An introduction to x-ray fluorescence microscopy

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Outline

- The physics and nomenclature of x-ray fluorescence
- Calculating fluorescence rates
- Detecting fluorescence
- Comparison with electron microprobes
- Radiation dose and damage, and cryo microscopy
- From 2D to 3D: fluorescence tomography, tomographic alignment, and self-absorption correction

The Bohr model of the atom

- Neils Bohr was inspired by Ernst Rutherford to think about the nature of atoms following Rutherford's discovery that they have a small, positively-charged nucleus.
- Bohr arrived at a model by a different means, but this is most easily undressed by the later picture of de Broglie standing waves for electrons.
- End result is quantized orbital energies of

$$E_n = -E_0 \frac{(Z - Z_{\text{screen}})^2}{(n - \delta_\ell)^2}$$

with E_0=13.6 eV.

- For rough estimates, we can ignore:
  - Z_{screen} which represents an approximate screening of the nuclear charge by other inner-shell electrons
  - δ_ℓ which represents a "quantum defect" for multi-electron atoms (especially alkali atoms).
- Unfortunately two notations for atomic orbitals developed in paralllel:
  - X-ray shells shells K, L, M (Barkla)
  - Principle quantum numbers n=1, 2, 3 (Bohr)

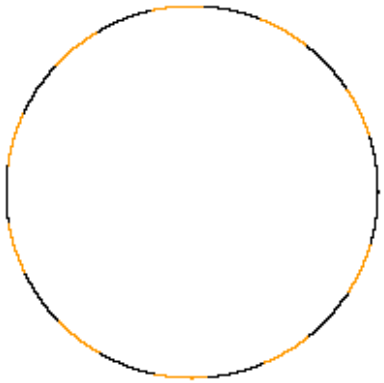
De Broglie waves

- Relationship between electron momentum p and wavelength λ of

$$\lambda = \frac{h}{p} = \frac{hc}{\sqrt{2m_e c^2 E_k}}$$

where h=Planck's constant, hc=1240 eV•nm, and the energy equivalent of the electron's mass is $m_e c^2$=511 keV.

n=3 (M shell) electron "standing wave" of circumference=3λ

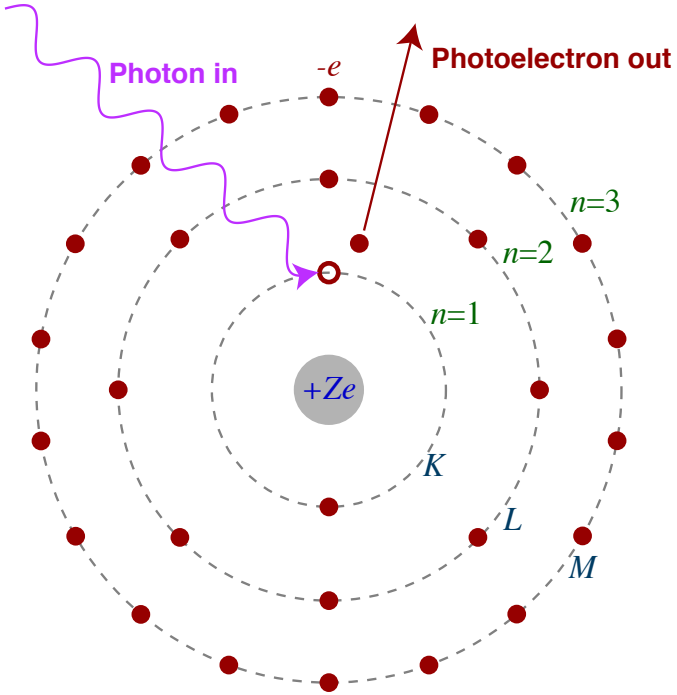


- For photons with energy E, the wavelength-energy relationship is

$$\lambda = \frac{hc}{E}$$

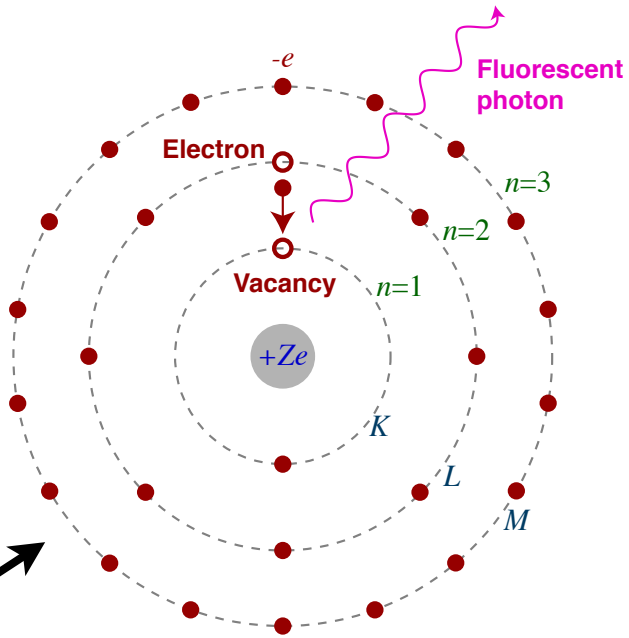
A cartoon of the process

$$E_2 - E_1 = E_0 Z^2 \left(\frac{1}{1^2} - \frac{1}{2^2} \right) = \frac{3}{4}(13.6 \text{ eV}) Z^2$$

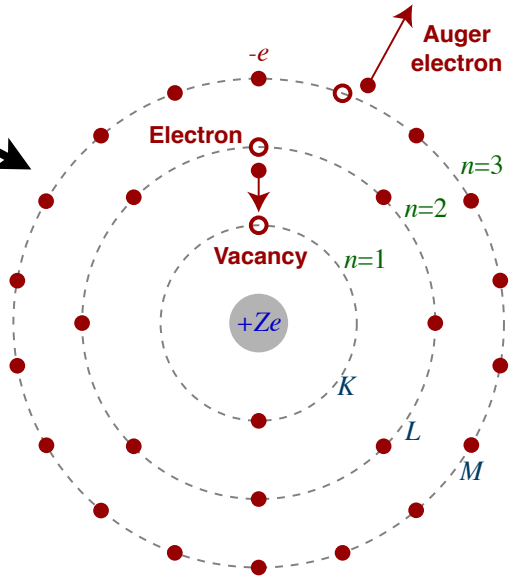


Absorb a photon; create an inner-shell vacancy. Must get rid of the energy!

Either/or



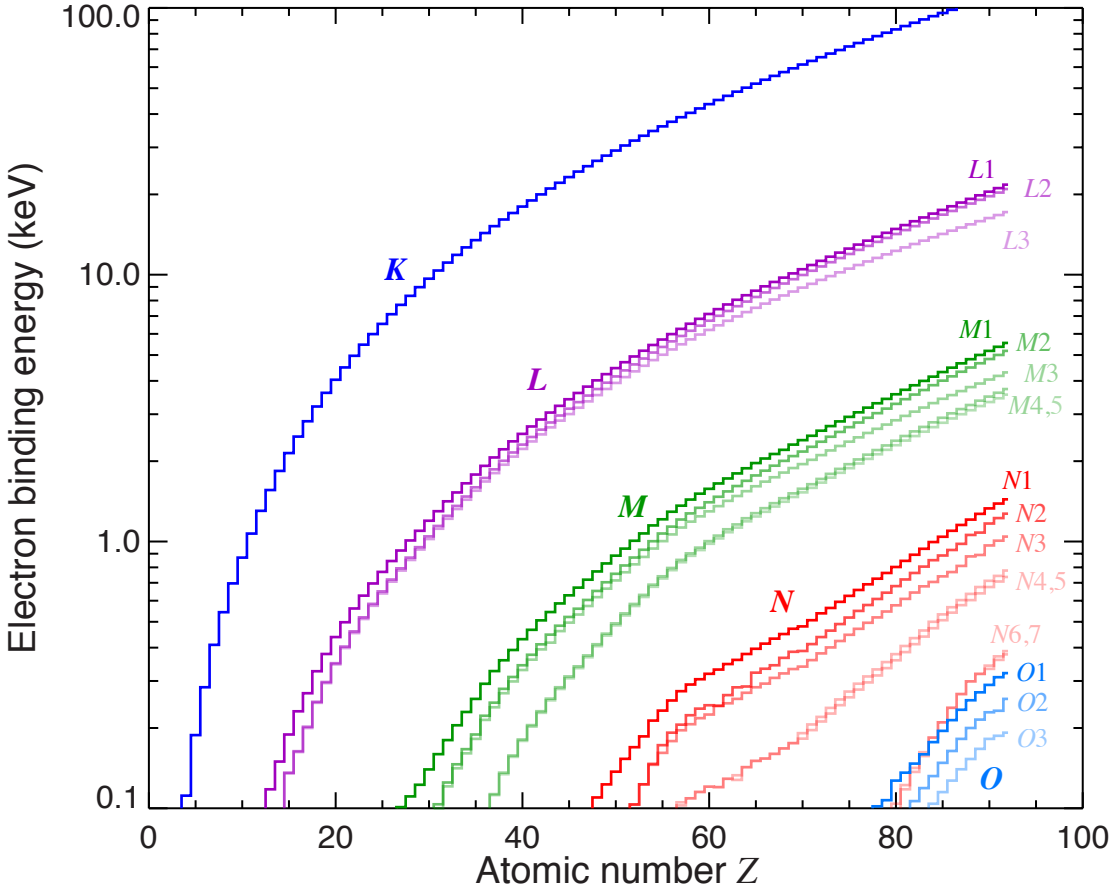
Fluorescence



Auger

Electron binding and Stokes shifts

- One must first remove an electron, which takes more energy.
- Fluorescence then follows from an electron dropping down from a less strongly bound orbital.
- This difference between absorption edges and fluorescence lines is called the Stokes shift.



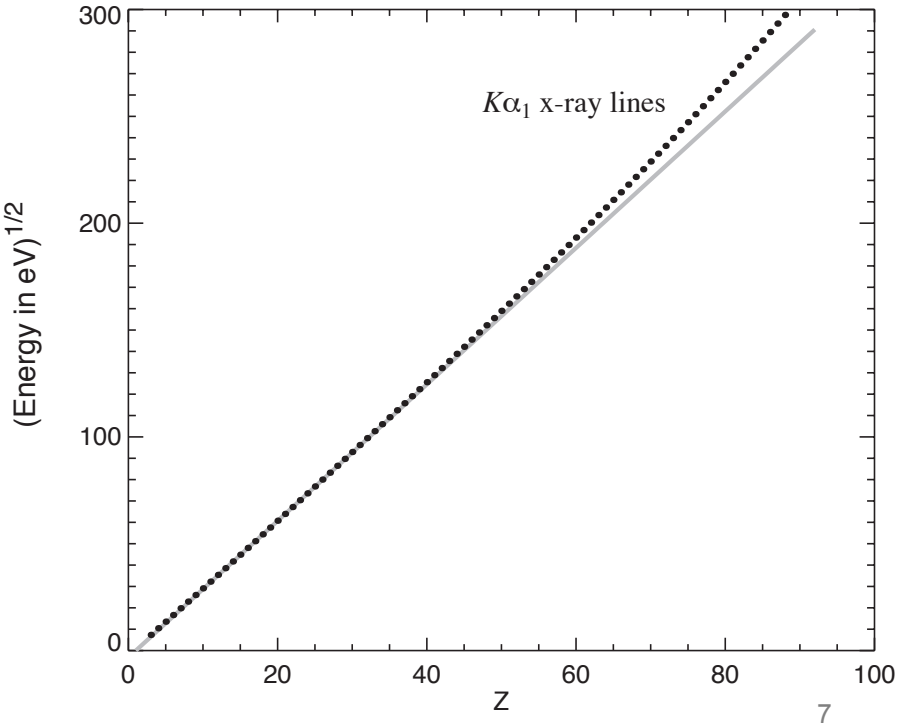
Tabulation: see *e.g.*, Elam, Ravel, and Sieber, *Radiation Physics and Chemistry* **63**, 121 (2002).

Python library xraylib: Schoonjans *et al.*, *Spectrochimica Acta B* **66**, 776 (2011)

Moseley's Law

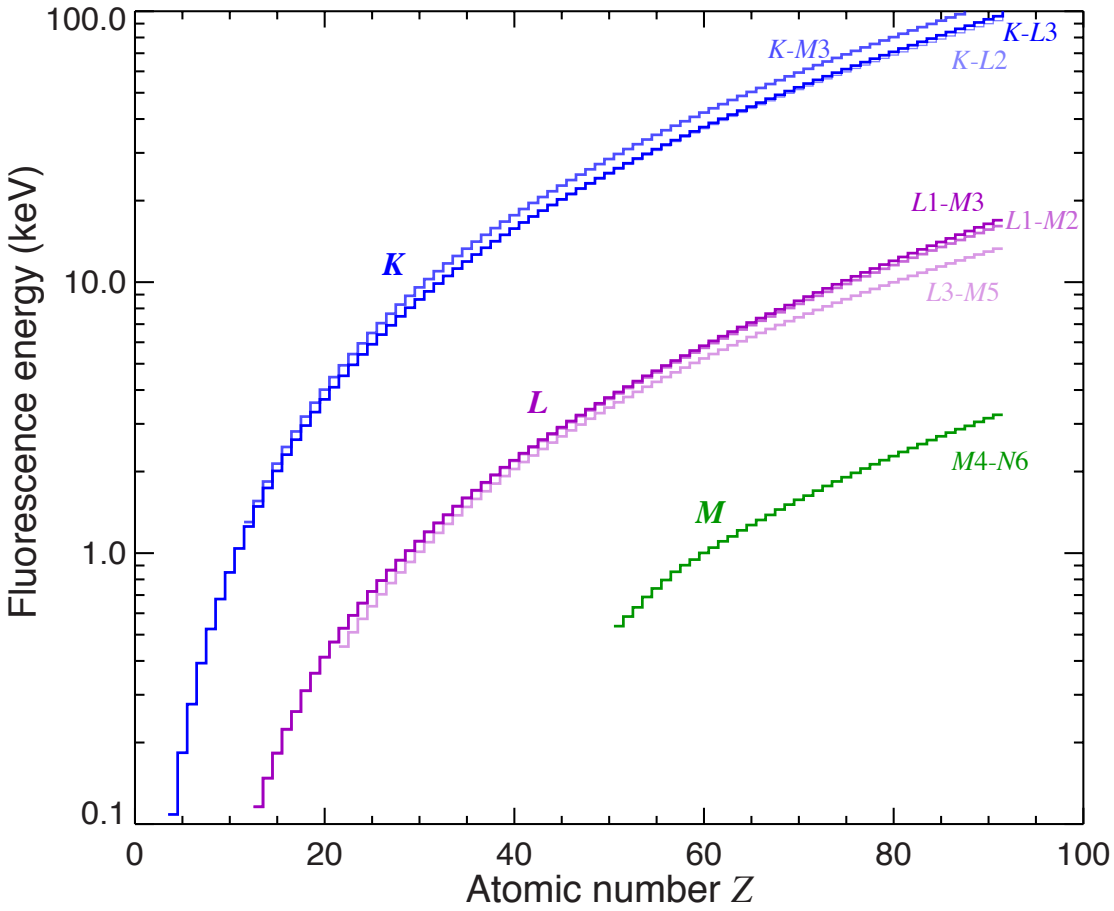
- Henry Moseley (in Rutherford's lab in Manchester) found in 1913 that the photon energy of x-ray emission lines go as Z^2.
- Predicted the existence of elements 43, 61, 72, and 75, and had a big influence on Bohr's theory.
- Killed by a sniper at Gallipolli, Turkey on Aug. 10, 1915.

$$n = 2 \quad \rightarrow \quad n = 1: \quad E = E_0 Z^2 \left(\frac{1}{1^2} - \frac{1}{2^2} \right) = \frac{3}{4}(13.6 \text{ eV}) Z^2$$



X-ray fluorescence energies

- Details of transitions on a later slide.



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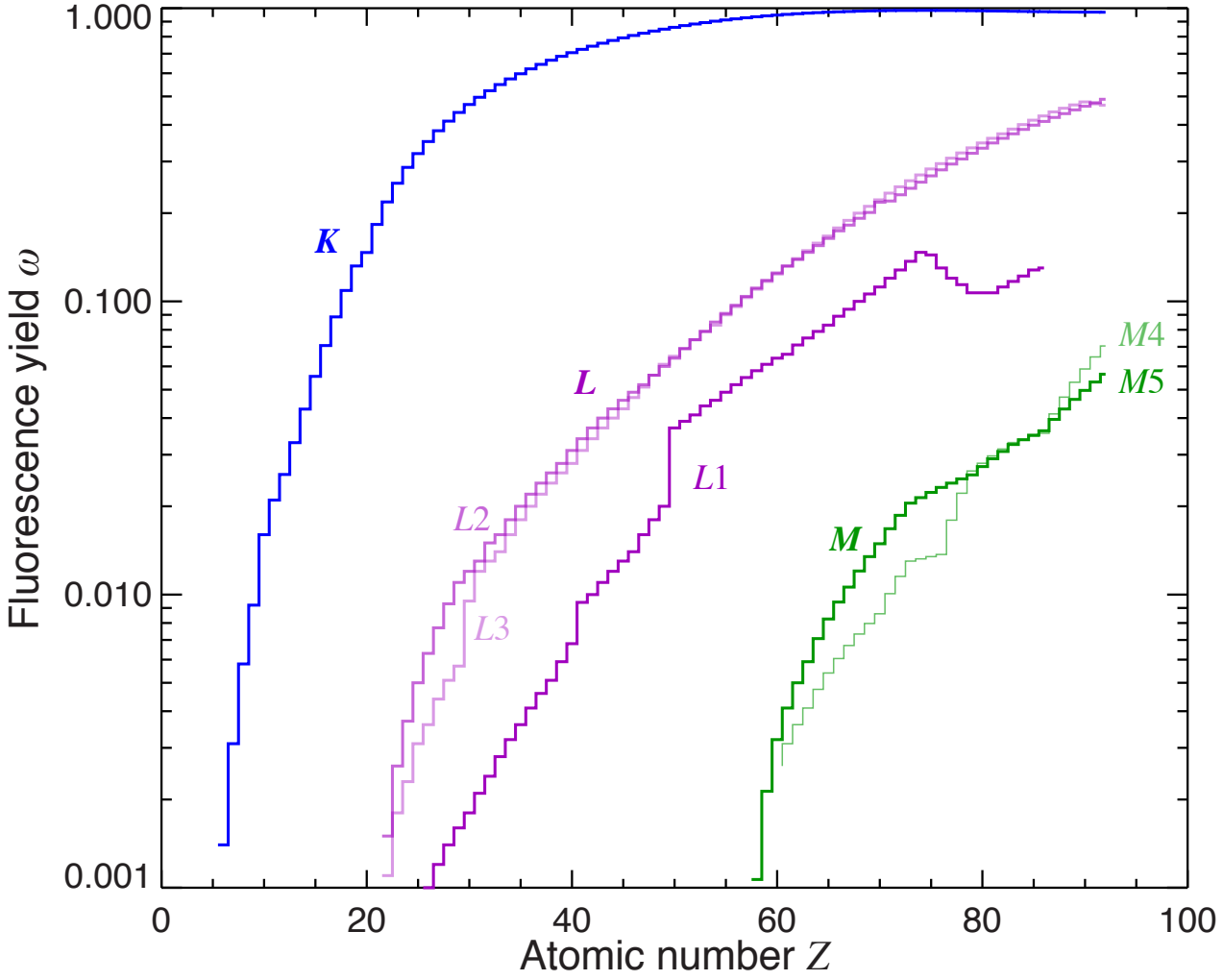
Python library xraylib: Schoonjans *et al.*, *Spectrochimica Acta B* **66**, 776 (2011)

Fluorescence yield ω

Fluorescence yield ω=fraction of time you get a fluorescent photon rather than an Auger electron

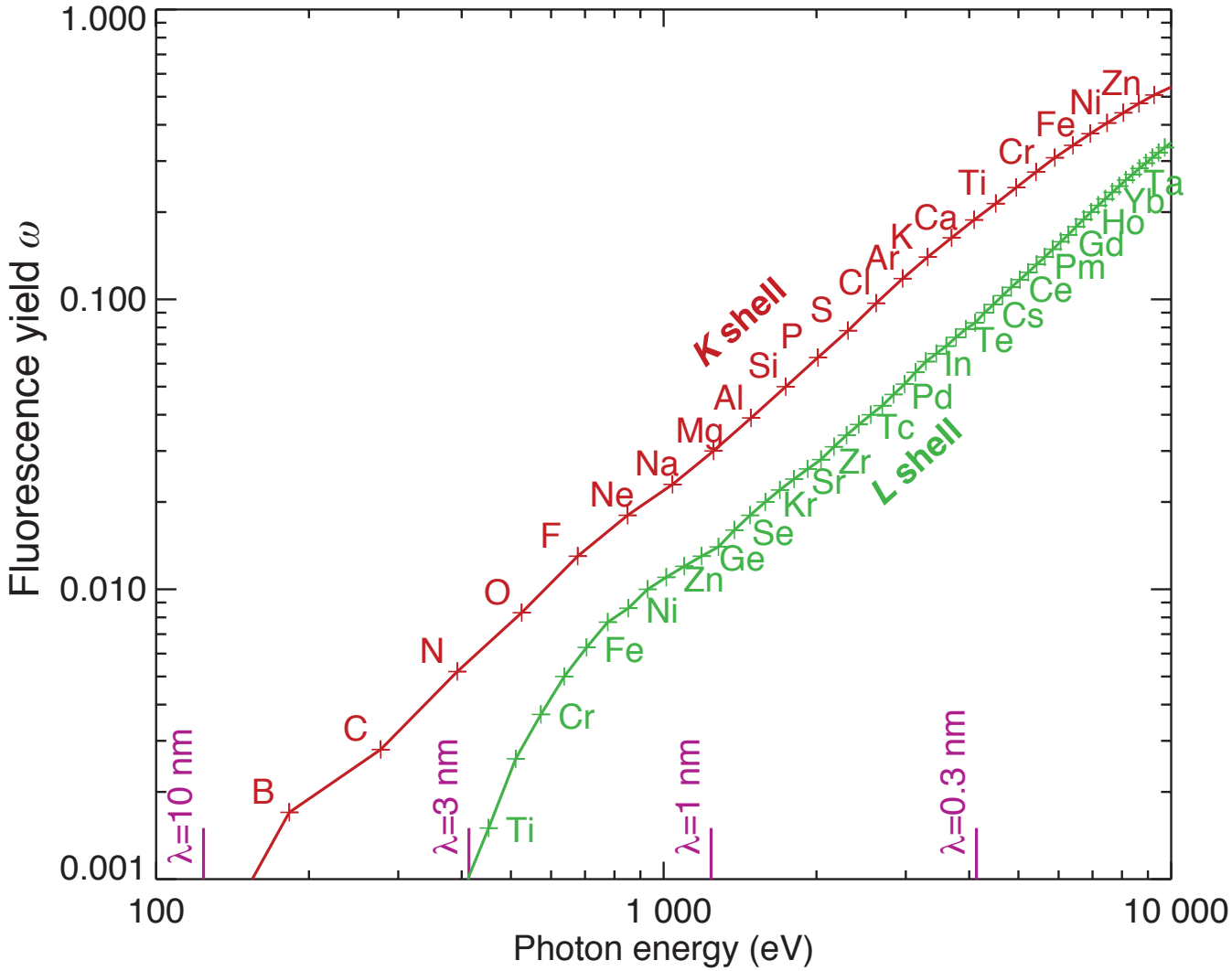
Tabulation: see *e.g.*, Elam, Ravel, and Sieber, *Radiation Physics and Chemistry* **63**, 121 (2002).

