Scanning x-ray microscopy

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Argonne Leadership Computing Facility (ALCF)

EMC: Electron Microscopy Center

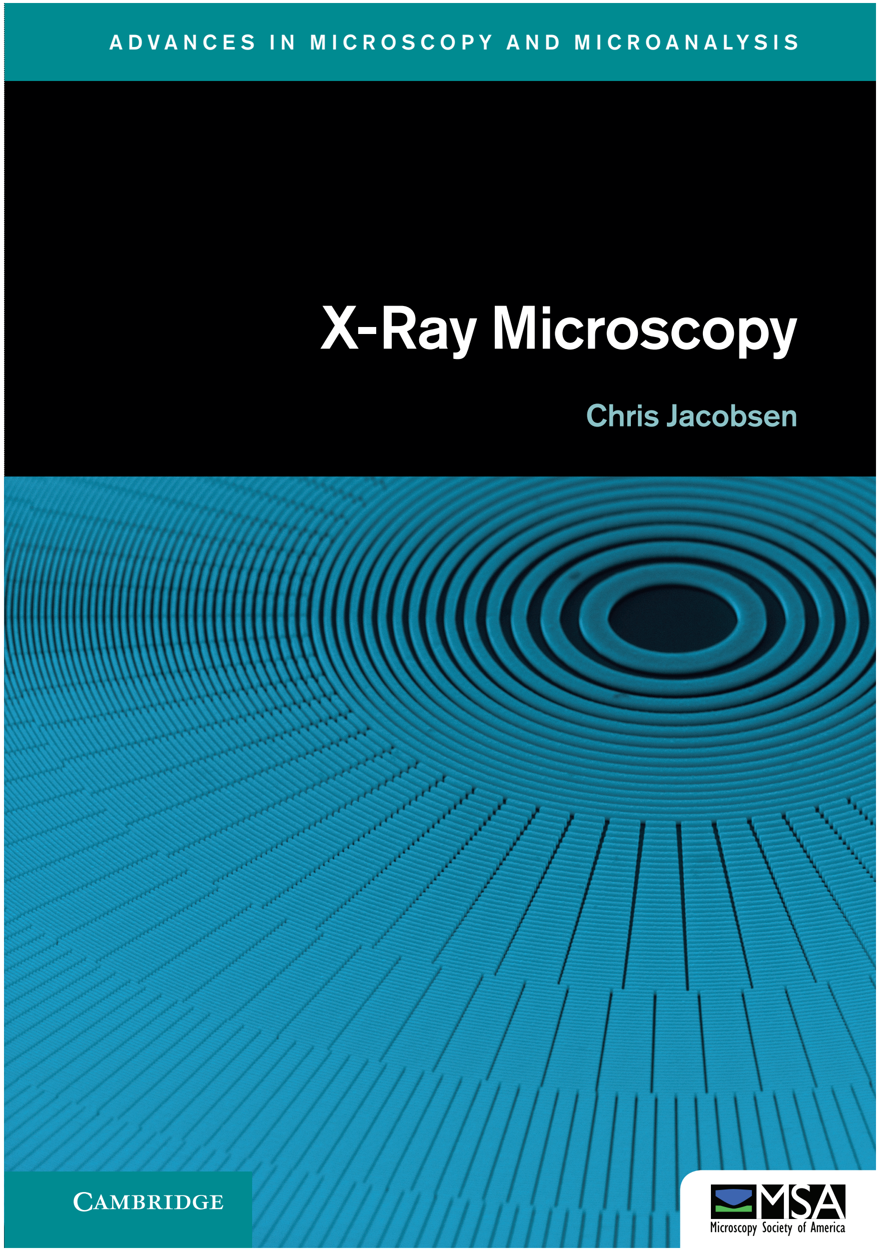
CNM: Center for Nanoscale Materials

Advanced Photon Source

APCF: Advanced Protein Crystallization Facility

X-ray Microscopy

- www.cambridge.org/Jacobsen
- 580 pages
- December 2019? January 2020?



ADVANCES IN MICROSCOPY AND MICROANALYSIS

X-Ray Microscopy

Chris Jacobsen

CAMBRIDGE

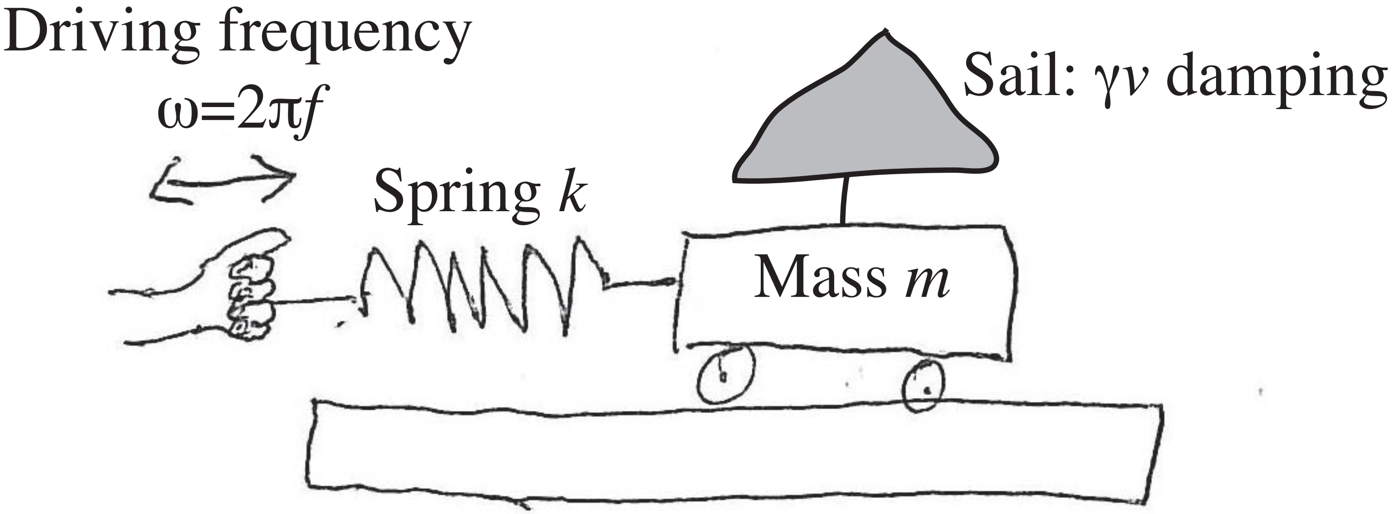
MSA
Microscopy Society of America

Outline

- **X-ray optics and x-ray lenses**
- Why scanning?
- Illumination considerations
- Instrumentation considerations
- Absorption spectromicroscopy
- CDI and ptychography

The refractive index

- Damped, driven harmonic oscillator



Driving frequency

ω=2π*f*

Spring *k*

Sail: *γv* damping

Mass *m*

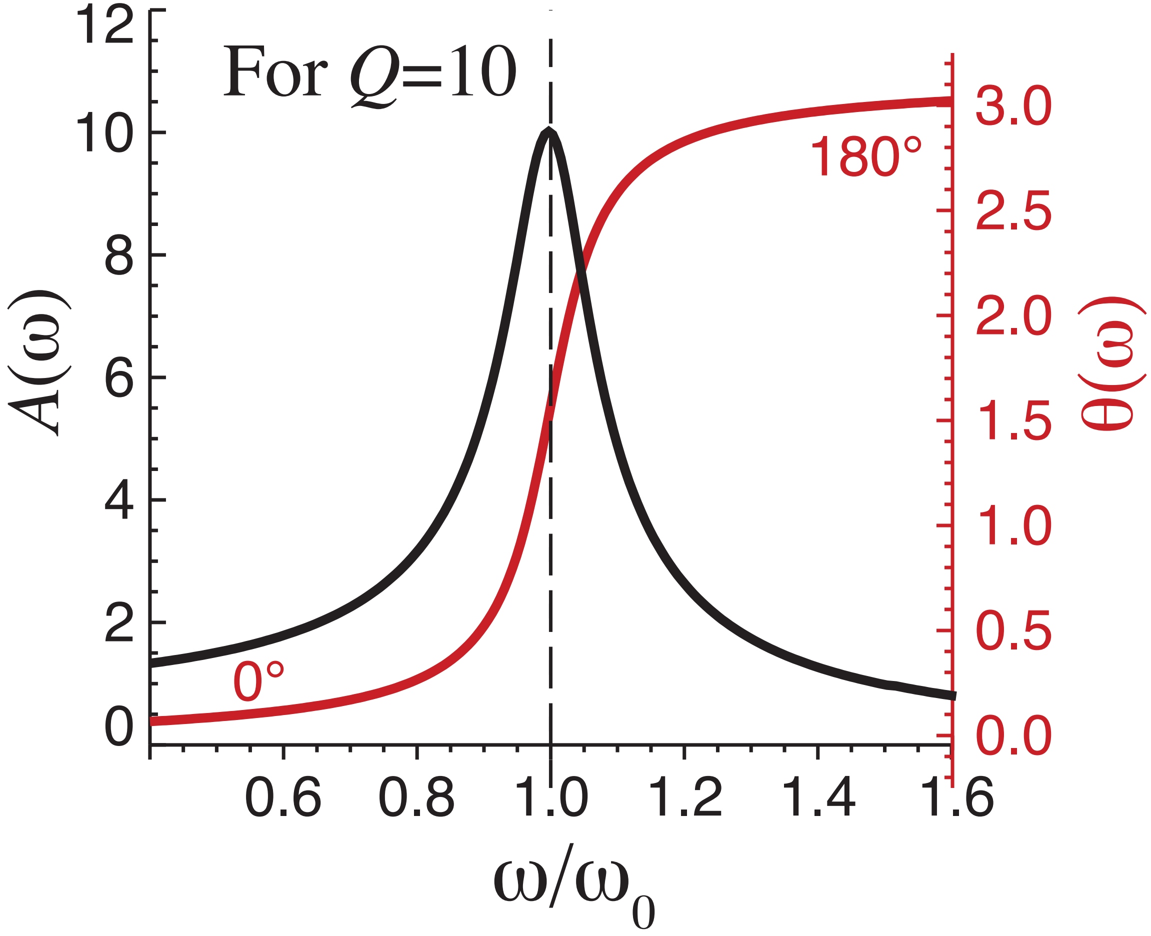
- Damped: scattering, absorption
- Driven: incident electromagnetic wave ω
- Harmonic oscillator: electronic quantum state with energy

$$\hbar\omega = \hbar\sqrt{k/m}$$

Damped, driven harmonic oscillator

- Single resonance: absorption peak, phase shift across resonance

$$Q = \omega_0/\gamma$$

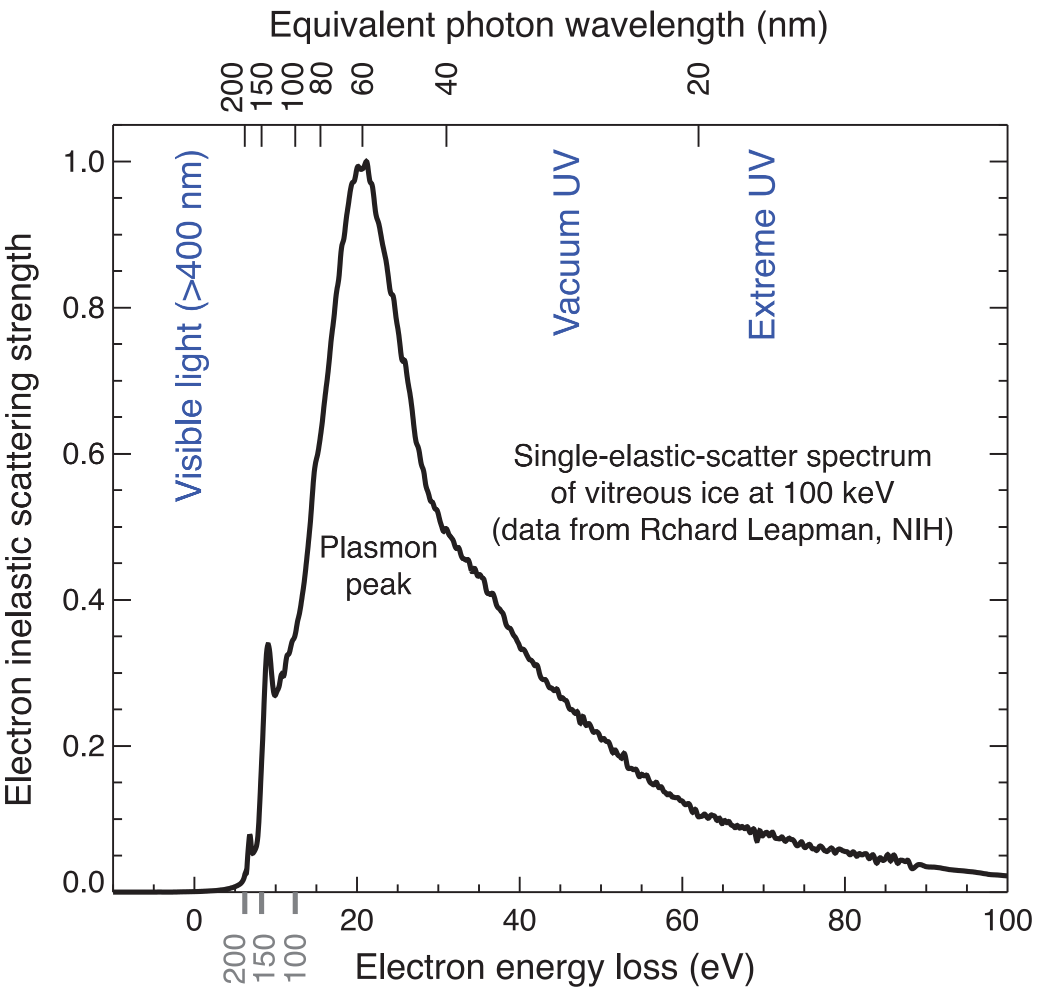


X-rays: the high frequency limit?

What's the dividing line between low and high frequency limits of refractive index? At what frequency are most of the oscillators?

Plasmon frequency

$\omega_p=(4\pi c^2 n_a r_e)^{1/2}$



Single-elastic-scatter spectrum of vitreous ice at 100 keV (data from Rchard Leapman, NIH)

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.

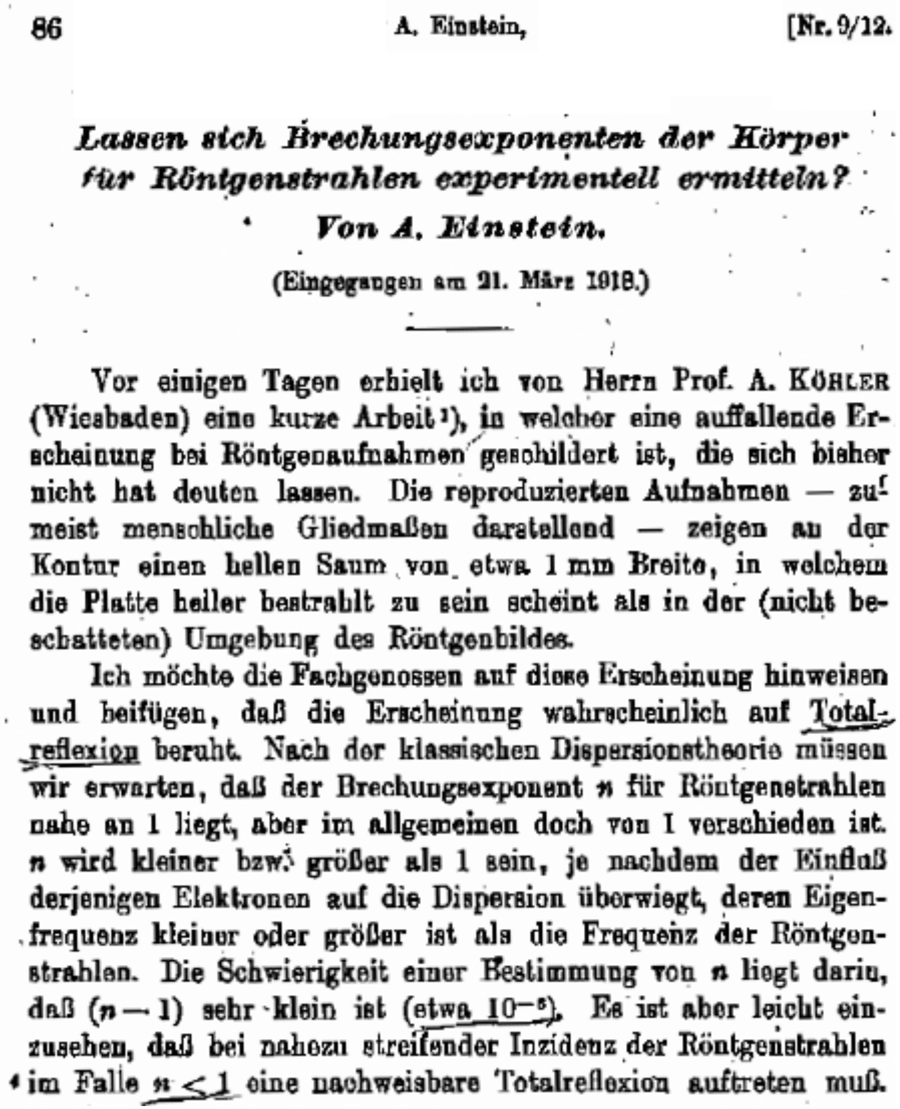
Also written as $n=1-\delta-i\beta$

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)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^{-6}). Es ist aber leicht einzusehen, daß bei nahezu streifender Inzidenz der Röntgenstrahlen im Falle $n < 1$ eine nachweisbare Totalreflexion auftreten muß.

X-ray refractive index

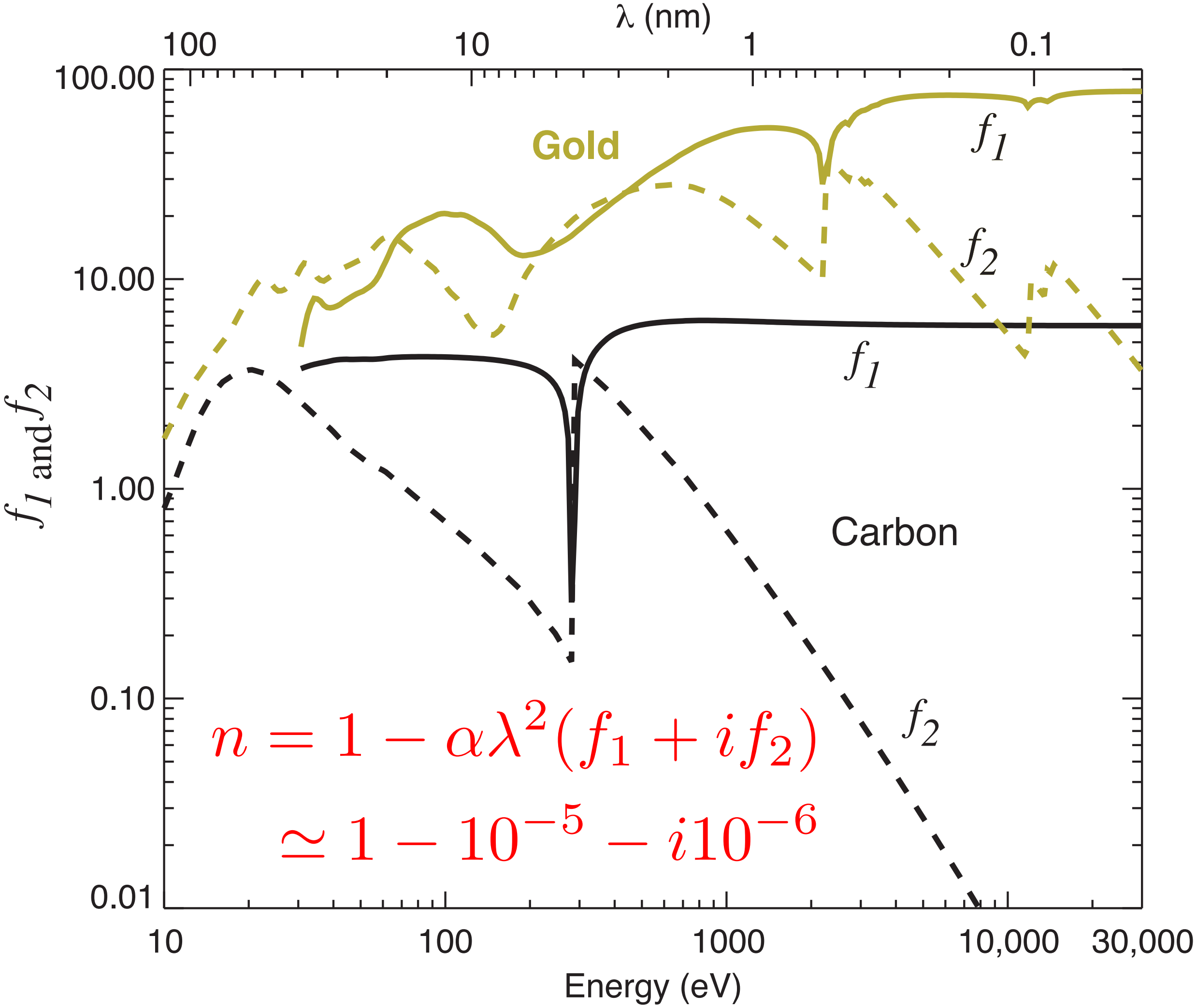
Refractive index of
$n=1-\alpha\lambda^2(f_1+if_2)$

Real part of oscillator strength f_1 tends towards atomic number Z

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

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

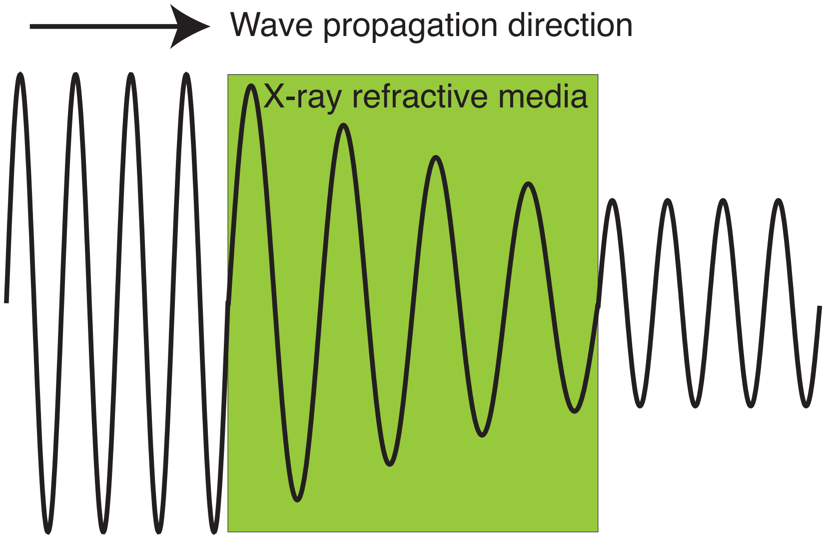
Intensity is decreased as exp[$-4\pi\alpha\lambda f_2$]

