School on X-Ray Imaging Techniques

ESRF, Grenoble, 5-6 February, 2007

Spectro-microscopies

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Imaging

taking a picture

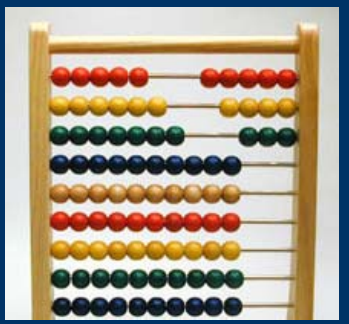
Spectro-microscopy ?

Spectroscopy

spread the light

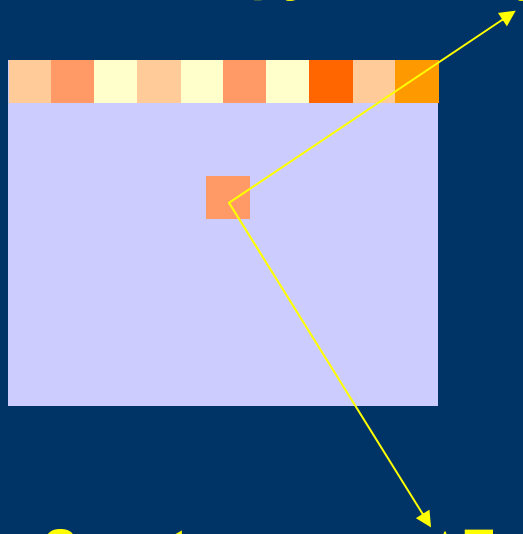
Photometry

measure how much light

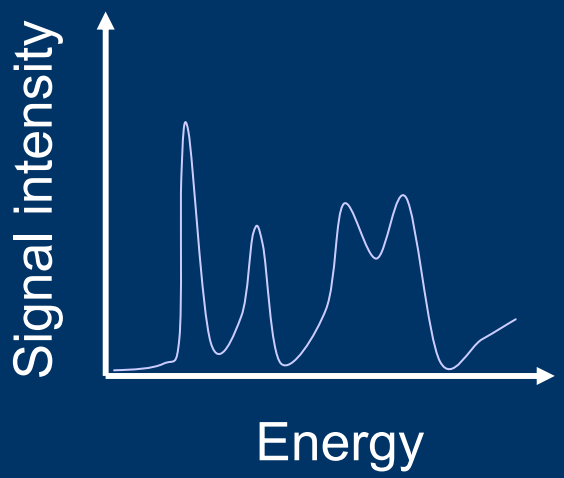


Spectro-microscopies

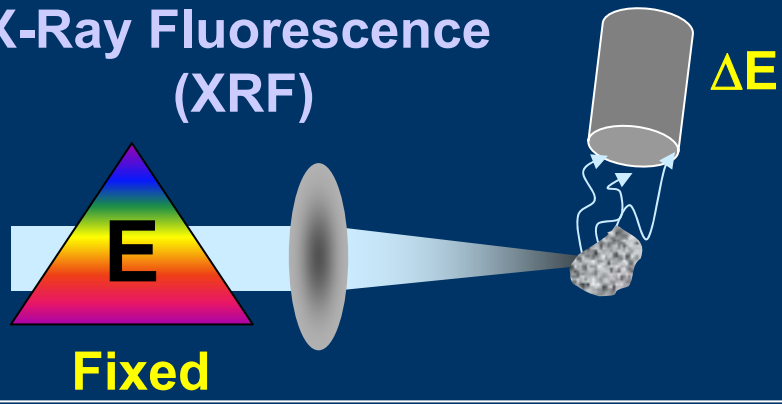
1 - Microscopy: < Δx, Δy < 1µm



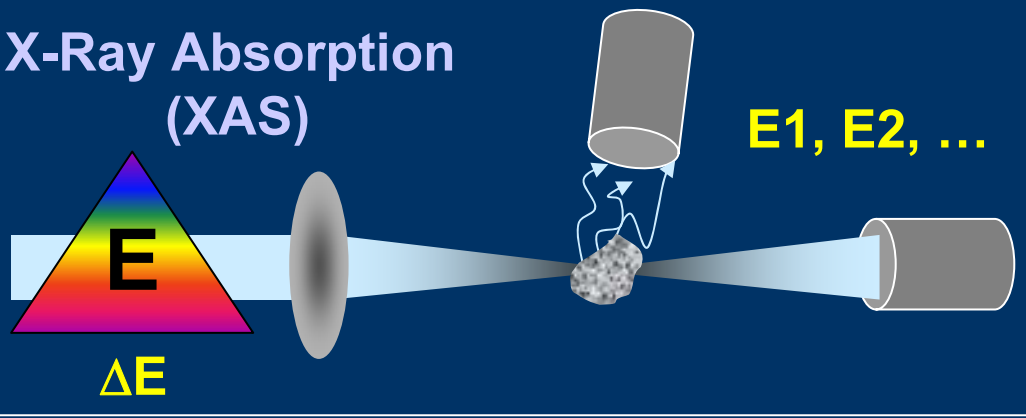
2 - Spectroscopy: ΔE



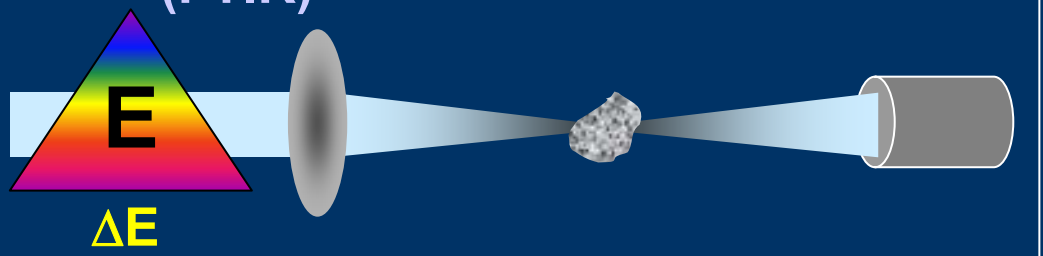
X-Ray Fluorescence (XRF)



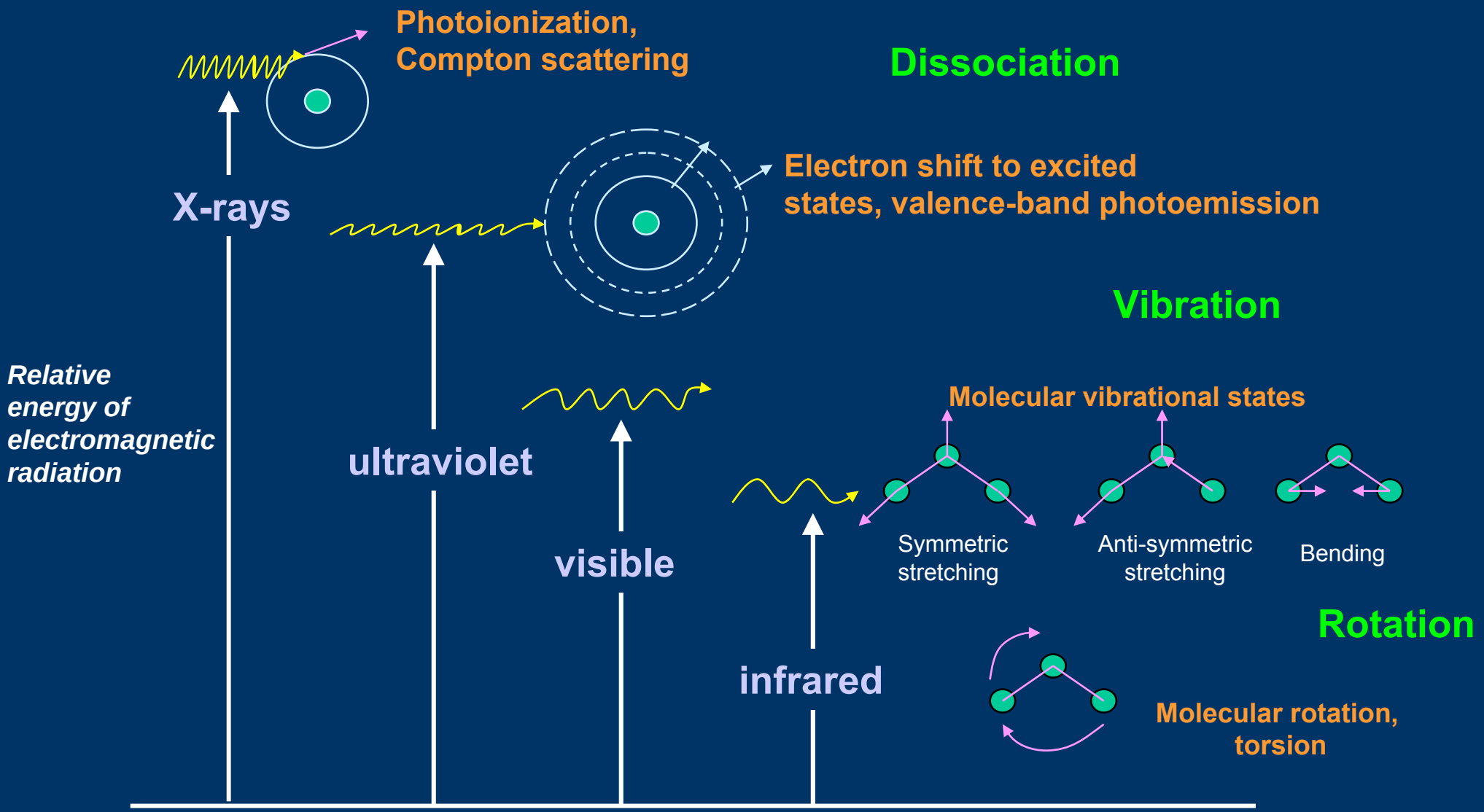
X-Ray Absorption (XAS)



Infrared Absorption (FTIR)



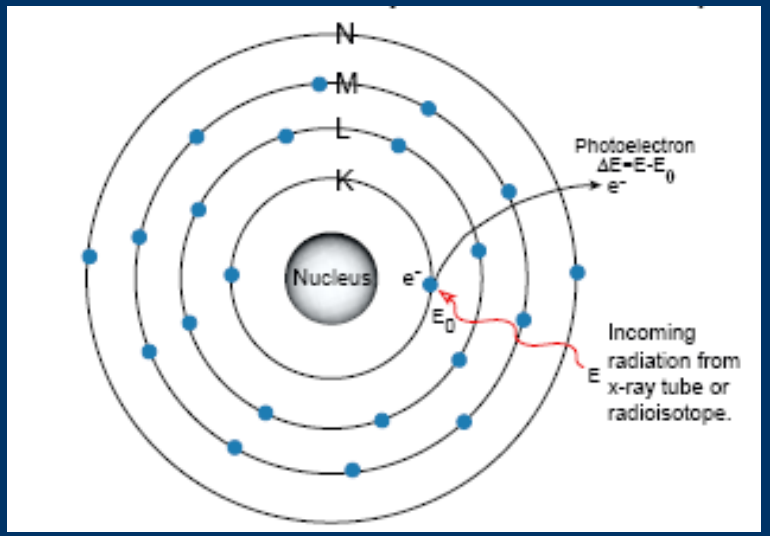
The interactions photons-matter provide several contrast mechanisms



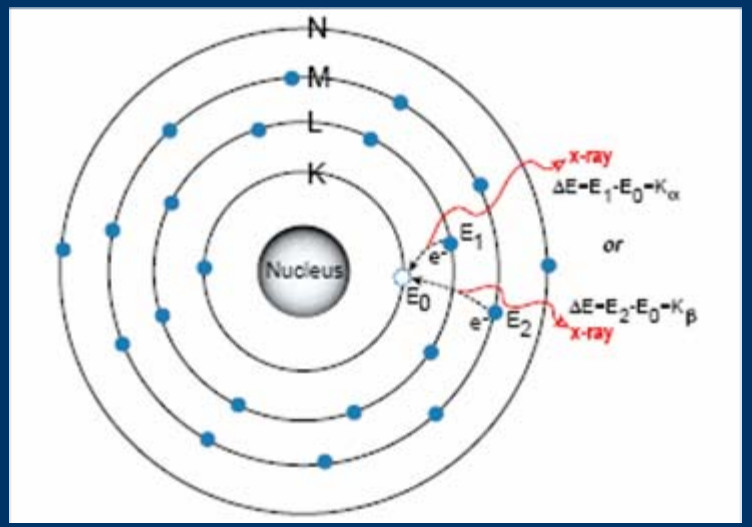
Relevant interactions of X-ray photons with matter

X-ray Absorption

✓ An electron in the given shell (e.g. K) is ejected from the atom by an external primary excitation x-ray photon, creating a vacancy.

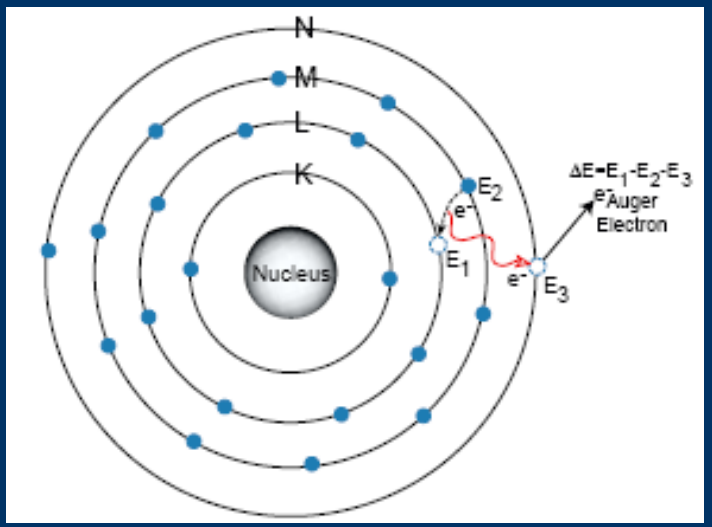


X-ray fluorescence photon



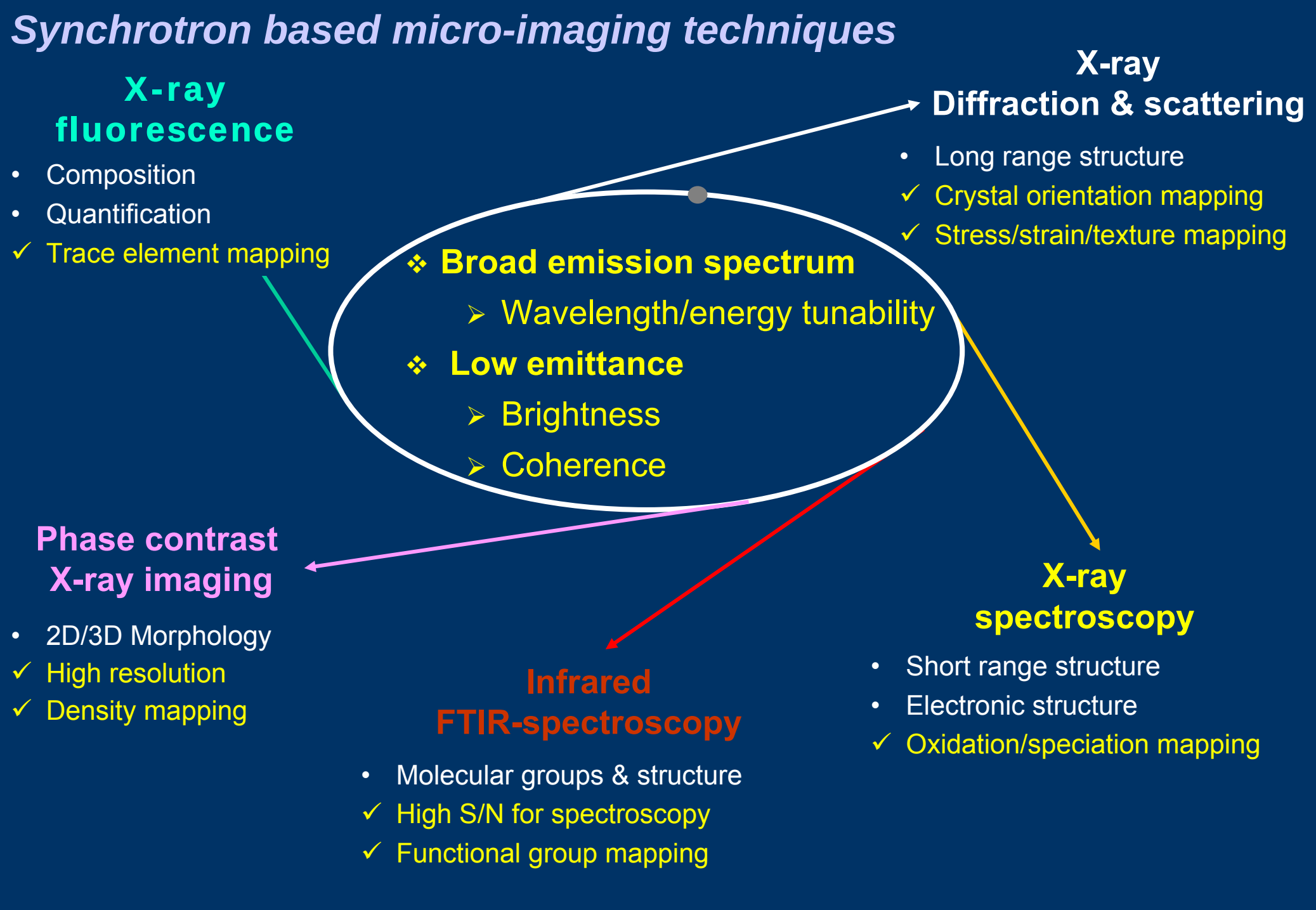
✓ Higher energy core electron fills empty electron level, and ejects an x-ray photon of fixed energy.

"Auger" Electron

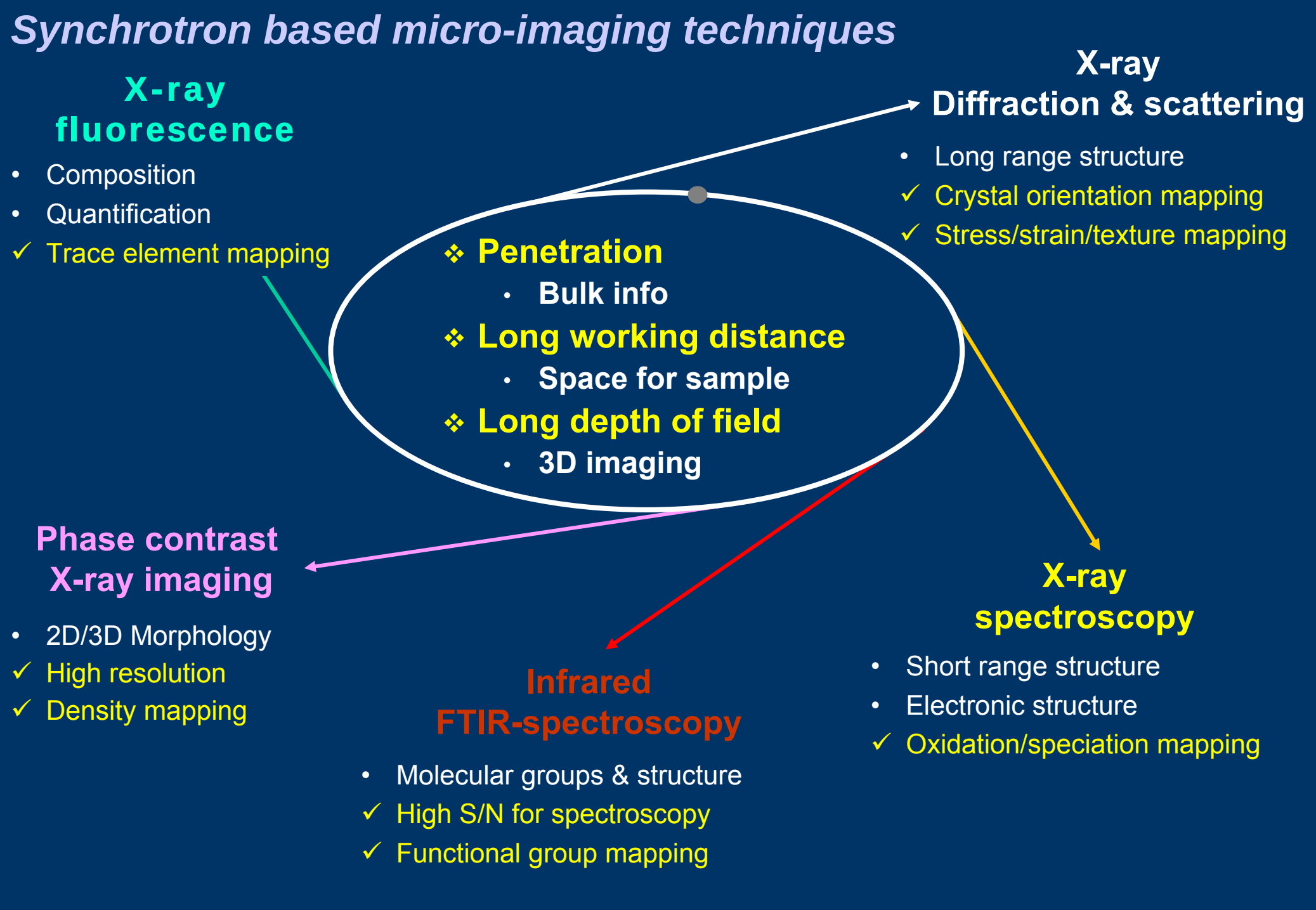


✓ The excitation energy from the inner atom is transferred to one of the outer electrons causing it to be ejected from the atom.

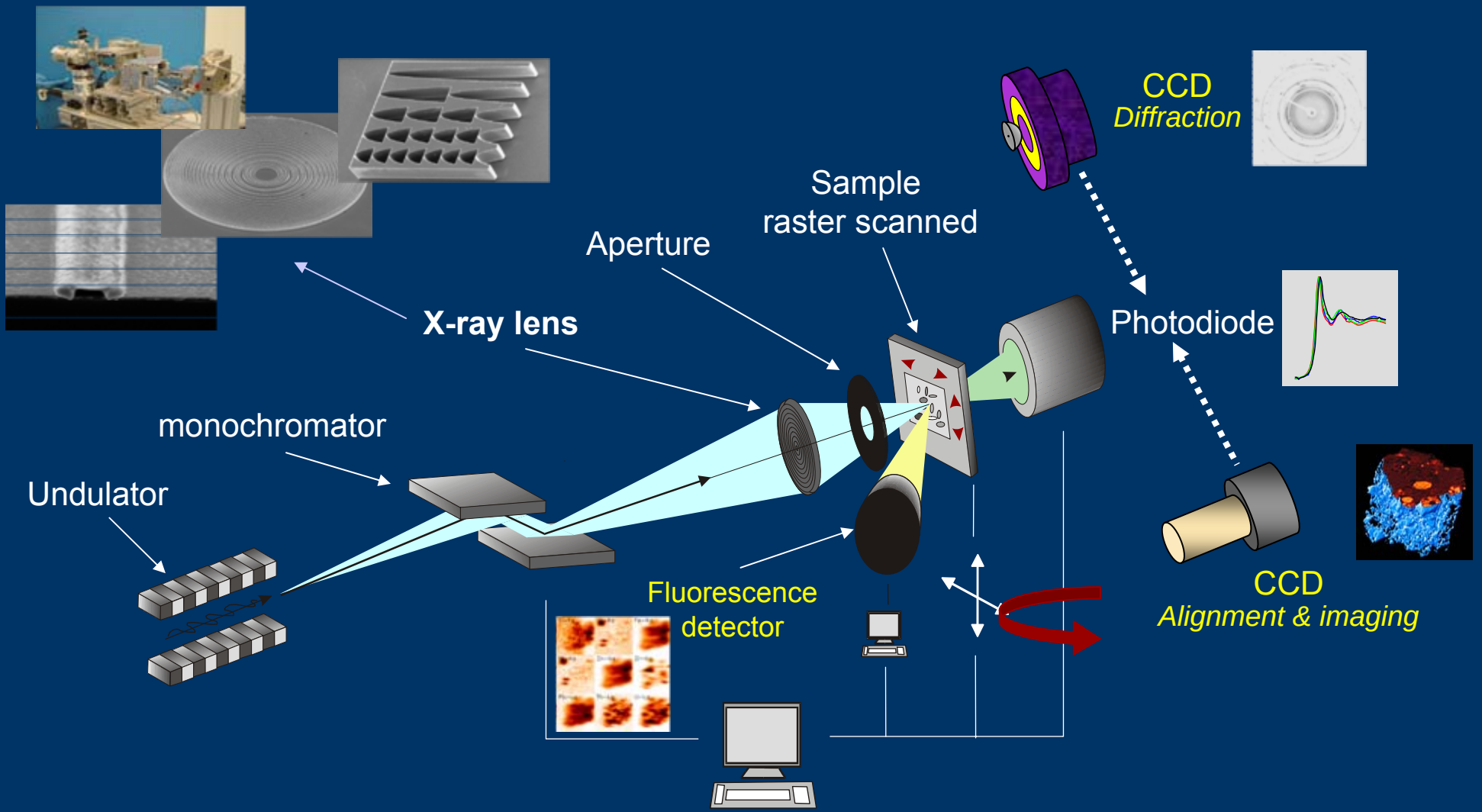
Synchrotron based micro-imaging techniques



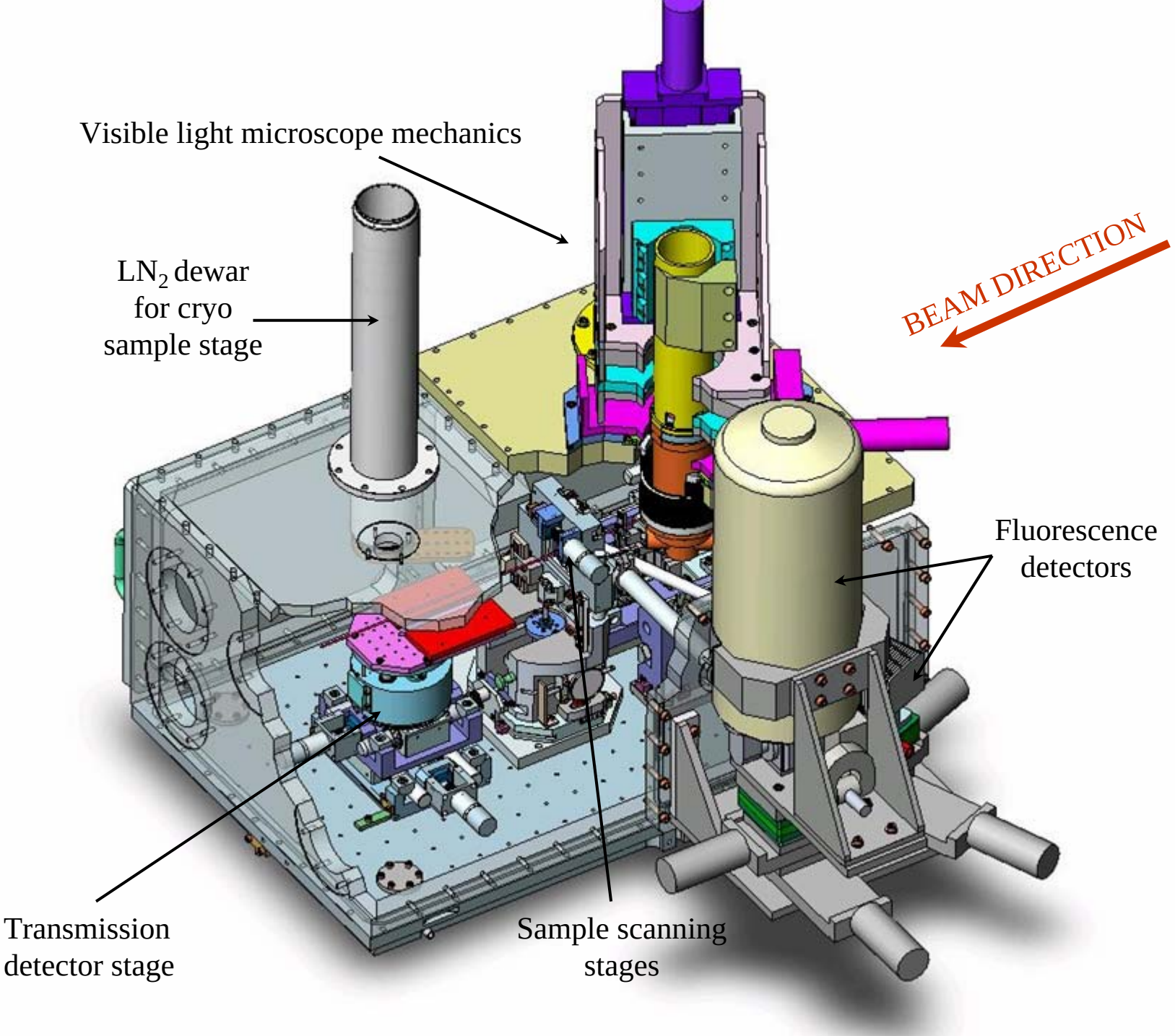
Synchrotron based micro-imaging techniques

