X-RAY IMAGING & X-RAY MICROSCOPY

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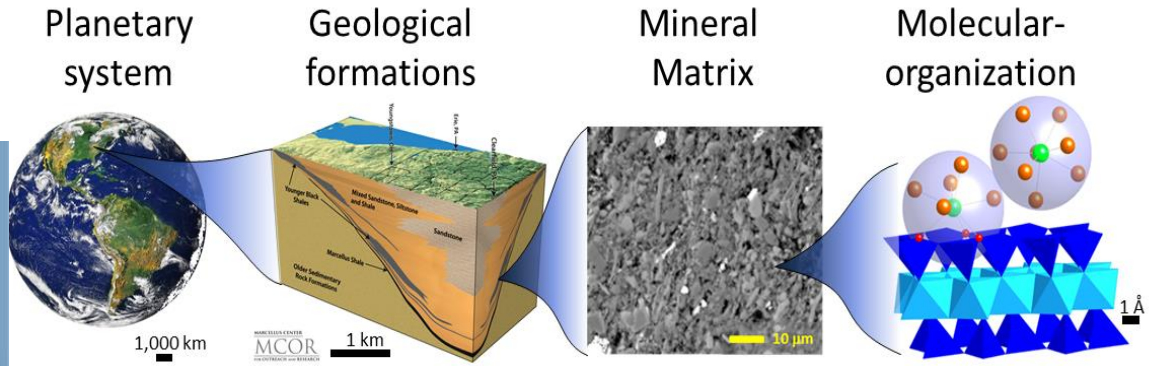
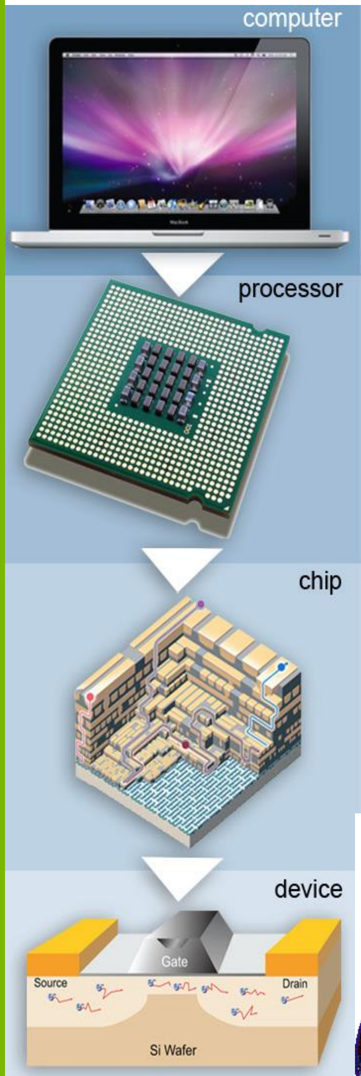
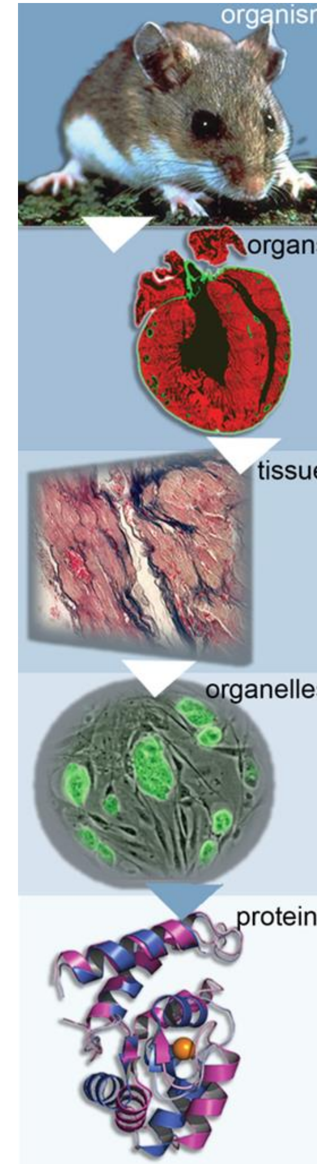
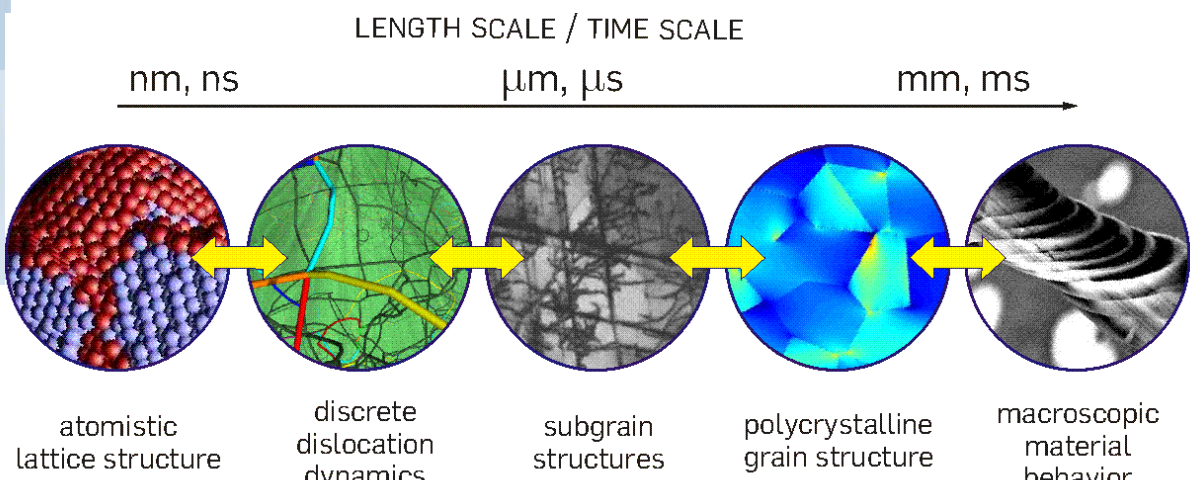






HIERARCHICAL STRUCTURE OF COMPLEX SYSTEMS
=>
NEED TO <u>VISUALIZE</u> <u>STRUCTURE</u> AND <u>FUNCTION</u> ON ALL RELEVANT LENGTHSCALES



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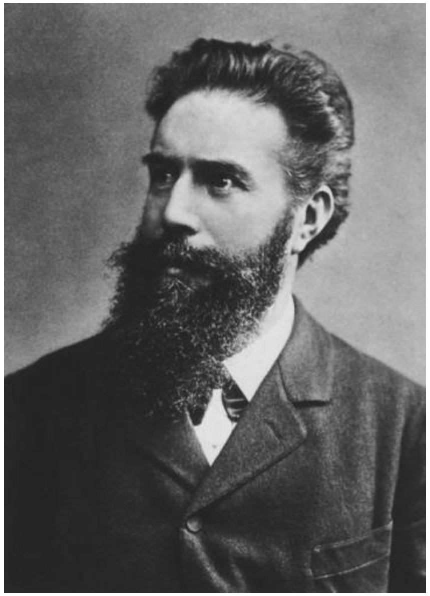
X-RAY IMAGING



Wilhelm Conrad Roentgen:

discovered x-rays 1895

Nobel Prize in Physics

Anna Roentgen's hand

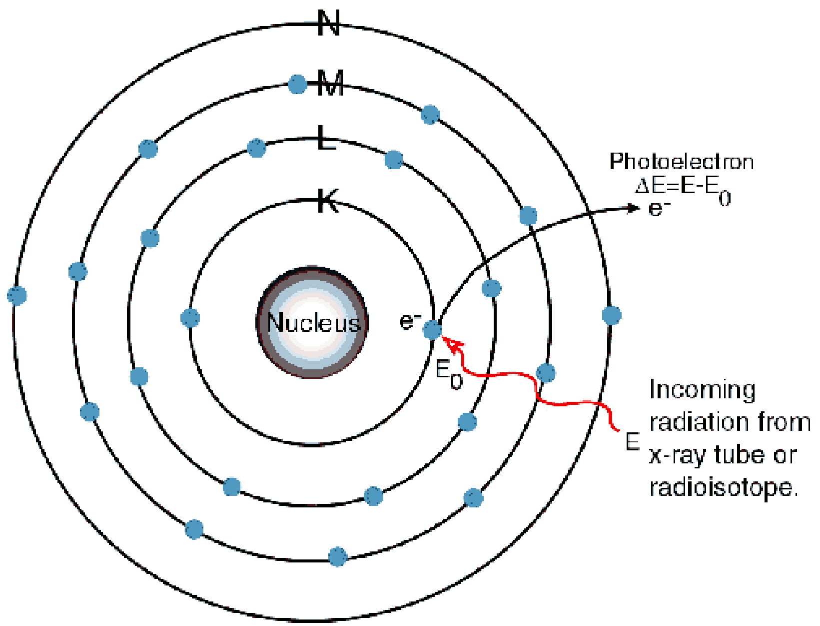
with wedding ring

Universität Würzburg

Dec. 1895

ABSORPTION OF X-RAYS

X-rays are absorbed by the *Photo-Electric Effect*



Albert Einstein

Nobel Prize in Physics

for Photo-Electric Effect

An x-ray has enough energy to kick out an electron bound to an atom

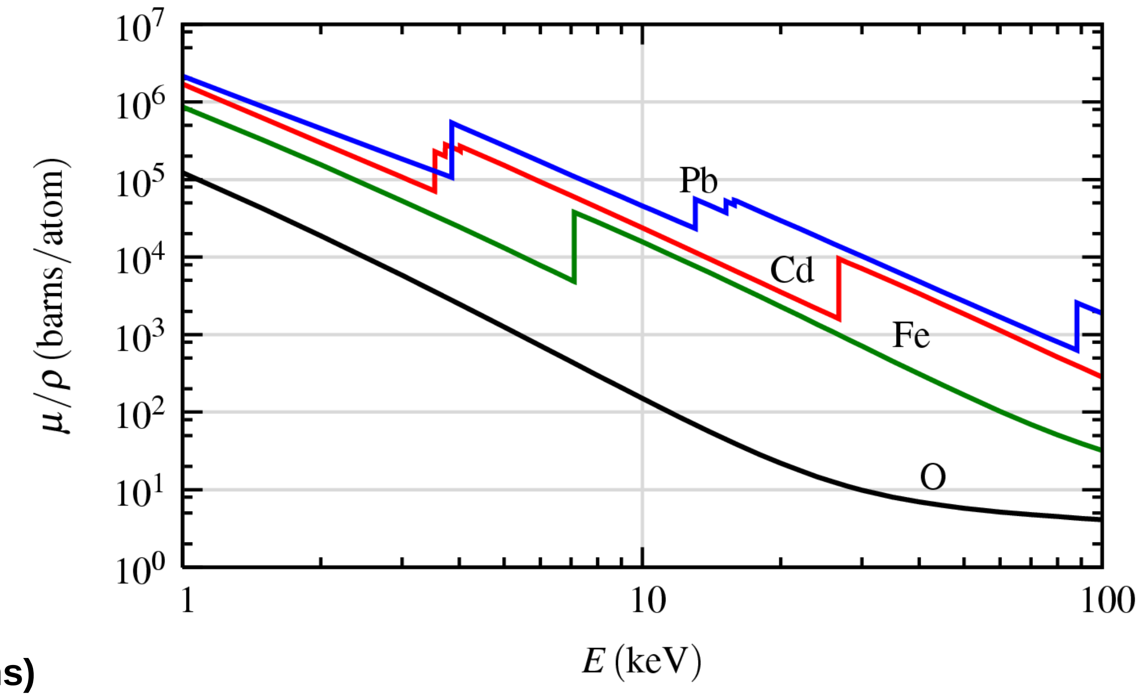
1. **the x-ray is absorbed**
2. **the core electron leaves the atom and becomes a *photo-electron***
3. **the atom is left without a core electron: in an *excited state***

X-RAY ABSORPTION COEFFICIENT

The x-ray absorption coefficient μ of a material depends *very strongly* on

1. **the density**
2. **the atomic composition**
3. **the energy of the x-ray**

$$\mu \sim \frac{\rho Z^4}{A E^3}$$



Where

ρ = sample density

Z = atomic number (# of electrons)

A = atomic mass

E = energy

$$I = I_0 \, e^{-\mu t}$$

This is why x-rays are used in medical imaging:

water (H_2O) is almost transparent to x-rays

bone ($CaCO_3$) is much more absorbing

lead is a really good x-ray absorber!

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Courtesy M. Newville

X-RAY ABSORPTION EDGES

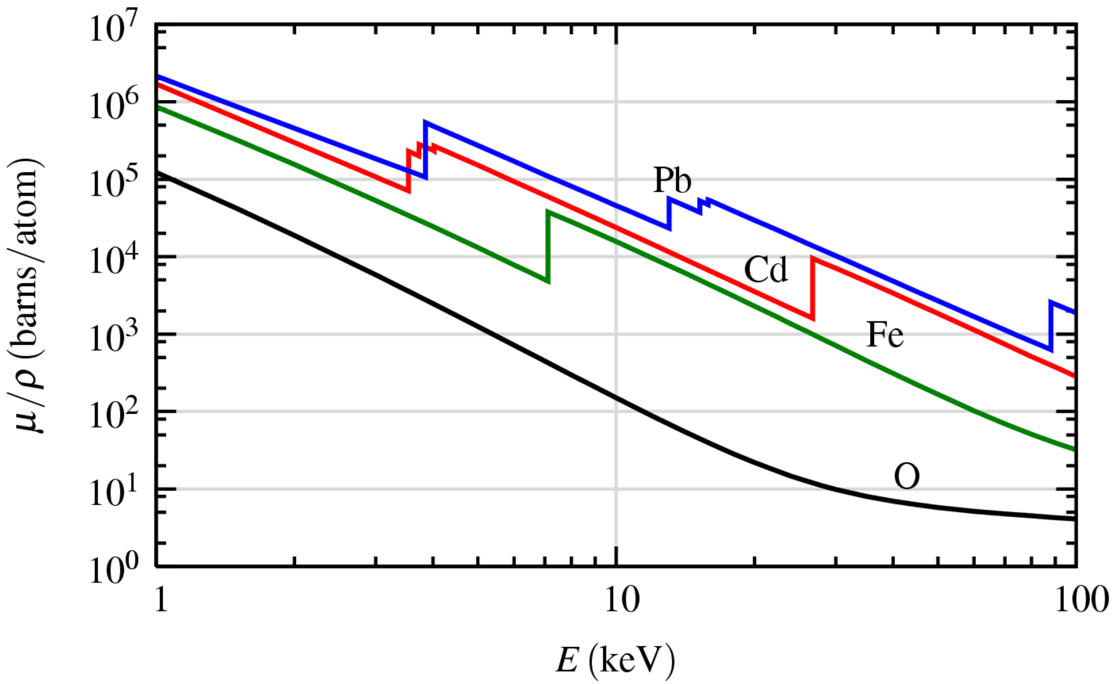
X-rays have energies comparable to ***binding energies*** **of electrons in atoms**

Notice the sharp jumps in Absorption:

Absorption Edges

These jumps occur at binding energies of core electrons:

x-rays have enough energy to kick out another bound electron



Some Binding Energies (eV)

H 1s	13.6
O 1s	545
Fe 1s	7112
Pb 1s	88005
Pb 2p3/2	13043

We can select energies to excite particular binding energy levels.

This lets us to adjust the contrast for detecting a particular element.

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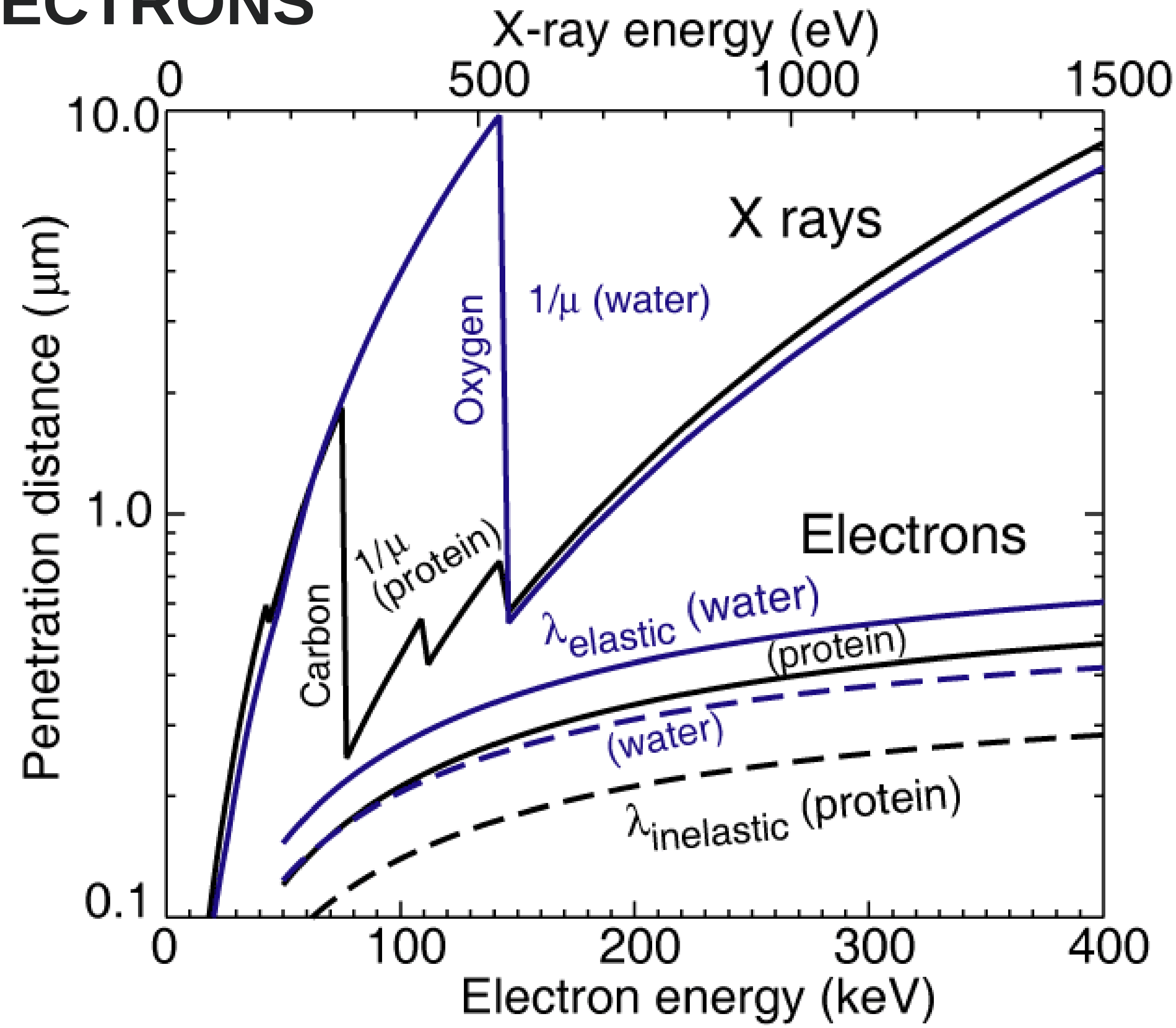
Courtesy M. Newville

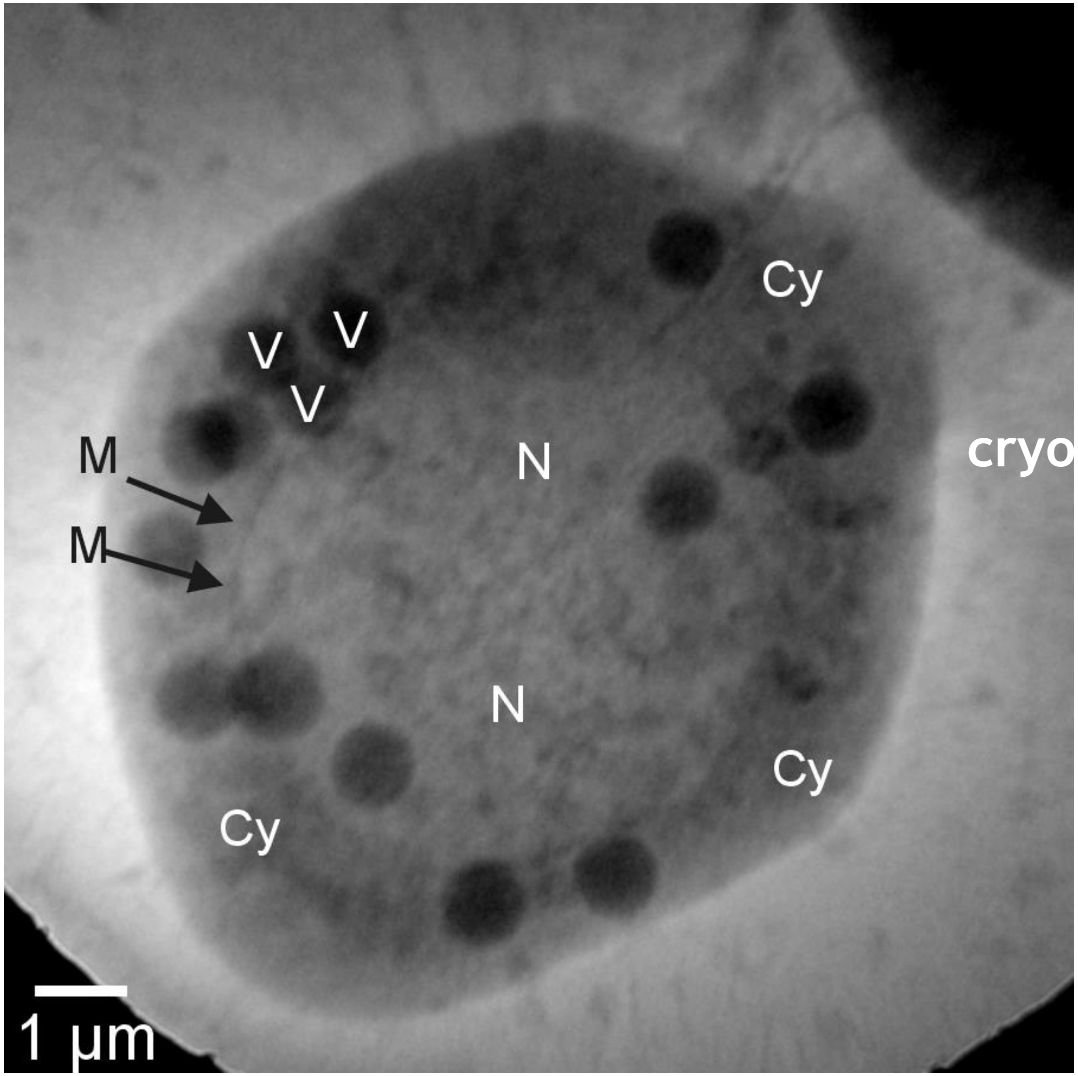
X RAYS AND ELECTRONS

Consider penetration distance: 1/*e* absorption length for x rays, scattering mean free paths for electrons



Courtesy C. Jacoben

X-RAY ABSORPTION CONTRAST IN THE WATER WINDOW

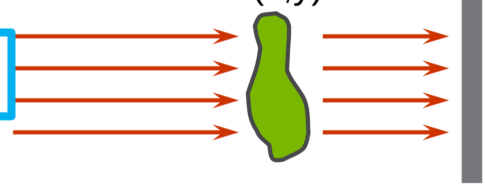


- *Drosophila melanogaster* cell, in vitrified ice, imaged @ 0.5 keV with the Goettingen TXM @ BESSY I. S. Vogt, *et al*

Cy: cytoplasm
V: vesicle
M: nuclear membrane
N: nucleus

DIRECT IMAGING VS SCANNING PROBE IMAGING

Direct Imaging



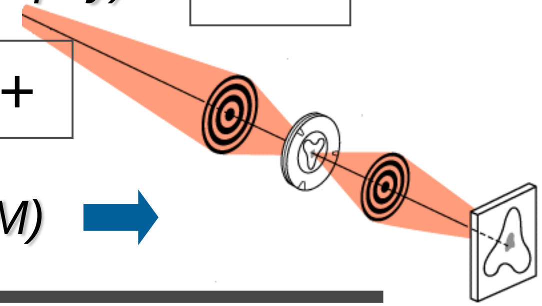
structure

Direct imaging (radiography) | +

Projection microscopy | +

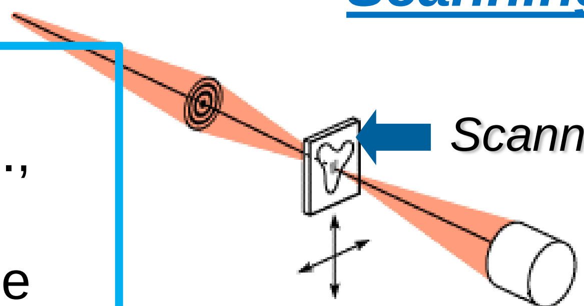
structure

- | Transmission microscope (TXM)



Scanning Probe

structure, elemental sens., chemistry, reciprocal space

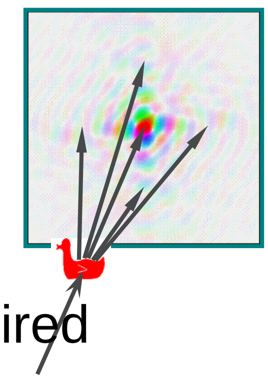


Scanning microscope (SXM) | ++

Ptychography | ++

structure, reciprocal space

++ | Coherent diffractive Imaging (CDI)



++	+	0	-

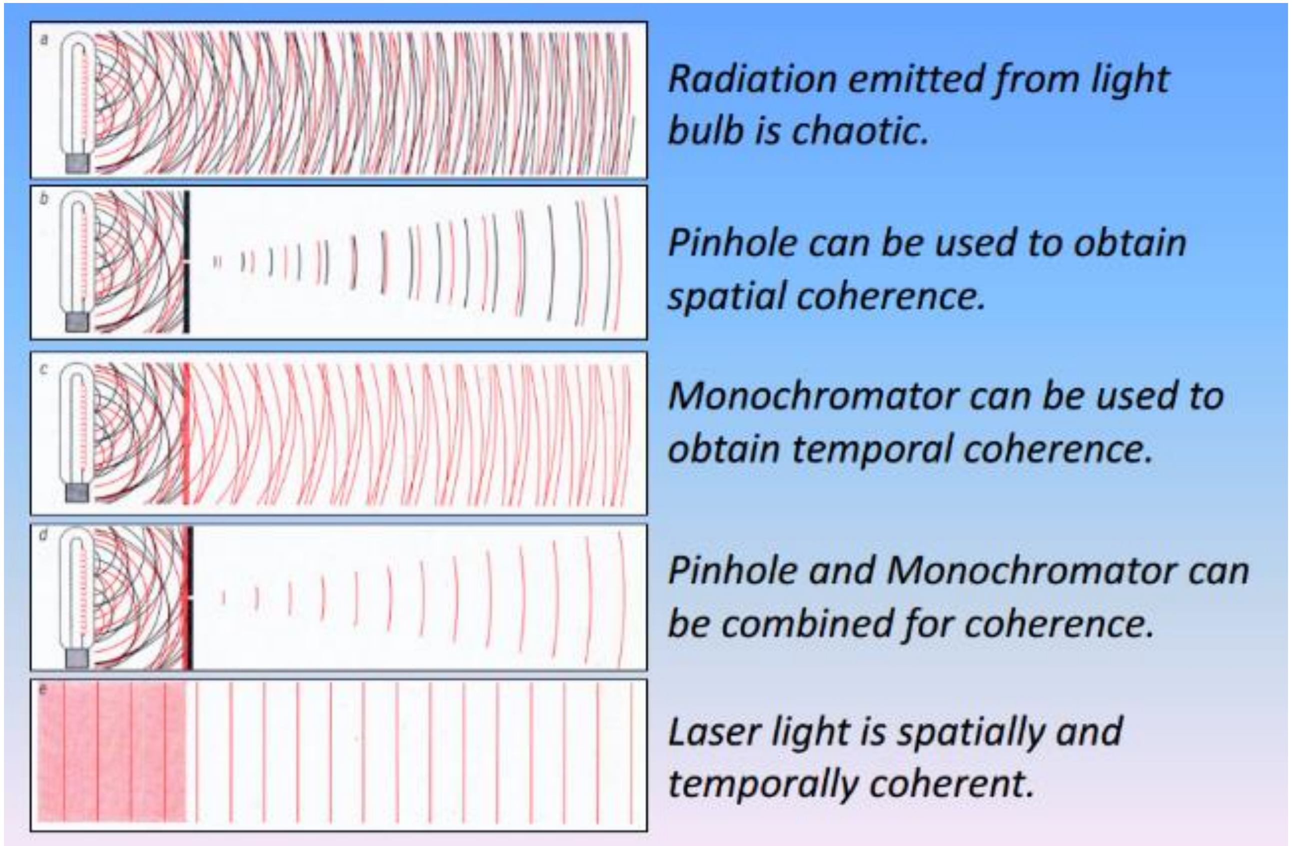
Level of coherence required / desired

X-RAY SOURCE

BRIGHTNESS (=BRILLIANCE) VS FLUX / INTENSITY, AND WHAT IS COHERENCE ?

