

3D imaging of whole cells:  
zone plate tomography,  
and diffraction microscopy

Enju Lima

ESRF

# Scientific Need

## Complementary microscopies:

- **Light microscopy:** up to 100 nm resolution in 3D imaging of *pre-selected* molecules (fluorescence). Dynamic single molecule studies.
- **Cryo EM:** up to ~8 nm 3D resolution with some labeling, but thickness limit is ~0.5 micron.
- **X-ray microscopy:** 30-50 nm today, <10 nm future goal, with some labeling. Able to image whole cells (10 microns thick or more).

## X-ray nanotomography:

- **Full-field imaging using zone plate optics:** becoming easy to do on bending magnets, with no special specimen restrictions! However, zone plates limit resolution (depth of focus) and increase radiation dose 20-100x.
- **Tomography in a scanning microscope:** no zone plate efficiency loss (upstream of specimen), so 10-20x dose reduction. Requires ultrabright beam, fast scanning.
- **Diffraction microscopy:** requires very coherent beam, “empty” specimen field or nearby pinhole (ptychography), but no optics limit to resolution or dose inefficiencies.

# User Demand

- Difficult to judge, in that there are many developments but few user facilities. But...
- **CryoEM tomography**: dozens of labs worldwide, at \$2-5M each.
- **National Center for X-ray Tomography at ALS** (Larabell, LeGros): soon to begin operation (zone plate tomography at ~50 nm resolution). >\$5M funding.
- **TXM at BESSY II** (Gerd Schneider): improved resolution (grating monochromator, capillary condenser). >\$3M investment; soon to begin operation.
- **COSMIC at ALS**: plan for dedicated beamline for cryo diffraction microscopy.

# Cell imaging at NSLS-II

- **“First 5” nanoprobe beamline**: if ~1 nm resolution really is reached, its application in biology will still be greatly complicated due to radiation damage limits. Will it have cryo transfer capabilities as required for bio studies?
- **Bio nanoprobe**: 10-100 nm resolution at ~10 keV, fluorescence detection for trace element analysis, phase contrast detector and fast scanning for thick cell tomography. Requires **3-10 keV undulator**, dedicated endstation.
- **Soft x-ray scanning**: besides spectromicroscopy (addressed separately), can also be used for tomography at 5-10x lower dose than full-field imaging. Speed required for tomography means a **0.2-2 keV undulator** is required.
- **Diffraction microscopy**: requires coherent beam (**undulator**) with ~10 micron footprint rather than ~200 micron footprint as for scanning microscopes. Other than that, specimen handling (cryo transfer, rotation) is the same.
- **Full-field tomography**: work at 520 eV (water window) or 3-6 keV (phase contrast). Sophisticated commercial systems (including cryo transfer robots) becoming available (Xradia; Zeiss?; Gatan?). Works very well with **bending magnet** sources; can be done at NSLS today!

# Current NSLS Programs

- No program in 10-100 nm tomography.  
Demonstration in 1998-2000: cryo scanning tomography of a whole cell at 100 nm (Stony Brook).
- Local experimenters with experience in phase contrast tomography (at APS); diffraction microscopy (at ALS); cryo transfer system design (at ALS, Xradia); zone plate fabrication (but no facilities at BNL's CFN!).

