X-ray nanotomography: seeing subcellular structure in 3D

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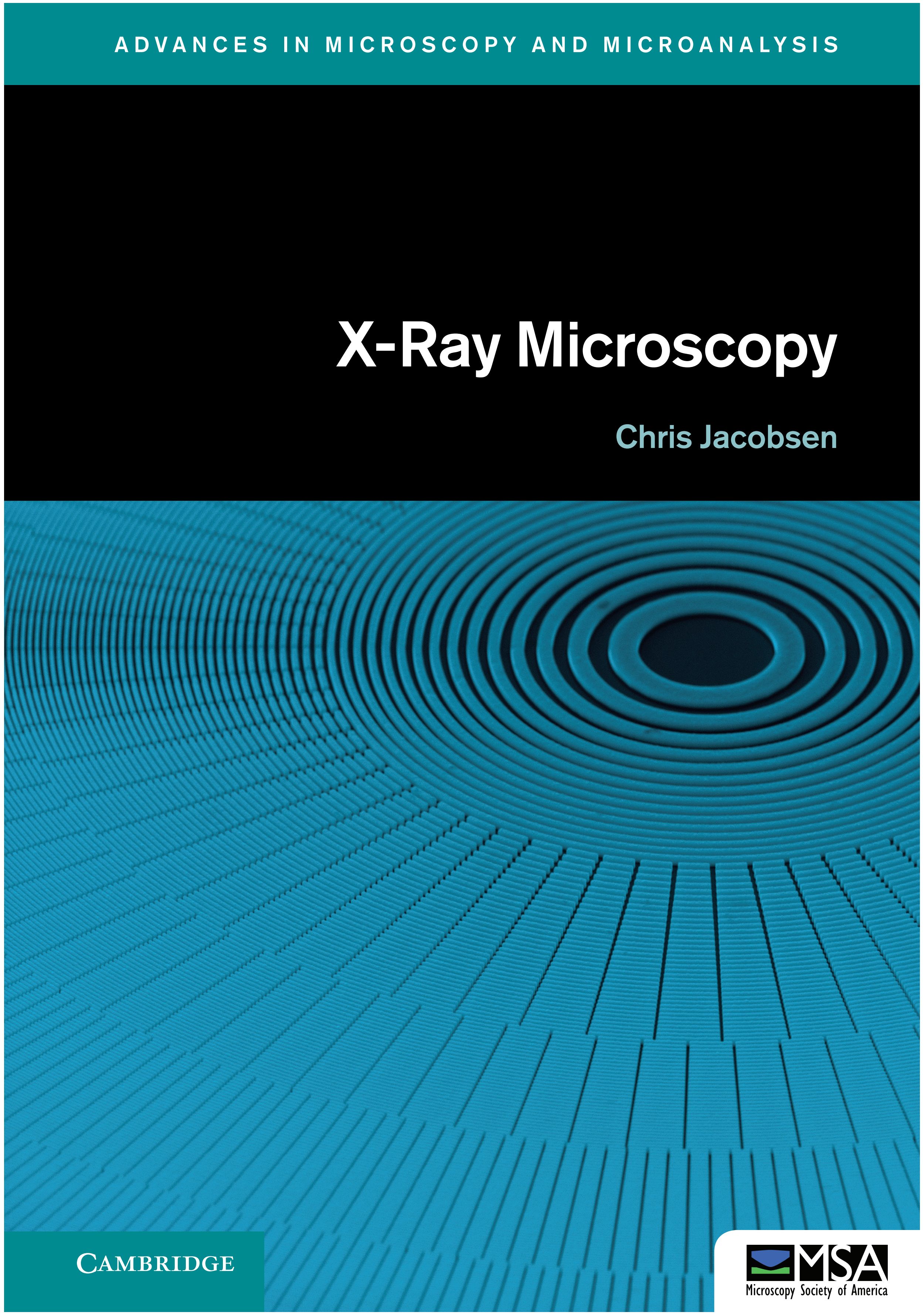
Support: Basic Energy Sciences, US Department of Energy; National Institutes of Health

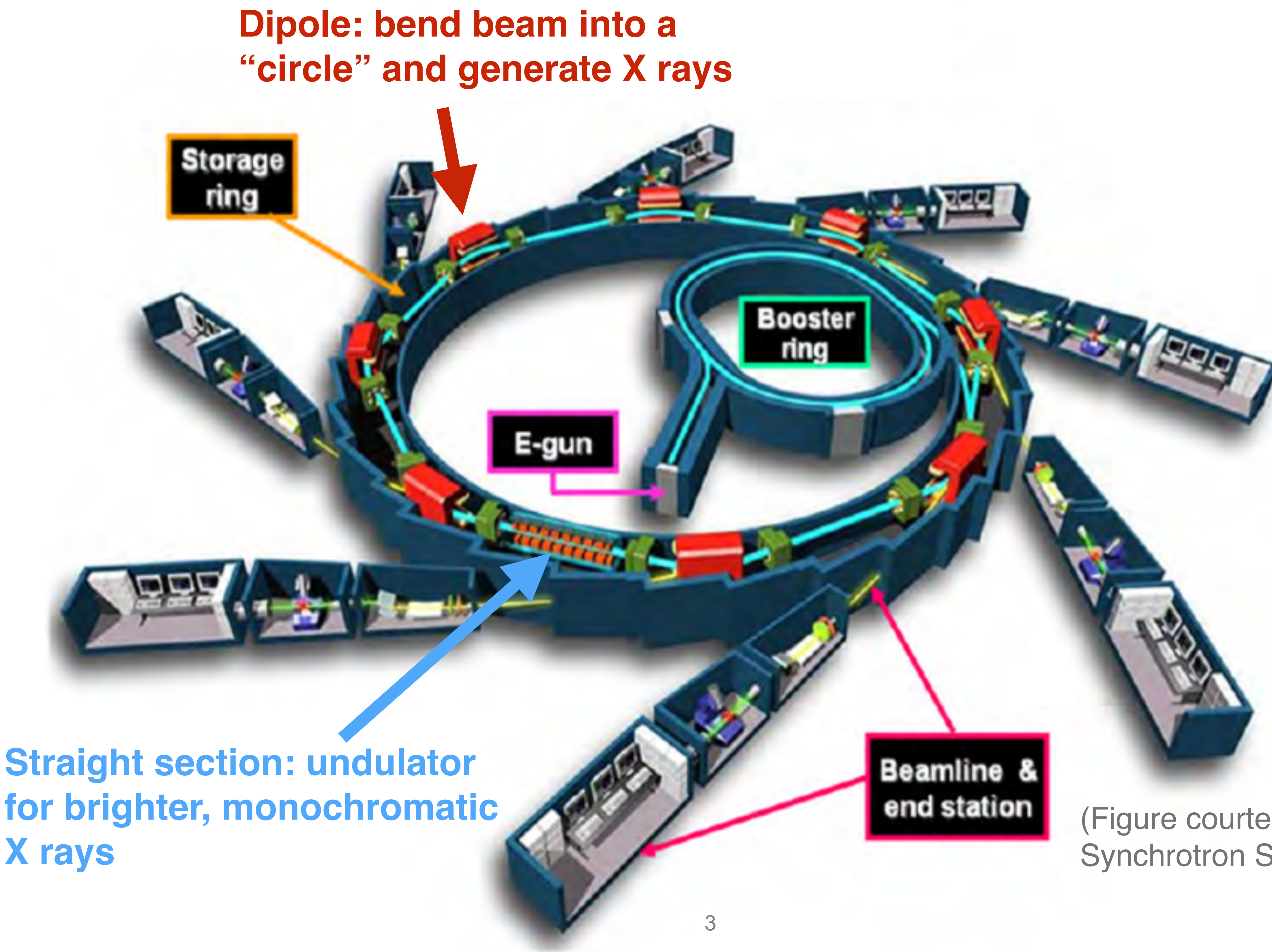
X-ray microscopy

- www.cambridge.org/Jacobsen



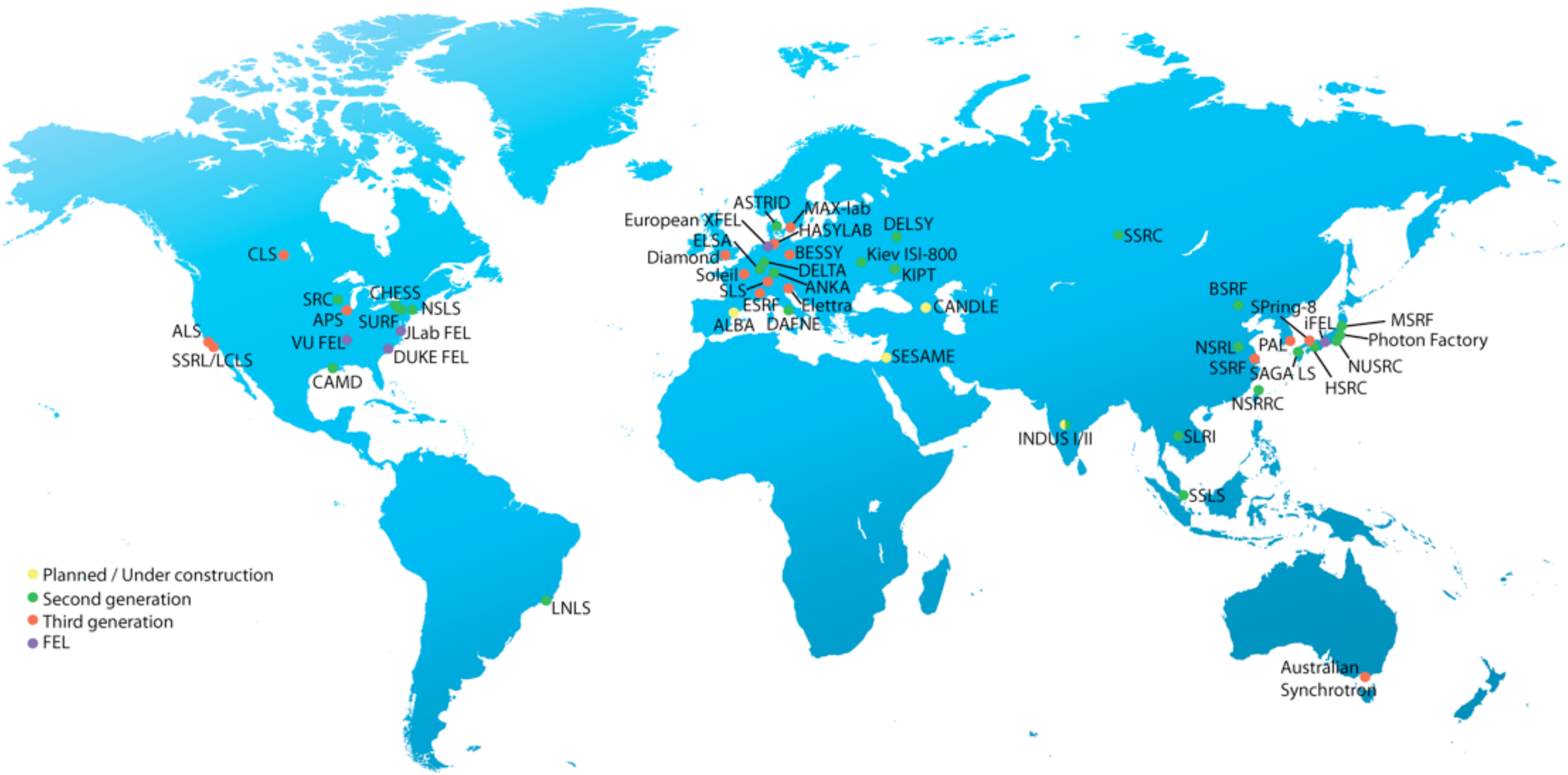
Synchrotron light sources

- Storage ring (constant beam energy) with “top-up” for steady current



(Figure courtesy Synchrotron Soleil)

Synchrotron light sources around the world



See for example www.lightsources.org

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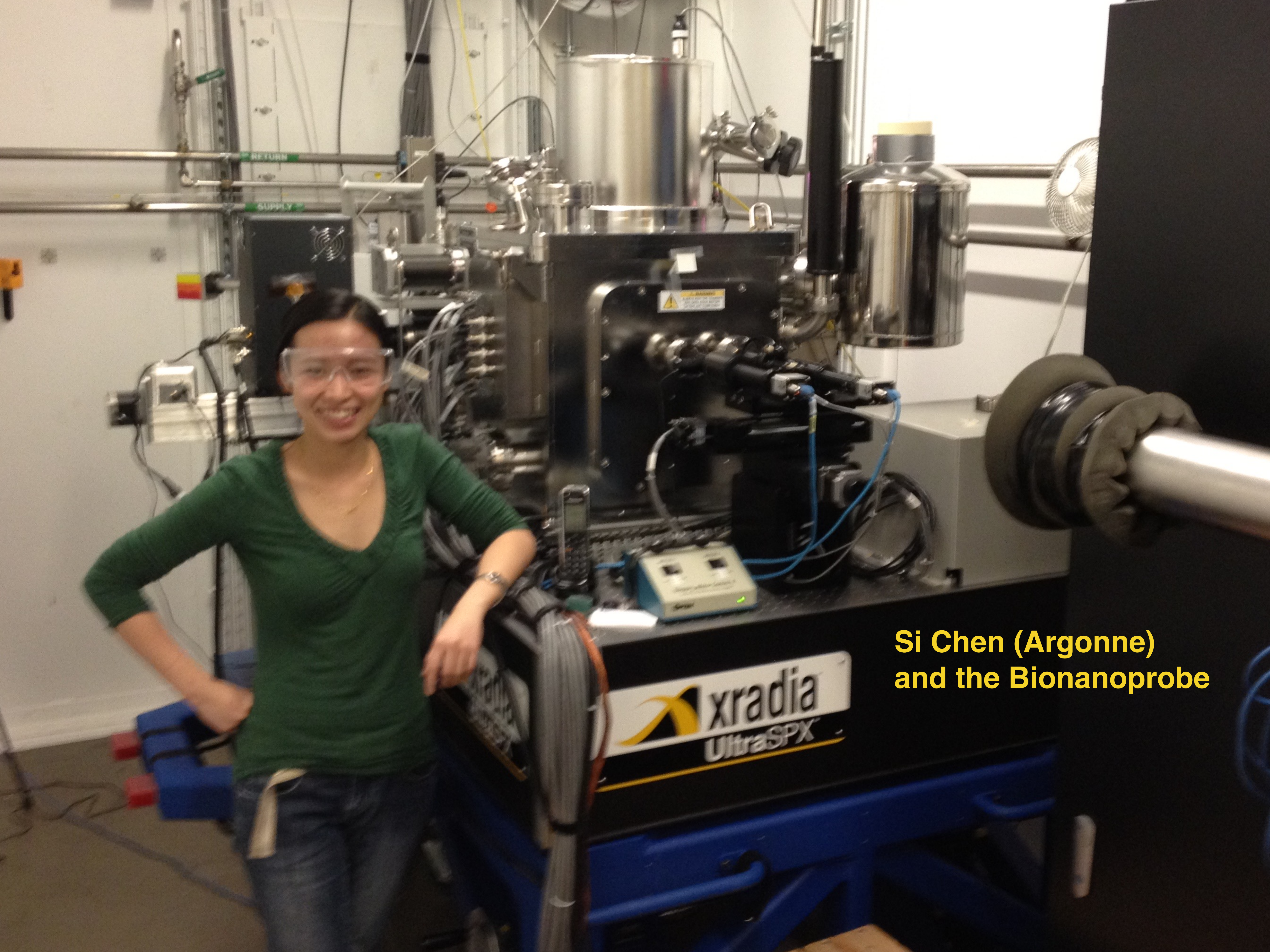
Advanced Photon Source at Argonne Lab: 7 GeV, ~10^{12} photons/sec (10^8 coherent) per experiment at 10 keV, ~65 simultaneous experiments, built ~1995.