100 W incandescent light bulb
(a lot of total flux / intensity, but goes into 4Pi sterradian

5mW laser pointer
Low total intensity, but very bright!!!
All light goes forward.

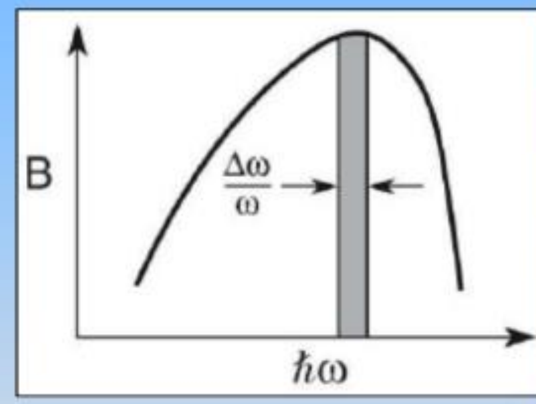


A. Schawlow (co-inventor of laser concept), Scientific Americans, 1968

- Coherent source: cannot distinguish the source from a point source
- You can make any source ‘coherent’ by putting it at infinity, or putting slits in front of it

=> For **microprobes**: need **coherent source to achieve diffraction limited spatial resolution**

Brightness



$$B = \frac{\text{Photons in unit spectral range in unit time}}{(\text{source size} \times \text{divergence})^2}$$

→ **Peak** / **Average**

Units: photons/s/mm^2/$mrad^2$/0.1%BW

13

=> For microprobes: brightness of sources determines amount of focussed flux on sample

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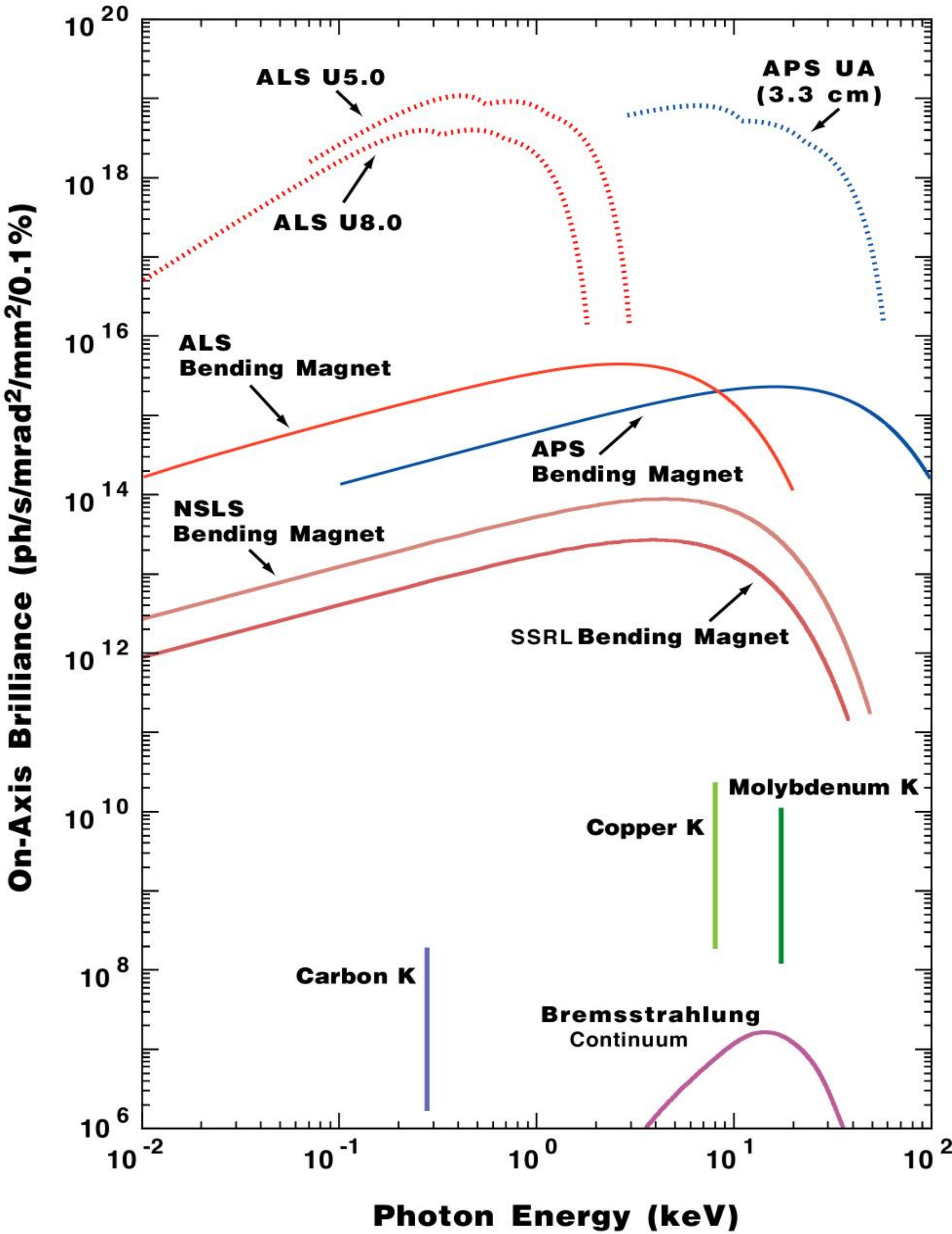
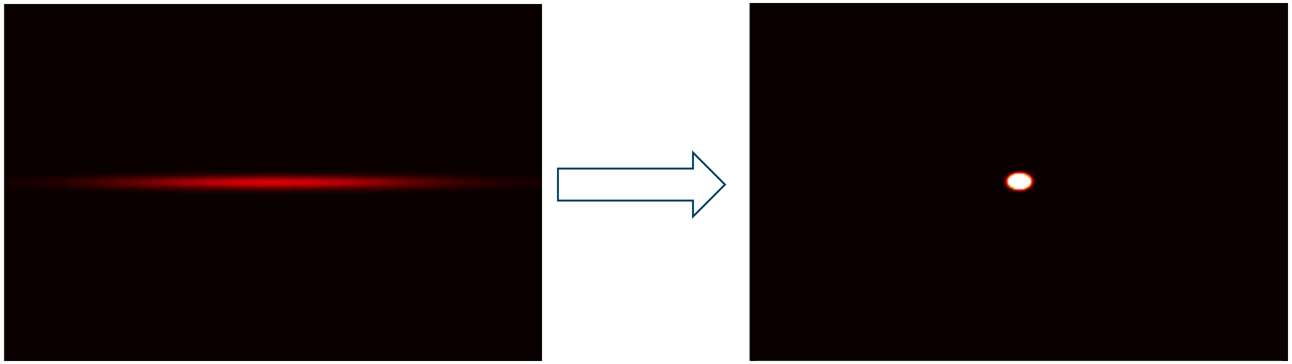
SR X-RAY SOURCES

- Typically, SR sources are large horizontally (~1mm), small vertically (~.05mm)
- source is imaged (demagnified) into the specimen – to achieve diffraction limited spatial resolution, need to use (horizontal) slits to define a small 'virtual' source (spatially coherent source)
- High brightness sources optimal for microprobes
- NB: typically, X-rays are polarized in horizontal direction
  - ⇒ Scattering in plane at 90 degrees is minimized, optimum position for XRF detector is at 90 degrees to the side of the incident beam

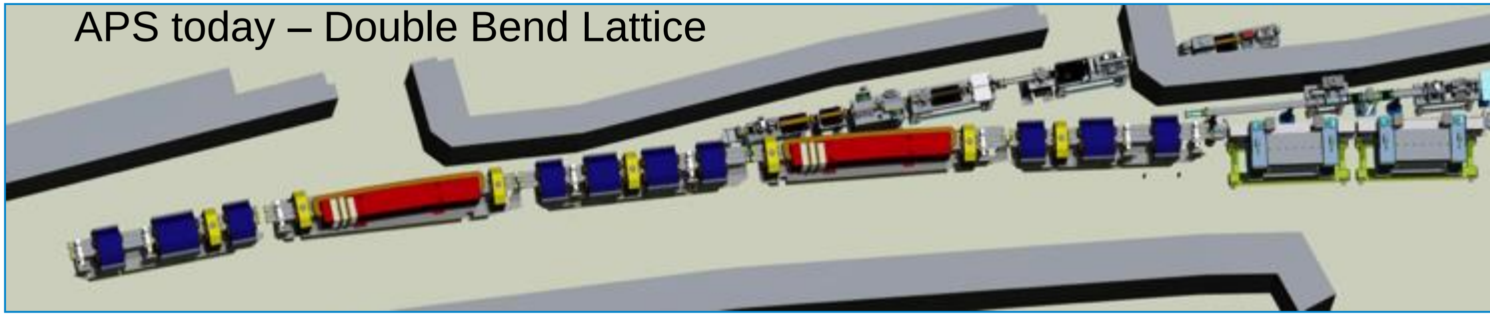


A REVOLUTION FOR SR SOURCES: MULTIBEND ACHROMATS

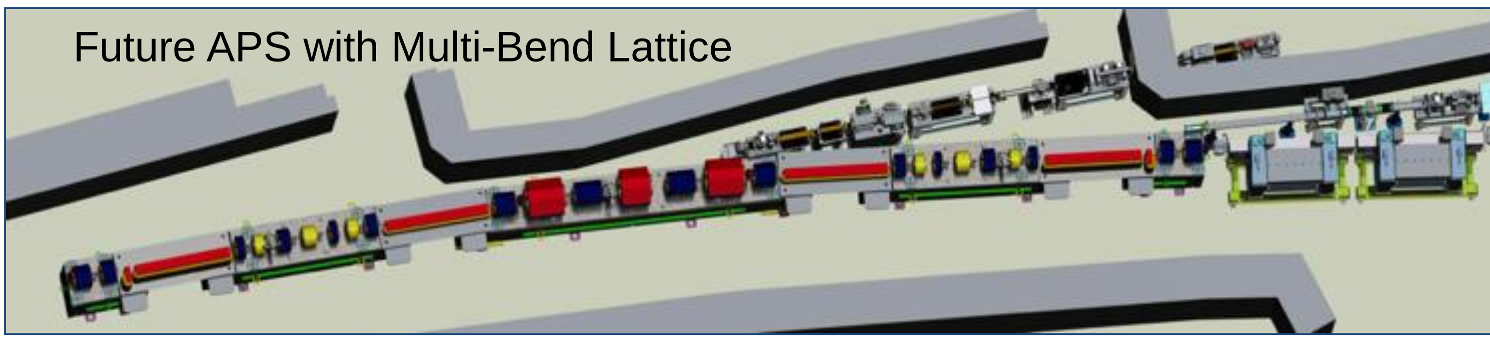


- Reduce Horizontal emittance to match vertical emittance (ie, round source)
- For example, can focus the full flux of APS into a ~250 nm spot!
- Can speed up u-XRF (and u-XRD) by factors of 100 - 1000x
- (Nearly) any technique can become a microscopic technique ….

APS Upgrade multi-bend achromat lattice concept



~50x reduction in horizontal emittance

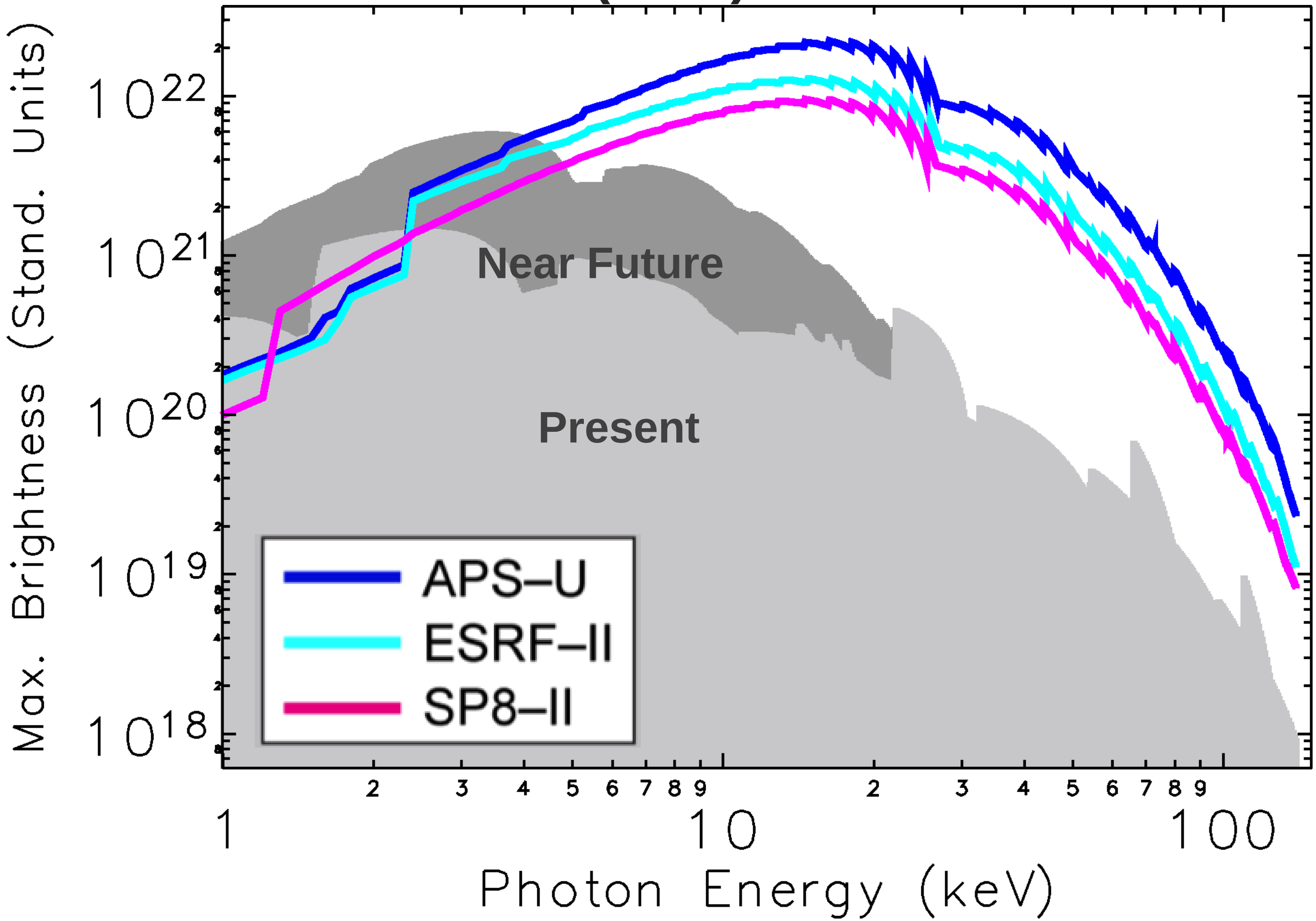


$$\varepsilon_x = C_L \frac{E^2}{N_D^3}$$

N_d = Number of dipoles per sector (N_d = 7 for APS MBA)
E = Beam energy (E = 6 GeV for APS MBA)

- increase current by 2x, also use optimized insertion devices
- work continues to further increase gains

EXCITING DEVELOPMENTS: MULTIBEND ACHROMATS (MBA)



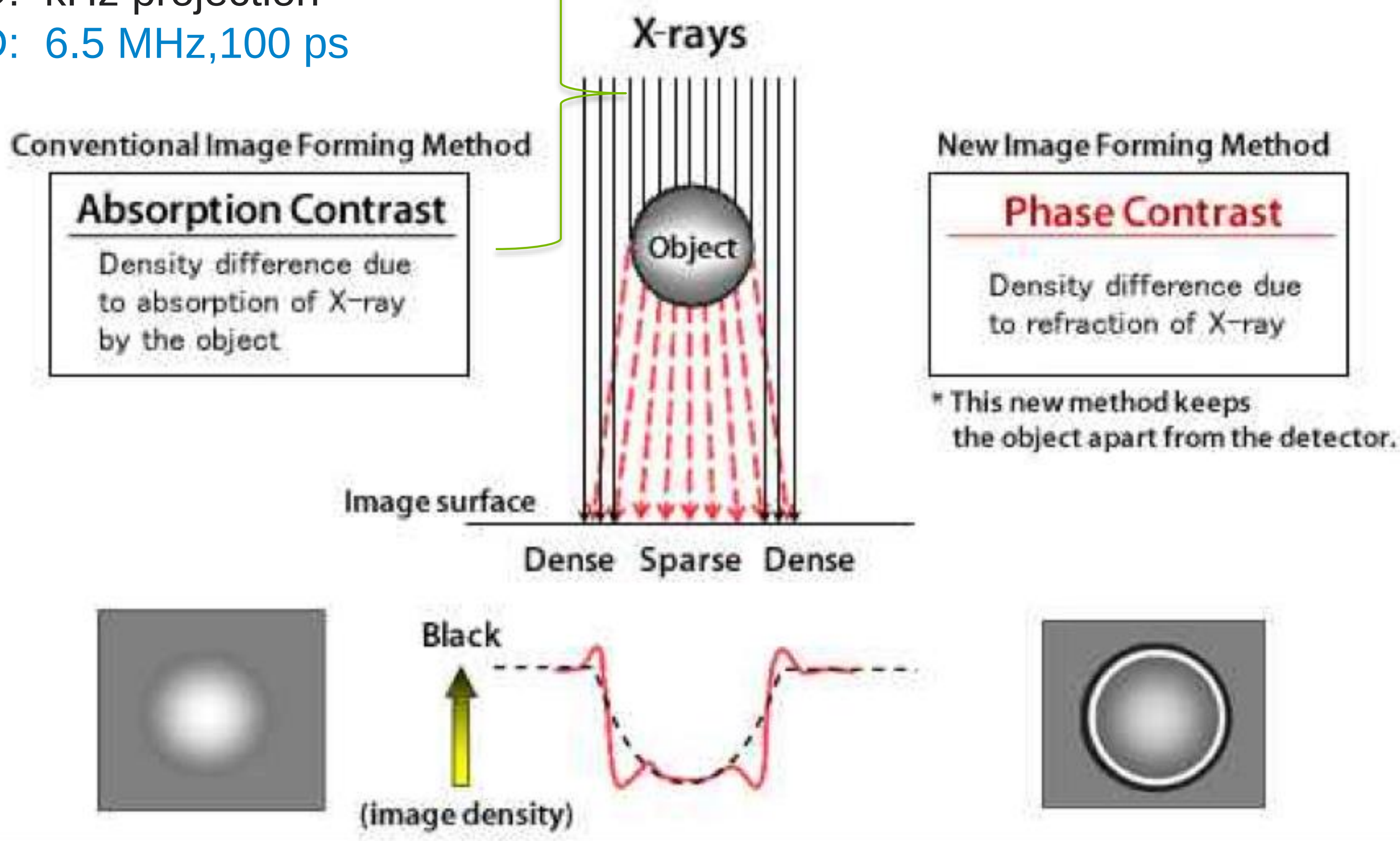
Curves for APS, ESRF and SP8 upgrades based on present designs, assuming identical undulators

FULL FIELD IMAGING

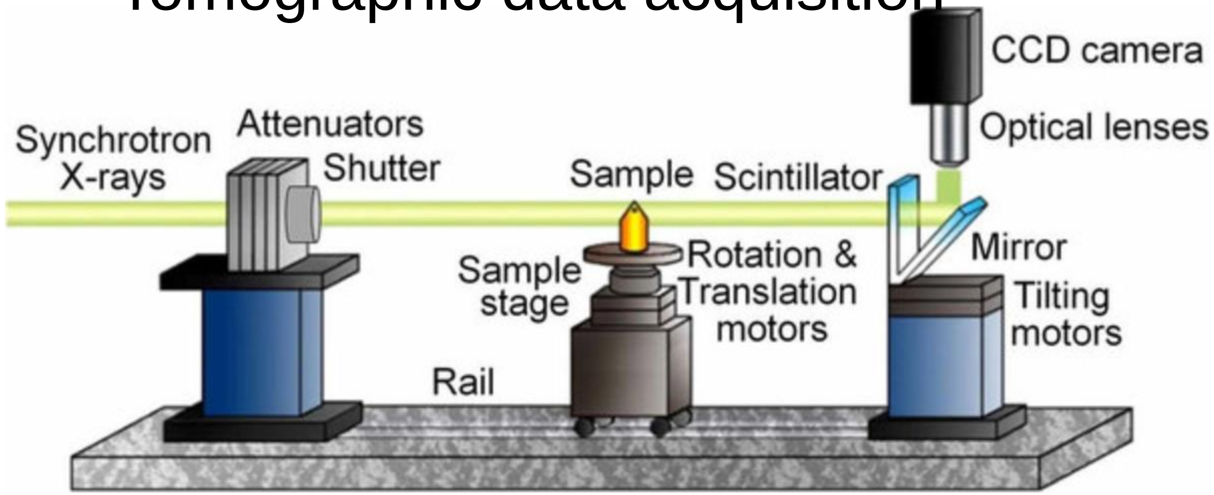
2D/3D/4D Imaging with the APS

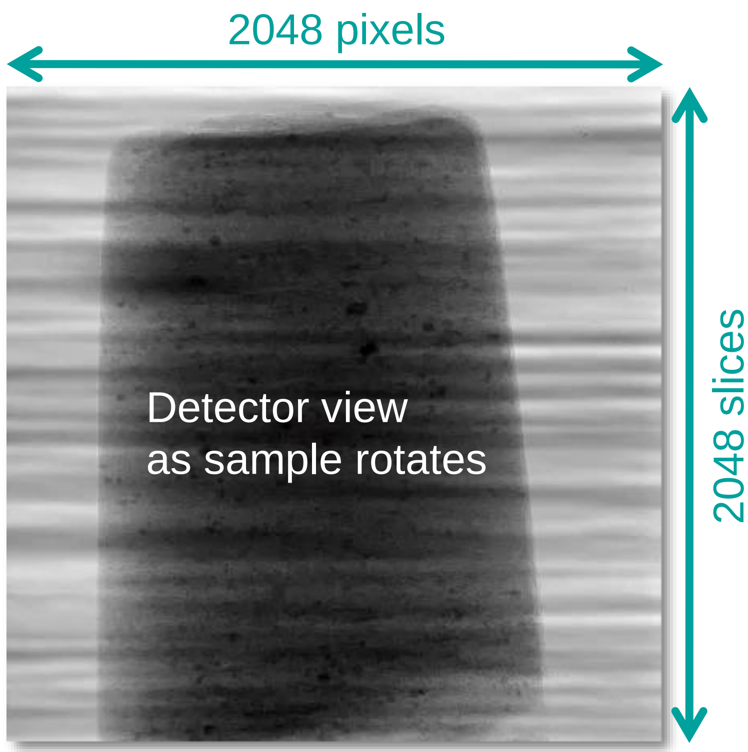
✦Parallel Beam Imaging (PBI)

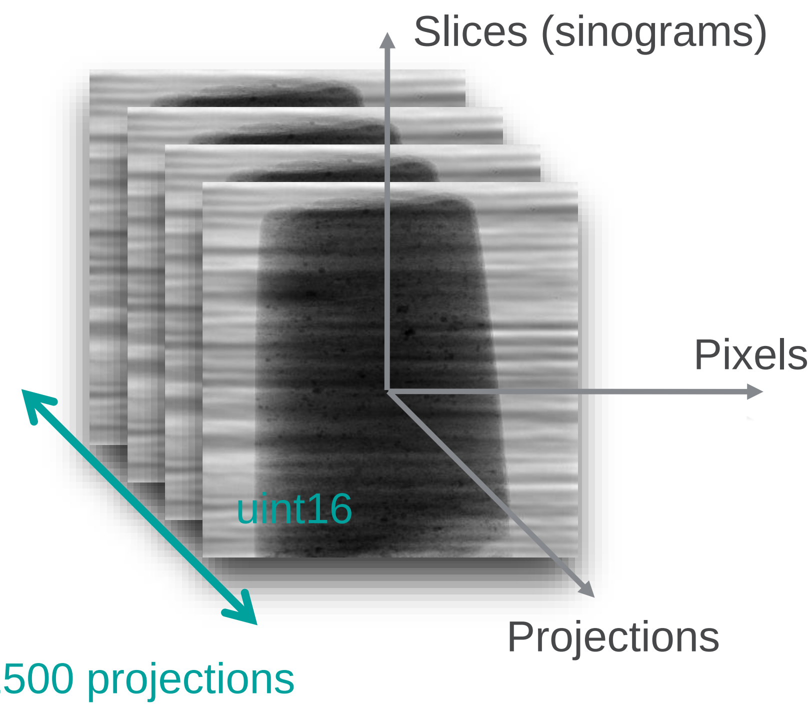
- Phase and absorption
- Spatial resolution: 1 µm
- Temporal resolution:
  - 3D: kHz projection
  - 2D: 6.5 MHz,100 ps



Tomographic data acquisition







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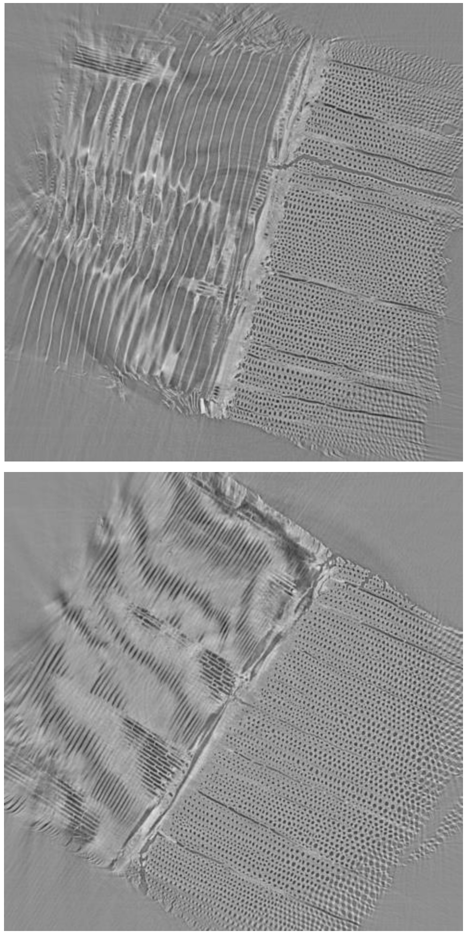
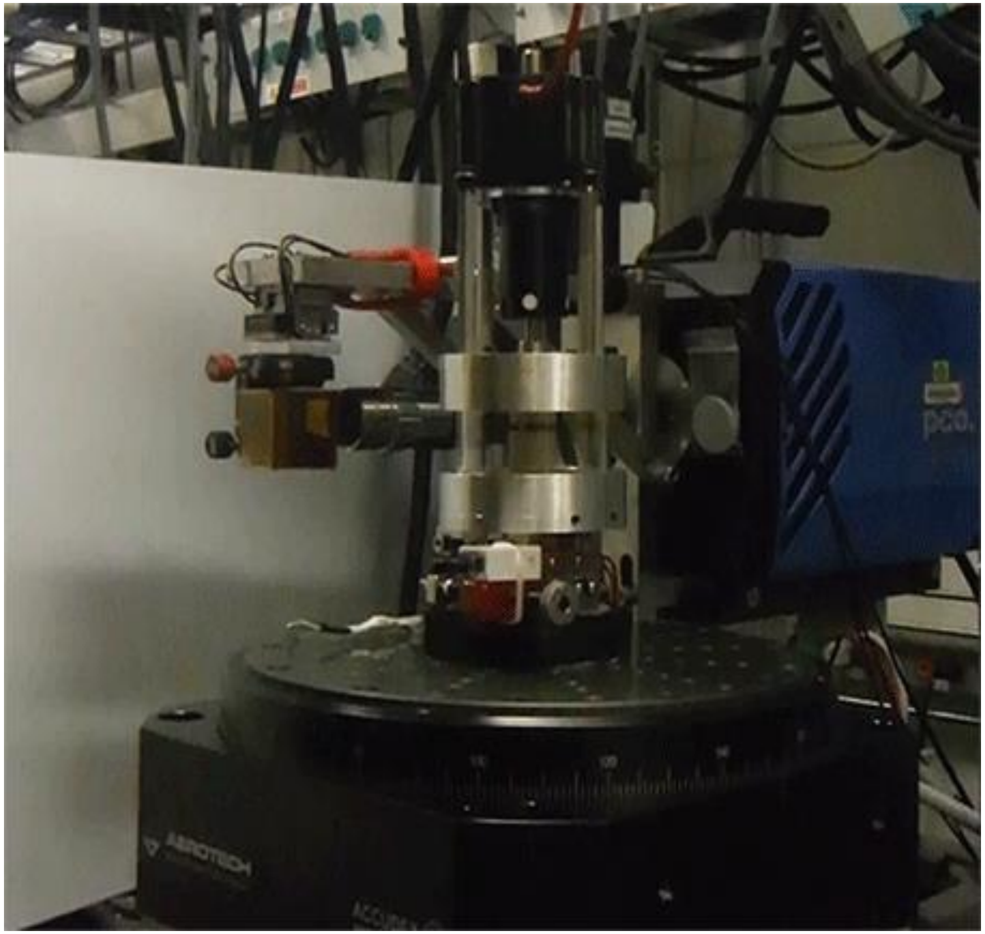
DYNAMIC IMAGING

