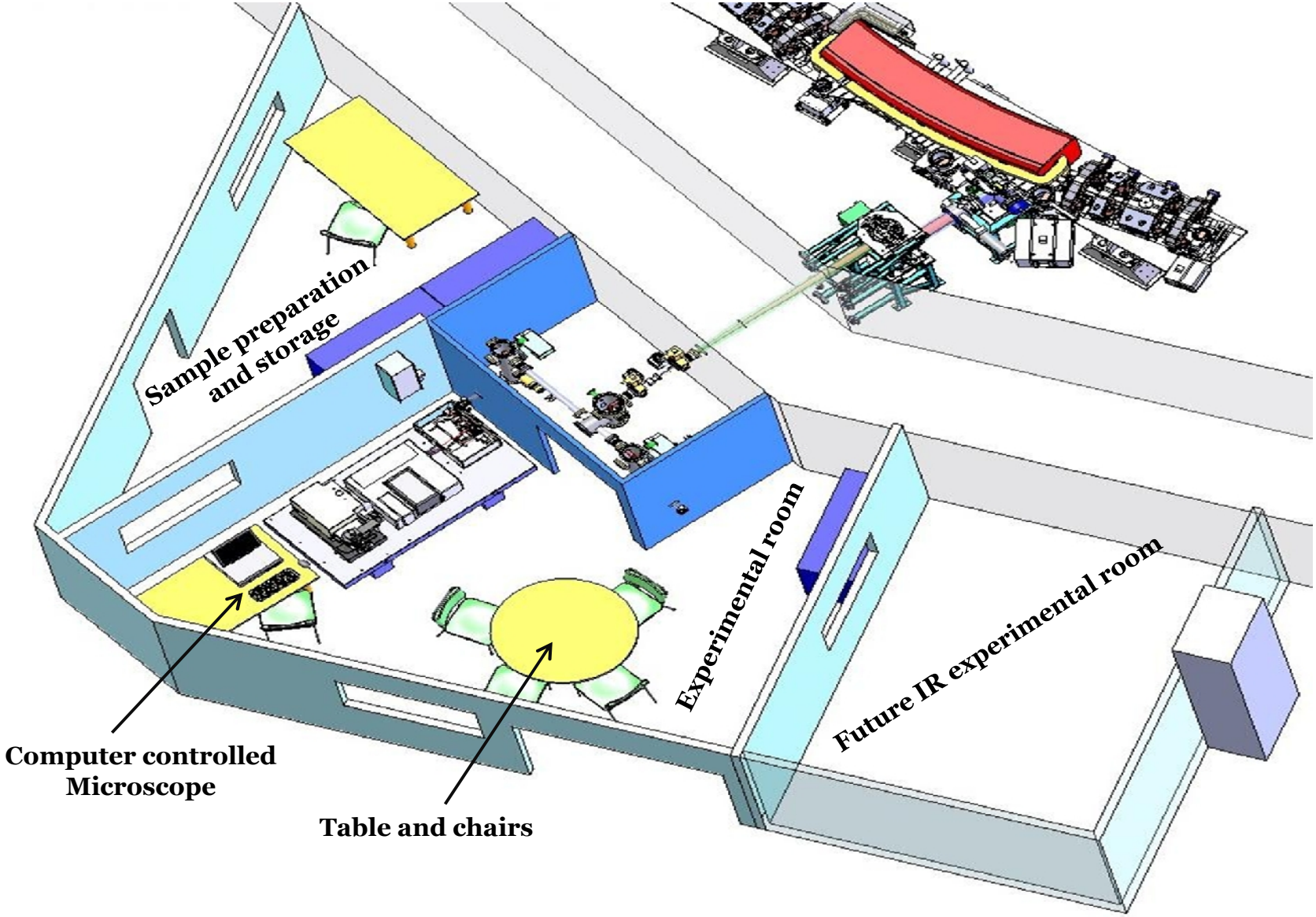
| | SRW | SpotX |
|---|---|---|
| At M1 with slot |  |  |
| At M2 - Toroidal |  |  |
| At M3 - Plane |  |  |
| At Focus – outside tunnel wall |  |   |

# Comparison with SOLEIL and other Synchrotrons




**Note: SOLEIL (black) 78mrad (H) x 20mrad (V); 500mA, DIAMOND (blue) and Australian Synchrotron (green).**

# Experimental Station for the IR Beamline



**I. Heavy metals accumulation in plants**

**II. Phytoremediation**

**III. Basic principles of Infrared Microspectroscopy**

**IV. Application in Environmental Science**

*a) Nickel hyperaccumulating and non-hyperaccumulating genotypes of* ***Senecio coronatus*** *from South Africa*

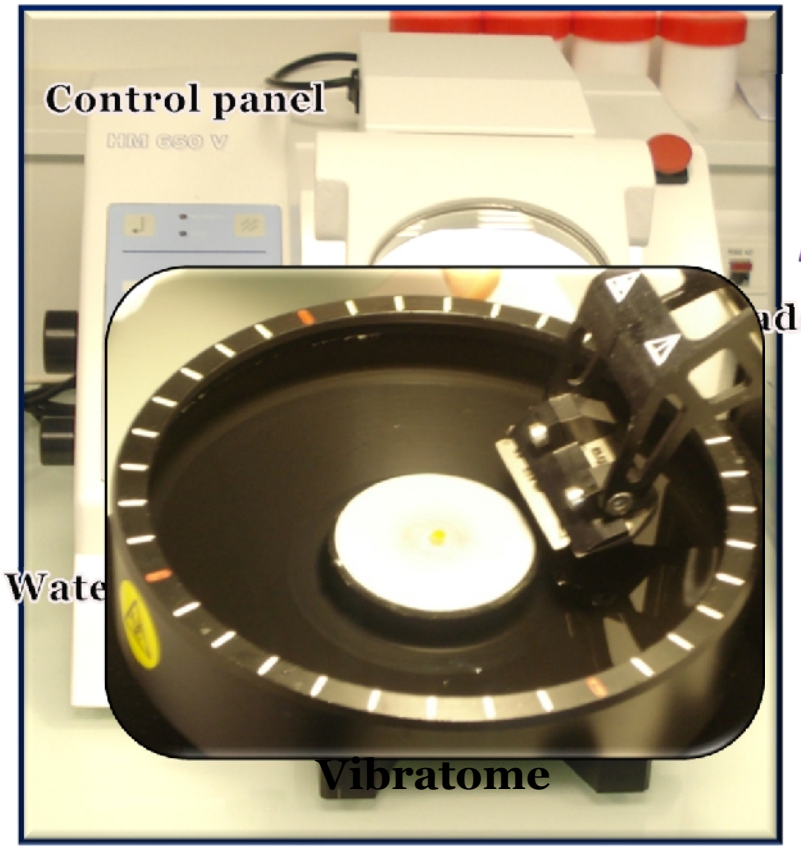
# Nickel Hyper-accumulator Roots from the Plant (*Senecio Coronatus*)