Python library xraylib: Schoonjans *et al.*, *Spectrochimica Acta B* **66**, 776 (2011)



Fluorescence versus Auger

Fluorescence yield ω=fraction of time you get a fluorescent photon rather than an Auger electron



Details of atomic orbitals

Electron orbital notation:

- n=principal quantum number
- ℓ=orbital angular momentum quantum number
- m_ℓ=z axis projection of ℓ
- s=electron spin
- j=total angular momentum

- M. Siegbahn, *The Spectroscopy of X-rays* (Oxford Press, London, 1925)

n	ℓ	m_ℓ	s	j	Occupancy	State	Siegbahn
1	0	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$1s$	K
2	0	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$2s$	L_1
2	1	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$2p_{1/2}$	L_2
2	1	-1,+1	$\pm\frac{1}{2}$	$\frac{3}{2}$	4	$2p_{3/2}$	L_3
3	0	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$3s$	M_1
3	1	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$3p_{1/2}$	M_2
3	1	-1,+1	$\pm\frac{1}{2}$	$\frac{3}{2}$	4	$3p_{3/2}$	M_3
3	2	-1,0,+1	$\pm\frac{1}{2}$	$\frac{3}{2}$	6	$3d_{3/2}$	M_4
3	2	-2,+2	$\pm\frac{1}{2}$	$\frac{5}{2}$	4	$3d_{5/2}$	M_5
4	0	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$4s$	N_1
4	1	0	$\pm\frac{1}{2}$	$\frac{1}{2}$	2	$4p_{1/2}$	N_2
4	1	-1,+1	$\pm\frac{1}{2}$	$\frac{3}{2}$	2	$4p_{3/2}$	N_3
4	2	-1,0,+1	$\pm\frac{1}{2}$	$\frac{3}{2}$	2	$4d_{3/2}$	N_4
4	2	-2,+2	$\pm\frac{1}{2}$	$\frac{5}{2}$	2	$4d_{5/2}$	N_5
4	3	-2,0,+2	$\pm\frac{1}{2}$	$\frac{5}{2}$	2	$4f_{5/2}$	N_6
4	3	-3,3	$\pm\frac{1}{2}$	$\frac{7}{2}$	2	$4f_{7/2}$	N_7
5	0	0	$\pm\frac{1}{2}$	$\frac{7}{2}$	2	$5s$	O_1
5	1	0	$\pm\frac{1}{2}$	$\frac{7}{2}$	2	$5p_{1/2}$	O_2
5	1	-1,+1	$\pm\frac{1}{2}$	$\frac{7}{2}$	2	$5p_{3/2}$	O_3

Detailed notation of x-ray transitions

- IUPAC: International Union of Pure and Applied Chemistry. R. Jenkins *et al.*, *Pure and Applied Chemistry* **63**, 735 (1991)
- M. Siegbahn, *The Spectroscopy of X-rays* (Oxford Press, London, 1925)

Initial state	Final state	IUPAC	Siegbahn
$2p_{3/2}$	$1s$	K-L_3	$K\alpha_1$
$2p_{1/2}$	$1s$	K-L_2	$K\alpha_2$
$2s$	$1s$	K-L_1	$K\alpha_3$ (forbidden)
$3p_{3/2}$	$1s$	K-M_3	$K\beta_1$
$4p_{3/2}$	$1s$	K-N_3	$K\beta_2^I$
$4p_{1/2}$	$1s$	K-N_2	$K\beta_2^{II}$
$3p_{1/2}$	$1s$	K-M_2	$K\beta_3$
$3d_{5/2}$	$2p_{3/2}$	L_3-M_5	$L\alpha_1$
$3d_{3/2}$	$2p_{3/2}$	L_3-M_4	$L\alpha_2$
$3d_{3/2}$	$2p_{1/2}$	L_2-M_4	$L\beta_1$
$4p_{5/2}$	$2p_{3/2}$	L_3-N_5	$L\beta_2$ (forbidden)
$4d_{3/2}$	$2p_{1/2}$	L_2-N_4	$L\gamma_1$
$4p_{1/2}$	$2s$	L_1-N_2	$L\gamma_2$
$4f_{7/2}$	$3d_{5/2}$	M_5-N_7	$M\alpha_1$
$4f_{5/2}$	$3d_{5/2}$	M_5-N_6	$M\alpha_2$

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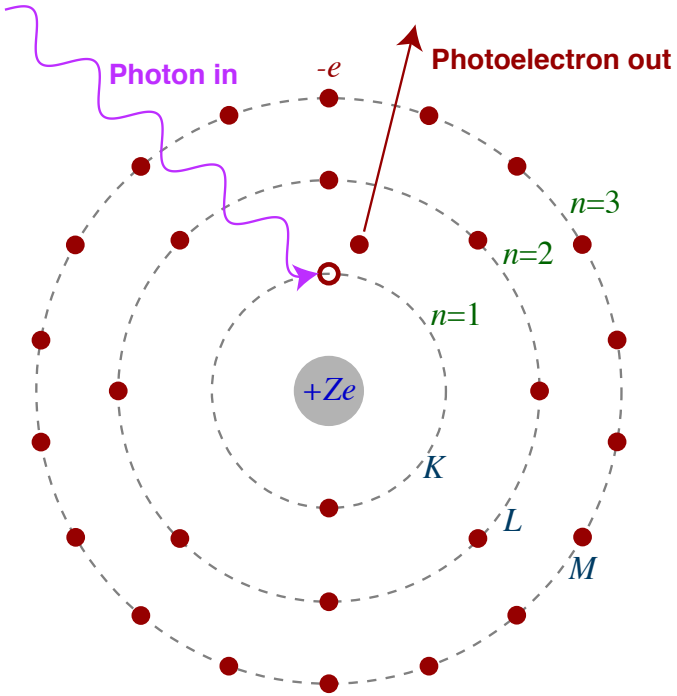
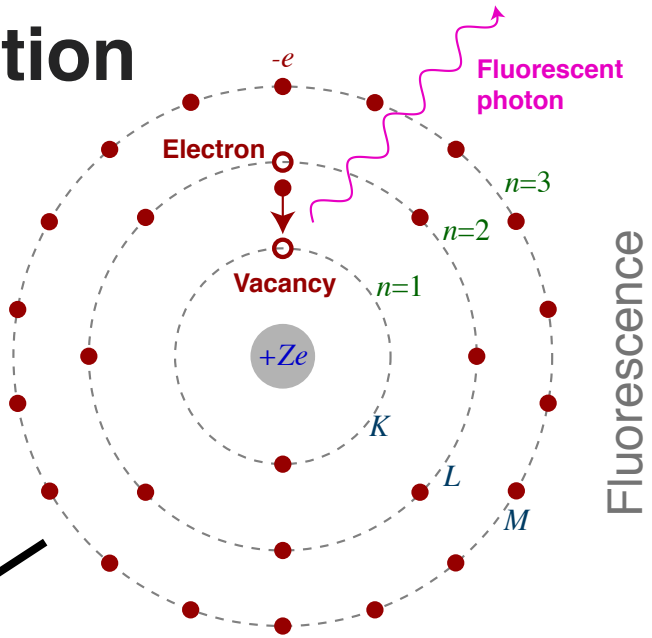
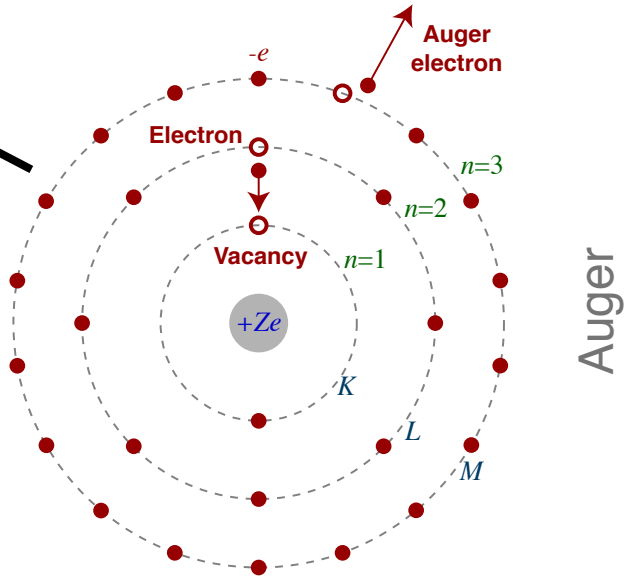
Calculating fluorescence fraction

- Absorption by many atoms, and many orbitals.
- What is the *extra* absorption connected with a particular fluorescence line?



Either/or

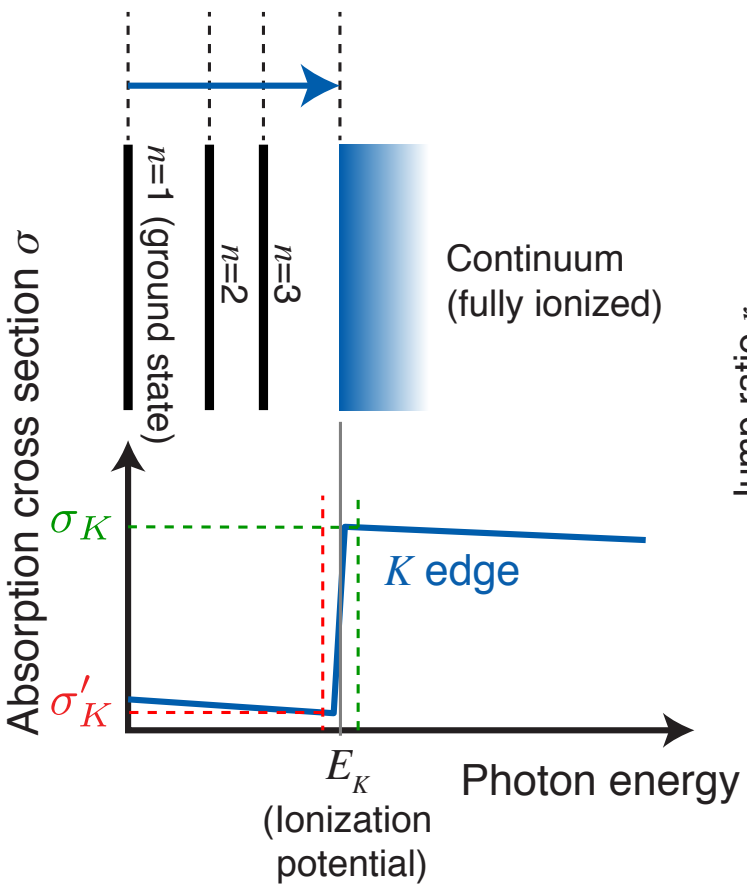


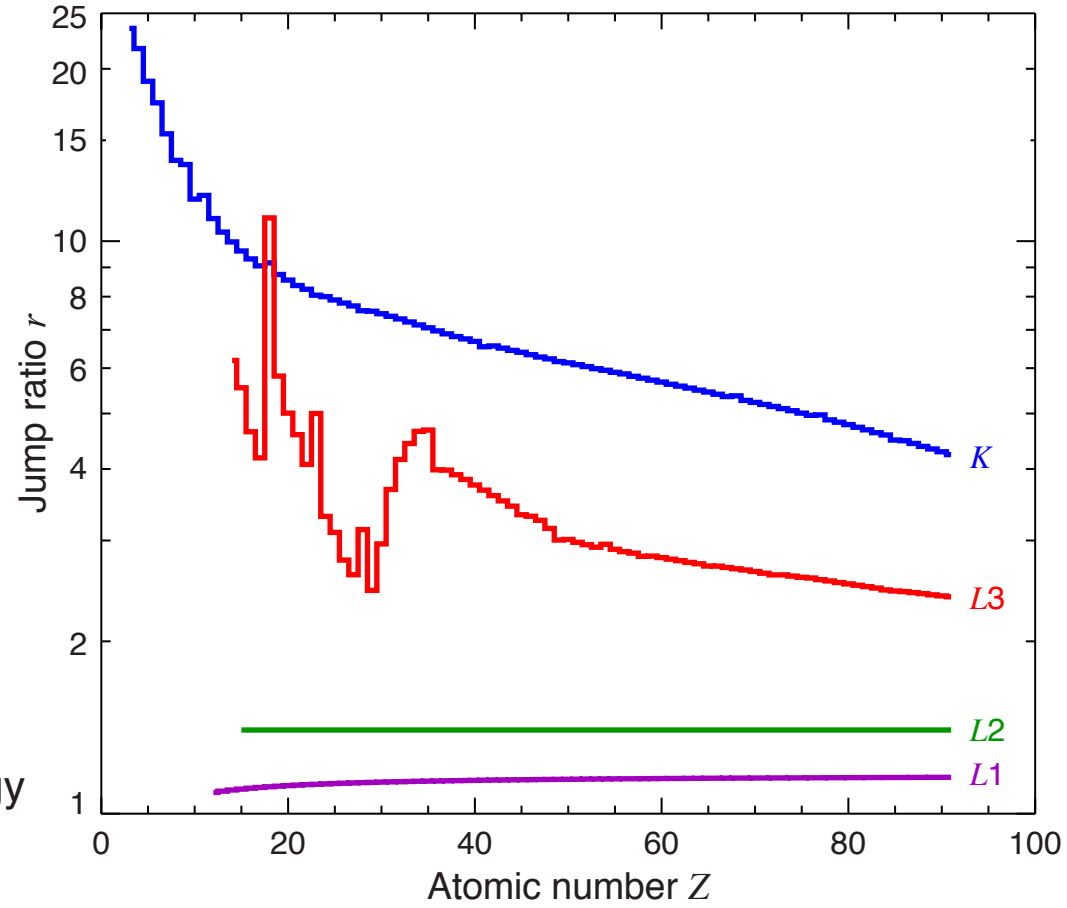


Absorb a photon; create an inner-shell vacancy. Must get rid of the energy!

Calculating fluorescence

- What is the *extra* absorption in a particular electron orbital?
- Jump ratio r gives extra fraction as (1-1/r), with $r = \dfrac{\sigma}{\sigma'}$





Tabulation of r: see *e.g.*, Elam, Ravel, and Sieber, *Radiation Physics and Chemistry* **63**, 121 (2002)

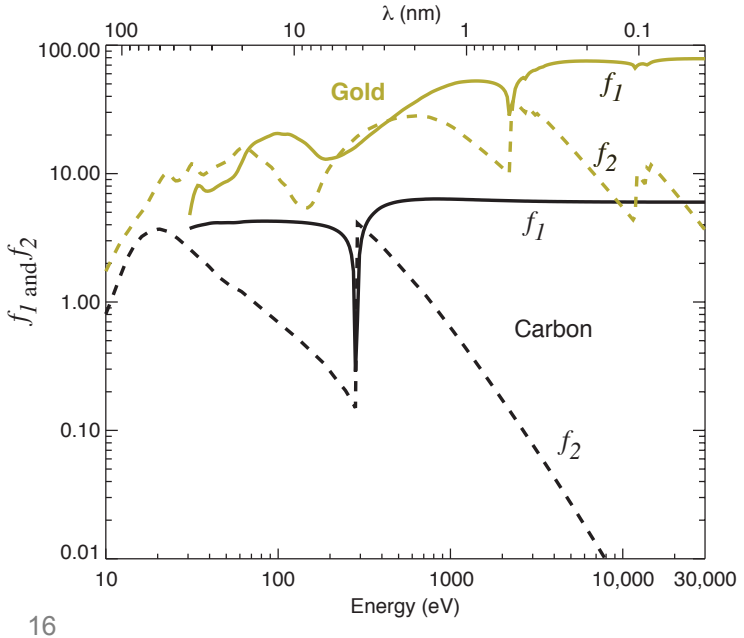
X-ray linear absorption coefficient μ

- X-ray refractive index: $n = 1 - \delta - i\beta = 1 - \alpha\lambda^2(f_1 + if_2)$
- Absorption as $I = I_0 \exp[-2k\beta t] = I_0 \exp[-\mu t]$
- Linear absorption coefficient for a single material::

$$\mu = 2\lambda\, n_a\, f_2 = 2\lambda \frac{\rho N_A}{A} f_2(\lambda)$$

$$\alpha = \frac{r_e}{2\pi}\frac{\rho N_A}{A}$$

- where μ=linear absorption coefficient (often in 1/micrometers), λ=x-ray wavelength (=hc/E), n_a=number density of atoms (*e.g.*, atoms/cm^3), and $f_2(\lambda)$=complex part of number of oscillation modes at wavelength λ.
- And of course ρ=density, A=atomic mass, N_A=Avogadro's number, and r_e=2.818×10-15 m (classical electron radius)



Tabulations of f_2: see *e.g.*, Elam, Ravel, and Sieber, *Radiation Physics and Chemistry* **63**, 121 (2002).

Python library xraylib: Schoonjans *et al.*, *Spectrochimica Acta B* **66**, 776 (2011)

X-ray mass-per-area absorption coefficient μ'

- Mass per area of element Z is $\rho'_Z = \frac{m_Z}{\Delta^2}$

  where Δ^2=pixel area.

- Mass absorption coefficient $\mu'_Z(E)$ for element Z at energy E is

$$\mu'_Z(E) = 2r_e \frac{N_A}{A_Z} \frac{hc}{E} f_{2,Z}(E)$$

  where r_e=2.818×10^{-15} m is the classical radius of the electron, N_A is Avogadro's number, and A_Z is the atomic mass of element Z.

$$\lambda = \frac{hc}{E} \quad \text{with} \quad hc = 1240 \text{ eV} \cdot \text{nm}$$

X-ray fluorescence intensity

Intensity I of fluorescence emission into the $\mathrm{K}_{\alpha 1}$ line is given by

$$I_{K\alpha 1}(E) = \omega_K \cdot F_{K\alpha 1} \cdot T_{K\alpha 1} \cdot \left(1 - \frac{1}{r_K}\right) \left[1 - e^{-\rho' Z \mu' Z(E)}\right] I_0(E)$$

Terms:

- ω_K=fluorescence yield (versus Auger yield) for K shell x-ray emission
- $F_{K\alpha 1}$=fraction of K shell x-ray emission into the α_1 line (other options include β_1 or β_3, for example)
- $T_{K\alpha 1}$=electron-hole transfer fraction (=1 for K shells)
- r_K=jump ratio for K edge absorption
- $I_0(E)$=incident flux at photon energy E

Zinc:

K absorption edge	$E_K = 9.659$ keV
Absorptive part of the oscillator strength	$f_2 = 3.694$ at 10 keV
Jump ratio (Eq. 3.8)	$r_K = 7.543$
Fluorescence energies	$E_{K\alpha 1} = 8.637$ keV, $E_{K\alpha 2} = 8.614$ keV
Net K fluorescence yield	$\omega_K = 0.46937$
Fractional yields F	$F_{K\alpha 1} = 0.57606$, $F_{K\alpha 2} = 0.29435$
Electron-hole transfer factor T	$T = 1$

Detected fraction: solid angle Ω

- Solid angle of an enclosing sphere is $\Omega=4\pi$ steradians

$$\Omega = \int_{\varphi=0}^{2\pi} \int_{\theta'=0}^{\theta} \sin\theta' \, d\theta' \, d\varphi = 2\pi(1-\cos\theta) \simeq \pi\theta^2$$

- Detector with a radius of r at a distance z collects a fraction of

$$\frac{\Omega_{\text{detector}}}{\Omega_{\text{sphere}}} = \frac{r^2}{4z^2}$$

- Detector solid angles vary (obviously), but $\Omega_{\text{detector}} \simeq 0.2$ is not uncommon

Example

- N_Z=10^4 zinc atoms in an area of (Δ=50 nm)^2, incident flux of 10^9 photons/sec at 10 keV, per-pixel time of 100 msec, detector solid angle of 0.2 sr gives 58 zinc fluorescence photons detected per 100 msec.
- If sample is 5 μm thick carbon, the pixel has 1.4×10^9 carbon atoms and 10^4 zinc atoms represents 7.1 parts per million, and mass is 1.1 attograms.
- Example: actual minimum detection limit for zinc at ESRF ID22NA is 2 attograms.
- "Skin dose" D to dominant material (the "matrix") in sample is given by

$$D = \bar{n}\frac{E\mu}{\rho\Delta^2}$$

where $\bar{n}$ is incident photons per pixel, μ is linear absorption coefficient of the dominant material, ρ=density, and E=incident photon energy.