3D imaging of dynamic systems



Wood adhesive bondline swelling and shrinking
J. Jakes, USDA Forest Service, Forest Products Laboratory, Madison, WI



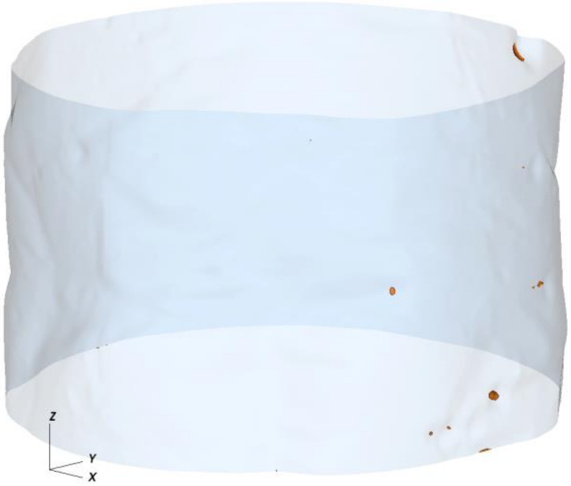
Xianghui Xiao, APS Imaging Group

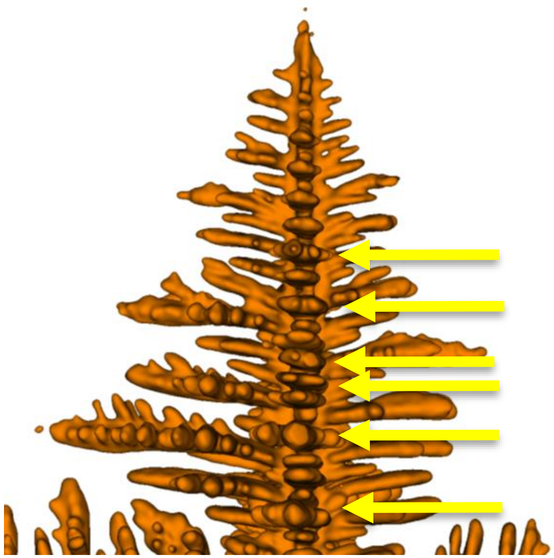
DYNAMIC IMAGING

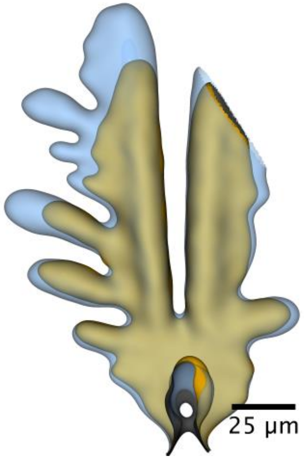
3D imaging of dynamic systems

- Parallel Beam Projection
  - Phase and absorption
  - Spatial resolution: 1 µm
  - Temporal resolution:
    - 3D: 1000 projection/s

Growth of Al-rich dendrite in Al-Cu alloy
Cooling rate 1K/min from 550 K
3D tomographic dataset in 1.6 s







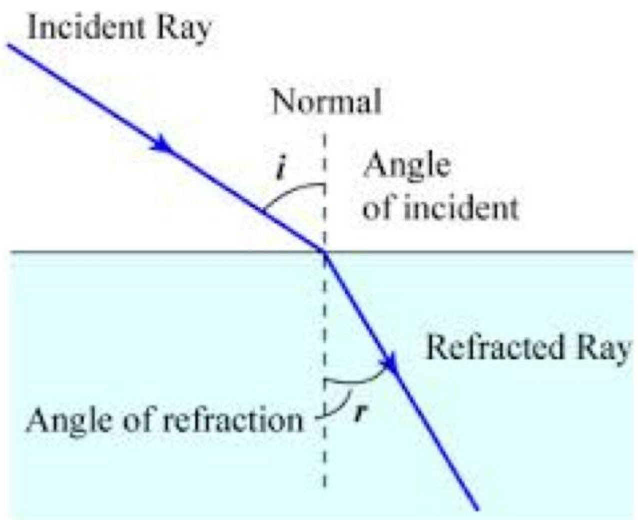
3.6 s

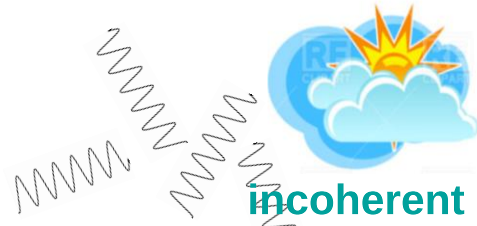
J.W. Gibbs, K.A. Mohan, E.B. Gulsoy, A.J. Shahani, X. Xiao, C.A. Bouman, M. De Graef, P.W. Voorhees, "The Three-Dimensional Morphology of Growing Dendrites," Sci. Rep. 5, 11824-1-11824-9 (2015).

K. Aditya Mohan, S.V. Venkatakrishnan, John W. Gibbs, Emine Begum Gulsoy, Xianghui Xiao, Marc De Graef, Peter W. Voorhees, Charles A. Bouman, "TIMBIR: A Method for Time-Space Reconstruction From Interlaced Views," IEEE T. Comp. Imaging 1 (2), 96-111 (2015).

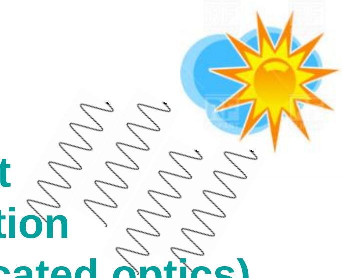
Phase contrast imaging

Refraction of waves:

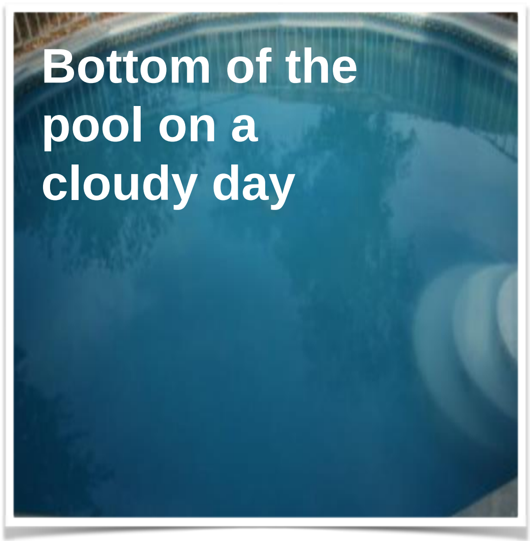


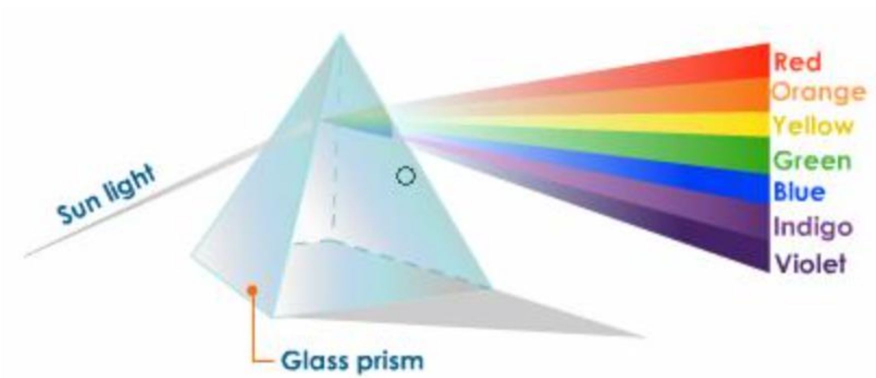


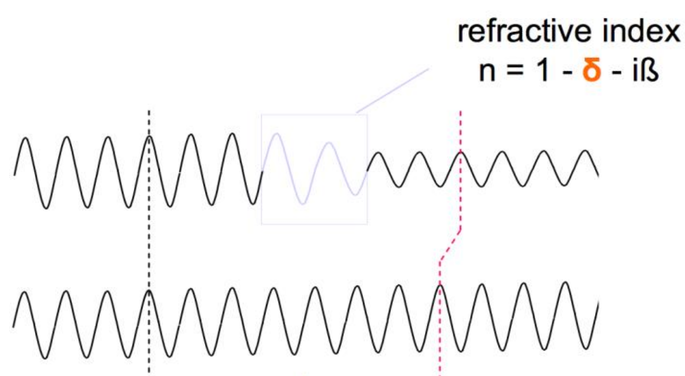
incoherent illumination



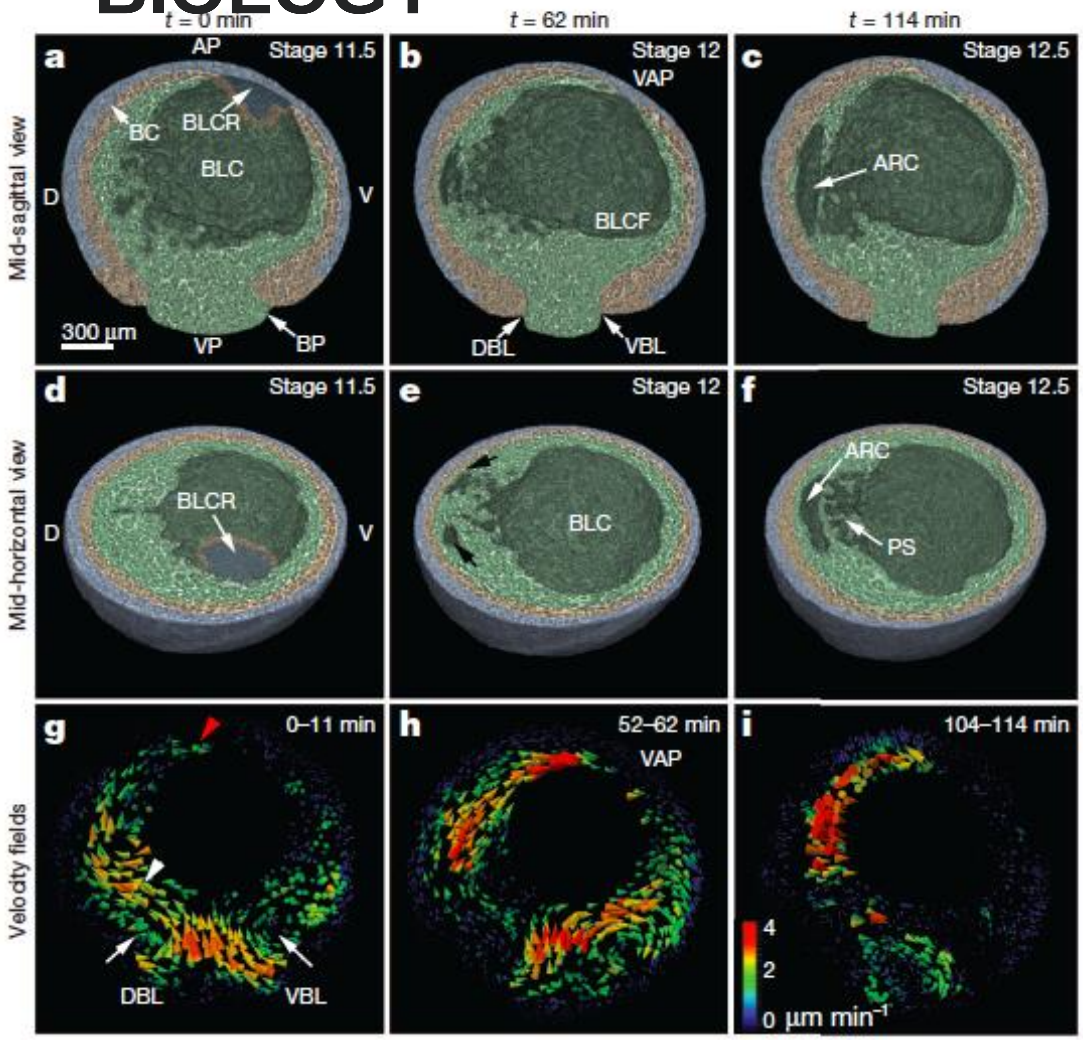
partially coherent Illumination (or dedicated optics)

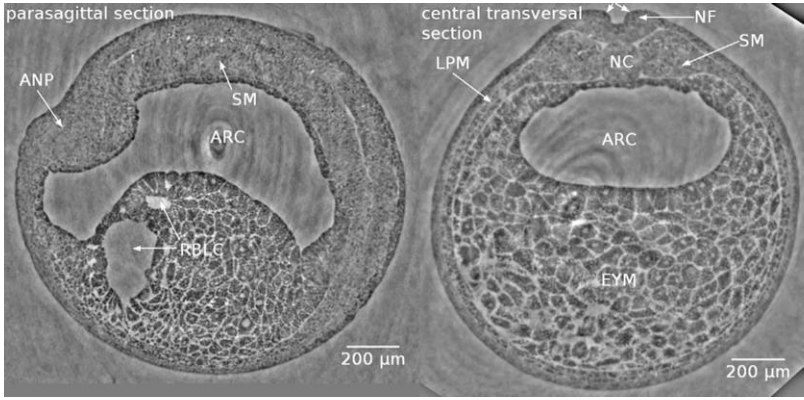






IN VIVO X-RAY PHASE-CONTRAST MICROTOMOGRAPHY FOR DEVELOPMENTAL BIOLOGY





- During gastrulation: series of dramatic, coordinated cell movements drive reorganization of a simple ball or sheet of cells into a complex multi-layered organism.
- Use time resolved x-ray tomography to follow structural reorganization during embryonic development

Moosmann *et al*, *Nature* **497**, 374–377 (16 May 2013)

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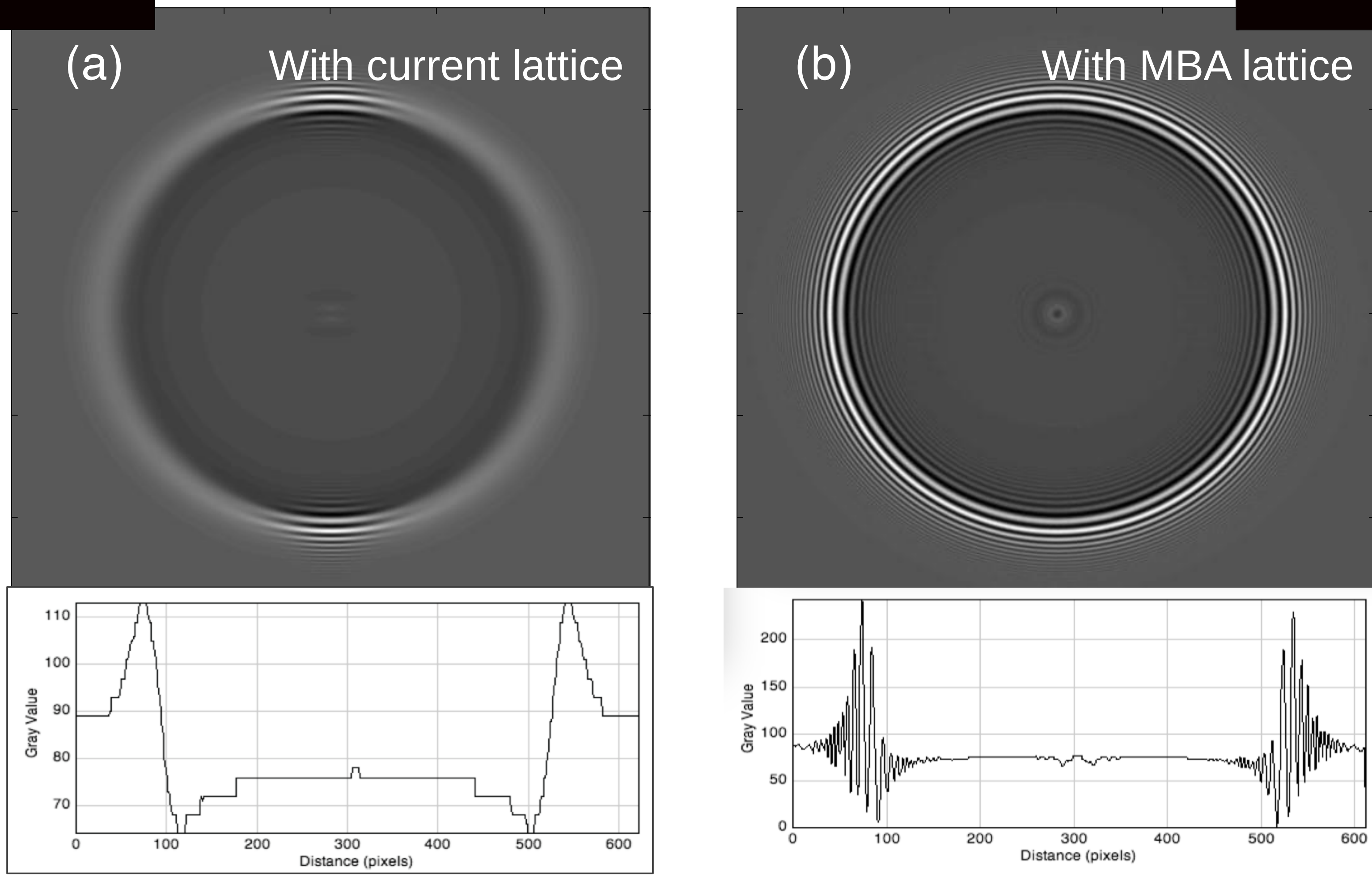
PARALLEL BEAM PROJECTION IMAGING WITH APS-U



Simulated images of a water drop (0.4 mm diameter) with an air bubble inside (2 µm diameter) at 15 keV. (a) With current APS lattice, (b) with future MBA lattice.

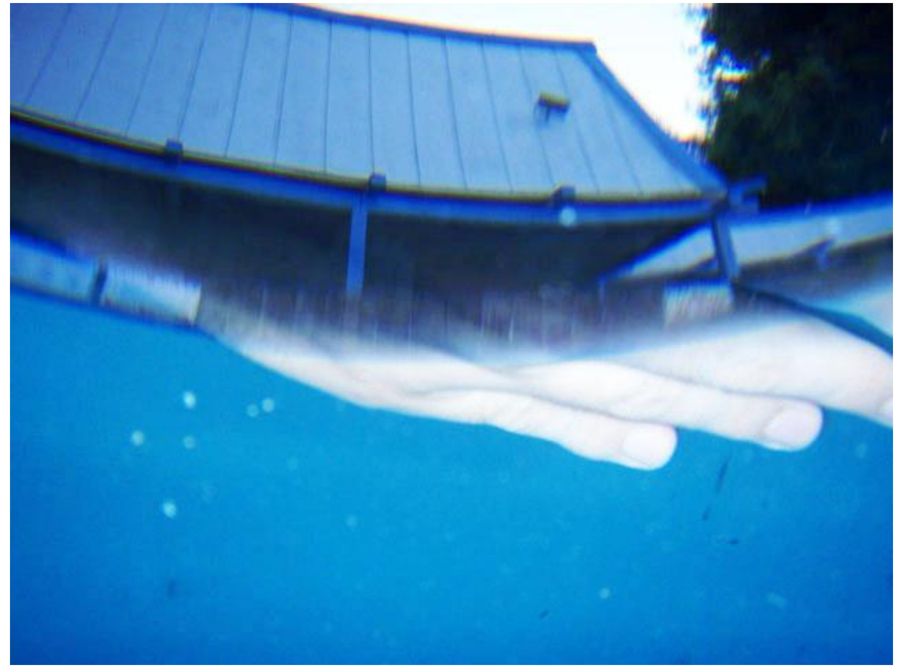
Round source => Significantly improved contrast in the horizontal direction

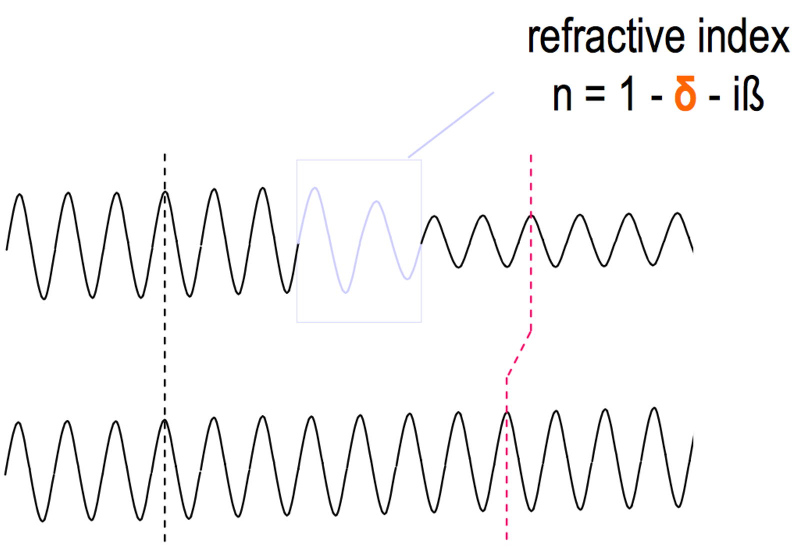
Courtesy APS Imaging group

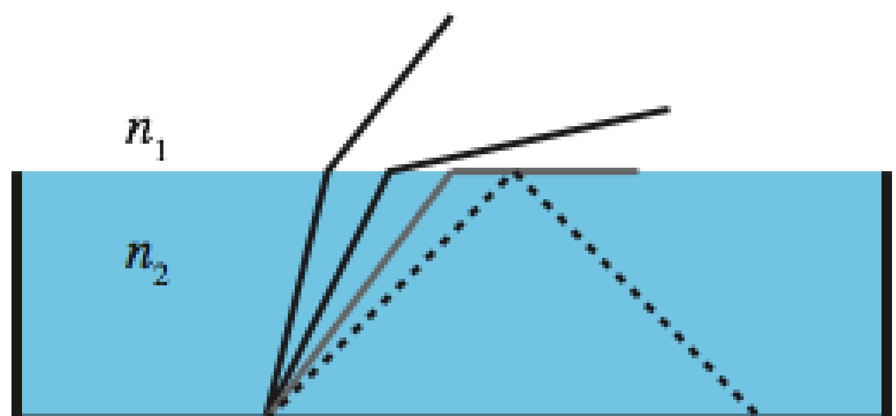
HOW TO FOCUS X-RAYS ?

INDEX OF REFRACTION FOR X-RAYS

- Because $n<1$ (!) in media, total internal reflection in the visible is total *external* reflection for X rays.
- Because (1-*n*) is small, grazing reflection angles only.



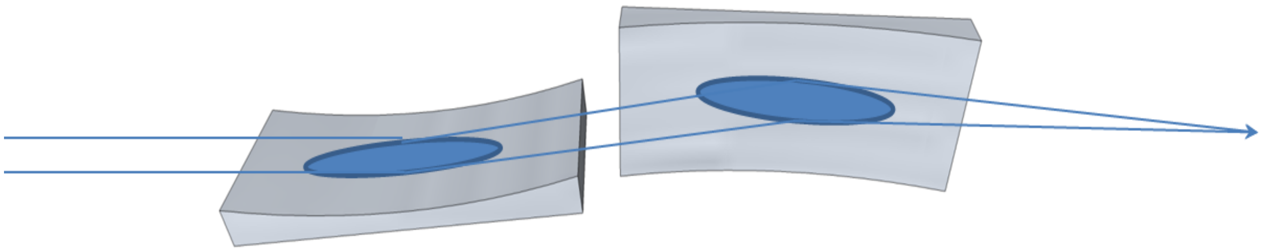




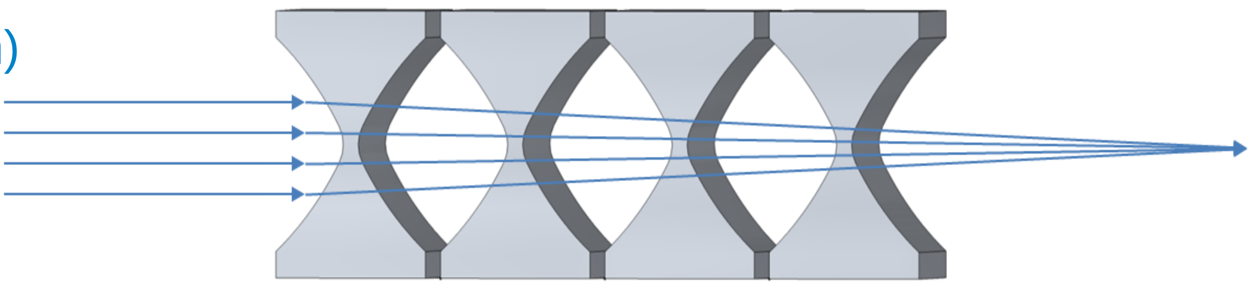
Courtesy C. Jacobsen, D Gursoy

FOCUSING FOR SYNCHROTRON X-RAY MICROSCOPY

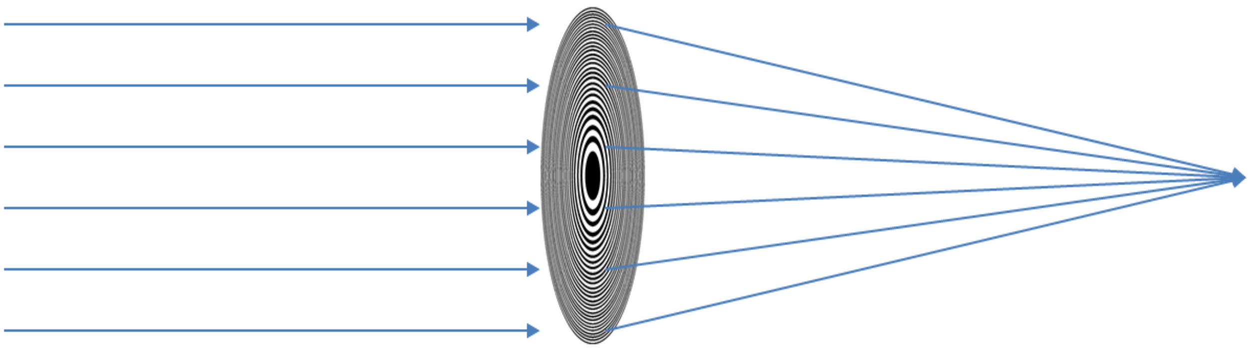
- **Reflective optics**
  - Efficiency ~90%
  - Achromatic focus
  - Spots down to ~100nm (limited by figure error)
  - Used for 5-20keV



- **Refractive optics**
  - Large working distance for microfocus (0.5-10m)
  - Mechanically robust
  - Spots down to ~100nm (limited by NA, absorption)
  - Used for 5-200keV



- **Diffractive optics**
  - Spots down to ~20nm limited by outer zone width (~1.22dr)
  - Compact optic
  - Efficiency ~2-30%
  - Chromatic focus
  - Used for 0.2-30keV



Courtesy Martin Holt

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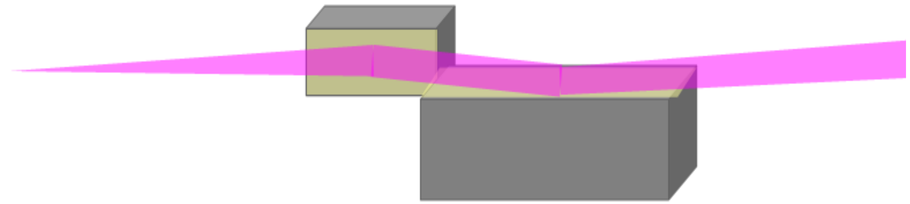
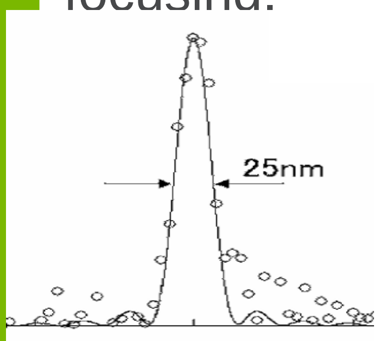
REFLECTIVE X-RAY OPTICS

- **Mirror optics are inherently achromatic, ie, focus position is independend of incident energy**
- **High efficiency: gain of ~10^5 with high ref**

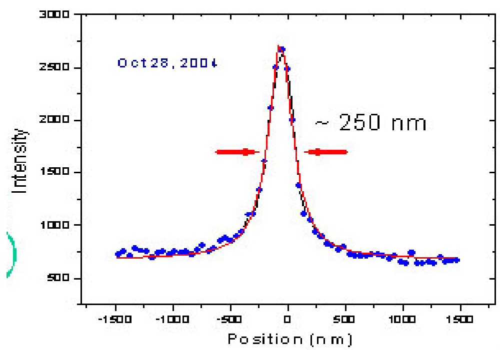
Many efforts have been made in recent years to use achromatic K-B mirrors for hard x-ray sub-100 nm focusing.



