Soft X-ray microscopy with diffractive optics

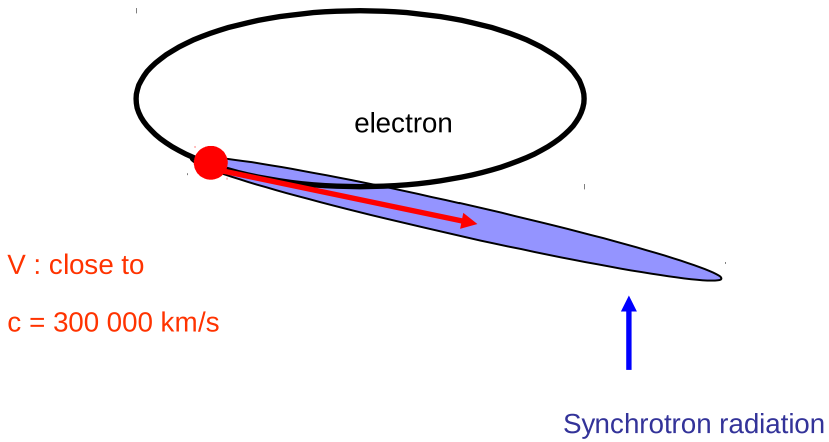
E. Pereiro

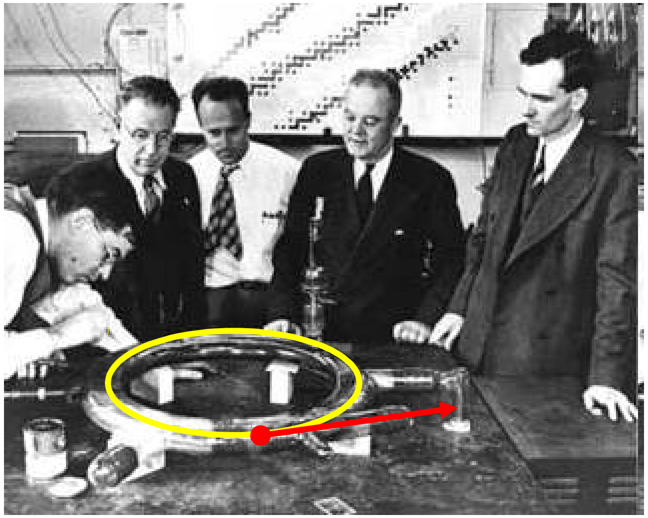
Outline

- Brief description of a synchrotron
- X-rays, what for?
- Soft X-ray microscopy with diffractive optics
- Two common microscopes (TXM & STXM)
- Challenges: radiation damage, depth of focus limitation
- 2 applications with TXM and STXM

Light for Science

A synchrotron is a piece of equipment that produces synchrotron light.





Synchrotron radiation is a set of electromagnetic waves emitted by charged particles that move within a curved trajectory at velocities close to the speed of light.

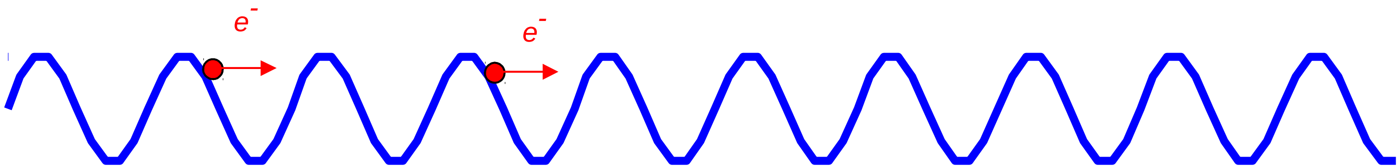
How does it work ?

An **electric field** accelerates an electron and increases its speed.

LINAC

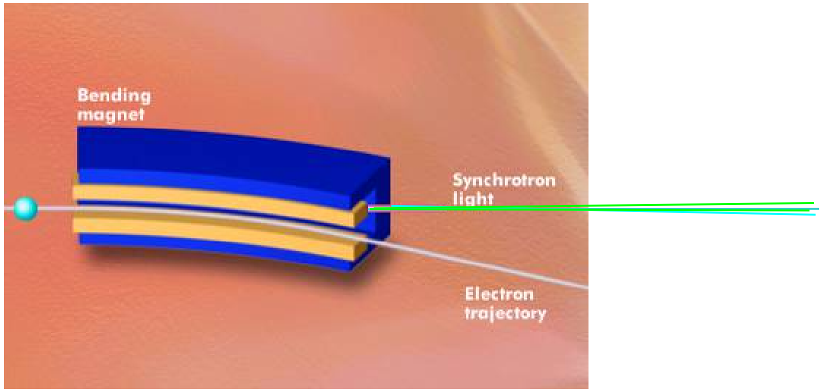
Booster

RF Cavities



When electrons are deflected through a **magnetic field** they emit synchrotron light.

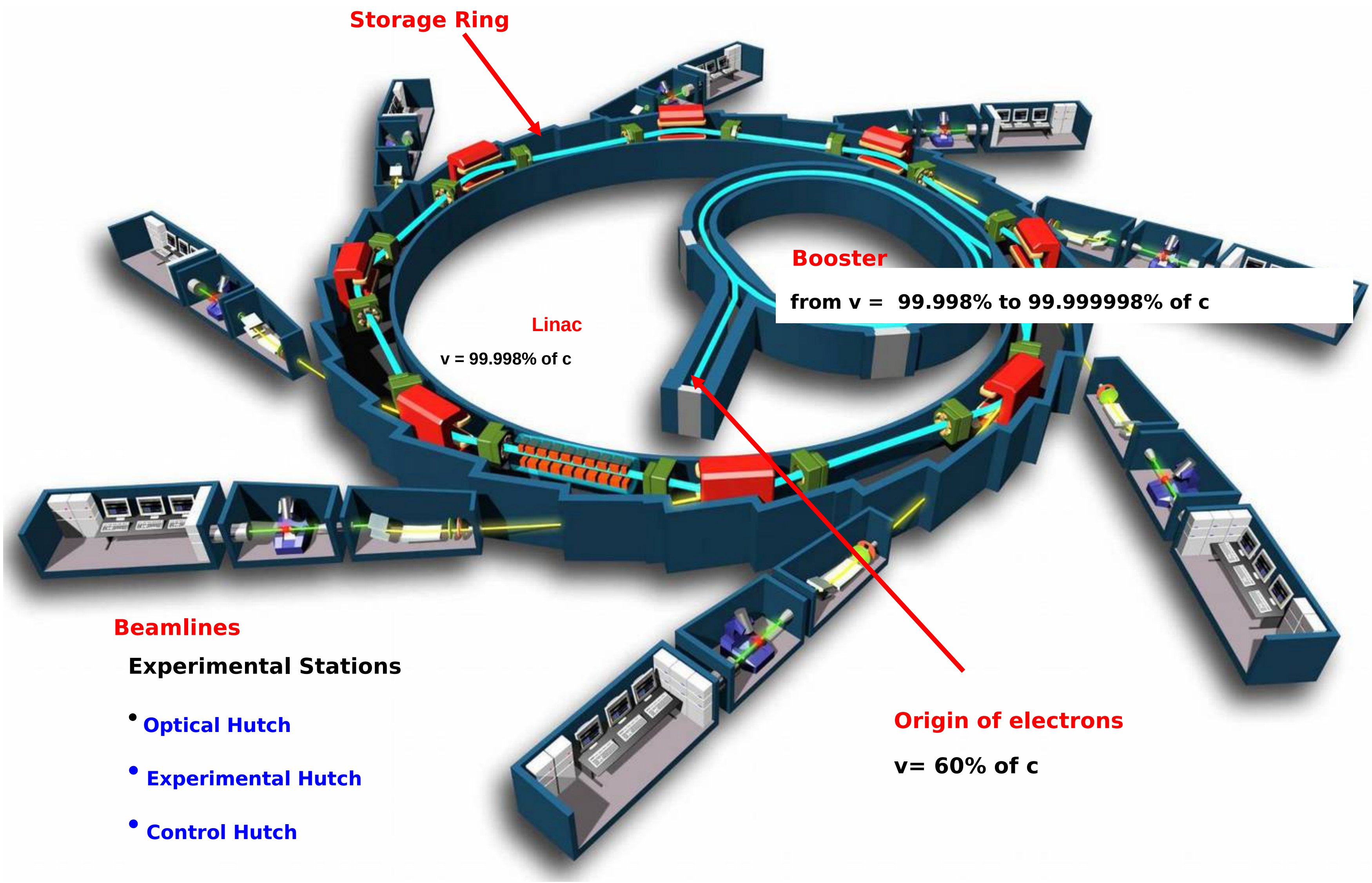
Bending Magnets



Synchroton Light:

IR, VL, UV, X-ray etc.

Anatomy of a Synchrotron



LINAC

The Linac is the first of the accelerators that accelerates the e^- up to 100 MeV

v from 60% to 99.998% of c

Booster and Storage Ring

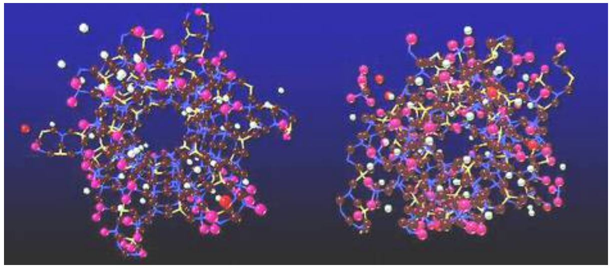
Science with synchrotron light

A SYNCHROTRON IS A TOOL THAT IS USED TO STUDY THE STRUCTURE OF MATTER

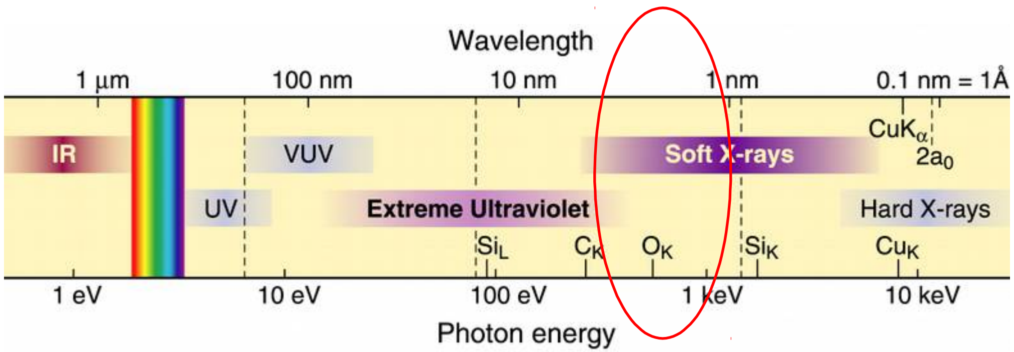
- Atomic
- Molecular
- Nano-, Micro-, Milli-metric

APPLICATIONS

- Chemistry
- Physics
- Biology
- Materials science
- Geology
- Cultural Heritage
- Environment
- Industry
- Medicine

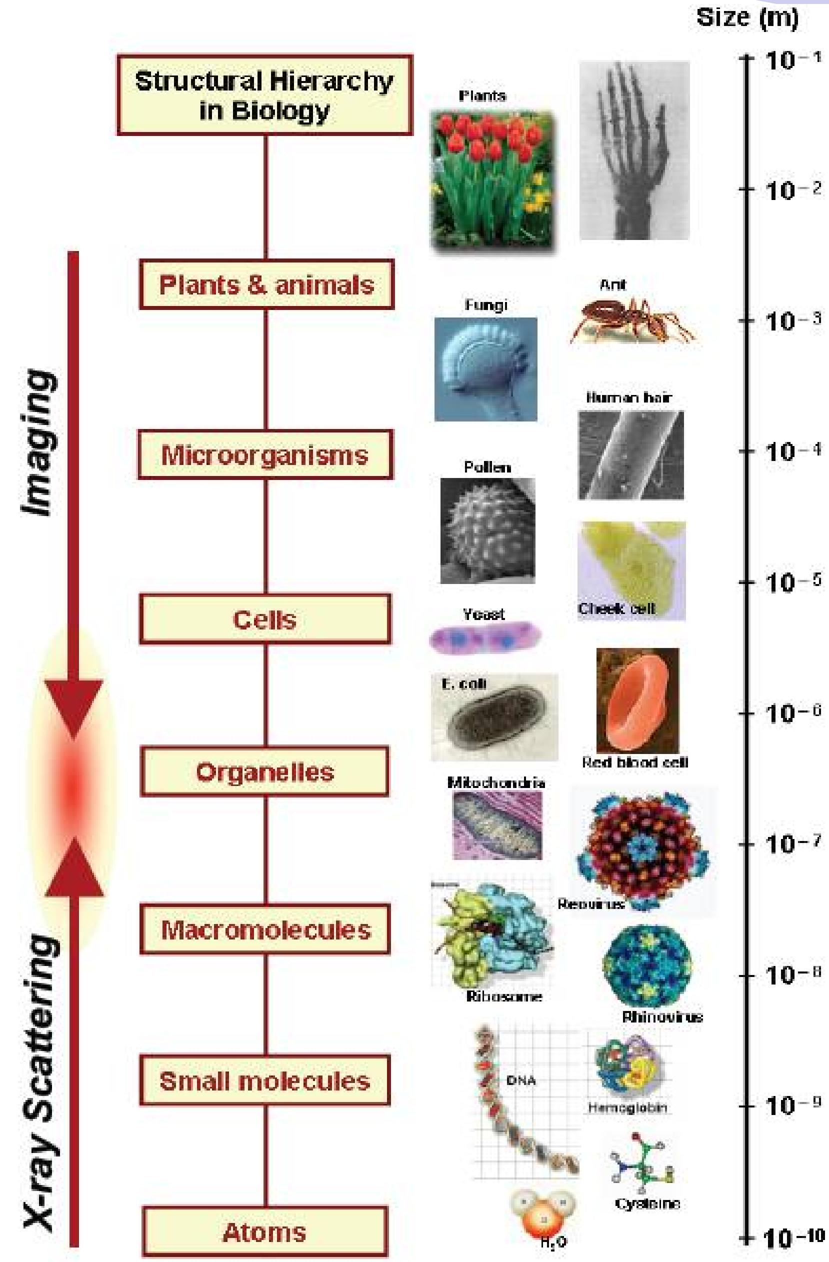


Why imaging with X-rays?



