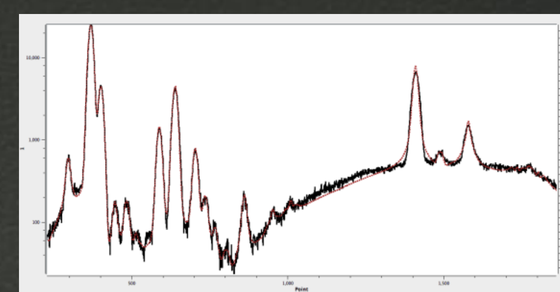
# First c[illegible]tion of dinosaur skin layers using synchrotron radiation

Mauricio Barbi
Department of Physics, University of Regina



CAP Congress, June 14, 2018

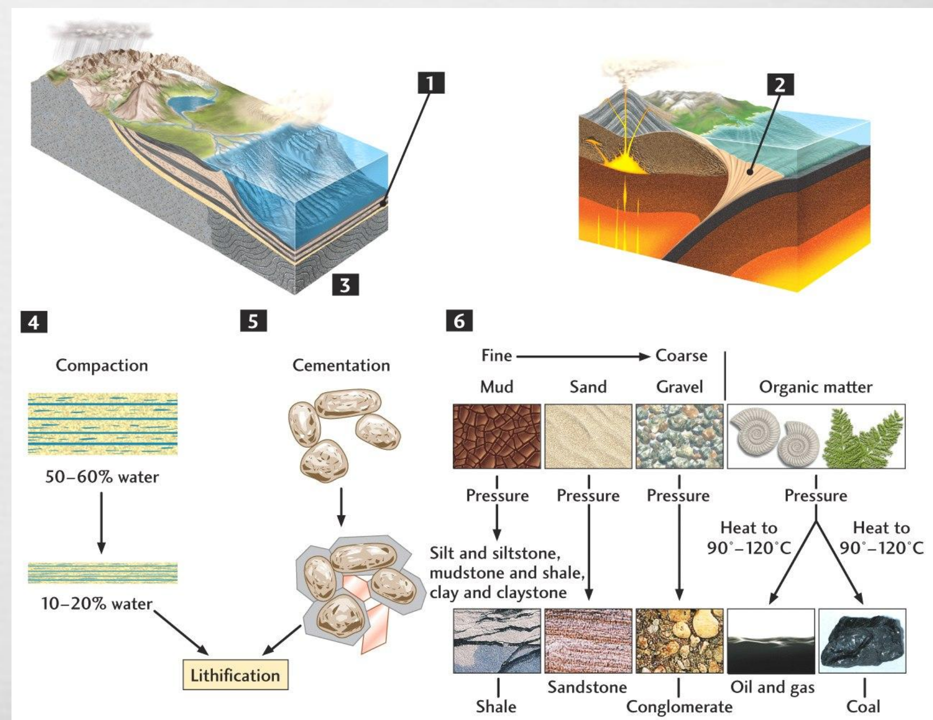
# Overview

- Dinosaurs: How to tell their stories?
  - Bones and evidences of preserved structures
- Applying synchrotron radiation and other techniques to studies in paleontology
- One of a kind discovery in Alberta: A well preserved 3-D hadrosaur skin
- Have we, unequivocally, observed preserved skin layers in a hadrosaur skin?
- Final Remarks

# Fossilization and Diagenesis


- Minerals replace organic matter

- Modifications and exchange with the surroundings as time flows

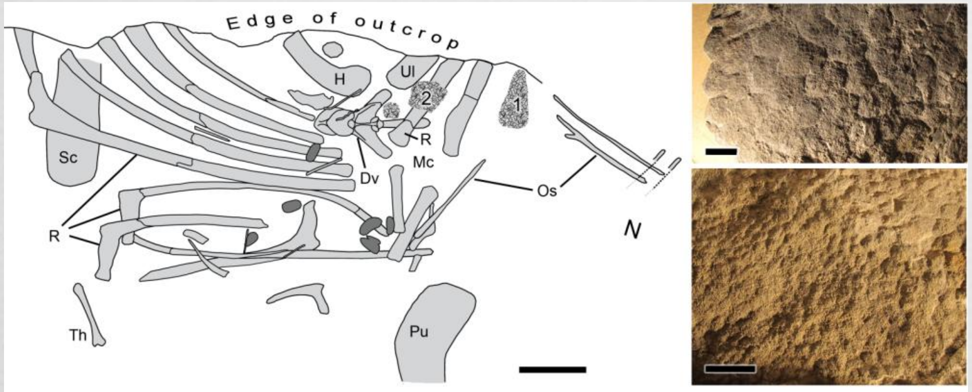



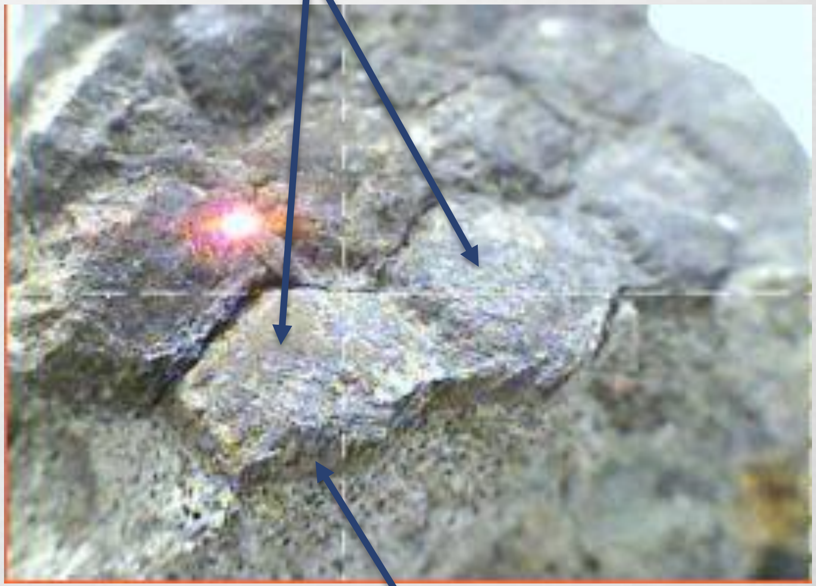
# 3-D skin

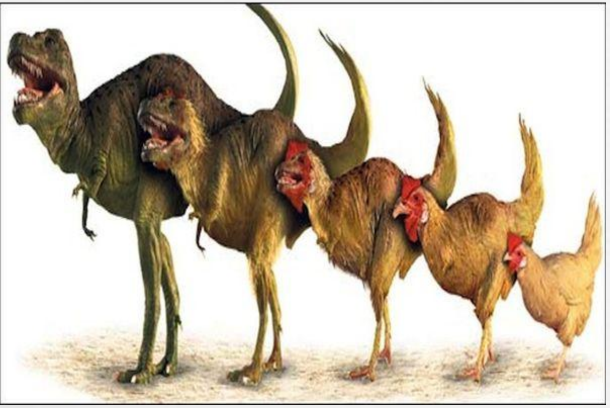
Discovery (2012) of a partially well preserved hadrosaur by Phil Bell – Grande Prairie, Alberta

3-D skin structures (initially thought to be just imprints).

Skin patches near the forelimb





# Methods and Analysis

- What could be preserved?
  - Original organic matter?
  - Pigmentation?
  - DNA????
  - Skin structures?
  - Nothing?

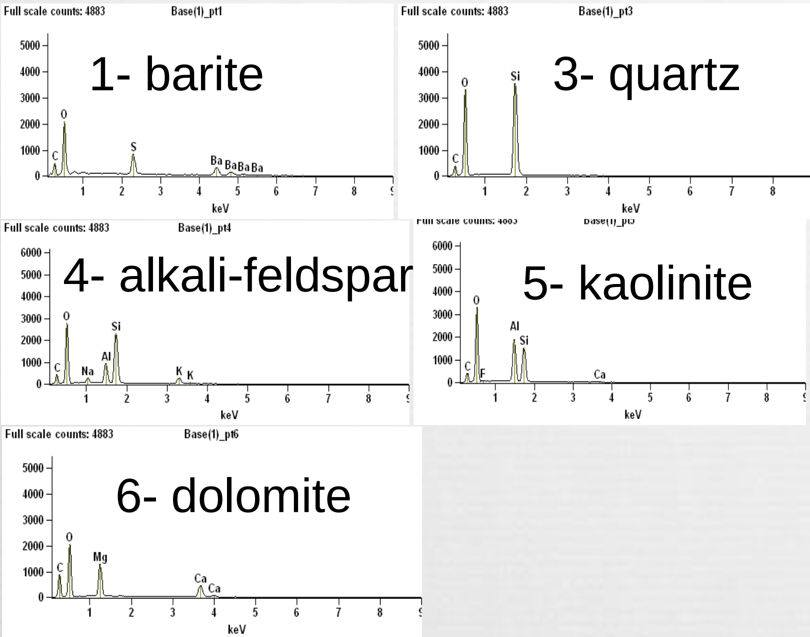
- If there are preserved skin structures, can we compare it to avian species?
  - A direct comparison of this kind would be the first ever to be realized (evolutionary path).

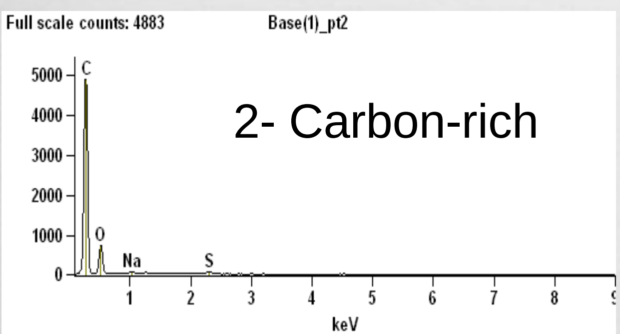
- Want to study the sample at microscopic scale → structural and chemical composition
  - Is it just an imprint?
  - Does it differ in composition from the sedimentary matrix?

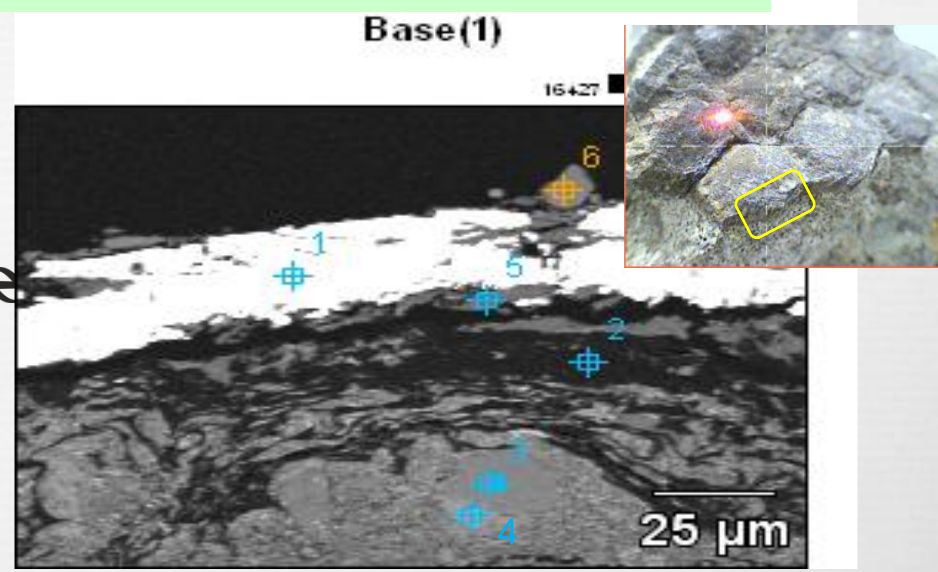
- The following techniques were used:
  - Synchrotron Radiation (SR)
  - Scanning Electron Microscopy (SEM)
  - Optical Microscopy

# (Scanning Electron Microscope – SEM analysis – 20 *μm* section)

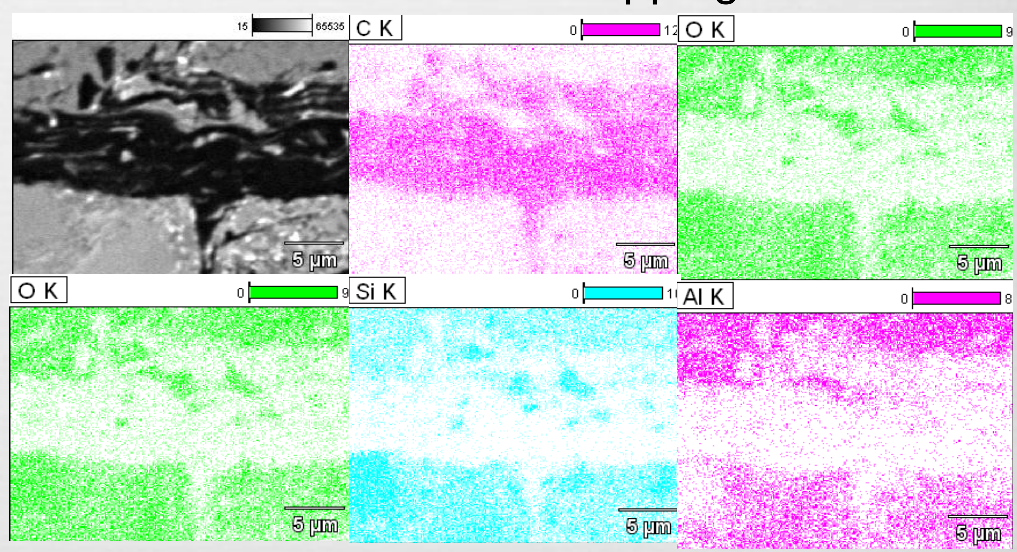
X-ray spectra were taken from each numbered point in the sample.




Elemental Mapping

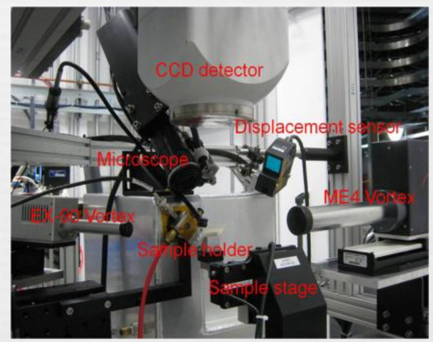



Strong correlation between carbon distribution and darker area in the BES image

# Chemical Signatures (Canadian Light Source - CLS)

- Fingerprinting the skin
  - Mapping chemical elements (VESPERS beamline)
- Organic traces (MidIR)
- Observing few chemical states (SXRMB and SM beamlines)

CLS



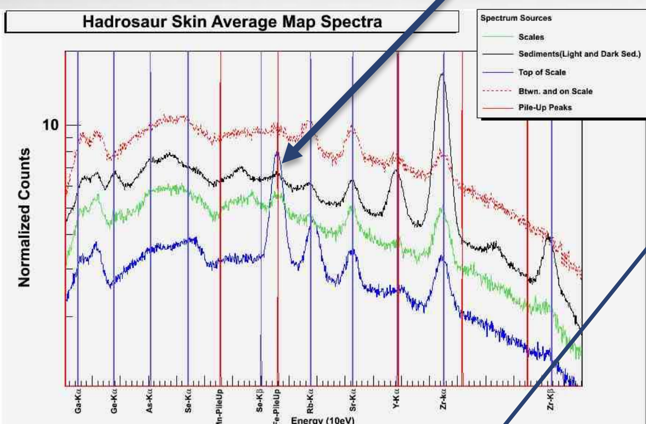

VESPERS

SXRMB

# XRF map : side of the sample scanning from skin into sediment

15 $\mu m$ step; 0.5 eV resolution

Fe




Skin;
Fe marks skin

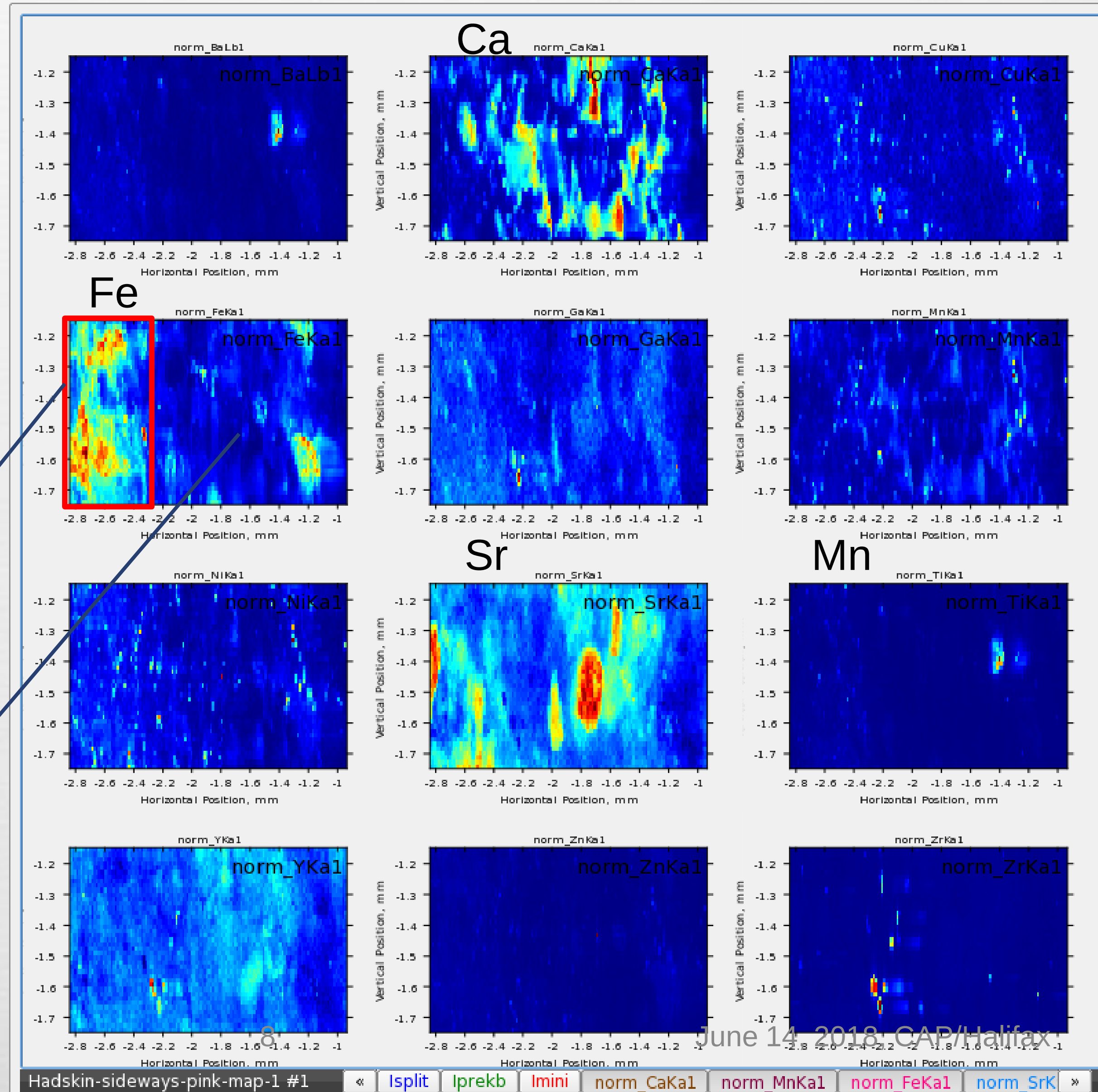
Sediment

Ca

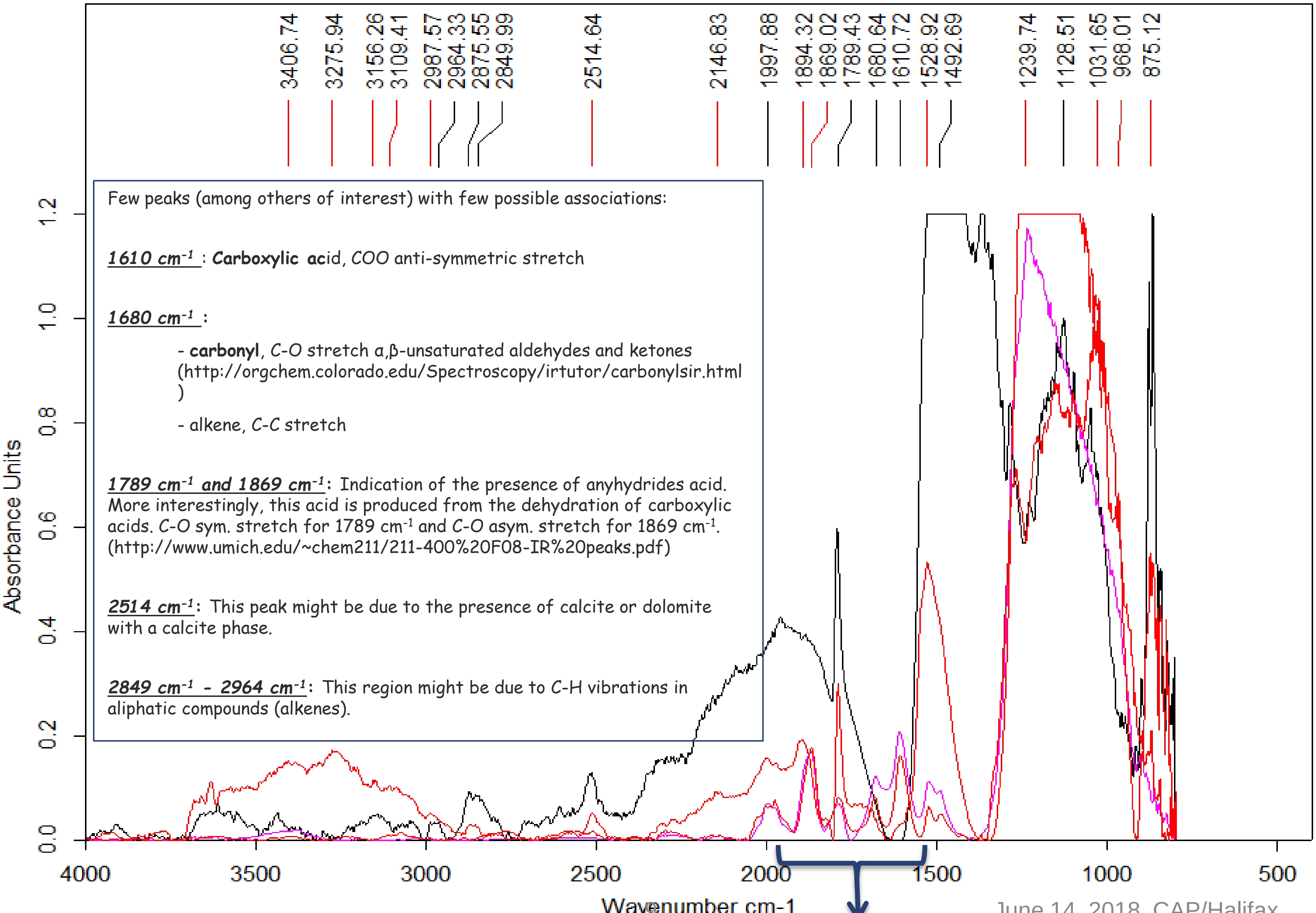
Fe

Sr

Mn



# Infrared spectrophotometry




Few peaks (among others of interest) with few possible associations:

*__1610 cm$^{-1}$__* : **Carboxylic aci**d, *COO* anti-symmetric stretch

*__1680 cm$^{-1}$__* :

- **carbonyl**, *C-O* stretch α,β-unsaturated aldehydes and ketones (http://orgchem.colorado.edu/Spectroscopy/irtutor/carbonylsir.html )
- alkene, *C-C* stretch

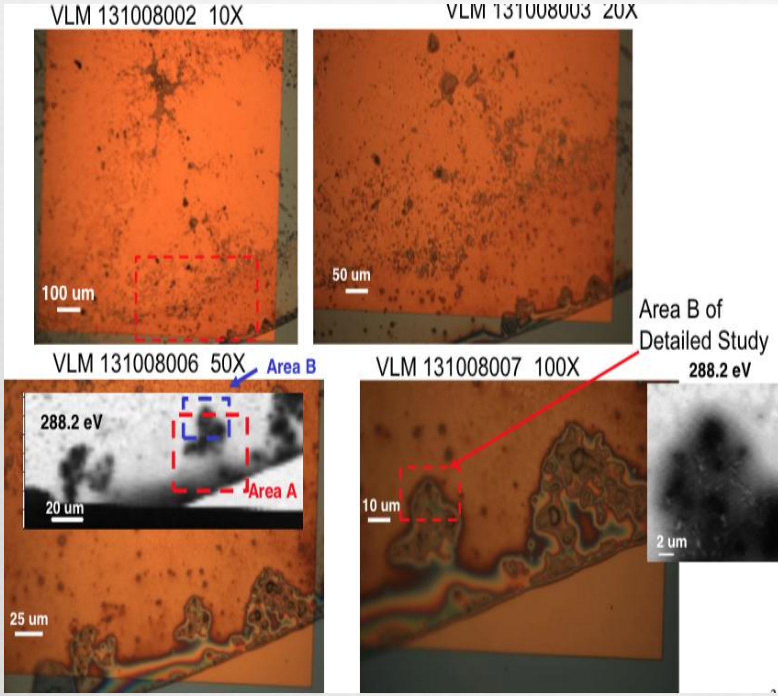
*__1789 cm$^{-1}$ and 1869 cm$^{-1}$__*: Indication of the presence of anyhydrides acid. More interestingly, this acid is produced from the dehydration of carboxylic acids. *C-O* sym. stretch for 1789 cm$^{-1}$ and *C-O* asym. stretch for 1869 cm$^{-1}$. (http://www.umich.edu/~chem211/211-400%20F08-IR%20peaks.pdf)

*__2514 cm$^{-1}$__*: This peak might be due to the presence of calcite or dolomite with a calcite phase.

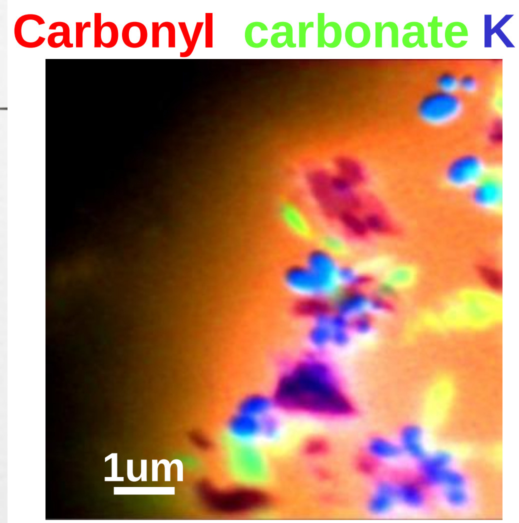
*__2849 cm$^{-1}$ - 2964 cm$^{-1}$__*: This region might be due to *C-H* vibrations in aliphatic compounds (alkenes).

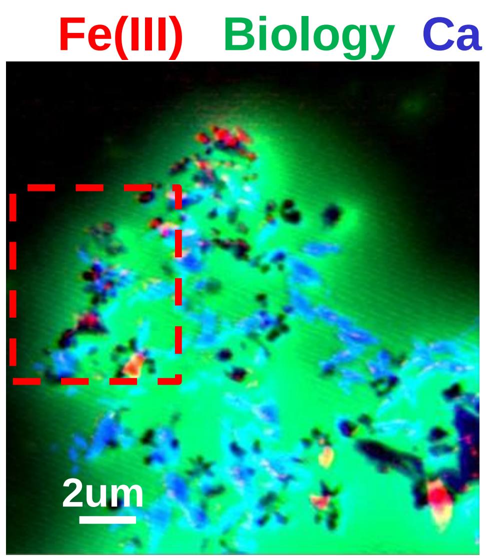
Region of the spectrum corresponding to organic signature

# “Biology” map (carbon K-edge scan)



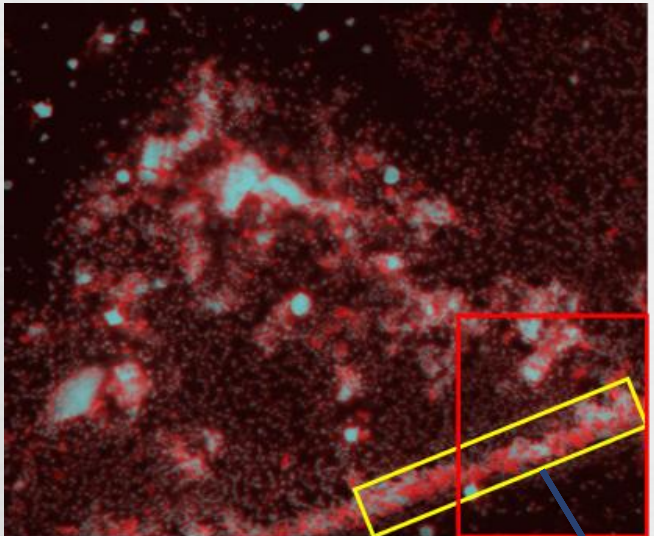

- These measurements seems to corroborate those from FTIR.
- Ketone can be produced from the breakdown of fat.
- Carbonyl might be remnant of carboxylic acid



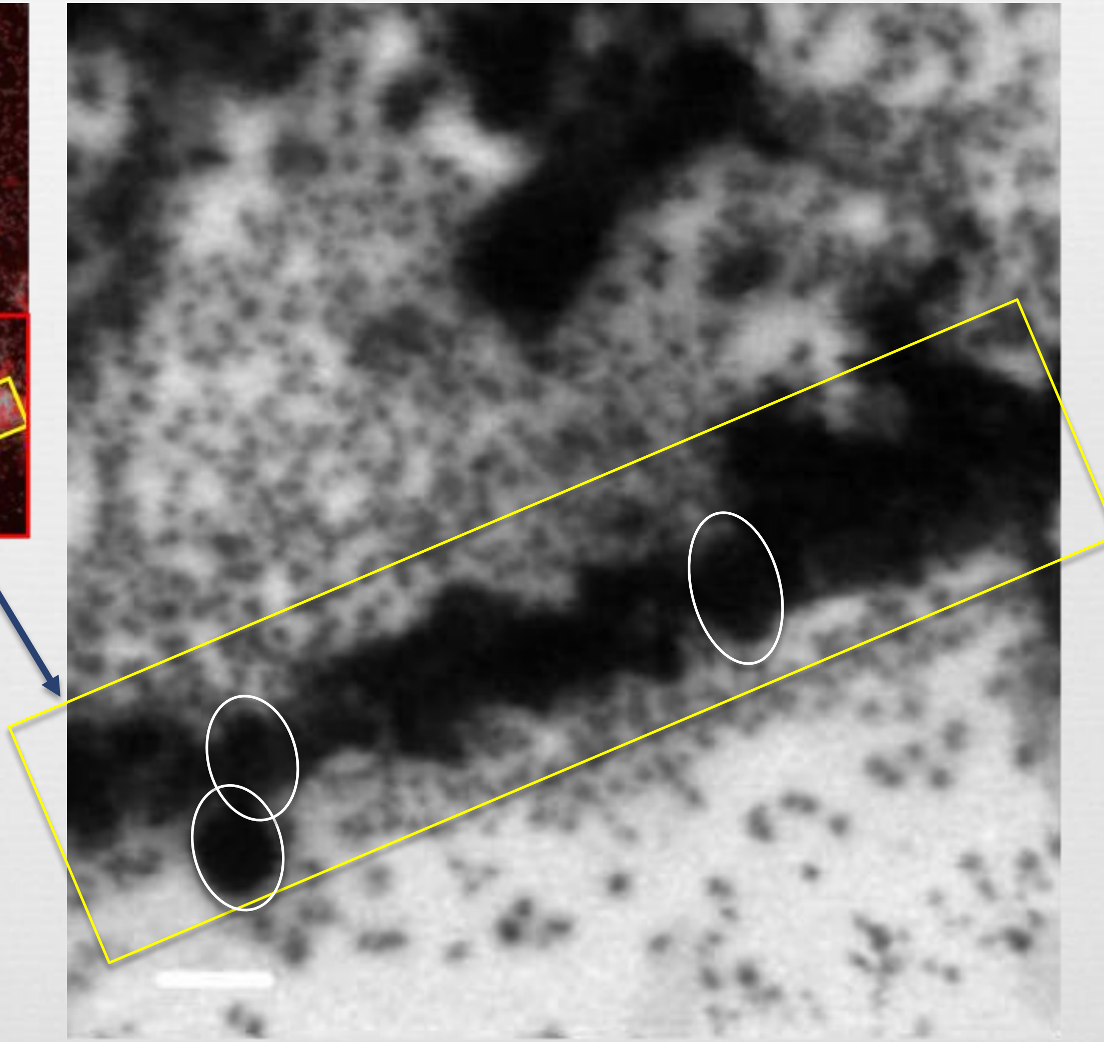




# Carbonyl map

Carbonyl (red) other compounds (cyan) map in a 65 x 50 µm area of the sample at 0.1 µm steps

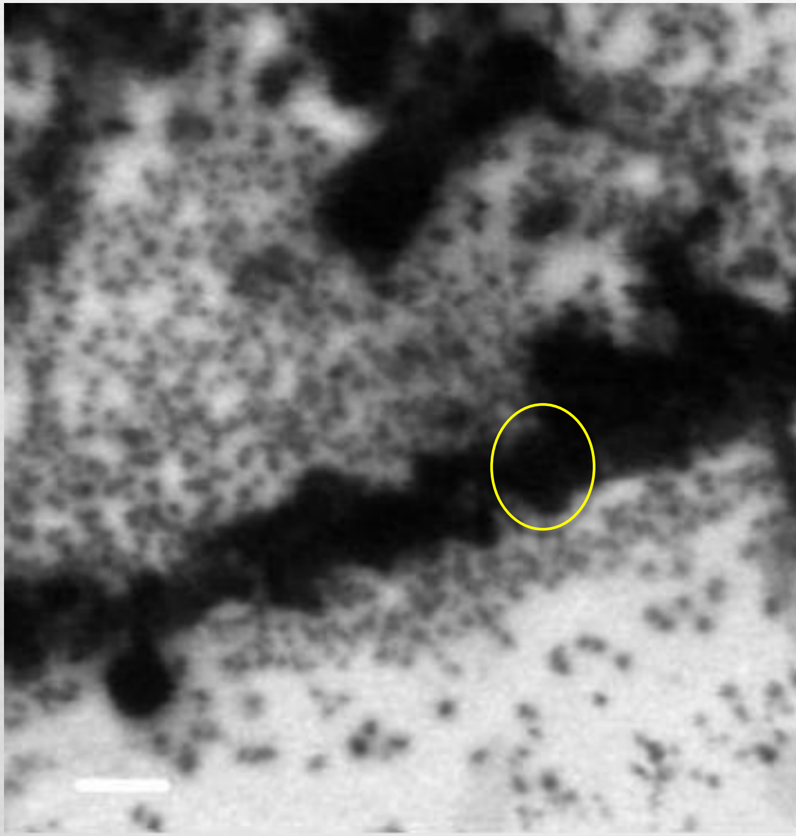


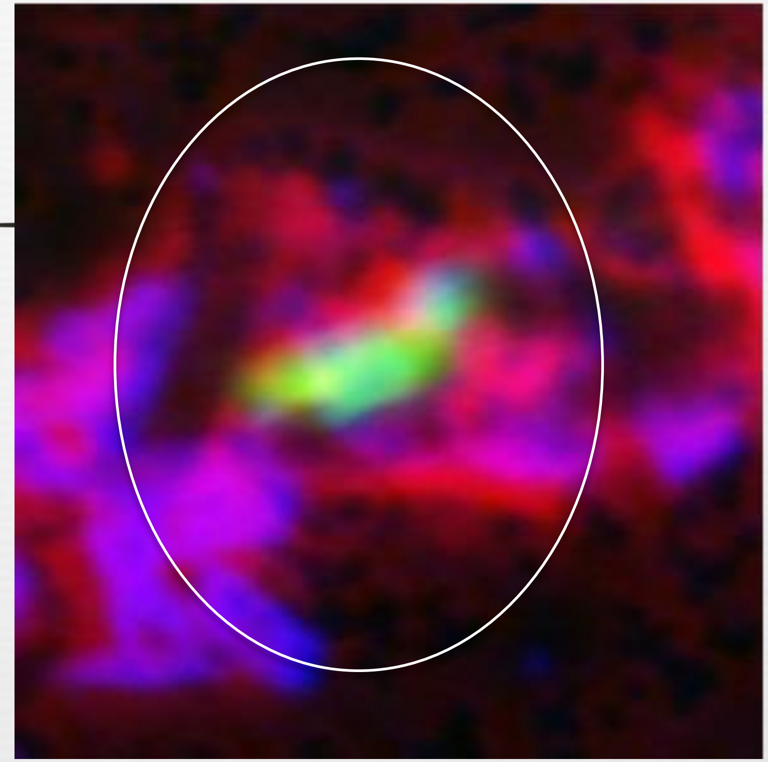
Carbonyl-only map



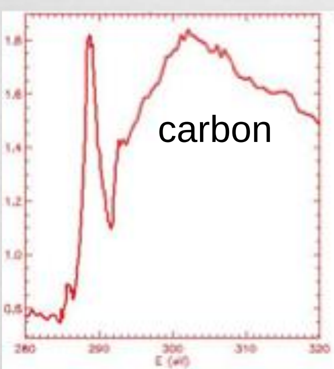
- Clear carbonyl-rich layer
- Visible substructures form the layer

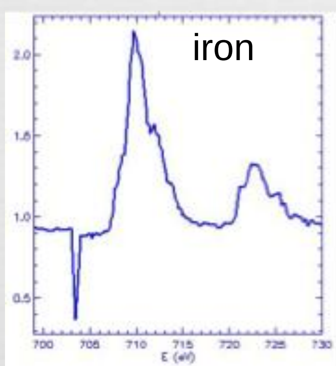
# Skin layer single substructure Ca, Fe and C map

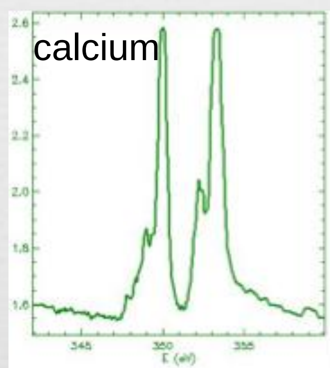




**Carbon**
**Iron**
**Calcium**



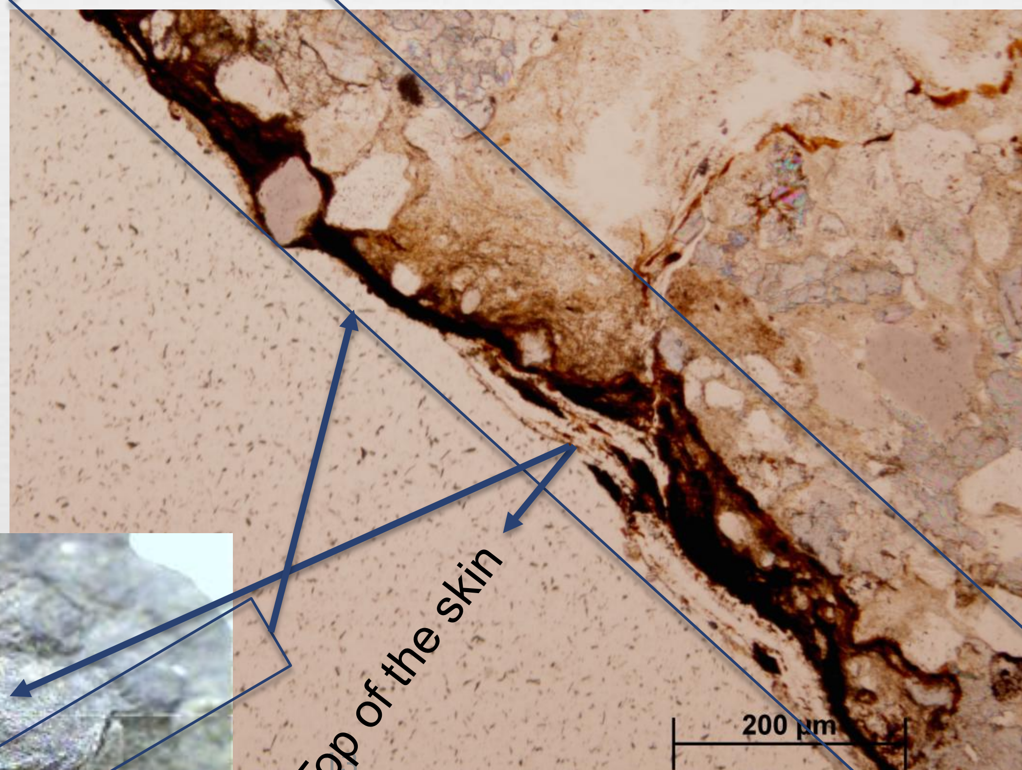




- Calcium (calcite or vaterite, $CaCO_3$) delineates substructure
- Iron (siderite) concentrated at the center of the substructure
- Siderite found to contribute to the preservation of soft tissue (M. Schweitzer et al, *Proceedings of the Royal Society B* 281, no. 1775, January 22, 2014)

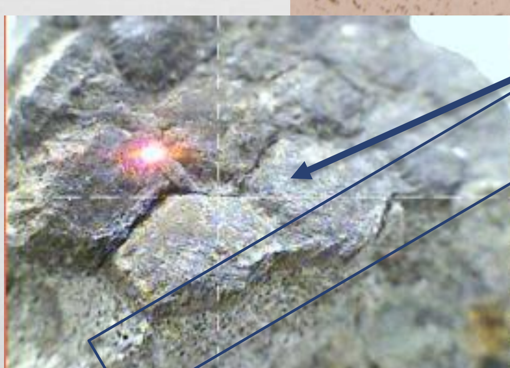
# Optical Analysis: 20 μm section (same used for SEM)

Overview




Top of the skin

Darker areas correspond to carbon-rich regions



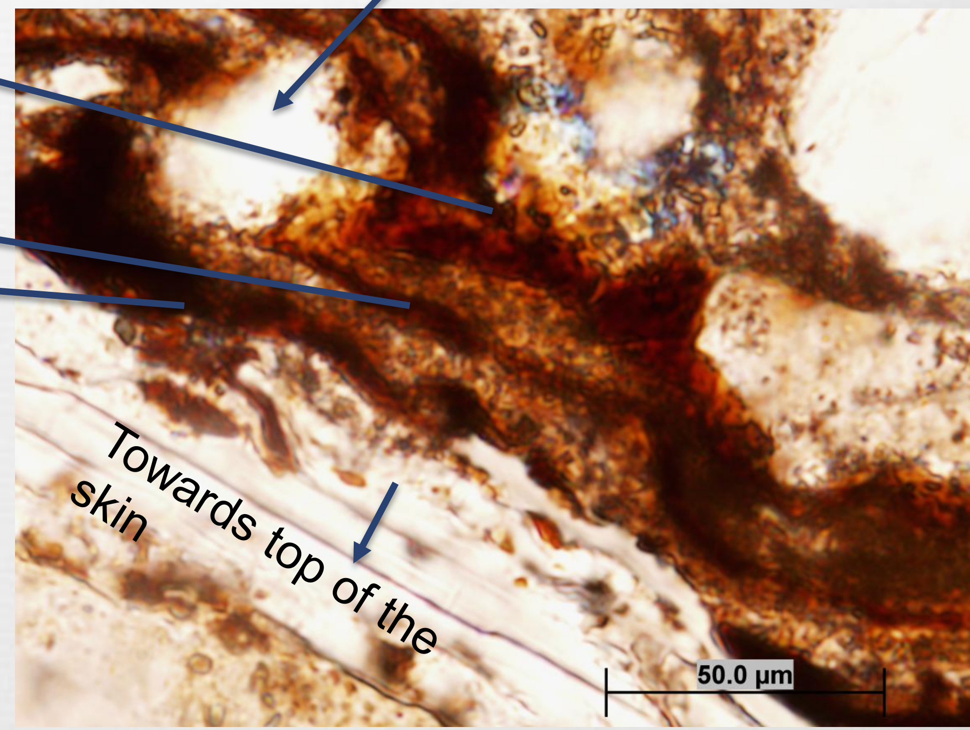
# Evidence of three preserved layers (Epidermis)