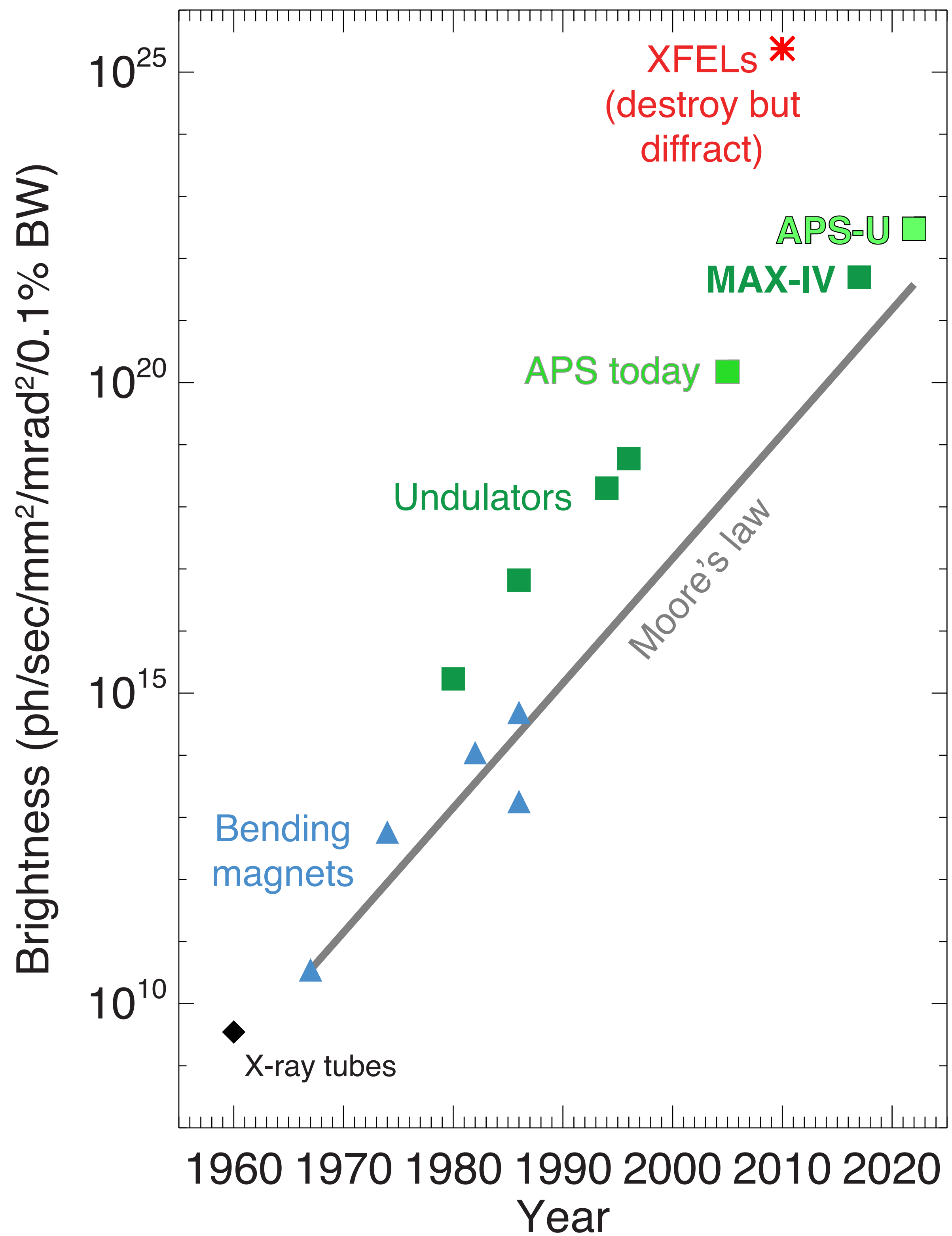
$800M upgrade planned 2021-2023.



Si Chen (Argonne)
and the Bionanoprobe

We are living in revolutionary times!

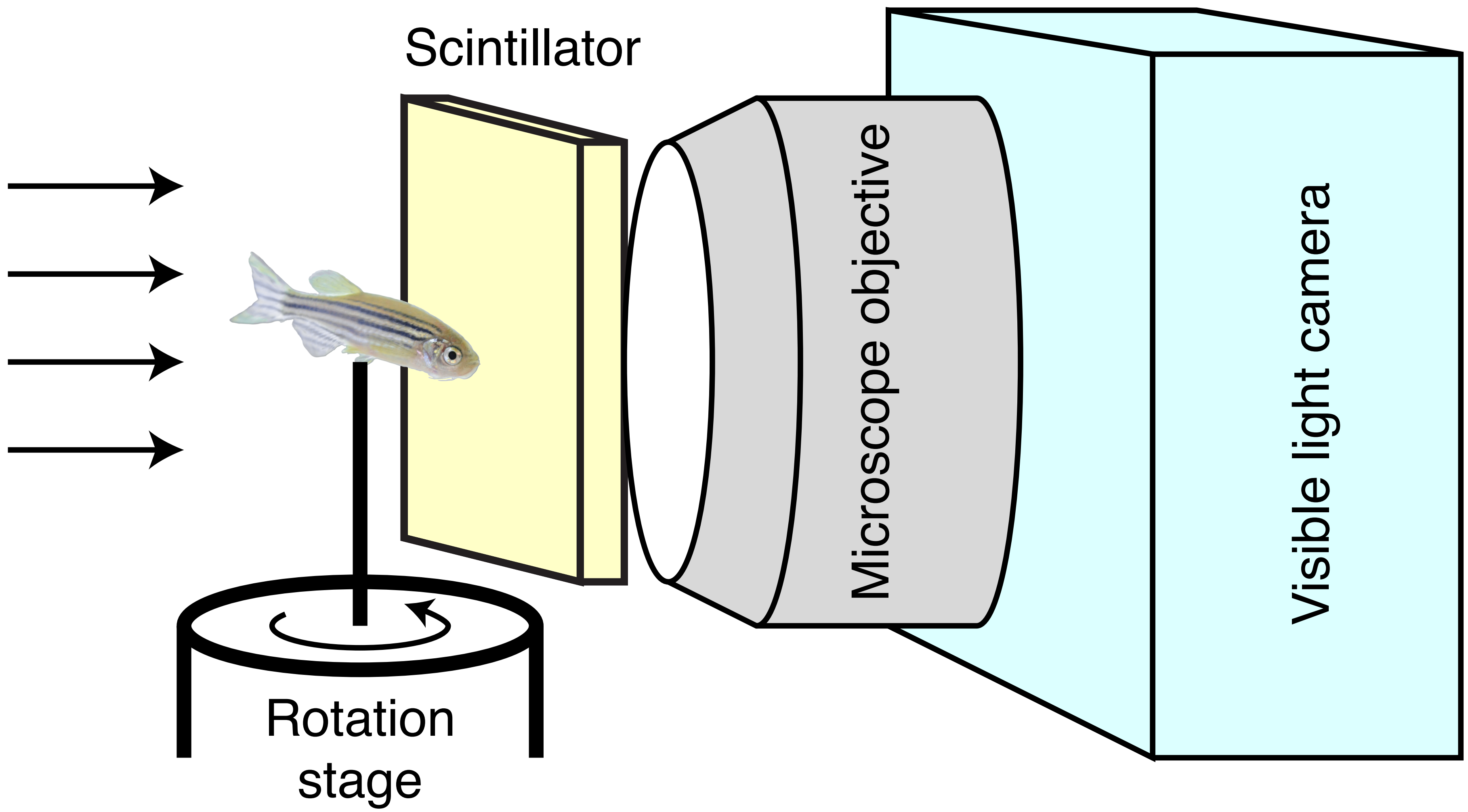
- X-ray brightness has been increasing faster than Moore's law!
- Spatially coherent flux is (Brightness)•λ^2. In typical experiments, we get 10^8-10^9 coherent photons/second today.
- New accelerator designs, with many weak dipoles, will give a 100-1000 fold increase in coherent flux.
- See for example Eriksson *et al.*, *Journal of Synchrotron Radiation* **21**, 837 (2014).



This plot: C. Jacobsen and M. Borland
doi:10.3254/978-1-61499-732-0-35

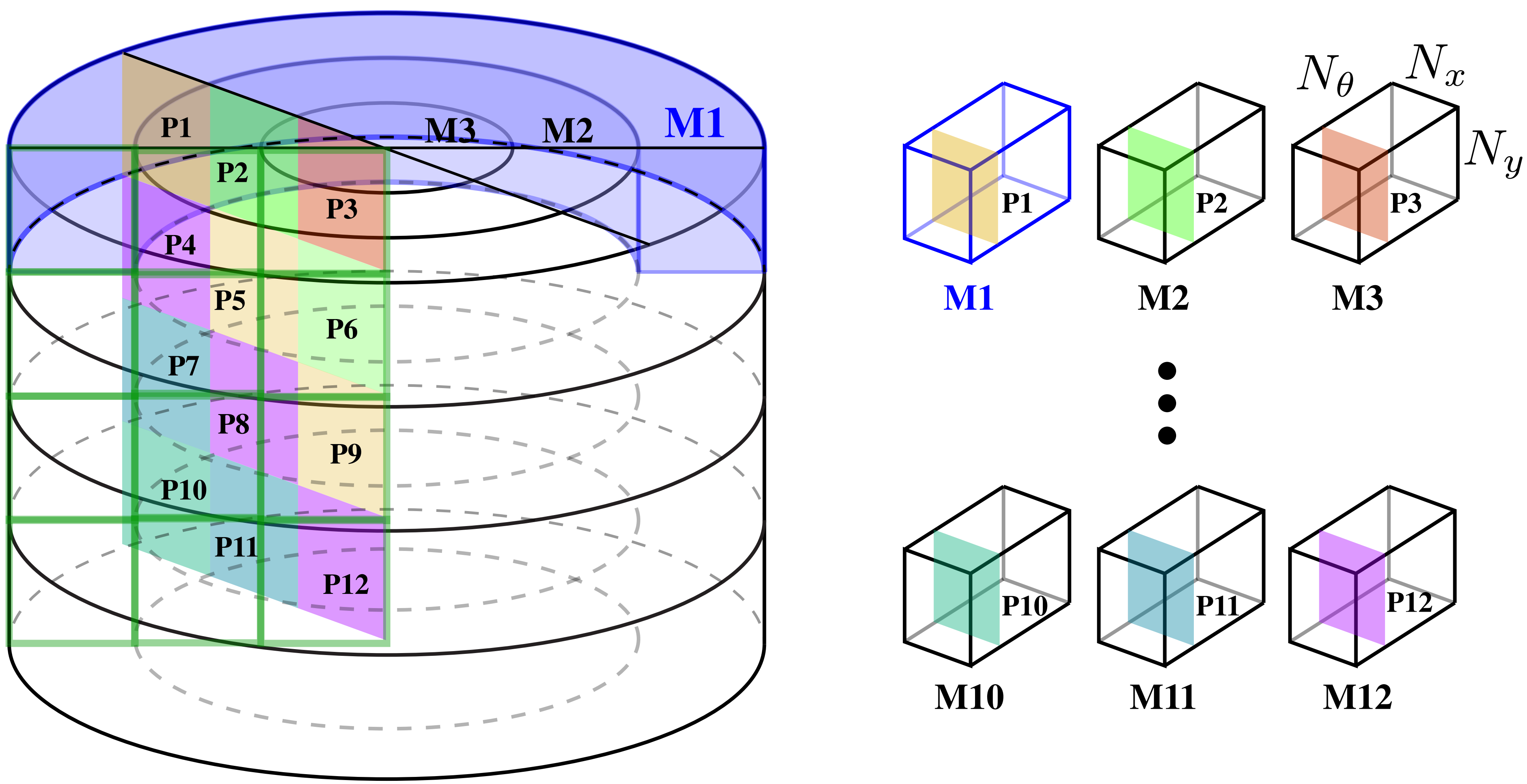
Let's think really big

- Tomography at ~1 µm resolution: scintillator, visible light objective, visible light camera
- See Flannery *et al*, *Science* **237**, 1439 (1987).



Mosaic tomography approach

- Synchrotron x-ray beams are ~1 mm in size. How to image a 10 mm sample?
- Mosaic of 11x12 tomograms (move, then rotate; repeat) to yield ~(22,000)³ voxels
- Data set size: (130x2500x2000x4500) pixels, 32 bit=10 TeraBytes
- Reconstruction volume at full resolution: (22,000)³ voxels=10,600 Gigavoxels, 32 bit=38 TeraBytes
- Rafael Vescovi, Ming Du, Vincent de Andrade, William Scullin, Doğa Gürsoy, Chris Jacobsen, *J. Synchrotron Radiation* **25**, 1478 (2018)



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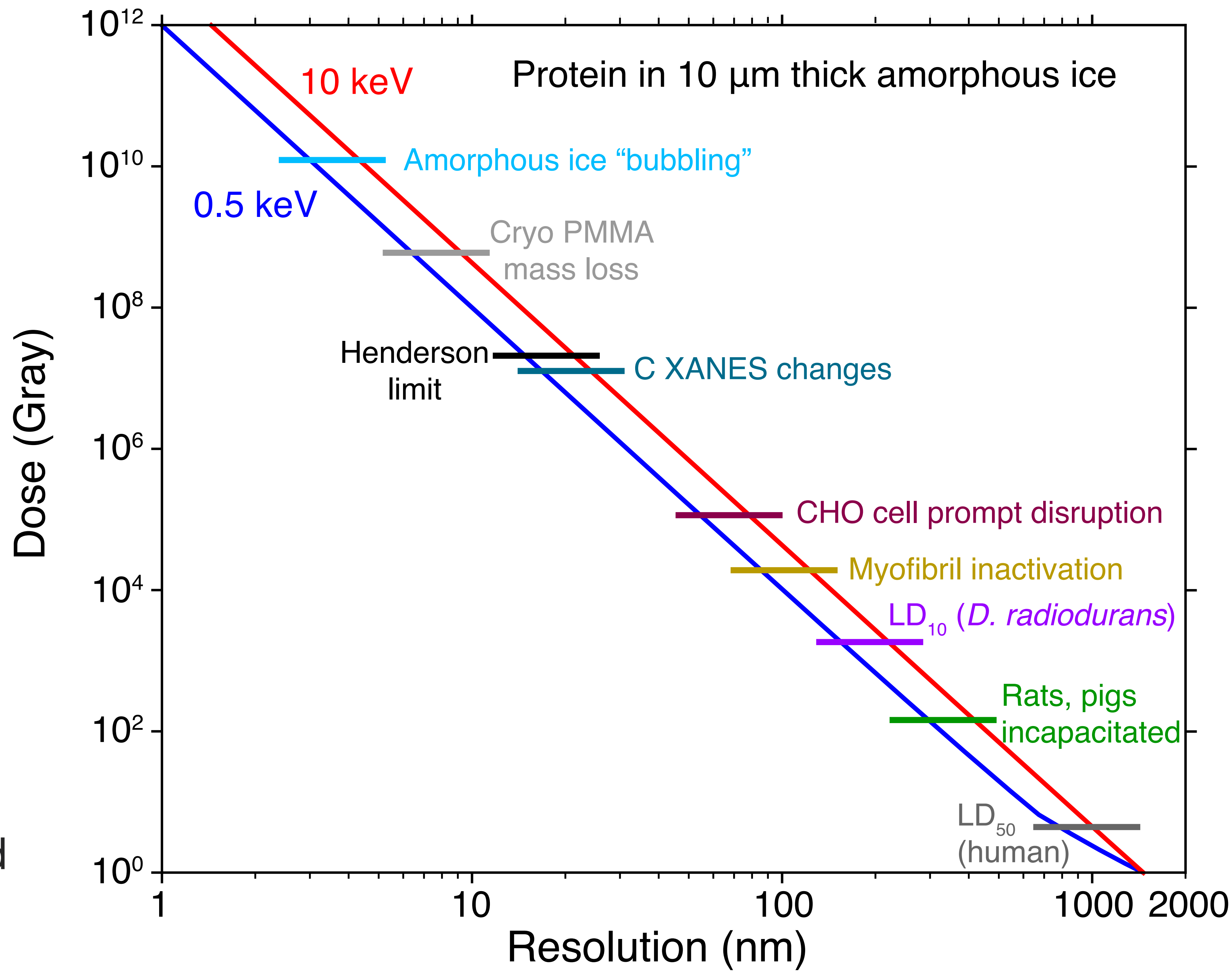
Mosaic tomography of a whole mouse brain

Bobby Kasthuri, Rafael Vescovi, Ming Du, Vincent de Andrade *et al.* 25 keV tomography (Os stain, Epon). NIH U01 BRAIN initiative project 2015-2018.

Resolution: 1-2 μm

Dose versus resolution for x-ray imaging

- For X rays, 1 Gray≃1 Sievert
- Calculation of radiation dose using best of phase, absorption contrast and 100% efficient imaging
- Things that can be done wet at room temperature:
  - bacteria at 50 nm resolution
  - small animals at micrometer resolution (followed by sacrifice)

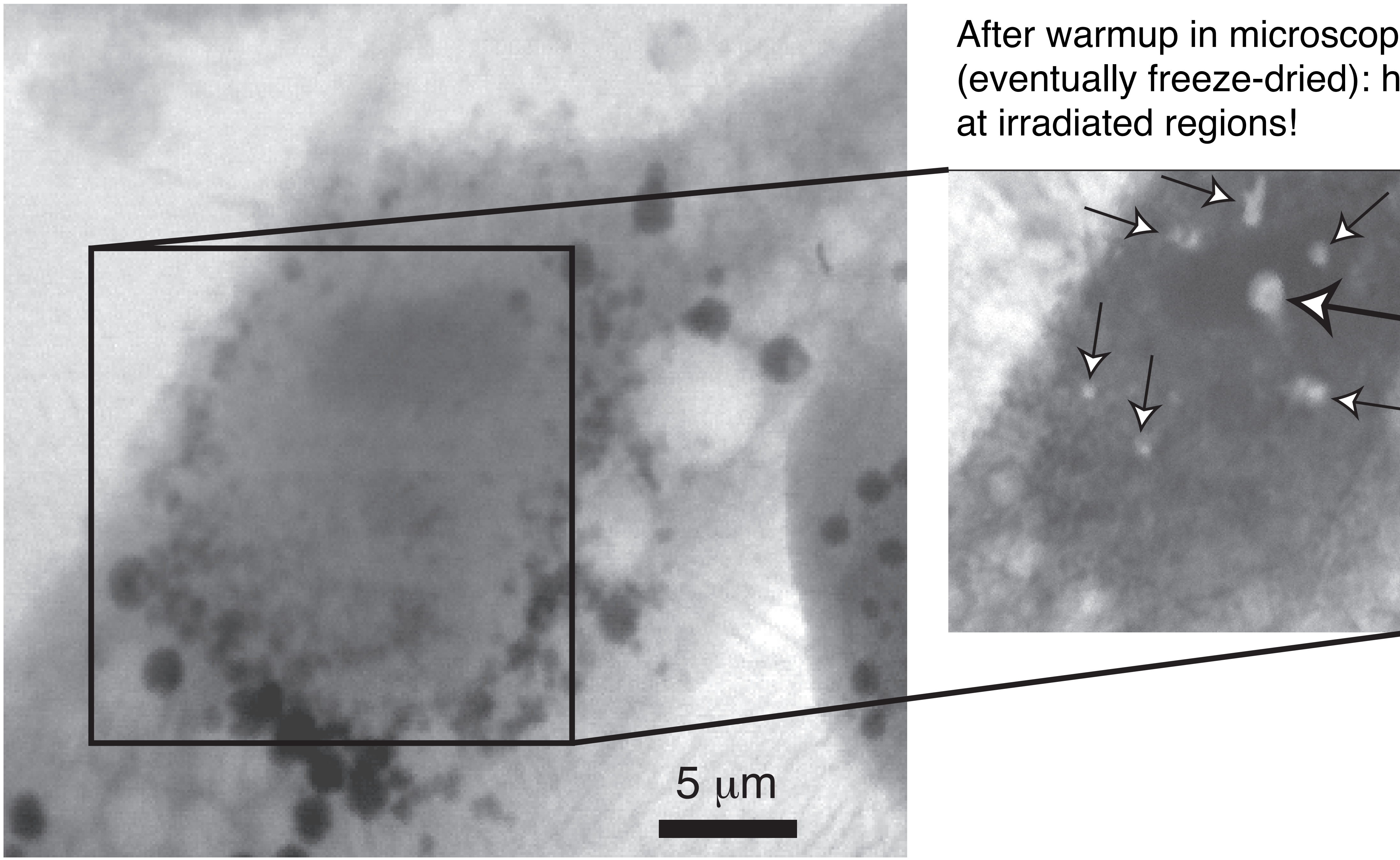


Du and Jacobsen, *Ultramicroscopy* **184**, 293-309 (2018).