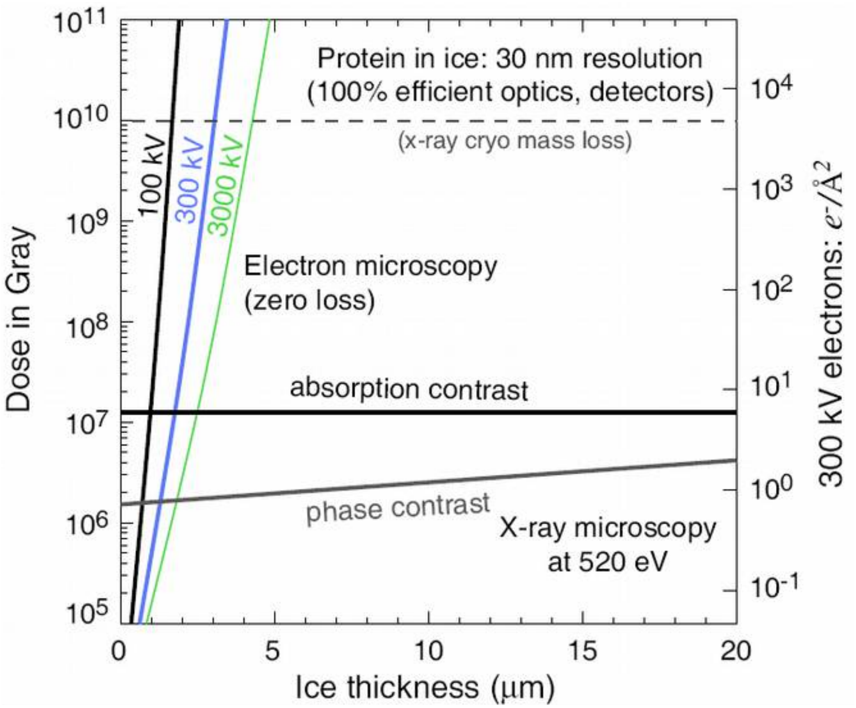
- **See small features in “thick” samples (3D)**

- **Element and chemical sensitivity**

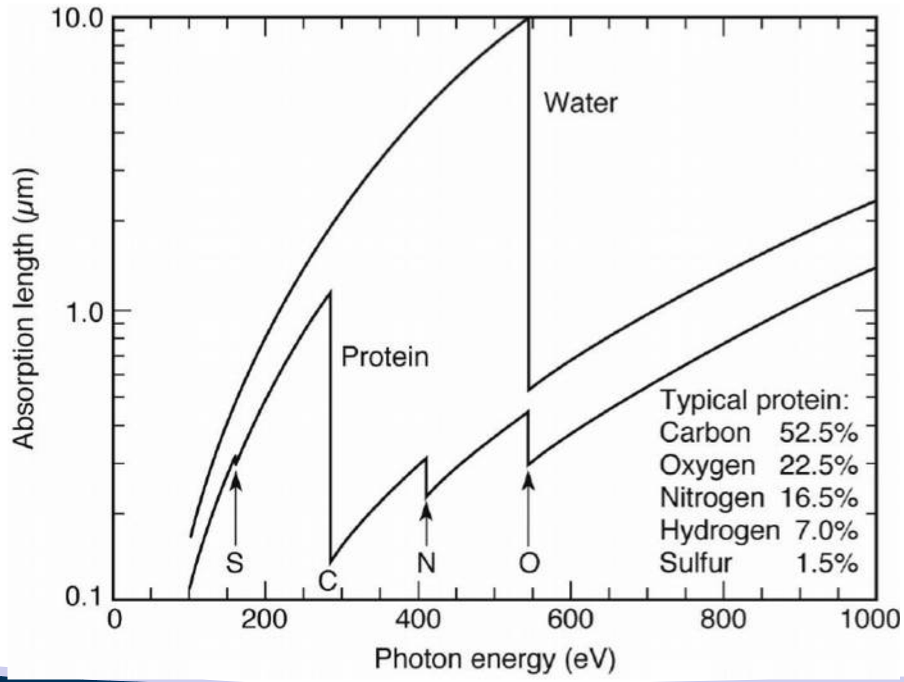


Advantages of X rays compared to electrons

- thick samples
- water window: natural contrast of wet samples
- tomography in statistical numbers
- spectroscopic imaging



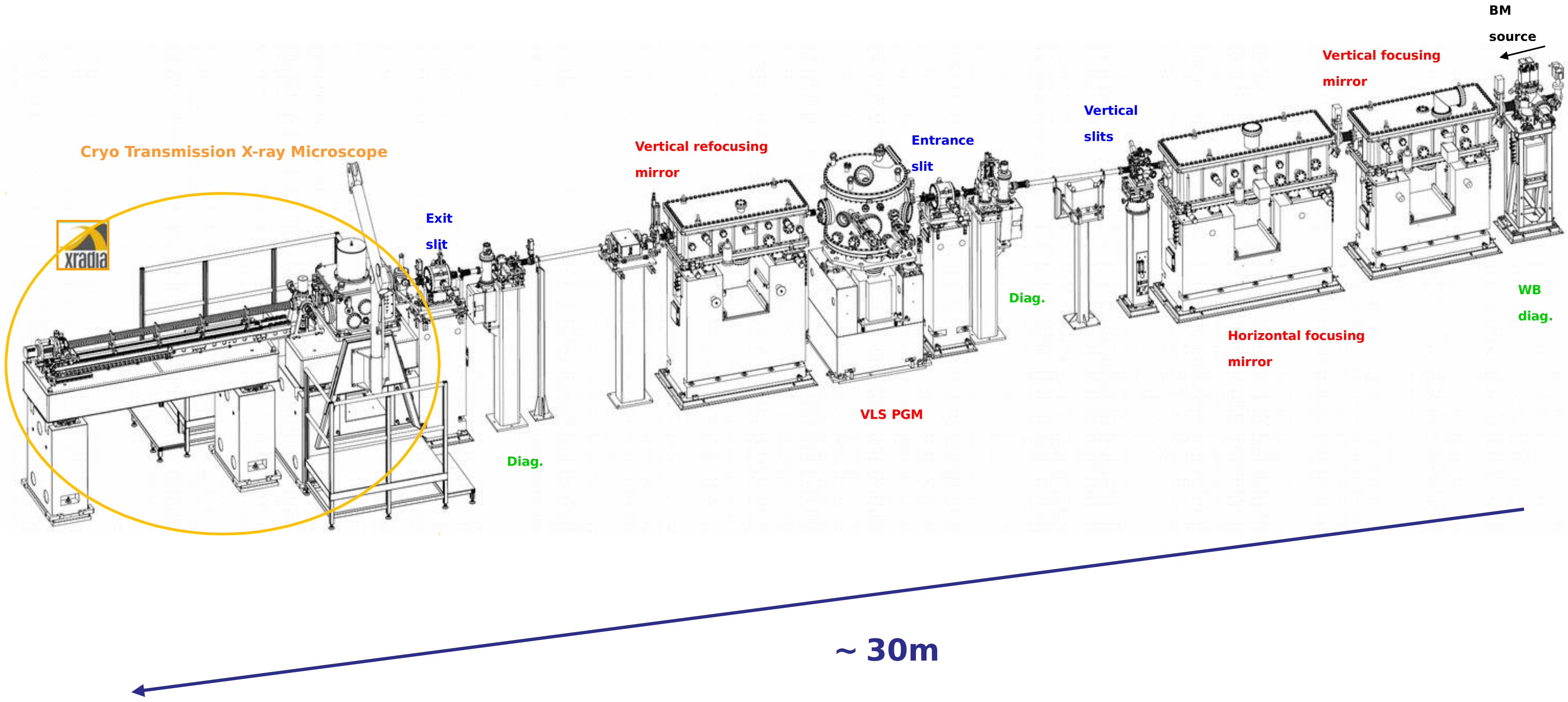
✭ **radiation damage of both electrons & X-rays**



XRM complementary to TEM and light microscopy

A beamline

MISTRAL Beamline @ ALBA



Soft X-ray microscopy

with diffractive optics

Ref: **Soft X-rays and Extreme Ultraviolet Radiation. Principles and Applications**.

David Attwood

Soft X-ray microscopy

What for?

- imaging the internal structure of a sample.
- spectroscopic imaging to map chemical states.

Transmission signal through a sample

- absorption contrast
- phase contrast

How to form an image with SXR wavelengths (0.3-5 nm)?

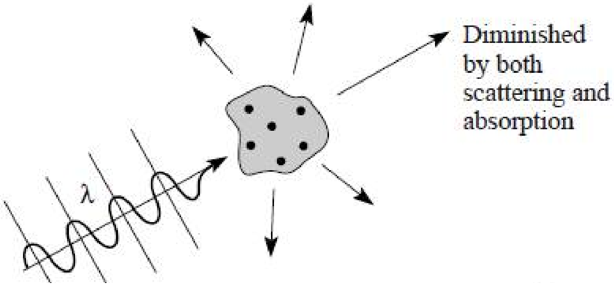
- ***Refraction***, as in optical microscopy (n=1.2-1.5), is impractical as $n=1-\delta+i\beta$ is too close to unity (refraction is too weak).
- ***Glancing incidence total external reflection*** with curved optics works but the image resolution is significantly compromised by aberrations.
- ***Diffraction*** allows forming images at high resolution - tens of nm.

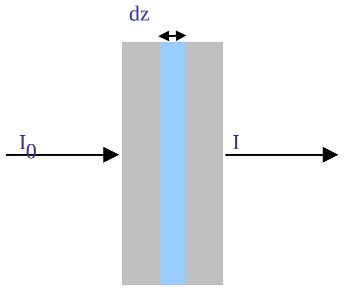
Contrast mechanism: absorption

Basic absorption and emission processes

The total cross section describes the likelihood of interaction between particles (absorption and scattering).

$$\sigma_T = \sigma_{abs} + \sigma_s$$





$$I = I_0 \exp(-\rho \mu z) = I_0 \exp(-n_a \sigma_{abs} z)$$

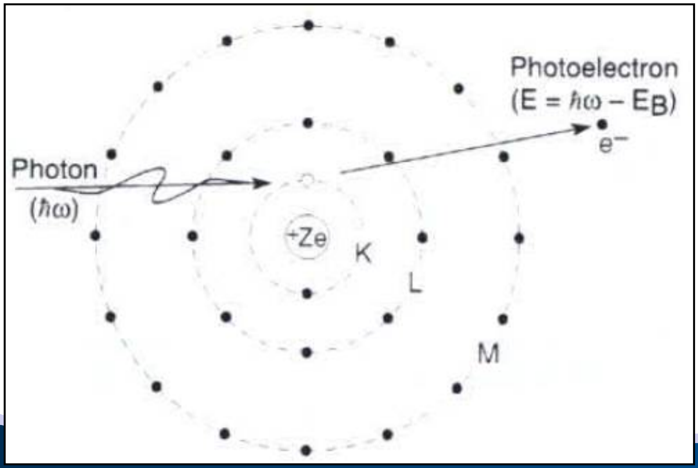
$$\mu = \mu(E, Z)$$

ρ: mass density

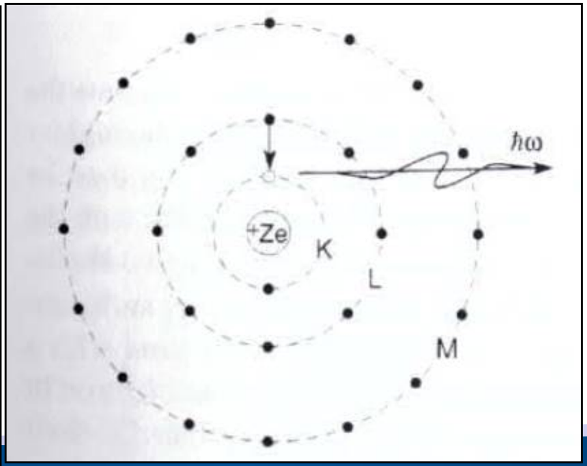
μ: absorption coefficient

n_a: atomic density

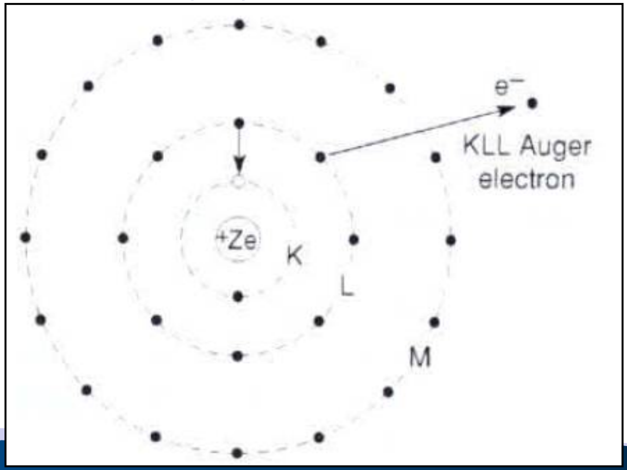
photoionization



fluorescent emission

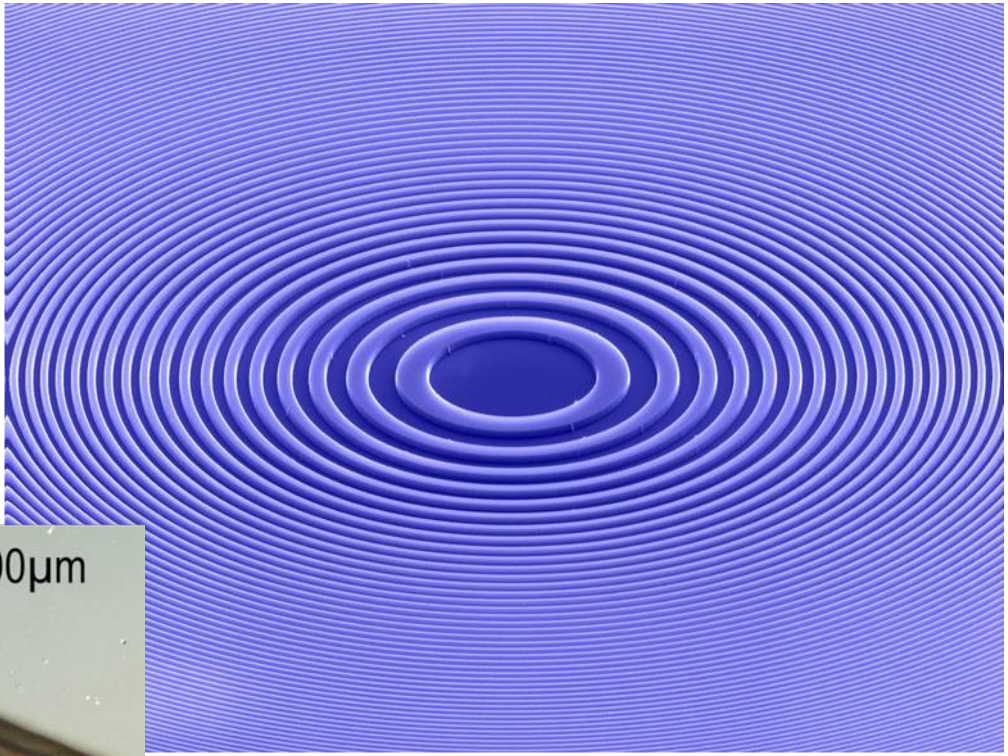


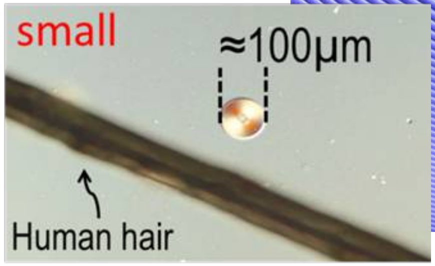
Auger process



Fresnel Zone Plate lens

Soft X-ray microscopy uses diffractive lenses.





Courtesy of Joan Vilà

DEFINITIONS

Scattering is a process by which incident radiation is redirected over a very wide angular range, generally by disordered systems or rough surfaces.

