# Soft and Hard X-ray Microscopy

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## **Cheiron School 2008**

**SPring-8** 

d ccode é acédét

## Outline

1. Fundamentals

resolution

contrast

sources

optics

- 2. Direct methods projection imaging full-field imaging scanning
- 3. Indirect methods microdiffraction coherent diffraction holography

References:

1. M. Howells, "Soft-x-ray microscopes," Physics Today 38, 22 (Aug. 1985).

2. J. Kirz, "Soft X-ray microscopes and their biological applications," Q. Rev. Biophys. 28, 1 (1995).

3. J. Als-Nielsen and D. McMorrow, *Elements of Modern X-ray Physics* (Wiley, New York, 2000).

4. D. Attwood, Soft X-Rays and Extreme Ultraviolet Radiation: Principles and Applications (Cambridge, 2007).

## Image formation as a scattering process



Ewald sphere is defined by conservation of momentum. Only spatial frequencies on the Ewald sphere are accessible to the imaging process, limiting attainable resolution.

## **Diffraction limits to resolution**



## What do we mean by "resolution"?



- Point sources are spatially coherent
- Mutually incoherent
- Intensities add
- Rayleigh criterion (26.5% dip)

Conclusion: With spatially coherent illumination, objects are "just resolvable" when

$$\operatorname{Res}|_{\operatorname{coh}} = \frac{0.61\,\lambda}{\mathrm{NA}} = 1.22\,\Delta r$$

## **Coherence**

## longitudinal coherence



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## Coherent vs. incoherent



#### Scanning and full-field microscopy are *incoherent* methods

- Transfer function is linear in the field intensities
- Characterized by sloping function down to 2NA

#### Diffraction and holographic microscopy are *coherent* methods

- Transfer function is linear in the field *amplitudes*
- Characterized by flat top, sharp cutoff at limiting NA

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# Why use x-rays?

- Penetration through whole cells and thick materials with minimal multiple scattering: "clean" images and spectra
- Short wavelengths, so high spatial resolution is possible
- Short pulses, so high time resolution is possible
- Coupling to core-level electrons: element detection, "clean" measurement of electronic and chemical states
- Coupling to electronic spin via polarization: probe magnetic states and ordering

But: ionizing radiation, so repeated high-resolution imaging of radiation-sensitive samples (e.g. live cells) is not possible.

# Contrast mechanisms in x-ray microscopy

#### Access a wealth of information

- Absorption measure electron density
   Phase measure real part of refractive index
   Fluorescence measure elemental distribution
   Spectroscopy extract chemical state, spin state
   Diffraction reveal structure, strain, mag. charge
- Natural sample contrast is possible; staining not required
- Image structure of thick samples, sectioning not required
- More penetrating, less damage, less charging than with electrons

## **Refractive index and contrast**

$$n = 1 - \delta - i\beta = 1 - \frac{r_e}{2\pi}\lambda^2 \sum_i n_i f_i(0)$$

$$A = A_0 \exp(-inkt)$$
$$k = 2\pi / \lambda$$

- Absorption contrast: sensitive to Im(n) $\sim 2\pi\delta(x,y)t/\lambda$
- Phase contrast: sensitive to Re(n) $\sim 4\pi\beta(x,y)t/\lambda$

Ni Density=8.902 delta (dash) beta (solid) 100 100 Photon Energy (eV)

## Elemental sensitivity at absorption edges





## The "water window"



# Fluorescence spectroscopy



## Why use synchrotrons?

Synchrotron sources offer

- Brilliance (small source, collimated)
- Tunability (IR to hard x-rays)
- Polarization (linear, circular)
- Time structure (short pulses)

# > Undulators produce the most brilliant x-ray beams available

**B** = photons/source area, divergence, bandwidth





### **Undulator radiation**

- SR sources (except FEL) are incoherent, but highly forward directed due to relativistic effects  $\theta \sim 1/\gamma$
- Spatial and temporal filtering (pinholes, monochromators) is needed to select the coherent flux  $F_c \sim \lambda^2 B$
- Only the coherent flux can be focused into a diffraction-limited spot or be used to form interference fringes



