Some comments on dose, signal to noise, damage, and heating

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Signal to noise and required number of photons

- Simple photon statistics with known contrast:

$$\mathrm{SNR} = \frac{\mathrm{Signal}}{\mathrm{Noise}} = \frac{\bar{n}|I_f - I_b|}{\sqrt{(\sqrt{\bar{n}I_f})^2 + (\sqrt{\bar{n}I_b})^2}} = \sqrt{\bar{n}}\frac{|I_f - I_b|}{\sqrt{I_f + I_b}} = \sqrt{\bar{n}}\Theta$$

  where Θ=contrast parameter, I_f=intensity of feature, I_b=intensity of background.

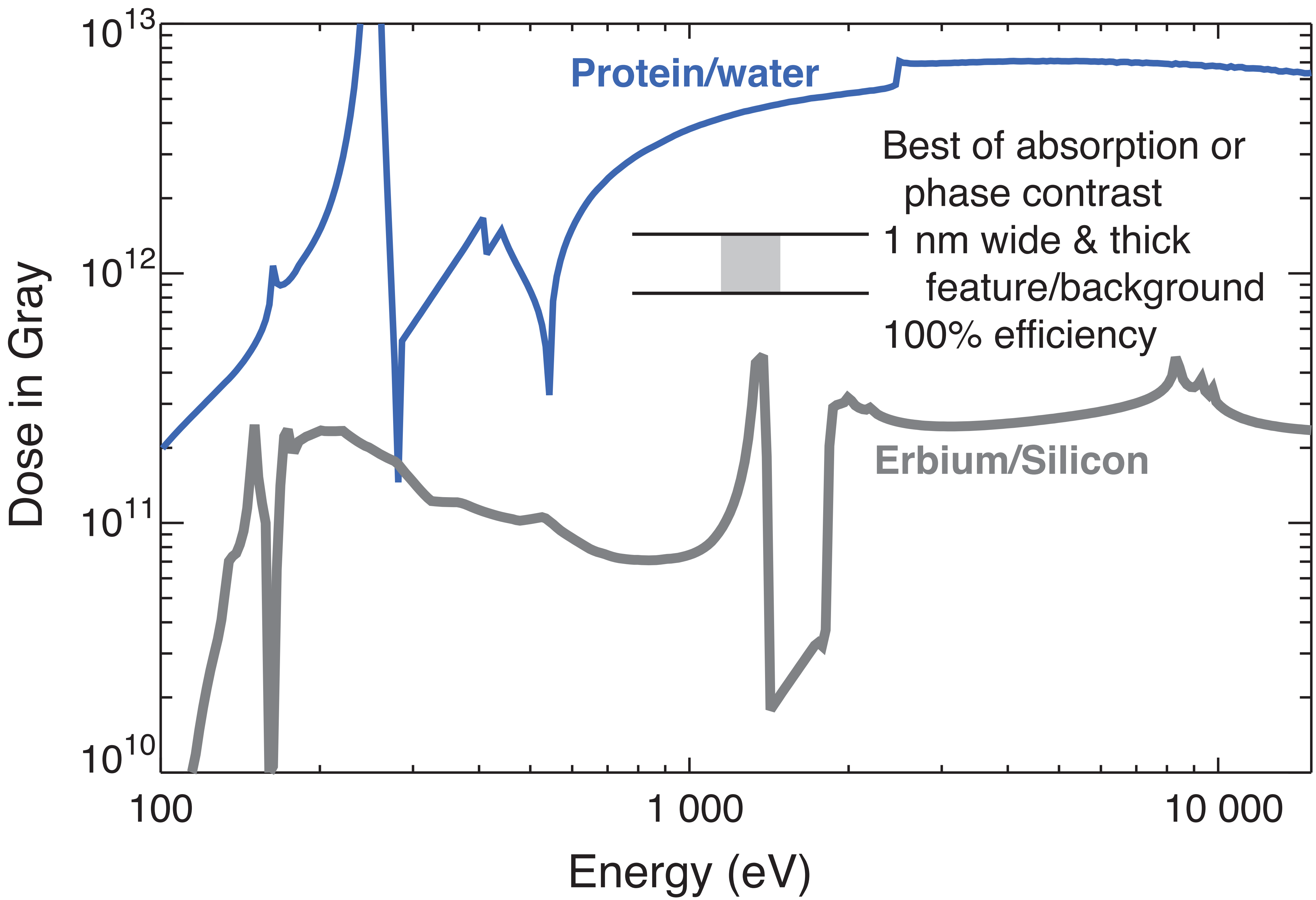
- Thus required number of incident photons $\bar{n}$ is

$$\bar{n} = \frac{\mathrm{SNR}^2}{\Theta^2}$$

- Example: 10 nm protein in ice at 520 eV via absorption contrast
  - Protein has linear absorption coefficient (LAC) of 1/9.900 μm, so 10 nm has I_f= exp[-0.010/9.900]=0.99899
  - Ice has LAC of 0.717 μm, so 10 nm has I_b= exp[-0.010/0.717]=0.98615
  - Contrast parameter is Θ=(.99899-.98615)/(.99899+.98615)$^{1/2}$=.00911
  - So with SNR=5 one requires $\bar{n}$=(5)^2/(.00911)^2=3x10^5 incident photons
- See e.g., Sayre *et al.*, *Ultramicroscopy* **2**, 337 (1977); Sayre *et al.*, *Science* **196**, 1339 (1977)

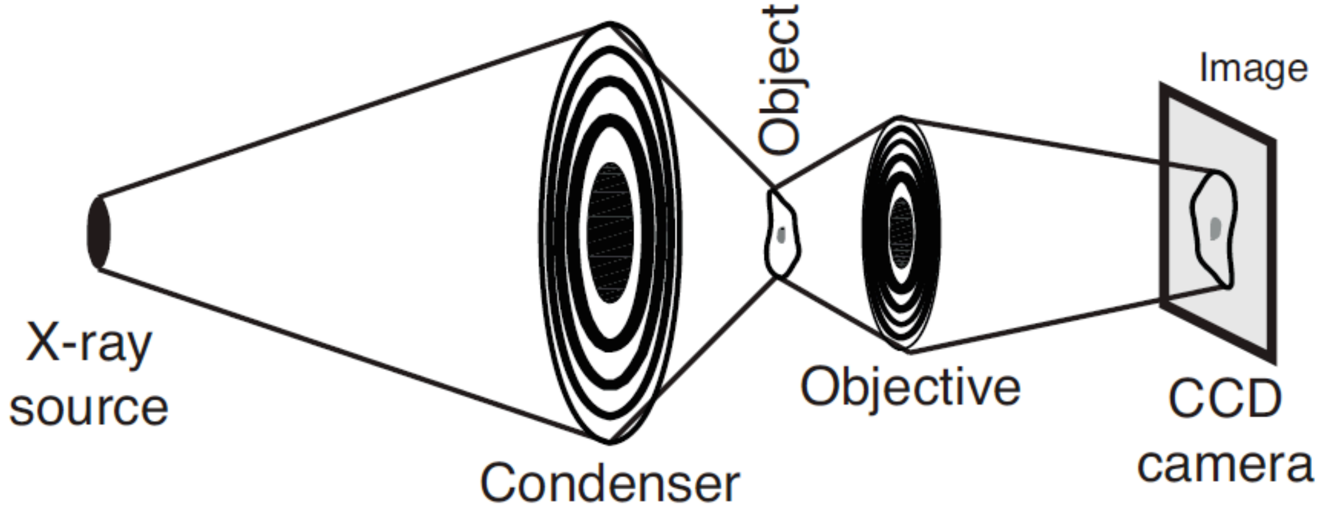
Estimates of dose for 1 nm resolution imaging

Absorbed dose correlates well with damage across a wide spectrum of ionizing radiation. Example: VUV, e-beam, x-ray exposure of photoresists.

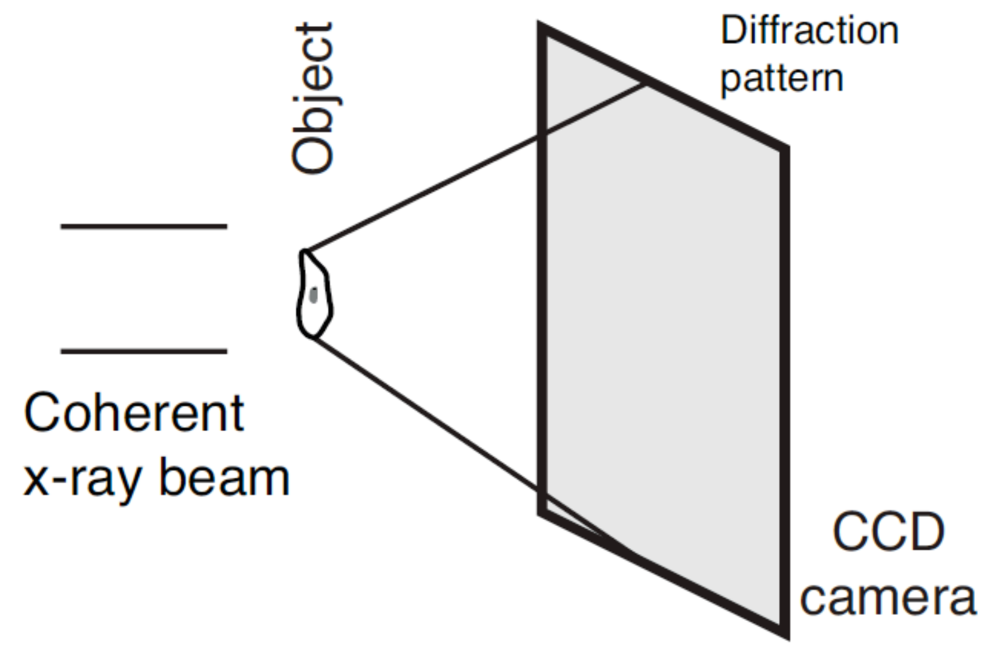


Comparison: TXM versus XDM

- TXM: assume zone plate with 20 nm outermost zone width (mean MTF ~20%), 10% efficiency. Net throughput: ~2%.
- XDM: assume 100% efficient detector.