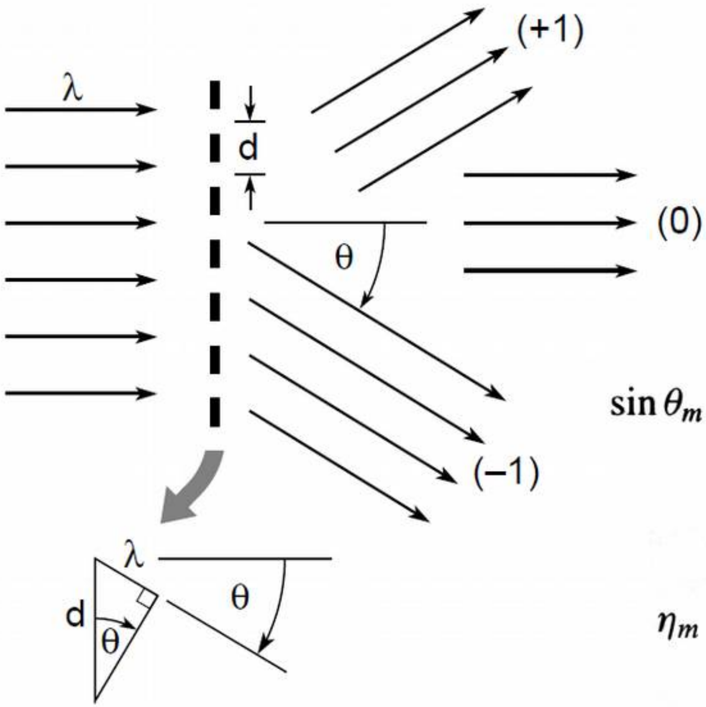
Diffraction is the process by which radiation is redirected into well-defined directions by ordered arrays of scatterers. As the radiation propagates away, it interferes with nearby undiffracted radiation, producing dark and bright bands known as interference patterns.

Example: diffraction of X-rays by a crystal

Transmission grating

Constructive interference occurs at angles where the path length is increased by one λ or m λ.

The fraction of incident E diffracted into the various orders depends on the nature of the periodic structure.



$$\sin\theta_m = \frac{m\lambda}{d}\,; \quad m = 0,\, \pm1,\, \pm2,\, \pm3,\, \ldots$$

$$\eta_m = \begin{cases} \frac{1}{4} & m = 0 \\ 1/m^2\pi^2 & m \text{ odd} \\ 0 & m \text{ even} \end{cases}$$

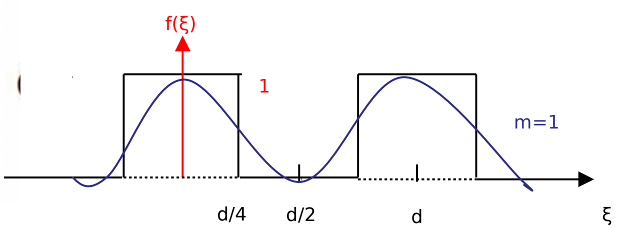
50% absorbed

transmission function:

$$f(\xi) = \sum_{-\infty}^{\infty} c_m \cos(2\pi m\xi / d)$$

$$c_m = \frac{\sin(m\pi/2)}{m\pi}$$

$$I_m = I_0 |c_m|^2 = \eta_m I_0$$



For small structures, diffracted radiation propagates at angles θ~λ/d.

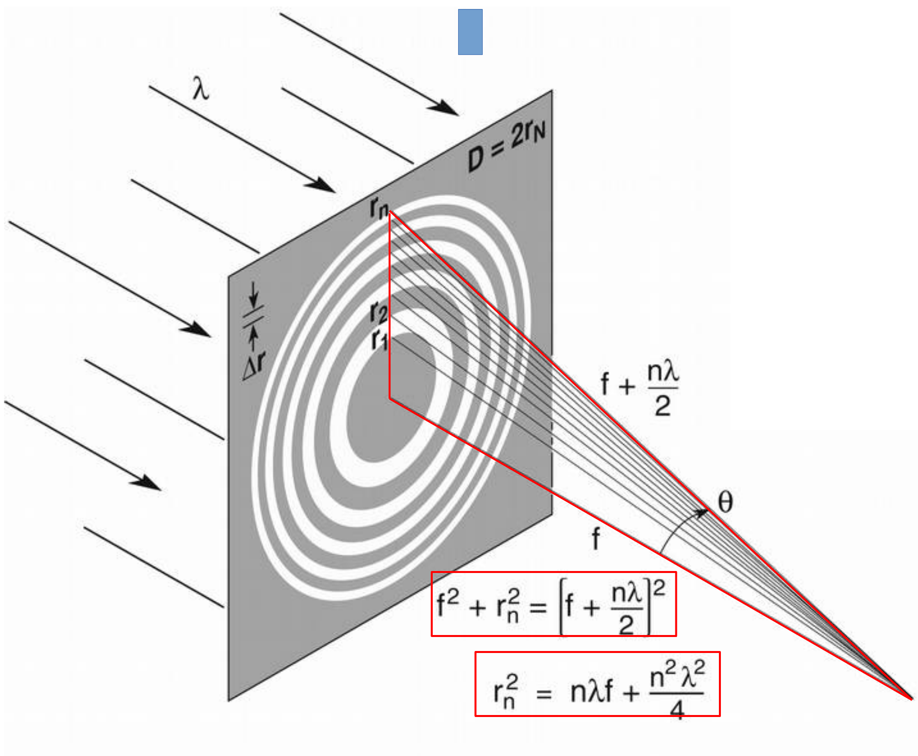
With repetitive structures, positive interference in certain directions can lead to a very strong redirection of E.

Fresnel Zone Plates

1) FZP can focus radiation

Consider a circular transmission grating with the zonal periods adjusted so that at increasing radius from the optics axis the periods become shorter, thus θ becomes larger allowing for a real focus.

The radial zones are located such that the increased path lengths through sequential transparent zones differ by one λ each and thus add in phase at the image point.



For f >> nλ/2, which corresponds to a small NA lens

$$NA = \sin\theta = \lambda/_{2\Delta r} << 1$$

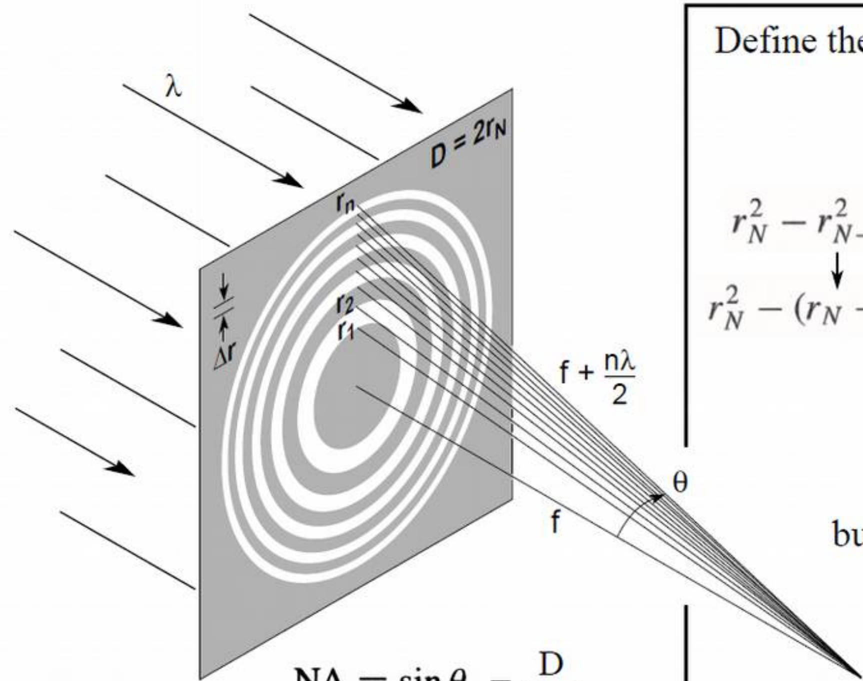
the radius of the nth zone is given by:

$$r_n \approx \sqrt{n\lambda f}$$

- A real first focus is achieved when successive zones increase in radius by $\sqrt{n}$
- FZP are highly chromatic

Fresnel Zone Plates

Relationships for *f* and *D* in terms of λ, Δr and *N*



$$\text{NA} \equiv \sin\theta = \frac{D}{2f}$$

$$\boxed{\text{NA} \simeq \frac{\lambda}{2\,\Delta r}} \qquad (9.15)$$

$$\text{F}^{\#} \equiv \frac{f}{D} \simeq \frac{\Delta r}{\lambda} \qquad (9.16)$$

Define the outer zone width for n → N,

$$\Delta r \equiv r_N - r_{N-1} \qquad (9.11)$$

$$r_n^2 \simeq n\lambda f$$

$$r_N^2 - r_{N-1}^2 = N\lambda f - (N-1)\lambda f = \lambda f$$

$$r_N^2 - (r_N - \Delta r)^2 = 2r_N\,\Delta r - \cancel{(\Delta r)^2} \simeq 2r_N\,\Delta r$$

$$2r_N\,\Delta r \simeq \lambda f$$

$$D\,\Delta r \simeq \lambda f \qquad (9.12)$$

$$\text{but } \lambda f = \frac{r_N^2}{N} = \frac{D^2}{4N} \qquad \text{(from 9.10)}$$

$$\therefore\ D\,\Delta r \simeq \frac{D^2}{4N}$$

$$\boxed{D \simeq 4N\,\Delta r} \qquad (9.13)$$

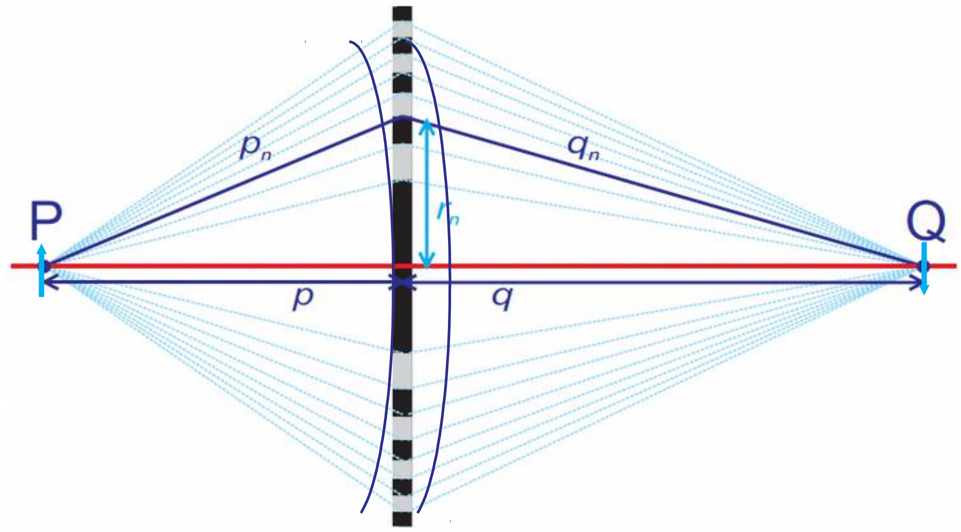
and from (9.12) above

$$\boxed{f \simeq \frac{4N(\Delta r)^2}{\lambda}} \qquad (9.14)$$

Important relationship for the design of FZP showing that *f* scales directly with *N*, with the square of the outer zone width (which sets the resolution) and inversely to λ, introducing a strong chromatic effect.

Fresnel Zone Plates

2) FZP can form a real image



Successive zones, alternately transmissive and opaque, are constructed so as to add λ/2 to successive path lengths, so that

$$q_n + p_n = q + p + n\lambda/2 \quad (1)$$

where for NA small,

$$p_n = \sqrt{p^2 + r_n^2} \approx p + r_n^2/2p \quad (2)$$

$$q_n = \sqrt{q^2 + r_n^2} \approx q + r_n^2/2q \quad (3)$$

Combining (1), (2) and (3) with $f = r_n^2/n\lambda$:

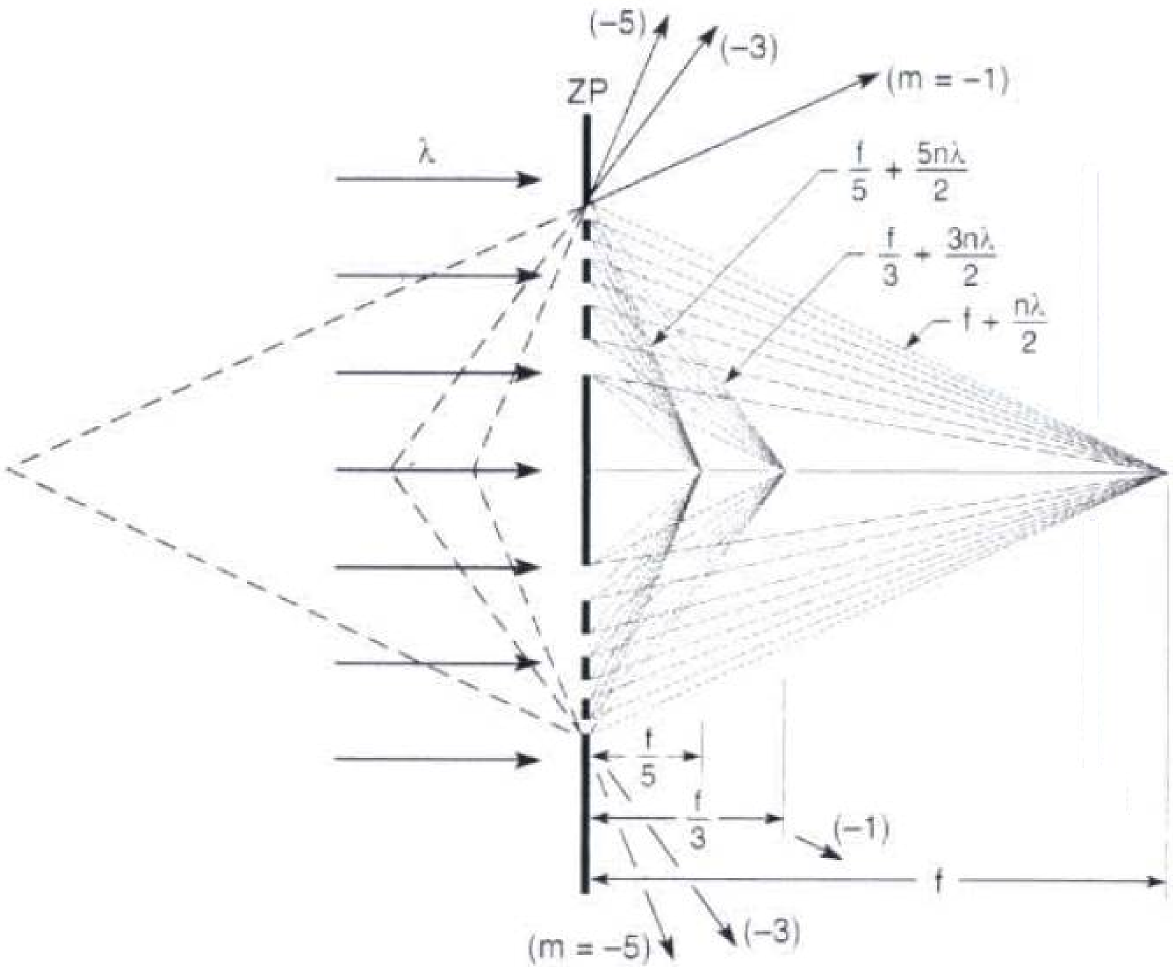
$$\frac{1}{q} + \frac{1}{p} \approx \frac{1}{f}$$

and

$$M = \frac{p}{q}$$

Fresnel Zone Plates

3) FZP: higher diffractive orders



FZP generates many diffractive orders (m).

Only a fraction of light goes to the 1st order.

