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.
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.
Experimental Stations

Origin of electrons
$v = 60\%$ of $c$

Linac
$v = 99.998\%$ of $c$

Booster
from $v = 99.998\%$ to $99.999998\%$ of $c$

Storage Ring

Beamlines
Experimental Stations

- Optical Hutch
- Experimental Hutch
- Control Hutch

Origin of electrons
$v = 60\%$ of $c$
The Linac is the first of the accelerators that accelerates the $e^-$ up to 100 MeV from 60% to 99.998% of c.
Booster and Storage Ring

Accelerates the e⁻ from 100 MeV to 3 GeV
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
Cryo Transmission X-ray Microscope

Vertical focusing mirror

Entrance slit

Vertical refocusing mirror

VLS PGM

Diag.

WB diag.

BM source

Vertical focusing mirror

Horizontal focusing mirror

Exit slit

~ 30m

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).

\[
\sigma_T = \sigma_{abs} + \sigma_{s}
\]

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

\[\mu = \mu(E, Z)\]

\(\rho\): mass density

\(\mu\): 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 $\lambda$ or $m \lambda$.

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

For small structures, diffracted radiation propagates at angles $\theta - \lambda/d$.

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

transmission function:

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

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

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

50% absorbed

$\sin \theta_m = \frac{m\lambda}{d} ; \ m = 0, \pm 1, \pm 2, \pm 3, \ldots$

$\eta_m = \begin{cases} 
\frac{1}{4} & m = 0 \\
\frac{1}{m^2\pi^2} & m \text{ odd} \\
0 & m \text{ even}
\end{cases}$
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 \( \lambda \) each and thus add in phase at the image point.

\[
f \gg n\lambda/2, \text{ which corresponds to a small NA lens}
\]

\[
NA = \sin \theta = \frac{\lambda}{2\Delta r} \ll 1
\]

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
Importantly, the relationship for the design of FZPs shows that the focal length $f$ scales directly with $N$, with the square of the outer zone width (which sets the resolution) and inversely to $\lambda$, introducing a strong chromatic effect.
2) FZP can form a real image

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

$$q_n + p_n = q + p + \frac{n\lambda}{2}$$  \hspace{1cm} (1)

where for NA small,

$$p_n = \sqrt{p^2 + r_n^2} \approx p + \frac{r_n^2}{2p}$$  \hspace{1cm} (2)

$$q_n = \sqrt{q^2 + r_n^2} \approx q + \frac{r_n^2}{2q}$$  \hspace{1cm} (3)

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

$$\frac{1}{q} + \frac{1}{p} \approx \frac{1}{f}$$  \hspace{1cm} and  \hspace{1cm} $$M = \frac{p}{q}$$
FZP generates many diffractive orders (m).

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

For higher orders, \( mn\lambda/2 \) is added to the path length and

\[
r_n^2 \approx mn\lambda f_m
\]
\[
f_m = \frac{f}{m}
\]
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 \( \eta_m \) depends on the material thickness and \( n=1-\delta-i\beta \).

The extra phase change introduced by the material reinforces \( \eta_m \) when material and zone thickness are conveniently chosen.
5) FZP diffraction

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

$$\frac{I_1(\theta)}{I_0} = N^2 \left| \frac{2J_1(ka\theta)}{ka\theta} \right|^2$$  \hspace{1cm} (9.45)

$$r_{null} = \frac{0.61\lambda}{NA}$$  \hspace{1cm} (9.46)

$$k = \frac{2\pi}{\lambda}$$

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 $\lambda$.