Radiation damage resistance in cryo microscopy

Frozen hydrated fibroblast image after exposing several regions to $\sim 10^{10}$ Gray

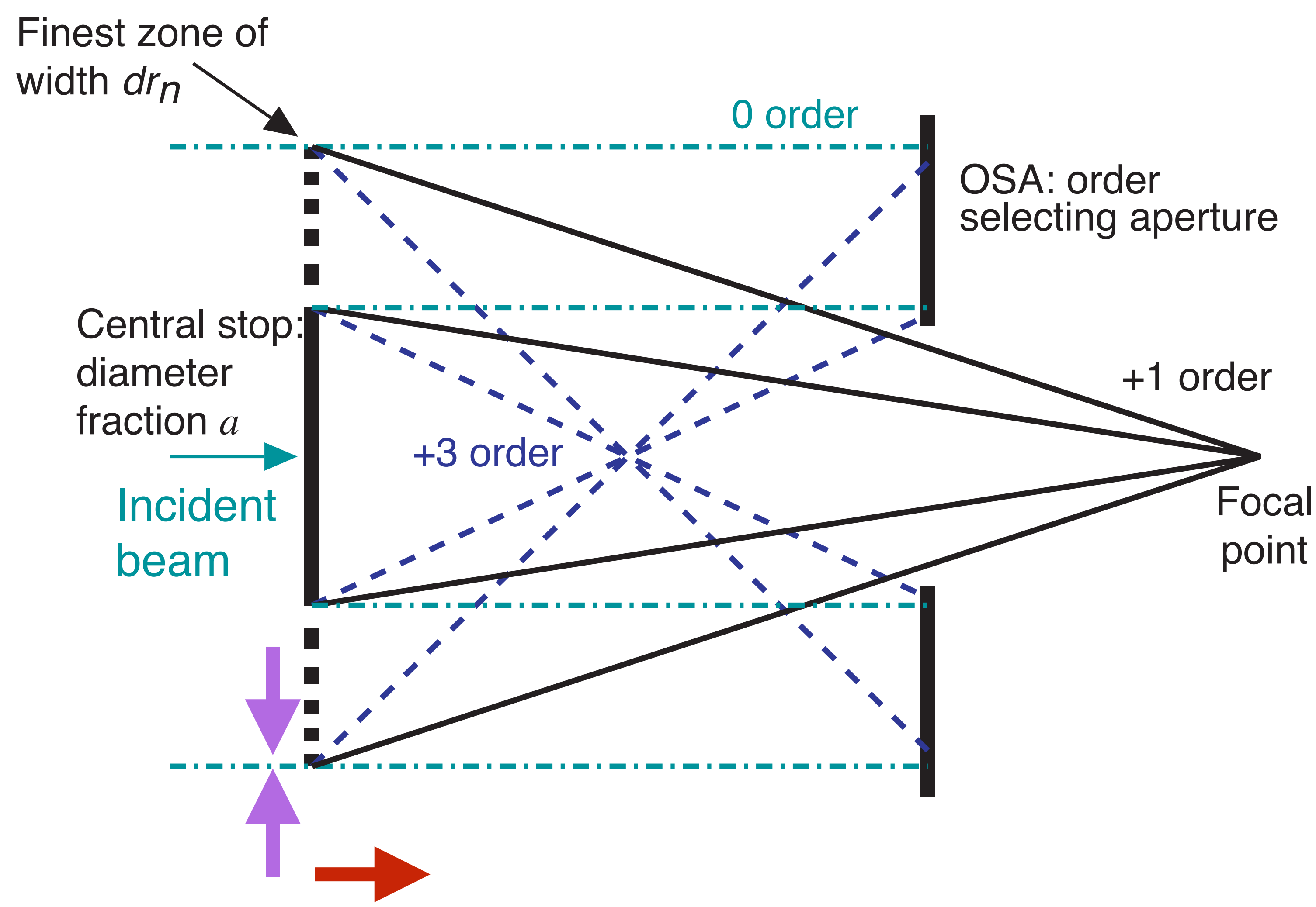
After warmup in microscope (eventually freeze-dried): holes at irradiated regions!



Maser, Osanna, Wang, Jacobsen, Kirz, Spector, Winn, and Tennant, *J. Micros.* **197**, 68 (2000)

X-ray focusing: Fresnel zone plates

- Diffractive optics: radially varied grating spacing
- Spatial resolution limited to **width** dr_N of finest, outermost zone.
  - 20-40 nm in practice
  - <10 nm in demonstrations
- Zones must be **thick** enough along beam direction to produce a phase shift of π:
  - about 100 nm at 0.5 keV
  - several 1000 nm at ~10 keV!

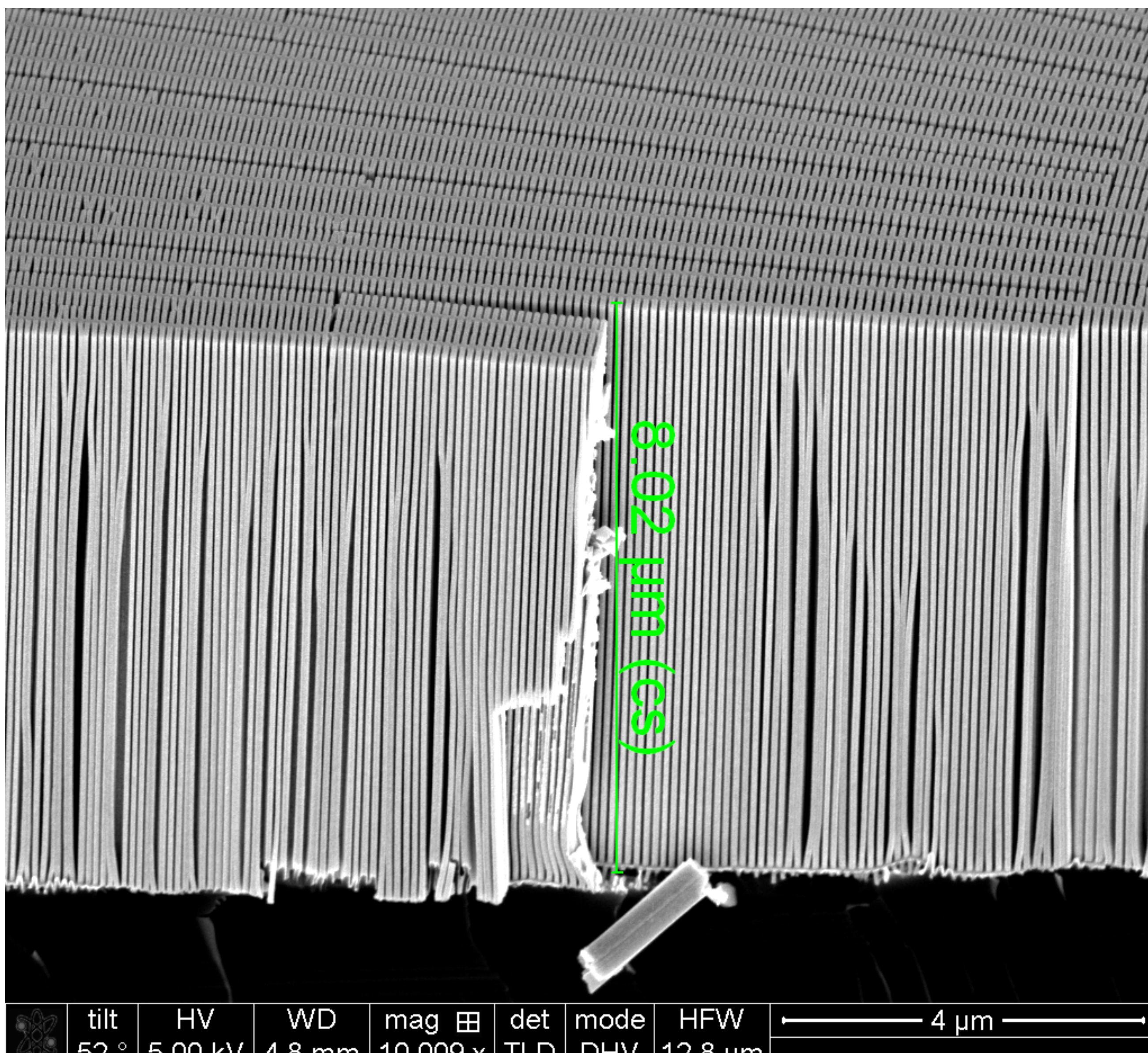


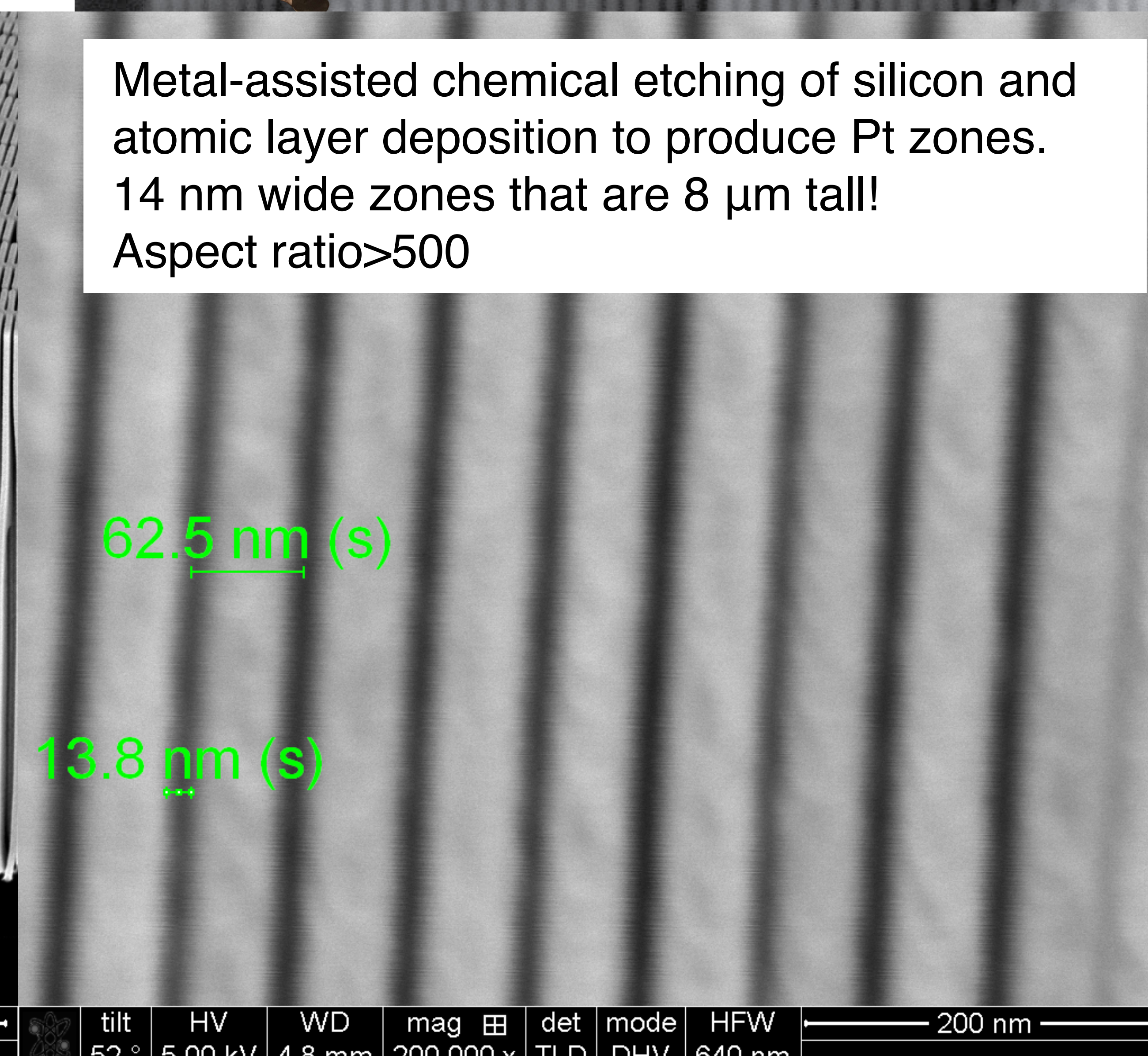
High aspect ratio nanofabrication!

Fresnel zone plates for hard x-ray nanofocusing

14 nm zone width in Pt, up to 8 µm tall (aspect ratio=500). 6% efficient at 20 keV in preliminary tests; resolution tests underway.

Kenan Li, M. Wojcik, R. Divan, L. Ocola, B. Shi, D. Rosenmann, and C. Jacobsen, *J. Vac. Sci. Tech. B* **35**, 06G901 (2017)

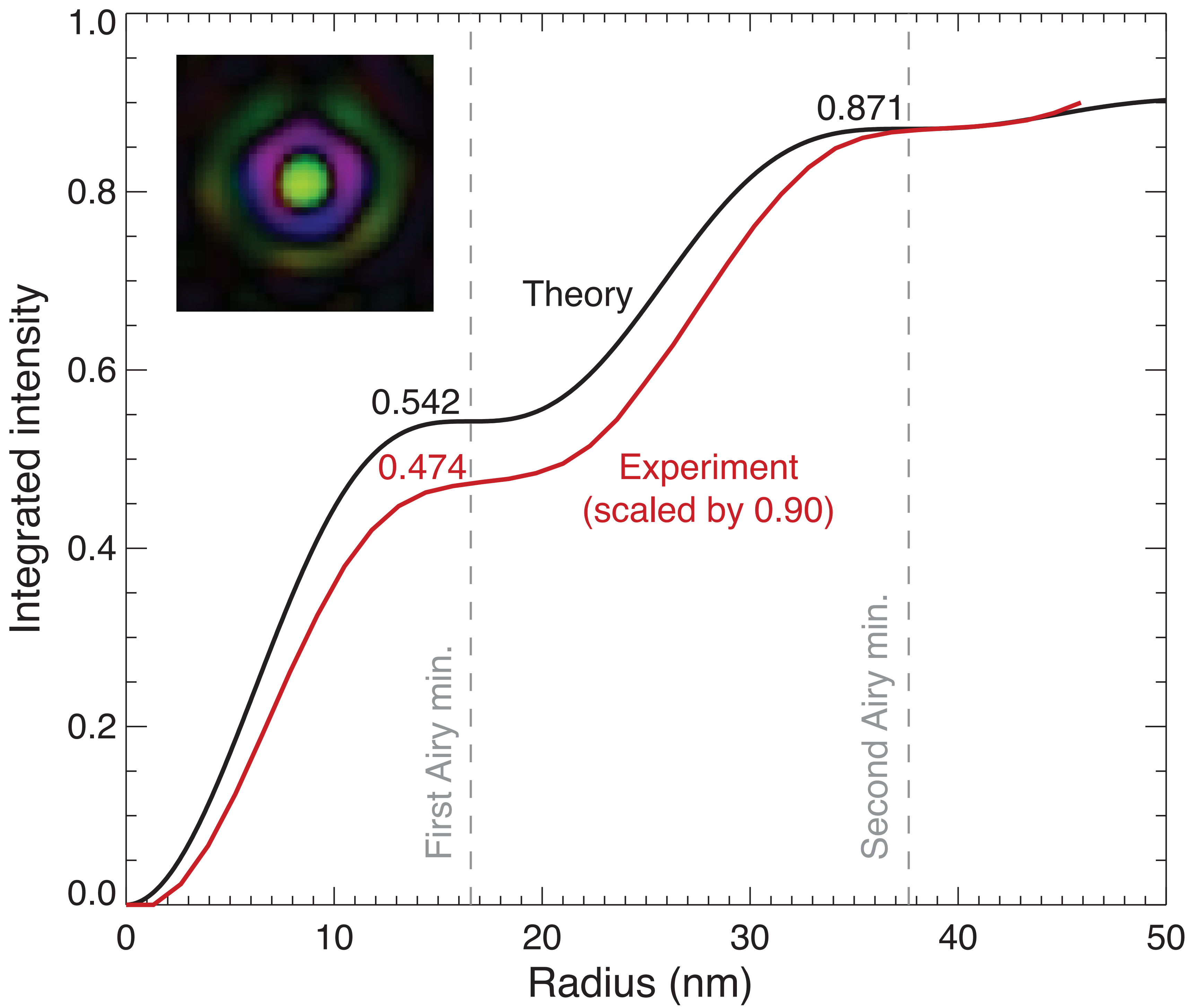




Metal-assisted chemical etching of silicon and atomic layer deposition to produce Pt zones. 14 nm wide zones that are 8 µm tall! Aspect ratio>500