TXM: transmission x-ray microscope



XDM: x-ray diffraction microscope

X. Huang *et al.*, *Optics Express* **17**, 13541 (2009)

SNR from unknown objects

- What if we don't know I_f and I_b?
- Let's say we have two noisy images I_1 and I_2, but we know they are of the same object.
- Calculate correlation r:

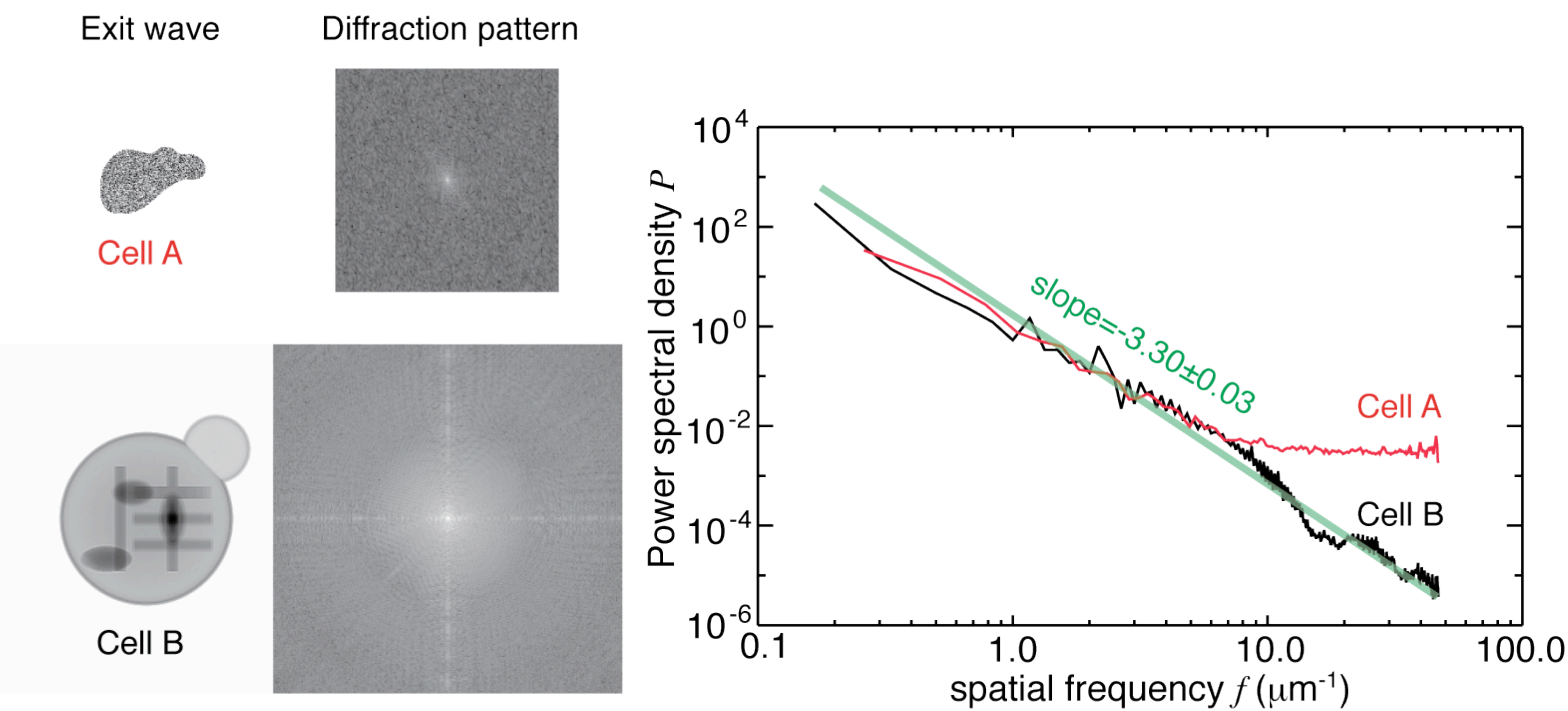
$$r = \frac{\langle (I_1 - \langle I_1 \rangle)(I_2 - \langle I_2 \rangle)^* \rangle}{\sqrt{\langle (I_1 - \langle I_1 \rangle)^2 \rangle \langle (I_2 - \langle I_2 \rangle)^2 \rangle}}$$

- We find $\mathrm{SNR} = \sqrt{r/(1-r)}$
- Note: square root of expression of Frank and Al-Ali, *Nature* **256**, 376 (1975)

X. Huang *et al.*, *Optics Express* **17**, 13541 (2009)

Two simulated "cells" at 540 eV

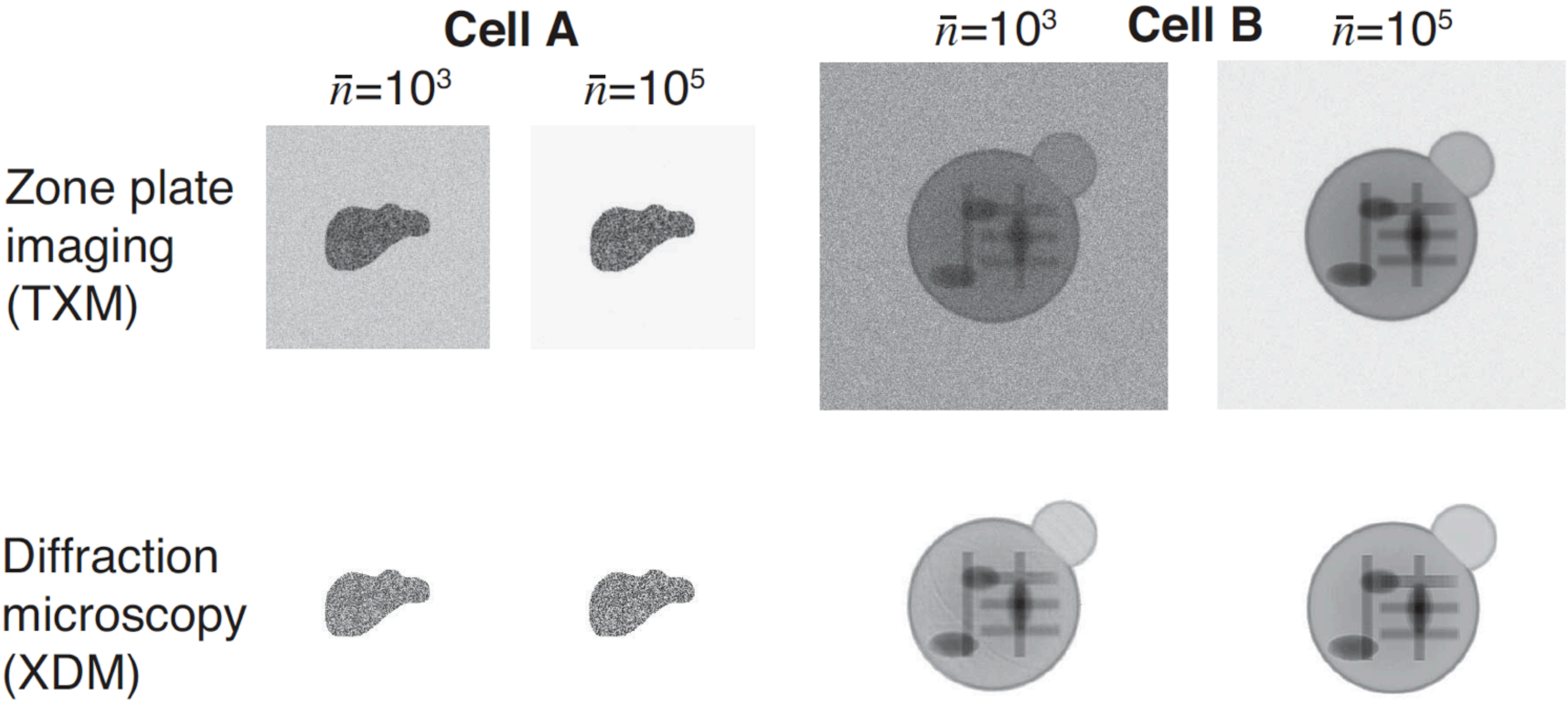
- Cell A: 0-500 nm random protein thickness
- Cell B: protein spheres, bars in ice (3D exit wave)