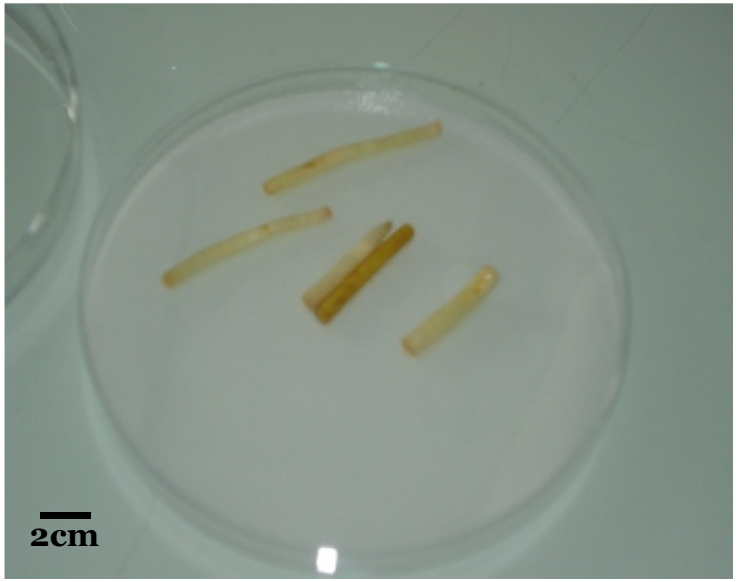
✓ **Whereby living plants are used for soil contamination uptake.**

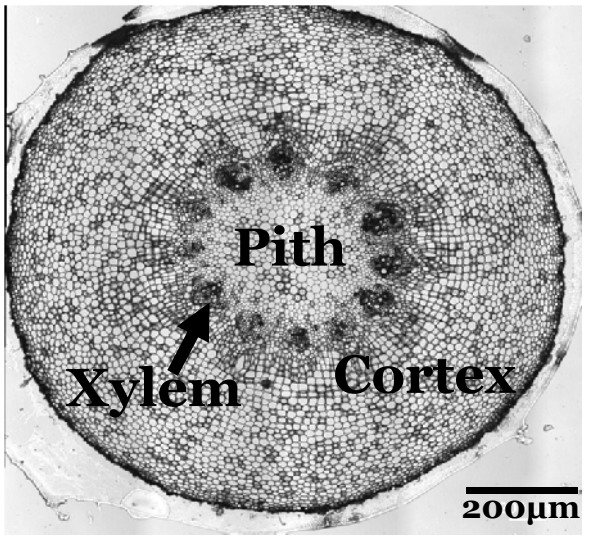
Collaboration: Jolanta Mesjasz-Przybyiowicz, Alban Barnabas
**IThemba Labs, South Africa**

# Sample Preparation

- **Sectioning roots with thickness of 30 microns**



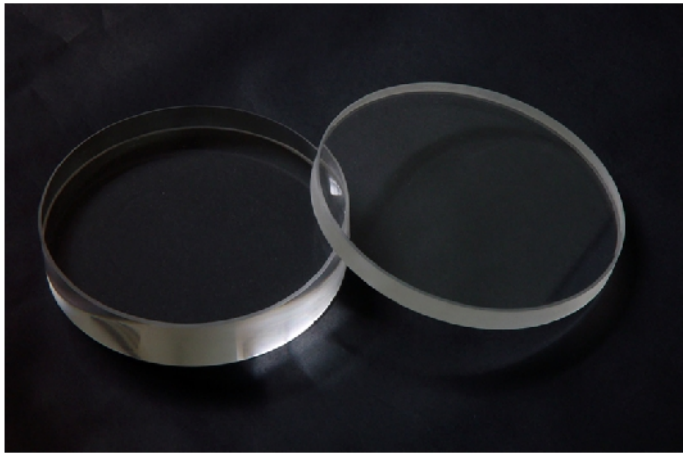




**Microscopic view of the root Cut-cross section**

# Experiment

BaF2 substrates

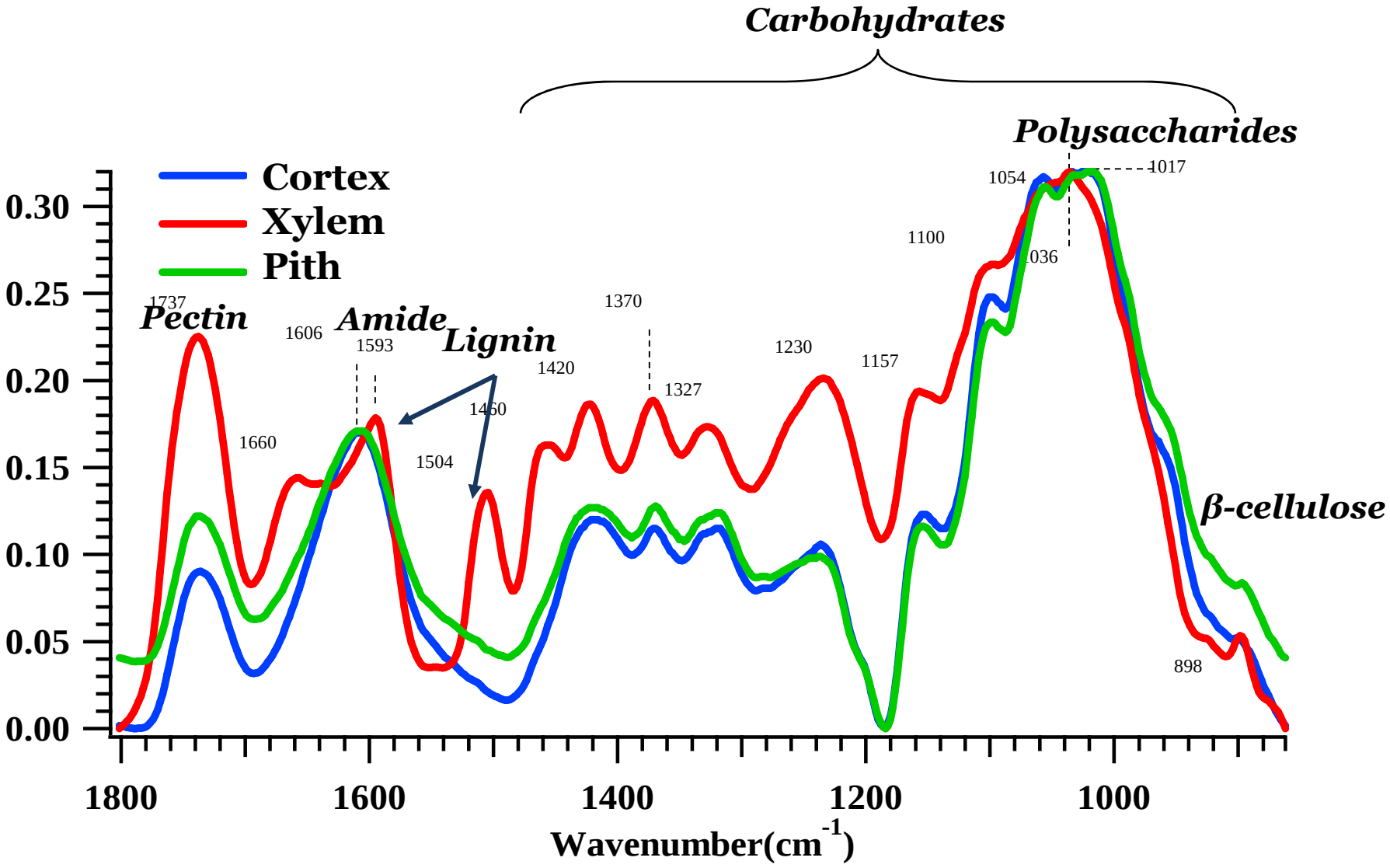


FTIR Microscope Setup


- Aperture size of 10µm²
- 4000-800 cm-1 mid-infrared
- Spectral resolution of 4 cm-1
- and step size of 5µm²

Data analysis using OMNIC software

# Band assignments



# *Infrared band assignments for the cellulosic fibres.*

- The C=O ester band at ~1737 $cm^{-1}$, from ***pectin***.