Recent tests at Brookhaven Lab

- 14 nm FWHM probe size at 12 keV, with 10^8 photons/second in the focus
- Northwestern University: Kenan Li, Sajid Ali, Chris Jacobsen
- Argonne Lab: Michael Wojcik
- Brookhaven Lab: Xiaojing Huang, Hanfei Yan, Yong Chu, Ajith Pattammattel, Evgueni Nazaretski



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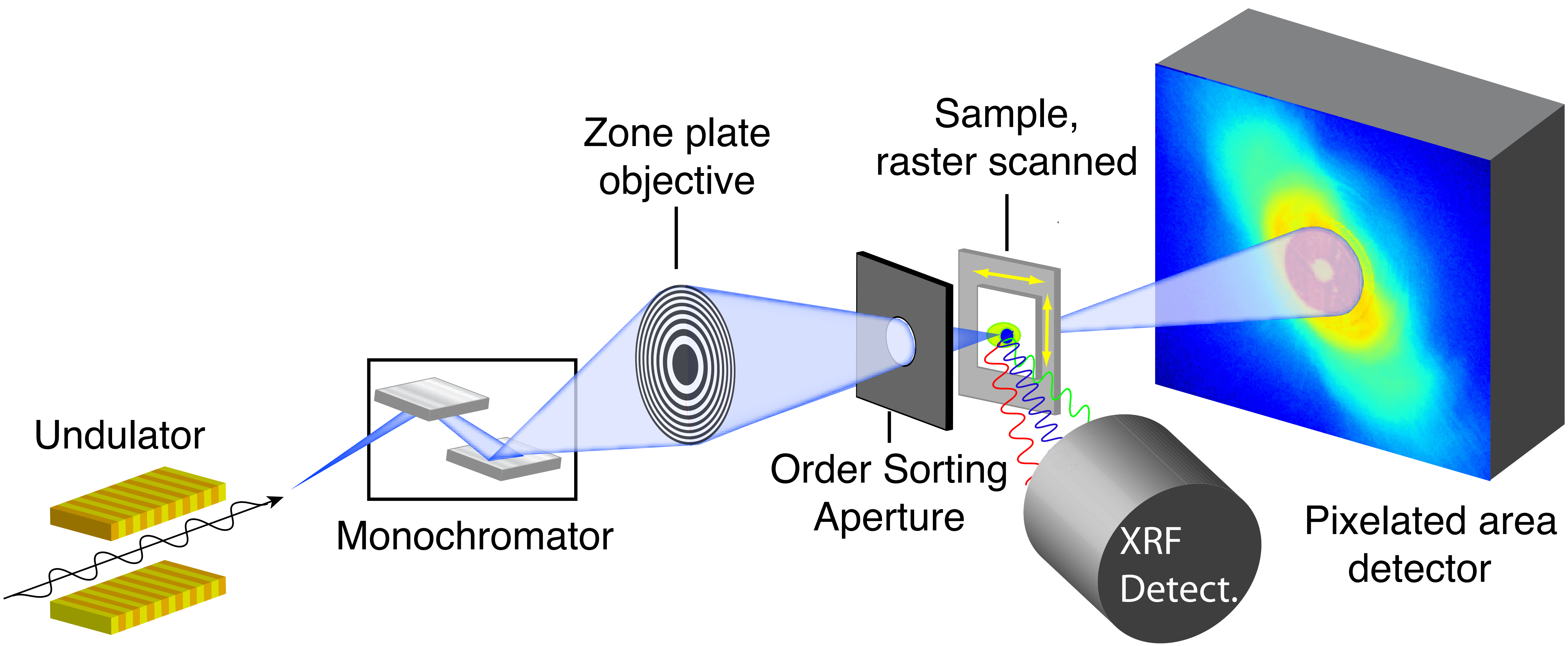
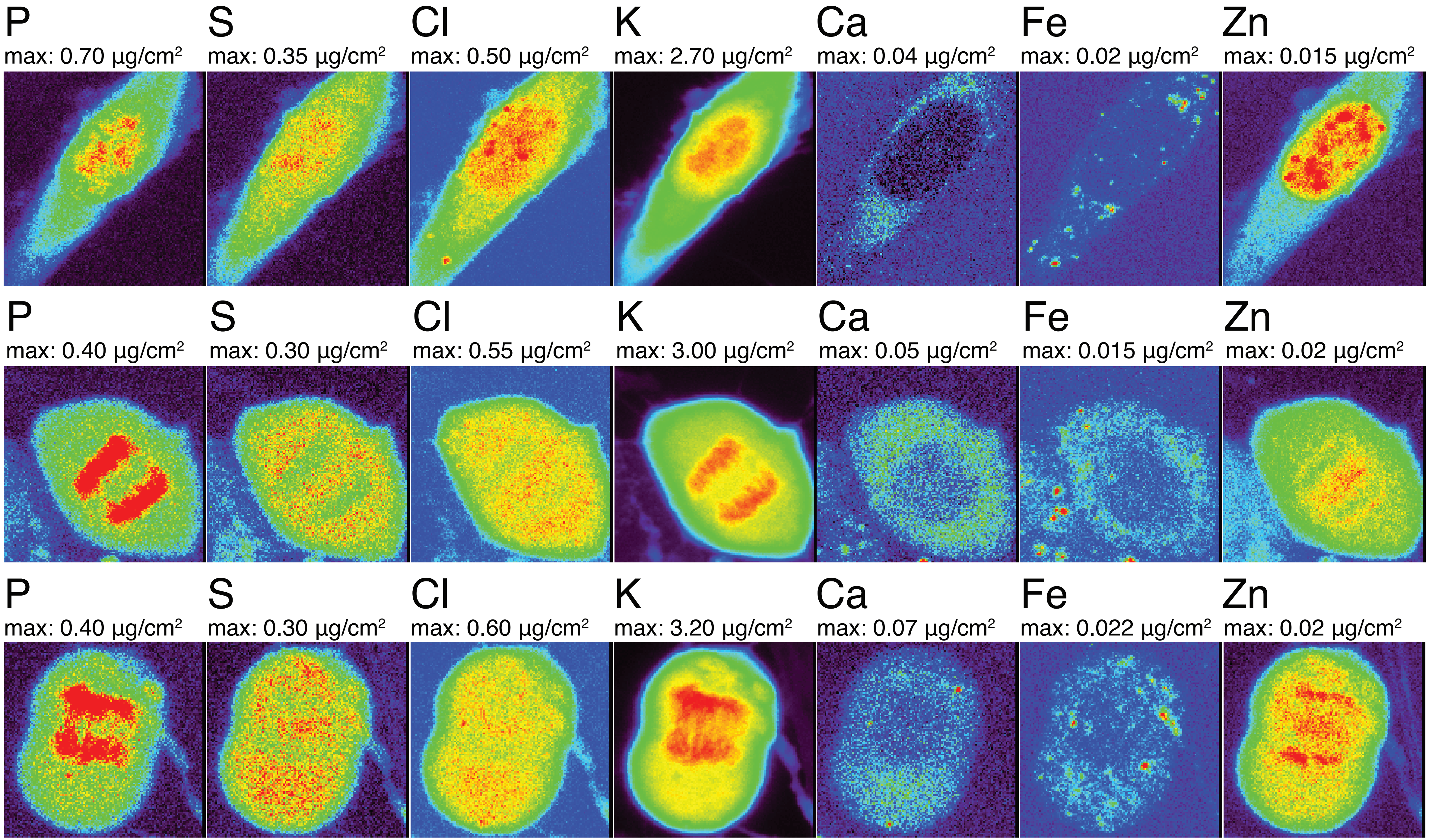
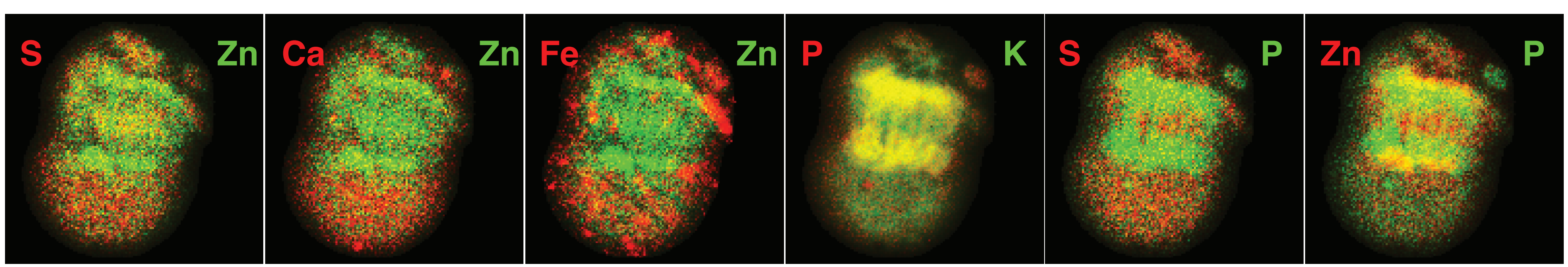
Combining x-ray transmission, and fluorescence

- One can record multiple imaging types simultaneously!



Metals in cell division





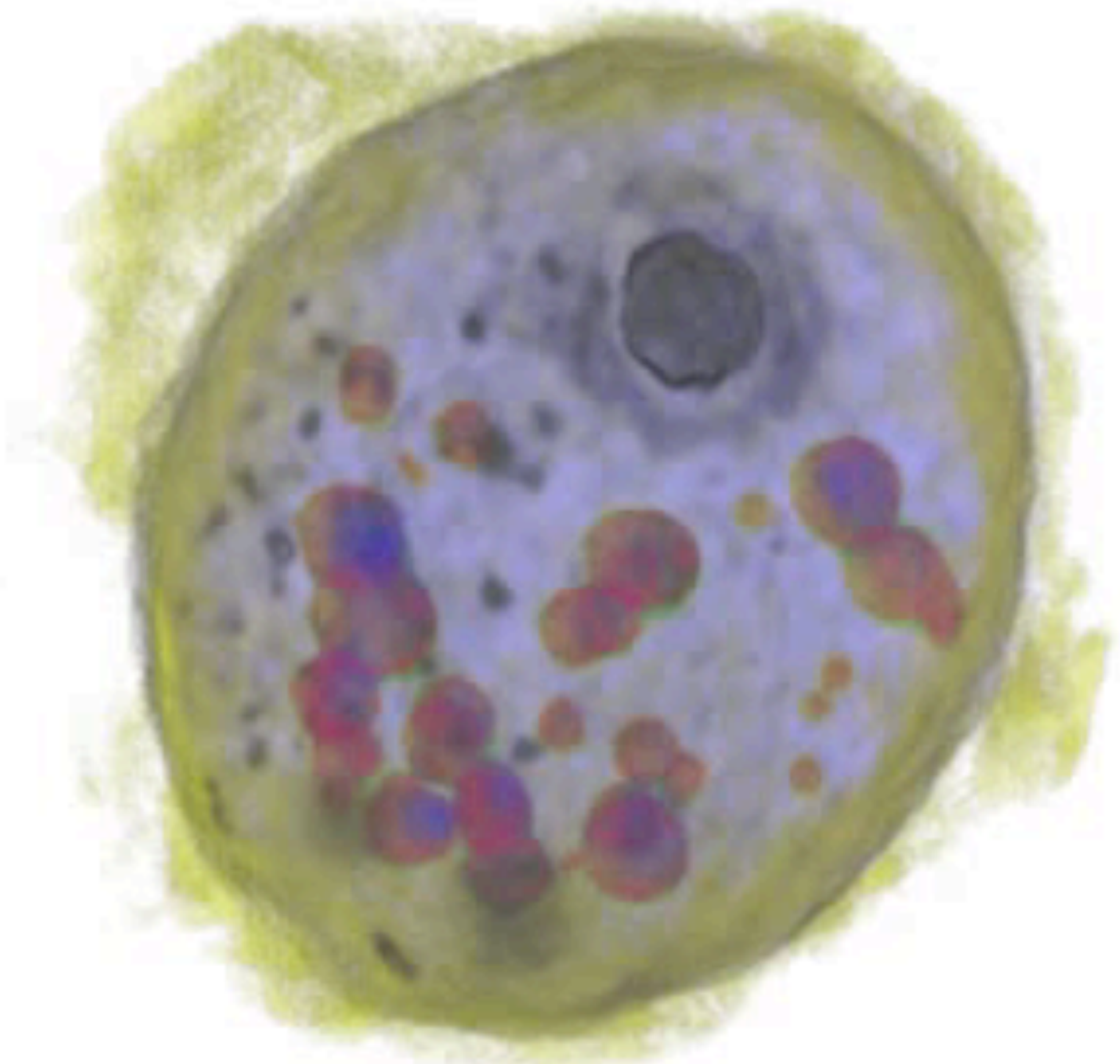
Qiaoling Jin,
Everett Vacek,
Si Chen, Barry Lai,
Tom O'Halloran,
Chris Jacobsen

Frozen hydrated alga in 3D

- Deng, Lo, Gallagher-Jones, Chen, Pryor Jr., Jin, Hong, Nashed, Vogt, Miao, and Jacobsen, *Science Advances* **4**, eaau4548 (2018)
- 3D resolution of about 100 nm

Removing/re-adding in turn:

Cl
S
K
P
Ca



Mysteries of the x-ray refractive index

Write refractive index as

$$n = 1 - \frac{n_a r_e}{2\pi}\lambda^2(f_1 + i f_2)$$
$$= 1 - \alpha\lambda^2(f_1 + i f_2)$$

where n_a=# atoms/volume, and

r_e=2.818x10^{-15} m is the classical radius of the electron. Assumes exp[$-i(kx-\omega t)$] for forward propagation.

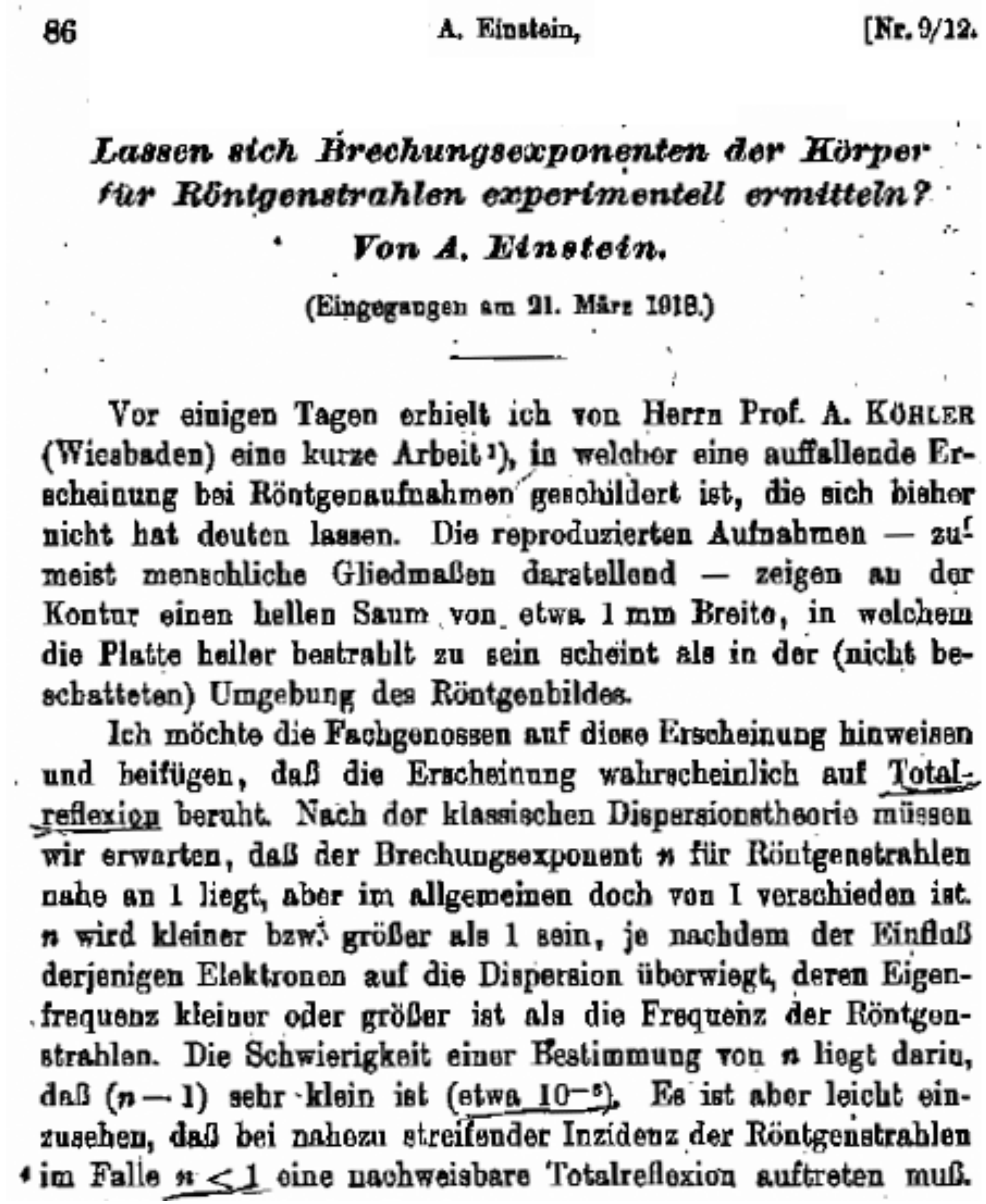
Also written as n=1-δ-iβ

Phase velocity is

$$v_p = \frac{\omega}{k} \simeq c(1 + \alpha\lambda^2 f_1)$$

Group velocity is

$$v_g = \frac{d\omega}{dk} \simeq c(1 - \alpha\lambda^2 f_1)$$



86 A. Einstein, [Nr. 9/12.

Lassen sich Brechungsexponenten der Körper für Röntgenstrahlen experimentell ermitteln?