Vascular canal

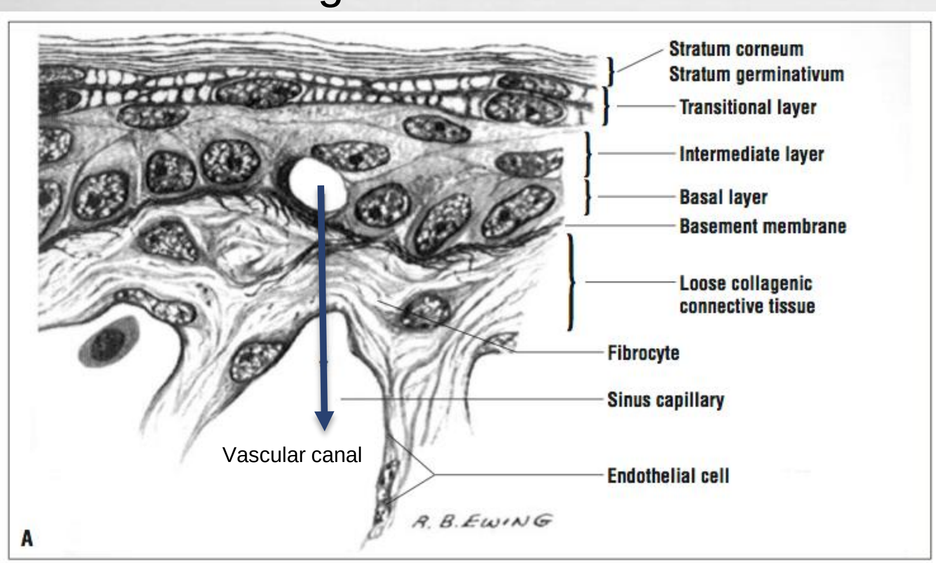
Stratum germinativum (basale)

Stratum granulosum

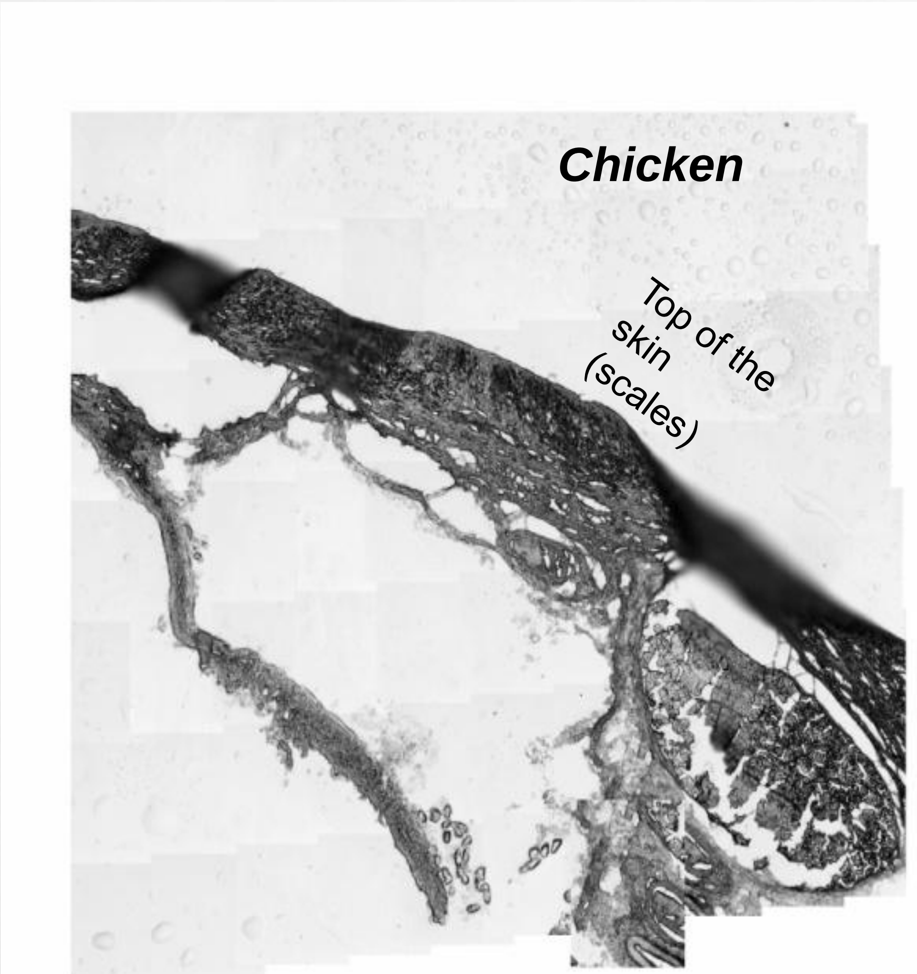
Stratum corneum

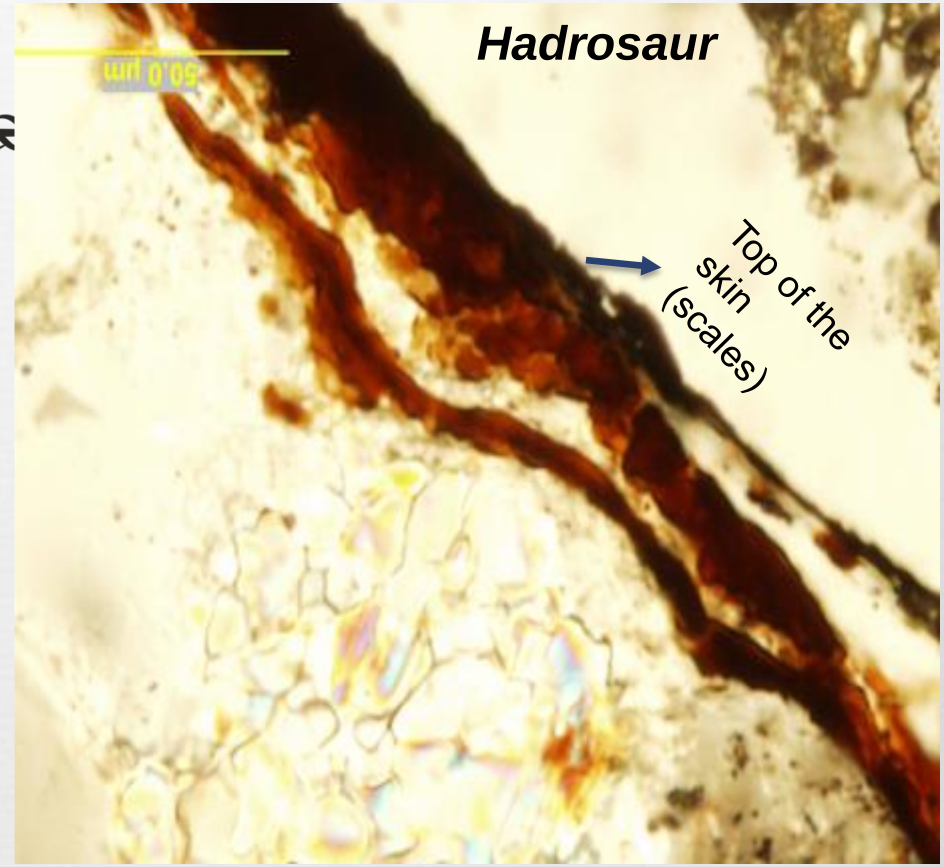



Avian Integument

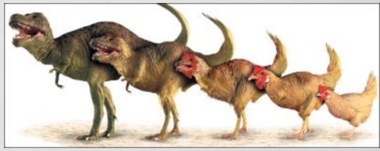


# Comparing to a chicken leg



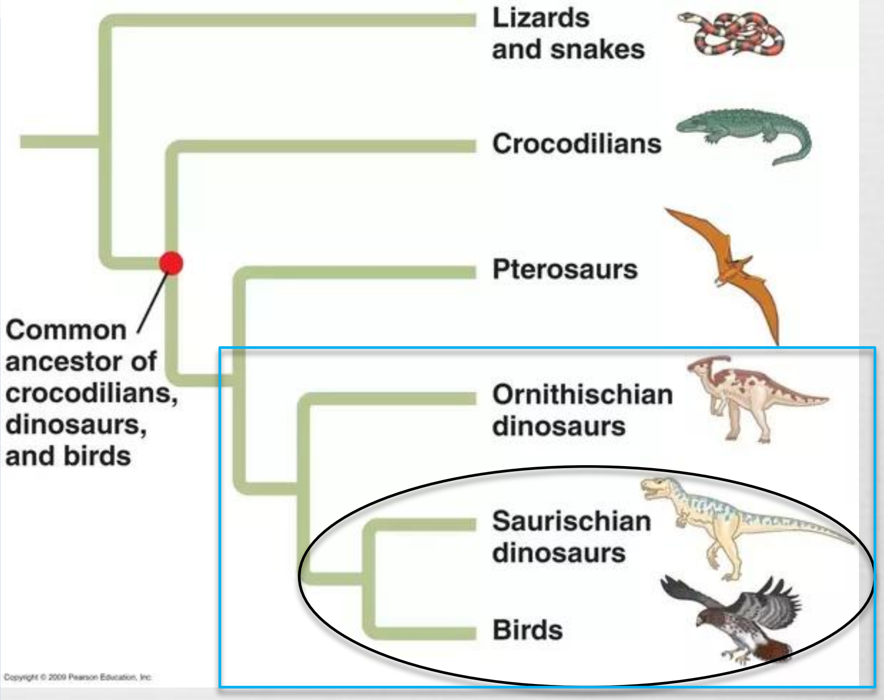




- Remarkable similarities
- Strongly support the evolutionary correlations between birds and dinosaurs



# Summary

- Several independent and different techniques used
- Skin layers formed of carbonyl-rich substructures with iron in the form of siderite concentrated in their center.
  - Iron probably correlated with this remarkable skin preservation
- First observation of preserved skin layers in a dinosaur skin
- Also, the first ever direct comparison between dinosaur and modern avian skin
  - Support to the evolutionary theory of birds and dinosaurs as coming from the same ancestor




Avians are indeed dinosaurs

# Collaborators (I didn't do all this alone)

- Phil Bell (Paleontology, University of New England, Australia) → Dinosaur discovery, preparation and taphonomy
- James Dynes (CLS) → Measurements at the SM beamline
- Josef Buttigieg (Biology, UofR) → Chicken skin preparation and comparisons (including the hypothesis about colour)
- Federico Fanti (Geology, University of Bologna, Italy) → Stratigraphic study
- Anezka Kolaceke (Physics, UofR, PhD student) → Data collection at CLS

# Still not Convinced?

Different techniques show:

- SEM: Apparently well-organized layers of carbon-rich distributions in the sample
- SXRMB: Reduced sulphur in the region corresponding to the “skin” – Very possibly of biological nature
- MidIR: tantalizing  peaks that might correspond to organic compounds, mostly remarkably carbonyl.
- SM: Clear “biological” region defined by carbonyl in similar carbon-rich region observed with SEM. Carbonyl fingerprints a organized layered structure
- Skin layers formed of carbonyl-rich substructures
- Optical microscopy: remarkable similarities between chicken skin and hadrosaur “skin” – comparable morphology
- Evidences of 3 layers that might correspond to preserved epidermis cell layers

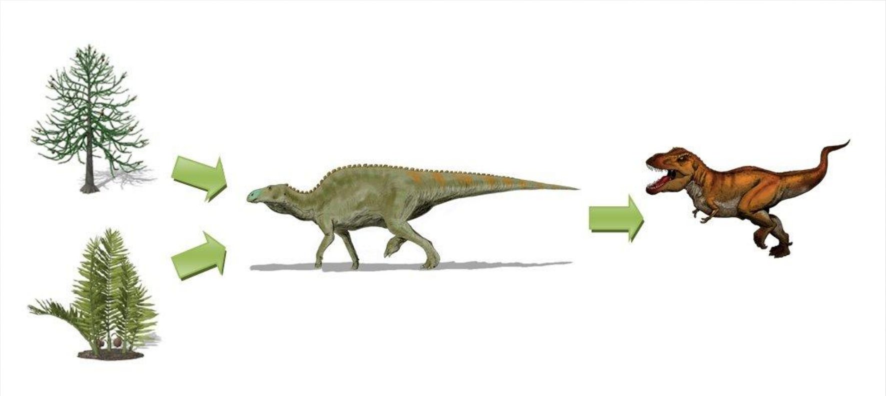
# Speculations (why not?)

- No pigments found (yet). However:
- Hypothesis that *hadrosaurs* could display colour by flushing blood through the skin.
- Evidence (and just that for now) of different skin thickness:
  - Thicker areas → more brownish
  - Thinner areas → more reddish

# Backup Slides

# Life and death of dinosaurs

- Plants absorb minerals from soil
- Herbivorous eat plants
- Carnivorous eat herbivorous
- Both eventually die one way
  or another

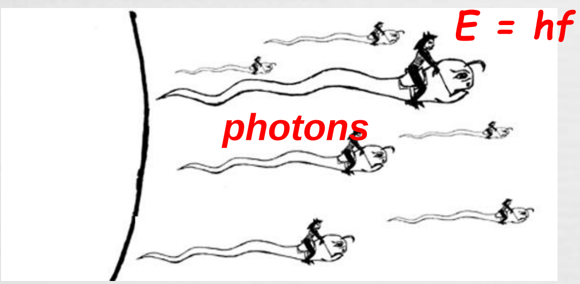


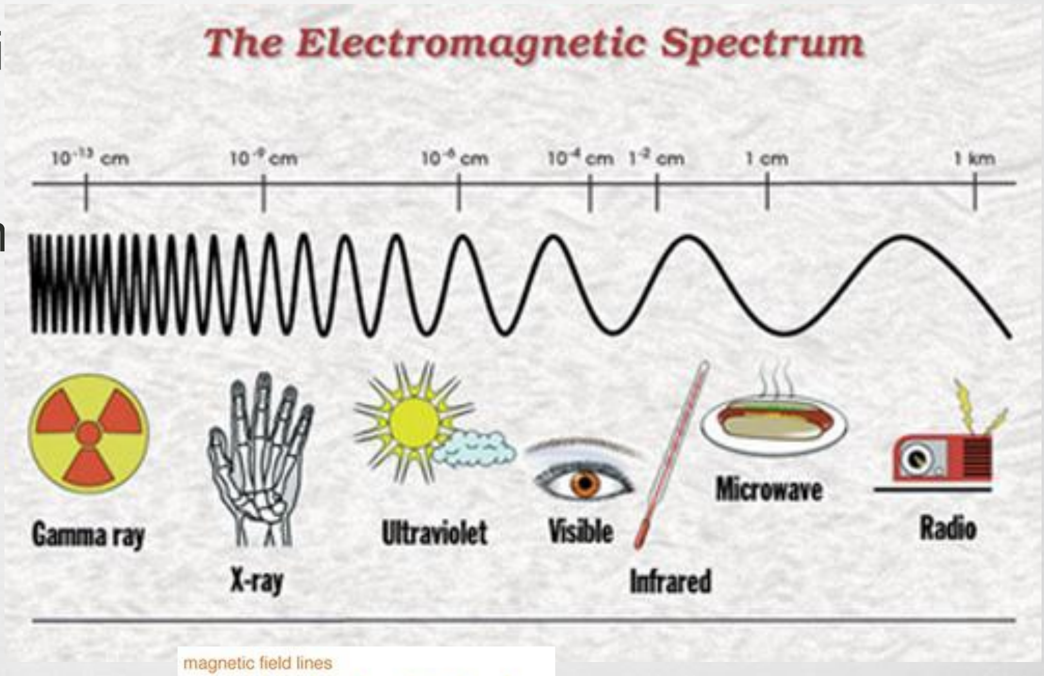
- Some animals are completely destroyed (eaten, etc)
- Others are preserved under special circumstances

# Synchrotron Radiation (SR) in a Nutshell

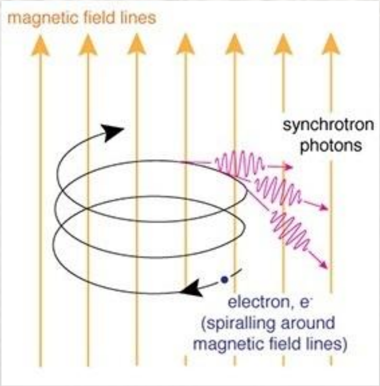
- Radiation is an electromagneti (EM) waves

- EM waves are made of photon (quantum of light)



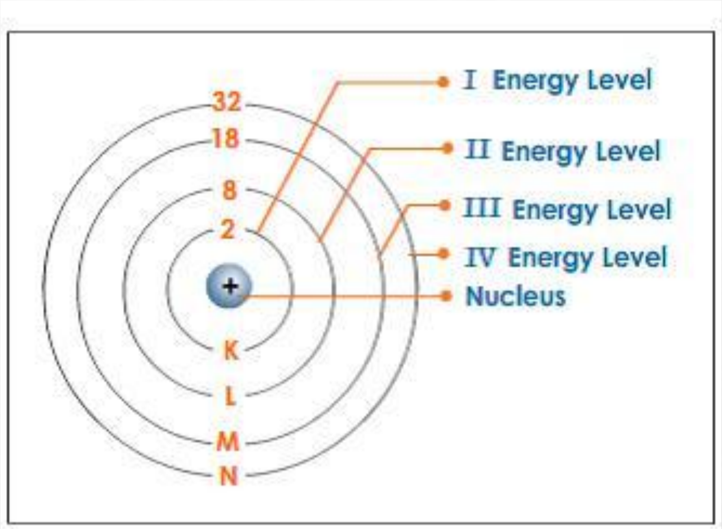

- Synchrotron Radiation is produced by accelerated charged particles

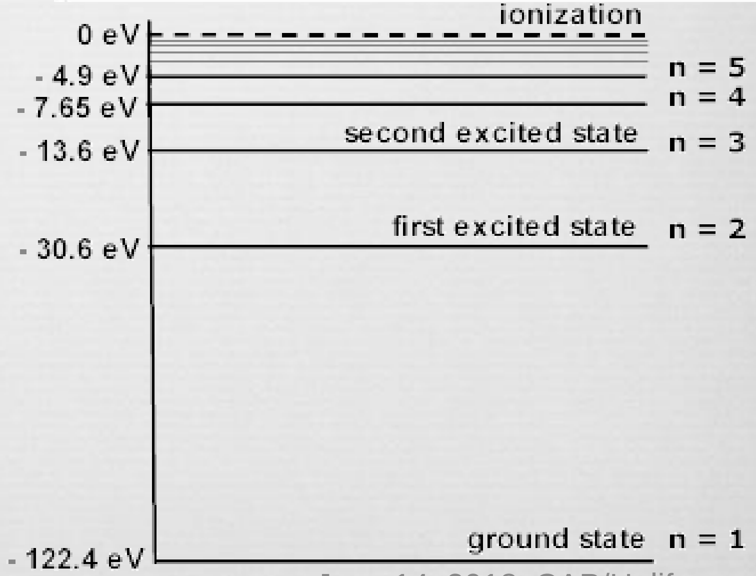


# How do we use SR?

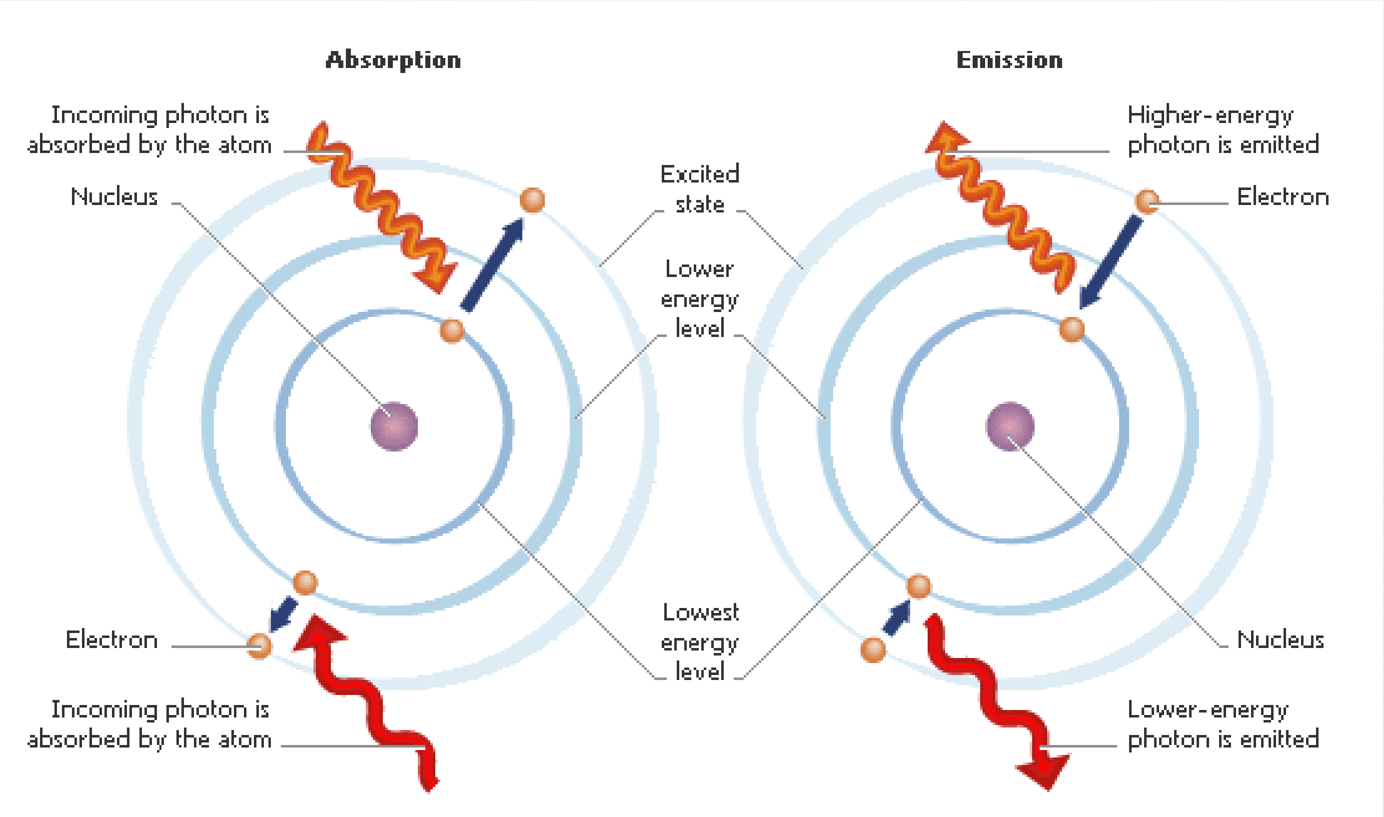
**Identifying chemical elements**

- The atom of an element is constituted of a nucleus (protons and neutrons) and electrons
- Quantum mechanics tells us that the electrons in an atom are found in discrete states of energy
- Each atom has its own set of discrete and characteristic energy levels → fingerprint.

