Phase Contrast Microscopy with Soft and Hard X-rays Using a Segmented Detector

Benjamin Hornberger

BNL Instrumentation Seminar, 28 March 2007
# Fluorescence Trace Element Map of Phytoplankton Cell

<table>
<thead>
<tr>
<th>Element</th>
<th>Max</th>
<th>Min</th>
<th>ug/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>7.86</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>1.68</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>0.398</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>8.59</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>0.0177</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.223</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.004</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.0200</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.343</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>s_a</td>
<td>57.67</td>
<td>40.2</td>
<td></td>
</tr>
</tbody>
</table>

Sample: Stephen Baines, Stony Brook Marine Sciences
Outline

• Introduction
  – X-ray Microscopy 101
  – Phase Contrast 101

• A Segmented Detector for Hard X-ray Microprobes
  – Segmented Silicon Chip
  – Charge Integrating Electronics

• Differential Phase Contrast (DPC)
  – Comparison with Amplitude Contrast and DPC Examples
  – Integration of the DPC Signal

• Quantitative Amplitude and Phase Reconstruction
  – Reconstruction Scheme
  – Simulations and Experiments with Soft and Hard X-rays

• Summary and Outlook
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X-ray Interactions: Wave Propagation

- Complex **index of refraction**: \( n = 1 - \delta - i\beta \)
- \( \delta, \beta \): small positive numbers (\(10^{-4}, \ldots, 10^{-9}\), tabulated values)
- Wave propagation through material with refractive index \( n \):
  \[
  \psi(z) = \psi_0 \exp(-inkz) = \psi_0 \exp(-ikz) \exp(+i\delta k z) \cdot \exp(-\beta k z)
  \]

Diagram:
- Vacuum propagation
- Phase Advance
- Complex specimen function
- Absorption

Material with \( n = 1 - \delta - i\beta \):
- \( \lambda_{\text{vac}} \) and \( \lambda_{\text{mat}} \)
- Phase shift \( \Delta\phi \)
X-ray Interactions: Fluorescence

(a) Photoelectron (E = hν - E₀)
(b) Fluorescence emission

Data from Krause (1979)
Synchrotrons

Advanced Photon Source (APS), Argonne Nat'l Lab, Illinois

National Synchrotron Light Source (NSLS), Brookhaven Nat'l Lab, New York
Scanning Transmission X-ray Microscope (STXM) and Fluorescence Microprobe

Sample (scanned in x and y)

Spatial resolution:
NSLS X1A: 40 nm (sub-keV)
APS 2-ID-B: 55 nm (1-4 keV)
APS 2-ID-E: 250 nm (7-17 keV)
Fresnel Zone Plates

- Circular diffraction gratings with radially decreasing line width
- **Spatial resolution:** 1.22 × outermost zone width
- Usually produced by electron-beam lithography / etching / plating

<table>
<thead>
<tr>
<th>Energy</th>
<th>500 eV</th>
<th>4 keV</th>
<th>10 keV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>2.5</td>
<td>0.31</td>
<td>0.12</td>
</tr>
<tr>
<td>Diameter</td>
<td>160 um</td>
<td>160 um</td>
<td>320 um</td>
</tr>
<tr>
<td>Out. zone wid.</td>
<td>30 nm</td>
<td>50 nm</td>
<td>100 nm</td>
</tr>
<tr>
<td>Focal length</td>
<td>1.9 mm</td>
<td>26 mm</td>
<td>270 mm</td>
</tr>
<tr>
<td>Thickness</td>
<td>200 nm</td>
<td>450 nm</td>
<td>1600 nm</td>
</tr>
<tr>
<td>Material</td>
<td>Nickel</td>
<td>Gold</td>
<td>Gold</td>
</tr>
<tr>
<td>Efficiency</td>
<td>12%</td>
<td>15%</td>
<td>30%</td>
</tr>
</tbody>
</table>

![Diffraction Limited Point Spread Function](image)
**Phase Contrast Motivation**

- Lower energies: Imaging at the low energy side of an absorption edge can **lower the radiation dose**

- At higher energies: **Phase contrast dominates**
  - Combine with fluorescence
  - PC to image ultrastructure
  - Quantitative PC $\rightarrow$ thickness $\rightarrow$ trace element concentrations

\[
\frac{\delta}{\beta} \propto E^2
\]

---

**Absorption vs. Phase Shift: Protein in Water**

- Data from Henke et al.