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 \( \lambda \) and NA.
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

\[
\Delta z = \pm \frac{1}{2} \frac{\lambda}{(NA)^2} \quad (9.50)
\]

\[
\Delta z = \pm 2F'\lambda = \pm 2(\Delta r)^2/\lambda \quad (9.51)
\]

\[
\frac{\Delta \lambda}{\lambda} \leq \frac{1}{N} \quad (9.52)
\]

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**Diagram**

- Focal plane, \( z = f \) = \( \frac{\lambda}{2(NA)^2} \)
- Two depths of focus away, \( z = f - \frac{\lambda}{(NA)^2} \)
- Four depths of focus away, \( z = f - \frac{2\lambda}{(NA)^2} \)

**Graph**

- 80 nm Rayleigh resolution
- 40 nm
- 20 nm
- 10 nm

**Energy (keV)**

**DOF (μm)**
Fresnel Zone Plates

DoF scales as $\lambda/NA^2$

For small $\theta$

$$\tan \theta \sim \sin \theta = NA$$

$$\therefore \text{DOF} \approx \frac{1.2\lambda}{NA^2}$$

in the text, eq. 9.50:

$$\text{DOF} = \pm \frac{1}{2} \frac{\lambda}{(NA)^2}$$
Optical Transfer properties with partially coherent illumination

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

\[ \sigma = \frac{\text{NA}_{\text{illum.}}}{\text{NA}_{\text{obj.}}} = \frac{\sin\theta_{\text{illum.}}}{\sin\theta_{\text{obj.}}} \quad (\text{for } n = 1) \]
Nanofabrication of Fresnel Zone Plates

e-beam lithography is used to manufacture FZP.

1. Expose

2. Develop

3. Cryogenic ICP Etch

4. Plate

5. Strip Resist

6. Strip Si₃N₄ and Cr/Au Plating Base

Courtesy of E. Anderson, A. Liddle, W. Chao, D. Olynick, and B. Harteneck (LBNL)
Fresnel Zone Plates: summary

\[ \lambda = 2.3843 \text{ nm for } E = 520 \text{ eV}, \Delta r = 40 \text{ nm} \]

\[ \text{NA} = \frac{\lambda}{2\Delta r} = 0.03 \]

\[ f = \frac{4N(\Delta r)^2}{\lambda} = 2.516 \text{ mm} \]

\[ \delta_{x,y} \big|_{\text{coherent}} = \frac{1.22\Delta r_N}{m} = 48.8 \text{ nm for } m=1 \]

\[ \delta_z = \pm \frac{2(\Delta r_N)^2}{m^2\lambda} = \pm 1.342 \mu\text{m for } m=1 \]
To sum up!

increasing spatial resolution implies decreasing DOF ($\lambda/NA^2$)

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

Two common soft X-ray microscopes
The scanning X-ray microscope (STXM)

1. least radiation dose
2. high spatial resolution ($\Delta r_N$)
3. requires spatially coherent radiation
4. longer exposure time
5. spectroscopic capabilities
6. allows detecting fluorescent emission
The transmission X-ray microscope (TXM)

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

Illumination can be provided by a FZP or a glass capillary.
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. Ultramicro. 84 (2000)

3. Drosophila melanogaster cell

4. cryo-tomography of a yeast
The cryo-Transmission Soft X-ray Microscopes

HZB-BESSY II U41-XM
Berlin (Germany)

ALBA-MISTRAL
Barcelona (Spain)

National Center for X-ray Tomography - XM2 - ALS (Berkeley, USA)
Challenges: radiation damage

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

critical dose for bond breaking $\sim 15 \times 10^6$ 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

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

- $\mu$ = the voxel intensity absorption coefficient
- $h \nu$ = the photon energy
- $r_e$ = the classical electron radius
- $\lambda$ = the photon wave length
- $\rho$ = the voxel electron density
- $\varepsilon$ = 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

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.

\[ 40 \text{ nm ZP} \quad \rightarrow \quad \sim 2.6 \mu \text{m depth of field @ 520 eV} \]

\( \text{z-dependence blurring is worse than missing wedge (both happen)} \)

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

Applications: 3D imaging & spectroscopic imaging
Soft X-ray tomography of a cryo-fixed cell

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

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

\( \text{a, c, d, e & f} \) acquired with 25-nm ZP at 510 eV
\( \text{b} \) acquired with 40-nm ZP.

Pixel sizes and slice thicknesses are 9.8 nm \((\text{a, c -f})\) and 15.6 nm \((\text{b})\).

Scale bars = 0.39 µm.

Selenium and zinc are necessary for motility and thus are often associated with infertility.
Thank you for your attention!
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)