# Upgraded/New NSLS Programs

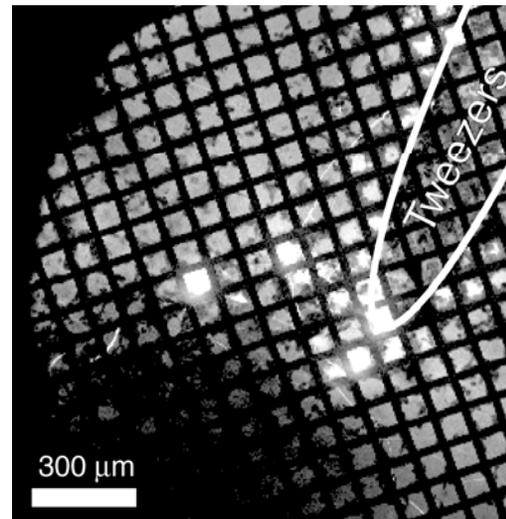
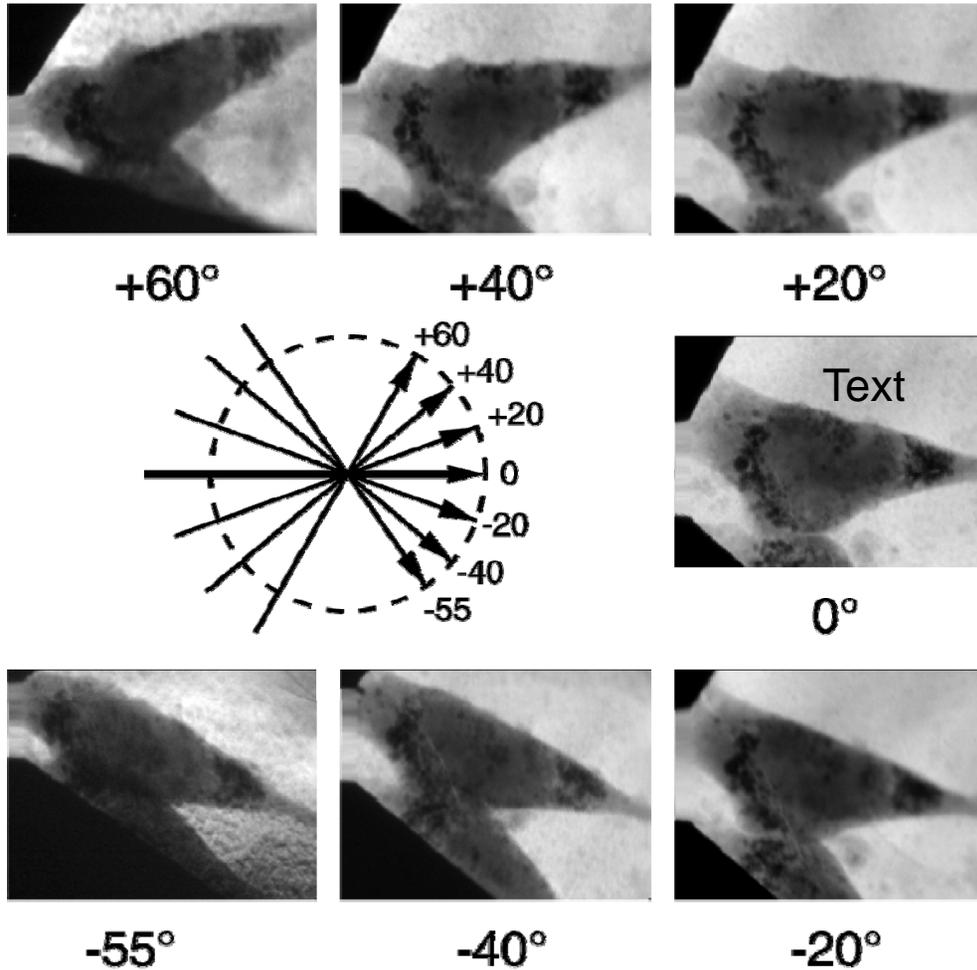
- **Upgraded program:** soft x-ray cryo scanning microscope. Testbed for fast scanning as required for NSLS II, cryo specimen preparation and transfer, and phase contrast tomography. Would also serve spectromicroscopy, environmental science, soft matter studies. Directly transferable to NSLS II.
- **New program:** tomography using full-field microscope with zone plates. Testbed for cryo specimen preparation and transfer, and phase contrast tomography. Directly transferable to NSLS II.
- **New complementary program:** cryo fluorescence microscopy (3-5x lower bleaching so higher resolution, experience in cryo specimen preparation and handling). See Schwartz *et al.*, *J. Micros.* **227**, 98 (2007); Sartori *et al.*, *J. Struct. Bio.* **160**, 135 (2007).