Von A. Einstein.

(Eingegangen am 21. März 1918.)

Vor einigen Tagen erhielt ich von Herrn Prof. A. Köhler (Wiesbaden) eine kurze Arbeit[1]), in welcher eine auffallende Erscheinung bei Röntgenaufnahmen geschildert ist, die sich bisher nicht hat deuten lassen. Die reproduzierten Aufnahmen — zumeist menschliche Gliedmaßen darstellend — zeigen an der Kontur einen hellen Saum von etwa 1 mm Breite, in welchem die Platte heller bestrahlt zu sein scheint als in der (nicht beschatteten) Umgebung des Röntgenbildes.

Ich möchte die Fachgenossen auf diese Erscheinung hinweisen und beifügen, daß die Erscheinung wahrscheinlich auf Totalreflexion beruht. Nach der klassischen Dispersionstheorie müssen wir erwarten, daß der Brechungsexponent n für Röntgenstrahlen nahe an 1 liegt, aber im allgemeinen doch von 1 verschieden ist. n wird kleiner bzw. größer als 1 sein, je nachdem der Einfluß derjenigen Elektronen auf die Dispersion überwiegt, deren Eigenfrequenz kleiner oder größer ist als die Frequenz der Röntgenstrahlen. Die Schwierigkeit einer Bestimmung von n liegt darin, daß $(n-1)$ sehr klein ist (etwa 10^{-5}). Es ist aber leicht einzusehen, daß bei nahezu streifender Inzidenz der Röntgenstrahlen im Falle $n < 1$ eine nachweisbare Totalreflexion auftreten muß.

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X-ray refractive index

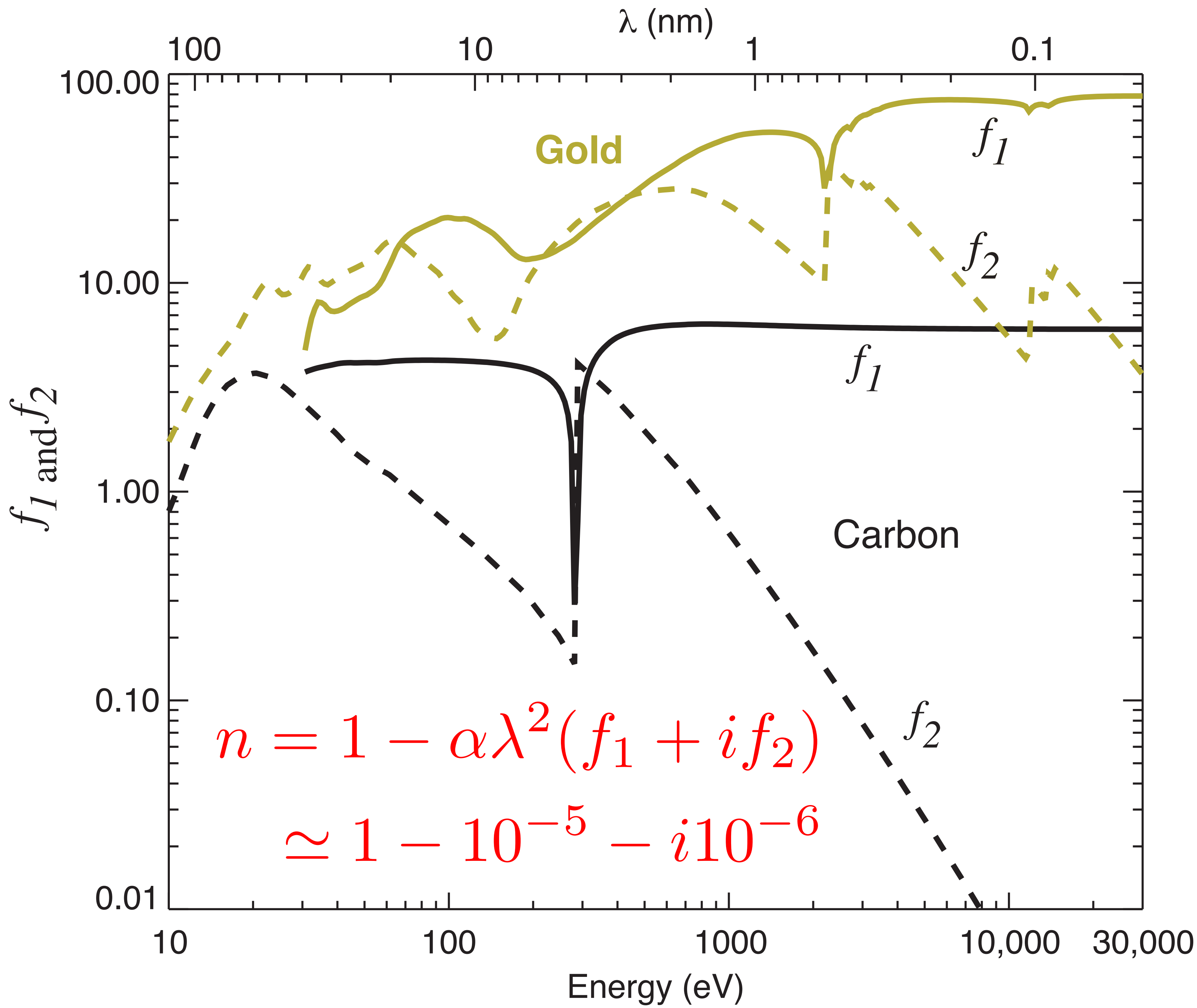
Refractive index of
$n=1-\alpha\lambda^2(f_1+if_2)$

Real part of oscillator strength f_1 tends towards atomic number Z

Imaginary part of oscillator strength f_2 declines as E^{-2}

f_1: phase $\exp[-ink]$ is advanced relative to vacuum by $2\pi\alpha\lambda f_1$

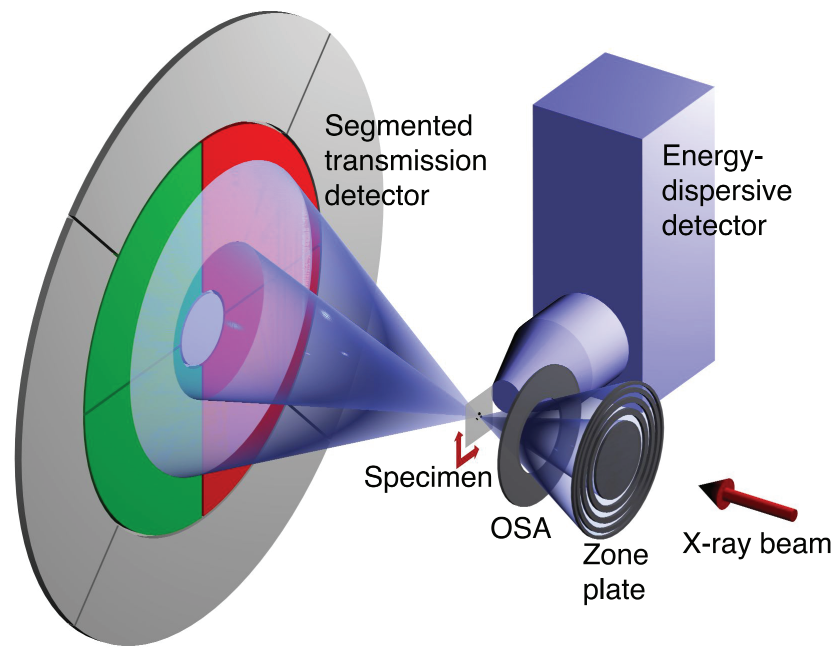
f_2: intensity is decreased as $\exp[-4\pi\alpha\lambda f_2]$

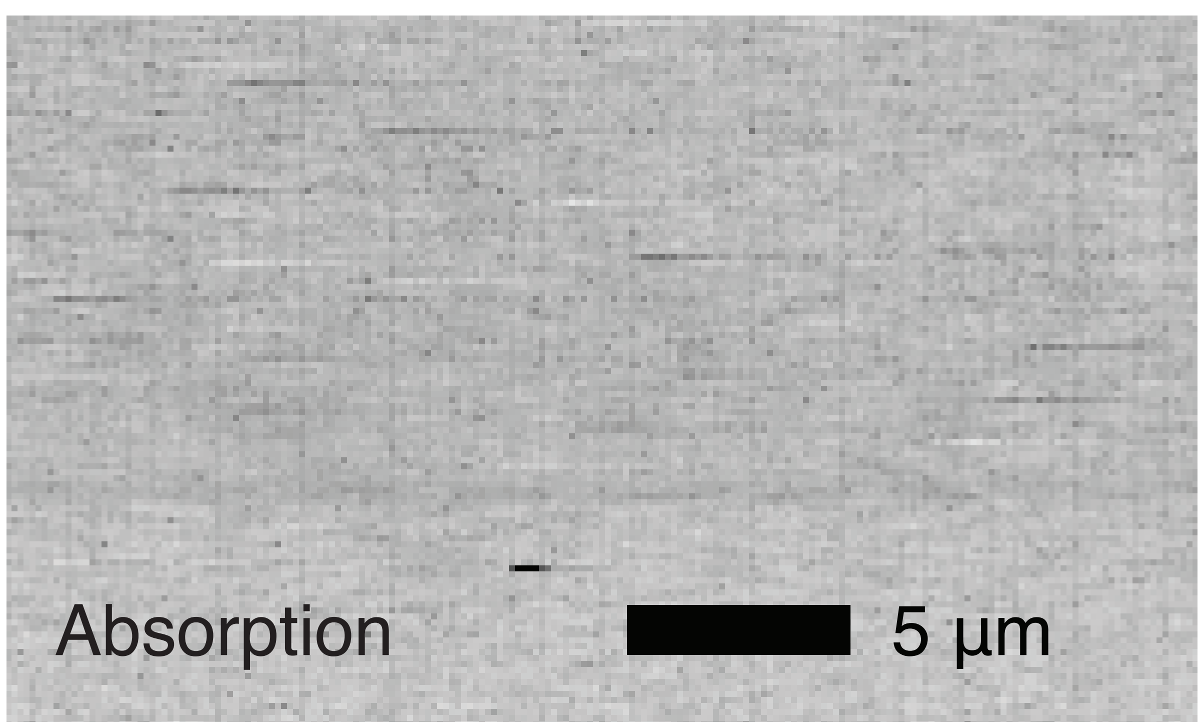


Data from http://henke.lbl.gov/optical_constants/

Transmission imaging: isn't it just x-ray attenuation?

- Image of a diatom taken using 10 keV X rays. Hornberger *et al.*, *J. Synchrotron Radiation* **15**, 355-362 (2008)





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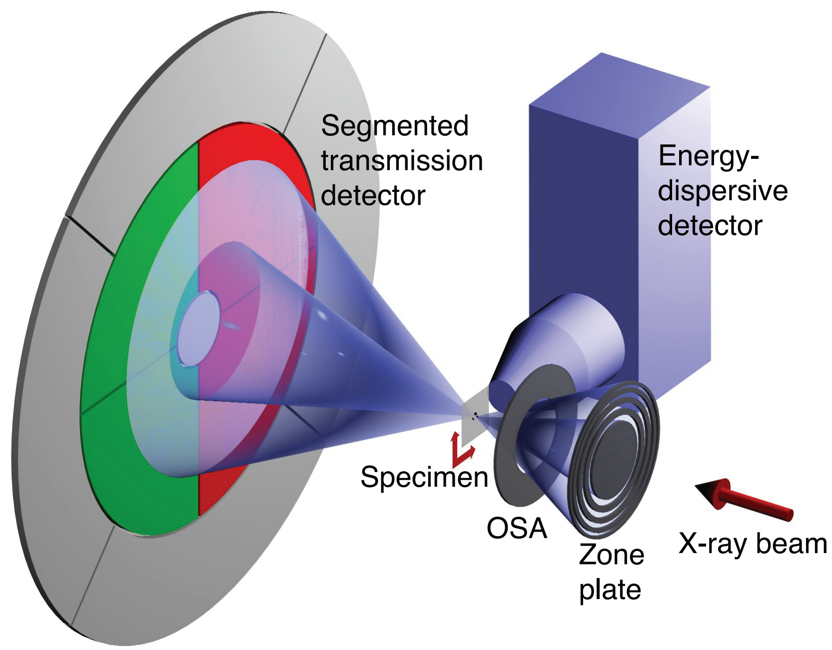
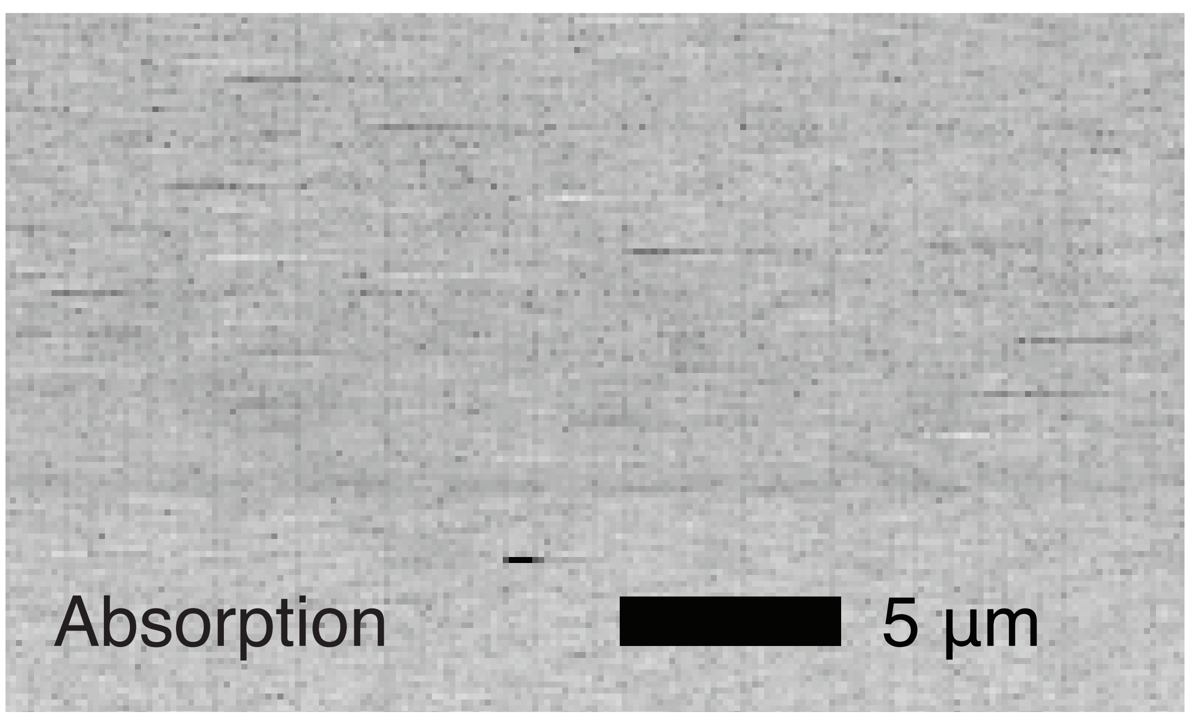
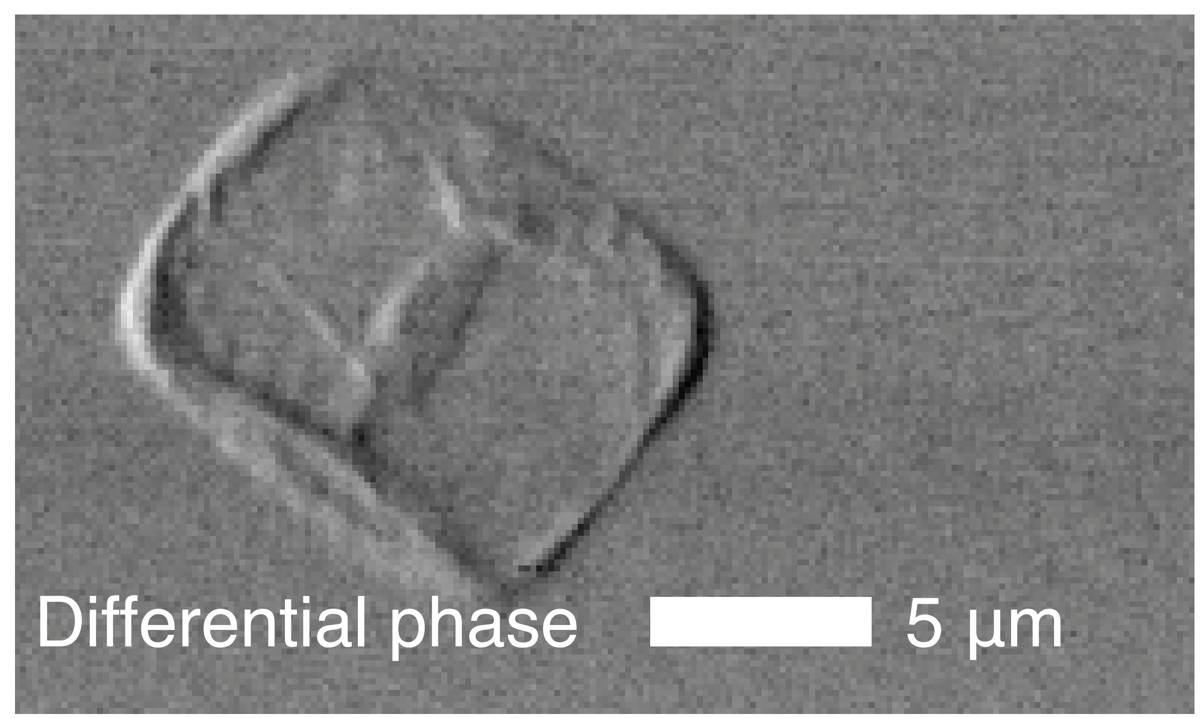


