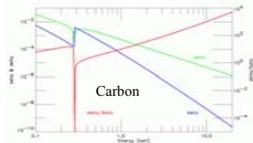
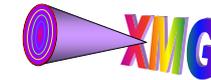


### PHASE CONTRAST MICROSCOPY

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#### ABSTRACT

We are developing powerful new phase contrast techniques at Sector 2 of APS that uniquely capitalize on the high brilliance of APS x-ray sources. They include absolute determination of the phase of materials science specimens such as AFM tips using coherent full-field imaging [1], differential phase contrast of weakly absorbing biological samples using configured detectors in scanning x-ray microscopes [2], and phase contrast enhancement of defects in weakly diffracting matter such as protein crystals [3]. These sensitive x-ray phase contrast methods, in addition to being advantageous for imaging weakly absorbing or scattering features with minimum attendant radiation dose, are also useful for fundamental measurements of wavefields.

#### QUANTITATIVE PHASE RETRIEVAL

Theoretical approach [4] using the transport of intensity equation to retrieve the quantitative phase component from intensity measurements at 3 focus locations.

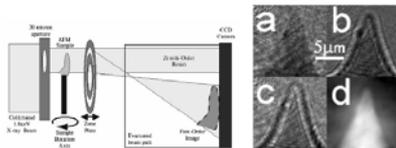


Fig. 1. Schematic of the full field imaging x-ray microscope used in the measurements [1]. Sample was an atomic force microscope tip composed of silicon: (a) Typical in-focus absorption image; (b) 1 mm over-focus; (c) 1 mm under-focus; (d) phase projection retrieved from intensity measurements. Images were acquired with a monochromatic 1.83 keV at beamline 2-ID-B.

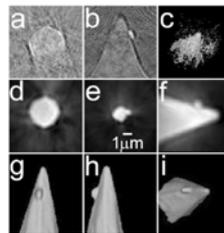


Fig. 2. Quantitative reconstructions of the real part of the refractive index phase of the AFM tip: (a) recovered horizontal slice through in-focus data set; (b) vertical slice in-focus; (c) volume rendering of absorption contrast (d) horizontal slice of retrieved phase; (e) horizontal slice which includes a 900 nm bump; (f) vertical slice through phase tomogram; (g - i) different views of volume rendering.

#### MOTIVATION

The advent of highly coherent x-ray beams at modern synchrotrons has revolutionized phase-contrast microscopy. At high X-ray energies the real part of the complex index of refraction of low Z elements is several orders of magnitude larger than the imaginary part. Consequently, x-ray imaging of low density samples such as thin sections of mammalian cells, bacteria or protein crystals can benefit significantly from phase contrast methods.

#### DIFFERENTIAL PHASE CONTRAST

Use a configured or segmented detector to obtain differential phase contrast on scanning x-ray microprobes. Collaboration with State University of New York, Stony Brook [5].

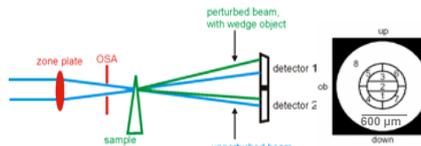


Fig. 3. Diagram of zone plate scanning microscope with the addition of a segmented detector to allow differential phase contrast. Advantages: simultaneous collection of bright field, dark field and differential phase contrast at msec dwell times; filters out intensity variations during a scan e.g. Fig 5. compare ABS and DPC images.

Sum of all segments (Bright field) Max = 11.7  
Segments 4d+5d-6d-7d Max = 0.683  
Max = 0.777  
Min = -1.62

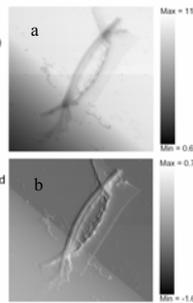


Fig. 4. Scanning microscope images of a diatom: (a) Bright field image, the sum of all segments; (b) Differential phase contrast image, showing horizontal contrast by subtracting right (6 and 7) from left (4 and 5) segments. Scans were taken with 1.8 keV monochromatic beam at 2-ID-B.

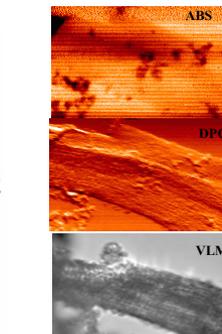


Fig. 5. Scans of a cardiac myocyte: ABS absorption contrast image; DPC differential phase contrast; VLM visible light micrograph. DPC image gives a significantly better representation of the biological mass of the specimen. Scans were taken with 10 keV monochromatic beam at 2-ID-E.

#### DIFFRACTION PHASE CONTRAST

Enhance the minute contrast due to crystalline defects in very weakly diffracting samples (e.g., protein crystals).

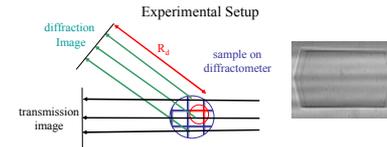


Fig. 6. Bragg condition achieved by orienting the sample in a diffractometer. Transmission image is used to align the sample and check the integrity of the sample. The white boundaries are due to absorption phase contrast. Sample to detector distance,  $R_d$ , is adjusted to optimize the diffraction contrast from various parts of the sample (see Fig. 6).

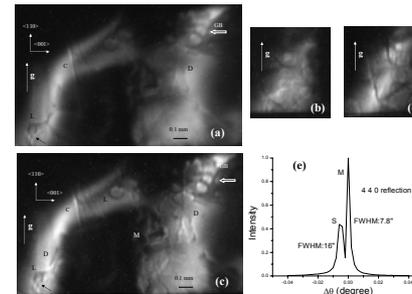


Fig. 7. (440) Diffraction Images of a lysozyme crystal taken at  $R_d$ : (a, b) 7 cm; (c, d) 25 cm; (e) rocking curve. The peak M and S in the rocking curve corresponds to the middle and outer part of the crystal. The images taken at 25 cm reveal much greater detail due to various defects: GB, growth boundaries; D, dislocation; L, dislocation loop. The images were collected using 13 keV monochromatic beam at 2-BM [3].

#### SUMMARY

Various forms of phase contrast microscopy are rapidly expanding in use in synchrotron science, especially for biological applications. We are developing the instruments and techniques to incorporate phase contrast and retrieval in the x-ray microscopy group.

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