# Funding

- Program could serve biology, soft condensed matter, environmental science. Joint funding?
- NIH NCRR: Bio imaging resource? There have been attempts to pursue this

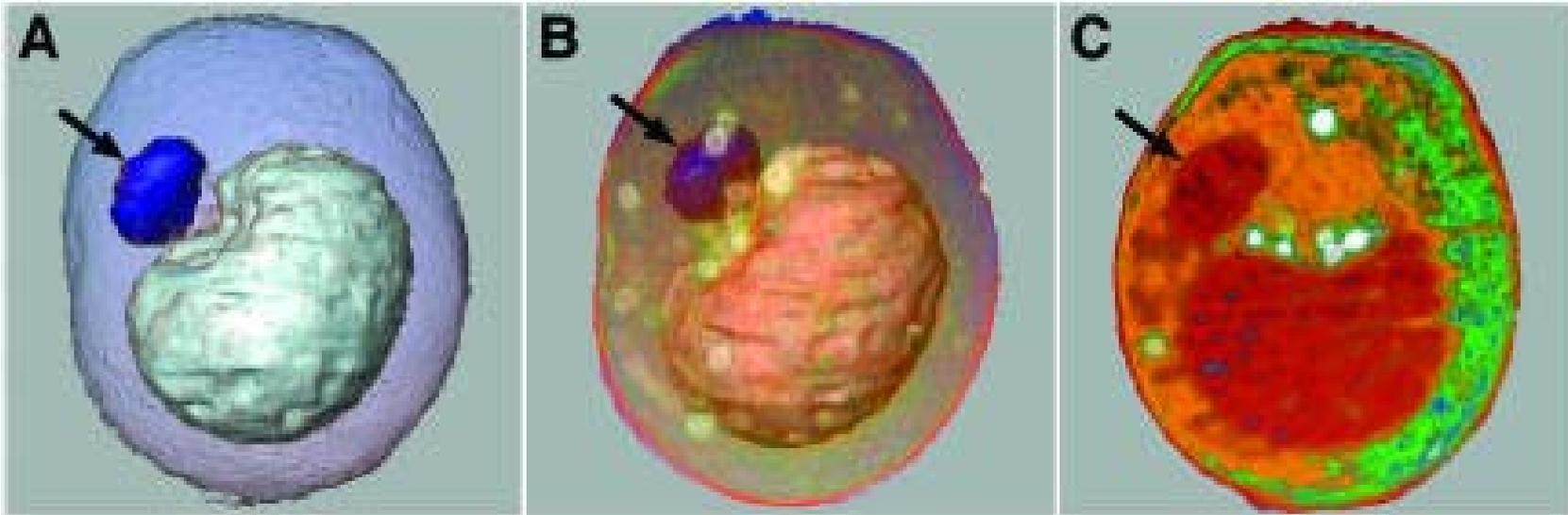
# Tomography: projections in a microscope

Projections of a frozen hydrated 3T3 fibroblast. Y. Wang *et al.*, *J. Microscopy* **197**, 80 (2000)



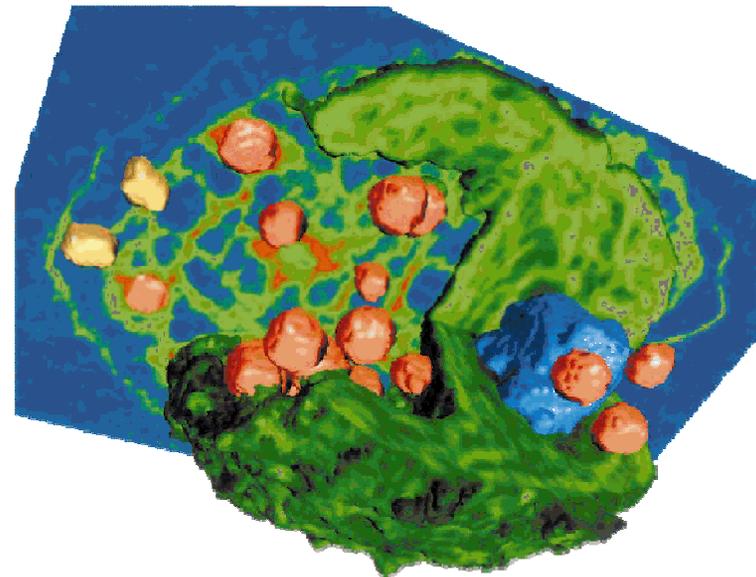
Maser *et al.*, *J. Micros.* **197**, 68 (2000)

