3D Coherent Diffractive Imaging of Materials and Whole Cells

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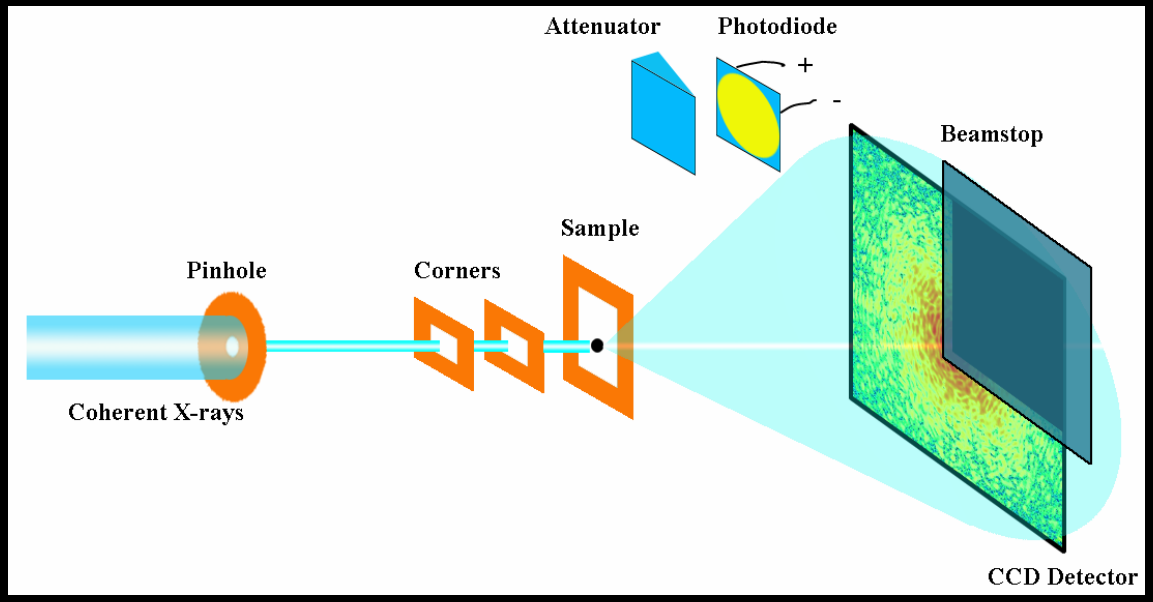
Outline

- ✓ 3D Coherent Diffractive Imaging at 3rd generation synchrotron facility

- ✓ Cryogenic Coherent Diffractive Imaging with hard X-rays
  sample preparation, experimental setup, data collection, and recent results

Coherent Diffractive Imaging (CDI)



$$\lambda = \frac{h \times c}{e \times V} \qquad \lambda = d \times 2 \times \sin(\theta)$$

- ✓ Resolution ~ maximum scattering angles measurable
- ✓ Insensitive to the displacement of sample
- ✓ Reduced radiation dose

BL29XUL at SPring-8

- ✓ RIKEN SPring-8 Center:

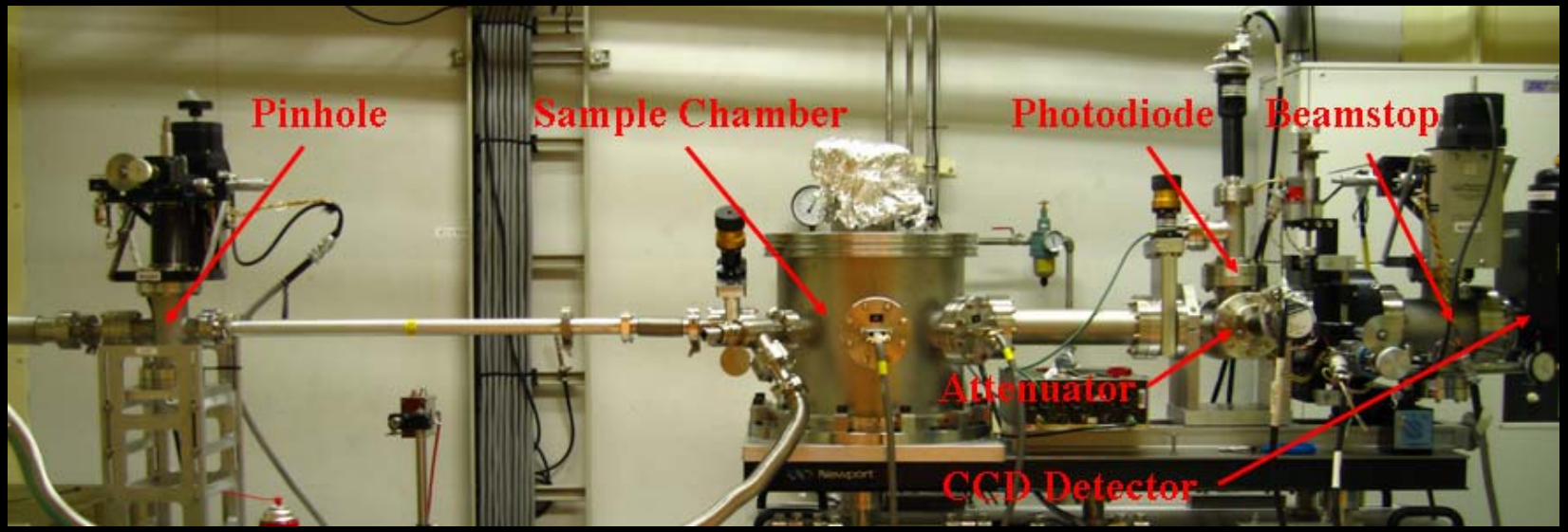
  Doctor Tetsuya Ishikawa
- ✓ Initiative Research Unit :

  Doctor Changyong Song
- ✓ BL29XUL:

  Doctor Yoshiki Komura

BL29XUL is an one-kilometer beamline with a long undulator (140 periods). Energy range from 4.9 to 18.7 keV by tuning the gap. A pair of platinum-coated vertical reflecting mirrors for higher harmonics reduction and vertical focusing. Photon flux is 6×10^{13} photon/s (@10 keV). The estimated photon flux at sample is ~1×10^{9} photon/s (@ 5 kev).

