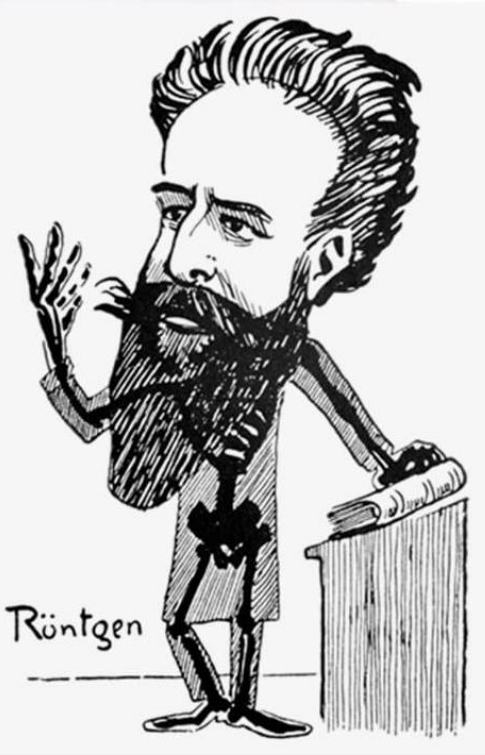
bio

An overview of imaging beamlines and techniques at MAXIV

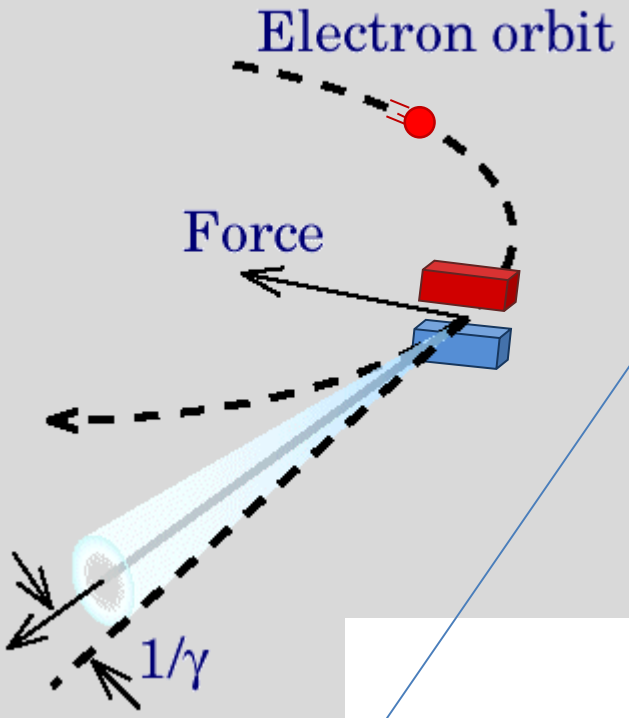
Karina Thånell

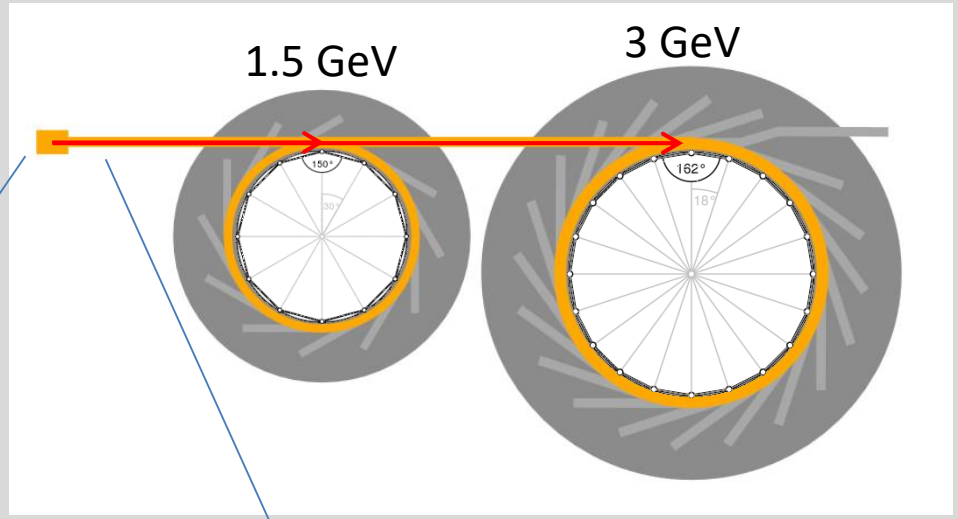
1. Intro MAX IV & beamlines
2. SoftiMAX: STXM
3. NanoMAX: nano-XRF
4. MedMAX: Phase contrast & tomography
5. Radiation: dose and damage
6. Sample preparation considerations

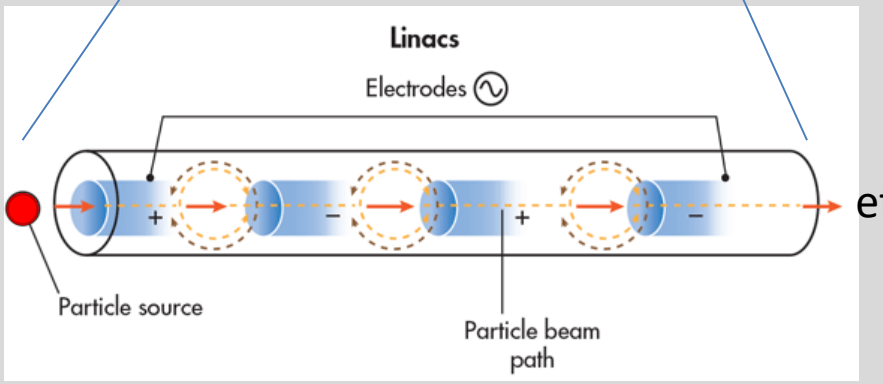
STXM: scanning transmission X-ray microscopy
XRF: X-ray Fluoresence
sSAXS: scanning small angle X-ray scattering
XAS: X-ray absorption spectroscopy



Synchrotron - principle

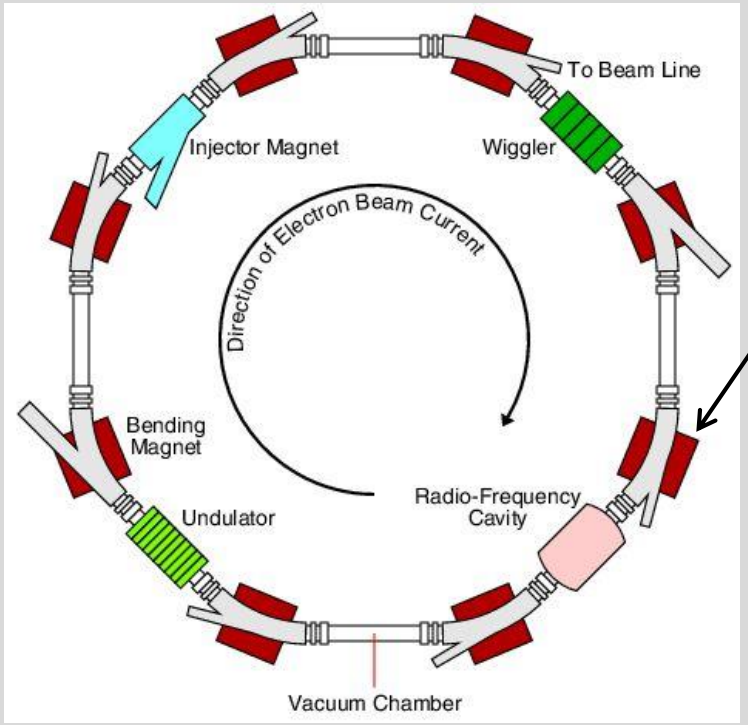




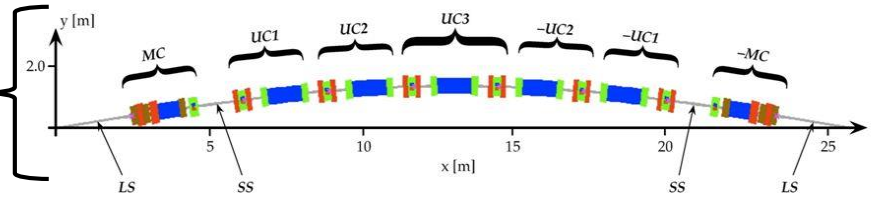


etc.

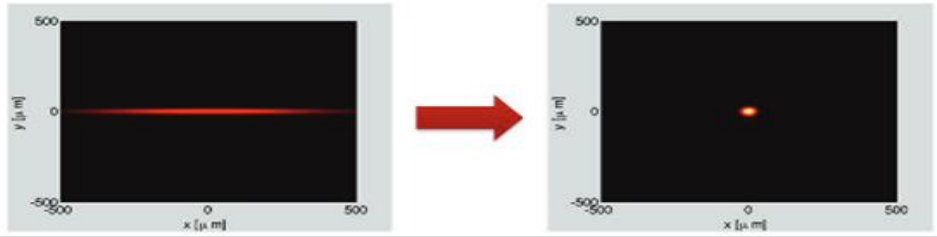
MAX IV Synchrotron

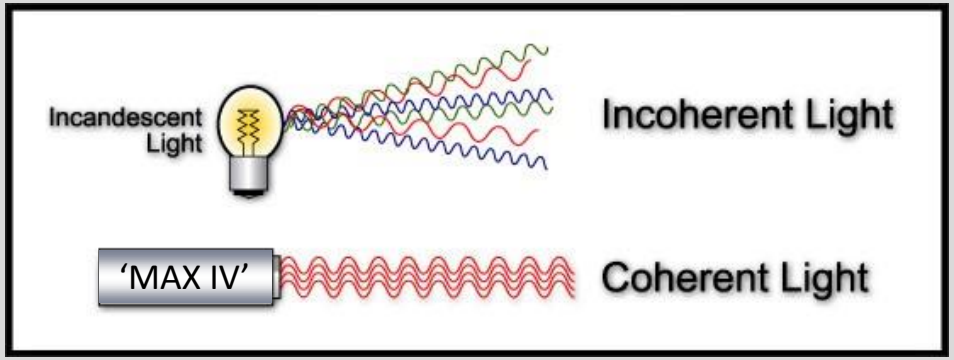


The MAX IV 3 GeV ring lattice

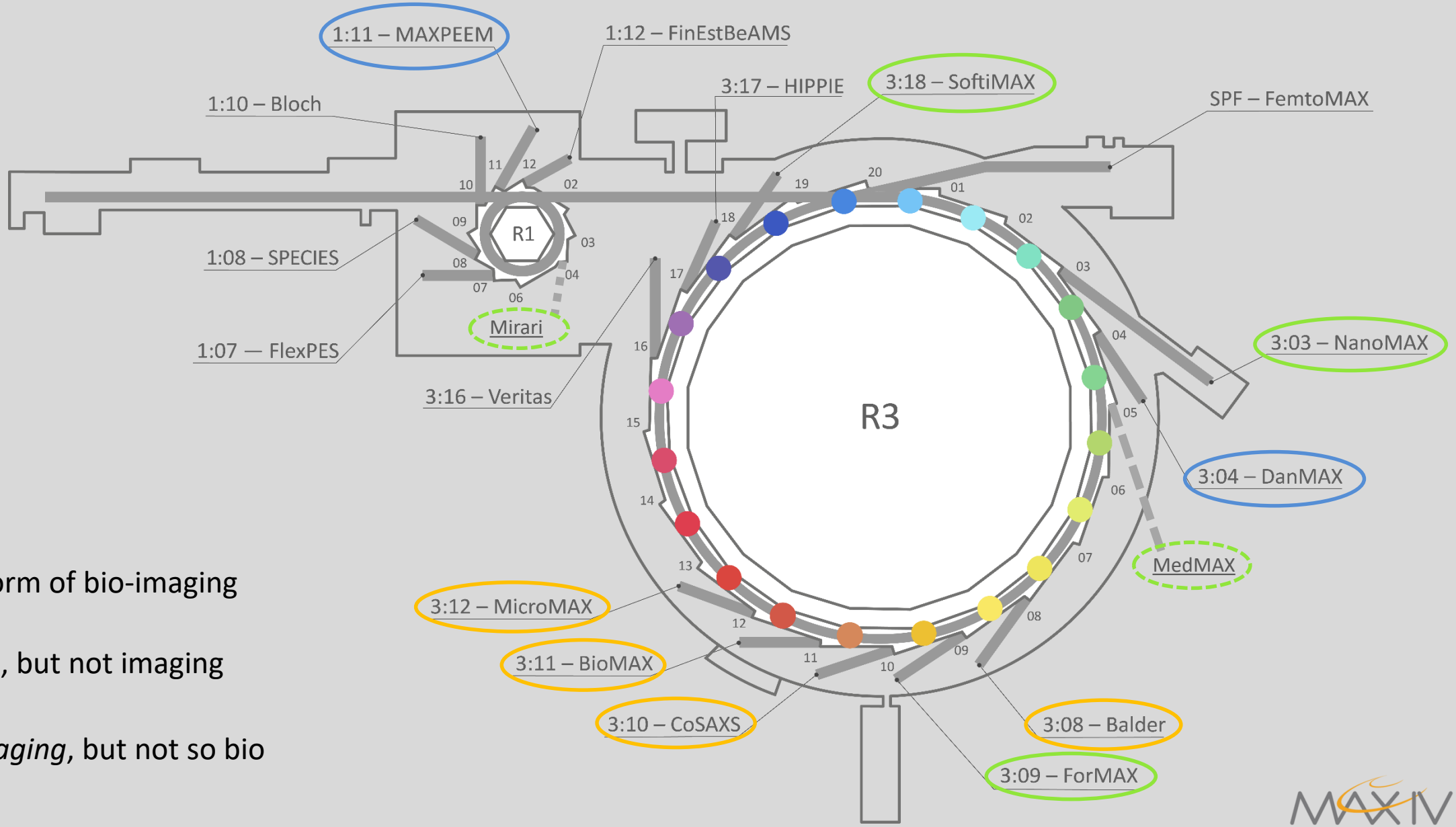


- 7 bend achromat consisting of five unit cells and two matching cells.





Which beamlines?



Some form of bio-imaging

Also *bio*, but not imaging

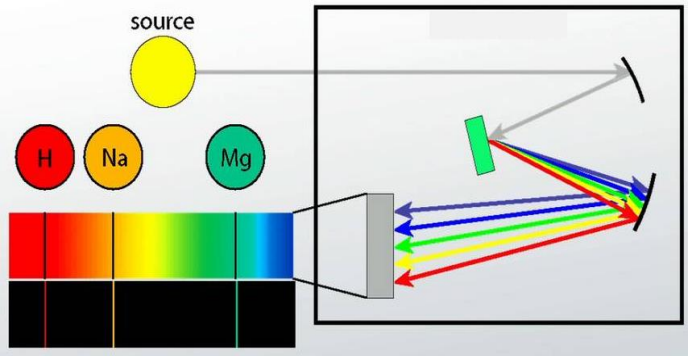
Also *imaging*, but not so bio

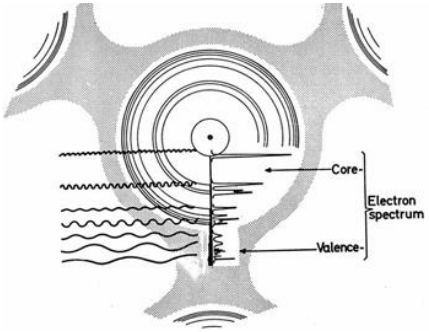
X-ray imaging

No staining/labelling necessary: distinction comes directly from X-ray interaction with elements (XRF, STXM, also IR) or X-ray scattering/refraction properties (sSAXS/phase contrast).

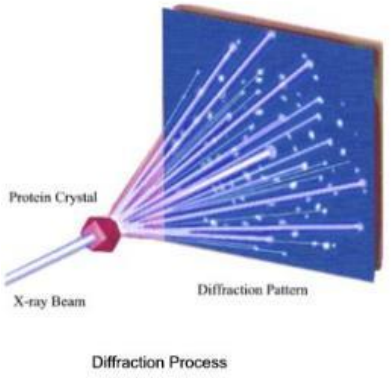
Resolution: either determined by X-ray spot size (XRF, STXM) - *Diffraction Limited Resolution* $R \approx \lambda/2$ or coherence of the light + detector (phase contrast). sSAXS combines real/reciprocal space resolution

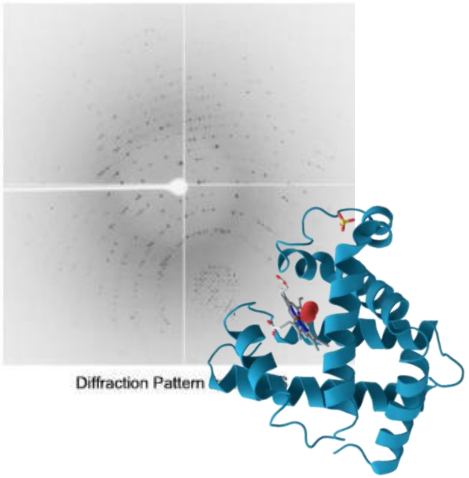
Soft & hard x-rays: absorption contrast is stronger in soft X-rays (STXM): more sensitive & better energy resolution, hard x-rays have better properties for XRF process, and can also penetrate deeper: thicker samples possible