## Focusing optics for x-rays

### Achieving high NA is challenging because x-rays interact weakly

 $n = 1 - \delta - i\beta$   $\delta, \beta \sim 10^{-3}$  to  $10^{-6}$   $\Rightarrow$   $|n| \approx 1$ 

Refractive (compound refractive lenses)~ 50 nmLow efficiency, highly chromatic, aberrations

Reflective (Kirkpatrick-Baez mirrors)~ 40 nmHigh efficiency, achromatic, limited to ~10 nm

Diffractive (Fresnel zone plates, MLLs) ~ 20 nm Moderate efficiency, limited to ~10 nm except MLL

Waveguides

Low efficiency, 2D is challenging

~ 20 nm





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### THE X-RAY MICROSCOPE

#### by Paul Kirkpatrick

- It would be a useful complement to 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

# 2. Direct methods





## Different regimes of x-ray imaging



## Phase contrast: tool of choice for low-absorption samples





(a)Dust mite and (b) tomographically reconstructed fly leg joint, recorded with  $\sim$ 8 keV x-rays and propagation phase contrast.

S. Mayo, Opt. Express 11, 2289 (2003)





Small fish, recorded with three-grating method and a standard x-ray tube (40 kV/25 mA). (a) Transmission. (b) Differential phase contrast.

F. Pfeiffer, Nature Phys. 2, 258 (2006)

Moth wing, recorded with 4 keV x-rays. (a) Bright-field. (b) Differential interference contrast.

B. Kaulich, T. Wilhein, J. Opt. Soc. Am. A19, 797 (2002)

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## Full-field and scanning x-ray microscopes



# XM-1 microscope at the ALS



## Wet specimens can be studied by x-ray microscopy



ħw = 520 eV
32 μm x 32 μm
Ag enhanced Au labeling of the microtubule network, color coded blue.
Cell nucleus and nucleoli, moderately absorbing, coded orange.
Less absorbing aqueous regions coded black.

W. Meyer-Ilse et al. J. Microsc. <u>201</u>, 395 (2001)

#### Whole, hydrated mouse epithelial cell

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## Cryo-preparation of sample mitigates radiation damage





## Interconnects in chips are more "hardy"



## Circular dichroism contrast using polarized x-rays



## Magnetic x-ray microscopy



## 3D imaging

R ≈ 0.61 λ / NA DOF ≈ 1.22 λ / (NA)<sup>2</sup> = 2 R<sup>2</sup> / (0.61λ)

 $|n| \approx 1 \implies NA << 1 \implies DOF << R$ 

#### Synthesize larger NA with multiple views



**Cannot improve R, only DOF by tomography** 

## **Computed tomography**

1 Record many projections through sample over wide angular range. Projections at angles *q* contain:

$$I(x, y, \theta) = I_0 e^{-\int \mu_{\theta}(x', y', z') dz'}$$

**2** Reconstruct 3D sample density from suite S of projections

Invert 
$$S\{I(x, y, \theta)\} \Rightarrow \mu(x, y, z)$$

## Soft x-ray nanotomography



Frozen hydrated yeast Saccharomyces cerevisiae C. Larabell, M. Le Gros, *Mol. Biol. Cell* **15**, 957 (2004)

Frozen hydrated alga *Chlamydomonas reinhardtii*: D. Weiß, G. Schneider, *et al.*, *Ultramicroscopy* **84**, 185 (2000)



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## Quantitative phase tomography

- Defocus series (a, b, c) and phase (d) of a silicon AFM tip
- Quantitative 3D reconstructions of real part of refractive index from ±70° tomographic projections through tip



Calculated δ = 5.1 x 10<sup>-5</sup>

**Measured**  $\delta$  = 5.0 ± 0.5 x 10<sup>-5</sup>





P. McMahon et al., Opt. Commun. 217, 53 (2003)

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## Full-field microscopy with hard x-rays



## Beamline for scanning microscopy (soft x-ray)



## 1-4 keV scanning x-ray microscope at APS 2-ID-B



## **Defects in buried interconnects**



**Example: Ta-lined Cu interconnect, W vias** 



Z. Levine et al., *J. Appl. Phys.* 95, 405 (2004)

**10** μm

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## X-ray spectromicroscopy is valuable to study P speciation

Marine sediments contain complex, heterogeneous P chemistry. P typically shows little covariance with most other elements.