Coherent Diffractive Imaging (CDI) at SPring-8



Hard X-ray Beamline BL29XUL

X-ray Energy: 5 Kev (λ = 0.25nm)

Sample-Detector Distance: 1.3m

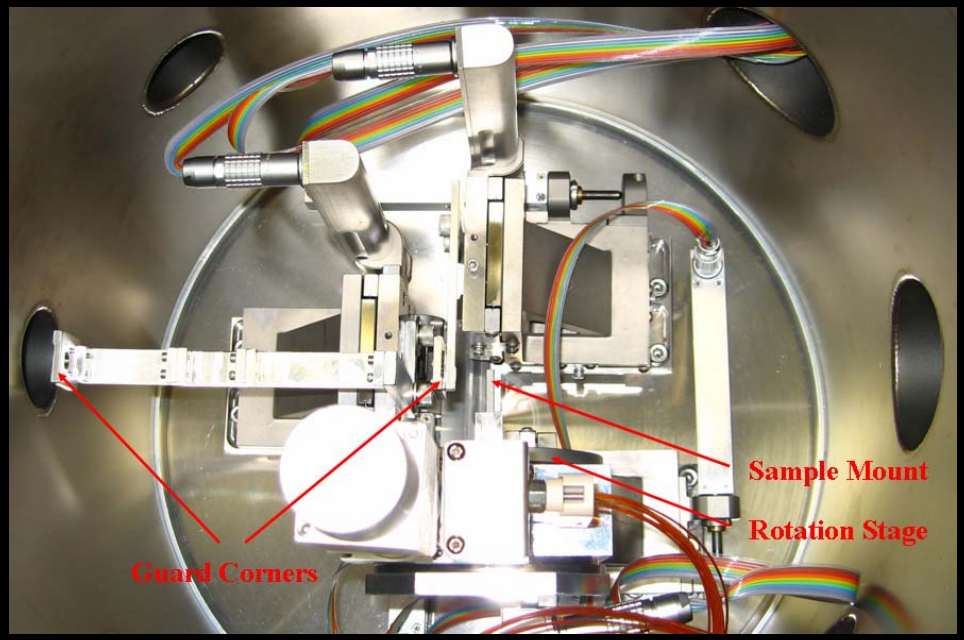
CCD Detector:

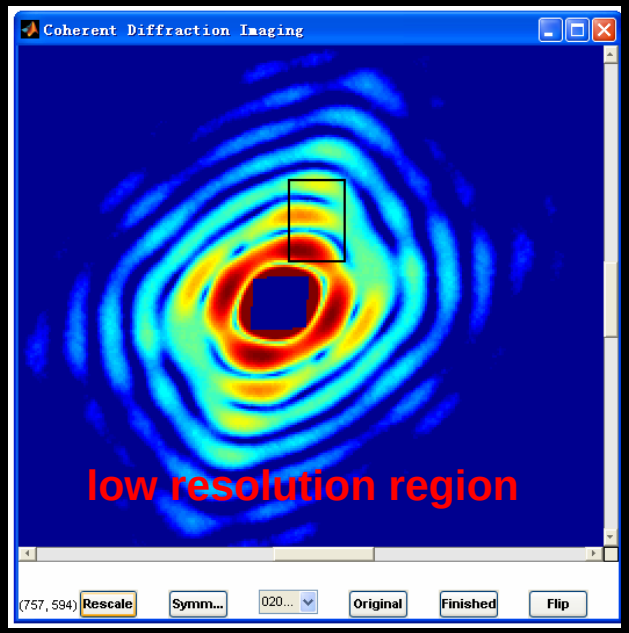
Princeton Instruments PI-LCX1300

1300×1340 pixels

Pixel size: 20um×20um

Coherent Diffractive Imaging (CDI) at SPring-8





In-vacuum piezo-actuator coupled motion stages (Newport CMA-25), controlled by a program written by LabView

The collected diffraction signal, after analysis by a program written by Matlab

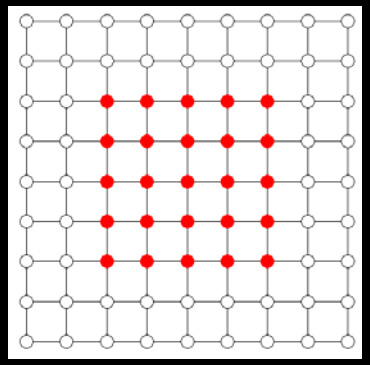
R. Xu, S. Salha, K. S. Raines, H. Jiang, C.-C. Chen, Y. Takahashi, Y. Kohmura, Y. Nishino, C. Song, T. Ishikawa, J. Miao, *J. Synch. Rad.* **18**, 293-298 (2011).

Oversampling Phasing Method

finite

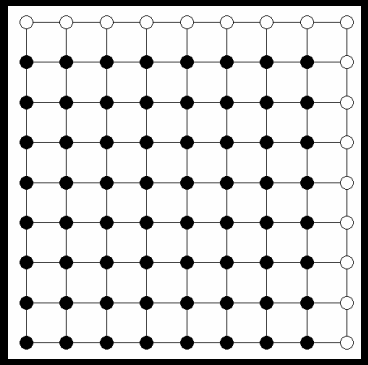
non-crystalline

sample



FT →

← **IFT**



oversampled

diffraction

intensity

oversampling is special attribute associated with Fourier transform

$$\begin{bmatrix} 0 \\ \cdot \\ \cdot \\ x[i] \\ \cdot \\ \cdot \\ 0 \end{bmatrix} \times \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & \cdot & \cdot & 1 \\ 1 & W & W^2 & W^3 & W^4 & \cdot & \cdot & W^{N-1} \\ 1 & W^2 & W^4 & W^6 & W^8 & \cdot & \cdot & W^{N-2} \\ 1 & W^3 & W^6 & W^9 & W^{12} & \cdot & \cdot & W^{N-3} \\ \cdot & & & & & & & \\ \cdot & & & & & & & \\ 1 & W^{N-1} & W^{N-2} & W^{N-3} & W^{N-4} & \cdot & \cdot & W \end{bmatrix} = \begin{bmatrix} f[0] \\ f[1] \\ f[3] \\ f[4] \\ \cdot \\ \cdot \\ f[N-1] \end{bmatrix}$$

forward model

More oversampling of the diffraction pattern, larger non-density region, meanwhile the number of unknowns remains the same. That is to say, if the forward model is Fourier transform, oversampling in reciprocal space corresponds to zero padding in real space

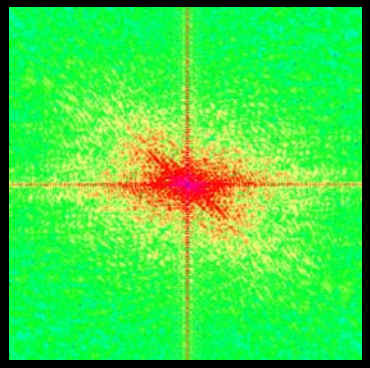
2D Phase Retrieval Algorithm

Constraint A

(Projection A)

FT →

← IFT

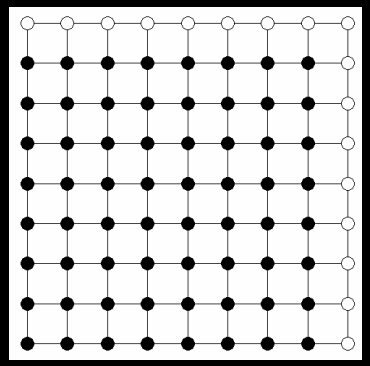


Constraint B

(Projection B)

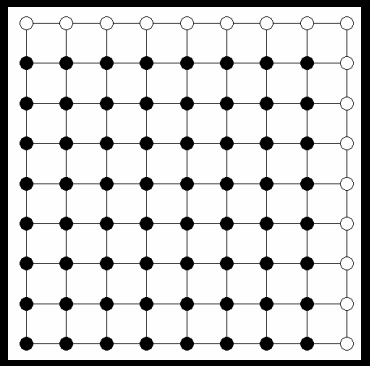
A mapping from Cartesian grid to Cartesian grid