Chemistry

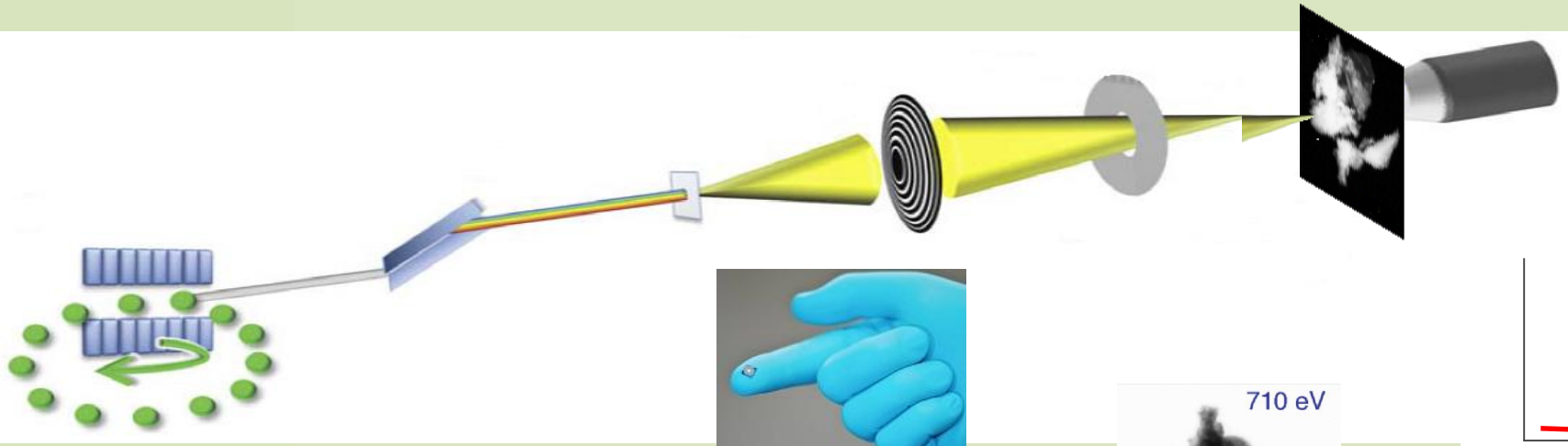


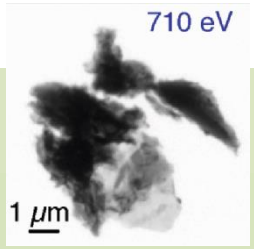


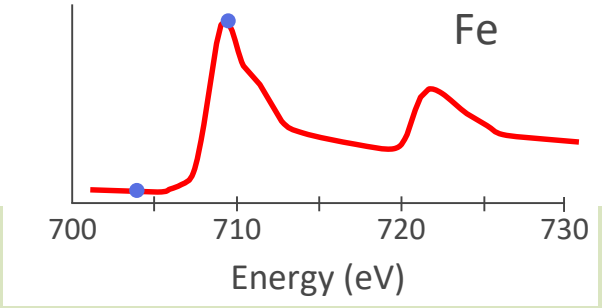
Structure

Typical STXM at a typical beamline…

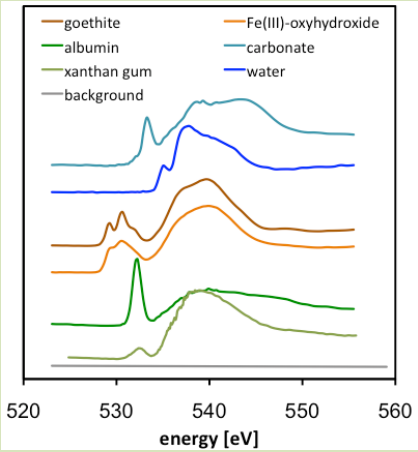
- The beamline provides **coherent monochromatic x-rays** onto a zoneplate.
- The **zoneplate** projects a nano-sized beam onto the sample.
- The sample is **scanned** through the beam.
- The intensity of the **transmitted** x-ray beam is measured in each spot.
- The x-ray energy is scanned through an **absorption edge**.

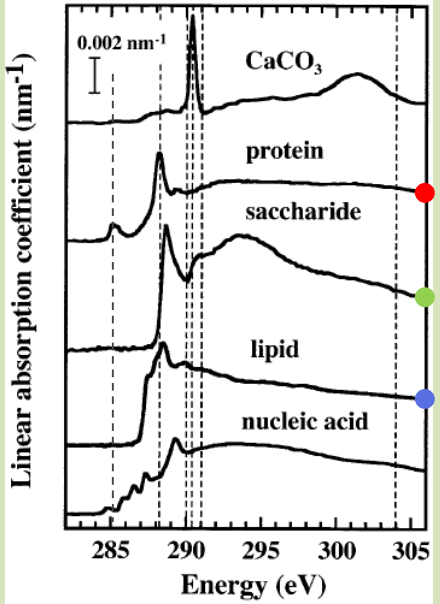


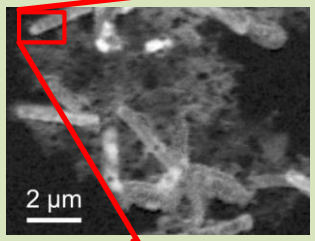




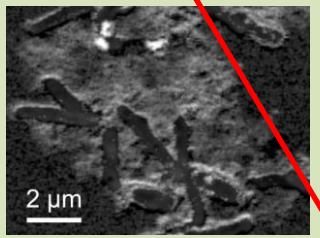
Quantitative chemical contrast



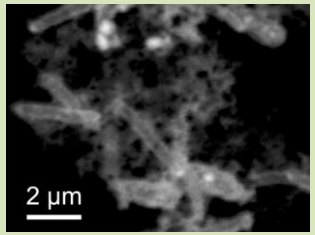




protein



polysaccharide



lipid

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21	24	25	27	30	27	26	31	35	37	46	58	68	77	89	95	100
29	30	33	38	38	33	31	38	46	54	70	87	93	93	102	106	108
27	33	43	53	56	51	47	55	62	73	87	101	105	104	106	101	102
31	40	50	57	62	64	68	75	82	89	99	110	117	120	113	104	102
36	46	52	52	55	67	85	100	109	107	112	116	125	129	122	110	106
35	41	43	45	55	75	105	129	135	125	119	113	124	132	128	115	109
35	38	45	55	75	103	130	148	147	134	119	107	117	130	134	122	112
32	33	42	59	89	129	157	162	157	143	130	116	118	131	132	115	97
28	30	36	53	87	132	156	163	158	150	145	136	127	118	100	74	52
27	28	31	47	78	112	137	153	155	143	132	121	99	77	50	33	24
25	26	26	34	55	78	106	123	130	120	96	79	61	44	29	26	27
24	26	27	27	38	53	69	72	72	66	50	43	36	31	25	24	25
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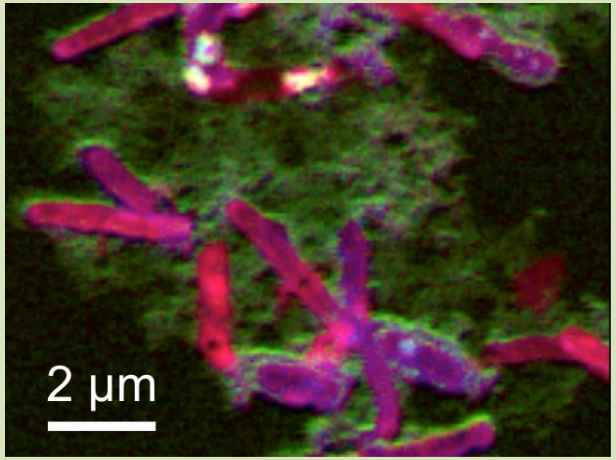
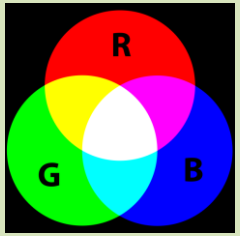
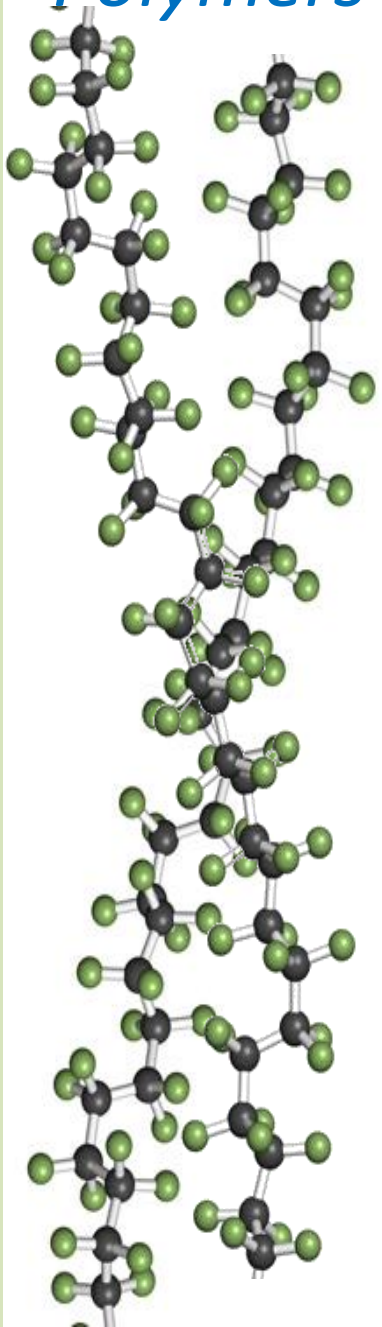
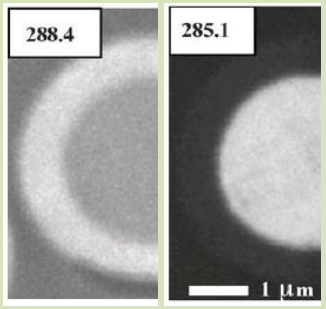
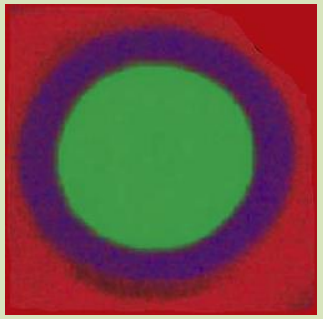


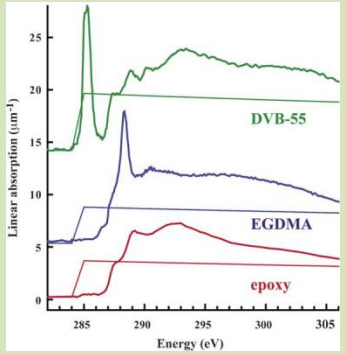


Polymers



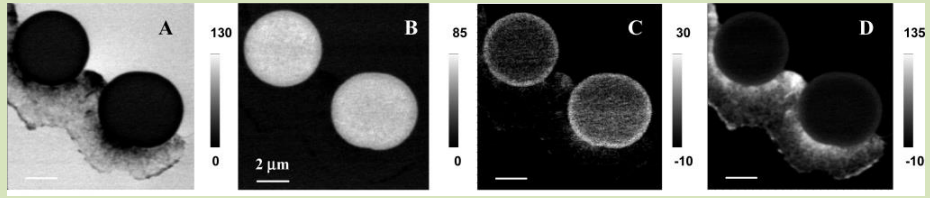


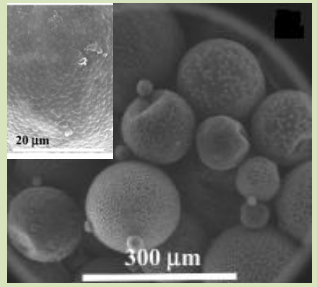


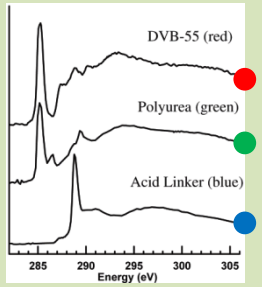


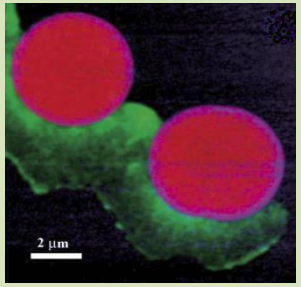
Core shell microspheres

A.P. Hitchcock *et al.*,
J. El. Spec. & Relat. Phen.
144-147, 259 (2005)



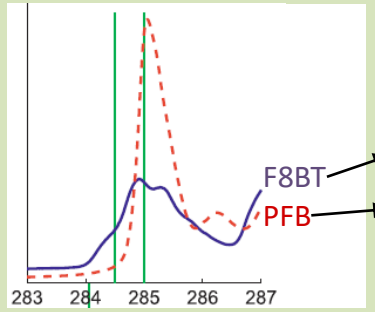


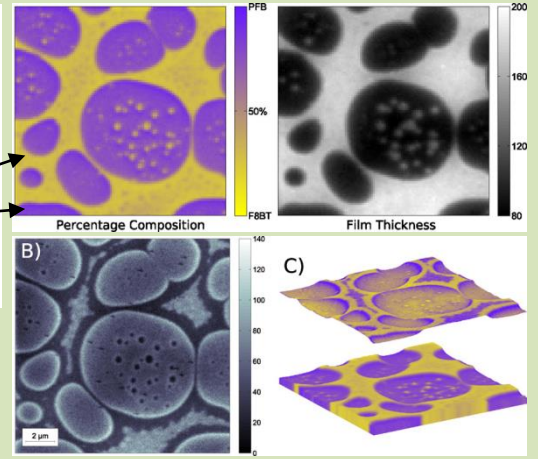




Composite Tectocapsules Containing Porous Polymer Microspheres

L.M. Croll, *et al.,* Macromolecules **38**, 2903 (2005)





Organic semiconductor thin film structures

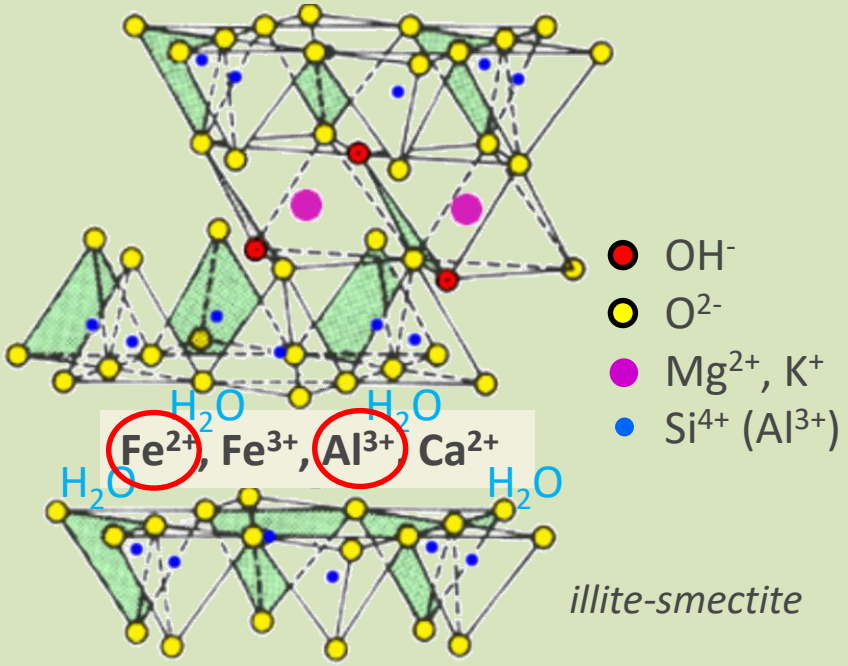
B. Watts *et al.*,
Synth. Met. **161**, 2516 (2012)

Antibacterial clay

"Some natural clays, when hydrated, can kill human pathogens including antibiotic resistant strains.

Only certain clays are bactericidal:

- contain soluble **reduced metals**
- **expandable** clay minerals that absorb cations
- capacity for extended **metal release**/toxic **hydroxyl** radicals"

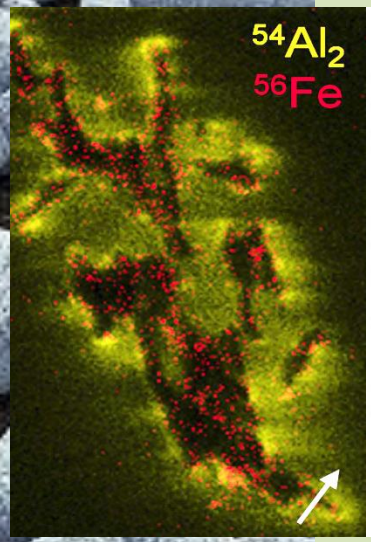


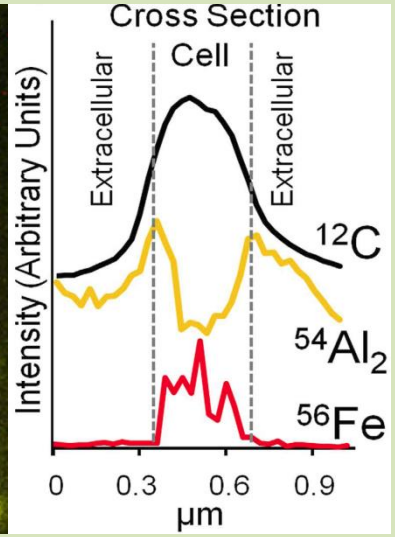
illite-smectite

K.D. Morrison, R. Misra, L.B. Williams, *Nature Sci. Rep*. **6**, 19043 (2016)