For higher orders, mnλ/2 is added to the path length and

$$r_n^2 \approx mn\lambda f_m$$

$$f_m = f/m$$

Fresnel Zone Plates

4) FZP efficiency

In theory…

50% of the incident energy is absorbed by the opaque zones.

25% is transmitted in the forward direction: m = 0.

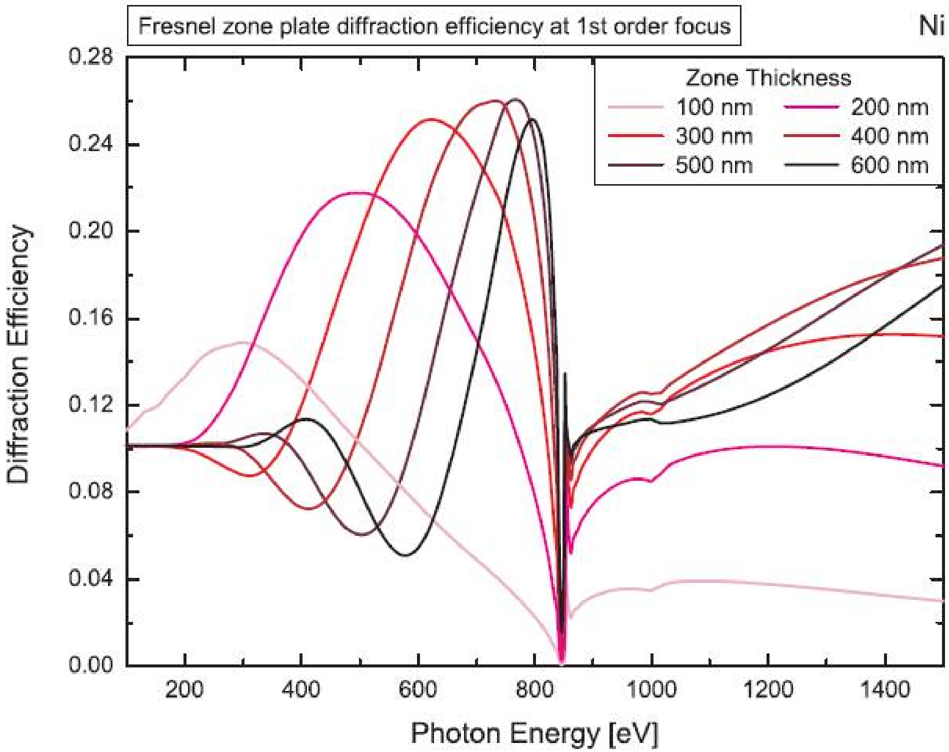
10% is focus onto m = 1 and 10% onto m = -1.

<5% is focus onto higher orders.

$$\eta_m = \begin{cases} 1/4 & m=0 \\ 1/m^2\pi^2 & m \text{ odd} \\ 0 & m \text{ even} \end{cases}$$

…but opaque zones are difficult to manufacture and therefore η_m depends on the material thickness and $n=1-\delta-i\beta$.

 the extra phase change introduced by the material reinforces η_m when material and zone thickness are conveniently chosen.



Joan Vilà, thesis

Fresnel Zone Plates

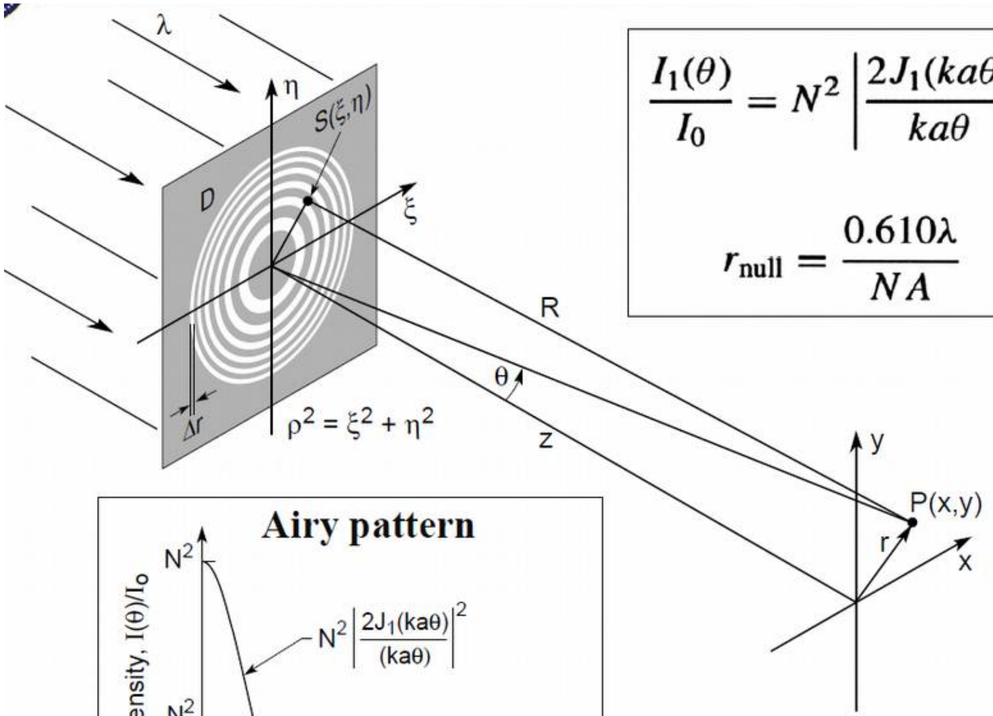
5) FZP diffraction

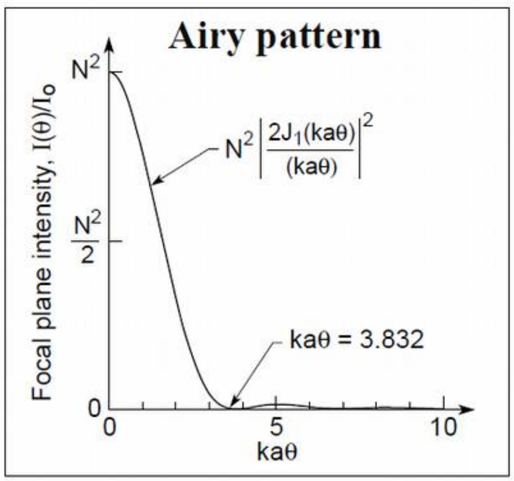
Intensity distribution for a coherently illuminated FZP.

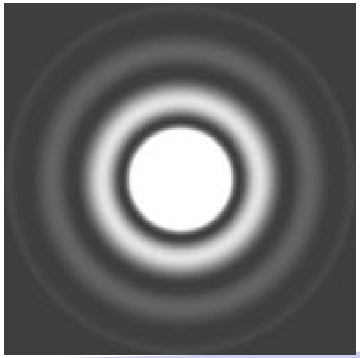
$$\frac{I_1(\theta)}{I_0} = N^2 \left| \frac{2J_1(ka\theta)}{ka\theta} \right|^2 \qquad (9.45)$$

$$r_{\text{null}} = \frac{0.610\lambda}{NA} \qquad (9.46)$$

$$k = 2\pi / \lambda$$







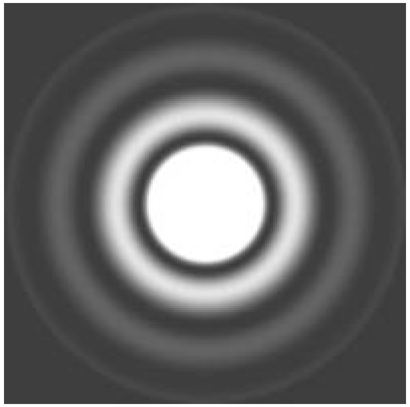
The FZP forms an Airy pattern in the focal plane with characteristic lateral dimension.

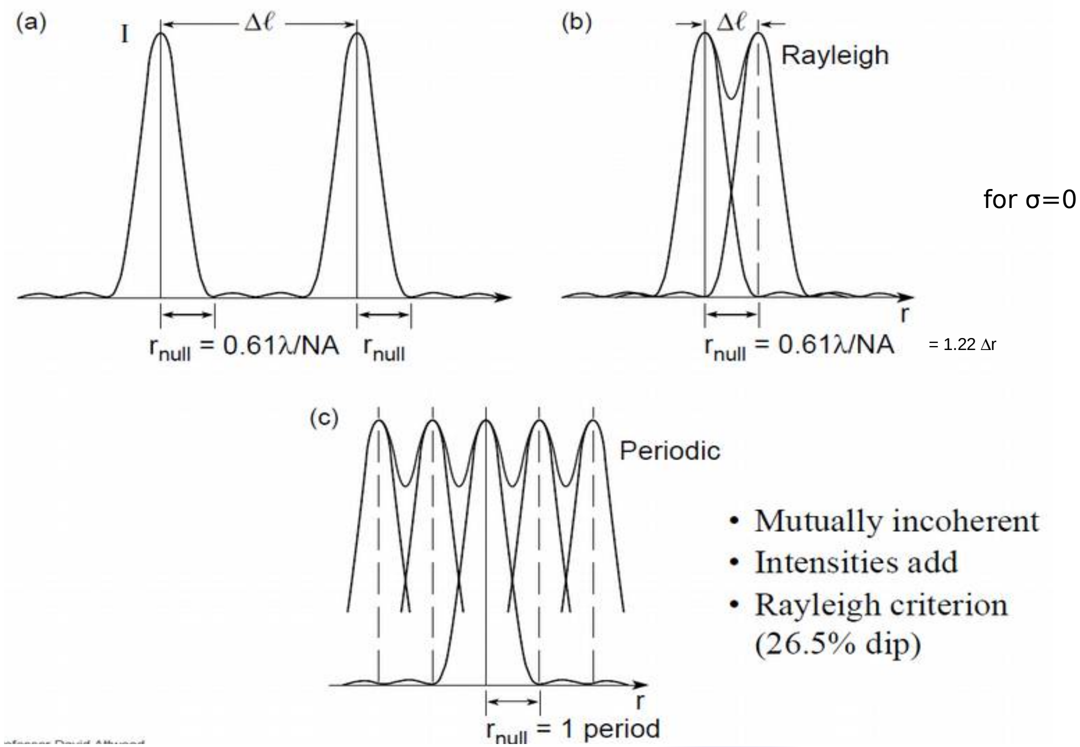
The resolution of an ideal lens is limited by NA and λ.

The Airy pattern carries 85% of the power in the first ring.

6) Spatial resolution: resolving 2 point sources

One measure of the resolution of a lens is the minimum discernible separation of 2 mutually incoherent point sources. This depends on the **point spread function**, that is the image plane intensity distribution due to a distant point source. For an ideal lens, including FZP, the PSF is an Airy pattern whose lateral extent (spread) depends on both λ and NA.

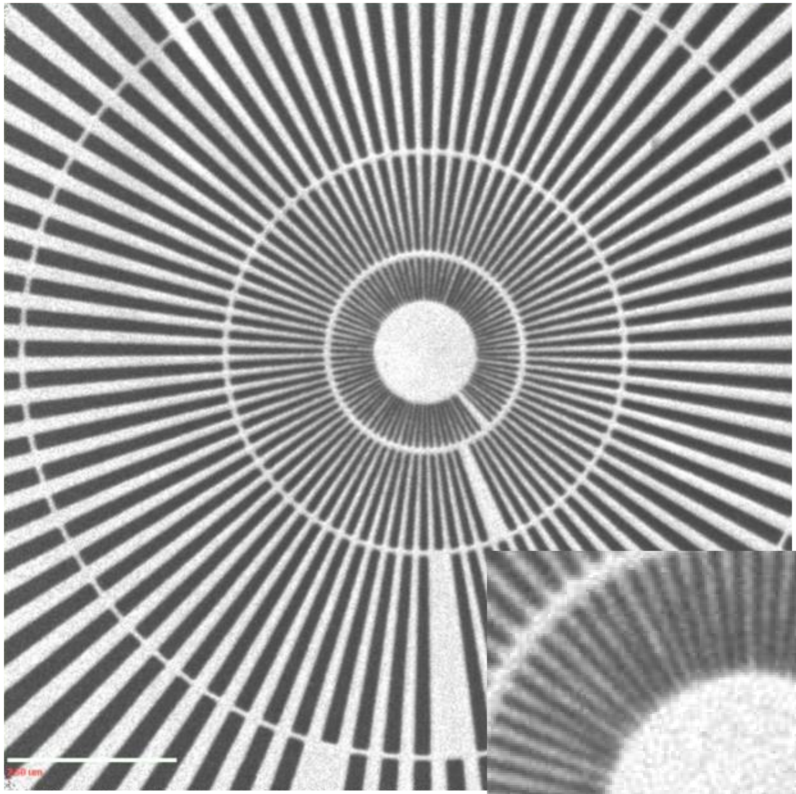




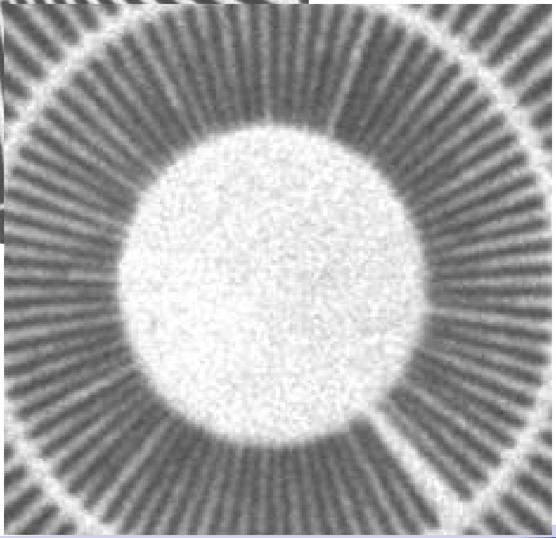
Fresnel Zone Plates

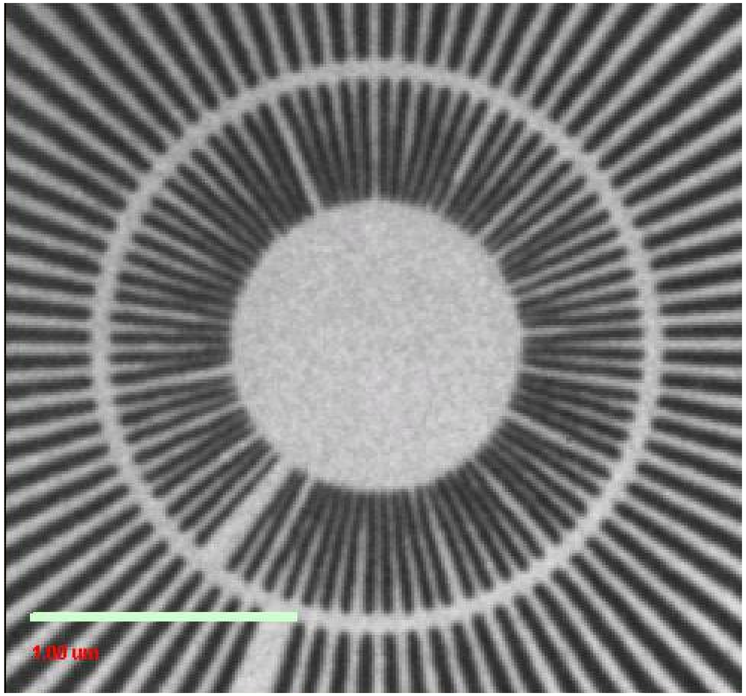
6) Spatial resolution: resolving 2 point sources

Au Siemens star with 30 nm smallest features.



