Microscopy for biomedical, biofuels, and environmental science research

Bjorg Larson
Chris Jacobsen
Department of Physics & Astronomy
Stony Brook University, USA
http://xray1.physics.sunysb.edu

environmental science?

Biomedical research on cells and tissues

- Need to work with micrometer-thick, wet, organic specime
- 3D imaging/tomography
- Cryo capabilties to minimize radiation damage
- Fluorescence for trace element mapping
- Spectroscopy for chemical speciation
- Analysis software to deal with complexity

Biofuels (*e.g.*, ethanol from lignocellulosic materials
bioreactor materials for hydrogen production): **sam
needs!**

Organic environmental science (*e.g.*, organic coatin
on soil particles, bacteria that change metal and

XM

coherent illumination; flux not
ightness. Fast at present
ources!

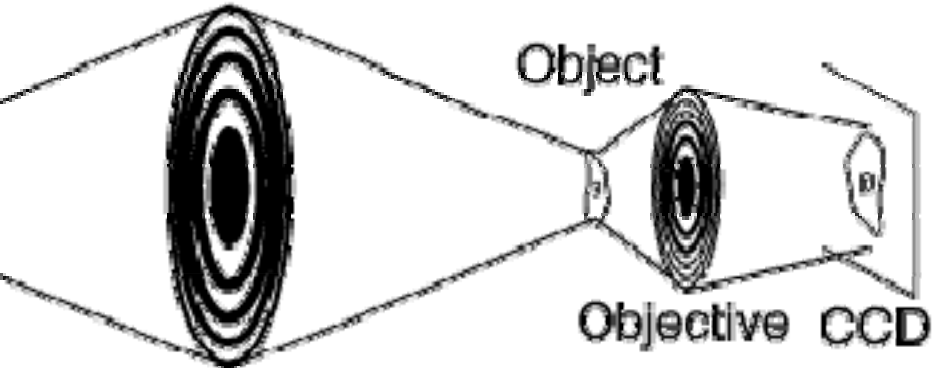
oderate spectral resolution if
ondenser zone plate is used:
/(ΔE) ≈300-1000.

ondenser zone plates are large
-5 mm dia.) and are usually of
maller NA than objective;
ompromise on resolution

STXM

- Coherent illumination; brightnes
NSLS II can make for fast imagi
times! Requires new approache
scanning.
- 10x lower dose because zone p
before specimen not *after*
- Better suited to conventional gra
monochromator: E/(ΔE)≈3000-5
- Detector plays role of condense
use optimum NA

ansmission x-ray microscope



STXM: scanning transmission x-ra
microscope

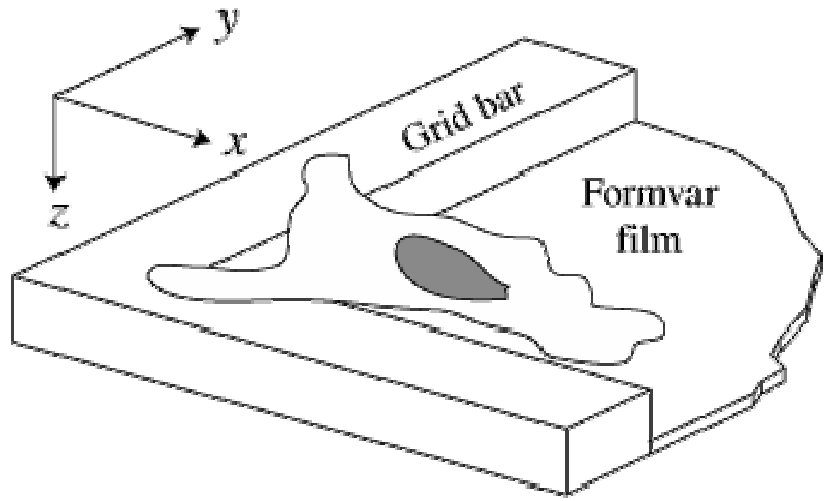
Tomography in a STXM

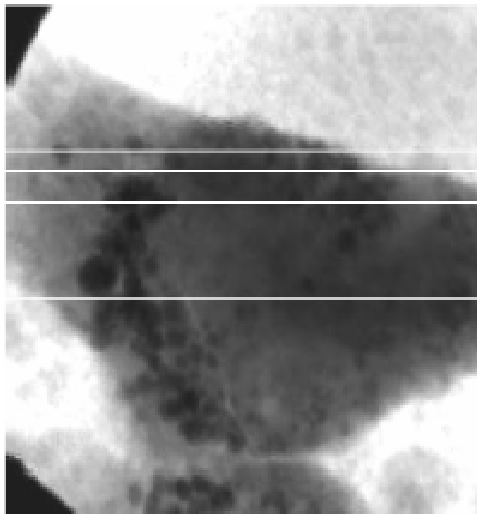
Ms have been used for
graphy! First
onstration of soft x-ray
graphy in any
oscope: Haddad *et al*.,
nce **266**, 1213 (1994);
y Brook STXM at X1A

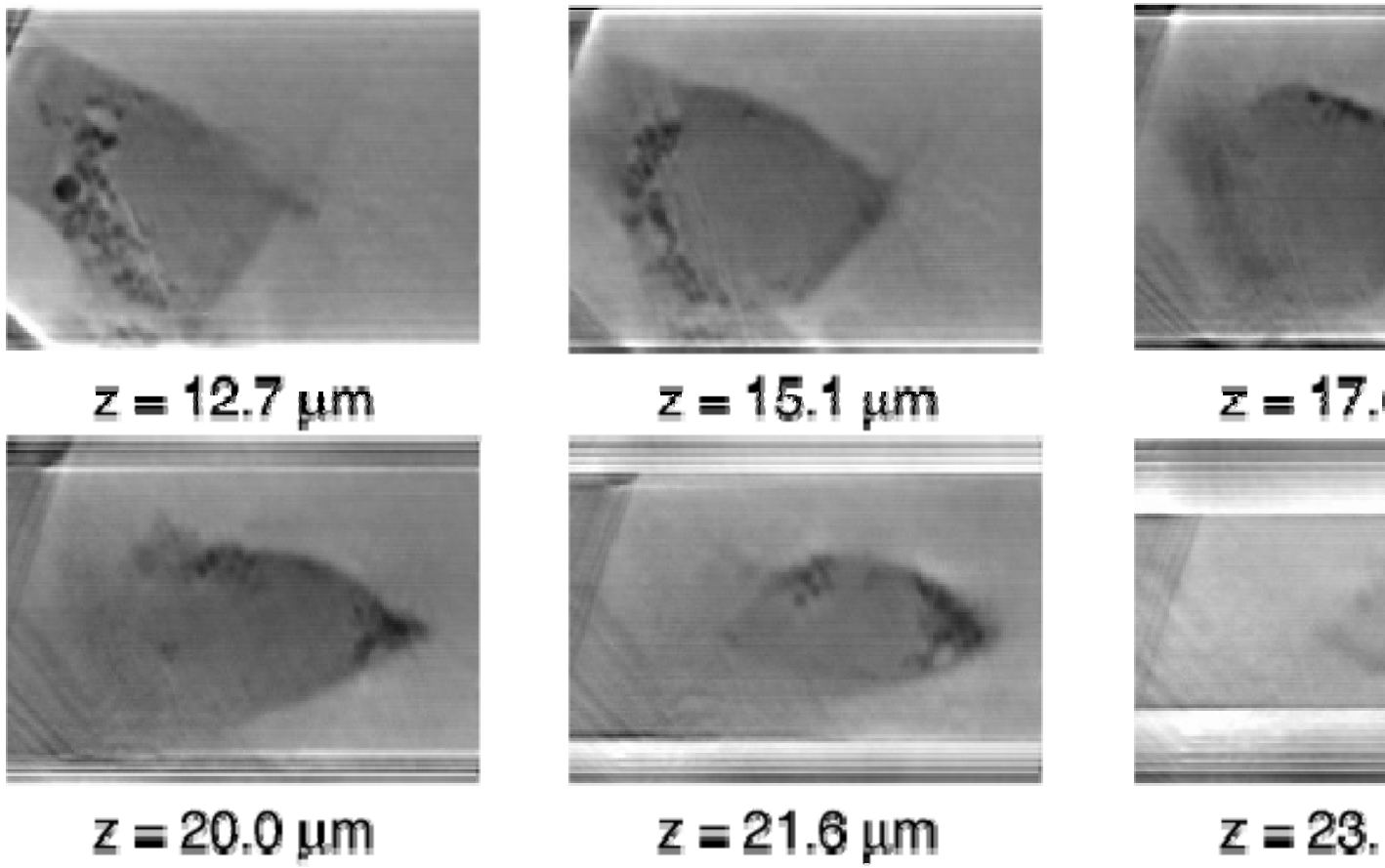
example: first
graphy of a frozen
ated mammalian
ryote. Y. Wang *et al*., *J.*
oscopy **197**, 80 (2000);
STXM at X1A. But it was
and used TEM-type
e...