Traditional sequential K-B arrangement
(Kirkpatrick and Baez 1948)



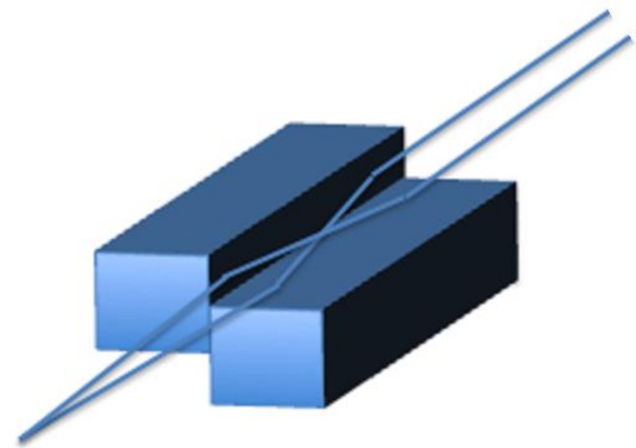
diffraction-limited, 1-km beamline, Spring-8 (2009)



white beam at 34-ID, APS

What is Montel (or nested K-B) mirror optics?

Two mirrors, mounted side-by-side and perpendicular to each other. Some rays strike one mirror first while others strike the other mirror first.



Nested K-B (Montel) arrangement
(Marc Montel 1957)

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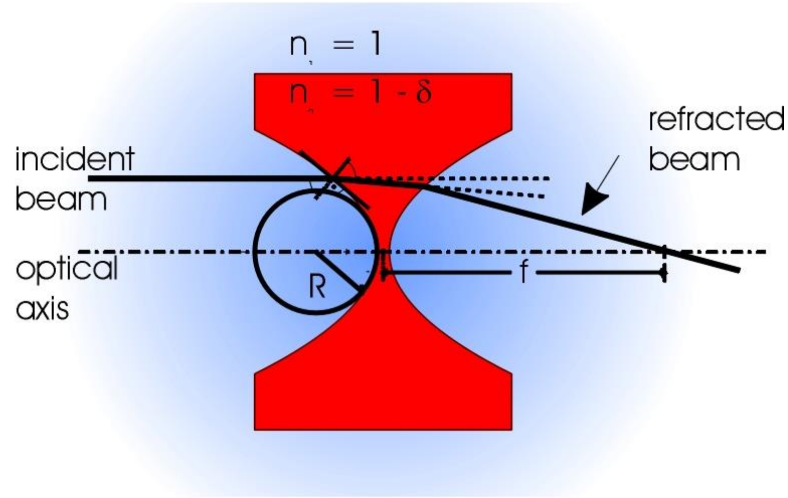
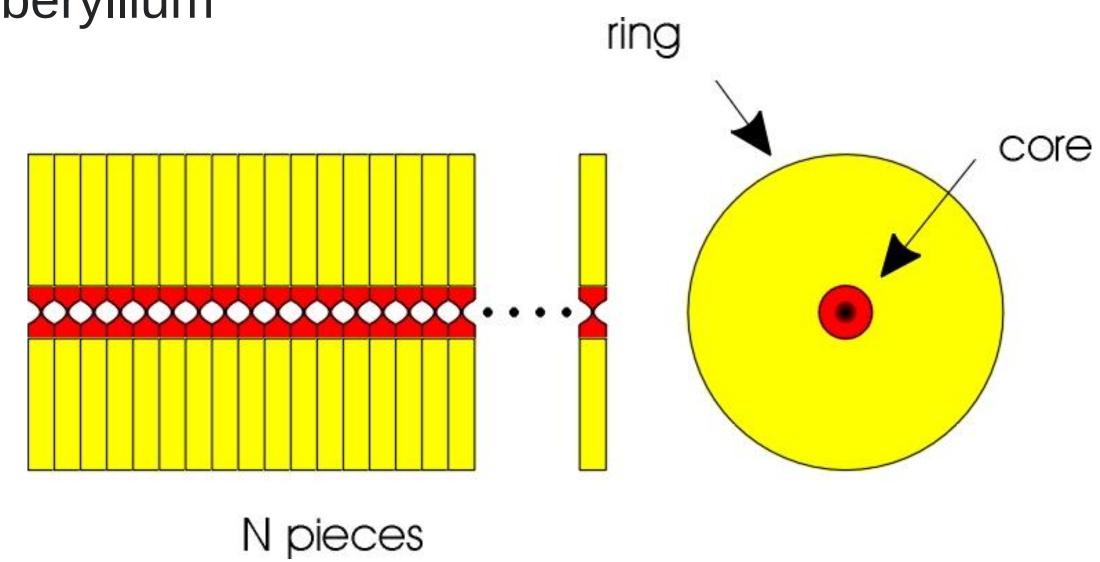
COMPOUND REFRACTIVE LENSES

- Röntgen tried to make lenses, but found no focusing.
- Focal length of one lens is long – so combine many lenses! Tomie; Snigirev *et al*., *Nature* **384**, 49 (1996); Lengeler *et al*., *J. Synch. Rad.* **9**, 119 (2002).
- Resolution approaching 100 nm at 5-10 keV with parabolic beryllium lenses



Compound refractive lenses at Universität Aachen



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Courtesy C. Jacobse

STANDARD DIFFRACTIVE OPTICS: FRESNEL ZONE PLATES

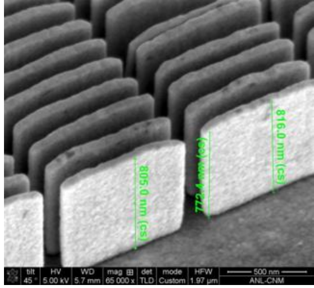
- Circular diffraction grating
  - Radially increasing line density
  - Numerical aperture related to outermost zone width dr_n.
  - Chromatic

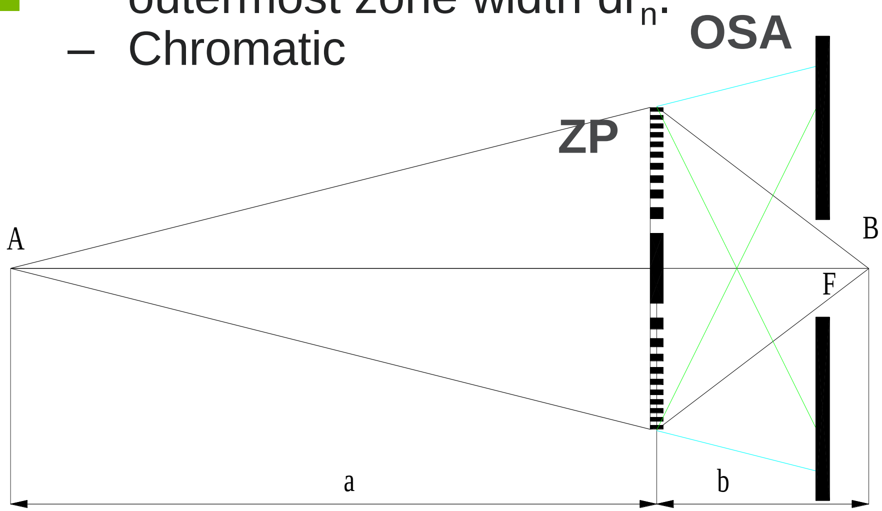
Typical Parameters, E = 10 keV:

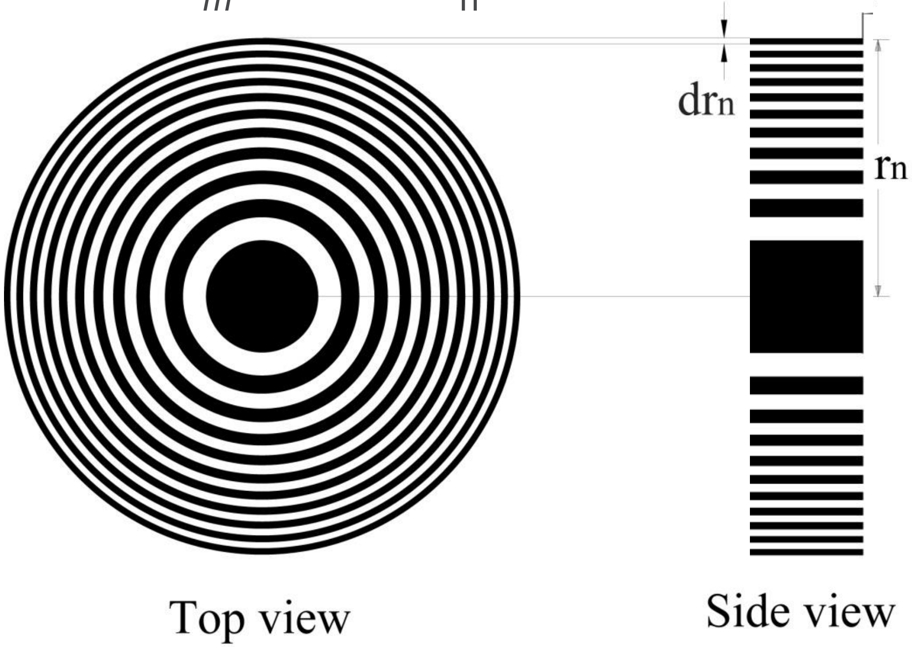
dr_n = 100 nm, r_n = 160 μm

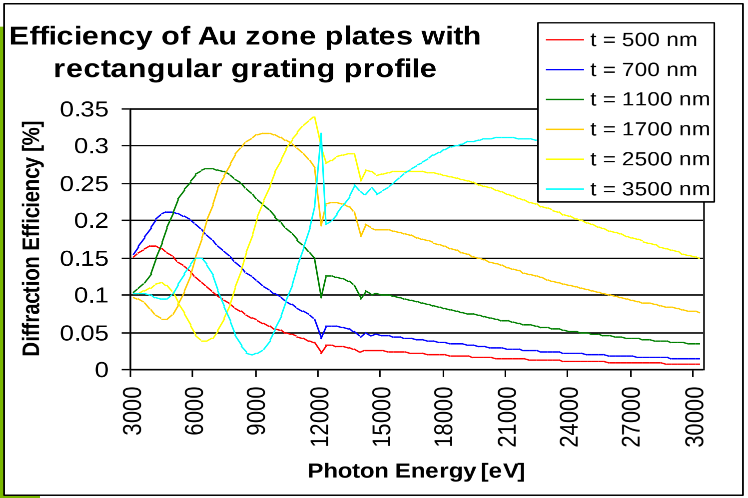
t = 1.6 μm, (Aspect ratio 16)

Resolution $\delta_m = 1.22\, dr_n/m$









Outermost zone width determines spatial resolution, thickness determines efficiency (at a given energy)

$$NA = \frac{r_n}{f} = \frac{\lambda}{2dr_n}$$

$$\delta_R = 1.22\,\lambda/NA = 1.22 \cdot dr_n$$

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DOES IT WORK - FRESNEL ZONE PLATE IMAGES

- R. W. Wood (1898): zone plate figure drawn with a pen and a compass! Photographically reduced

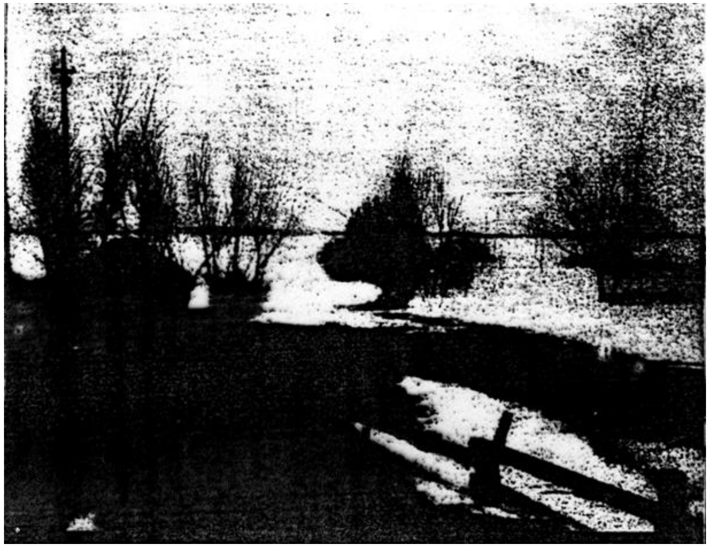


PLATE 2. ZONE-PLATE, FROM A DRAWING.

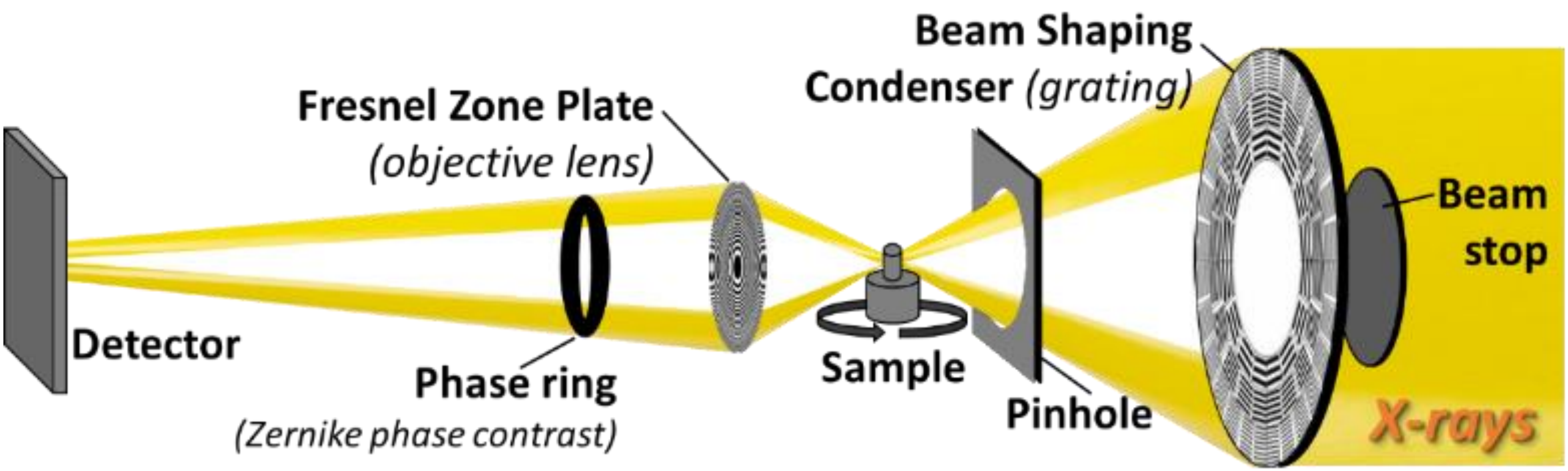
Courtesy C. Jacobse

FULL FIELD NANO IMAGING

3D imaging at 20 nm

- Transmission X-ray Microscope (TXM)
- Resolution: typically 60 nm (30 nm voxels) down to 20 nm
- Energy range: 6 to 12 keV, $\Delta E/E = 10^{-4}$
- Multi-scale approach with an integrated µ-CT module
- In situ: compatible in a wide range of samples environments

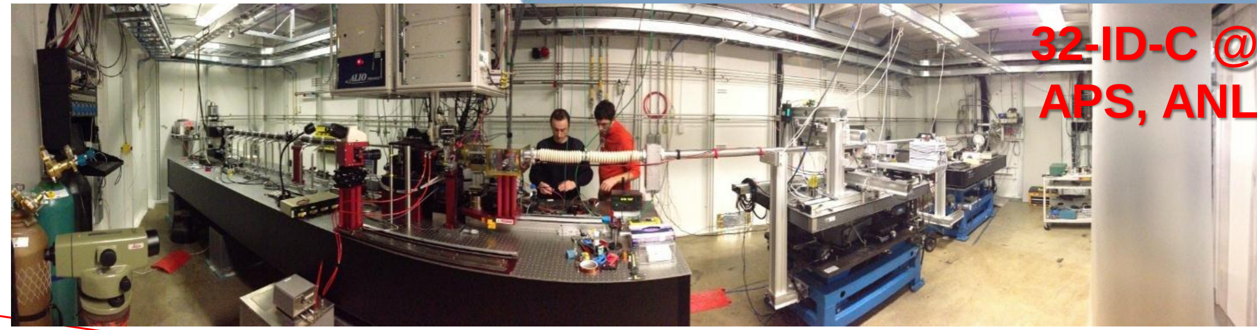
  (T = ambient to 1500 ^{o}C, P up to 100 GPa), chemical bath, etc.



Vincent De Andrade, APS Imaging Group

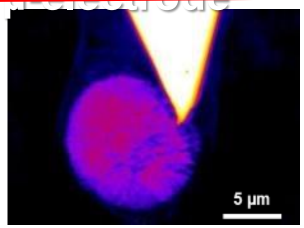
NANO IMAGING

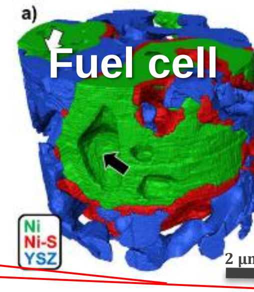
3D imaging at 20 nm

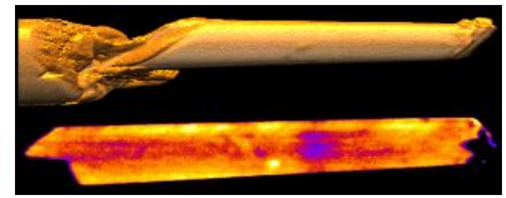


Energy Science

- Fuel cell
- Battery
- UMo nuclear fuel

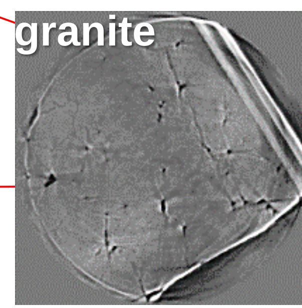


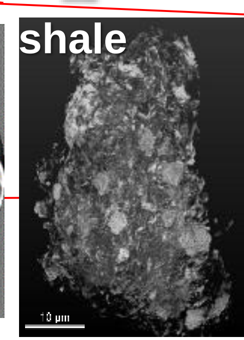


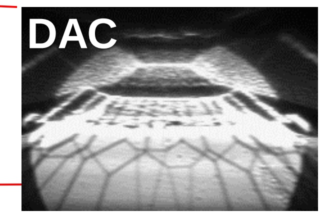


Earth and Environmental

- Melt formation
- Rock fracking
- High pressure experiments with DAC
- CO_2 storage
- Pollution / remediation

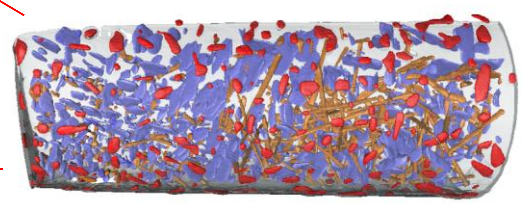






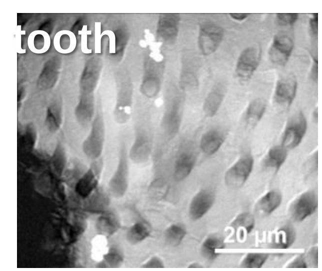
Material Science

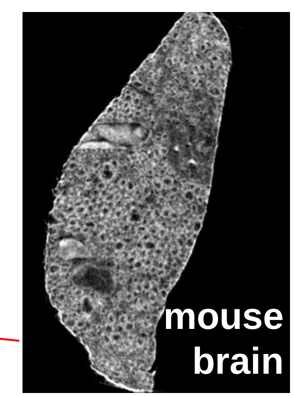
- Metallurgy
- Photonics
- Electronic industry
- Supraconductors



Biology

- Biomaterials
- Wood preservation
- Biology (neurosciences)





Vincent De Andrade, APS Imaging Group

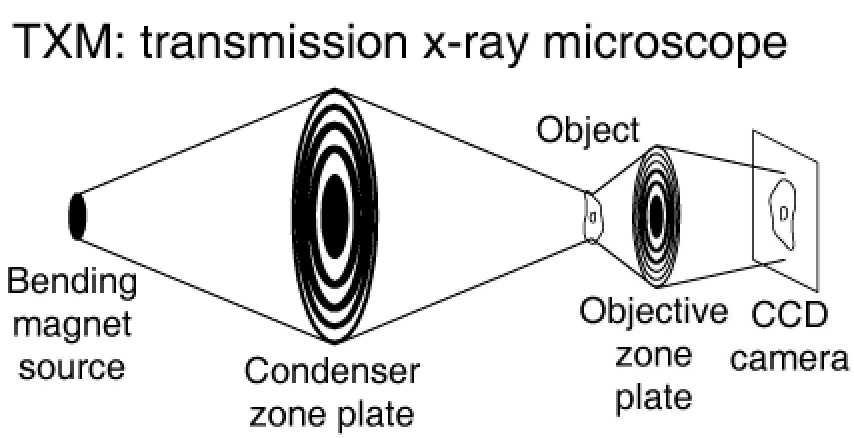
X-RAY MICROSCOPES

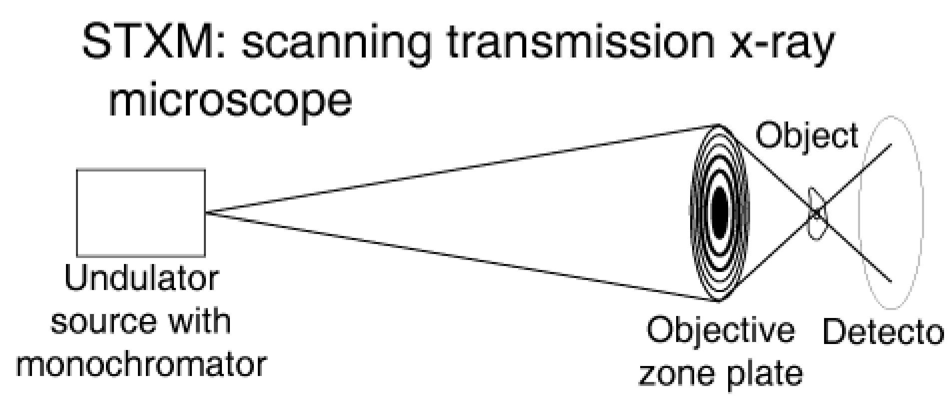
Transmission X-ray Microscope

- Full field
- Incoherent illumination; works well with a bending magnet (or lab source), with fast imaging
- More pixels (e.g., 2048^2)
- Moderate spectral resolution in most cases

Scanning X-ray Microscope

- Coherent illumination; works best with an undulator
- Less dose to sample (~10% efficient ZP)
- Well suited for spectroscopy
- Microprobes: fluorescence etc.





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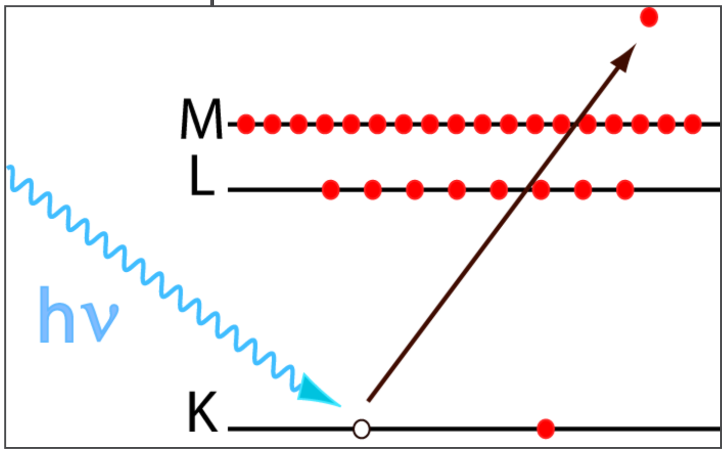
Courtesy C. Jacobse

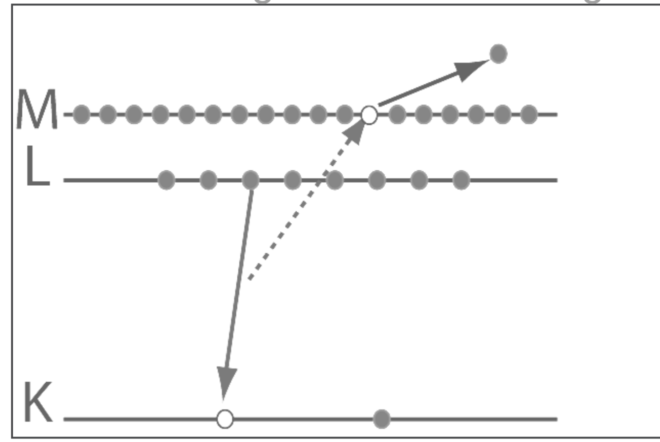
MICROPROBES:
ADDING TRACE ELEMENTAL SENSITIVITY WITH X-RAY FLUORESCENCE

X-RAY INDUCED X-RAY FLUORESCENCE – A BRIEF REMINDER

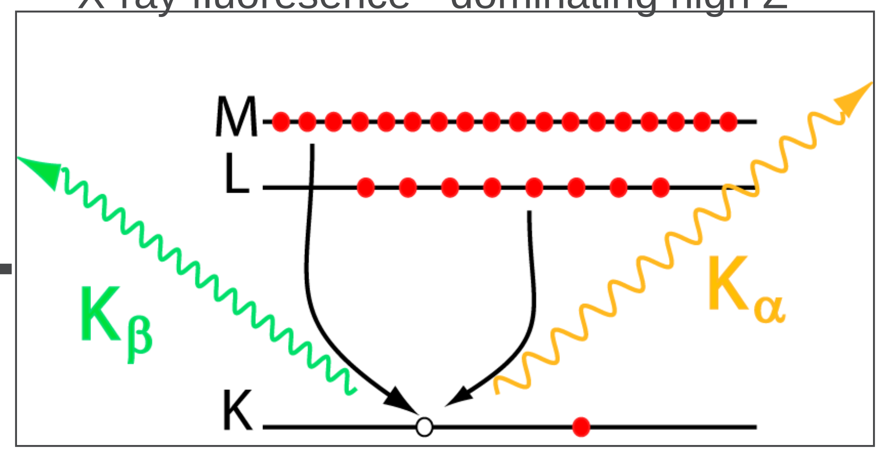
photo-electric absorption of incident hard X-ray
emission of photo-electron



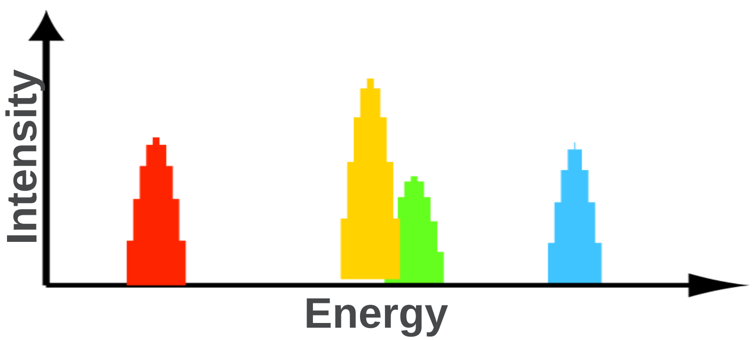
Emission of Auger e^- - dominating low Z



X-ray fluoresence - dominating high Z



Detect XRF using energy dispersive detector



- Energy of X-ray fluorescence photons is characteristic for each element
- XRF is quantitative, i.e., number of XRF photons is directly related to quantity of element
- Photo-electric absorption crossection straightforward to calculate (monochomatic incident beam)

