Microscopy with Synchrotron Radiation: Phase Contrast Imaging and Micro-spectroscopy

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Outline

• Basic interactions
• Contrast imaging:
  - Absorption
  - Phase contrast
  - Chemical contrast
  - Fluorescence

• Micro-spectroscopy
  FFI
  STXM
  Applications
Why synchrotron light?

• High brightness
• Tunability
• Polarisation
• Time structure
• Coherence

Why X-rays instead of charged particles?

• Higher penetration power
• Great variety of spectroscopies
• Variety of imaging contrast
• Less sensitive to sample environment
Interaction of X-ray with matter: redirection, absorption (⇒X-ray transmission) and photons and electron emission
Background info: X-ray microscopy types

Scanning

- Monochromatic light
- Sample (scanned)
- Detector
- Photo-emission detector

- ZP
- OSA

Full-field imaging

- Monochromatic light
- Sample (fixed)
- Aperture
- CCD

- ZP

+ versatile detectors can run simultaneously;
+ easier optics set-up;
- long exposure time;
- complex electronics.

Ideal for spectromicroscopy

+ short exposure time;
+ higher resolution
- static system;
- complex optical alignment.

Ideal for dynamic studies and tomography
Full-field X-ray imaging or “one shot” X-ray image acquisition can be considered as the optical analogon to a visible light transmission microscope

BUT

Refractive index $n$ is very close to unity and smaller than unity!!!

$$n = 1 - \delta(\lambda) - i\beta(\lambda) < 1$$
Which lenses to use to focus X-rays?

When Roentgen discovered X-rays, he immediately tried to focus them with refractive lenses but did not succeed!

Why? ...

\[ f = \frac{R}{2\delta} \]

For example: Al lens with \( R=500\mu m \), \( E=10\text{keV} \), \( \delta=5.46 \times 10^{-6} \):

\[ f = 92m \]
The complex refractive index

\[ n = 1 - \frac{n_a r_e \lambda^2}{\pi} \left( f_1 + i f_2 \right) \equiv 1 - \delta - i \beta \leq 1 \]

“Conventional refractive index” describing phase change:

\[ \varphi(z) = \frac{2\pi}{\lambda} \delta z \]

Exploitation of phase contrasts possible using X-rays? Lower radiation damage?

Describing photoelectric absorption with coefficient:

\[ \mu = \frac{4\pi}{\lambda} \beta \]

Consequence:
Emission of Auger, photo-electrons and fluorescence photons, but also causes radiation damage (energetic secondary electrons!)
Refractive index and X-ray contrast techniques

X-ray contrast is generated by differences in the complex scattering factor per unit volume

\[ n(\lambda) = 1 - \delta(\lambda) - i\beta(\lambda) = 1 - \frac{n_ar_e\lambda^2}{2\pi} \left(f_1(\lambda) - f_2(\lambda)\right) \]

\[ \delta(\lambda) = \frac{n_ar_e\lambda^2}{2\pi} f_1(\lambda) \]

\[ \beta(\lambda) = \frac{n_ar_e\lambda^2}{2\pi} f_2(\lambda) \]

\( \delta(\lambda) \): Phase sensitive
\( \beta(\lambda) \): Absorption ...
\( n_a \): average atom density
\( r_e \): classical electron radius
\( f_1, f_2 \): atomic form factors

Scattering, refraction:
- Zernike phase contrast
- Differential phase contrast
- Differential interference contrast
- Dark-field imaging
- Magnetic phase contrast

Absorption:
- Bright-field imaging
- Chemical contrast techniques
- Magnetic absorption contrast
Delta versus beta

Delta is orders of magnitude larger !!!
**Absorption mode**

X-ray photons are selectively absorbed by the material according to its density and thickness (ex. radiography)

\[ I = I_0 e^{-mx} \]

**Phase contrast mode**

Absorption can produce little contrast for light (transparent) materials or for materials with similar atomic number (similar attenuation factors).

Moreover as the energy increases the contrast diminishes (absorption coefficient \( \propto 1/E^3 \))

Phase contrast is more sensitive to edges and borders in the sample

Contrast techniques using the real, phase-shifting part of the complex refractive index are in many cases superior to absorption contrast because:

(i) the x-ray dose can be reduced dramatically
(ii) the throughput is higher (the phase shift dominates the absorption in the x-ray regime)
Basic principles of contrasts

- Contrast is not an inherent property of the specimen, but is dependent upon interaction of the specimen with light AND the efficiency of the optical system to record the image to the detector.

- Human eye needs at least about 2% image contrast to distinguish between image and background.

- Values might vary for other detectors.

