Cryo diffraction microscopy: ice conditions and finite supports

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Abstract. Cryo specimen conditions greatly alleviate radiation damage in x-ray microscopy. In diffraction microscopy with plane-wave illumination, a finite support constraint is required in order to obtain a reconstructed image, but this demands that there be an area outside the specimen which is free of optical response. We report here observations based on our early attempts in cryo diffraction microscopy, where ice scatter from outside the cell and ice deposition during data taking have been problems. We outline solutions that are being implemented for future experiments.

1. Introduction
X-ray diffraction microscopy offers an approach to 2D and 3D cell imaging with no lens-imposed limits on spatial resolution or efficiency of data recording [1, 2], and our group has used the approach to image freeze-dried yeast cells [3]. However, when using plane wave illumination and far-field data recording, one must use a finite support constraint in order to obtain a reconstructed image [4]. This finite support constraint demands that part of the real-space image plane sampled in the data recording be optically “empty,” with no significant variations in absorption or phase shift. While detailed studies are required to understand the phase tolerance of the “empty” background requirement, one would expect that phase shifts that are very small compared to the Rayleigh quarter wave criterion (i.e., ice thickness variations that are very small compared to $\lambda/(4\delta) = 1260$ nm) are required in the “empty” area.

While radiation damage is a potential limitation in any microscopy with ionizing radiation, working with the specimen at cryogenic temperatures can greatly reduce this limitation [5, 6]. In x-ray microscopy, this allows specimens to tolerate radiation doses of up to about $10^{10}$ Gray before structural changes are observed at the 30–100 nm resolution level [7, 8] (though latent changes can be detected spectroscopically at lower doses [9]). A common method for preparing frozen hydrated specimens is to place cells on a thin membrane (such as ~50 nm thick formvar), blot off the excess liquid, and rapidly plunge into liquid ethane or propane in order to rapidly
freeze the sample and avoid ice crystal formation. With sufficiently thin water layers, one can obtain a vitreous or amorphous ice layer with very uniform transmission as observed in cryo electron microscopy [6], and the use of solutions such as glucose can increase the thickness that can be prepared in an amorphous state. We have already used this approach to obtain some preliminary reconstructions of frozen-hydrated yeast cells [10]. Smooth ice layers are important for cryo x-ray diffraction microscopy, where one wishes to obtain an isolated cell with a uniform, structureless ice layer outside the cell so that a finite support constraint can be applied and a real-space reconstruction can be achieved.

2. Observations of ice buildup
We describe here some initial observations on working with frozen hydrated specimens in x-ray diffraction microscopy. Our experiments were carried out using a homebuilt vacuum chamber system employing a standard transmission electron microscope type goniometer stage (JEOL) and side-entry cryo transfer holder (Gatan) [11]. This chamber is also equipped with a liquid-nitrogen-cooled copper plate of about 80 cm² total surface area at a temperature of about -190°C, although the nearest corner of this plate is about 8 cm distant from the specimen. This plate was cooled down about two hours before each experiment reported here. In one test, we loaded formvar-coated but otherwise empty specimen grids into the cryo holder while at room temperature, inserted them into the specimen chamber, and then cooled the grid while in the microscope chamber. After the temperature of the grid stabilized at around -160°C, we took a diffraction pattern of an initially blank area every minute. Surprisingly, a doughnut-shaped ring clearly appeared in the pattern after some time (see Fig. 1). In repeated experiments, the time for this ring to form was highly dependent on the vacuum pressure in the chamber; Fig. 1 shows the azimuthally averaged diffraction signal obtained after 60 minutes when the base pressure was at $3 \times 10^{-6}$ Torr only a few hours after the chamber had been open to room air, as well as the signal when the chamber had been under high vacuum for more than a day so that the base pressure was at about $1 \times 10^{-7}$ Torr. We have reasons to believe that the “doughnut” is due to the scattering from either the ice crystals formed in the chamber, or a “wavy” surface of an amorphous ice layer (see [12] for further discussion of possible explanations for this “doughnut” shape). Given that it takes more than one hour to collect the data (especially when going to 3D imaging, where at ALS beamline 9.0.1 the data collection time could be well over several hours), this scattering from ice formed during data collection could prevent us from reconstructing diffraction patterns.

![Image of diffraction pattern](inset image)
using a finite support constraint, since the “empty” ice area outside the cell might not be so optically empty to the x-ray beam.

3. Discussion

The above experiments were carried out with a very simple anticontamination plate. When cooling down this anticontaminator plate, the base pressure in the chamber would often drop by a factor of ten, so it certainly was effective in capturing residual vapors in the system. However, ice contamination is well recognized as a potential limit in electron microscopy, where the cryo specimen is often surrounded by plates in very close proximity to the sample—like a few millimeters away. In one example, Cheng et al. have achieved a contamination rate of only 0.13 nanometers/hour [13]. While rebuilding our chamber to improve a number of its capabilities, we are replacing our first version of a poorly-adjustable “cryo box” of this type with one that fits both more tightly around the specimen and is also easily adjustable using motorized positioners. When this upgrade is completed, we hope to move forward in the imaging of frozen hydrated specimens using cryo x-ray diffraction microscopy.

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