ography plus
tromicroscopy:
nsson, Hitchcock et al.,
aster/ALS

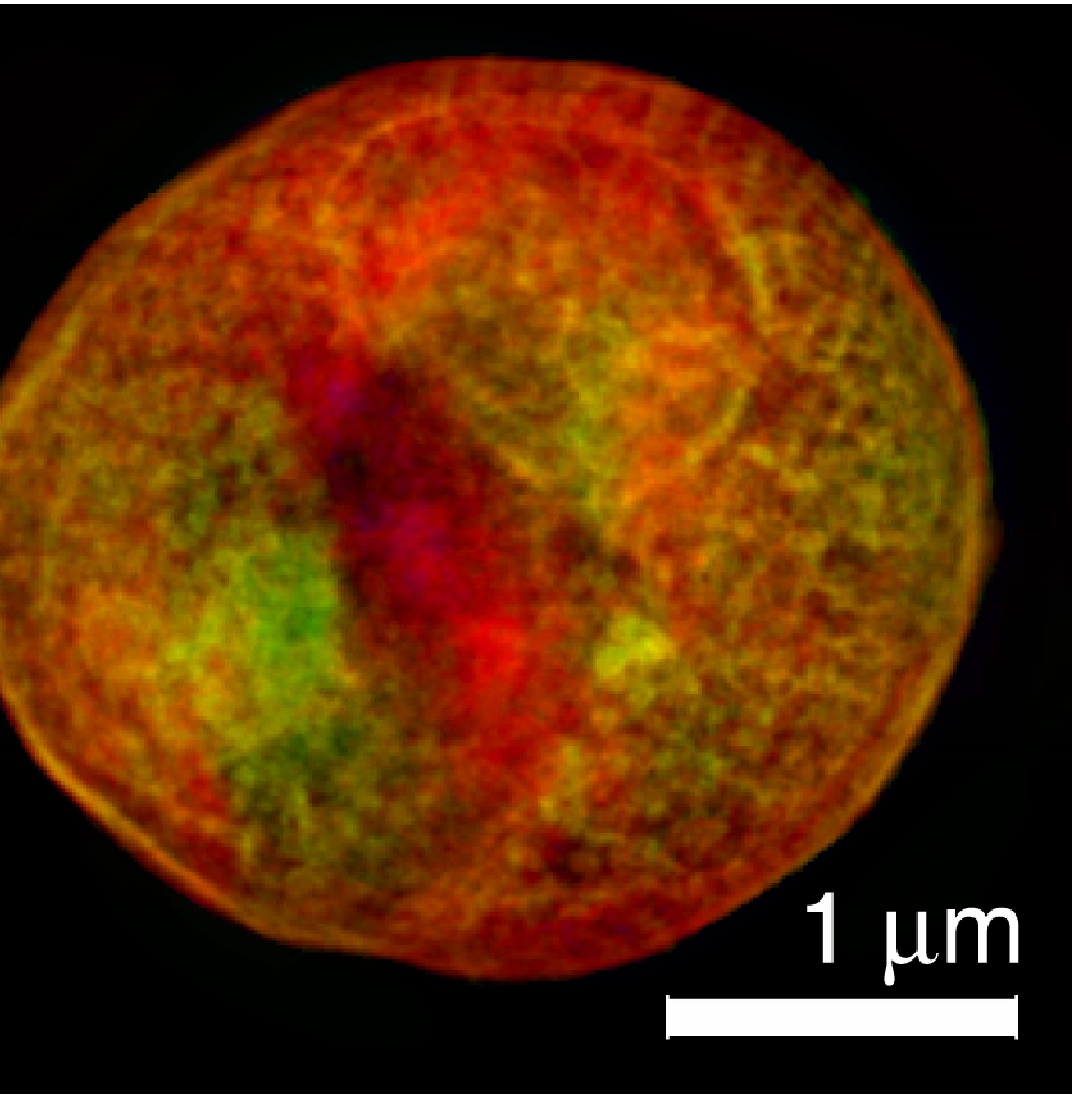
the right cryo/scanning
gn, tomography with