- The C=C in plane aromatic, vibrations at ~1593 $cm^{-1}$ and ~1504 $cm^{-1}$ from ***lignin.***

- The C-C ring breathing band at ~1157 $cm^{-1}$ and the C-O-C glycosidic ether band at ~1100 $cm^{-1}$, both of which arise from the ***polysaccharide*** components. that is, largely ***cellulose.***

- Bands at 1420, 1370 and 1327 $cm^{-1}$ are characteristics of structural ***carbohydrates***.

- The homogalacturonan content of ***pectin*** bands assigned at 1100 and 1017$cm^{-1}$.

- At 1036$cm^{-1}$ the band arises from ***glucan*** [(1→4) β-glucan].

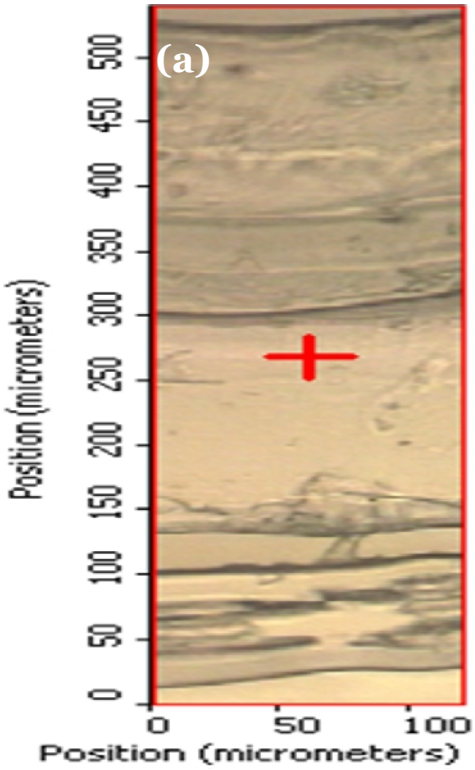
- That at 898$cm^{-1}$ to the β configuration of ***cellulose***.

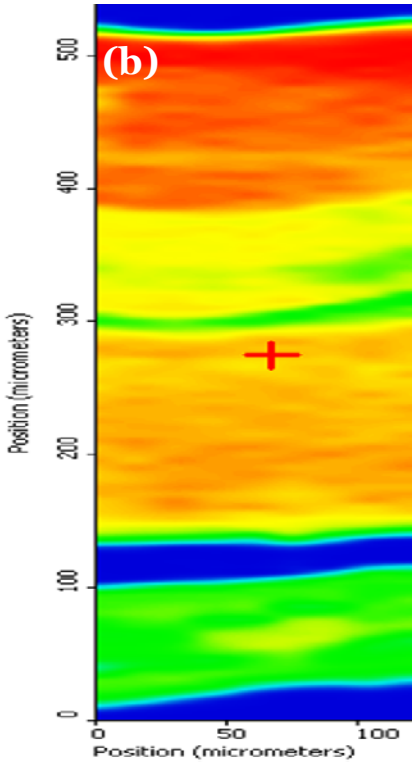
- The bands at 1740-1745$cm^{-1}$ (carbonyl C=O stretching of ester), 1370$cm^{-1}$ [C-H in -O(C=O)-CH3] and 1230 $cm^{-1}$ (C-O stretching of acetyl group) provide evidence of ***acetylation***.

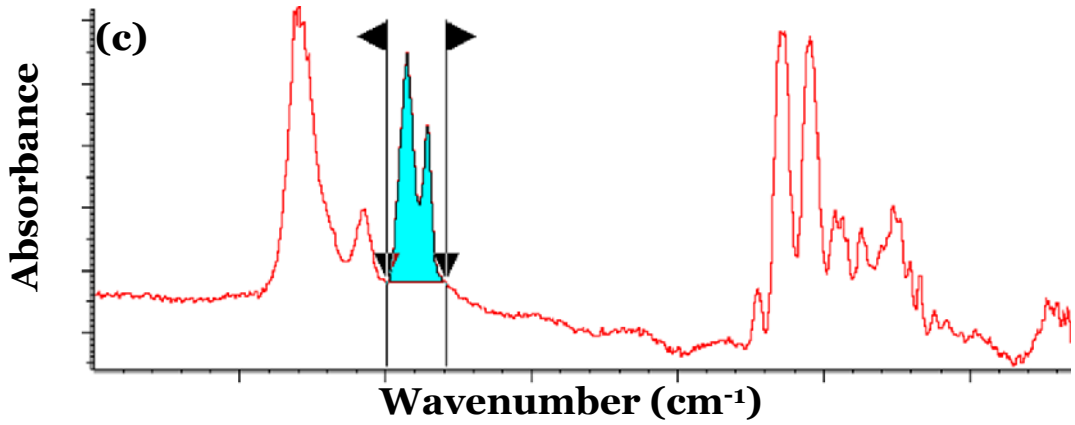
- Although it is possible to quantify ***quinones,*** for example, separately, the C=O and C=C bands appear around 1660 $cm^{-1}$, where several other compounds, such as hexenuronic acids, may be contributing as well.

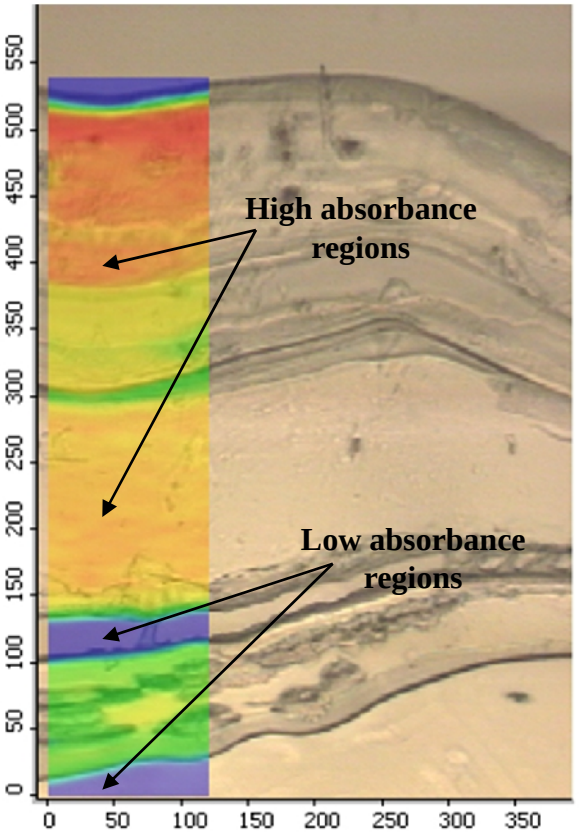
| Position/ $cm^{-1}$ | Assignment |
|---|---|
| **~ 1737** | **ν(C=O) ester** |
| **~ 1660** | **C=O, C=C** |
| **~ 1606** | |
| **~ 1593** | **ν(C=C) aromatic in-plane** |
| **~ 1504** | **ν(C=C) aromatic in-plane** |
| **~ 1460** | **δ(C-H); δ(C-OH) 1° & 2° alcohol** |
| **~ 1433** | **aromatic C=C stretch** |
| **~ 1420** | **δ(C-H)** |
| **~ 1370** | **δ(C-H)** |
| **~ 1335** | **δ($CH_2$) wagging** |
| **~ 1327** | **C-C and C-O skeletal stretch** |
| **~ 1230** | **δ(C-OH) out-of-plane** |
| **~ 1157** | **ν(C-C) ring breathing, asymmetric** |
| **~ 1100** | **ν(C-O-C) glycosidic** |
| **~ 1054** | **ν(C-OH) 2º alcohol** |
| **~ 1036** | **ν(C-OH) 1º alcohol** |
| **~ 1017** | |
| **~ 898** | **ν(C-O-C) in plane, symmetric** |