Antibacterial clay

STXM

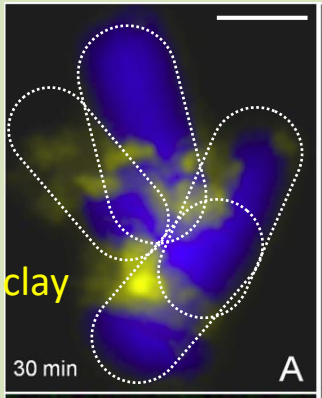


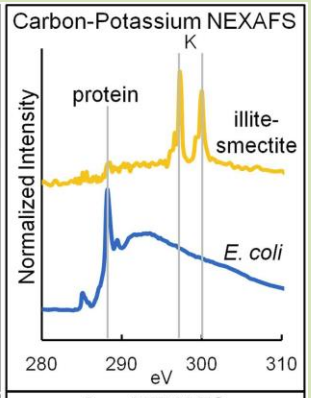


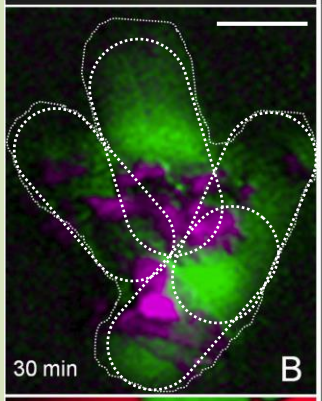
Monitor *e. coli*, and clay

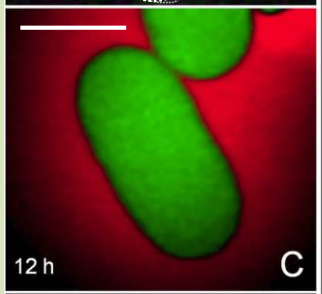
Monitor Fe in same

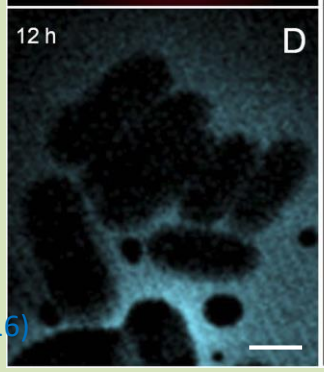
Monitor in time:
in and out of cells

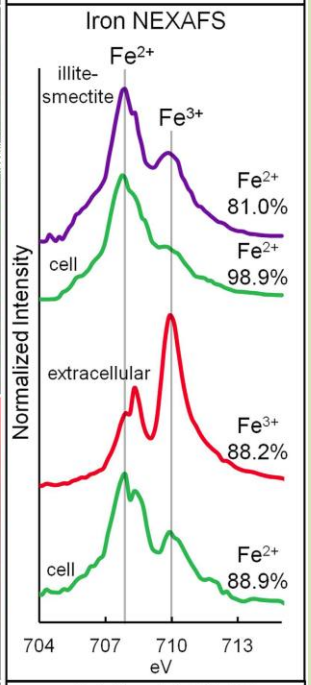


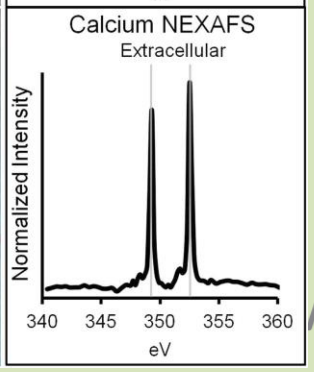




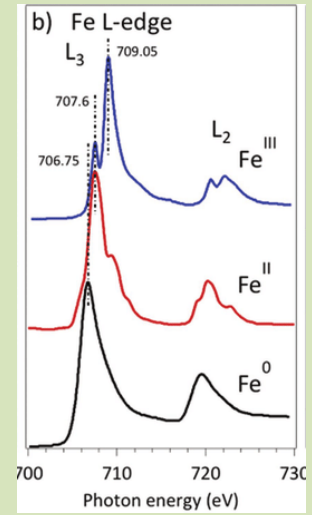








Reference



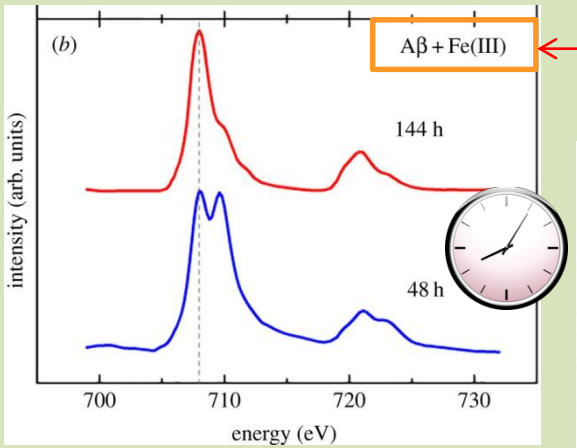
One metallic element— Fe^{2+} , which in small amounts is required by a bacterial cell for nutrition—tricks the cell into opening its wall. Then another element, Al^{3+} , props the cell wall open, allowing a flood of iron to enter the cell. This overabundance of iron then poisons the cell, killing it as the reduced iron becomes oxidized to Fe^{3+} ."

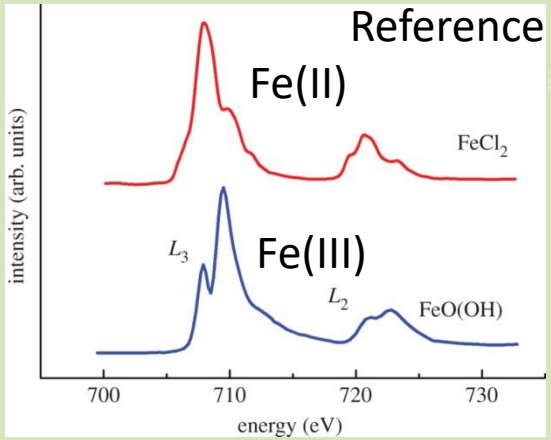
K.D. Morrison, R. Misra, L.B. Williams, *Nature Sci. Rep*. **6**, 19043 (2016)

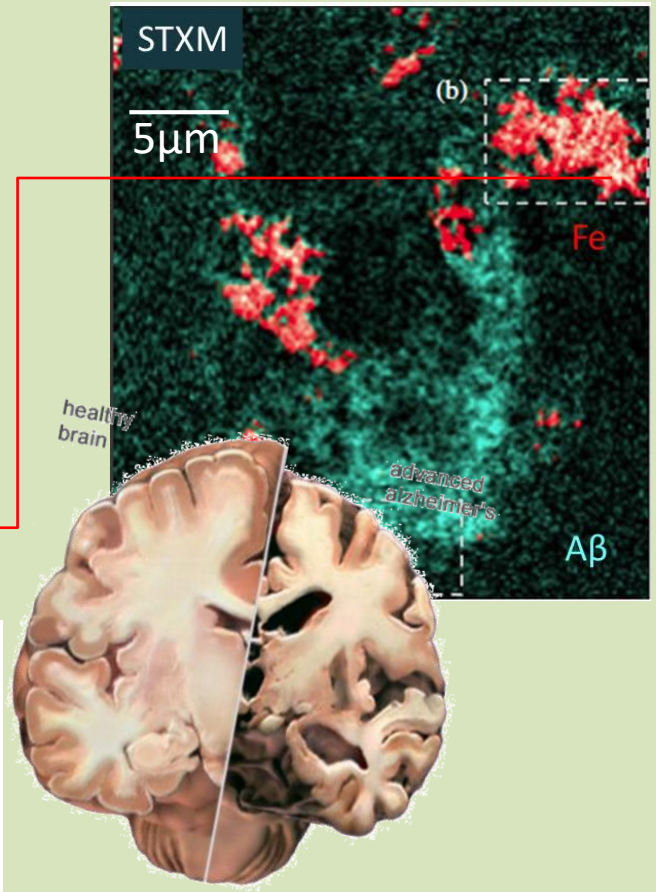
Co-aggregation of Aβ and Fe(III) → Fe(II)

*STXM spectroscopy study of Fe and Al and their co-aggregation with **beta-amyloid protein***

- For decades, a link between increased levels of iron and areas of **Alzheimer's disease** pathology has been recognized.
- Study interaction between **β-amyloids (Aβ)** and synthetic **iron(III)**, reminiscent of ferric iron in the brain:
- Iron(III) accumulates within Aβ-aggregates → Aβ-mediated reduction of iron(III) to a **redox-active iron(II)** phase with time.
- Aluminium is a further catalyst for this process





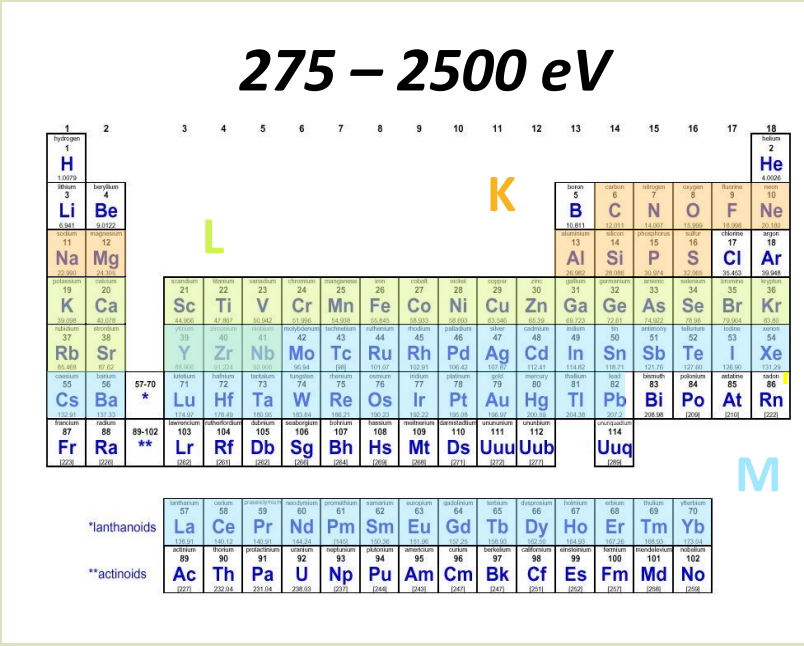


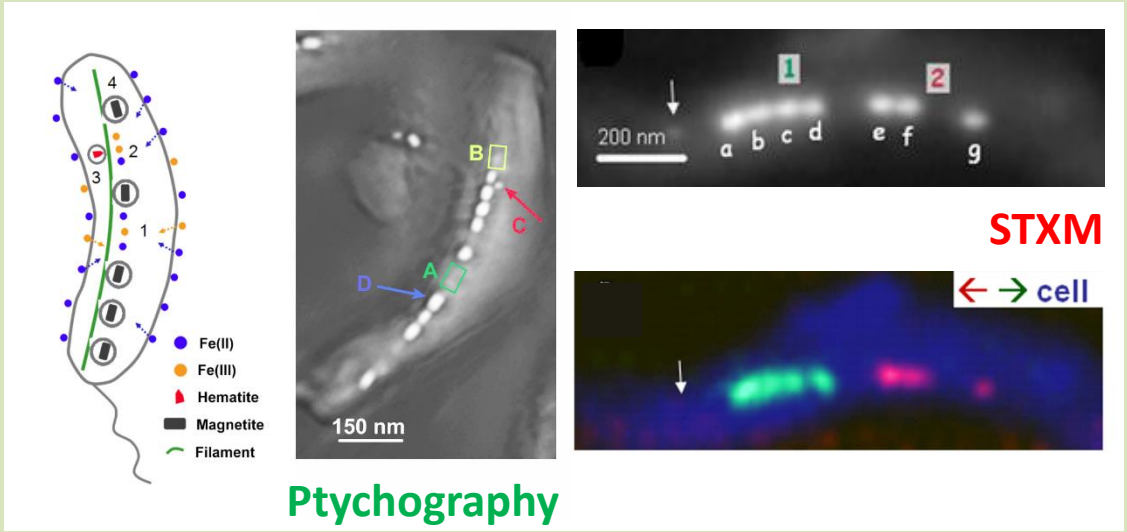
C. Everett, *et al*. J. Royal Soc. Interface **11**, 20140165 (2014)

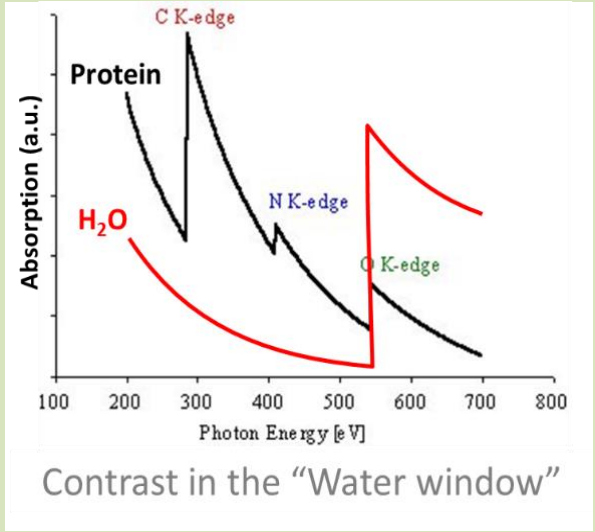
SoftiMAX: open for first users!

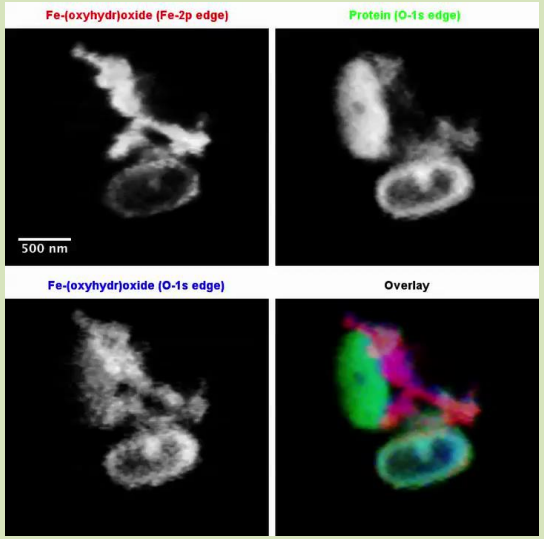
Keep in mind:

- *Thin* samples, FoV 1x1 mm^2, 'standard' resolution: 20 nm & up
- Accessible energies & edges: includes C & N
- Quantitative measurement, sensitivity ≈ 1-10mg/g
- Below 525 eV: hydrated possible
- 2D is the norm, 3D possible, but cumbersome
- Closely related: ptychography!
- Magnetic contrast









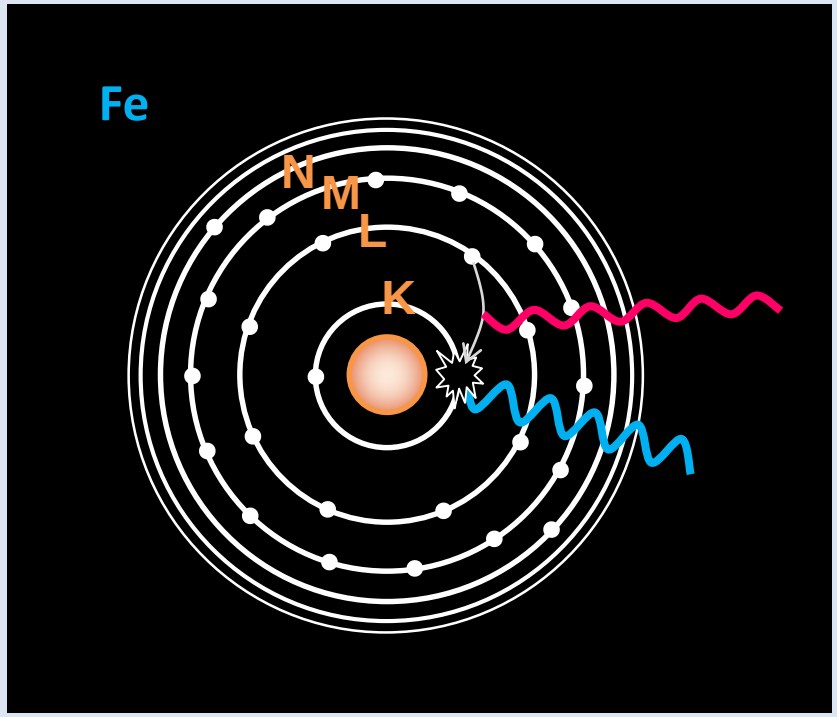
X. Zhu, *et al*., PNAS **113**, E8219 (2016) S. Kalirai, *et al*., PLoS ONE **8**, e53368 (2013)

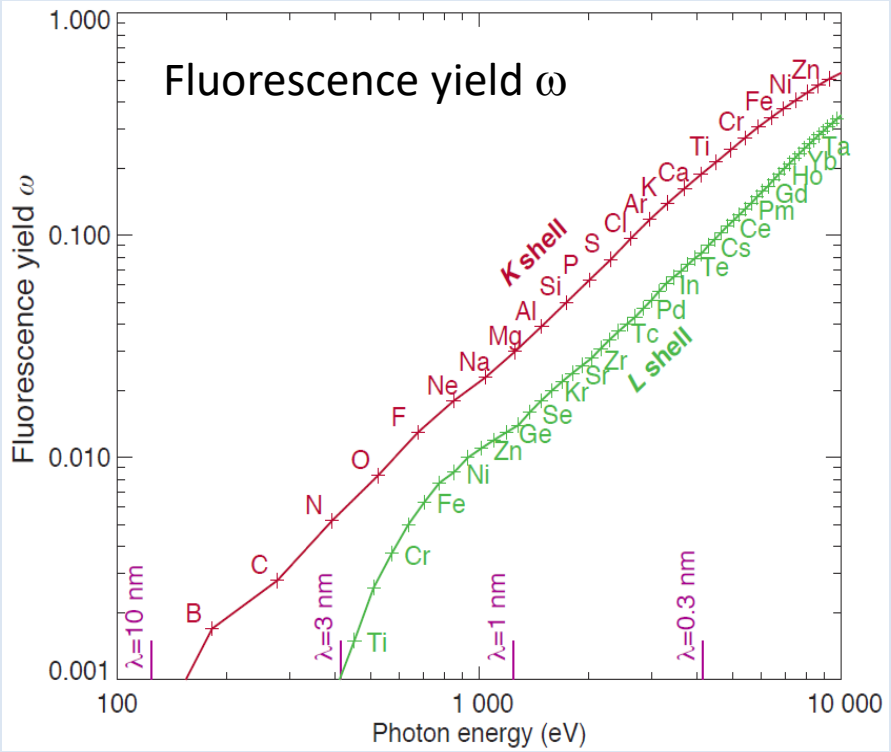
G. Schmidt, *et al*., Geobiology 12, 340 (2014)

X-ray Flu·o·res·cence

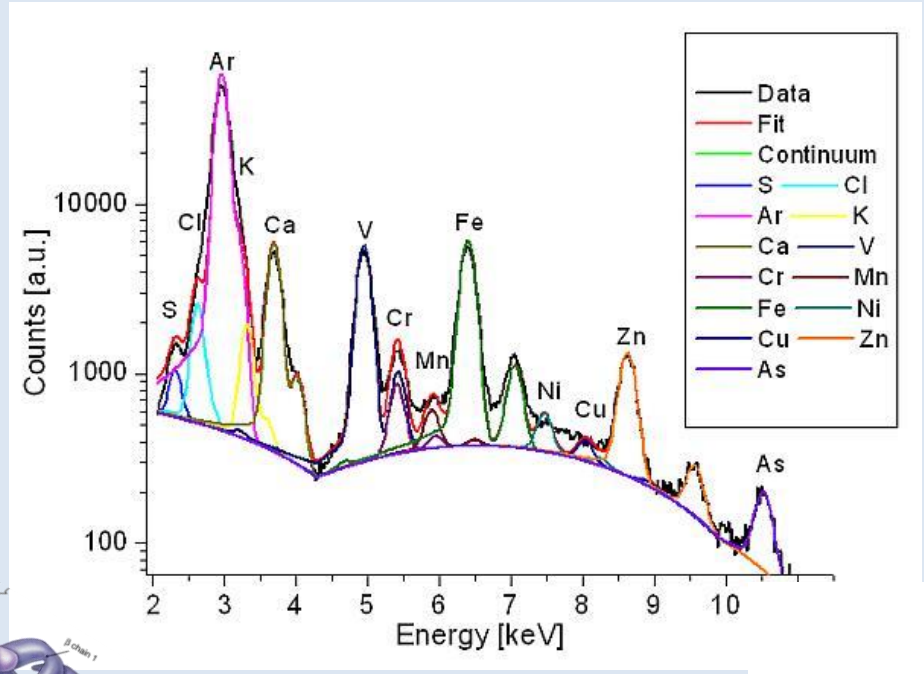
/eks-rey flʊəˈrɛs(ə)ns,flɔːˈrɛs(ə)ns/

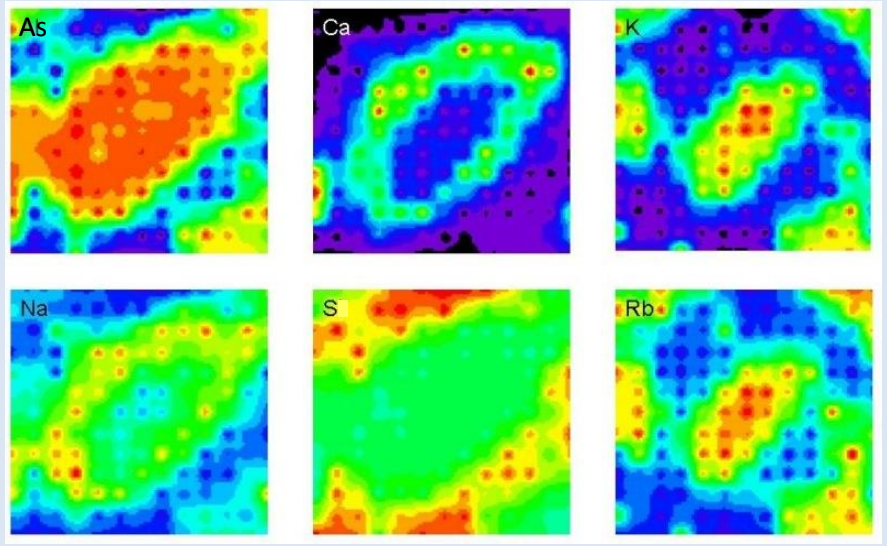
X-ray Fluorescence is the emission of characteristic, secondary X-rays by the atoms of a material which have absorbed X-rays. The emitted light always has a longer wavelength, and therefore lower energy, than the absorbed radiation.