- Occasionally find odd P-containing minerals with distinctive P-XANES that do not match closely with any known AI-phosphate mineral
- Previously unknown in marine samples Riverine or Aeolian source?

J. Brandes et al., Marine Chem. 103, 250 (2007)

Use phase contrast for biological specimens

 $\begin{array}{c} \text{Complex transmission function:} & exp(i \ \delta \ kt) \ exp(- \ \beta \ kt) \\ & \text{phase shift} \\ & \text{absorption} \end{array}$ 



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## STXM: differential phase contrast



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## **Pennate diatoms**



2535 eV, 30 nm steps, 5 ms dwell (3 h scan) -10 -5 0 5 10 Specimen position [ $\mu$ m]

## Hard x-ray scanning flourescence microscope



## Copper is an essential trace element for all life forms

• Catalyzes production of highly reactive oxygen species

 $\Rightarrow$  oxidative damage to lipids, proteins, DNA, etc

- Defects in regulatory processes may led to:
  - ✓ Menkes syndrome
  - ✓ Wilson's disease
  - ✓ Amyotropic lateral sclerosis (ALS)
  - ✓ Alzheimer's disease



Mouse fibroblast cell + 150 µM CuCl<sub>2</sub>

- Need to be understand cellular uptake, trafficking, storage of Cu
- Novel Cu(I) fluorescent sensor (CTAP-1) was recently developed

⇒ Does it reflect the true cellular distribution?

## *μ-XANES indicates Cu(l) is present (reducing environment)*



#### L. Yang et al., Proc. Natl. Acad. Sci. 102, 11179 (2005)

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## Arsenic distribution and speciation in rice grains

A. Meharg, E. Lombi, P. Williams, K. Scheckel, J. Feldmann, A. Raab, Y. Zhu, and R. Islam, *Environ. Sci. Technol.* 42, 1051 (2008)

- There is worldwide concern over elevated arsenic concentrations in regions where rice paddy fields are irrigated with As contaminated groundwater.
- Rice production increasingly occurs in As and metal contaminated soils in Asia
- S-XRF was utilized to locate As in polished (white) and unpolished (brown) rice grains from the United States, China, and Bangladesh
- As dispersed in white rice but localized in the pericarp and aleurone layer of brown rice - Cu, Fe, Mn, and Zn localization followed that of As in brown rice
- µ-XANES and bulk extraction revealed the presence of mainly inorganic As and dimethylarsinic acid (DMA)
- Percentage of DMA present in the grain increased along with total As.



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# 3. Indirect methods

## Microdiffraction

- Scanning (monochromatic/Bragg)
- 3D differential-aperture x-ray microscopy (white/Laue)
- Coherent diffraction imaging
- Holography

## Is there lattice strain in zinc oxide nano-rings?

Termination at Zn (+) and O (-) results in spontaneous polarization along the c-axis resulting in formation of nano-rings

Internal lattice strain and distribution needed to study balance between mechanical and electronic configurations for nano-sensor applications.



X-ray fluorescence (Zn K $\alpha$ ) map









Diffraction of Zn (110) from a section of the ring. Patterns were taken at angles  $\theta$  across the rocking curve width

## X-ray nanodiffraction explains strains

Image (220) reflections from ZnO nanoring with CCD using 10 keV x-rays



Patterns cover a  $\theta$  angular range of 0.6°

- Might expect that coiling stress would cause a continuous strain distribution from compressive to tensile across the ring width.
- The two branches of the diffracted intensity indicate existence of two strain domains, with as much as 1.8% difference in the (220) plane spacing.
- Data show the structure either collapses into distinct domains across the ring width, or exhibits strain that varies little in width but continuously up to 2.4% along the ring arc.