(special properties: fast,unitary,isometric, that facilitate algorithm)



FT →

← IFT



Algorithms: ER, HIO, GHIO, DM, RAAR, ASR, HPR …

3D Reconstruction Method

Data acquisition:

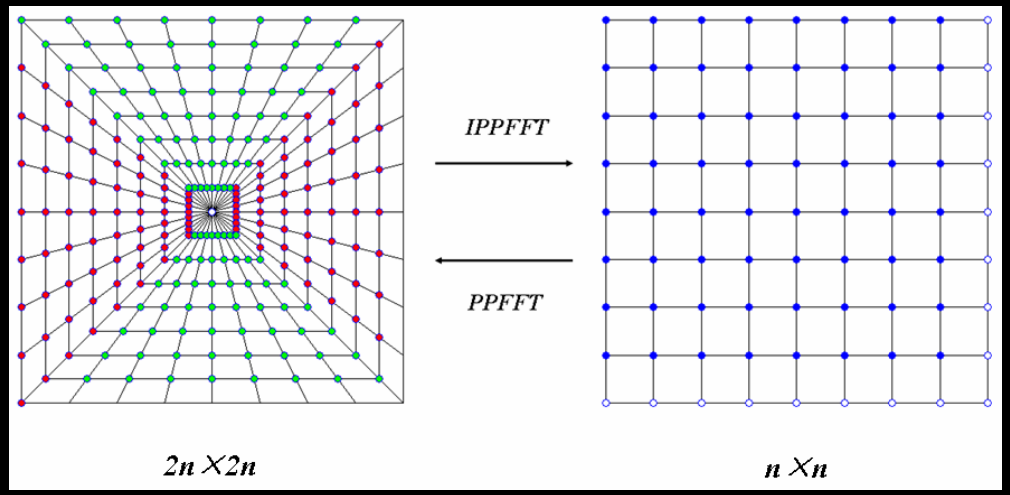
a tilt series of 2D diffraction patterns

Reconstruction approach:

- ✓ Independent 2D phase retrieval, consequently perform the tomographic reconstruction.

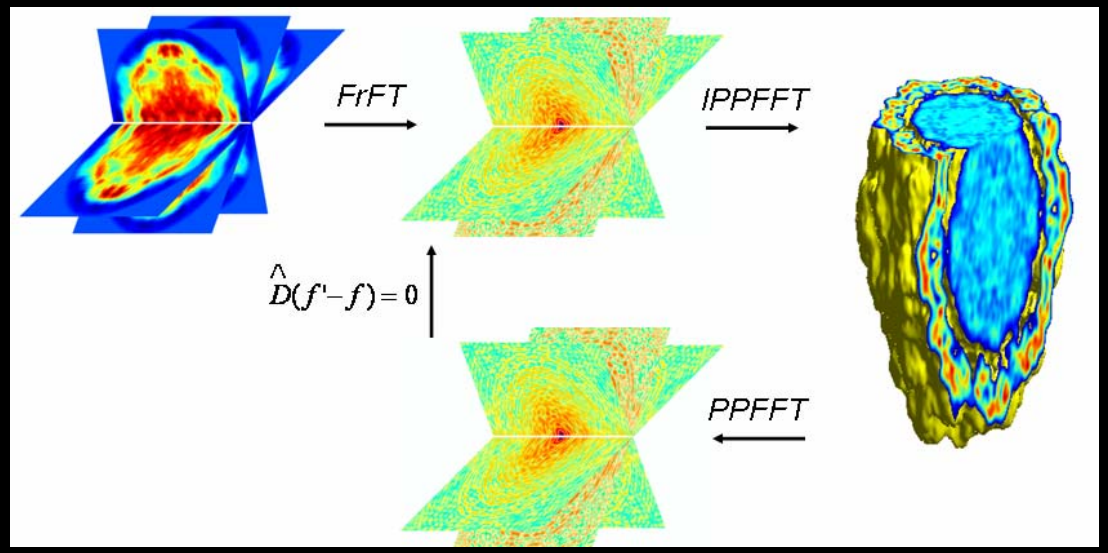
- ✓ Assemble 3D diffraction ensemble from the obtained series of 2D diffraction patterns, consequently perform 3D phase retrieval.

Pseudo-Polar Fourier Transform



A mapping between Pseudo-Polar grid and Cartesian Grid, which is rapidly computible(o(NlogN)), algebraically exact, geometrically faithful and invertiable.

Equally-Sloped Tomography (EST)



Inputs are parallel projections (Projection Slice Theorem holds). The algorithm iterates back and forth between Fourier and object domain.

Miao et al, Phy. Rev. B 72, 052103 (2005)

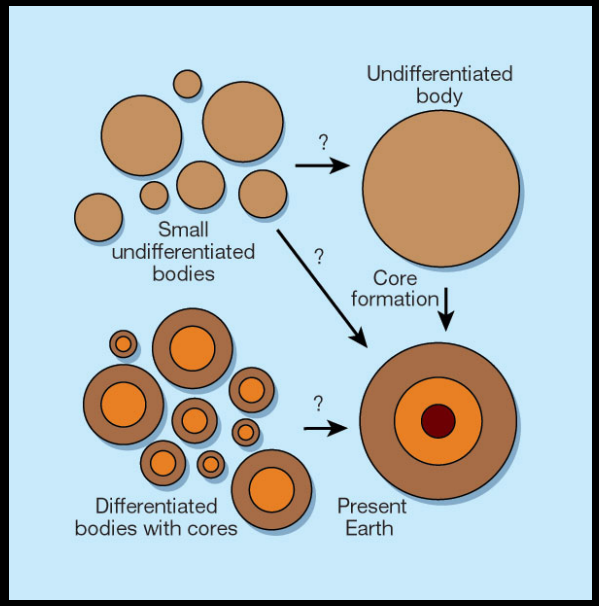
Equally-Sloped Tomography (EST)

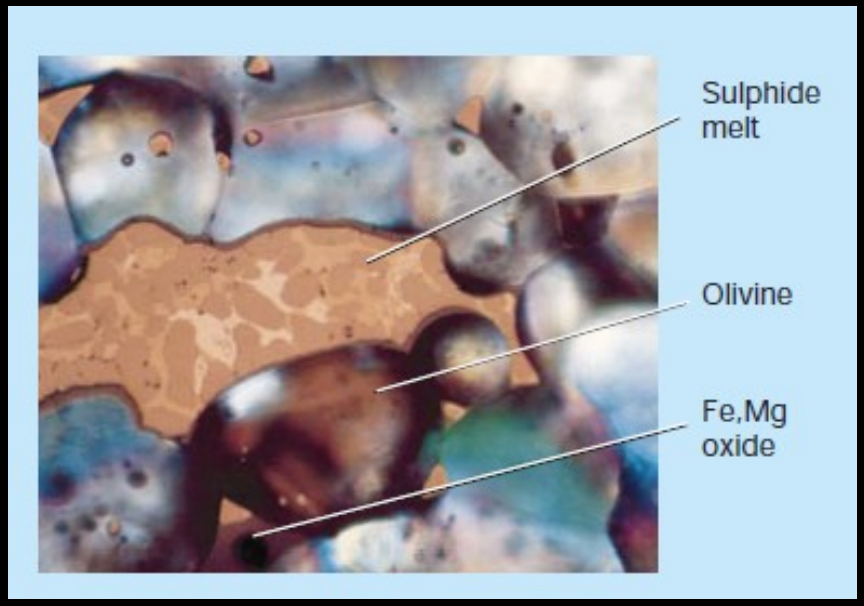
- ✓ To perform tomographic reconstruction from a limited number of noisy projections in an accurate manner by avoiding direct interpolation.