- With each detector, the signal to noise ratio must be large enough to be interpreted in terms of the formation of an image.
**Definition of contrast**

**Often applied definition:**

Contrast is defined as the difference in light intensity between the image and the adjacent background relative to the overall background intensity.

\[
C = 100 \cdot \frac{(I_S - I_B)}{I_B}
\]

- \(I_S\): Specimen intensity
- \(I_B\): Background intensity

**Definition used for XRM:**

Contrast is defined as the difference in maximum and minimum light intensity normalized to the sum of maximum and minimum light intensity.

\[
C = \frac{(I_{\text{max}} - I_{\text{min}})}{I_{\text{max}} + I_{\text{min}}}
\]

- \(I_{\text{max}}\): Max. image intensity
- \(I_{\text{min}}\): Min. image intensity
Natural amplitude contrast between water and organic matter

The “Water Window”:

Due to dramatic difference in the f2 values of two materials, especially water and organic matter between the C and O K-absorption edges.

Note the penetration distance compared to electrons !!!
Bacteria and clay dispersion: Destruction of associations of clay particles by soil microbes

X-ray images acquired with the full-field imaging microscope at BESSY I @ 520 eV

Samples analysed in the natural hydrated state:
→ no alteration of the environment of the sample

J. Thieme et al., IRP, Uni Goettingen / G. Machulla, Uni Halle, D
Brightfield imaging at higher photon energies

Characterization of morphology and defects in modern semiconductors with a full-field imaging microscope (@ 1.8 keV, XM1/ALS)

Sample preparation:
Back side thinning of Si wafer

G. Schneider et al., BESSY II
Material sciences: Electromigration in modern Cu interconnects

SEM micrograph

X-ray micrograph imaged at 1.8 keV

X-rays

Via with metal line as dark shadow

thickness approx. 1 - 2 μm

HVTEM (0.8 MeV electrons)  TXM (1.8 keV photons)

Grain boundaries

Void

200 nm

1 μm

Discontinuities due to absorption
The absorption occurs when the incoming X-rays are matching the electron binding energies
**Absorption edges are fingerprints ⇒ they can be used to identify the chemical elements**
By taking two images, one above and one below a specific absorption edge, the correspondent chemical element will give a high contrast difference in the two images
Across edge imaging

701eV

706.3eV / 701eV

706.3eV

Environmental science: Analysis of air particulate matter

P. Barbieri et al.,
Dept. of Chem., Univ. Trieste, I
Amplitude and phase contrast for a model protein $\text{C}_{94}\text{H}_{139}\text{N}_{24}\text{O}_{31}$

Absorption contrast

Mostly used for chemical studies in combination with XANES and XRF

Phase contrast techniques

- tremendous reduction of dose applied to object (dose $\sim t^{-4}$ with spat. resolution $t$)
- additional transmission information on low side of absorption edges (XANES, XRF !)

Courtesy of G. Schneider et al. BESSY, D
Basics of Zernike phase contrast

\[
A_{\text{specimen}} = A_{\text{surr}} e^{i\Phi} = A_{\text{surr}} e^{i \frac{2\pi \Delta t}{\lambda}} \approx A_{\text{surr}} (1 + i\Phi)
\]

\(\Phi << 1\)

Phase plate in “back-focal” plane: Phase of \(A_{\text{surr}}\) can be shifted by +/- \(\pi/2\) !!!
Phase differences are converted in amplitude differences !!!
Zernike phase contrast in full-field imaging X-ray microscopy
Zernike phase contrast in X-ray microscopy

Contrast and dose for a model protein $\text{C}_{94}\text{H}_{139}\text{N}_{24}\text{O}_{31}\text{S}$
Zernike phase contrast with multi-keV X-rays

Images acquired with the FFIM at ID21, ESRF

Cu interconnect structures imaged at 4 keV photon energy
60nm spatial resolution

90 deg shift (pos.)  270 deg (neg.)
TXM images of *S. cerevisiae* at 5.4 keV

a) in absorption contrast (b) in Zernike phase contrast

Zernike phase contrast in X-ray microscopy

Amplitude and Zernike phase contrast images of an alga *Euglena gracilis*

$E = 500$ eV, accumulated dose is $3 \times 10^6$ Gray

Amplitude: 3 s
Phase contrast: 15 s

Drawbacks of Zernike phase contrast:

- Halos around structures
- Quantitative analysis difficult
- Limitation in spatial resolution
- Not all spatial frequencies are treated equally

Darkfield or darkground imaging

Darkfield illumination requires blocking out of the central light which ordinarily passes through and around (surrounding) the specimen, allowing only oblique rays from every azimuth to "strike" the specimen.