40 nm ZP at E=520 eV, t=1s.





25 nm ZP at E=776 eV, t=4s

Fresnel Zone Plates

7) FZP depth of focus and spectral bandwidth

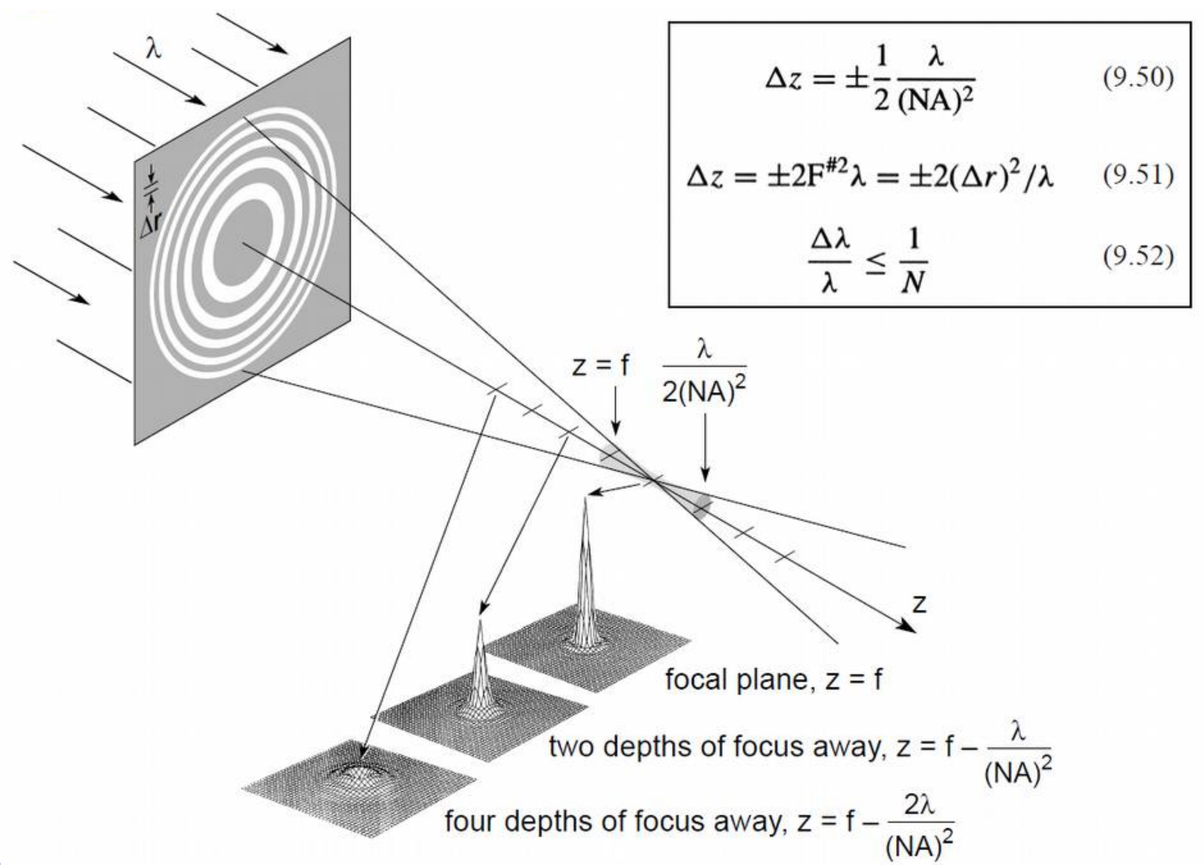
DoF = sample thickness that can be imaged allowing for only a 20% on-axis intensity decrease

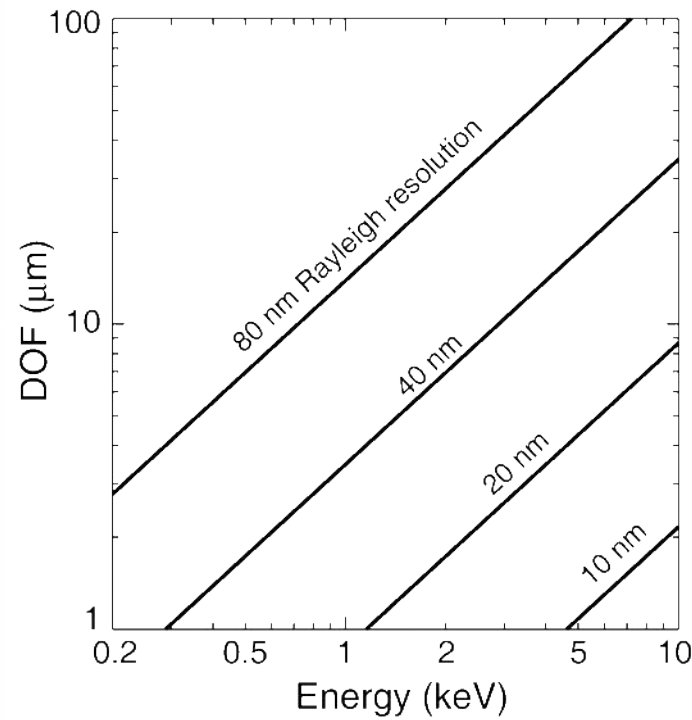
FZP are highly chromatic: for precise imaging spectral bandwidth illumination should be restricted

$$\Delta z = \pm\frac{1}{2}\frac{\lambda}{(\mathrm{NA})^2} \qquad (9.50)$$

$$\Delta z = \pm 2\mathrm{F}^{\#2}\lambda = \pm 2(\Delta r)^2/\lambda \qquad (9.51)$$

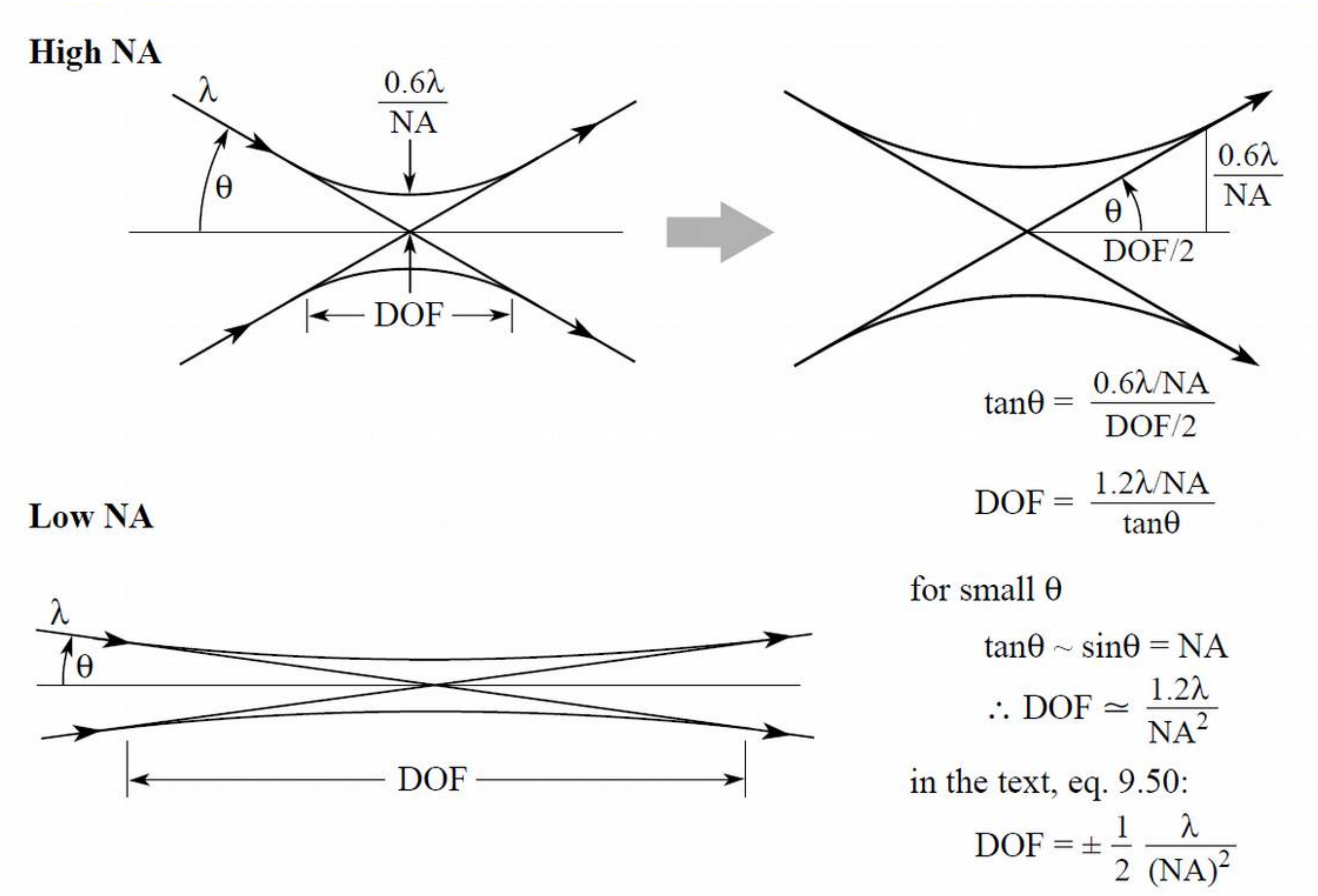
$$\frac{\Delta\lambda}{\lambda} \le \frac{1}{N} \qquad (9.52)$$



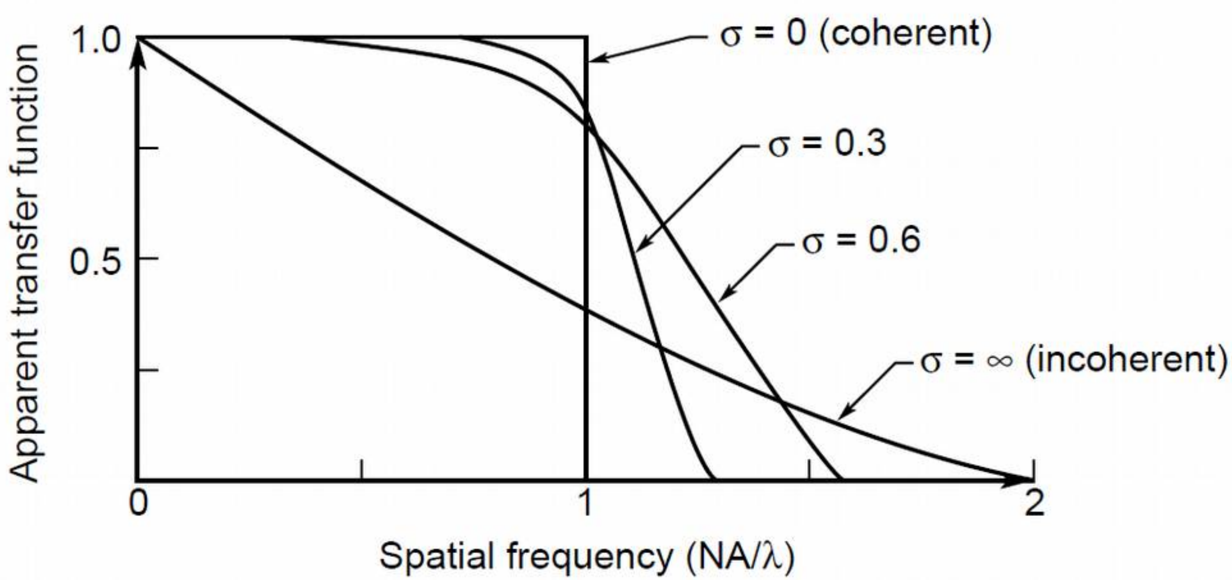


Fresnel Zone Plates

DoF scales as λ/NA^2



Optical Transfer properties with partially coherent illumination

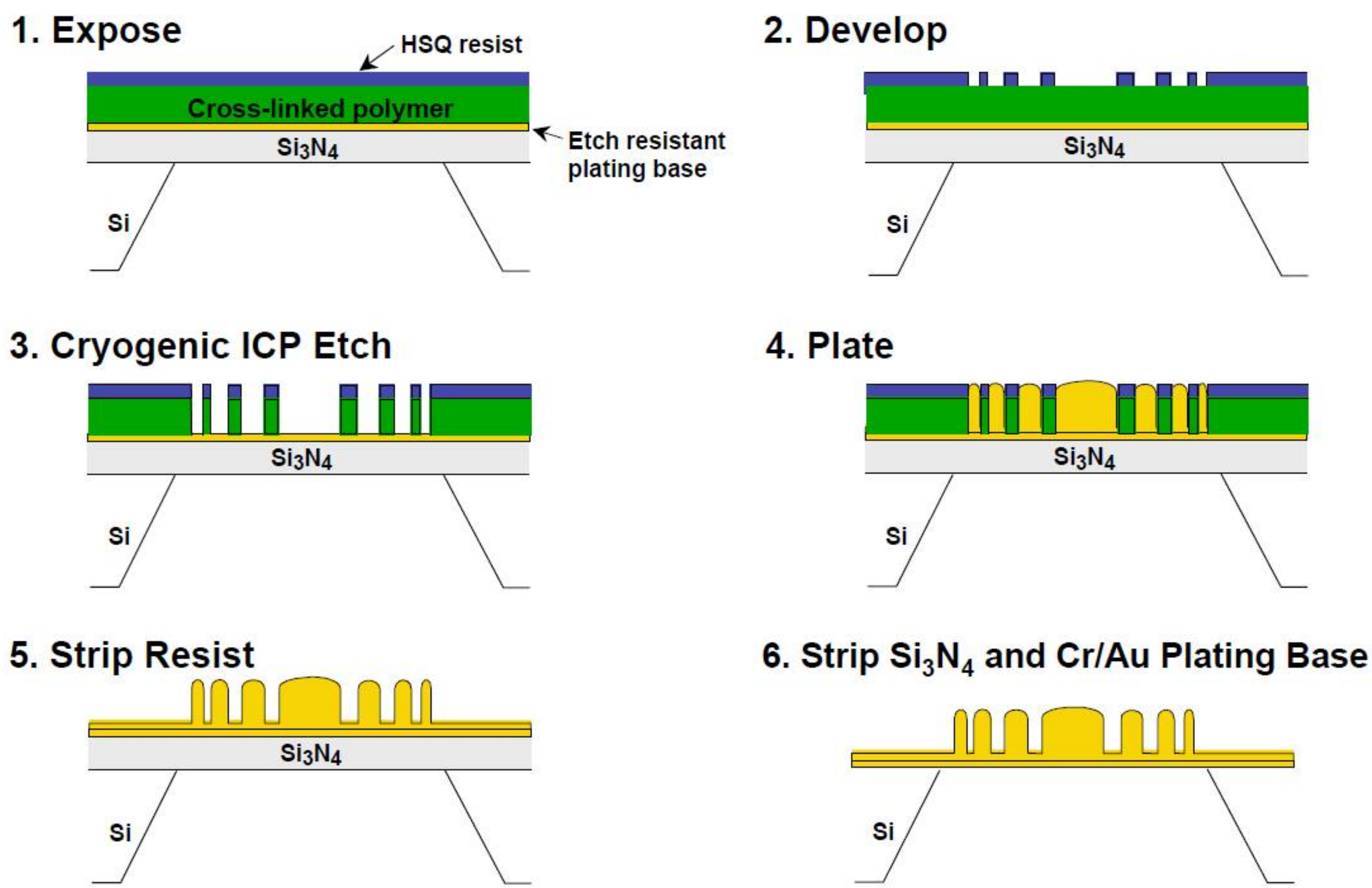


$$\sigma \equiv \frac{NA_{illum.}}{NA_{obj.}} = \frac{\sin\theta_{illum.}}{\sin\theta_{obj.}} \quad (\text{for } n = 1)$$

Depending on the coherence of the illumination you can have better resolution than the Rayleigh one (1.22Δr).