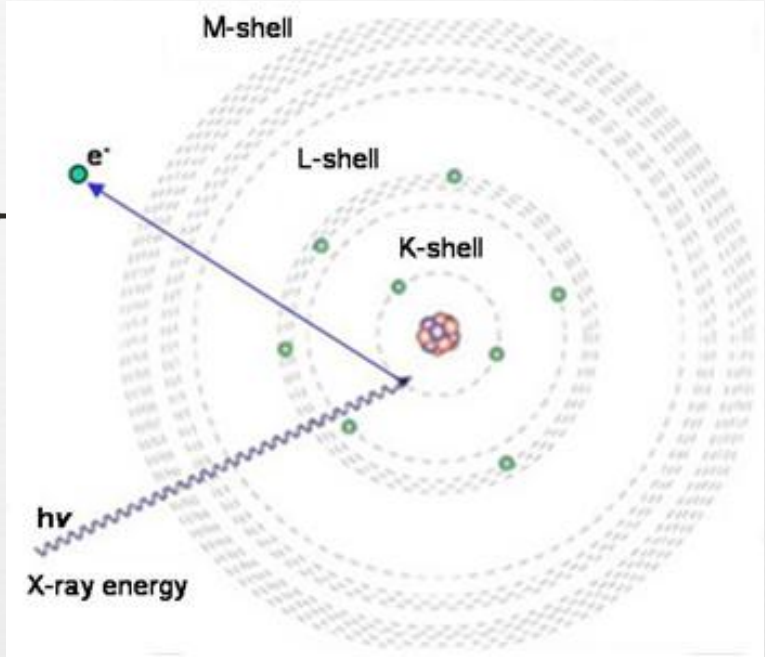


- Electrons can move between energy levels → absorption or emission of photons.



- They can also be removed from the atom (ionization process)

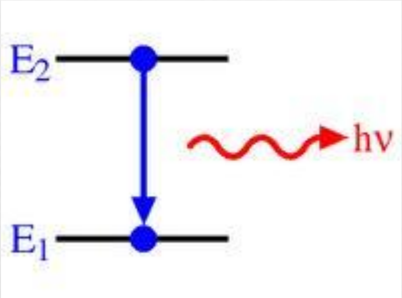



- Electrons from higher orbits will want to move to the lower vacant orbit created by ionization

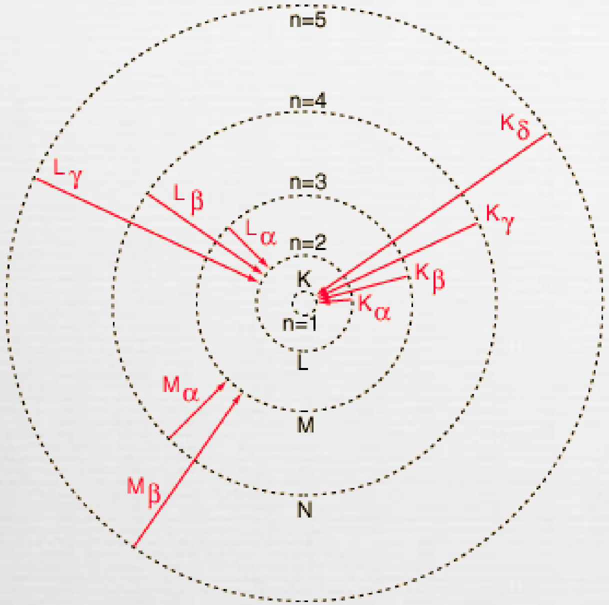
➔ emission of photon in the process.

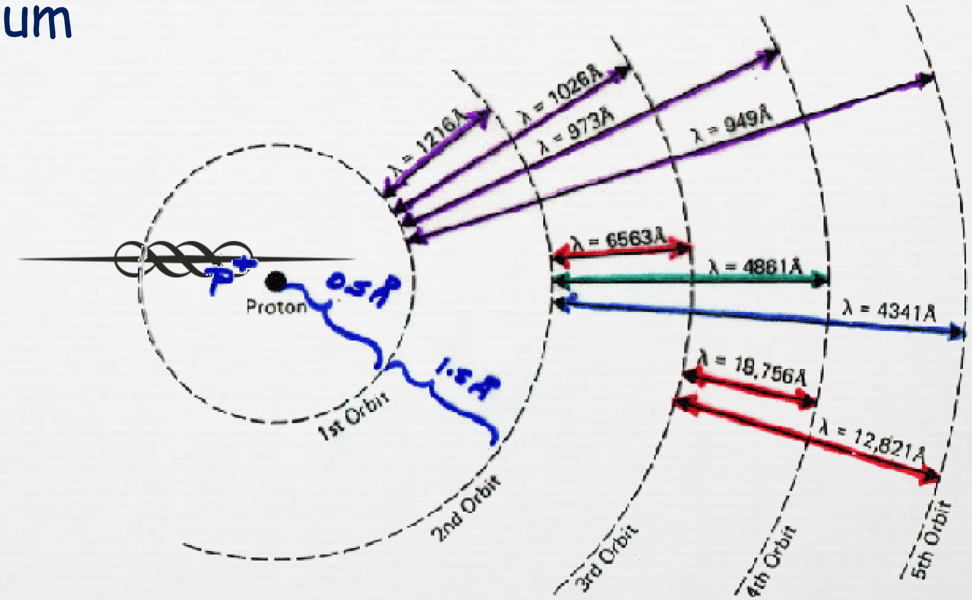
Emitted photon energy given by $hf = \Delta E_{mn} = E_n - E_m$

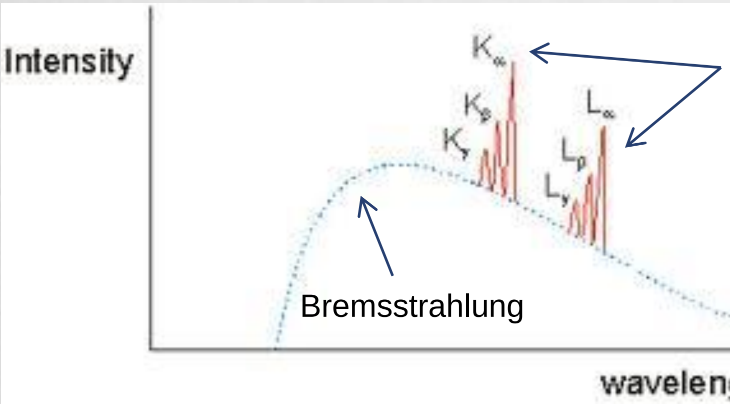
➔ Characteristic of each atom



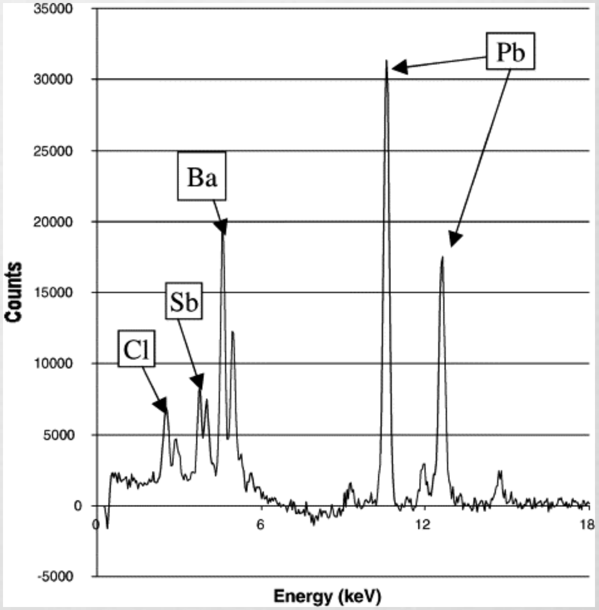
# Characteristic spectrum



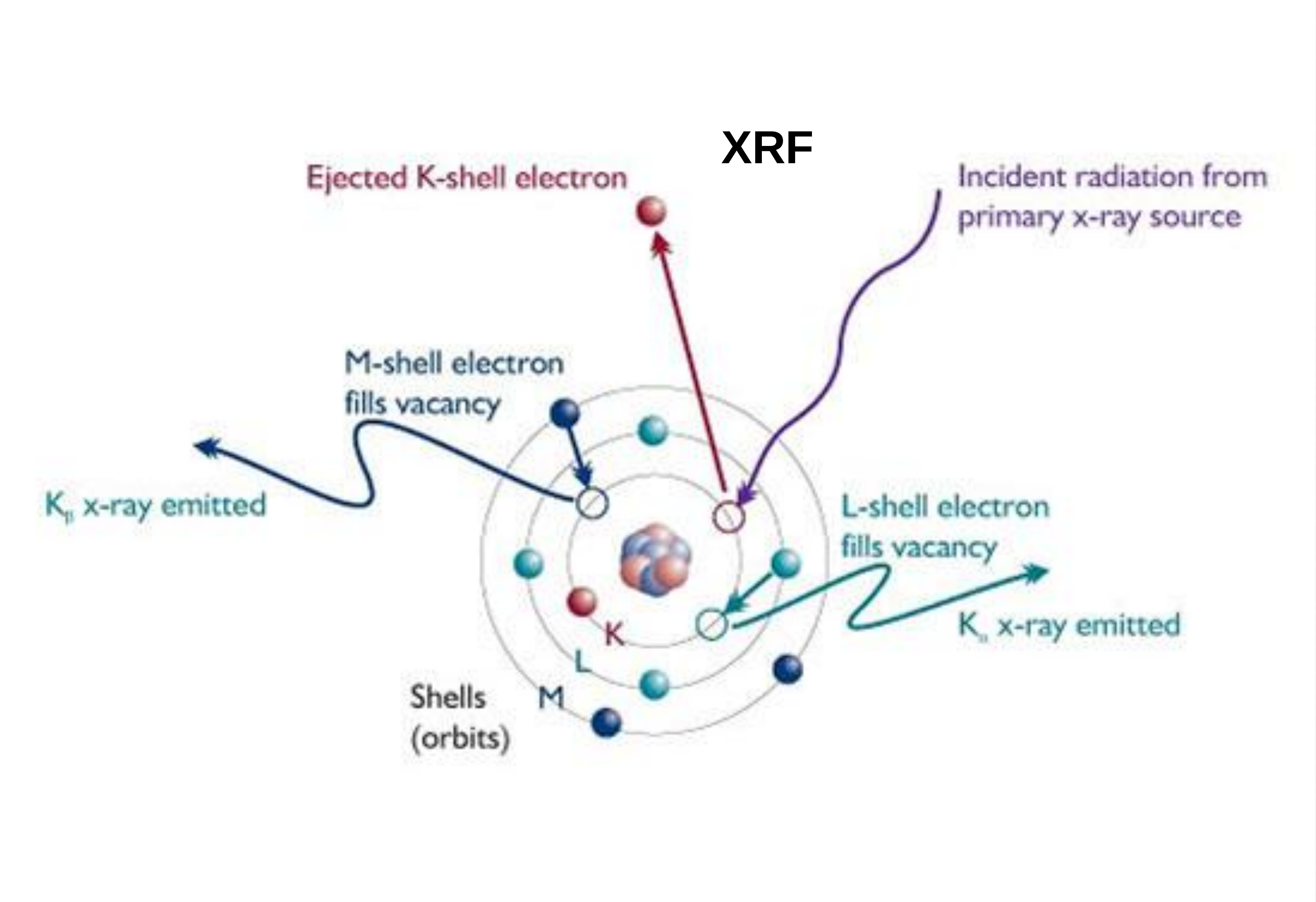




Characteristic spectral lines (peaks) come with different probabilities given by quantum mechanics (some will be more intense than others).

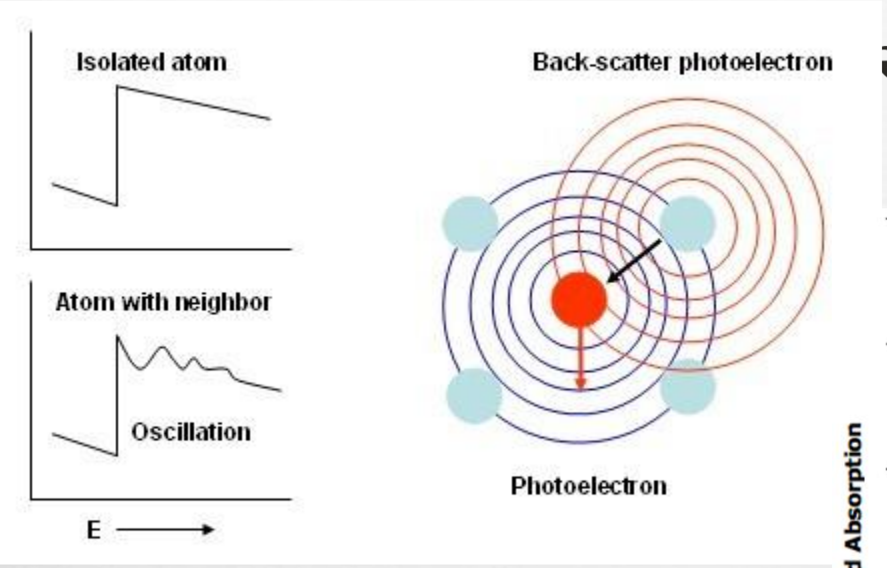


Several techniques available such:

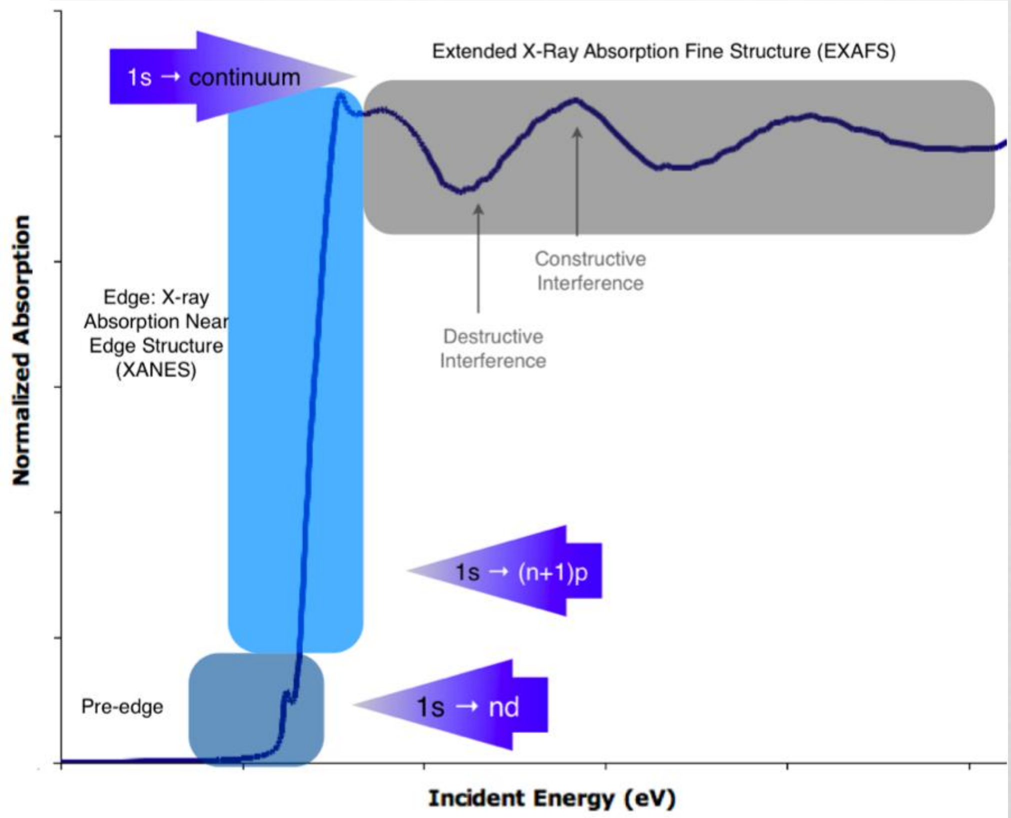
- X-ray Fluorescence Spectroscopy (XRF) : for elemental identification



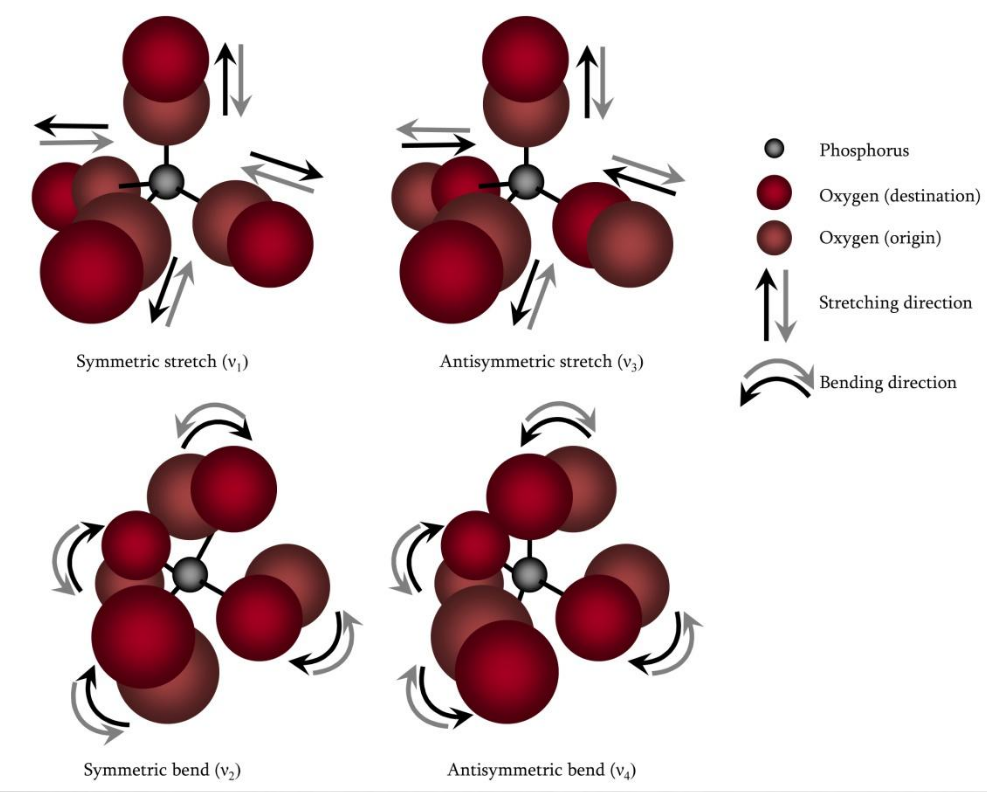
- X-ray Absorption Spectroscopy (XAS): for elemental speciation



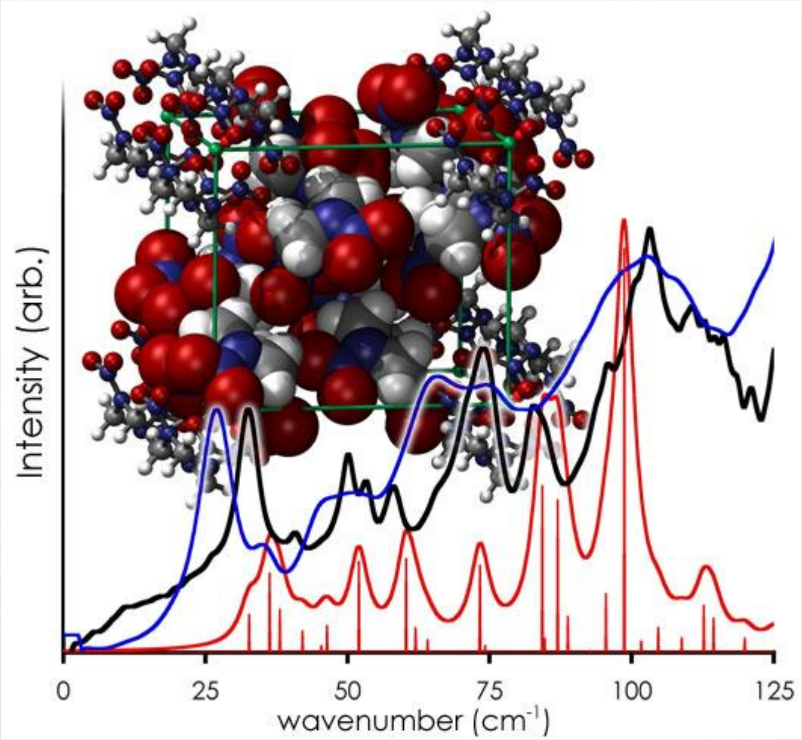

XAS



- Fourier Transform Infra-red Spectroscopy (FTIR) : probe complex molecular structure such as organic matter