- Dose to carbon in this example is D=6.1×10^3 Gray.

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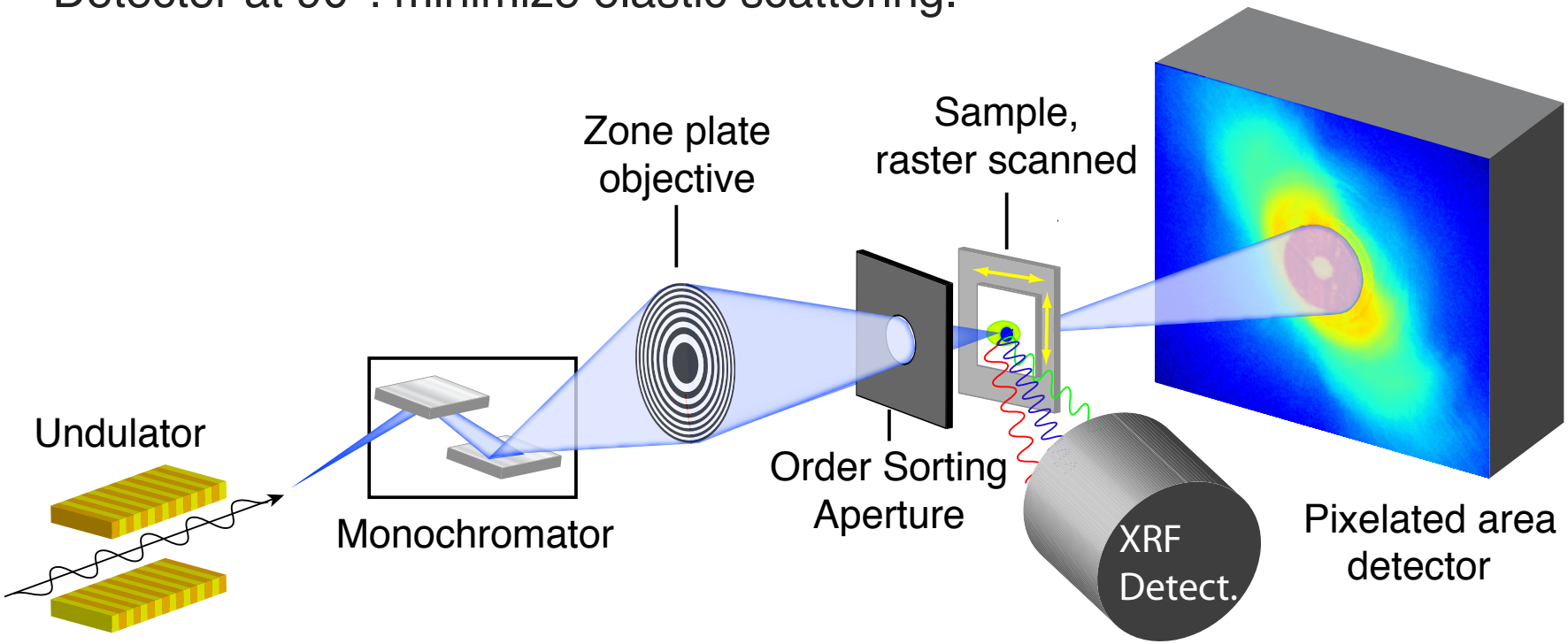
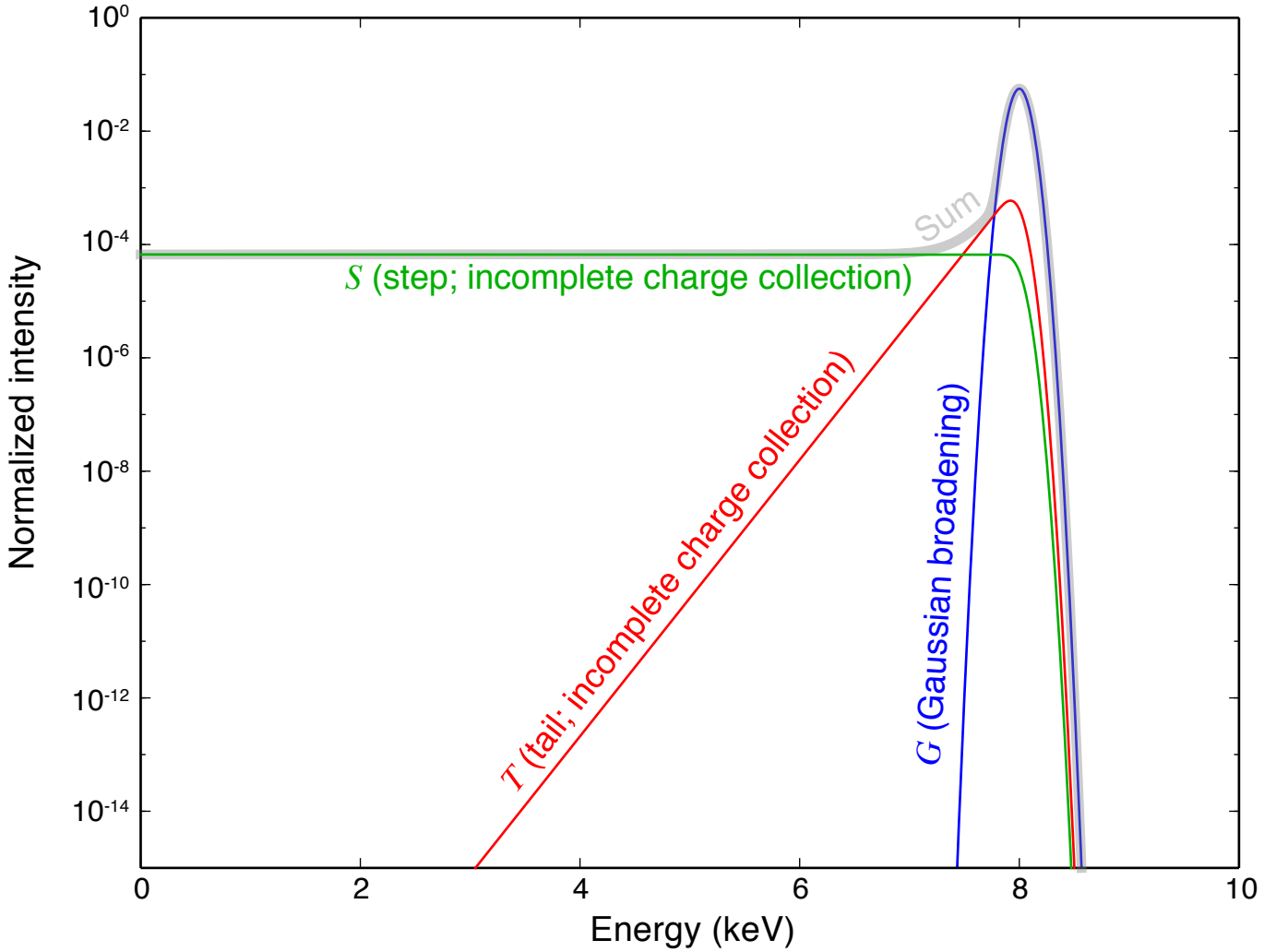
X-ray fluorescence microprobes

- Energy dispersive detectors: 3.65 eV per electron-hole pair separation in Si.
- 10 keV photon produces 2740 electrons. Fluctuations: $(2740)^{1/2}=52$, so uncertainty is (52/2740)*10,000 eV=190 eV. Fano factor reduces this to ~130 eV.
- Detector at 90°: minimize elastic scattering.

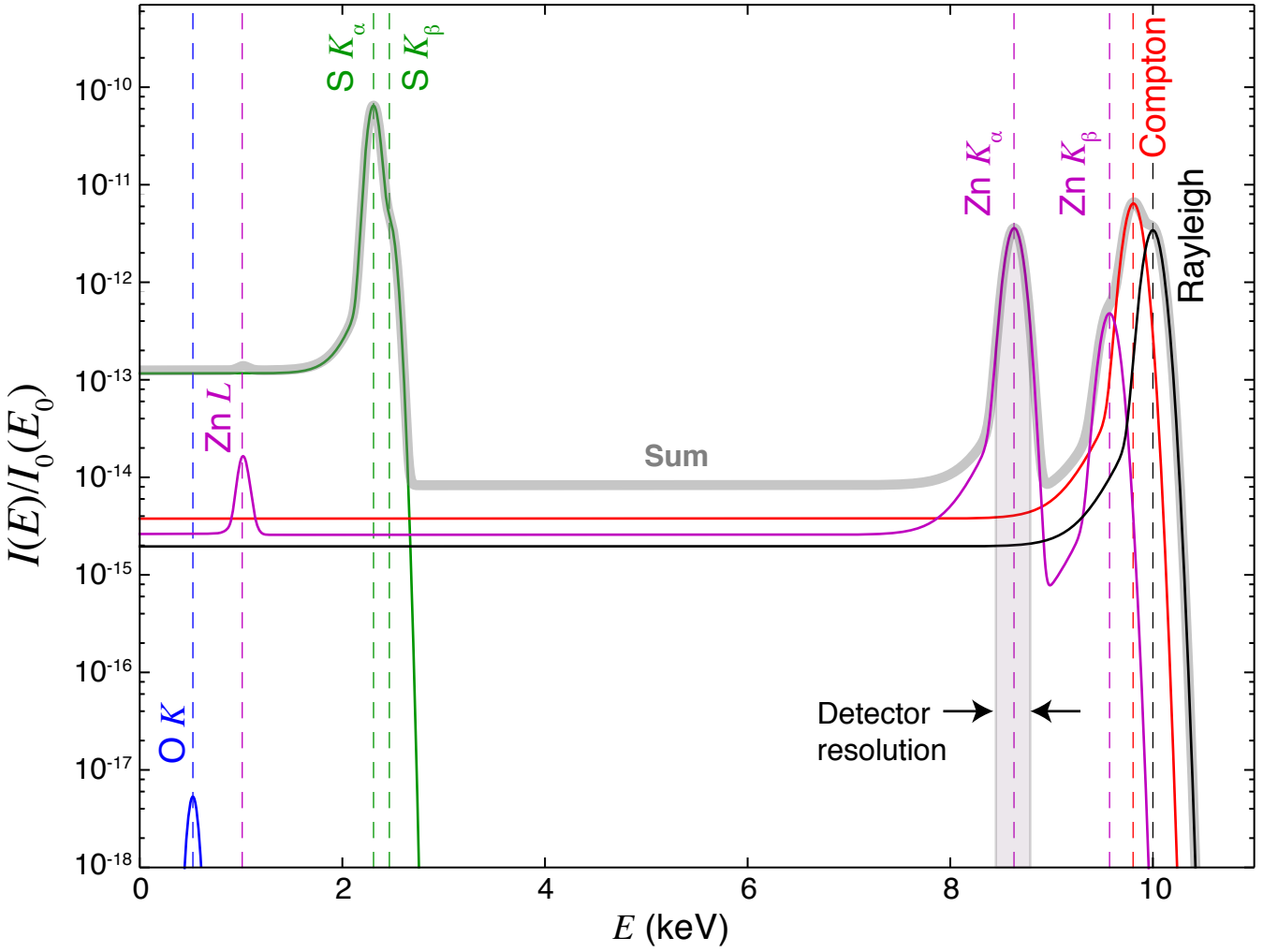


Fluorescence detector response to a 8 keV photon



Van Grieken and Markowicz, *Handbook of X-ray Spectrometry* (CRC Press, 2001).
This plot from Sun, Gleber, Jacobsen, Kirz, and Vogt, *Ultramicroscopy* **152**, 44 (2015)

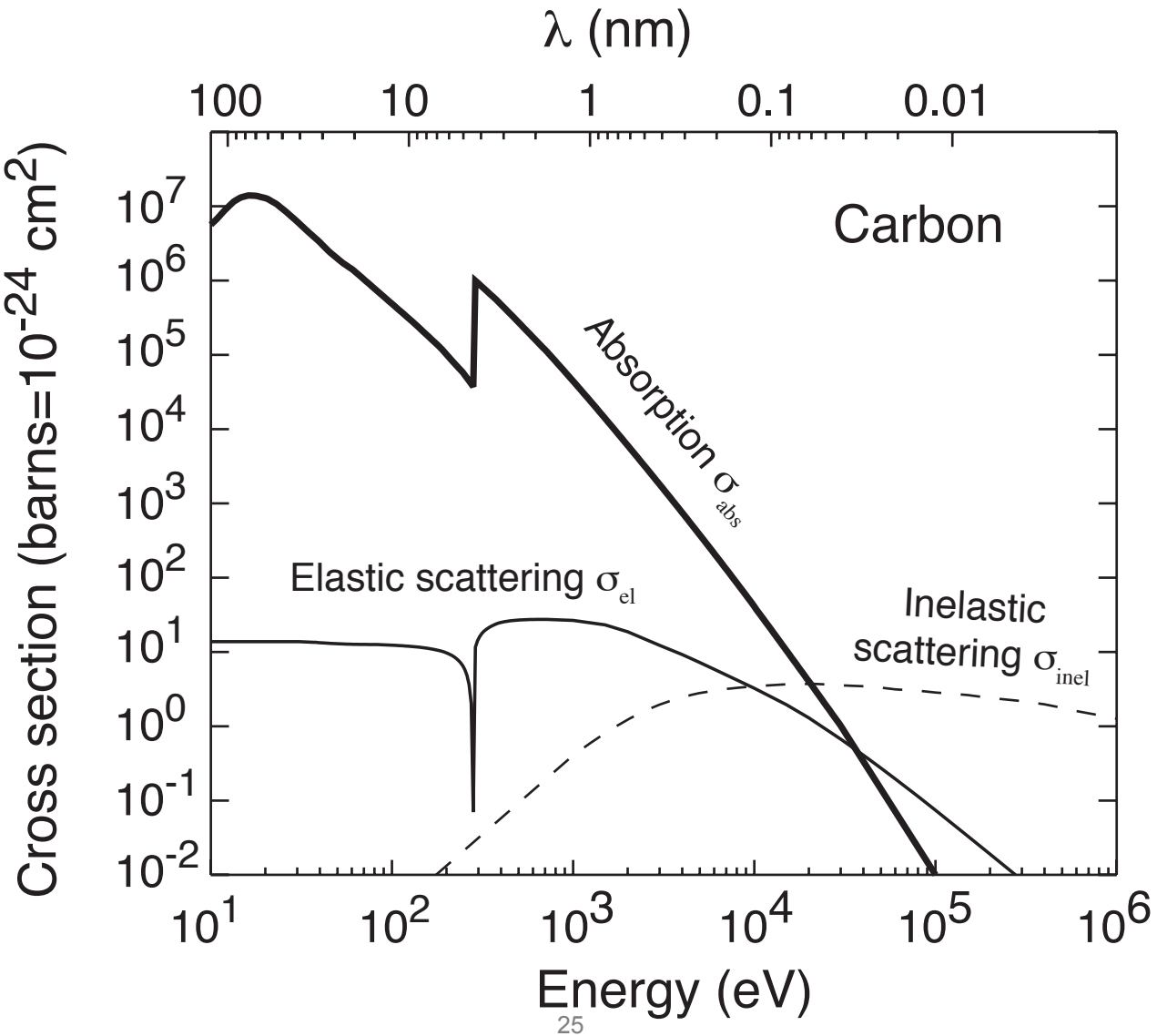
Example calculated x-ray spectrum



Sun, Gleber, Jacobsen, Kirz, and Vogt, *Ultramicroscopy* **152**, 44 (2015)

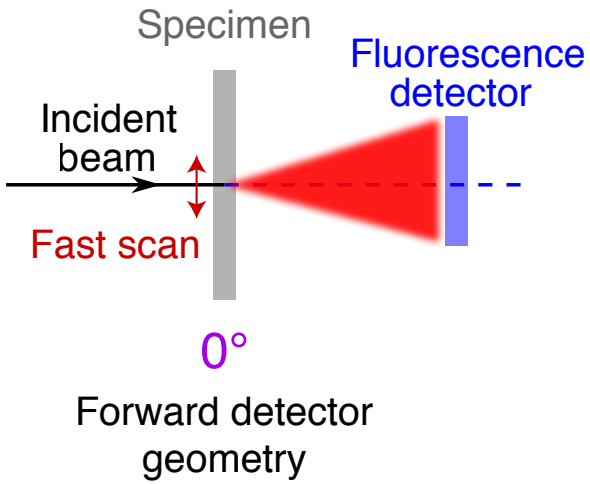
X-ray interaction cross sections

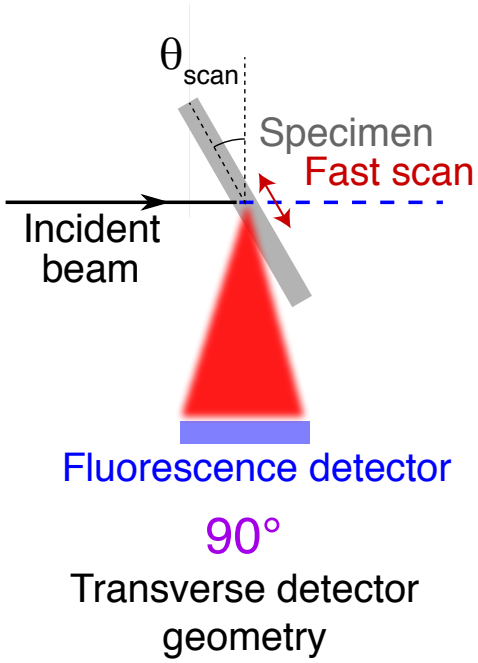
- There are no cloudy days for X rays!

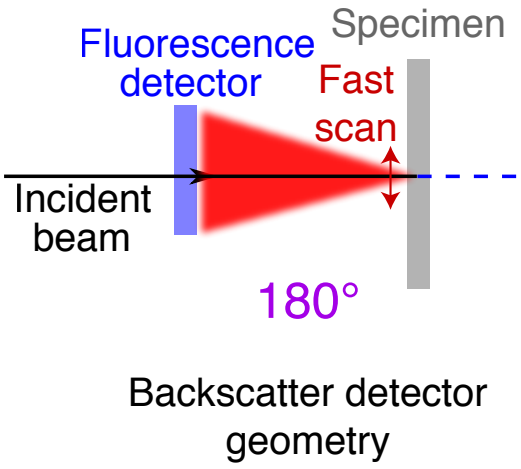


Detector geometries vary!

- Elastic and Compton scattering profiles have angular dependencies



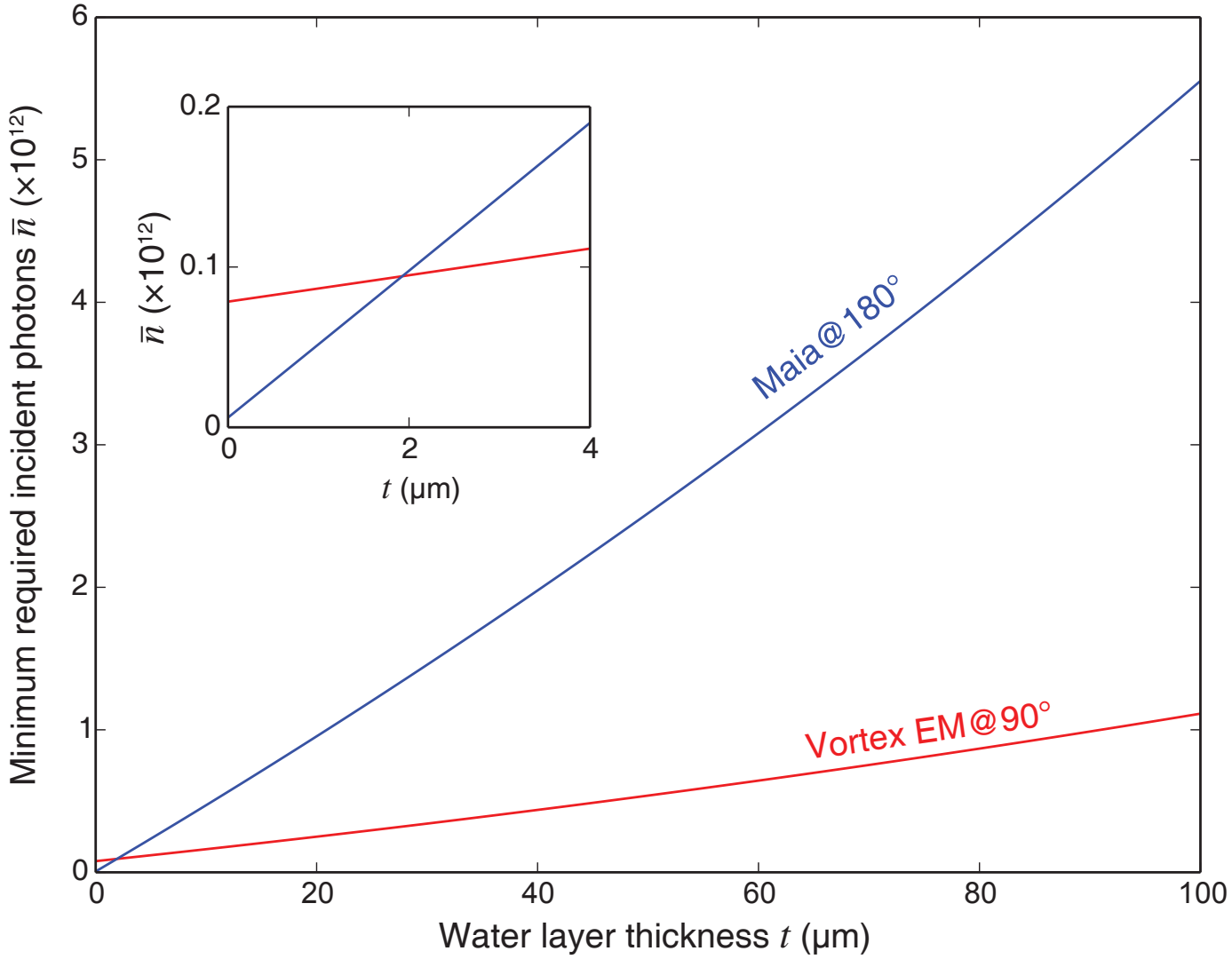




Sun, Gleber, Jacobsen, Kirz, and Vogt, Ultramicroscopy 152, 44 (2015)

