Overview and summary: This proposal was to develop cryogenic x-ray diffraction microscopy (cryo-XDM) for frozen-hydrated biological samples. Utilizing high-penetration power of hard x-rays and imaging sensitivity of XDM, cryo-XDM has a potential to provide noninvasive high-resolution imaging capability to large biological samples [1]. We proposed, originally, a Cat. II beamtime, including 3D imaging, to achieve the full capability of cryo-XDM and we were awarded Cat. I beamtime according to the P10 operation schedule. During the allocated beamtimes, we successfully demonstrated the feasibility of cryo-XDM in two dimensions (2D) through two user beamtime experiments in April and December 2011. The following summarizes the progress and the results of the experiments.

April 21-26, 2011: This beamtime was primarily devoted to commissioning cryo-XDM data collection at P10, in collaboration with M. Sprung and A. Zozulya, since this type of experiment was never performed before at the beamline. Figure 1A shows a zoom-in image of the commissioned sample environment where, from right to left, are shown an on-axis optical microscope, a vertical sample stage with a CryoLoop™ (Hampton Research), and a narrow pipe housing an entrance window to a vacuum vessel. Behind the sample stage is shown a nozzle head for the cryogenic gas jet system. Cryogenic sample preparation of plunge-freezing cultured cells into liquid ethane was performed on-site to minimize the risk of sample damage during cryo-handling. A shaking incubator was provided by Max Planck Institute, Hamburg, and a plunge-freezer was available to us from the P06 beamline, for which we are very thankful. The experiment resulted in several high-quality cryo-XDM data in 2D (an example is shown in Fig. 1B). The spatial frequency in the measured data extended to high-resolution signals, corresponding down to 31 nm, which was limited by the detector size. Despite the high quality XDM data collected from frozen-hydrated D. radiodurans, the data analysis confirmed that the missing center area behind the beamstop was too large for a successful reconstruction. With the given pixel size (55 microns) of a MAXIPIX detector and the sample-to-detector distance of 5 meters, one can obtain diffraction patterns of ~3 oversampling ratio, at 8 keV x-ray photon energy, when the sample size is ~4 microns, a typical size of D. radiodurans. The missing data behind the beamstop in Fig. 1B covered an area of ~20 \times 23 pixels, from which, given the speckle size of D. radiodurans, the low spatial frequencies in the reconstruction were underconstrained, which presented difficulties in finding the
right support for a fidelity reconstruction. The commissioned cryo-XDM setup and our data analysis helped to improve the experimental conditions for the following, including those of other users at P10.

**December 8-13, 2011:** For the second beamtime in December, we provided a suitable beamstop, which reduced the missing region significantly, to an area of $10 \times 10$ pixels. The experiment was conducted at 7 keV to increase oversampling ratio of XDM data. However, this beamtime was particularly challenging due to the necessary commissioning of an in-house onaxis microscope with a limited field-of-view of a few-hundred microns. The setup required manual positioning of the frozen samples loops onto the stage with a precision of ~100 microns, which was often difficult to achieve with frozen-hydrated samples. Despite the difficulties, we collected several high-quality cryo-XDM data with a stereo pair as shown in Fig. 2A and 2B. For the given short time, we have reached a preliminary reconstruction that resembles a frozen-hydrated *D. radiodurans* in tetra configuration with further data analysis underway.

Figure 2. December 2011 experiment. (A, B) measured diffraction patterns of total 438x438 array with missing center 10x10 pixel area. 15 degree rotation from each other (C) preliminary reconstruction of *D. radiodurans* from data (B).

References