# Chemical imaging



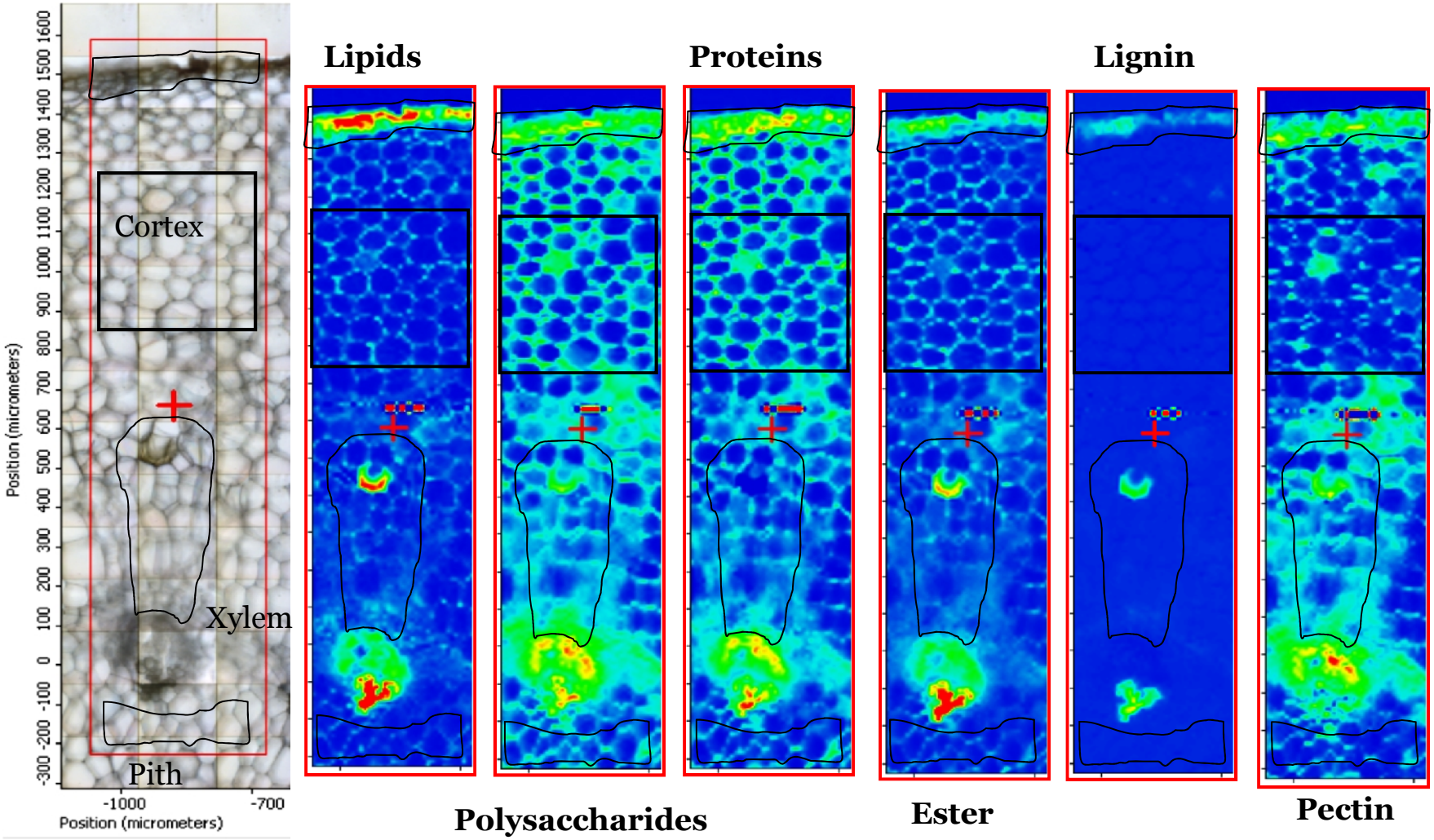



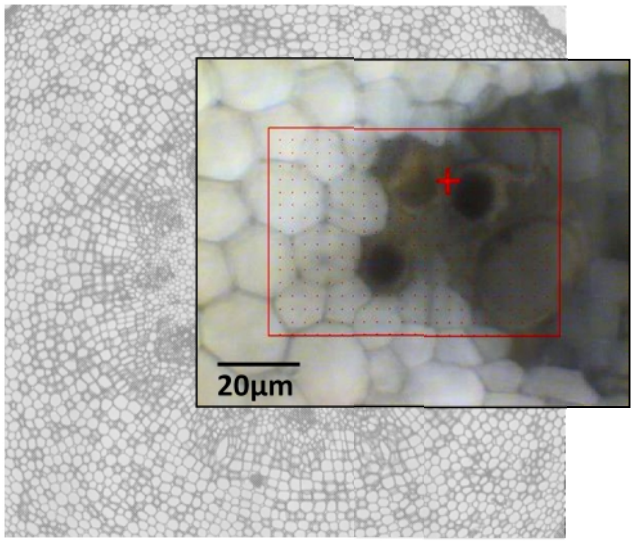

**Superimposition of IR chemical image with the visual image illustrates the zones of absorbance.**

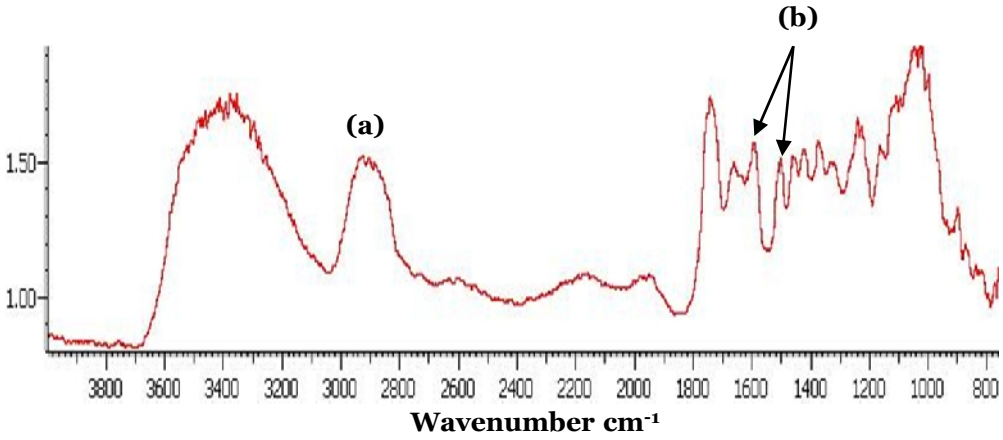
# Chemical imaging

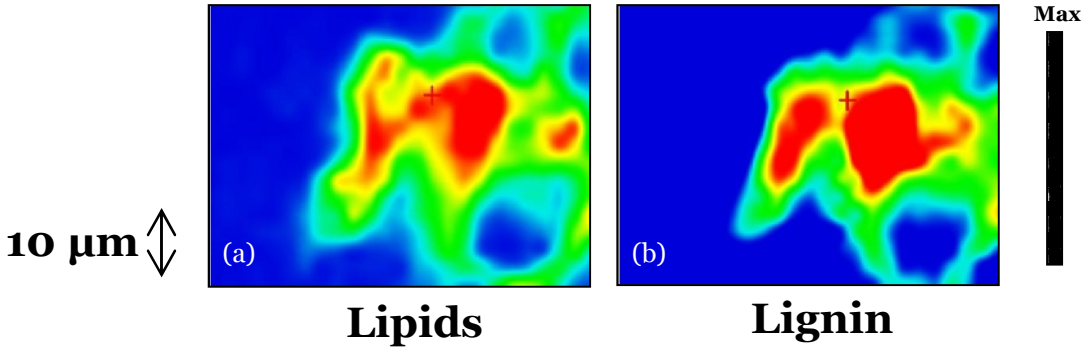



**Specialized structures in roots**

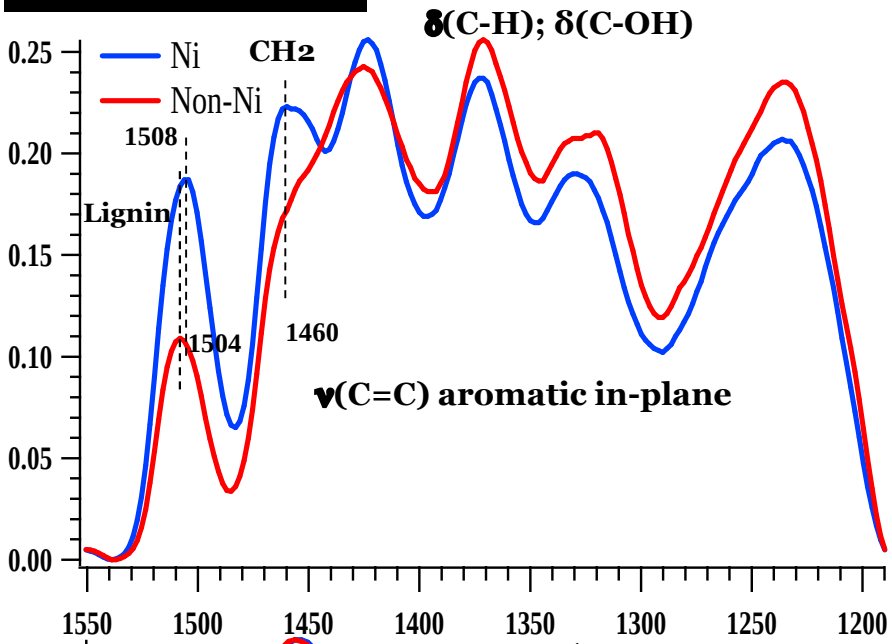
# Chemical imaging in the Xylem with high spatial resolution