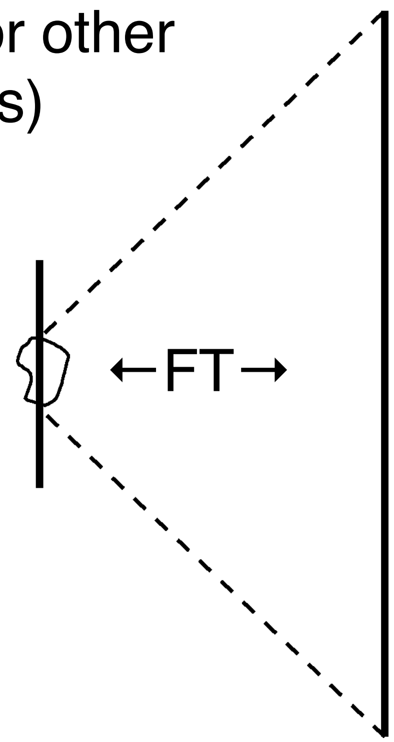




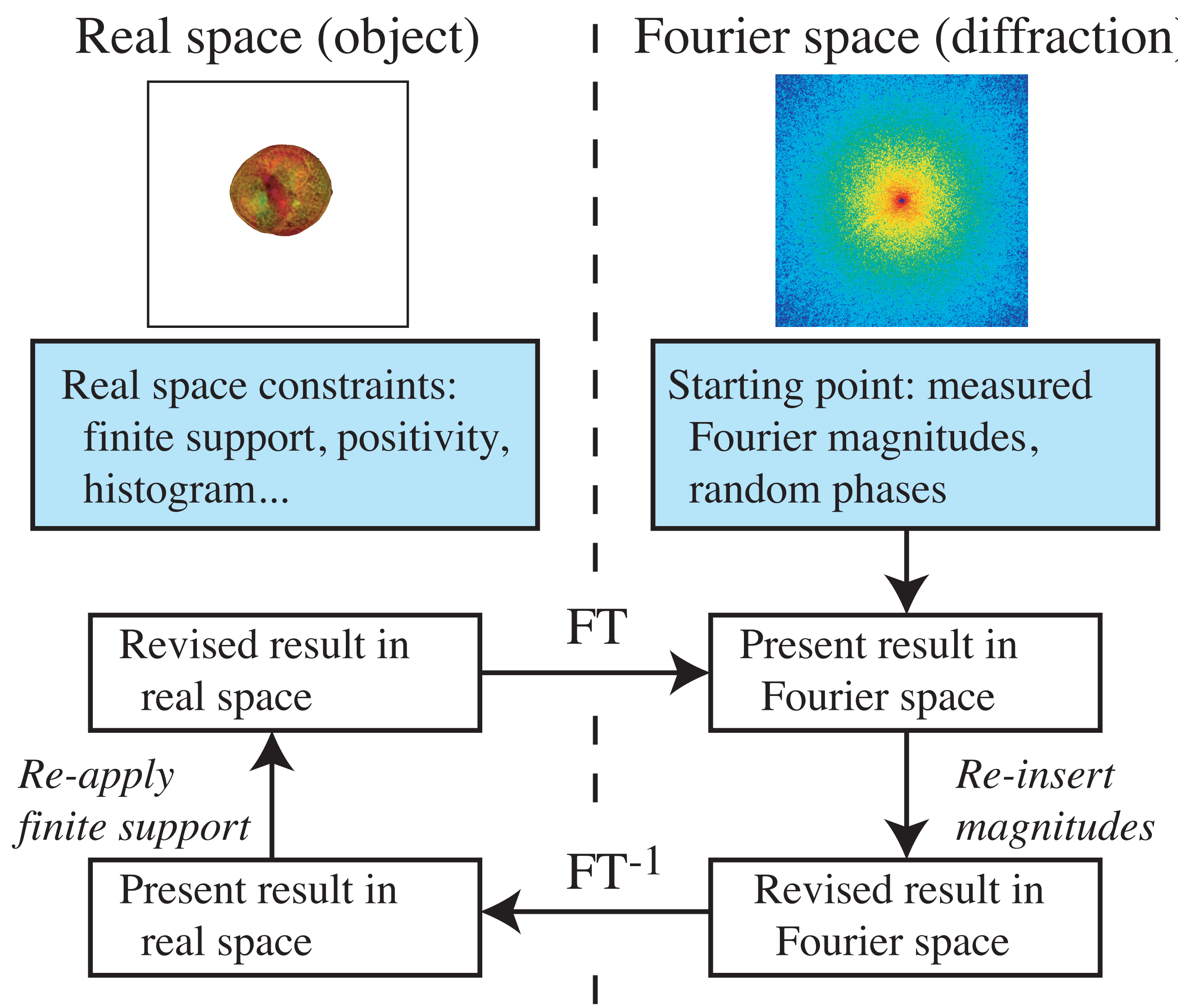
Imaging without lenses

- Avoid losses of lens efficiency and transfer function
- Must phase the diffraction intensities

Real space: finite support (or other constraints)



Fourier space: magnitudes known, but phases are not

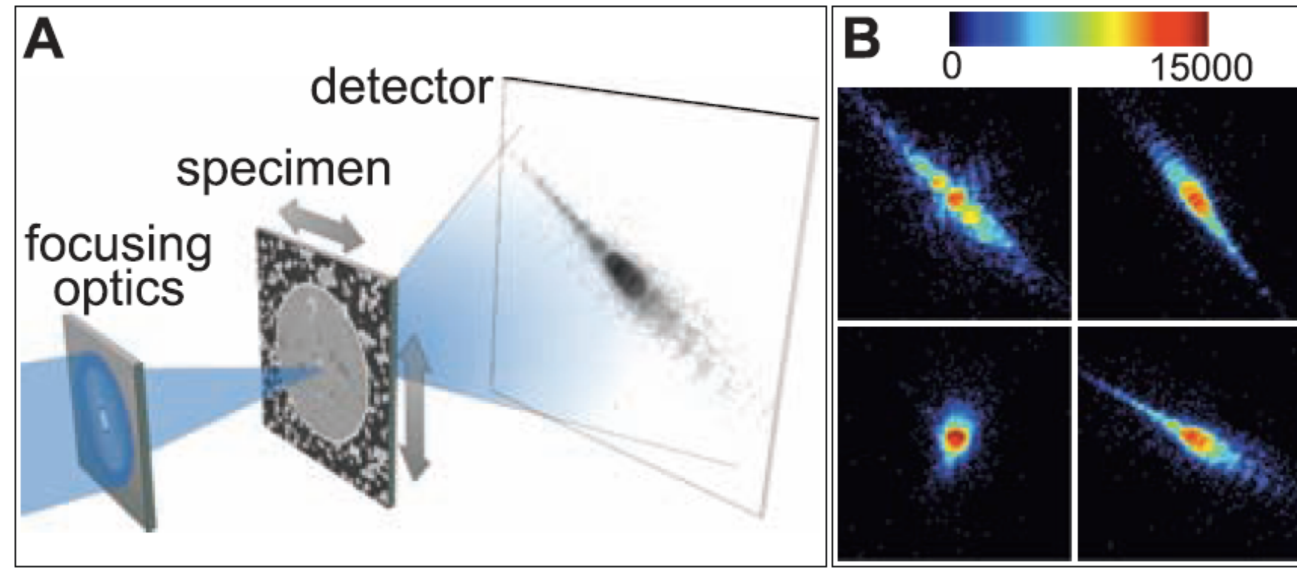


Phasing algorithms: Gerchberg and Saxton, *Optik* **37**, 237 (1972); Feinup, *Opt. Lett.* **3**, 27 (1978). First x-ray demonstration: Miao *et al.*, *Nature* **400**, 342 (1999).

Ptychography: overlapping illumination spots ease phasing

- First discussion: Hoppe, *Acta Cryst. A* **25**, 495 (1969)
- Iterative phase retrieval from single diffraction patterns: Fienup *et al.*, *Opt. Lett.* **3**, 27 (1978)

- Iterative ptychography algorithms: Faulkner and Rodenburg, *Phys. Rev. Lett.* **93**, 023903 (2004); Thibault *et al.*, *Science* **321**, 379 (2008).
- X-ray demonstration of different ptychography algorithms: Rodenburg *et al.*, *Phys. Rev. Lett.* **98**, 034801 (2007); Thibault *et al.*, *Science* **321**, 379 (2008)



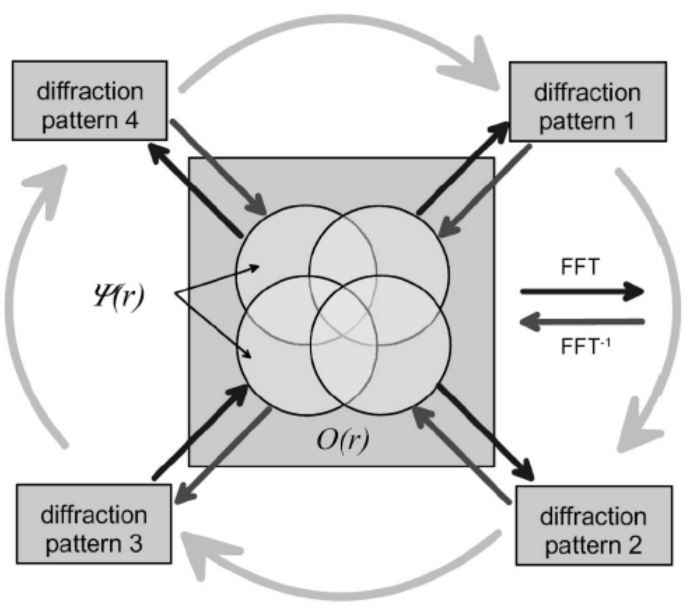
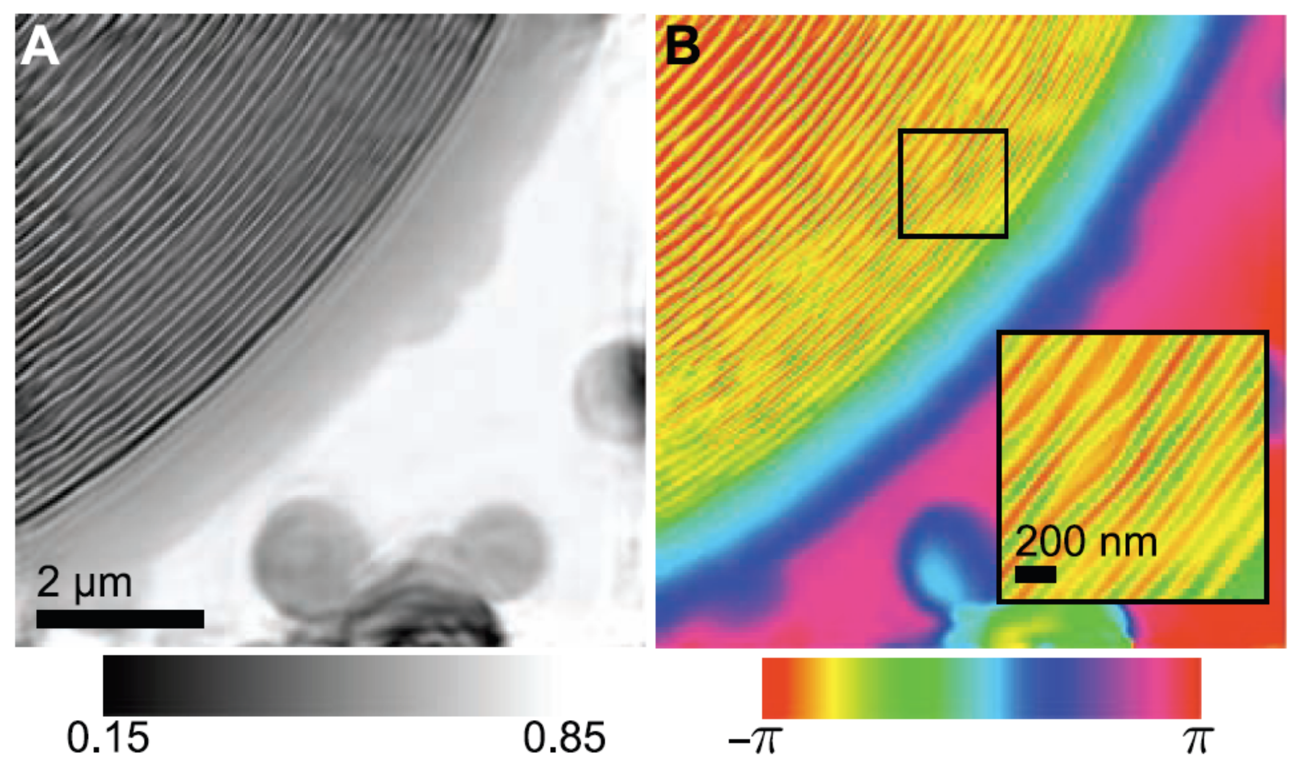


FIG. 2. Diagram of the phase-retrieval algorithm. The outer circular arrows indicate the position stepping within one iteration. The arrows within indicate (inverse) Fourier transforms and the desired input-output information.

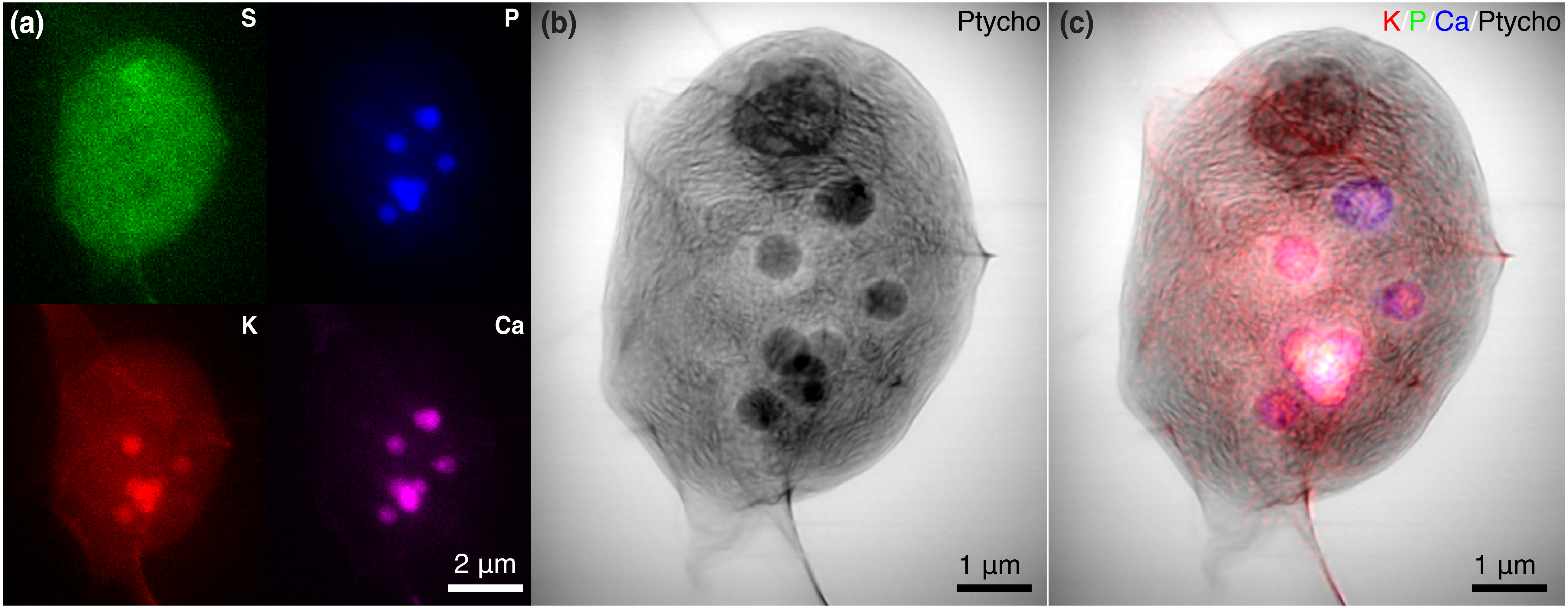
Rodenburg 2007



Thibault 2008

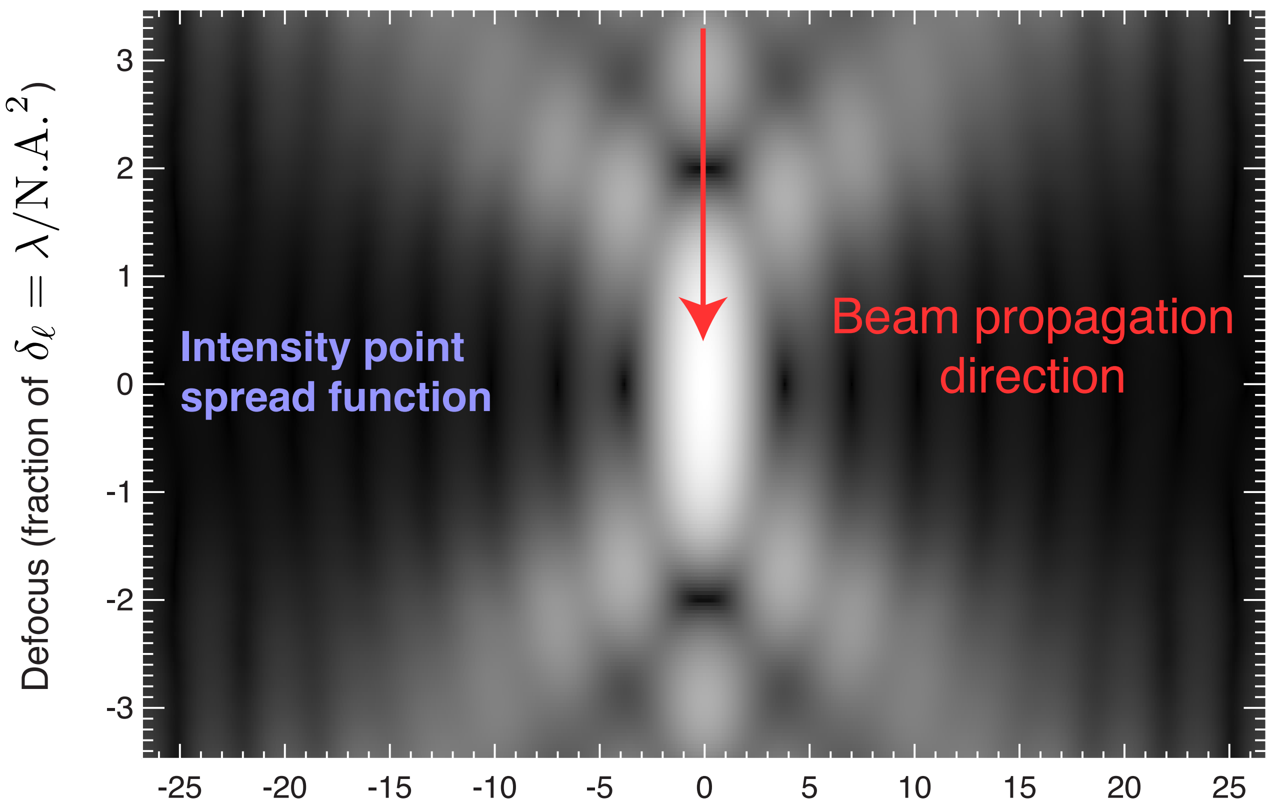
Combined x-ray ptychography and fluorescence