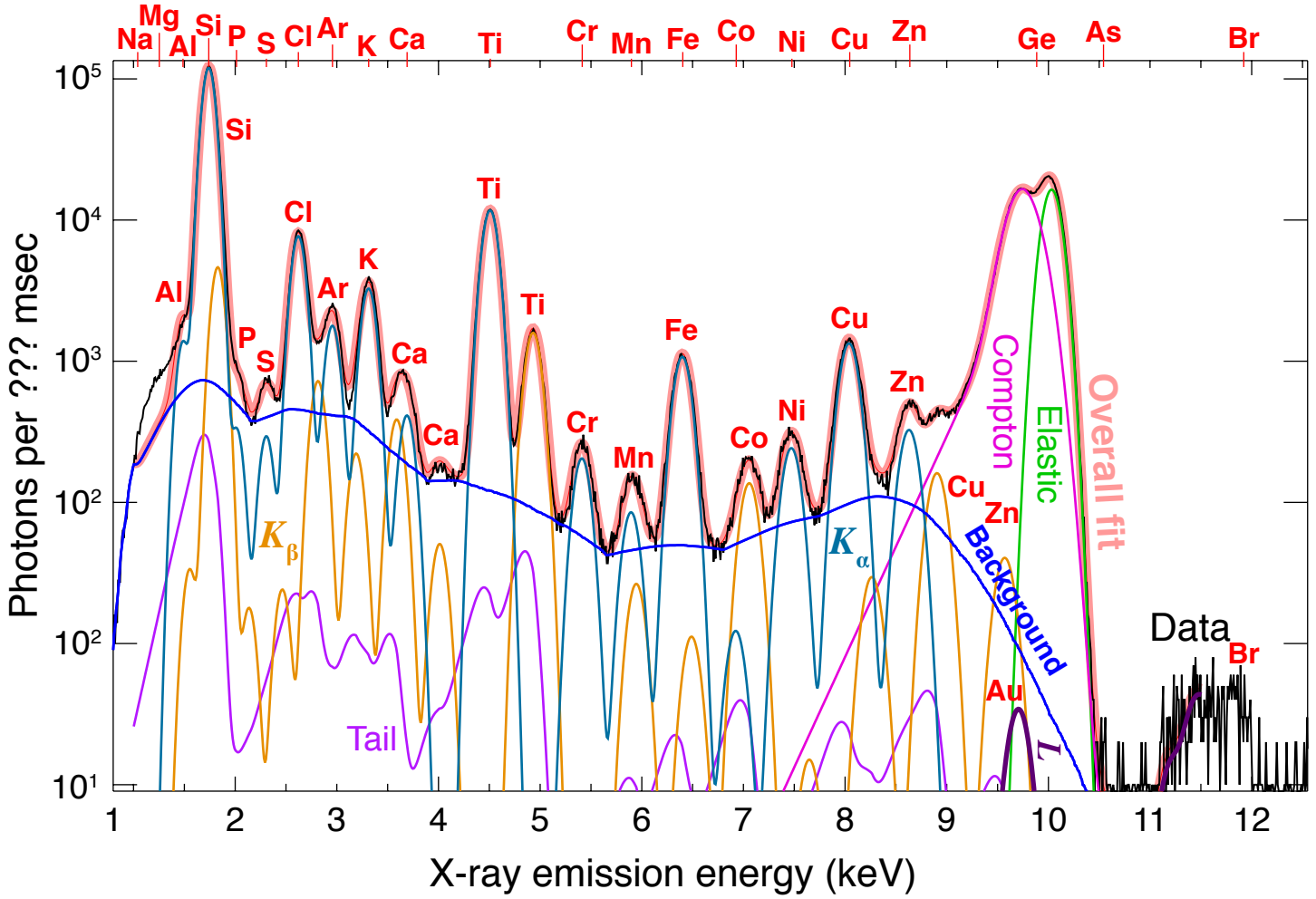
Sensitivity for two detector geometries

- Bio sample with 0.01% Zn in a 20 nm thick protein layer within ice/ water, using 10 keV photons.
- Vortex: solid angle fraction is 0.0034.
- Maia in backscatter geometry.



Sun, Gleber, Jacobsen, Kirz, and Vogt, *Ultramicroscopy* **152**, 44 (2015)

Example x-ray spectrum and fit



Spectrum: Olga Antipova, APS. Fit: S. Vogt, *J. Phys. IV France* **104**, 635 (2003). See also Ryan and Jamieson, *Nucl. Inst. Meth. B* **77**, 203 (1993); and Solé *et al.*, *Spectrochimica Acta B* **62**, 63 (2007).

Quantitation

- While one should be able to calculate concentrations from first principles, there are many complicating factors:
  - Details of scattering and absorption (including Argon in air; 3.0 and 3.2 keV)
  - Details of detector response
  - Exact detector distance
- Therefore the usual practice is to use a spectral standard:
  - A “matrix” of a pure material (a polymer, or a glass) with scattering properties similar to the sample under study.
  - Into which are mixed in known quantities of elements to be measured, at concentrations similar to those expected in the specimen.
- Fit the spectrum to known peaks and detector response, and normalize the concentrations relative to the spectral standard.

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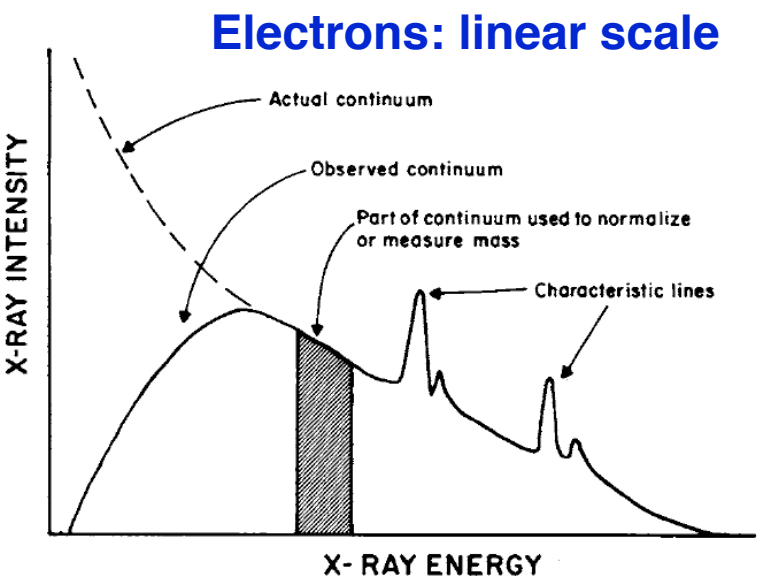
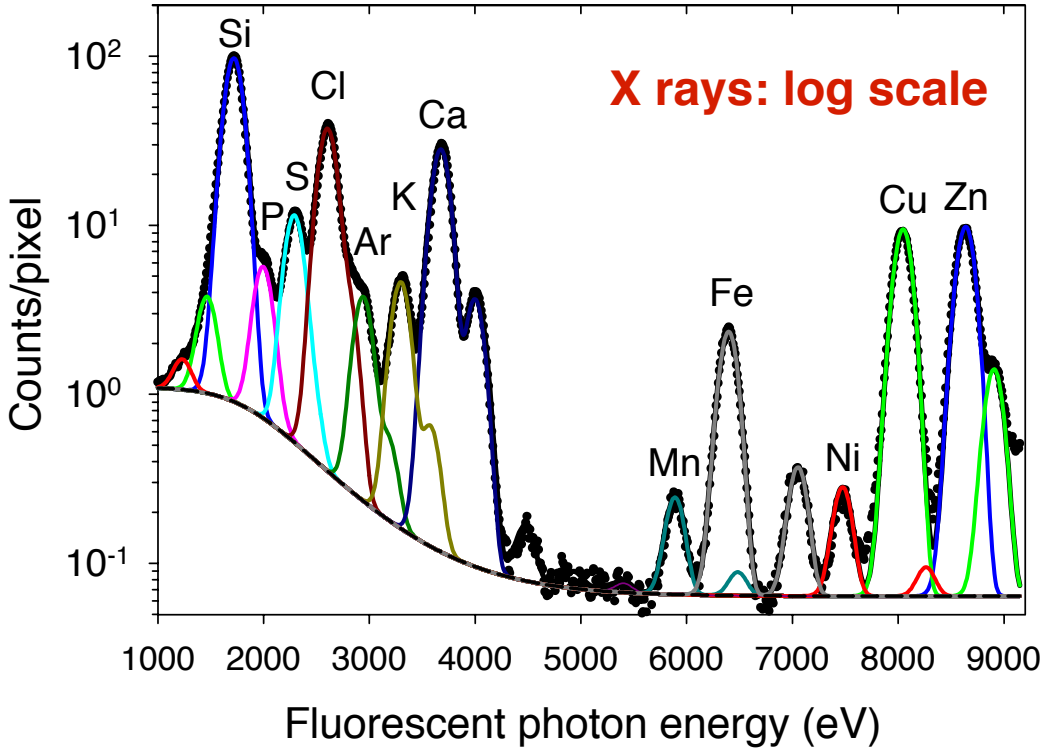
Exciting x-ray fluorescence

X rays and protons produce a dramatically lower continuum background, increasing sensitivity (but proton microprobes induce much more damage)

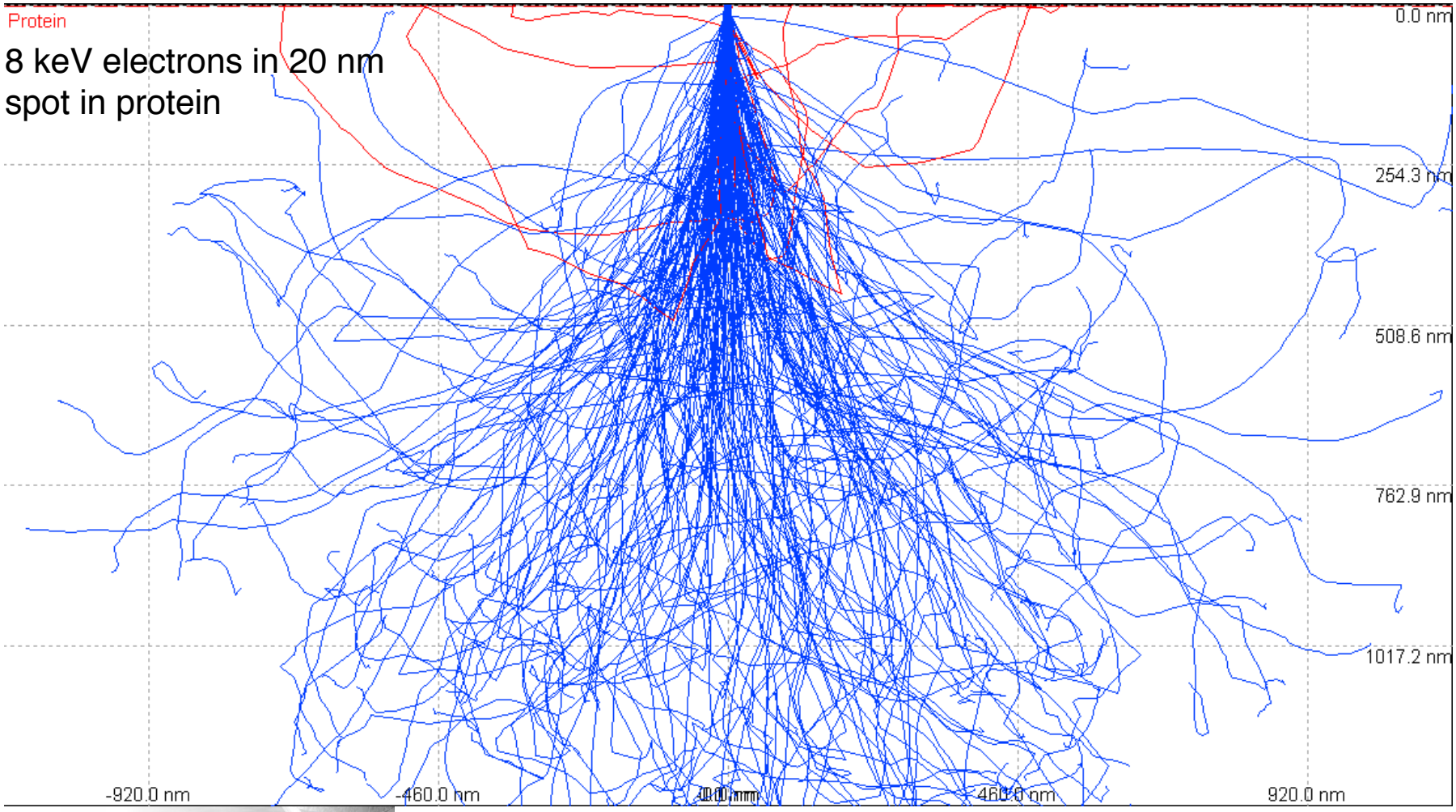




LeFurgey and Ingram, *Environmental Health Perspectives* **84**, 57 (1990)

Twining *et al.*, *Anal. Chem.* **75**, 3806 (2003). Analysis approach: Vogt, Maser, and Jacobsen, *J. Phys. IV* **104**, 617 (2003).

Electron scattering in soft matter solids



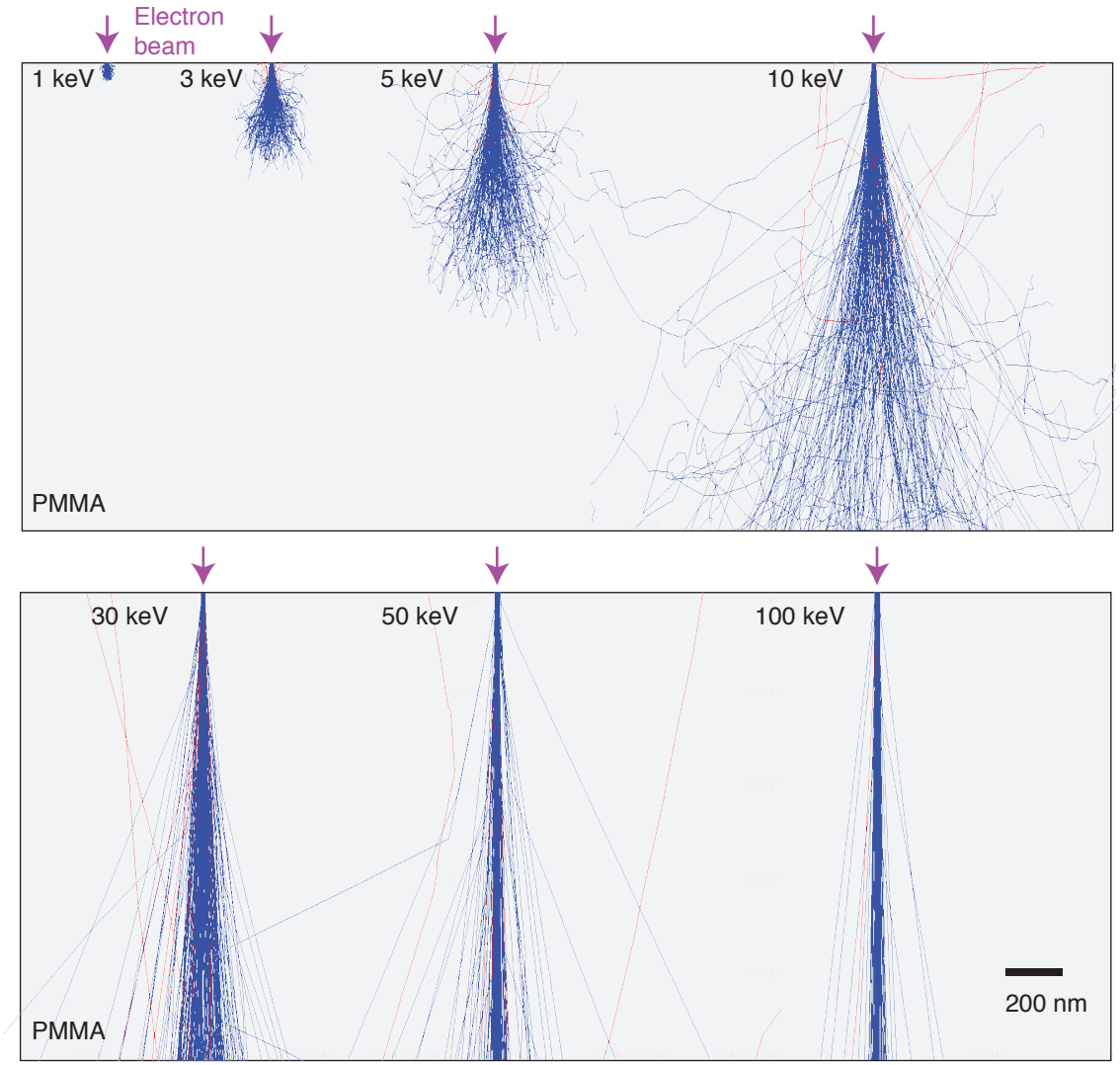
8 keV electrons in 20 nm spot in protein

Casino
monte CArlo SImulation of electroN trajectory in sOlids

http://www.gel.usherbrooke.ca/casino/

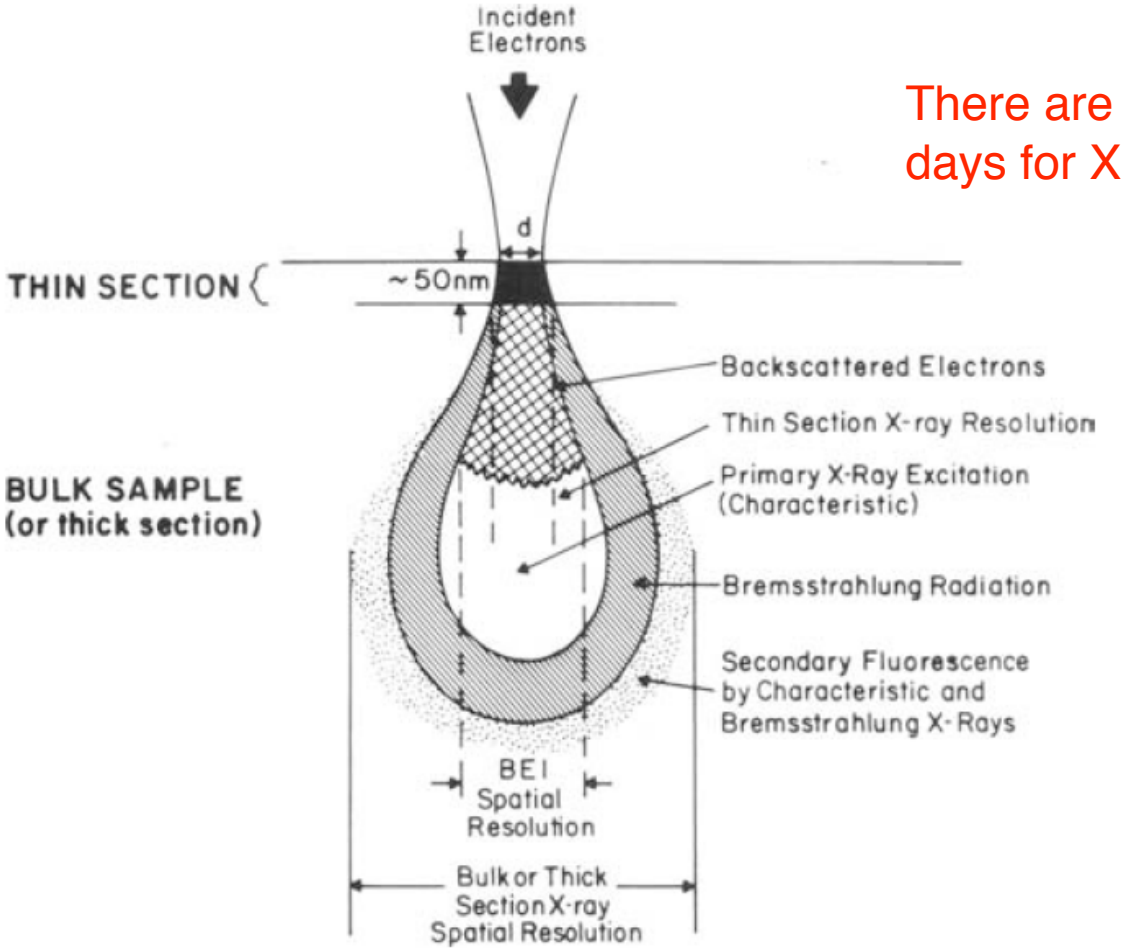
Argonne NATIONAL LABORATORY

Electron scattering versus incident electron energy



http://www.gel.usherbrooke.ca/casino/

Electron probes in thick specimens



There are no cloudy days for X rays!