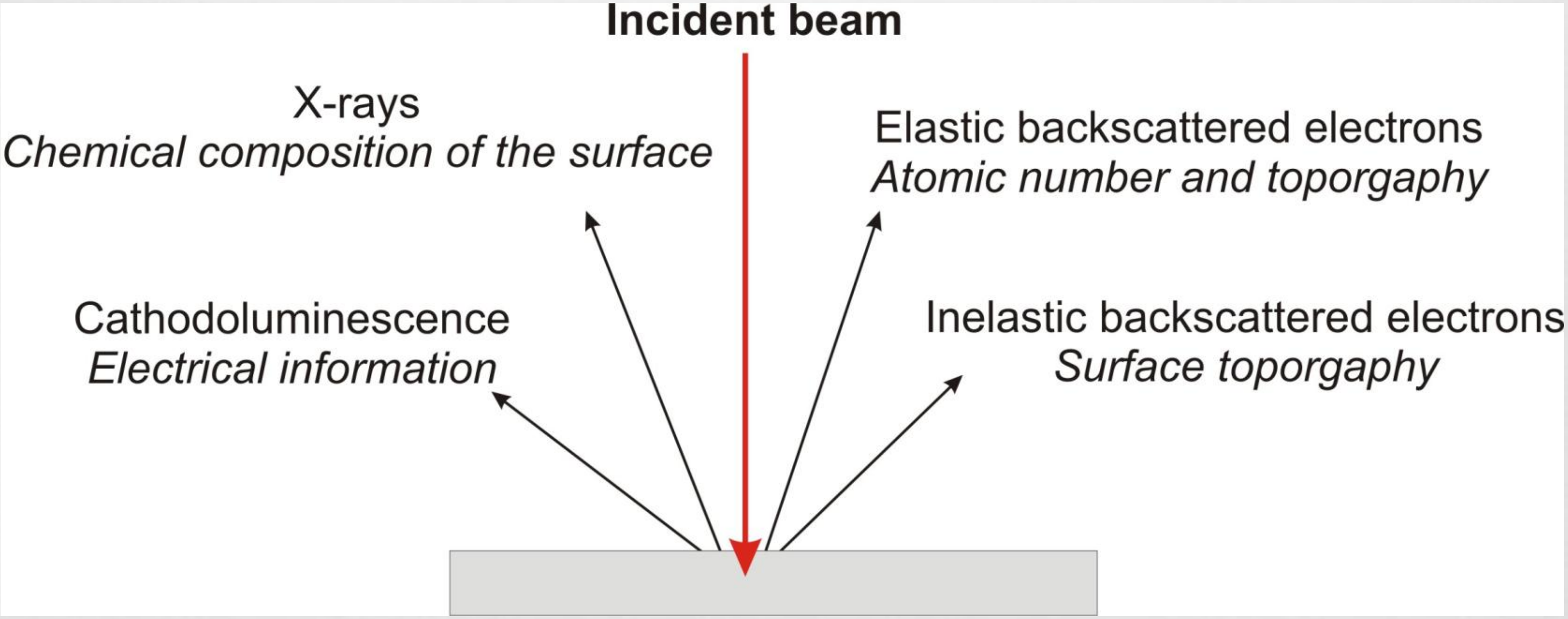



Like electrons in atoms, each molecule has a characteristic spectral line representing a given vibration mode



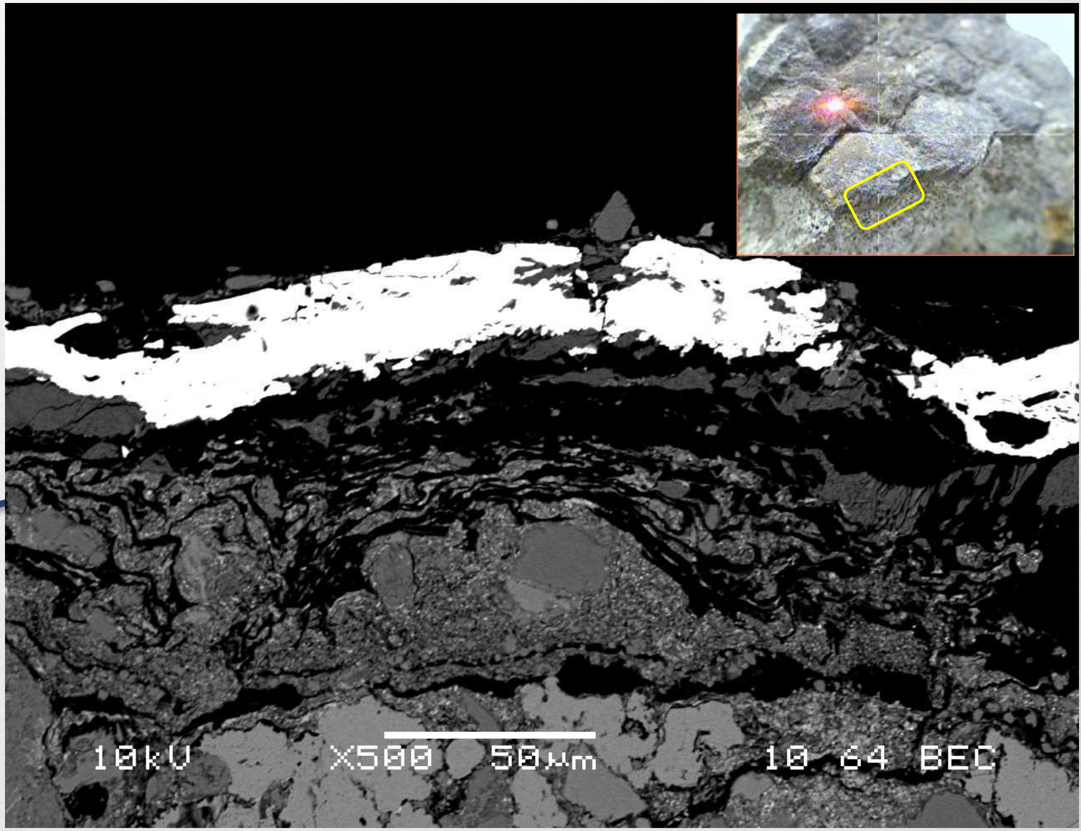
# Scanning Electron Microscopy (SEM)

Electron beams are accelerated at very high voltages and aim at a sample



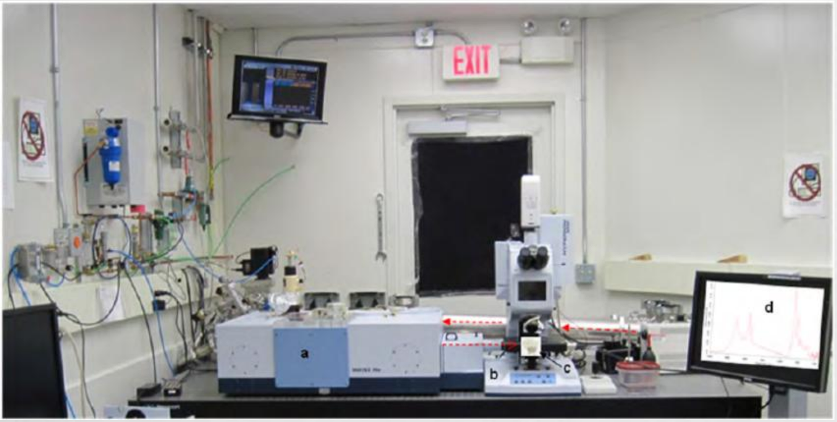
# Identifying mineral contents (Scanning Electron Microscope – SEM analysis – 20 *μm* section)

- Sample was coated with a thin layer of Carbon
- Backscattered electrons
  - <u>Brighter</u> areas represent <u>higher Z</u> material.
  - <u>Lower Z</u> structures are expected to show as <u>darker</u> areas
- The white top layer is the top of a skin scale
- Thin darker areas under this white layer and above another sedimentary region can be observed



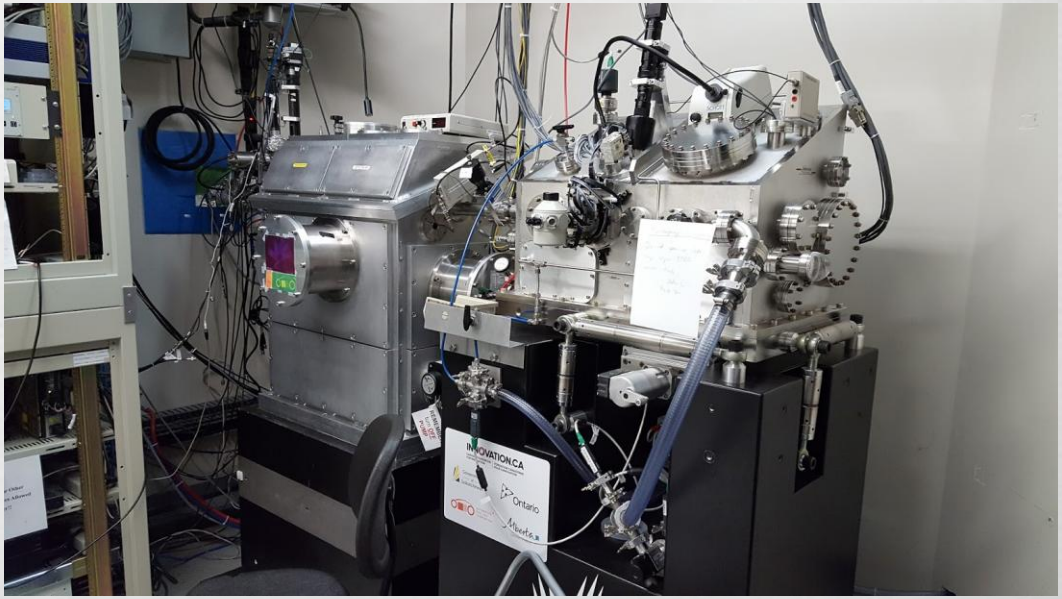
# Complex Molecules And Chemical States of Fe, Ca, C, etc

- Want to probe possible organic compounds (MidIR beamline, CLS)
- Map the sample using chemical speciation (Spectromicroscopy (SM) beamline, CLS)



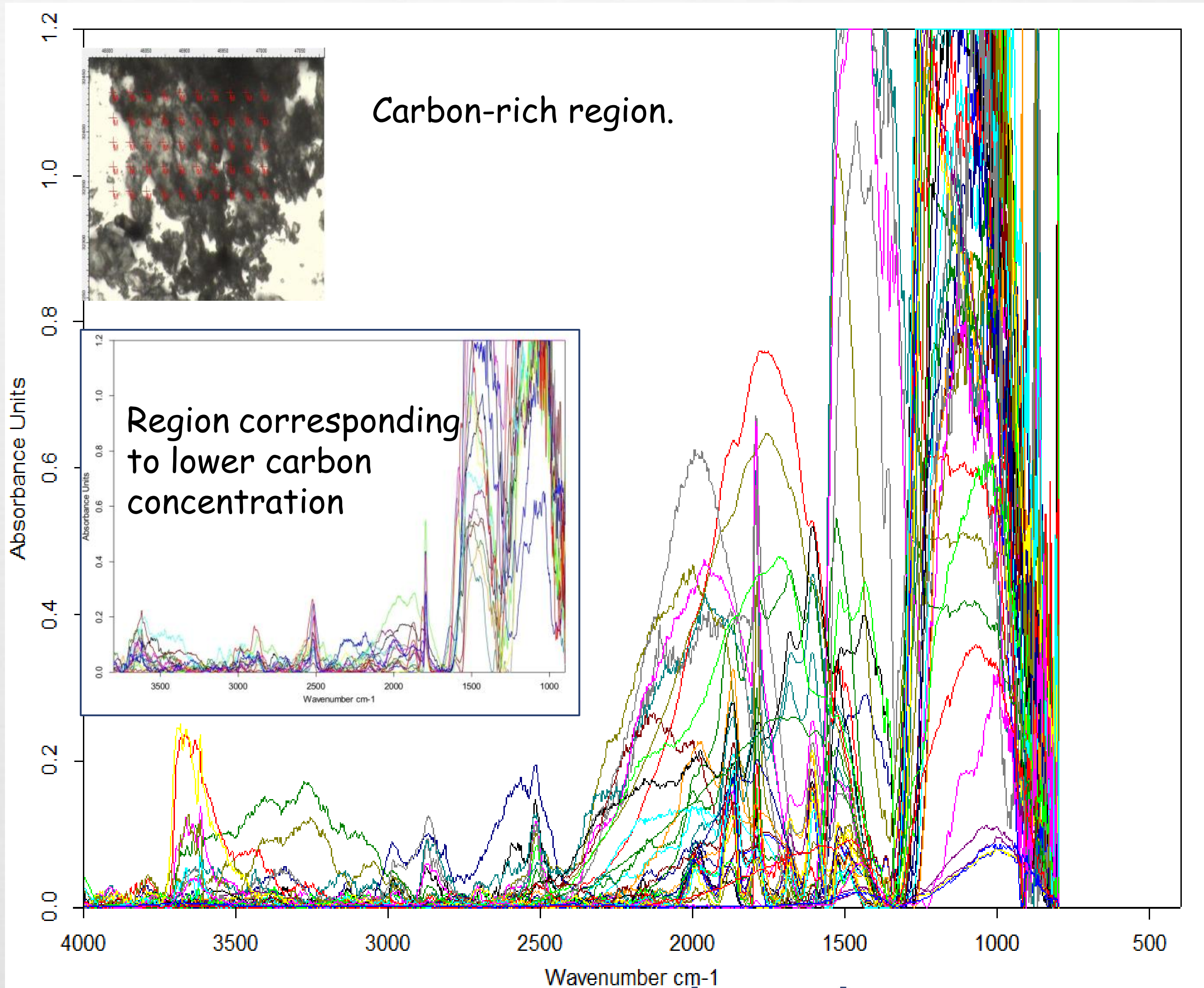

MidIR

SM



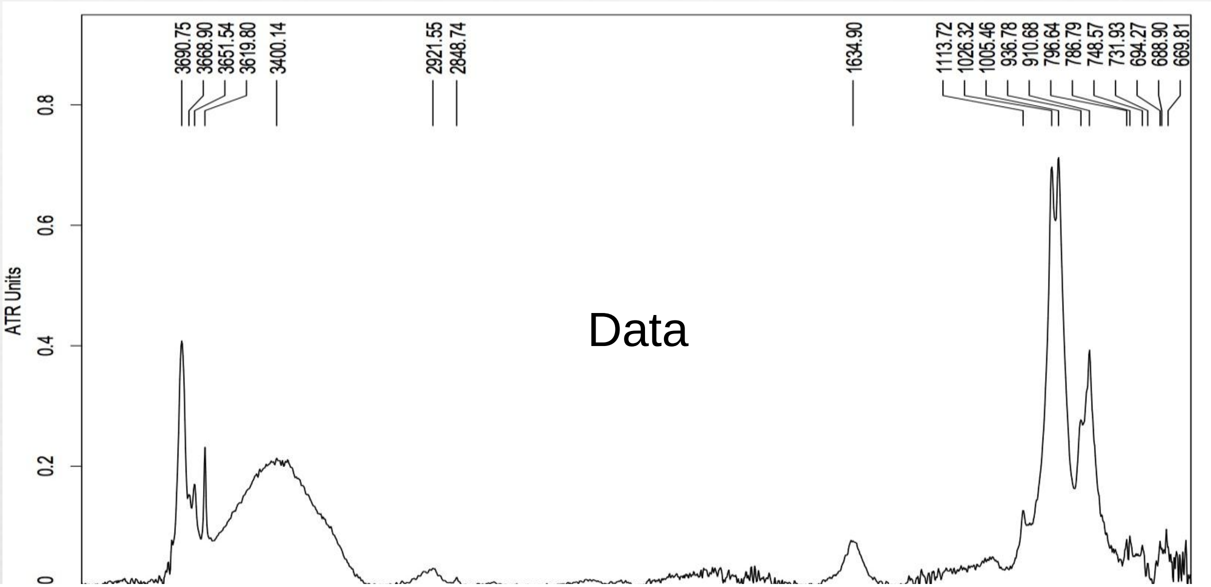
# Fourier Transform Infrared (FTIR) spectrophotometry

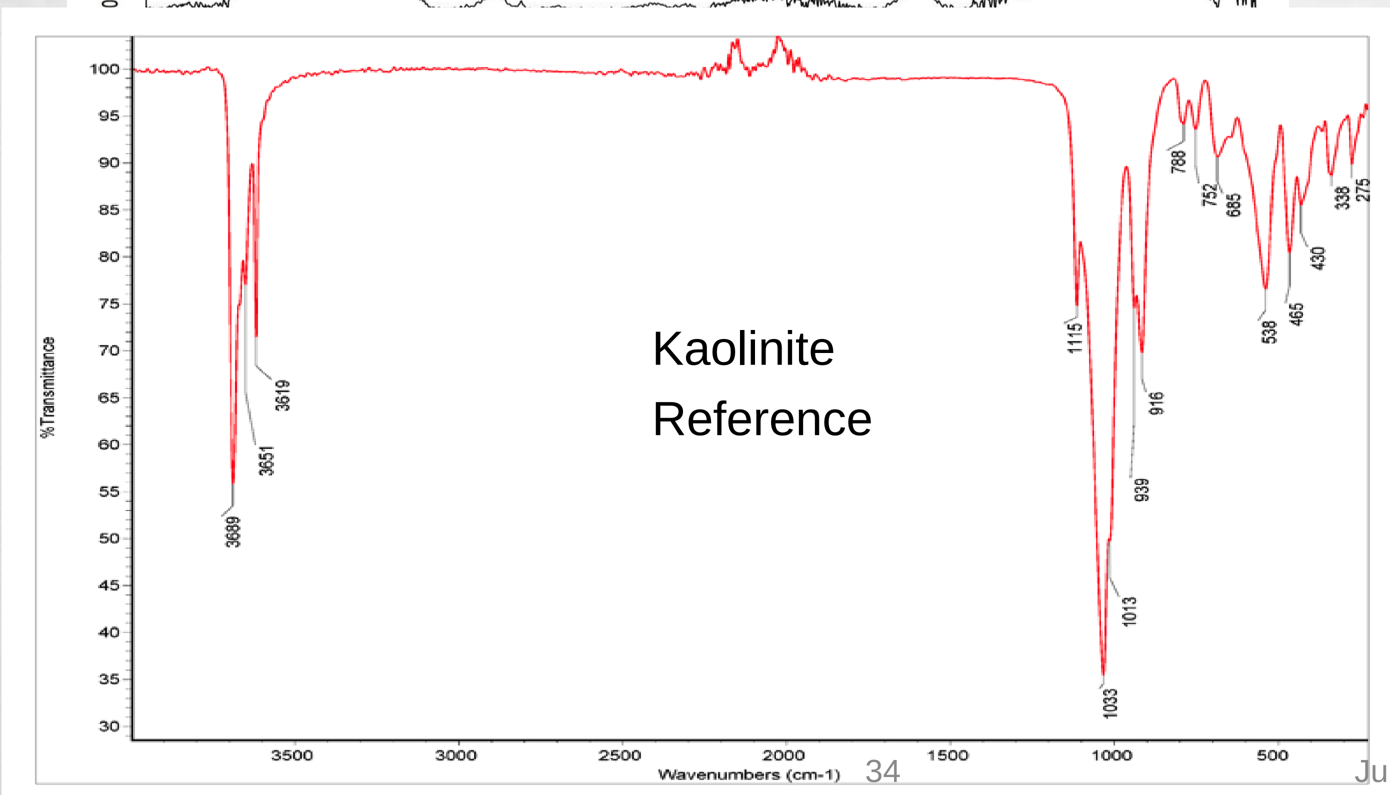
- Region corresponding to carbon-rich area in the sample.
- Each cross represents a position used to collect a FTIR spectrum.