Differential-aperture x-ray microscopy

## 3D depth-resolved, white-beam Laue diffraction technique



## B. Larson et al., Nature 415, 887 (2002)

## 3D diffraction microscope at APS beamline 34-ID-E



(wire scan, ~ 200 mm above sample surface)

Sample stage

K-B focusing mirrors



## DAXM illuminates the 3D structure of aluminum



W. Yang, et al., *Micron* 35, 431 (2004)

## Coherent diffraction and holography: "lensless" imaging



**Coherent Diffraction** 

- Object wave (diffraction) is detected directly
- Diffraction intensity corresponds to autocorrelation of object





- Coherent reference wave interferes with object wave to form hologram
- Hologram intensity corresponds to convolution of object and reference

Resolution:	transverse	~ λ/NA
	longitudinal	~ λ/(NA)²
Contrast:		$\propto  f_1^2 + f_2^2 $

## **Coherent diffraction x-ray imaging**

Lensless method - resolution limited only by wavelength, signal

- Two-step process: record coherent diffraction pattern, recover object structure numerically (iterative phase retrieval)
- Sensitive to phase as well as absorption of the specimen
- Get 3D by tomographic methods; no depth of field limit
- But: must assume some a priori information to recover phase, e.g. known object extent or illumination profile







resolution ~  $\lambda$  / angular size

J. Miao, et al., Nature 400, 342 (1999)

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## Iterative phase retrieval



R. Gerchberg and W. Saxton, *Optik* 35, 237 (1972); J.R. Fienup, *Appl. Opt.* 21, 2758 (1982)

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# Freeze dried yeast cell imaged by coherent x-ray diffraction



Diffraction reconstruction (data taken at 750 eV; absorption as brightness, phase as hue).

Stony Brook/NSLS STXM image with 45 nm Rayleigh resolution zone plate at 520 eV (absorption as brightness)

#### D. Shapiro et al., PNAS 102, 15343 (2005)

# **Diffraction microscopy in 3D**



Bragg gratings that diffract to a certain angle represent a specific transverse and longitudinal periodicity (Ewald sphere)

Data collection over a series of rotations about an axis fills in 3D Fourier space for phasing

# 3D coherent diffraction imaging



(a) SEM of pyramidal indentation in a 100-nm Si<sub>3</sub>N<sub>4</sub> membrane lined with 50-nm Au spheres. (b) 3D image reconstructed from 123 diffraction projections spanning -57° to +66°, using reality and positivity constraints. (c) Large DOF projection. (d) Enlarged region of (c).

#### H. Chapman et al., JOSA A23, 1179 (2006)

## **Coherent x-ray diffraction can reveal strain in crystals**

- Double crystal monochromator selects temporal coherence
- Precision slits select spatially coherent fraction of hard x-ray beam
- Diffractometer is used to locate Bragg peaks from an isolated nanocrystal
- Rocking curves around Bragg peak are recorded to obtain 3D data



Setup at APS 34-ID-C beamline

# Mapping crystal strain in 3D by CDI

## 0.5 µm Pb crystal grown on SiO<sub>2</sub> substrate









# Map series of diffraction patterns about (111) Bragg peak

Diffraction data reconstructed by HIO

## M. Pfeifer et al., Nature 442, 63 (2006)

## CDI setup at APS beamline 2-ID-B (1-4 keV)



## Buried structures can be probed with element specificity

In contrast to weak segregation theory, Bi is locally concentrated in Bi-doped Si crystals



- (a) Image below Bi M5 edge (2550 eV)
- (b) Image above the Bi M5 edge (2595 eV)
- (c) Difference
- (d) SEM image

## C. Song et al., PRL 100, 025504 (2008)

## **Curved object illumination aids unique phase recovery**



- Lens illuminates object with curved wavefront, defines field of view
- Object illumination is reconstructed by back-propagation, used to retrieve phase of object wave by iterative methods

## K. Nugent et al., PRL 91, 203902 (2003)

... and converges faster

# Original





# curved beam illumination

plane wave illumination

## FCDI setup



## Fresnel coherent diffraction imaging of a gold test pattern



(a)

## Images reconstructed by FCDI



Magnitude with color-encoded phase

STXM image (1.8 keV)

## G. Williams et al., PRL 97, 022506 (2006)

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## "Keyhole" FCDI

Use reconstructed illumination profile to determine support in extended sample

H. Quiney, *Nature Physics* 2, 101 (2006)







Ability to study extended samples is essential for many real-world problems!