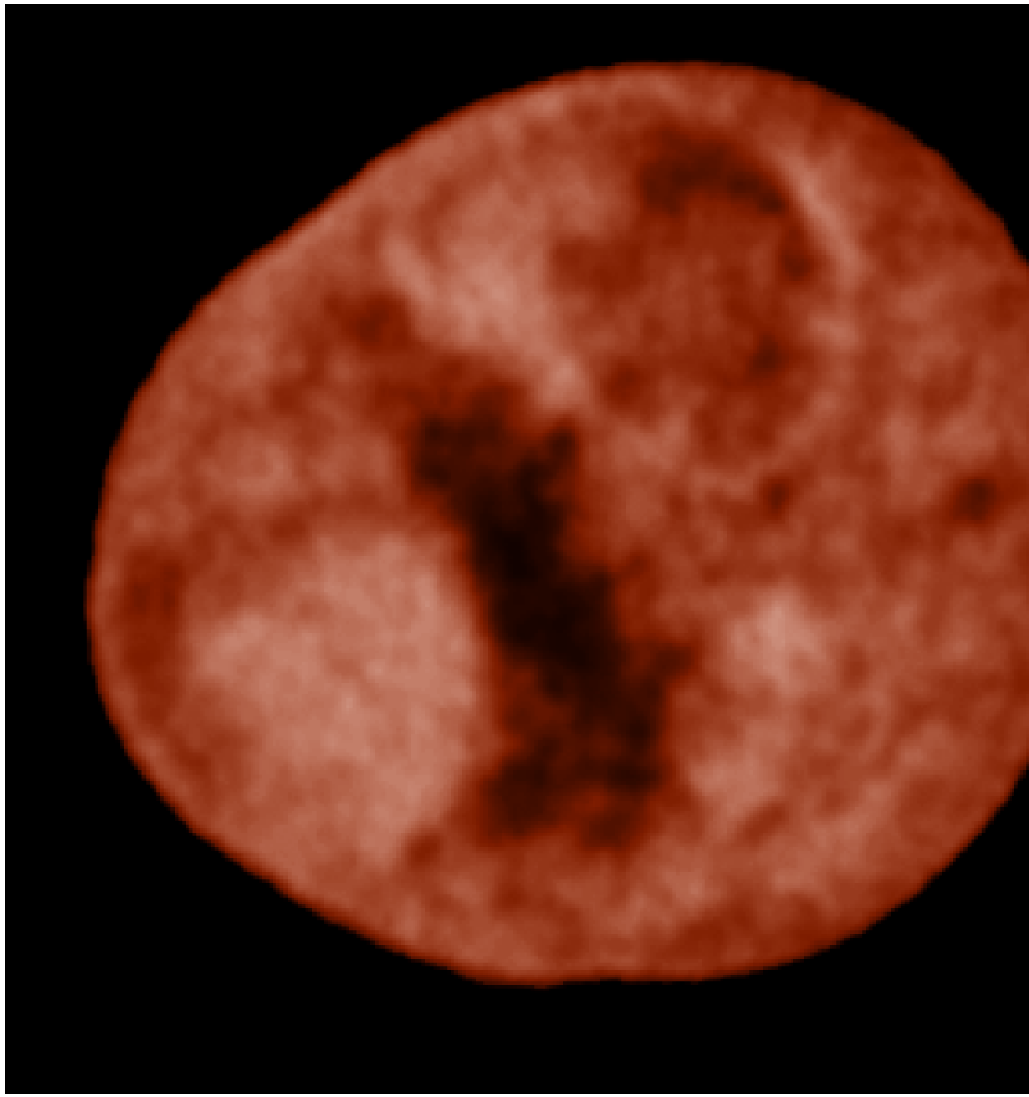




Freeze dried, unstained yeast

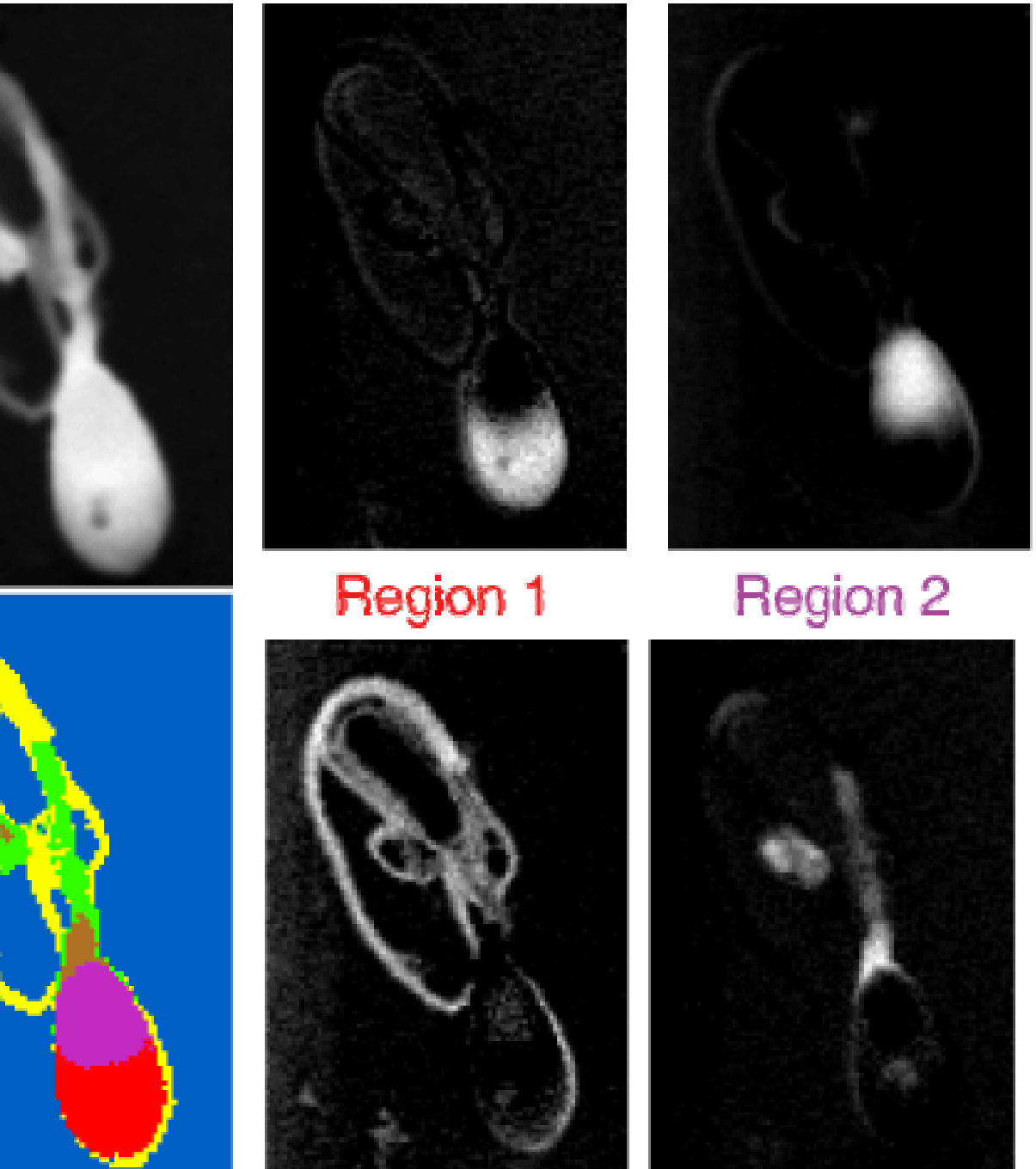


iffraction reconstruction (data
ken at 750 eV; absorption as
rightness, phase as hue).

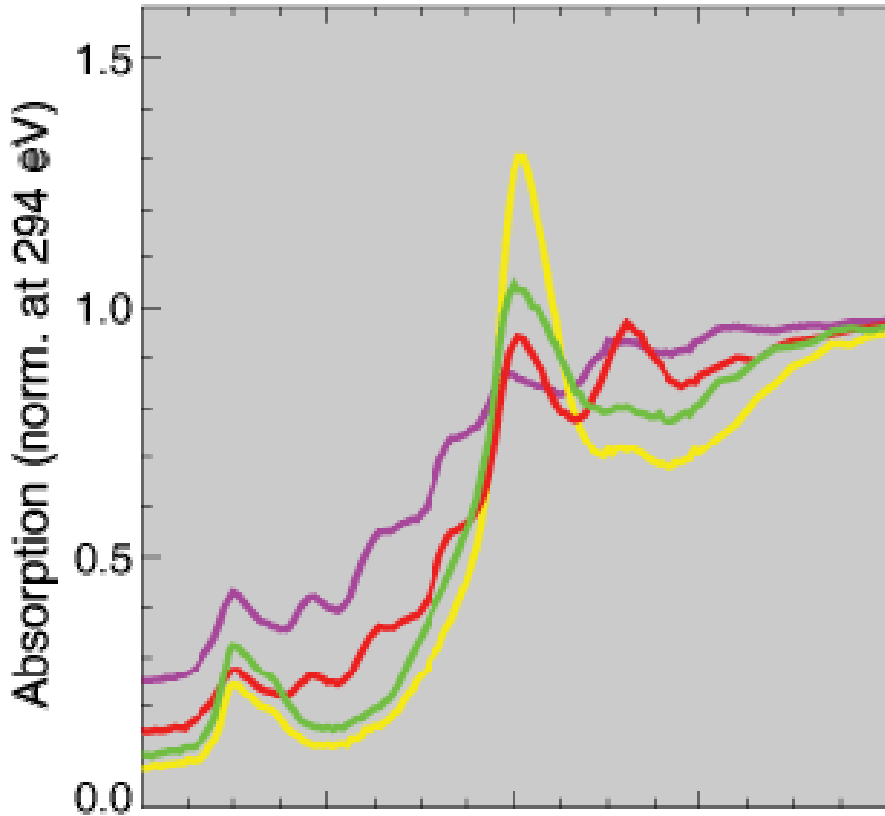


Stony Brook/NSLS STXM image
with 45 nm Rayleigh resolution
zone plate at 520 eV (absorption
as brightness)

ge series near carbon absorption edge (XANES/NEXAFS)
hemical organization of sperm revealed directly from data:
yme-rich region, DNA, mitochondria and flagellar motor, lipid