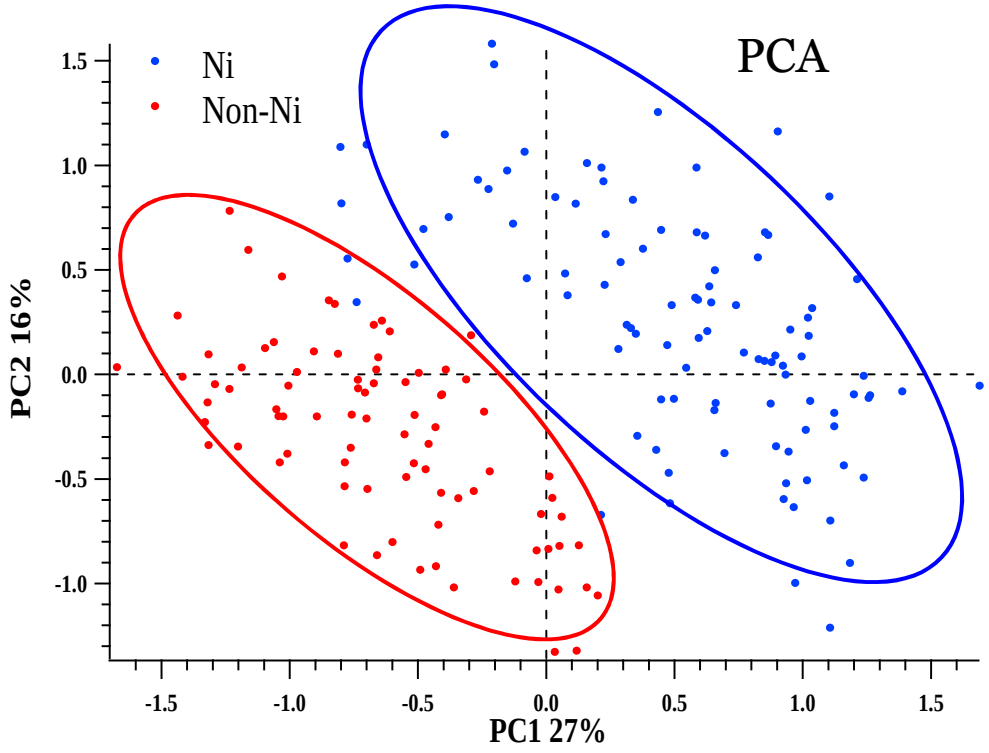


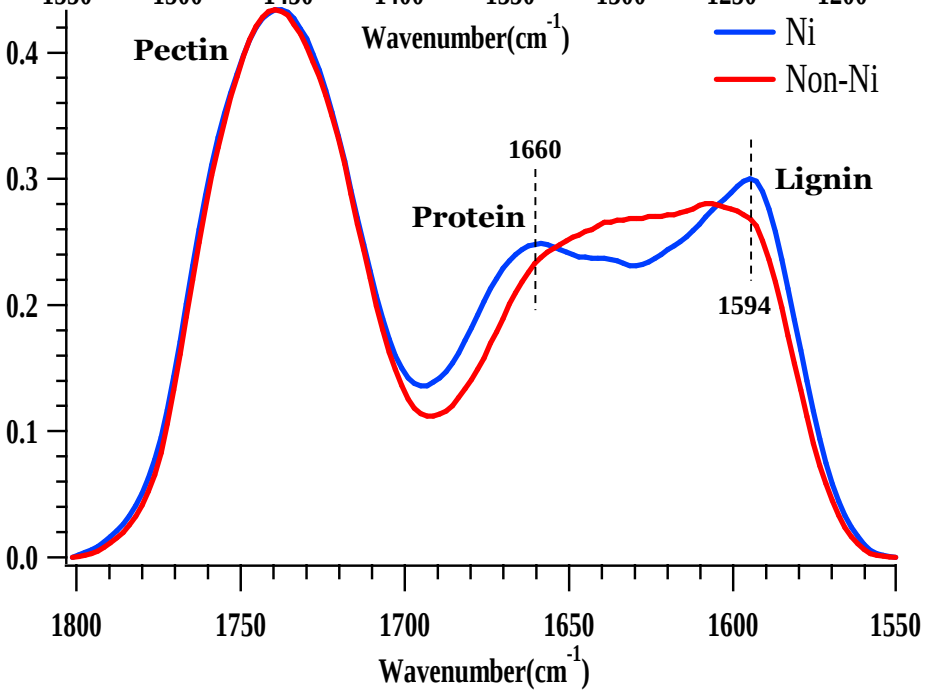



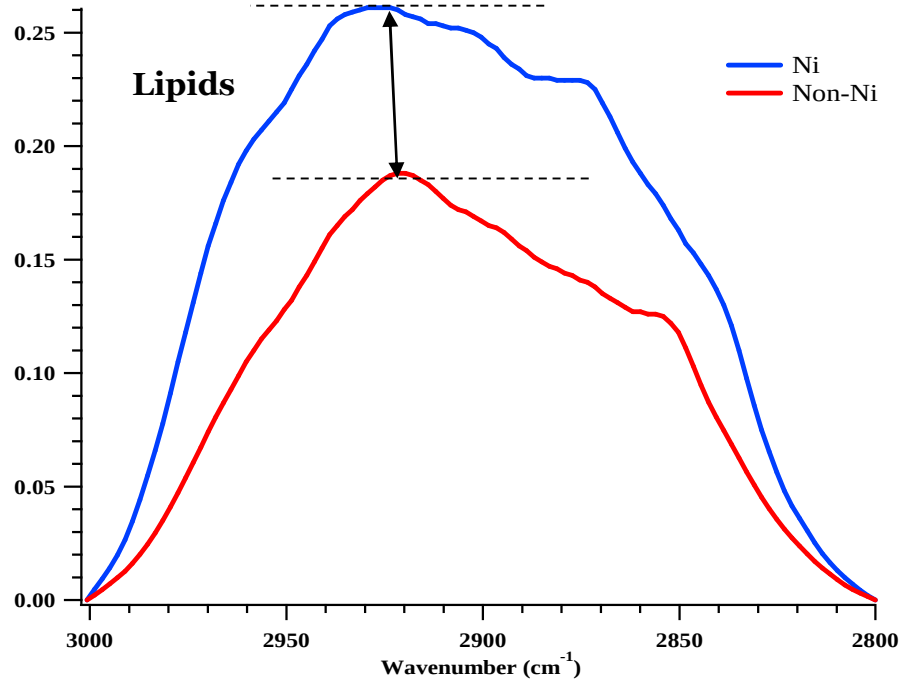



# Xylem

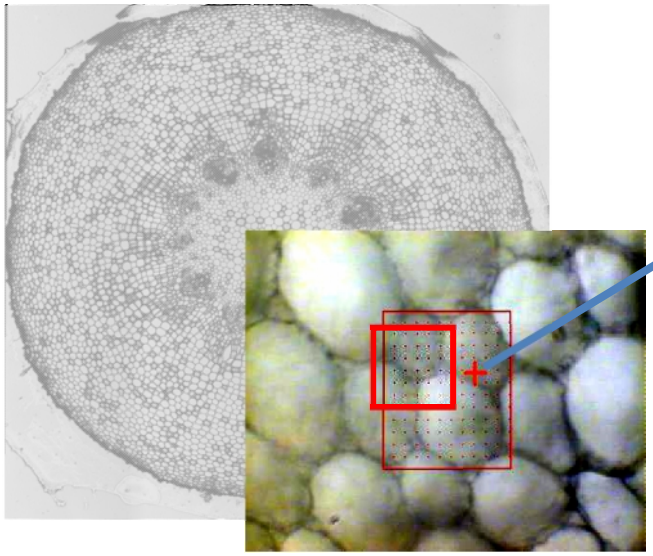

δ(C-H); δ(C-OH)
Ni
Non-Ni
CH2
1508
Lignin
1504
1460
ν(C=C) aromatic in-plane
0.25
0.20
0.15
0.10
0.05
0.00
1550
1500
1450
1400
1350
1300
1250
1200
Wavenumber(cm-1)

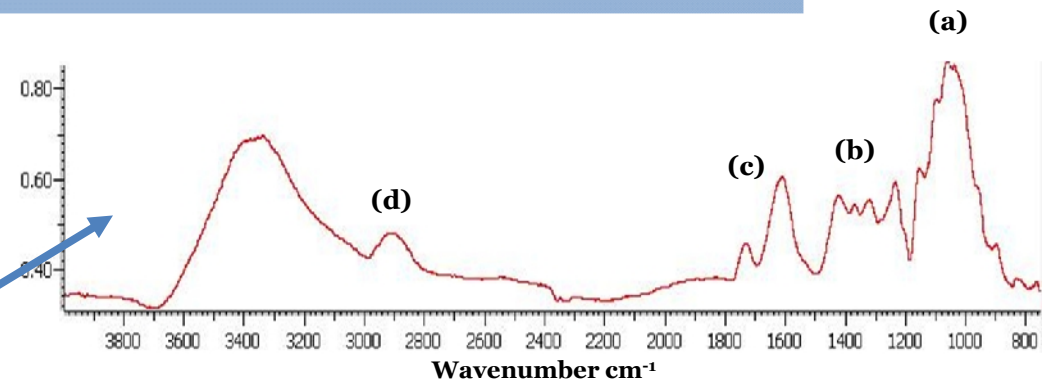

PCA
Ni
Non-Ni
PC2 16%
PC1 27%
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5


Pectin
Ni
Non-Ni
1660
Protein
Lignin
1594
0.4
0.3
0.2
0.1
0.0
1800
1750
1700
1650
1600
1550
Wavenumber(cm-1)