- ✓ Allow for the implementation of the mathematical regularization schemes, such as total variation (TV) constraints, and the incorporation of various physical constraints, such as finite support, non-negativity

- ✓ Applicable to other imaging modalities to obtain radiation dose reduction and image enhancement

3D Imaging of Materials Samples

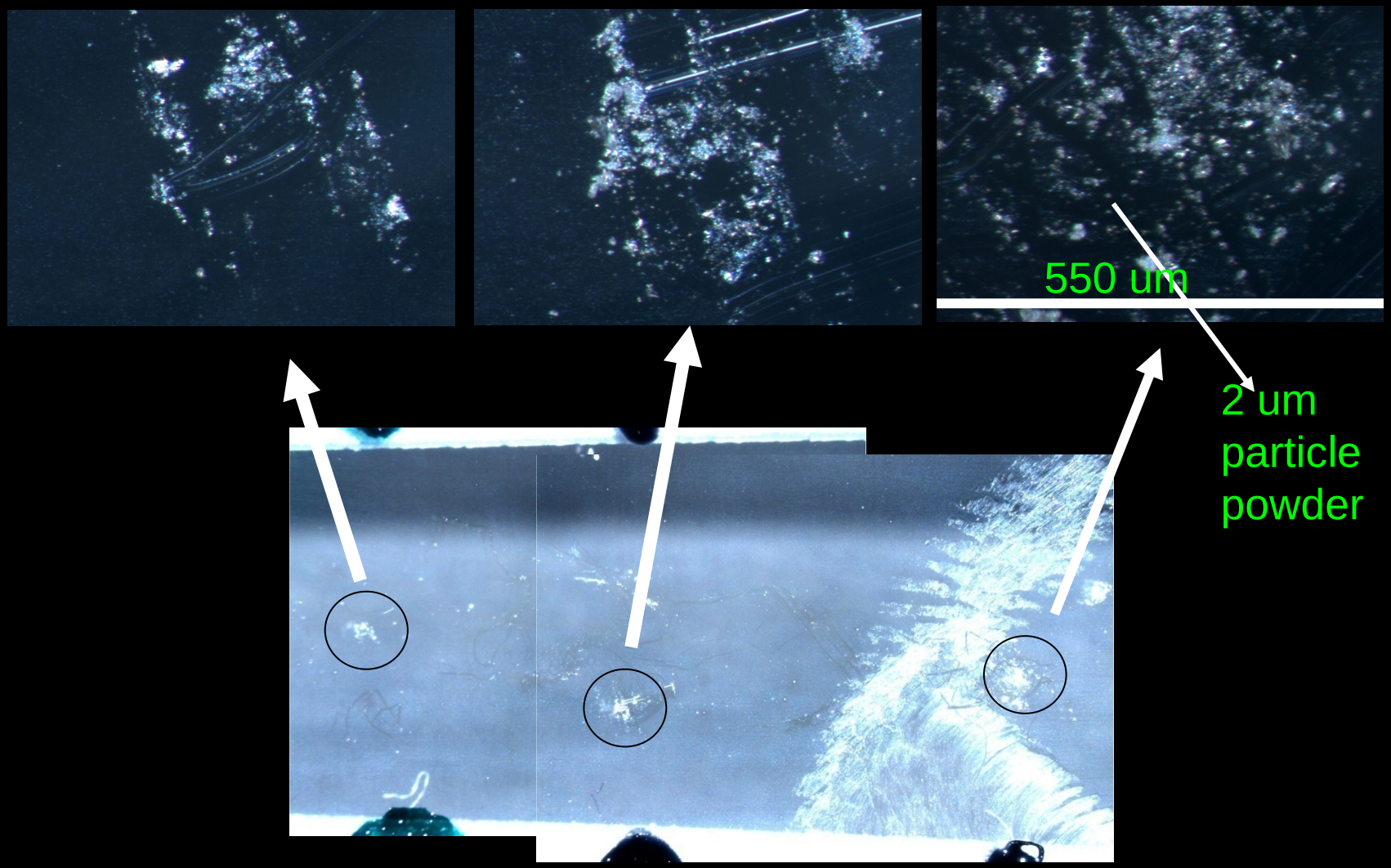




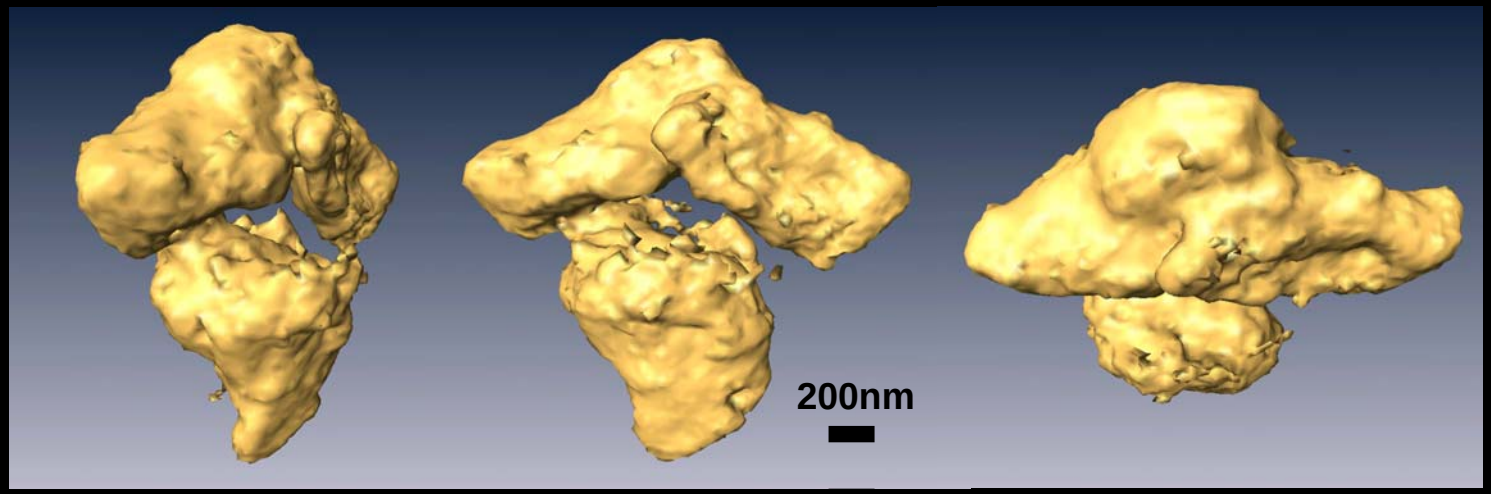
To visualize the three-dimensional structure of the materials under extreme environments.

To understand the properties of the materials and the process of forming.