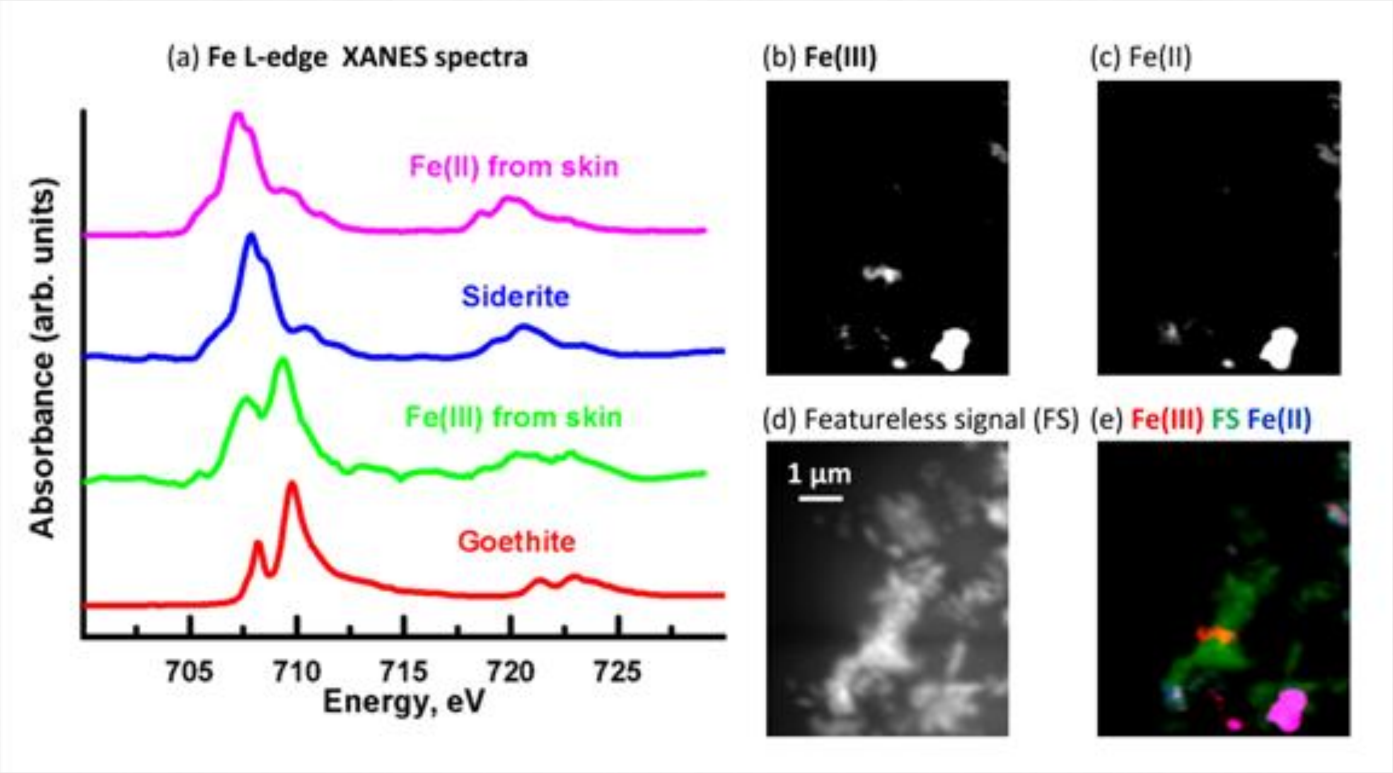

Region of the spectrum corresponding to organic signature

You can see spectra corresponding to minerals



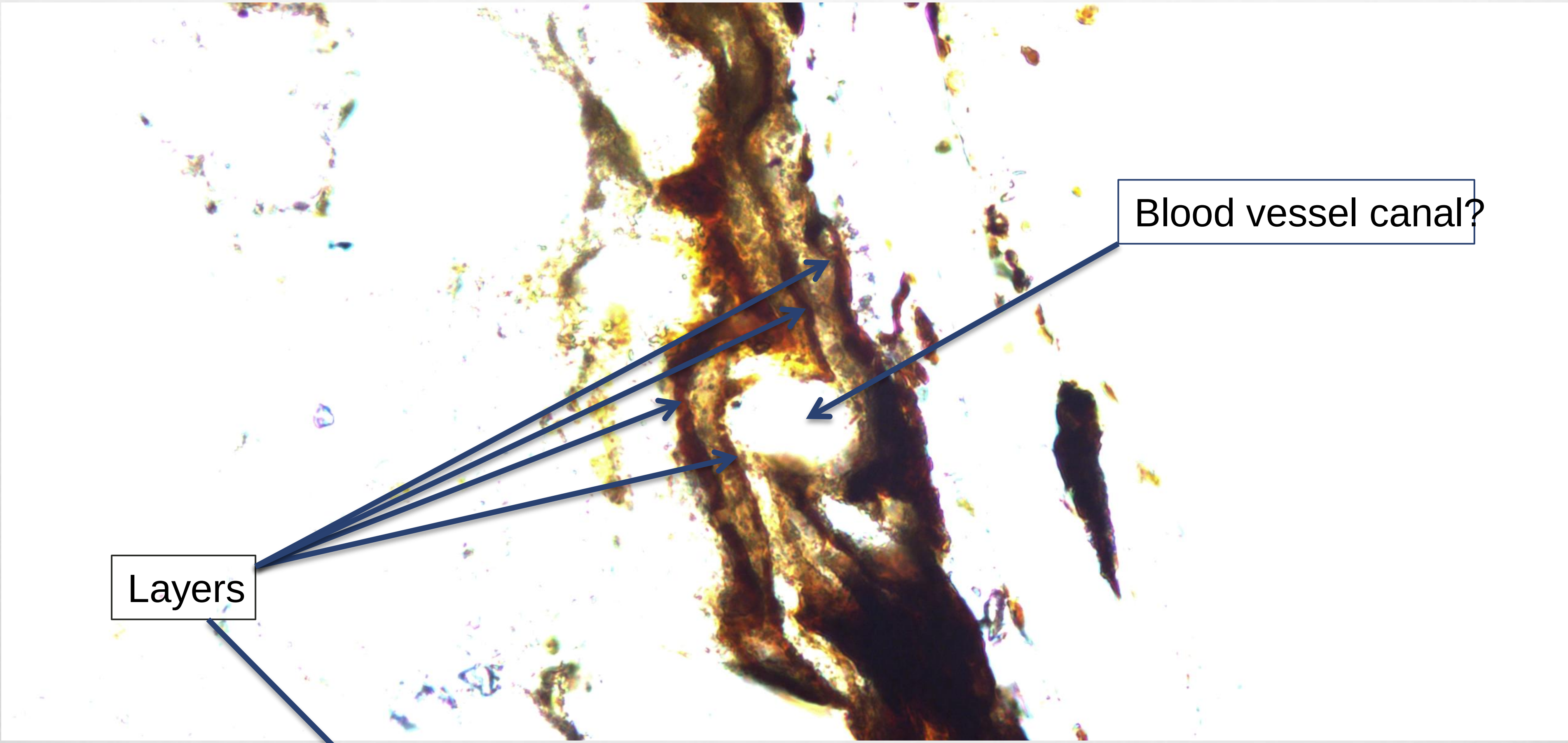



# Fe map K-edge scan



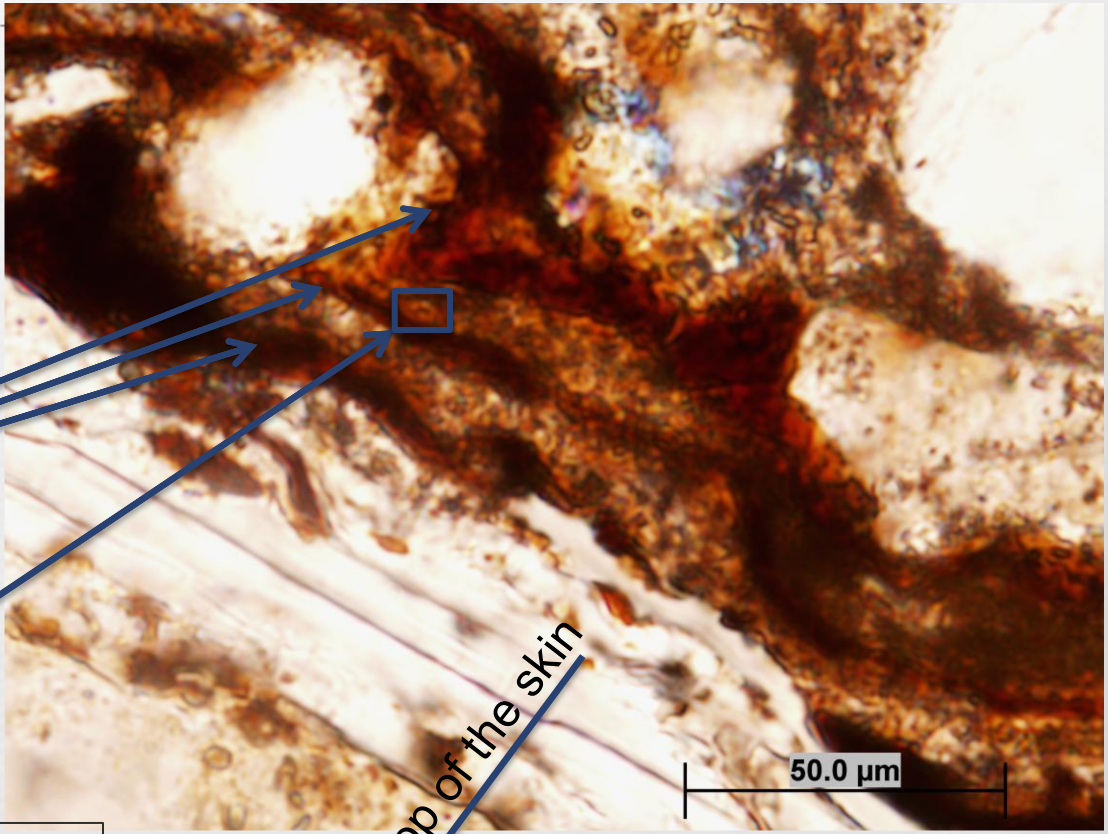

- Fe in the form of siderite (iron carbonate with oxidation state Fe(II)) is found in the skin
- Goethite also present in the form of Fe(III)

# Zooming in




Compatible with observation from CLS

# Zooming further in

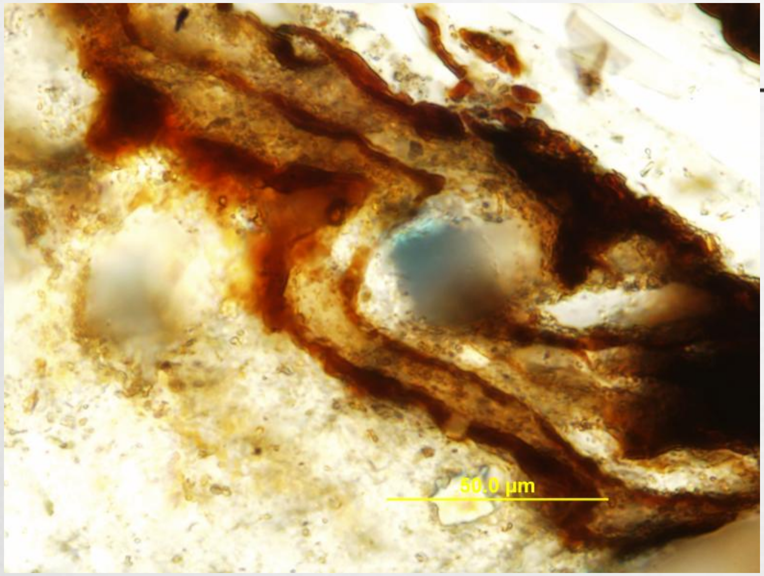


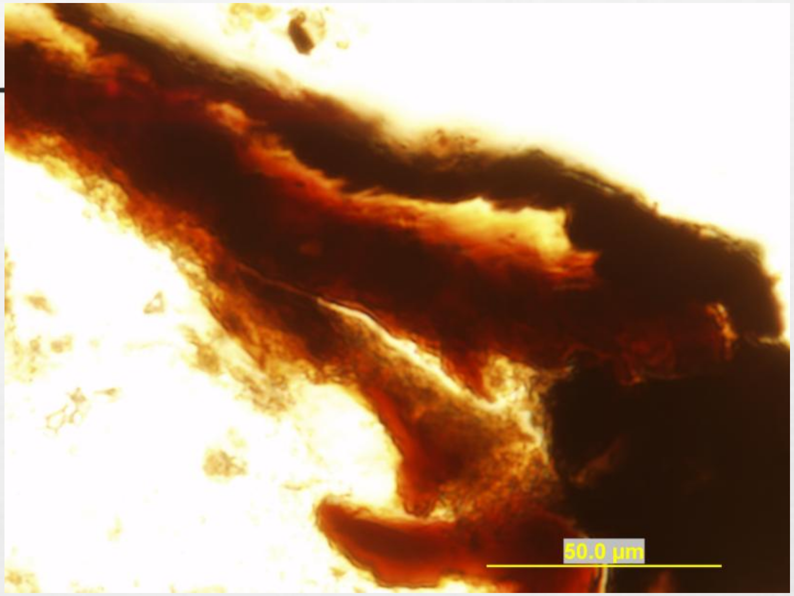

Layers

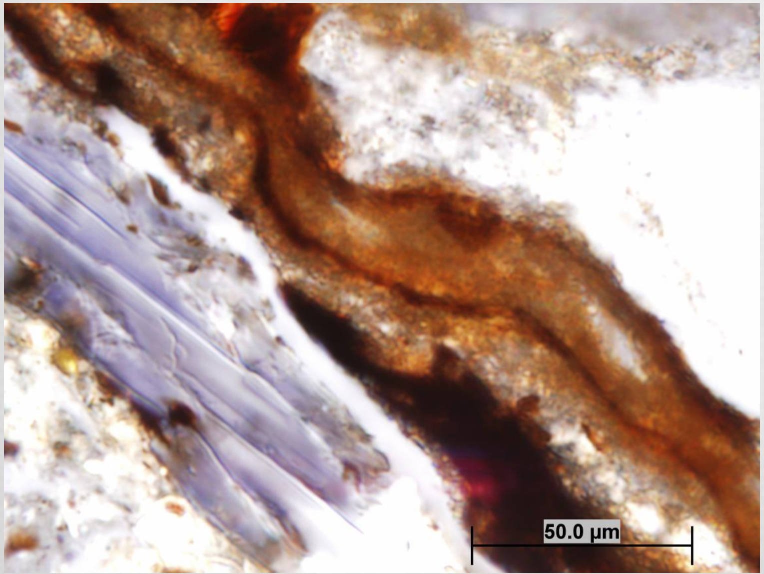
Substructures or optical effects?? → More likely substructures → compatible with observations from CLS

Towards top of the skin

# Few more optical images




- Consistent structure throughout the C-rich layer
- However, some regions seem thicker than others

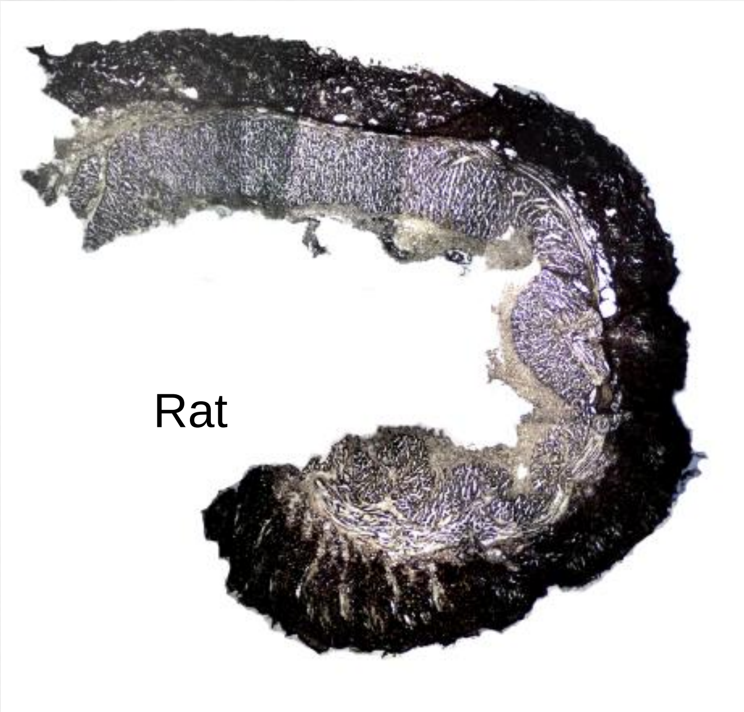
# How does it compare to a … chicken (extant avian dinosaur)

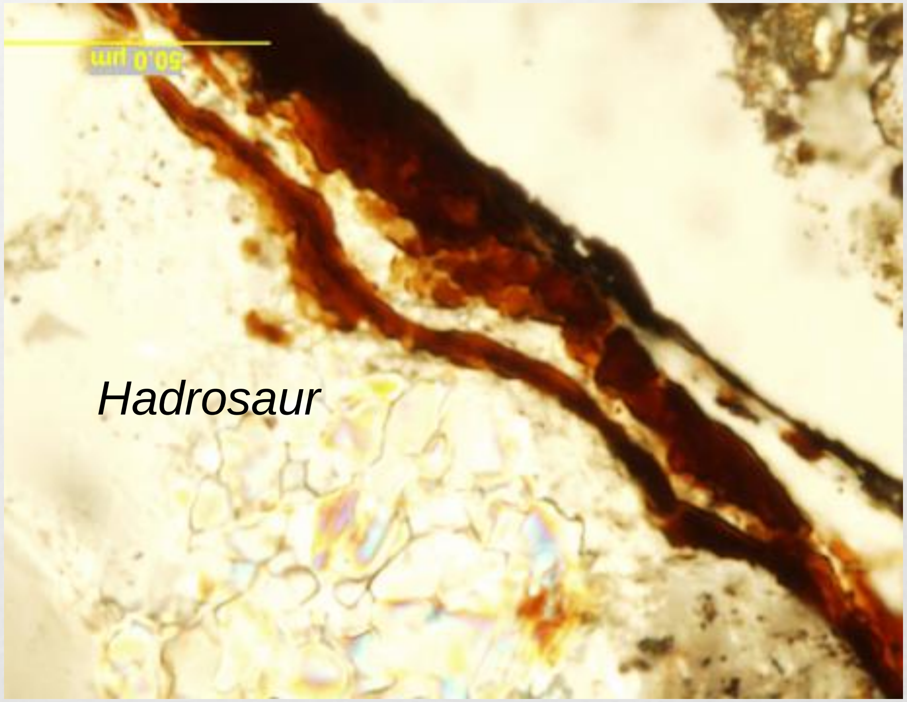
- "Extra Foods" chicken leg (featherless area) → similar scale structures

- The rest of the chicken was eaten by one of my students

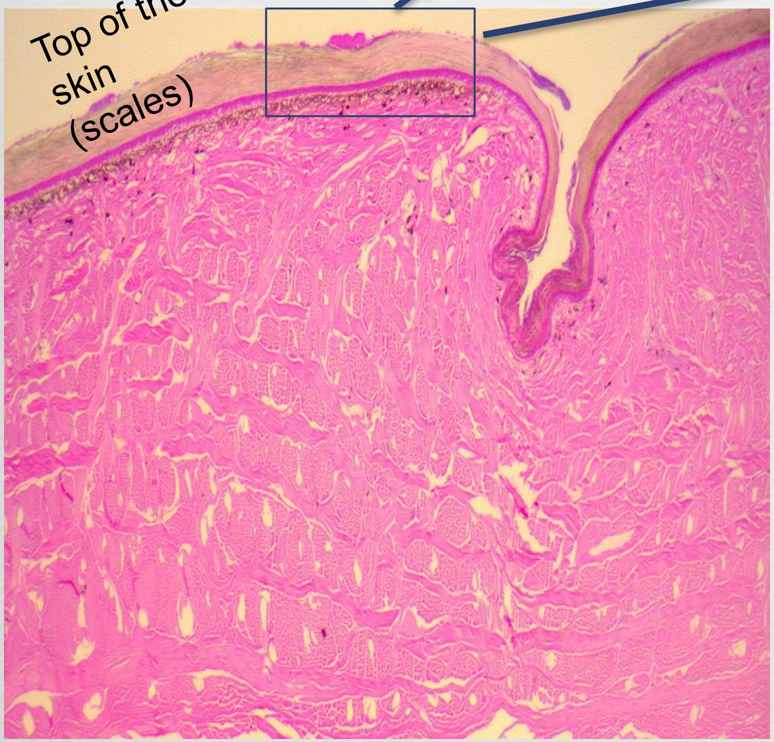

# But, first, how does it compare to a mammal

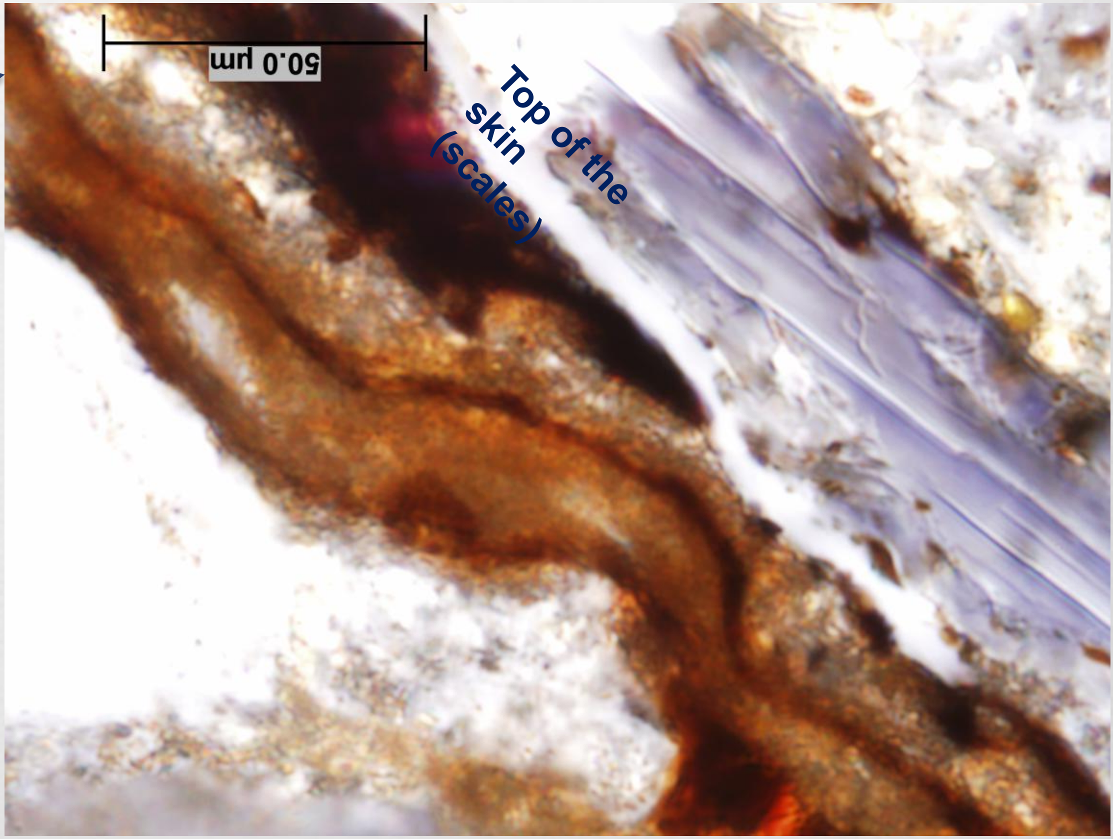
Structures are very different



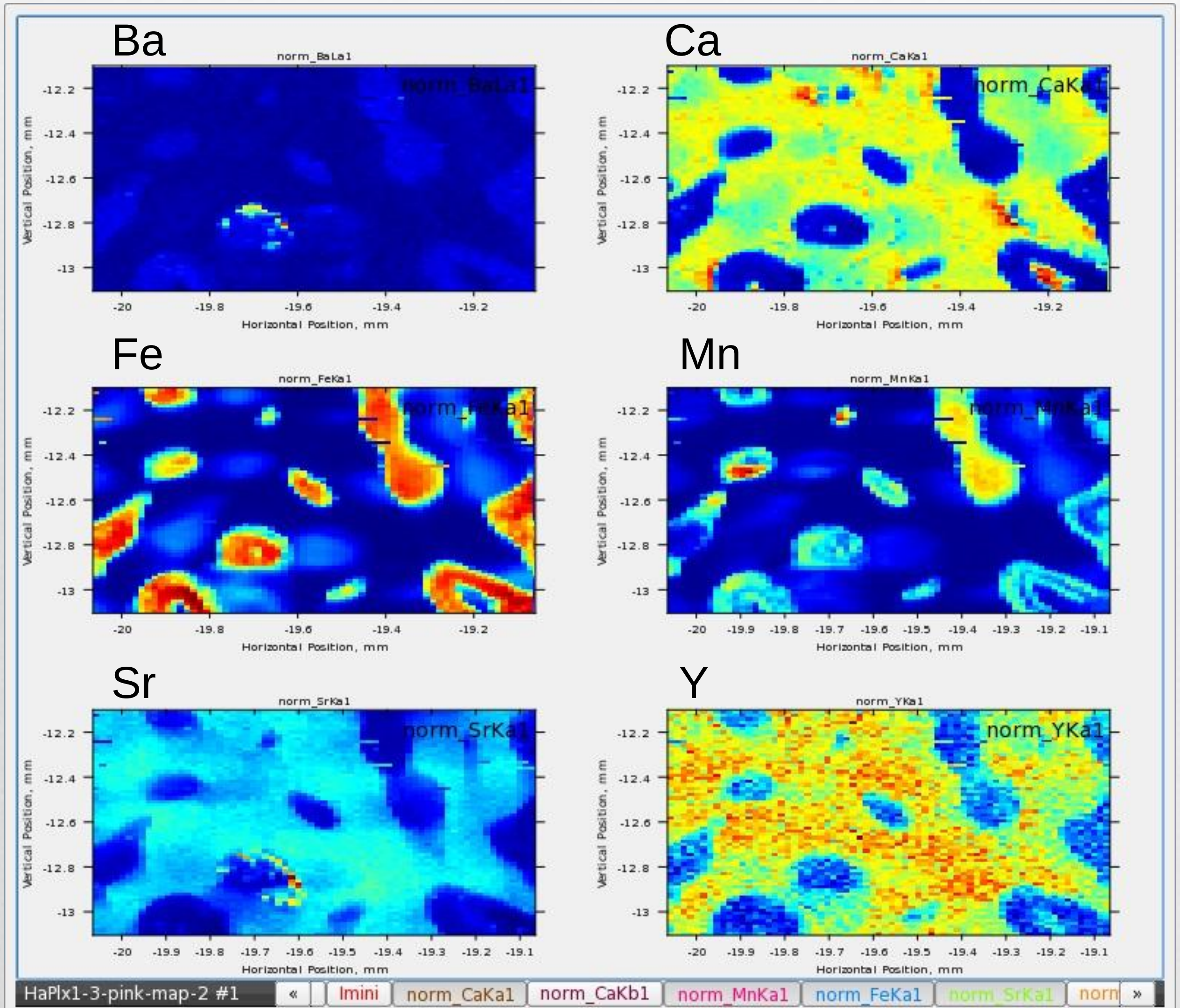
# Comparison to Salt Water Crocodile




There are also some similarities

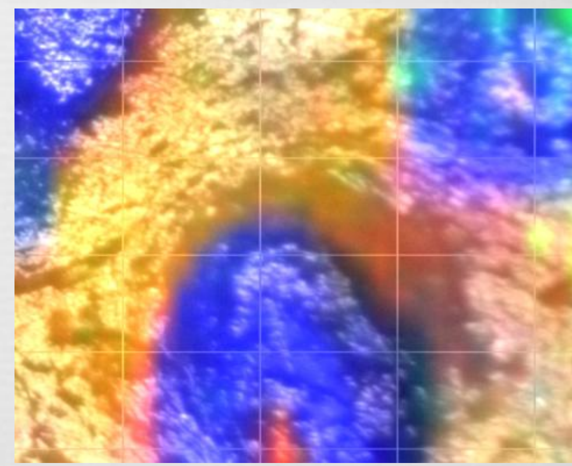
# Map of few key elements – a more comprehensive analysis