# Imaging with lenses: very successful!



Frozen hydrated yeast *Saccharomyces cerevisiae*. C. Larabell and M. Le Gros, *Mol. Biol. Cell* **15**, 957 (2004). ALS/UC San Francisco.

Frozen hydrated alga *Chlamydomonas reinhardtii*: D. Weiß, G. Schneider, *et al.*, *Ultramicroscopy* **84**, 185 (2000). Göttingen/BESSY I. Newer results at BESSY II.

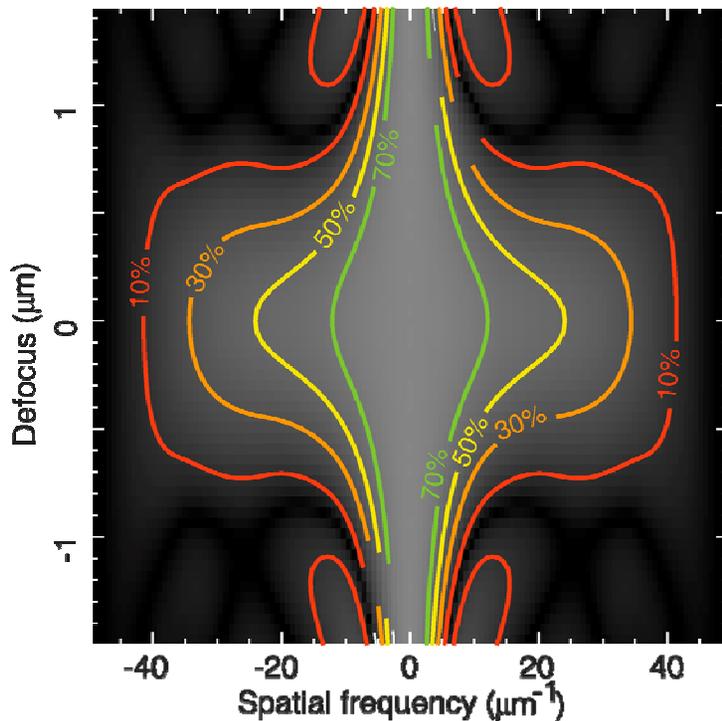


# 3D imaging with lenses

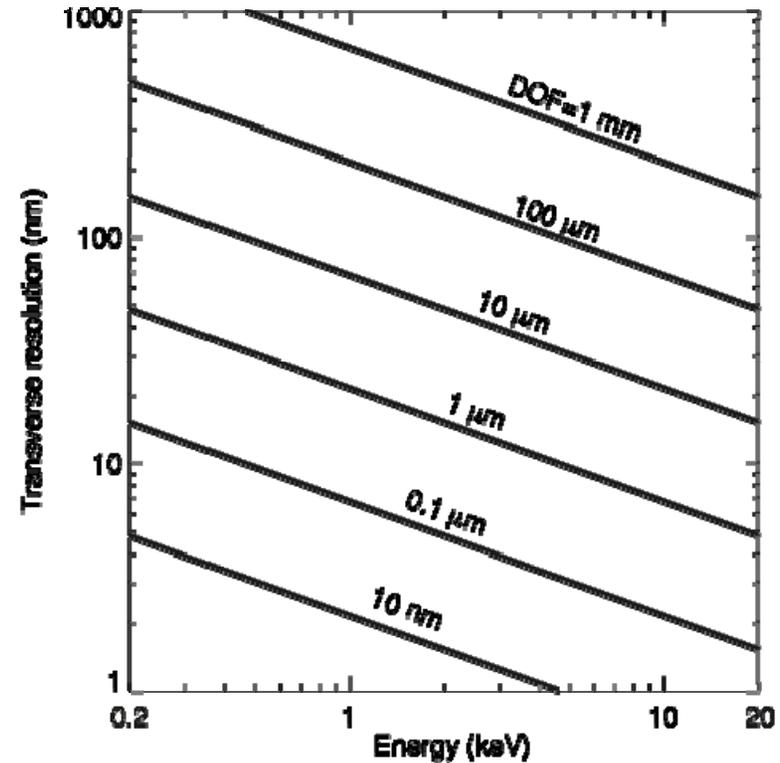