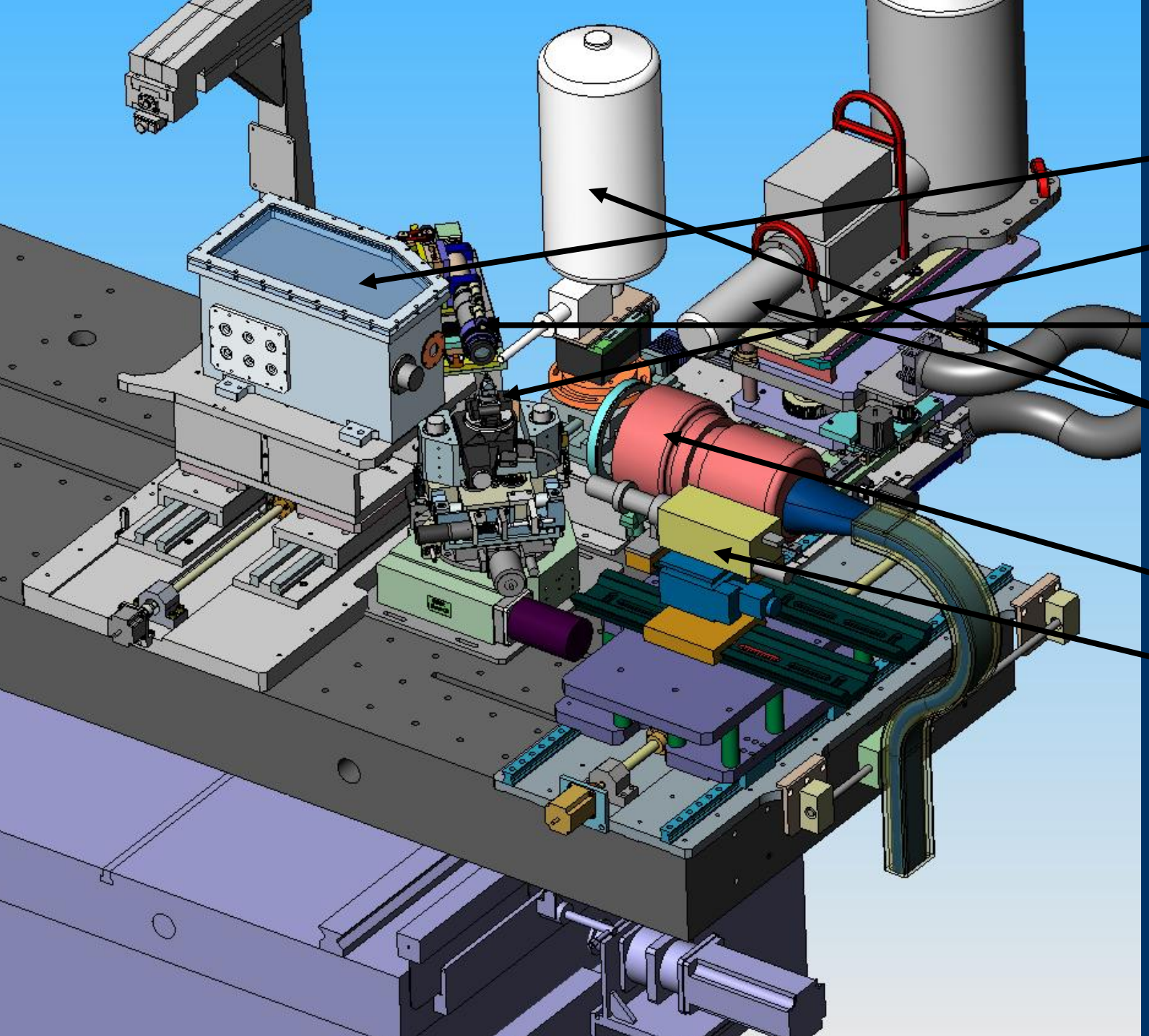


Synchrotron based hard X-ray microprobe



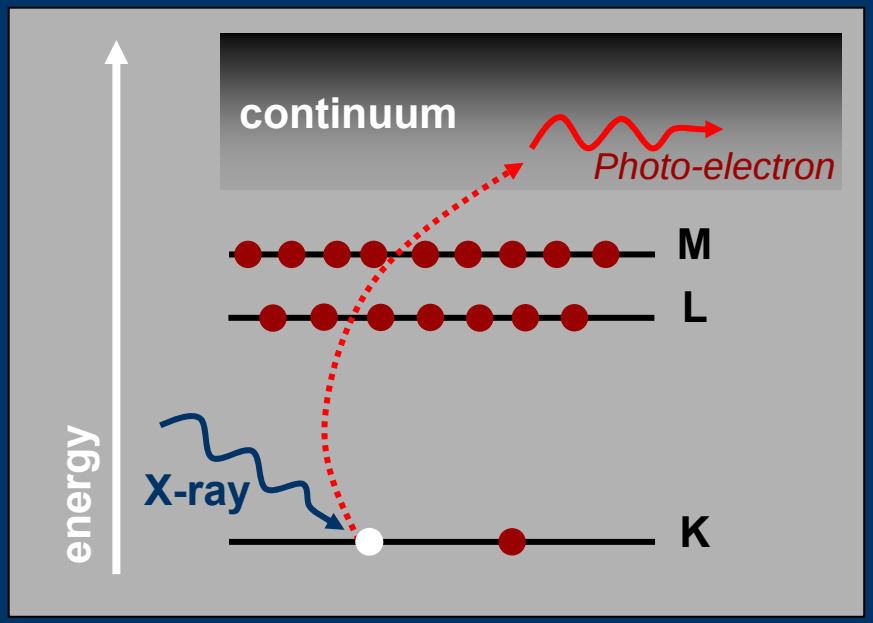
- **Spatial resolution : 0.05-2µm**
- **Spectral resolution : $\Delta E/E \sim 10^{-2} - 10^{-4}$**
- **Averaged flux : $10^{9} – 10^{13}$photons/s/µm^2**

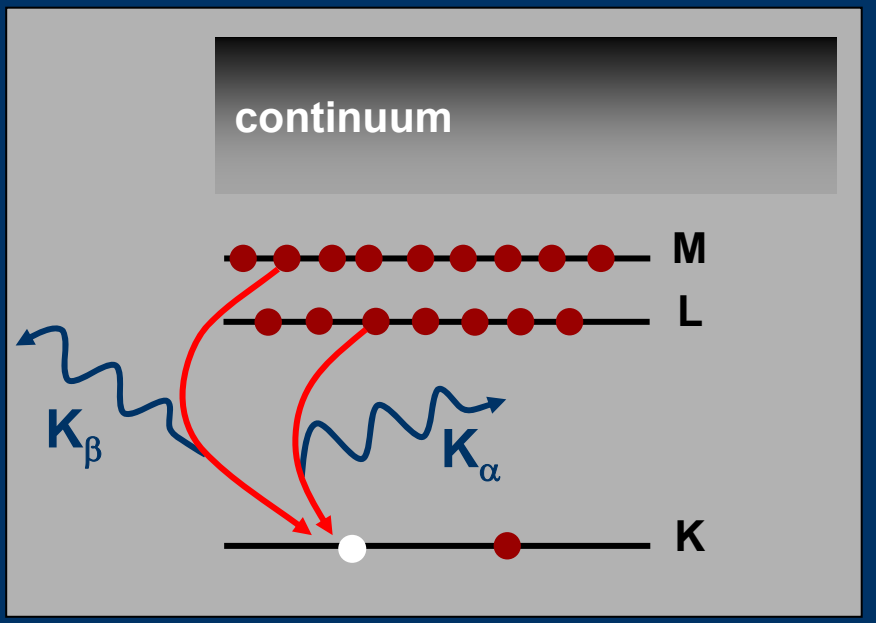

Visible light microscope mechanics
LN_2 dewar for cryo sample stage
BEAM DIRECTION
Fluorescence detectors
Transmission detector stage
Sample scanning stages

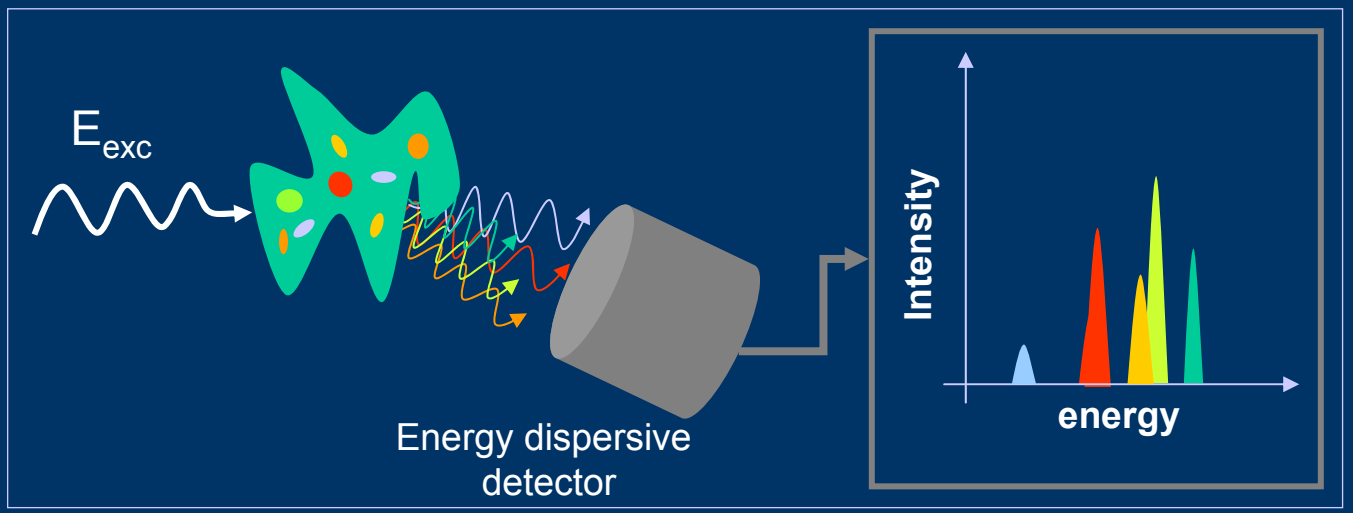


- KB mirror system
- Sample stage
- Video microscope
- Si(Li) 1 element
- Si(Li) 13 elements
- Diffraction camera
- Imaging camera

X-ray Fluorescence: a brief reminder

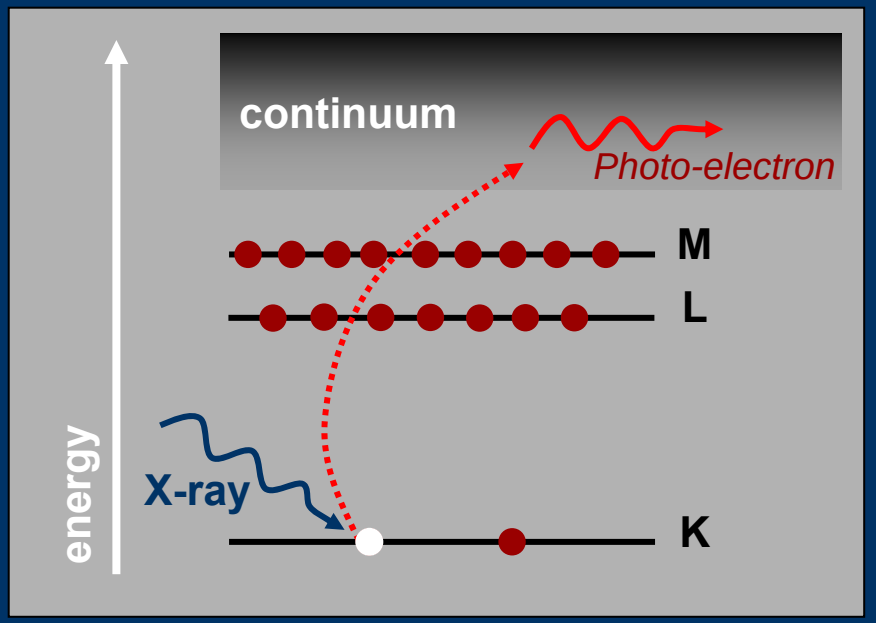


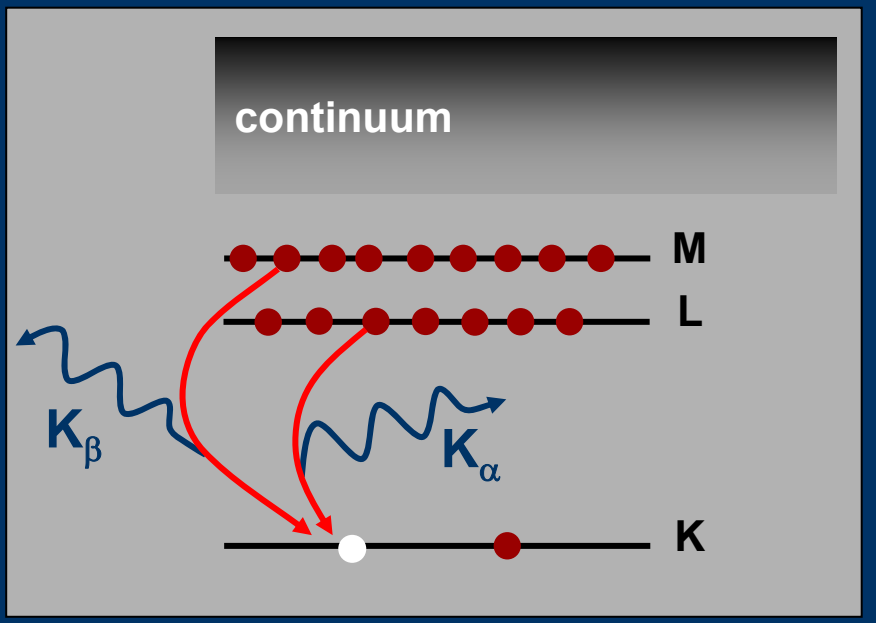


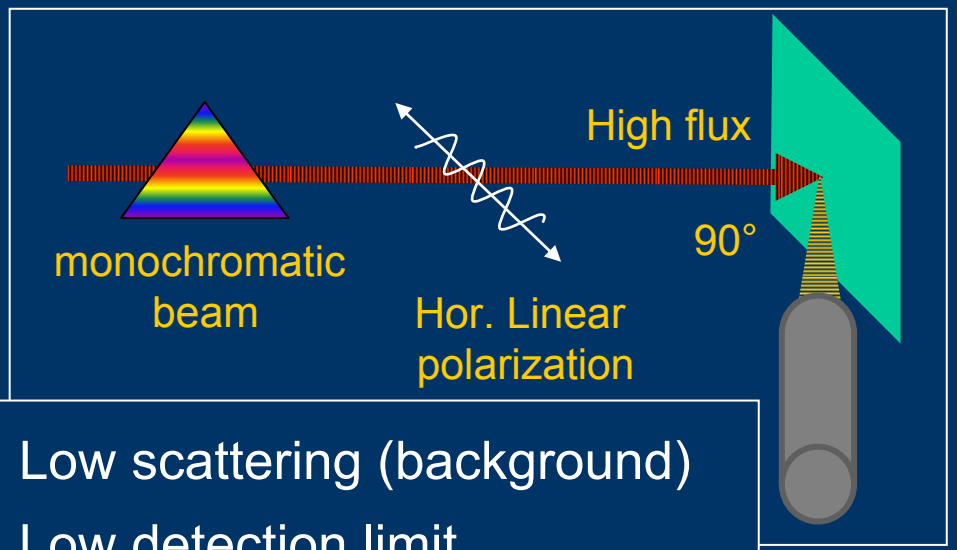


- ✓ **Element specific**
- ✓ **Co-localization**
- ✓ **Quantification**

X-ray Fluorescence: a brief reminder







- Low scattering (background)
- Low detection limit

XRF geometry on synchrotron beamline

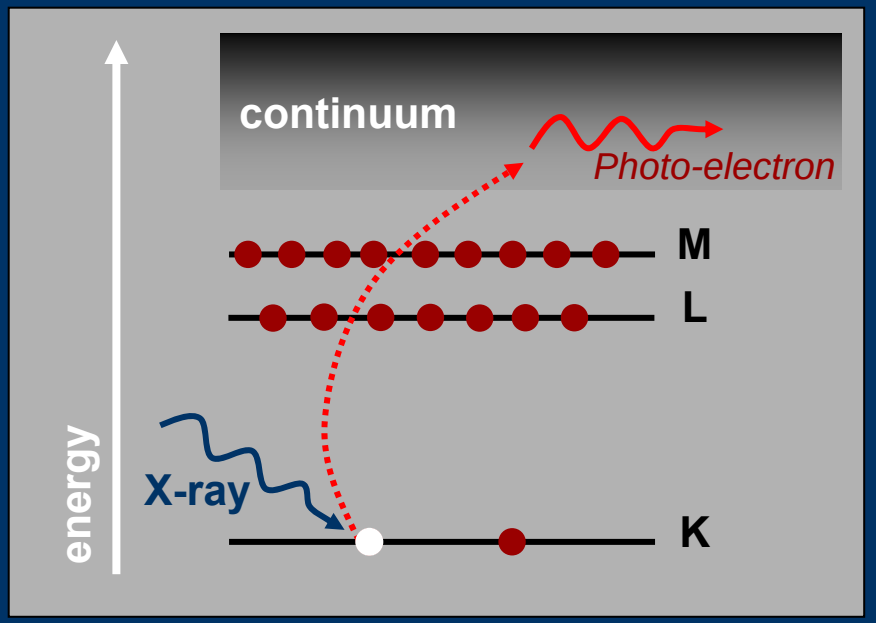
- X-ray absorption spectroscopy

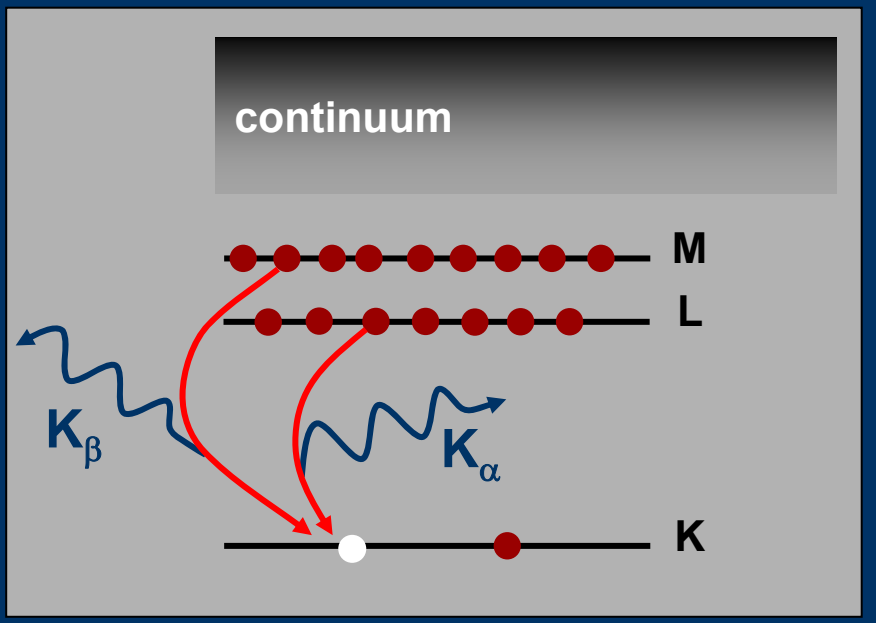
✓ **Element specific**

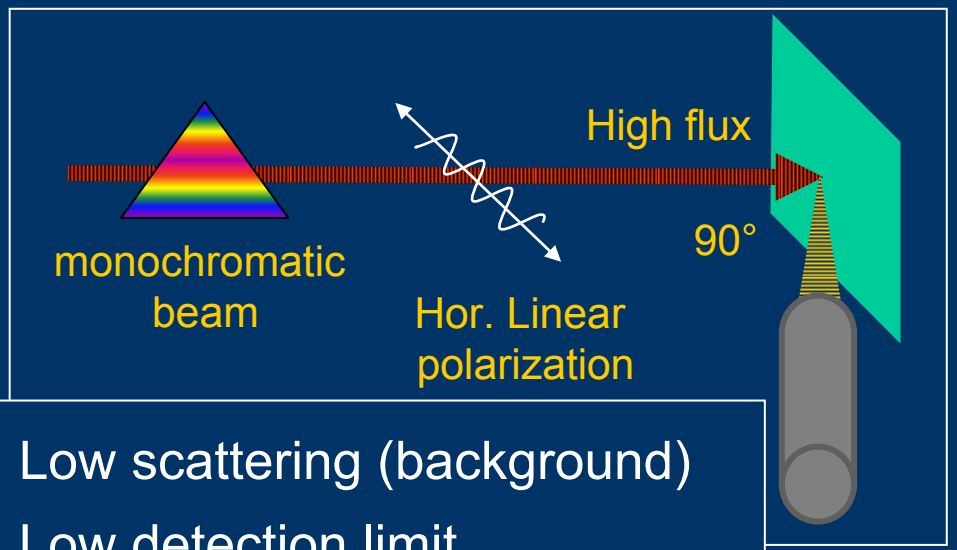
✓ **Co-localization**

✓ **Quantification**

X-ray Fluorescence: a brief reminder



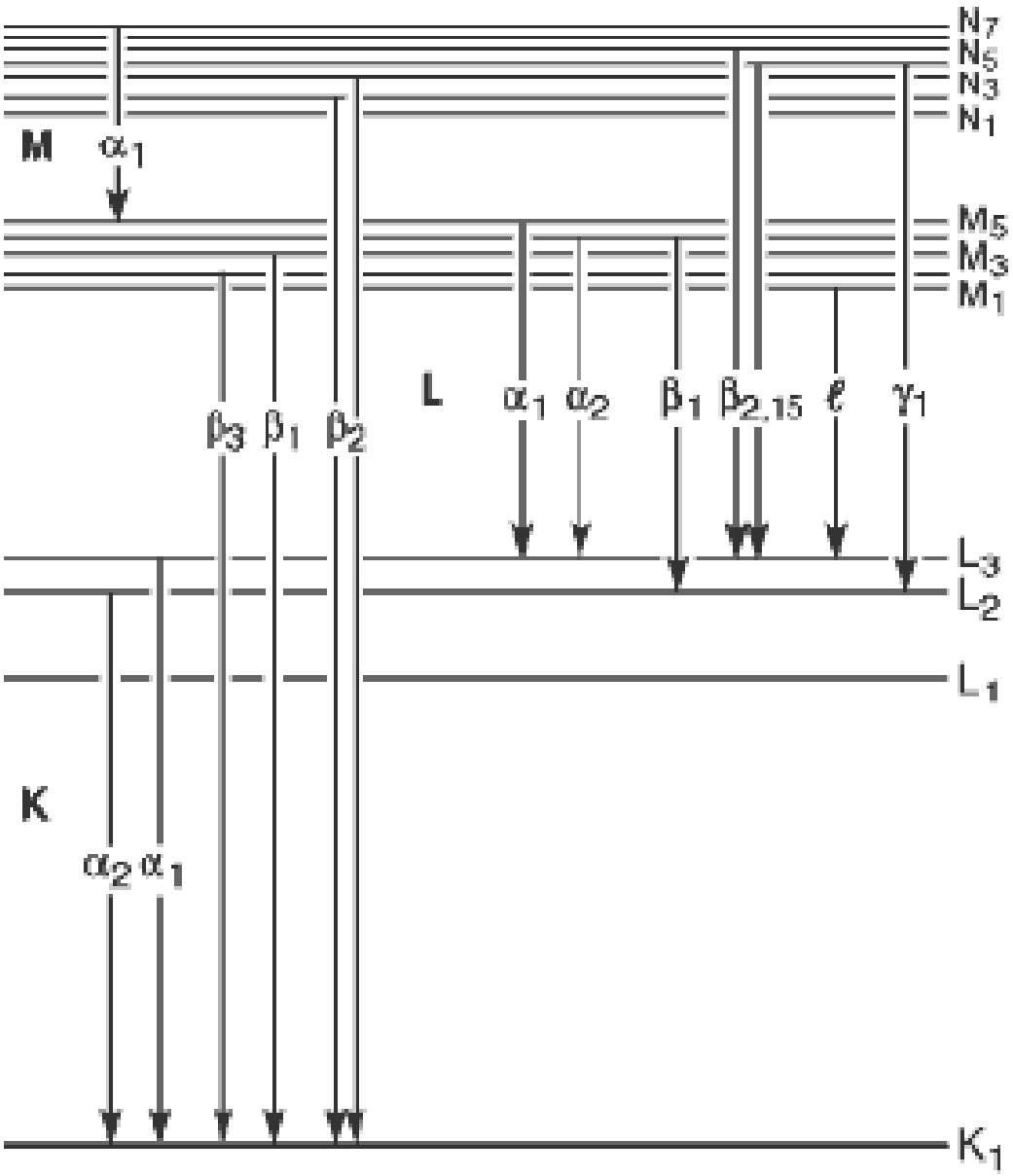




- Low scattering (background)
- Low detection limit
- X-ray Absorption spectroscopy

- ✓ **Element specific**
- ✓ **Co-localization**
- ✓ **Quantification**
- ✓ **High sensitivity**
- ✓ **Chemical info.**

X-ray Lines and Transitions



J. A. Bearden,
Rev. Mod. Phys. **39**, 78 (1967)

Photon energies, in electron volts, of principal K-, and L- shell emission lines

Element	Kα_1	Kα_2	Kβ_1	Lα_1	Lα_2	Lβ_1	Lβ_2	Lγ_1	Mα_1
3 Li	54.3								
4 Be	108.5								
5 B	183.3								
6 C	277								
7 N	392.4								
8 O	524.9								
9 F	676.8								
10 Ne	848.6	848.6							
11 Na	1,040.98	1,040.98	1,071.1						
12 Mg	1,253.60	1,253.60	1,302.2						
13 Al	1,486.70	1,486.27	1,557.45						
14 Si	1,739.98	1,739.38	1,835.94						
15 P	2,013.7	2,012.7	2,139.1						
16 S	2,307.84	2,306.64	2,464.04						
17 Cl	2,622.39	2,620.78	2,815.6						
18 Ar	2,957.70	2,955.63	3,190.5						
19 K	3,313.8	3,311.1	3,589.6						
20 Ca	3,691.68	3,688.09	4,012.7	341.3	341.3	344.9			
21 Sc	4,090.6	4,086.1	4,460.5	395.4	395.4	399.6			

From http://xdb.lbl.gov/xdb.pdf

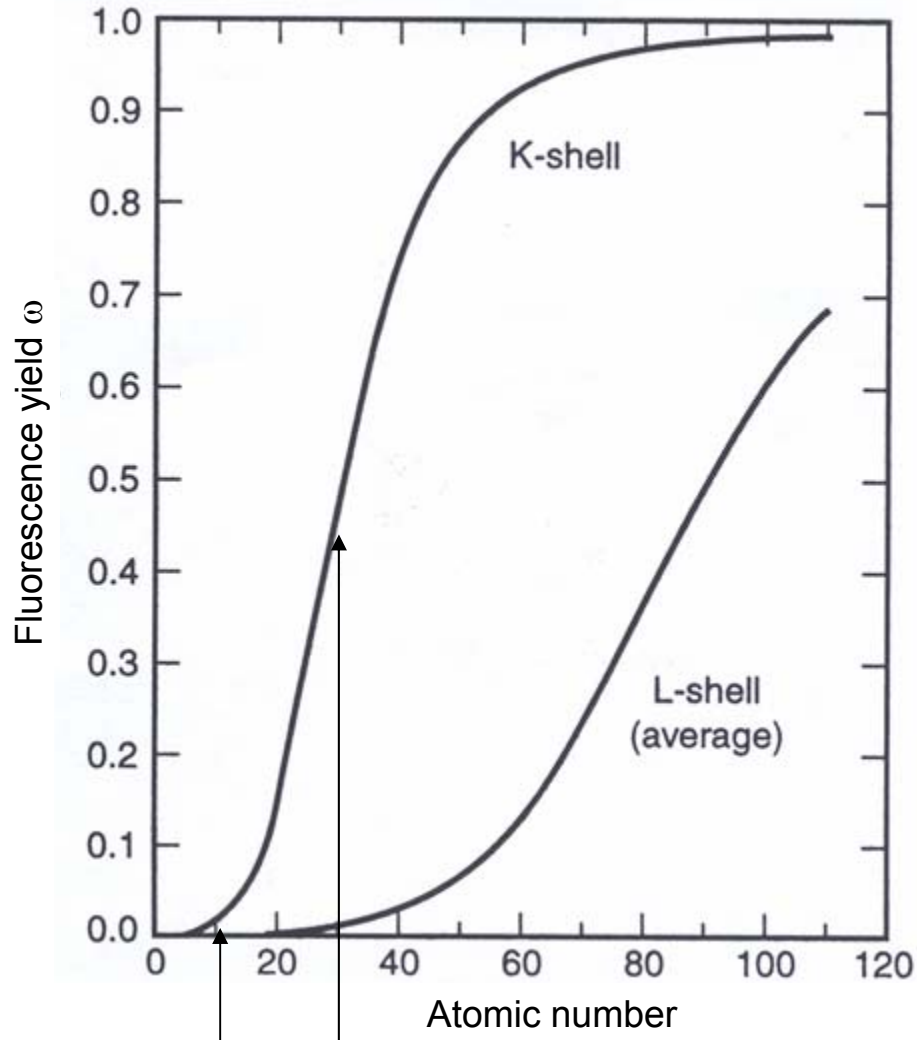
The fluorescence yield ω

✓ ω is the probability that an x-ray photon will be emitted as a result of ionization of a specific shell. For a given series of X-ray lines (e.g., the K series), ω is numerically equal to the ratio of K photons escaping from the atom to the ratio of original K-shell ionizations.

✓ The fluorescence yield for K lines increases monotonically as a function of atomic number, and algebraic models accurately predict the empirical data.

✓ This factor means that the sensitivity of the x-ray method decreases for the lighter elements. The decrease, however, is partly compensated by the higher photo-absorption cross sections in light elements.