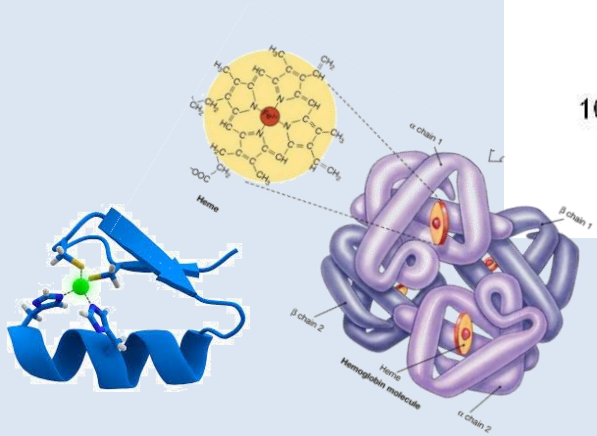


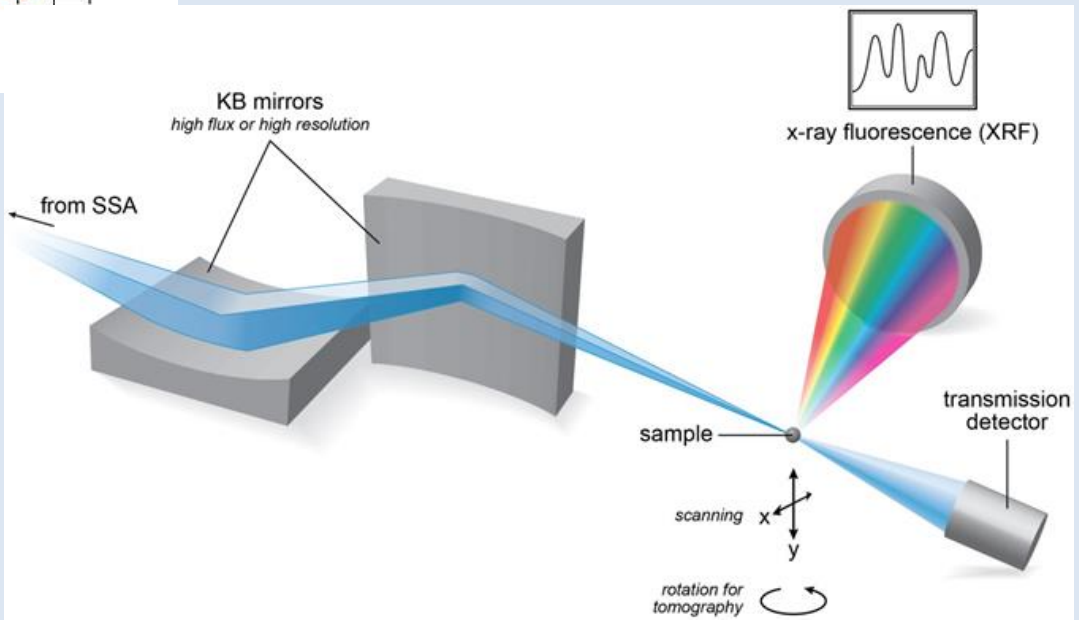


Nano-XRF mapping









Trace metals in the life sciences:

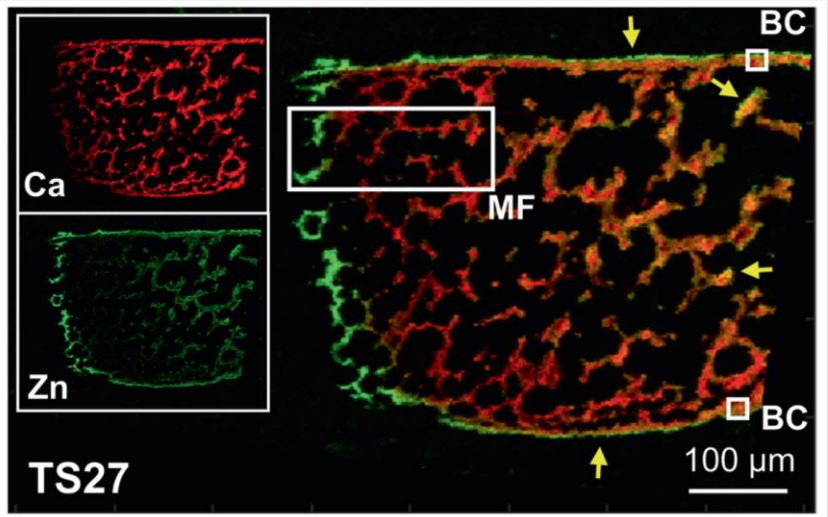
- 1/3 of all known proteins contain metal cofactors
- Endogenous dysregulation & deficiency are linked to disease
- Used in medicine and diagnostic agents
- Pollution markers/bio-remediation

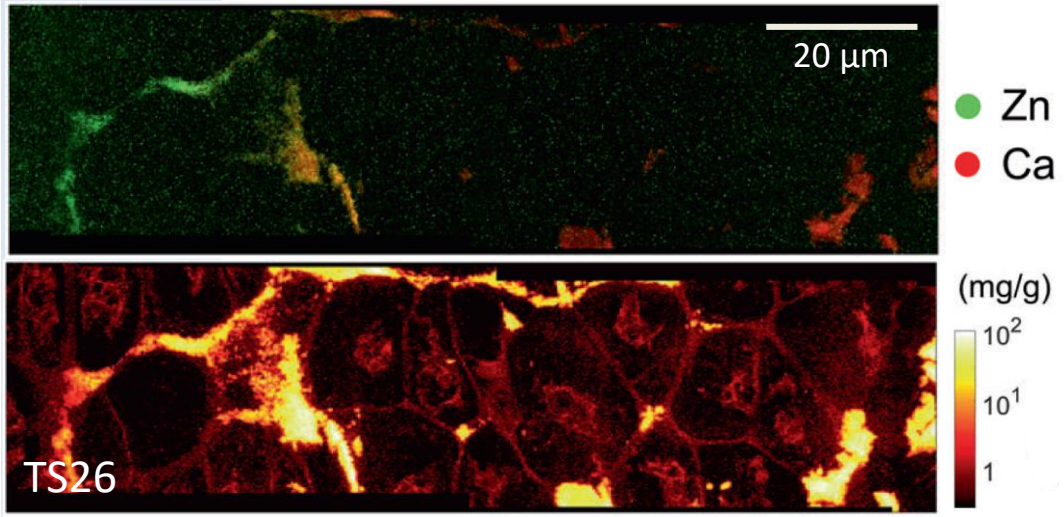
Embryonic bone mineralization

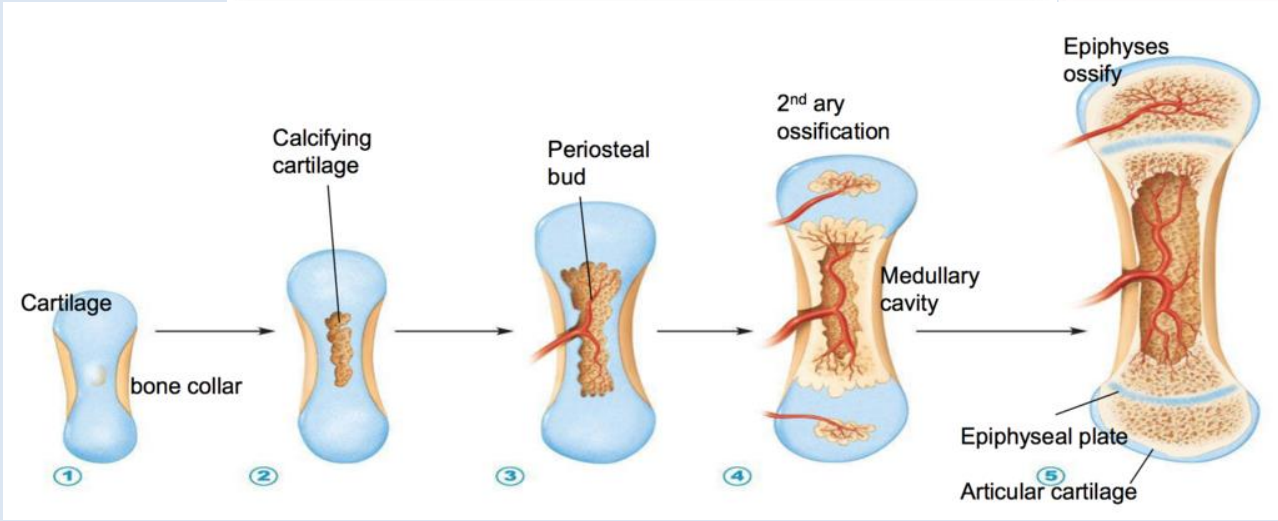
- Zn @ leading edge of mineralization front

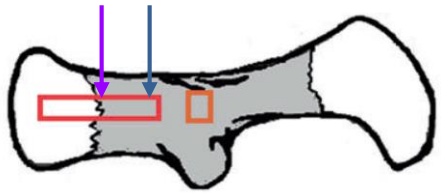
- Ca concentration in bone center increases during bone maturization

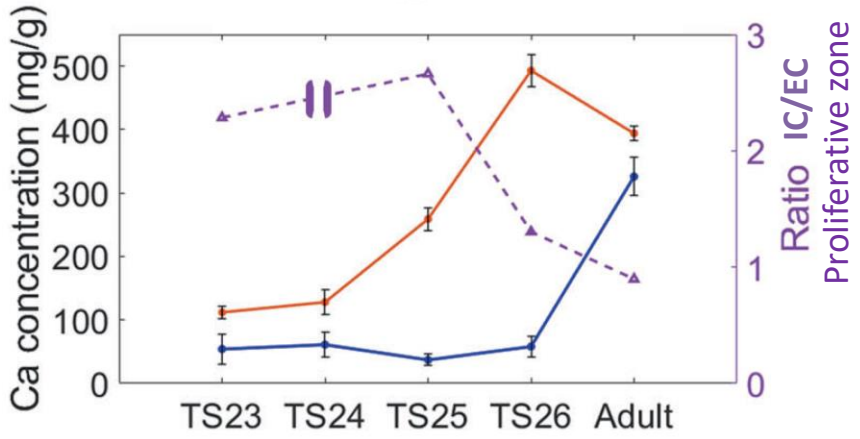
- Non-ordered Ca deposits: not hydroxyapatite before bone formation











Isabella Silva Barreto *et al.*, Adv. Sci. **2020,** 2002524

Tattoos: marked for life

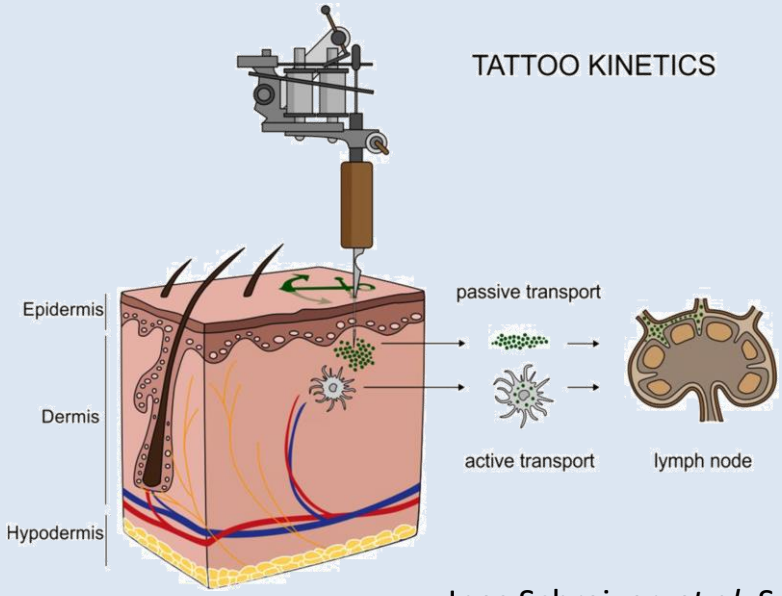
TISSUE

Tattoo: deposit insoluble ink in the dermal skin layer, where they eventually form pigment particles up to several micrometers in size

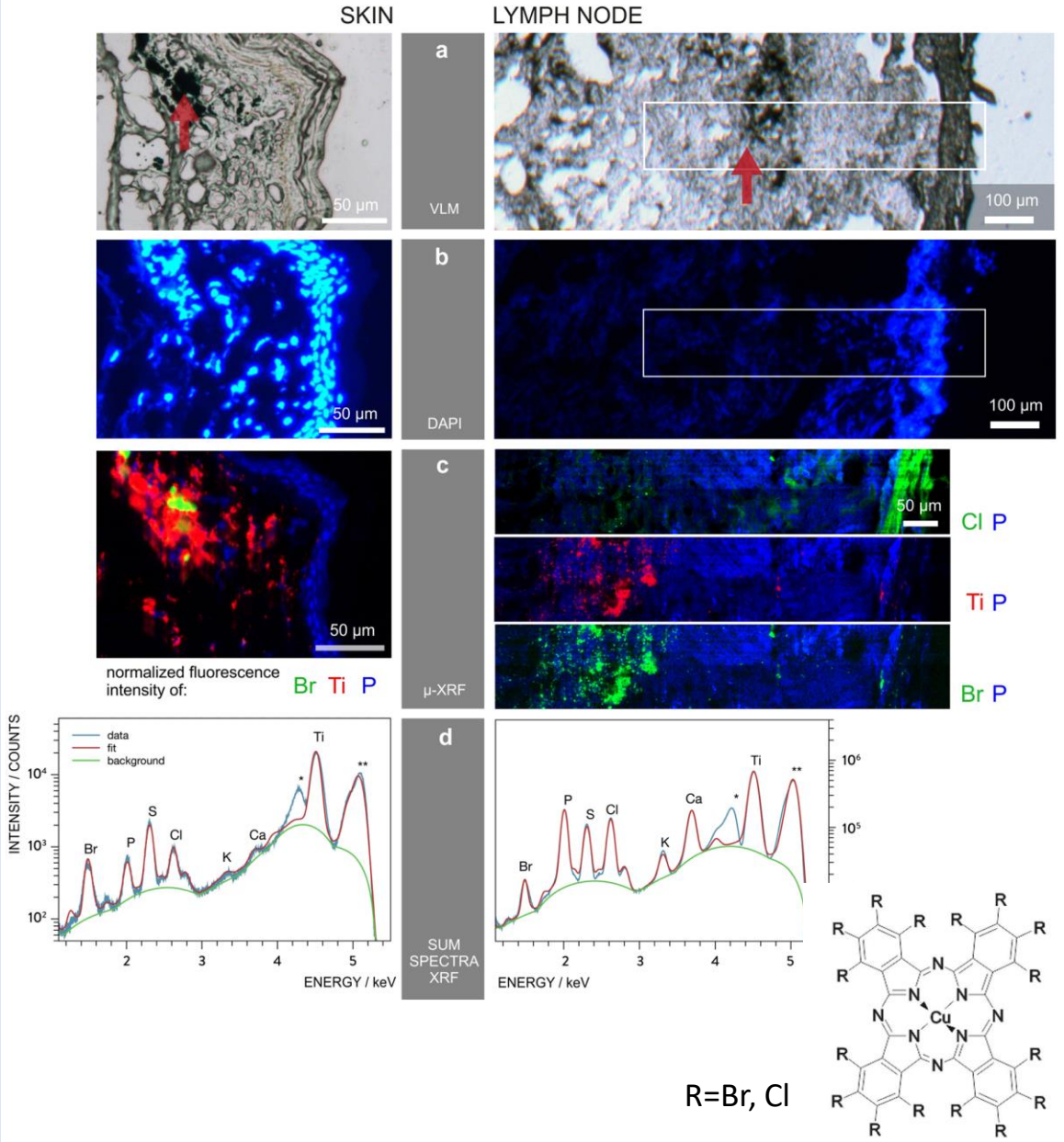
In lymph nodes only smaller (nano)particles were found. The exact size limit preventing this translocation is unknown yet.

The deposit of particles leads to chronic enlargement of the respective lymph node and lifelong exposure.

The signatures can be linked to the pigments and provide strong analytical evidence for migration of pigments from the skin towards regional lymph nodes in humans.