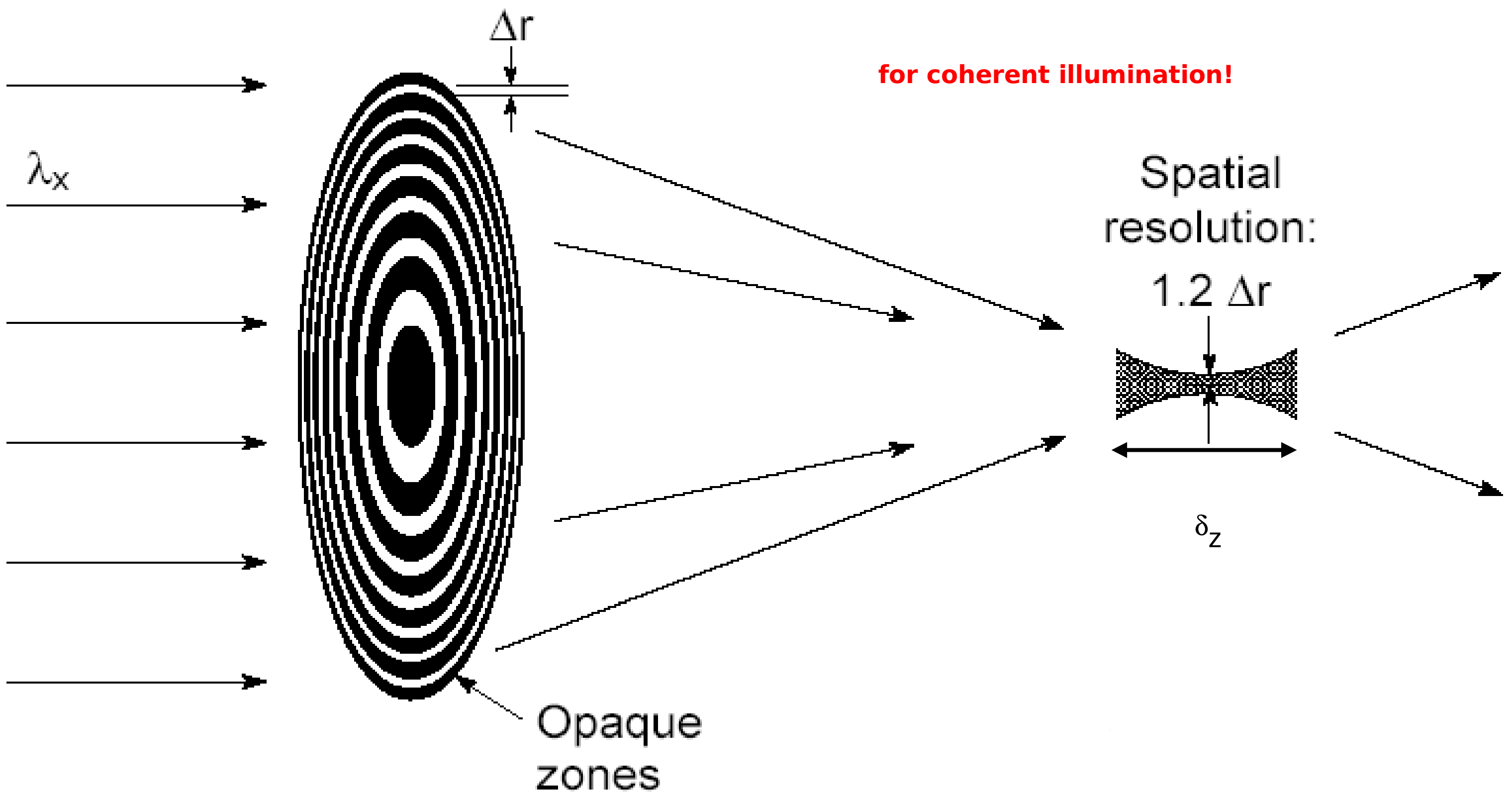
Nanofabrication of Fresnel Zone Plates

e-beam lithography is used to manufacture FZP.



Courtesy of E. Anderson, A. Liddle, W. Chao, D. Olynick, and B. Harteneck (LBNL)

Fresnel Zone Plates: summary



for coherent illumination!

$$\lambda = 2.3843\ nm\ for\ E = 520\ eV, \Delta r = 40\ nm$$

$$\delta_{x,y}\Big|_{coherent} = \frac{1.22\Delta r_N}{m}$$ = 48.8 nm for m=1

NA = λ /2Δr = 0.03

$$\delta_z = \pm\frac{2(\Delta r_N)^2}{m^2\lambda}$$ = ± 1.342 μm for m=1

$$f \simeq \frac{4N(\Delta r)^2}{\lambda}$$ = 2.516 mm

Fresnel Zone Plates

To sum up!

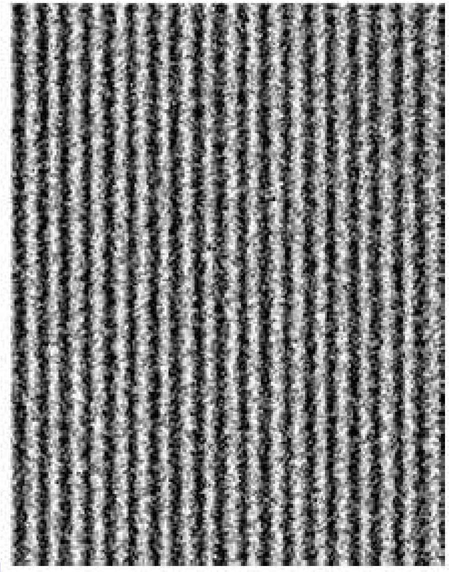
increasing spatial resolution implies decreasing DOF (λ/NA^2)

higher resolution with reasonable DOF @ multi-keV

✯ low efficient (high aspect ratio)

✯ sensitive to heat load

✯ few suppliers



State of the art

“Cr/Si multilayers with 15.1 nm half-period imaged with 15 nm ZP”

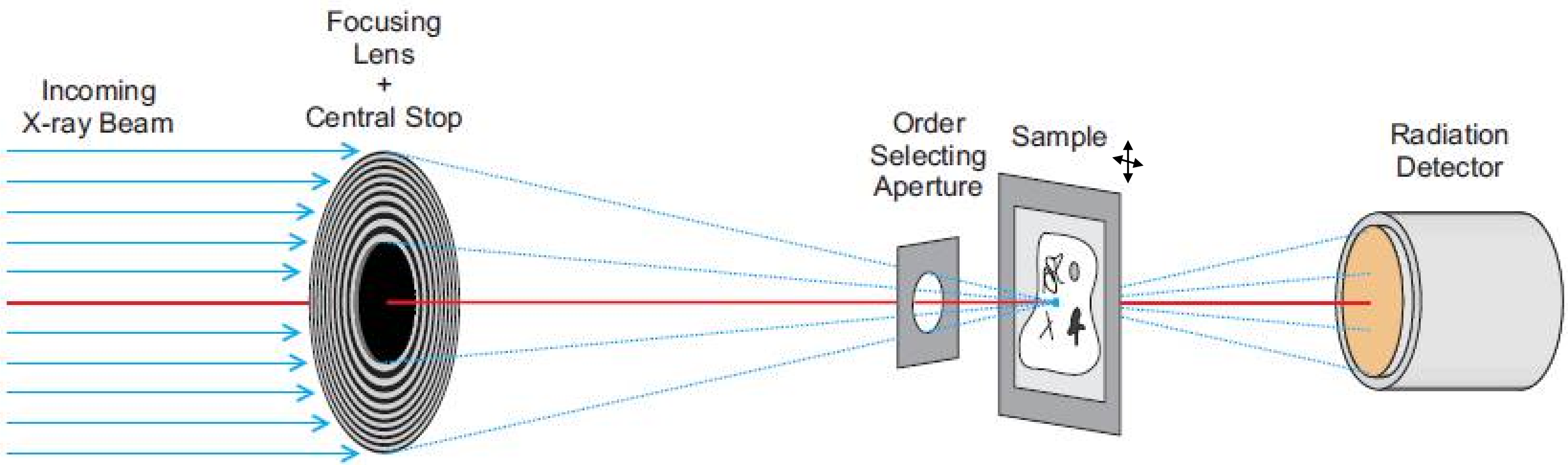
- efficiency ~3%
- focal length at C edge: 100 µm

by Chao *et al.* Nature 435 (2005)

Two common soft X-ray microscopes

The microscopes

The scanning X-ray microscope (STXM)

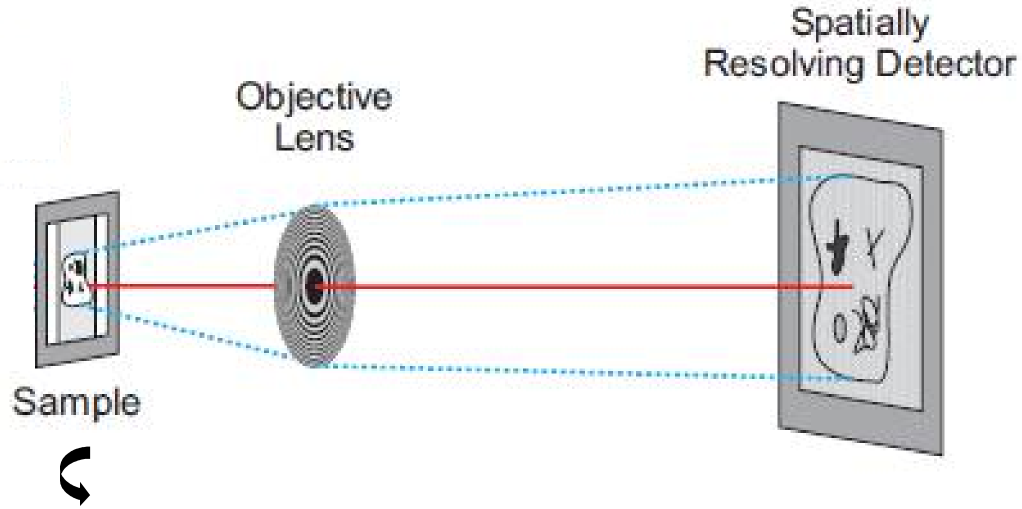


1. least radiation dose
2. high spatial resolution (Δr_N)
3. requires spatially coherent radiation
4. longer exposure time
5. spectroscopic capabilities
6. allows detecting fluorescent emission

The microscopes

The transmission X-ray microscope (TXM)

1. high spatial resolution (Δr_N)
2. short exposure time (snapshot)
3. higher radiation dose
4. fast 3D imaging

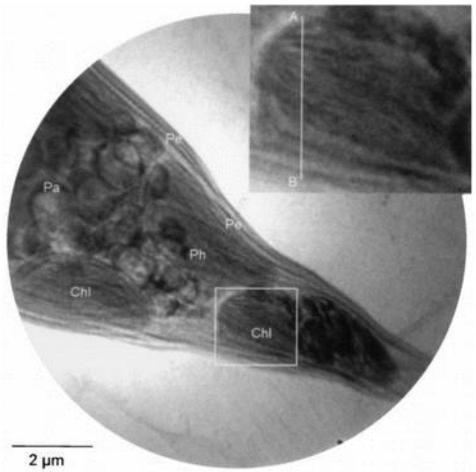


Illumination can be provided by a FZP or a glass capillary.

A chronology of cryo Transmission X-ray Microscopy

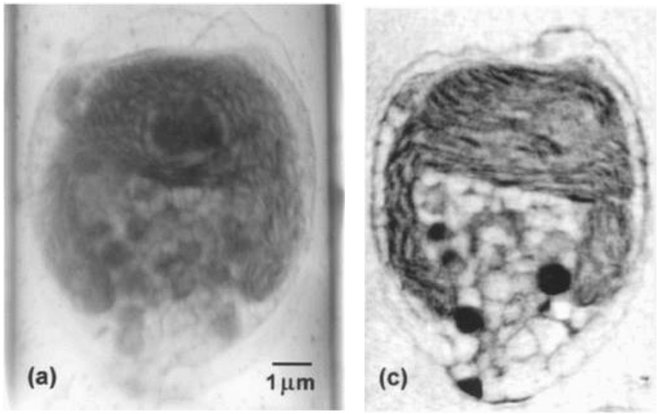
1. X-ray projection of frozen hydrated alga

G. Schneider, Ultramicroscopy 75 (1998)

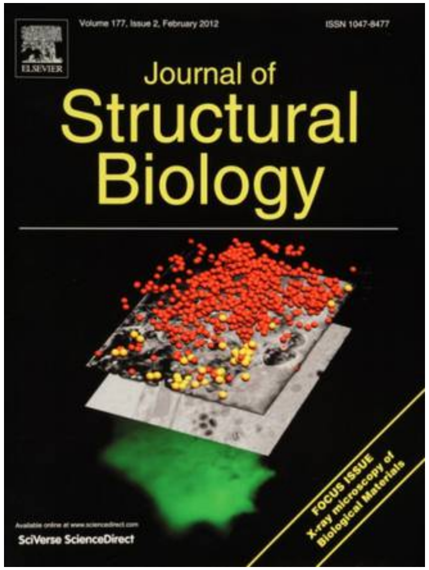


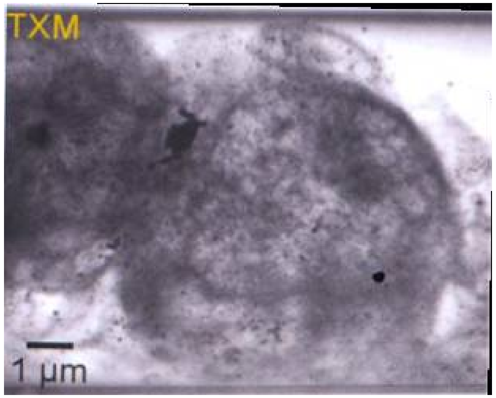
2. 1st cryo-tomo of a frozen alga

D. Weiss *et al.* Ultramicr. 84 (2000)



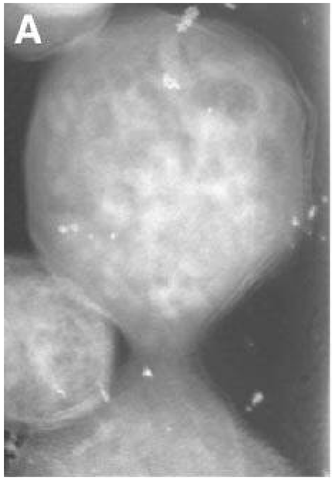
XRM focused issue (2012)