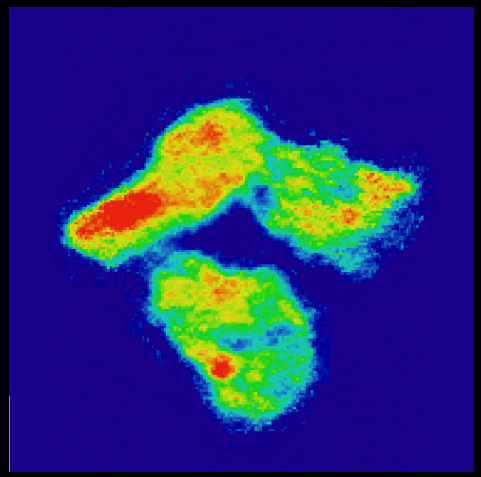
Sample: San Carlos + 10wt% Fe-S, 6GPa and 1800 C for 1 hour



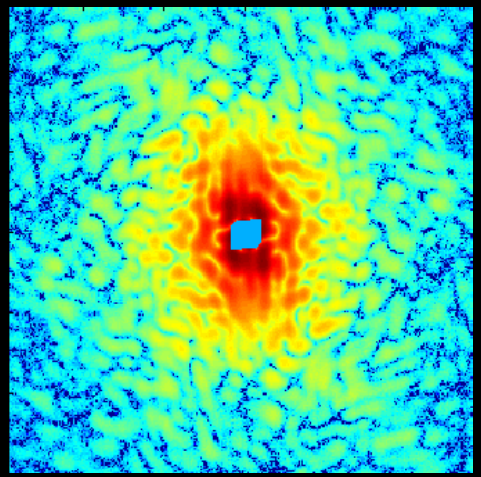
3D Imaging of Materials Samples



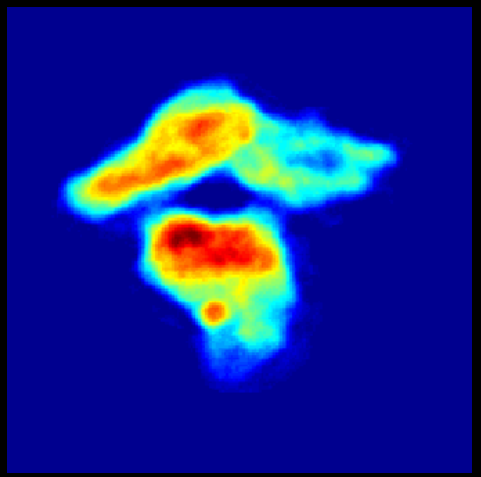
Iso-surface



Central slice



Pattern at 0 deg

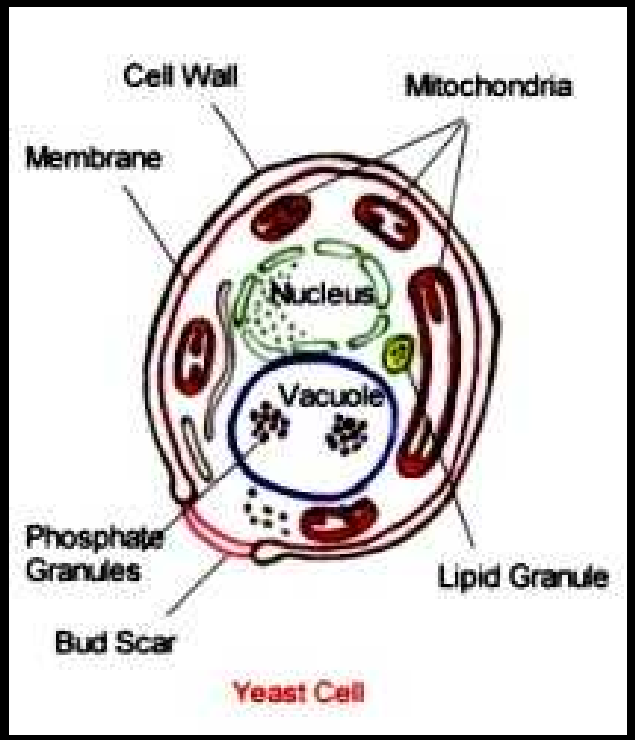


Projection at 0 deg.

3D Imaging of Whole Cells

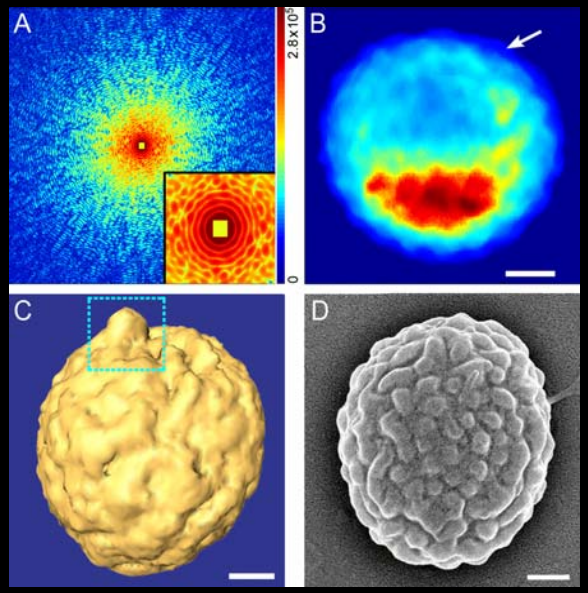
To visualize the three-dimensional structure and interaction of sub-cellular organelles and macromolecular complexes within an micron-size whole cell.

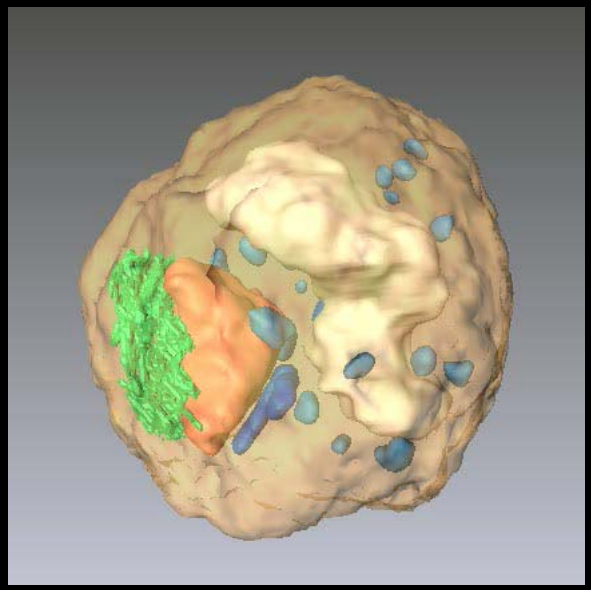
To understand of cellular processes, such as cell division and physiology.



3D Imaging of Whole Cells

3D architecture of a yeast spore cell by CDI that are too thick for EM.





H. Jiang, C. Song, C.-C. Chen, R. Xu, K. S. Raines, B. P. Fahimian, C.-H.Lu, T. K. Lee, A. Nakashima, J. Urano, T. Ishikawa, F. Tamanoi, and J. Miao, *Proc. Natl. Acad. Sci. USA* **107**, 11234-11239 (2010).