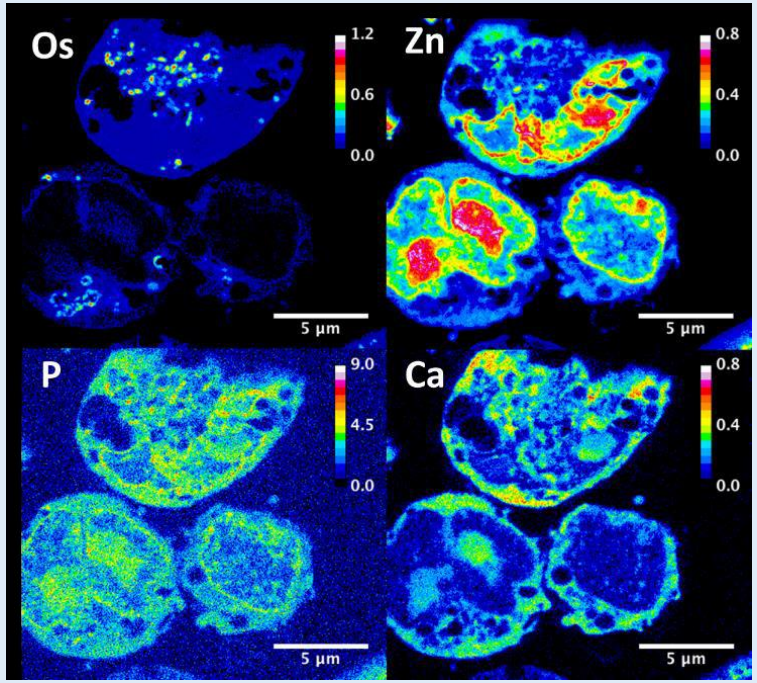


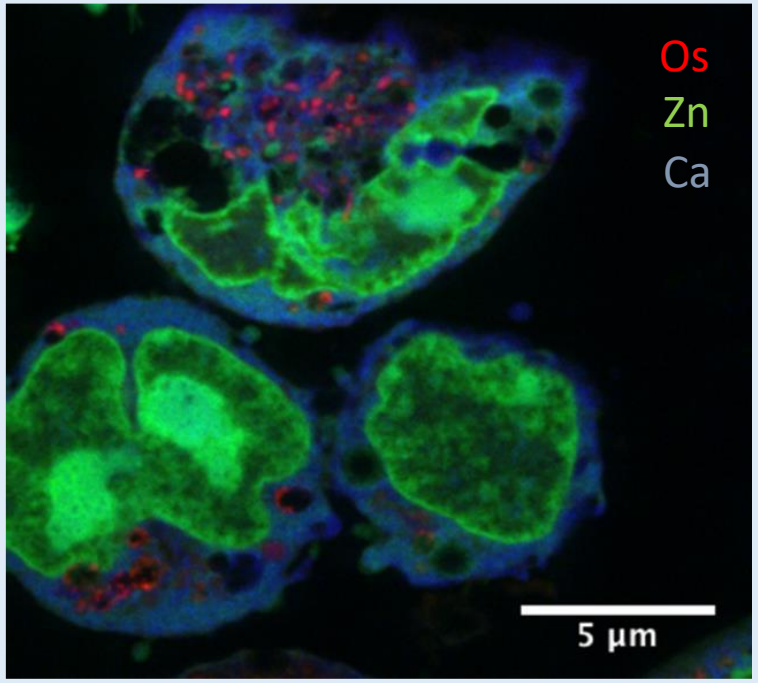
Ines Schreiver, *et al*. Sci. Reports **7**, 11395 (2017)

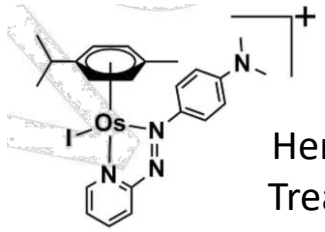


Target sites for Os complex in human ovarian cancer cells

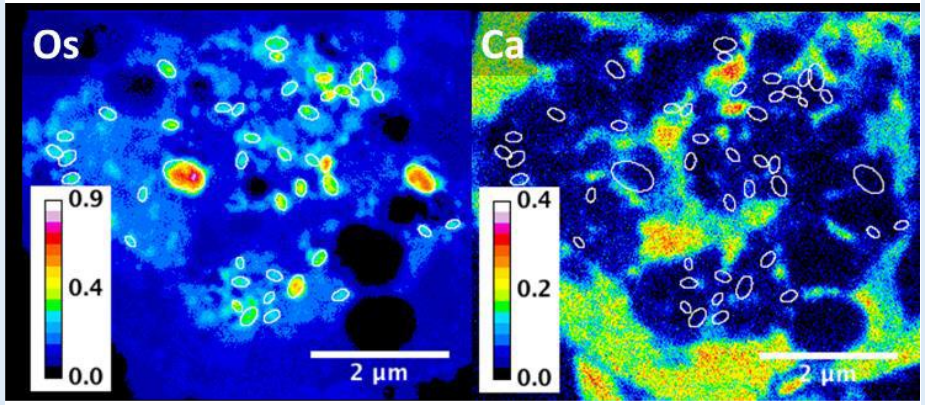
- Cancer chemotherapy drugs contain Pt complexes: severe side effects (Pt resistance)
- Try other metals & complexations: Ru, Rh, Gd, Nb, Co, Ti, V -> **Os^{II} arenes**: 49x more potent than cisplatin towards a range of 809 cancer cell lines
- XRF: Pt-based *DNA-intercalators* concentrate in nuclei, while *cisplatin* is evenly distributed in the cell
  Gd complexes maybe in mitochondria: inconclusive micro-XRF (and was done at higher concentrations than 'humane')







Here, concentrations 0.16-1µM
Treatment 24 hr

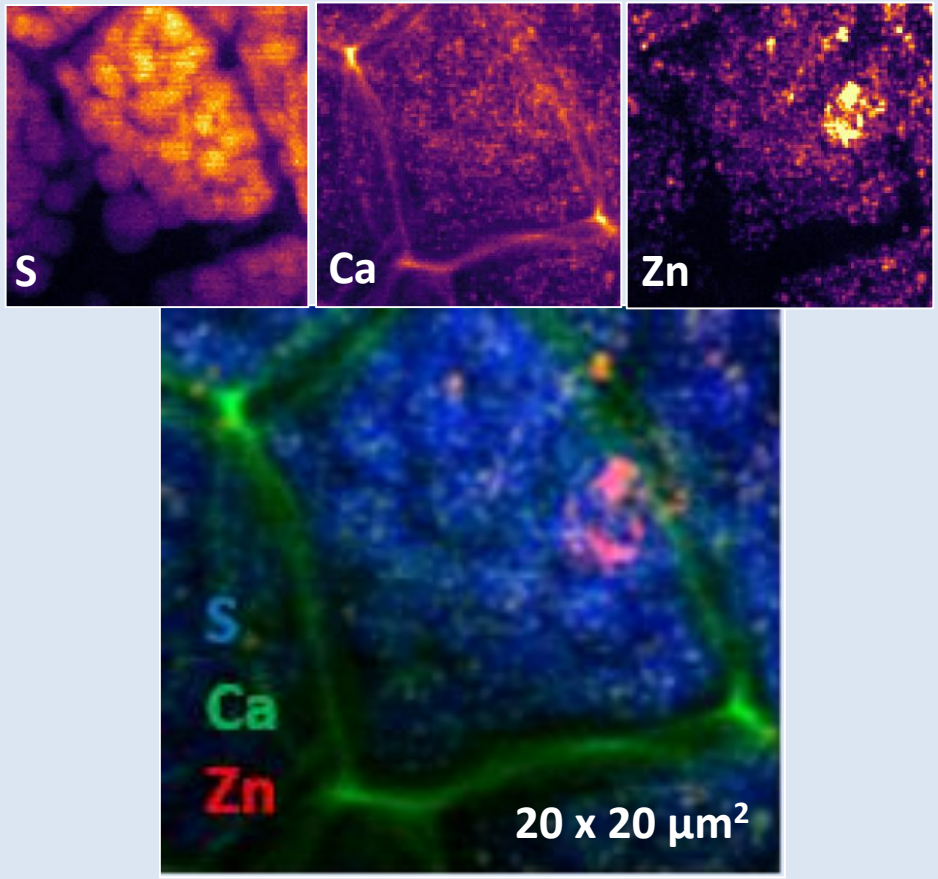


- Osmium not localized in cell nuclei (no overlap with Zn, marginal with P) and suggests that DNA is **not** a major target.
- IC-PMS and XRF indicate **Os^{II} arenes** instead located in mitochondria.
- Ca outside of ER may indicate apoptosis.

Carlos Sanchez-Cano *et al.*, Chem. Eur. J. **23**, 2512 (2017).

Keep in mind:

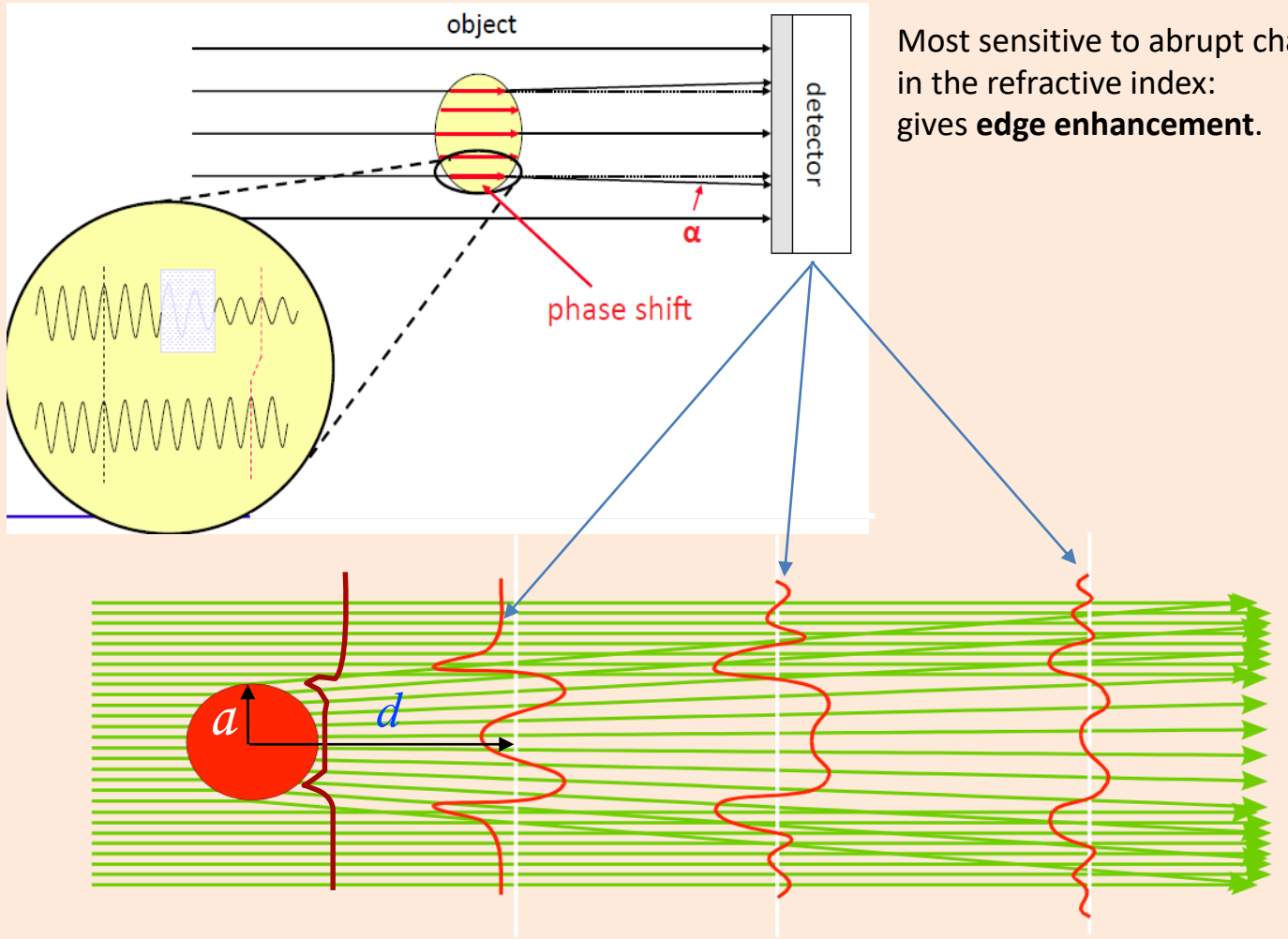
- Small areas per scan (≤ 100 x 100 µm2), resolution 50nm and up: from 20 min - x hrs per scan
- 3D information is lost: penetration depth OK, but keep samples thin (≤ 2µm) to avoid blurring (or do tomo!)
- Semi-quantitative: compare to XRF standards (NB: surrounding 'matrix') or theory
- Sensitivity: down to ≈1-100 µg/kg, but depends a lot on element & local concentration!
- Not for elements below P, and vacuum helps
- Tissue/organ: use alternate slices for optical microscopy
- NB: sample preparation – contamination and wash out of elements!



Nano-XRF at NanoMAX:
Zn in plant seeds

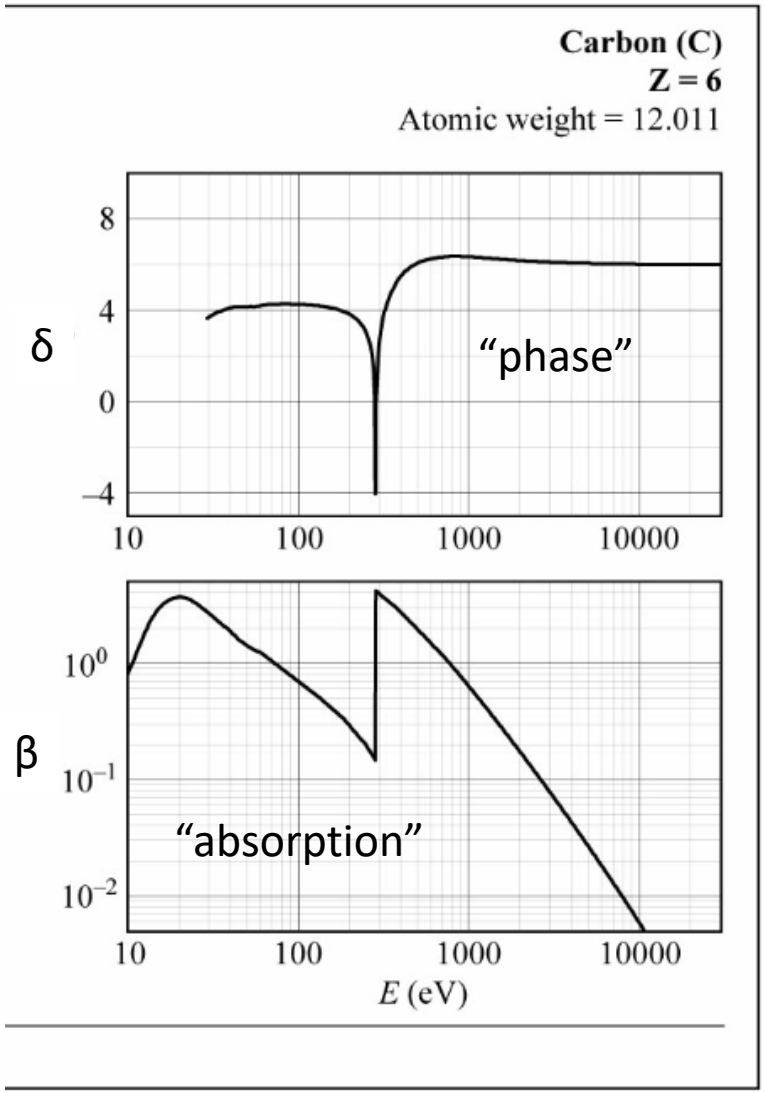
MedMAX

Phase contrast tomography

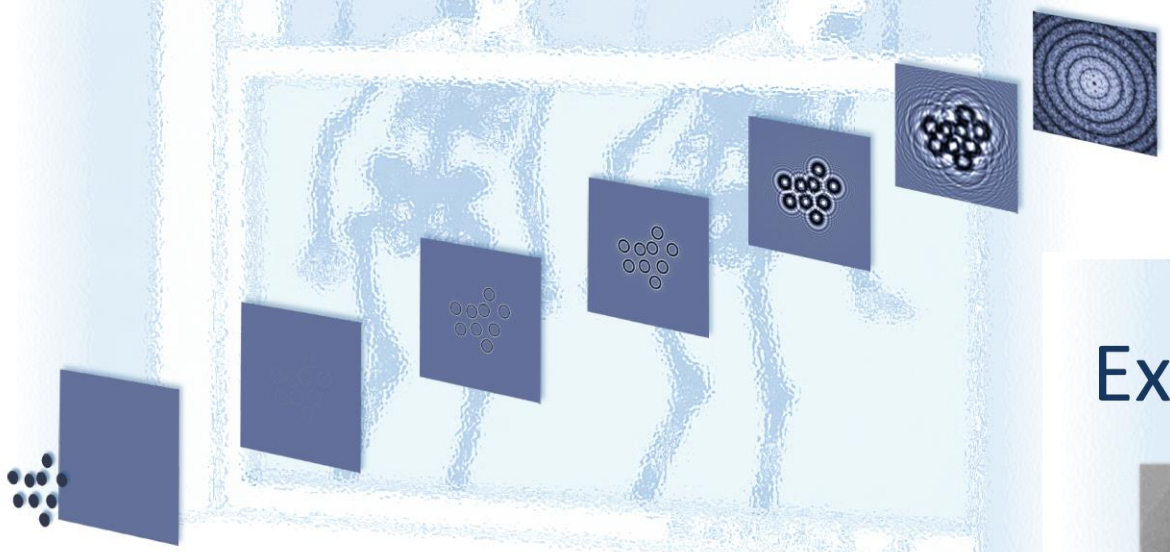


Most sensitive to abrupt changes in the refractive index: gives **edge enhancement**.