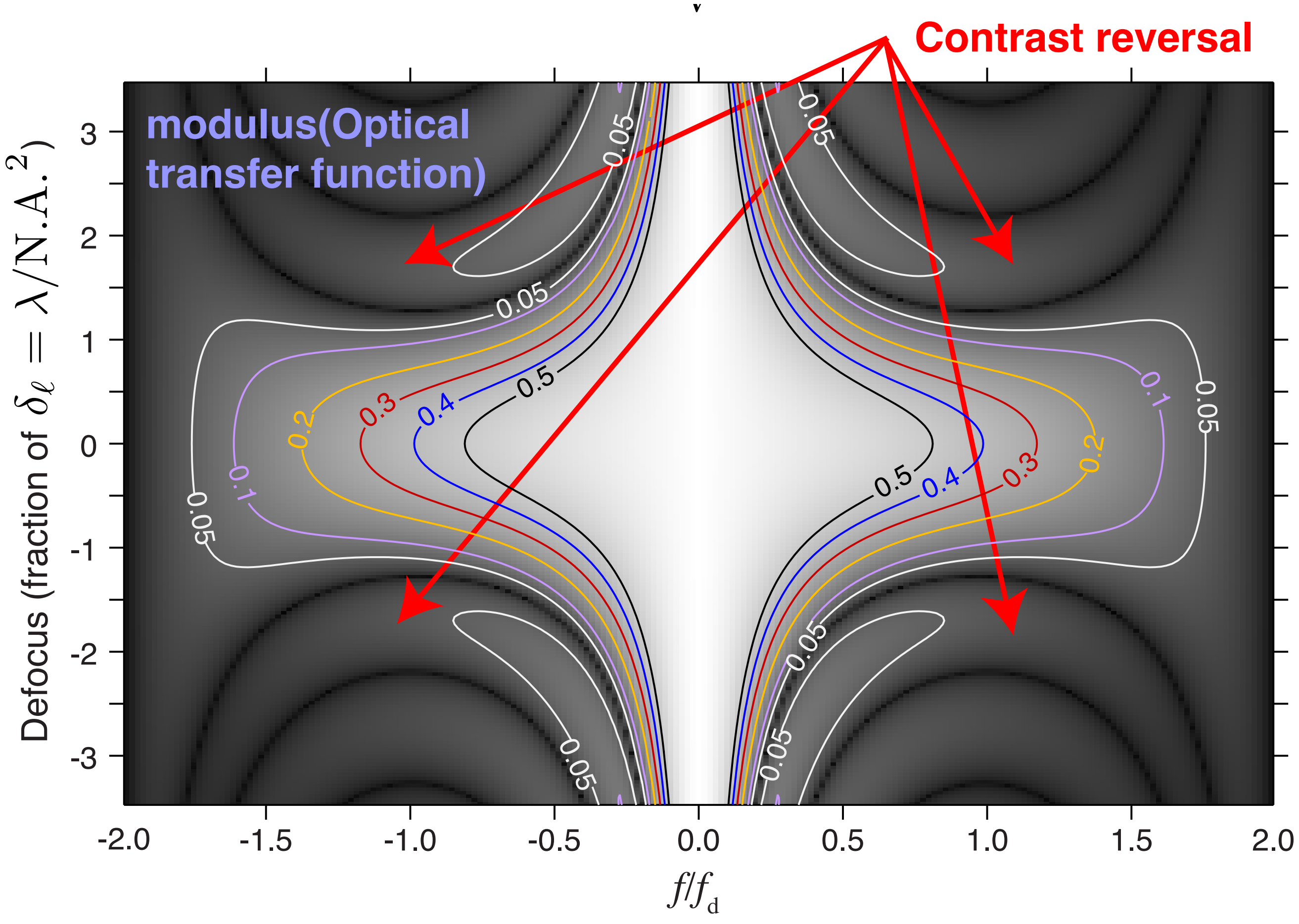
- *Chlamydomonas reinhardtii*, frozen in <0.1 msec from the living state, imaged whole under cryogenic conditions.
- 80 nm resolution in fluorescence, 18 nm resolution in ptychography
- Deng, Vine, Chen, Jin, Nashed, Peterka, Vogt, and Jacobsen, *Scientific Reports* **7**, 445 (2017)



Going beyond the depth of focus

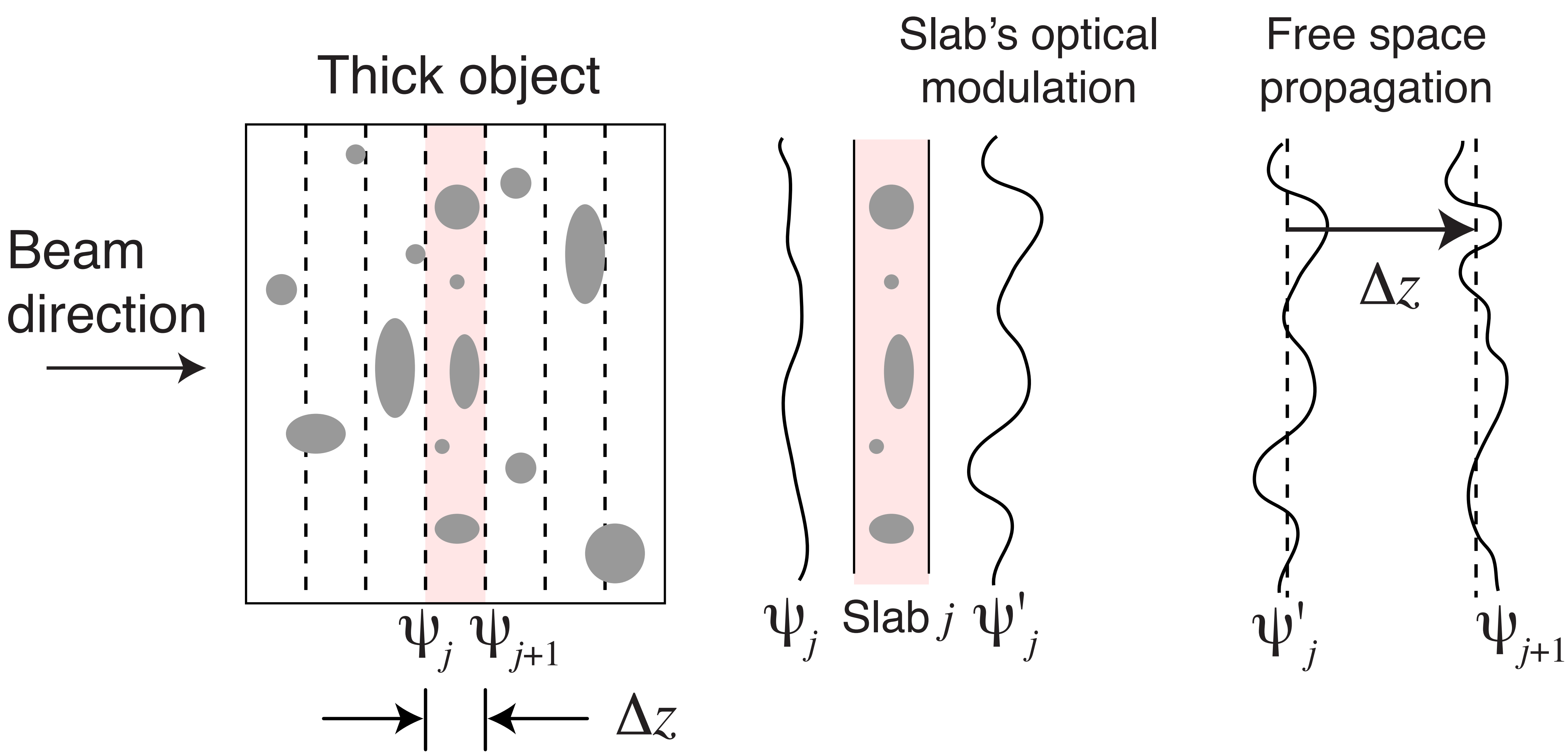
- Classical optics: depth of focus goes like ~5(transverse resolution)²/λ
- Past work: 5•(1 μm)² @ 25 keV=10 cm
- Future: 5•(30 nm)² @ 25 keV=90 μm
- Tomography normally ignores this, and assumes one records a pure projection through the specimen with no diffraction effects (or multiple scattering).





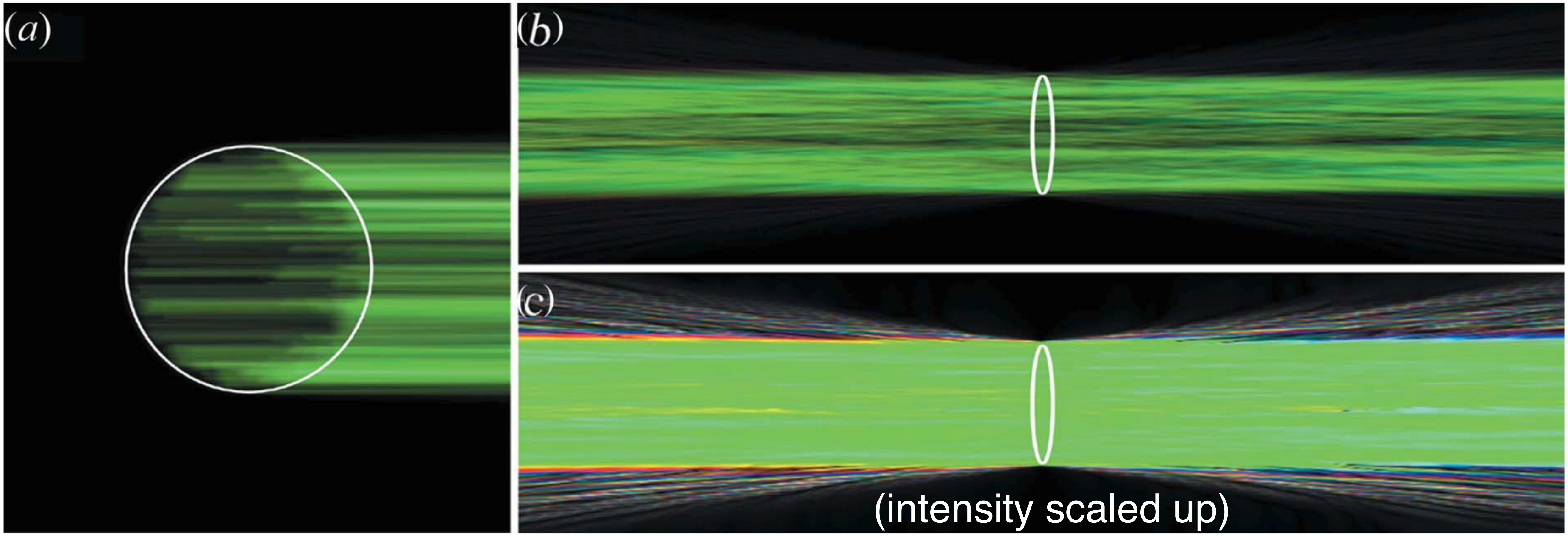
The multislice method

- Multislice method: Cowley and Moodie, *Proceedings of the Physical Society of London B* **70**, 486 (1957); *Acta Crystallographica* **10**, 609 (1957).
- Visible light: beam propagation method. Van Roey *et al.*, *JOSA* **71**, 803 (1981).
- Accounts for multiple scattering effects, but not backscattering.
- It even reproduces mirror reflectivity and waveguide effects in x-ray optics! Li and Jacobsen, *Optics Express* **25**, 1831 (2017).



Beyond the depth of focus, and Kierkegaard

- Søren Kierkegaard (1813-1855): “life must be lived forwards, but it is best understood backwards”
- Wavefield through a simulated cell: Thibault, Elser, Jacobsen, Shapiro, and Sayre, *Acta Cryst. A* **62**, 248 (2006).



Forward multislice propagation of a wave through a simulated cell

Backward propagation of the correctly-phased far-field wavefield

Numerical optimization approach

- If you know the forward model A (multislice), you can recover the object x from the data y with the help of regularizers R.

$$||y - Ax|| + \lambda R$$

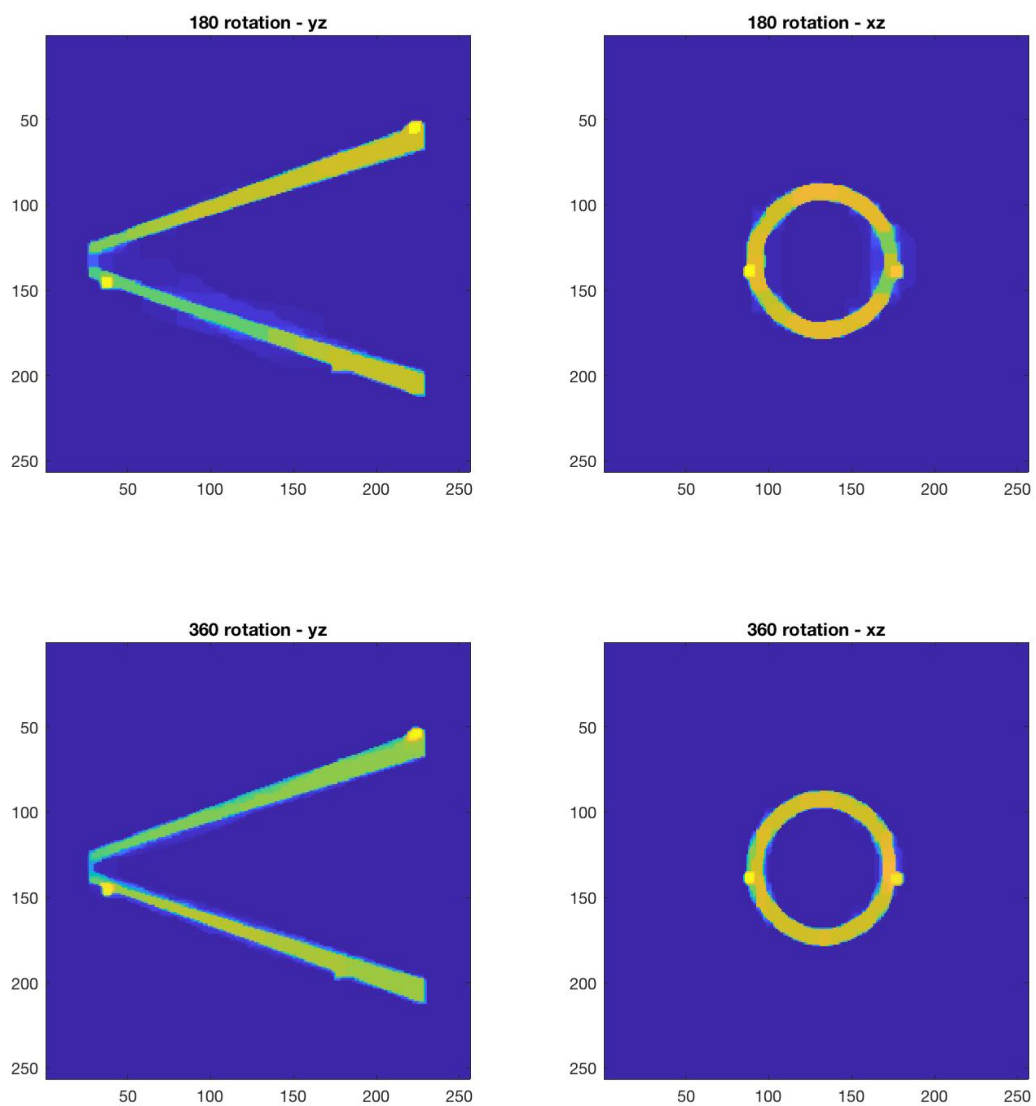
- No need for phase unwrapping.
- Examples of optimization approach for visible light diffraction tomography:
  - Kamilov *et al.*, *IEEE Transactions on Computational Imaging* **2**, 59 (2016).
  - Kostencka *et al.*, *Biomedical Optics Express* **7**, 4086 (2016)
  -