X. Huang *et al.*, *Optics Express* **17**, 13541 (2009)

Fake "cells" via TXM and XDM

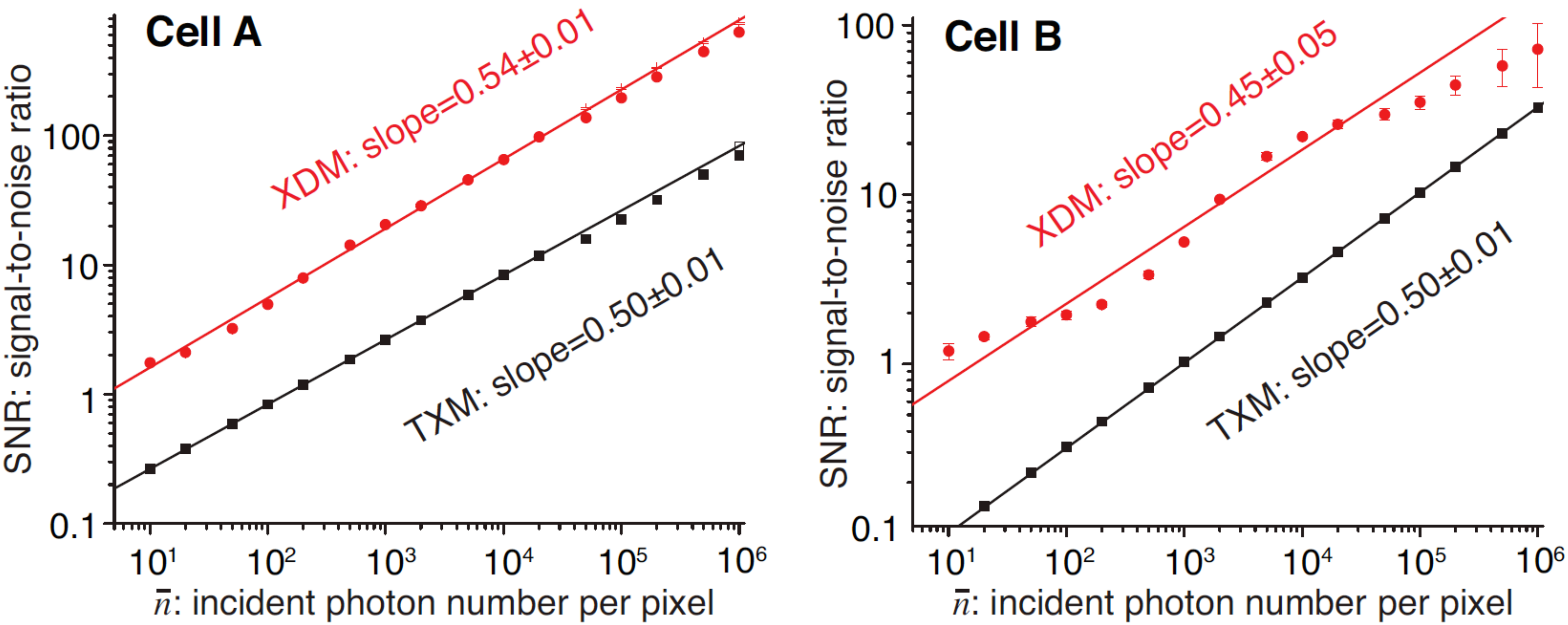
- XDM: assume perfect support



X. Huang *et al.*, *Optics Express* **17**, 13541 (2009)

SNR versus exposure: results

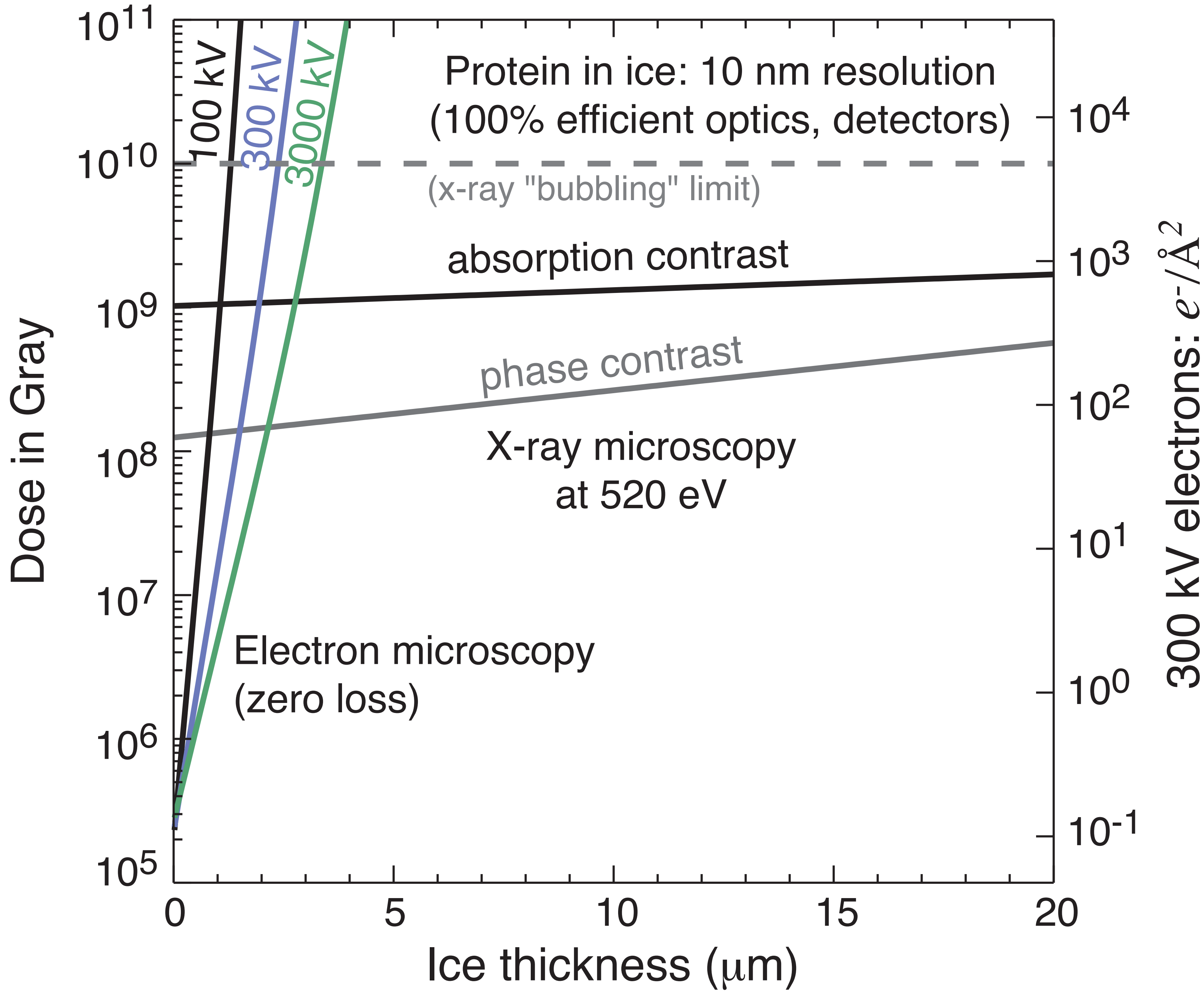
- TXM: net throughput ~2%, or 1/50. Expect SNR to be ~√50 or ~7 times lower.



X. Huang *et al.*, *Optics Express* **17**, 13541 (2009)

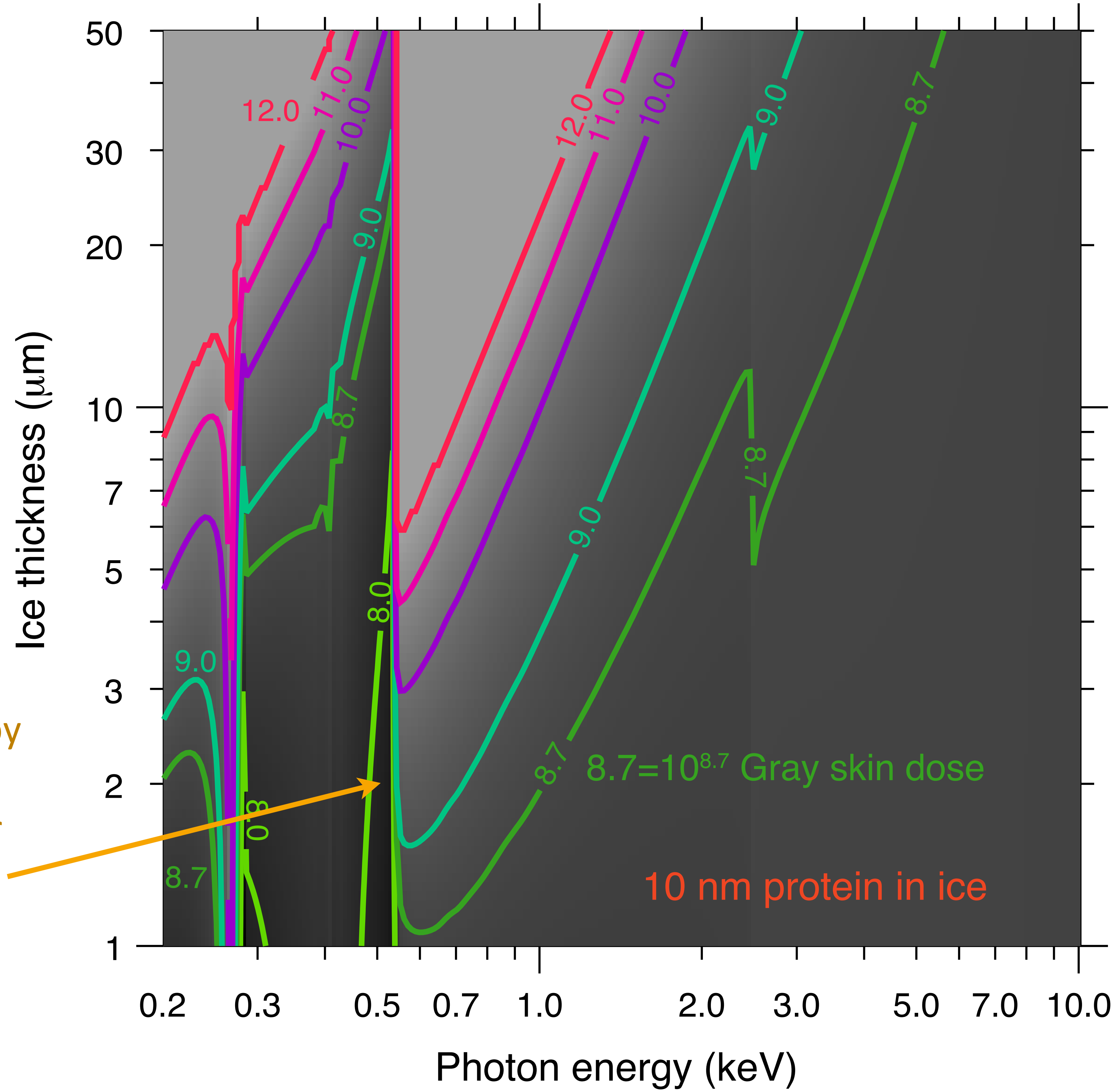
X rays are better than electrons for thick bio specimens

- Electrons are better for <500 nm thick specimens
- X rays are better for whole cells



This plot: based on Jacobsen, Medenwaldt, and Williams, in **X-ray Microscopy & Spectromicroscopy** (Springer, 1998). See also Sayre *et al.*, *Science* **196**, 1339 (1977).