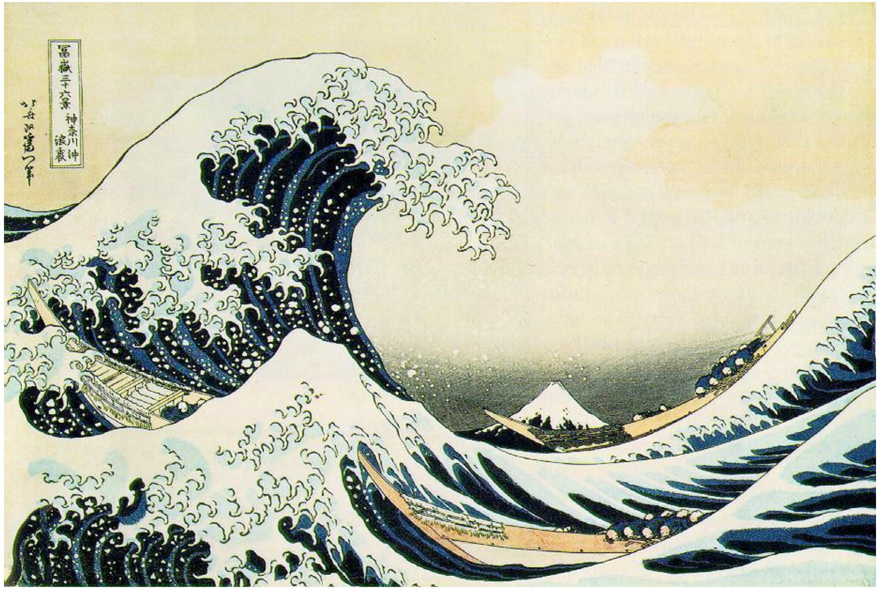


$$n = 1 - \alpha\lambda^2(f_1 + if_2)$$
$$\simeq 1 - 10^{-5} - i10^{-6}$$

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

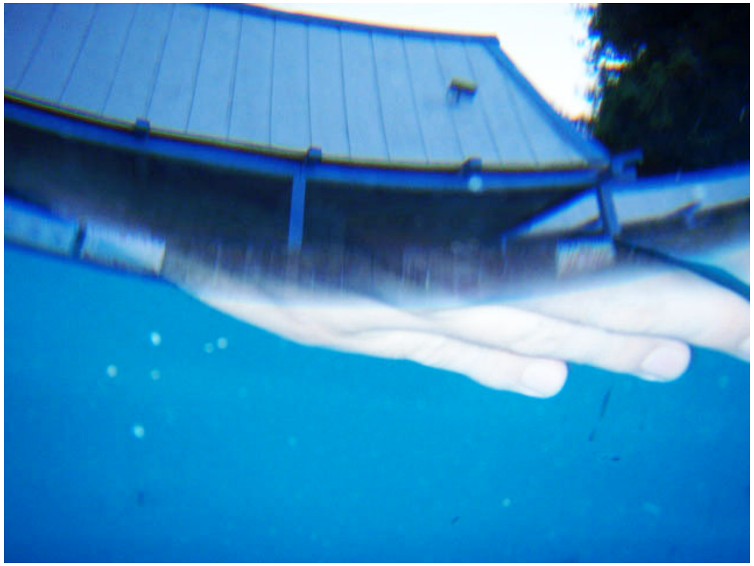
X rays in media

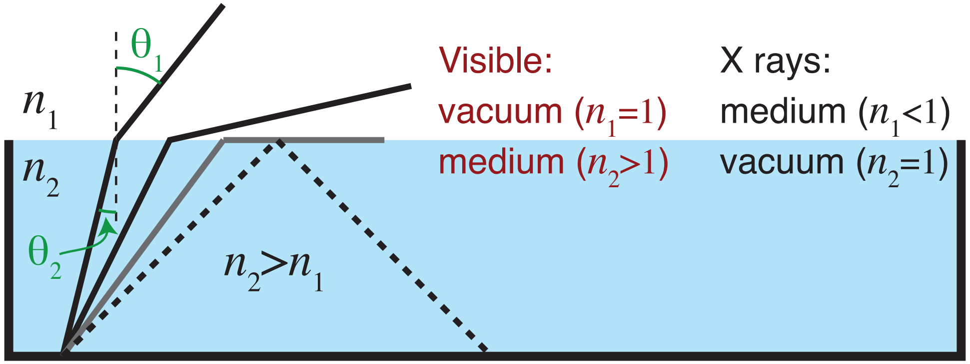


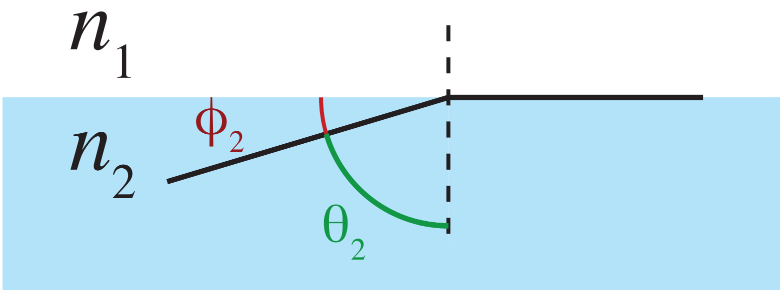


X-ray mirrors use total *internal* reflection!

- Total internal reflection happens when θ_1=90° in $n_1\sin\theta_1=n_2\sin\theta_2$, or θ_2=asin(n_1/n_2).

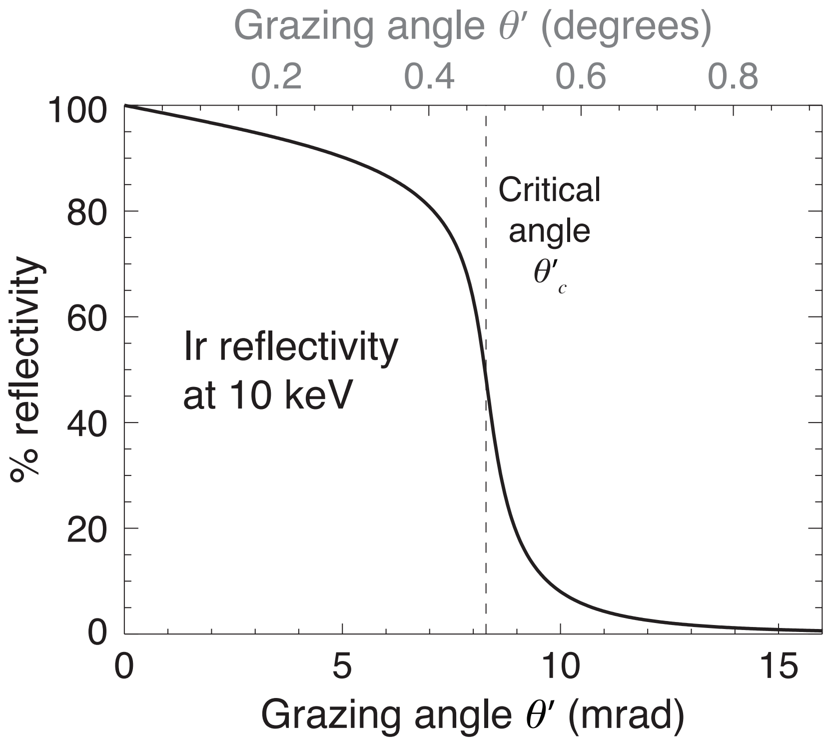


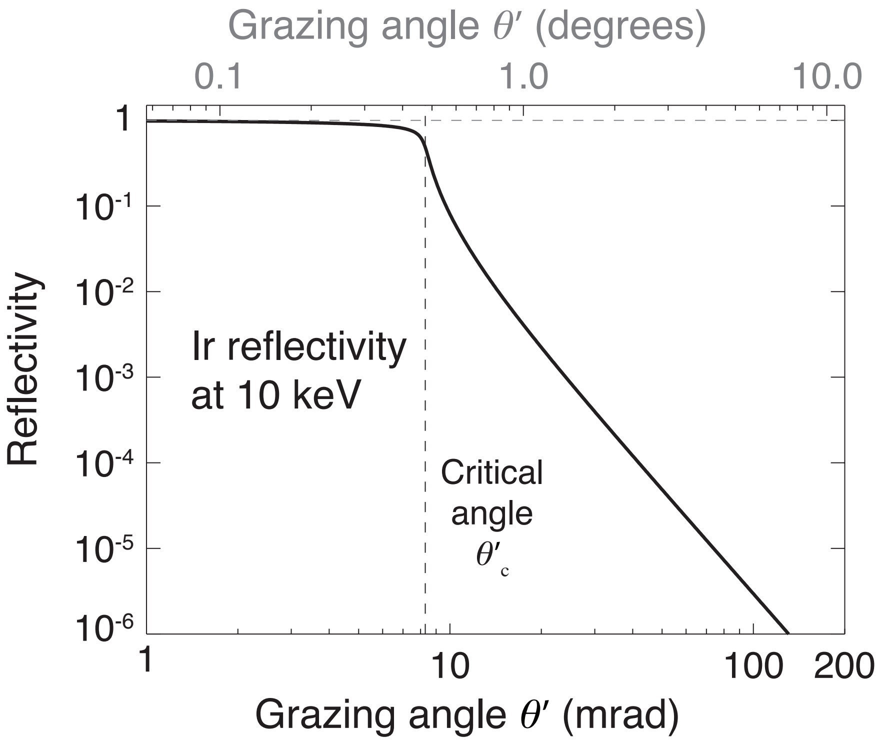




- Switch from angle θ_2 relative to normal incidence, to angle ϕ_2 relative to grazing incidence, or sin(θ_2)=cos(90°-ϕ_2)=sin(ϕ_2)
- We then have $n_1=n_2\sin(\phi_2)$ or with $n_2=1$ and $\sin(\phi_2)\approx1-(\phi_2)^2/2$ we have a grazing incidence critical angle of $\phi_2=\lambda(2\alpha f_1)^{1/2}$
- Note diffraction resolution limit of ϕ_2/λ is (almost) independent of wavelength!

X-ray mirror reflectivity

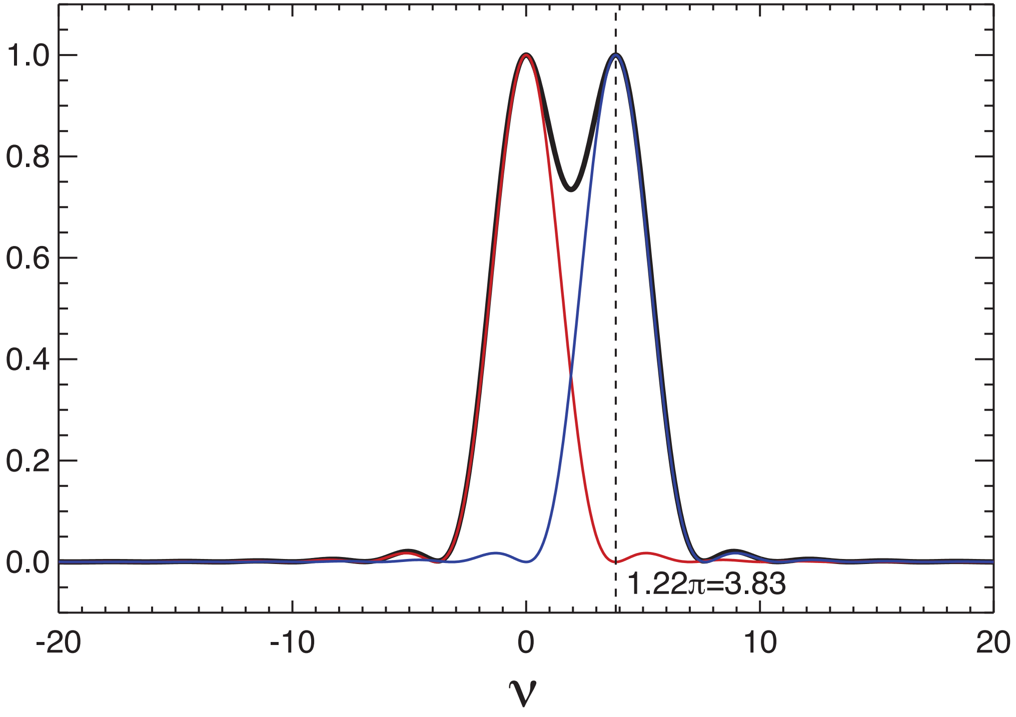




Lord Rayleigh (John William Strutt) and telescopes

- Diffraction from a circular aperture limits the angular resolution of telescopes
- Diffraction is described by the Airy function with a first minimum at ν=1.22π:

$$[2J_1(\nu)/\nu]^2$$



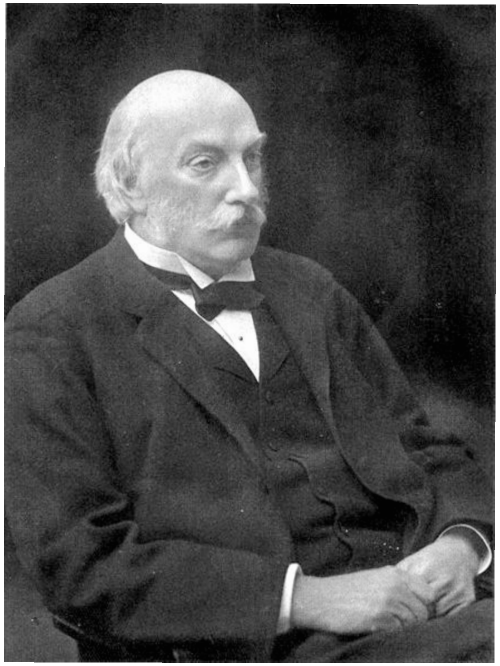
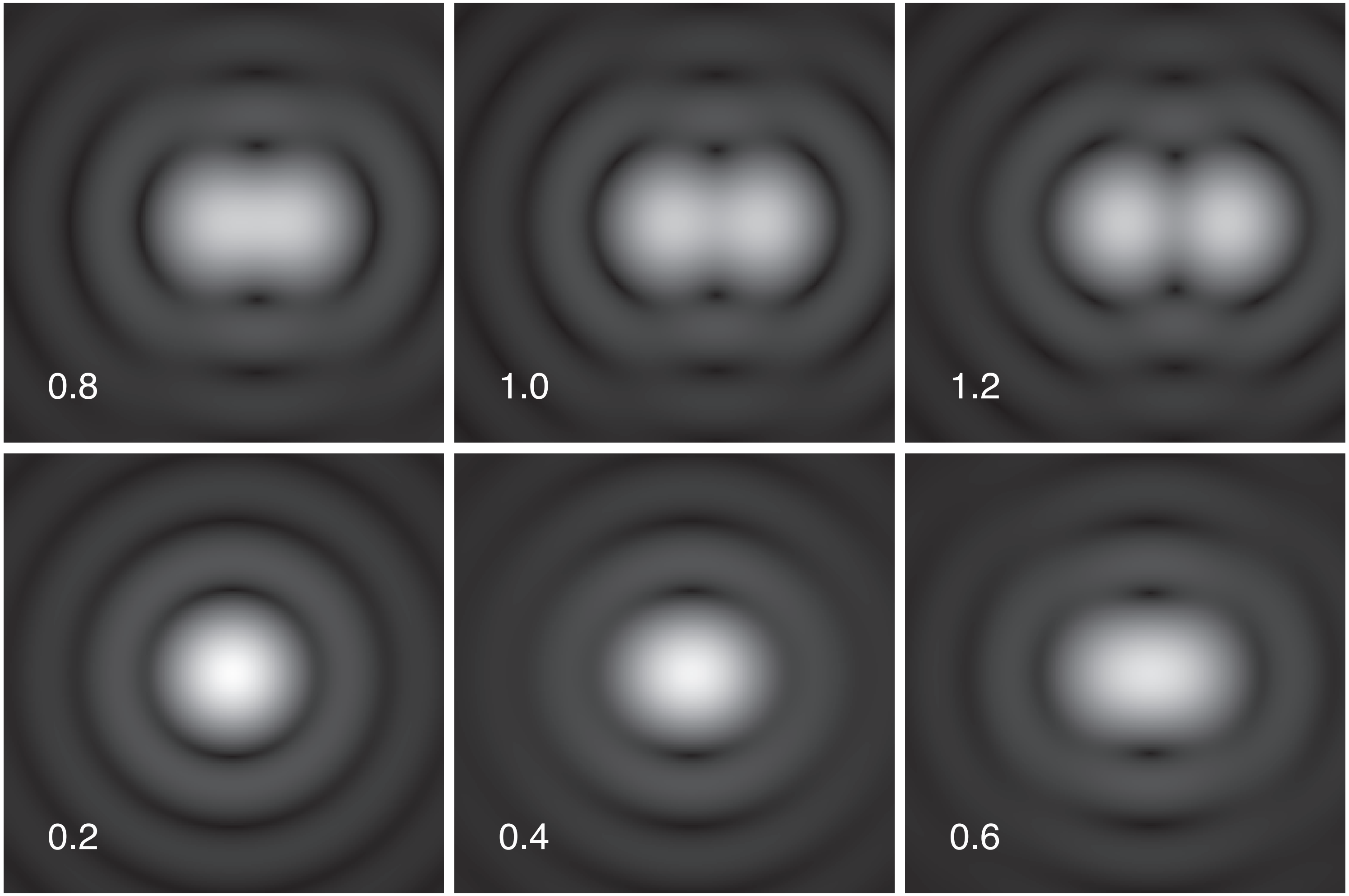
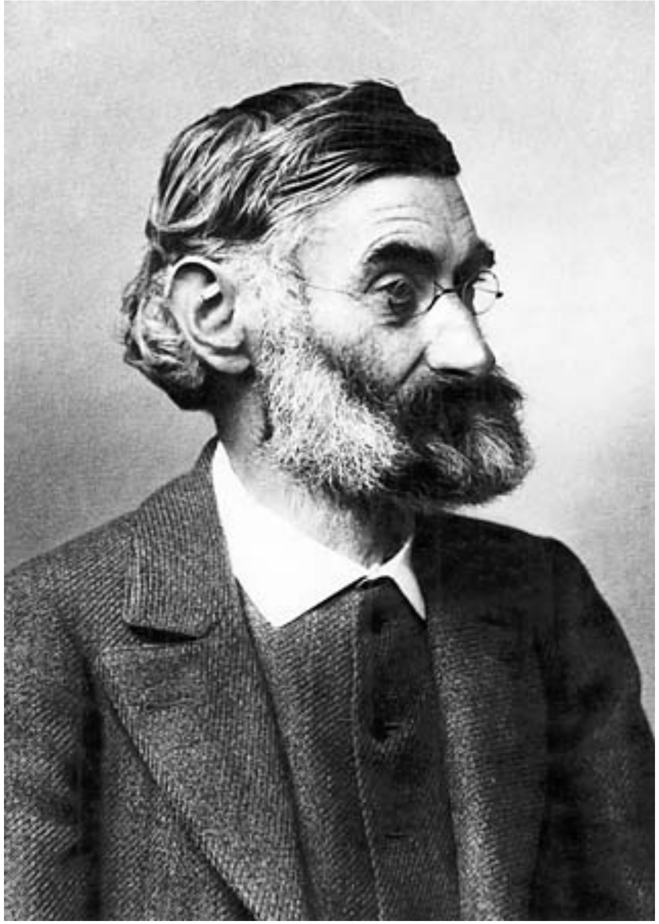


Illustration of Rayleigh resolution

- Separation of two spots as a fraction of the Rayleigh resolution



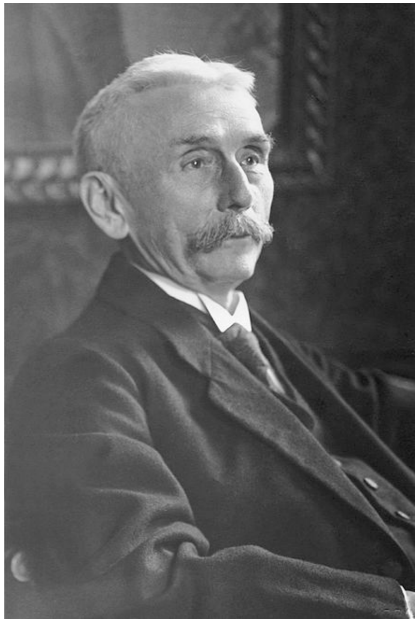
Ernst Abbe and Carl Zeiss, plus Otto Schott