Visible light micrographs of silica skeletons from a small marine protozoan (radiolarian)
Darkfield imaging in scanning X-ray microscopy

Technique is especially suited for small, strongly scattering particles as for example a few 10nm diameter labelling spheres

Brightfield image of a cell with Au labelling spheres overlayed with a darkfield image

Images acquired with STXM at the NSLS

Detector based contrast technologies in scanning X-ray microscopy:

Acquired with Andor Ixon DV860A
Frame transfer back-illuminated Electron Multiplying CCD with shutter and light converting system (128x128px, 5 Mhz, 110f/s)
Computational extraction of contrasts by masking:

Raw data acquisition of first diffraction order image for each pixel of the raster scan

Applying different masks:

- Bright field
- Differential phase and absorption
- Darkfield

A. Gianoncelli et al., Appl. Phys. Lett.
Marine biology: Imaging of giant diatoms

Planktonic diatom Coscinodiscus sp.  
(A. Beran, Laboratory for Marine Biology, Trieste, I)
Brightfield and differential phase contrast images acquired simultaneously with configured detector

Planktonic diatom “Casciodiscus sp.” (provided by LBM, Trieste, I)

Images acquired in STXM mode (TwinMic Microscope ELETTRA) with FRCCD camera; E=1320 eV, 200x190 px, 50ms dwell/px
Methods for imaging phase variations

Crystal interferometer

Free space propagation

Crystal analyzer

Grating interferometer

From C. Beck, PhD thesis
Crystal analyser

Crystal for angular filtering

-X-ray scattered by more than some tens µrad are rejected

- Small misalignments -> investigation of phase shift effects (refraction angle is roughly proportional to the gradient of d)

-Bigger misalignments -> primary beam almost totally rejected -> pure refraction images

- Large field of view

Ingal et al. (1995) Appl. Phys. 28
Blood Vessels: Depiction at Phase-Contrast X-ray Imaging without Contrast Agents in the Mouse and Rat

**Crystal interferometer**

- I crystal -> splits the monochromatic beam into two beams with the same phase
- II crystal -> acts as a mirror
- III crystal -> recombines the two beams
- Phase shift produced by the presence of the sample
- The beams re-combined generate an interference pattern recorded by the detector
- Extremely sensitive to subtle phase variations
- Limited field of view

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Phase contrast  
Absorption contrast

Momose et al, Radiology 2000; 217:593–596
Grating interferometry imaging:

- Large field of view
- Moderate spatial resolution

Grating interferometry imaging:

Polychromatic X-ray tube operated at 40 kV/25 mA

Free space propagation imaging:

Polystyrene foam at E=18 keV

- It exploits the high spatial coherence of the X-ray source and needs a high-resolution detector.
- $z = 0$ -> absorption image
- For $z > 0$ -> interference between diffracted and un-diffracted wave produces edge and contrast enhancement; it makes use of Fresnel diffraction.
- Real space information
- Edge enhancement

Chemical/Magnetic contrast

XANES = X-ray Absorption Near Edge Spectroscopy

Resonances with unfilled states.

XANES: tuning on molecular orbitals
XMLD: imaging antiferromagnets,
XMCD: imaging ferromagnets
Chemical contrast

Outlining the lateral distribution of PS/PMMA

Transmission x-ray micrographs

C 1s → \pi^*_{C=C}

C 1s → \pi^*_{C=O}

Continuum

Absorption coefficients, \alpha (\mu m^{-1})

Photon Energy (eV)

Pre C edge

PMMA

PS

H. Ade, SUNY-SB STXM at the NSLS
Photoionization

**X-ray absorption**
(through photoelectric effect)

The primary X-ray photon causes the ejection of electrons from the inner shells, creating vacancies.

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**X-ray Fluorescence**

The vacancy created by the primary X-ray photon is filled by an electron coming from an outer shell causing the emission of a characteristic X-ray photon whose energy is the difference between the two binding energies of the corresponding shells.

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**Auger effect**

The vacancy created by the primary X-ray photon is filled by an electron coming from an outer shell and the energy is transferred directly to one of the outer electrons, causing it to be ejected from the atom.
Chemical sensitivity of X-rays: Elemental mapping

Electron binding energies for relevant materials

Photon energy
Z Number

B C N O F Ne Na Mg Al Si P S P S Cl Ar K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn Ga Ge As As Se Br Kr Rb Sr Y Nb

K 1s L 1 2s L 2 2p 1/2 L 3 2 3/2
Fluorescence contrast

- Microprobe focused on the sample
- Sample raster scanned
- Analysis of each spectrum in the raster scan
- Construction of an X,Y map for each chemical elements present in the spectrum
Full field Imaging mode

- Similar to conventional visible light microscope
- Analysis of morphology in transmission
- Fast imaging, dynamics, microtomography
Human biology and virology

Analysis of changes of biomechanics in cells infected with a virus by tagging specific proteins with metals (here HOS 2e11 cell line)

Collaboration with A. Marcello (ICGEB Trieste, I)
Scanning X-ray microscope (STXM)

Zone plate that forms a microprobe

Specimen raster-scanned across the microprobe

Multi-element emission detector system

Configured transmission detector system with visible light converter
Scanning transmission mode
Differential phase contrast with a fast read-out CCD camera

Simultaneous acquisition of:
- Absorption or transmission
- Differential phase contrast
- Darkfield images
The benefit of a fast read-out, electron-multiplied CCD as configured detector in STXM

- Projection imaging for alignment purposes
- Simultaneous acquisition of absorption, differential phase contrast and darkfield imaging
- Diffraction imaging as ptychography
- XANES by across-edge imaging

Low-energy X-ray fluorescence for elemental analysis:

Detecting trace elements:

X-ray fluorescence: ~1000x better sensitivity than electrons for trace elemental mapping (ion concentrations etc.).