3D Coherent Diffractive Imaging of Frozen-Hydrated Biological Samples

X-ray imaging of whole cells offers:

Long penetration length, valuable for imaging of whole cells (a few micrometers thick).

Promises high resolution (~10nm), valuable for the identification and quantitative analysis of structural features within a cell, such as organelles.

Toward 3D Coherent Diffractive Imaging of Frozen-Hydrated Biological Samples

J. Miao et al, *Proc. Natl. Acad. Sci*. (2003)

Shapiro et al, *Proc. Natl. Acad. Sci*. (2005)

Jiang et al, *Phys. Rev. Lett.* (2008)

Song et al, *Phys. Rev. Lett.* (2008)

Nishino et al, *Phys. Rev. Lett.* (2009)

Lima et al, *Phys. Rev. Lett.* (2009)

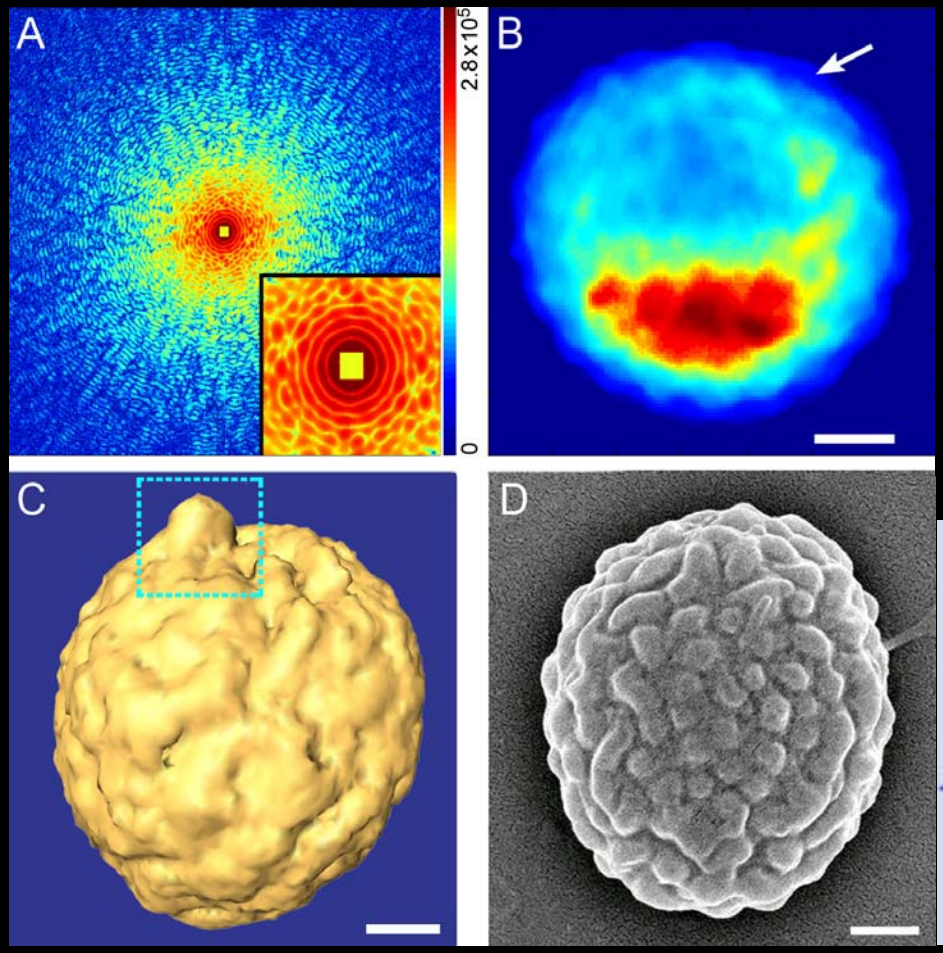
3D Coherent Diffractive Imaging of Frozen-Hydrated Biological Samples

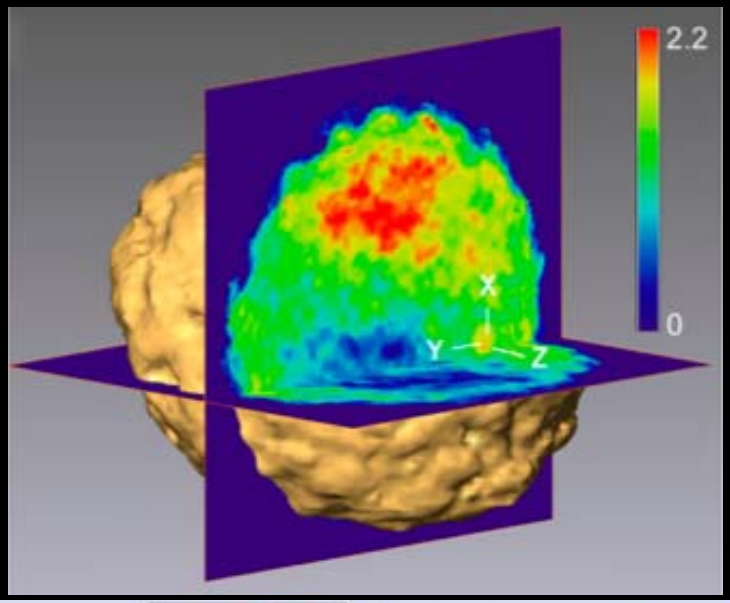
Stability of biological samples in 3D CDI is limited by x-ray radiation damage.

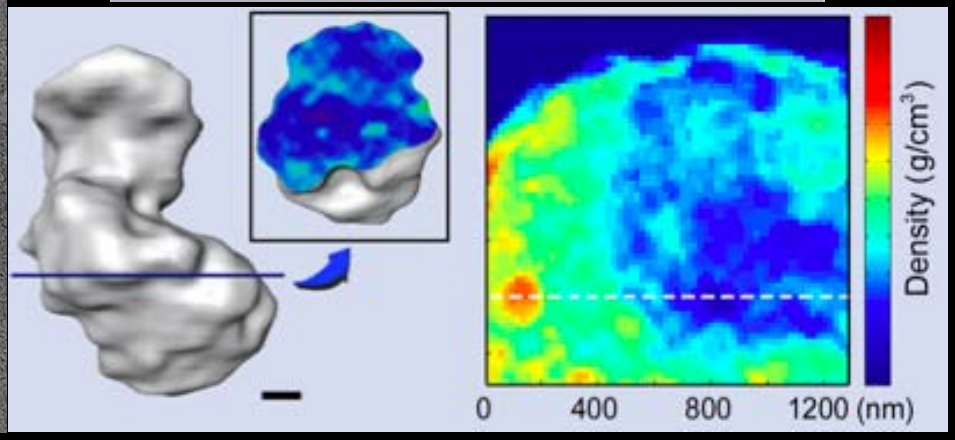
Substantial improvement in lifetime of samples under study can be achieved by cryogenic freezing: frozen-hydrated samples.