Ernst Abbe

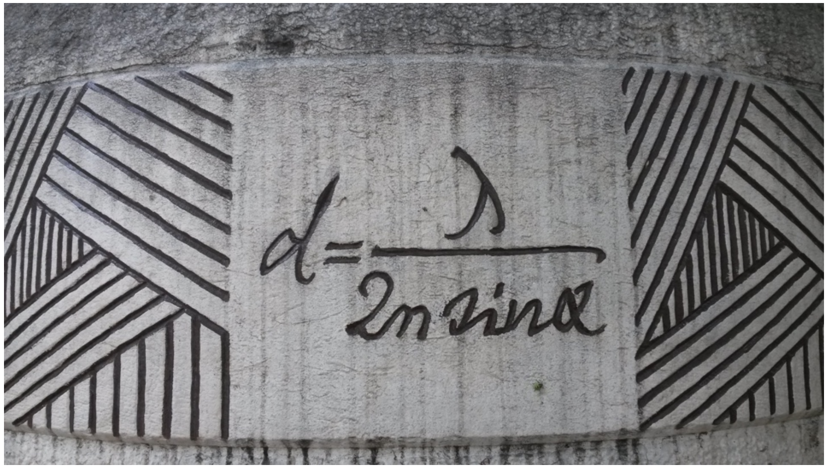
Carl Zeiss

ZEISS



Otto Schott

Microscope resolution: Rayleigh, plus index *n*



d=resolution

n=refractive index of medium between lens and specimen

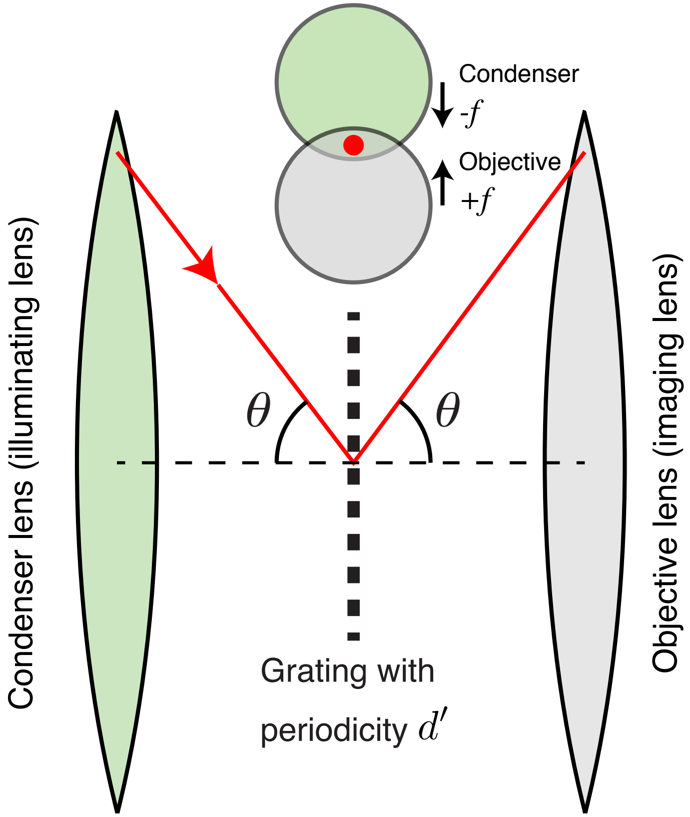
θ=semi-angle of lens

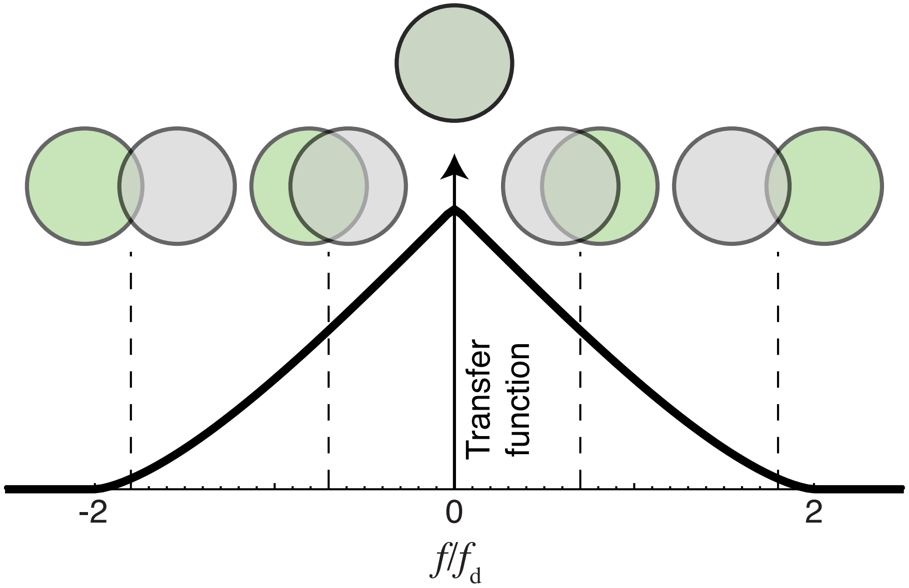
Microscope resolution: Rayleigh, plus index *n*

Abbey Road by The Beatles

Modulation transfer function for incoherent brightfield

- Lens semi-angle θ (NA=$n$sinθ): θ=λ/d'
- Coherent imaging cutoff at spatial frequency of f_d=1/d'
- Incoherent imaging goes to twice the spatial frequency, but with losses





Paul Kirkpatrick and Albert Baez, 1948

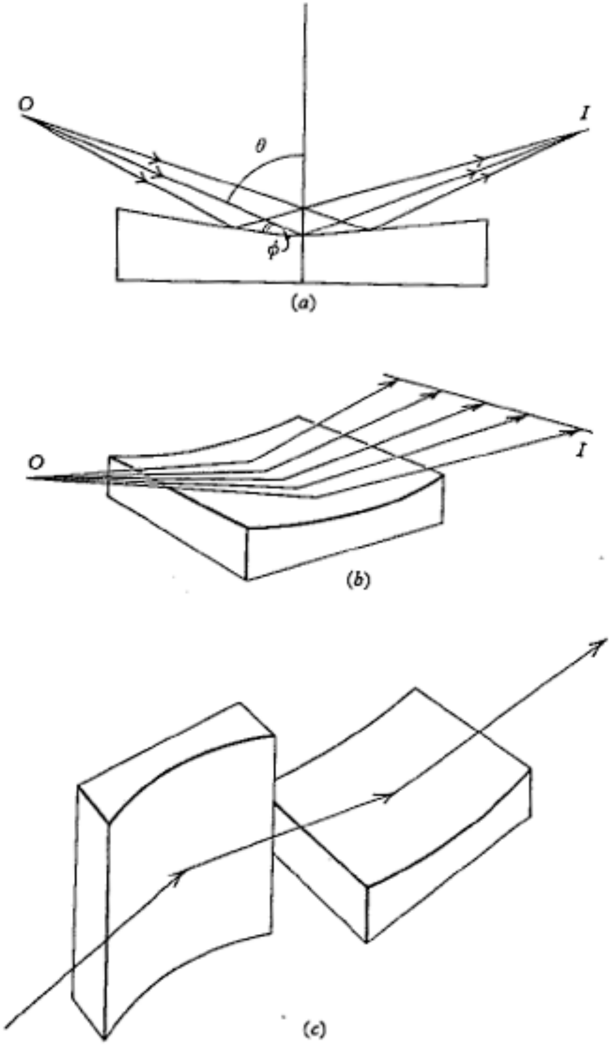


Fig. 1.2. Reflexion X-ray microscopy. (*a*) and (*b*) X-rays diverging from a source *O* are focused by a cylindrical surface to form an astigmatic image *I*; (*c*) arrangement of two cylindrical mirrors for eliminating astigmatism. (Kirkpatrick & Pattee, 1953.)

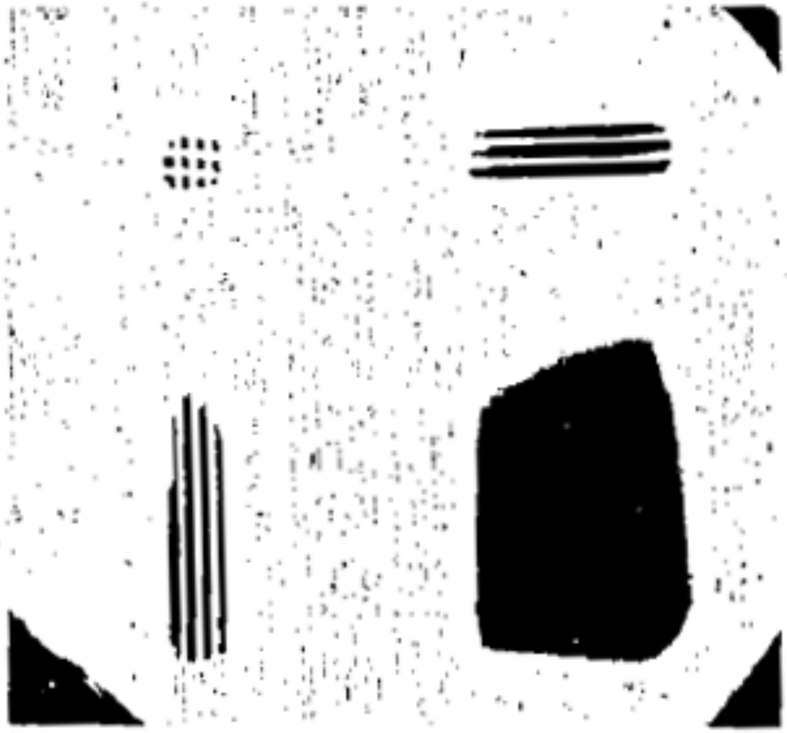
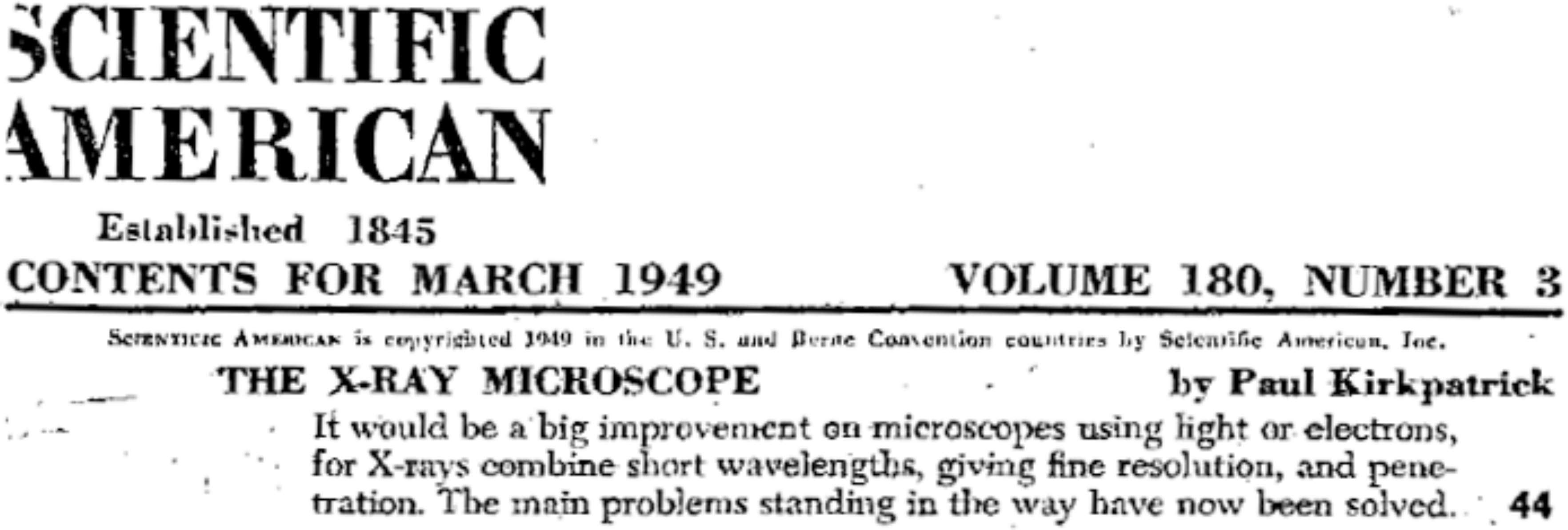


FIG. 12. Pattern produced by mirrors arranged as in Fig. 11. Object was a monel screen having 350 meshes per linear inch. In addition to the full image of the screen two partial images, each formed by one mirror, and a large spot caused by direct radiation appear above.



SCIENTIFIC AMERICAN

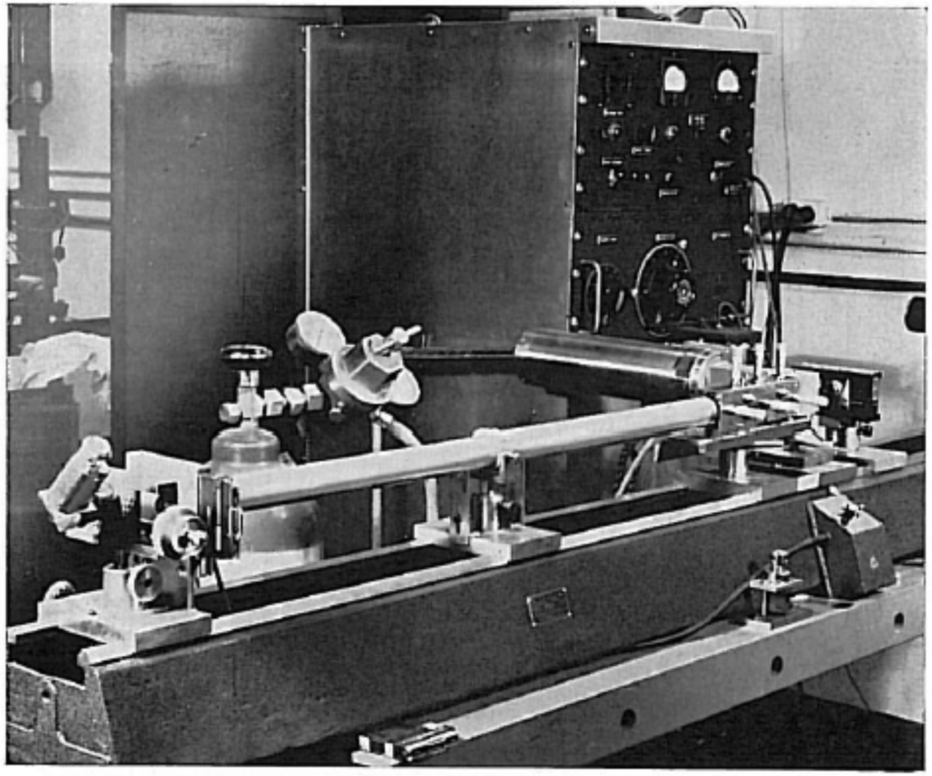
Established 1845

CONTENTS FOR MARCH 1949 VOLUME 180, NUMBER 3

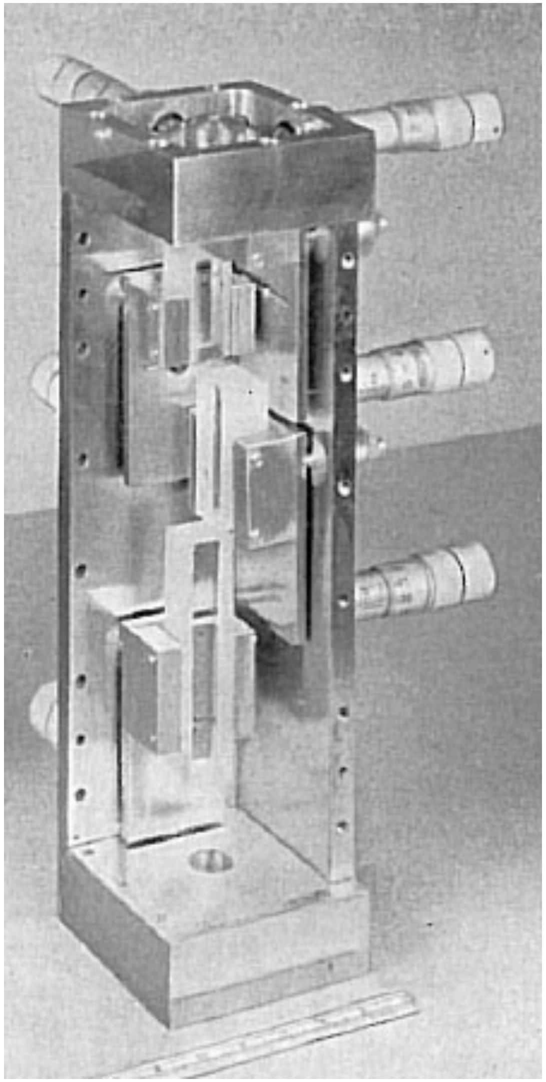
THE X-RAY MICROSCOPE by **Paul Kirkpatrick**

It would be a big improvement on microscopes using light or electrons, for X-rays combine short wavelengths, giving fine resolution, and penetration. The main problems standing in the way have now been solved. **44**

Kirkpatrick and Pattee, 1953

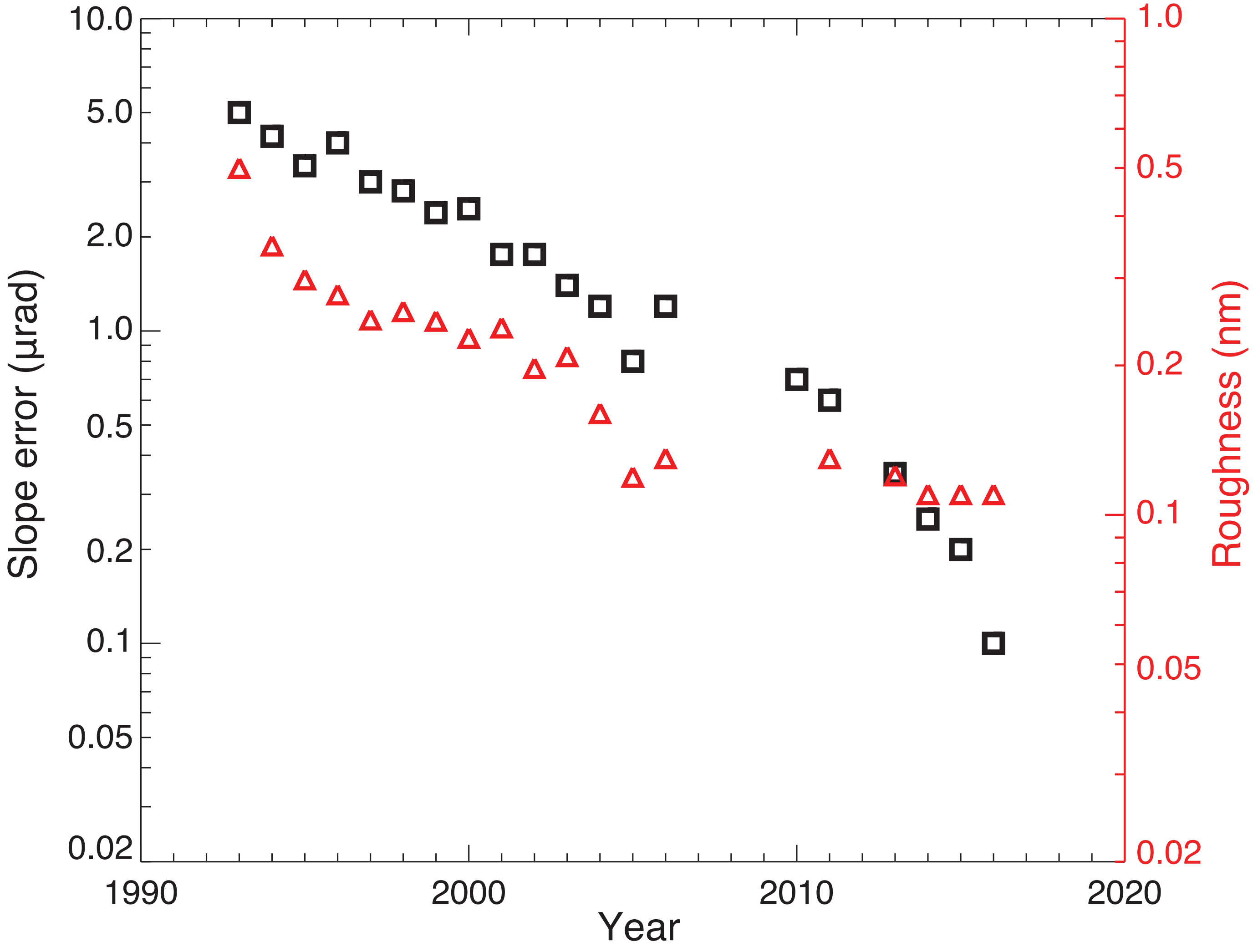


Reflexion X-ray microscope of Kirkpatrick and Pattee, incorporating two pairs of mirrors. (Kirkpatrick & Pattee, 1953.)