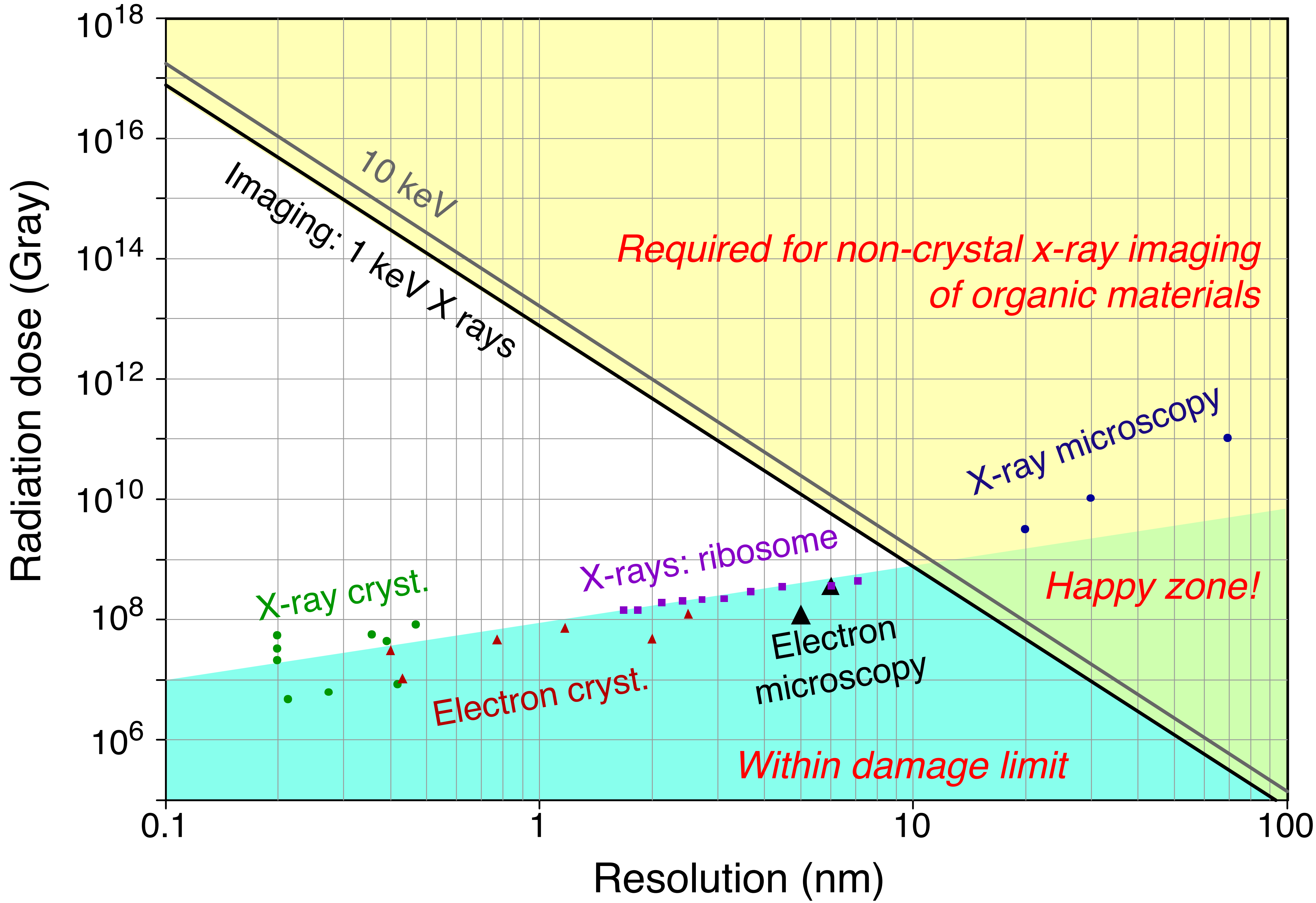
Another look at dose versus energy and ice thickness



Higher energies: easier with Born approximation

Lowest dose, by 1 order of magnitude, for <10 microns thick

What's the limit for cells?

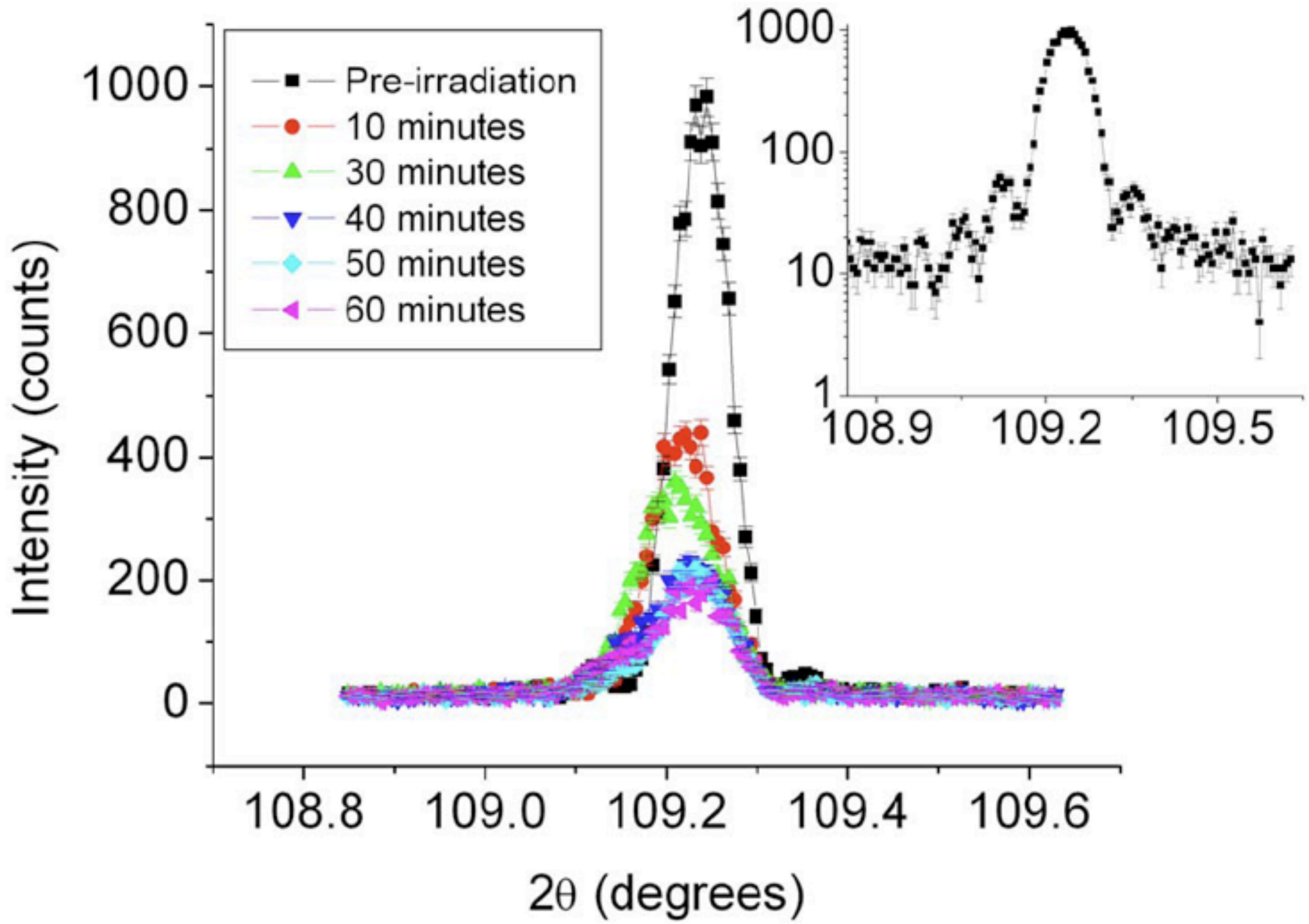


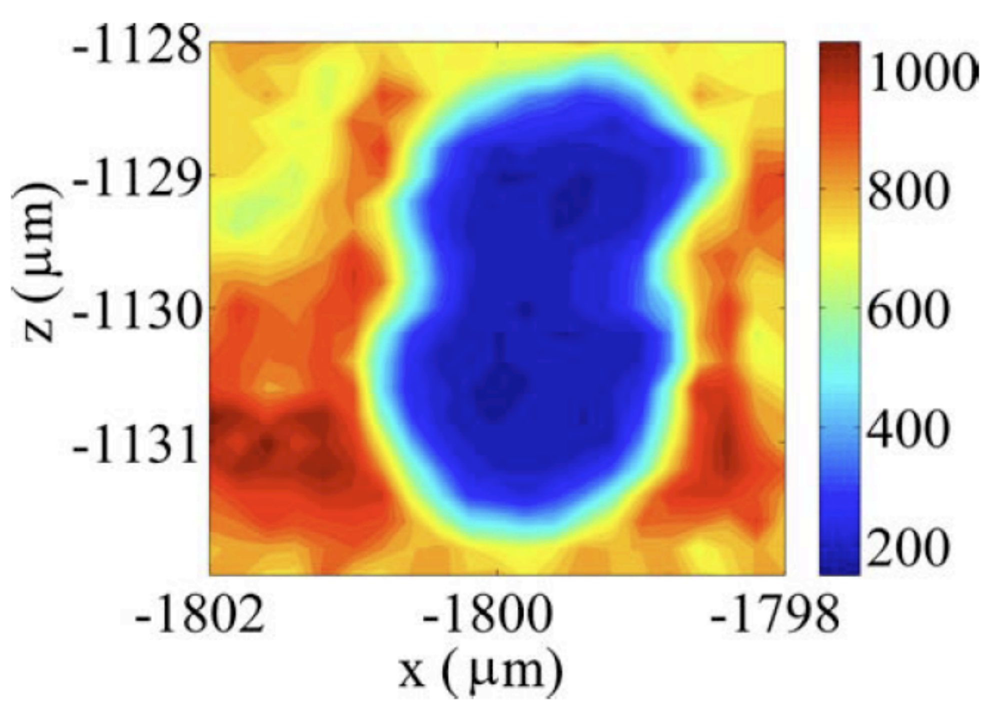
Howells et al., *JESRP* 170, 4 (2009)

See also Shen et al., *J. Sync. Rad*. **11**, 432 (2004)