H. Fleckenstein, M. Lerotic,
Sheynkin *et al*., Stony Broo
Cluster analysis: Lerotic *et*
Ultramic. **100**, 35 (2004).

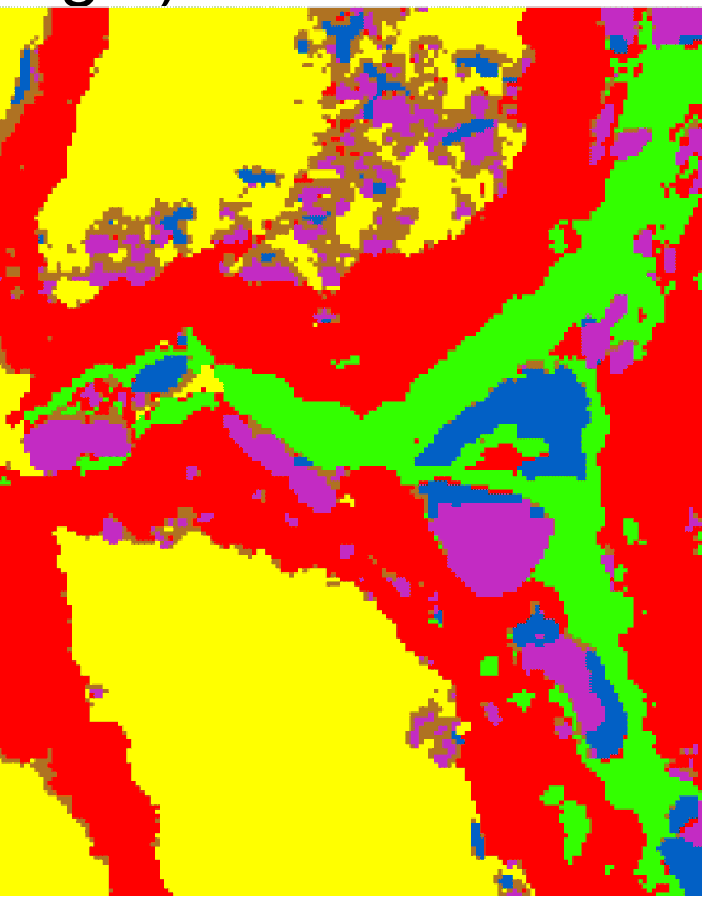


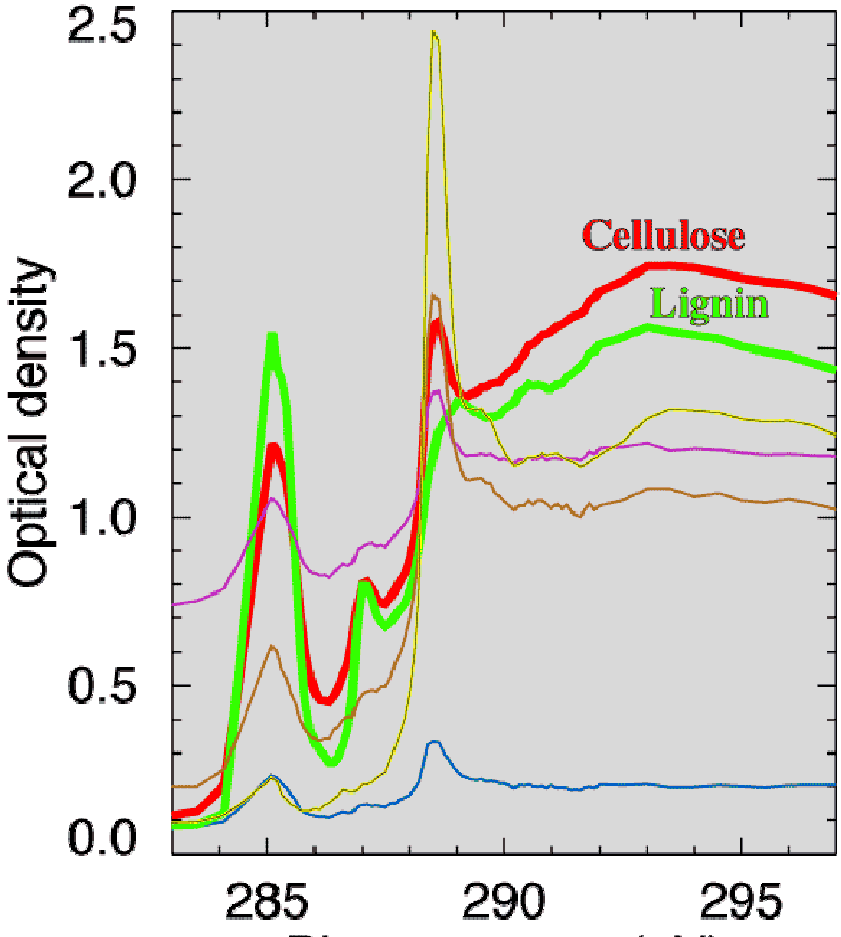
nol from lignocellulose materials is promising: large fraction of total
ass, easier cultivation.

here are great challenges in economically separating cellulose from

x-ray spectromicroscopy can map cellulose and lignin so that one c
ffects of various enzymes.

proposal with David Wilson (Cornell microbiology), George Cody
negie)





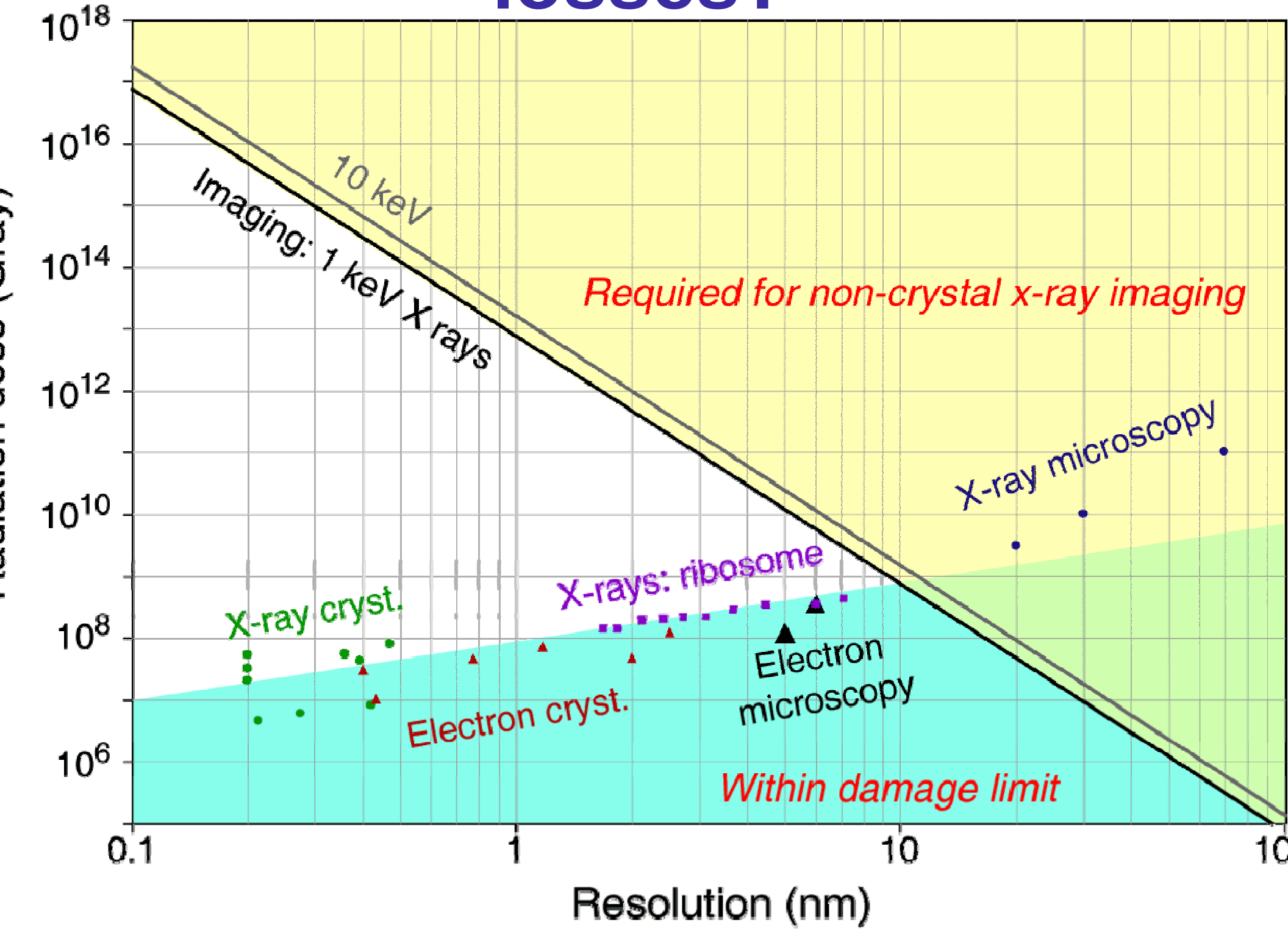
Lignin and cellulo
in 400 million yea
old chert: Boyce
al., *Proc. Nat. Ac*
Sci. **101**, 17555
(2004), with
subsequent patte
recognition analy
by Lerotic *et al.*,
Ultramicroscopy
35 (2004).

Coherent microscopy at NSLS II

NSLS II will provide new opportunities for scanning microscopy: fast enough for practical tomography with reduced dose, spectroscopic sensitivity to biochemical organization

Diffraction microscopy provides a path to reduced dose imaging beyond lens resolution limits and without depth of focus limitations.

losses?



Howells et al., JESRP (submitted)

specimen

hapman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rose
e, Spence, Weierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. A*
3, 1179 (2006)

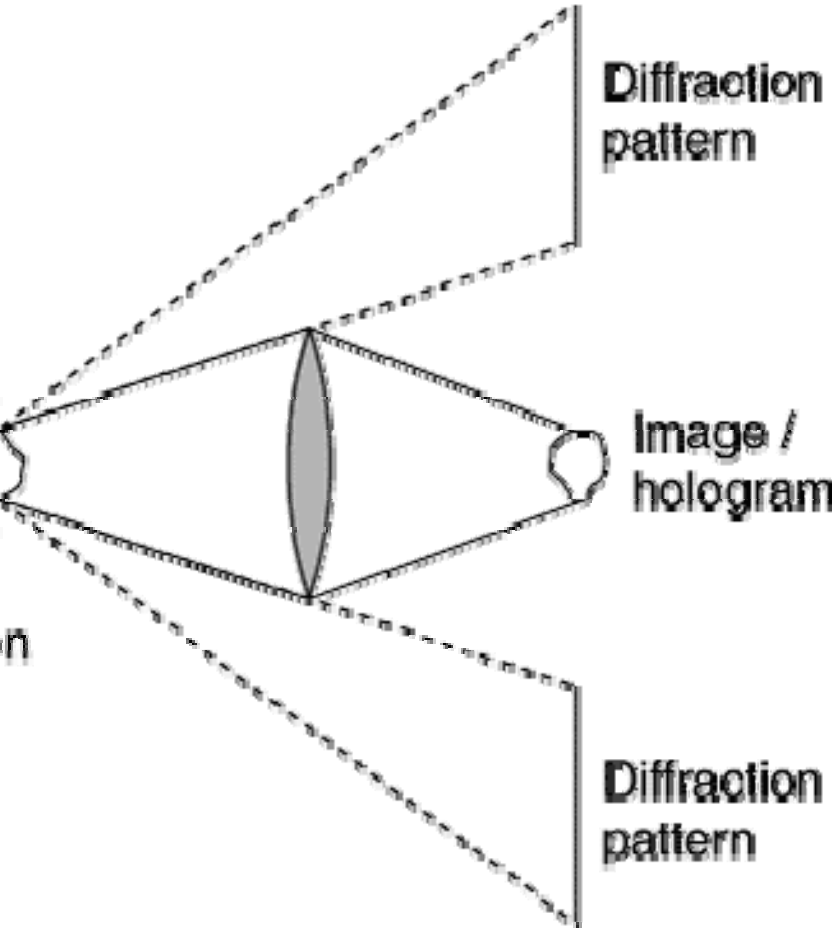
esolution ~10x10x50 nm on 50 nm gold spheres in hollowed-out
FM tip