$$n(\omega) = 1 - \delta + i\beta$$

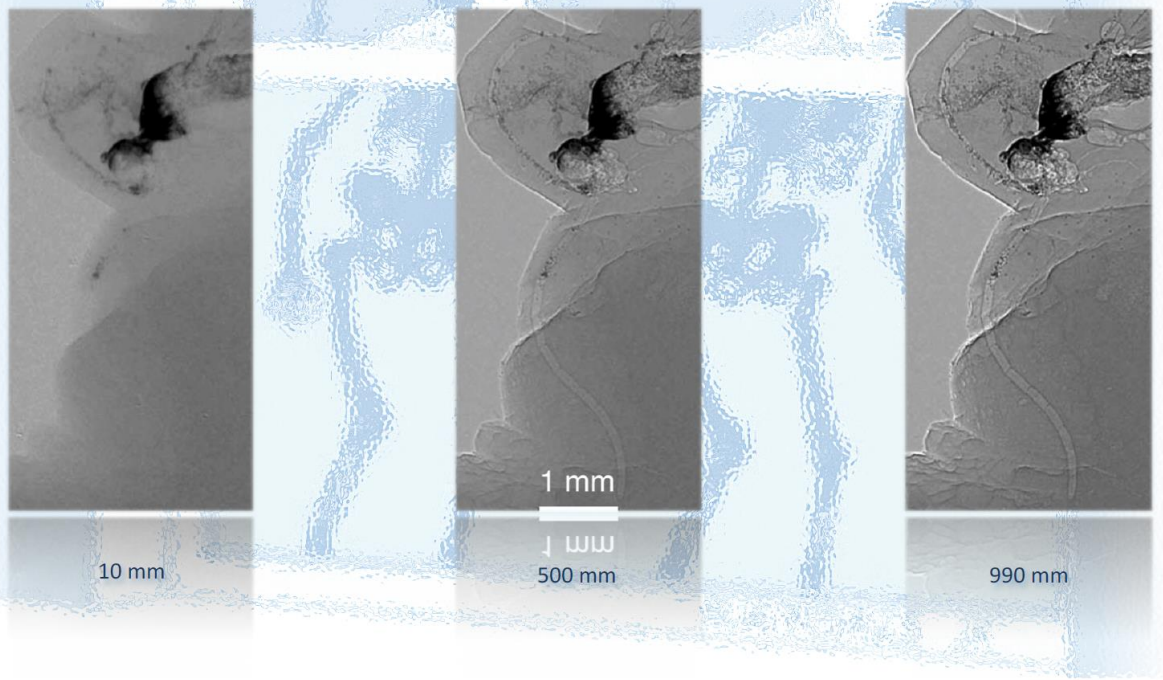


Not too near and not too far

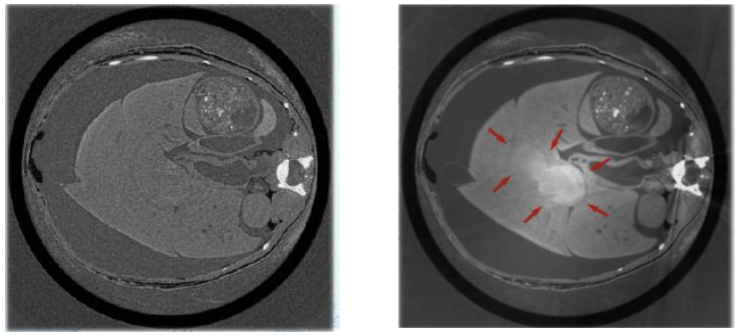


Phase contrast needs coherent beam
→ MAX IV has that!

Example – cretaceous insect in amber

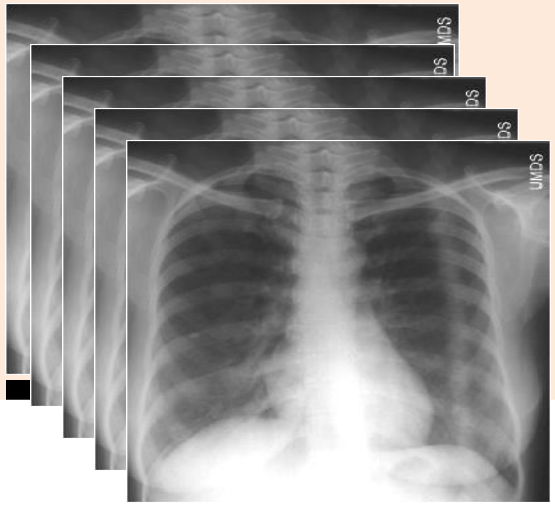


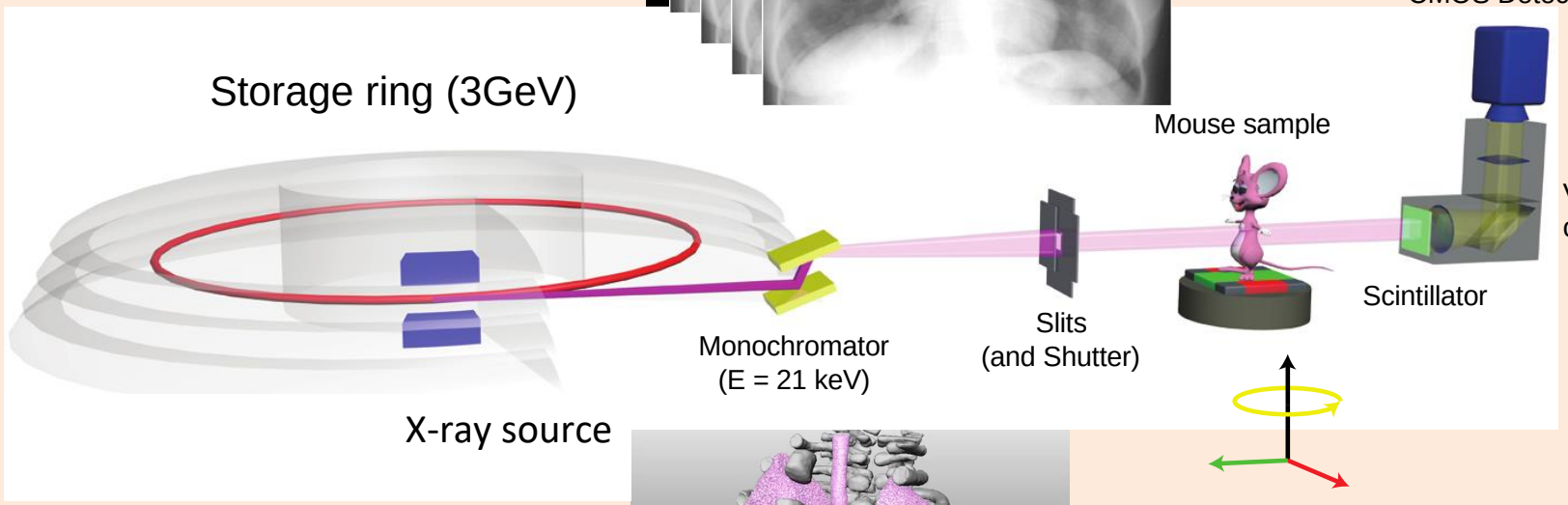
Tumour tissue highlighted



From: Lecture given by Phil Willmott, SLS Switzerland

Principles of tomography

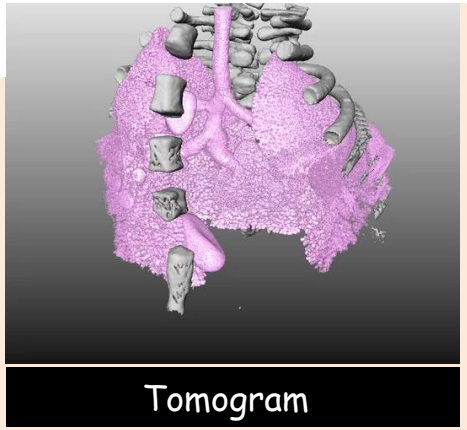




Projections: 300–2000

Exposure times: 0.1–300 ms

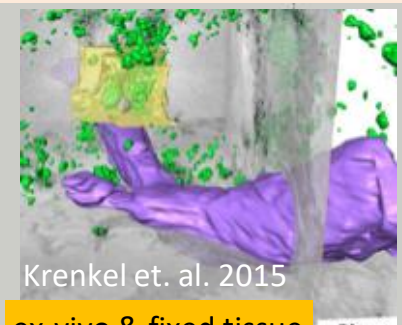
Total scan time: 0.05 to 500 s



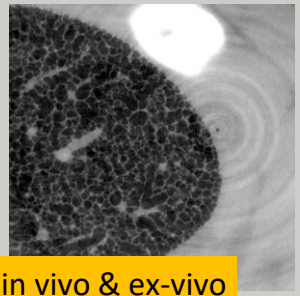
Pixel size: 11 -> 0.3 µm

FOV: 22 x 22 -> 1 x 1 mm^2

MedMAX concept

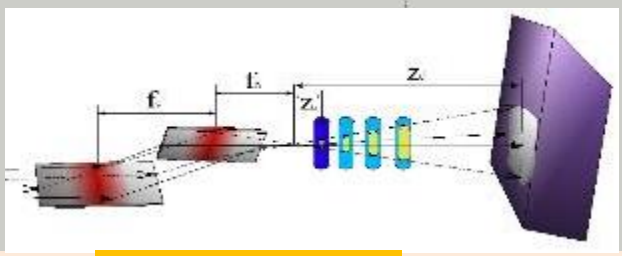


ex-vivo & fixed tissue

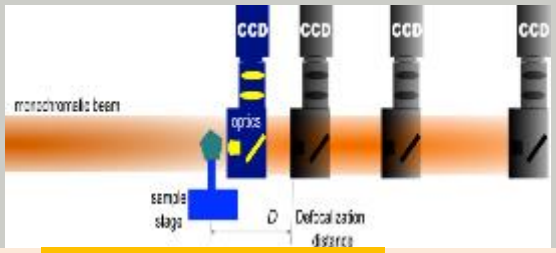


terminal in vivo & ex-vivo

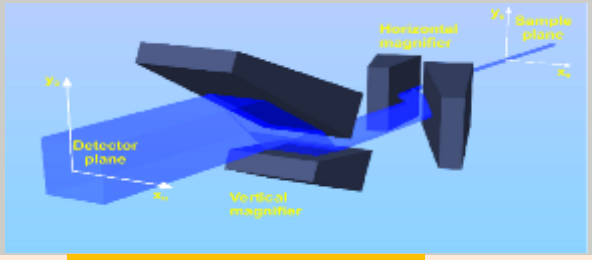
Longitudinal in vivo



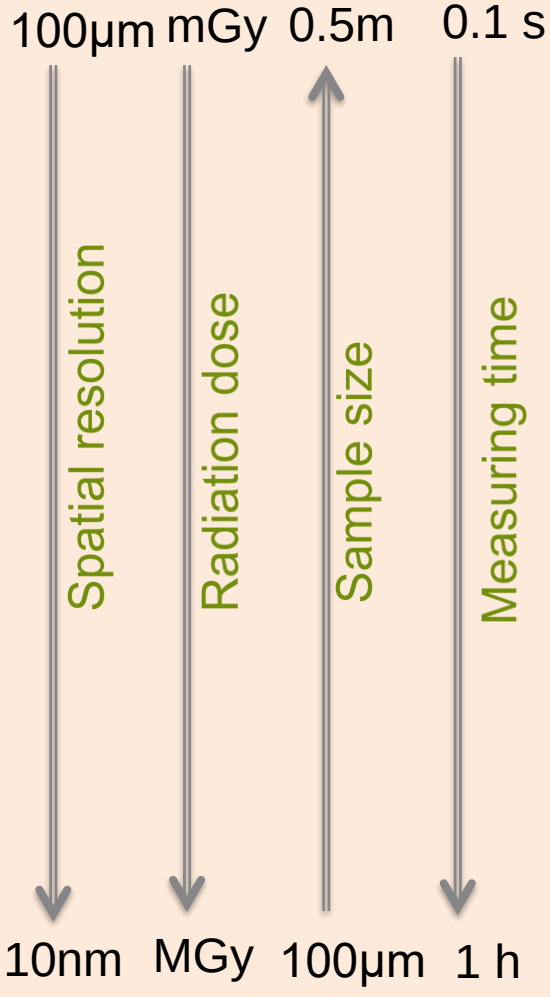
Nanoscale projection microscopy



High resolution parallel beam tomography



Medium resolution in 4D

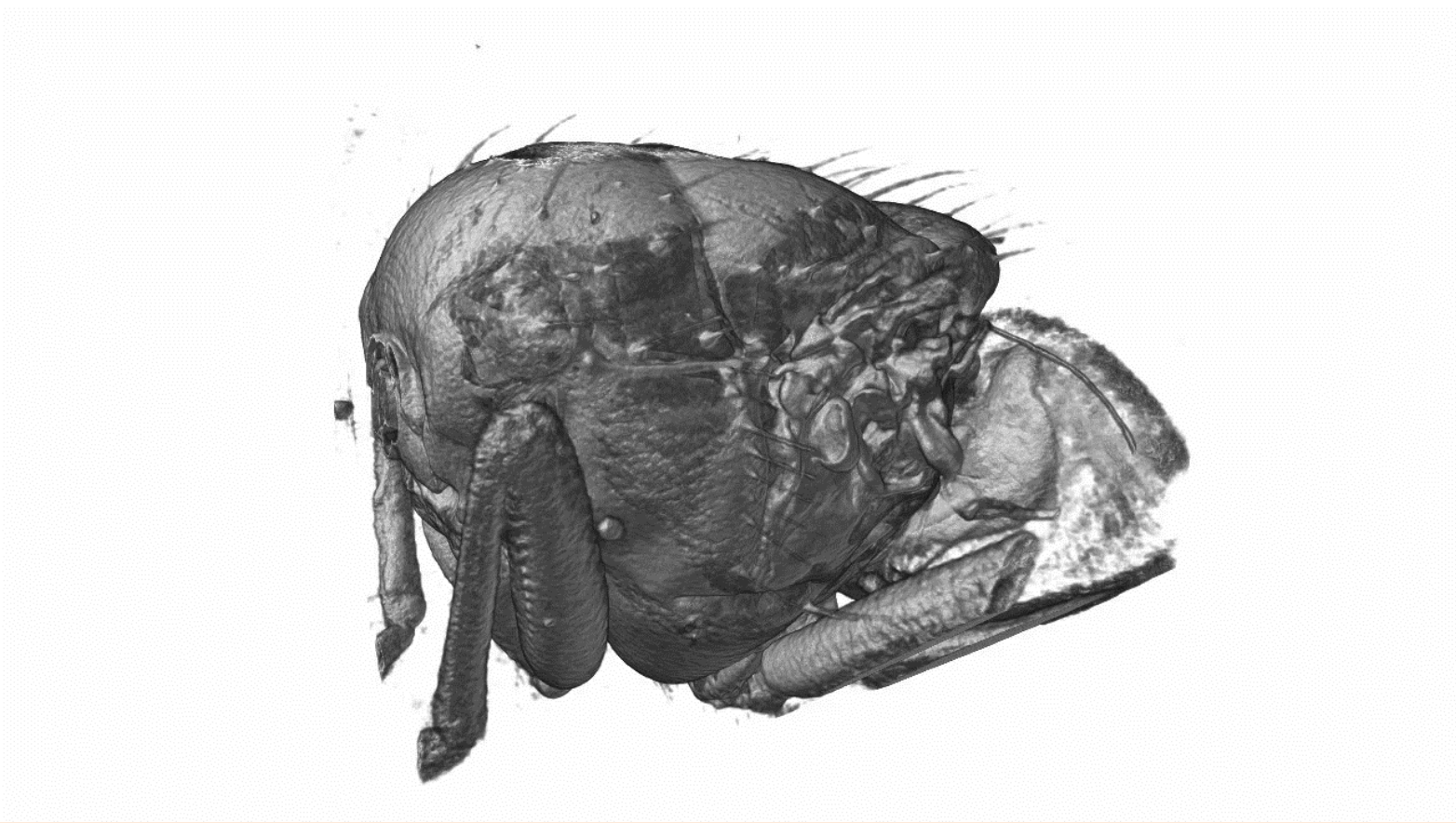


How will MedMAX be unique?

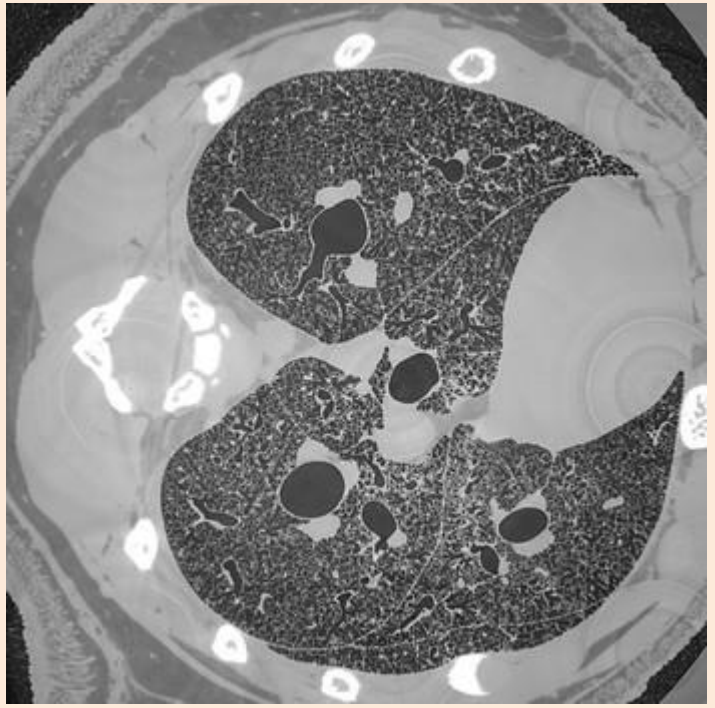
- 3D zooming in and out from the organ level to sub-cellular level with the same instrument
- Bright x-ray beam of MAX IV is necessary for optimizing image resolution/contrast vs. sample radiation dose. Coherence enables phase imaging and shorter exposure times.
- Dynamic studies will enable following biological processes in living organisms in 3D
- On-site Comparative Medicine Unit provides context for longitudinal pre-clinical imaging on mice and rat models
- Extensive user support in image analysis to interpret multidimensional datasets to quantitatively support the scientific outcome

MedMAX: A high-speed 3D imaging beamline for pre-clinical medical studies, study of physiological processes in cell biology, tissues, and zoology, biomaterials, and cultural heritage science

In vivo time-resolved microtomography reveals the mechanics of the blowfly flight motor.
Walker, SM, Schwyn, DA, Mokso, R, Wicklein, M, Müller, T, Doube, M, Stampanoni, M, Krapp, HG, Taylor, GK
PLoS Biol 12(3): e1001823 (2014).