X-ray damage: Silicon in Silicon-on-insulator (SOI)

Non-recoverable fading of Si 008 diffraction peak in 140 nm thick SOI layer, using 11.2 keV X rays





Beam spot: ~0.3 μm
Damage radius: ~1.8 μm

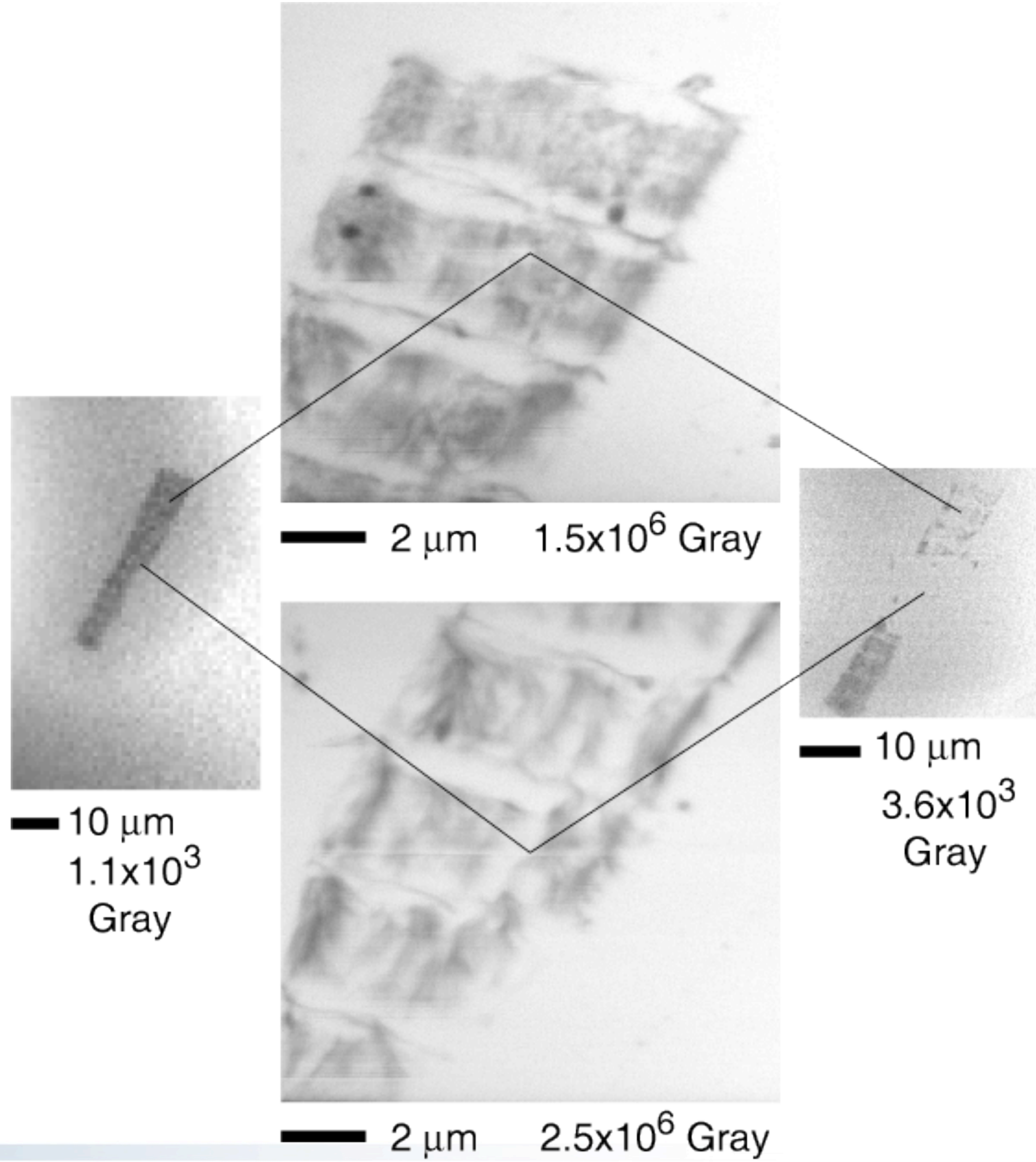
1.9×10^{8} photons/sec into 0.25×0.30 μm, or

$\sim6\times10^{9}$ Gray per 10 minutes

Polvino, Murray, Kalenci, Noyan, Lai, and Cai, *Appl. Phys. Lett.* **92**, 224105 (2008)

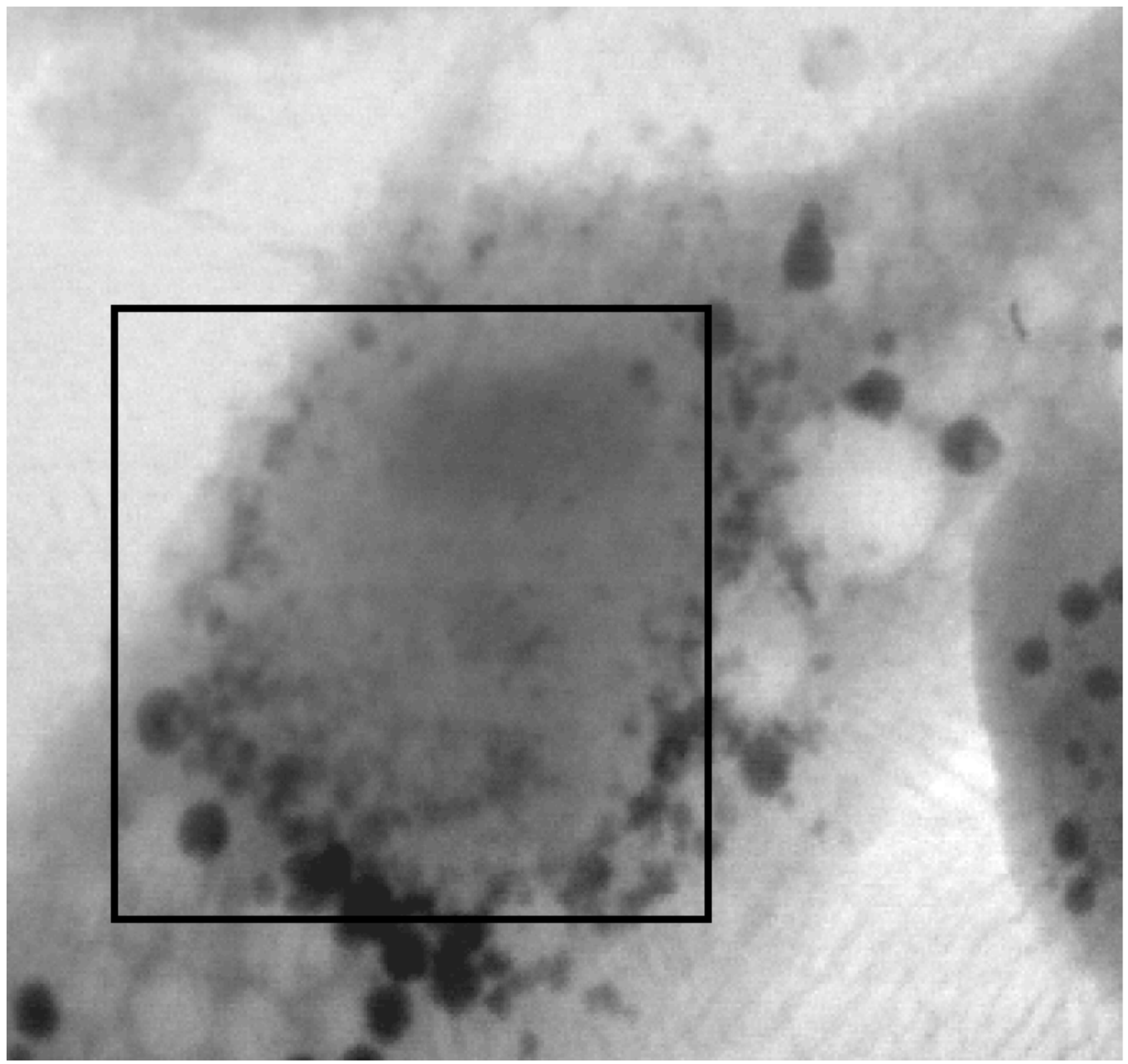
Muscle damage

- Images: dragonfly flight muscle, with Clara Franzini-Armstrong
- At 10^4 Gray, myofibrils stop contracting in the presence of ATP. Bennett *et al.*, *J. Micros.* **172**, 109 (1993)