**Required Carbon Thickness**

- 1/e absorption
- $\pi/2$ phase shift

Data from Henke et al.
Differential Phase Contrast

- Refraction model – effect of **phase gradient** (like prism for visible light):
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• Summary and Outlook
Why not use a CCD?

- Slow (serial) readout (tens of ms to sec) vs. ms pixel dwell times
- huge amounts of data
- statistical significance of a single detector pixel
- fast readout pixel detectors in the future?
Review: Segmented Detector Version 1

- **Collaboration** with
  - BNL Instrumentation (P. Rehak, G. De Geronimo)
  - Max Planck Semiconductor Lab (L. Strüder, P. Holl)
- **For NSLS STXM:**
  - 200-800 eV, $10^6$ photon/sec
- **Segmented silicon chip**
  (high quantum efficiency)
  - rotational symmetry
- **Charge integrating**
  electronics (high count rates)
  - Simultaneous recording of all segments (**various contrast modes**)

Electronics: 10 channels
# Modifications for Hard X-Rays (APS)

<table>
<thead>
<tr>
<th>Beamline</th>
<th>Flux</th>
<th>Photon Energy</th>
<th>Current</th>
<th>Dwell Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSLS X-1A</td>
<td>$10^6$/s</td>
<td>200 – 800 eV</td>
<td>1-20 pA</td>
<td>1-10 ms</td>
</tr>
<tr>
<td>APS 2-ID-B</td>
<td>$10^8$/s</td>
<td>1 – 4 keV</td>
<td>1-100 nA</td>
<td>0.5-5 ms</td>
</tr>
<tr>
<td>APS 2-ID-E</td>
<td>$10^9$/s</td>
<td>7-17 keV</td>
<td>0.1-1 μA</td>
<td>sub-ms – sec</td>
</tr>
<tr>
<td>Nanoprobe</td>
<td>$10^{10}$/s</td>
<td>10 ( - 30) keV</td>
<td>0.5-5 μA</td>
<td>sub-ms – sec</td>
</tr>
</tbody>
</table>

- **APS 2-ID-B:**
  - One NSLS detector modified with larger feedback capacitance

- **APS 2-ID-E:**
  - Used 15-20 layers of Al foil in front of detector to absorb > 99.5 % of the photons
  - Decouple detector integration time and pixel dwell time
To be detected, photons must be absorbed in (active region of) chip

At higher energies, thickness limits quantum efficiency

At lower energies (< 1 keV), absorption effects in surface oxide layer

Data from Henke et al.
**Segmented Silicon Chip**

- Produced by Max Planck Semiconductor Lab
- 300 to 450 μm thick n-type silicon
- segments: shallow p-implant with current readout
- Ohmic junction on back side for bias voltage
- Can illuminate front or back side
- Extremely low leakage current

~7 mm
Radiation Damage

- Front side is radiation-sensitive
- Increase of leakage current with exposure
- Repair by annealing
- **Problems:**
  - Adds to signal → Calibration
  - Uses up part of dynamic range
- **Solution:**
  - Soft x-rays: Back side illumination
  - Hard x-rays: Regular annealing

<table>
<thead>
<tr>
<th>Seg.</th>
<th>Leakage Current (pA)</th>
<th>3 days exp.</th>
<th>annealed</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
<td>15</td>
<td>0.7</td>
</tr>
<tr>
<td>5</td>
<td>1.9</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>1.1</td>
<td>7.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Charge Integrating Electronics