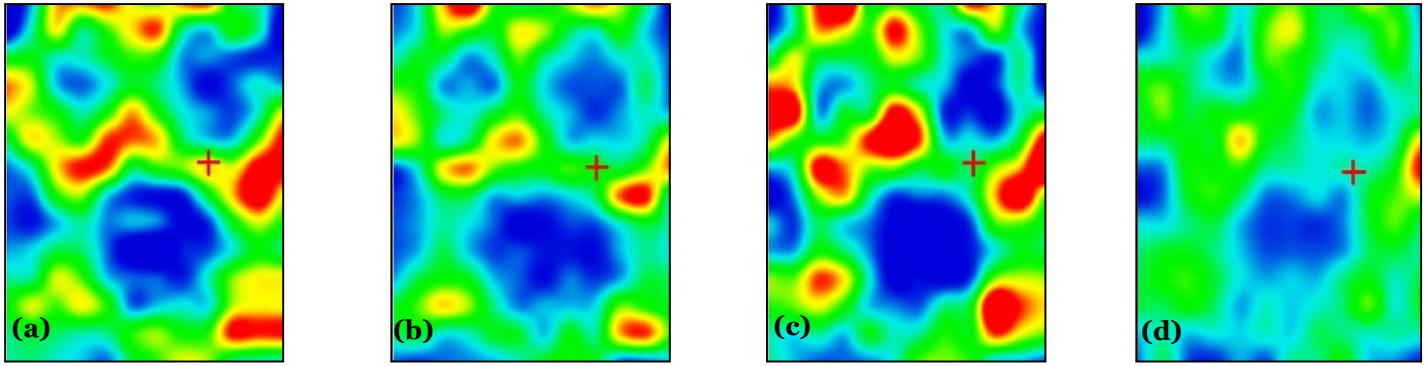

Lipids
Ni
Non-Ni
0.25
0.20
0.15
0.10
0.05
0.00
3000
2950
2900
2850
2800
Wavenumber (cm-1)

# Chemical imaging in the Cortex with high spatial resolution

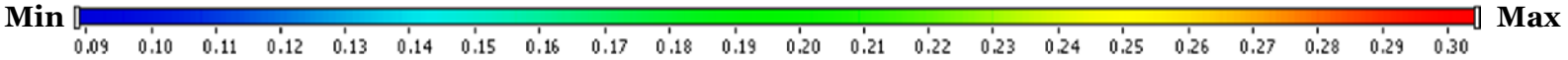





10 µm

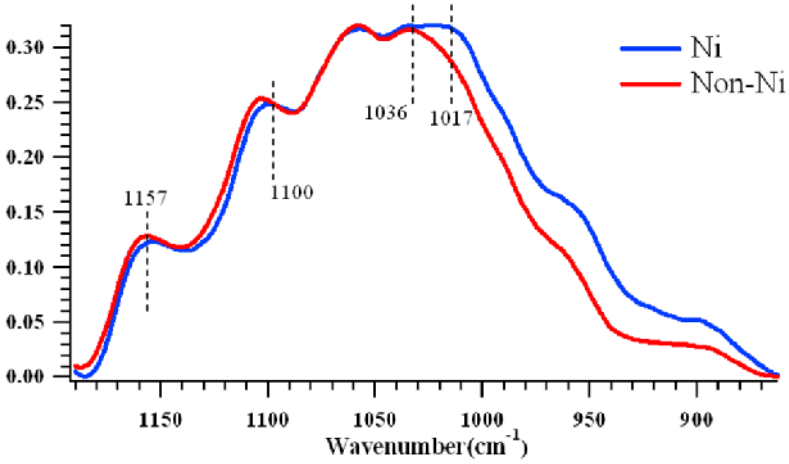



Polysaccharides | Carbohydrates | Protein | Lipids

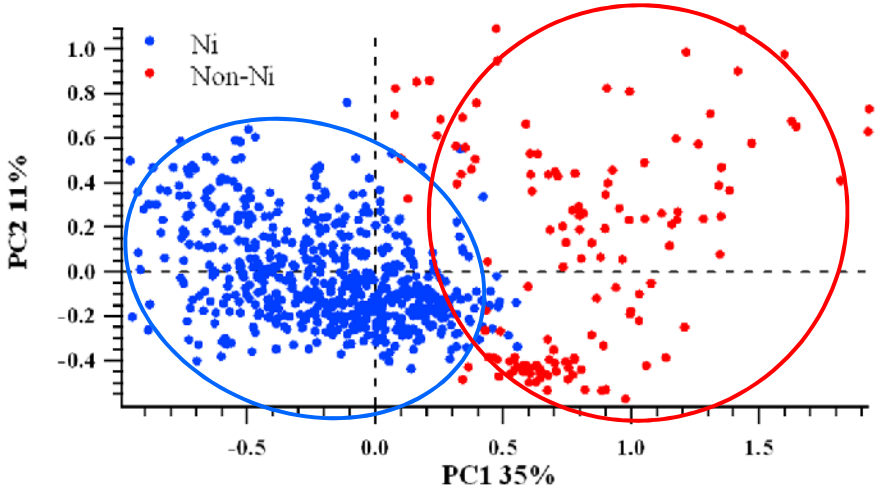


# Cortex

This region is dominated by ring vibrations overlapped with stretching vibration of (C-OH) side groups and the (C-O-C) glycosidic bond vibration.