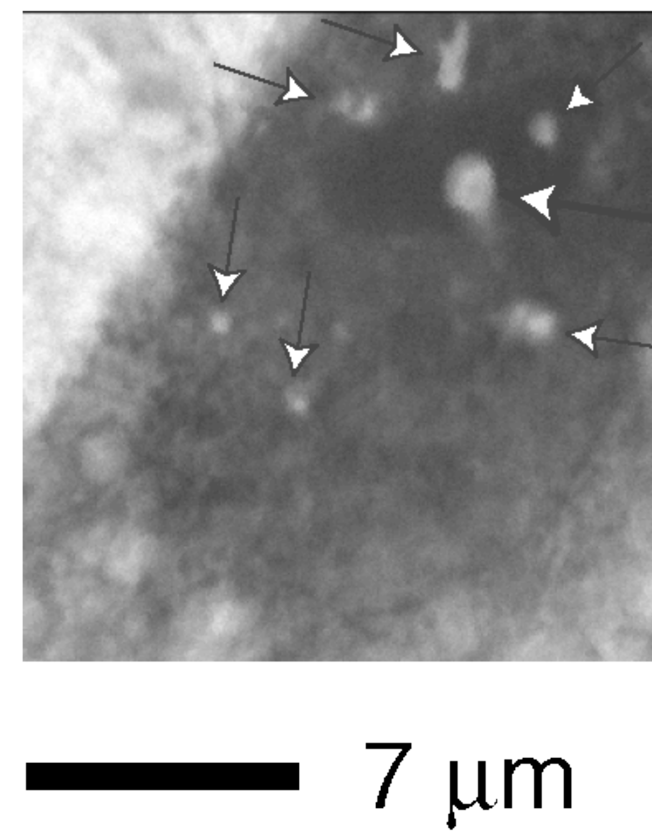
Radiation damage resistance in cryo

Left: frozen hydrated image after exposing several regions to ~10^{10} Gray

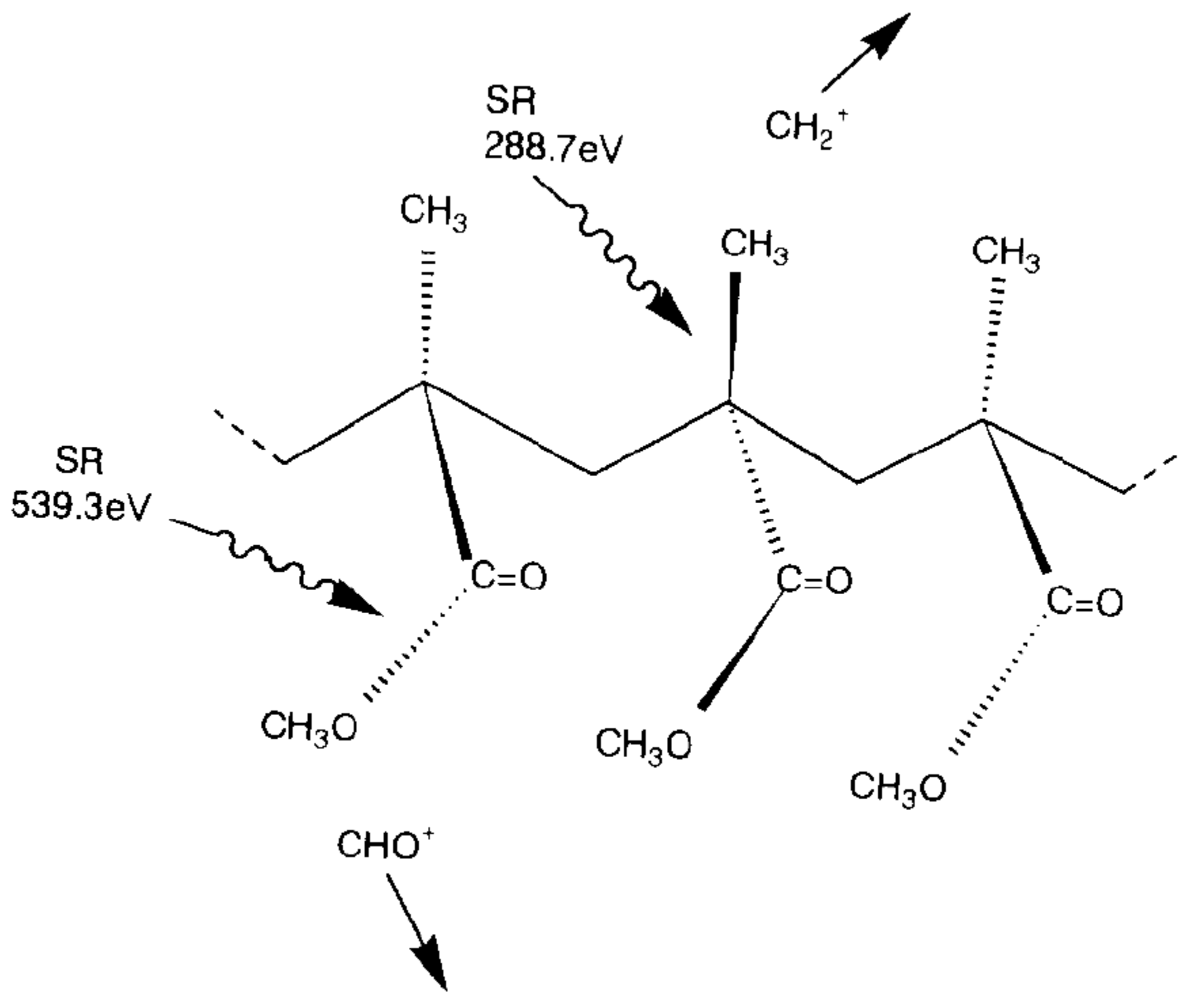


Right: after warmup in microscope (eventually freeze-dried): holes indicate irradiated regions!

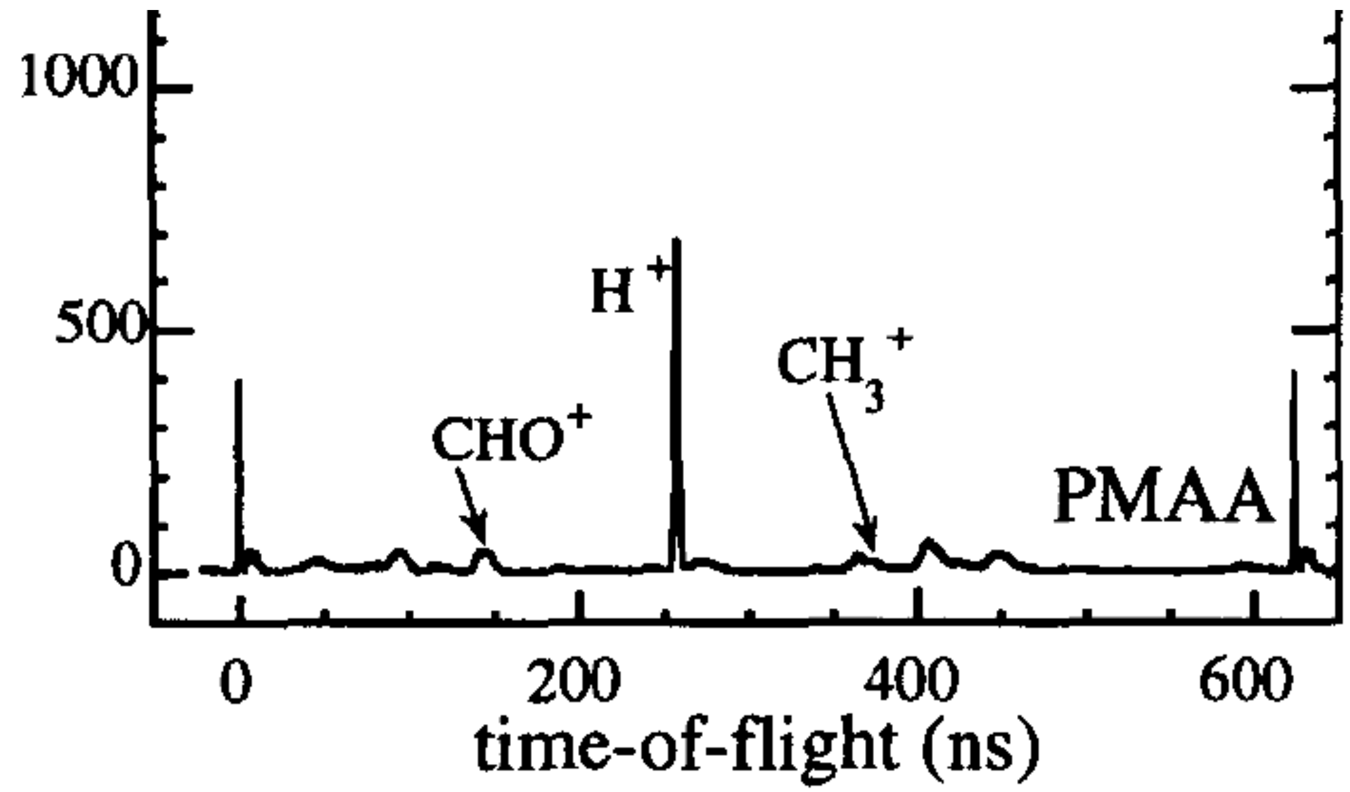
Maser *et al.*, *J. Micros.* **197**, 68 (2000)



Radiation damage studies: poly (methyl methacrylate) or PMMA



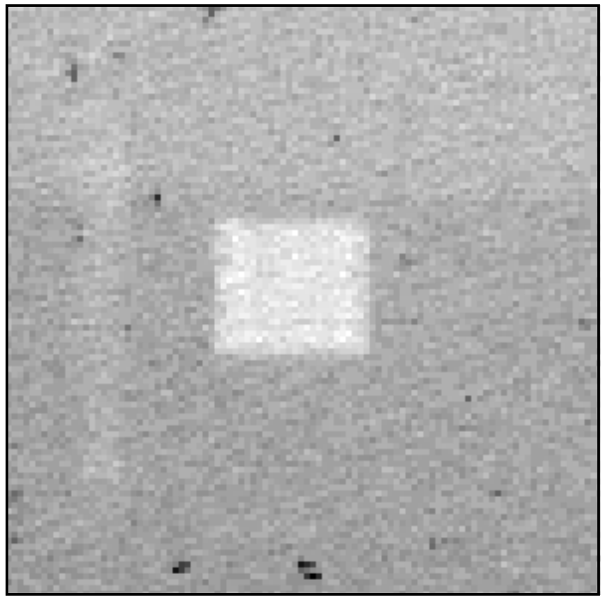
Tinone *et al.*, *Appl. Surf. Sci.* **79**, 89 (1994)



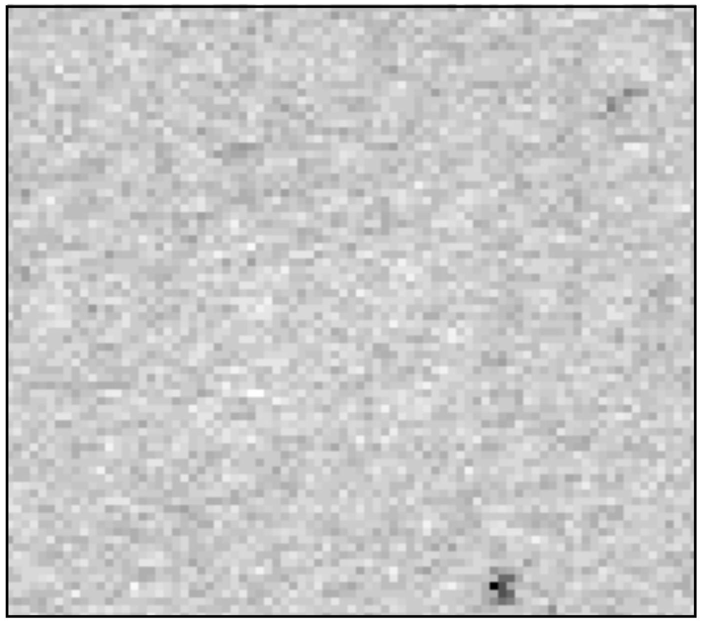
Tinone *et al.*, *J. Electron Spect. Rel. Phen.* **80**, 117 (1996).