- 10 channels for up to 10 segments
- Current amplifier (adjusted to signal rate)
- Integrator (adjusted to dwell time)
- Sample and hold for readout
- Dead time ca. 10 μs

\[ U_{\text{bias}} (+) \]

\[ \text{Det} \]

\[ \text{X-rays} \]

\[ R_f \]

\[ R_c \]

\[ \text{Inverting Amplifier} \]

\[ S2 \]

\[ C_f \]

\[ \text{Integrating Amplifier} \]

\[ S3 \]

\[ C_{\text{s/h}} \]

\[ \text{Sample / Hold} \]

\[ \text{output} \] (to Analog to digital converter)
Integration Cycle

- Trigger to ADC
- S/H control pulse
- Integrator
- Reset pulse
- S/H output
Interfacing with Microscope Electronics

- Two scan modes:
  - Step scan (slow)
  - Fly scan (fast)
- Two signal types
  - Digital (pulse train)
  - Analog (voltage)
    - Voltage to Frequency converter (V2F)

- Operation in fly scan mode:
  - Scan pixels and detector integration in sync
  - Read voltage directly
- Operation in step scan mode:
  - Pixel dwell time >> integration time
  - Use V2F
Detector Calibration

- Measure amplifier output voltage, want photon flux
- Need to know
  - Photon energy (monochromatic illumination!)
  - Charge created per photon: 3.6 eV per e/h pair
  - Calibration constant between input charge and output voltage (amplifier gains, integrating capacitor)
  - Charge integration time (pixel dwell time)
  - Leakage current (measure signal with no x-rays incident for several dwell times)
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• Summary and Outlook
**Cardiac myocyte** (heart muscle cell)

**Diatoms** (phytoplankton).
Sample: Stephen Baines, Stony Brook Marine Sciences.

5 µm **Polystyrene spheres**
At Lower Energies

**Diatom** at 2-ID-B (1.8 keV)

**Polymer** spheres in polymer matrix @ 286.4 eV (NSLS STXM) (sample provided by Gary Mitchell, Dow Chemical)
Combination with Fluorescence

But can we do something more quantitative?
DPC Integration – Noise-Free Simulations

- Sphere: max. phase shift 0.1 rad, no absorption
- Image simulated with “true” wave propagation
- No noise
Simulations with Noisy Data

DPC image

bi-directional integration

one-directional integration

two orthogonal bi-directional integrations
Integration of DPC Data

- 5 μm diameter polystyrene spheres
- E = 10 keV
- expected δkt = 0.60
DPC – Conclusions

- Vastly improved contrast for weakly absorbing specimens at multi-keV energies
- Easily available with segmented detector (real-time)
  - Quick orientation images (finder scans)
  - High-resolution images of sample morphology

- Hard to interpret
  - Differential signal
  - Directional dependence
  - Hard to quantify
  - Simple integration doesn't work well
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Image Formation in a Scanning Instrument

- **Wave propagation** from source to detector plane
- Segmented detector
Contrast Transfer Functions

• Complex specimen function: \( \Psi_{\text{out}}(r) = h(r - r_0) \cdot \Psi_{\text{in}}(r) \)

• Weak specimen approximation: 
  \( h(r) = 1 + h_r(r) + i h_i(r) \)

  \[ h_r(r) = -\beta(r) k \, dz \quad h_i(r) = \delta(r) k \, dz \]

• Image recorded by detector segment \( k \) (Fourier space)

  \[ S_k(f) = c_k \cdot \delta(f) + H_r(f) \cdot T_r^k(f) + i H_i(f) \cdot T_i^k(f) + N_k(f) \]

• Contrast Transfer Functions depend on
  – P: Zone plate pupil function
    (assume coherent illumination)
  – R: Detector response function
Calculated Contrast Transfer Functions

- **Real part CTFs:**
  - even symmetry
  - CTFs for opposing detector segments are identical
- **Imaginary part CTFs:**
  - odd symmetry
  - CTFs for opposing detector segments are opposite in sign

→ **Sum** of opposing segments shows only *

absorption contrast*

→ **Difference** of opposing segments shows *

differential phase contrast*
Comparison of Detector Geometries

\[ T_{r,i}^{\text{tot}}(f) = \sum_k T_{r,i}^{(k)}(f) \]
Amplitude and Phase Reconstruction