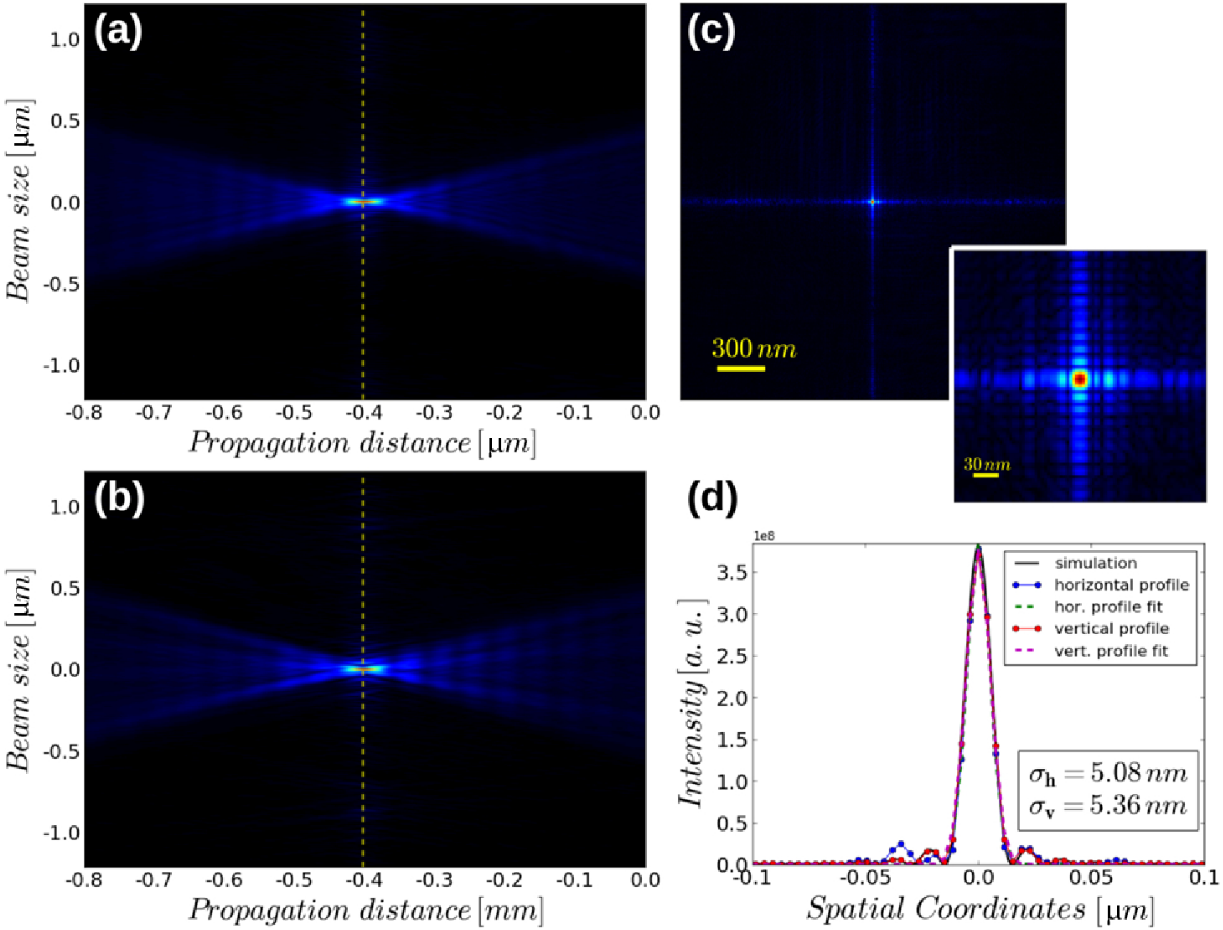
Improving the figure and roughness of mirrors

- Data provided by Lahsen Assoufid, head of the Optics Group at the APS

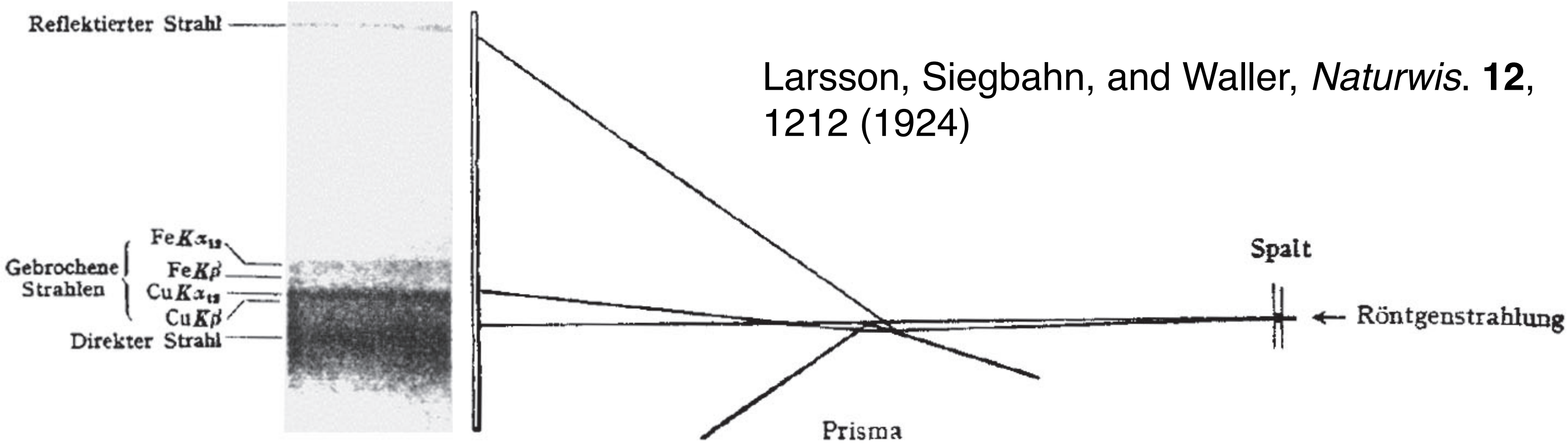


Nanofocusing with multilayer-coated KB mirrors

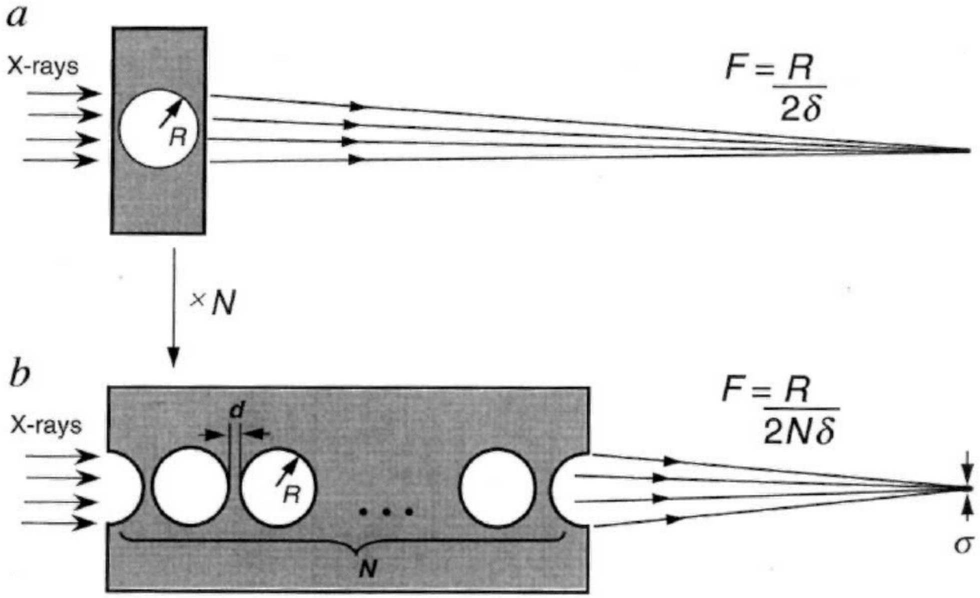
- da Silva, Pacureanu, Yang, Bohic, Morawe, Barrett, and Cloetens, *Optica* **4**, 492 (2017)
- FWHM of 12.0 nm (H) and 12.6 nm (V) at 17 keV



X-ray refraction



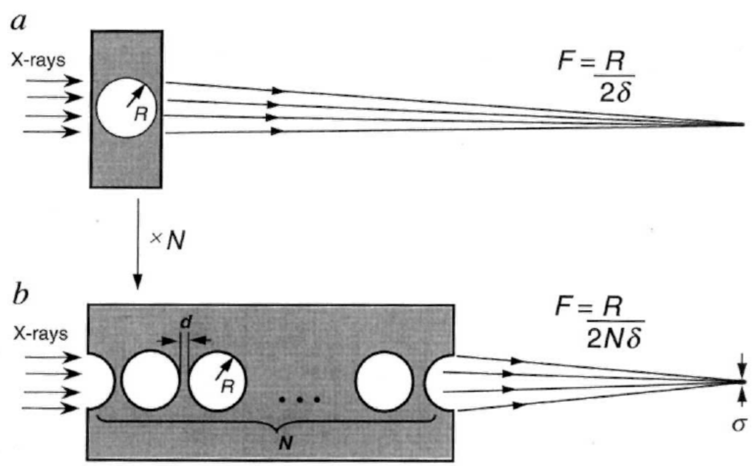
Larsson, Siegbahn, and Waller, *Naturwis*. **12**, 1212 (1924)



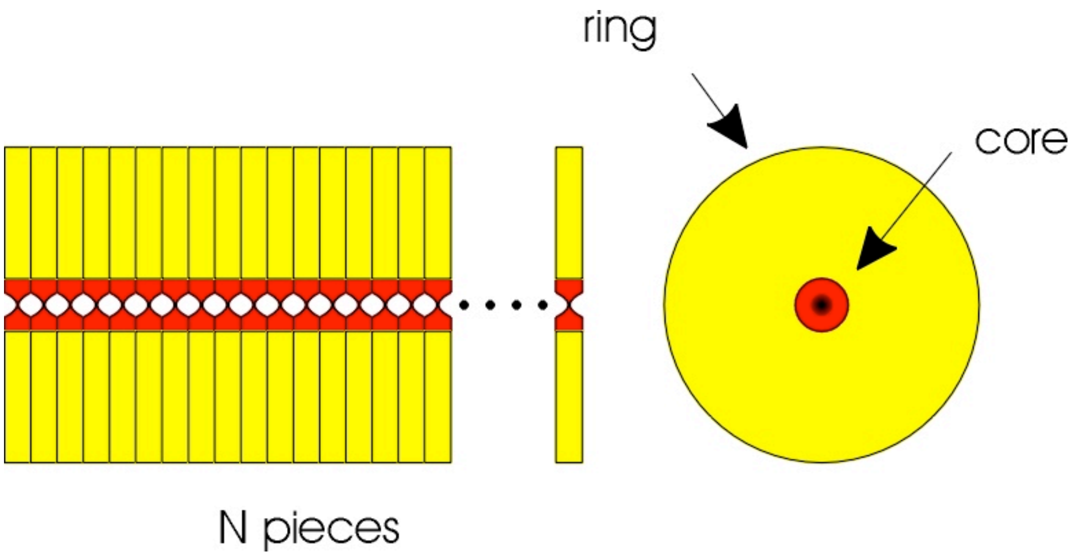
Snigirev, Kohn, Snigireva, and Lengeler, *Nature* **384**, 49 (1996). Earlier patent by T. Tomie.

Compound refractive lenses

- Röntgen tried to make lenses, but found no focusing.
- Focal length of one lens is long – so combine many lenses! Tomie; Snigirev *et al.*, *Nature* **384**, 49 (1996); Lengeler *et al.*, *J. Synch. Rad.* **9**, 119 (2002).
- Resolution approaching 60 nm at 5-10 keV with parabolic beryllium lenses.
- Refractive lenses are especially good at 20 keV and higher.

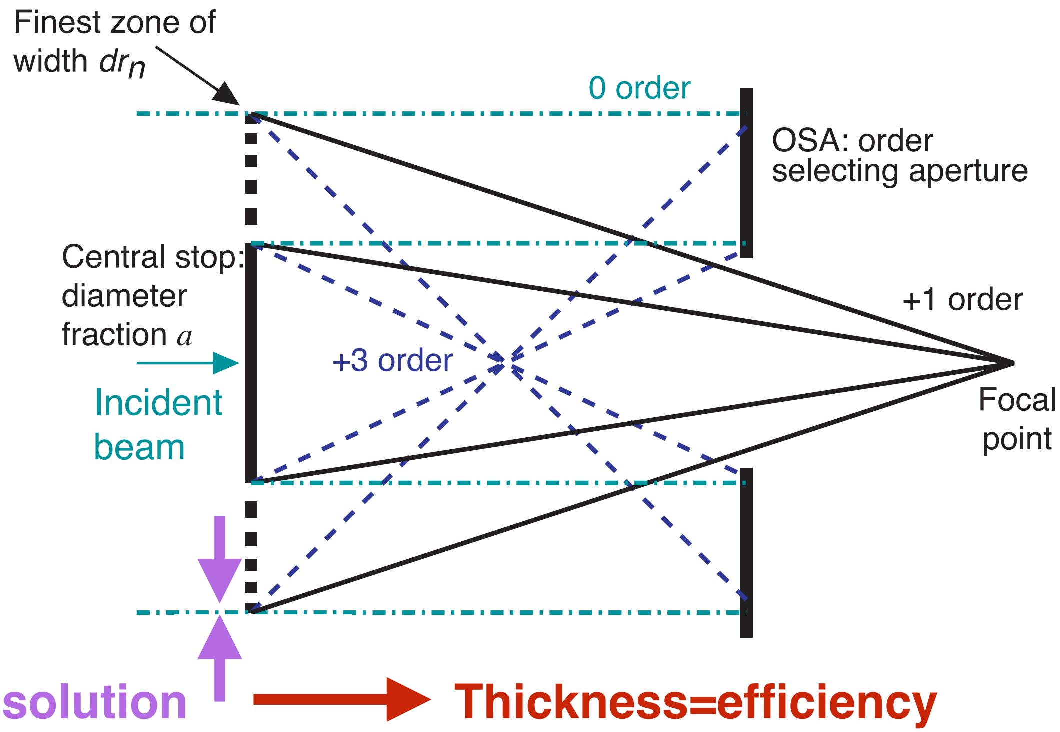


Compound refractive lenses at Universität Aachen



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!



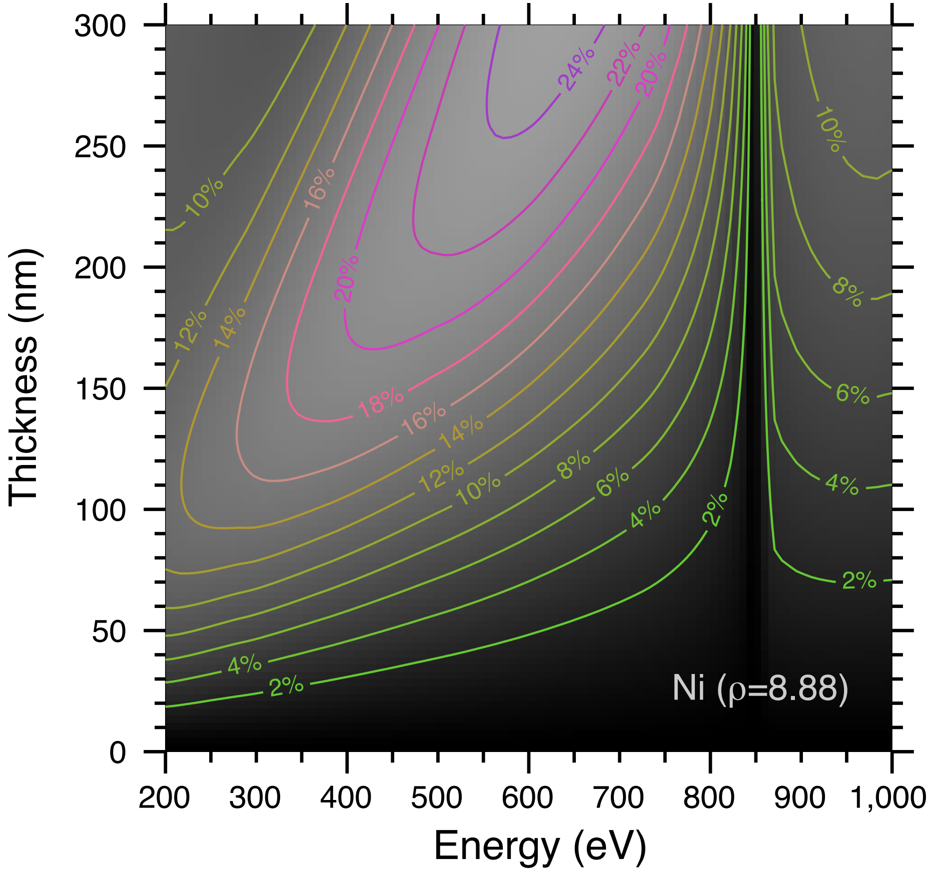
Width=resolution

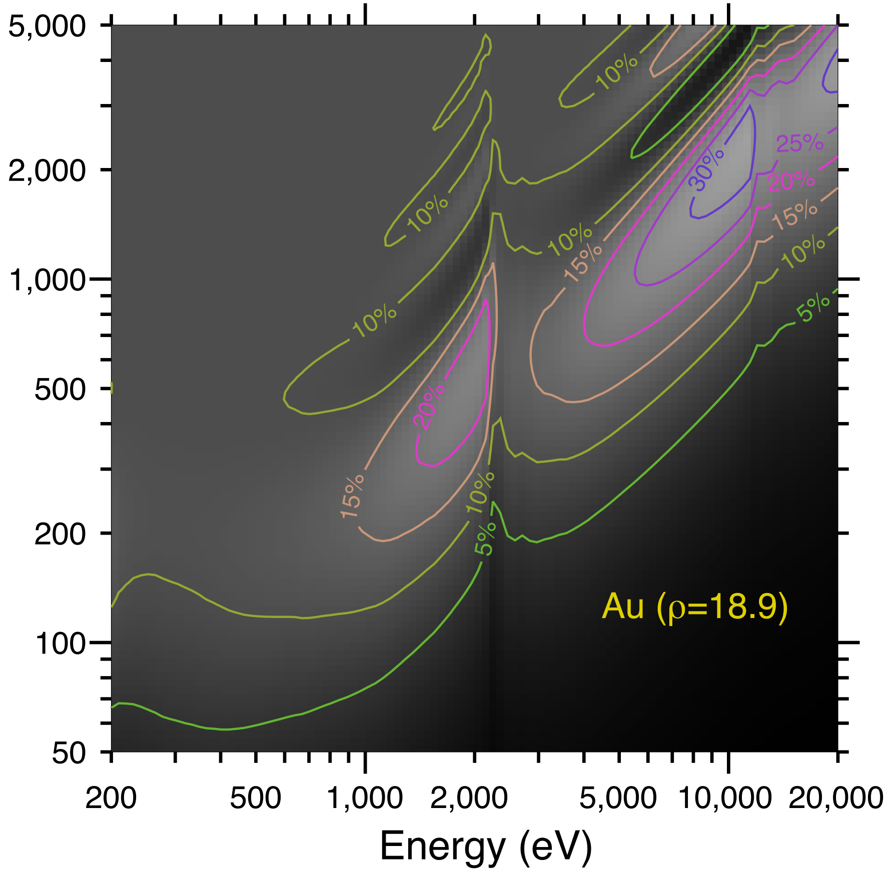
Thickness=efficiency

High aspect ratio nanofabrication!

Zone plate efficiency and thickness

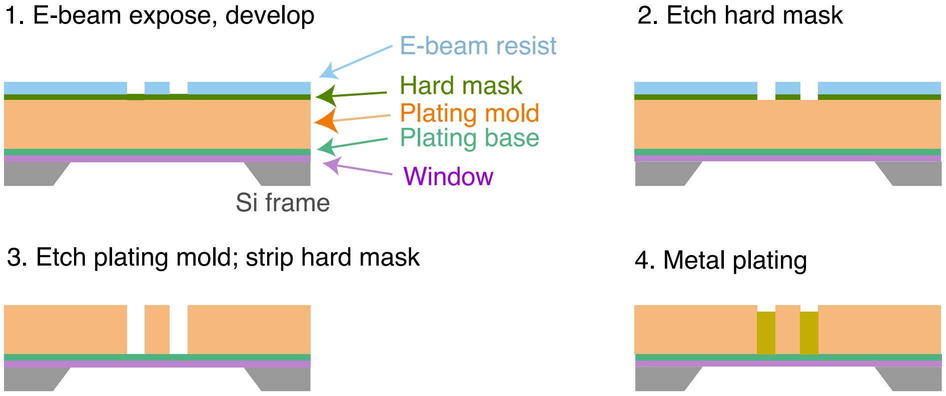
For binary zones, 1:1 mark:space ratio.
See Kirz, *J. Opt. Soc. Am.* **64**, 301 (1974)





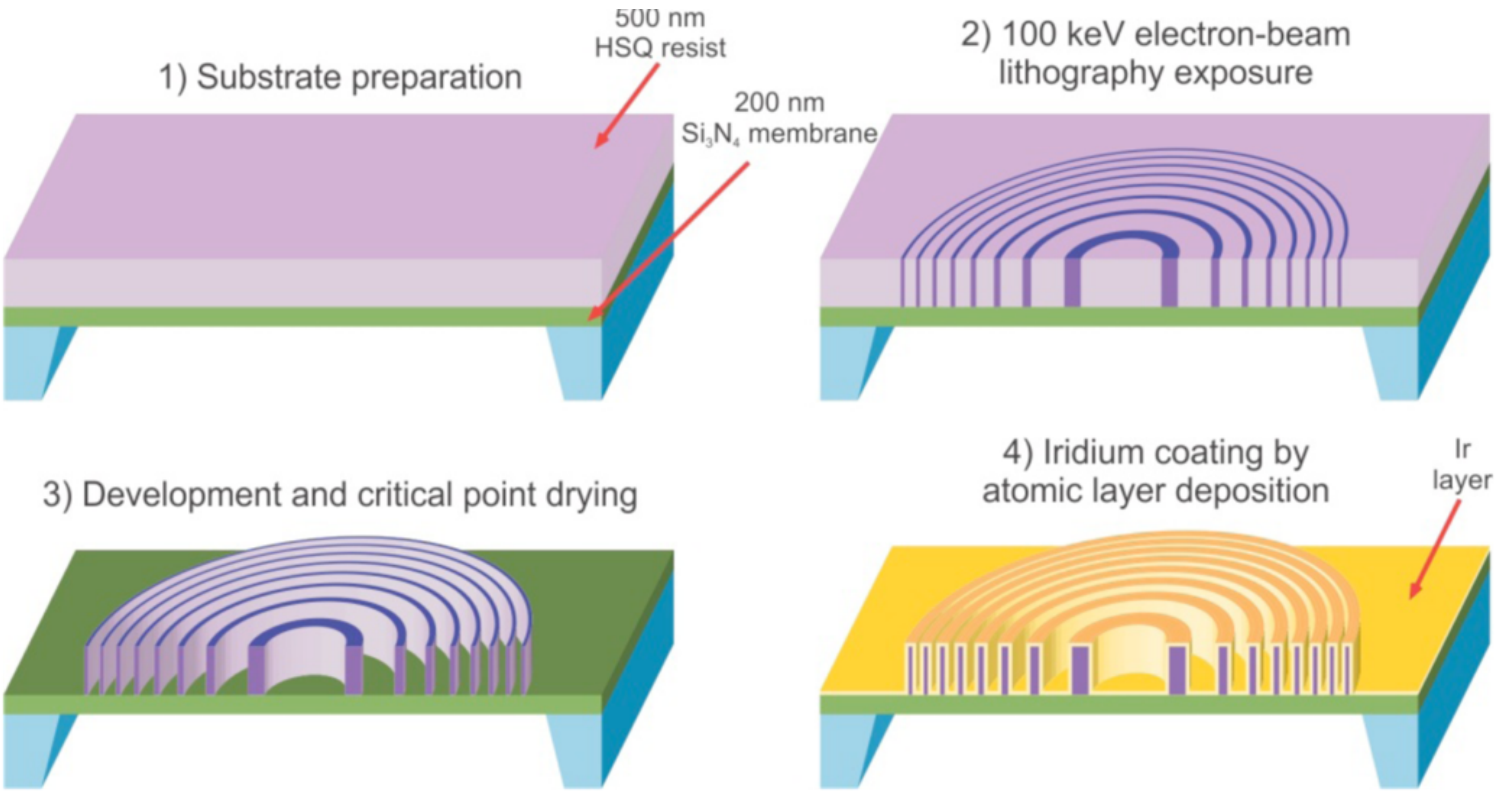
Zone plates by electron beam lithography