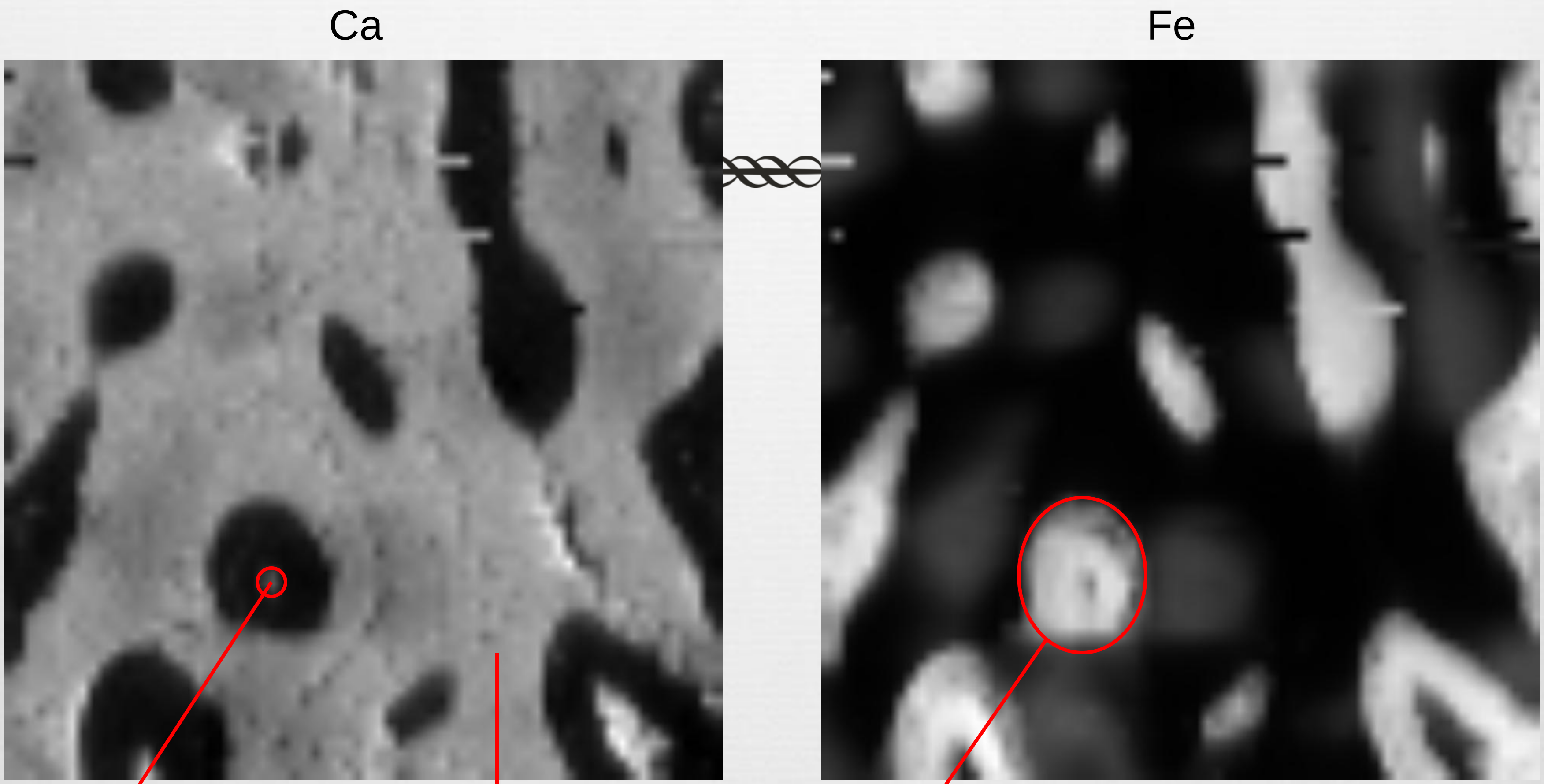

- Mapping in 10 µm steps
- one spectrum per each point in the map

- Superposition of chemical map and microscopic image:

- red = Ca ; green = Sr ; blue = Fe
  yellow = superposition of Sr and Ca ↓



## Imaging using chemical maps

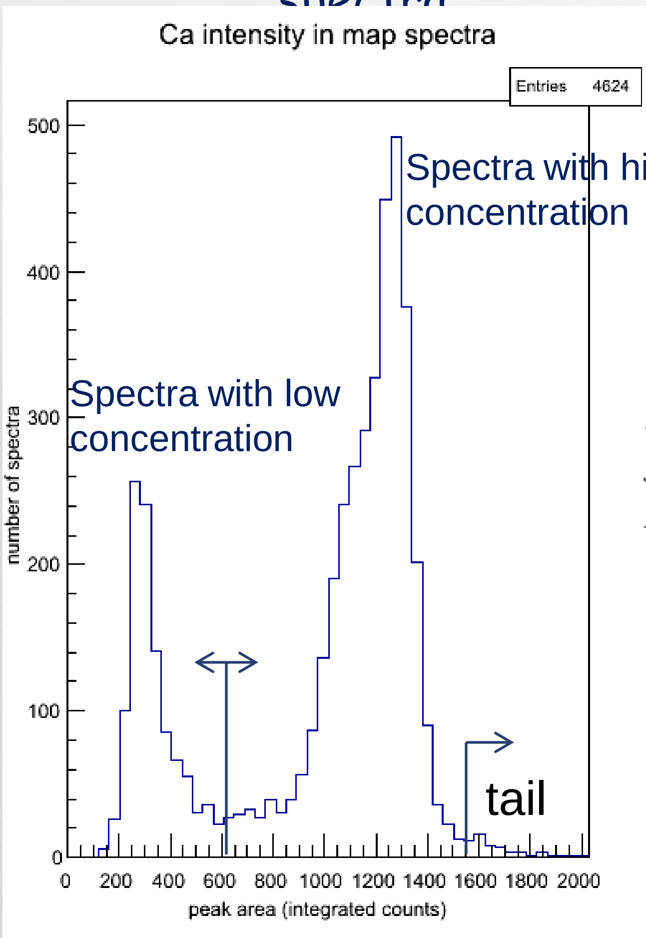


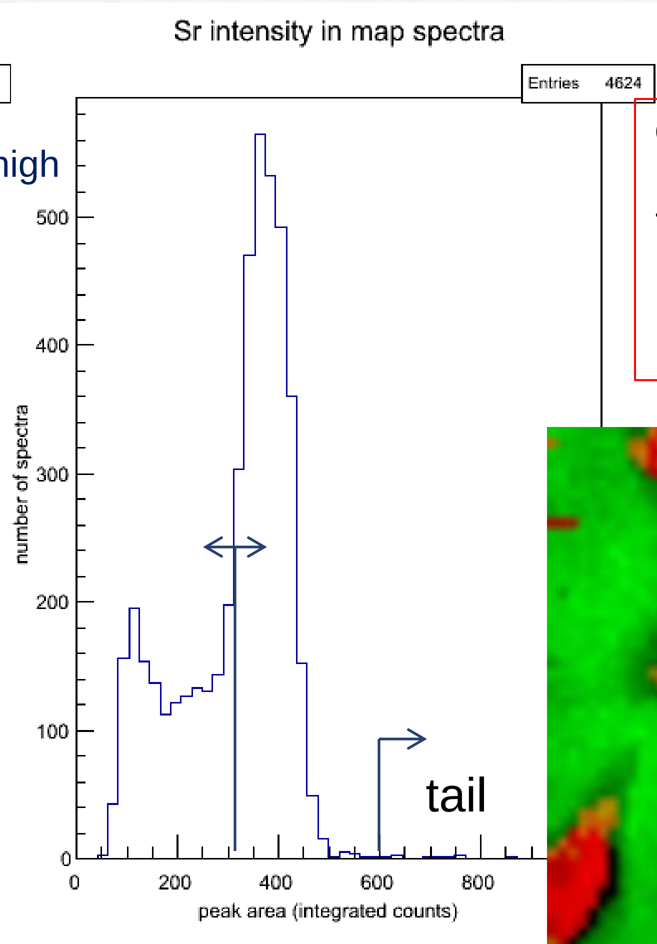

Ca marks bone (apatite?)

Fe marks osteons (what is its state?)

Ca also seems to mark Harvesian canals (calcite?)

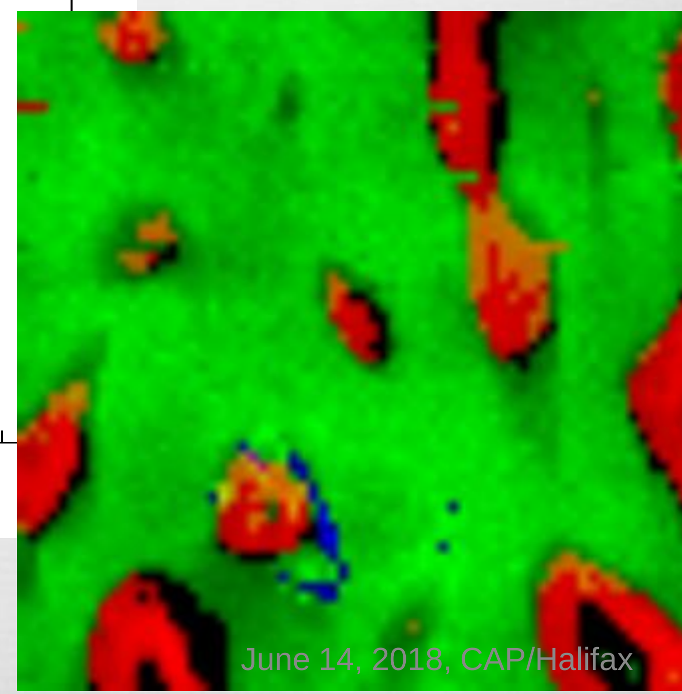
- Analysis of individual concentrations for each element ➔ area under each respective peak per spectrum versus number of spectra:



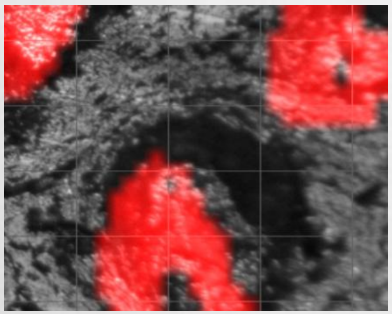

Chemical imaging

- red = High Fe ; green = high Sr ; blue = tail Sr ; orange = superposition of Sr and Fe

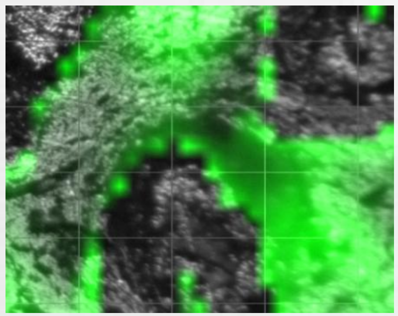


- Repeat chemical map superimposed to microscopic image for different concentration levels (zooming on a small area of the map):

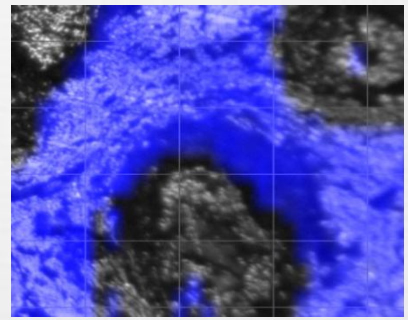
Only spectra with high **Fe** concentration



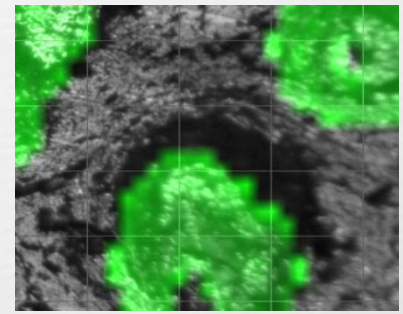
Only spectra with low **Fe** concentration



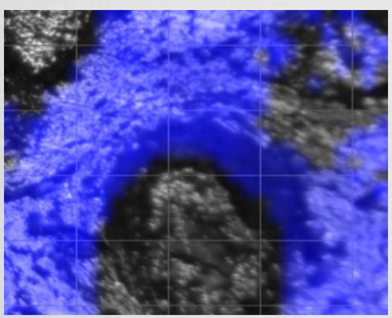
Only spectra with high **Ca** concentration



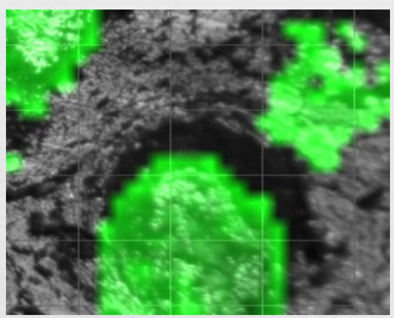
Only spectra with low **Ca** concentration



Only spectra with low **Sr** concentration



Only spectra with low **Sr** concentration

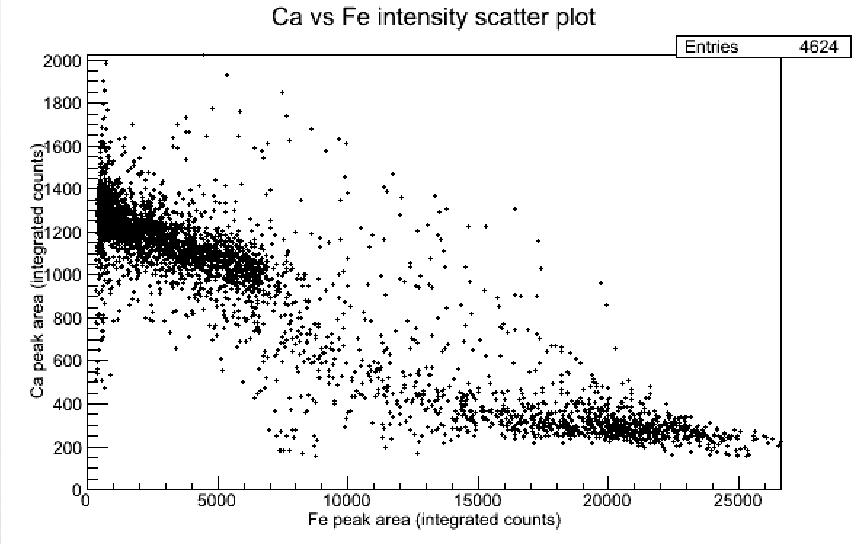


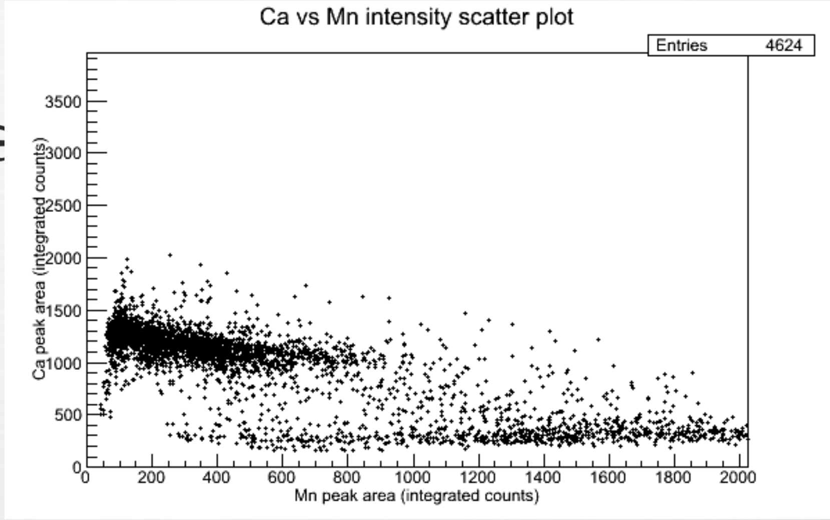
Etc…

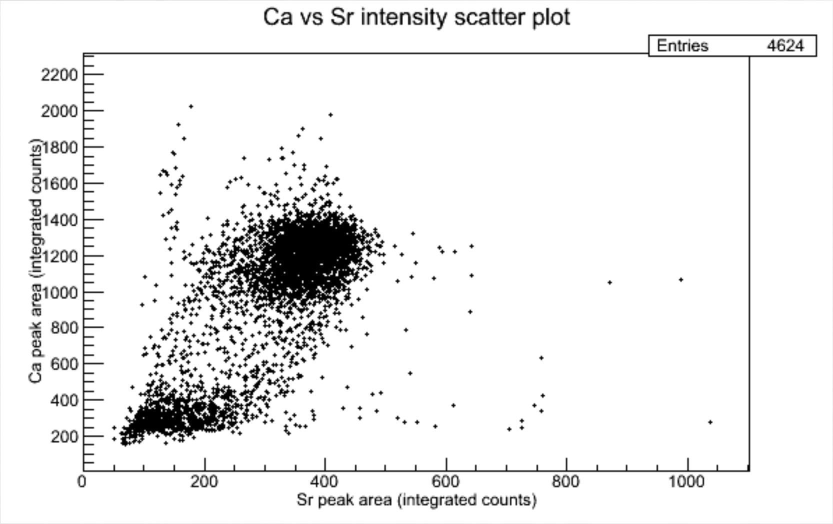
**Ba** follows distributions similar to that of **Ca** and **Sr**.

