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Quantitative scanning differential phase contrast microscopy

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Abstract. Differential phase contrast can be realised in the scanning transmission x-ray microscope by use of a detector with an appropriate configuration of detector elements. Use of such a configured detector significantly improves the ability to image specimens with low absorption contrast. A natural application is found in scanning fluorescence x-ray microprobe studies of biological specimens. We discuss the technique and several applications.

1. Introduction

The scanning fluorescence x-ray microprobe can be used to map elemental distributions, to obtain spatially-resolved chemical information by means of XANES measurements, and to obtain longer-range bonding and co-ordination information from XAFS measurements. The spatial resolution of such measurements ranges from several micrometres down to around 50 nm using present synchrotron facilities. Virtually any format of specimen can be measured, with limitations coming mainly from mechanical constraints and the desire to avoid imaging artifacts. Studies have been performed on geological, environmental, and soil specimens and a wide range of biological specimens, including cells, plants, and whole organisms.

While the microprobe can be used to investigate distributions on one side of a bulk specimen, the high penetration of hard x-rays recommends the application to a particular class of specimens with low absorption. In this regime the incident probe beam maintains its intensity as it traverses the specimen. Quantitative studies are possible when fluorescence x-rays escape with only minimal absorption [1]. While such studies can be performed on thin sections of specimens with high linear attenuation coefficient, a popular alternate regime is the measurement of thick specimens with low linear attenuation. This regime is particularly applicable to the imaging of trace metals in a biological context, and is ideally suited to measure almost any specimen coming from the biological community.

The high penetration of x-rays is required to produce high-fidelity and quantitative fluorescence maps; however, the same condition leads to transmission images having extremely low contrast. The framework of a cell or organism is largely composed of lipid, protein, and water. These components absorb high-energy x-rays only weakly, and so studies involving biological specimens are usually blind to the biological ultrastructure in which the trace metals are situated. Detailed ultrastructural knowledge can help an investigator identify various organelles within the organism and thereby contextualise the elemental maps. While it has long been known that phase offers stronger contrast at high x-ray energies, phase contrast methods have only recently been developed for the STXM.

2. Differential phase contrast imaging in the STXM

Sensitivity to phase structure is possible in the scanning microprobe simply by employing a detector with spatial sensitivity [2–4]. Two different methods for reconstructing quantitative values of the phase for scanning x-ray microprobe have been reported recently [5, 6]. Although the detector configurations used for these demonstrations were identical, the analytical approaches are completely different. The method of Hornberger [5] determines the CTF appropriate to the detector elements, and inverts this CTF to determine the phase advance and absorption of the specimen. This method is most general, and can be applied to any configuration of detector. The method of de Jonge [6] expands the specimen function in a Taylor series and demonstrates that the first-order effect of a specimen on the intensity distribution in the detector plane is (1) to attenuate according to the mean optical thickness under the beam footprint (according to the usual assumptions of STXM imaging) and (2) to shift the x-ray beam in proportion to the mean refractive index gradient under the beam footprint. The next most significant term in the Taylor expansion describes a novel differential interference contrast effect, but this is small when the focal spot is small, when the absorption gradient is modest, and when the specimen is fully within the focal plane. The centre-of-mass of the intensity distribution can be used to gauge the shift of the intensity distribution when that distribution is well defined. We determine the centre of mass by examining the normalized differences in the detector halves (see Fig. 1); for example, the vertical component of the phase derivative DPC_V is gauged using $DPC_V = (I_{top} - I_{bottom}) / (I_{top} + I_{bottom})$.In this article we use this method to visualize the DPC.

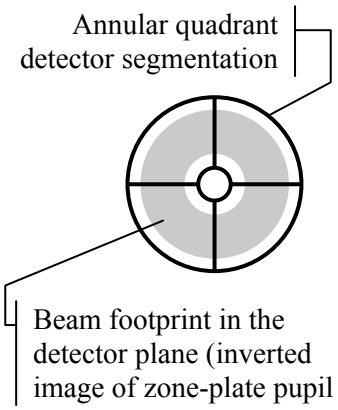

Figure 1. Detector segmentation and intensity distribution in the detector plane.