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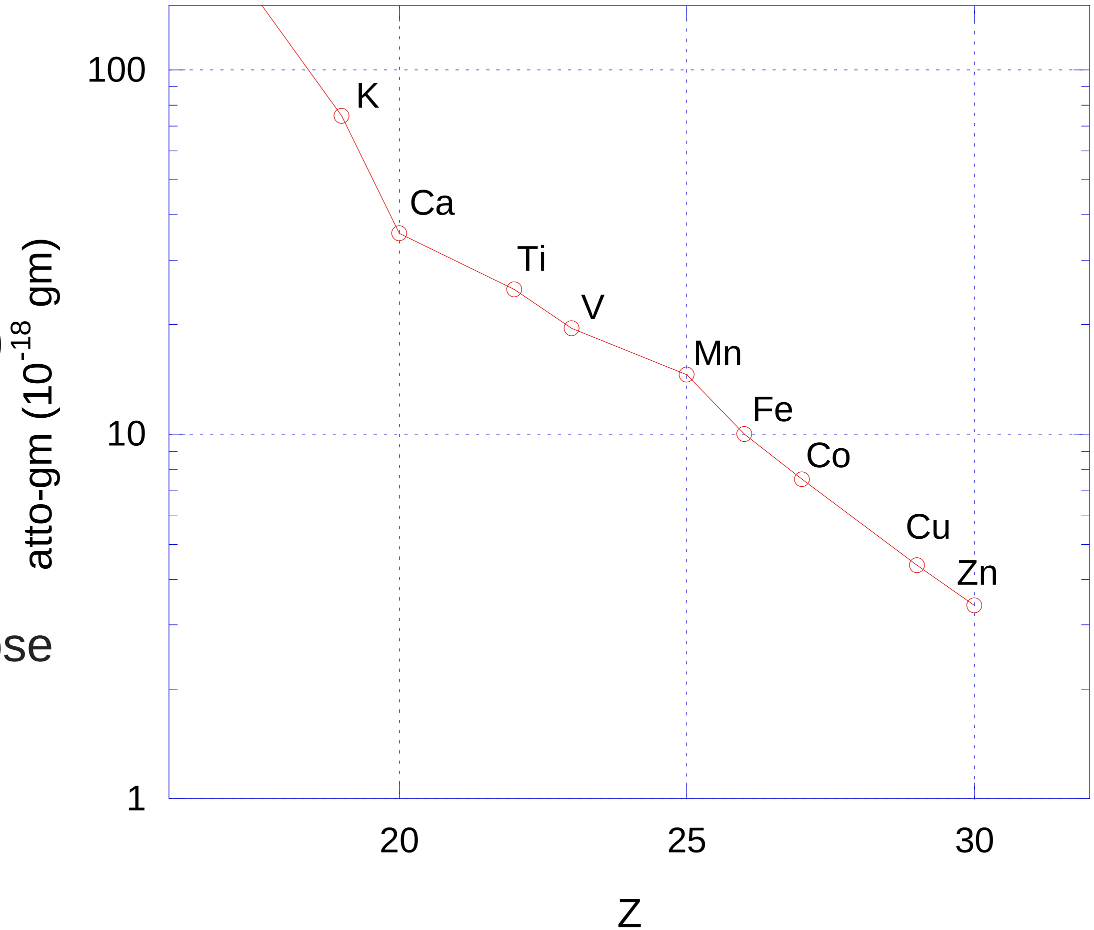
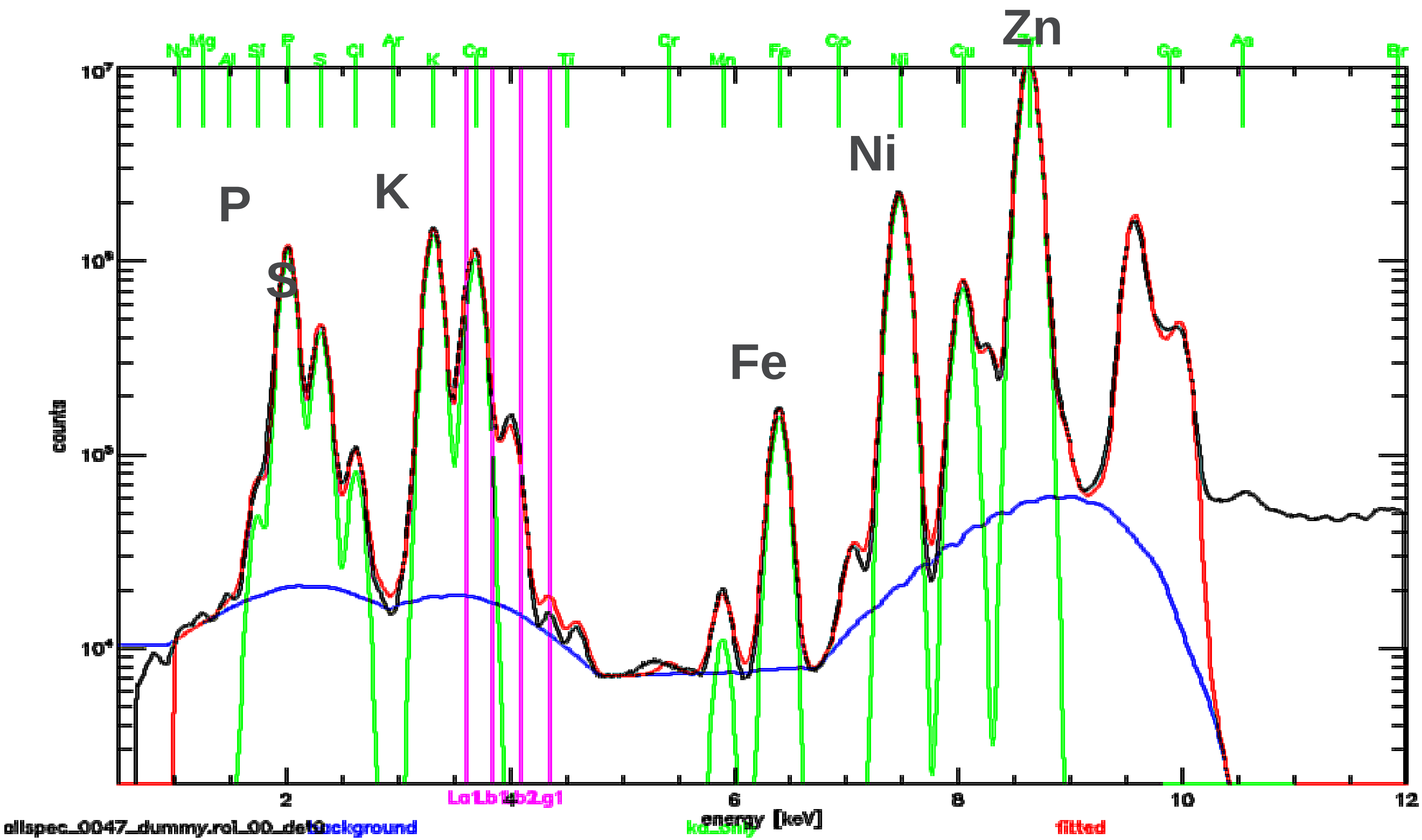
WHY USE X-RAY-INDUCED FLUORESCENCE TO STUDY TRACE METALS?

- Simultaneously map 10+ elements
- No dyes necessary
- High signal/background ratio
  - sub-ppm (part-per-million) sensitivity, increasing with Z
- Large penetration depth (~> 100 µm)
  - study whole cells, w/o sectioning
  - study ‘thick’ tissue sections
  - possibility to study hydrated “natural” samples using cryo
- monochromatic incident beam: choose at which Z to stop excitation (e.g., excite As but not Pb)
- straightforward quantification
- Microspectroscopy / Spectromicroscopy: Map chemical states by u-XANES
- Little radiation damage *



Detection Limit for Transition Elements: for 1 sec. acquisition time, 0.2 x 0.2 µm² spot, E=10 keV

A TYPICAL X-RAY FLUORESCENCE SPECTRUM



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Periodic table highlighting X-ray fluorescence

K-line Fluorescence typically used

L-line Fluorescence typically used

Major/minor elements in Biological Systems

‚Natural' Trace elements

Toxic / carcinogenic elements

Used in Imaging, Diagnosis, Therapy, ...

H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	18 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110	111	112	113	114	115	116	117	118

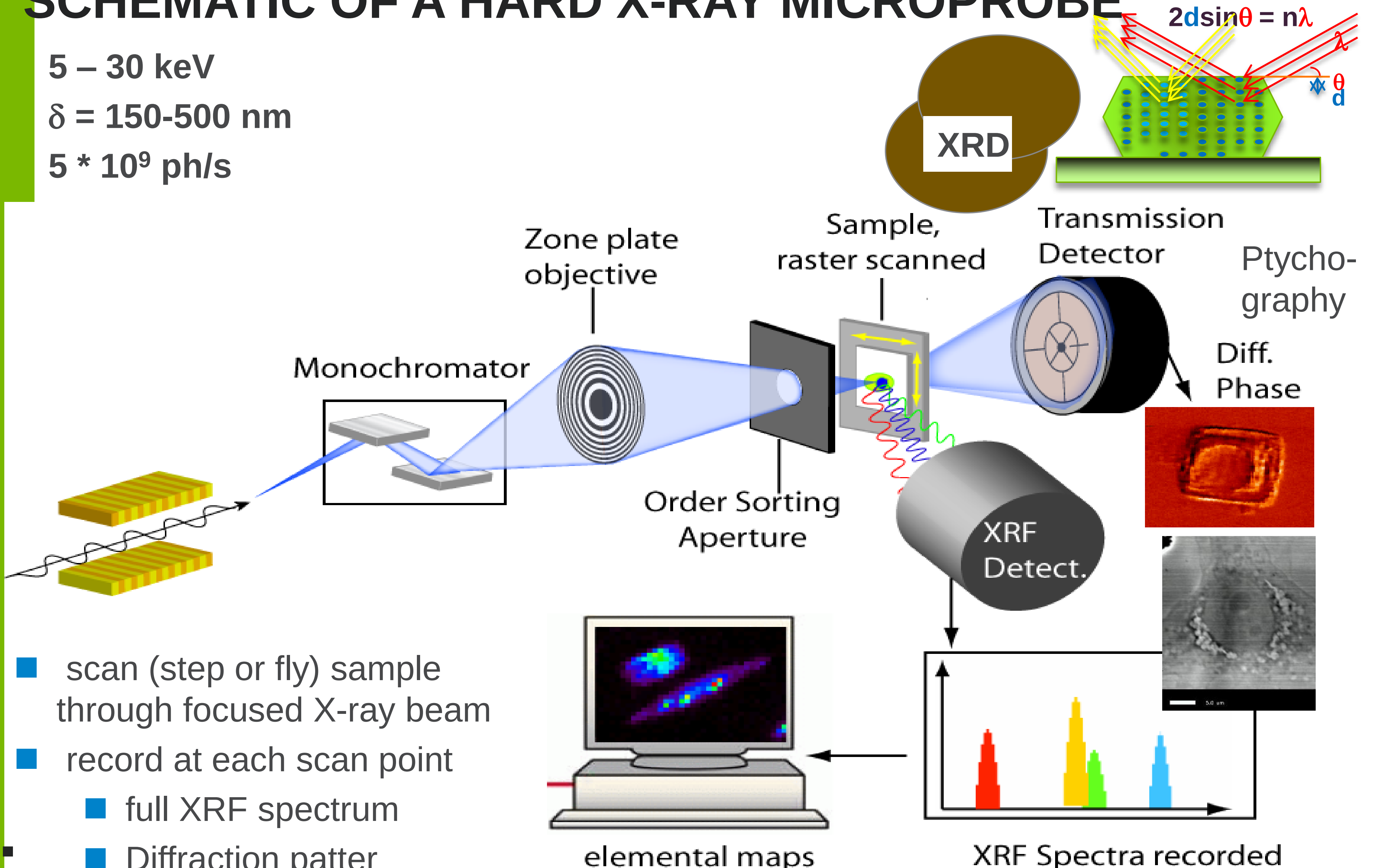
57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb
89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No

SCHEMATIC OF A HARD X-RAY MICROPROBE

5 – 30 keV

δ = 150-500 nm

5 * 10^9 ph/s

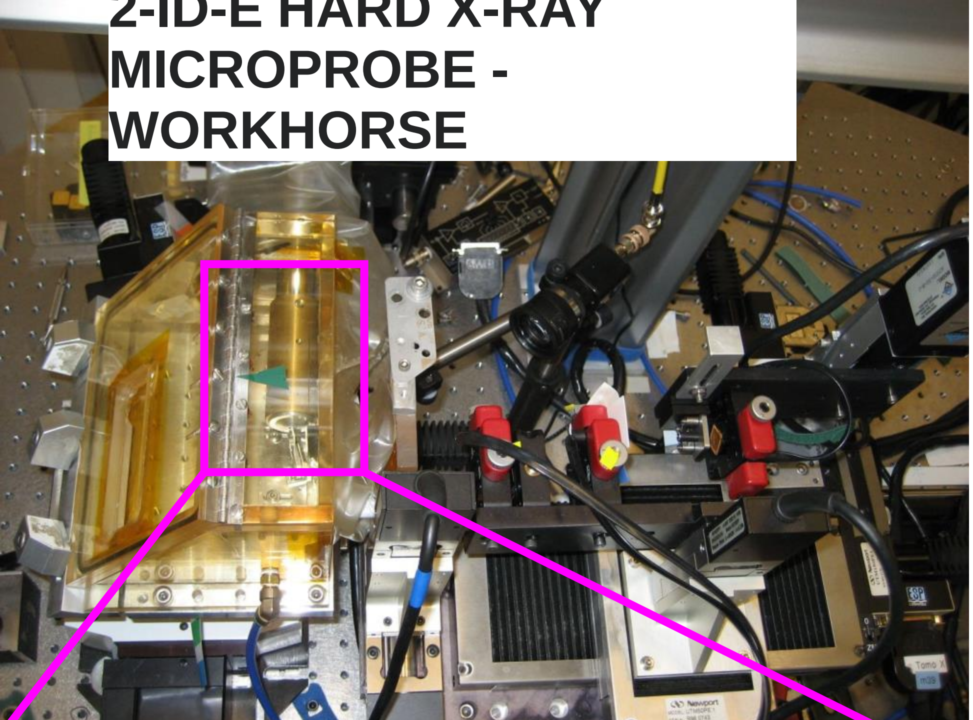


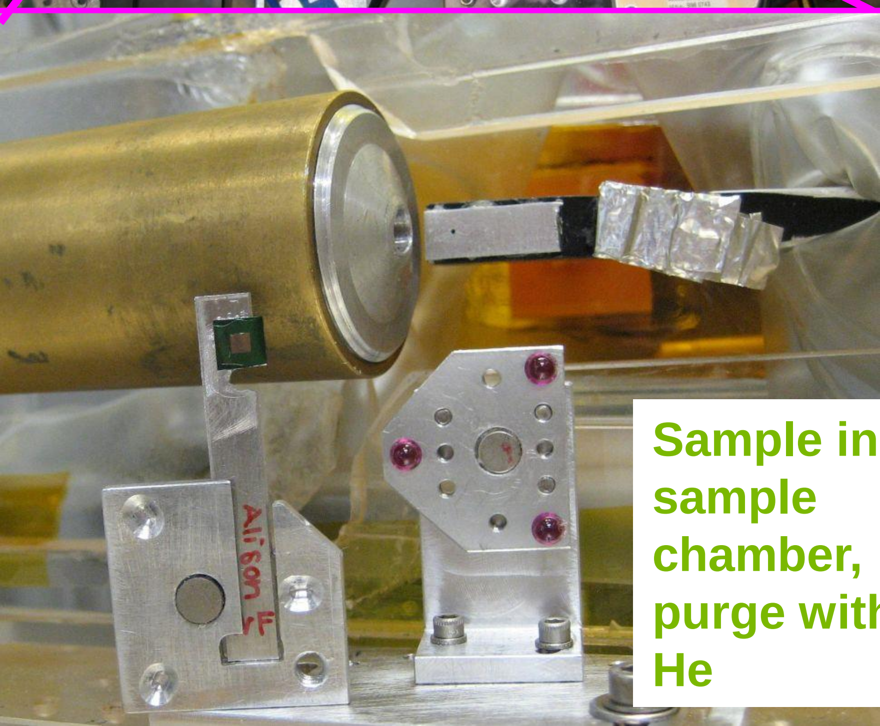
- scan (step or fly) sample through focused X-ray beam
- record at each scan point
  - full XRF spectrum
  - Diffraction patter
  - Ptychography

schematic NOT to scale !!

* B. Hornberger *et al*, J Synchr. Radiat **15**(Pt 4), 2008
* de Jonge *et al*, Phys Rev Lett **100**(16), 2008
* Holzner et al, Nature Physics 2011

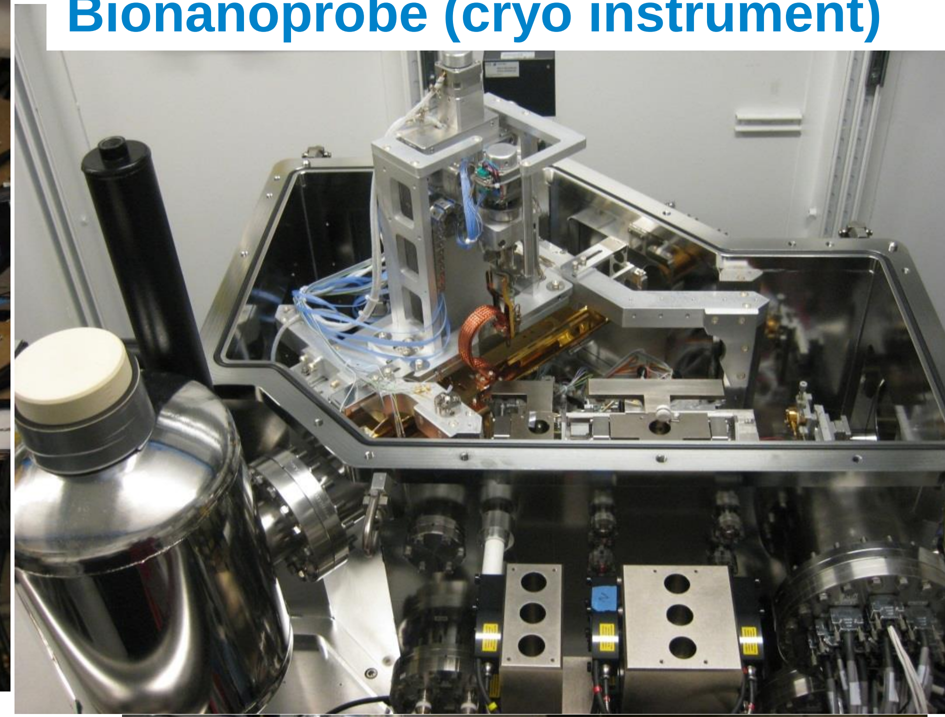
2-ID-E HARD X-RAY MICROPROBE - WORKHORSE

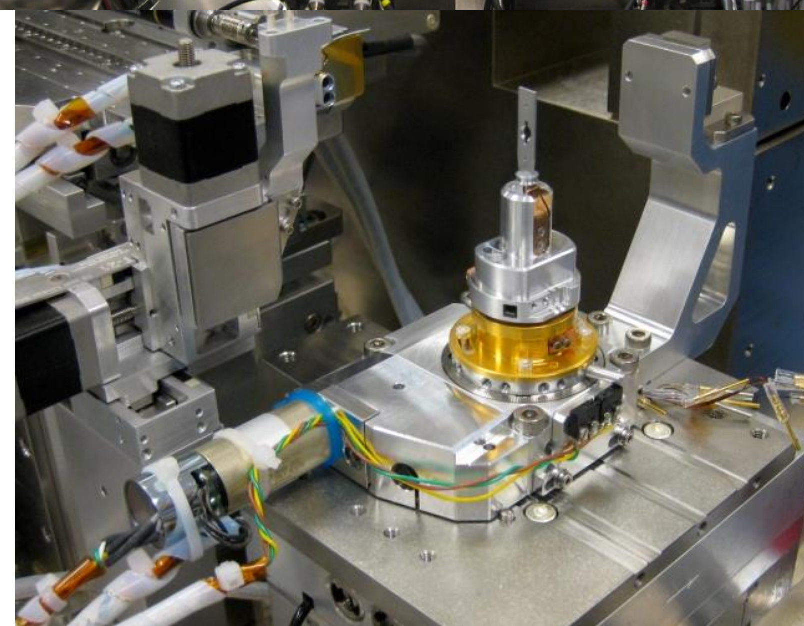




Sample in sample chamber, purge with He

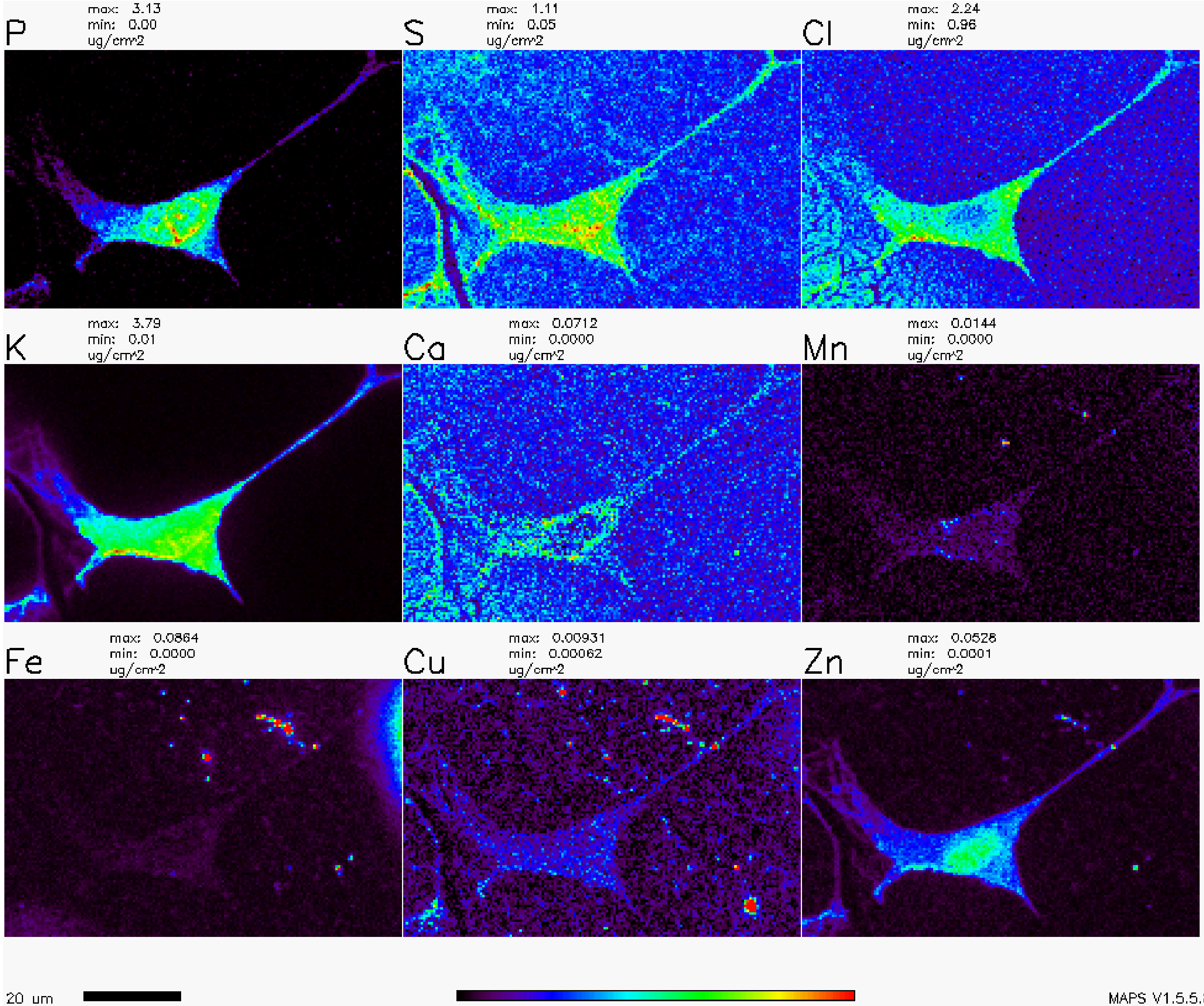
Bionanoprobe (cryo instrument)





ELEMENTAL CONTENT OF AN HMVEC CELL

Overview Image of a full HMVEC cell (plunge frozen in liquid ethane, freeze dried), 2 hours after initiating angiogenesis. Cu is localised strongly to areas outside of the cell, comparison to other timepoints suggests the Cu is transported out of the cell, and after a few hours back into the cell.



See also: L. Finney et al, PNAS **104**(7): 2247-52. (2007

GREAT TOOL, BUT IS IT THE RIGHT TOOL FOR THE JOB ?

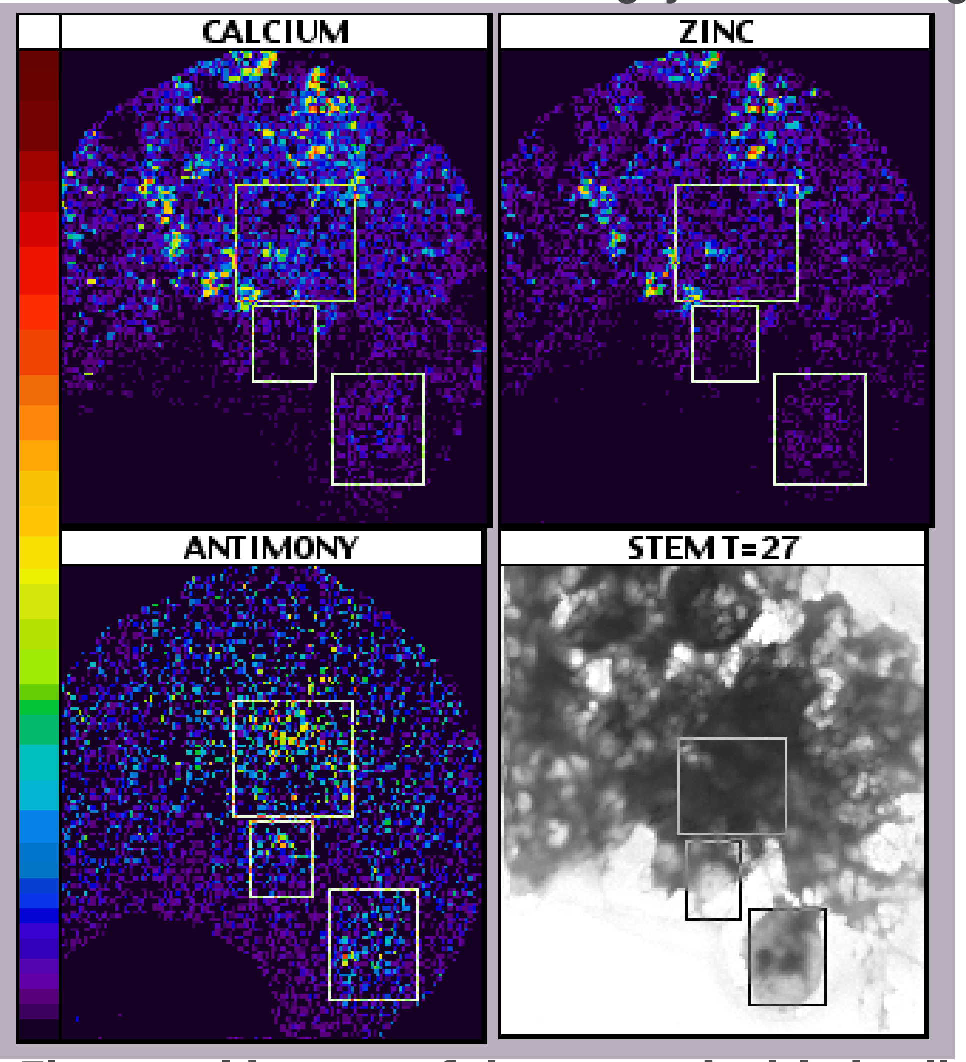
from http://www.cartoonstock.com/

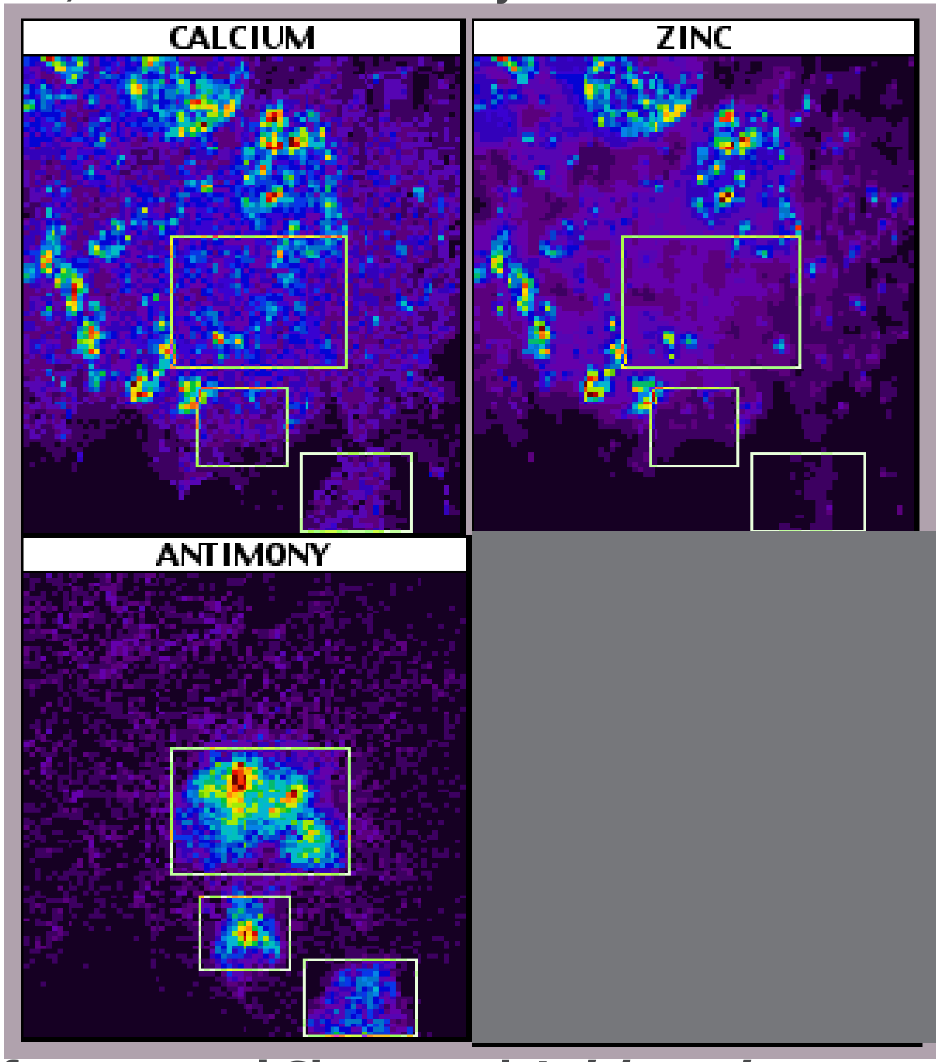
COMPARISON OF SOME OTHER TECHNIQUES FOR TRACE ELEMENT MAPPING:

	Spatial Resol.	object thick.	Res. Limit.	Advantages/Disadvantages
Light-microsc.	200 nm	30 µm	Wave-length	+ changes in living cells can be monitored, but competition w. proteins +/- only see ions (in solution), and not total content - need dyes - quantification difficult
Hard X-ray-micropr.	**200 nm-20nm**	**10 µm**	**Curren-tly Optics**	**+ no dyes, visualize total elemental content** **+ very high sensitivity, low background, selective excitation** **+ simultaneously detect >10 elements** **+ µ-XANES for chemical state mapping / - slow**
Analytical Electron-micropr.	20 nm	0.1 µm	object thickn.	+ high spatial resolution + simultanously detect >10 elements - thick samples very difficult, sectioning necessary - slow - radiation damage
EELS/ EFTEM	2 nm	0.005 - 0.05 µm	Rad. Damage	+ very high spatial resolution - require ultrathin sections - only some elements readily accessible (e.g., P, Fe) -co-registration can be difficult (EFTEM), slow (EELS)
Proton Micropr. (PIXE)	~1um	~50um	Rad. damage Flux limit	+ simultanously detect >20 elements + high sensitivity - slow - radiation damage

B. Twining *et al*, J Eukaryot. Microbiol. 55(3), 2008 *analytical electron microscope* *hard X-ray microscope*

Collaboration with Ann LeFurgey and Peter Ingram, VA & Duke University





Elemental images of the same air-dried cells from several Sb-treated *Leishmania* amastigotes. Sb is much clearer visible in the x-ray microscope due to its greater sensitivity. Scan width: 10µm.