- Electron beam lithography: produces the finest possible structures (other than what nature can be persuaded to make by itself)
  - Example: JEOL JBX-9300FS: 1 nA into 4 nm spot, 1.2 nm over 500 μm, 100 keV
- Electrons scatter within resist, so highest resolution is only within ~100 nm thickness.
- Use directional etching methods like reactive ion etching for thick structures

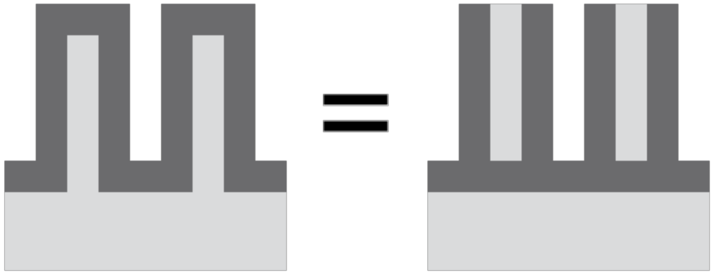


A. Stein and JBX-9300FS

Improving single zone plates: zone doubling



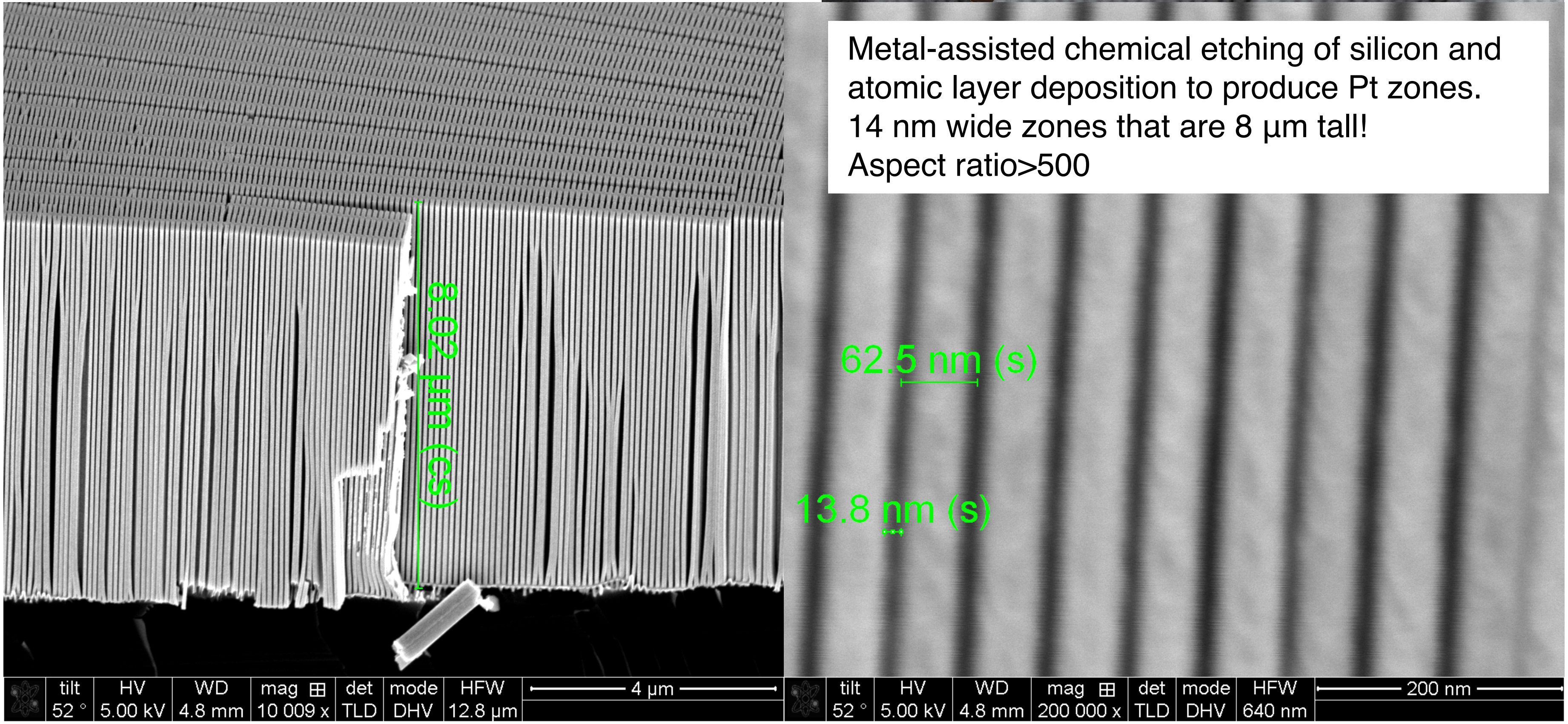
Jefimovs *et al.*, *Phys. Rev. Lett.* **99**, 264801 (2007).



Fresnel zone plates for x-ray nanofocusing

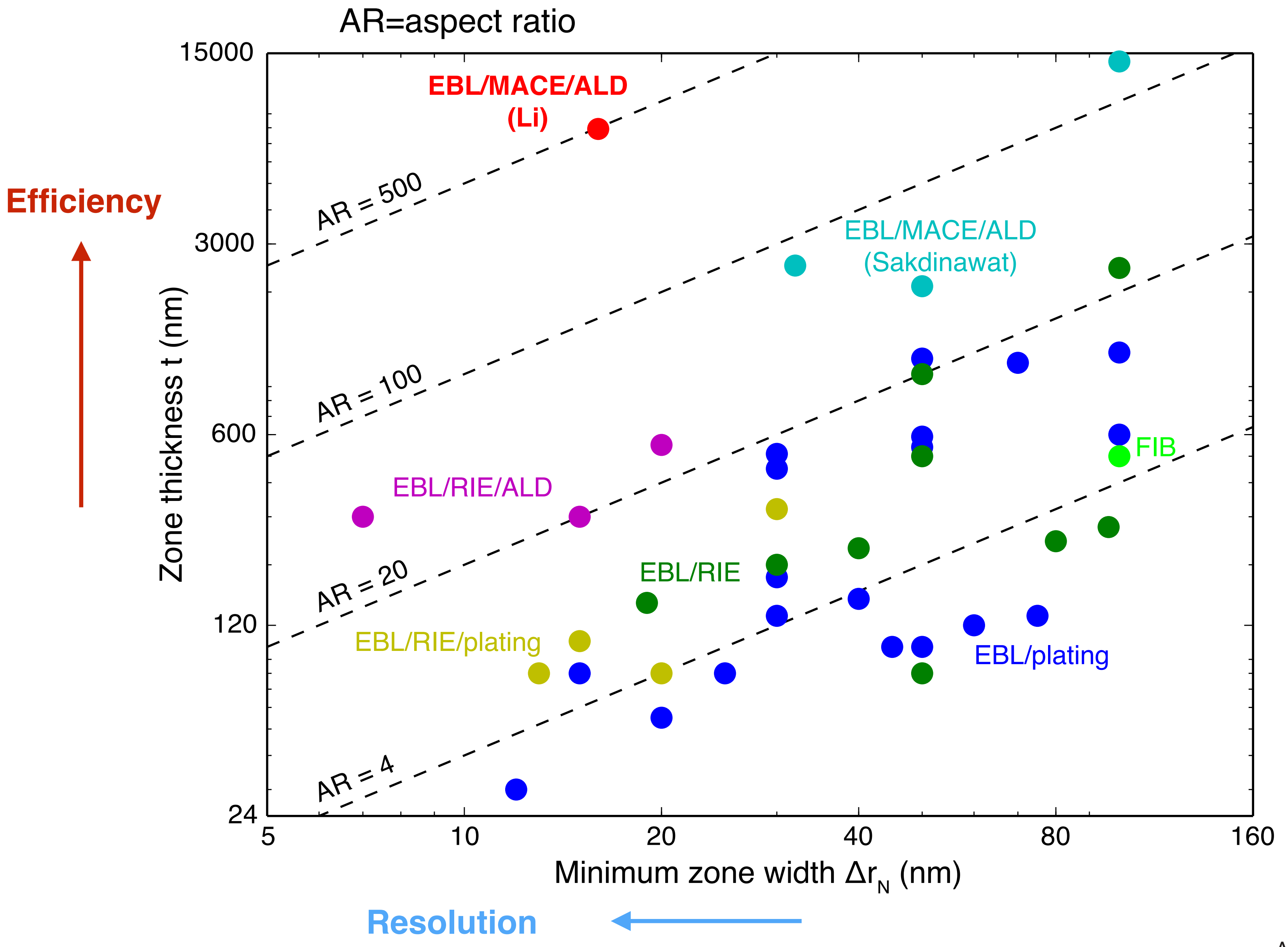
14 nm zone width in Pt, up to 8 µm tall (aspect ratio=500). 6% efficient at 20 keV in preliminary tests; resolution tests underway.

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



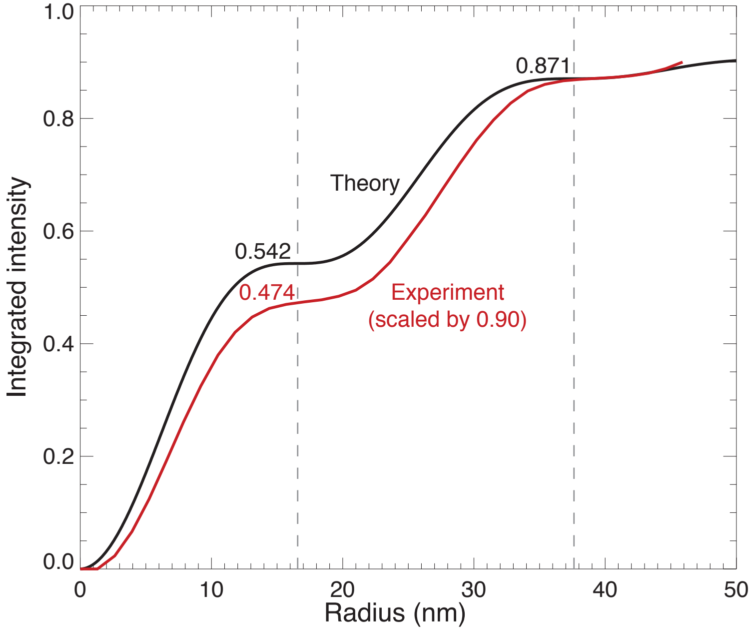
Metal-assisted chemical etching of silicon and atomic layer deposition to produce Pt zones.
14 nm wide zones that are 8 µm tall!
Aspect ratio>500

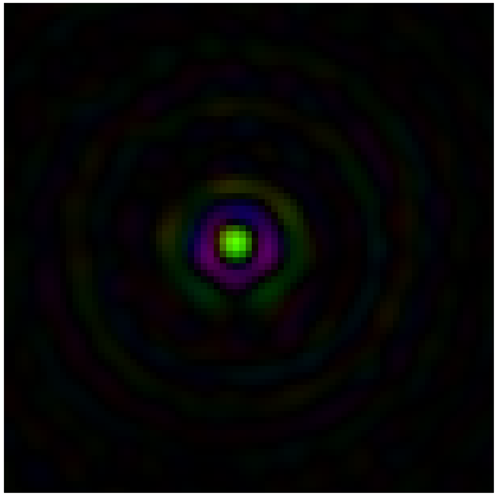
Recent APS/CNM/NU results: off the charts, on a log scale!

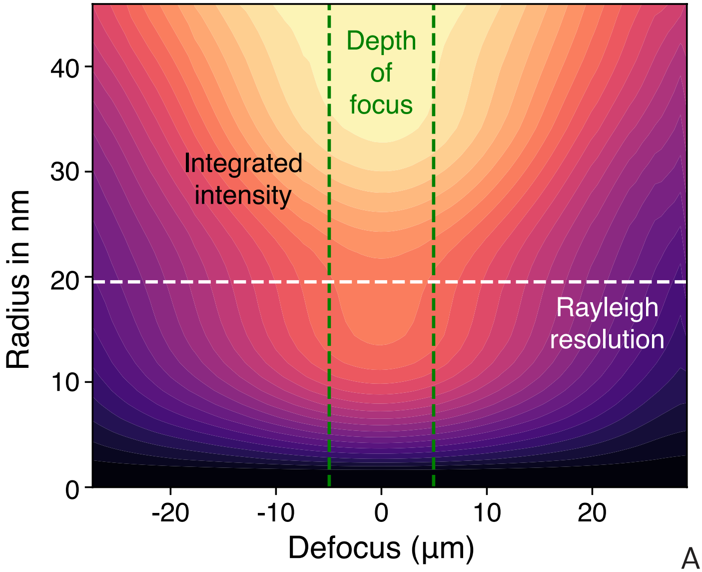


Recent tests at Brookhaven Lab

- 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

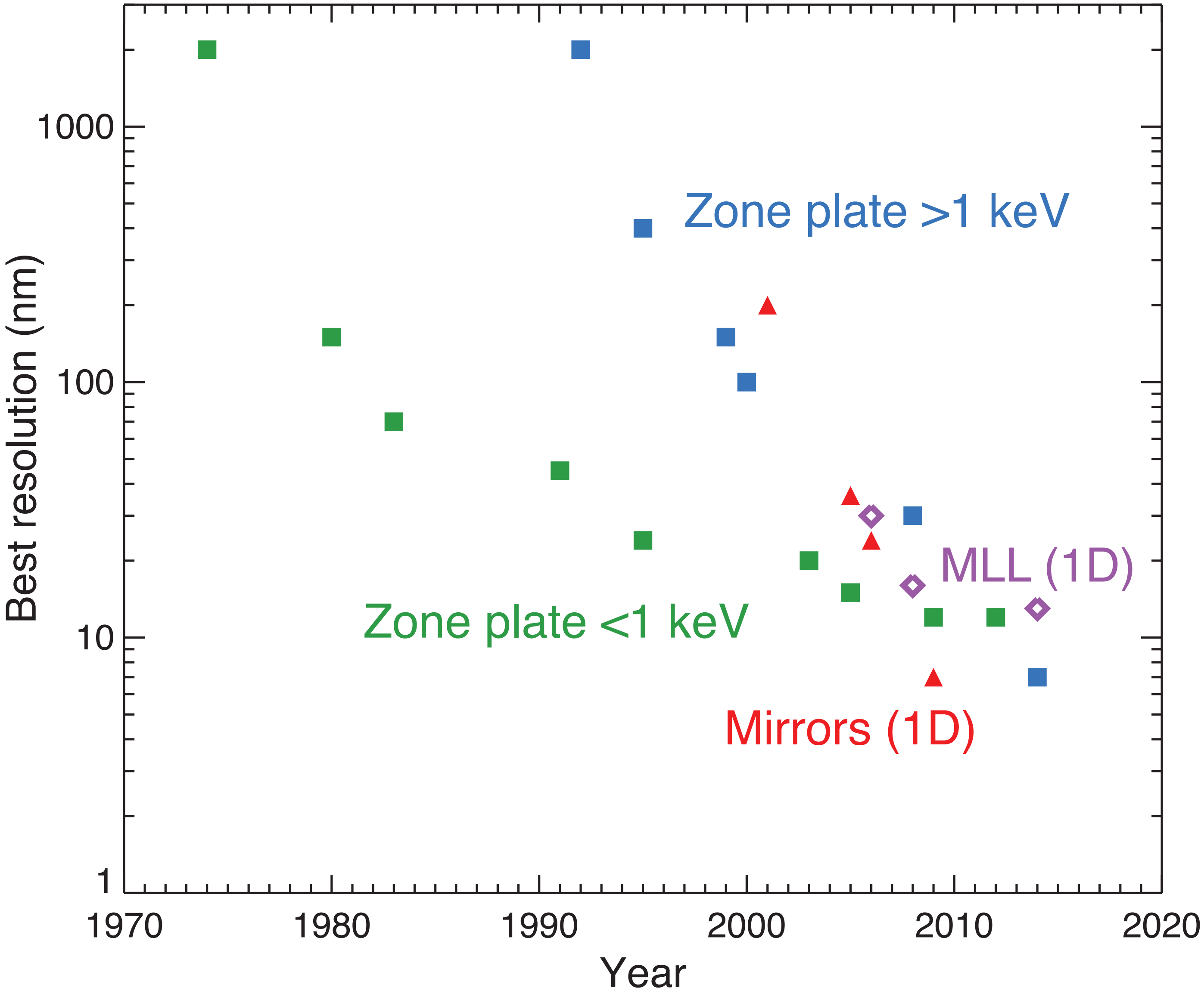






X-ray nanofocusing approaches are improving

X-ray nanofocusing has improved over time - but resolution in practical experiments is still limited to 20-30 nm



Outline

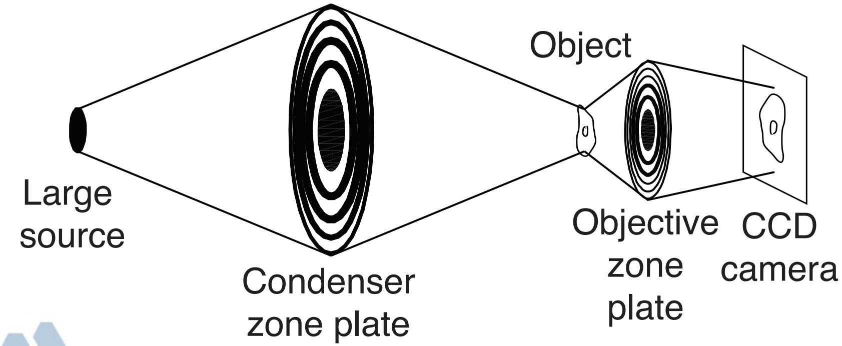
- X-ray optics and x-ray lenses
- **Why scanning?**
- Illumination considerations
- Instrumentation considerations
- Absorption spectromicroscopy
- CDI and ptychography

Zone plate microscopes

Full-field: transmission x-ray microscope (TXM)

- Incoherent illumination; works well with a bending magnet or a laboratory source
- Inefficient zone plate is *after* the sample (higher radiation dose)
- Faster (pixels in parallel)
- If zone plate condensers are used as monochromators, poor spectral resolution
- Transmission or reflection imaging

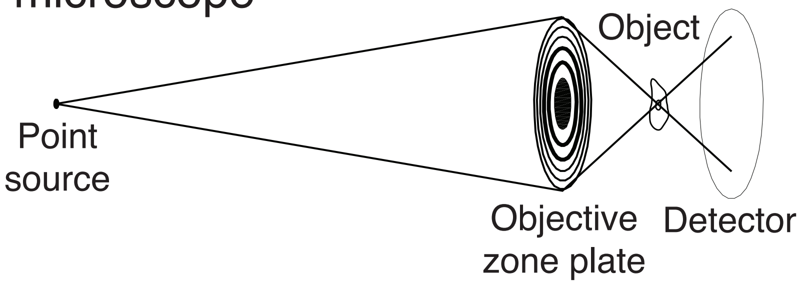
TXM: transmission x-ray microscope



Scanning transmission x-ray microscope (STXM)

- Coherent illumination; works best with an undulator
- Inefficient zone plate is *before* the sample (lower radiation dose)
- Slower (pixels one-by-one)
- Unlimited field of view and magnification (scanning stages)
- Better suited to high resolution monochromators
- Flexible modalities: fluorescence etc.