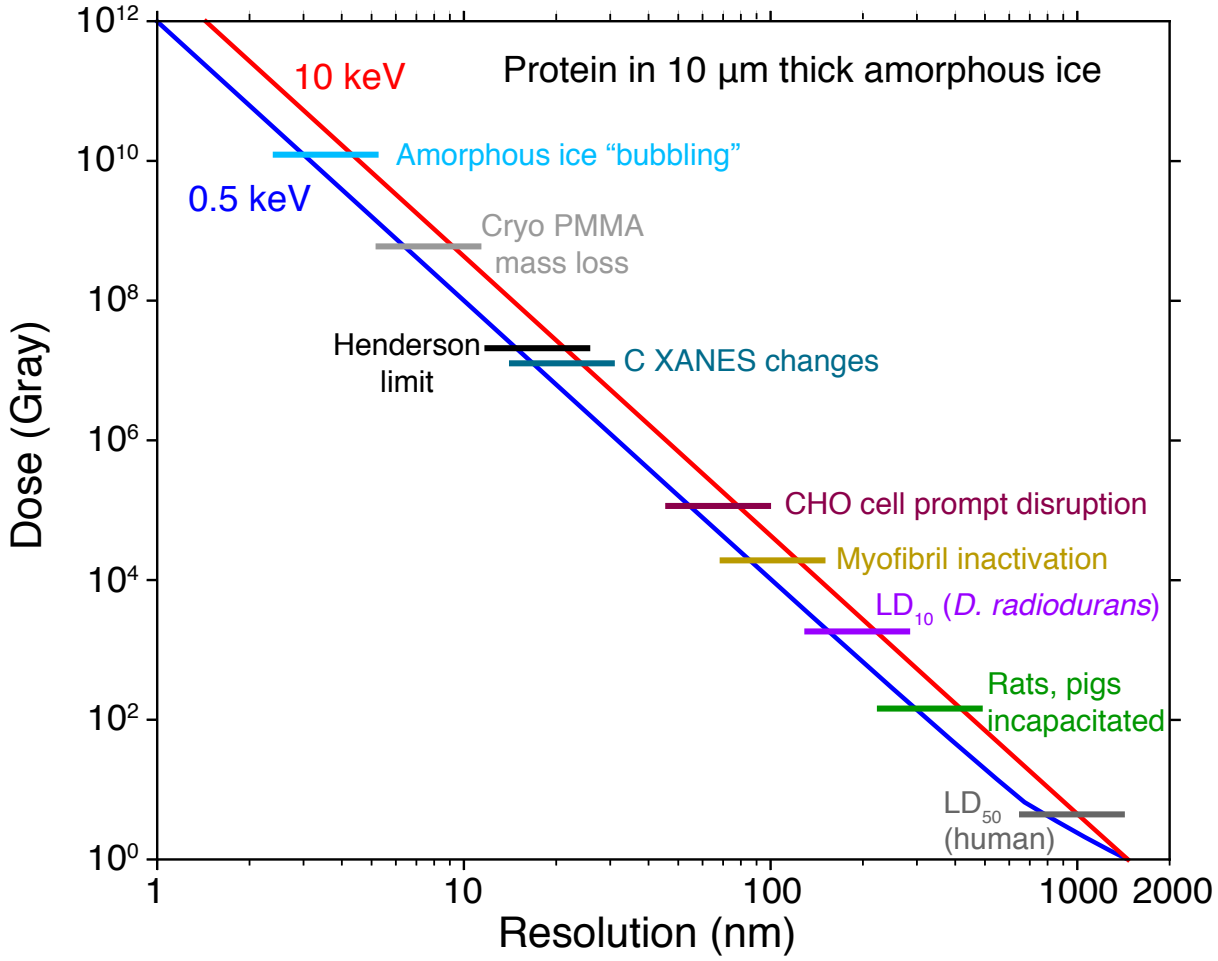
Electron beams broaden in thick specimens due to sidescattering; x-ray beams do not. LeFurgey and Ingram, *Environmental Health Perspectives* **84**, 57 (1990).

Outline

- The physics and nomenclature of x-ray fluorescence
- Calculating fluorescence rates
- Detecting fluorescence
- Comparison with electron microprobes
- Radiation dose and damage, and cryo microscopy
- From 2D to 3D: fluorescence tomography, tomographic alignment, and self-absorption correction

Dose versus resolution for transmission x-ray imaging

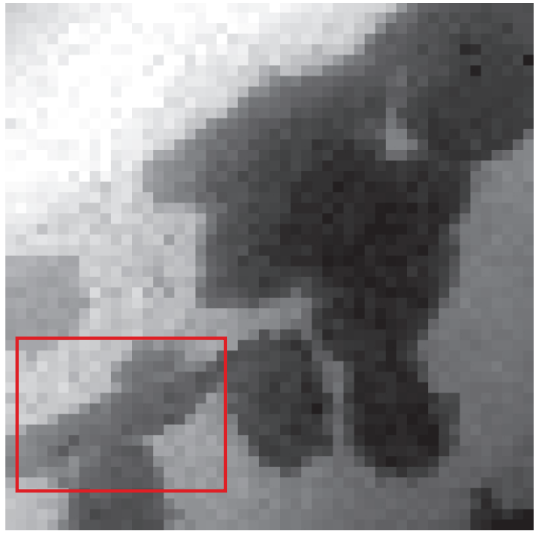
- Calculation of radiation dose using best of phase, absorption contrast and 100% efficient imaging



Du and Jacobsen, *Ultramicroscopy* **184**, 293-309 (2018).

X-ray microscopy of initially living cells

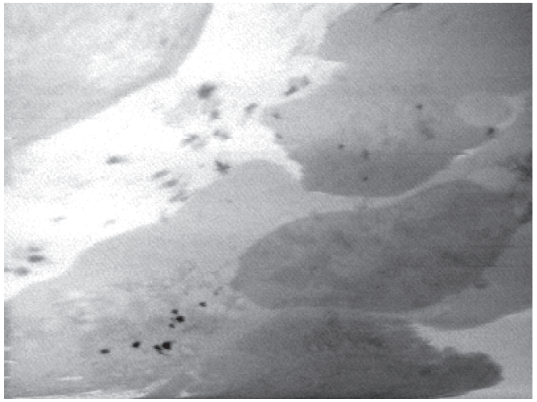
- Radiation dose in high resolution x-ray, electron microscopy is ~10^6-10^8 Gray
- 5 Gray (really 5 Sievert) kills people!
- Chinese hamster ovarian (CHO) fibroblasts in culture medium with periodic reflow.
- "Red for dead" fluorescent dyes used to confirm viability over hours with no x-ray exposure.
- Imaged in a soft x-ray scanning transmission microscope (NSLS X1A)



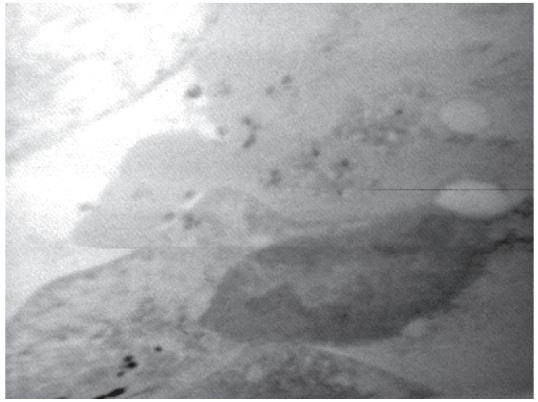
10 μm
6.0×10^2 Gray, ET=2 minutes

Experiment by V. Oehler, J. Fu, S. Williams, and C. Jacobsen, Stony Brook using specimen holder developed by Jerry Pine and John Gilbert, CalTech. In Kirz, Jacobsen, and Howells, *Quarterly Reviews of Biophysics* **28**, 33 (1995).

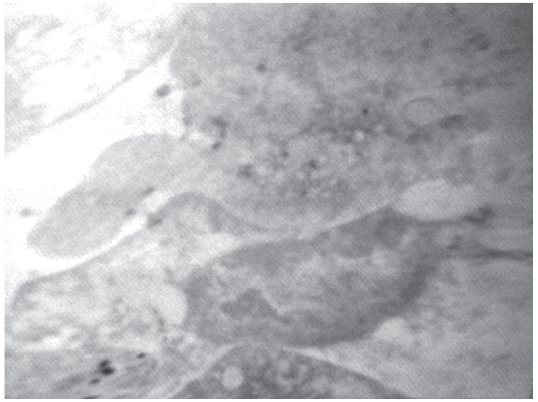
1 Gray=1 Joule/kg absorbed
1 Sievert=1 Gray•RBE (relative biological effectiveness of radiation type)



5 μm
1.2×10^5 Gray, ET=9.5 min



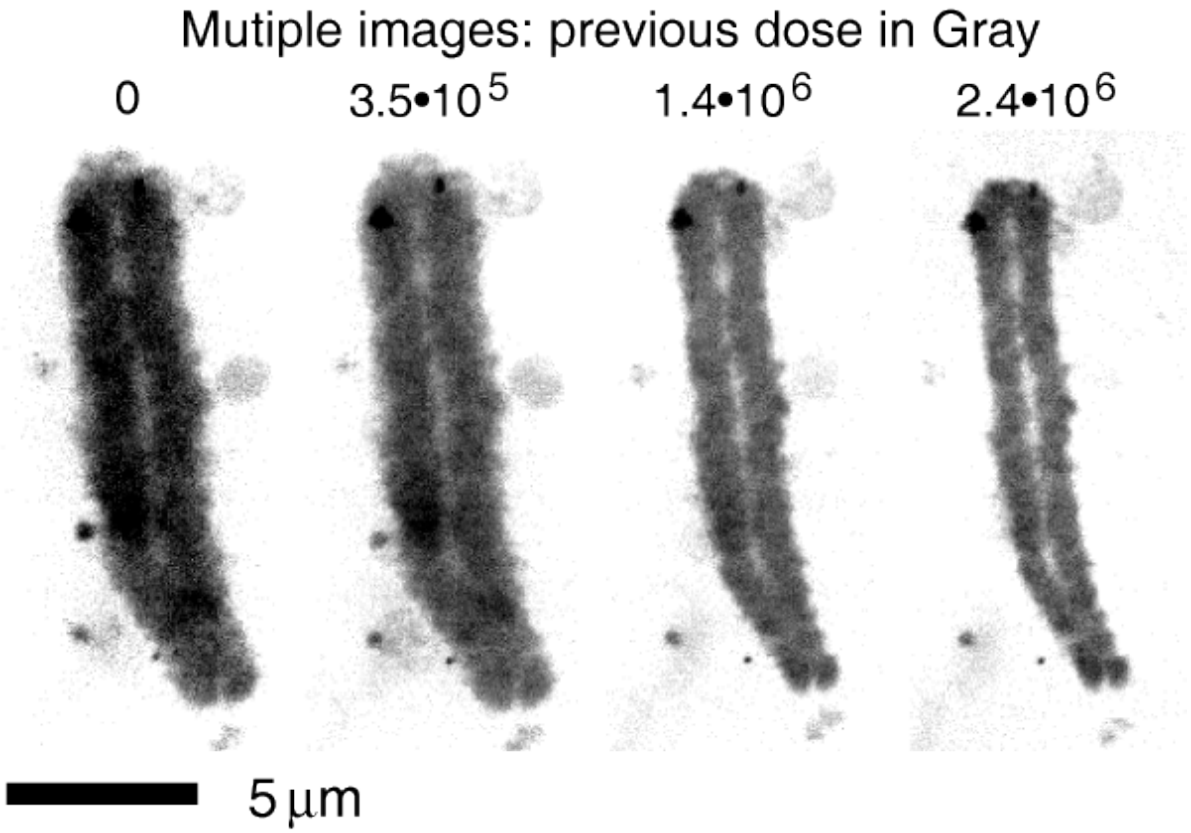
5 μm
2.4×10^5 Gray, ET=17 min



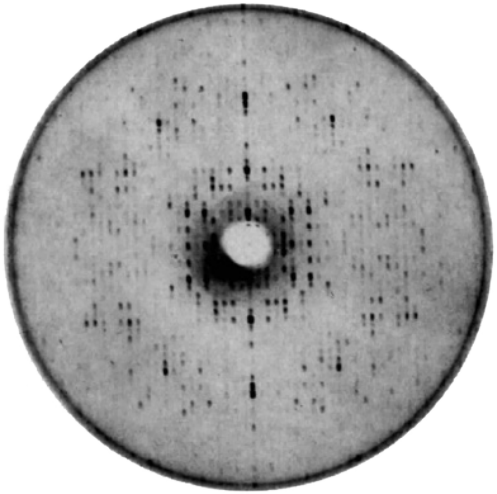
5 μm
3.7×10^5 Gray, ET=24.5 min

Wet, fixed samples: one image is OK

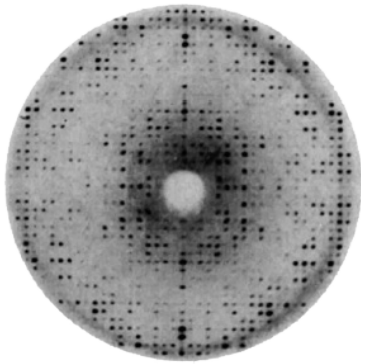
- Chromosomes are among the most sensitive specimens.
- *V. faba* chromosomes fixed in 2% glutaraldehyde. S. Williams *et al.*, *J. Microscopy* **170**, 155 (1993)
- Repeated imaging of one chromosome shows mass loss, shrinkage



Cryo crystallography



25°C



-75°C

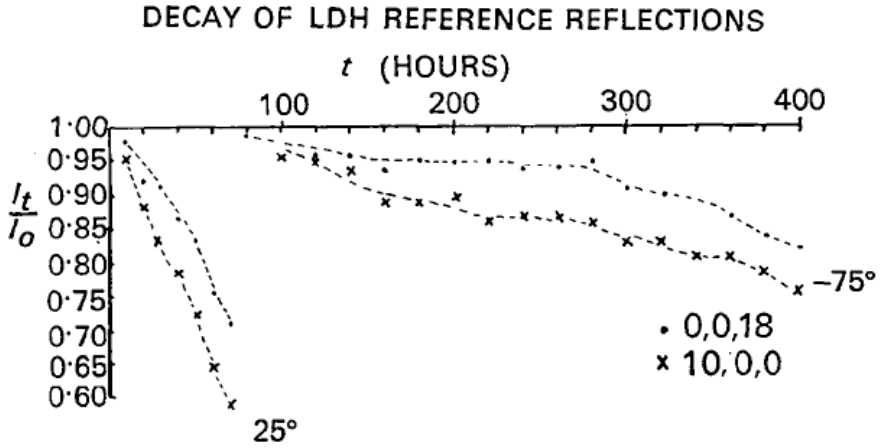


Fig. 5. The ratio I_t/I_0 for two reference reflections plotted as a function of exposure time for a typical native and frozen crystal. I_t represents the intensity at time t. Results for 0,0,18 and 10,0,0 are shown with dots and crosses respectively.

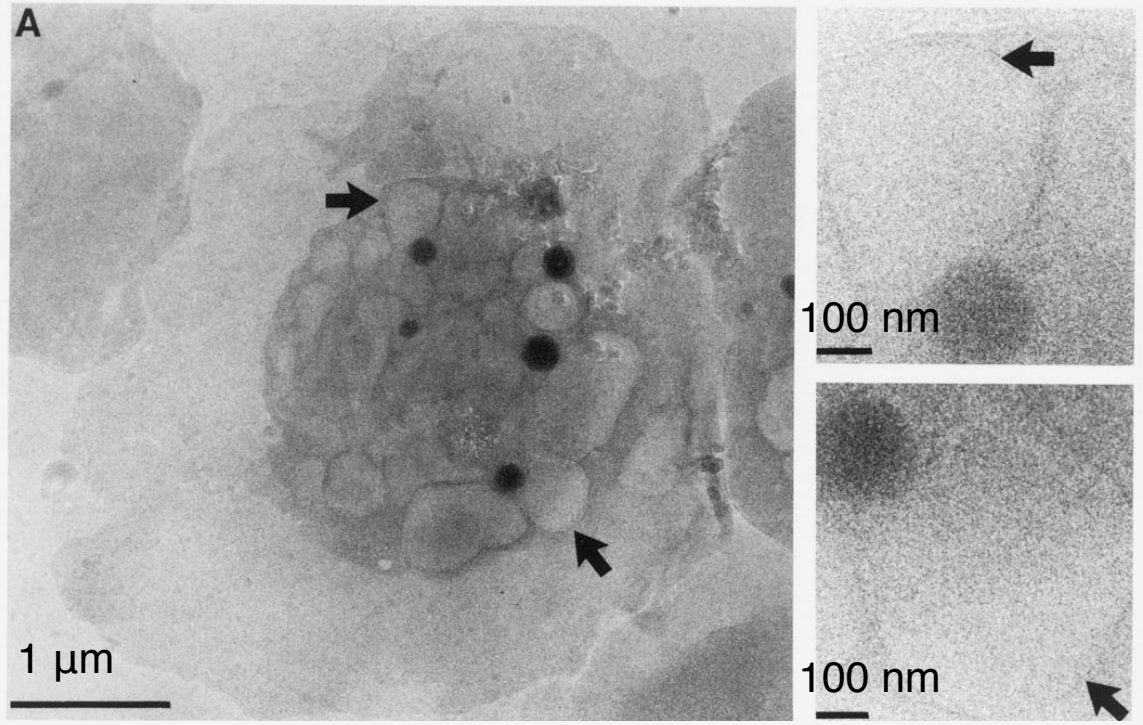
Acta Cryst. (1970). B**26**, 998

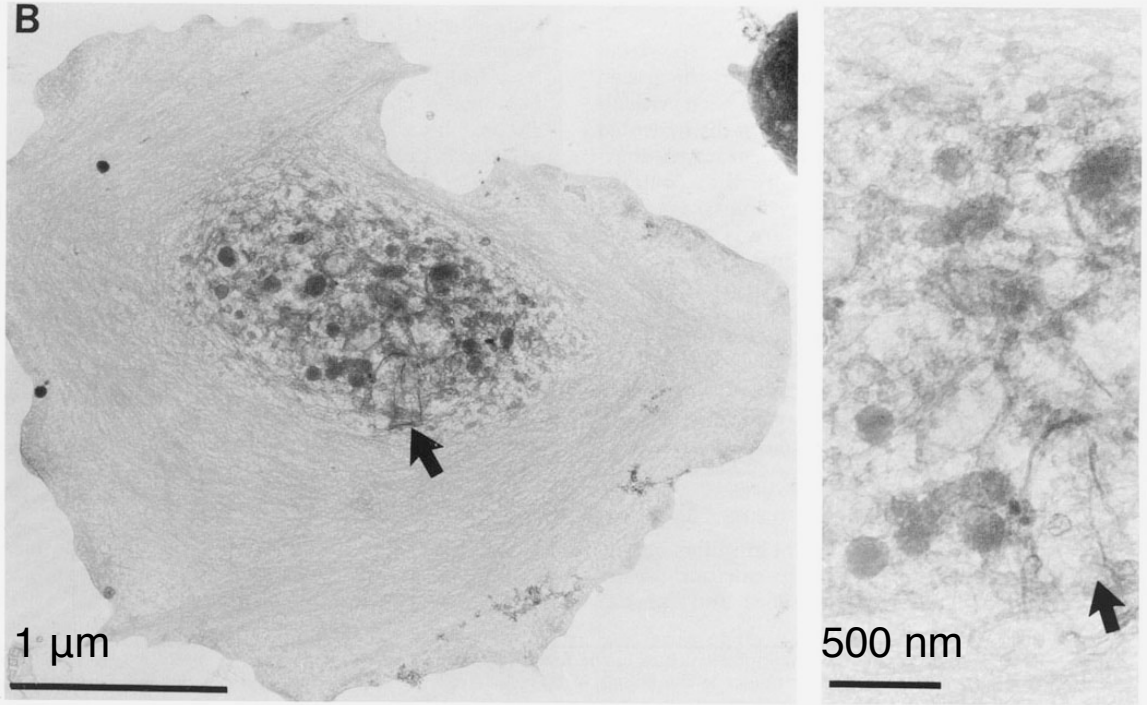
Crystallographic Studies on Lactate Dehydrogenase at -75°C

BY DAVID J. HAAS* AND MICHAEL G. ROSSMANN

Crystals of lactate dehydrogenase (LDH) were frozen by equilibration in a sucrose–ammonium sulfate solution, and then dipping into liquid nitrogen. The rate of radiation damage for frozen crystals was tenfold less than for crystals at room temperature. The physical properties of frozen crystals are discussed. Analysis of 3·5 Å data collected at −75°C for native LDH and two heavy atom derivatives showed that these derivatives retained their isomorphism in the frozen state.