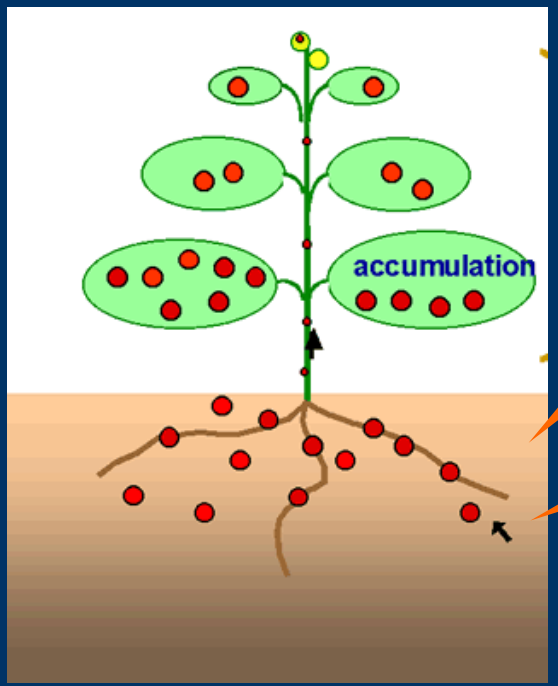
M.O. Krause, *J. Phys. Chem. Ref. Data*, 89,(1979)



Example:

- **zinc (Z=30) ω_k = 0.45**
- **sodium (Z=11) ω_k = 0.02**

Phytoextraction in hyper accumulator plants



Anthropogenic activities

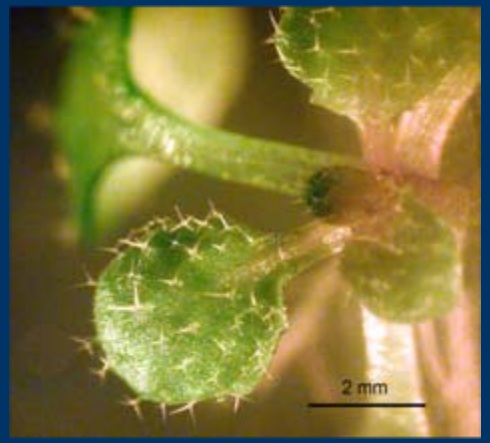
Cesium (Cs)
Nuclear activities

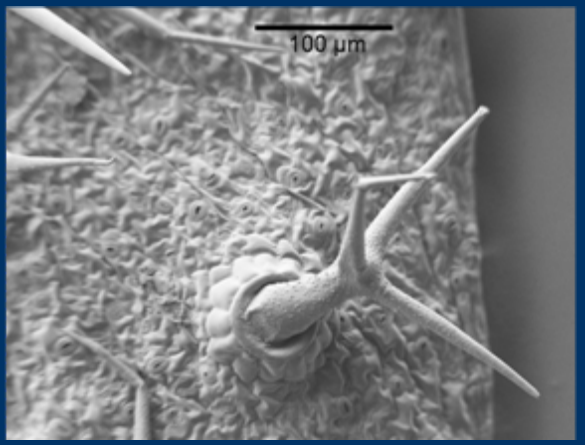
Cadmium (Cd)
Industrial (mining), agricultural activities (fertilizers)

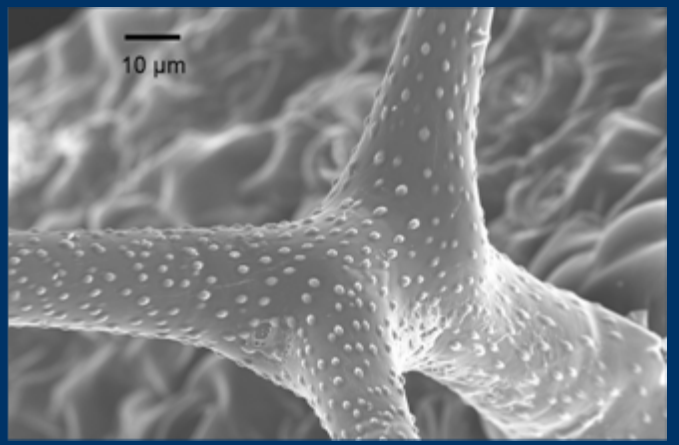
- Green and low cost strategy for soil cleaning
- Requires knowledge on the mechanisms of metal accumulation

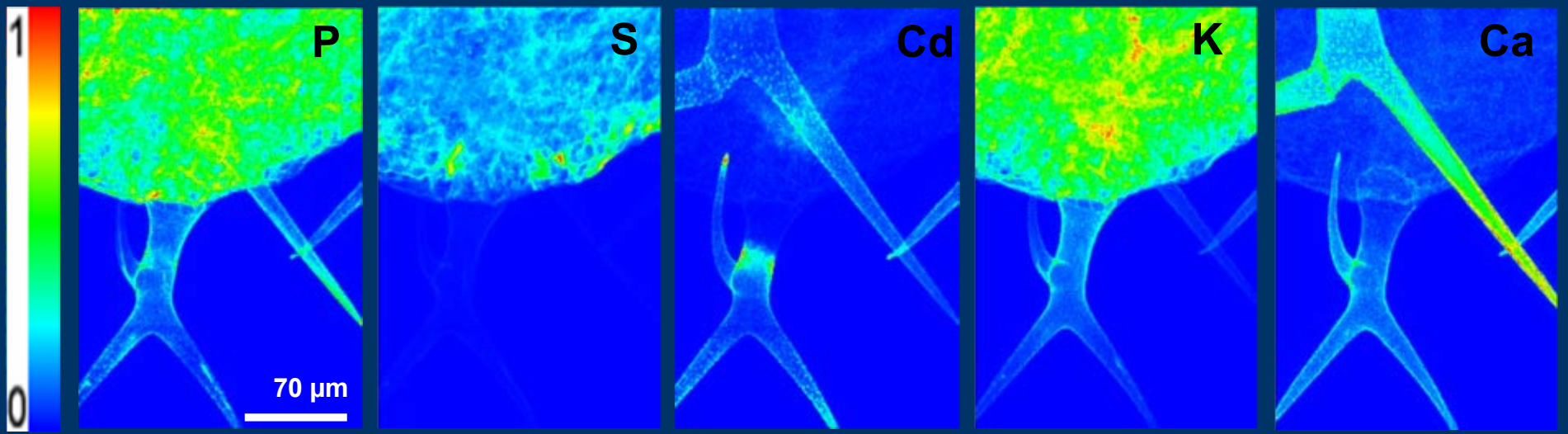
M.P. Isaure *et al*., *Biochimie*, 2006

XRF mapping in Trichomes of Arabidopsis Thaliana





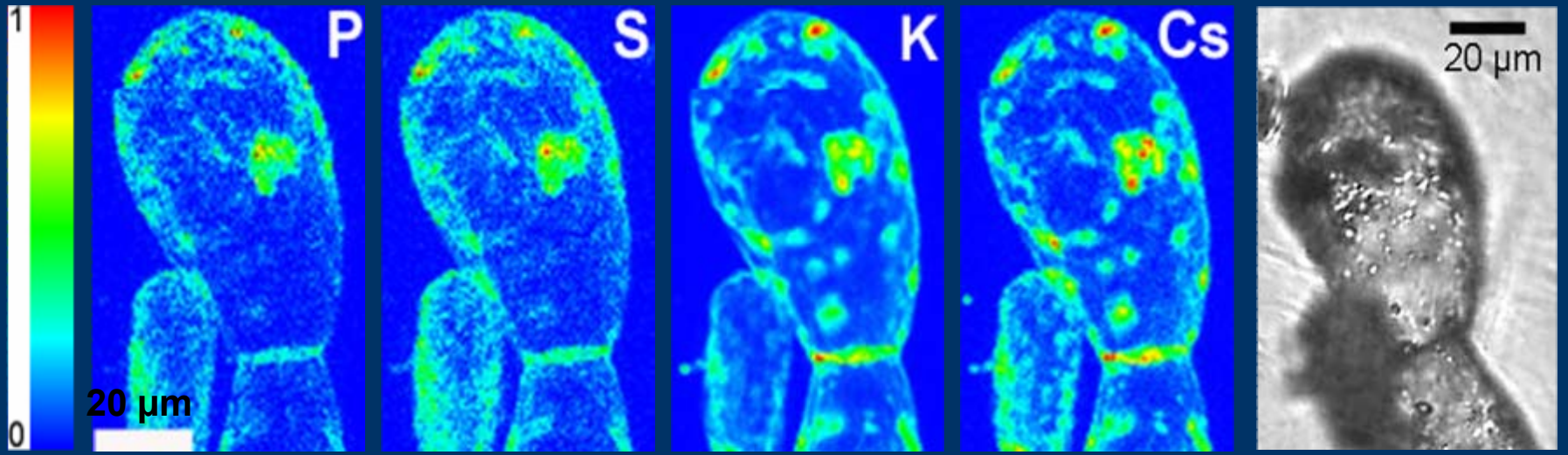


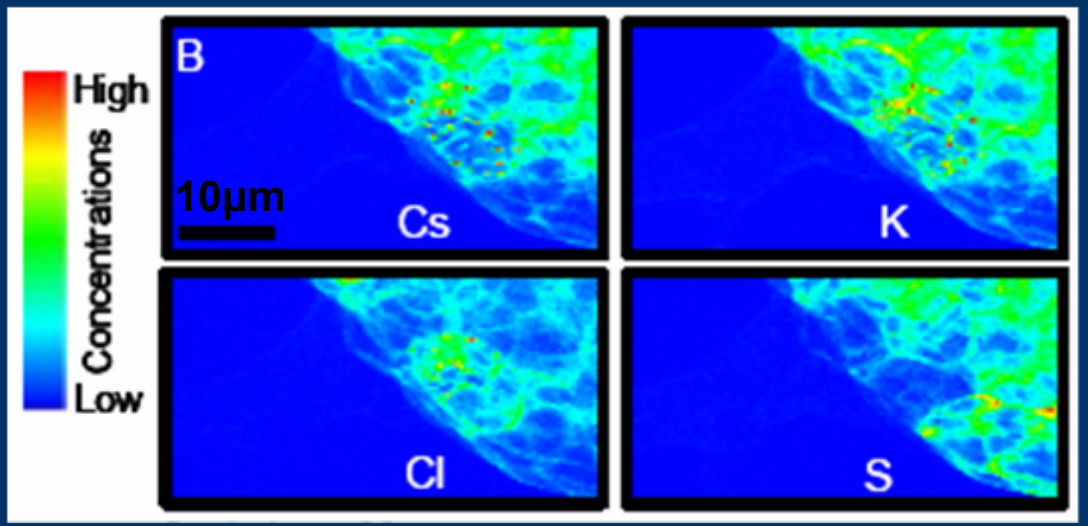


E_{ex}: 5.8 keV, probe size: 0.4x0.2µm^2, dwell time: 800 ms/pixel.

M.P. Isaure *et al*., *Biochimie*, in press

Cs accumulation in Arabidopsis Thaliana





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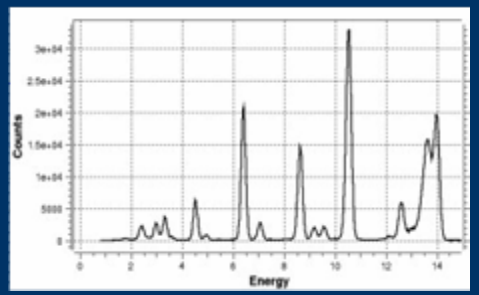
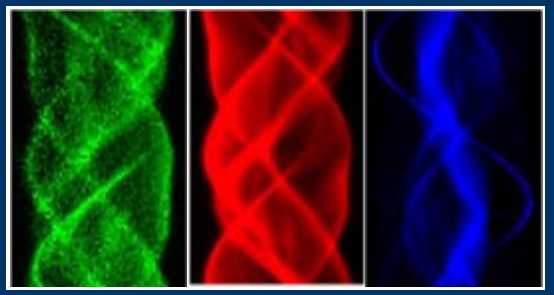
M.P. Isaure *et al.*, *Biochimie*, 2006

Fluorescence tomography (3D-µXRF)

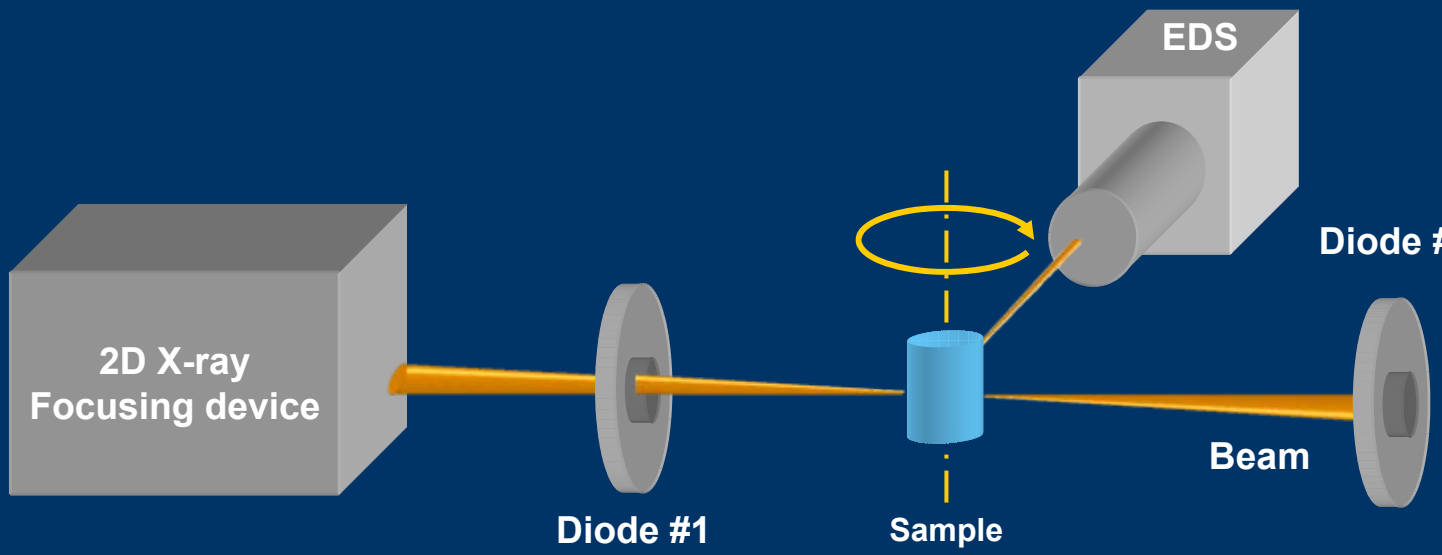
Pixel- by-pixel acquisition



Sinogram(s)

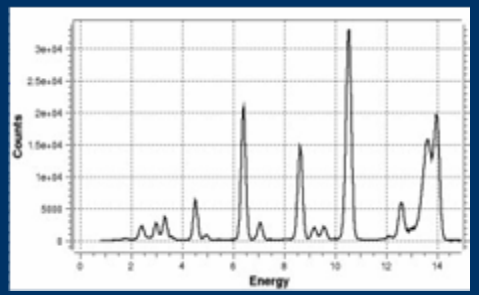


2D-Slice or 3D-Volume

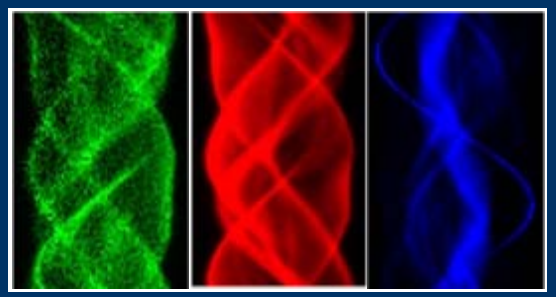


Fluorescence tomography (3D-µXRF)

Pixel- by-pixel acquisition



Sinogram(s)

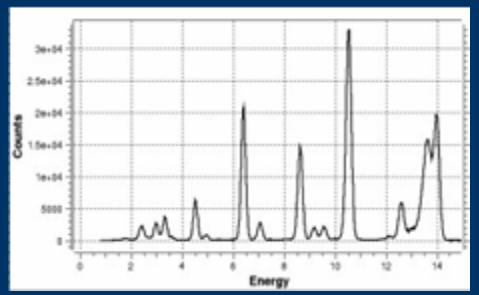


2D-Slice or 3D-Volume

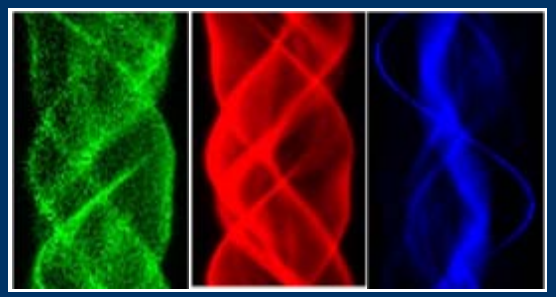
- The reconstruction problem is far more difficult compared to transmission tomography
  - self absorption corrections
  - $\mu(E_a, x)$ is a priori unknown
  - weak fluorescence signal for light elements

Fluorescence tomography (3D-μXRF)

Pixel- by-pixel acquisition



Sinogram(s)



2D-Slice or 3D-Volume

Algorithmic solution:

Optimal estimation of attenuation maps by combination of transmission, fluorescence and Compton tomographies

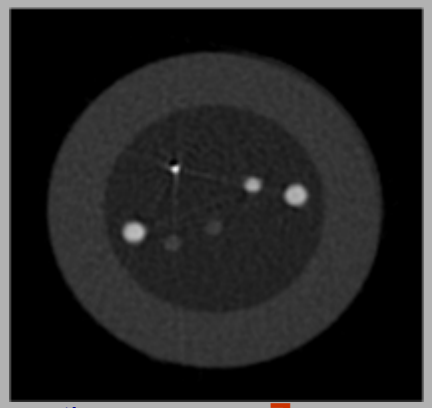
- B. Golosio *et al., J. Appl. Phys. 94*(1), 145 (2003)

Geometrical solution:

Collimation of the detection angle to define a voxel: confocal geometry

- B. L. Vincze et al., Anal.Chem., 76(22) (2004)

Combining several signals



section $\sigma_{Compton} \sim Z$

Compton tomography

- provides information on the ***electronic density*** spatial distribution

Transmission tomography

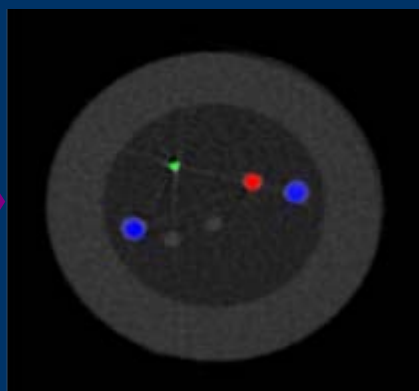
- Provides the ***absorption coefficient*** distribution $\mu(E_0, \mathbf{x})$

Ph… absorption: $\sigma_{photo} \sim Z^4$

Fluorescence tomography

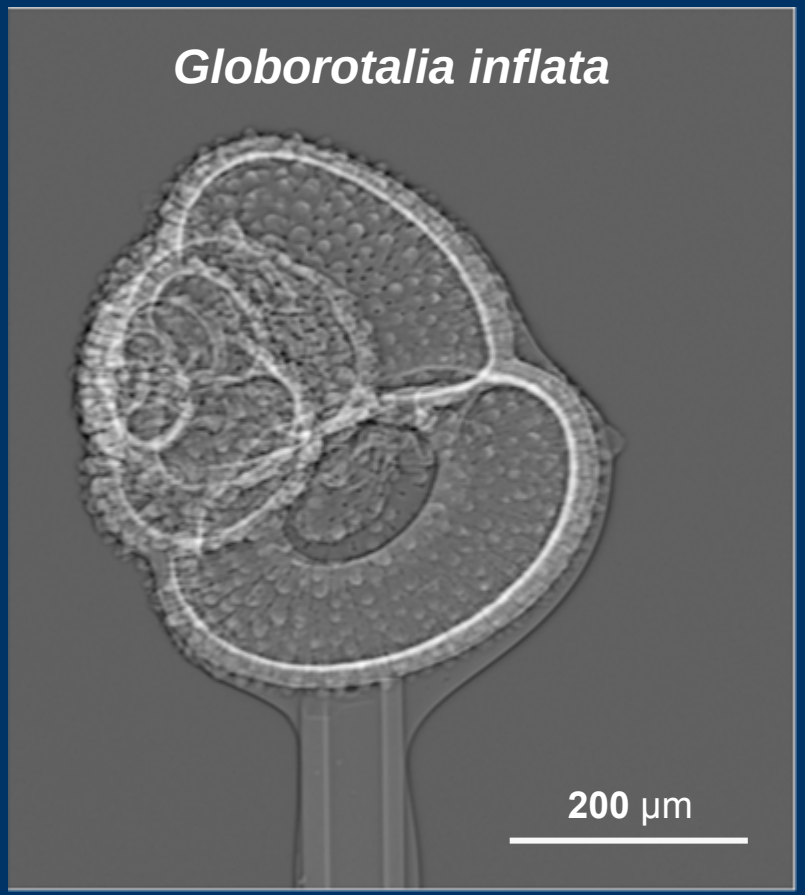
- information on the internal ***spatial distribution of specific elements***

QUANTIFICATION?
Integration of the information
(transmission+Compton+fluorescence)



B. Golosio *et al., J. Appl. Phys. 94*(1), 145 (2003)

X-ray fluorescence tomography



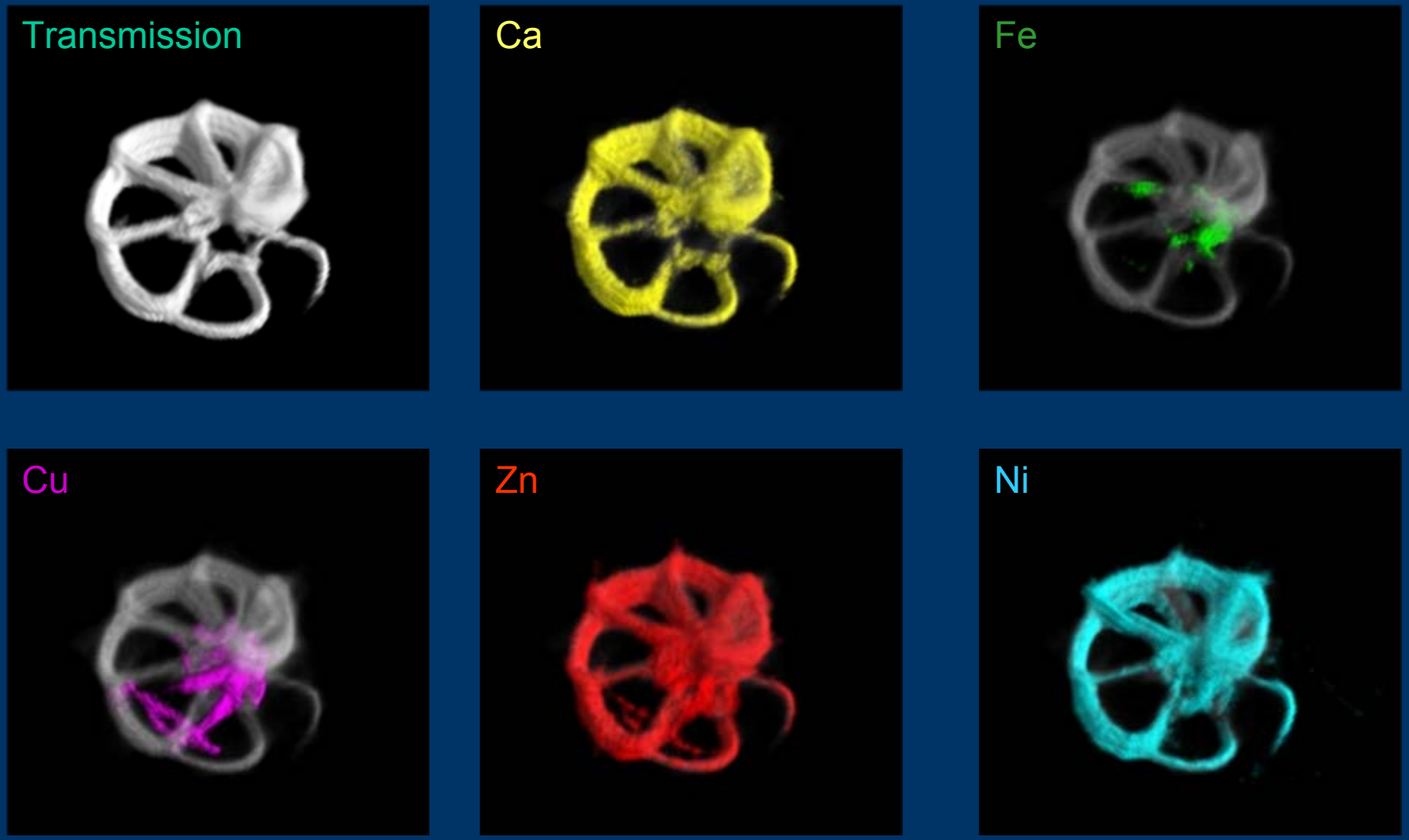
X-ray radiograph

- **Foraminifera**
  - ✓ Single-cell marine animals
  - ✓ Fossilized calcium carbonate shells
  - ✓ Proxies for past oceanic conditions (climate)

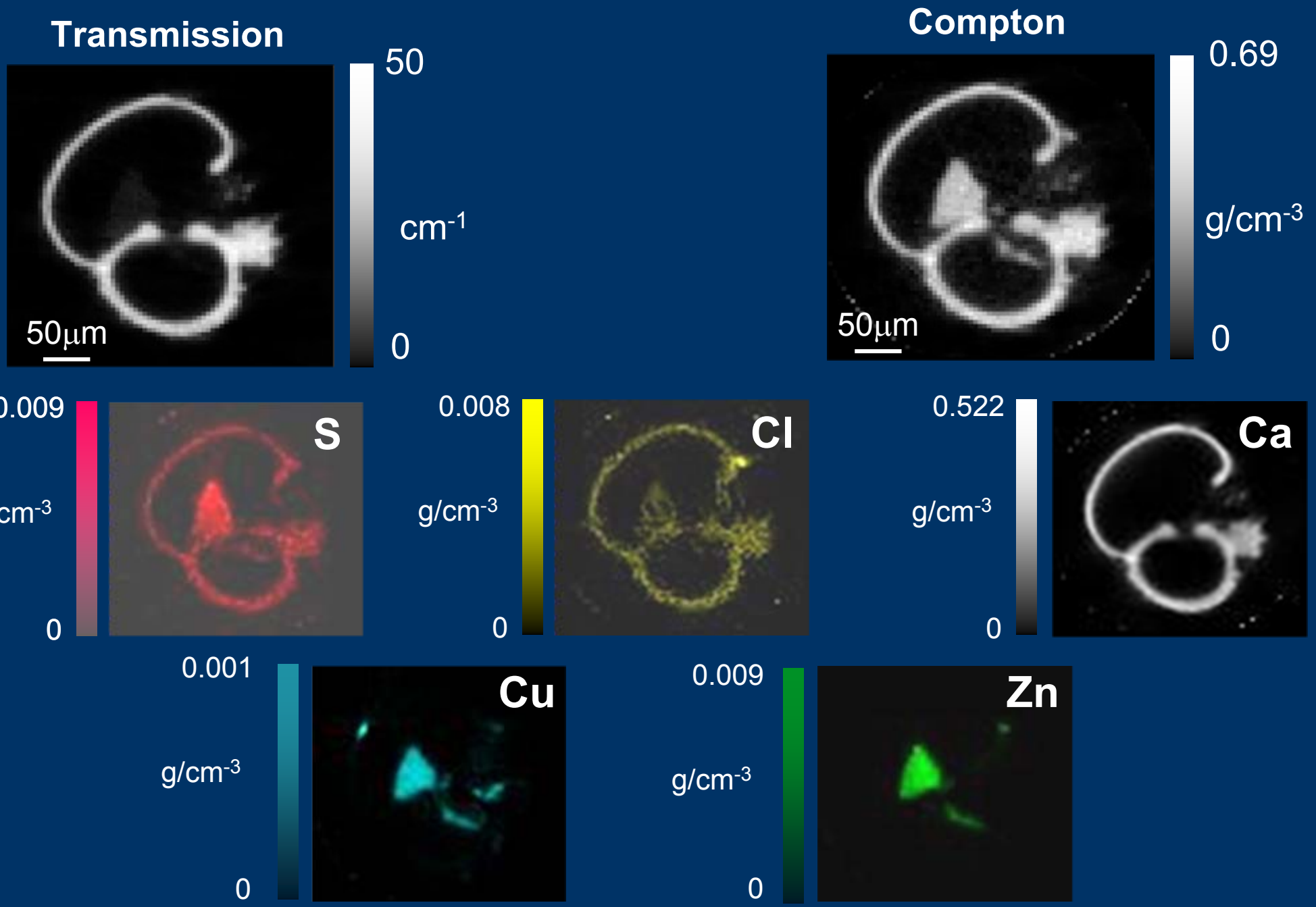
P. Bleuet *et al., SPIE, 2006*

Fluo-tomography: 3D volume rendering

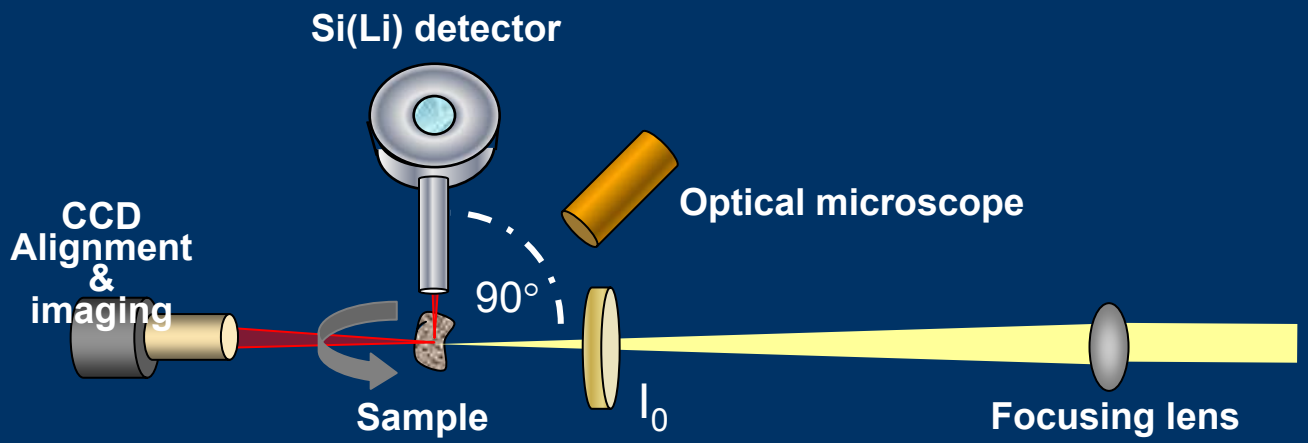
Foraminifera (*Globorotalia inflata*)