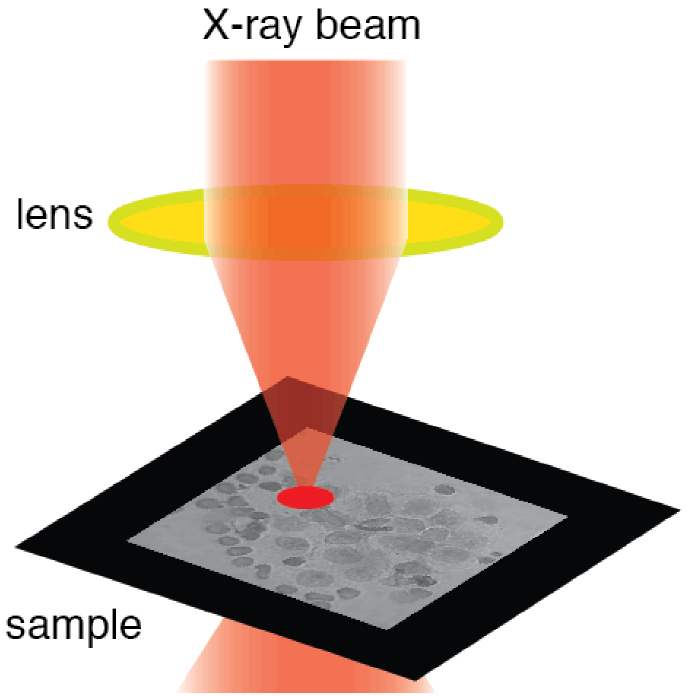
APPROACHES TOWARDS SCANNING

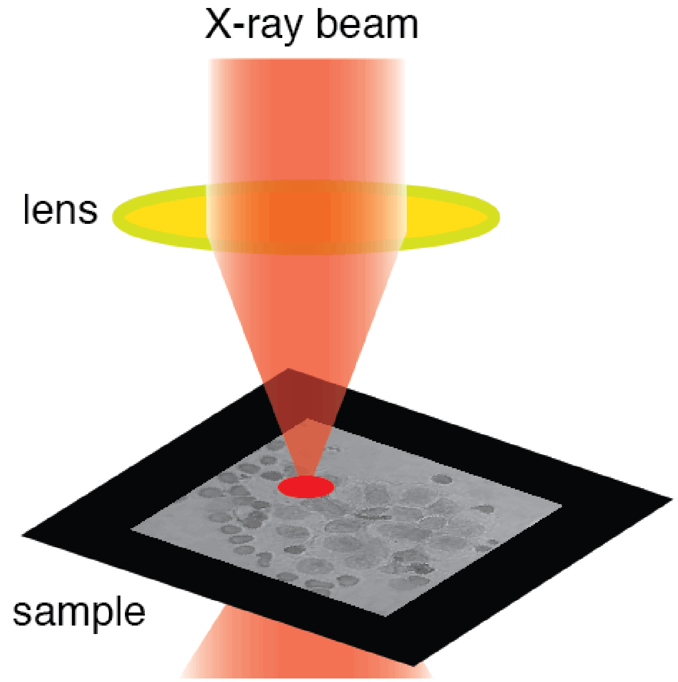
Step scans:

- **Move to measurement point, settle, start detectors, read out detectors, go to next point.**
- **Typical overhead ~100 ms/pixel, 1s/line**
- **Beam utilization ~80%**
- **Appropriate for long dwell times**

Fly scans:

- **Move sample continuously through focus,** reading out detectors 'continuously', **synchronization via hardware triggers.**
- **Typical overhead none/pixel, 1s/line**
- **Beam utilization ~99%**
- **Permits tweaking spatial resolution vs sensitivity AFTER data acquisition, to optimize results**

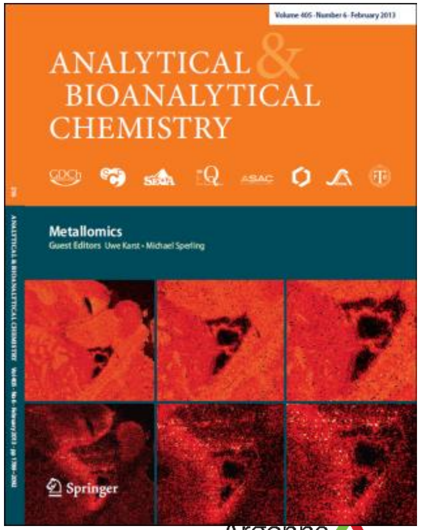




After David Vine

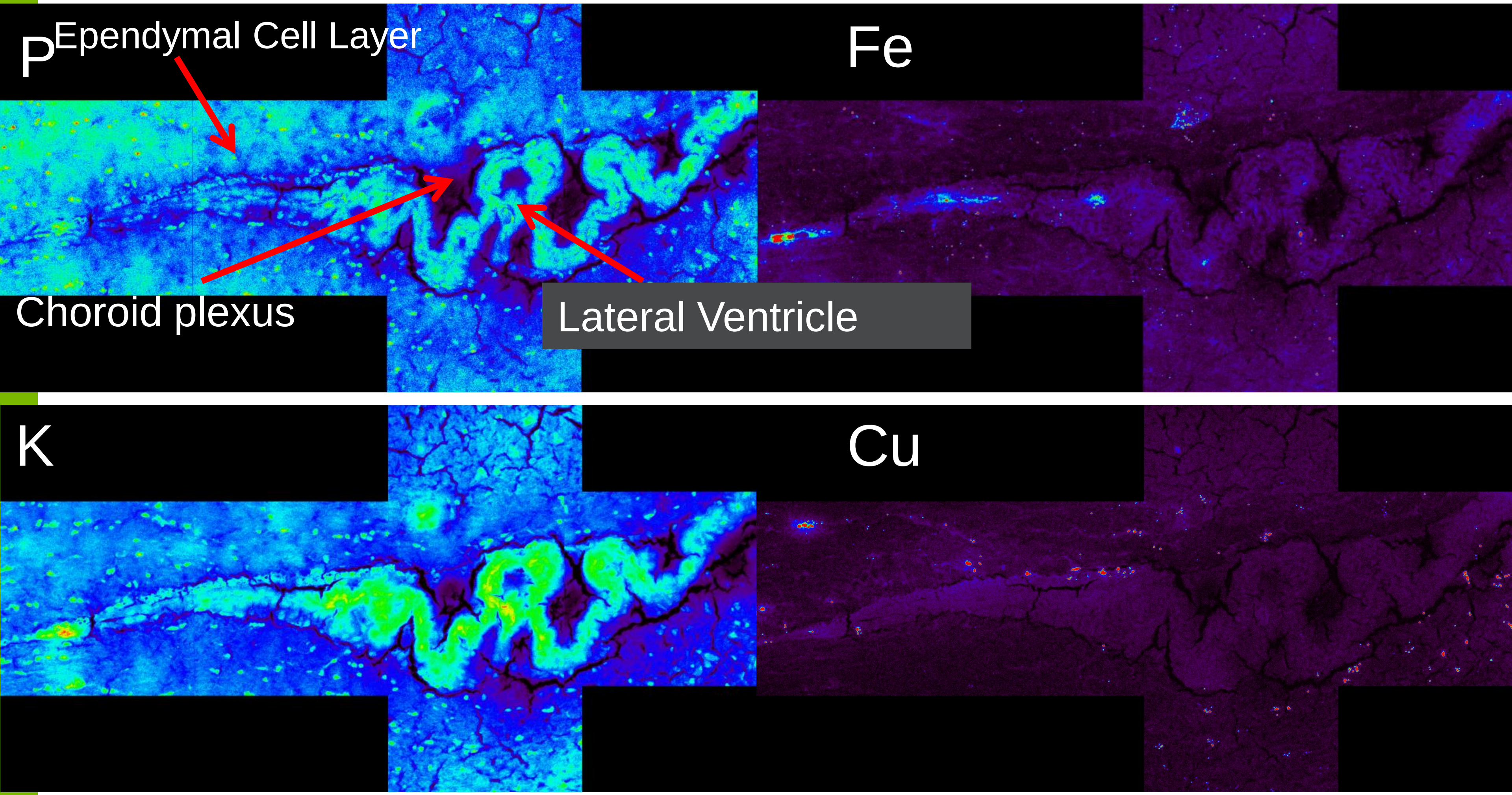
Fly scanning has been a game changers, uniquely enabled by detector developments:

- enable both high resolution and large field of view
- allow trading spatial resolution against signal to noise ‘after’ data acquisition
- Essential for X-ray fluorescence tomography with full spectral fidelity



Vogt S. & Ralle, M. (2013) Opportunities in multidimensional trace metal imaging: taking copper-associated disease research to the next level. Anal Bioanal Chem. 405(6):1809-1820.

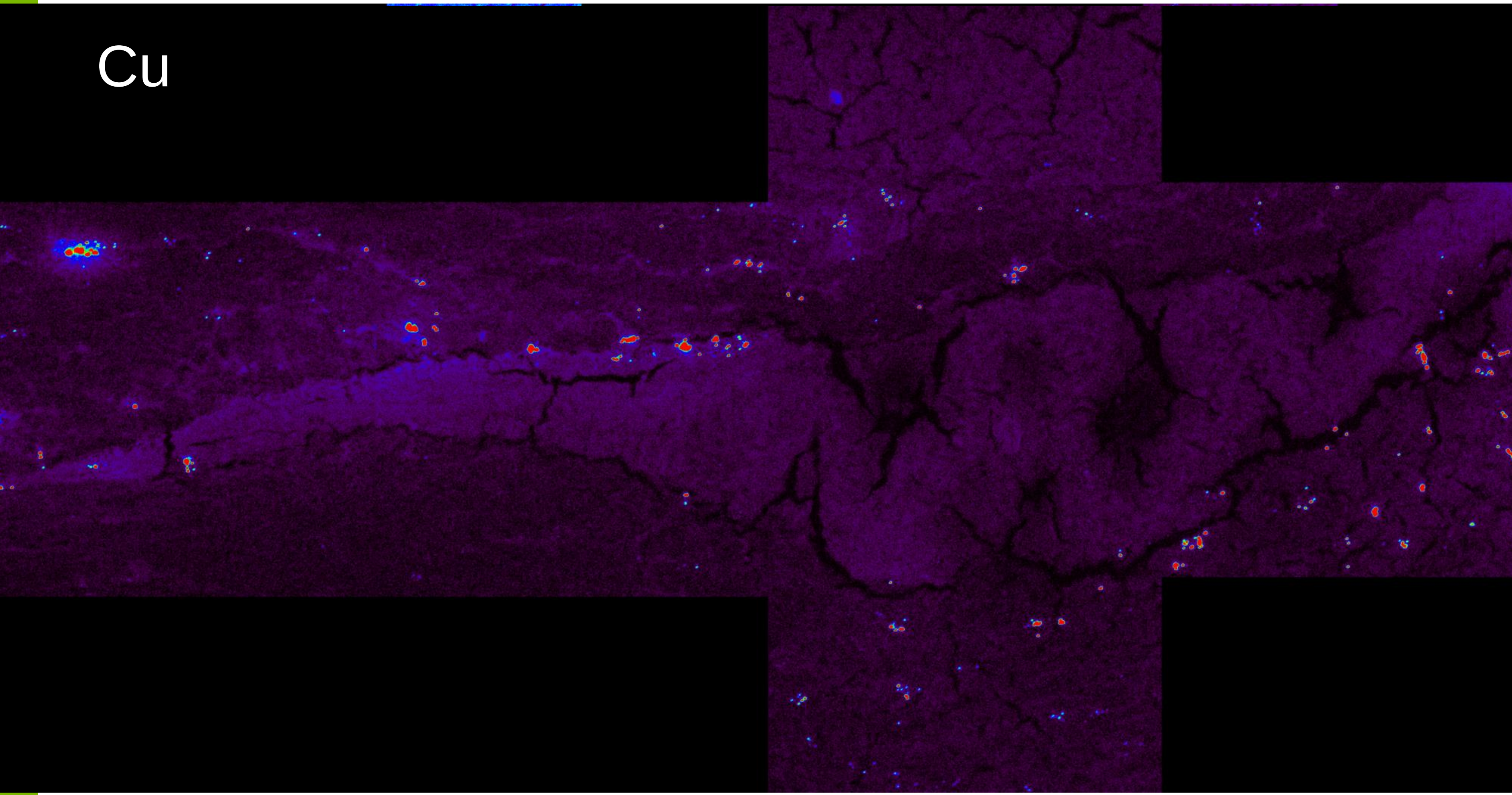
YES, IT IS A GAME-CHANGER FOR NANOIMAGING!



Scan of mouse brain section, M Ralle, OHSU

780x400um, 3900x2000 pixel, 200 nm, 10 ms -> 20h

YES, IT IS A GAME-CHANGER FOR NANOIMAGING!



Highest resolution ➔ 'Needle'

Large field of view ➔ 'Haystack'

780x400um, 3900x2000 pixel, 200 nm, 10 ms -> 20h

- Finding the 'needle' in the 'haystack' requires both capabilities

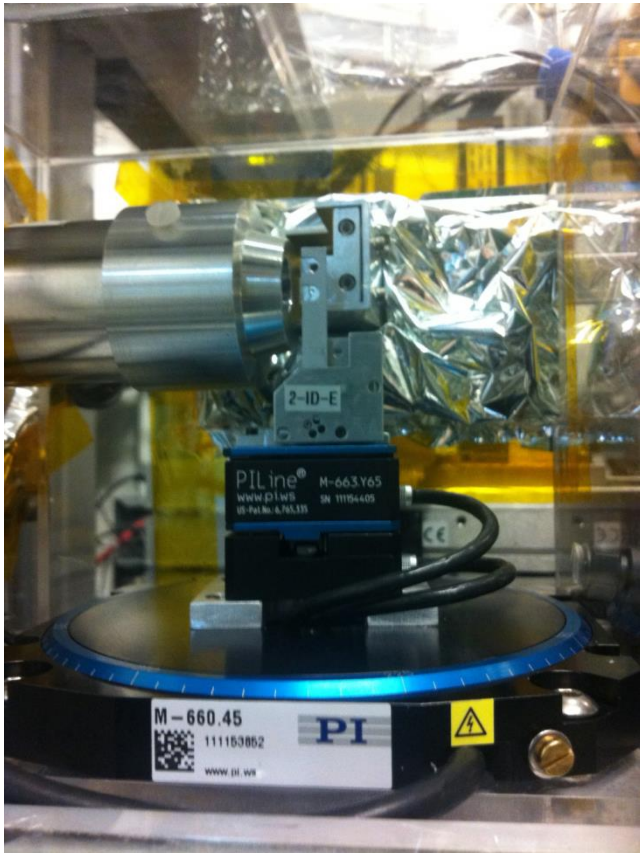
APS-U enables full, contextual imaging with nanometer resolution

Just one example, applies for stitching nano-CT, … -> leads to big data opportunities

TOMOGRAPHY TO VISUALISE 3D STRUCTURE

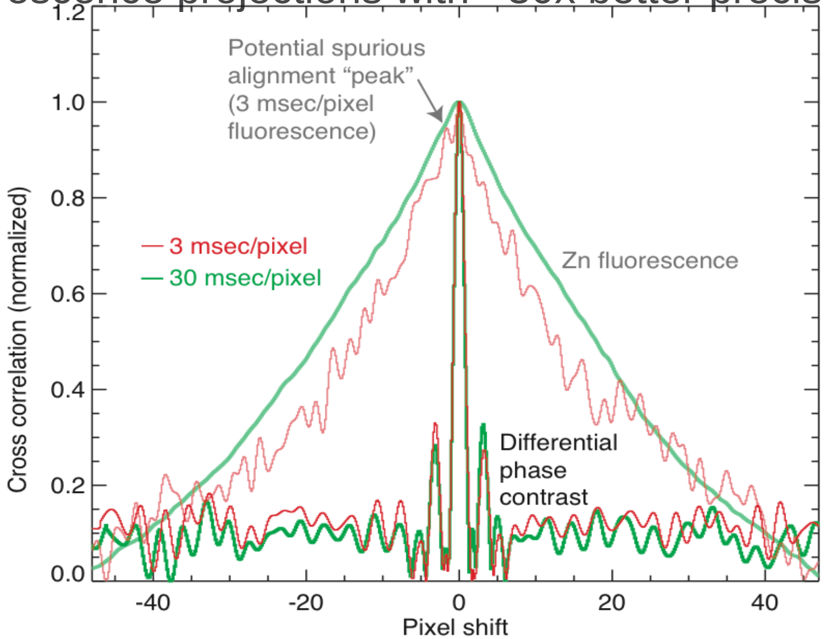
- 3D resolution: $\delta = D\alpha$ D specimen size, α tilt angle interval (Crowther et al 1970)
  - For 10um thick sample, 20 nm desired resolution, need 1600 projections
    -> need automation, must use dose fractionation

  Use diff. phase contrast for alignement, or fiducials



New tomography setup at 2-ID-E
Sophie-Charlotte Gleber, et al

- Dose fractionation [Hegerl and Hoppe, *Z. Natur.* **31**, 1717 (1976)] provides a way to do fluorescence tomography at higher speed and with lower dose: divide the signal needed for a 2D view among all the 3D projections!
- Differential phase contrast allows you to align low-dose fluorescence projections with ~30x better precision.



Y.P. Hong et al, J Synchrotron Rad (2014). **21**, 229-234

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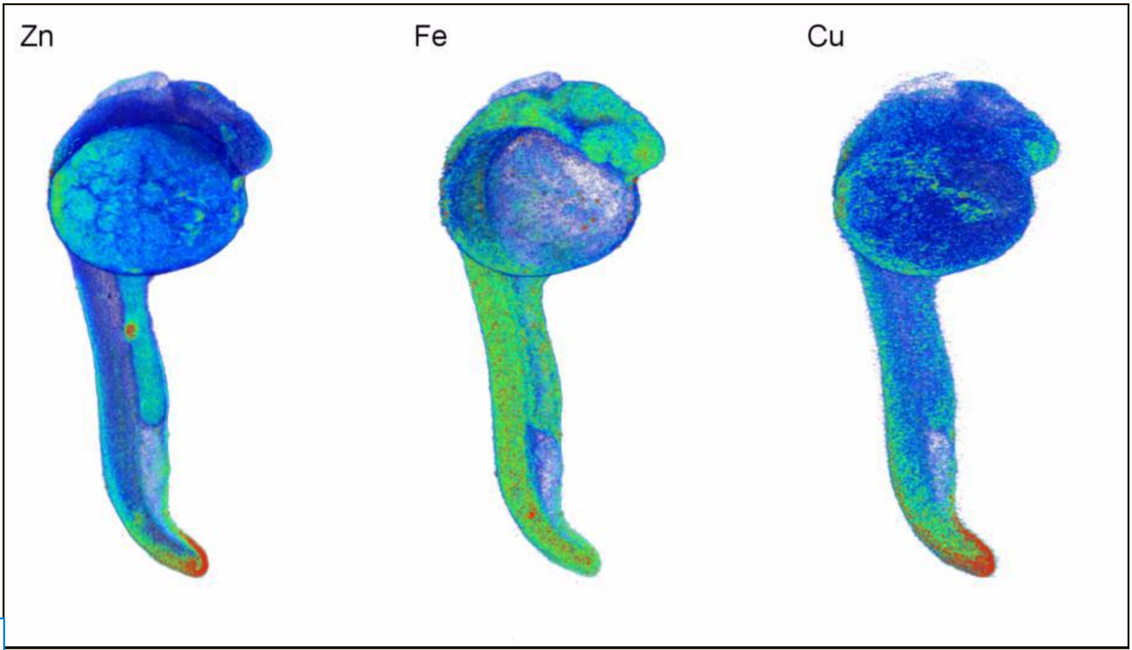
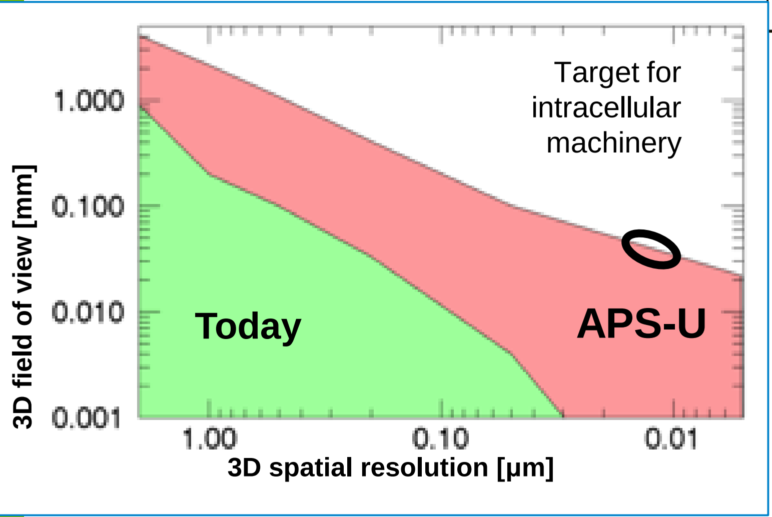
LOOKING AT TRACE METALS IN ZEBRAFISH DEVELOPMENT

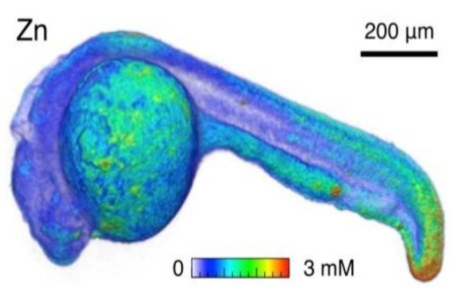
- XRF tomography becoming routine. Data acquisition fairly automated.
- Field of view ~800x1500um, 400x750 pixels, 60 projections, dwell:10 ms/pixel. Total data acquisition time: 3-4 days!
- Here resolution limited only by available flux (scan time).