See also Low, Chen, Berger, Singman, and Pletcher, *PNAS* **56**, 1746 (1966)



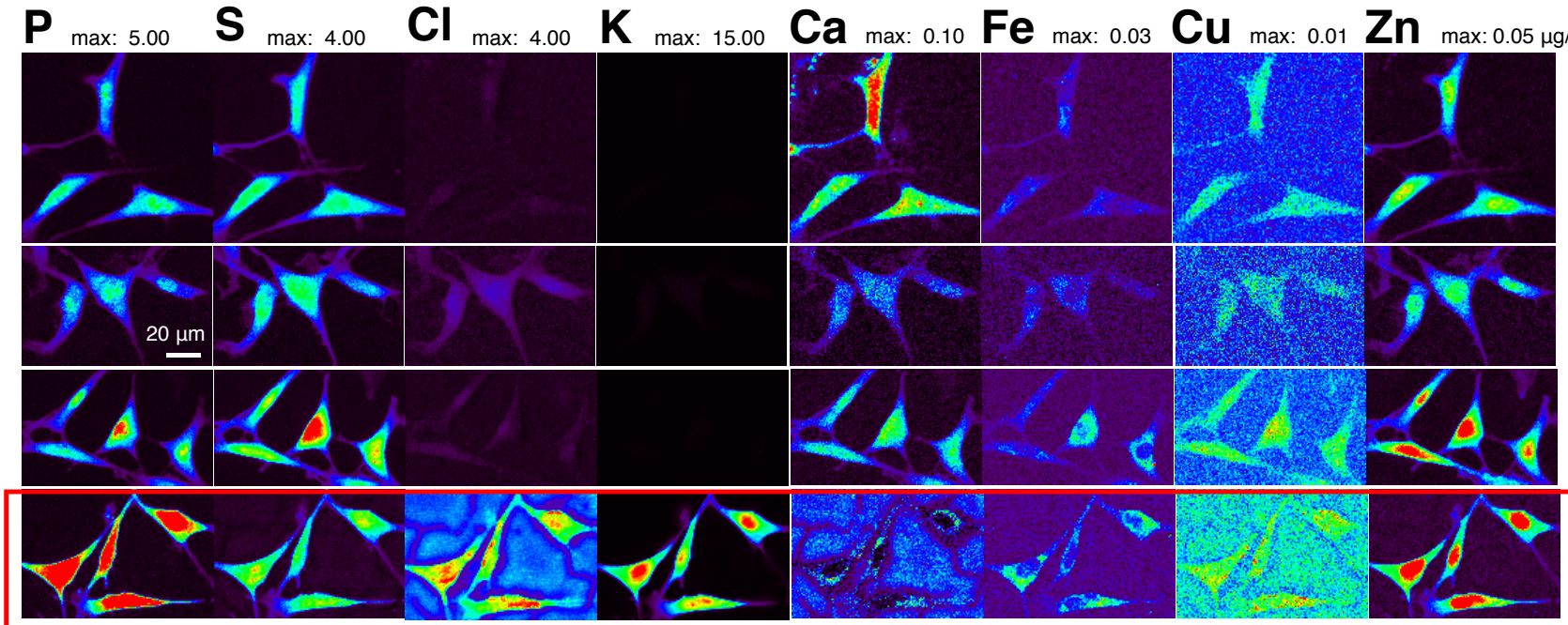


Frozen hydrated

- Human blood platelets
- 1 MeV transmission electron microscope (JEOL-1000)
- O'Toole, Wray, Kremer, and McIntosh, *J. Struct. Bio.* **110**, 55 (1993)

2% glutaraldehyde fix
1% OsO_4 postfix
critical-point dry

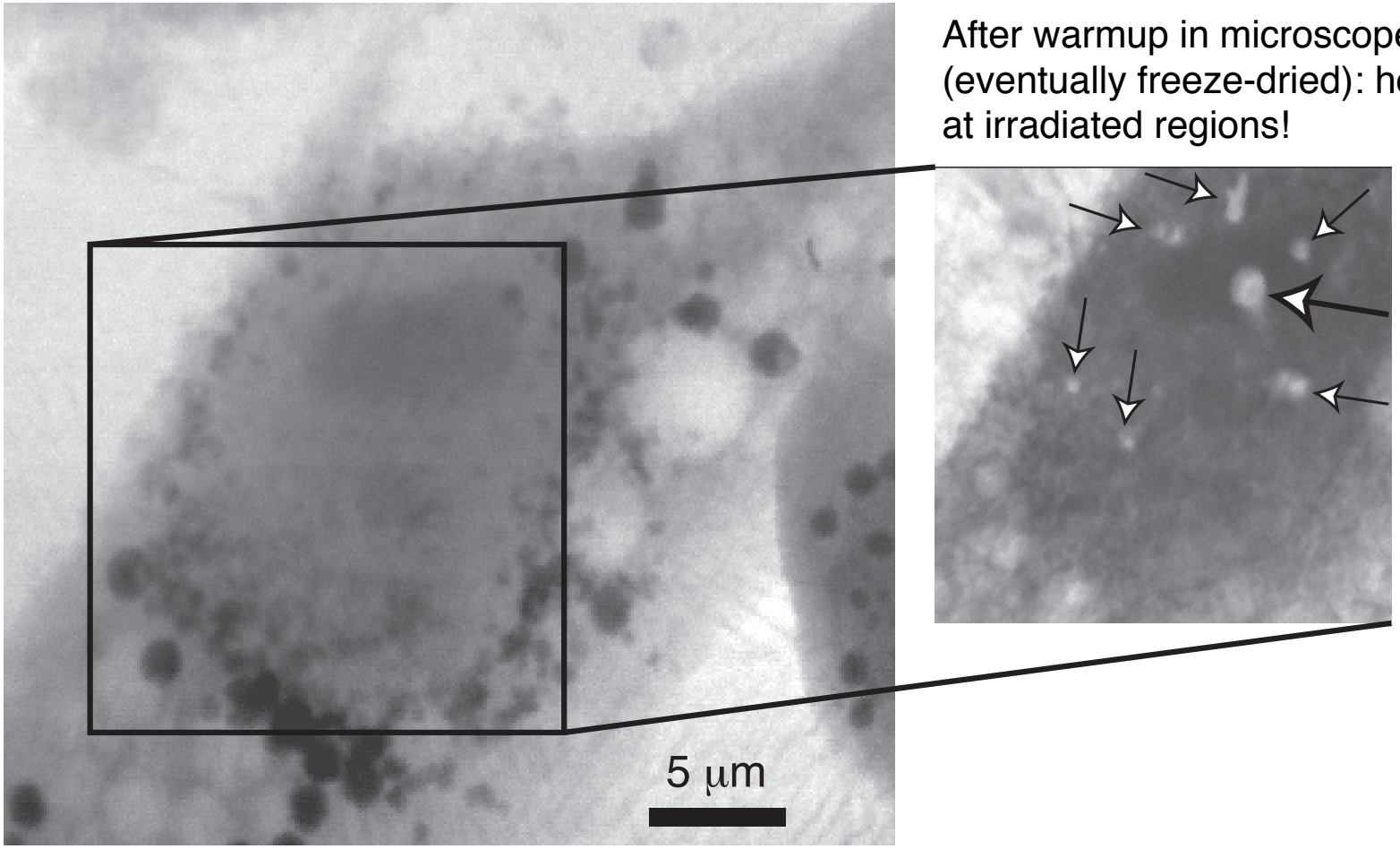
Cryo preservation keeps chemistry intact



- 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).

Radiation damage resistance in cryo microscopy

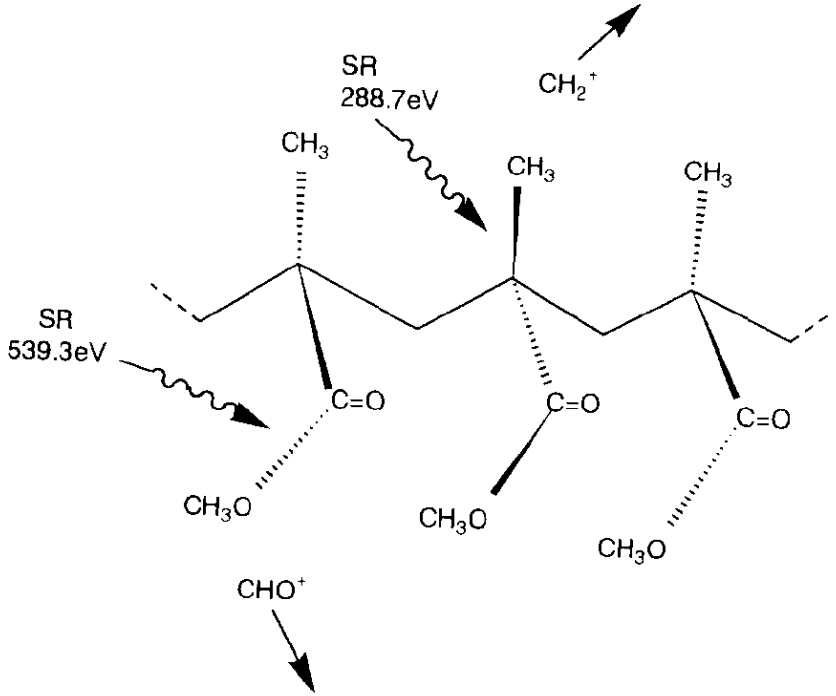
Frozen hydrated fibroblast image after exposing several regions to ~10^{10} Gray



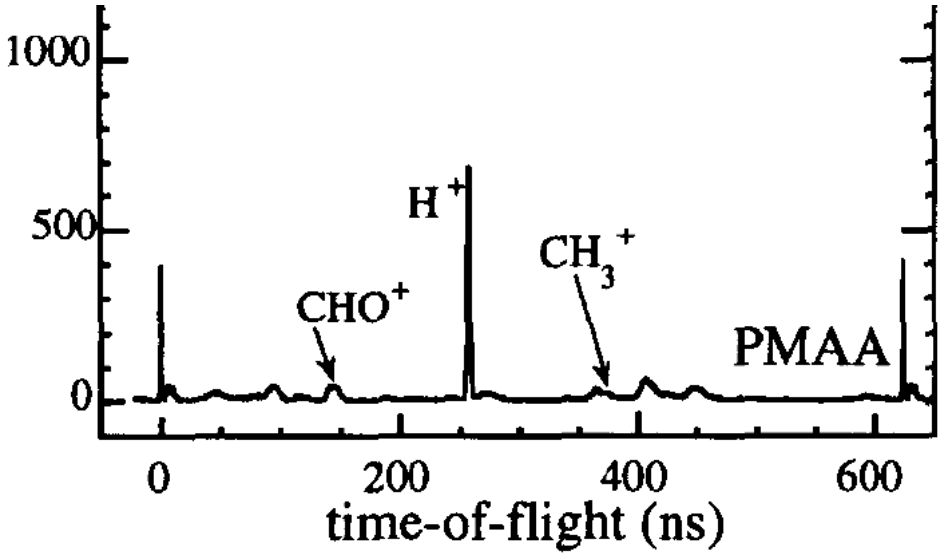
After warmup in microscope (eventually freeze-dried): holes at irradiated regions!

Maser *et al.*, *J. Micros.* **197**, 68 (2000)

Radiation damage studies: poly (methyl methacrylate) or PMMA



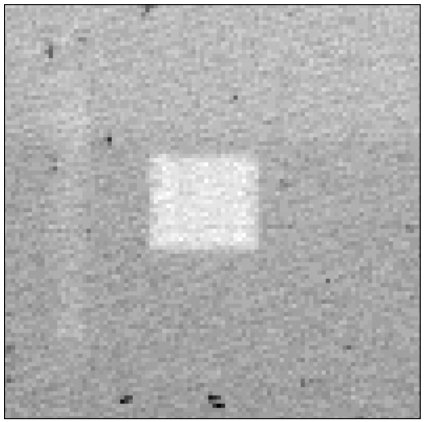
Tinone *et al.*, *Appl. Surf. Sci.* **79**, 89 (1994)



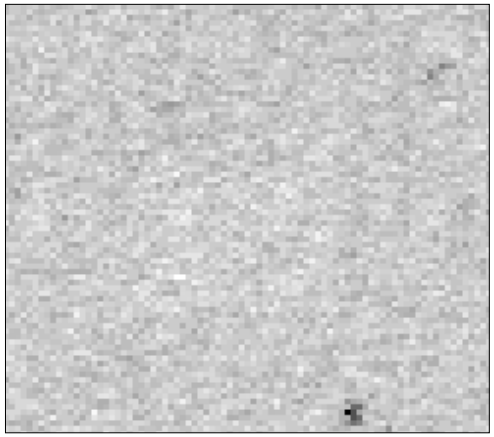
Tinone *et al.*, *J. Electron Spect. Rel. Phen.* **80**, 117 (1996).

PMMA at room, LN2 temperature

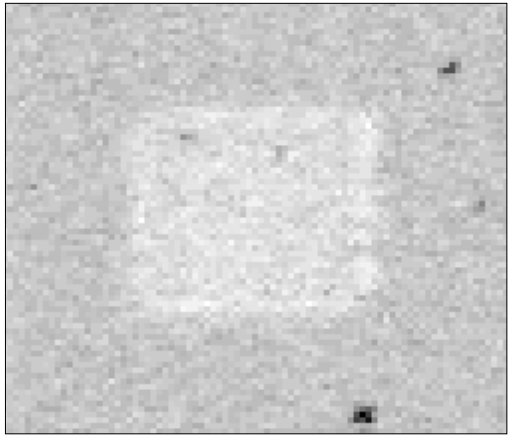
- Beetz and Jacobsen, *J. Synchrotron Radiation* **10**, 280 (2003)
- Repeated sequence: dose (small step size, long dwell time), spectrum (defocused beam)
- Images: dose region (small square) at end of sequence



Room temperature: mass loss immediately visible



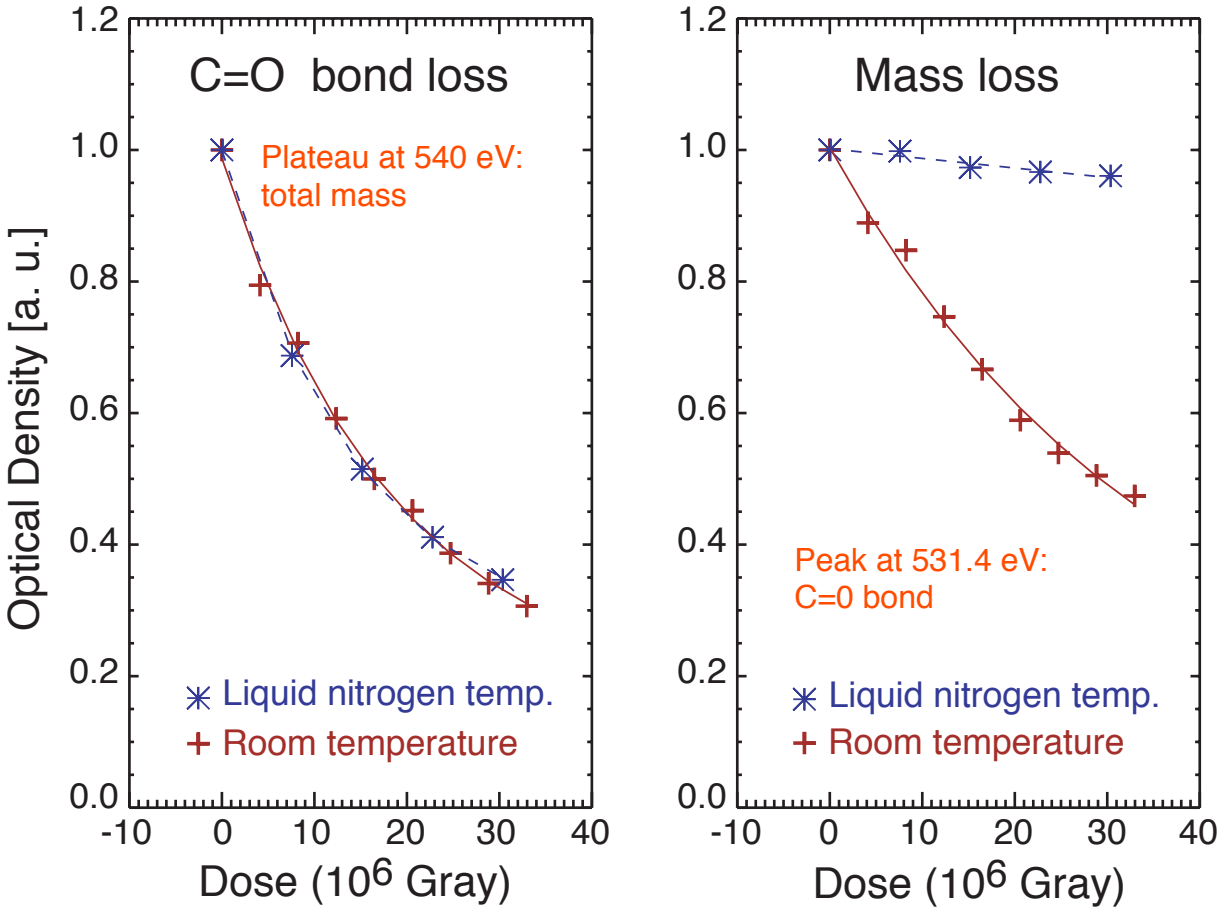
LN_2 temperature: no mass loss immediately visible



After warm-up: mass loss becomes visible

PMMA at 300 K and 110 K: chemistry and mass

LN_2 temp: protection against mass loss, but not against breaking bonds (at least C=0 bond in dry PMMA)



Beetz and Jacobsen, *J. Synchrotron Radiation* **10**, 280 (2003)

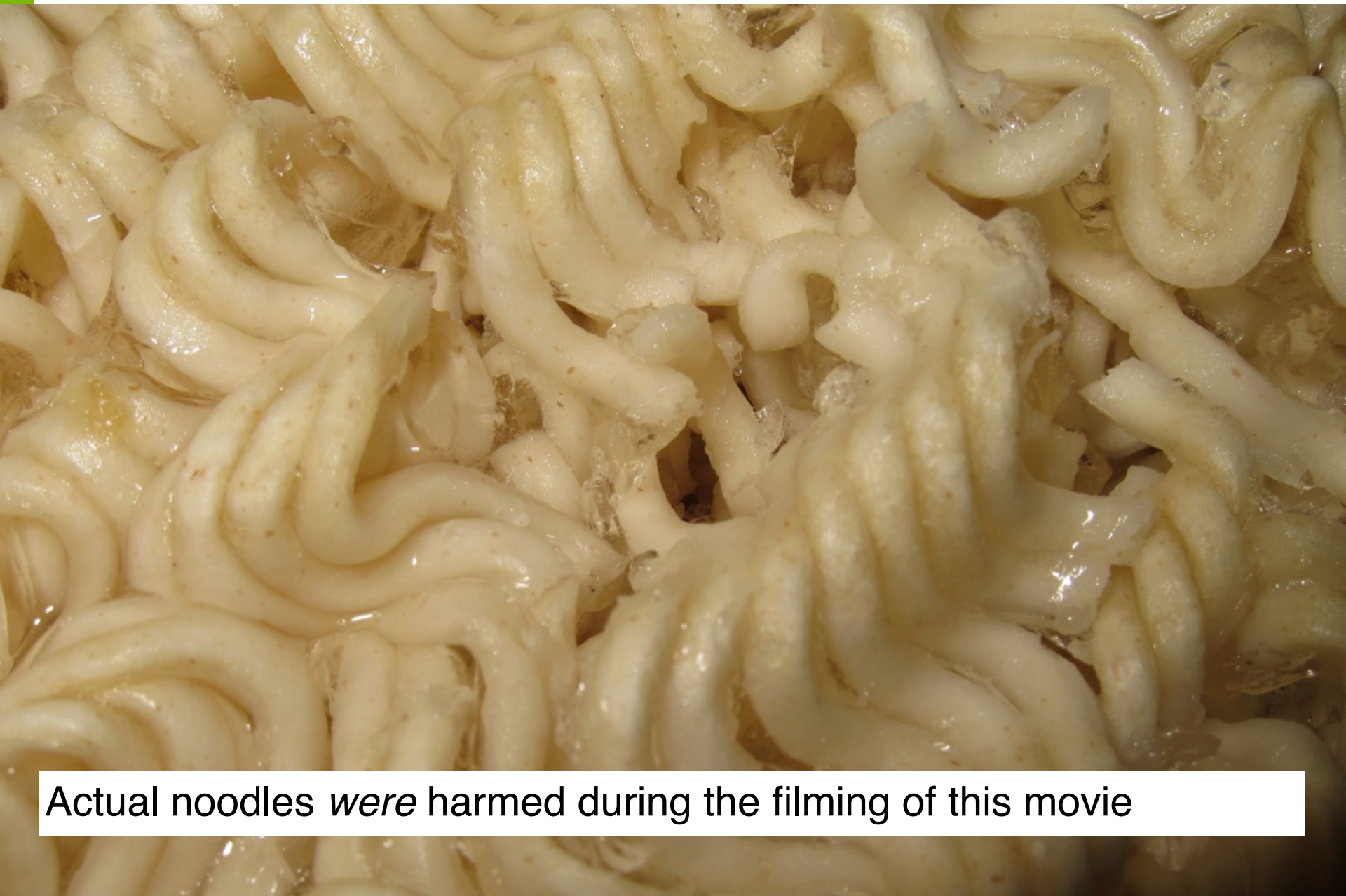
The Ramen noodle model of radiation damage

Macromolecular chains with no “encapsulating” matrix
(dry, room temperature wet)

The Ramen noodle model of radiation damage

Macromolecular chains in an “encapsulating” matrix
(frozen hydrated)

The Ramen noodle model of radiation damage



Actual noodles *were* harmed during the filming of this movie

Outline

- The physics and nomenclature of x-ray fluorescence
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- From 2D to 3D: fluorescence tomography, tomographic alignment, and self-absorption correction