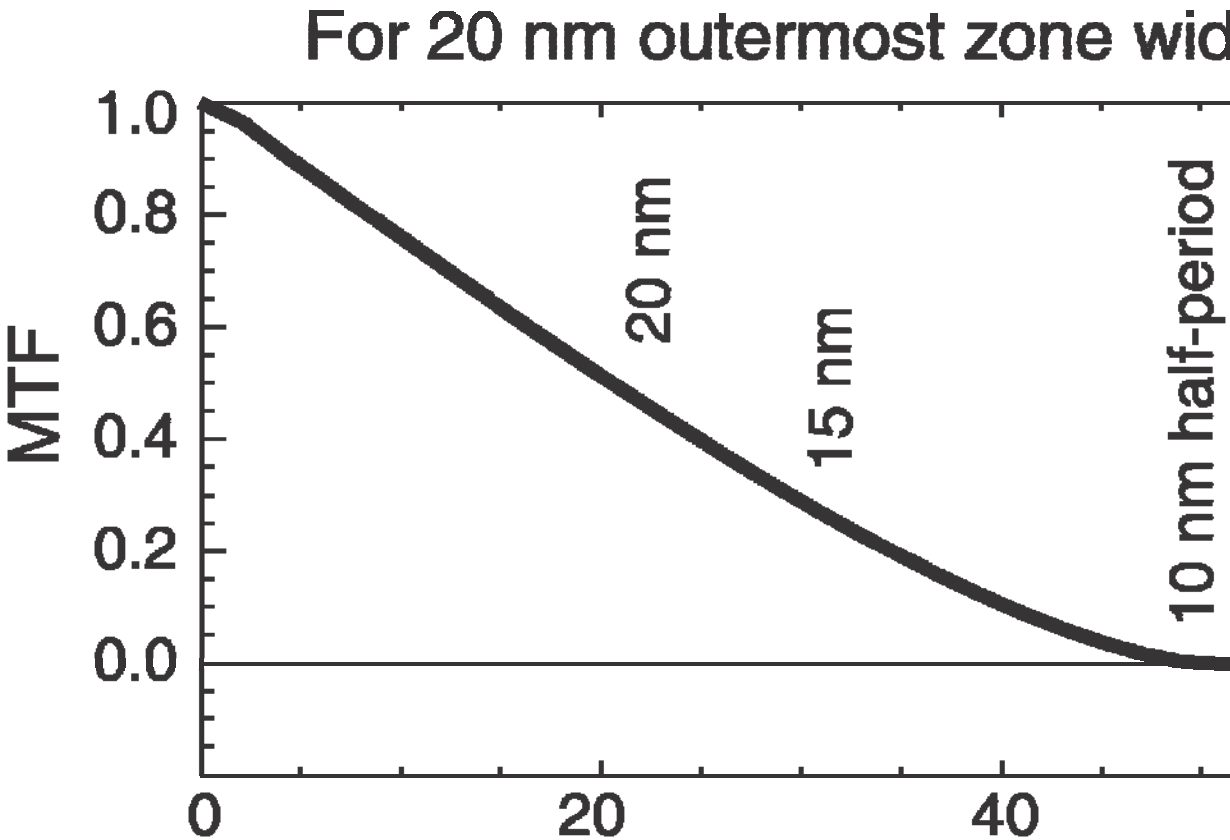
QuickTime™ and a
PNG decompressor
are needed to see this picture.

enses phase the signal, but lose the signal. Example: 20 nm zon
ate with 10% efficiency, 50% window transmission, 20% modula
ansfer function (MTF) for 15 nm half-period:

et transfer of 1% for high spatial frequencies

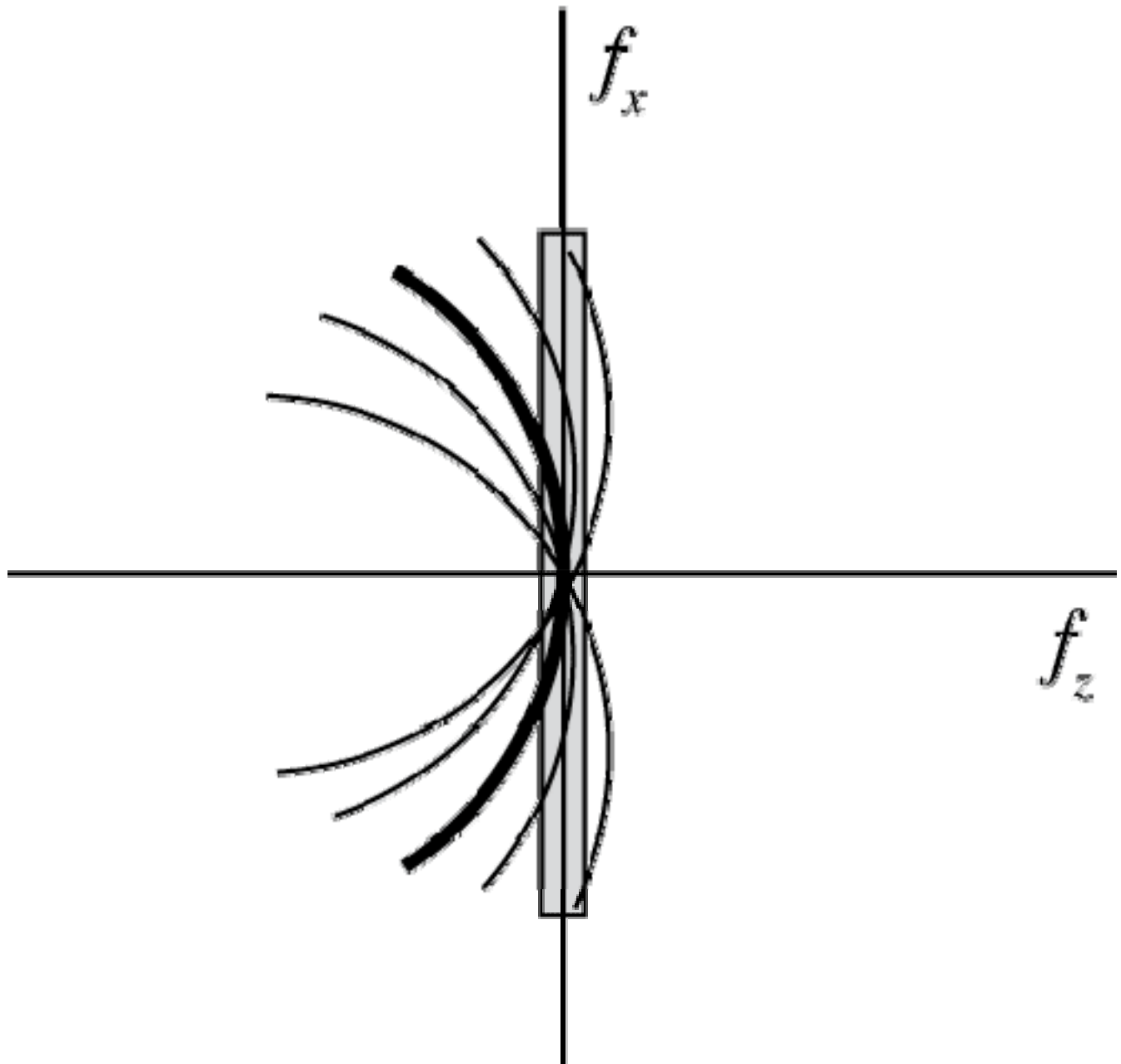
an we avoid this ~100x signal loss, and also go beyond numeric
perture limit of available optics?