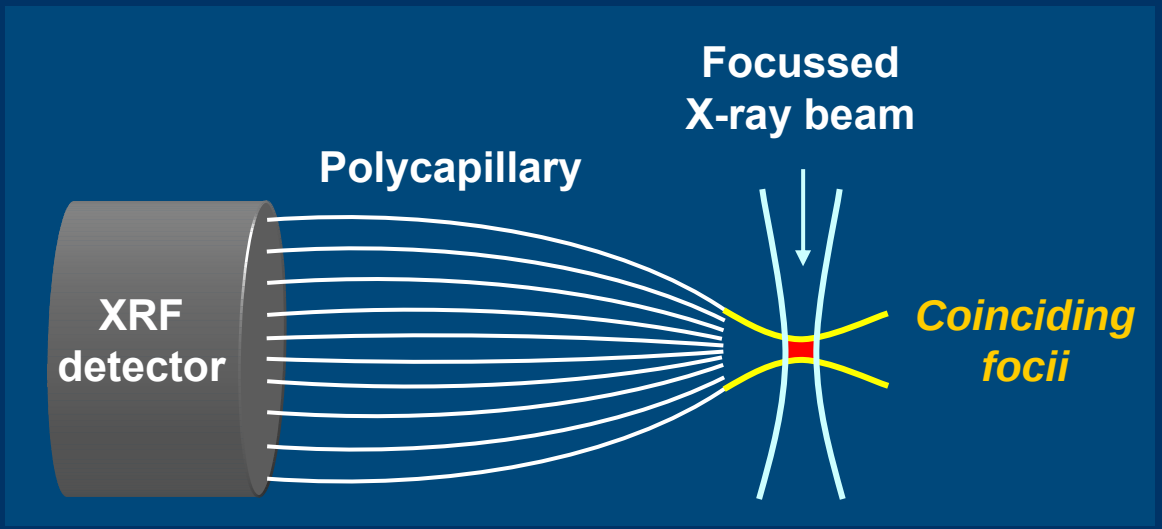


Quantification on 2D slices



X-ray Fluorescence Tomography – "confocal geometry"

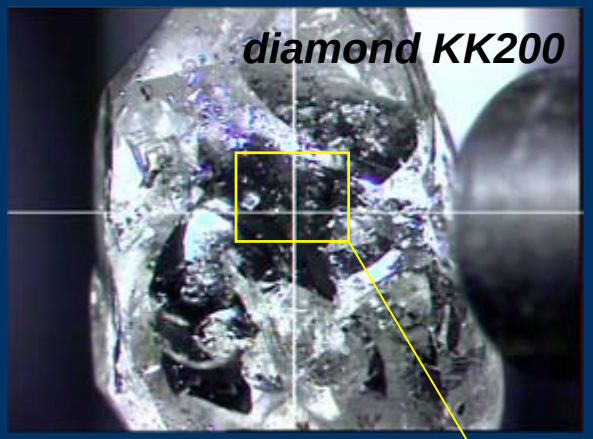


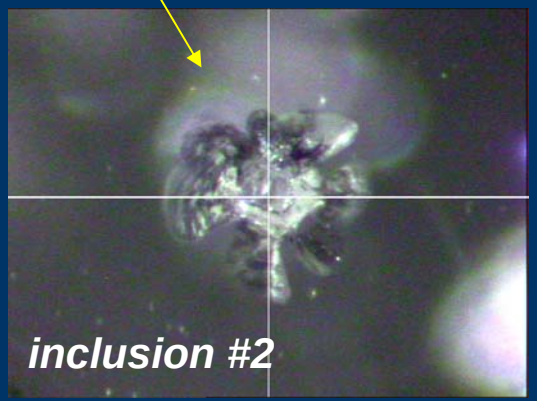


- **Spatial resolution: 5-15µm**

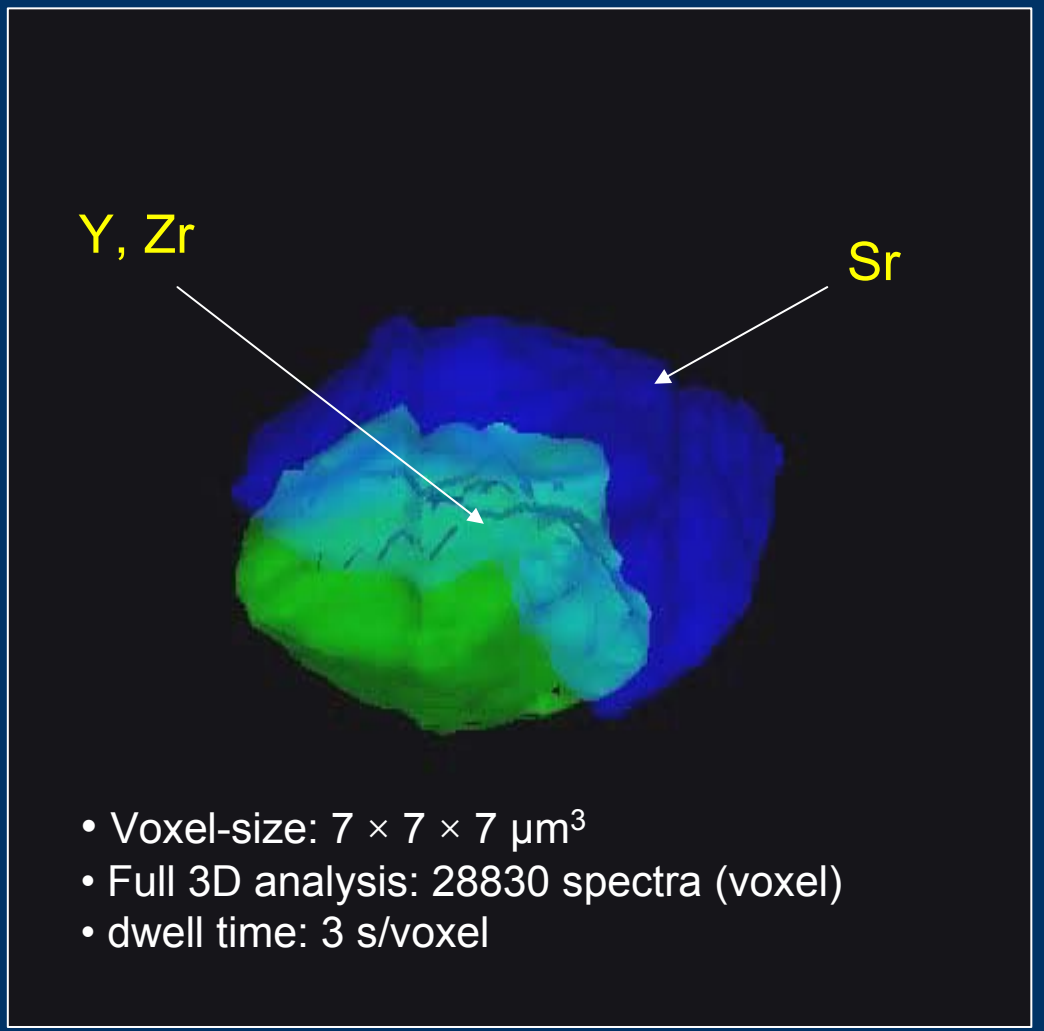
L. Vincze *et al.*, *Anal.Chem.*, 76(22) (2004)

3D-Confocal XRF for tomography





3D-rendering based on measured Sr, Y and Zr-K_α distributions



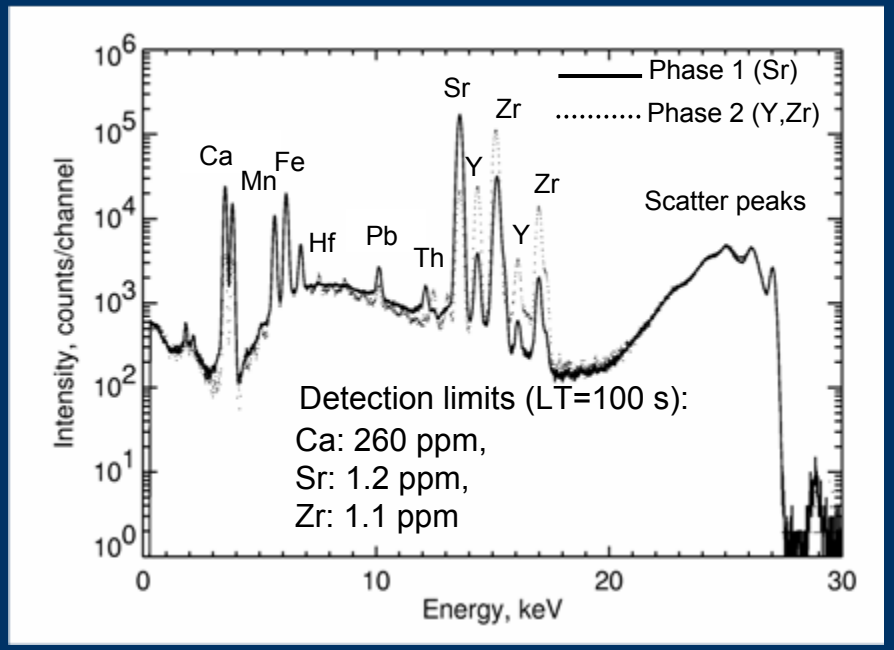
✓ Information on the geochemical environment and conditions in which the diamond was formed

✓ Chemistry at several 100km depth

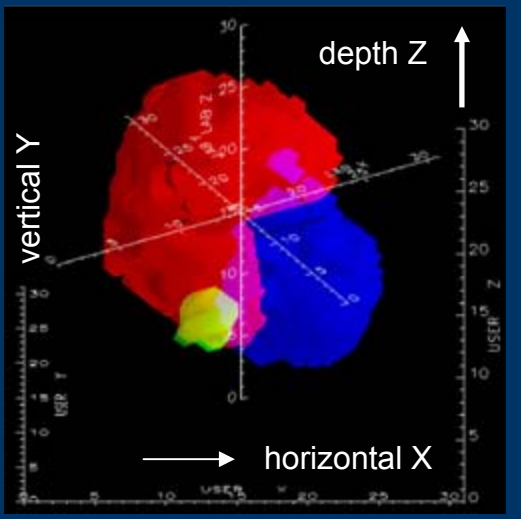
L. Vincze *et al., Anal.Chem.*, 76(22) (2004)

F.E. Brenker *et al.*, *EPSL*, 236, (2005)

From 3D-confocal XRF to quantification (Vekemans et al. JAAS, 2004)



Sr, Th, Y/Zr composed image



Quantification using NIST SRM 613

Element	Phase 1	Phase 2
Ca	29.6 %	18.6 %
Sr	431 ppm	48 ppm
Y	9 ppm	61 ppm
Zr	35 ppm	233 ppm

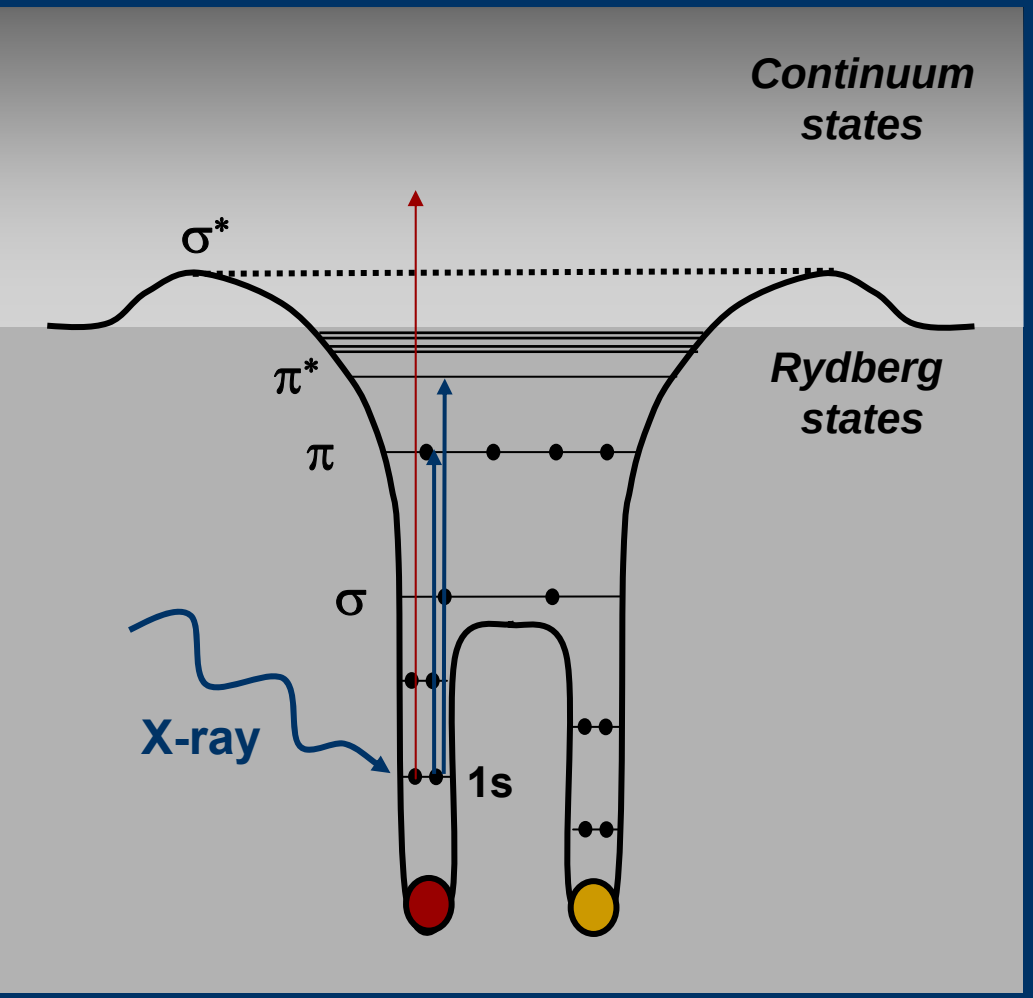
Sr-rich phase
Lamite Ca_2SiO_4

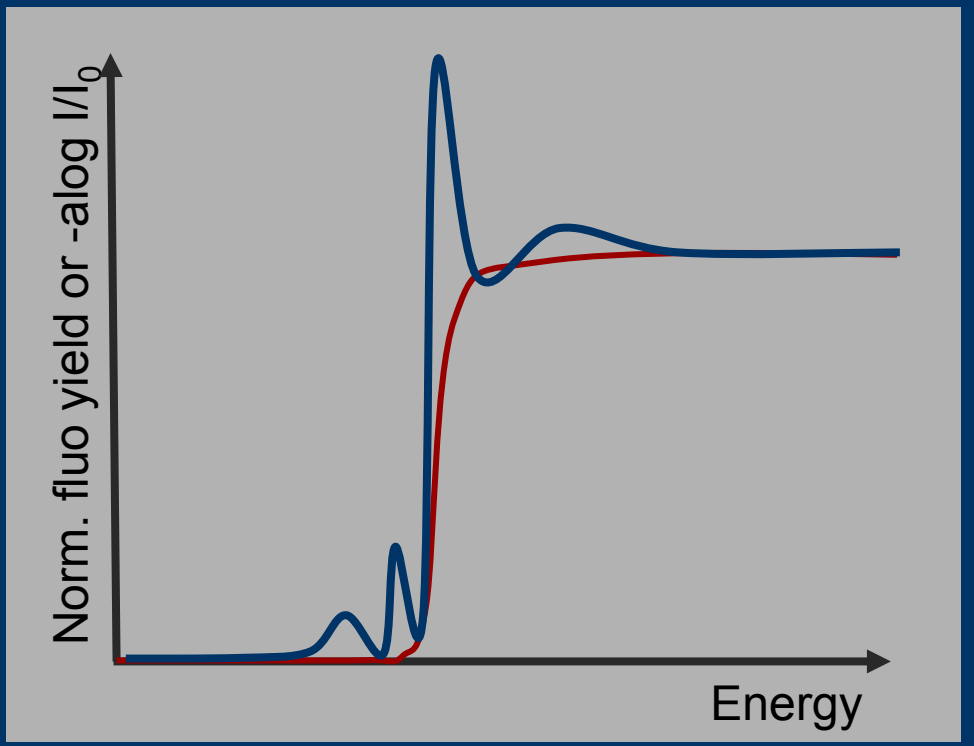
Zr,Y rich phase
Walstromite structured $CaSiO_3$

unusual high Ca concentration

“existence of a Ca-rich diamond reservoir at depth below 300 km”

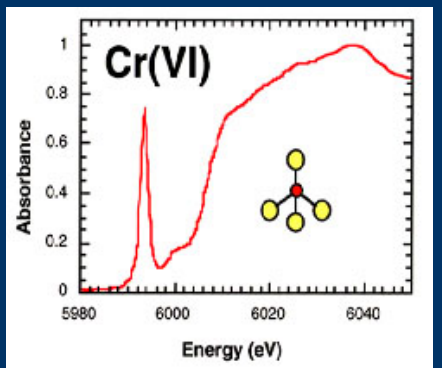
X-ray Absorption Near Edge Structure (XANES)

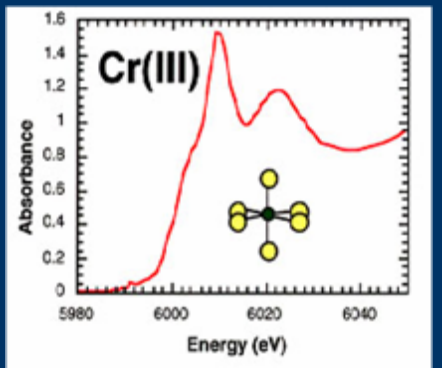




XANES:
electronic transitions to bound states, nearly bond states or continuum

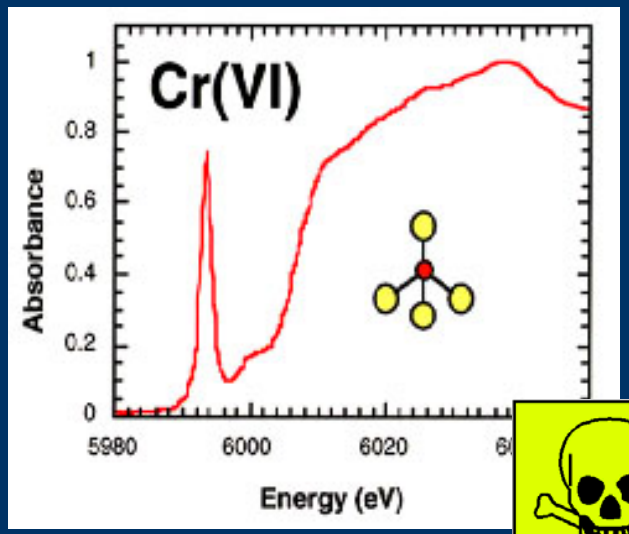
- **Local site symmetry**
- **Oxidation state**
- **Orbital occupancy**

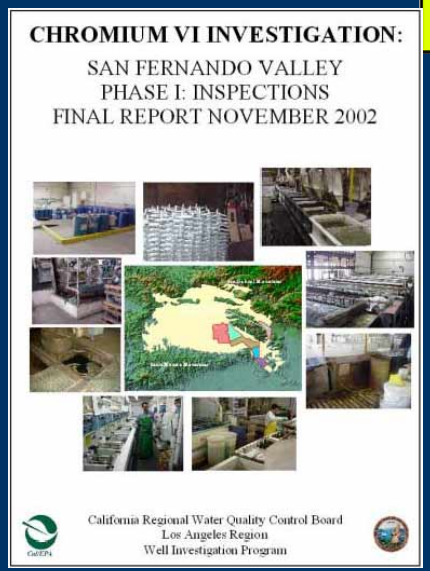


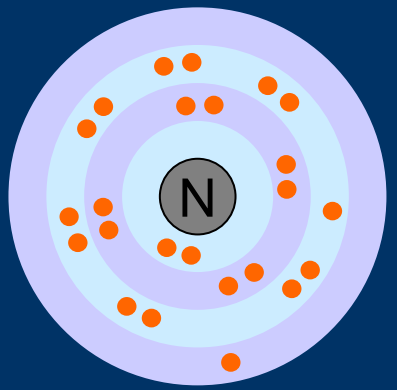


Chromium compounds

- 6 electrons
Cr VI (hexavalent)

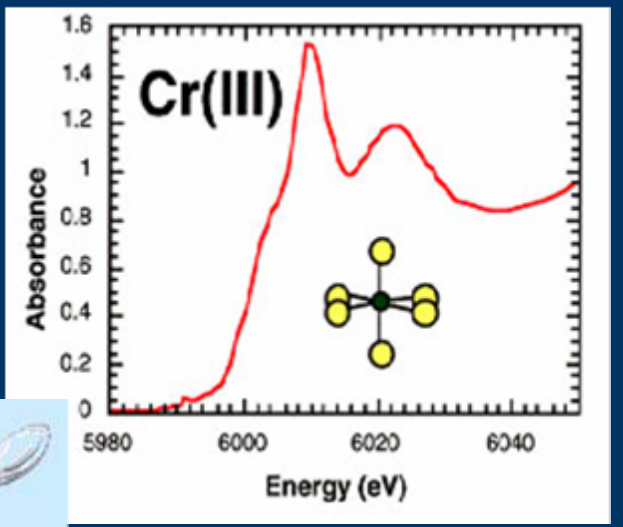






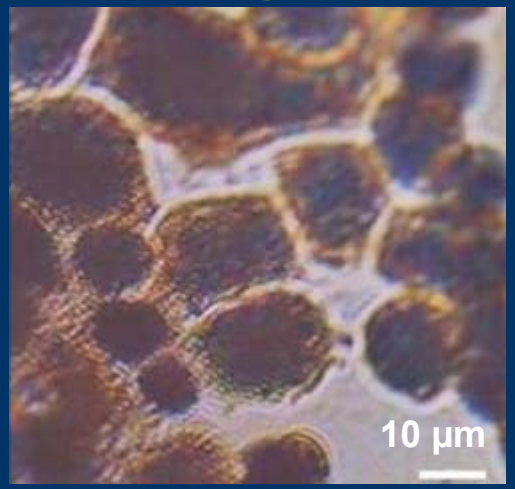
24 electrons

- 3 electrons
Cr III (trivalent)

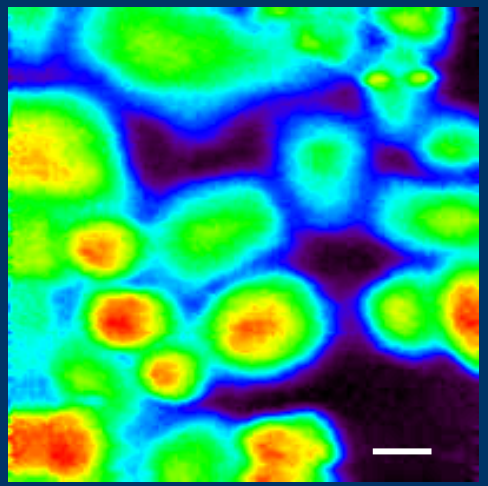


Chromium in cells

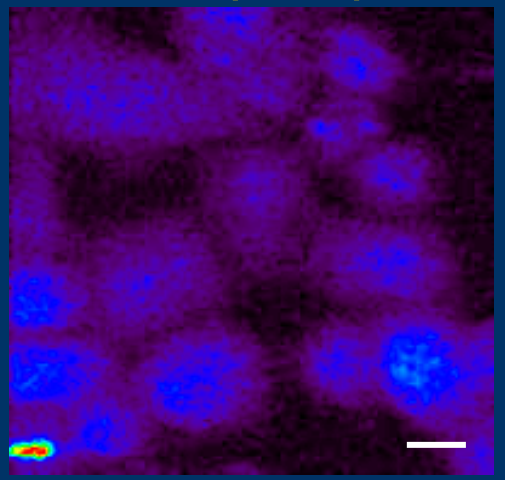
Micrograph

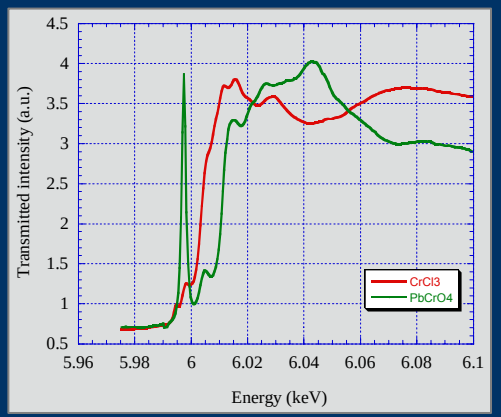


Potassium

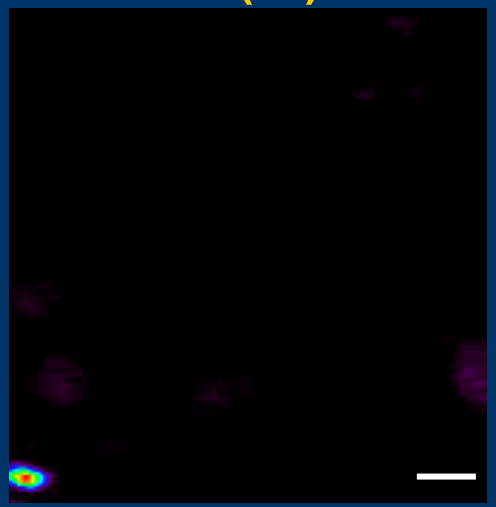


Cr (total)



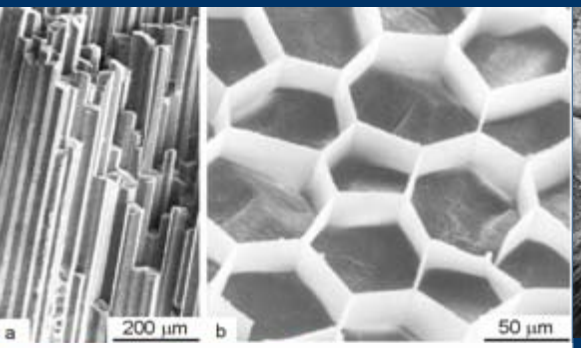


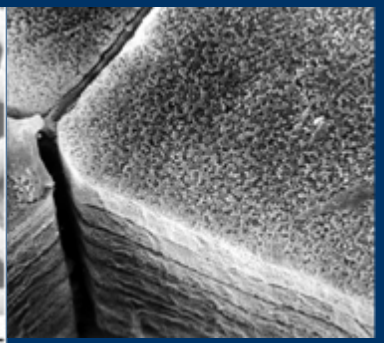
Cr(VI)



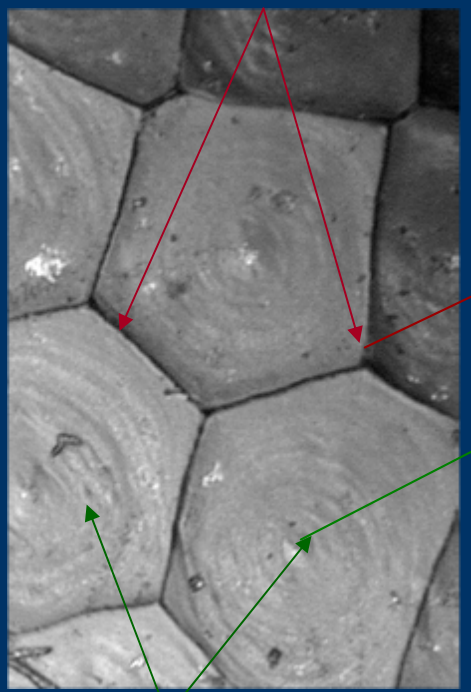
R. Ortega *et al.* , *Chem. Res. Toxicol.*, 5, (2005)