Transverse:  $\Delta_t \Rightarrow \frac{\lambda}{4\theta} = \frac{\Delta_r N}{2}$

Longitudinal:  $\Delta_l \Rightarrow \frac{\lambda}{\theta^2} = 4\Delta_r N \frac{\Delta_r N}{\lambda}$

Contrast versus defocus:  
 $\delta_r N = 20 \text{ nm}, \lambda = 2.5 \text{ nm}$



20 nm resolution at 520 eV: depth of field  $\sim 1 \mu\text{m}$

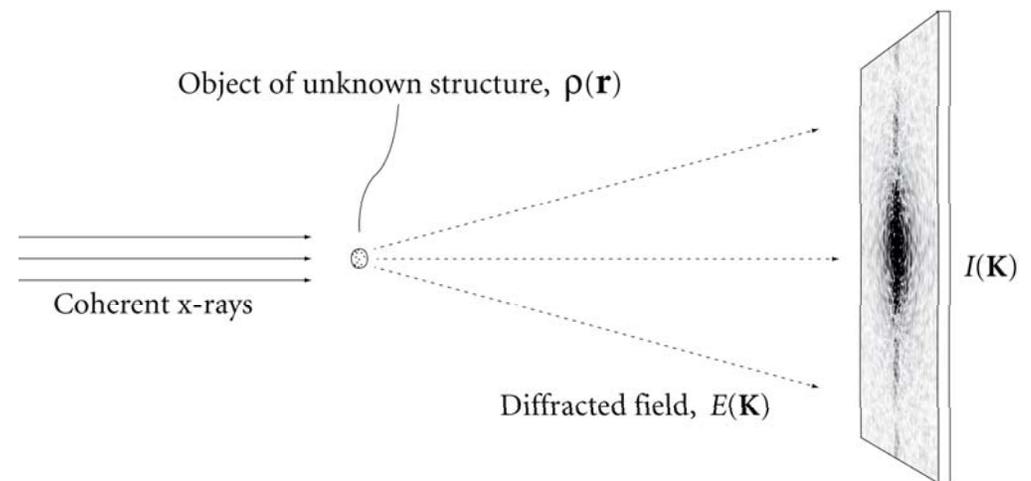
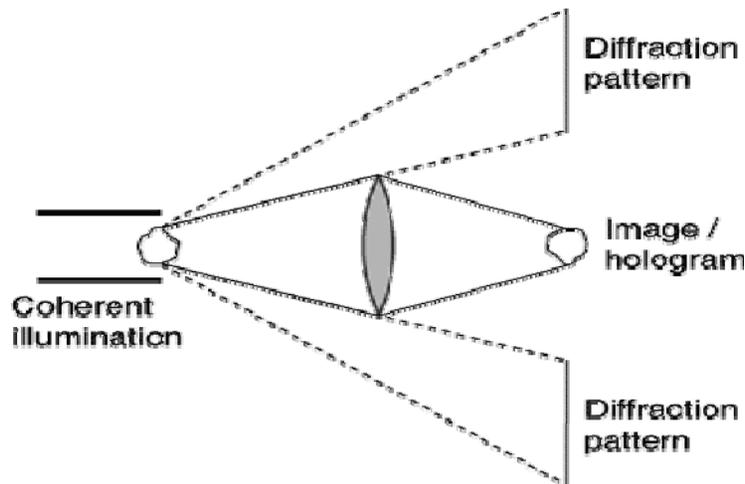