STXM: scanning transmission x-ray microscope

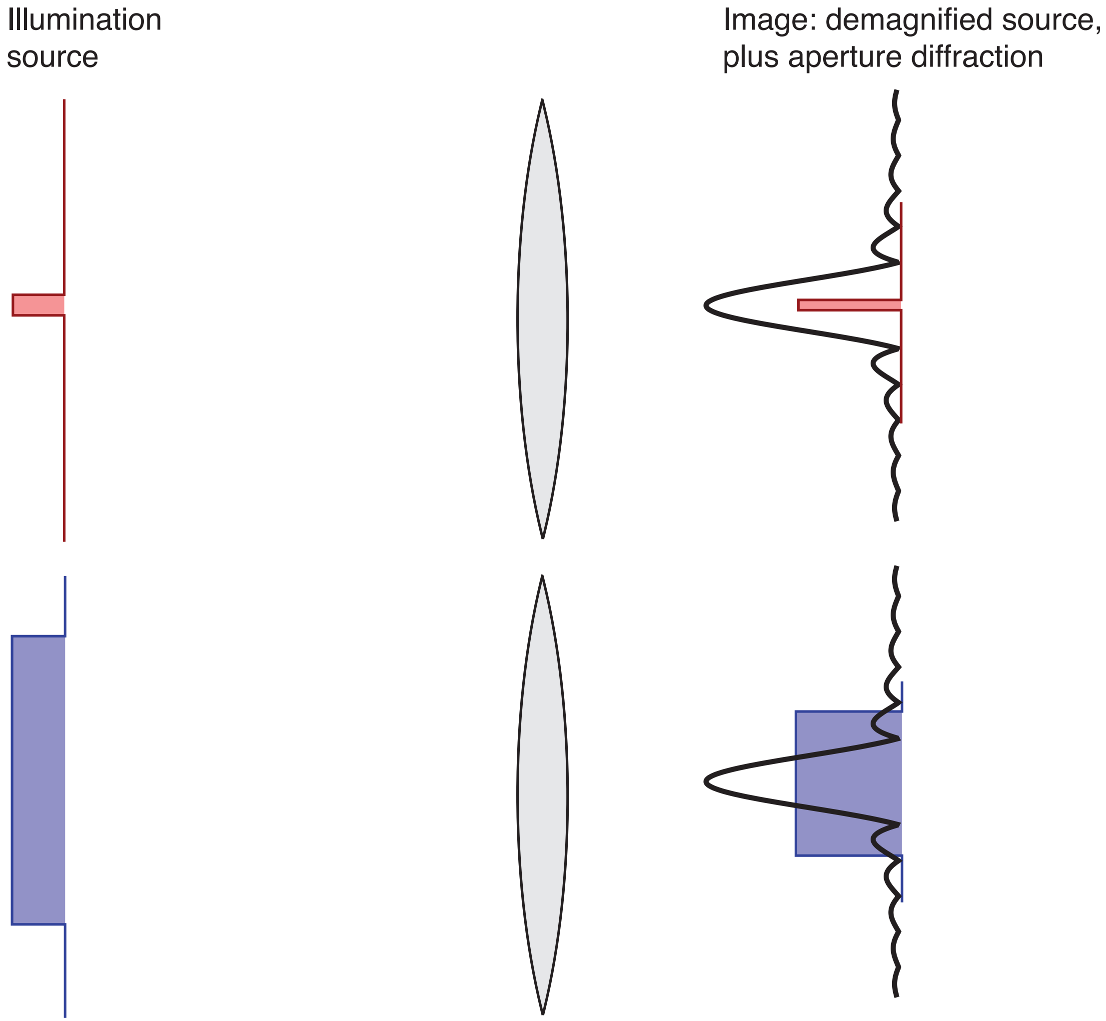


Outline

- X-ray optics and x-ray lenses
- Why scanning?
- **Illumination considerations**
- Instrumentation considerations
- Absorption spectromicroscopy
- Fluorescence microscopy

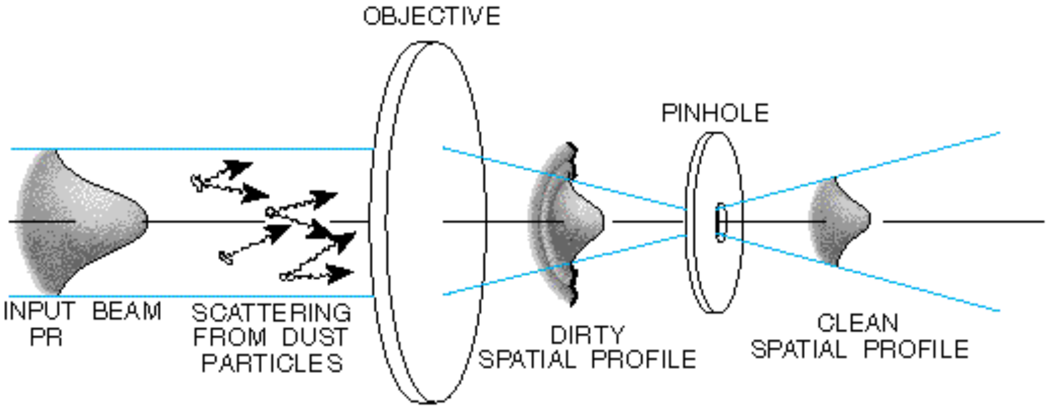
Nanofocusing requires coherent illumination

- Full-width, full-angle phase space of a diffraction limited lens with numerical aperture θ: (2θ)·(2·0.61λ/θ)=2.44λ
- Thus need to limit source phase space to ~λ both in x and y



Controlling spatial coherence

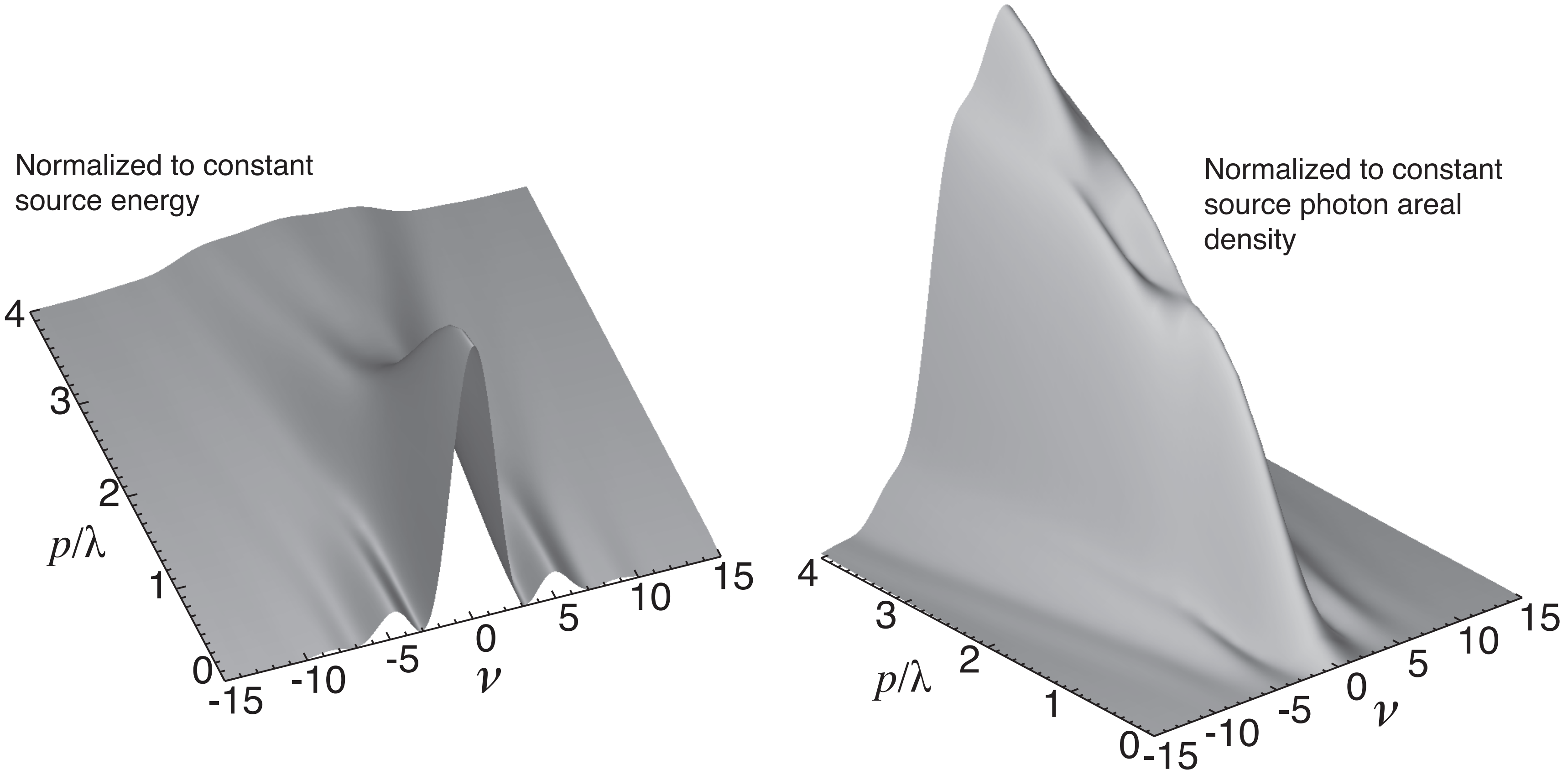
- Spatial filter: pinhole at the focus of a lens. Passes only the spatially coherent fraction of an incident beam.
- X-ray beamlines: image the source to a secondary position with an aperture, for a flux-versus-coherence tradeoff.



Diagram, photo from Newport catalog

Phase space area and probe focus

How close must p=(source diameter)•(optic's full subtended angle) be to λ? $p \approx 1 \cdot \lambda$ works pretty well!



Normalized to constant source energy

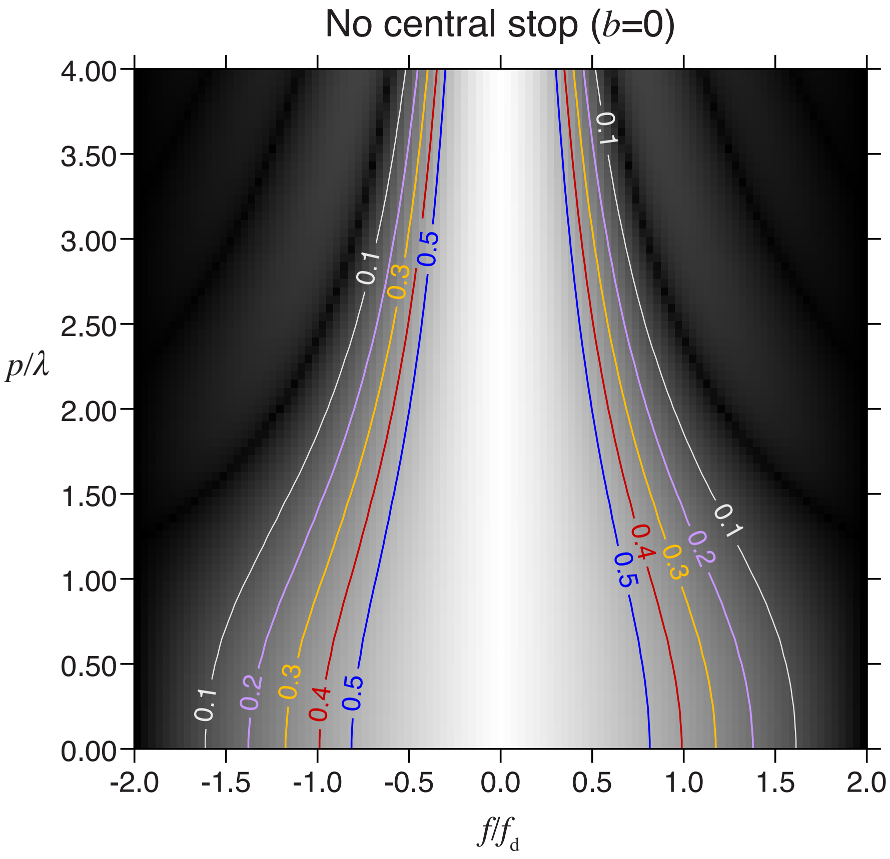
Normalized to constant source photon areal density

Effect on point spread function PSF (50% central stop)

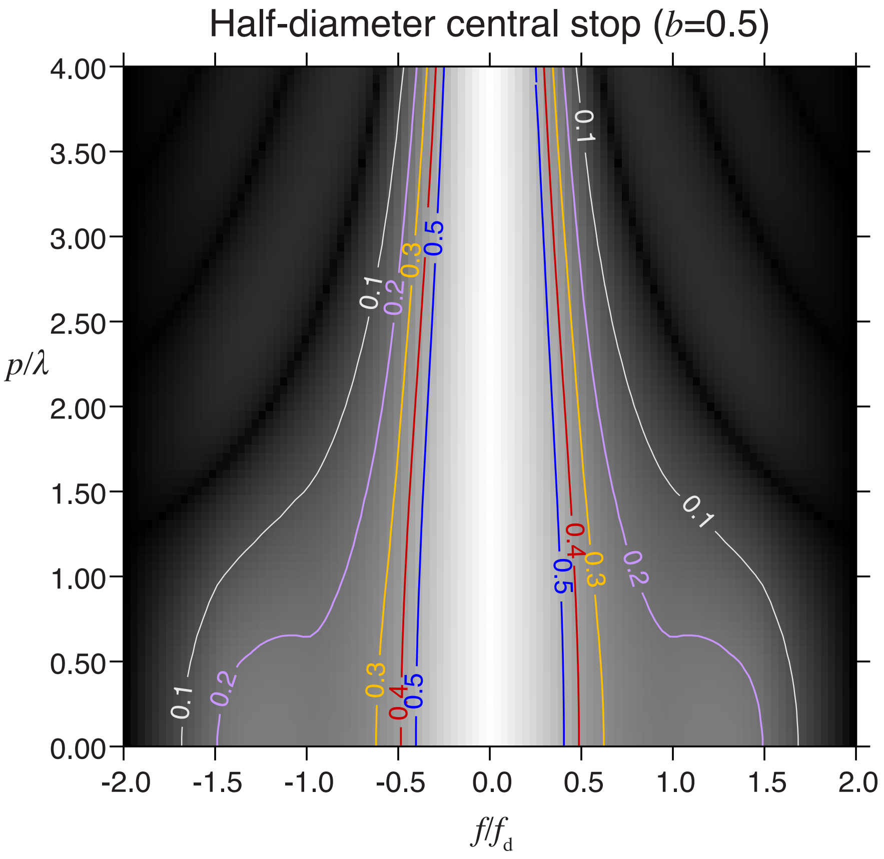
Jacobsen *et al.*, *Ultramicroscopy* **47**, 55 (1992); Winn *et al.*, *J. Synch. Rad.* **7**, 395 (2000).

Transfer function view of source phase space p

- Contours of transfer function value



No central stop (b=0)



Half-diameter central stop (b=0.5)

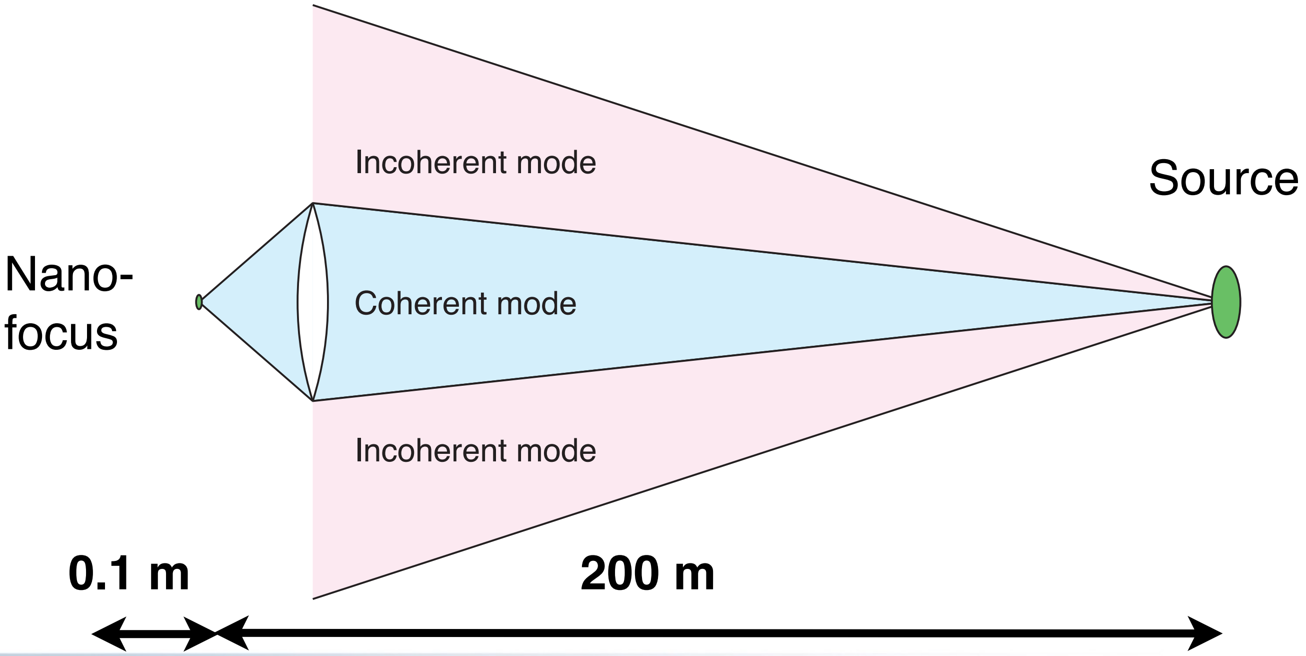
Coherence and nanoprobes

- You can only reach the diffraction limit (as opposed to the source-limited limit) of focusing from a lens when you accept a single coherent mode with phase space area ~1λ in each direction.
- If the phase space of your source is larger, you must throw away the incoherent modes! Example with the APS at Argonne:

Parameter	**electron beam**	**photon beam**	MBA Upgrade **combination**	**today**
Horizontal size	$\sigma_x = 21.5\ \mu\text{m}$	$\sigma_r = 5.5\ \mu\text{m}$	$\Sigma_x = 22.2\ \mu\text{m}$	$275.0\ \mu\text{m}$
Horizontal divergence	$\sigma'_x = 3.1\ \mu\text{rad}$	$\sigma'_r = 3.6\ \mu\text{rad}$	$\Sigma'_x = 4.8\ \mu\text{rad}$	$12.1\ \mu\text{rad}$
Vertical size	$\sigma_y = 4.0\ \mu\text{m}$	$\sigma_r = 5.5\ \mu\text{m}$	$\Sigma_y = 6.8\ \mu\text{m}$	$10.7\ \mu\text{m}$
Vertical divergence	$\sigma'_y = 1.7\ \mu\text{rad}$	$\sigma'_r = 3.6\ \mu\text{rad}$	$\Sigma'_y = 4.0\ \mu\text{rad}$	$6.2\ \mu\text{rad}$
Phase space parameter p_x	$(2.35\Sigma_x)(2.35\Sigma'_x)/\lambda$		4.69 at 10 keV	148.53
Phase space parameter p_y	$(2.35\Sigma_y)(2.35\Sigma'_y)/\lambda$		1.20 at 10 keV	2.95
$p_x \cdot p_y/\lambda^2$			5.7	438.1

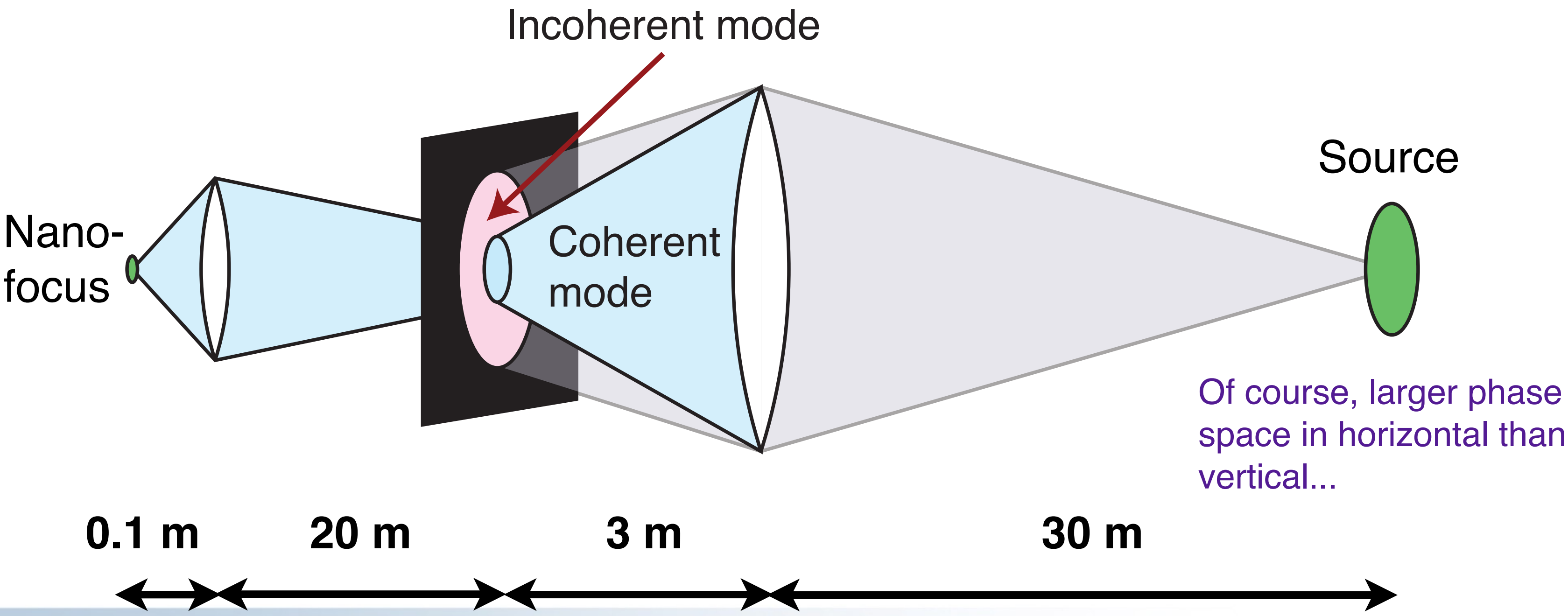
How to limit modes?

- Approach 1: distance, with a single nanofocusing optic.
  - Need to demagnify the source size in one step; for a fixed focal length (thus working distance) optic, you need a longer beamline.
  - Example: 40 µm to 20 nm in 200/0.1 meters. Total length: **200 m**.
  - For an optic with fixed diameter (and thus numerical aperture, and resolution), you have no flux-versus-resolution tradeoff.



How to limit modes?

- Approach 2: aperture at an intermediate focus.
  - Two-stage demagnification means you can work with a shorter beamline!
  - Example: demagnify source size from 40 µm to 4 µm in 30/3 meters, then 4 µm to 20 nm in 20/0.1 meters. Total length 30+3+20=**53 m**.
  - You can adjust your intermediate aperture and have a flux-versus-resolution tradeoff.



Of course, larger phase space in horizontal than vertical...