Micro-XANES at the Sulfur K-edge in Pinna Nobilis

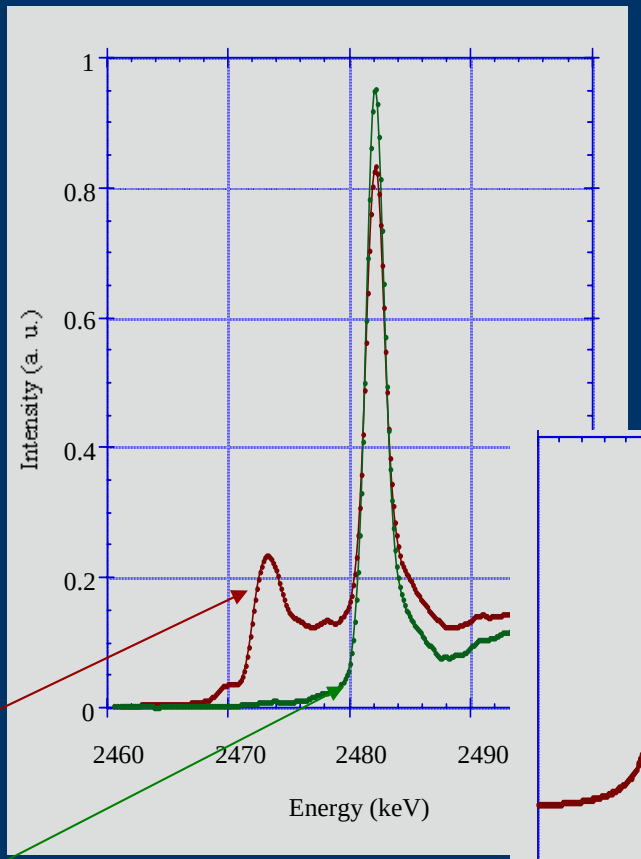


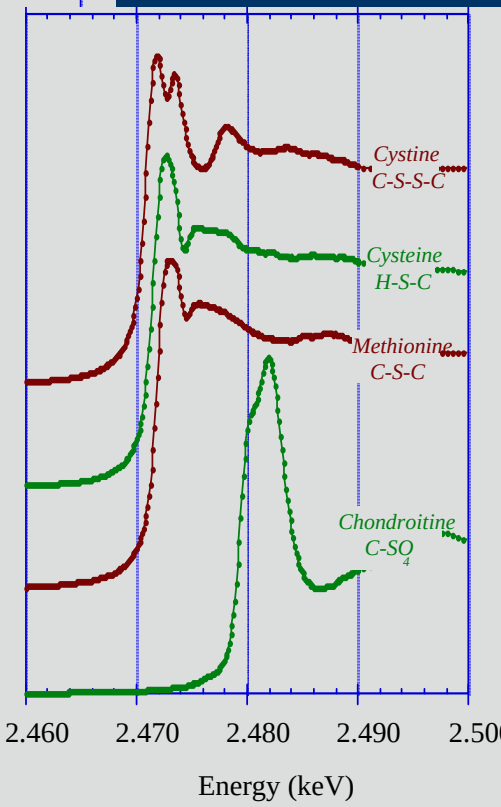


Organic matrix



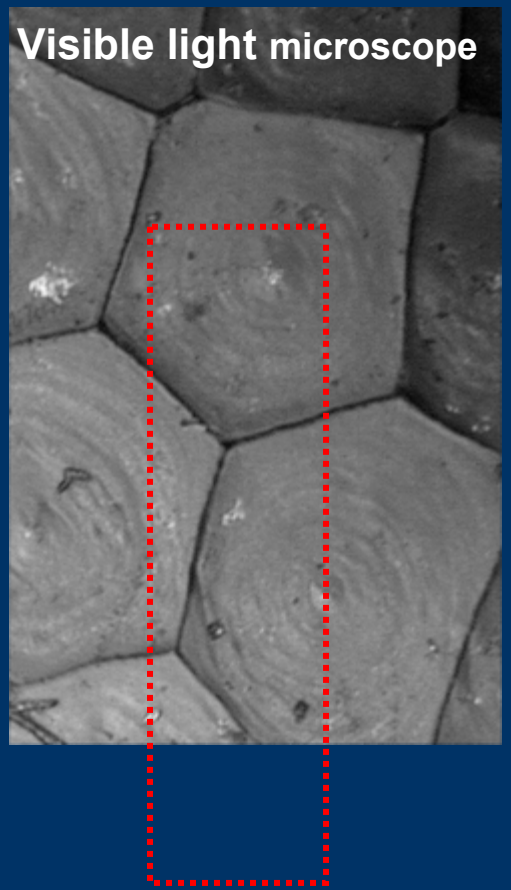
Mineral prisms

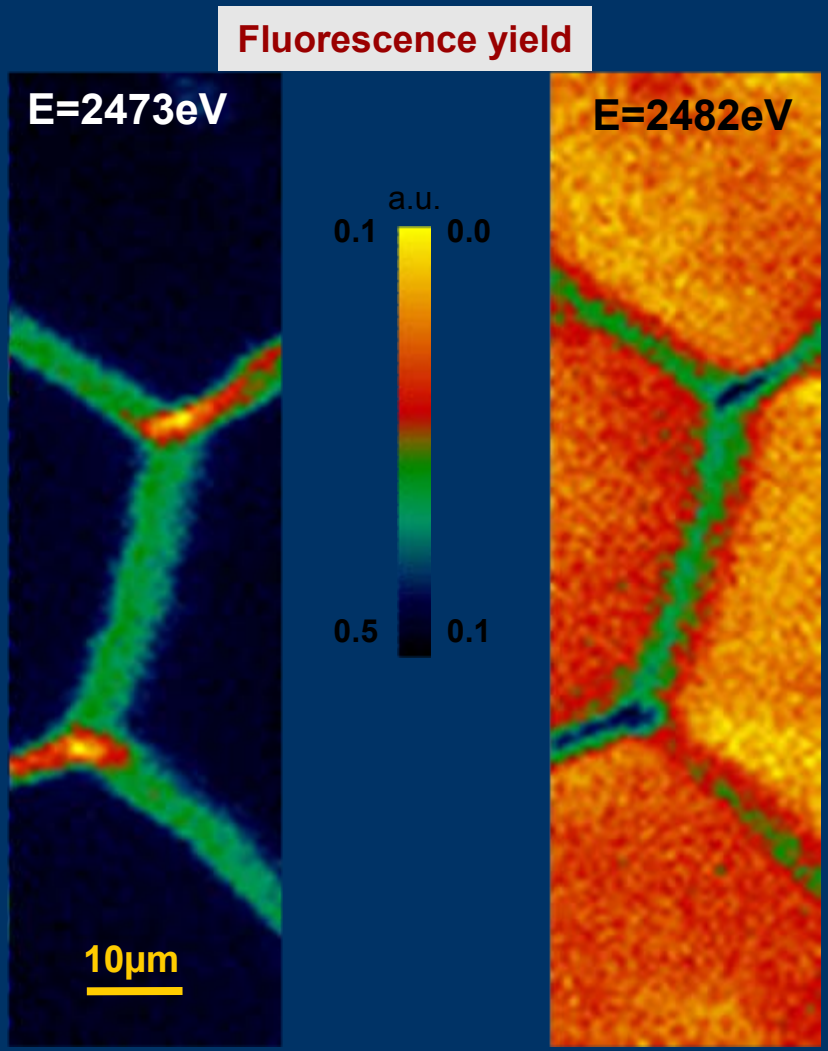




Y. Dauphin et al., *Comparative Biochemistry and Physiology*, Part A 132, (2002)

Chemical mapping of Sulphur species in Pinna Nobilis





Y. Dauphin et al., *Journal of Marine Biology*, 142, (2003)

Y. Dauphin et al., *J. Structural Biology,* 132, (2003)

- probe: 0.20x0.30 mm^2
- dwell time: 2 sec/pixel

Alternative strategy for fast and stable hard X-ray μ-XAS

❖ ***Two-crystal fixed exit scanning monochromator***

+ large energy range for EXAFS

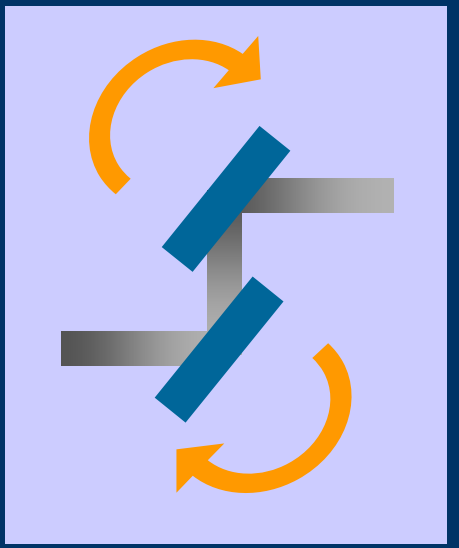
+ high flux (XRF)

+ mature technology

- source of non-statistical noises

(crystal motions, lack of beam stability, precision)

- relatively slow (10s-1s)



❖ ***Wavelength (energy) dispersive polychromator***

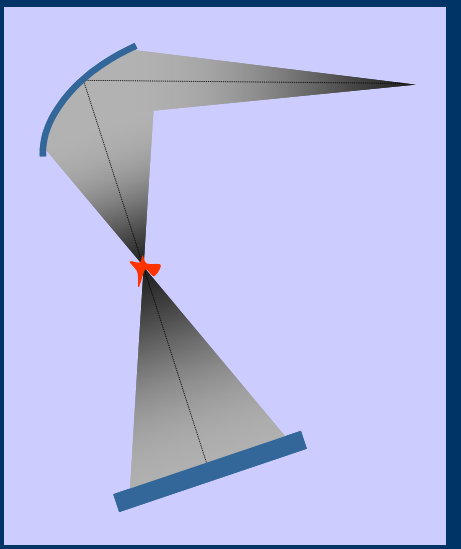
+ fast (1s-1μs)

+ stable

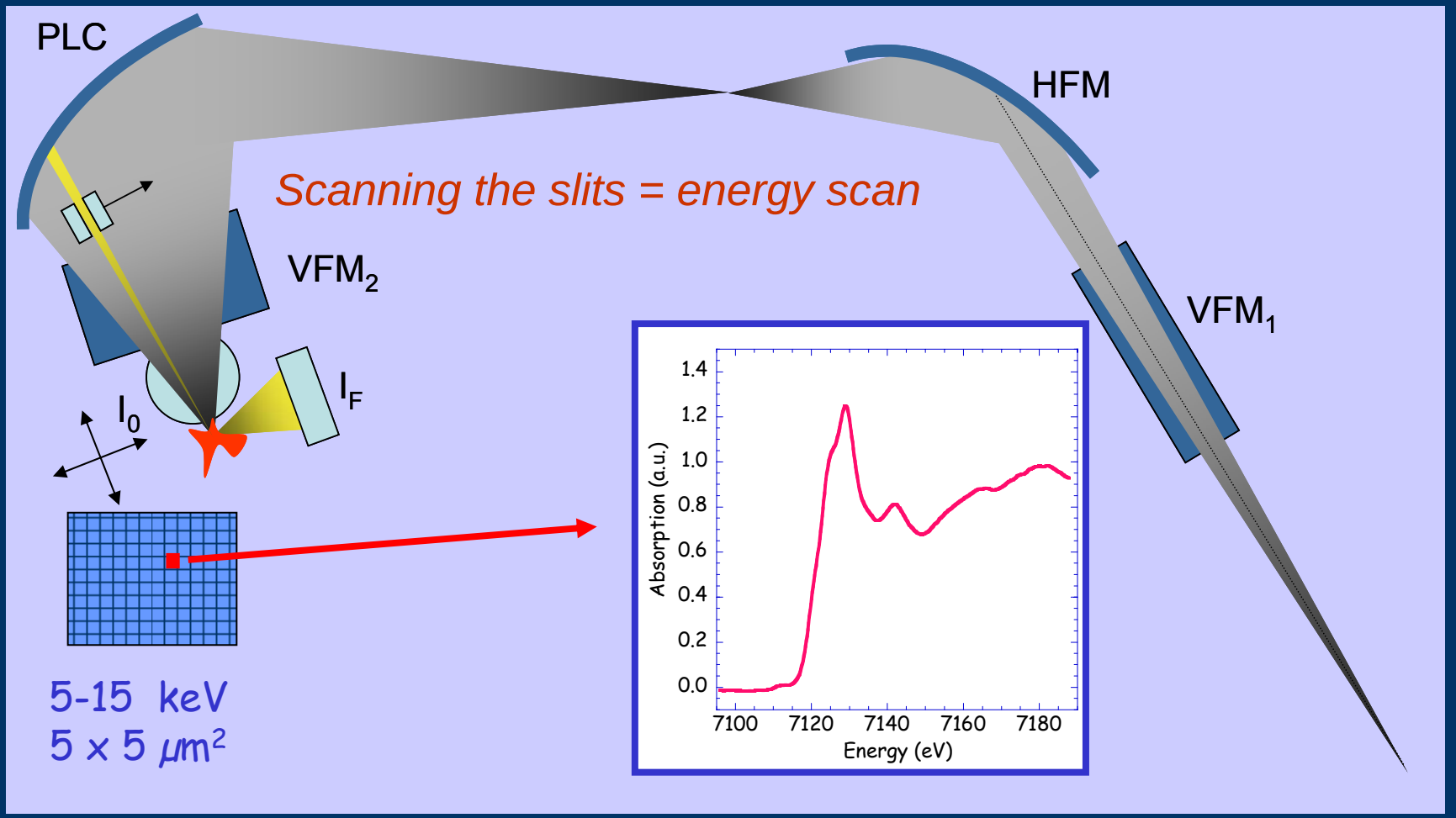
- low flux

- limited energy range and less flexible

- fluorescence more difficult



ID24-ESRF: an energy dispersive spectrometer on undulator source



Slits: 50mm/s -> 1000eV/s

$10^{-4} < \Delta E/E < 10^{-1}$

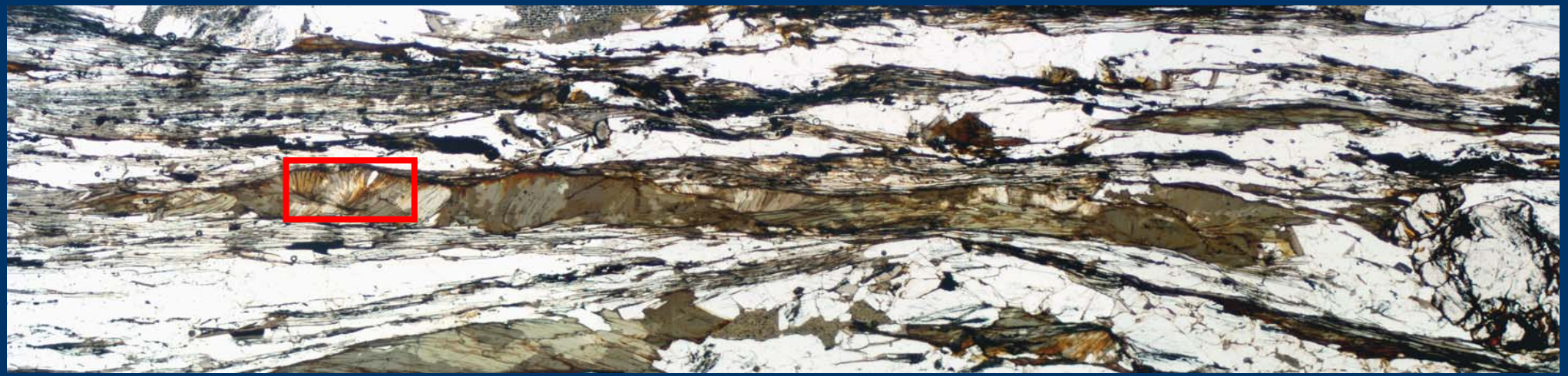
Tuning of flux vs $\Delta E/E$

1 pixel = 1 EXAFS or NEXAFS spectrum
100x100pixels = 10000 spectra in 2 hrs acquisition

With an optimized optical scheme: 300x300nm²

S. Pascarelli *et al., J. of Synchrotron Radiation*, 2006

Redox and speciation mapping of metamorphic rocks

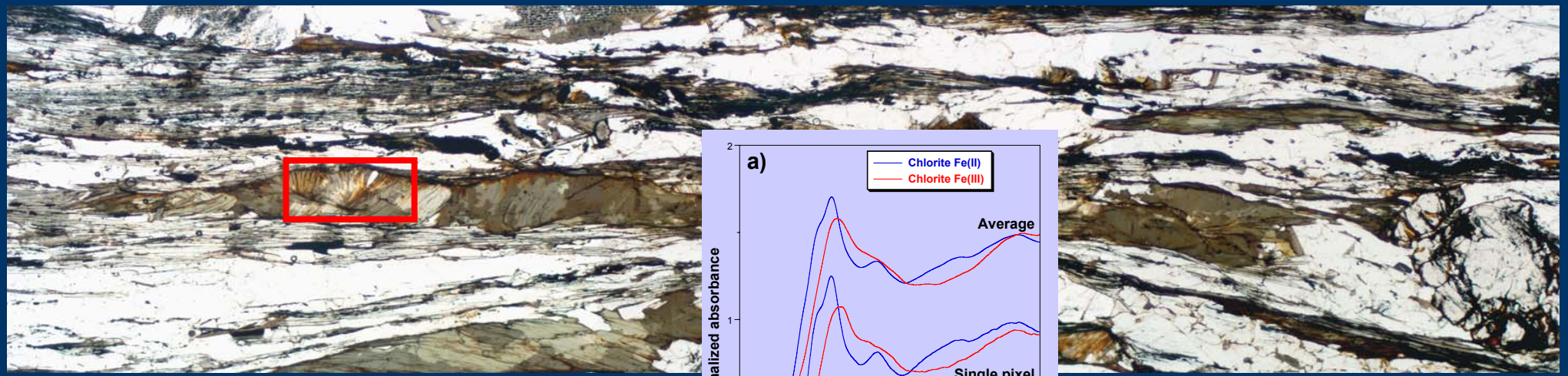


Sambagawa (Japan)

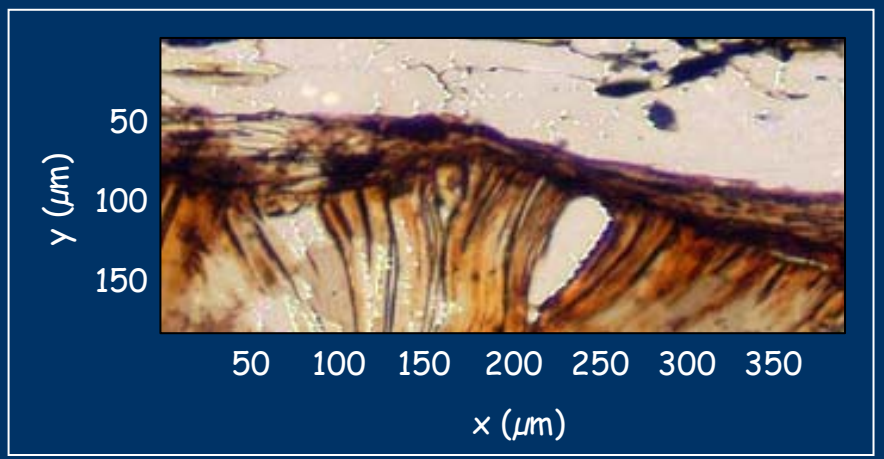
- ✓ P, T conditions of formation of rocks
- ✓ Thermodynamical models depend critically on oxidation state of Fe
- ✓ Need for quantitative map of Fe redox at the µm level

S. Pascarelli *et al.*, *J. of Synchrotron Radiation*, 2006

Redox and speciation mapping of metamorphic rocks



Sambagawa (Japan)

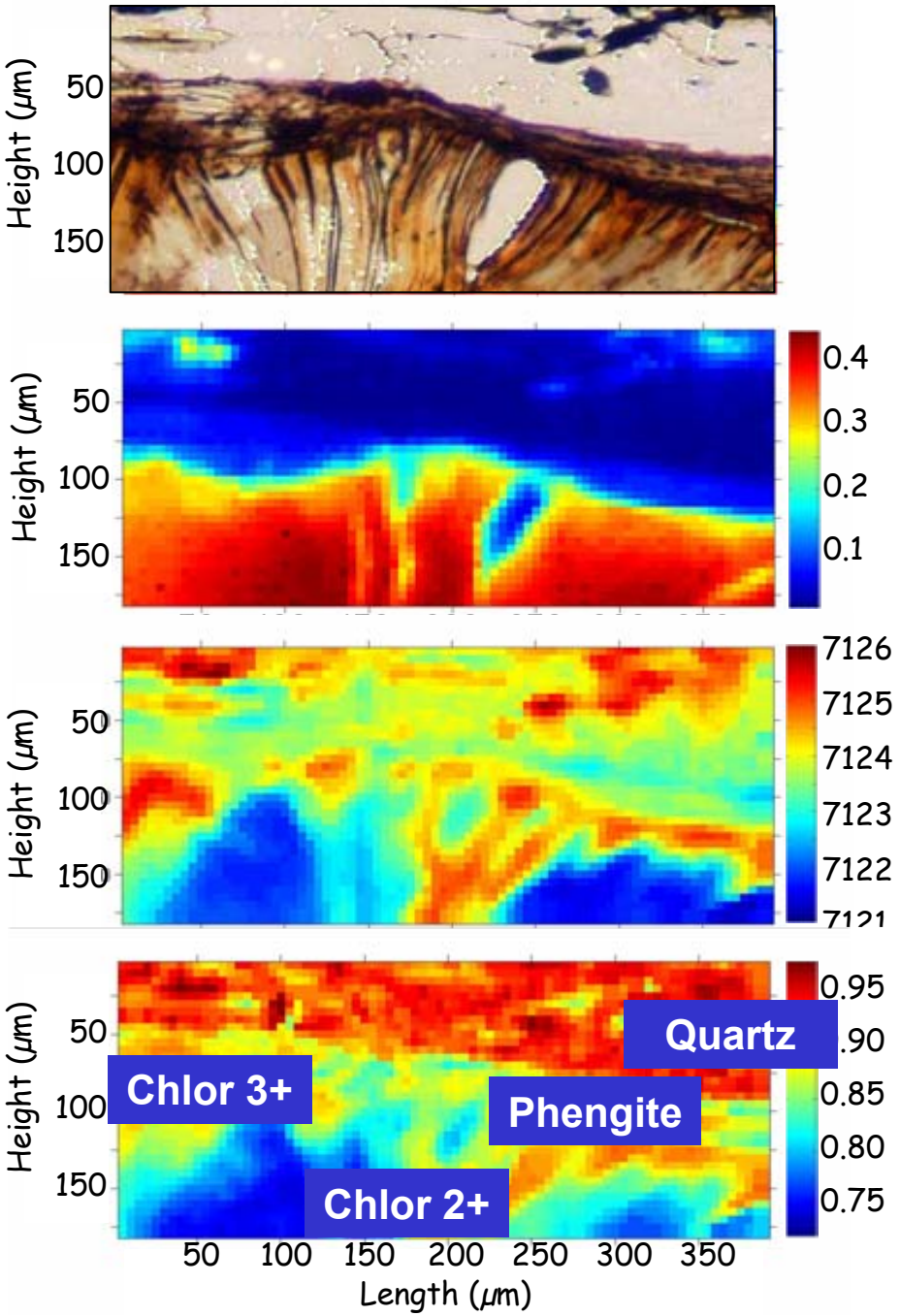


- Pixel size: 4x4µm^2
- 3000spectra (1.5hrs)

From spectra to speciation mapping ?

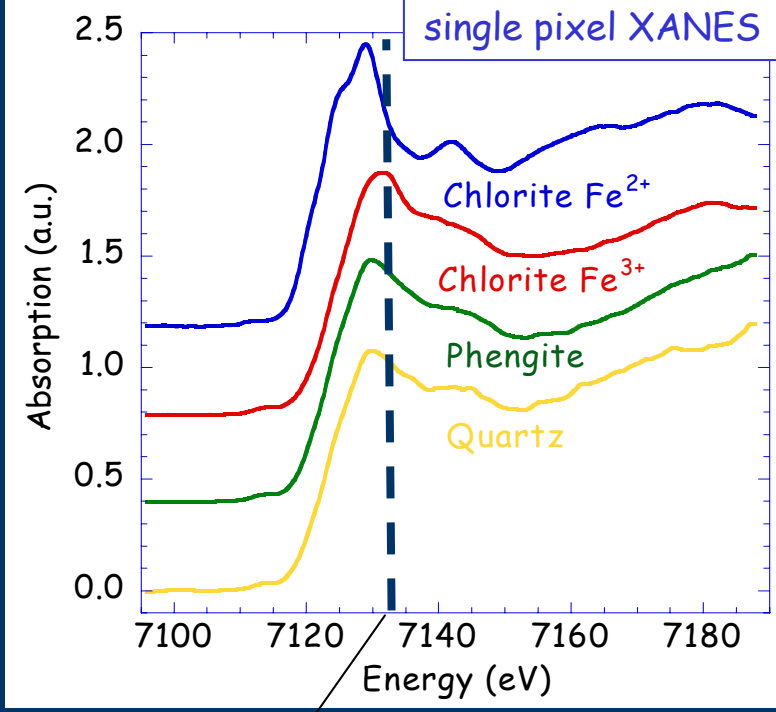
3 basic criteria:

- Edge jump: Fe content
- Edge position: Fe oxidation state
- Absorbance at a defined energy: Fe speciation



Edge jump
Fe content

Edge position
Fe oxidation state



Absorbance at a defined energy
Fe speciation

M. Muñoz *et al.*, *G-cubed*, (2006)

Blackening of Pompeian Cinnabar paintings

Red = cinnabar (HgS)

- **M. Cotte, J. Susini**, ESRF, Grenoble, France
- **A. Moscato & C. Gratziu,** Università di Pisa, Pisa,Italy
- **A. Bertagnini,** Istituto Nazionale di Geofisica e Vulcanologia, Pisa, Italy
- **M. Pagano,** Soprintendenza per i Beni Archeologici del Molise, Campobasso, Italy

Blackening of Pompeian Cinnabar paintings

Red = cinnabar (HgS)

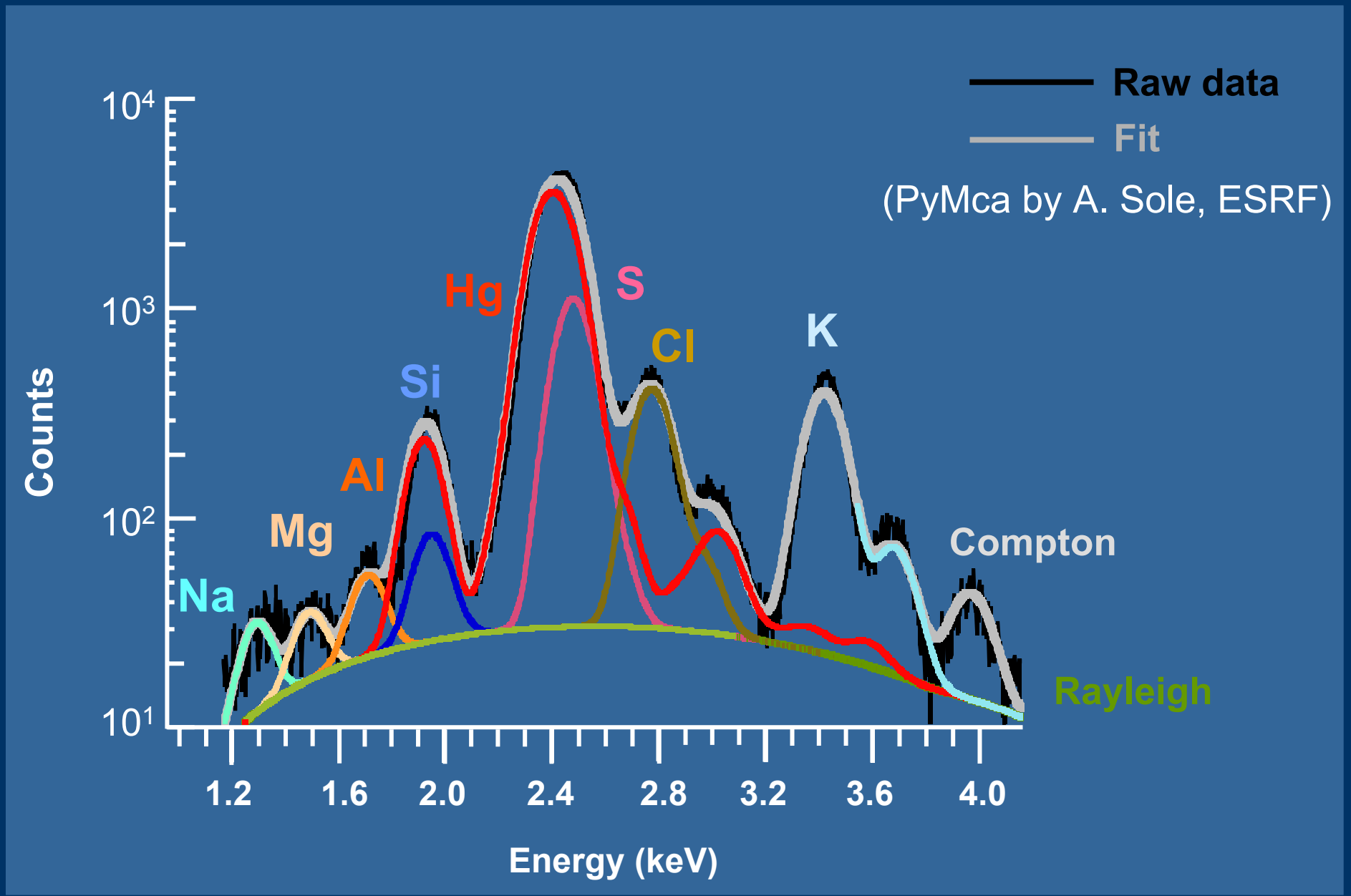
- Excavation started in 1988 and was completed in 1992
- Rapid blackening since 1990

Blackening of Pompeian Cinnabar paintings

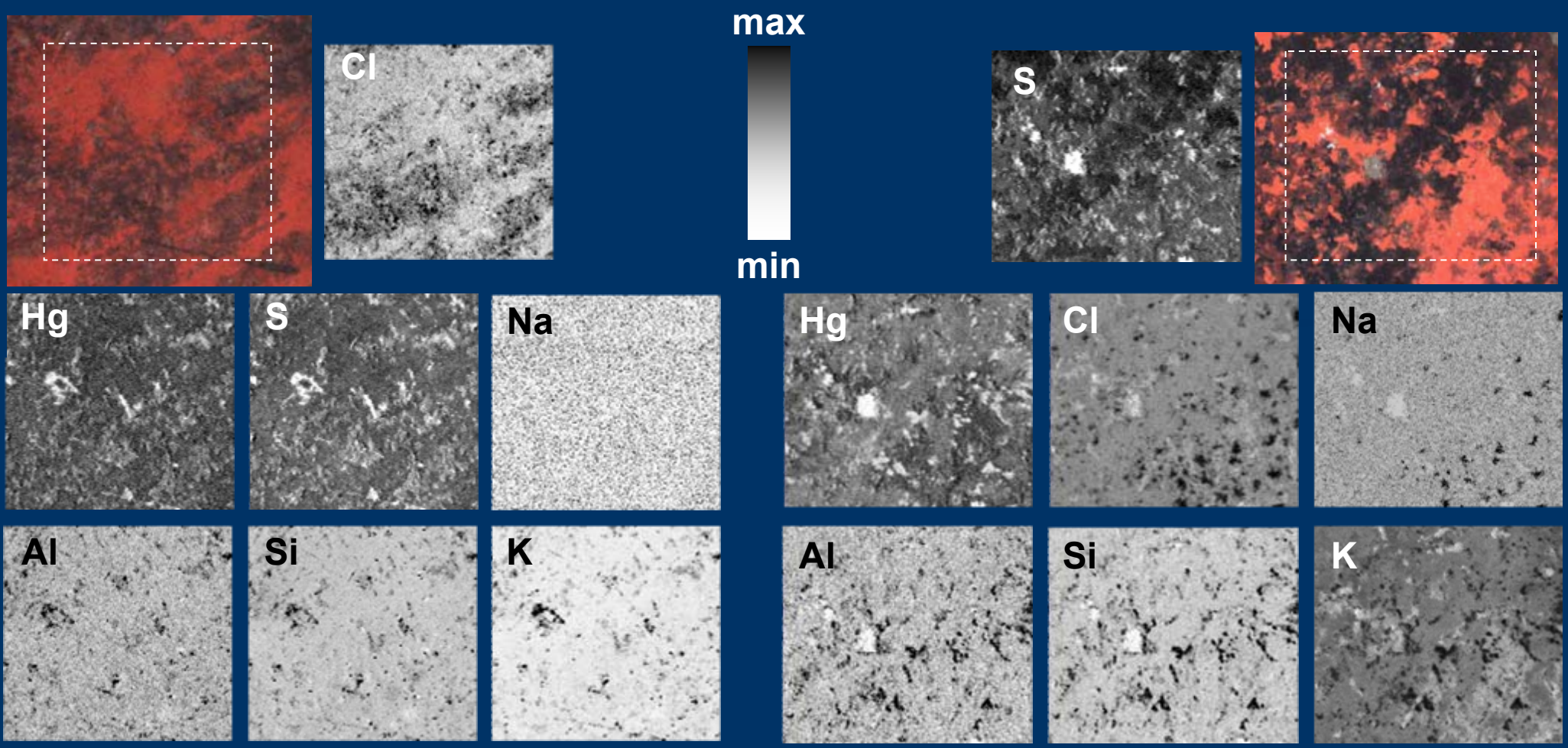
no alteration **low alteration** **high alteration**

- Cinnabar → β–cinnabar?
- Role of the lime substrate ($CaCO_3$)?
- Superficial or deep alteration?
- Role of sulfur?
- Other elements (e.g. *cera punica)* ?