Through-focus deconvolution with lenses:

- Confocal: fully incoherent (fluorescence)
- EM: phase only, coherent
- TXM: partially coherent, equal absorption and phase contrast, need for experimental CTF

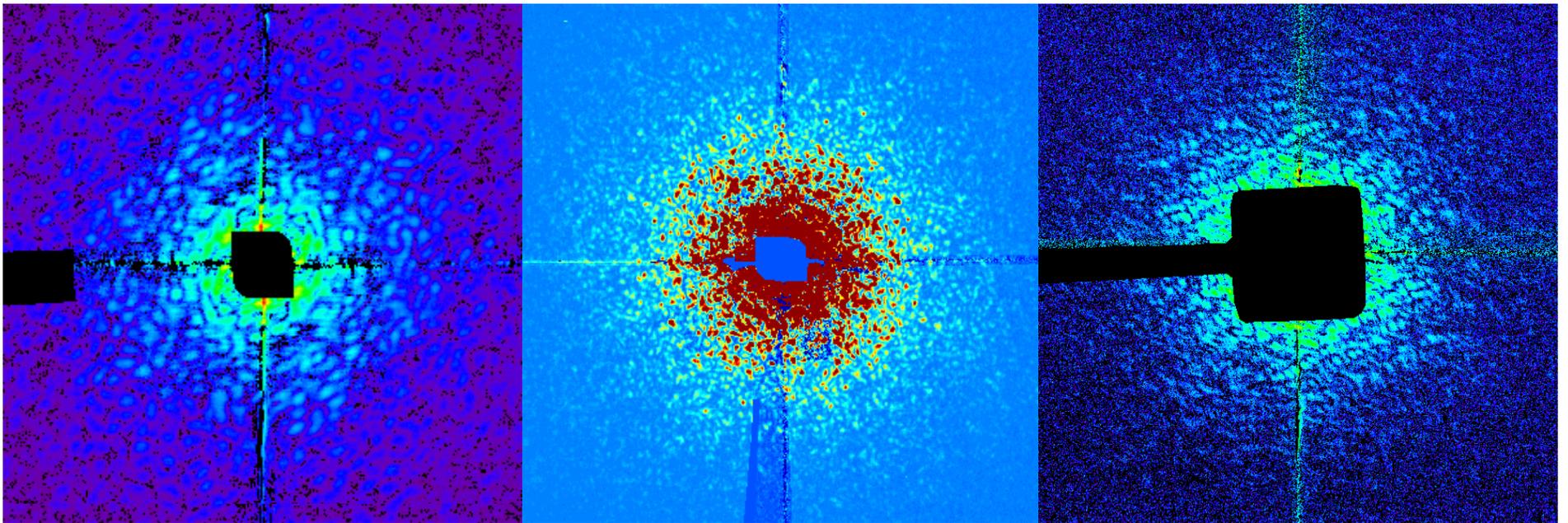
# Radiation damage sets the ultimate resolution limit

- For many specimens, radiation damage sets the ultimate limit on achievable resolution.
- Lenses phase the signal, but lose the signal. Example: 20 nm zone plate with 10% efficiency, 50% window transmission, 20% modulation transfer function (MTF) for 15 nm half-period:
- **net transfer of 1% for high spatial frequencies**
- Can we avoid this  $\sim 100x$  signal loss, and also go beyond numerical aperture limit of available optics?