Samples studied using 3D CDI:
Dried Yeast Spore cell (Spring-8)
Frozen-hydrated Neospora caninum (ESRF)

3D Imaging of Dried Yeast Spore Cells





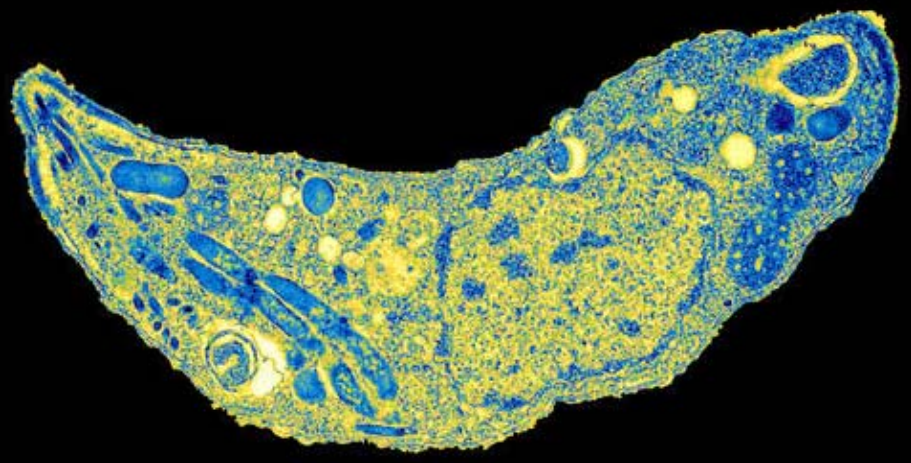


Jiang *et al*., *PNAS* **107**, 11234 (2010).

Toward a 3D structure of Frozen-Hydrated N. caninum

Sample and approach:

Neurospora caninum cells extracted from their host, Vero cells.



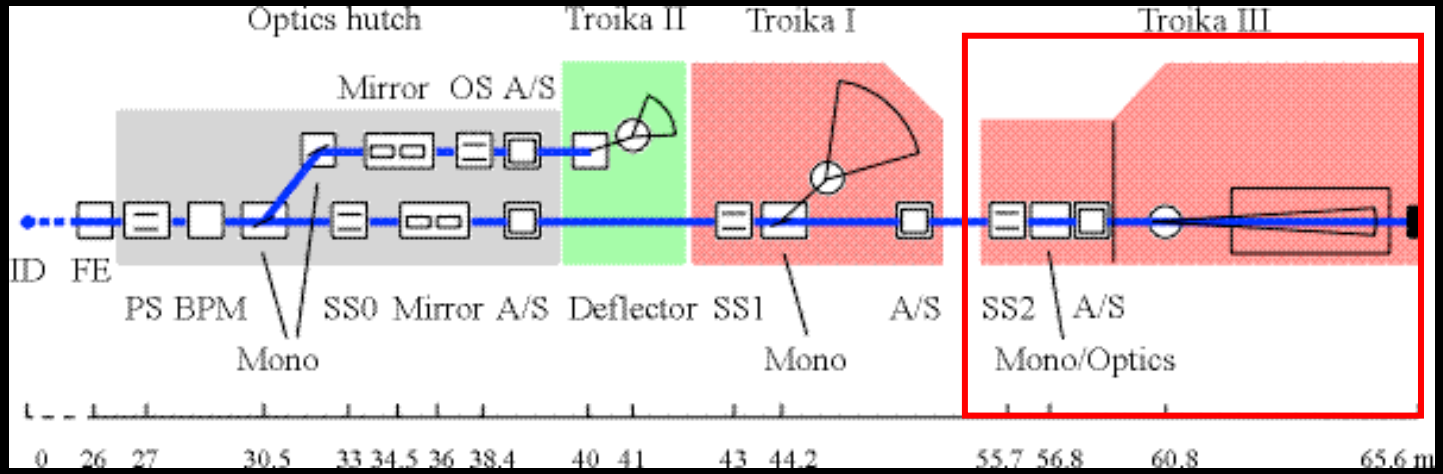
2x3 µm in size, wedge shaped parasites relatives of Toxoplasma.

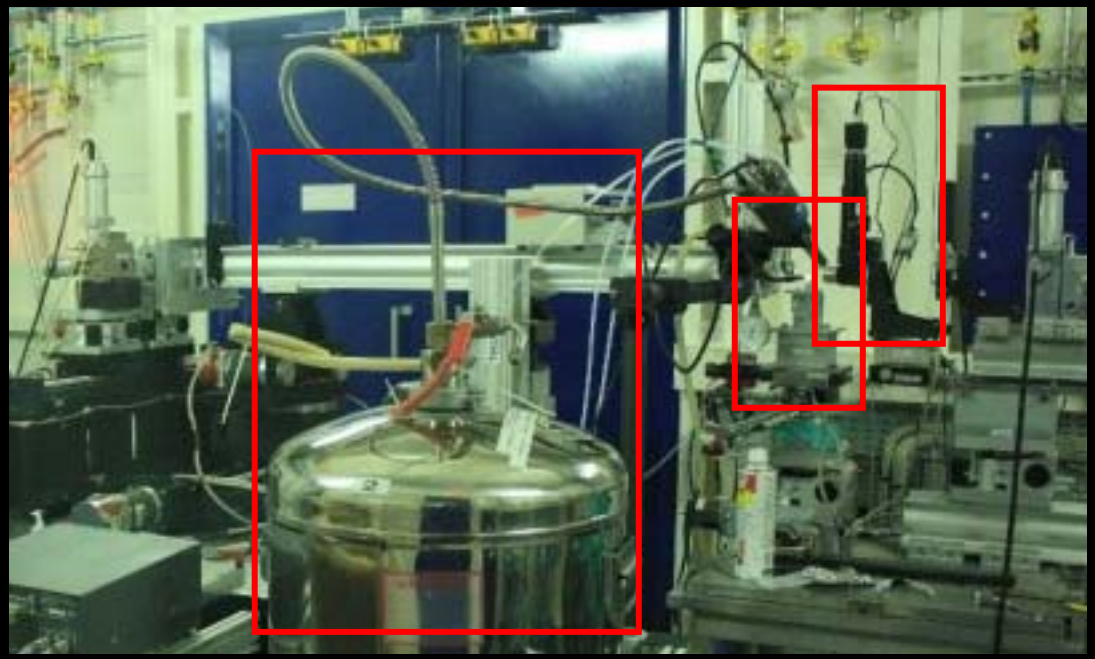
Live cells were extracted from parasitoporous vacuoles and plunge-frozen in cryo-protectant, on site.

Evaluated by in-line optical microscope.

Coherent Diffractive Imaging of Frozen-Hydrated B-cells

Experimental hutch setup at ID10C in ESRF.