PMMA at room, LN2 temperature

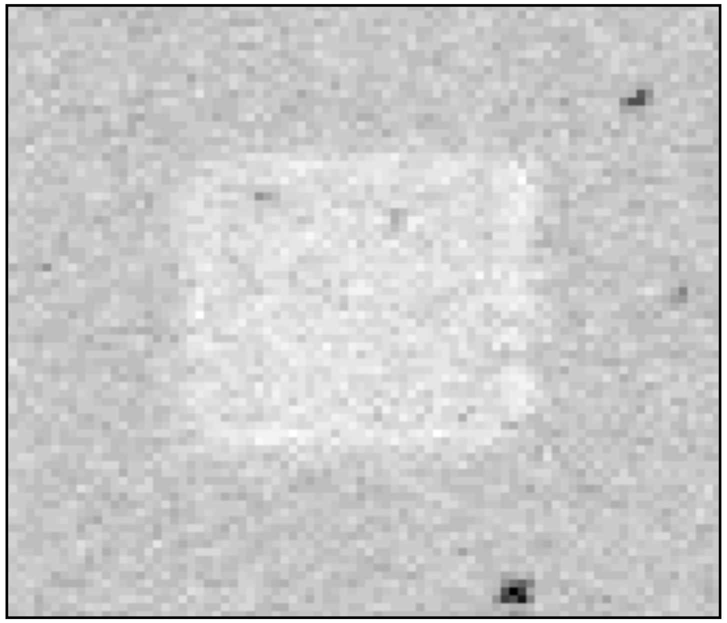
- Beetz and Jacobsen, J. Synchrotron Radiation **10**, 280 (2003)
- Repeated sequence: dose (small step size, long dwell time), spectrum (defocused beam)
- Images: dose region (small square) at end of sequence



Room temperature: mass loss immediately visible



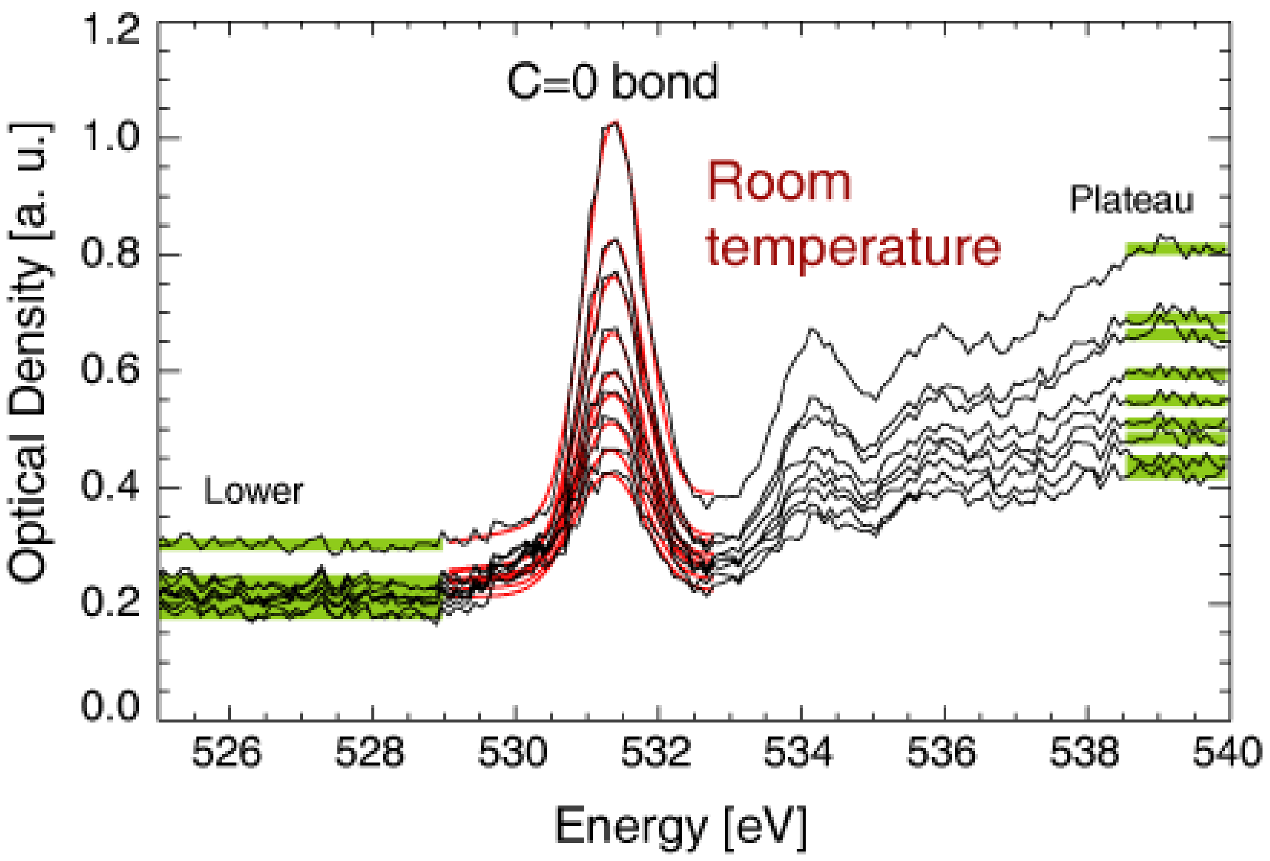
LN2 temperature: no mass loss immediately visible