Outline

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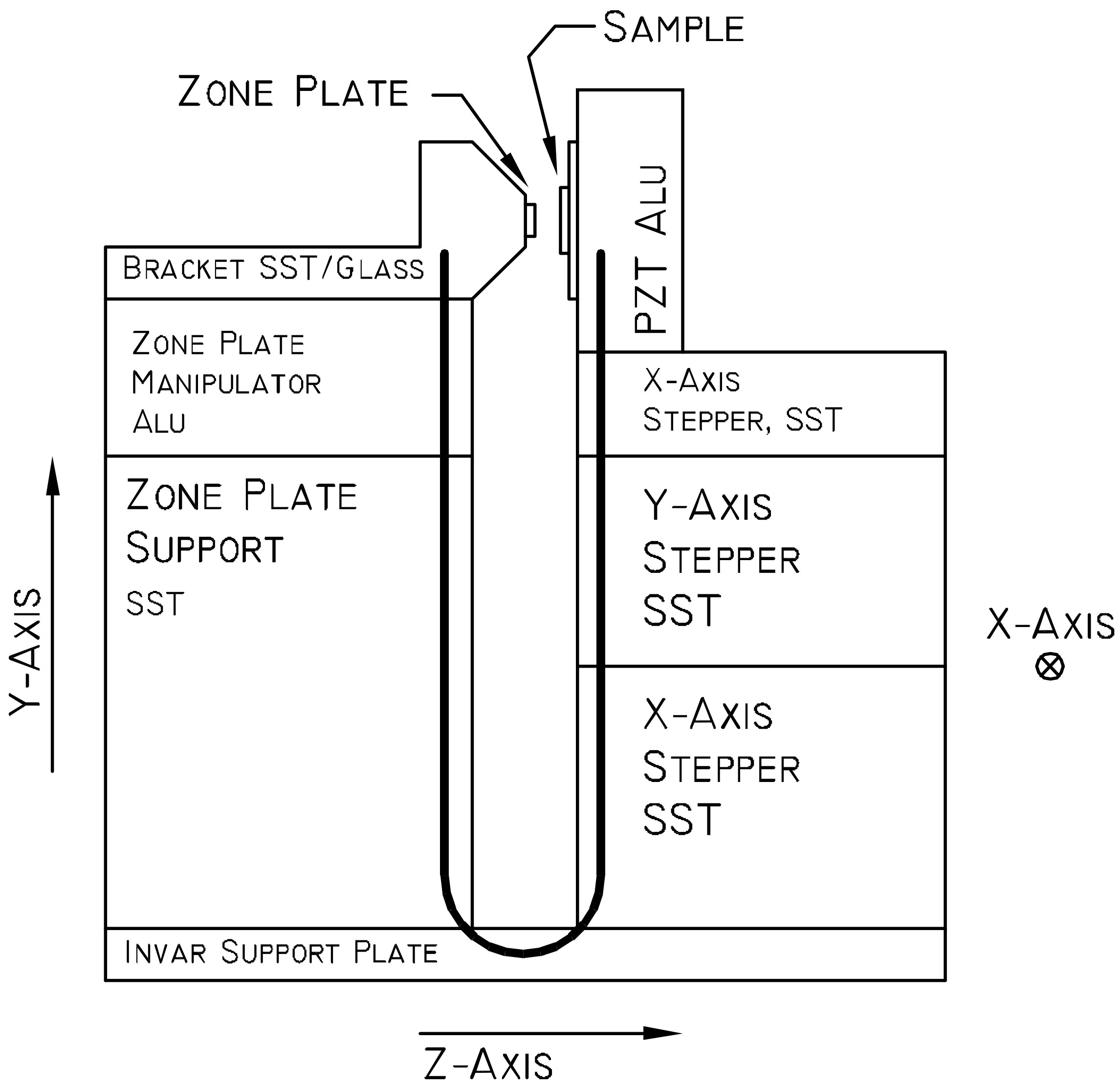
Scanning microscope instrumentation

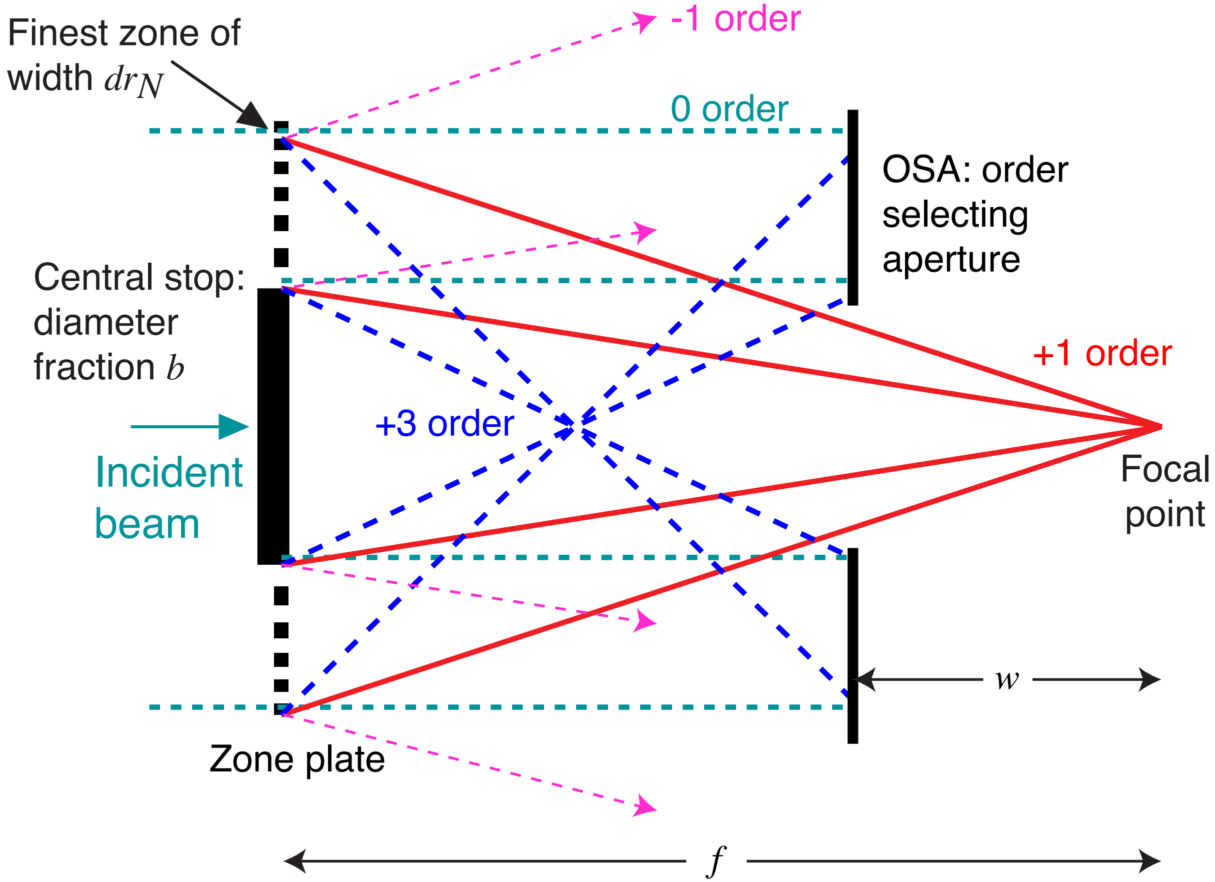
- Mechanical design principles: small, light and stiff, with resonant frequencies >100 Hz.
- Thermal expansion: many materials have ~10^{-5} per C which is 1 µm per 10 cm.
- Stepper motors on bearings for large range scans, and positioning (0.1 µm resolution over many mm).
- Piezoelectric pushers on flexure guides for fine scans (10 nm resolution over 20-100 µm).



M. Feser PhD dissertation: `tinyurl.com/kowp6vs`

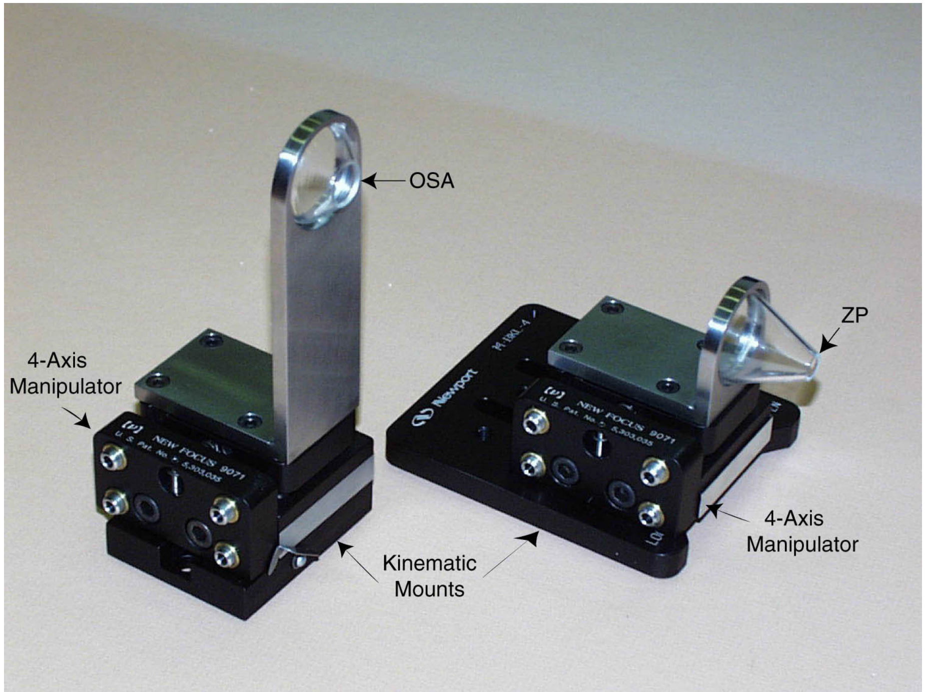
Zone plates and diffraction orders

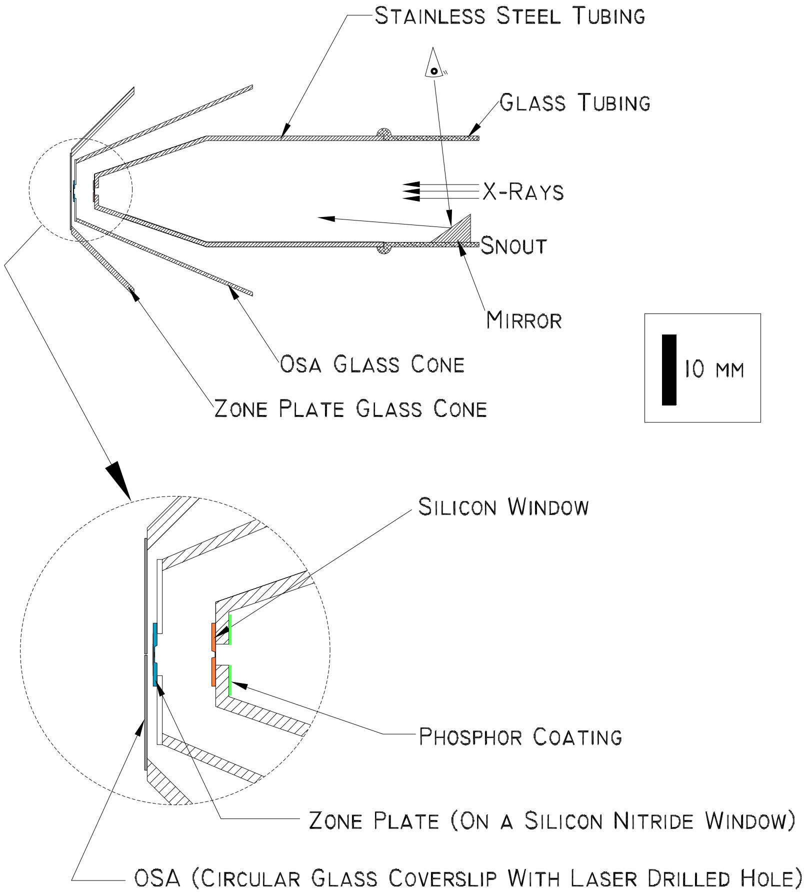
- Central stop fraction b is 0.5 for soft x-rays, and less with hard x-rays (longer focal length so more generous with working distance w)



Swappable zone plates and paired order selecting apertures (OSAs)

- Glass cones: see-through, low thermal expansion coefficient
- Low-cost (commercial) kinematic mounts and position/tilt adjusters





M. Feser PhD dissertation: `tinyurl.com/kowp6vs`

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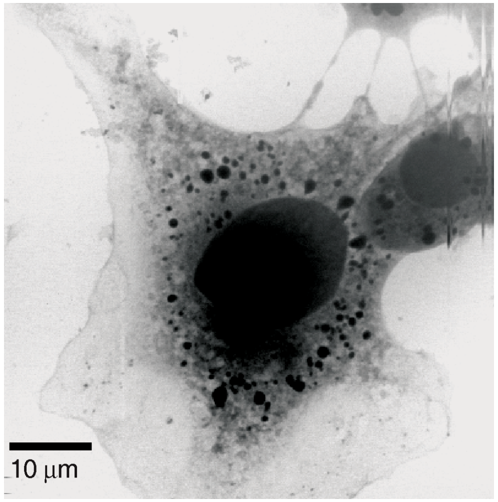
2D imaging with Stony Brook STXMs

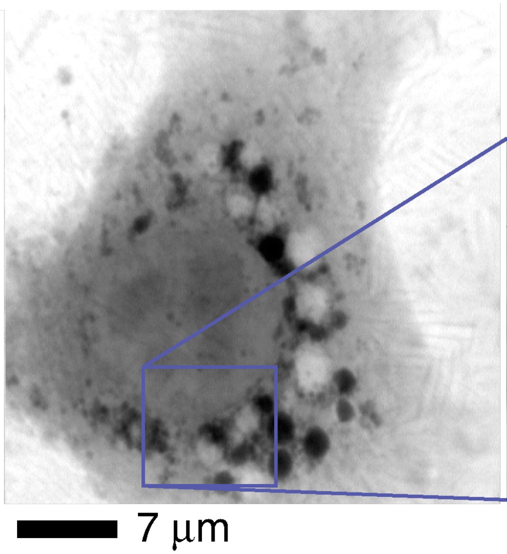
2D imaging is moderately useful but...

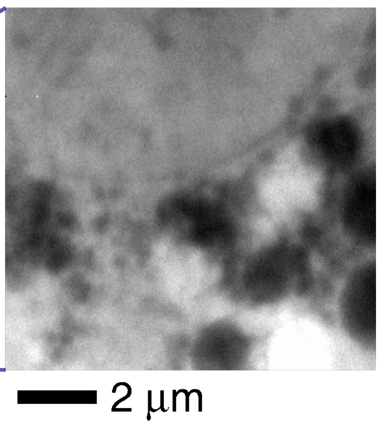
- Cannot track fluroescently-labeled proteins in living cells
- Resolution is inferior to cryoEM, though do not need to section
- Best utility may lie beyond simple 2D imaging



NIL 8 fibroblast (wet, fixed): Oehler *et al.*

Human sperm (unfixed): Wirick, Fleckenstein, Sheynkin *et al.*





Fibroblast (frozen hydrated): Maser *et al.*, *J. Micros.* **197**, 68 (2000)

Anilao, Philippines, 17-Oct-2010

Energy scales

- Chemistry: C-H bond is 104 kcal/mol or 435 kJoules/mol:

  $$(435\frac{\mathrm{kJ}}{\mathrm{mol}})\cdot(\frac{10^3\ \mathrm{J}}{\mathrm{kJ}})\cdot(\frac{1\ \mathrm{eV}}{1.602\times10^{-19}\ \mathrm{J}})\cdot(\frac{1}{6.02\times10^{23}\ \mathrm{molecules/mol}})$$

  or E=4.5 eV/molecule.
- Photon wavelength associated with 4.5 eV is

  $$E=\frac{hc}{\lambda}=\frac{1240\ \mathrm{eV}\cdot\mathrm{nm}}{\lambda}$$

  or λ=275 nm.
- More broadly, chemical bonds involve 300-900 kJ/mol, or 3-9 eV/molecule, or 130-400 nm light.
- Bohr model:

  $$E=-E_0\frac{Z^2}{n^2}=-13.6\ \mathrm{eV}\ \frac{Z^2}{n^2}$$

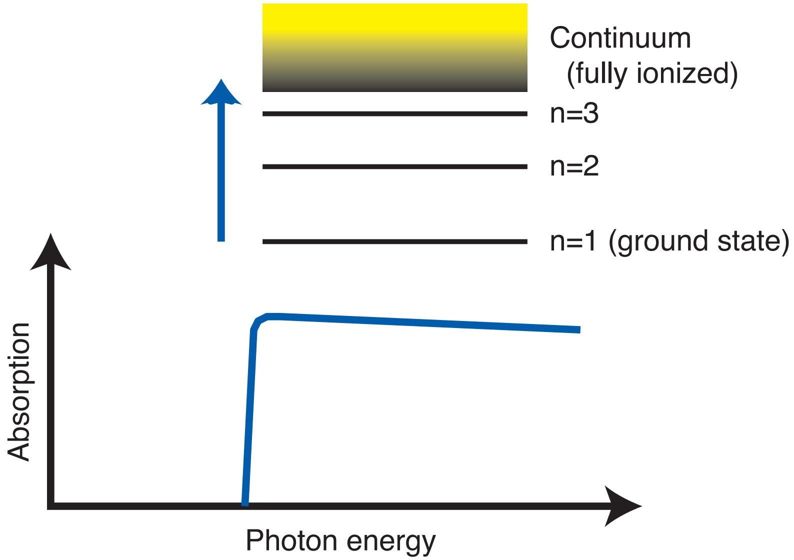
Absorption edges

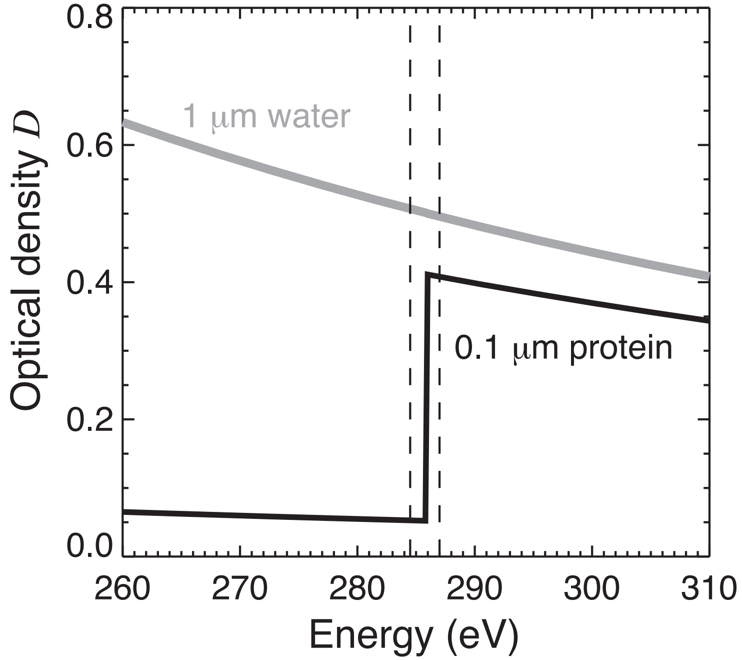
Lambert-Beer law: linear absorption coefficient μ

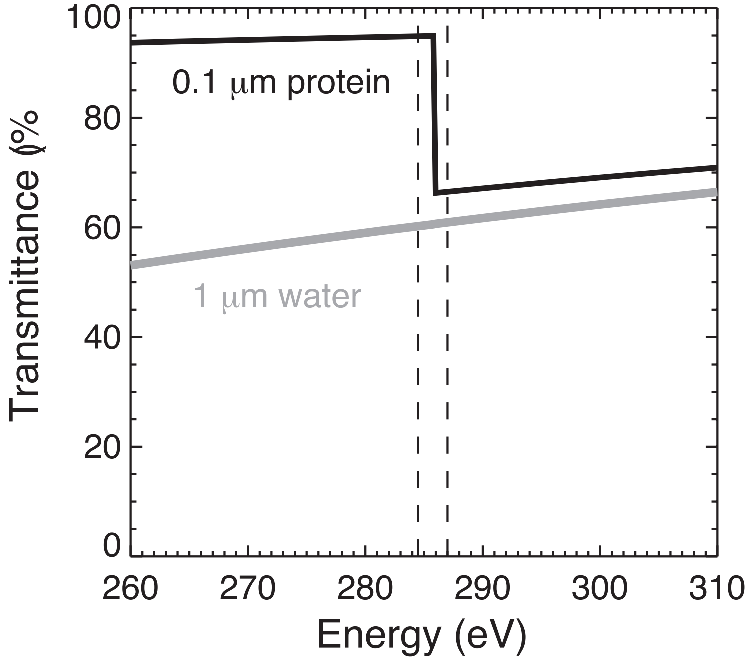
$$I = I_0 e^{-\mu(E)\cdot t} = I_0 e^{-D(E)}$$

This coefficient makes a jump at specific elemental absorption edges!

This example: 0.1 μm protein in water

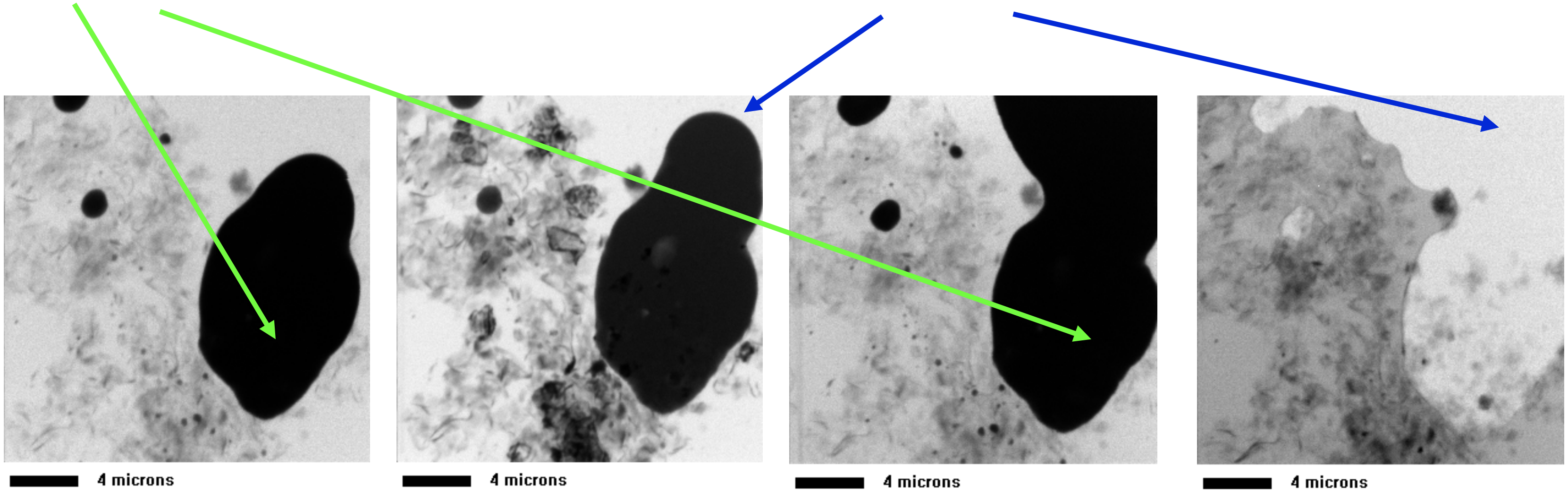






X-ray microscopy of colloids

- U. Neuhäusler (Stony Brook/Göttingen), S. Abend (Kiel), G. Lagaly (Kiel), C. Jacobsen (Stony Brook), *Colloid and Polymer Science* **277**, 719 (1999).
- Emulsion: water, oil droplets, clay, and layered double hydroxides (LDH).
- "Caged" part of oil droplet remains fixed; "uncaged" part can disperse.