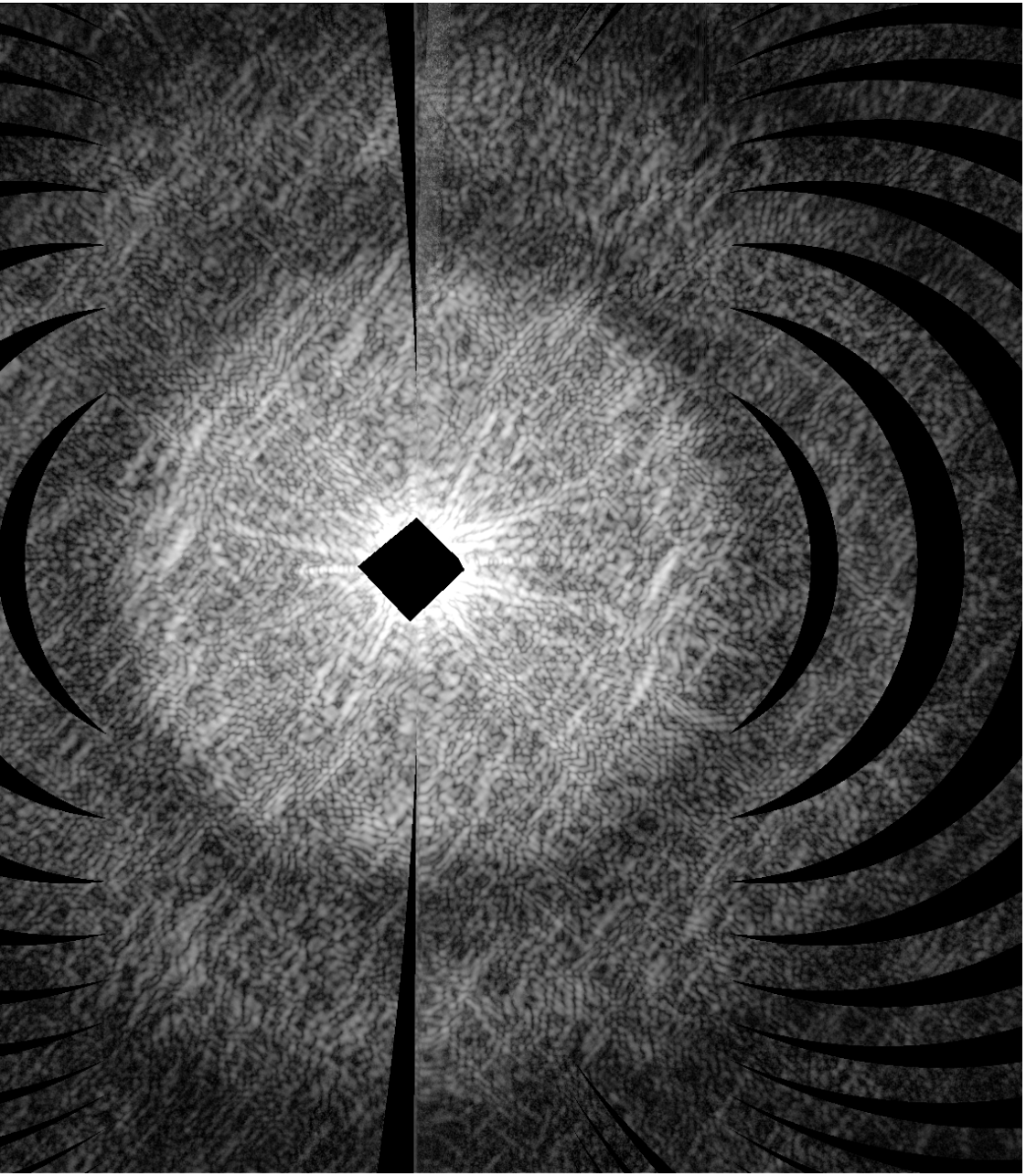


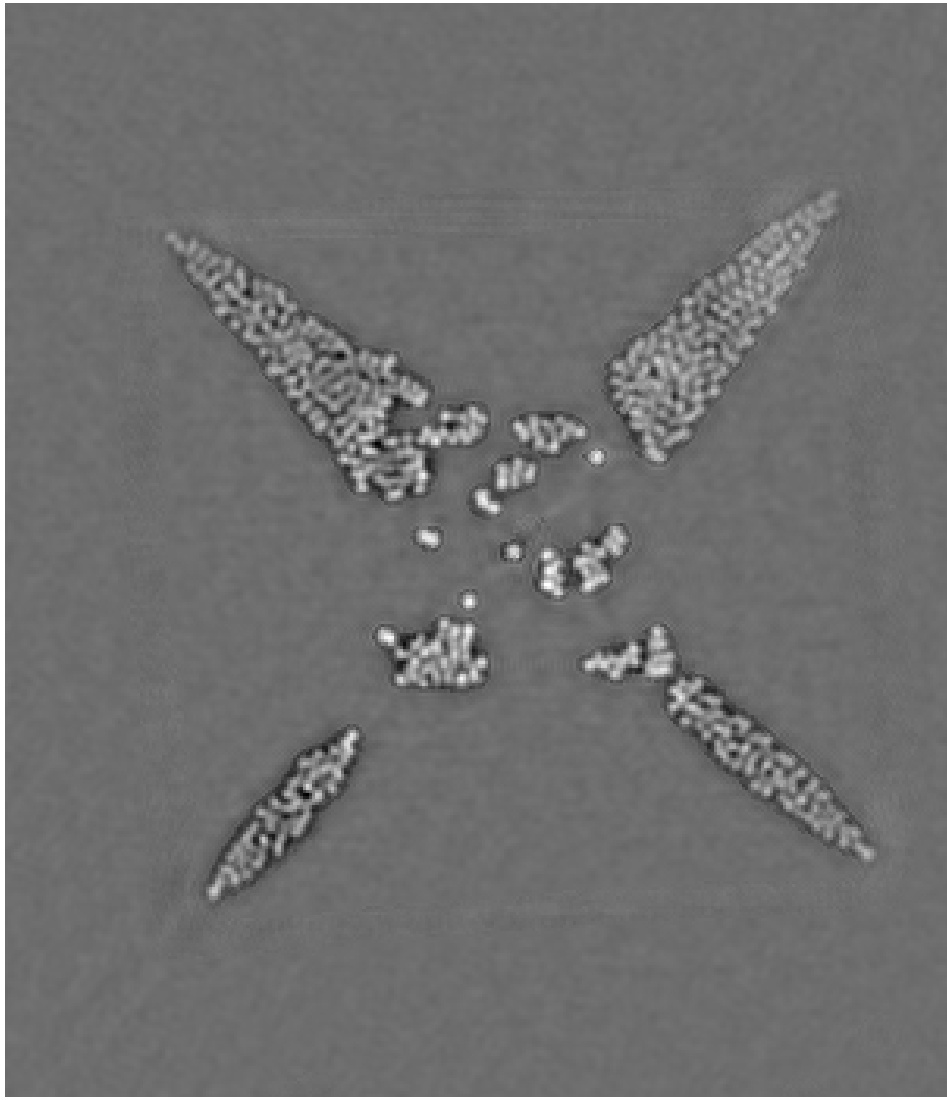


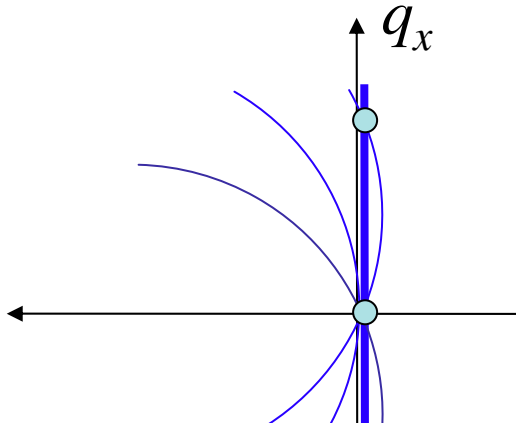
data

apman, Barty, Marchesini, Noy, Hau-Riege, Cui, Howells, Rosen, He, Spence,
ierstall, Beetz, Jacobsen, Shapiro, *J. Opt. Soc. Am. A* **23**, 1179 (2006)