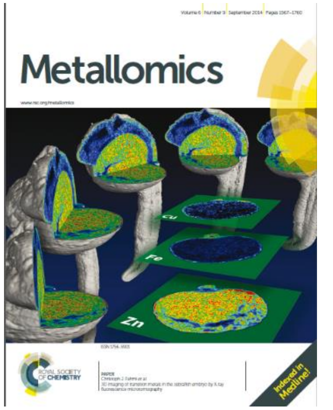


Zebra-fish: metalloprotein cofactor metal distributions correlated with characteristic anatomical features of embryonic development

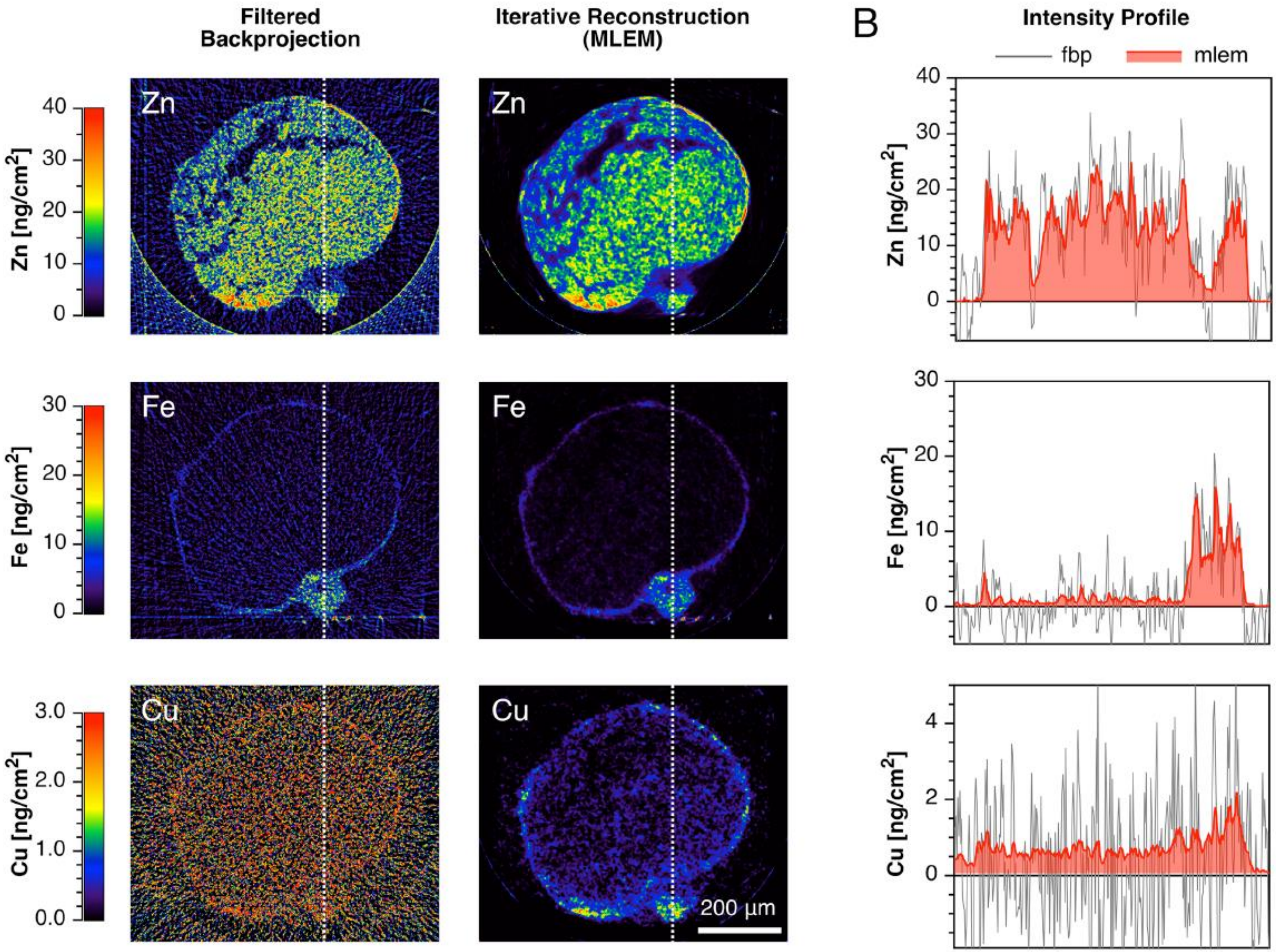
D. Bourassa et al, Metallomics, accepted



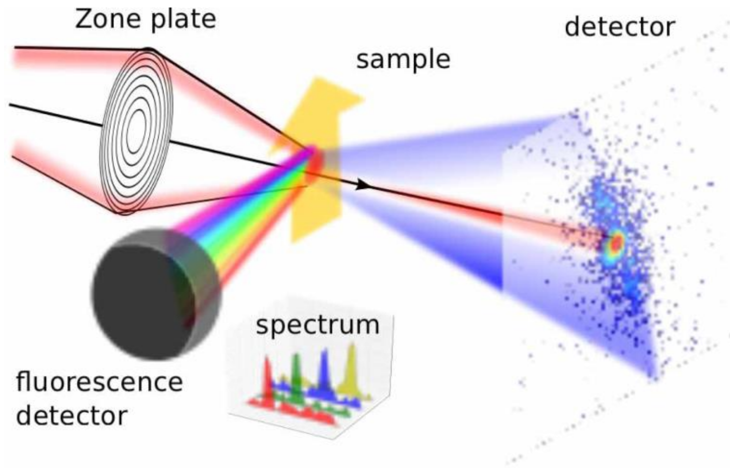




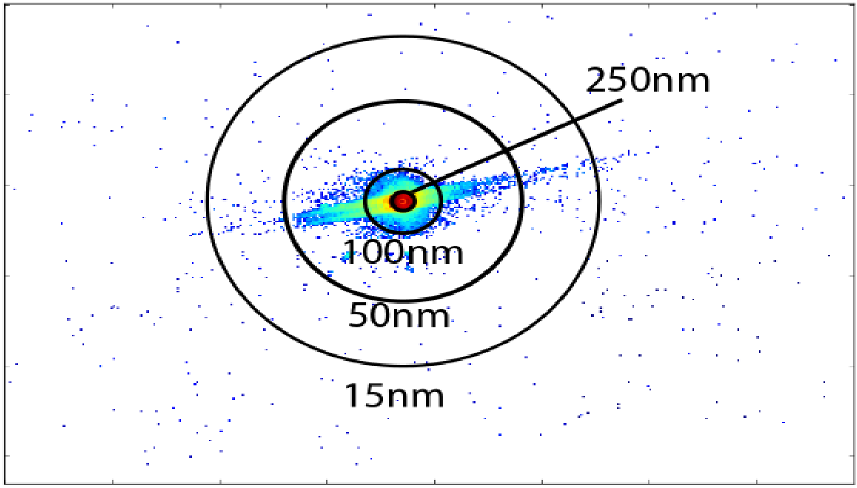
DATA ANALYSIS METHODS CAN MAKE A HUGE DIFFERENCE: FILTERED BACKPROJECTION VS ITERATIVE RECONSTRUCTION



COMBINE LENSLESS IMAGING WITH SCANNING MICROSCOPY: PTYCHOGRAPHY



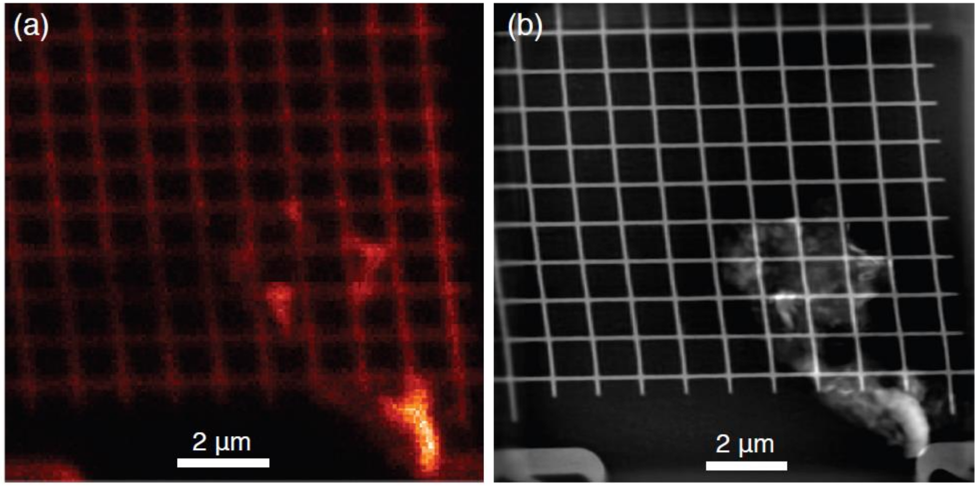
Detector plane:



- Scanning microscopy typically only utilises red area
- Additional information is then 'integrated over' and lost

- R. Hegerl, W. Hoppe, *Ber. Bunsenges. Phys. Chem* 74, 1148 (1970).
- J. M. Rodenburg, H. M. L. Faulkner, *Appl. Phys. Lett.* 85, 4795 (2004).
- P. Thibault et al., *Science* 321, 379 (200
- D. J. Vine, *et al* Opt. Express (2012);

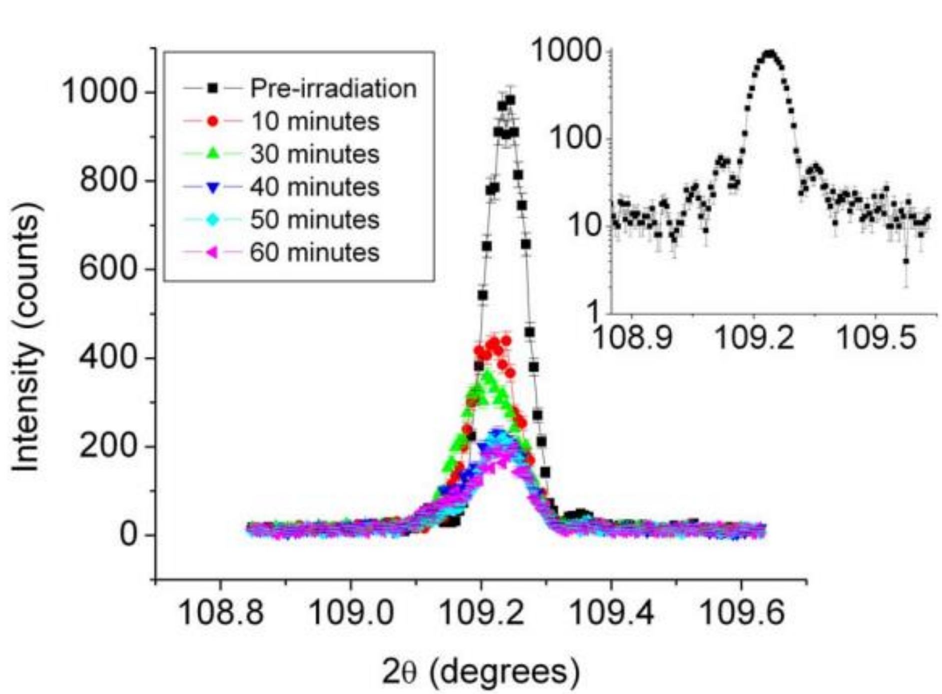
See presentation tomorrow, Ross Harder



Deng et al, submitted

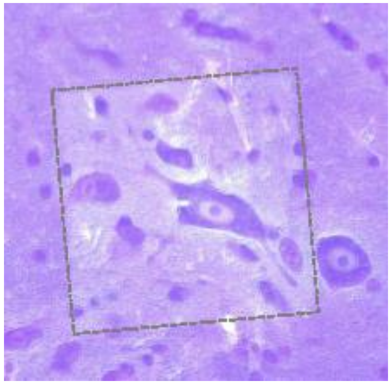
RADIATION DAMAGE:

- Exciting optics developments: <10 nm spatial resolution seems achieveable, but what about radiation damage ?
- In particular with focused x-ray and sensitive samples, radiation damage can be an issue that needs to be taken into account

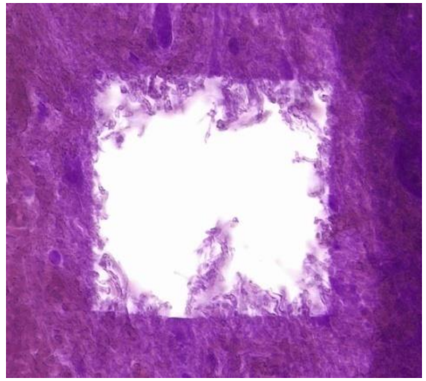


- Example for radiation damage in a SOI structure, Polvino et al, APPLIED PHYSICS LETTERS 92, 224105 2008

Fixed (p-formaldehyde), paraffin, scanned, rehydrated



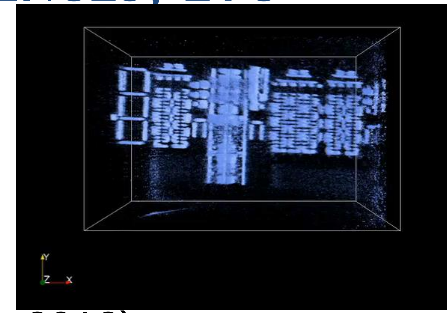
Freeze dried (unfixed), scanned, rehydrated



With 200x higher brightness, 10x10 better focusing, can have 10,000 higher flux densities.
Fast scanning becomes an absolute must:
ms -> us becomes requirement and opportunity

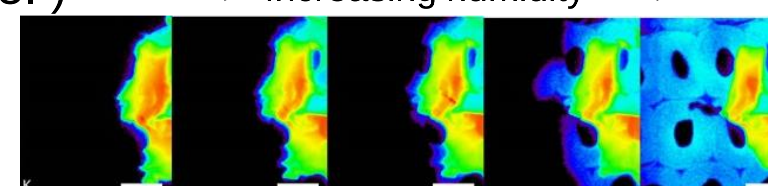
APPLICATIONS

IMAGING WITH ELEMENTAL CONTRAST: AT HIGH SPATIAL RESOLUTION (TRACE) METALS IN ENERGY, MATERIAL SCIENCES, ETC



- Metals can be contaminants that can severely impact device performance
  - in multi crystalline Solar cell materials (eg, Bertoni *et al.*, Energy Environ. Sci., 2011)
  - in organic photovoltaics (Nikiforov *et al*, Energy Environ. Sci., 2013)
- Metals play a significant role in the semiconductor industry (dopants, structures) (w. BAE systems)
- Metals are often the active component in catalysts their behaviour can improve design choices for materials.
  - ageing catalysts in the chemical industry
- Metals can be used as tracers, e.g., in Cultural Heritage (eg, Picasso paint: Casadio &Rose, Appl. Phys. A (2013))
- Facilitate R&D of construction materials
  - Geopolymer Science, eg, leaching of heavy metal contaminants (Langmuir 25 (2009) 11897, Cem. Conc. Res. 42 (2012) 855.)
  - Fastener corrosion and fungal decay in wood Diffusion of Ions through wood as a function of relative humidity (Zelinka *et al*, Holzforschung, in review)

→ Increasing humidity →

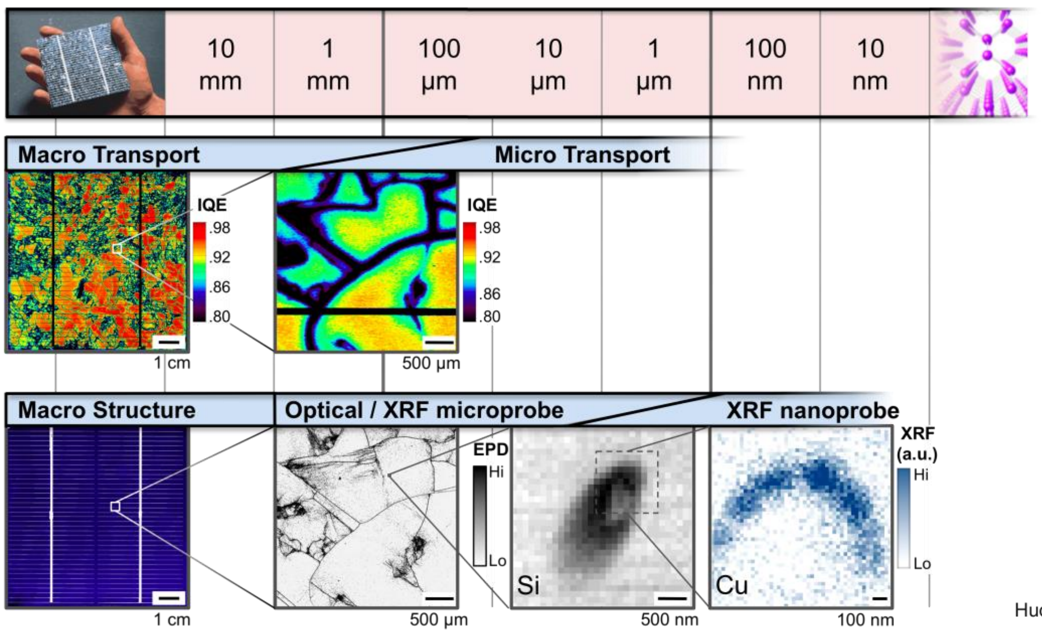


Moisture-induced chemical transport

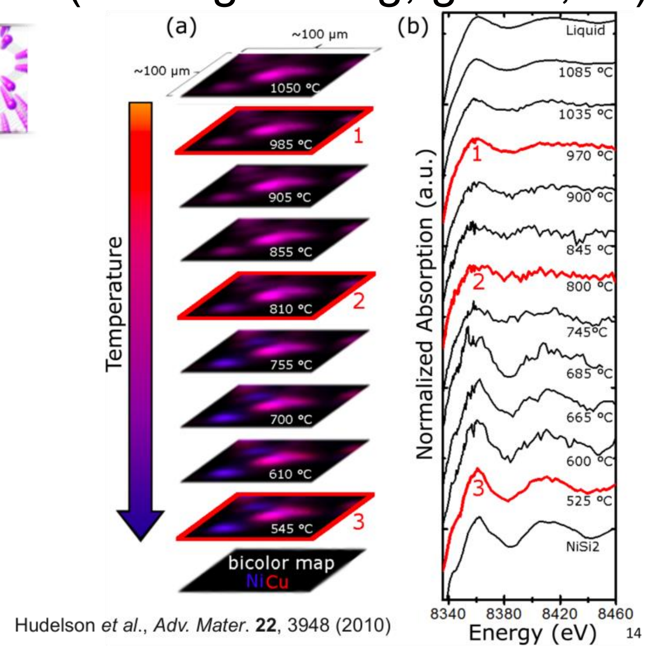
Argonne NATIONAL LABORATORY

PHOTOVOLTAICS: NANODEFECT ENGINEERING FOR HIGHER EFFICIENCY MC-SI SOLAR CELLS

- Multicrystalline solar cells have significant potential for inexpensive energy harvesting
- Small quantities of inhomogeneously distributed precipitates, and contaminants affect overall system performance
- High spatial resolution (and sensitivity) to detect smallest quantities of metal contaminants
- High efficiency to survey large sample areas
- Working distance to support in situ environments (heating/cooling, gases, …)



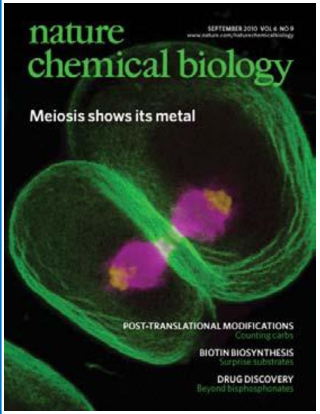
Bertoni, et al., Energy Environ. Sci. 2011,

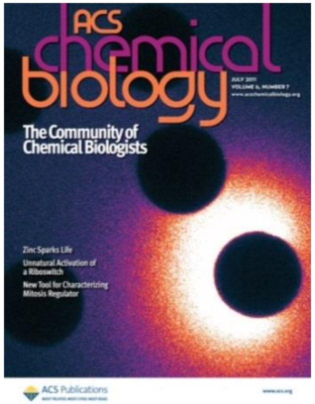


S. Hudelson *et al.*, Adv. Mater. **22**, 3948 (2010)

IMAGING WITH ELEMENTAL CONTRAST AT HIGH SPATIAL RESOLUTION: TRACE METALS IN THE LIFE SCIENCES

- Trace elements (metals) are fundamental, intrinsic components of biological Systems. estimated: 1/3 of all known proteins contain metalcofactors as integral, catalytic components, often with regulatory functions, e.g.,
  - Zn in Zinc finger proteins: transcription factors
  - Fe in Haemoglobin; and necessary in Chlorophyll synthesis
- Metals are linked to diseases
  - Endogenous dysregulation, e.g., Alzheimer's, ALS, Wilson disease (Cu accumulation)
  - Exogenous uptake, e.g., Pb, As, Hg (or lack thereof: e.g., Se deficiency)
  - Bio-remediation
- Metals in therapeutic drugs and diagnostic agents
  - Cis-platin in chemotherapy
  - Gd in Magnetic resonance imaging (MRI)
  - Novel bio-inorganic nanoparticles, in particular Nanomedicine: multifunctional nanovectors ideally combining targetting, therapy (e.g., Pt, TiO_2) and diagnosis



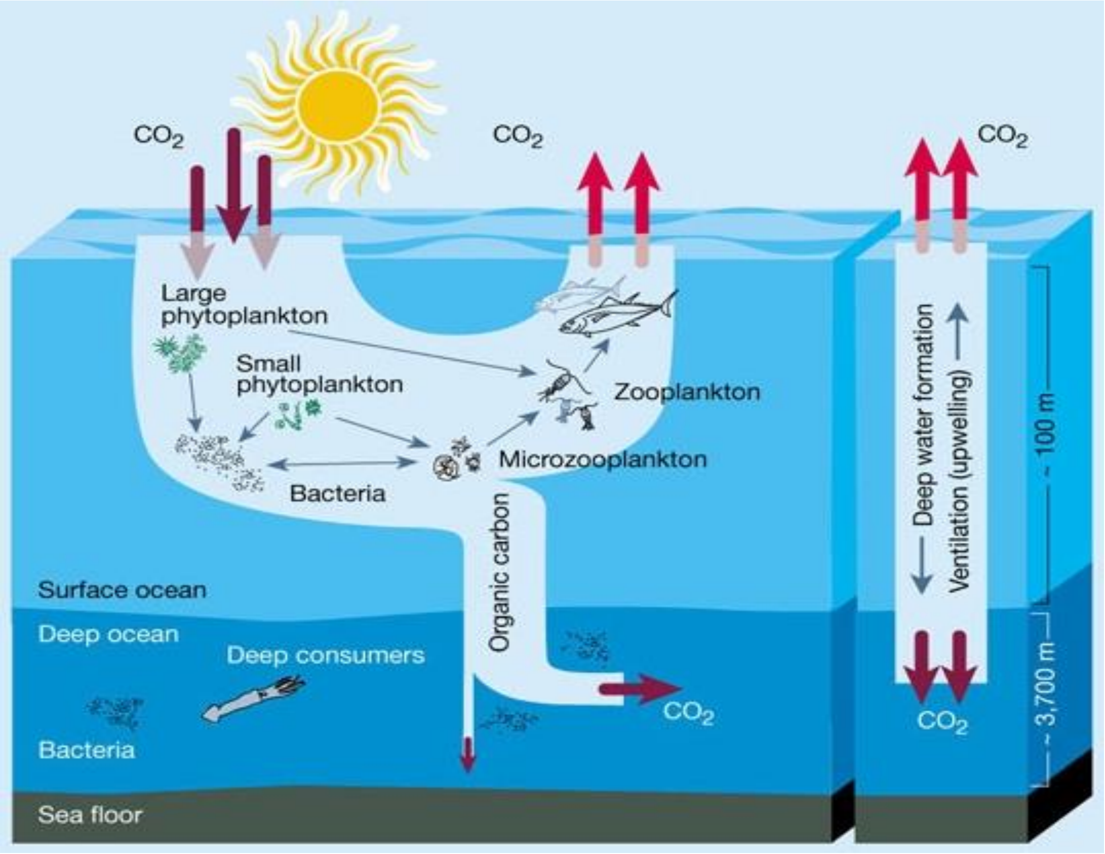


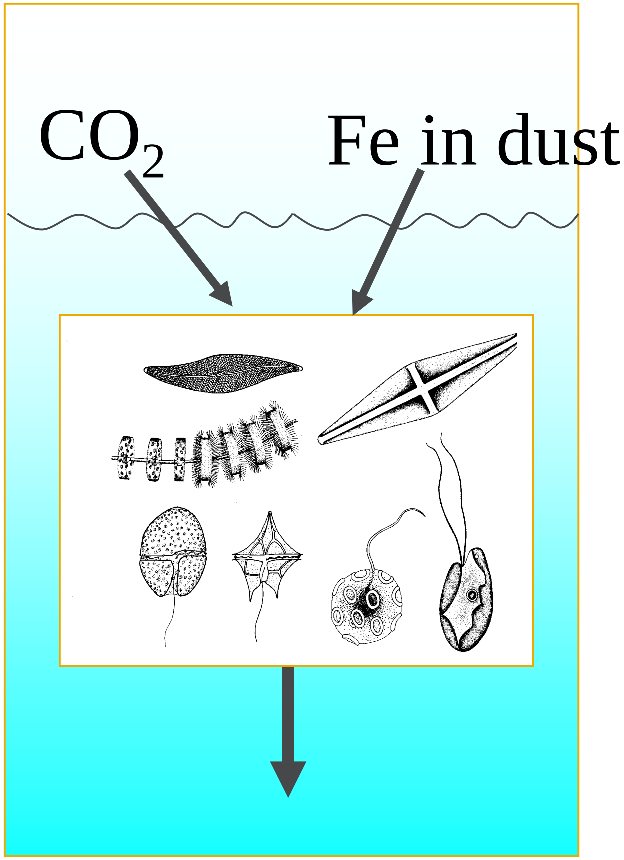
Zinc plays an unexpected role in oocyte maturation: Kim *et al*. Nat Chem Biol. 2010 **6**(9):674-81; Kim et al, ACS Chem Biol, 2011. **6**(7): p. 716-23.

Recent reviews of **XFM applications**:
Imaging: T. Paunesku *et al*., J Cell Biochem **99**(6), 2006
Spectroscopy: C. Fahrni, Curr Opin Chem Biol **11**(2), 2007

Review of **XFM tomography**:
M. de Jonge & S. Vogt, Curr Opin Struct Biol **20**(5), 2010

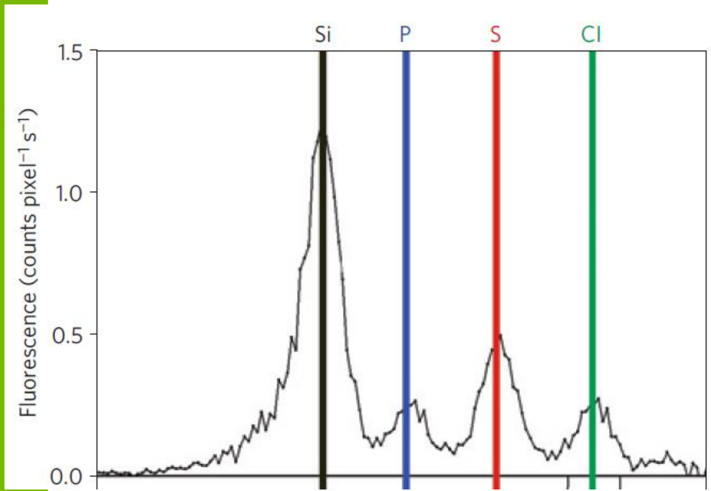
GLOBAL CARBON BALANCE - THE BIOLOGICAL PUMP

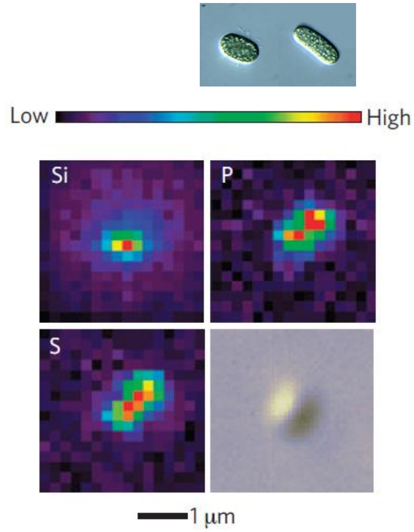


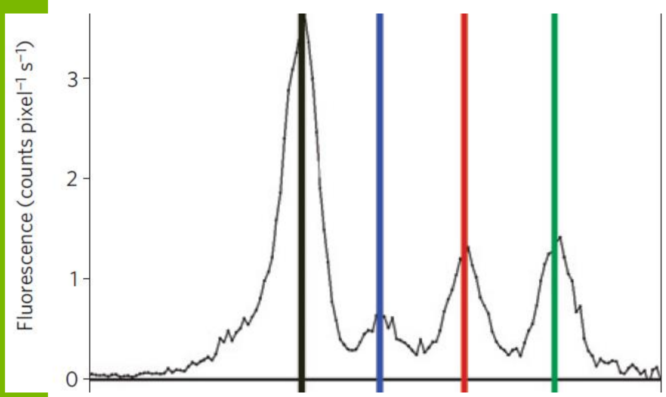


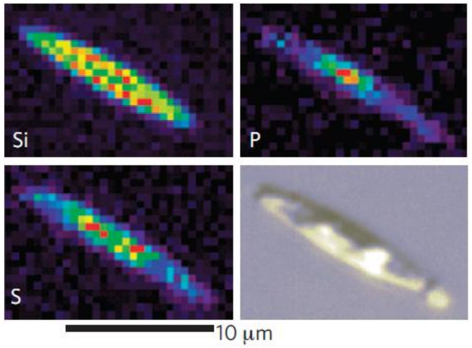
- Phytoplankton converts dissolved carbon into biomass.
- small fraction (~1%?) is exported from the surface waters into deep ocean (net loss for hundreds of years
- Key limiting factors: micronutrients (Fe), but also Silicon …

SURPRISING ROLE FOR PICOYANOBACTERIA





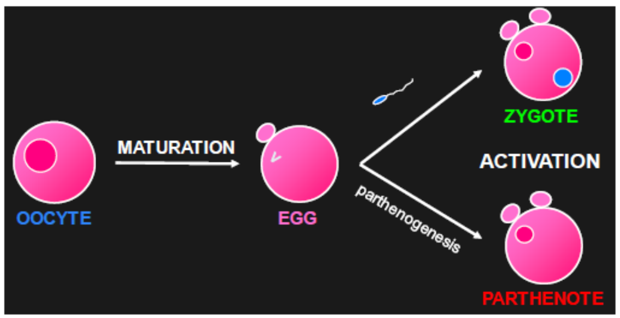


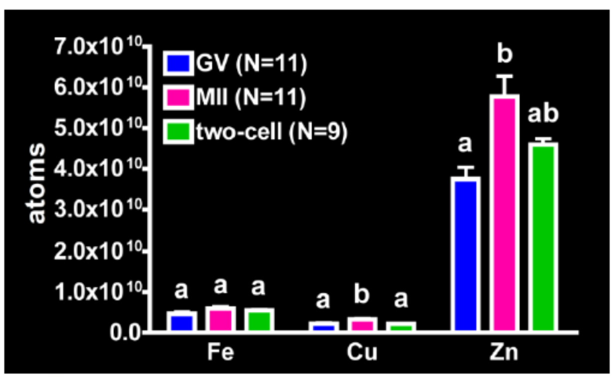


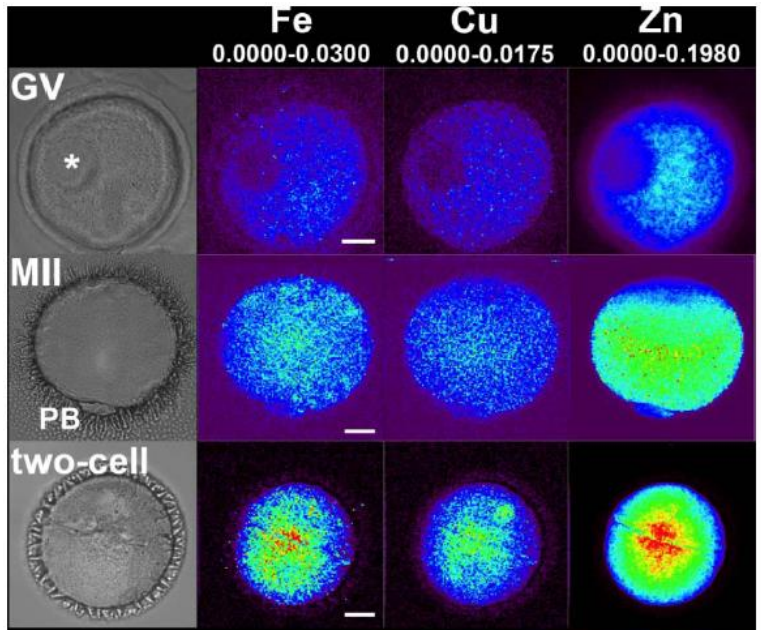
- Picocyanobacteria make up majority of organisms in ocean. 50+% (!) of O_2 generated by ocean.
- *Synechococcus* can show silicon ratios similar to diatoms
- significant, previously not know Si sink
- mechanism of Si accumulation is not yet known, in part because we cannot resolve the form and precise location of the Si associated with the cell.