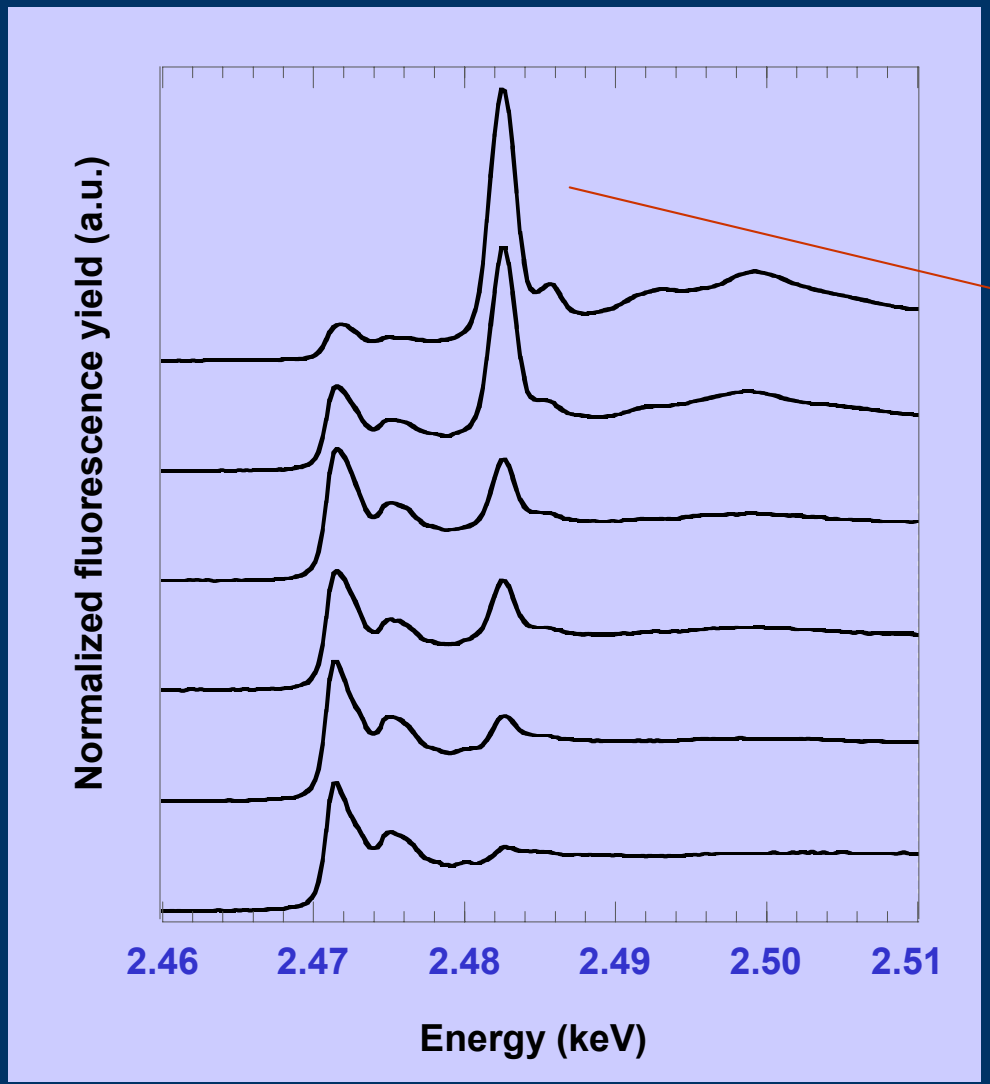
X-ray fluorescence and elemental mapping

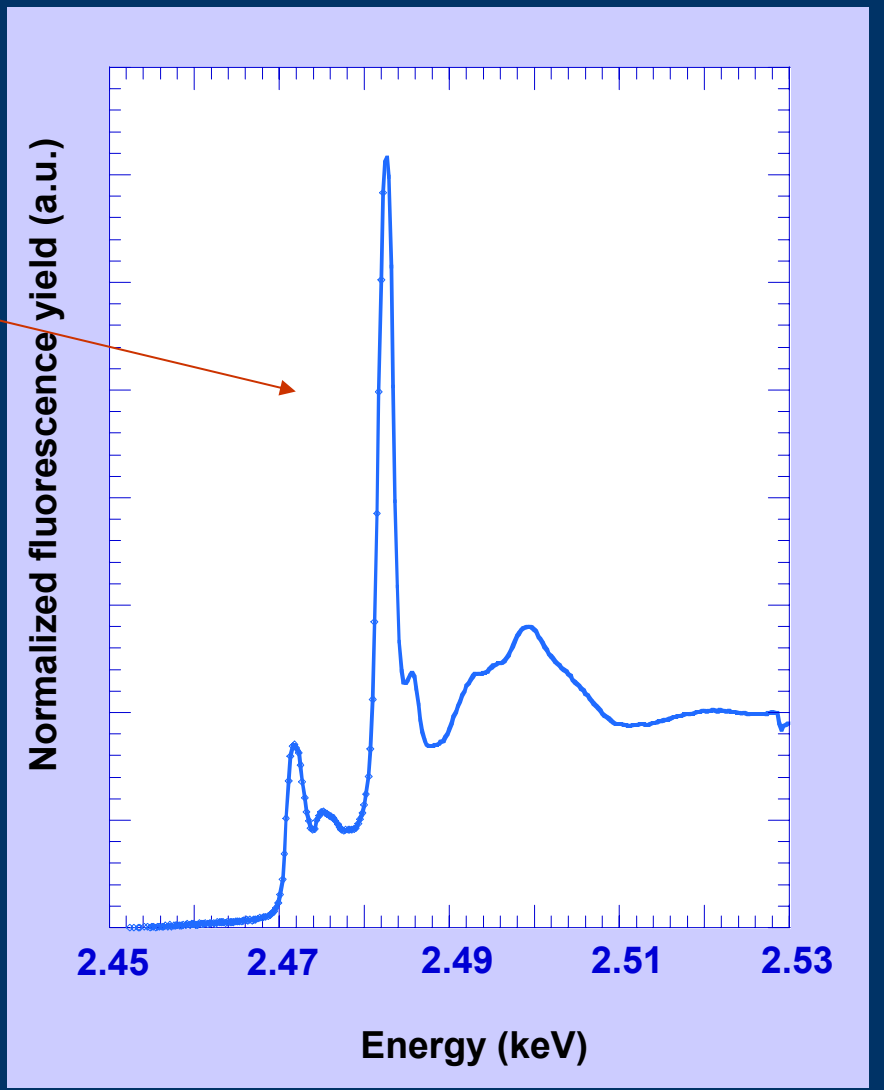


Composition maps by X-ray Fluorescence



Semi-quantification?

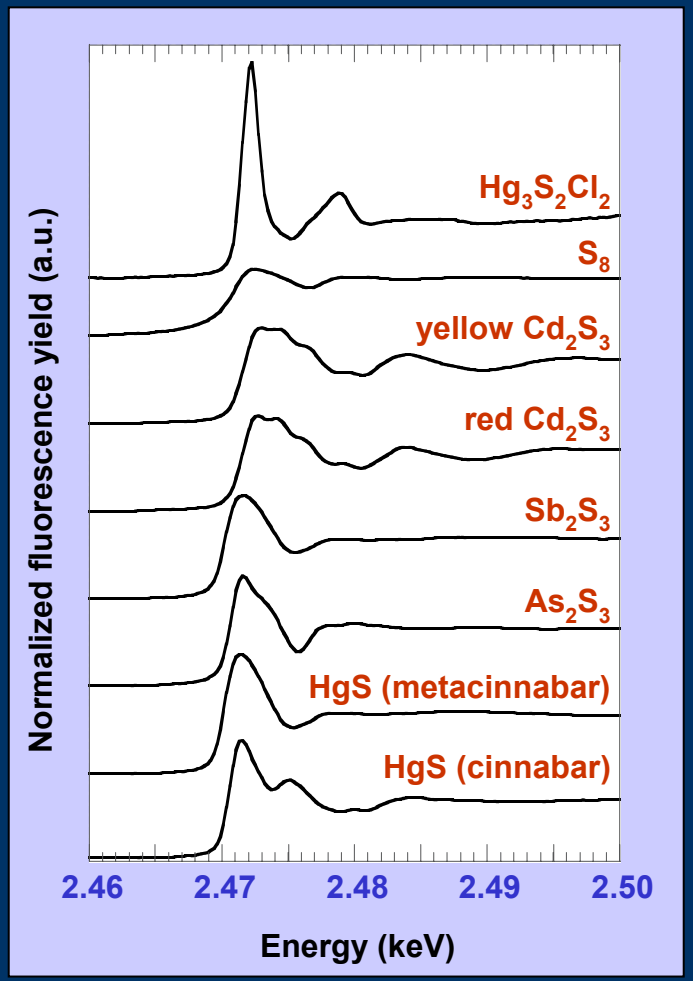




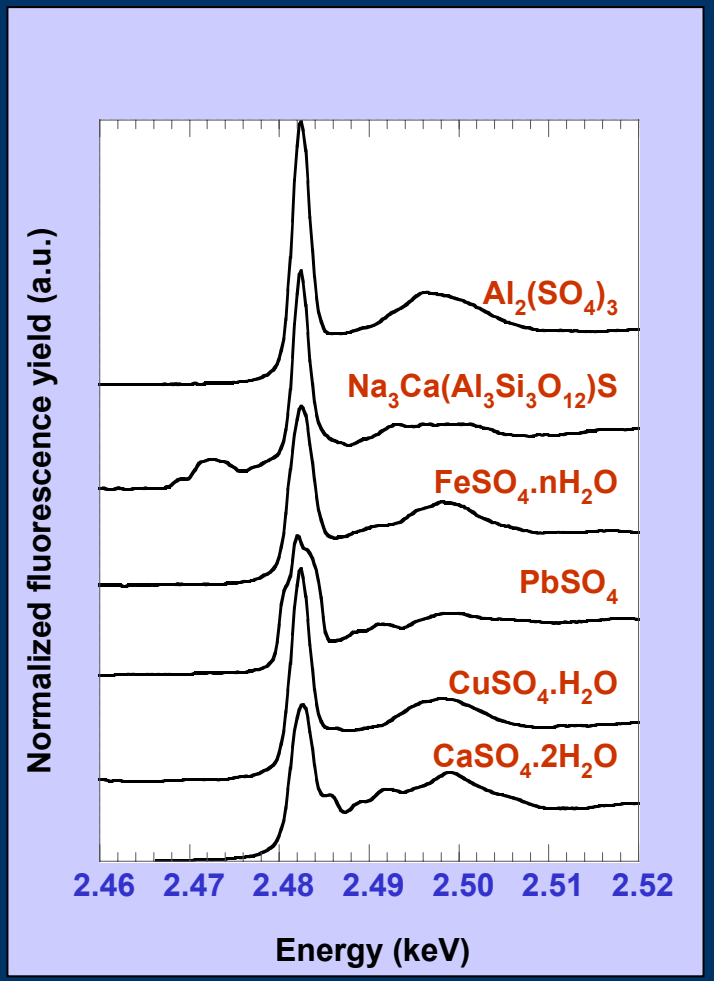
Sulfur K-edge XANES

XANES Sulfur K-edge

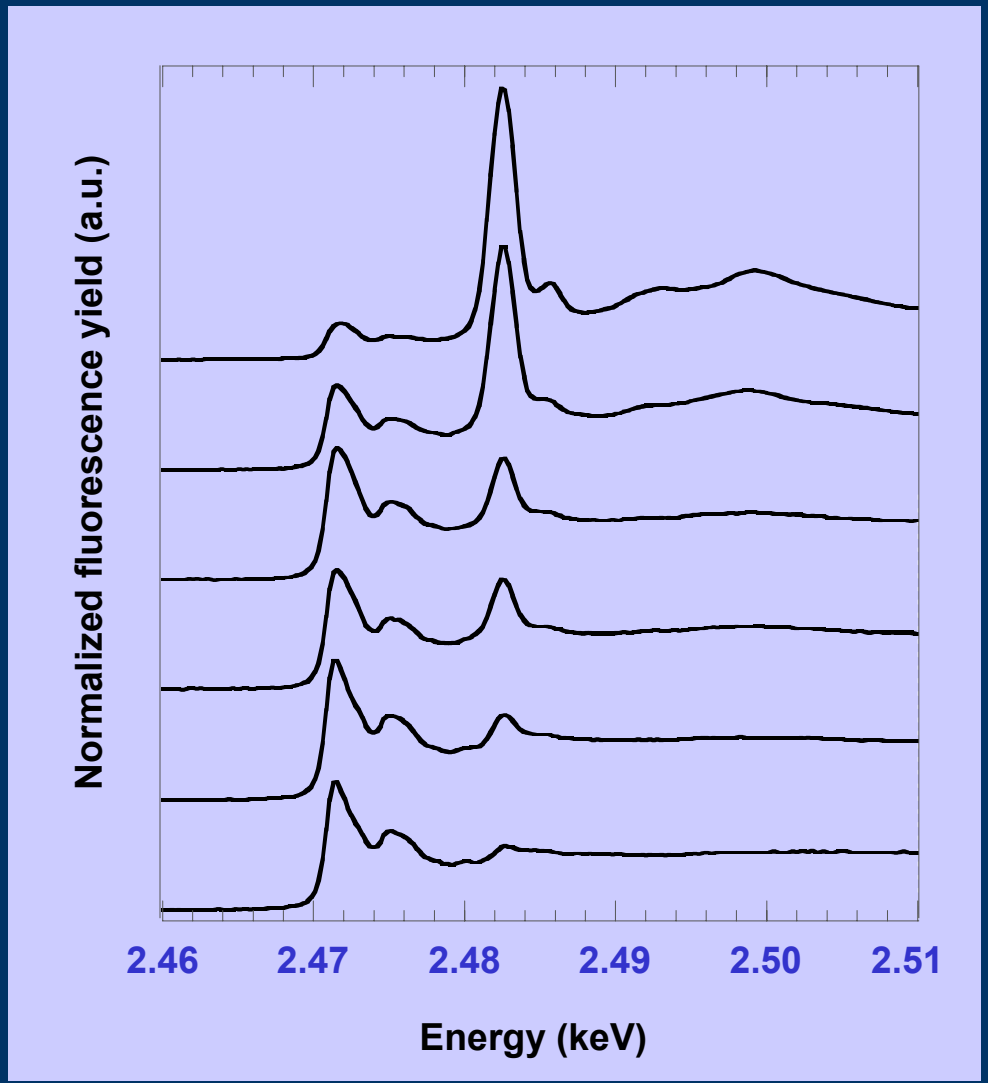
Sulfides

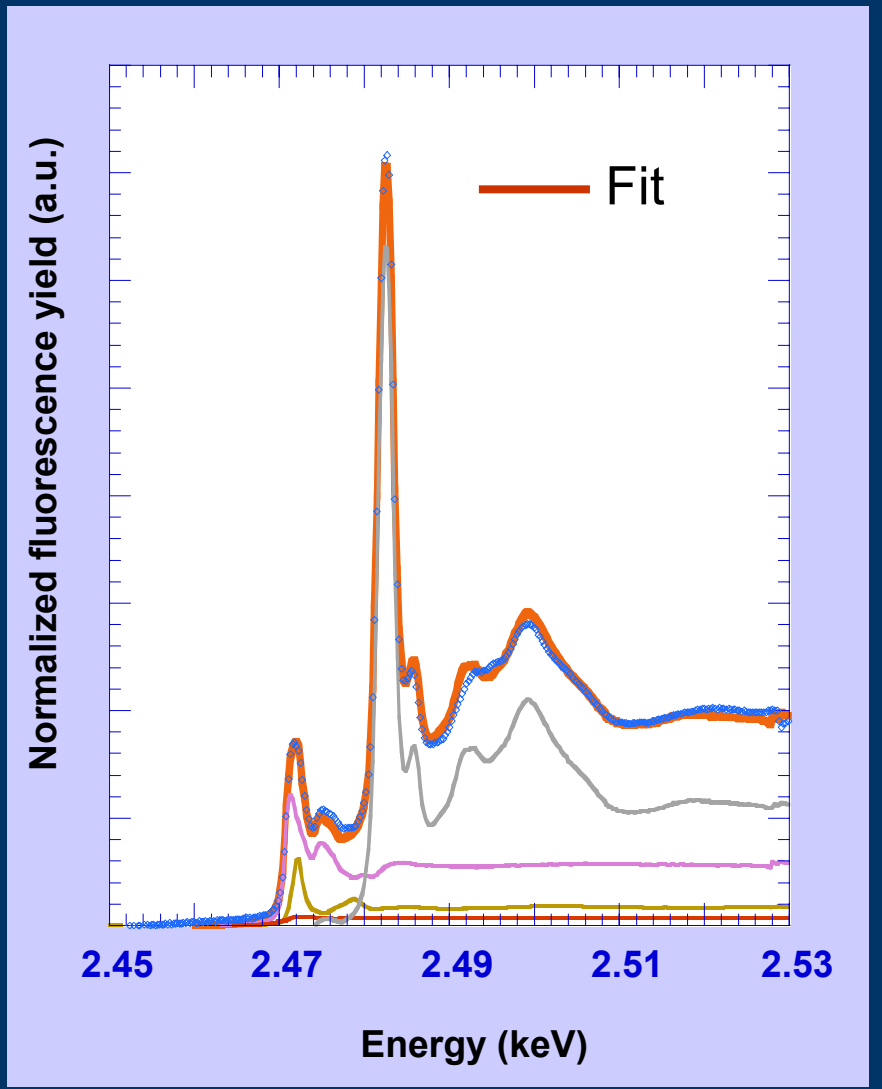


Sulfates

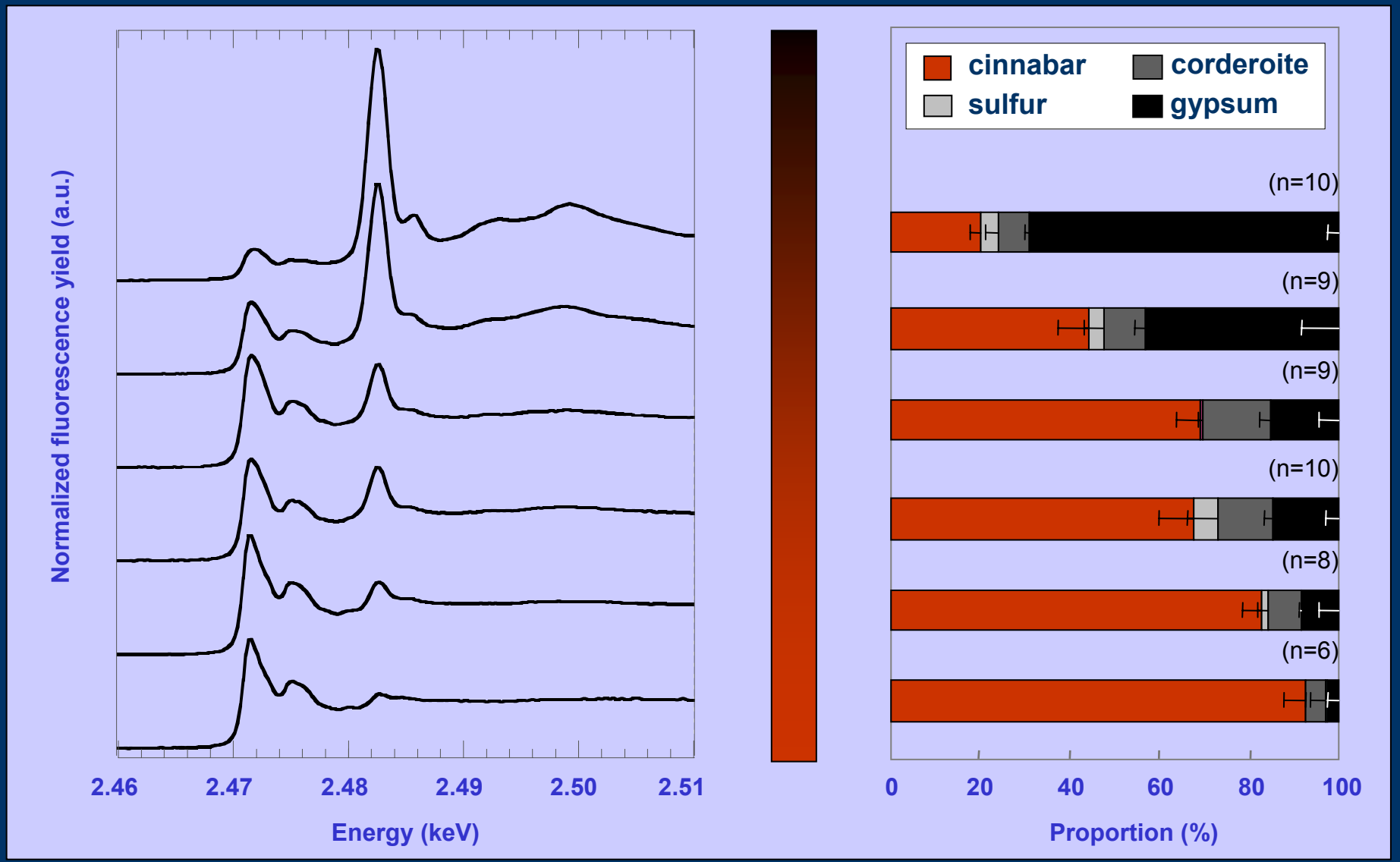


Semi-quantification?

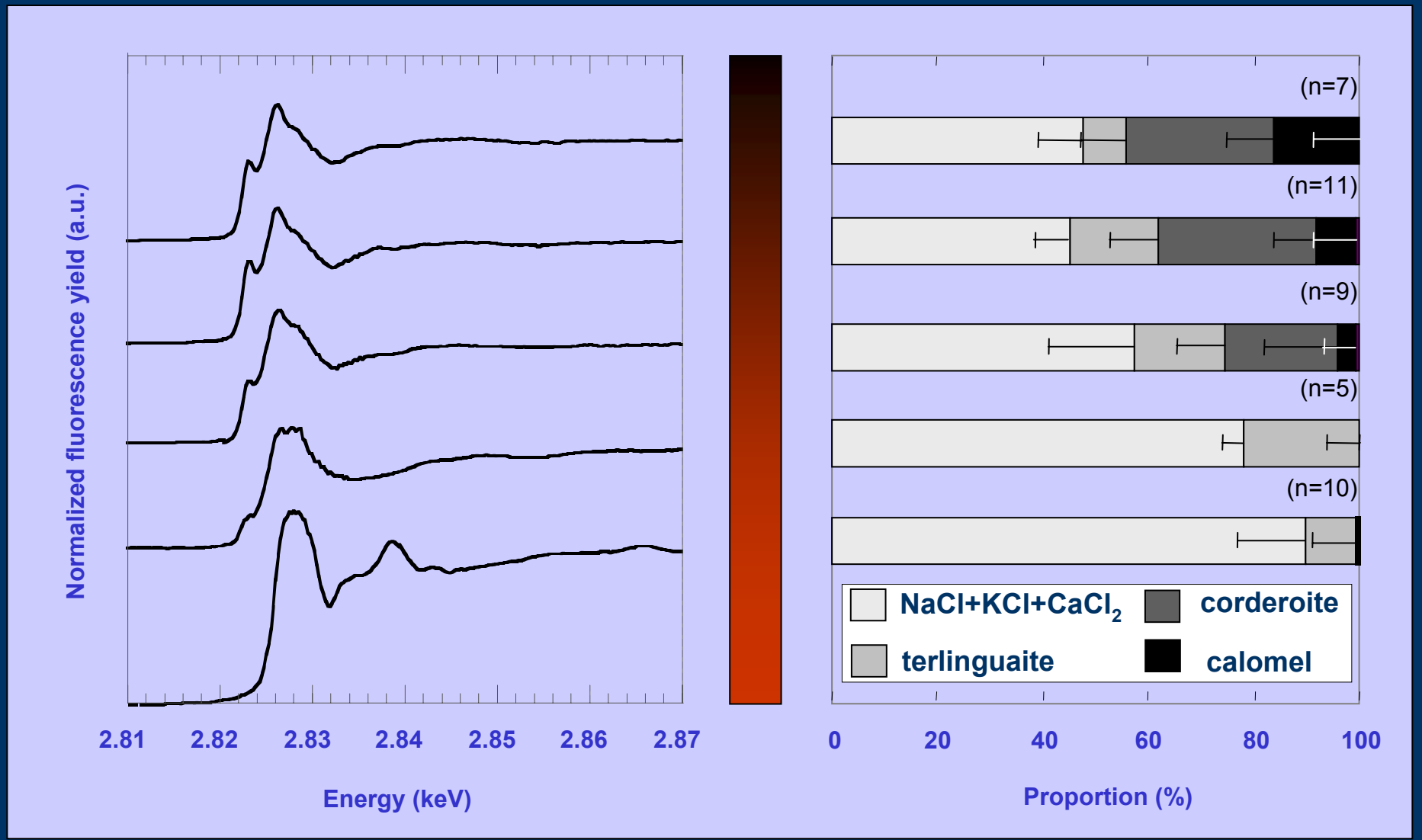




Semi-quantification: sulfur compounds



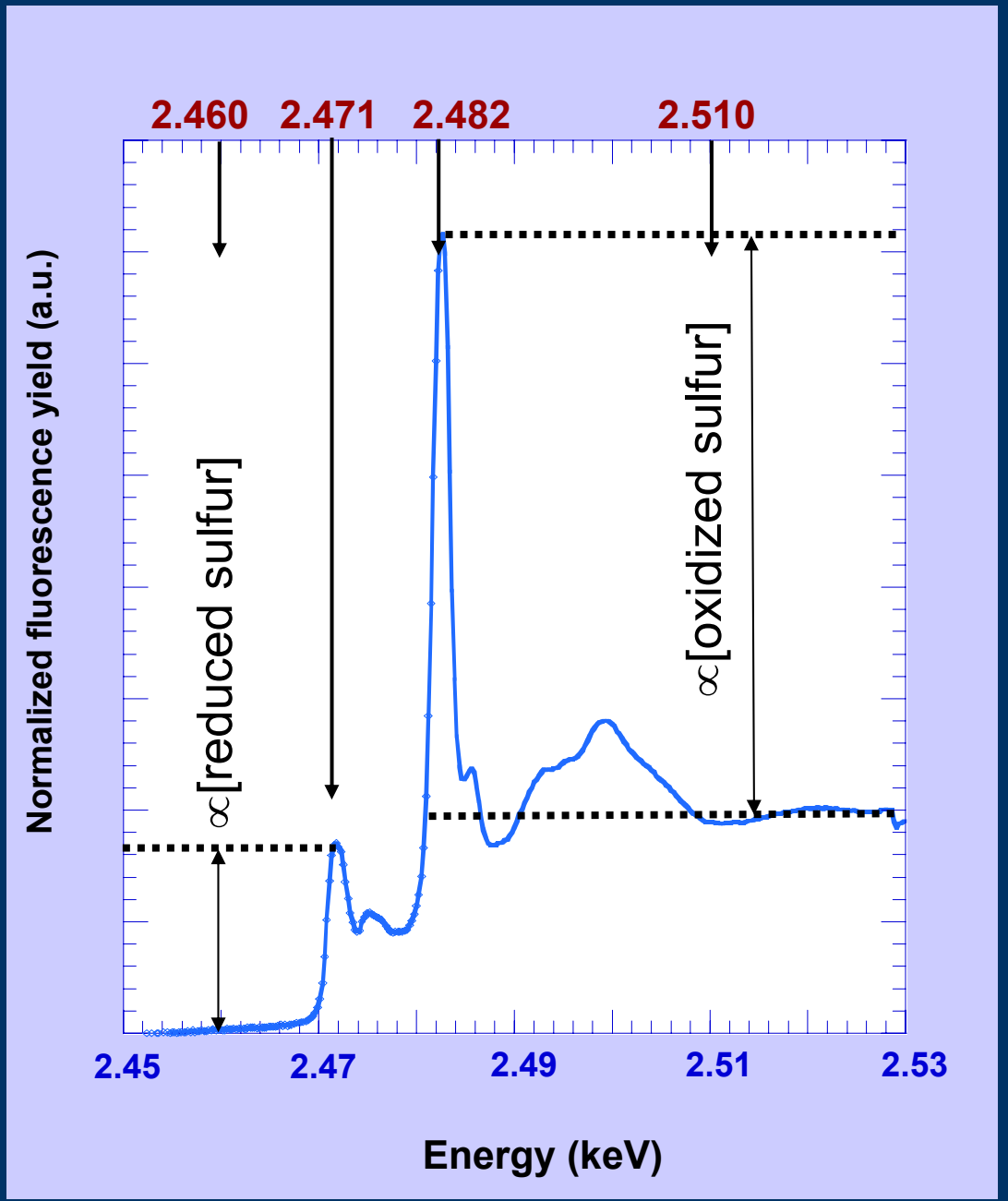
Semi-quantification: Chlorine compounds



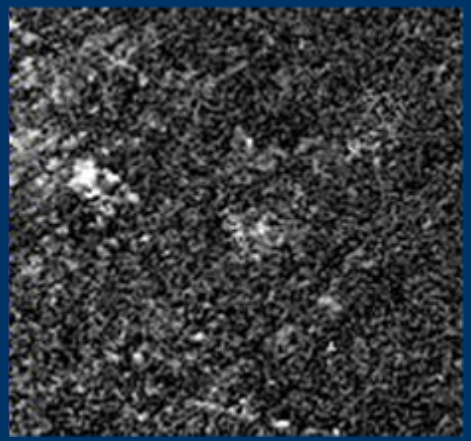
Chemical mapping?