Shedding light on metal-based nanoparticles in zebrafish by computed tomography with micrometer resolution
E. Cörek, *et al*., Small **16**(31), 2000746 (2020).



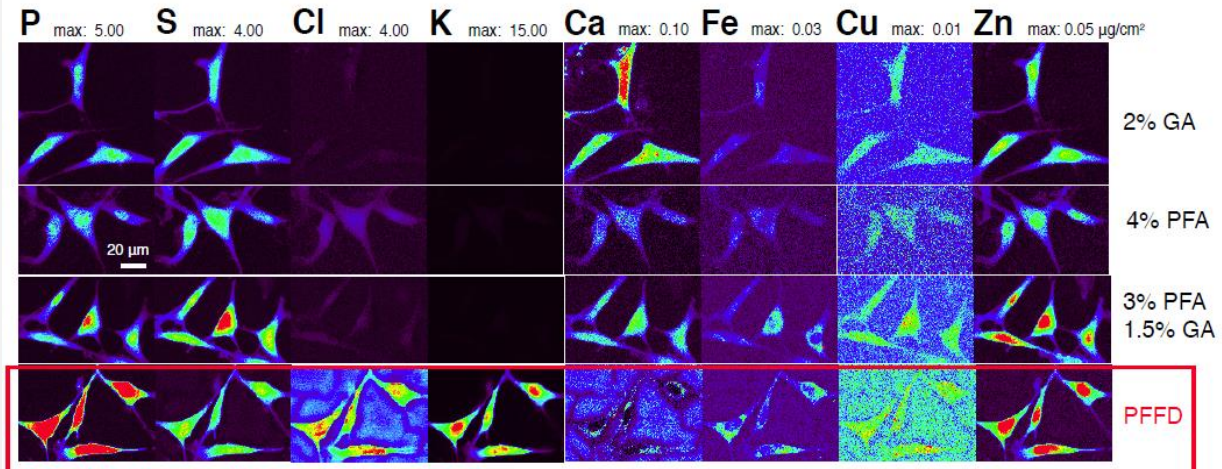
Micrometer-resolution X-ray tomographic full-volume reconstruction of an intact post-mortem juvenile rat lung
E. Borisova, *et al*., Histochemistry and Cell Biology (2020).

…and finally, a check-list

Before an experiment, think about:

1) Resolution needed
2) Information needed: which contrast mechanism is best?
3) Acceptable radiation dose?
4) Sample preparation
5) Combinations of BLs
6) Contact us to discuss!

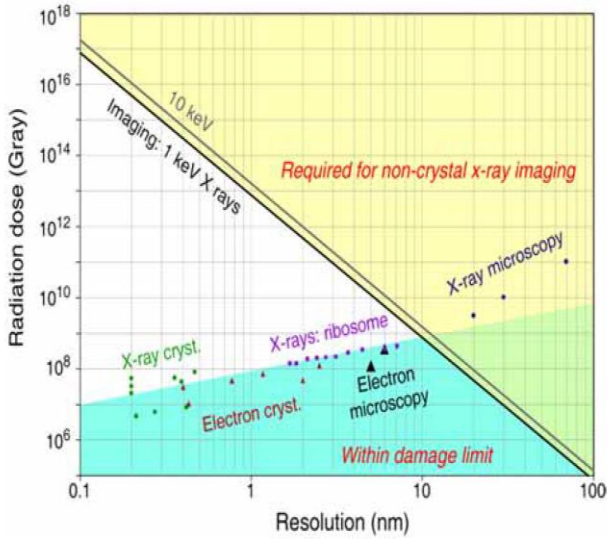
Cryo preservation keeps chemistry intact

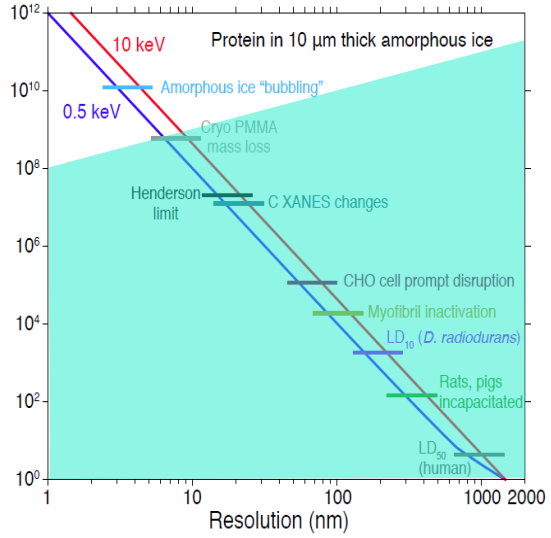


- Jin, Paunesku, Lai, Gleber, Chen, Finney, Vine, Vogt, Woloschak, and Jacobsen, *J. Microscopy* **265**, 81 (2017).
- See also Perrin, Carmona, Roudeau, and Ortega, *J. Analyt. Atom. Spectr.* **30**, 2525 (2015).

Dose versus resolution for transmission x-ray imaging

- Calculation of radiation dose using best of phase, absorption contrast and 100% efficient imaging





This plot: Howells *et al.*, *J. Electr. Spectr. Rel. Phen.* **170**, 4 (2009). See also Shen *et al.*, *J. Sync. Rad.* **11**, 432 (2004).

Ultramicroscopy **184**, 293-309 (2018).

Thank you!

MIRari:	anders.engdahl@med.lu.se
SoftiMAX:	karina.thanell@maxiv.lu.se
NanoMAX:	ulf.johansson@maxiv.lu.se
ForMAX:	kim.nygard@maxiv.lu.se
Tensor SAXS:	marianne.liebi@chalmers.se
MedMAX:	rajmund.mokso@maxiv.lu.se
	and martin.bech@med.lu.se
DanMAX:	Innokenty.kantor@maxiv.lu.se

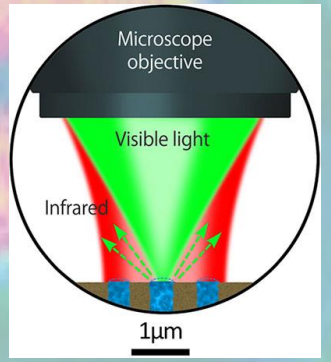
SAXS: eg. Organizational structure of (macro)molecules, like fibrils – distances, orientations.
TensorSAXS: a 3D implementation of this, both done at ForMAX. Regular SAXS also at CoSAXS (= open for users)
MIRari: using IR light for very sensitive molecular vibration contrast – characteristic resonances indicate eg. Phosphate, triglycerides – etc.
DanMAX: imaging like MedMAX (simplified) but the beamline is not dedicated to biological samples. **Open for first users 2021**

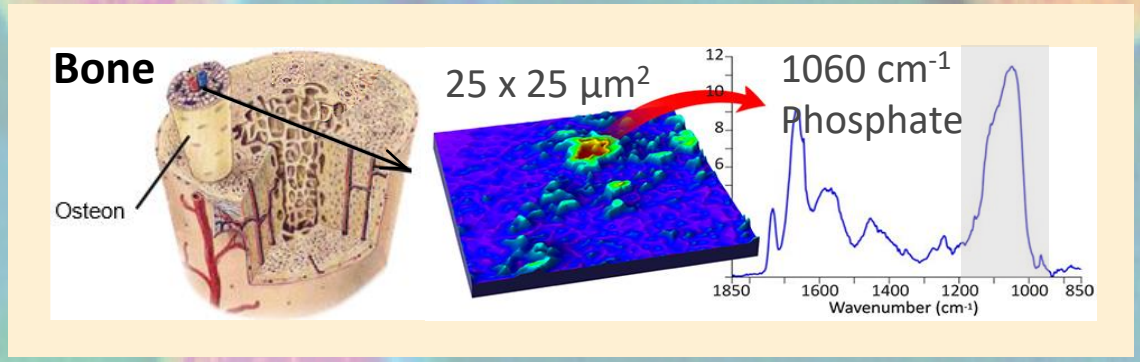
Wish list beamline

Microscopy for InfraRed And Rapid Imaging: MIRaRI

What? *An IR microscope for imaging, based on chemical contrast, for 2D imaging at the (sub) µm scale, of samples with complex chemistry.*

- *mIRage* - spatial resolution of 500 nm over the whole MIR region.
  Uses IR light for photothermal excitation + a visible laser for analysis.



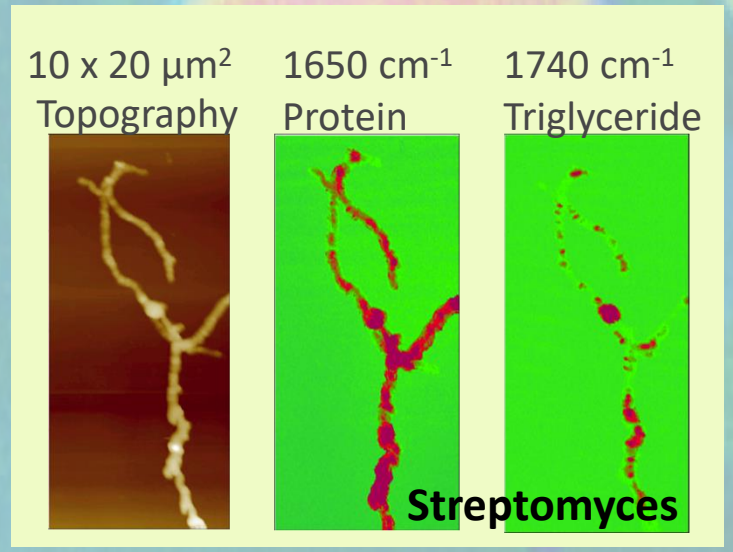


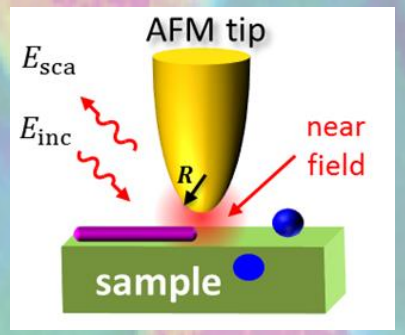
Microscopy for InfraRed And Rapid Imaging: MIRaRI

What? *An IR microscope for imaging, based on chemical contrast, for 2D imaging at the (sub) µm scale, of samples with complex chemistry.*

- *mIRage* - spatial resolution of 500 nm over the whole MIR region. Uses IR light for photothermal excitation + a visible laser for analysis.

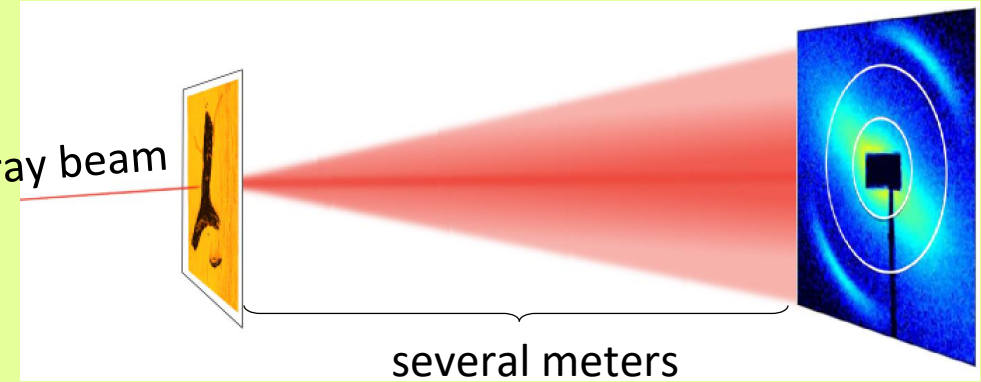
- *AFM-IR* (or s-SNOM): spatial resolution down to ~20nm. IR light is coupled to an AFM-tip, where the tip diameter sets the resolution.



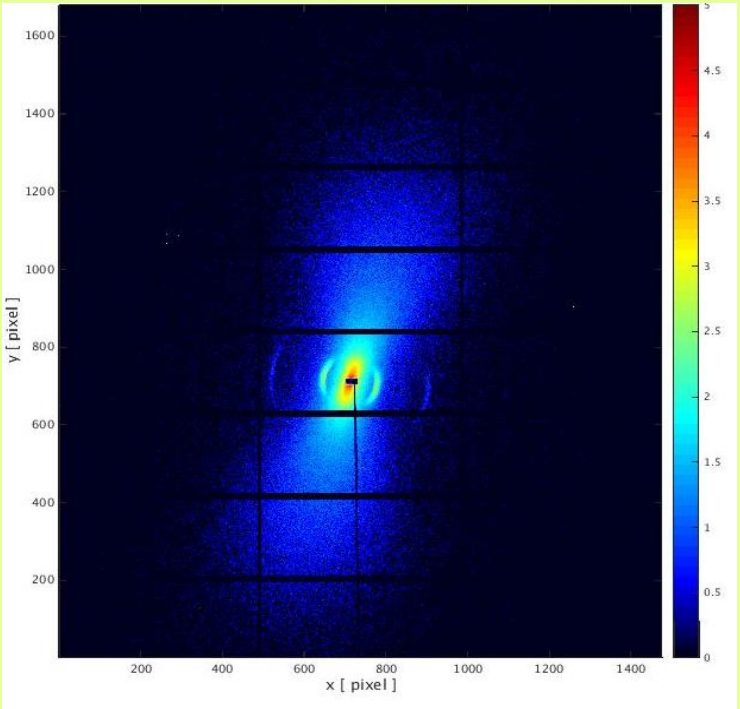


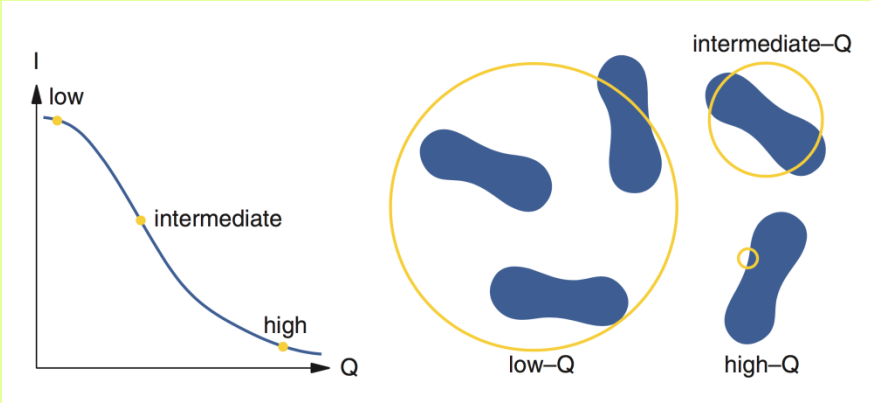
kim.nygard@maxiv.lu.se

Small-angle x-ray scattering on solid samples



Single photon counter





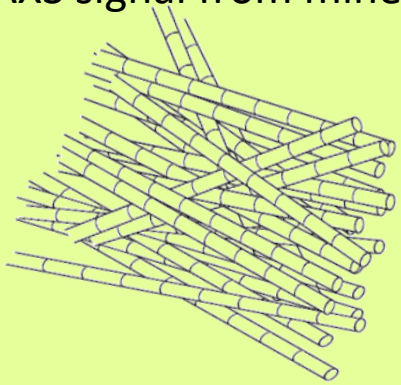
Willmott, P., John Wiley & Sons, Ltd: 2011.

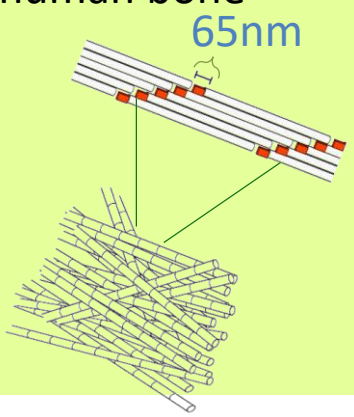
Combined SAXS/WAXS
q-range 6 x 10^{-4} – 6 $Å^{-1}$
Probing length scales between 1Å and 1µm