- Reconstruction of the complex specimen function by **Fourier filtering** detector images
- Proposed for scanning transmission electron microscopy (McCallum *et al.*, Optik 101(2) 1995)
- Similar to Wiener Filter
- **Best estimate** of complex specimen function:

\[ \tilde{H}(f) = \sum_k W_k(f)S_k(f) \]

- Images from det. segments
- Filter functions

- Calculate filter functions by minimizing reconstruction error
- Weak specimen approximation
- Account for noise
Reconstruction Filters

- Result for filter functions

\[ W_k(f) = \frac{T^*_r(f)}{\sum_l |T^l_r(f)|^2 + \beta_r^k(f)} + \frac{T^*_i(f)}{\sum_l |T^l_i(f)|^2 + \beta_i^k(f)} \]

- Noise parameter

\[ \beta_{r,i}^k(f) = \frac{\langle |N_k(f)|^2 \rangle}{|H_{r,i}(f)|^2} = \frac{\text{Spectral noise of segment } k}{\text{Specimen power spectrum}} \]
Soft X-ray Simulations of a Test Pattern

• Simulated weak and strong test pattern
• Conditions as in experiment (next slide)

<table>
<thead>
<tr>
<th></th>
<th>weak specimen</th>
<th>strong specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>simul.</td>
<td>recon.</td>
</tr>
<tr>
<td>$\beta_{kt}$</td>
<td>0.100</td>
<td>0.098</td>
</tr>
<tr>
<td>$\delta_{kt}$</td>
<td>0.100</td>
<td>0.103</td>
</tr>
</tbody>
</table>
Reconstruction of a Germanium Test Pattern

- Data acquired by Michael Feser @ 525 eV
- Recovered $\beta_{kz} \approx 0.35$, $\delta_{kz} \approx 0.99$ in good agreement with expected values
- More details in the phase image

• 5 μm Polystyrene spheres
• 10 keV photon energy
• invisible in amplitude contrast
• expected $\delta_{kz} \approx 0.6$
• reconstructed $\delta_{kz} \approx 0.43$
• Uneven zone plate illumination?
• Limited knowledge about zone plate?
• Independent verification of expected value?
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Summary

• Phase contrast is useful!
  – reduce radiation dose at lower energies
  – superior transmission contrast at higher energies
    • combination with fluorescence
    • high resolution images of specimen ultrastructure
    • fast finder scans

• Segmented detector for hard x-ray microprobes
  – simultaneous amplitude and phase contrast
  – installation in parallel with fluorescence detector
  – segmented silicon chip
  – 10 channel charge integrating electronics
  – adjustable dynamic range
  – wide range of pixel dwell times
  – absolute calibration
Summary (2)

- **Differential phase contrast**
  - vastly superior contrast at higher energies
  - easily available
  - not so good for quantitative interpretation
  - simple integration doesn't give good results

- **Quantitative amplitude and phase reconstruction by Fourier filtering**
  - quantitative phase contrast can give specimen mass / thickness
  - “invert” image formation process
  - includes noise filter
  - works great in simulations for weak specimens
  - good experimental results
  - more careful measurements and consideration of experimental conditions
Future Work

• Detector installation at more beamlines
  – APS 2-ID-B, 2-ID-D, Nanoprobe
  – Australian Synchrotron
  – Better incorporation in data acquisition system

• More investigations about the Fourier filtering algorithm

• Go beyond test specimens to “real” applications

• Future hardware improvements?
  – fast readout pixel detectors
  – germanium detectors for higher energies
Acknowledgements – Thanks!

- Christian Holzner, Chris Jacobsen, Michael Feser (Stony Brook)
- Detector development: Pavel Rehak (BNL Instrumentation)
- Soft x-ray experiments: Sue Wirick (NSLS X1A)
- Hard x-ray experiments: David Paterson, Stefan Vogt, Daniel Legnini, Martin de Jonge, Ian McNulty (APS)
- Detector chips: L. Strüder, P. Holl *et al.* (Max Planck Institute)
- Samples: B. Palmer (U. Vermont), M. Kissel (CEMS / Stony Brook), S. Baines *et al.* (Stony Brook Marine Sciences)