3. Drosophila melanogaster cell

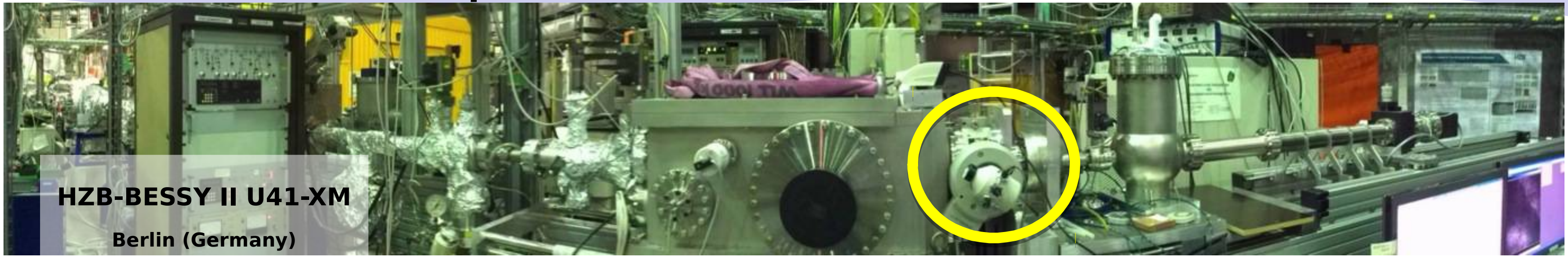
G. Schneider et al. Surf. Rev. Lett 9, 1 (2002)

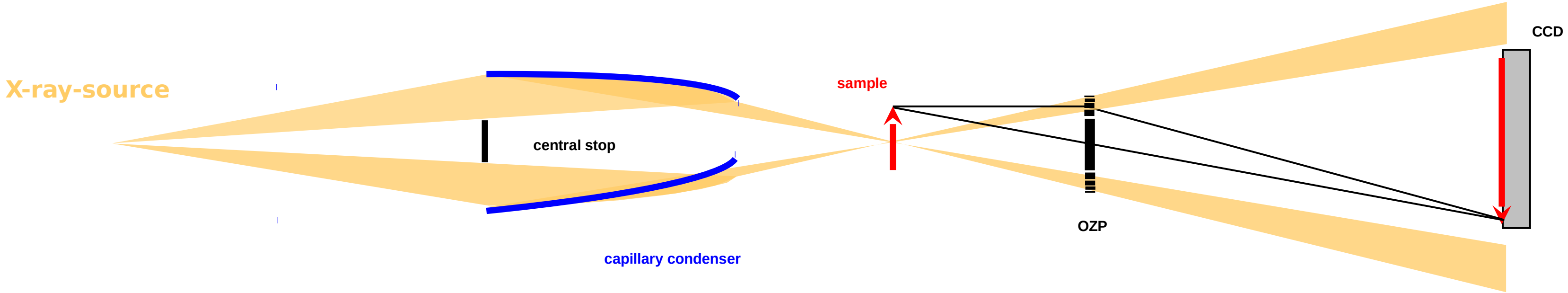


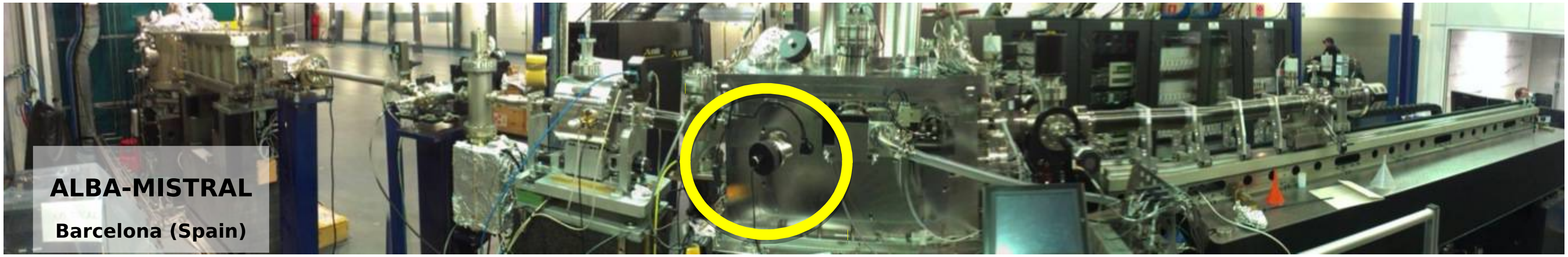
4. cryo-tomography of a yeast

C. A. Larabell *et al.*, Mol. Bio. Cell, **15** (2005)

The cryo-Transmission Soft X-ray Microscopes







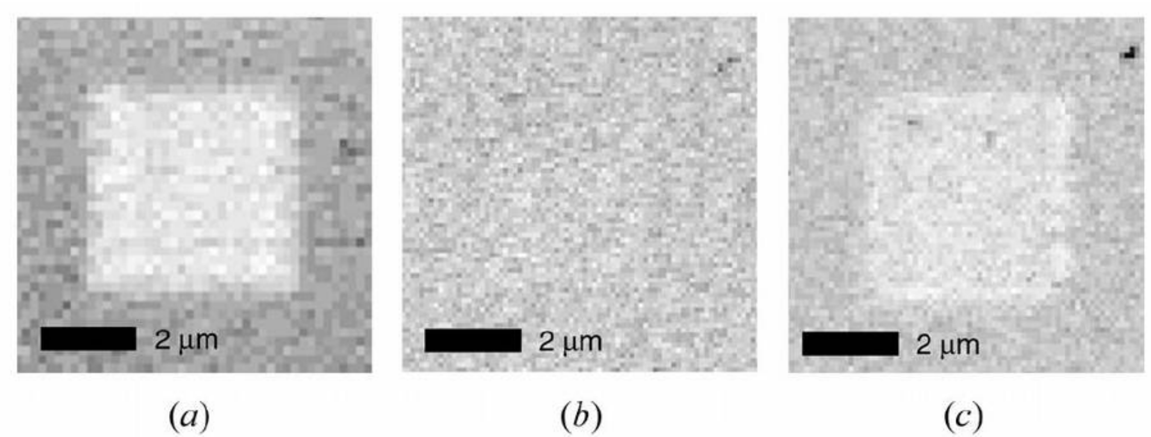
National Center for X-ray Tomography – XM2 – ALS **(Berkeley, USA)**

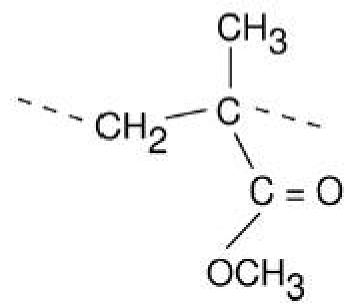
Challenges: radiation damage

Radiation damage

radiation damage studies using XANES spectroscopy in PMMA

by T. Breez & C. Jacobsen, *J. Synchr. Rad.* **10** (2002)





O 1s(C=O) → $\pi^*_{C=O}$

298 K

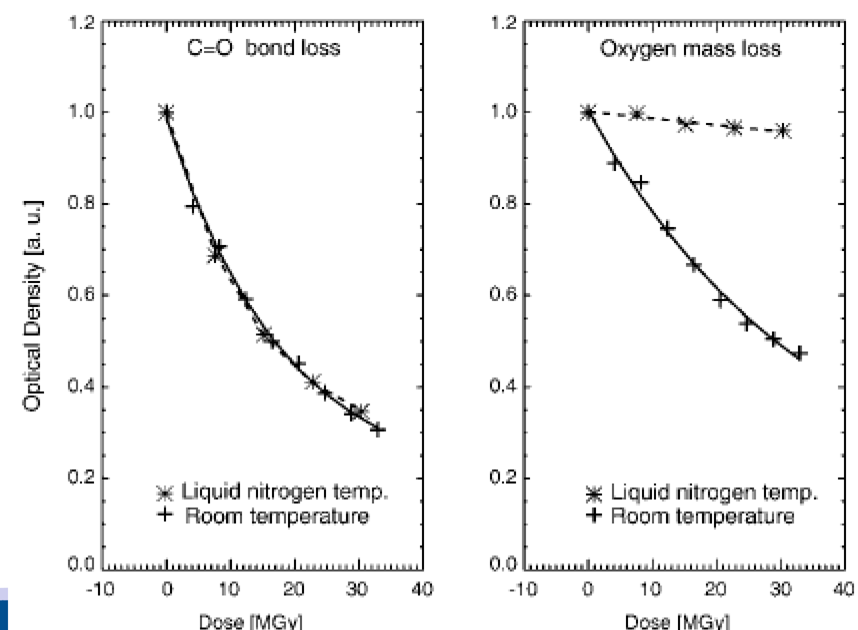
dose ~13×10^6 Gy

113 K

dose ~10^{10} Gy

warming up →

298 K



with cryo no mass loss up to 10^{10} Gy @ 50 nm

critical dose for bond breaking ~ 15×10^6 Gy

Dose and ultimate resolution

Calculation of dose & flux required for 3D imaging with a given resolution

- Calculation based on dose fractionation theorem (Hegerl and Hoppe (1976))
- The coherent scattering cross section of a cubic voxel is $r_e^2\, \lambda^2\, |\rho|^2 d^4$.
- Therefore the dose D and the flux F required to deliver P scattered x-rays into a detector with collection angle chosen for resolution d is

$$D = \frac{\mu P h\nu}{\varepsilon} \quad \frac{1}{r_e^2\, \lambda^2\, |\rho|^2\, d^4} \qquad\qquad F = \frac{P}{r_e^2\, \lambda^2\, |\rho|^2\, d^4}$$

μ = the voxel intensity absorption coefficient

$h\nu$ = the photon energy

r_e = the classical electron radius

λ = the photon wave length

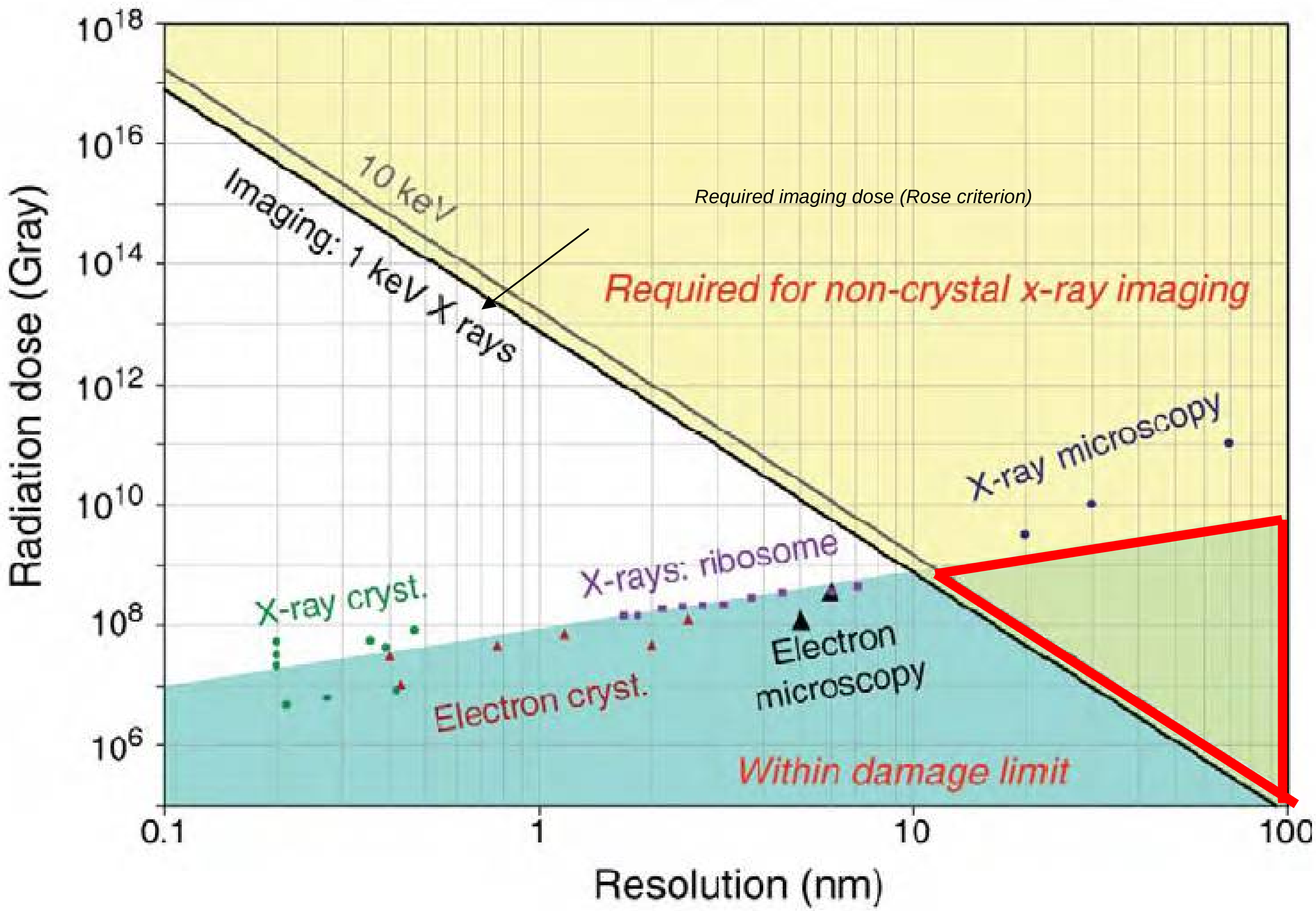
ρ = the voxel electron density

ε = the density

DOSE SCALES AS THE INVERSE FOURTH POWER OF THE RESOLUTION

radiation damage sets the ultimate resolution

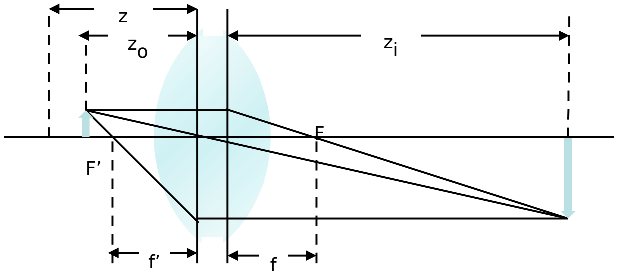
Dose-resolution relationship for 3D imaging of frozen-hydrated samples

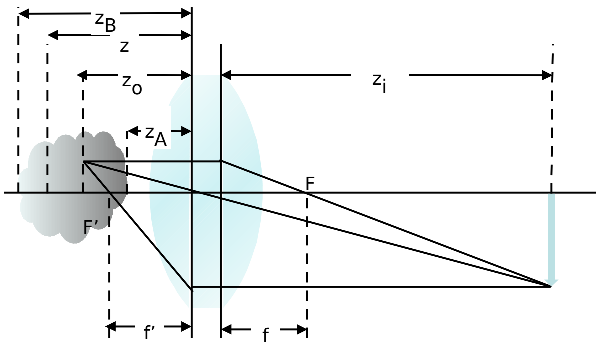


from Howells *et al*., JESRP (2005)

Challenges. Depth of focus limitation: what can be done?

Depth of field limitation in soft X-ray tomography





PSF

$$h(z, z_i) \equiv h(x, y, D(z, z_i))$$

Beer-Lambert absorption coeff.

$$\mu(x, y, z)$$

Blurring in soft X-ray tomography?

1) missing wedge (as in ET): “elongation” along Z axis

2) depth of field smaller than sample thickness: “elongation” along Z with a strong radial component.