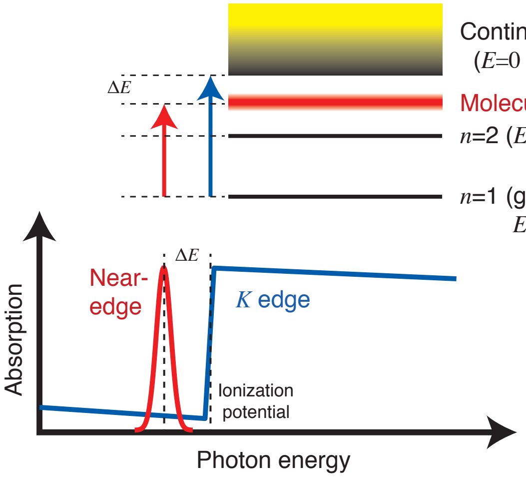
346 eV: calcium weakly absorbing. Clays and LDHs absorb equally

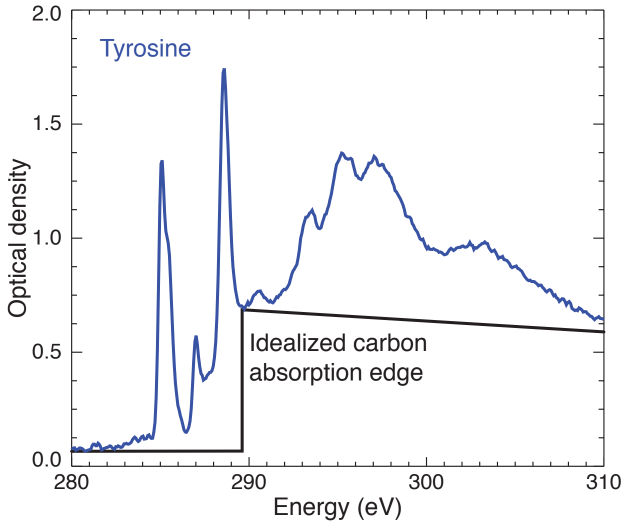
352.3 eV: calcium strongly absorbing. Calcium-rich LDHs are highlighted.

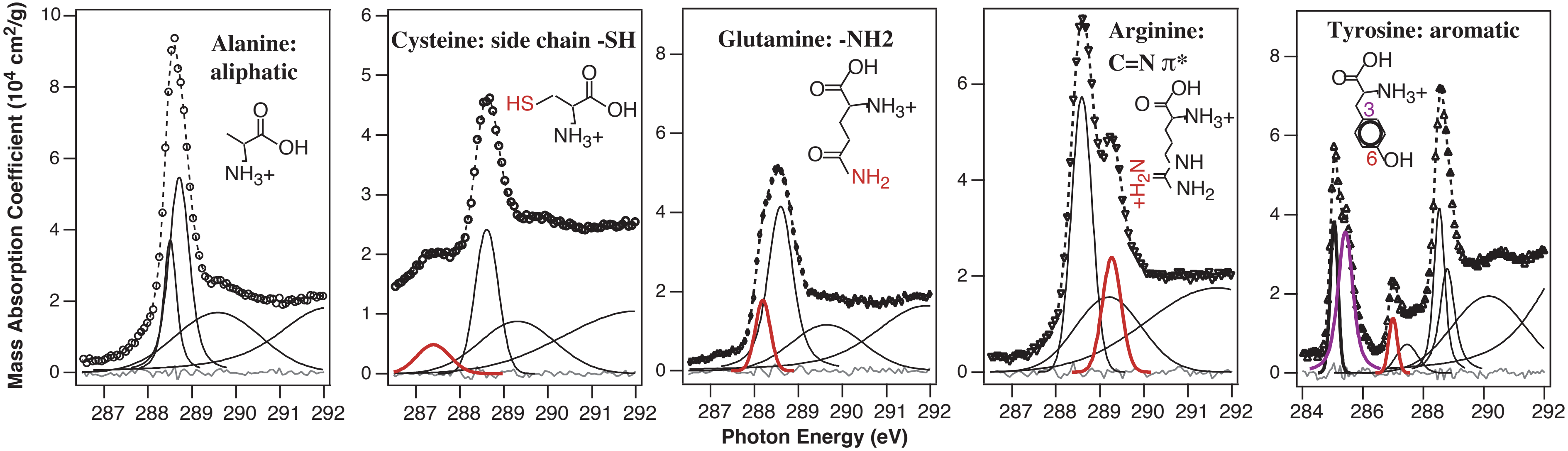
290 eV: carbon strongly absorbing

284 eV: carbon (oil drop) weakly absorbing

Near-edge absorption fine structure (NEXAFS) or X-ray absorption near-edge structure (XANES)







Amino acids example: K. Kaznacheyev *et al.*, *J. Phys. Chem.* **A 106**, 3153 (2002)

Near-edge spectroscopy: ELNES and XANES

ELNES (electron **E**nergy **L**oss)

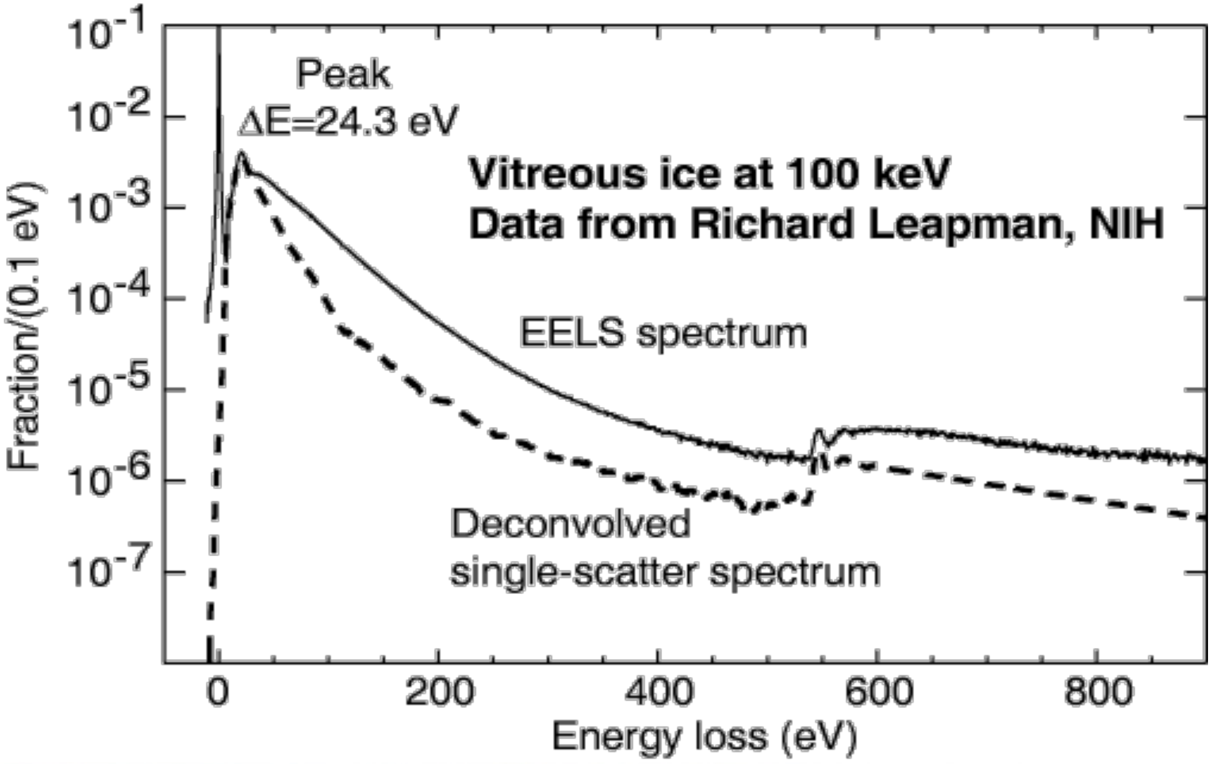
- Plural inelastic scattering
- Many elements at once - but plasmon modes are always excited (damage)
- Δ*E* was ~0.6 eV, but now <0.1 eV in some cases

XANES (**X**-ray **A**bsorption)

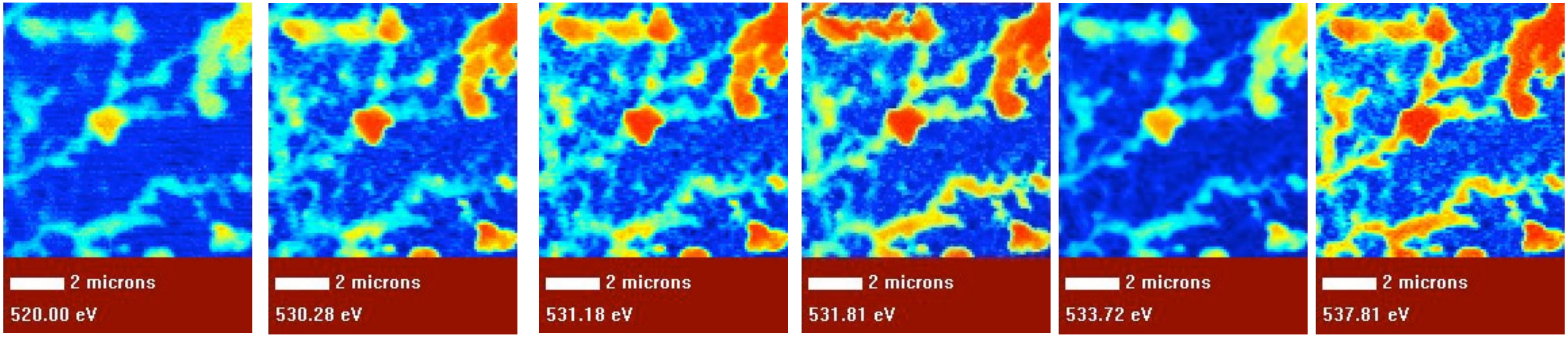
- No plural scattering
- One element at a time - slow but less damage
- Δ*E* of 0.05-0.1 eV is common

Electrons ~1000x more damaging:

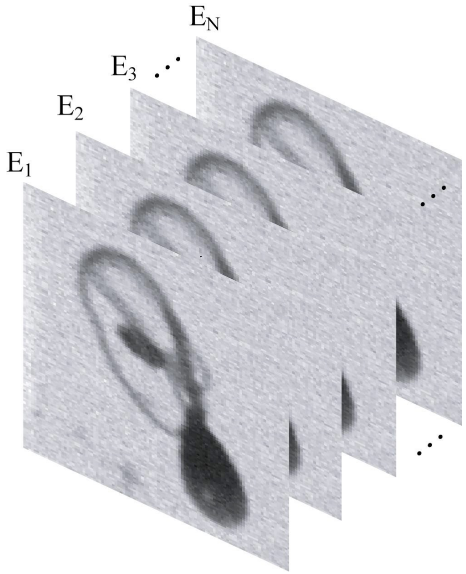
- Isaacson and Utlaut, *Optik* **50**, 213 (1978).
- Rightor *et al.*, *J. Phys. Chem. B* **101**, 1950 (1997).



Spectromicroscopy from image sequences



Lu in hematite (T. Schäfer)



Aligned spectral image sequences: Jacobsen *et al.*, *J. Microscopy* **197**, 173 (2000)

10^4-10^5 spectra! Too many to analyze “by hand.”

Complex mixtures *etc.*; life is not made up of uniform thin films.

How to deal with the complexity?

1. PCA for noise reduction, orthogonalization.
2. Cluster analysis to find patterns.
3. Non-negative matrix factorization for final analysis.

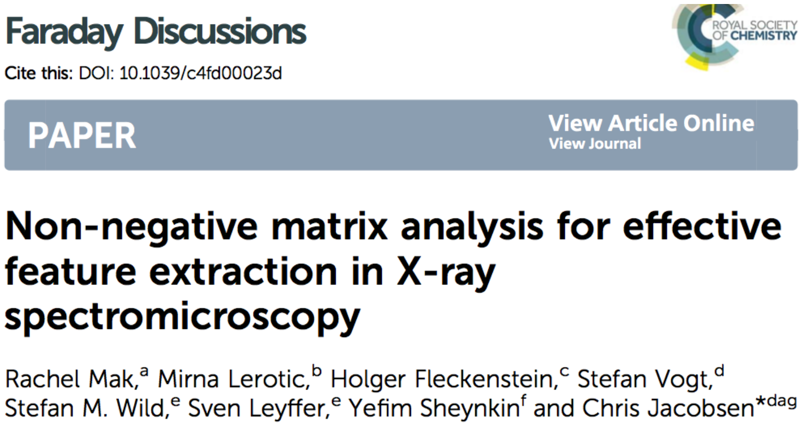
Optimization techniques in spectromicroscopy analysis

Global cost function F:

$$F(\mu, t) = ||D - \mu \cdot t||_2^2 + \lambda_{\mathrm{sparse}}||t||_1 + \lambda_{\mathrm{sim}}||\mu - \mu_{\mathrm{cluster}}||_2^2$$

- Data matching: reconstruction from spectra μ and images t should match optical density data D.
- Sparseness: maximize the separation of features in the images t. The L_1 norm provides a good measure of sparseness.
- Similarity: keep the spectra μ similar to those found in regions of the sample (μ_{cluster}).
- The regularization parameters λ are like a currency exchange rate to put all the costs on the same scale; increase λ until data matching error begins to grow significantly.
- Partial derivatives of each cost guide iterative solutions.
- Mak, M. Lerotic, H. Fleckenstein, S. Vogt, S. Wild, S. Leyffer, Y. Sheynkin, and C. Jacobsen, *Faraday Discussions* **171**, 357 (2014).

Optimized analysis



Faraday Discussions

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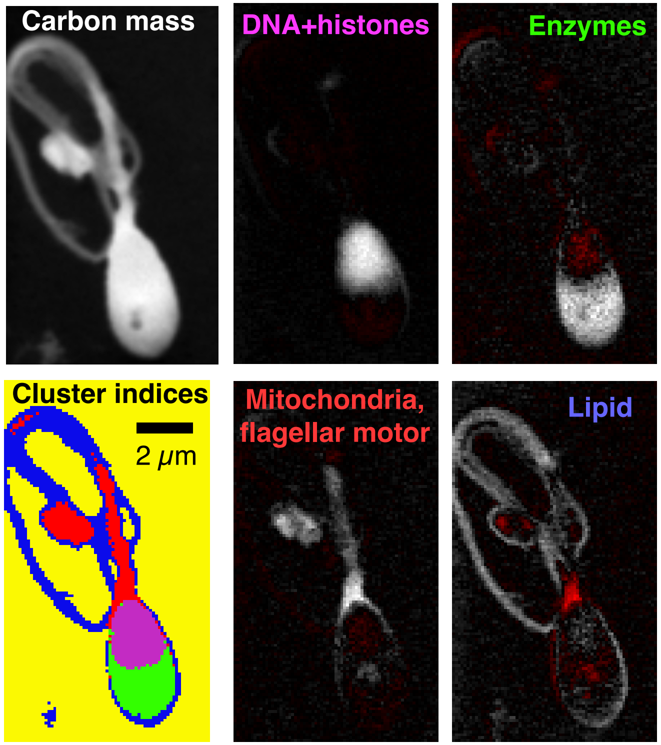
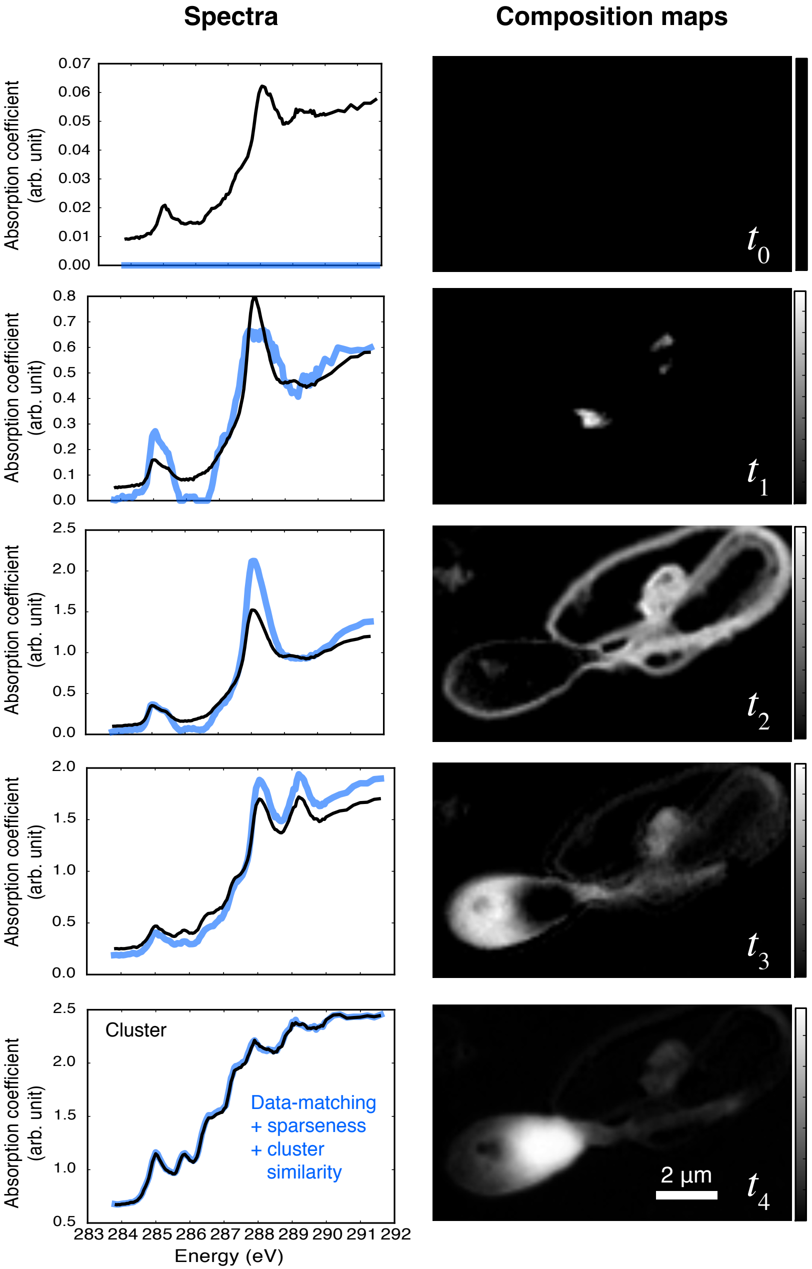
Non-negative matrix analysis for effective feature extraction in X-ray spectromicroscopy

Rachel Mak,[a] Mirna Lerotic,[b] Holger Fleckenstein,[c] Stefan Vogt,[d] Stefan M. Wild,[e] Sven Leyffer,[e] Yefim Sheynkin[f] and Chris Jacobsen*[dag]





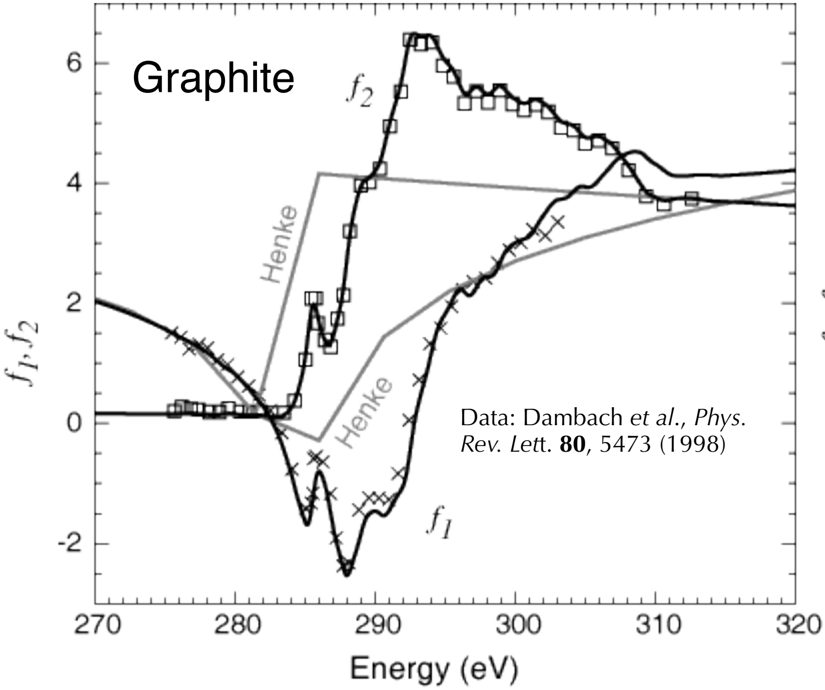
Phase contrast spectromicroscopy?

Kramers-Kronig transform:

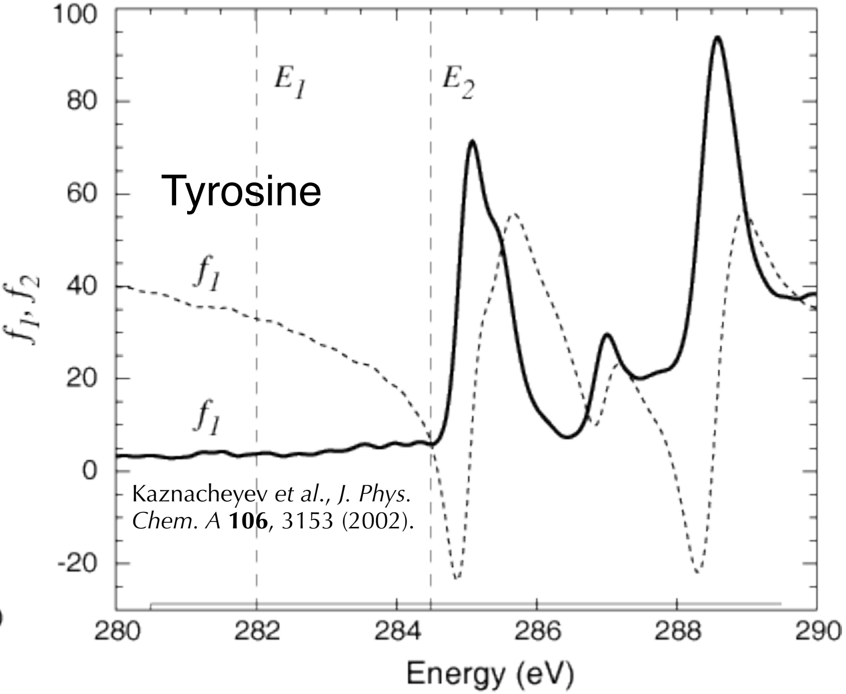
phase shift $f_1(E)$ from absorption $f_2(E)$

$$f_1(E) = Z + \frac{2}{\pi}\int_0^\infty \frac{\varepsilon f_2(\varepsilon)}{E^2 - \varepsilon^2} d\varepsilon - \Delta f_r,$$

Requires phase contrast imaging, such as in ptychography



Data: Dambach *et al.*, *Phys. Rev. Lett.* **80**, 5473 (1998)



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

See Jacobsen *et al*, *SPIE* **5538**, 23 (2004); Maiden *et al.*, *Nature Comm.* **4**, 1669 (2013).