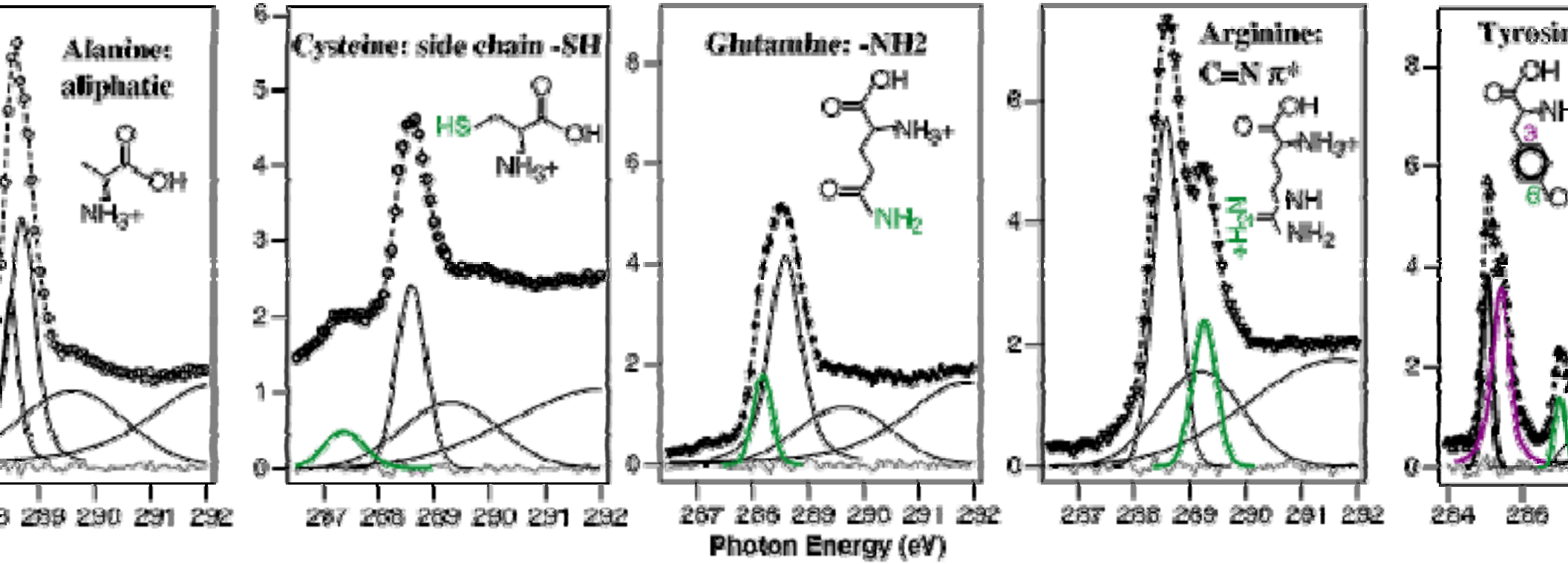
Barty, Marchesini, Noy, Hau-Riege, Cui, Howells,
Spence, Weierstall, Beetz, Jacobsen, Shapiro,
Am. A **23**, 1179 (2006)

C XANES of amino acids

. Kaznacheyev *et al*., *J. Phys. Chem.* **A 106**, 3153 (2002)

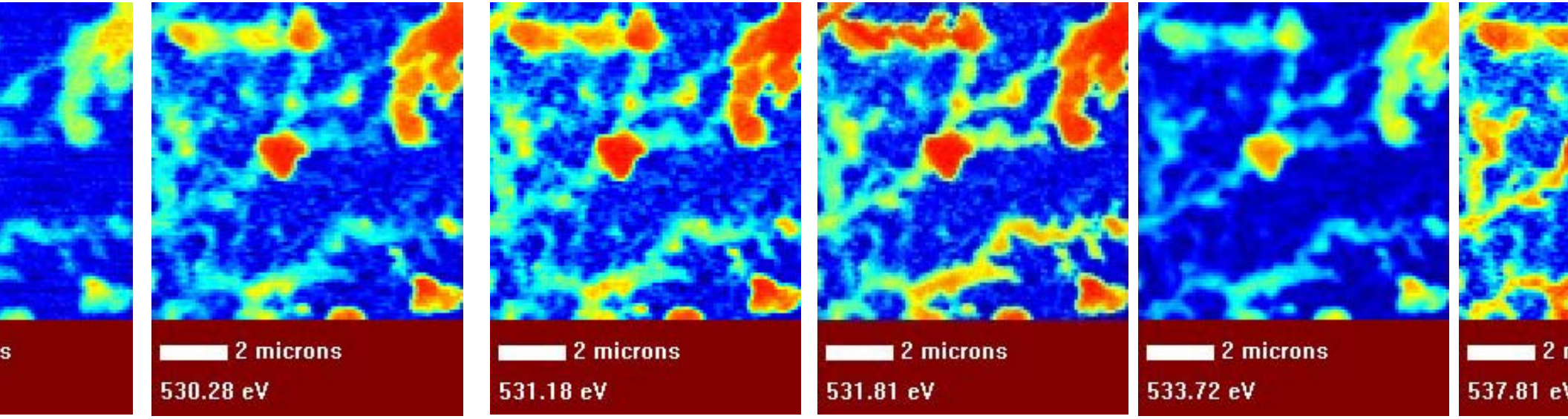
xperiment: K. Kaznacheyev *et al.*, Stony Brook (now CLS)

heory: O. Plashkevych, H. Ågren *et al.*, KTH Stockholm; A. itchcock, McMaster



Polymers: see e.g., Dhez, Ade, and Urquhart, *JESRP* **128**, 85 (2003)

Spectromicroscopy data are complex!



Lu in hematite (T. Sch

ectral image sequenes:
et al., J. Microscopy **197**

10^4-10^5 spectra!

Sometimes it's not so bad: domains of immis
pure substances for which you have referen
spectra

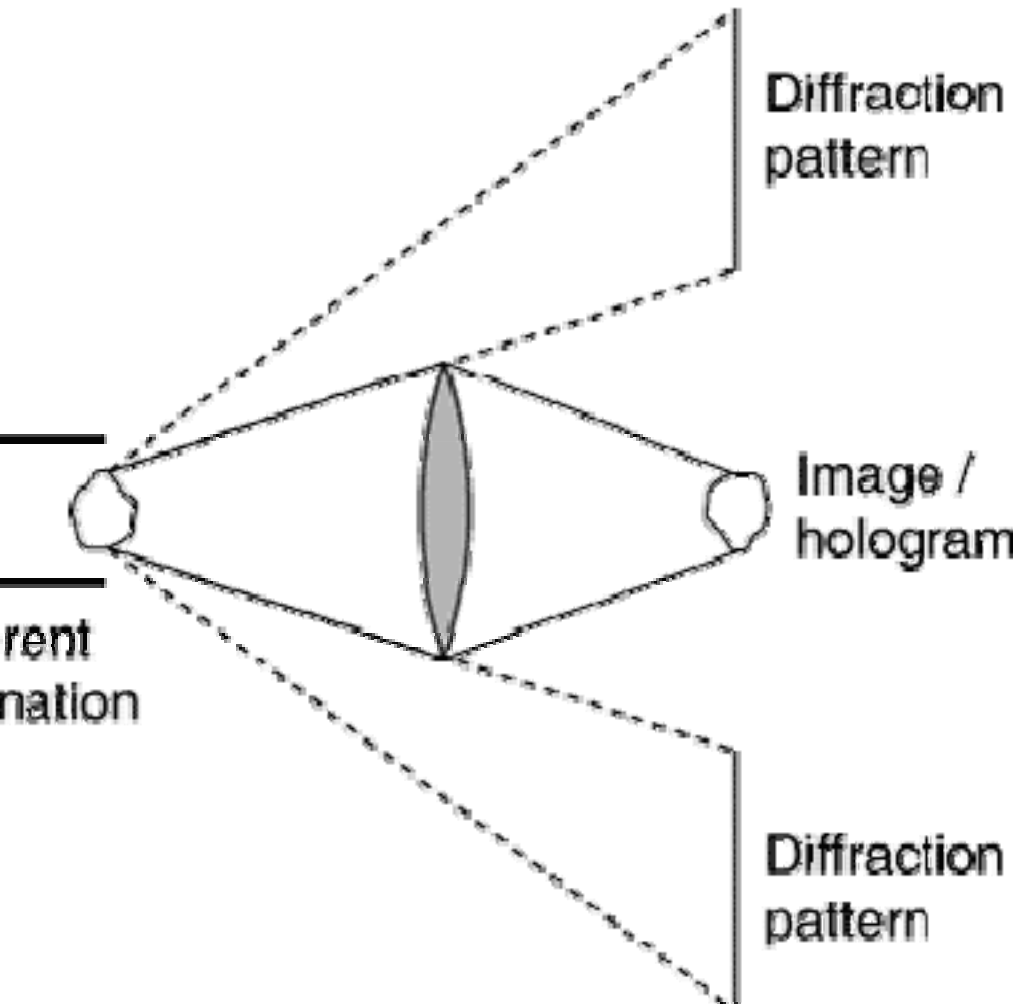
Sometimes it can seem hopelessly complex:
especially in biology or environmental scien

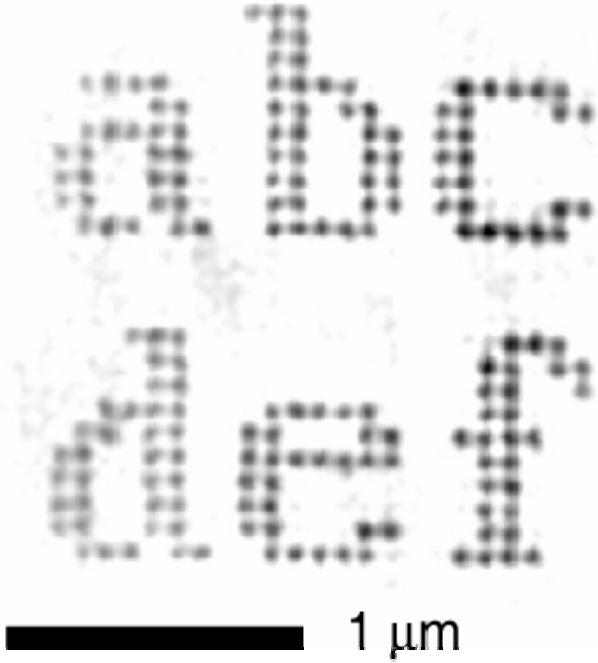
How can we deal with this complexity?