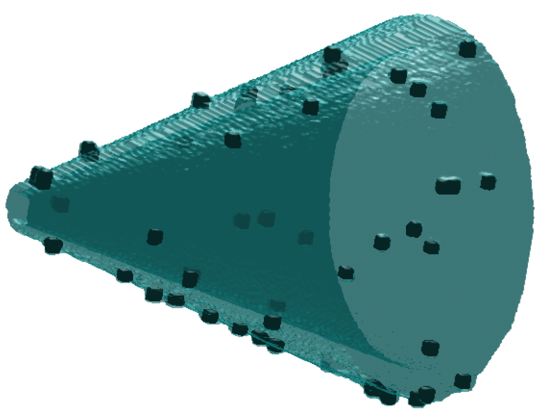
Multislice on continuous 3D objects

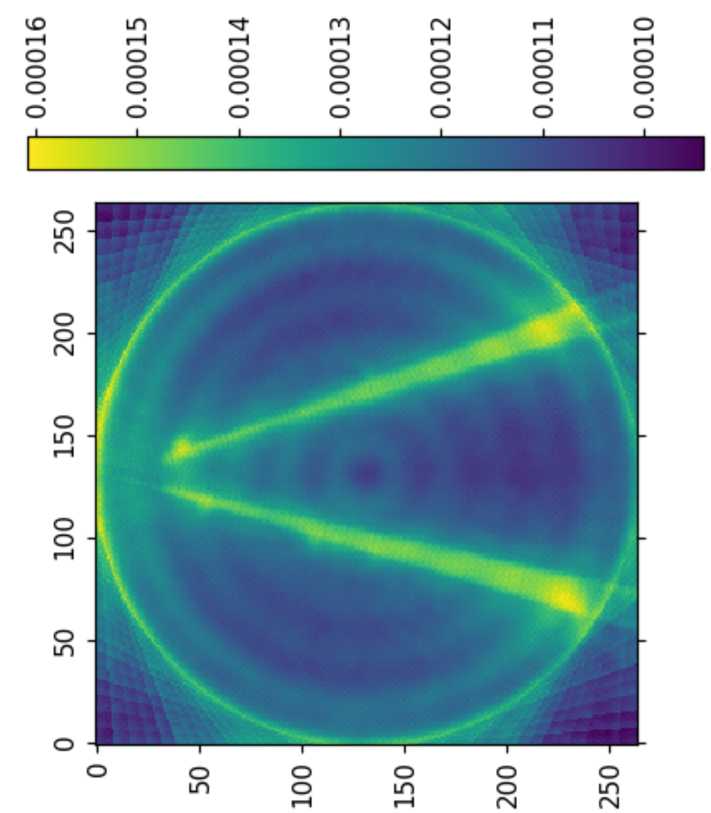
- Gilles, Du, Nashed, Jacobsen, and Wild, “3D X-ray imaging of continuous objects beyond the depth of focus limit,” *Optica* **5**, 1078 (2018).
  - 200k core hours for 256^3 with 23x23 probe positions, 70 viewing angles
  - Beyond pure projection approximation: important to rotate over 360° not 180°

180° rotation

360° rotation

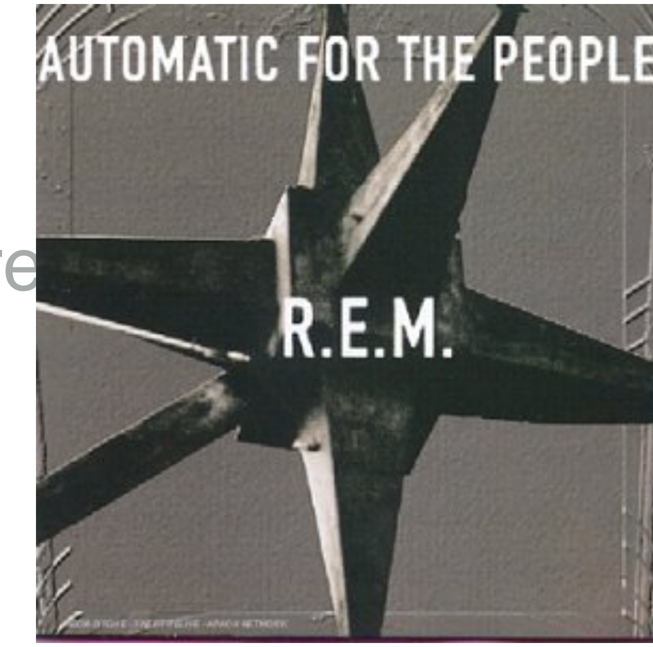






Standard ptychography reconstructions used as projections in tomography

Automatic for the people



- If you know the forward model A (multislice), you can re
  from the data y with the help of regularizers R.

$$||y - Ax|| + \lambda R$$

- Calculating derivatives on complex sequences of mathematical operations can be tedious!
- Let's let a computer do the work: calculate derivatives of operations expressed in computer code. Automatic Differentiation!
- Several toolkits available: TensorFlow (from Google), AutoGrad…
- Better performance on high performance clusters: Horovod version of TensorFlow (from Uber) which uses MPI instead of TCPIP for internode communication.

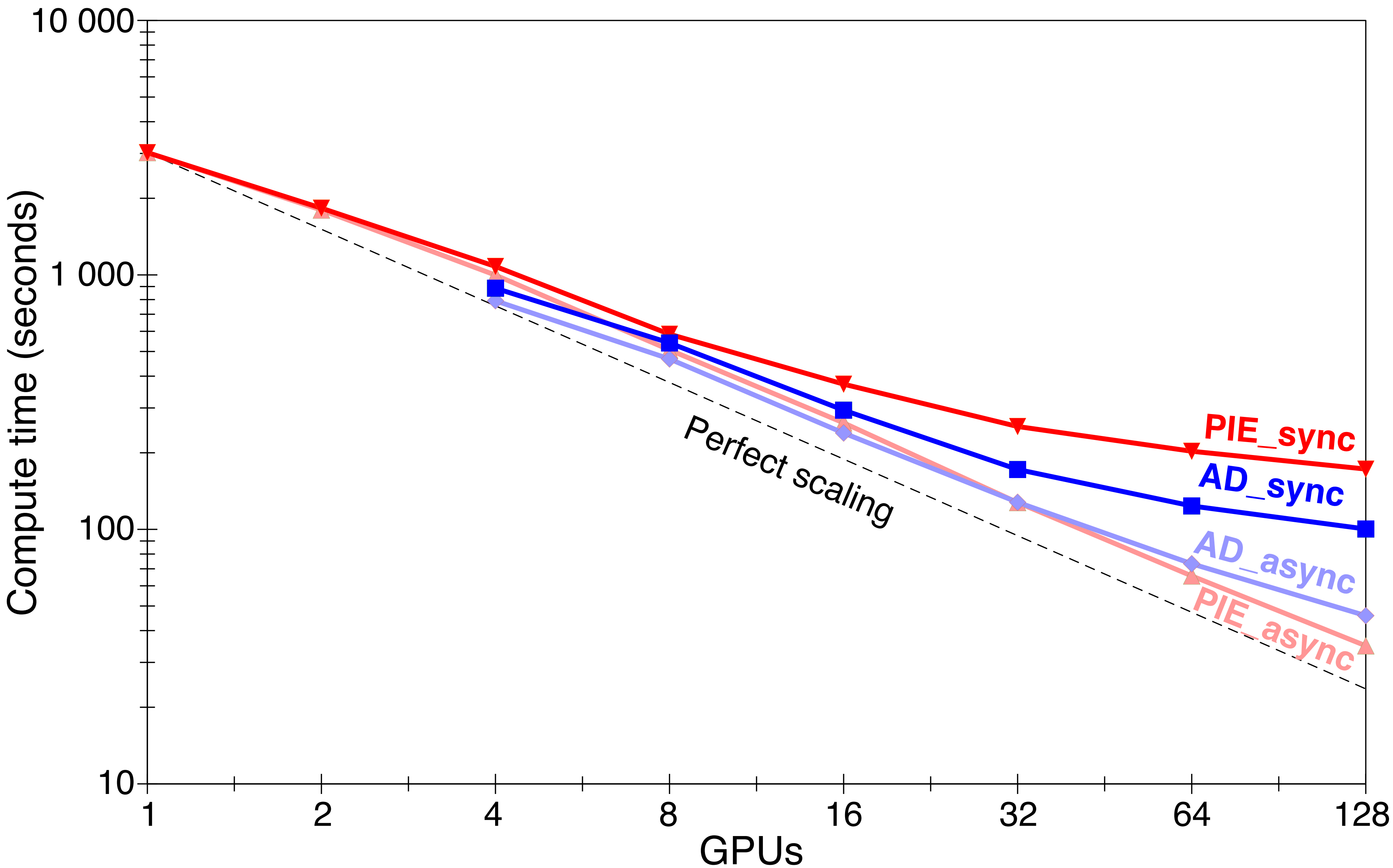
TensorFlow HOROVOD

Automatic differentiation is competitive!

- Y. Nashed, T. Peterka, J. Deng, and C. Jacobsen, *Procedia Computer Science* **108C**, 404 (2017). doi:10.1016/j.procs.2017.05.101
- PIE: Rodenburg and Maiden. AD: Tensor Flow's Automatic Differentiation
- **Sync: synchronize solutions from subregions at every iteration**
- Async: solve subregions (per computational node) and stitch together at the end

Simulation: 160x160 scan points with 90% overlap, 128x128 detector pixels (1.56 TBytes)

See also S. Kandel, S. Maddali, M. Allain, S. Hruszkewycz, C. Jacobsen, and Y. Nashed, *Optics Express* (accepted)



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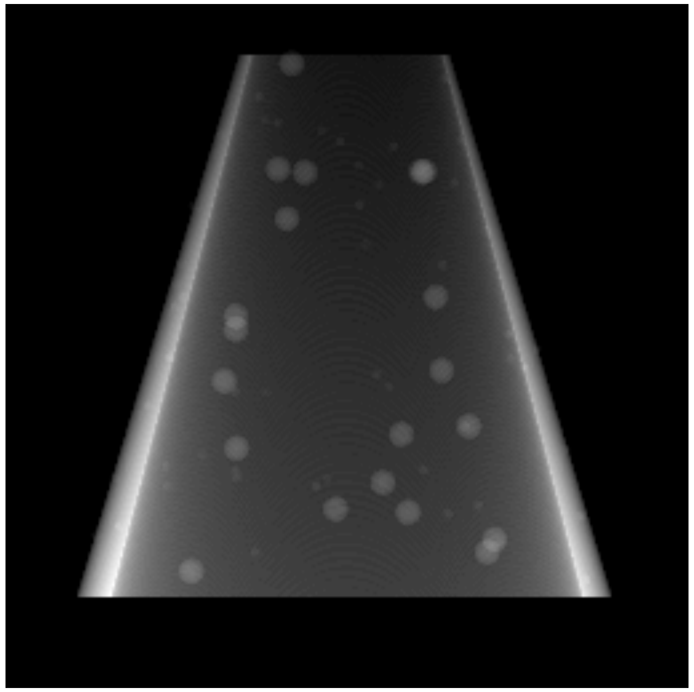
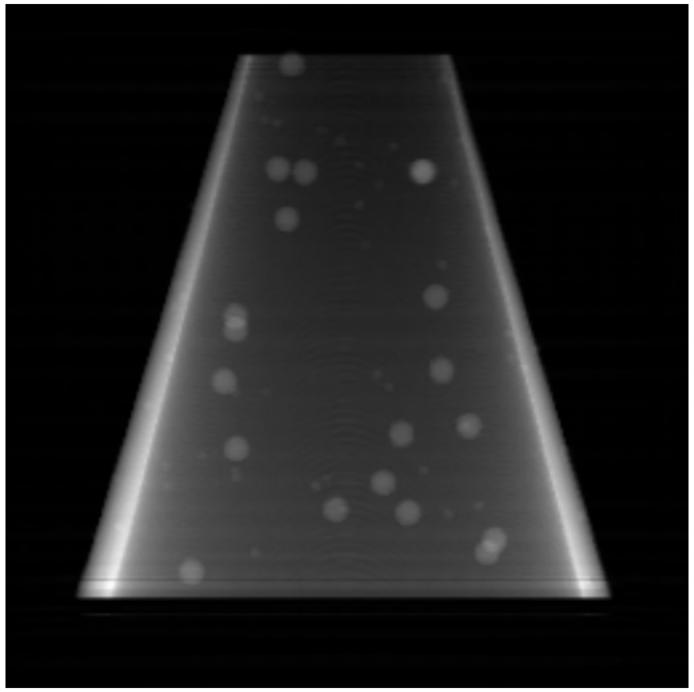
Recent activities in Automatic Differentiation

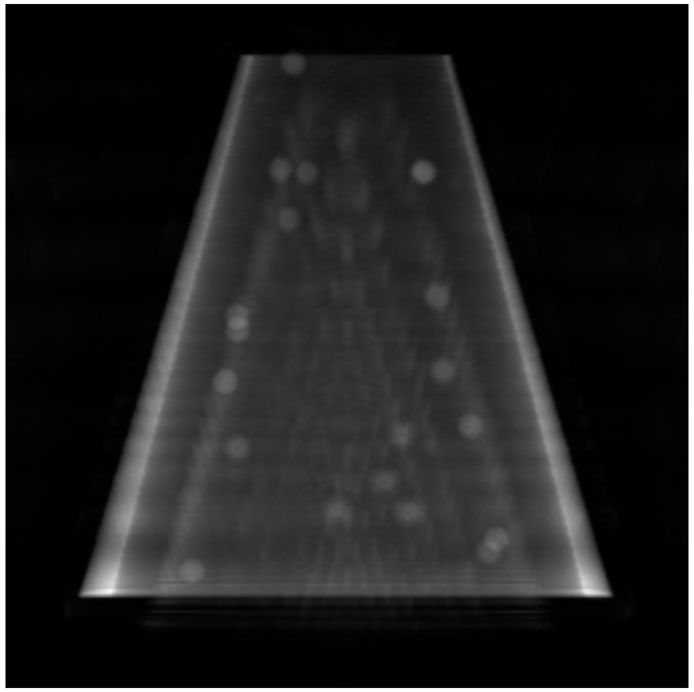
- Saugat Kandel: One framework can solve multiple inverse problems (far field, near field, Bragg diffraction). Kandel *et al.*, *Optics Express* **27**, 18653-18672 (2019).
- Saugat Kandel: second order methods in automatic differentiation (let the data determine the step size)
- Sajid Ali and Ming Du: alternative forward models based not on Fresnel propagation but the finite difference approximation to the Helmholtz equation
- Ming Du *et al.*: use Automatic Differentiation in TensorFlow to reconstruct 3D objects beyond the Pure Projection Approximation. 23x23 probe positions, 500 viewing angles, regularizers included. 16,500 core hours (46 clock hours) for $(256)^3$.



Projection through true object

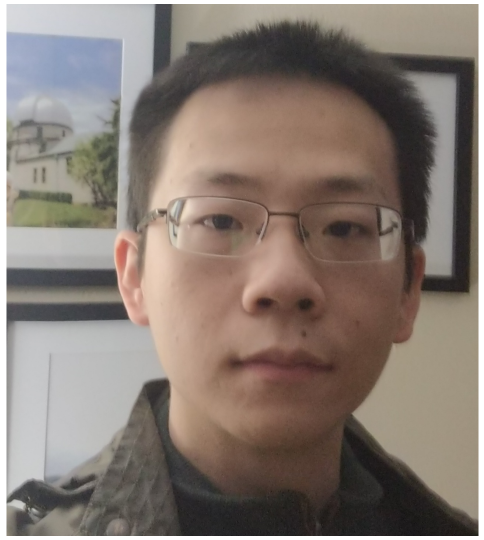


Projection through 3D reconstruction



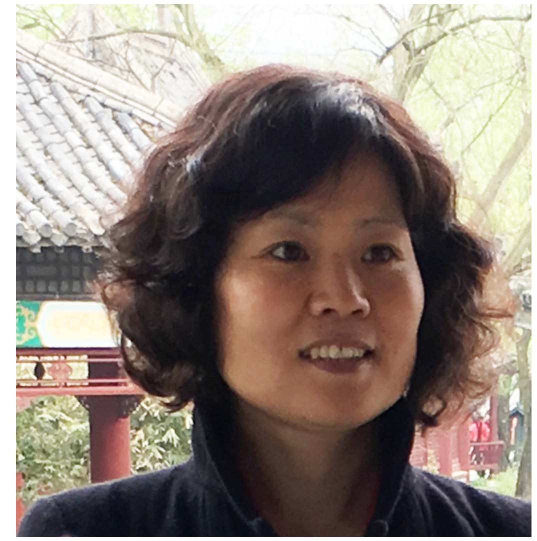
Conventional pure projection imaging

Sajid Ali
Applied Physics



Ming Du
Materials Science

Saugat Kandel
Applied Physics



Qiaoling Jin
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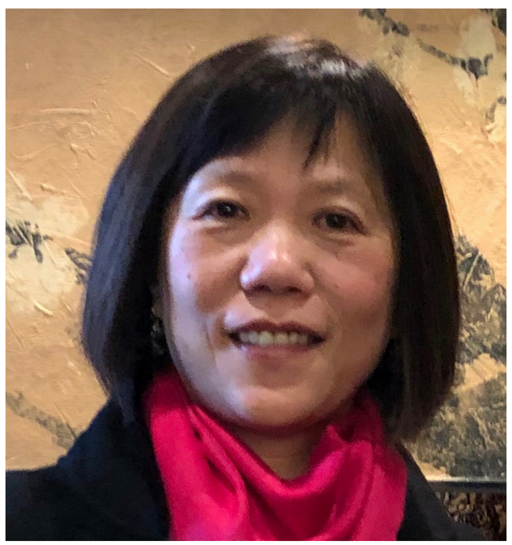

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