B. Abbey et al., Nature Physics 4, 394 (2008)

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## Recent results ("fuse bay" structure)



## Alternative approach: ptychography

R. Hegerl et al., Phys. Chemie 74, 1148 (1970)
H. Faulkner and J. Rodenburg, PRL 93, 023903 (2004)
J. Rodenburg et al., Phys. Rev. Lett. 98, 034801 (2007)
P. Thibault et al., Science 321, 379 (2008)







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# Holography



- Reference wave encodes magnitude and phase of object wave
- Reconstruct object wave by "re-illuminating" hologram with reference wave (or its C.C.)

# Gabor holography





6.0 μm

Visible light

Reconstructed hologram (right) of a NIL8 hamster neural fibroblast recorded with 656 eV x-rays. Estimated dose was 7.5 x105 Gy

#### S. Lindaas et al, J. Opt. Soc. A. 13, 1788 (1996)



X-ray

## Hard x-ray holotomography





Phase-sensitive image quantitative phase map, and volume rendition of polystyrene foam (18 keV x-rays)

P. Cloetens et al., Appl. Phys. Lett. 75, 2912 (1999)

## FT hologram formation



object wave

reference wave

$$I = |a+b|^2 = |a|^2 + |b|^2 + a^*b + ab^*$$

## hologram intensity
#### **Reconstruction**

Numerically take FT of hologram intensity to reconstruct

Spatially separated primary, conjugate object waves result

Weak curvature f(x,y) on object wave can be ignored

Image terms: 
$$a^*b+ab^* = \varphi(s\xi)F(\xi,\eta)+\varphi(s\xi)^*F(\xi,\eta)^*$$

where: 
$$F(\xi,\eta) = \frac{e^{ikz}}{i\lambda z} f(\xi,\eta) \iint a(x,y) f(x,y) e^{-\frac{ik}{z}(x\xi+y\eta)} dxdy$$
,

$$\varphi(s,\xi) = e^{-\frac{ik}{z}s\xi} \text{ and } f(\xi,\eta) = e^{\frac{ik}{2z}(\xi^2 + \eta^2)}$$
$$FT^{-1}\{a^*b + ab^*\} = f(x-s,y)a(x-s,y) + f(-(x-s),-y)^*a(-(x-s),-y)^*$$

# **CoPt** magnetic labyrinth nanostructures



side view

Sample: O. Hellwig (Hitachi)

MFM, top view



 $5~\mu m$  x  $5~\mu m$ 

continuous object

SiN<sub>x</sub> / Pt (24 nm) / [Co (1.2 nm) / Pt (0.7 nm)]<sub>50</sub> / Pt (1.5 nm)

perpendicular anisotropy

→ magnetic storage media

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# **UE56-SGM** beamline and ALICE



## Pinhole mask method



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#### Imaging resolution by x-ray holography



## What's in store for the future?

- Resolution of x-ray optics is improving steadily
  - 30 nm is routine
  - < 5 nm looks possible using MLLs</p>
- Commercial x-ray microscopes are proliferating
  - Precision full-field and scanning instruments
  - Multiple contrast mechanisms available
  - Cryo-preparation will become standard (as for TEM)
- Hybrid scanning (ptychography) and single-view (keyhole) coherent diffraction methods broaden range of applications
- Diffraction and holographic methods are attractive for x-ray lasers such as XFEL and LCLS !
  - Bypass radiation damage with a single few-fs flash
  - Use pump-probe methods to study ultrafast dynamics
  - No lens to limit resolution or get between sample, detector









### Ultrafast coherent diffraction with a free-electron laser

The reconstruction is carried out to the diffraction limit of the 0.26 NA detector



H. Chapman et al., Nature Physics 2, 839 (2006)

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### Now it can be done on a tabletop!





R. Sandberg et al., PRL 99, 098103 (2007)
J. Spence, *Nature* 449, 543 (2007)
R. Sandberg et al., PNAS 105, 24 (2008)



- Resolution, contrast, sources, and optics for x-ray imaging
- Direct methods: projection, full-field, and scanning
- Indirect methods: microdiffraction, coherent diffraction, holography

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