X-ray diffraction microscopy

pposed by Sayre (in Schlenker, ed., **Imaging and herence Properties in Physics**, Springer-Verlag, 198

rough 1999: experiments by Sayre, Kirz, Yun, Chapma ao



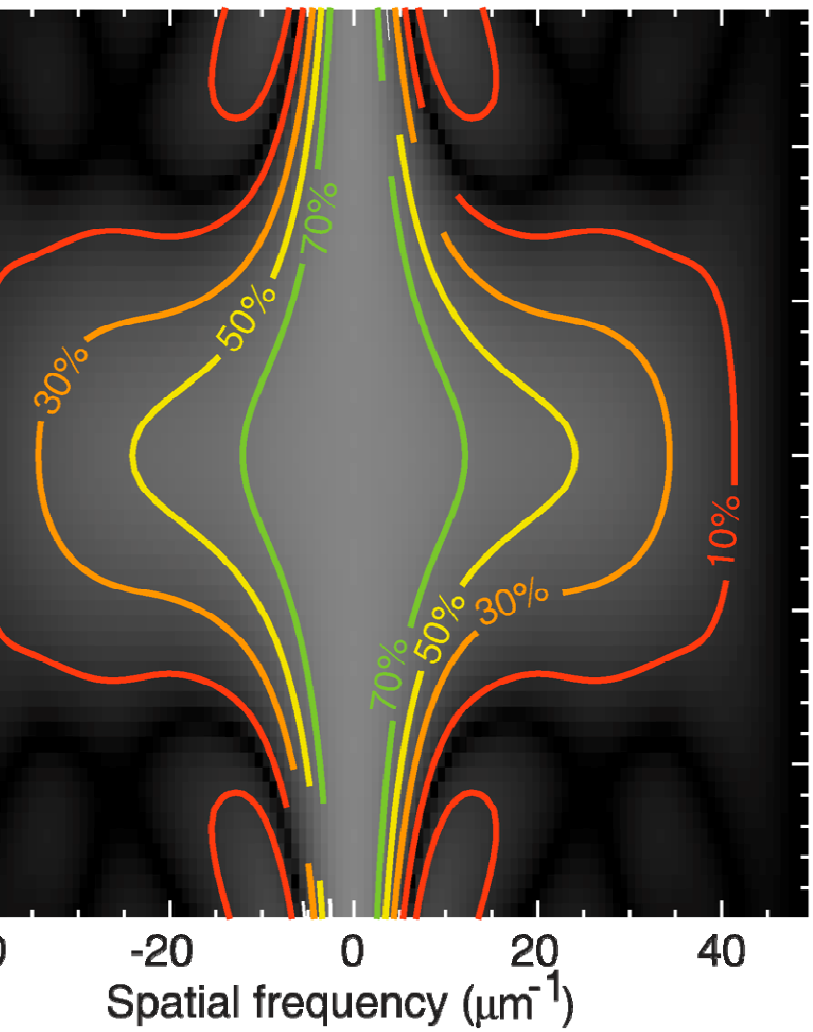


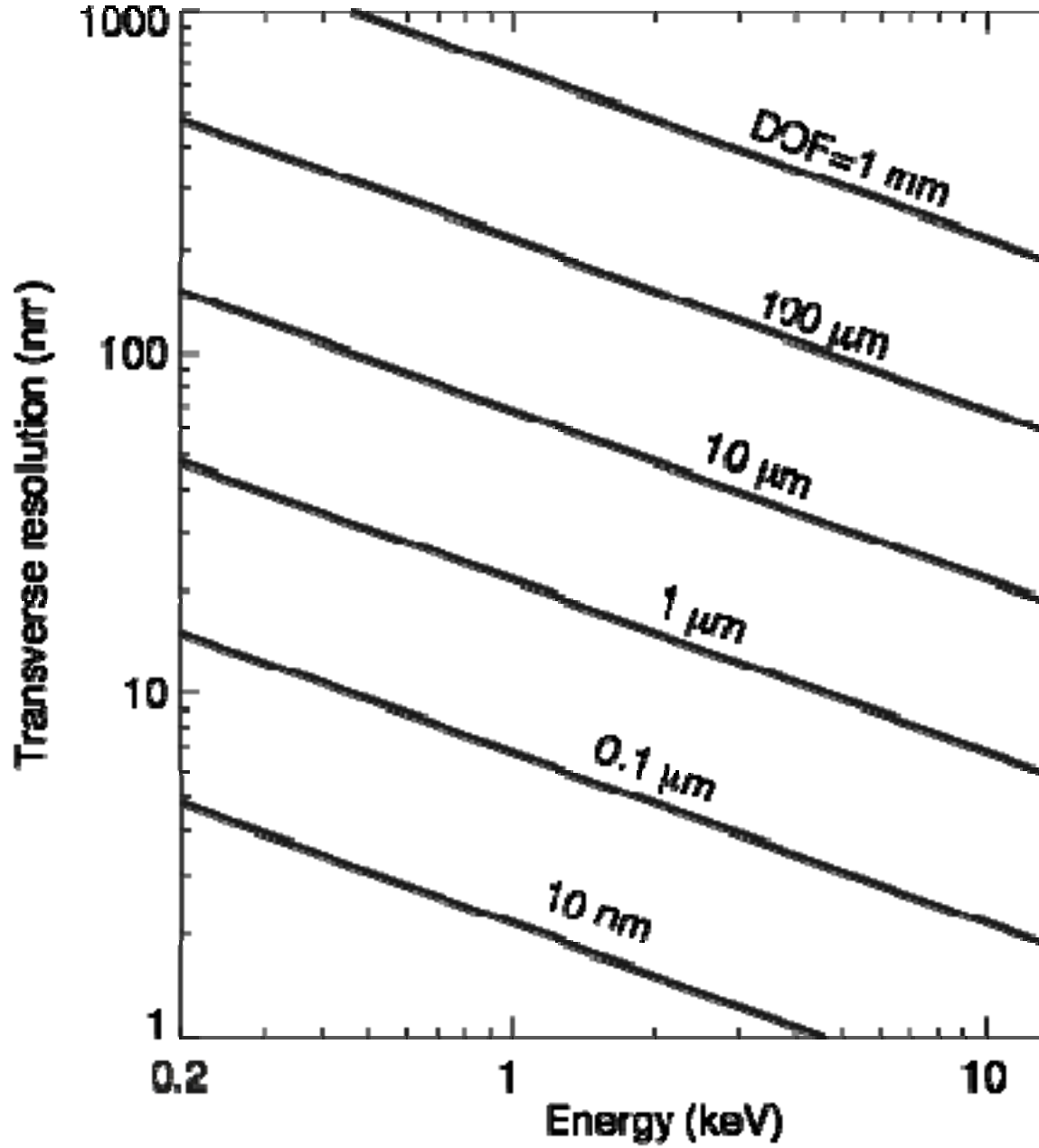
First x-ray reconstruction: Mia Charalambous, Kirz, and Say *Nature* **400**, 342 (1999)

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

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

Contrast versus defocus:
δ_{rN}=20 nm,λ=2.5 nm





Through-focus deconvolution with lenses:

- Confocal: fully incoherent (fluorescence
- EM: phase only, coherent
- TXM: partially coherent, equal absorptio
phase contrast, need for experimental C

electrons or photons?

eV photons

bout 100 absorption events per
astic scatter

bout 10 keV deposited per
sorption

herefore about 10^6 eV
eposited per elastic scatter

thousand scattered photons:
^{3}• 10^6 eV into (2 Å)^3, or 2×10^{13}
ray

- **100 keV electrons**
- About 2.5 inelastic scatters elastic scatter
- About 45 eV deposited per inelastic scatter
- Therefore about 10^2 eV dep per elastic scatter
- A thousand scattered electr 10^3•10^2 eV into (2 Å)^3, or 2×
Gray

Electrons are better than photons for atomic resolution imaging:
Breedlove and G. Trammel, *Science* **170**, 1310 (1970); R.
enderson, *Q. Rev. Biophys*. **28**, 171 (1995).

Crystallography's answer: spread the dose out over many
entical unit cells