40 nm ZP $\longrightarrow$ **~2.6 μm depth of field @ 520 eV**

z-dependence blurring is worse than missing wedge (both happen)

What can be done? We need to explore

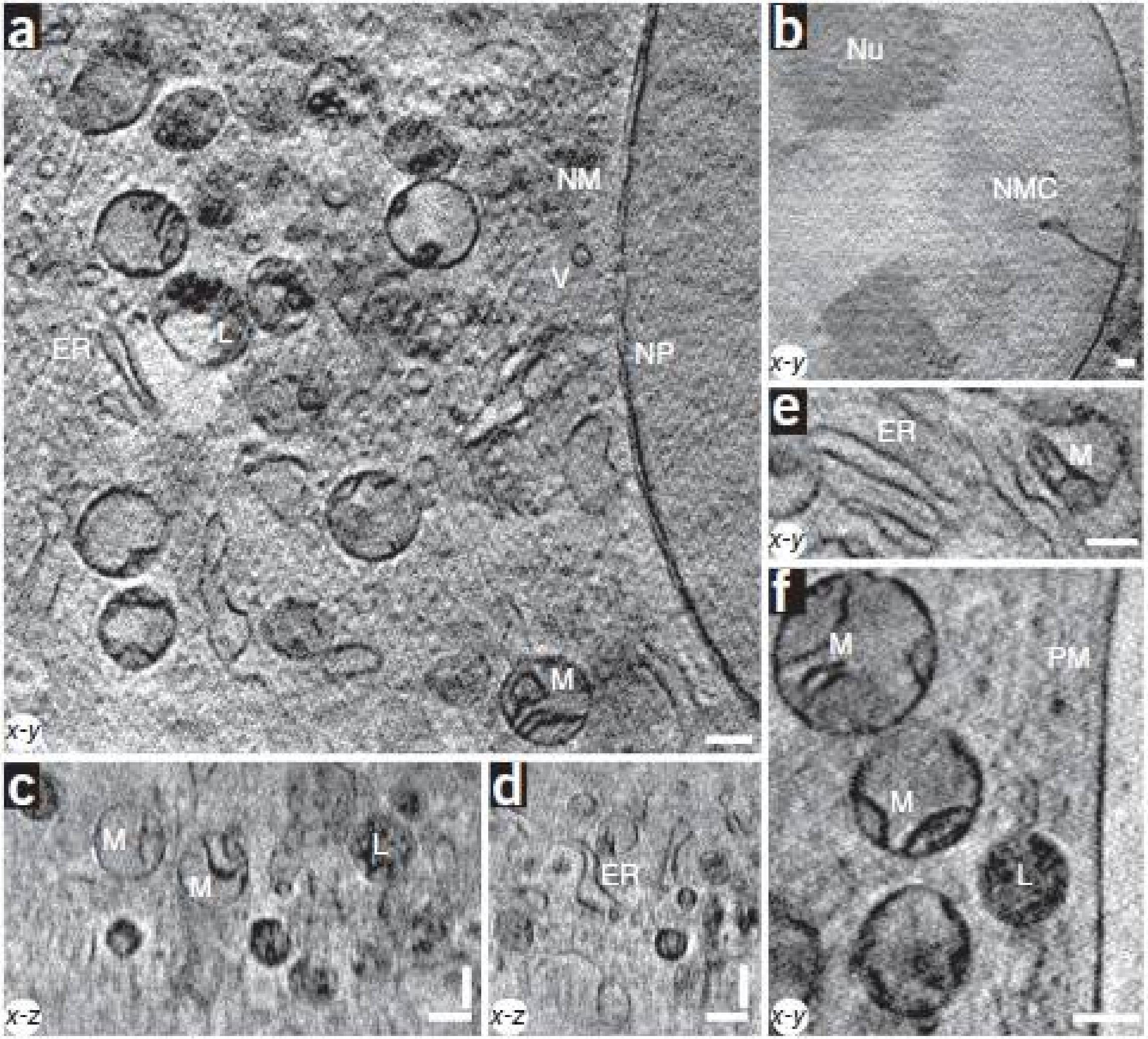
1. dual axis tomography could be explored
2. deconvolution of the Point Spread Function (PSF) of the lens

J. Oton *et al.* , *Image formation in cellular X-ray microscopy*, J. Struct. Biol. **178** (2012)

Applications: 3D imaging & spectroscopic imaging

Applications: 3D imaging

Soft X-ray tomography of a cryo-fixed cell



3D X-ray tomograms of mouse adenocarcinoma cells showing many subcellular organelles:

- mitochondria (M)
- lyosomes (L)
- endoplasmic reticulum (ER)
- vesicles (V)
- plasma membrane (PM)
- nuclear membrane (NM)
- nuclear pores (NP)
- nucleoli (Nu)
- nuclear membrane channels (NMC)

a, **c**, **d**, **e** & **f** acquired with 25-nm ZP at 510 eV

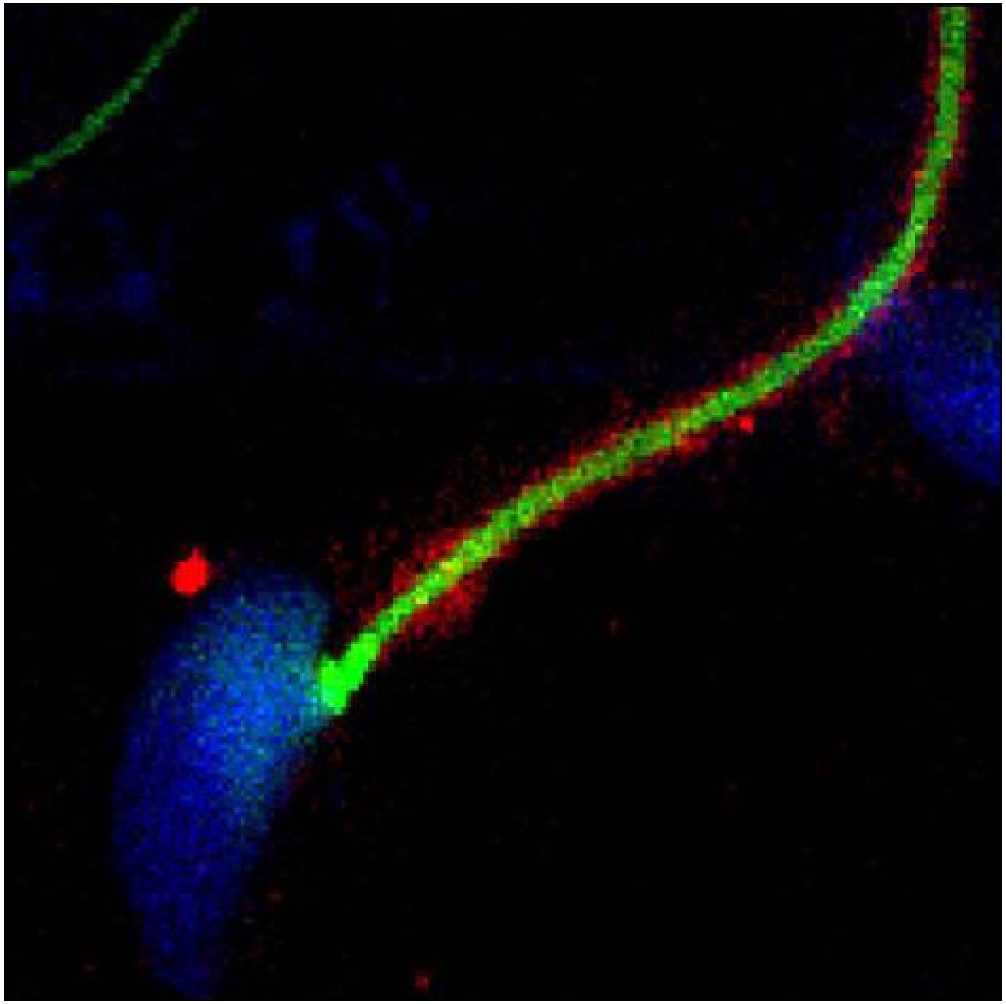
b acquired with 40-nm ZP.

Pixel sizes and slice thicknesses are 9.8 nm (**a**, **c -f**) and 15.6 nm (**b**).

Scale bars = 0.39 µm.

G. Schneider *et al.*, *Three-dimensional cellular structure resolved by X-ray microscopy*, **Nature Methods 7**, 985-987 (2010)

Hard X-ray 2D spectroscopic imaging



Mammalian spermatozoa

red: iron

blue: phosphorus

green: zinc

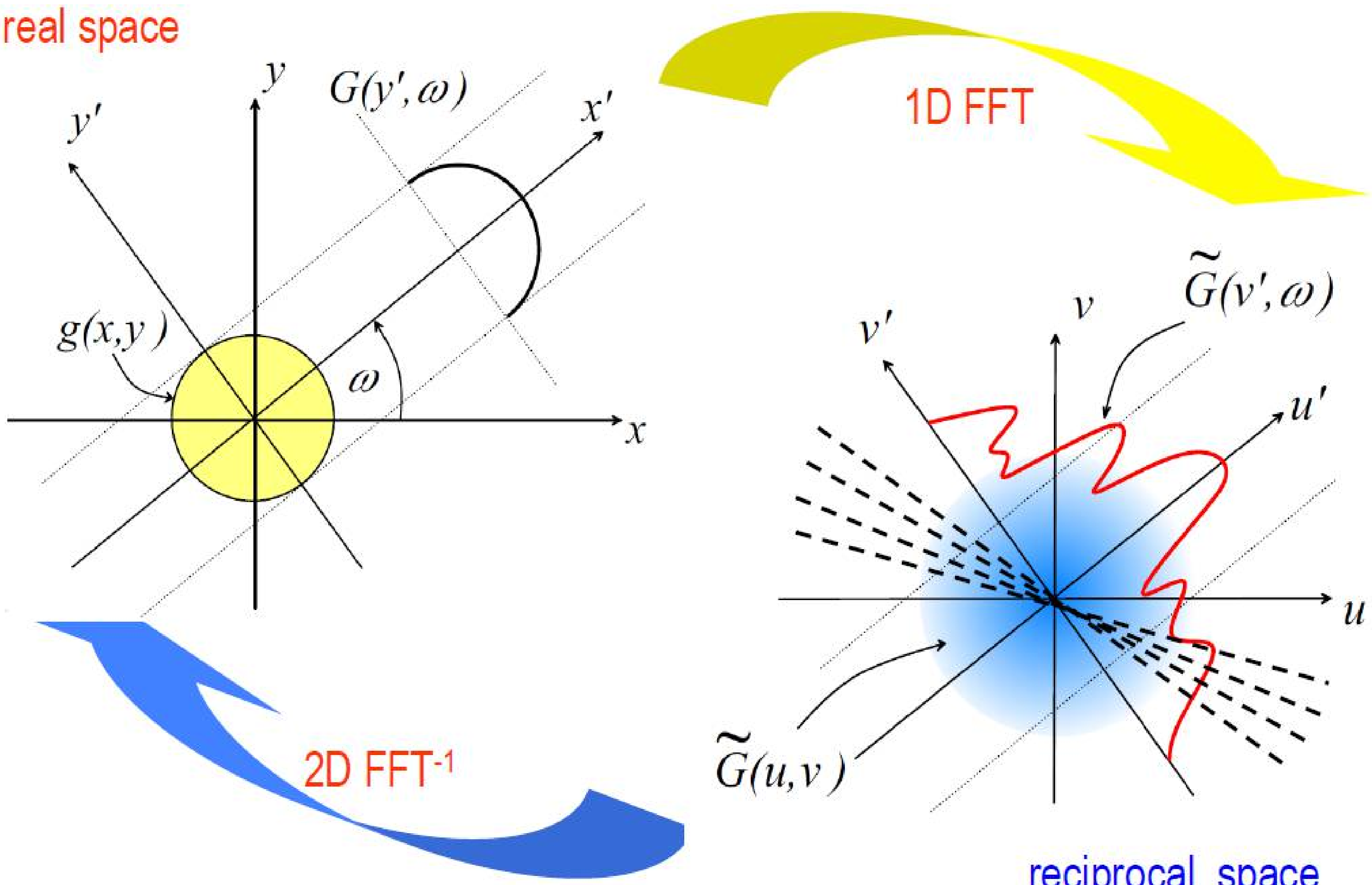
Selenium and zinc are necessary for motility and thus are often associated with infertility.

Bohic et al. J. Struct. Biology 177 (2012)

Thank you for your attention!

Computed Tomography

1D Fourier transform of a projection corresponds to a slice of the 2D Fourier transform of the original object



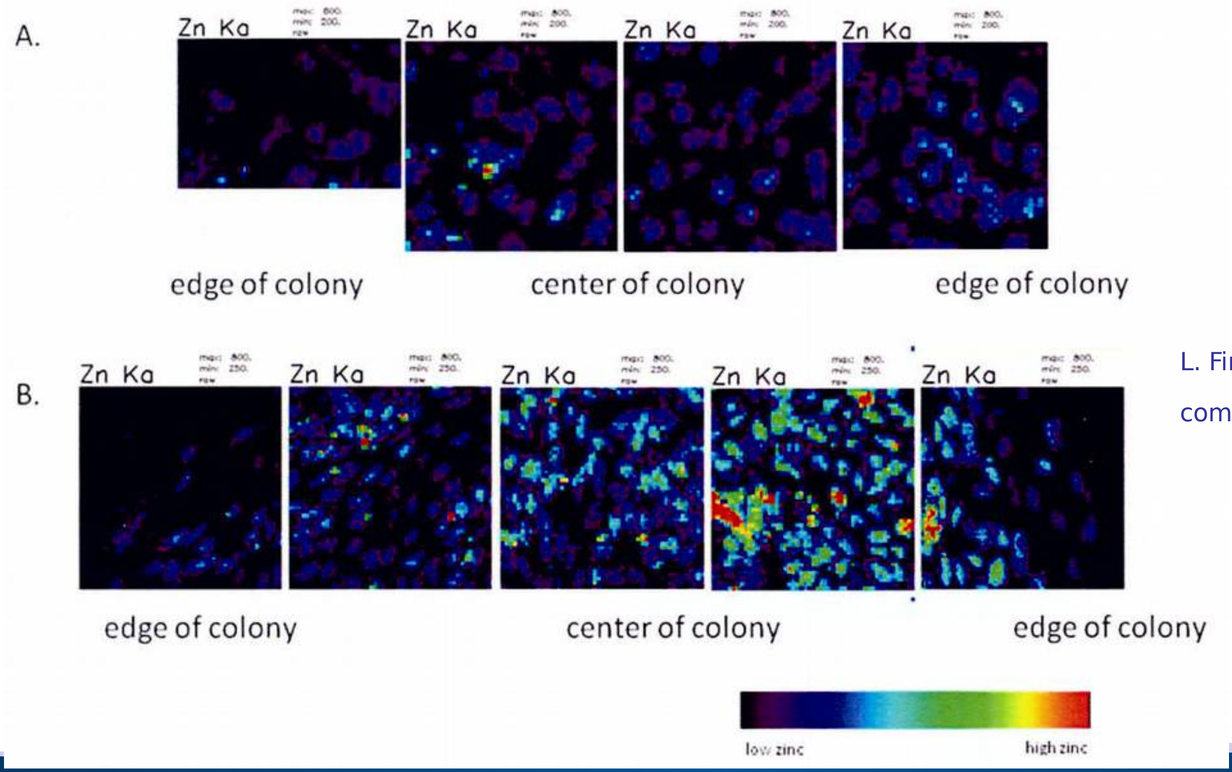
Applications: Spectroscopic imaging

Elements of living cells: H, C, N and O constitute 96% by weight

Na, Mg, P, S, Cl, K and Ca make up the remaining 4%

Examples of hard X-ray STXM:

- Subcellular X-ray fluorescence imaging as a tool to understand metal-induced pathologies.
- Zinc in stem cell differentiation



L. Finney *et al.*, XRM2010 communication (APS)