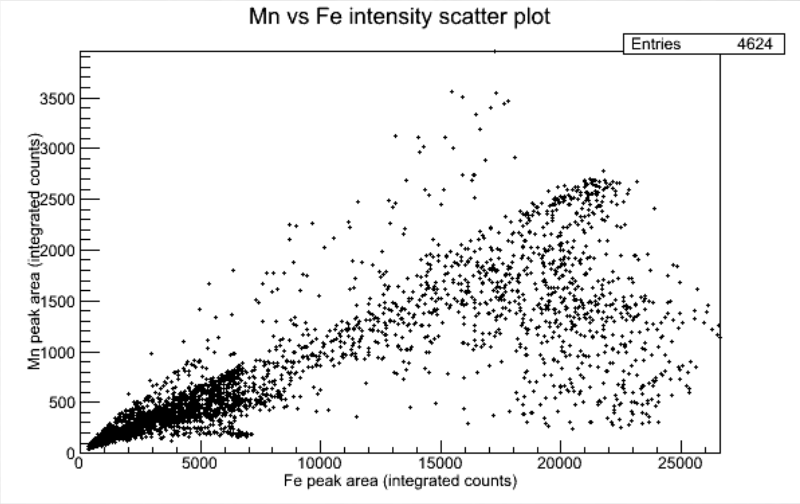
That is expected as **Sr** and **Ba** replaces **Ca** in apatite

# Correlations between concentrations of different elements (scatter plots)



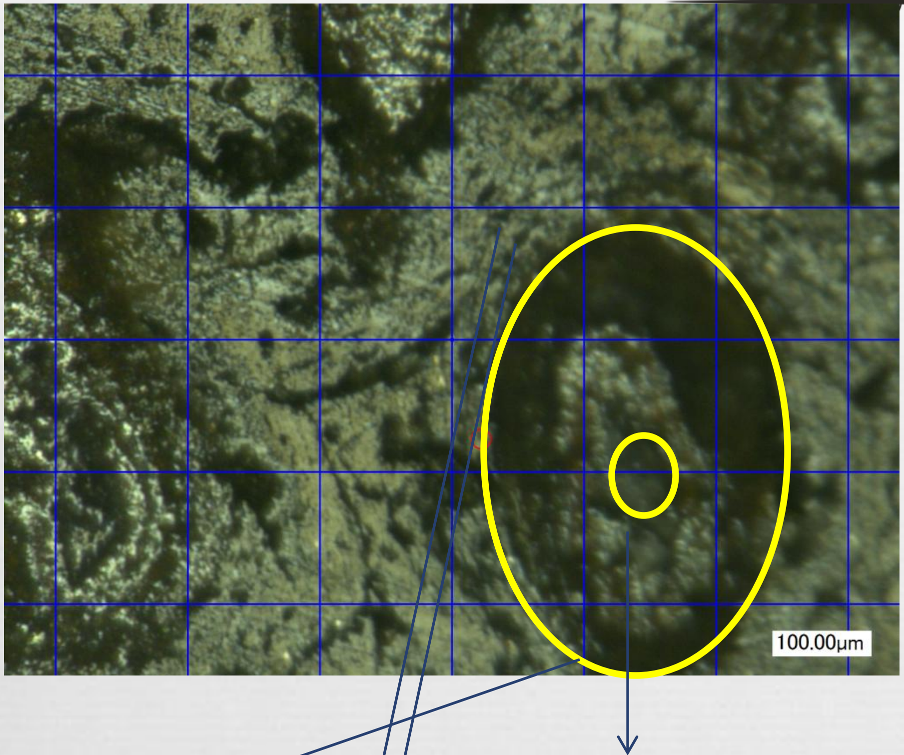



# A Close look at few dinosaur fossils

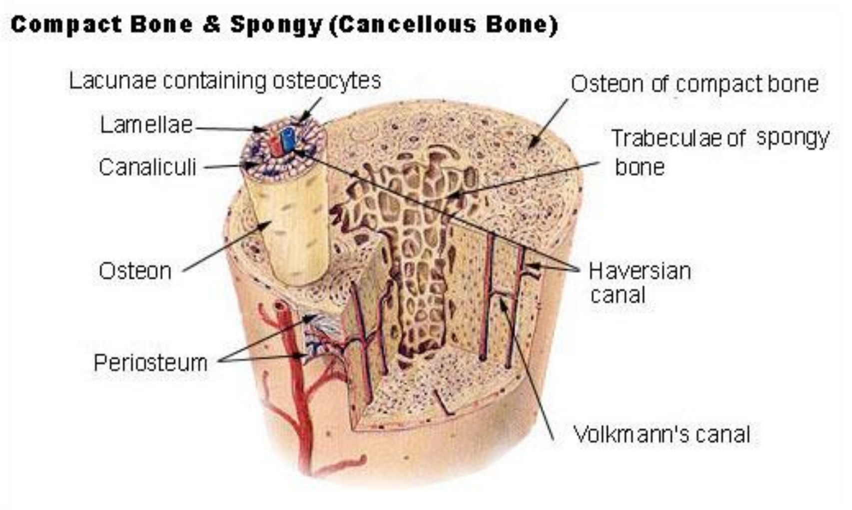
- XRF of a phalanx (toe bone) from a ~70 million-year old hadrosaur (duck-bill dinosaur), from Dinosaur Provincial Park, using the VESPERS (hard X-ray) beamline at CLS.




osteon

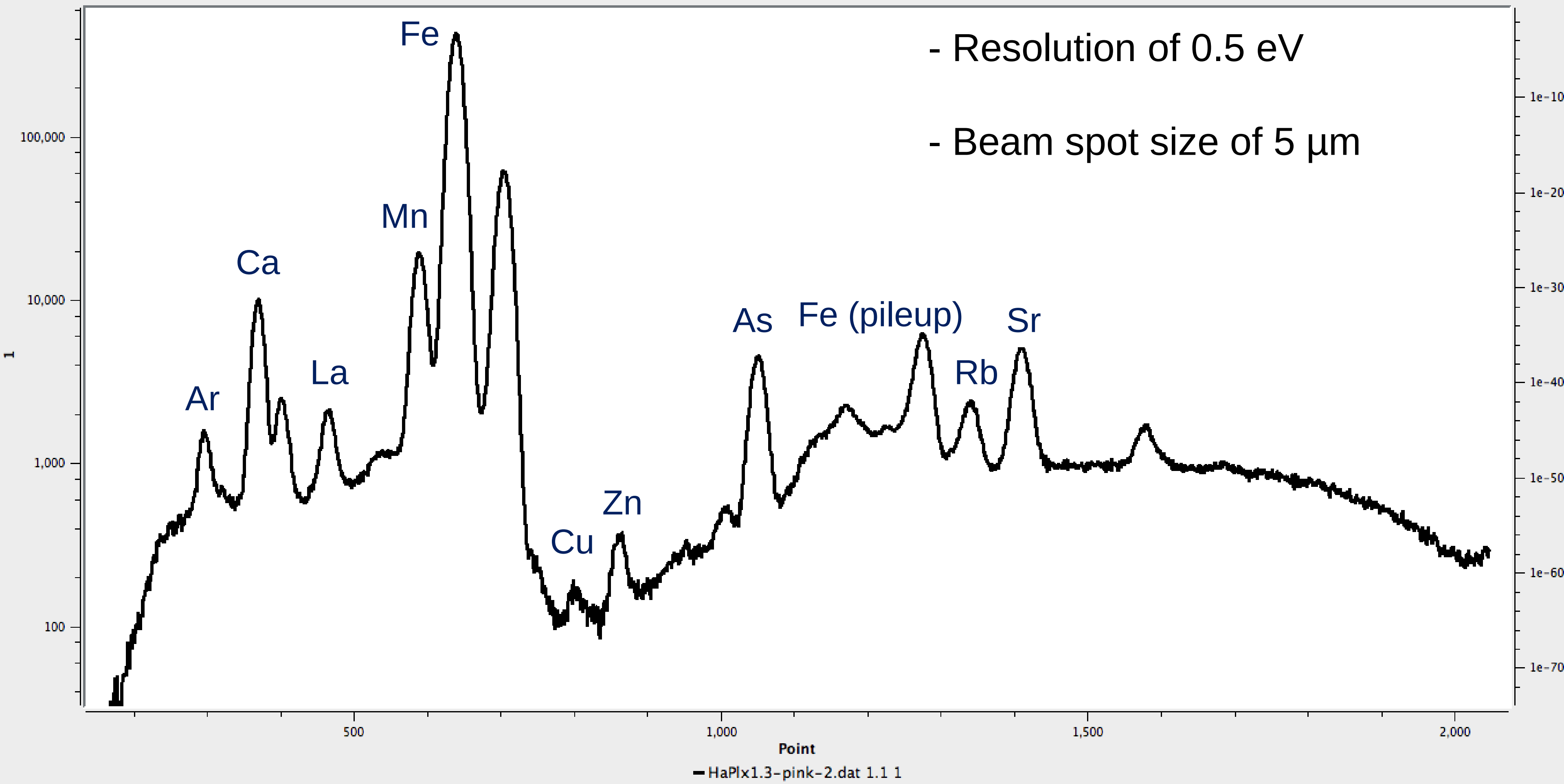
lamellae

Harvesian canal

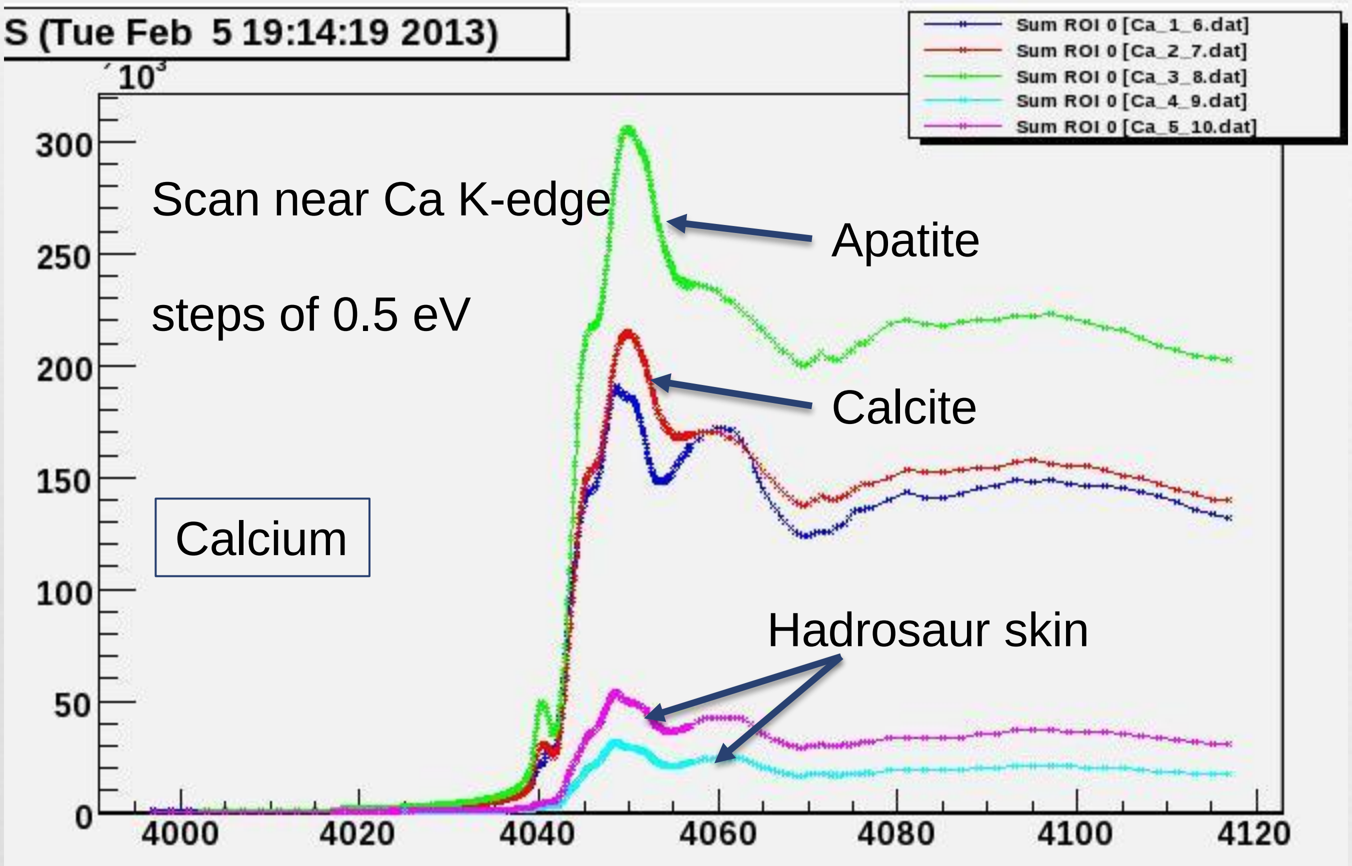


# A Close look at few dinosaur fossils

- Identification of elements (single point spectrum – not map)

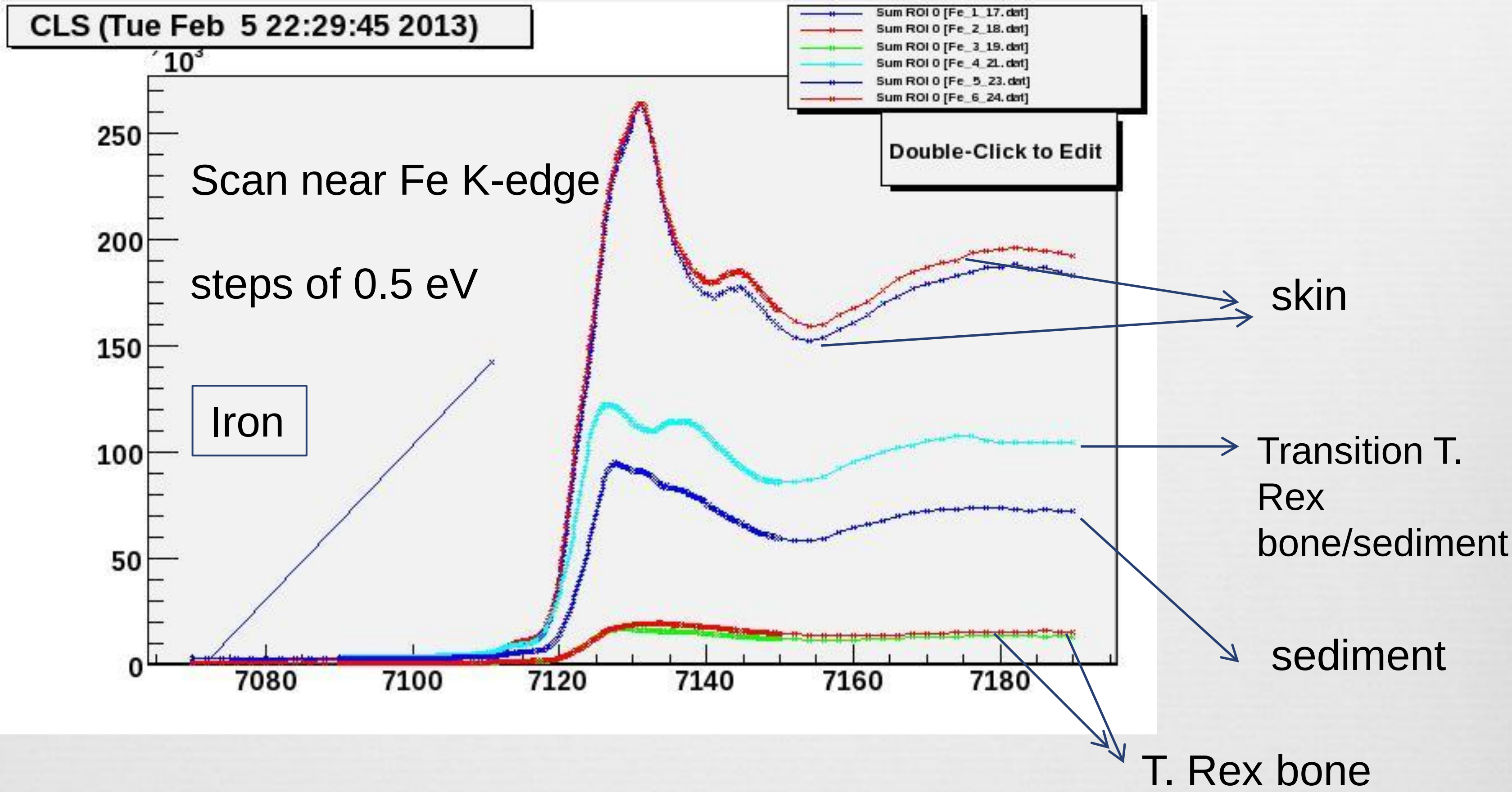


# XAS spectra : Use a T. rex bone (Scotty) and its matrix for comparison



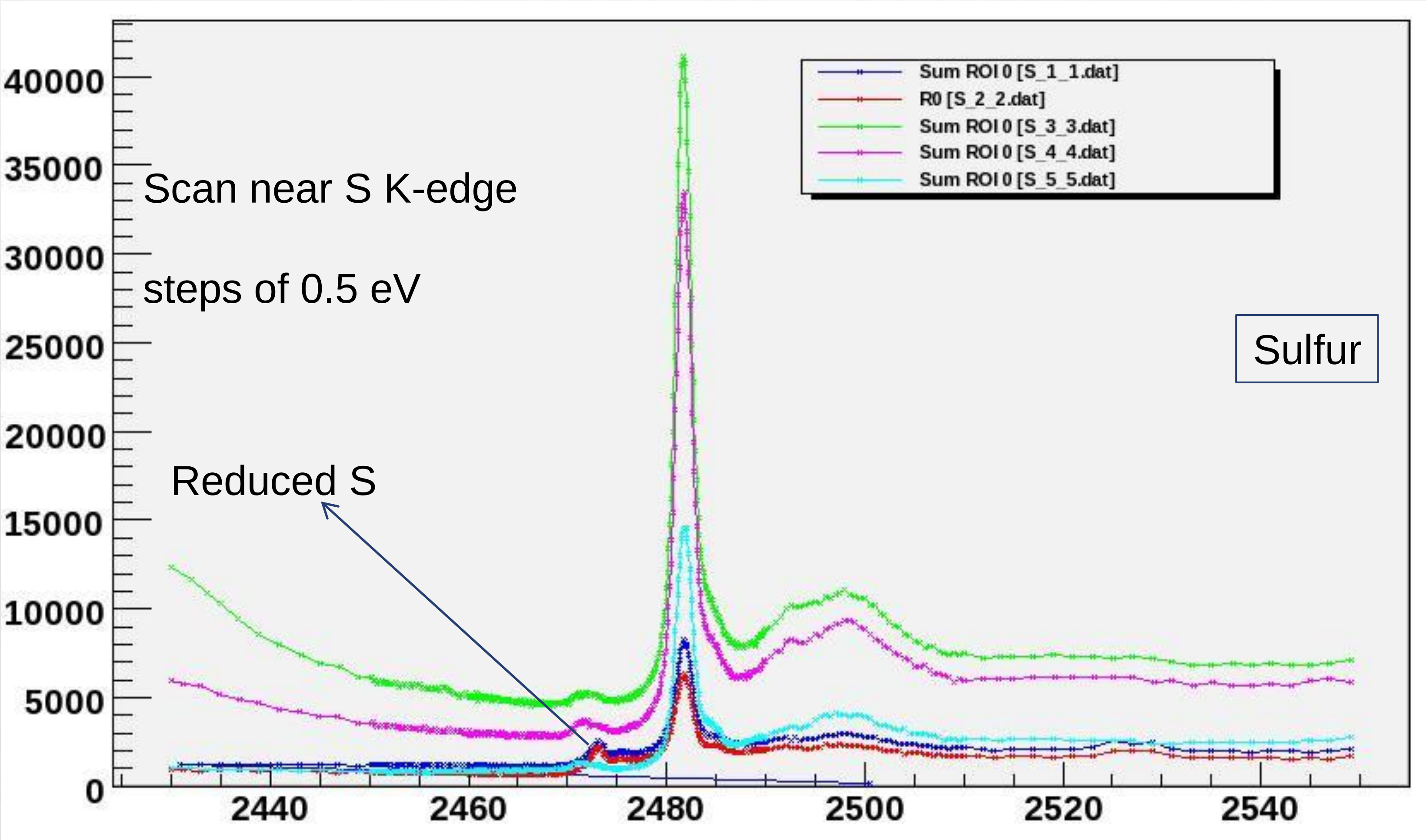

- Green is T. rex bone – compatible with apatite
- Blue is T. rex bone/sediment transition – still compatible wit apatite
- Red is sediment – compatible with calcite
- Magenta and cyan are hadrosaur skin – compatible with calcite

## XAS spectra : Use a T. rex bone and its matrix for comparison



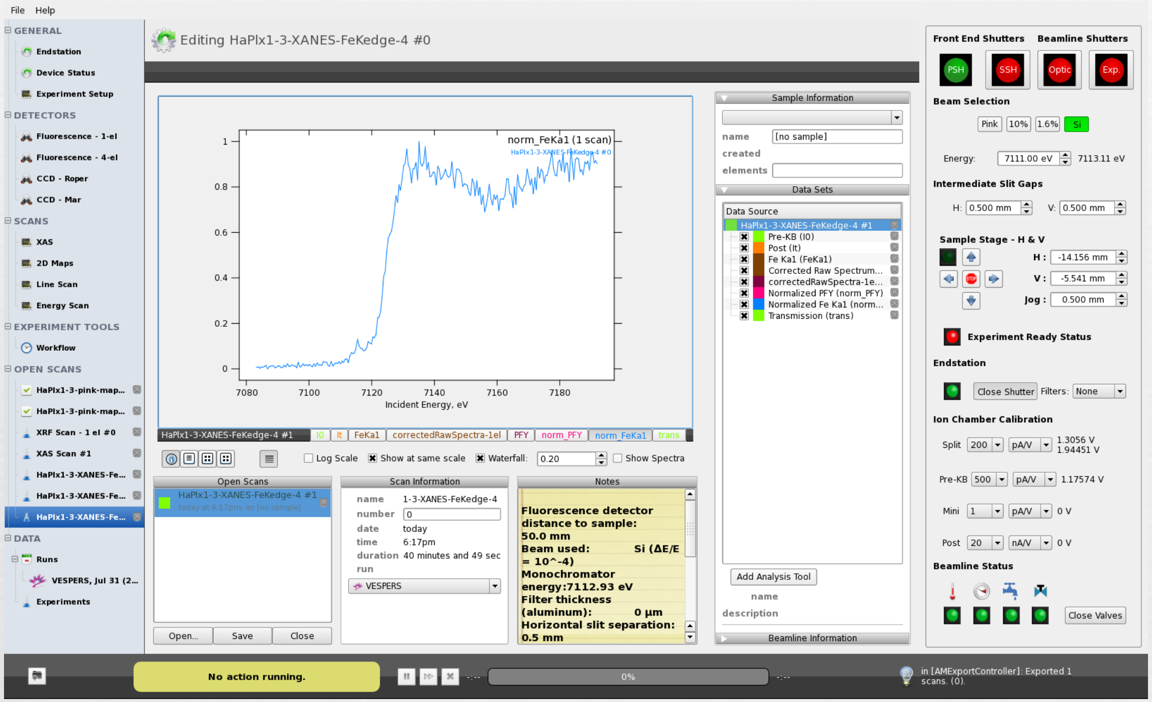

- Clear differences between all regions.
- It's evident that Fe, S have different signatures in the skin.

## XAS spectra : Using a T. rex bone and its matrix as references (SXRMB beamline, CLS)




- Blue and red are hadrosaur skin – presence of reduced S (sulfide; organic nature?)
- Magenta is T. rex bone/sediment transition – sulfate?
- Cyan is sediment – sulfate?

- XAS at K-edge of Fe using the SXRMB (soft X-ray) beamline




- Data yet to be analyzed, but Fe likely coming from hematite (my guessing based on the spectrum above).