3. Applications

3.1. Specimen visualisation

The low absorption contrast of biological specimens makes them difficult to find in the x-ray beam. One of the immediate uses of DPC imaging is to facilitate this process by providing high contrast images in real time. Figure 2 shows a scan of pennate diatoms. The absorption image (left) shows very low contrast; the diatoms are barely visible. The DPC image (right) shows very clearly the diatoms, including periodic structures along their length and potentially some internal organs. Fine hairs, possibly flagella, connect the two diatoms; these are likely to be protein fibres with estimated thickness of around 5 nm. Both of these images are generated form the one scan; the absorption image is generated from the sum of all detector segments, the DPC image from the normalized differences defined in Section 2.

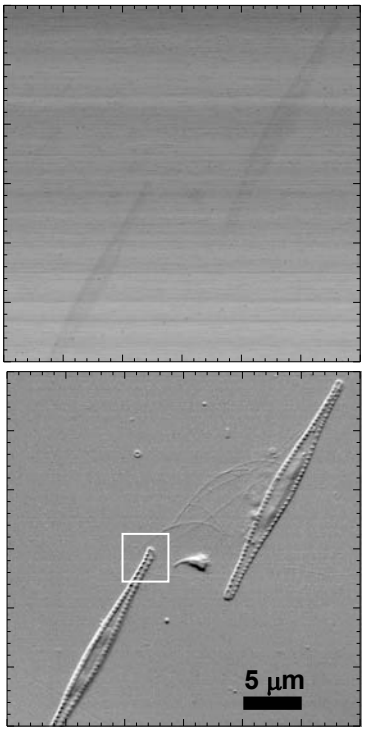

Figure 2. The absorption image (upper) barely resolves these pennate diatoms, but the vertical component of the DPC (lower) shows excellent structural detail. Thin flagella between the two diatoms have an estimated phase shift of $\pi/300$ radians, corresponding to around 5 nm of protein. The several 'doughnut' features are the result of beam damage caused by dwelling the focussed x-ray beam on the EM grid membrane. Theoretical sensitivity is estimated at better than 1 nm of protein for the STXM at 2-ID-B of the APS. Scan parameters: x-ray energy = 2.5 keV, step size = 30 nm, dwell = 5 ms, zone plate: radius = 80 μm, finest zone width = 50 nm.

3.2. Quantitative phase reconstruction

Quantitative phase reconstructions require a well characterised optical system because current reconstruction algorithms make assumptions about the optical parameters. For example, our use of a quadrant diode to measure the location of the centre of mass of the intensity requires assumptions about the source, the uniformity of the zone-plate illumination, and the uniformity of the zone-plate efficiency. Zone-plate and optical parameters are used to scale the phase gradient, and so these must be accurately known.

Figure 3 shows a reconstruction of the phase of the tip of a diatom (Fig. 2). Tooth-like structures are clearly visible. A line-out of the reconstructed phase along these structures shows that the phase sensitivity is easily better than $\pi/100$ radians; we estimate that the phase of the flagella to be of order $\pi/300$ – corresponding to about 1 nm of protein – demonstrating the extreme sensitivity of this imaging modality.

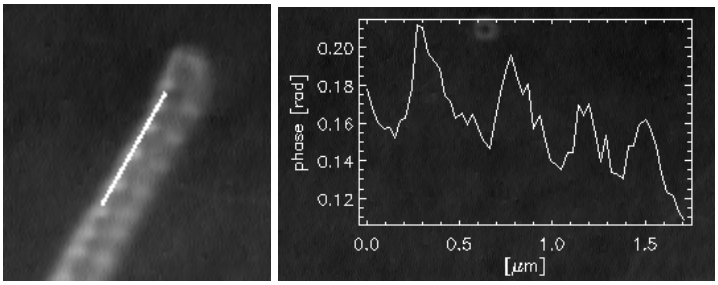

Figure 3. Phase reconstruction (left) of the tip of the pennate diatom indicated in Fig. 2. The frustule (or shell) of the diatom is comprised largely of SiO_2. A lineout along the indicated path is shown (right), clearly showing phase structures of order $\pi/100$ radians, corresponding to around 30 nm of SiO_2.

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