Fluorescence tomography

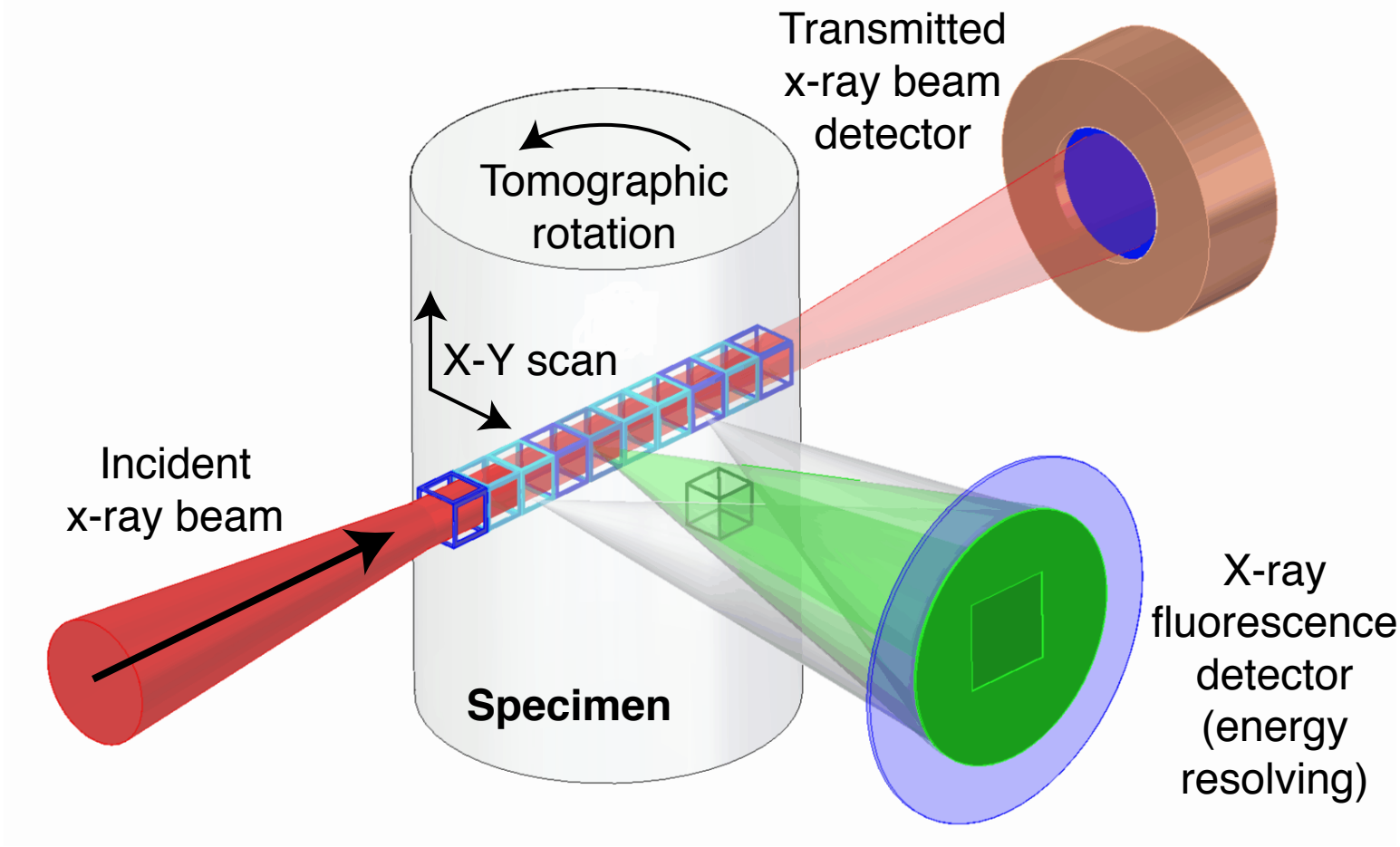
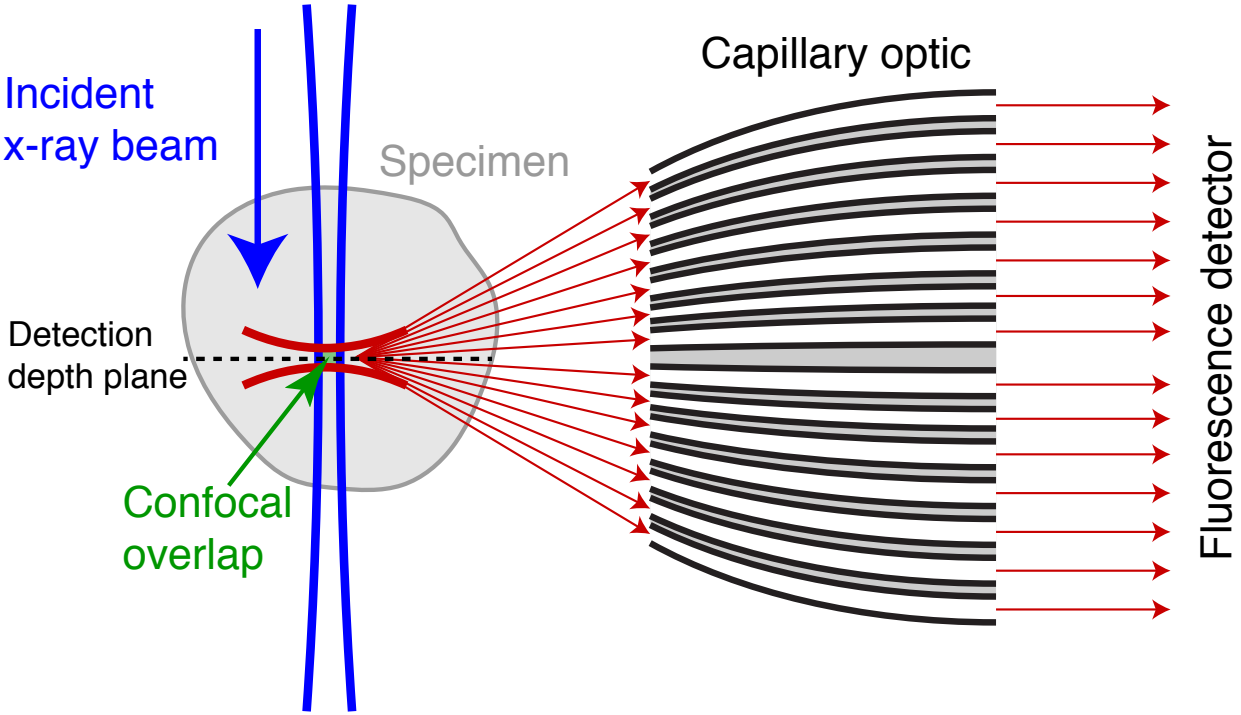


Figure from De Jonge and Vogt, *Current Opinion in Structural Biology* **20**, 606 (2010)

“Confocal” fluorescence

- Different than confocal light microscopy where focusing and detecting objectives are along same axis; with X rays, on orthogonal axes.
- Selective detection of one depth point along x-ray beam path.



Fluorescence tomography

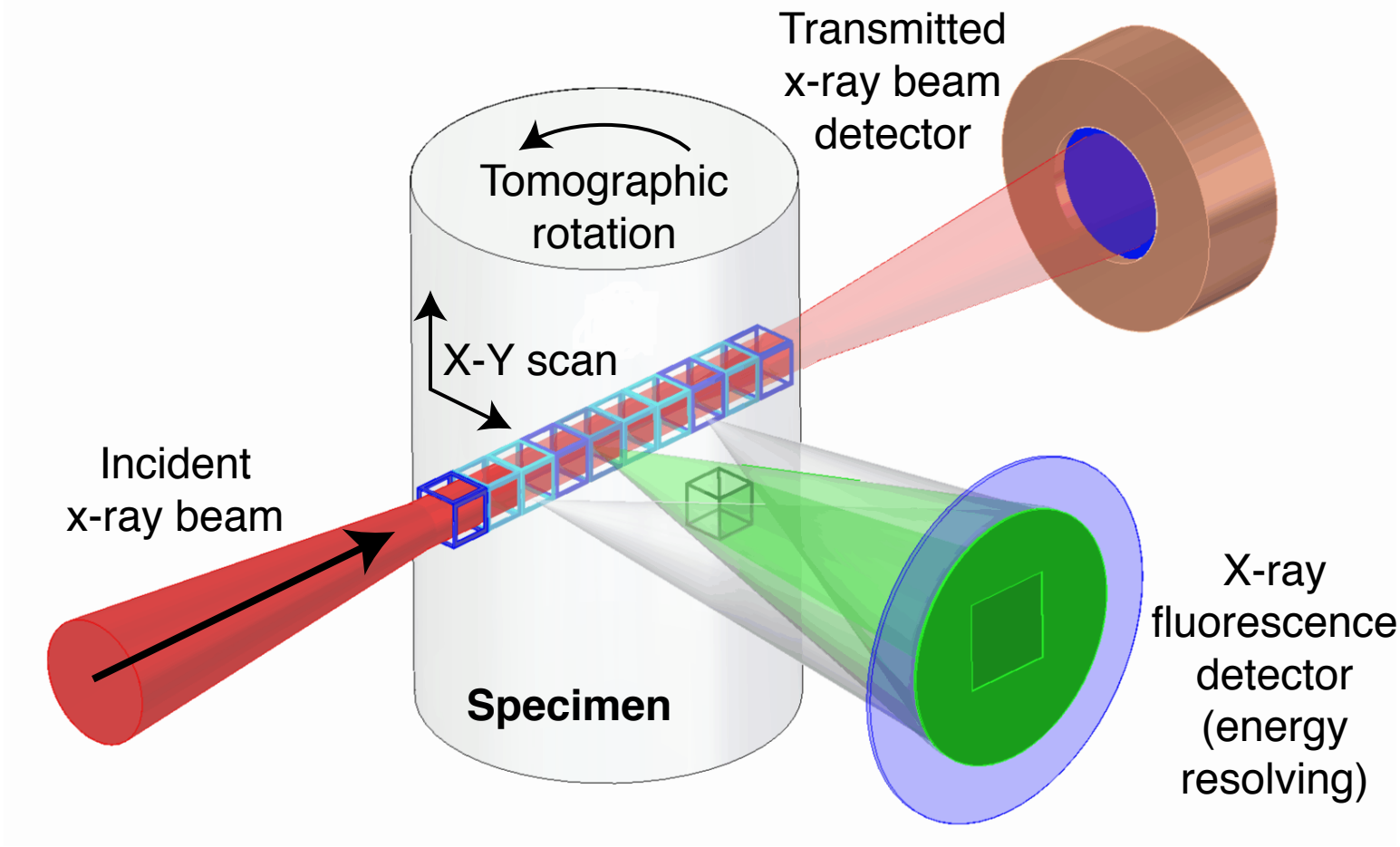
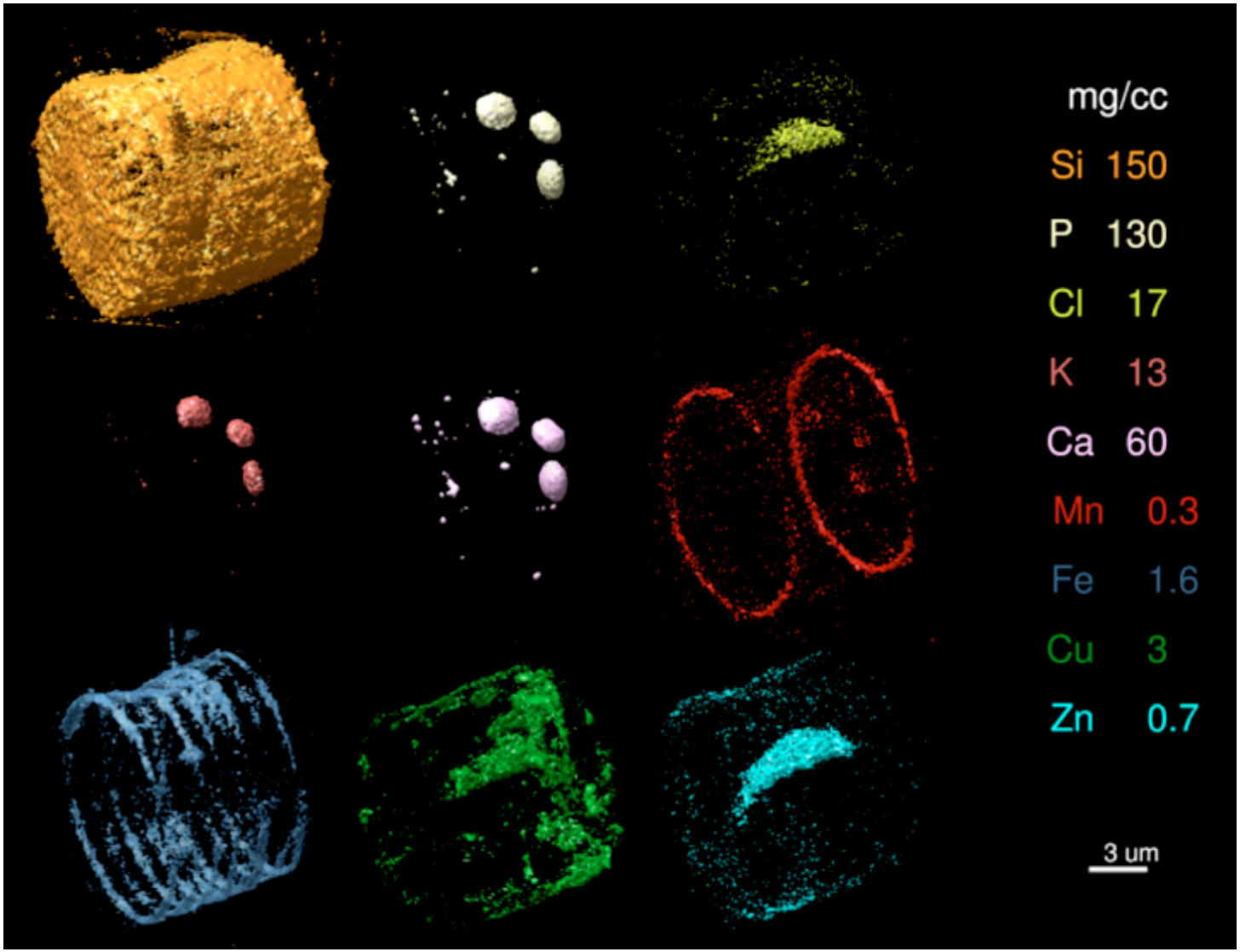


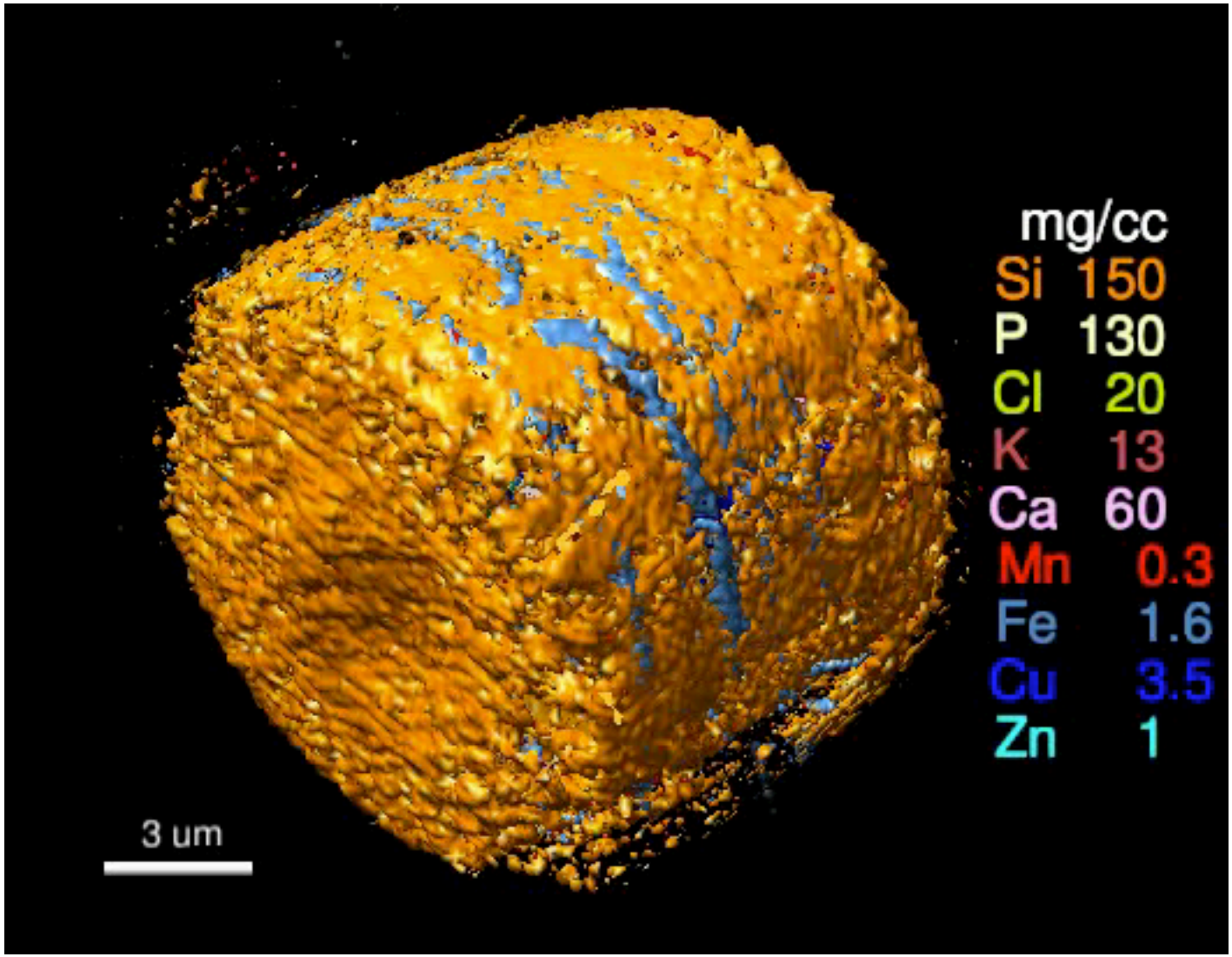
Figure from De Jonge and Vogt, *Current Opinion in Structural Biology* **20**, 606 (2010)

Quantitative 3D fluorescence of a diatom



M. de Jonge, C. Holzner, S. Baines, B. Twining, K. Ignatyev, J. Diaz, D. Howard, A. Miceli, I. McNulty, C. Jacobsen, S. Vogt, *Proc. Nat. Acad. Sci.* **107**, 15676 (2010)

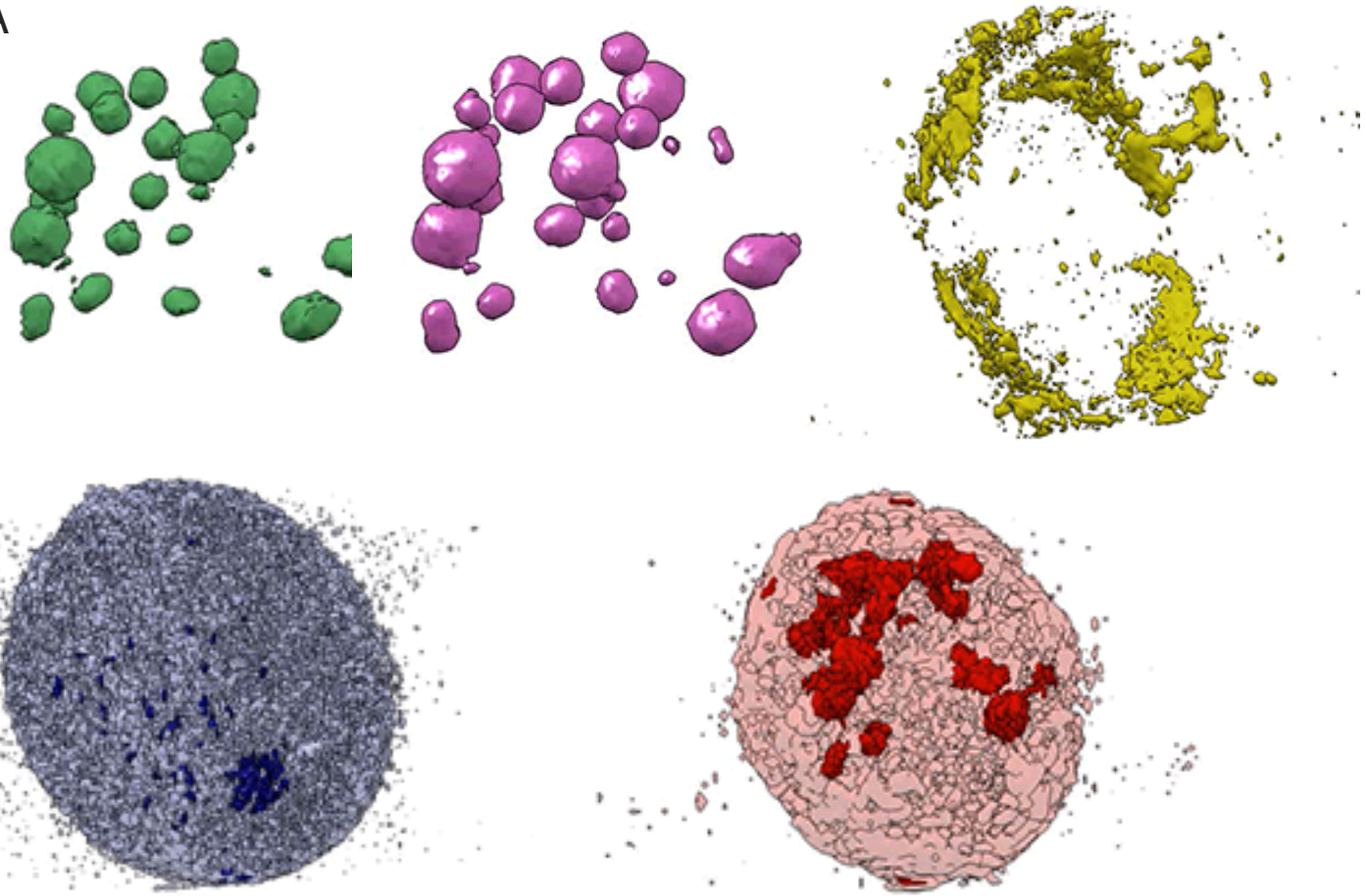
Fluorescence tomography



de Jonge *et al.*, *Proc. Nat. Acad. Sci.* **107**, 15676 (2010).