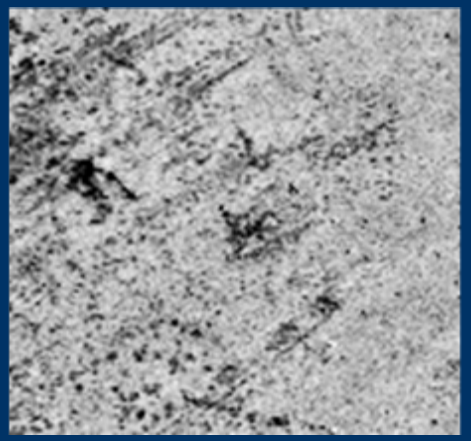
$$[\text{Sulfides}] \propto \frac{I(2.471) - I(2.460)}{I(2.510) - I(2.460)}$$

$$[\text{Sulfates}] \propto \frac{I(2.482) - I(2.510)}{I(2.510) - I(2.460)}$$



Chemical mapping: reduced vs oxidised Sulfur

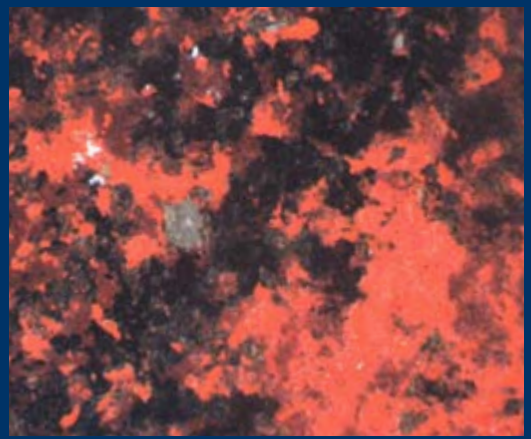


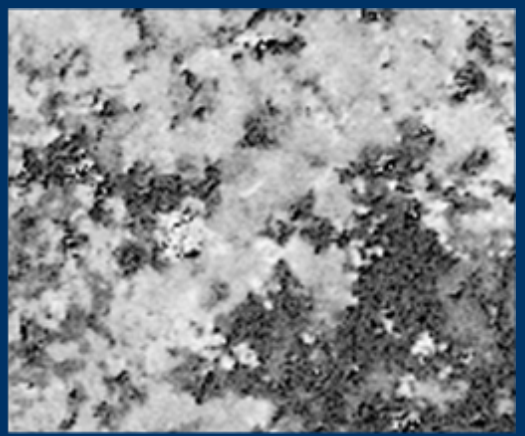


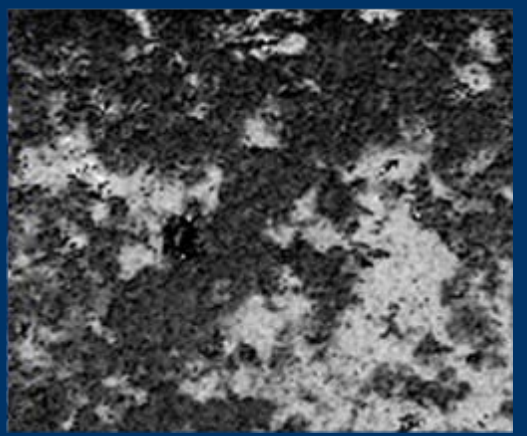
Light microscopy

Reduced sulfur

Oxidised sulfur







Summary

Attributes of multi-keV X-Ray spectro-microscopies

- **X-ray Fluorescence** → *Trace element detection & mapping*; *Quantitative fluorescence analysis*
- **Micro-spectroscopy (XANES)** → *Chemical state specificity*
- **Higher penetration**; **Phase contrast** → *Microscopy on thick samples*; *Lower radiation damage (?)*
- **Larger focal lengths (> 20mm)**; **Larger depth of focus (> 100µm)** → Space for sample environment; *3D imaging*

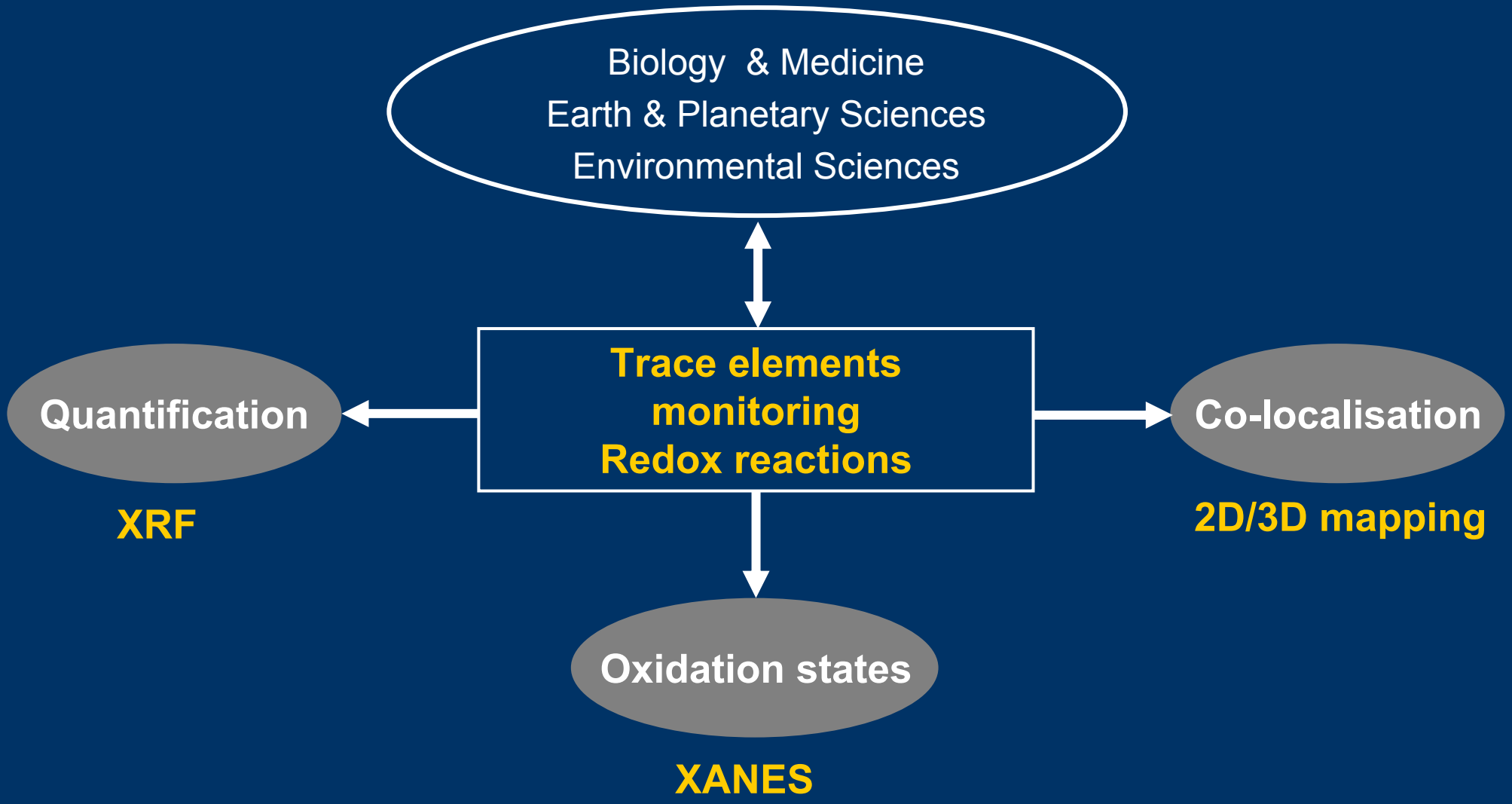
Multi-technique approach

- ***Micro-Fluorescence***
- ***Micro-diffraction***
- ***3D imaging***
- ***Spectroscopies***

⟷

In-situ experiments
controlled sample environment

A common scientific case : trace element analysis in heterogeneous systems



Infrared spectroscopy

CO_2

O = C = O

Asymmetric stretch
2350 cm^{-1}

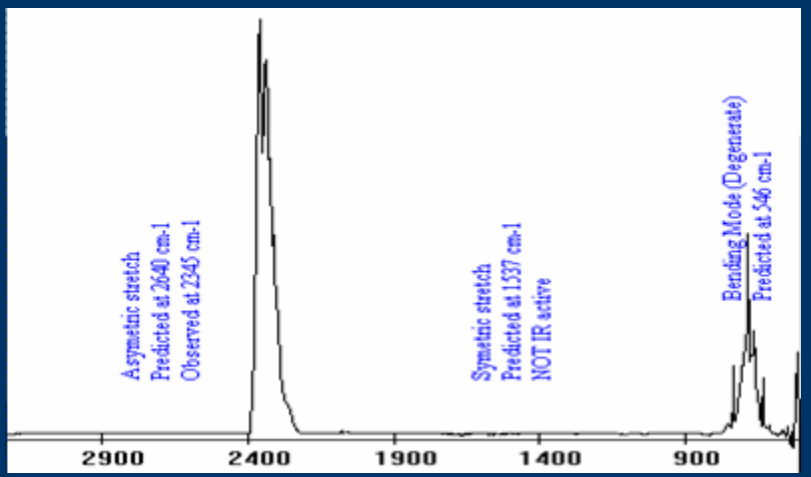
Symmetric stretch
(not IR active)

Vertical bend
666 cm^{-1}

Horizontal bend
degenerate mode same motion as above but rotated by 90°

- An IR active mode must involve a change in the dipole moment of the molecule
  = charge imbalance in the molecule

absorption =-log (I_t /I_o)



Infrared spectroscopy

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- Each functional group has an ensemble of motions (vibrational) specific of the molecular group (fingerprint)
- These motions (or vibrational frequencies) are detected under « resonant » excitation in the energy domain 0.495 eV-0.062eV or 2.5 to 20µm or 4000-500 cm^{-1}
- There are databanks of spectra, which allow a rapid search and identification.

Infrared spectroscopy: some figures…

- Vibration frequencies (and wave-numbers) are inversely proportional to atomic masses
  - C-H stretch (3000 cm^{-1})
  - C-O stretch (1000-1300 cm^{-1})
  - C-Br stretch (600 cm^{-1})

1 eV ~ 8100 cm^{-1}

Near-IR: 12500 – 4000 cm^{-1}

0.8 to 2.5 μm

Mid-IR: 4000 – 400 cm^{-1}

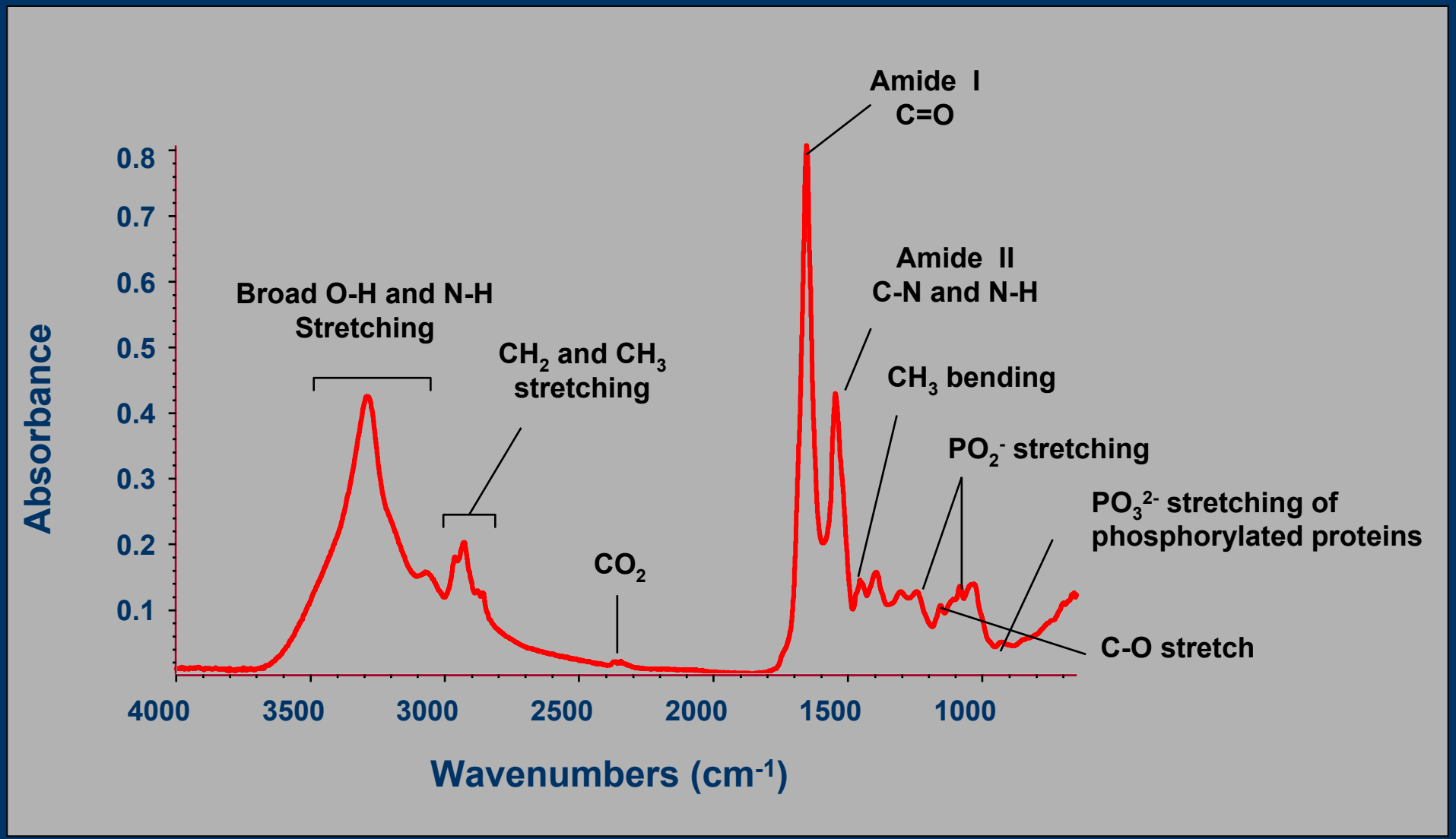
2.5 to 25 μm

Far-IR: 400 – 10 cm^{-1}

25 to 100 μm

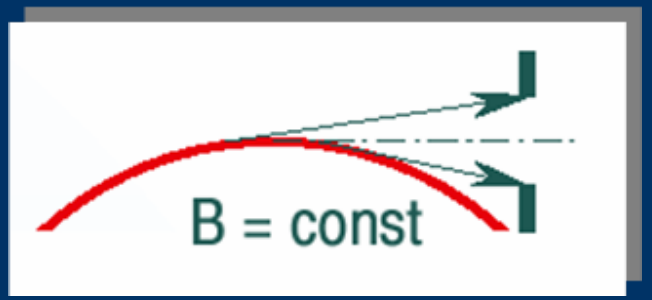
- Vibration frequencies (and wave-numbers) are proportional to bond strength
  - C-C stretch (1000 cm^{-1})
  - C=C stretch (1600 cm^{-1})
  - C≡C stretch (2200 cm^{-1})

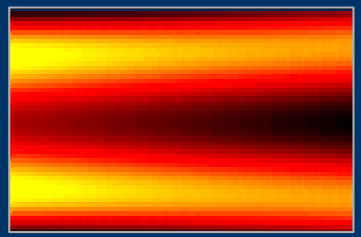
An example of spectrum (biological sample)



Synchrotron infrared radiation: Two modes of emission

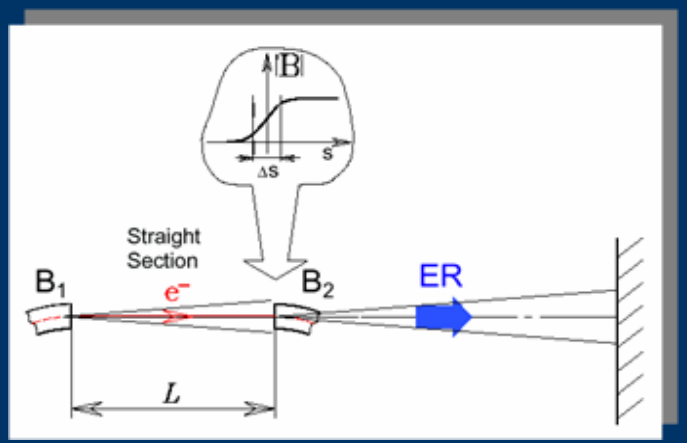
- **Bending magnet emission:**

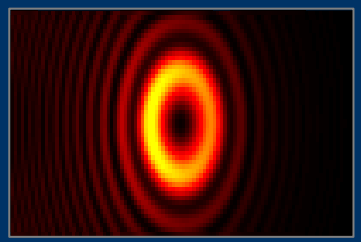




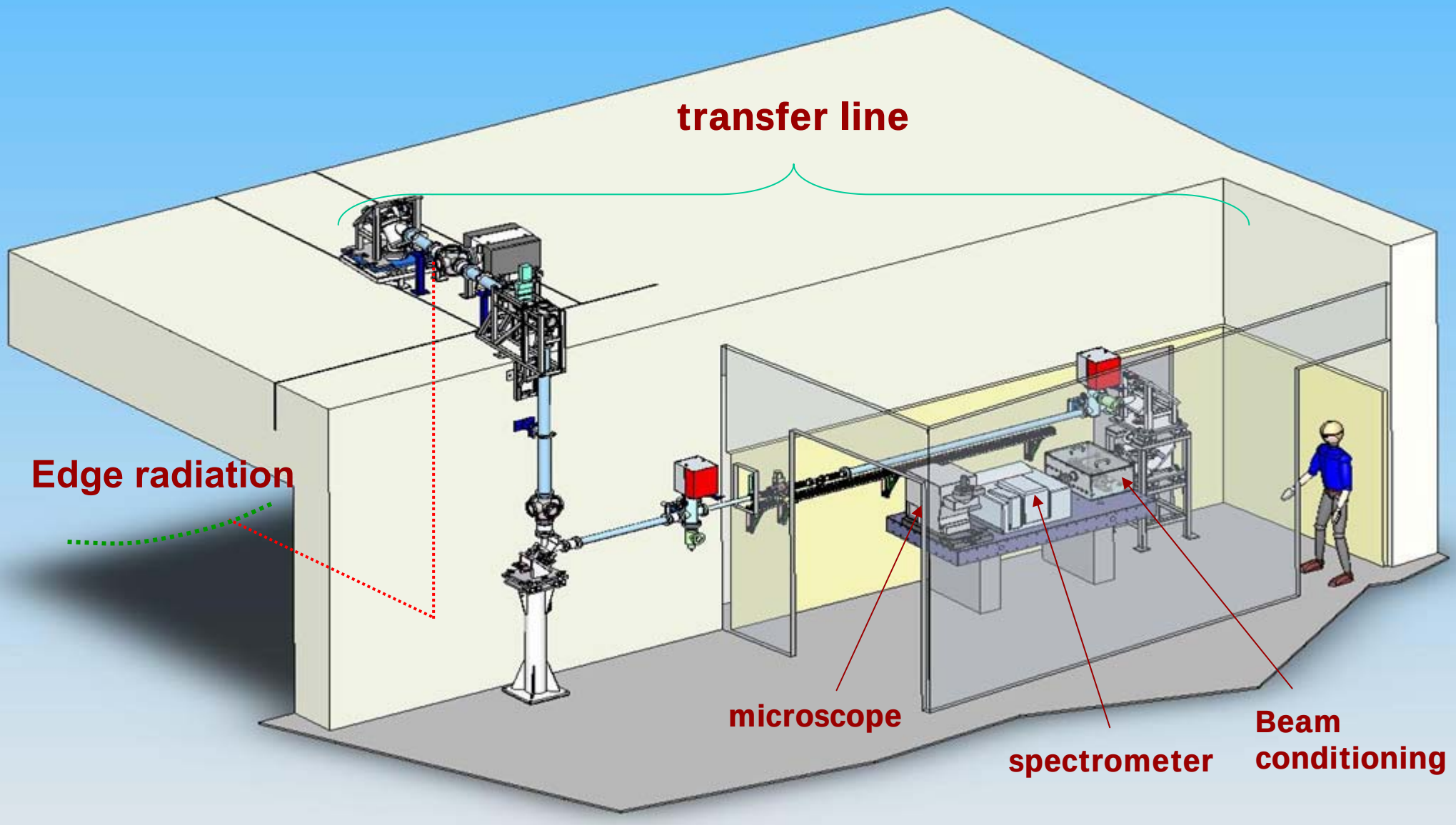
@ 10 μm

- **Edge emission:**

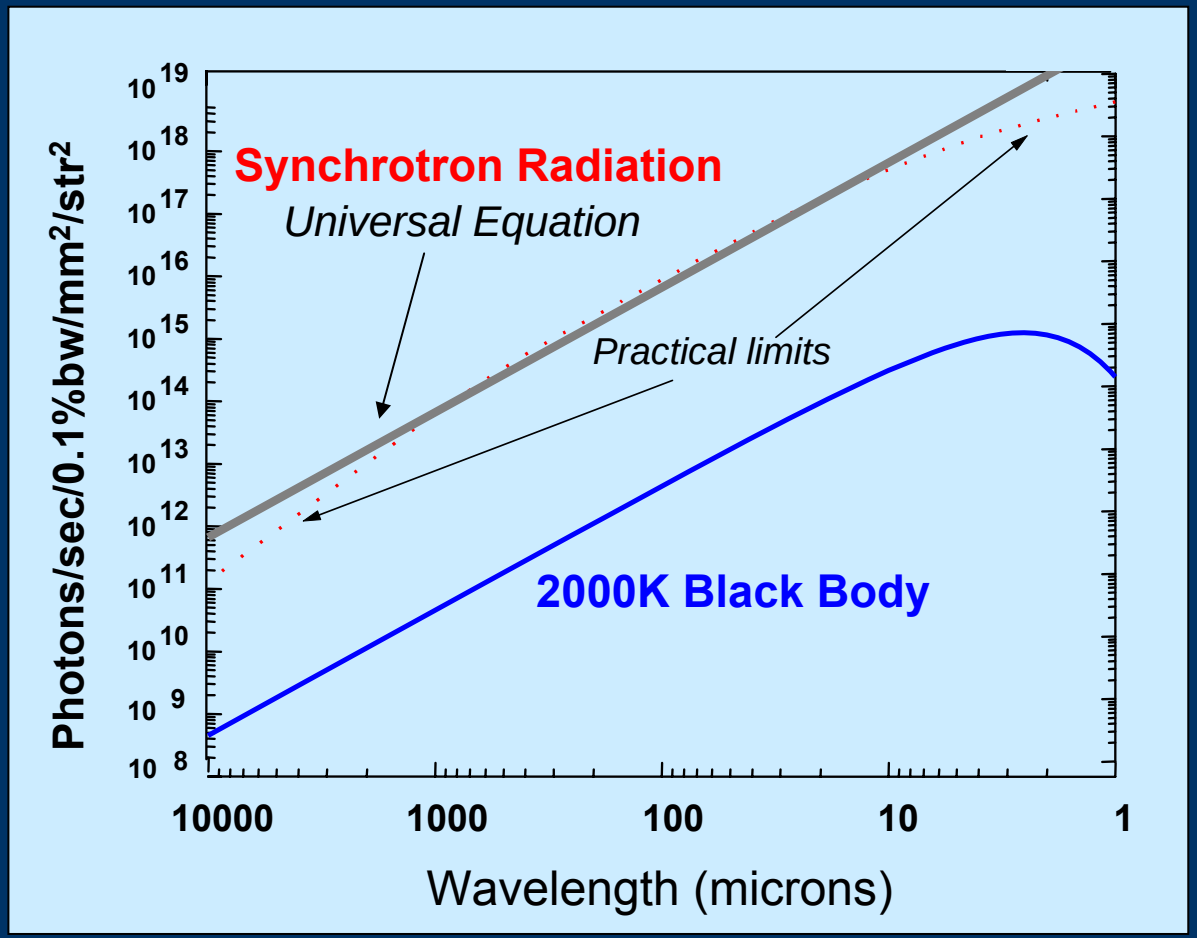




Synchrotron Infrared microscopy



Synchrotron source: brightness advantage



BRIGHTNESS

Signal-to-Noise

Data Collection

Spatial Resolution

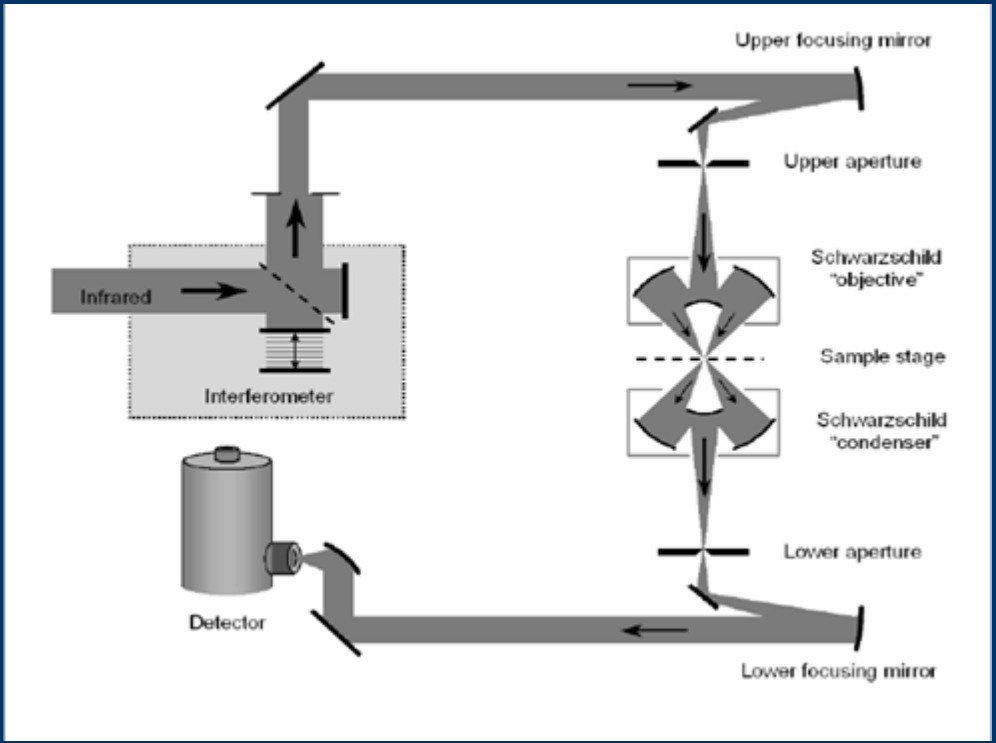
BROADBAND

Spectroscopy

Spectro-microscopy

Chemical mapping

A confocal microscope



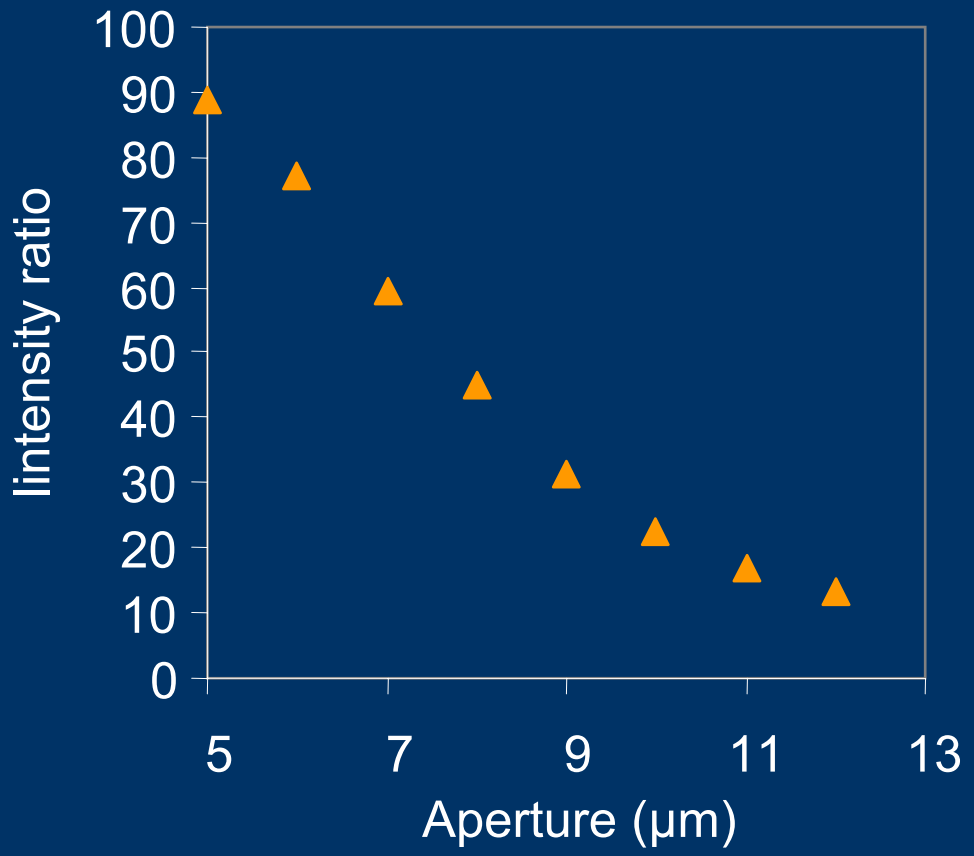
Two confocal Schwarzschild objectives:

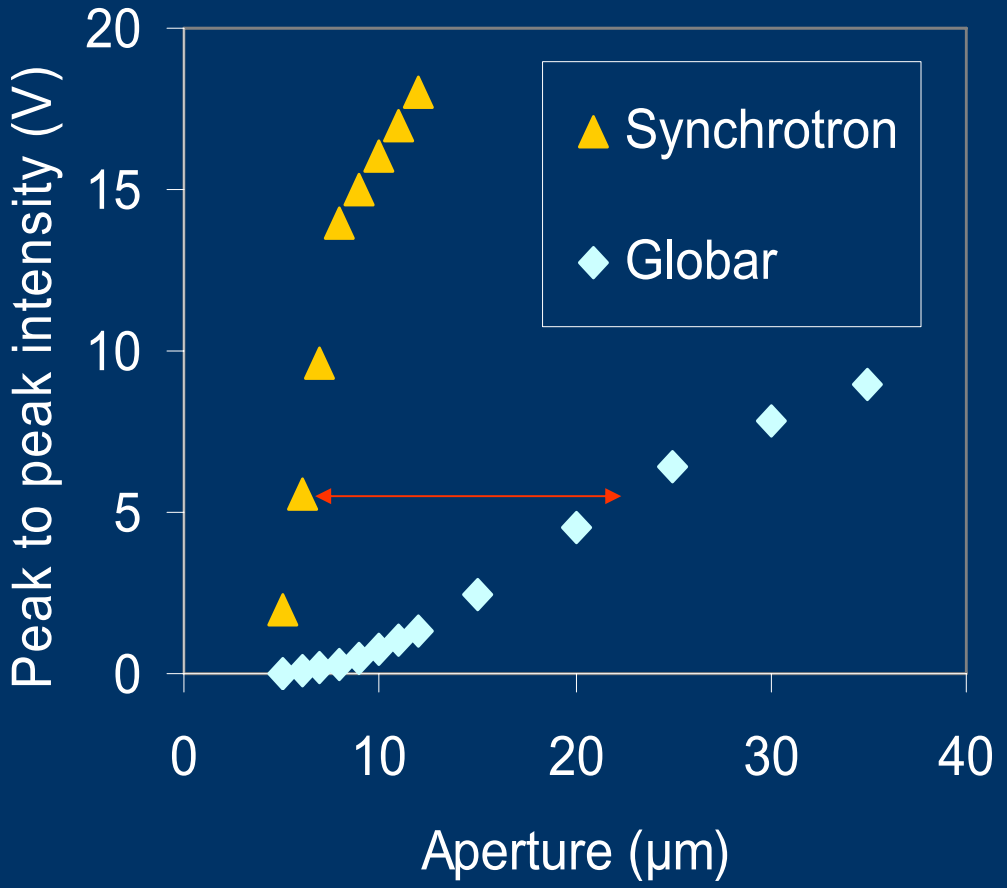
- focus the light onto the sample
- collect the light and relay it to the detector.

Diffraction-limited resolution of $\lambda/2$
(λ: 2→12μm)

G.L. Carr, *Rev. of Scientific Instruments*, **2001**, 72, 1613

Synchrotron vs Globar





Synchrotron

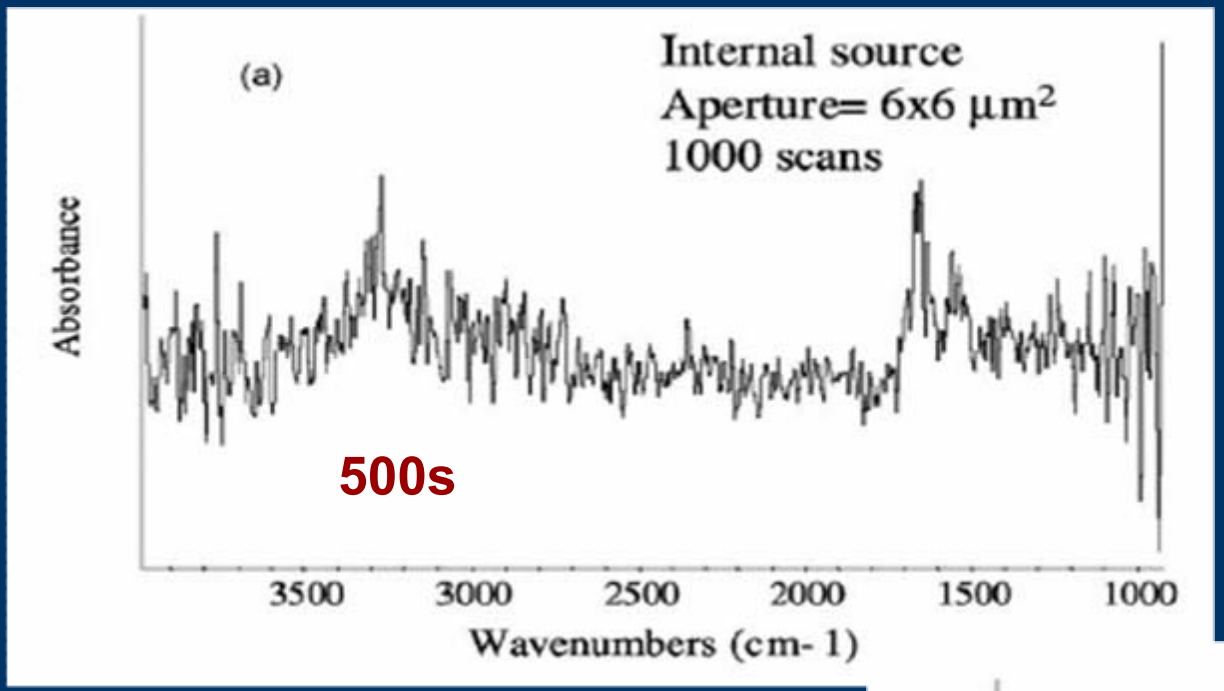
6×6 μm²

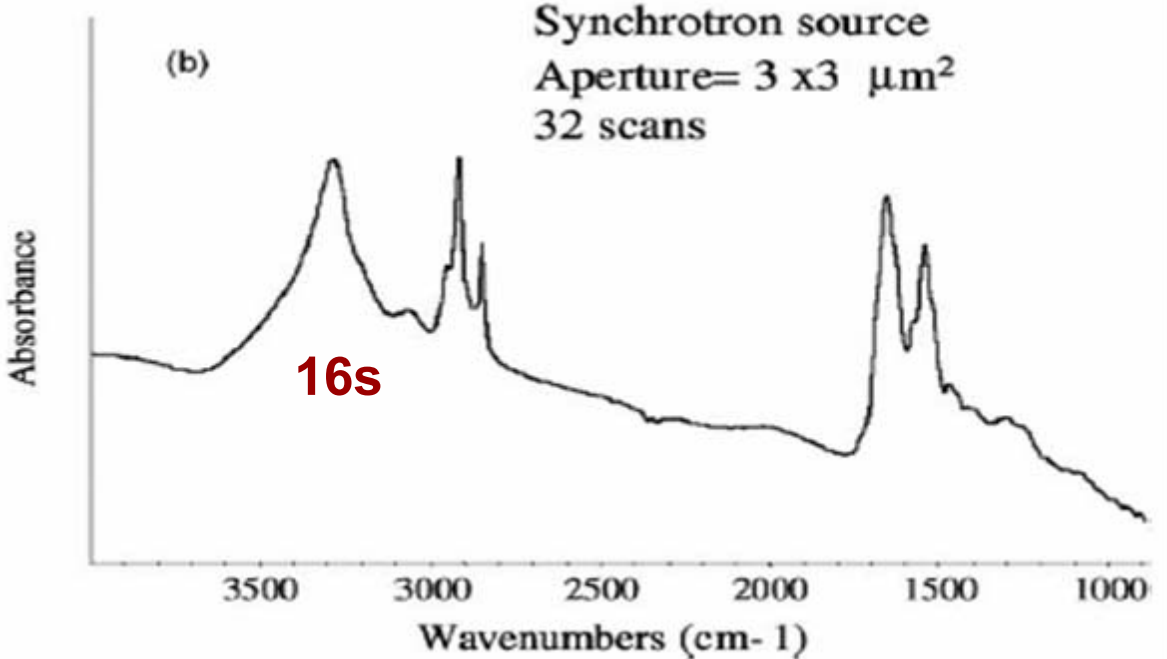
Globar

22×22 μm²

ESRF-ID21

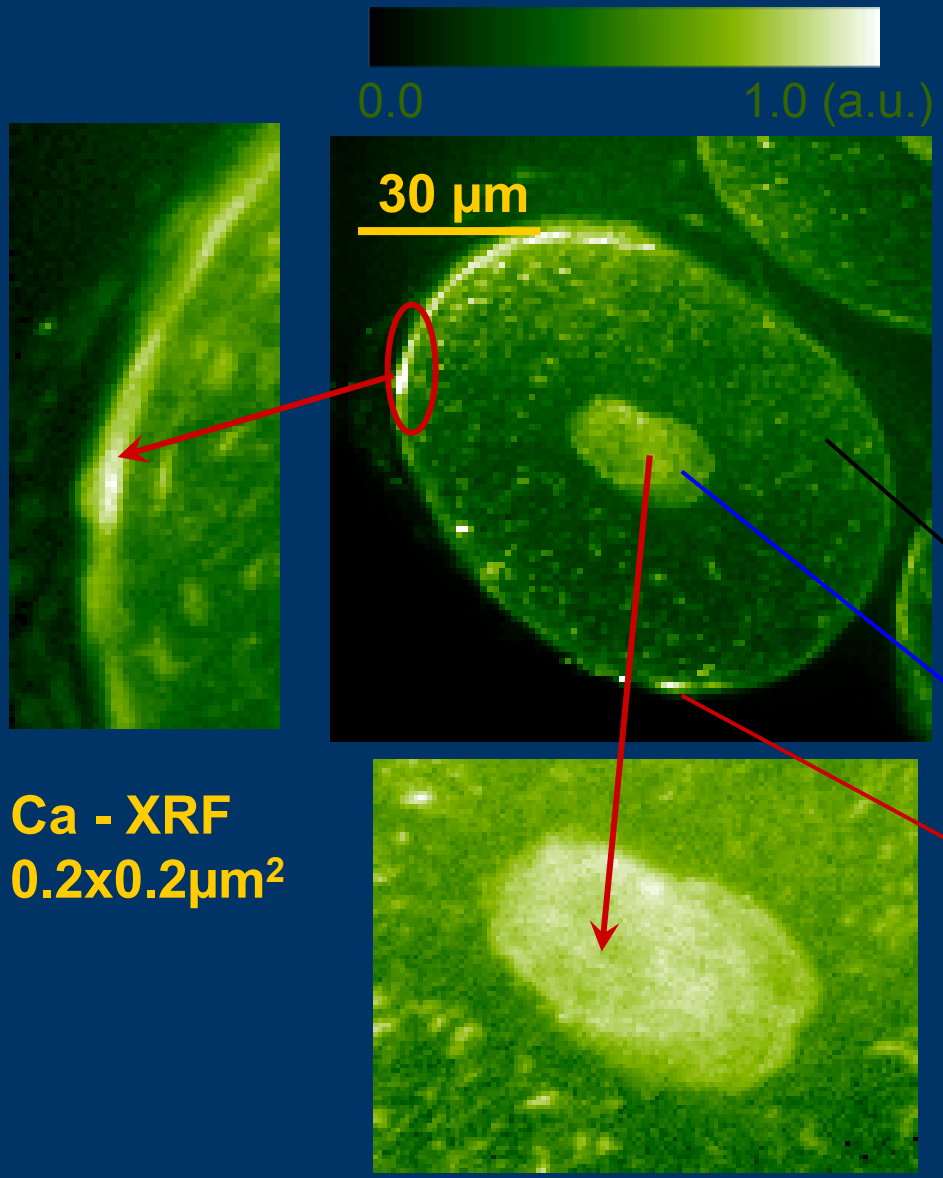
Synchrotron FTIR Microscopy

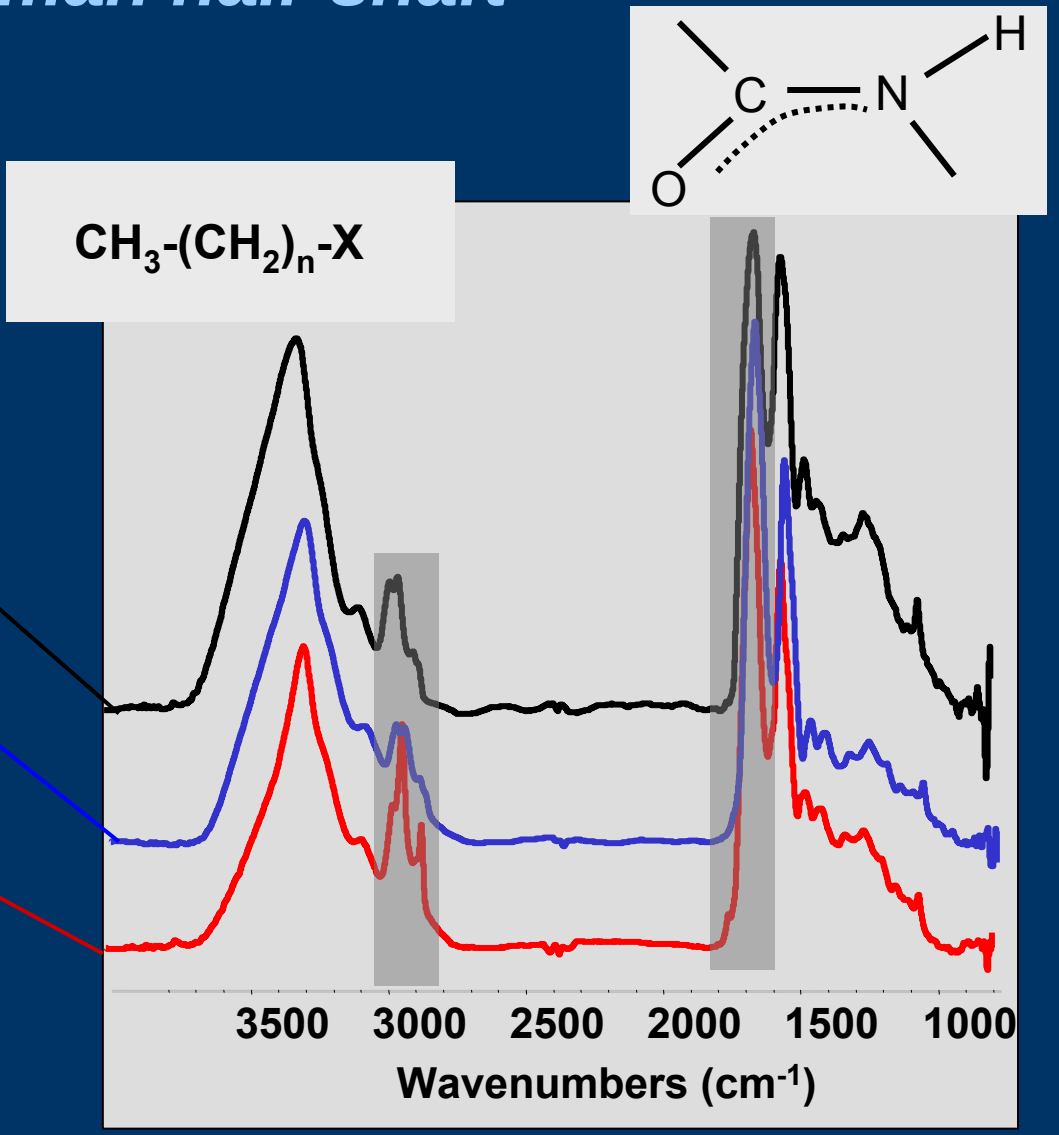




P. Dumas et al.
Vibr. Spectr. 32 (2003).

Various calcium sites in human hair shaft

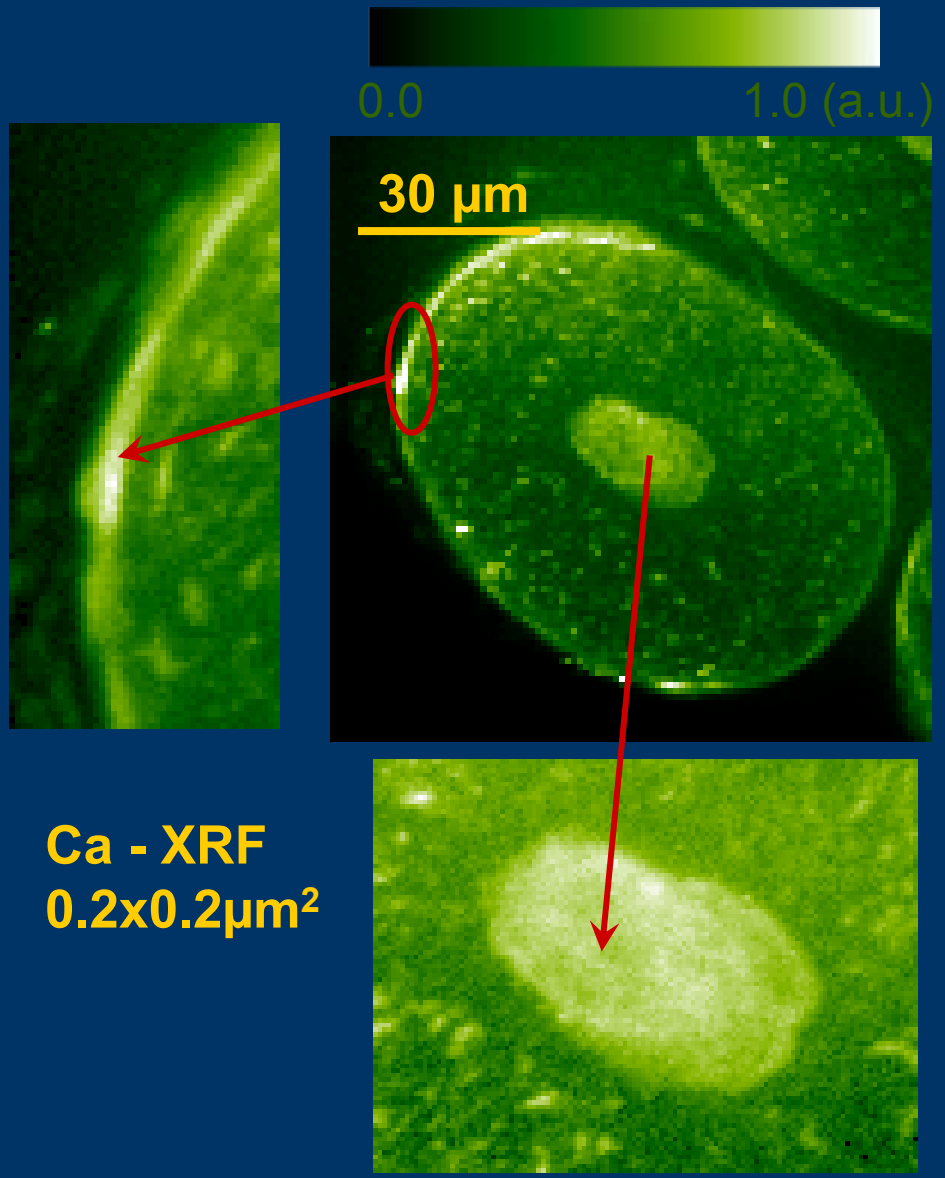




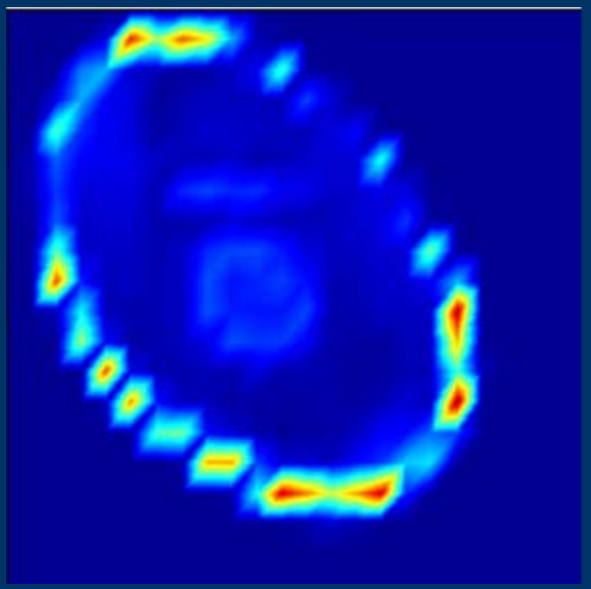
Infrared Spectra

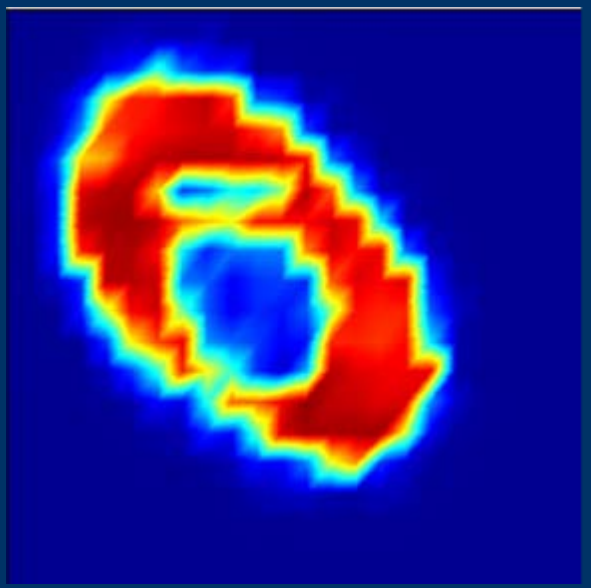
C. Merigoux *et al.*,
Biochimica & Biophysica Acta, 1619, 53, (2003)

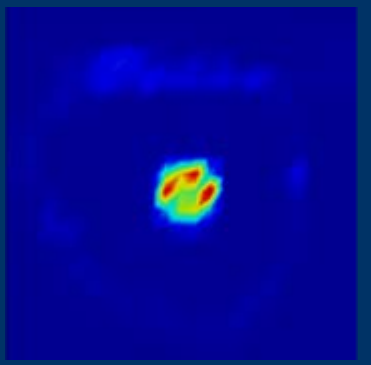
Various calcium sites in human hair shaft



Ca - XRF
0.2x0.2µm²





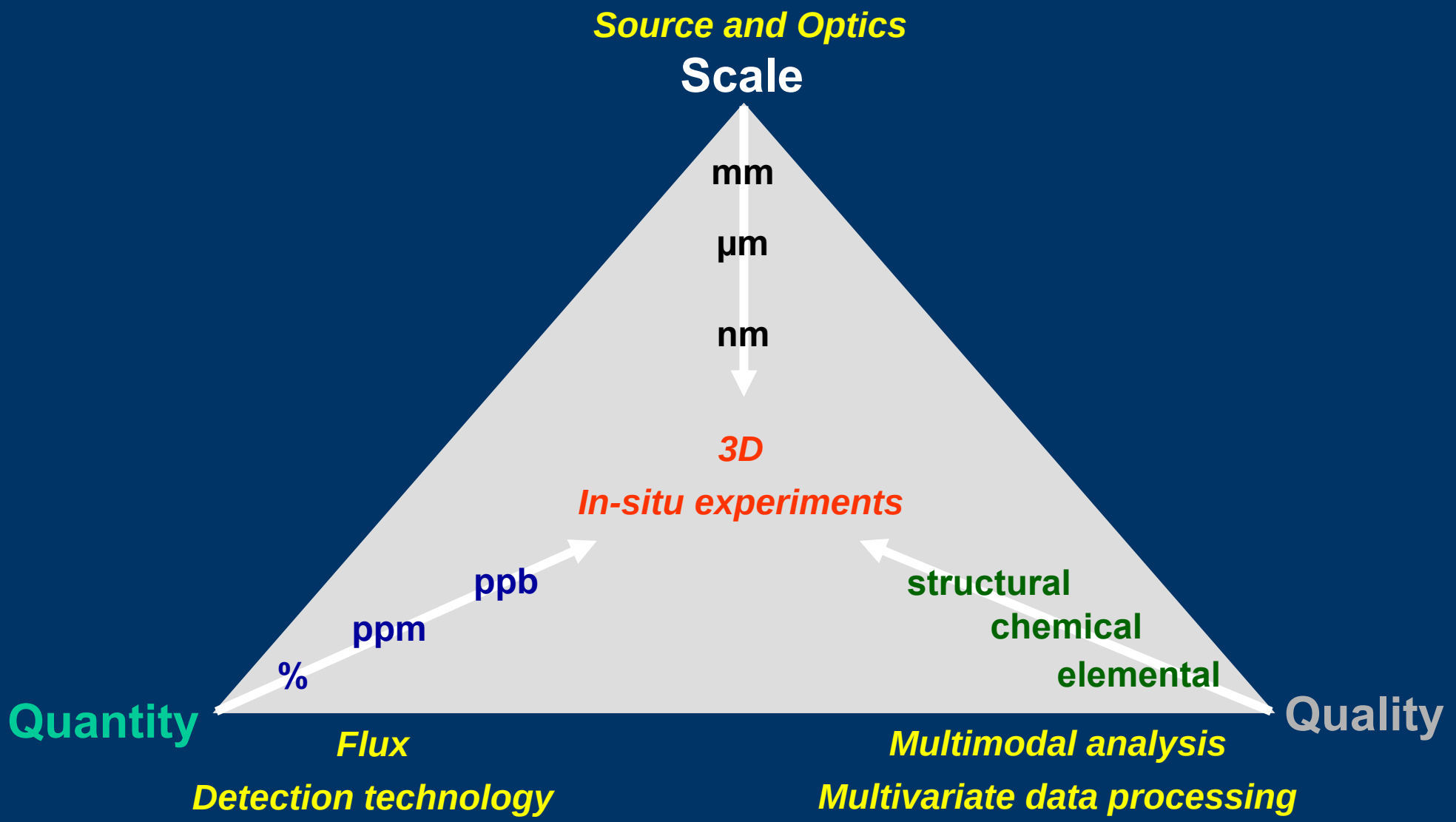


Two different « types » of lipids in cuticule and medulla

Protein distribution in cortex

C. Merigoux *et al.*,
Biochimica & Biophysica Acta, 1619, 53, (2003)

Outlook


Source and Optics
Scale
mm
µm
nm
3D
In-situ experiments
ppb
ppm
%
structural
chemical
elemental
Quantity
Quality
Flux
Detection technology
Multimodal analysis
Multivariate data processing