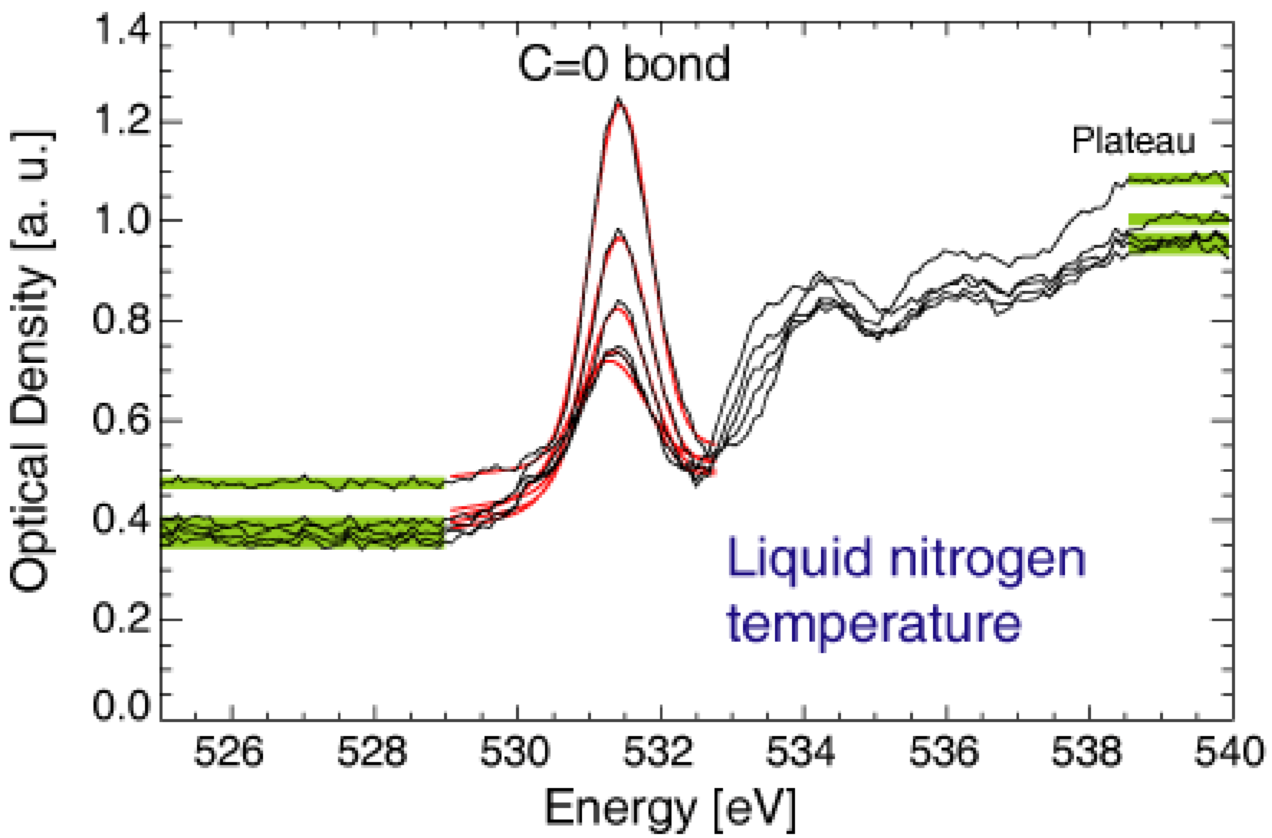


After warm-up: mass loss becomes visible

PMMA at LN2, room temperature: XANES spectra

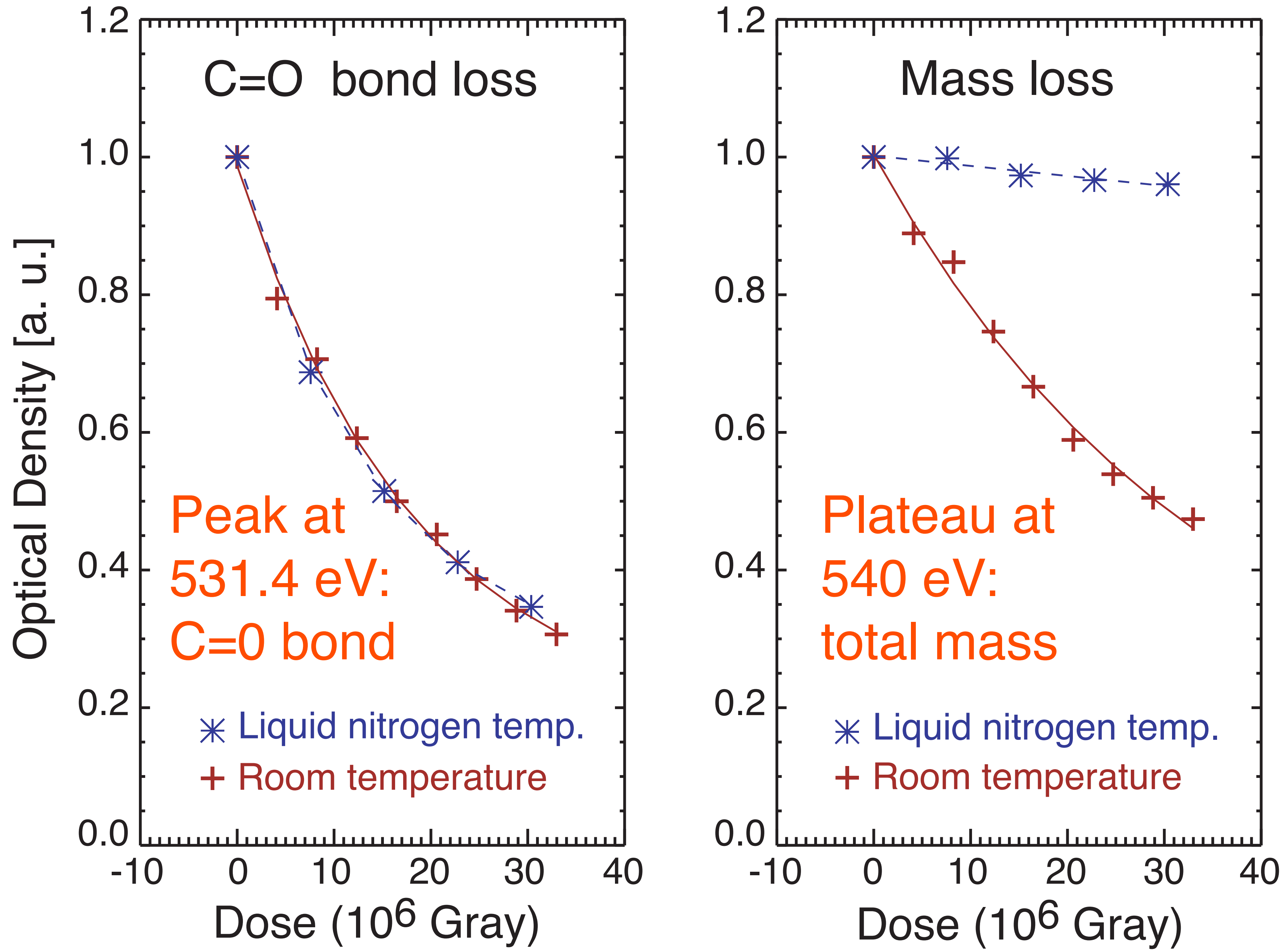
- Peak at 531.4 eV: C=0 bond
- Plateau at 540 eV: total mass (plus some emphasis on oxygen σ* bonds)
- Beetz and Jacobsen, *J. Synchrotron Radiation* **10**, 280 (2003)





Cryo does not work miracles

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

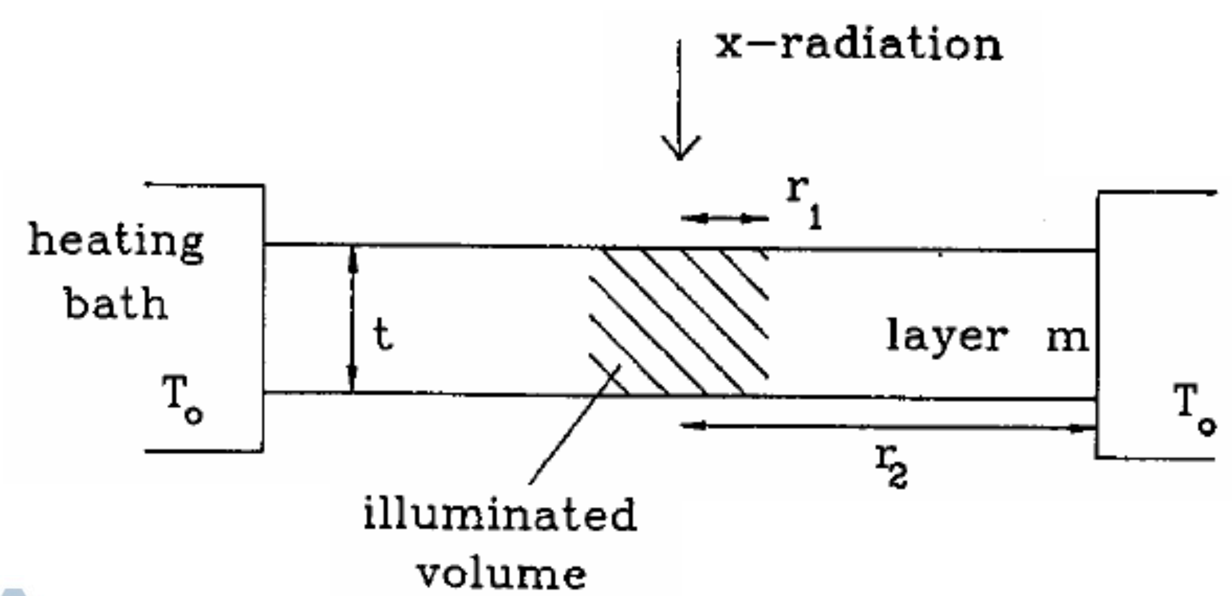
Macromolecular chains in an "encapsulating" matrix
(frozen hydrated)

The Ramen noodle model of radiation damage

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

Effects of 10^5 photons in $(10\ \text{nm})^3$

- With no cooling, the temperature rises due to absorption:
  - H_2O@500 eV ⇒ 2300K
  - H_2O@3 keV ⇒ 2200K
  - Si@10 keV ⇒ 7300K
- In scanning microscopes, localized heating with a thermal reservoir. Photon flux for ΔT=1K in 10 nm wide spot with r_2=100 μm:
  - H_2O@500 eV: 4×10^{10} photons/sec
  - Si@10 keV: 2×10^{12} photons/sec



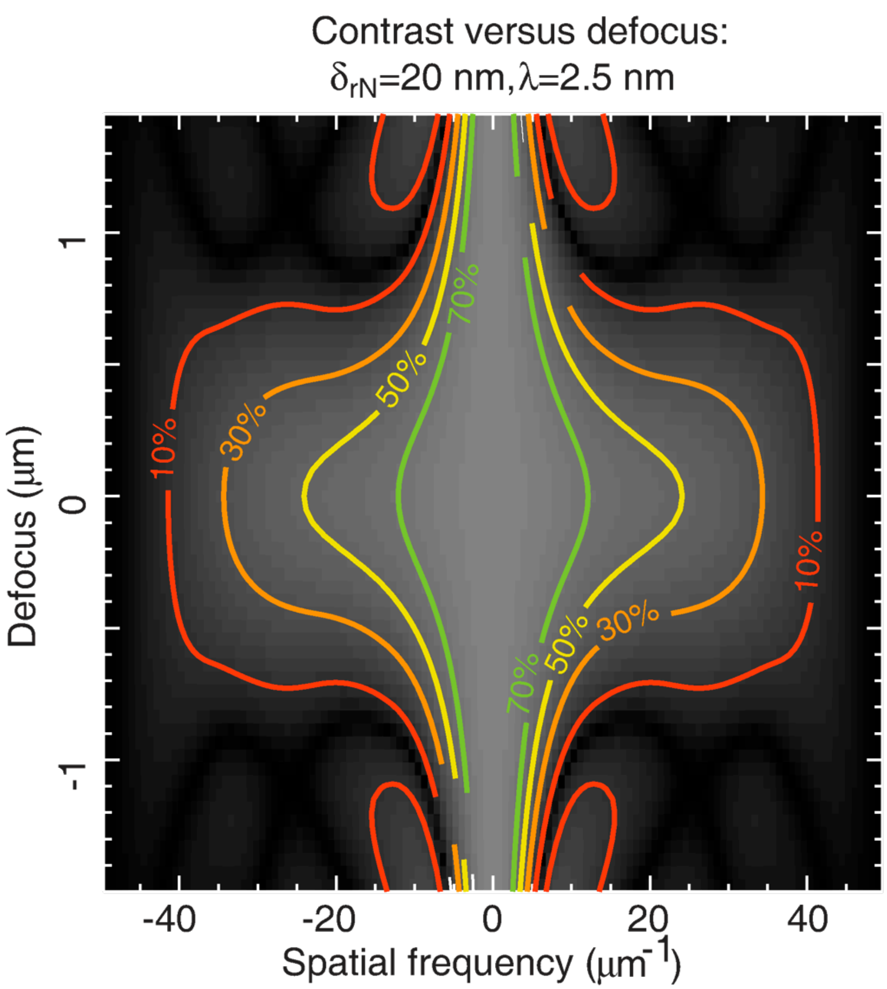
$$\Delta T = \frac{N}{t}\frac{h\nu\cdot\mu}{4\pi k}\left(1+2\ln\frac{r_2}{r_1}\right)$$

Greinke and Gölz, XRM 1991

Depth of focus in terms of zone plate/MLL Δ_{rN}

Transverse: $$\Delta_t \Rightarrow \frac{\lambda}{4\theta} = \frac{\Delta_{rN}}{2}$$

Longitudinal: $$\Delta_\ell \Rightarrow \frac{\lambda}{\theta^2} = 4\Delta_{rN}\frac{\Delta_{rN}}{\lambda}$$



Incoherent brightfield imaging;
50% central stop.
Partial coherence: better?