Ptychography and fluorescence in 3D - preliminary results on a frozen hydrated algae

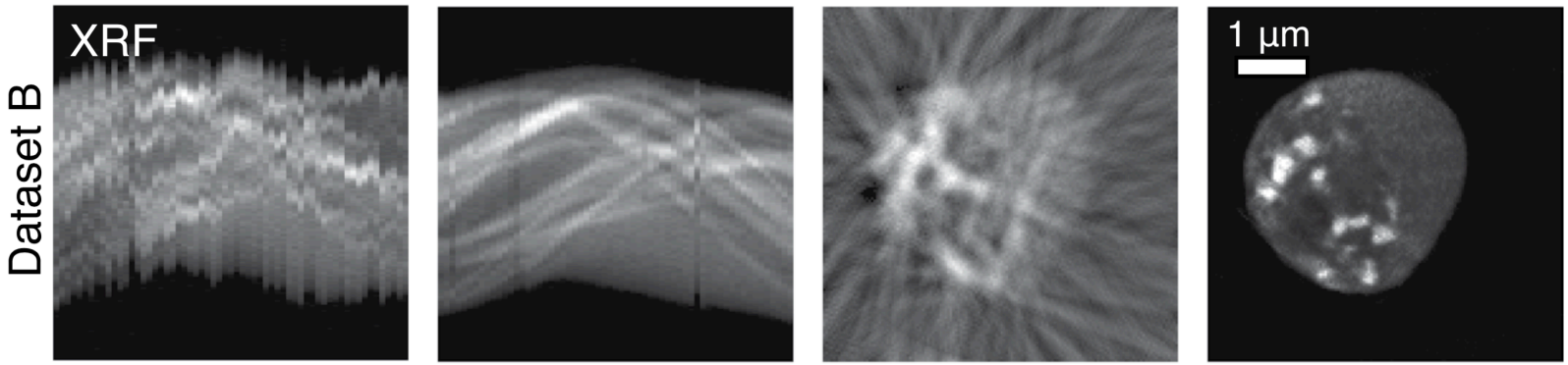
J. Deng, C. Jacobsen *et al.,* Argonne/Northwestern
J. Miao *et al.,* UCLA



Aligning tomographic datasets

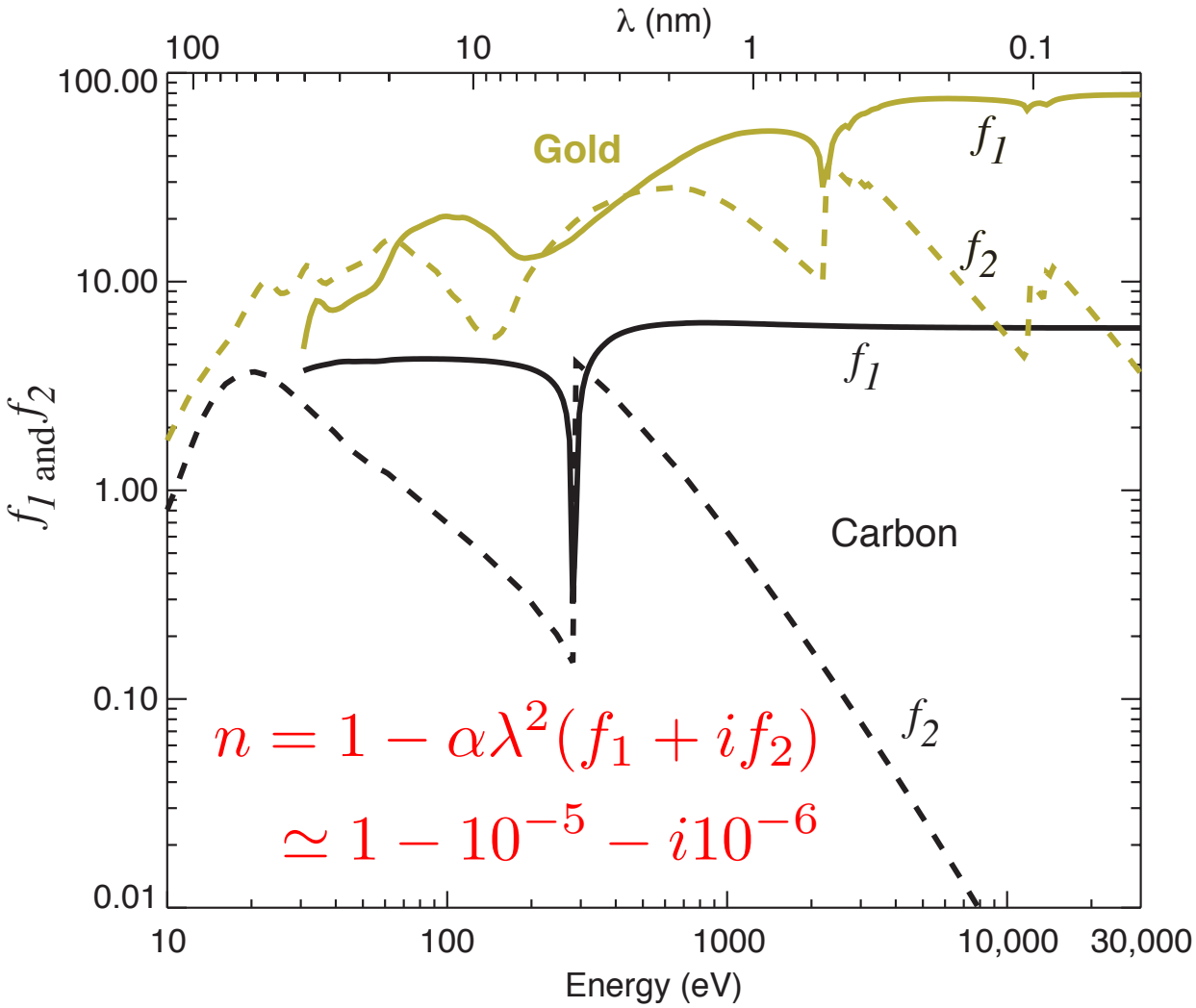
Iterative reprojection:

1. Reconstruct from poor alignment.
2. Align each projection to reconstruction
3. Re-do reconstruction.
   - Electron microscopy and filtered backprojection: Dengler, *Ultramicroscopy* **30**, 337 (1989)
   - Incorporation into advanced tomographic reconstruction algorithms: Gürsoy, Hong, He, Hujsak, Yoo, Chen, Li, Ge, Miller, Chu, De Andrade, He, Cossairt, Katsaggelos, and Jacobsen, *Scientific Reports* **7**, 11818 (2017)



X-ray refractive index

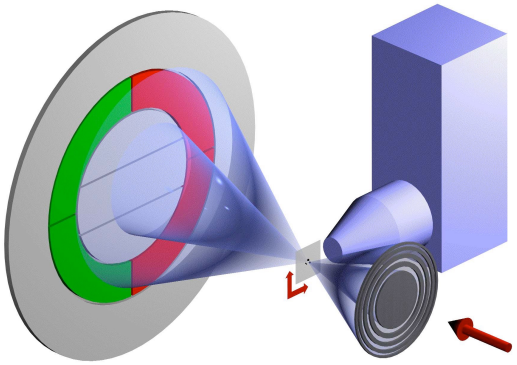
Refractive index of
$n=1-\alpha\lambda^2(f_1+if_2)$

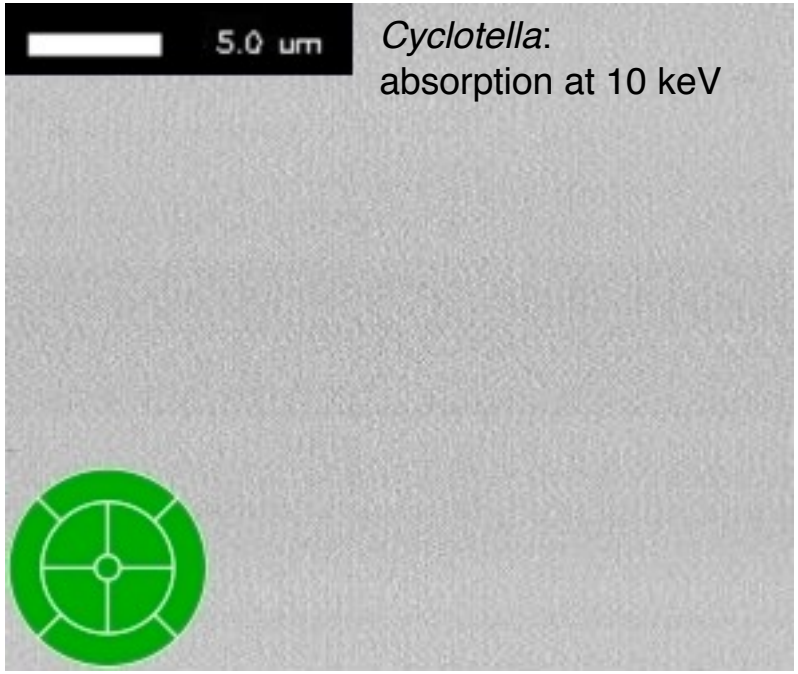


Data from http://henke.lbl.gov/optical_constants/

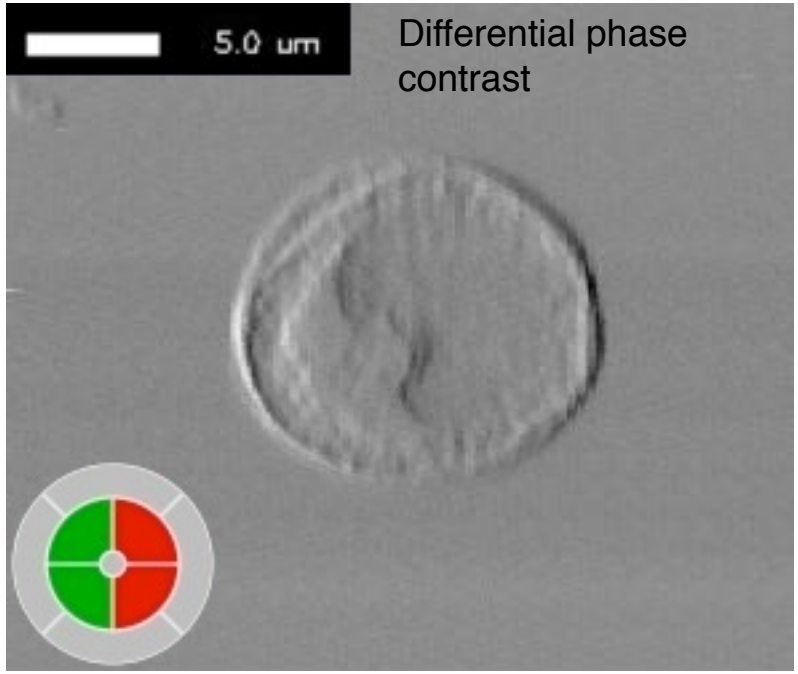
X-ray phase contrast can be dramatically stronger

- Imaging of a marine diatom using a segmented x-ray detector.
- Acquire simultaneously with fluorescence
- Fourier filtering, Fourier integration for absolute phase contrast.
- Sensitivity: ~π/180.





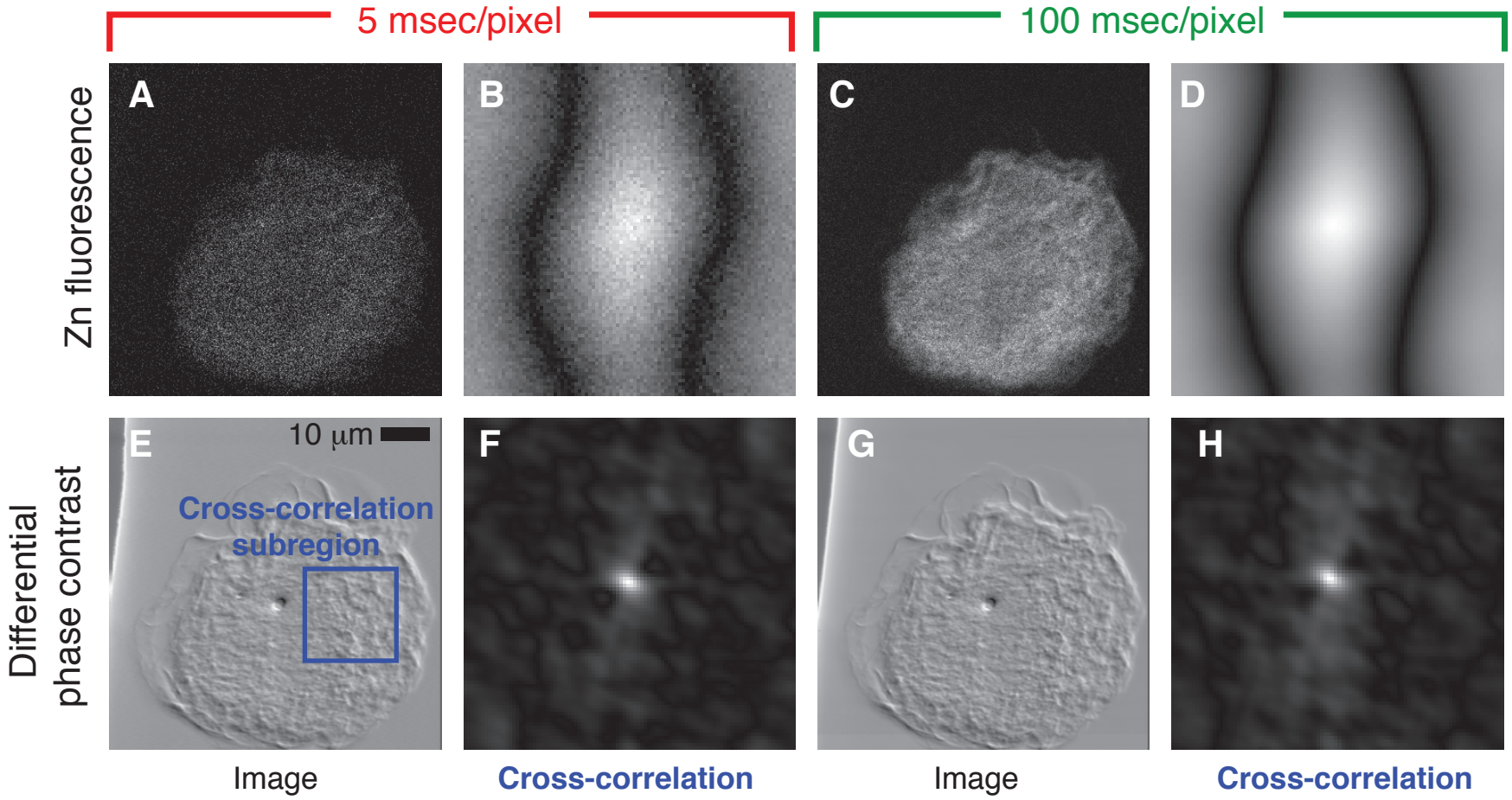
Cyclotella: absorption at 10 keV



Differential phase contrast

Hornberger *et al.*, *Ultramic.* **107**, 644 (2007); Feser *et al.*, *Nucl. Inst. Meth. A* **565**, 841 (2006); de Jonge *et al.*, *Phys. Rev. Lett.* **100**, 163902 (2008)

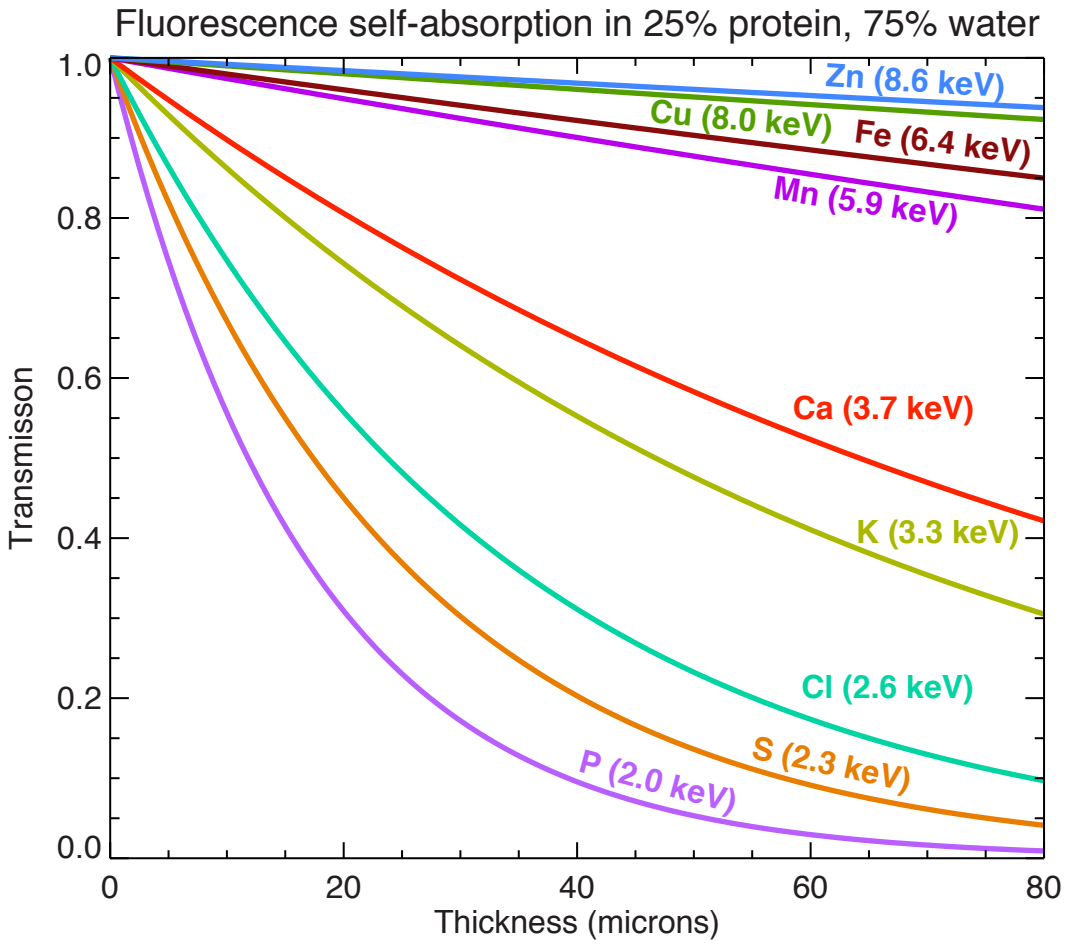
Using phase contrast to align tomography



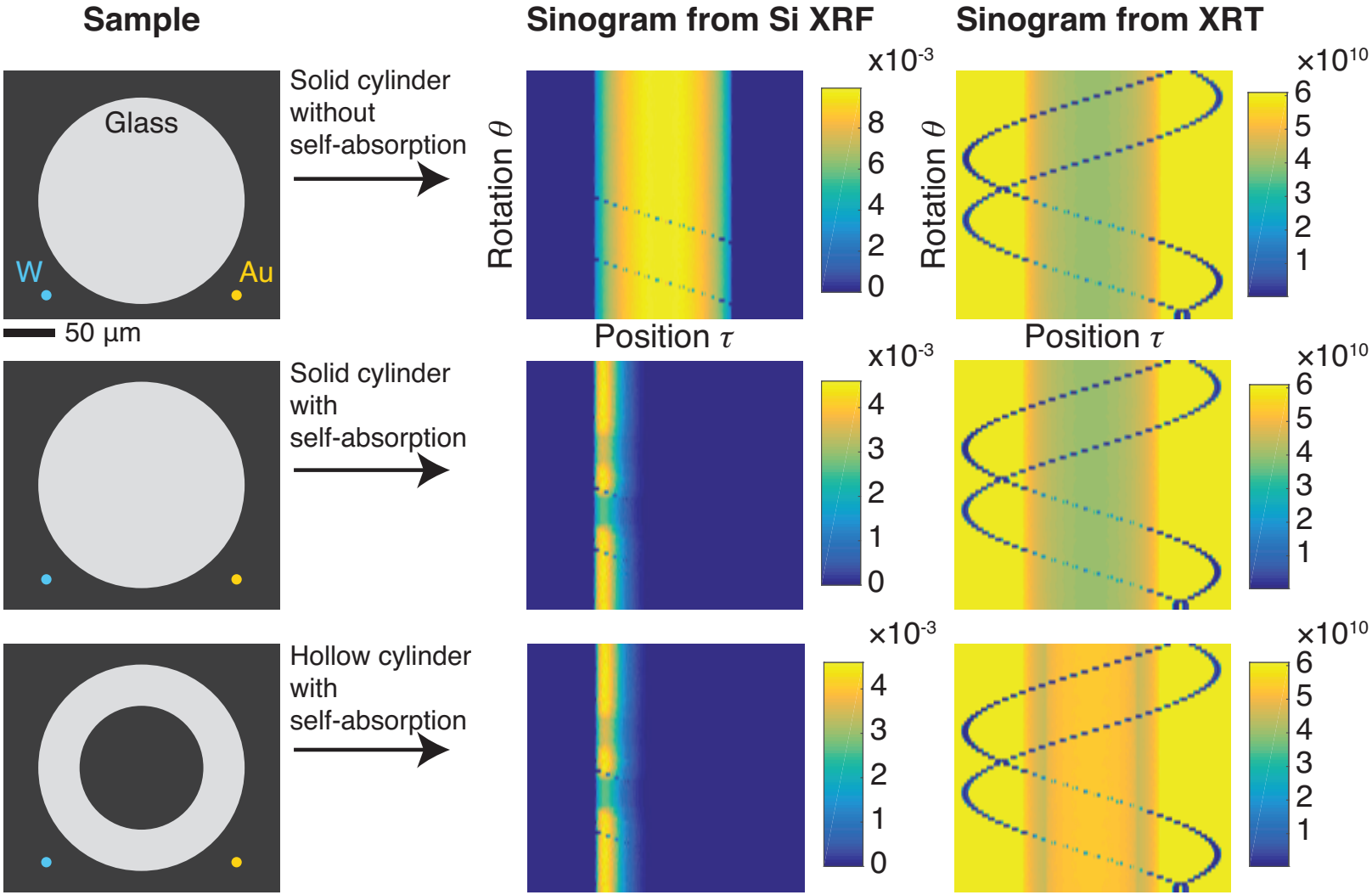
Hong, Gleber, O'Halloran, Que, Bleher, Vogt, Woodruff, and Jacobsen, *J. Sync. Rad.* **21**, 229 (2014)

Self absorption of x-ray fluorescence

- Fluorescent photons created within specimen, but some get re-absorbed on path through specimen to detector.



Self-absorption in fluorescence tomography



Di, Chen, Hong, Jacobsen, Leyffer, and Wild, *Optics Express* **25**, 13107 (2017)

Self-absorption correction

- Combined computational method using both x-ray fluorescence and x-ray transmission lets you correct for self-absorption.
- Di, Chen, Hong, Jacobsen, Leyffer, and Wild, *Optics Express* **25**, 13107 (2017)