Small-angle x-ray scattering on bone

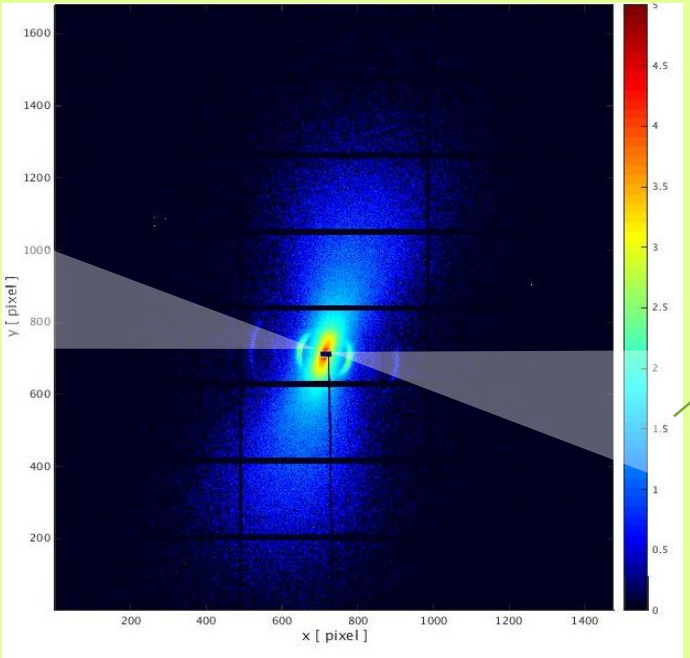
Bragg's law $n\lambda = 2d \sin\theta$

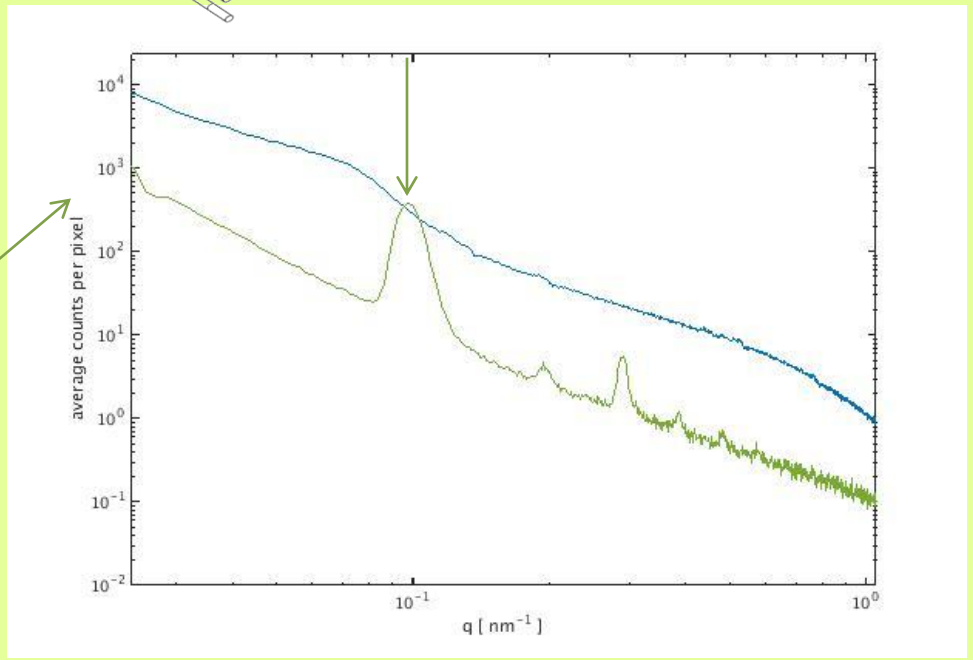
SAXS signal from mineralized collagen in human bone





spacing of gaps along collagen fibrils





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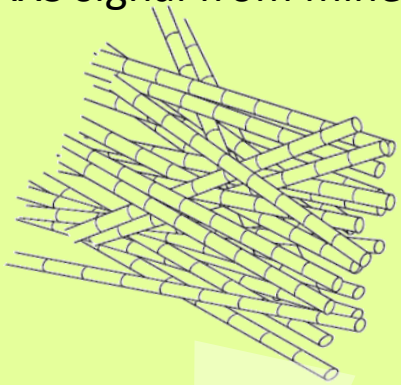
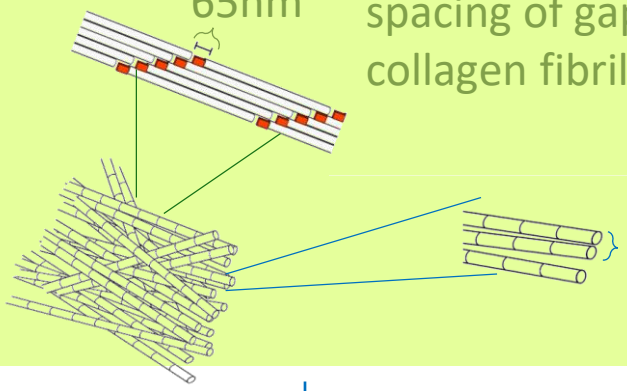
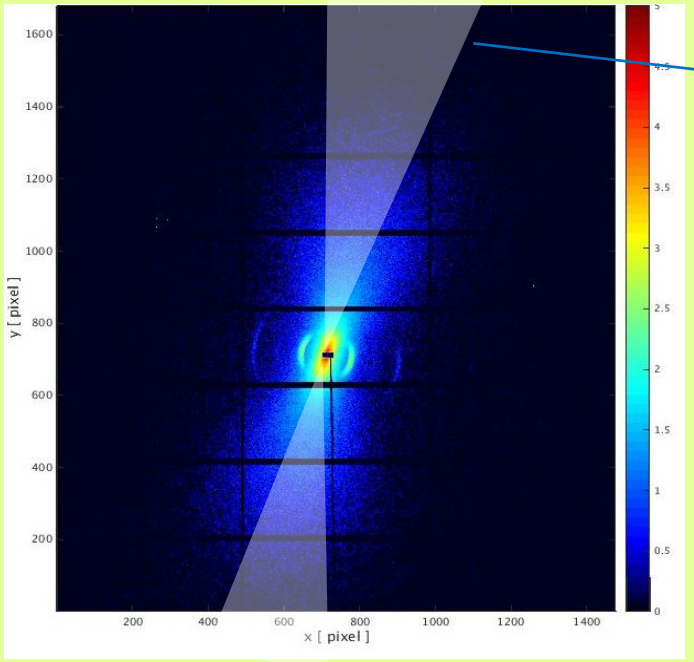


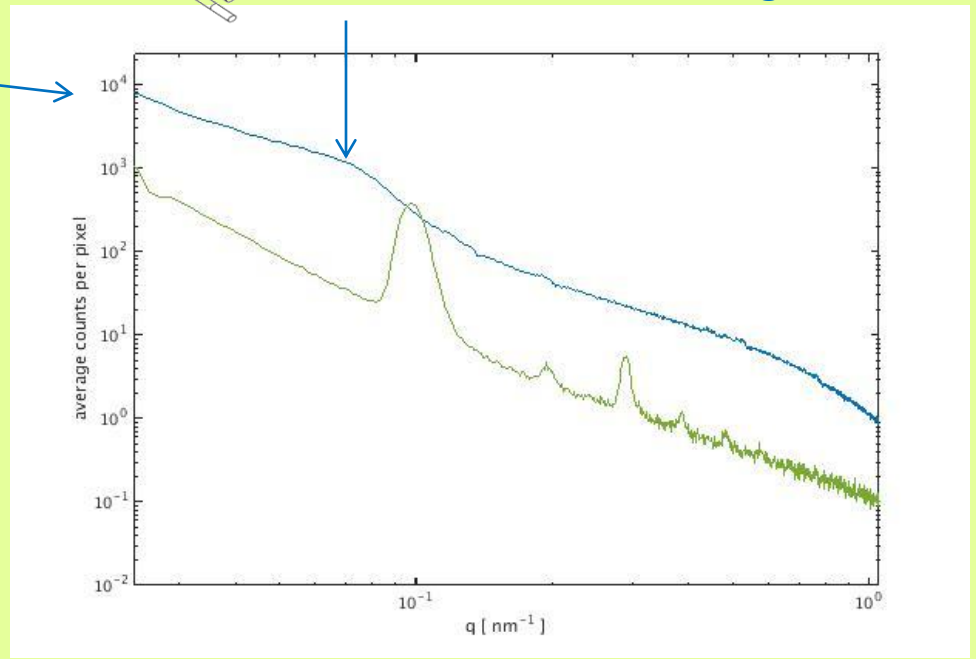
65nm

spacing of gaps along collagen fibrils



50 – 200 nm diameter of collagen fibrils



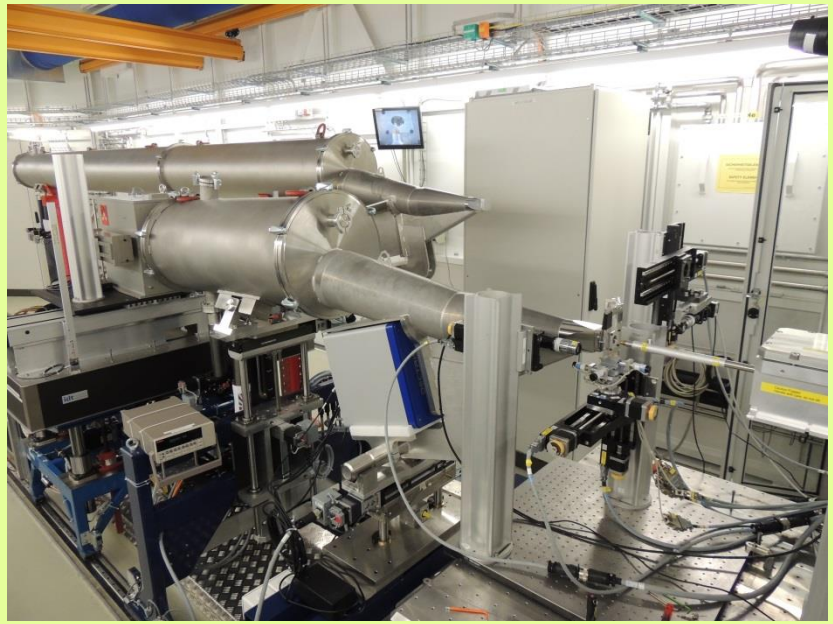


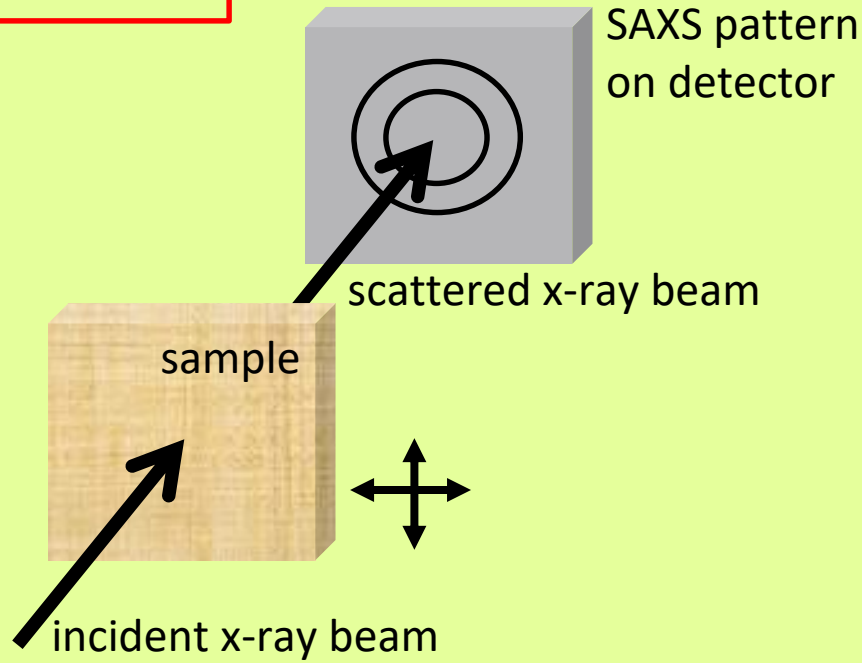
marianne.liebi@chalmers.se
kim.nygard@maxiv.lu.se

Scanning SAXS imaging

SAXS: Size, shape, & orientation of nanostructures

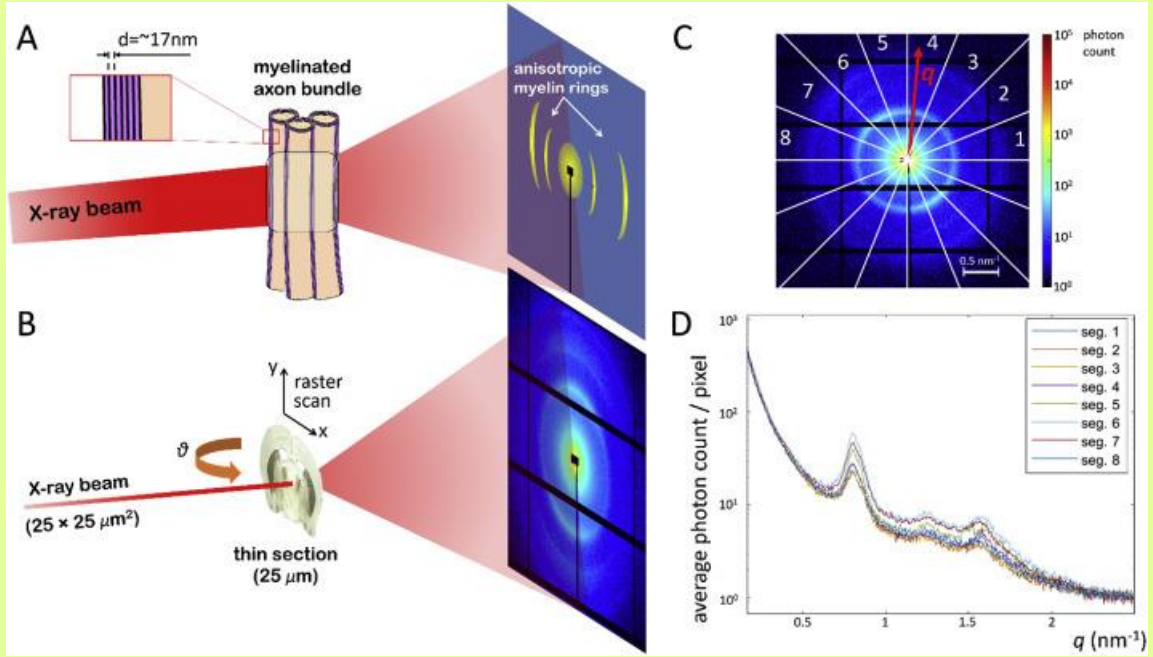
Scanning SAXS: Spatially resolved SAXS

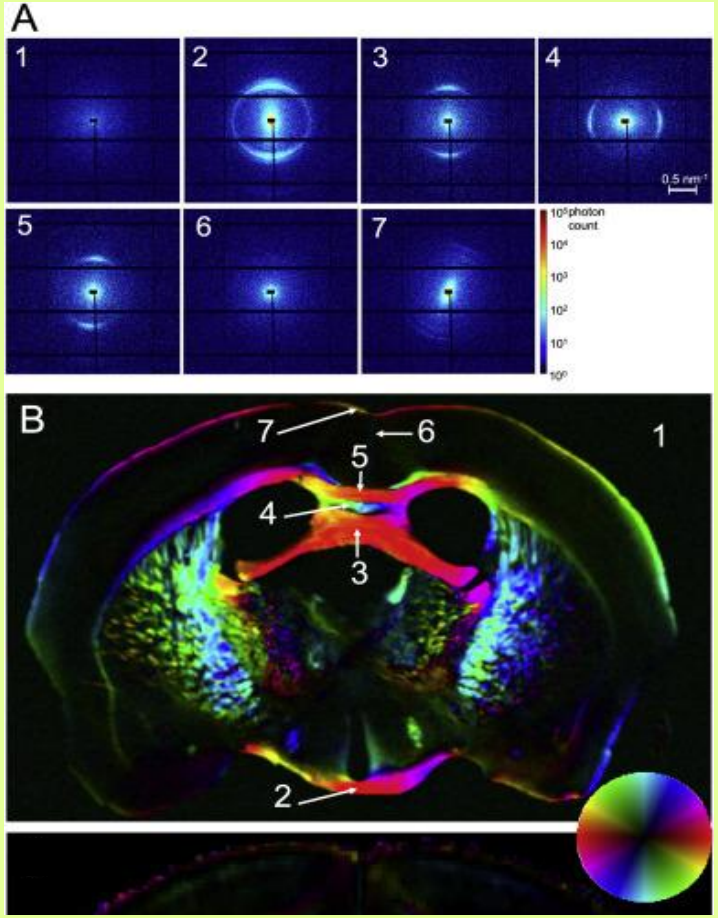




X-ray beam size → real-space resolution (≈ 1x1 μm^2 – 50x50 μm^2)

SAXS pattern → nano-scale features (≈ 1 – 500 nm)





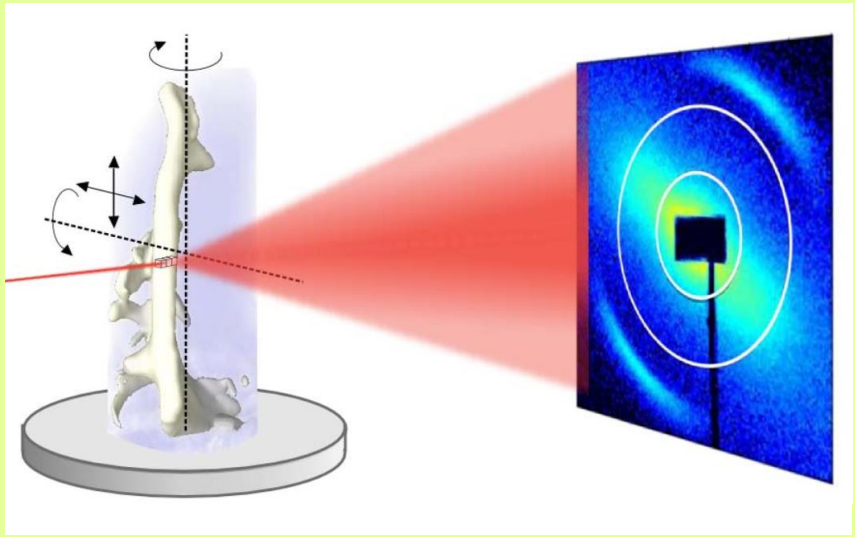
M. Georgiadis *et al*., NeuroImage **204**, 116214 (2020)

Small-angle X-ray scattering (SAXS) probes myelin sheath's periodic structure.

3D scanning SAXS (3D sSAXS) is applied on thin mouse brain sections, retrieving orientation distribution functions (ODFs) of myelinated axons per voxel.

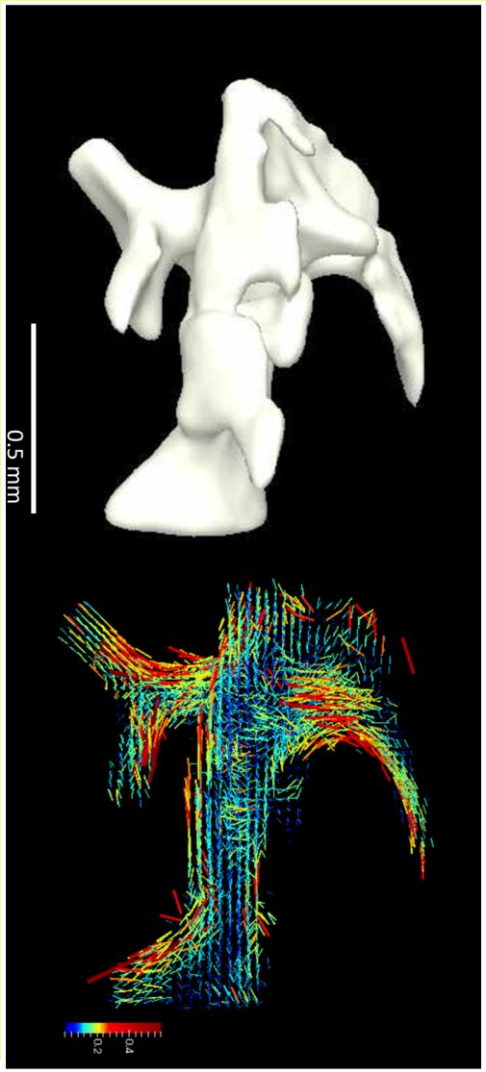
marianne.liebi@maxiv.lu.se

Also in 3D: SAXS tensor tomography



Length scales: ≈ nm to mm
Time scales: ≤ 1 minute (2D)
≤ 1 hour (3D)

Possible examples:
(1) Nanocomposite characterization
(2) 3D cellulose fibril structure in cell walls



Liebi *et al*, Nature **527**, 349 (2015)