PCA

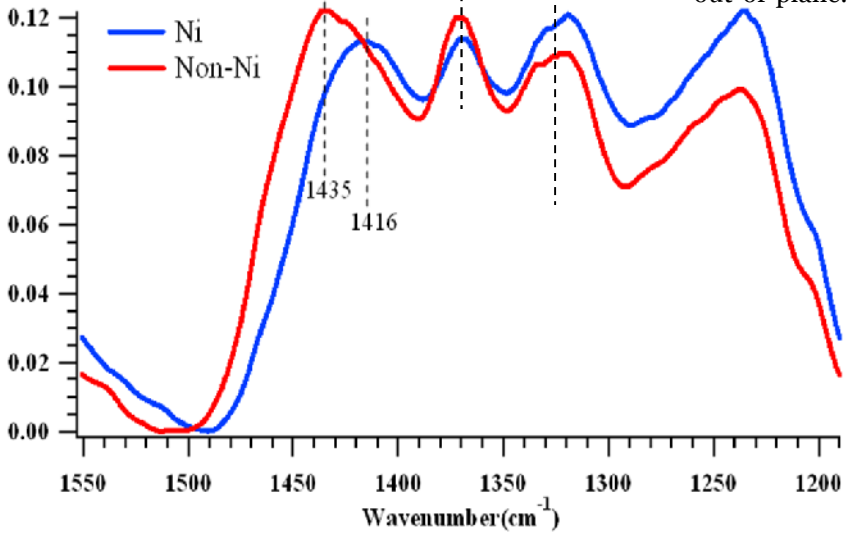



aromatic C=C stretch

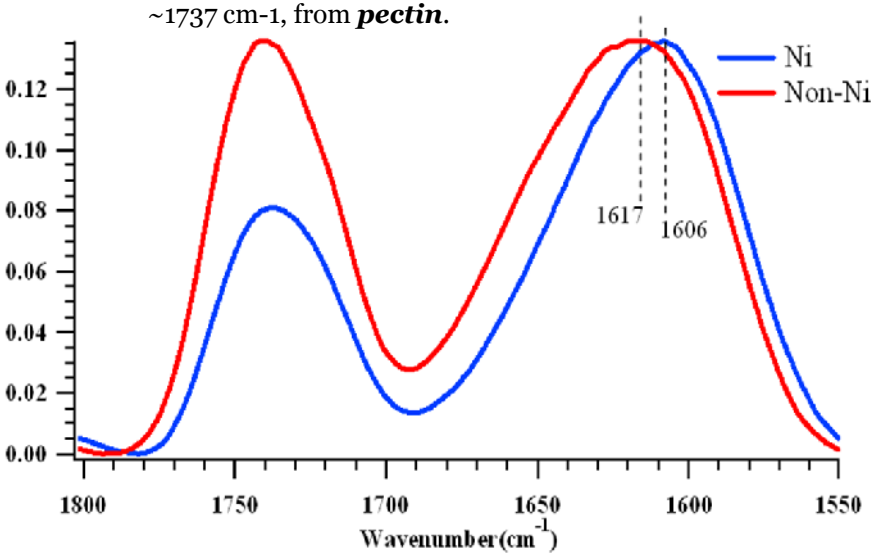
1370

1327

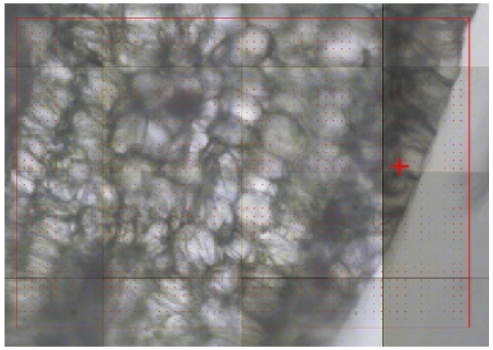
1230cm$^{-1}$ δ(C-OH) out-of-plane.



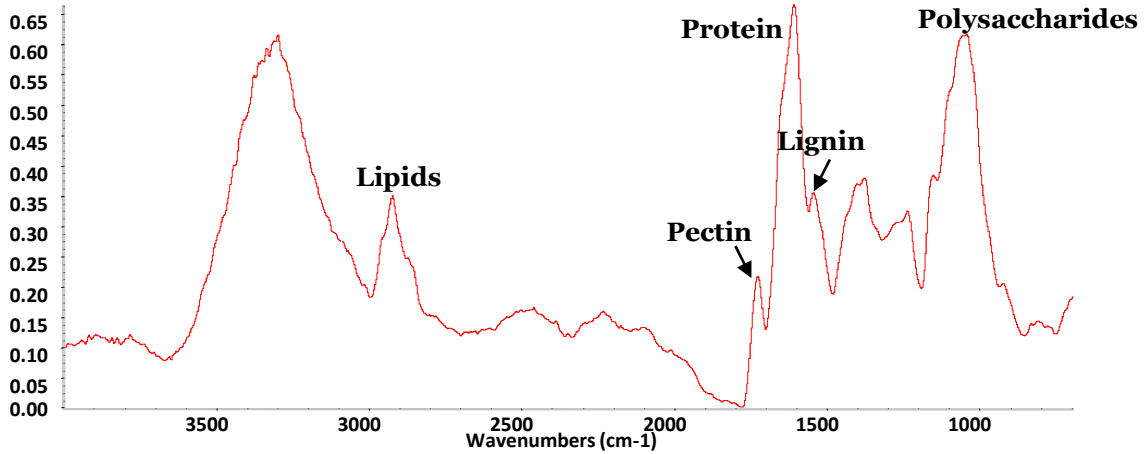

The C=O ester band at ~1737 cm-1, from ***pectin***.

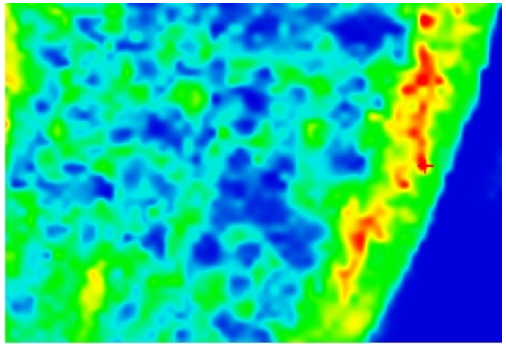


# FTIR Investigations on Leafs…

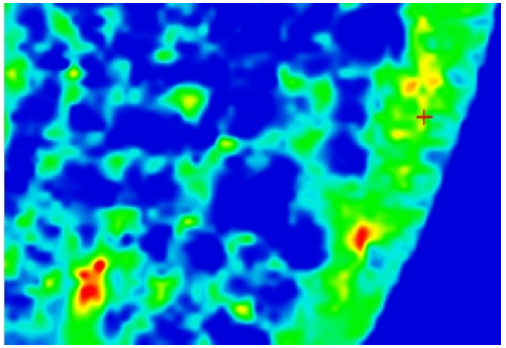


Visible image

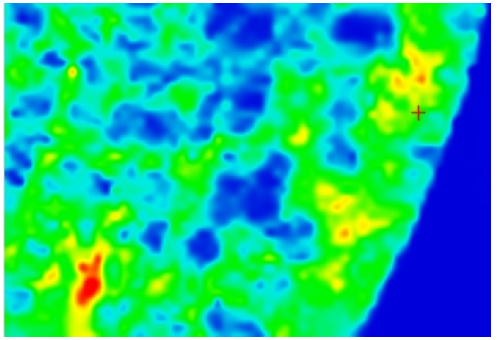


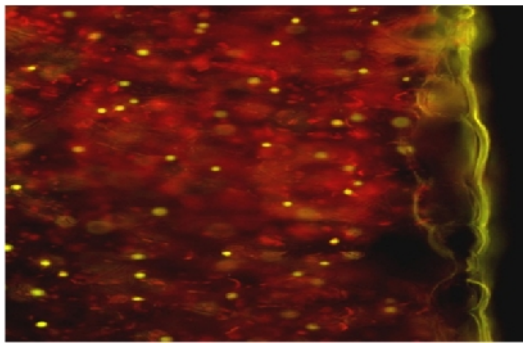
Lipids



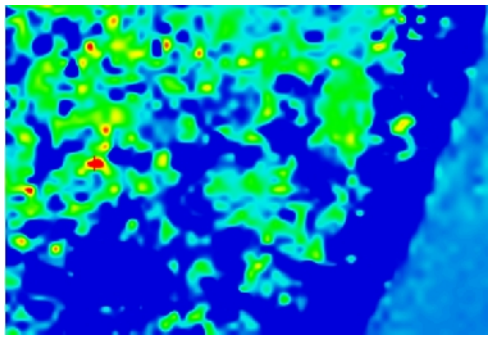
Pectin



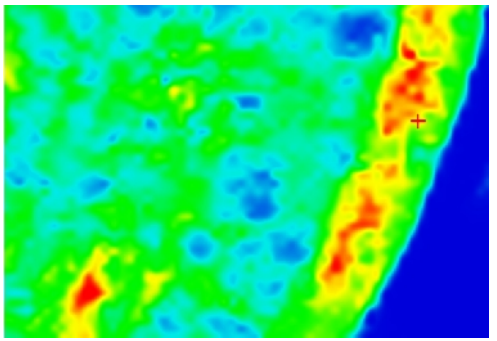
Protein



Histochemical staining (yellow spot could be lipids)



Lignine



Polysaccharides

# Summary

- Ni versus Non-Ni accumulator show chemical differences.

- Differences depend on the region of the root:
  - Results from Pith and Cortex parts are similar.
  - More significant differences are observed in Xylem.

- Frequency shift of Lignin and the aromatic C double bond stretch bands in Ni accumulator, structural changes: (***chemical bonding or rearrangement***).

- The concentrations of lignin, and lipids are higher in Ni accumulator (***natural response***).


Thank you for your attention!!