WHAT MAKES A GOOD EGG AND HEALTHY EMBRYO?

- Zinc plays an unexpected role in oocyte maturation: Zn content is an order of magnitude higher in eggs than Fe and Cu.
- Zn level increases by 50% during maturation. Zn depletion arrests the maturation process.
- One of the first studies to implicate zinc as a possible signaling molecule in a biological system, not just a protein cofactor
- Bulk analysis cannot be applied to rare cells such as mammalian oocytes







In the XFM image a mature (MII) eggs retains Zn while polar body is Zn low. This asymmetry is required for correct oocyte maturation. Scale bar 20 um.

Kim AM, Vogt S, O'Halloran TV, Woodruff TK. ***Nat Chem Biol. 2010*** *6(9):674-81.*
Kim, AM, ML Bernhardt, BY Kong, R.W Ahn, S Vogt, TK Woodruff, TV O'Halloran. ***ACS Chemical Biology 2011***

APPLICATION: CHROMIUM CARCINOGENESIS

- Cr(III): common dietary supplement, supposed essential role in insulin action. Often claimed to have value as a weight loss or muscle building agent. Dietary supplement: not regulated by FDA
  - US $100 million / year industry

- Cr(VI): has been designated as an established human carcinogen by the IARC.
  - Industrial carcinogen: lung cancer, sino-nasal cancer.
  - environmental exposure to Cr(VI), resulting from the poor disposal practices of Cr(VI) into unlined ponds

- Cr(V): Lab studies suggest Cr(VI) exerts its genotoxic effects via reduction into the reactive Cr(V) intermediate – more genotoxic than Cr(VI)?

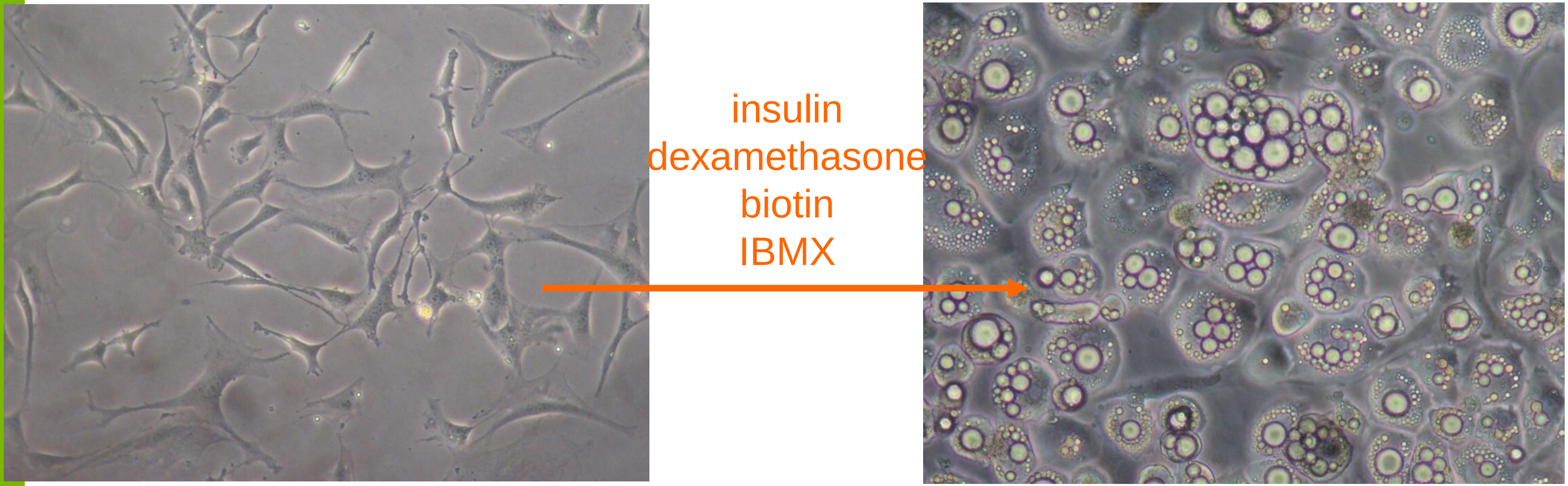
P. Lay *et al*, Univ Sydney

Erin Brockovich

Argonne NATIONAL LABORATORY

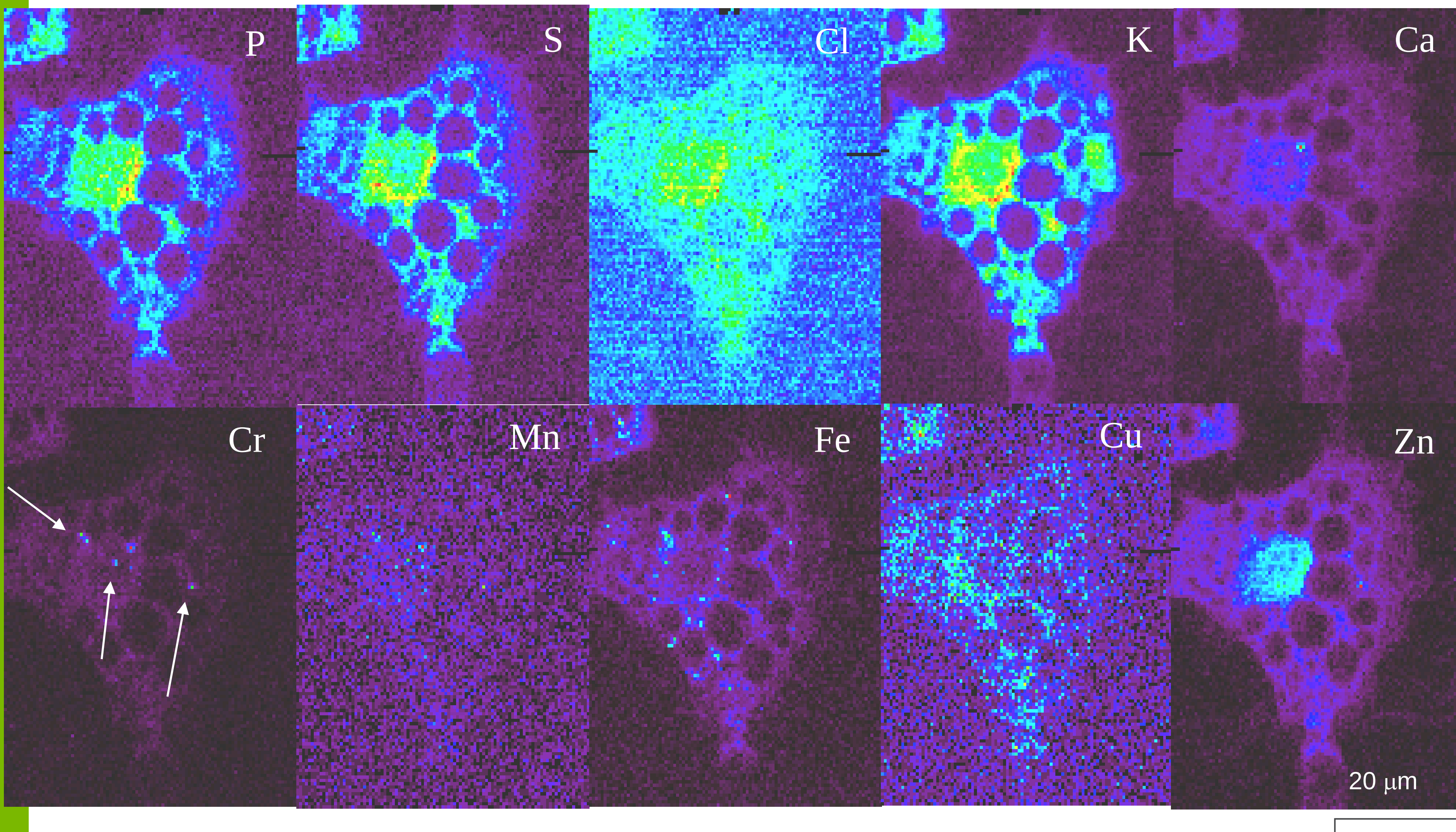
IN VITRO MODELLING OF CHROMIUM TREATMENT – 3T3-L1 ADIPOCYTES.

- 3T3-L1 adipocytes (fat cells) easily cultured.
- Do adipocytes take up Cr(III) ?
  – If so, does Cr(III) change its oxidation state ?

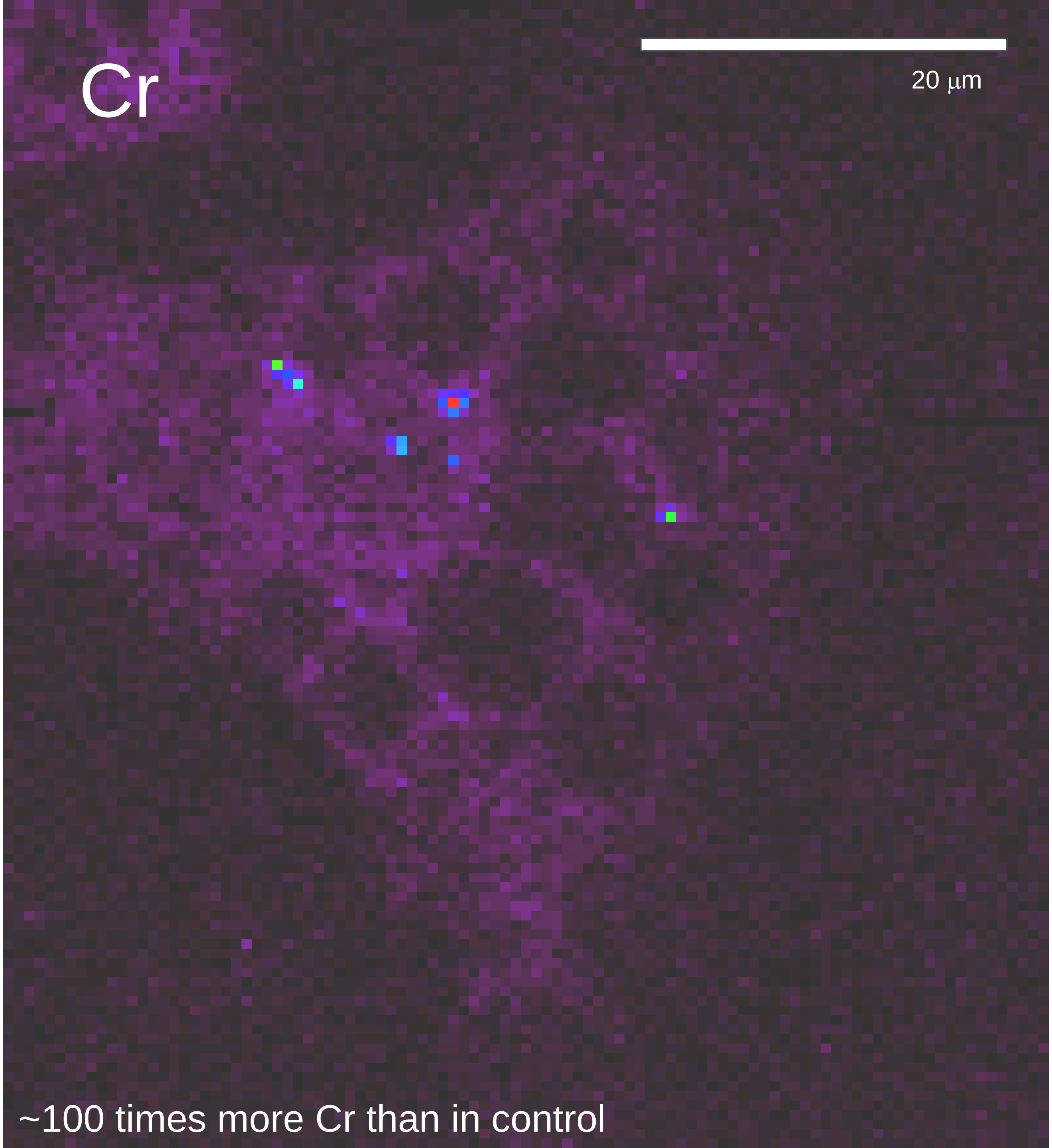


XRF ELEMENTAL IMAGING OF WHOLE ADIPOCYTES

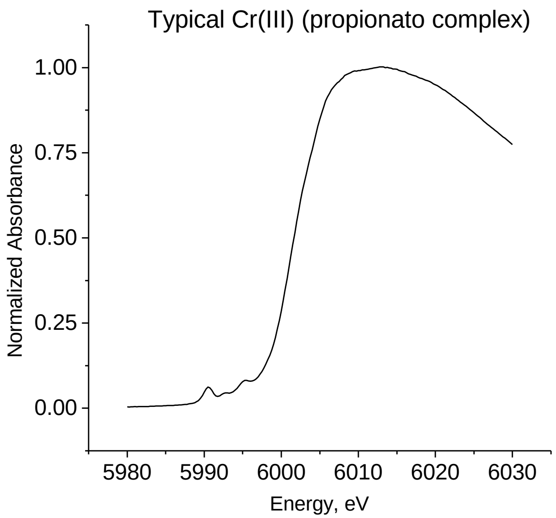
- What is the intracellular distribution of Cr in adipocytes treated with Cr?

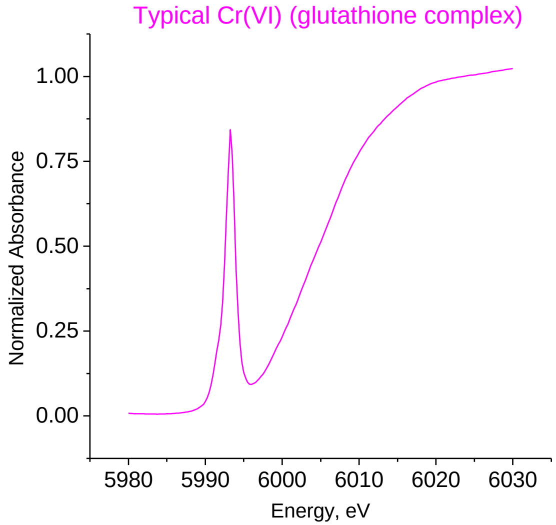


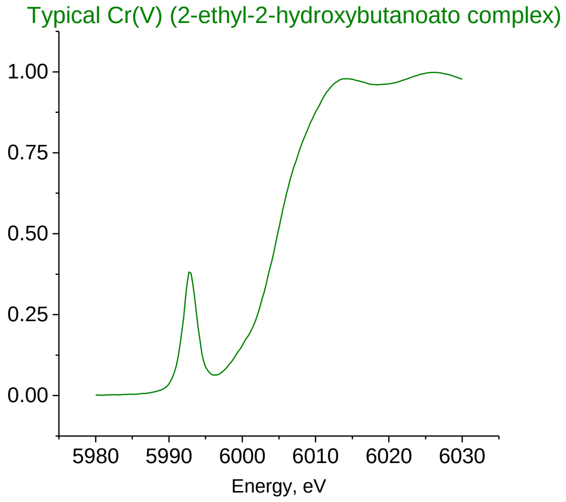
- Treated with 100 µM trinuclear Cr(III) propionate, 20 hours.
- Cells grown and fixed on silicon nitride windows

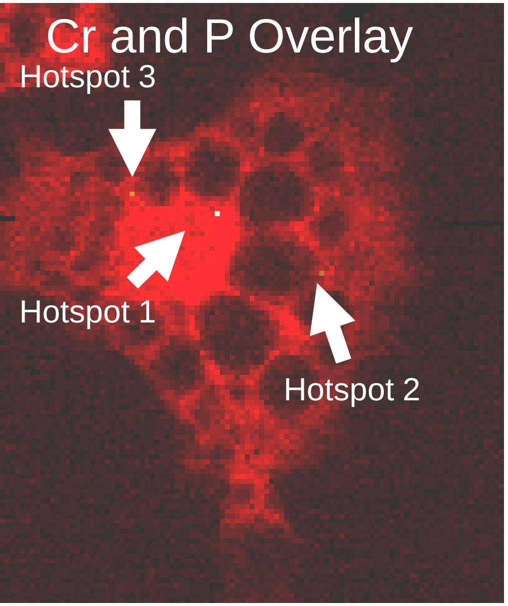

Cr
20 µm
~100 times more Cr than in control

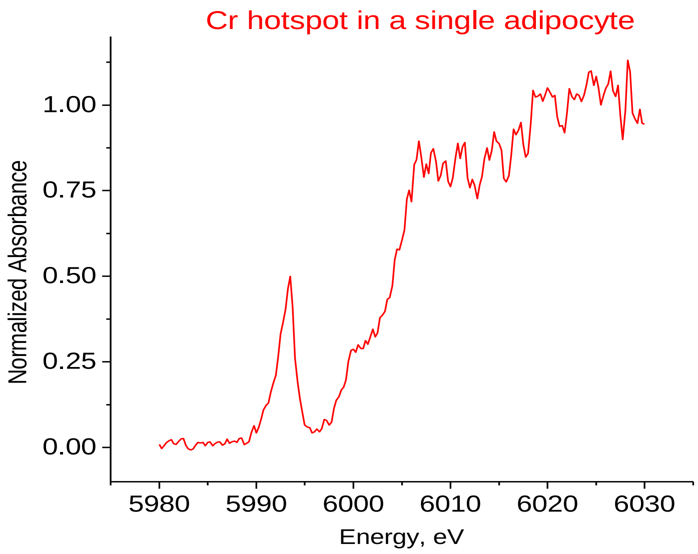
What sort of chromium is present? - Cr K-edge XANES









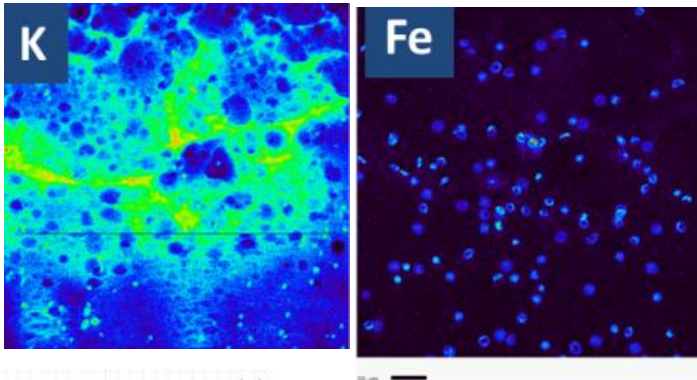


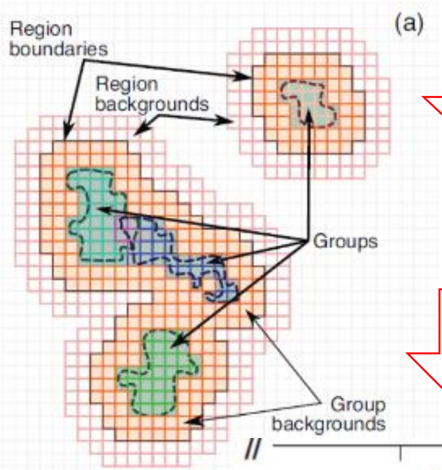
Up to 55% Cr(V)
Up to 36% Cr(VI)
(the carcinogenic form)

L. Wu *et al*, submitted

BIG DATA

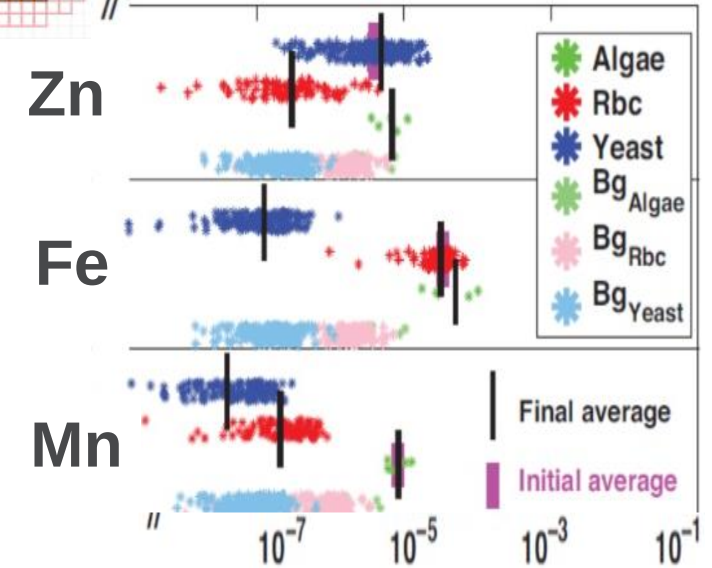
- Today
  - Manually moving, analyzing data.
  - Ad hoc tools that do not scale to the next generation of instruments
  - algorithms can be "dangerous" if not used carefully
- Tomorrow
  - Extensive toolset of scalable algorithms (e.g., machine learning, statistical)
  - Scientific knowledge integrated with analysis, visualization and simulation
  - Automatic Integration of data from multiple sources, cataloguing and transfer
  - Efficient data reduction strategies





Identification and Classification

Reduction and Visualization



Top: X-ray fluorescence maps of different cells. Middle: software automatically identifies and classifies 3 different cell types, enabling further analysis. Comparison of the resulting average elemental content per individual cell.

S. Wang, et al, J Synchrotron Radiation, accepted

HOW CAN YOU MAKE USE OF THESE RESOURCES ?

- beamtime is available on most beamlines at most synchrotrons to outside users through a competitive proposal process.
- Proposal submission deadlines typically 2 or 3 times a year.
- Typically 80% of ‘beamtime’ on any beamline is distributed
- Some types of proposal:
  - General User Proposals
    - Open to anyone, just have to write a good proposal. Proposals get reviewed by committee, assigned based on scores. Proposals that don’t quite make the score, ‘age’ so that they have a better chance next time.
    - Users typically come for experiments 3-4 days (9-12 shifts), carry out experiments with help of beamline scientist
    - No cost for beamtime, the expectation is that results will be published.
  - Proprietary Experiments
    - Are also possible. Proposals are rated differently, less detail needed. Results generally not published, but cost recovery of beamtime is required (APS, $373/h at the moment)
- Most importantly: try to identify possible beamlines in advance, and **contact the beamline scientist** well **before writing the proposal**

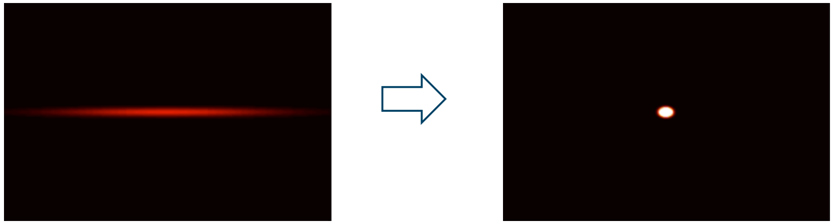
A general resource relating to synchrotron sources world wide:

http://www.lightsources.org/

SUMMARY

- Full field imaging, often in combination with tomography
  - Parallel Beam Imaging (PBI): Phase and absorption, 1 µm spatial resolution, and temporal resolution kHz (3D) to 6.5 MHz,100 ps (2D). No x-ray optics
  - Nanoimaging: spatial resolution limited by x-ray optics (typically zone plates, CRLs for higher energies), down to 60-20 nm. Time resolution ~Hz
- Scanning probe imaging
  - Resolution limited by x-ray optics (KBs: typically ~microns, can go down to 100 nm, ZPs typically 200 – 20 nm)
  - Typically slow (can only use coherent part of beam for high resolution, need to scan the sample)
  - Access to variety of contrast modes (absorption, phase, fluorescence, diffraction)
    - XRF for trace element detection
- Both can be combined with spectroscopy, but different sensitivities.

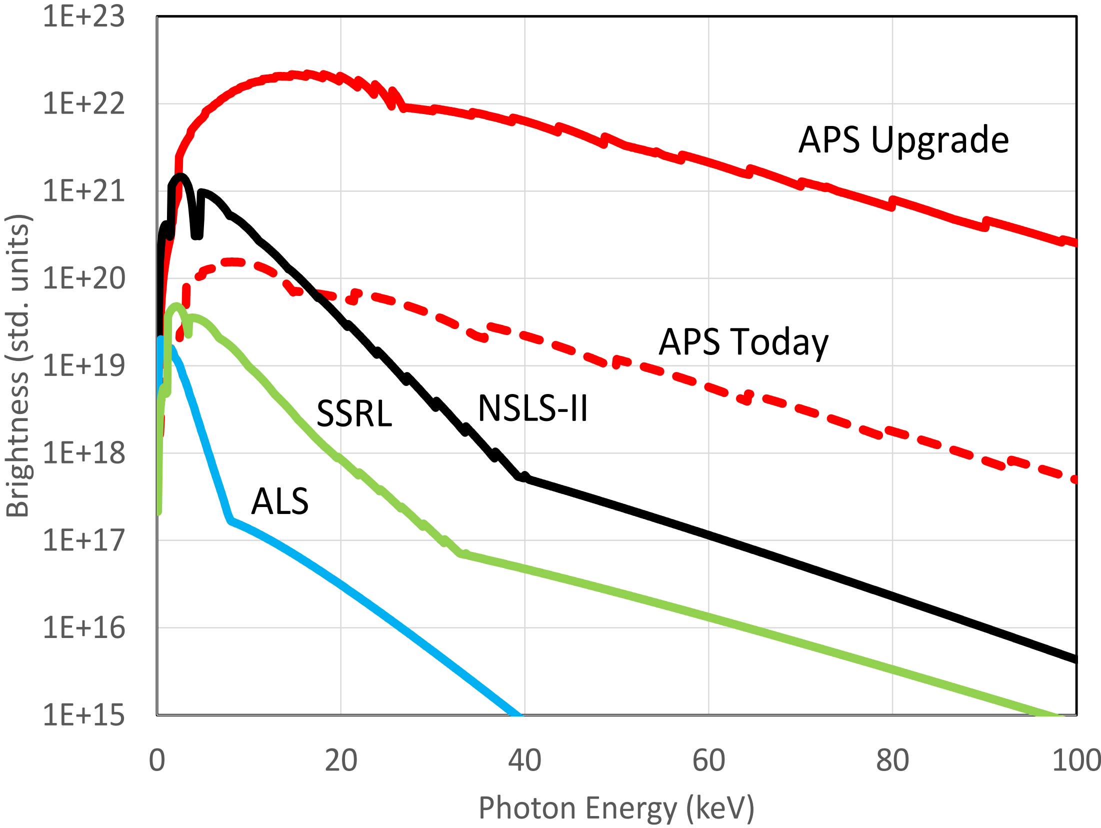
APS MBA UPGRADE: A BRIGHT FUTURE



- Brightness increases of 100x and more compared to what we have today
- Micro/nanoprobes directly brightness driven

⇒ possible to get nearly 100% of APS flux into a 0.3x0.25 um spot !!!

⇒ Upgrade: push for highest direct resolution <=10 nm and augment with CDI/ Ptychography



This upgrade will revolutionize scanning microscopies and lensless imaging techniques !!!

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Thanks!