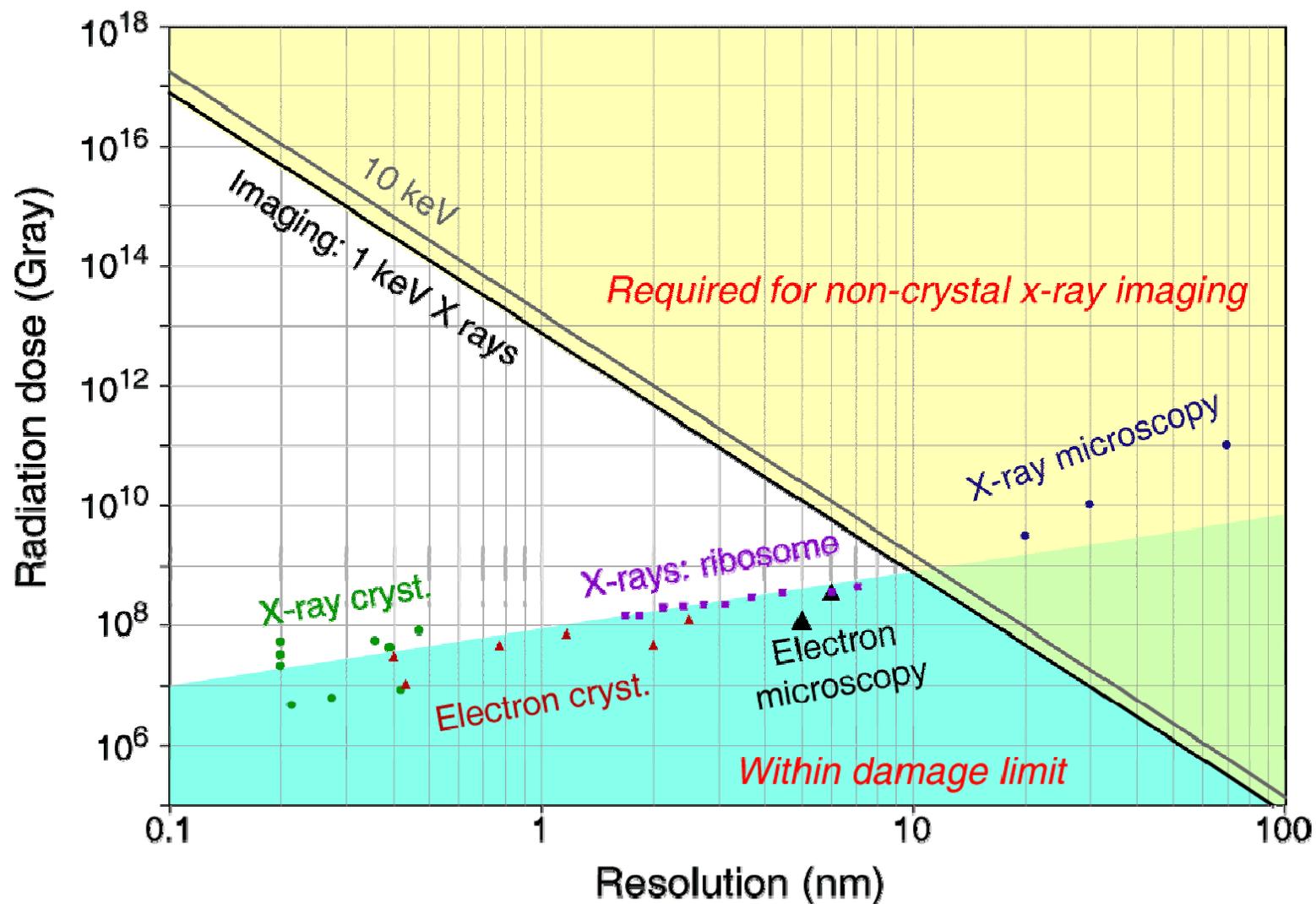


# Imaging without lenses:new

Speckles from unstained frozen-hydrated  
DR bacteria at 8 keV, ESRF



# What's the limit for cells?



Howells et al., *JESRP* (submitted).

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