Low fluorescence yields for soft X-rays! !!
Low-energy X-ray fluorescence

TwinMic LEXRF spectrum with unfocused beam of a test organic matrix on a metal shim

Dynamic range: up to 30 kcounts/s

Average FWHM energy resolution @ C- K edge: 69 eV

Electrochemistry: Development of fuel cells

Towards the development of a micro fuel-cell for in-situ spectromicroscopy

B. Bozzini et al., ChemSusChem 2010

Benedetto Bozzini, Uni Lecce/ Salento, I
Electrochemistry: Development of fuel cells

Spatial variations in the Fe concentration confirmed by the \( \mu \)-XAS Fe L\(_3\) spectra, measured in selected spots
Highly sensitive to the Fe chemical state

Fe signal attenuation approaching the edge of the Fe electrode, that reflects the increasing loss of Fe due to the corrosion process

The relative amount of the Fe species in the higher oxidation states and FeOOH is increasing in the heavily corroded areas and for areas closer to the edge

Electrochemistry: Development of fuel cells

The contrast in the 701.0 eV map taken below the Fe L₃ edge is dominated by the morphology (thickness variations).

Above the absorption edge (706.3 eV) dramatic intensity drop occurs in the Fe electrode region and in locations containing Fe species.

Division map: Fe concentration distribution map. Two very bright ‘cracks’ inside the Fe electrode and several ‘bubble-like’ brighter areas, resulting from localised corrosion.

The released Fe contributes to the gradually fainting darkness moving away from the electrode edge: diffusion of Fe species released from the electrode as a result of the electrochemical reactions.

Sulfonated based fluoropolymer-copolymer. Synthetic polymers with ionic properties. Considerable attention as a proton conductor for PEMFC for its excellent thermal and mechanical stability.

Electrochemistry: Development of fuel cells

Three different spectroscopies: AAEI, XANES, LEXRF

Benedetto Bozzini,
Lucia D’Urzo
Uni Lecce, I

Understanding the electrocorrosion in fuel cells that is the main life-time limiting factor

Food Science: Inside the wheat

Ivan Kreft, University Ljubljana

Functionality and toxicity of Zn in wheat and buckwheat analyzed on sub-cellular level

Ivan Kreft, Fac. of Biotechnology, University Ljubljana

Functionality and toxicity of Zn in wheat and buckwheat analyzed on subcellular level

Healthy control wheat

E=1686 eV
80 x 80 mm²
80 x 80 px
8 s dwell/ px
1 mm resolution
4 detectors

Ivan Kreft, 
Fac. of Biotechnology, 
University Ljubljana

Functionality and toxicity of Zn in wheat and buckwheat analyzed on subcellular level

TXM images acquired with a double-frequency ZP (15nm outermost zone width from J. Vila-Comamala (PSI)

1s dwell, 740 eV photon energy

Nanotoxicology: CoFe$_2$O$_4$ ENPs

G. Ceccone, P. Marmorato et al., EC Joint Research Center, Ispra, I

Localization of engineered nanoparticles (ENPs) inside a cell and on the possible effects on the cell metabolic behaviour

CoFe$_2$O$_4$ in mouse 3T3 fibroblast cells, E=2019 eV, 60um x 60 um, 80 x 80 pixels, 15s/pixel

Nanotoxicology: CoFe$_2$O$_4$ ENPs

Control

Exposed to 500µM

Exposed to 40µM

Clinical medicine: Asbestos in lung tissue

M. Melato,
Monfalcone Hospital
L. Pascolo,
Sincrotrone Trieste

Mesothelioma and differentiation of lung tissue due to asbestos; the role of Mg

E=2019 eV, 50mm x 50 mm, 100 x 100 pixels, 15s/pixel LEXRF

Biotechnology: Al in tea leaves

In young tea leaves the preferential accumulation of Al occurs at the end of the transpiration stream, in the epidermal cell walls.

Conclusions

- Synchrotron radiation facilities provide state of the art techniques
- Synchrotron radiation microscopy techniques offers high spatial resolution and high chemical sensitivity
- Wide range of applications
- Complementariness to other techniques (laboratory, SR...)