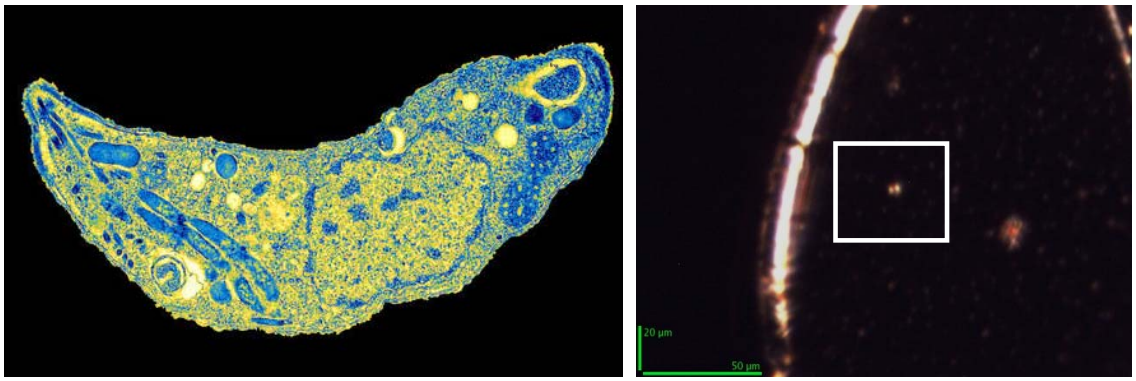
Experiment Report

Jose Rodriguez[1], Huaidong Jiang[2], Zhifeng Huang[1], Chien-Chun Chen[1], Daewoong Nam[3], Federico Zontone[4], Yuriy Chushkin[4], Changyong Song[3] & Jianwei Miao[1]
[1]*Department of Physics and Astronomy and the California NanoSystems Institute, University of California, Los Angeles, CA 90095, USA.*
[2]*State Key Laboratory of Crystal Materials, Shandong University, Jinan 250100, China.*
[3]*RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan.*
[4]*European Synchrotron Radiation Facility, Grenoble, France*

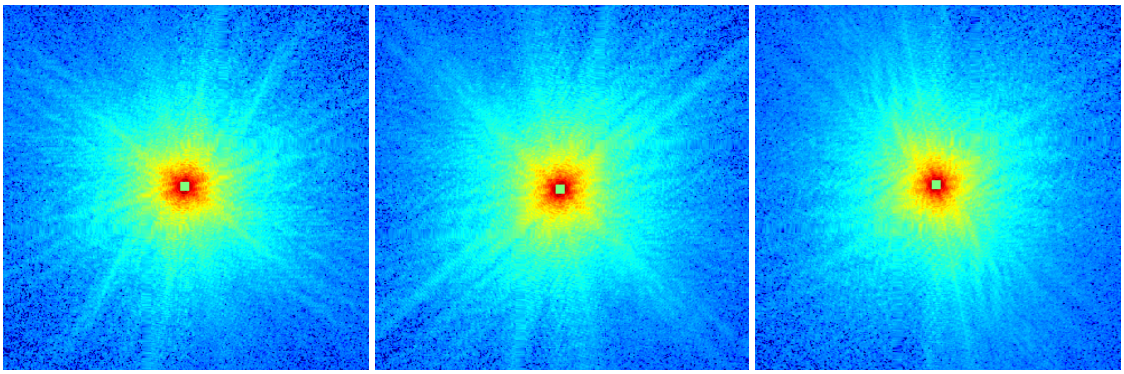
We were granted 18 shifts on beamline ID10C at the ESRF in June 2011. Our experiment focused on the imaging of a small non-pathogenic parasitic eukaryote, *Neospora Canninum*, cryogenically preserved in an aqueous state. Using the *N. canninum* cells we brought to the ESRF from our home institution, we prepared a limited number of samples. This was due to a limited number of available supplies for cryogenic sample preparation including the cryo-loops and magnetic bases used to hold whole intact cells suspended in vitreous ice. This was also due to the intrinsic limitations inherent to imaging live specimens, which must be kept alive outside their ideal environment, which could not be replicated on site. The cryo-CDI instrument at ID10C was well suited for obtaining diffraction patterns from cryo-preserved specimens, and with the addition of silicon-nitride corners, allowed us to obtain a clean background signal, several diffraction patterns of distinct *N. canninum* cells, and a tilt-angle series of 24 diffraction patterns of a pair of frozen-hydrated *N. canninum* cells. We have now preformed a preliminary three-dimensional reconstruction from the collected diffraction patterns that show the pair of cells. During our next experiment at the ESRF we intend to install additional corners to remove background scattering, and will bring our own cryogenic preservation and mounting supplies and so be able to prepare a larger number of frozen samples for use throughout the experiment.

Neospora canninum are single celled eukaryotic organisms closely related to the malaria causing *Plasmodium falciparum* and the causative agent of toxoplasmosis, *Toxoplasma gondii*. Besides not being pathogenic to humans, *N. canninum* are used ubiquitiously as model organisms for the study of malaria and toxoplasmosis given their similar mechanism of infection and cellular architecture. Being only two to three micrometers in length and one to two micrometers in diameter, *N. canninum* make ideal subjects for the investigation of sub-cellular structures. However, a high-resolution three dimensional structure is unattainable using conentional techniques. Such a three dimensional reconstruction of a frozen-hydrated *N. canninum* cell would provide the first visualization of the complex cellular architecture of a eukaryotic cell and of a model pathogenic organism, providing clues that could help further our understanding of parasitic infection in diseases such as malaria and toxoplasmosis.



(a) **(b)**

Figure 1. a, TEM image of a sectioned and fixed *Neospora canninum* cell. **b**, In-line optical microscope image of frozen-hydrated *N. canninum* cells mounted during an experiment on ID10C at the ESRF.



0° 14° 29°

Figure 2. A tilt series of 23 coherent X-ray diffraction patterns were acquired from frozen-hydrated *N. caninum* cells using a cryo X-ray diffraction microscope, mounted on ID10C at the ESRF. Three representative diffraction patterns at 0°, 14° and 29° are shown here.

To obtain a detailed three dimensional structure of a whole frozen-hydrated *N. canninum* cell, we harvested the parasitic organisms from infected host cells and purified them to obain a single cell suspension in the presence of cryoprotectant. Single cells and cell clusters were mounted on cryo-loops and plunge-frozen at the ESRF, then preserved at cryogenic temperatures in the beampath via a cold nitrogen stream. Visual inspection of the samples allowed for alignment of the *N. canninum* cells to the incident beam (Fig 1).
A pair of cells was chosen for a tilt-series data acquisition scheme according to the equally sloped requirement. In total, 23 coherent X-ray diffraction patterns were collected with a constant exposure time capitalyzing on the high dynamic range MaxiPix detector recently installed on ID10C (Fig 2). From these, a preliminary three dimensional reconstruction has been obtained, using the GHIO reconstruction algorithm and choosing the best reconstruction from 50 distinct random seeds after 2000 iterations (Fig 3). We will finalize the reconstruction prior to our next experiment at the ESRF. *As 3-D coherent diffraciton imaging of frozen hydrated biological specimens has not been demonstrated before, we anticipate this work will represent an experimental milestone in this fast-growing field..*

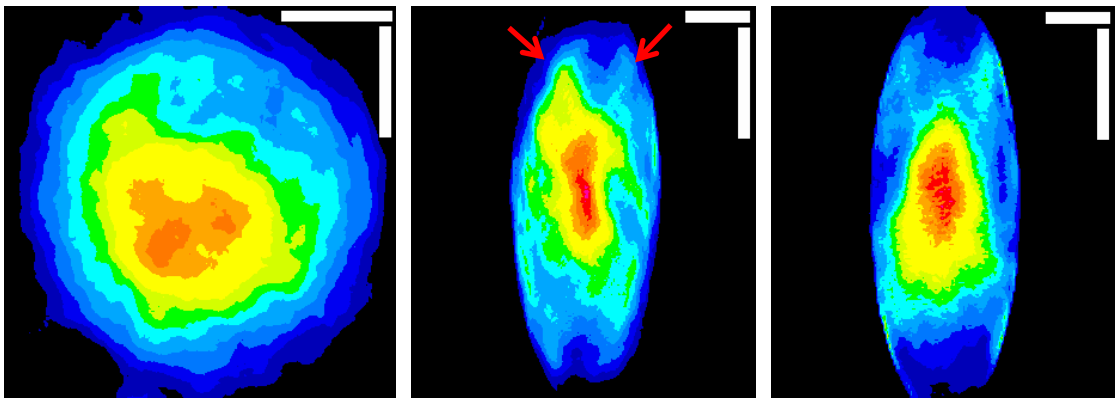


Figure 3. Preliminary 3D reconstruction of *N. canninum* from the measured coherent X-ray diffraction patterns. Two dimensional projections of the reconstruction are sown in the direction parallel to the beam (left), and both directions perpendicular to the beam (center, right). Red arrows denote the outlines of two cells. The different spatial resolution parallel and perpendicular to the beam direction is due to the limited number of projections. Scale bars are 2 μm.