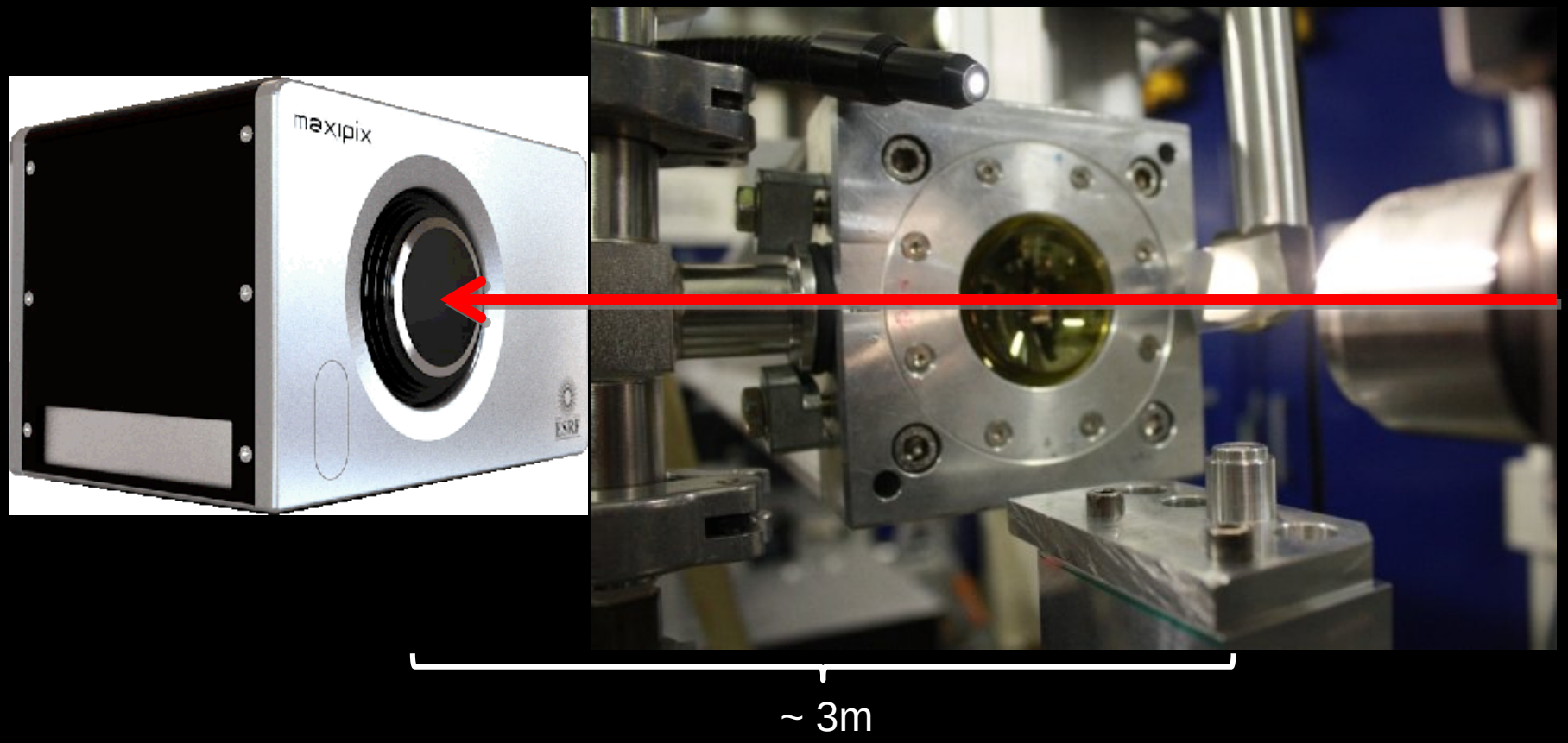




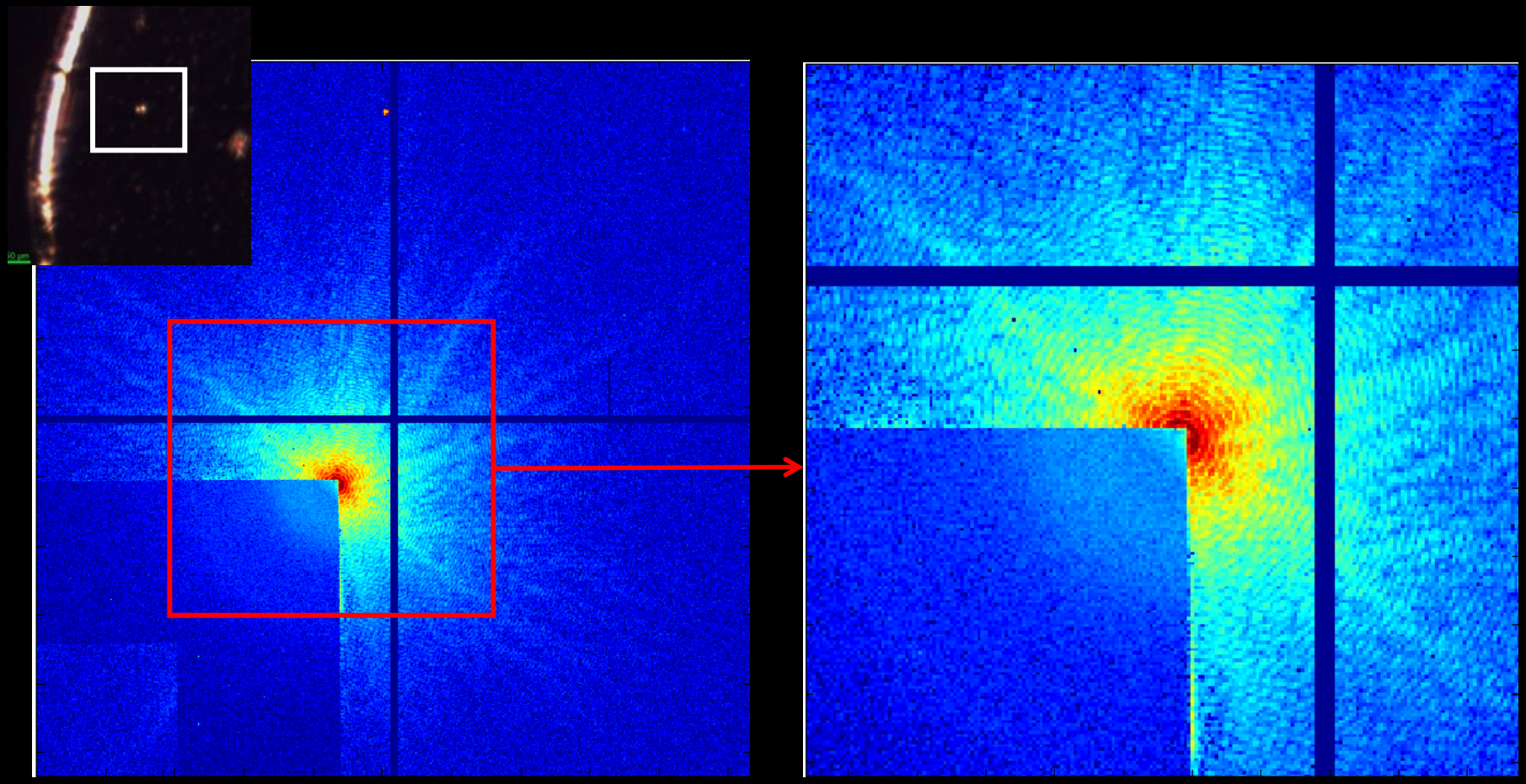
Equipped with an LN2 stream for sample cryo-protection, an in-air stage for sample manipulation, and an in-line microscope.

Coherent Diffractive Imaging of Frozen-Hydrated B-cells

Experimental hutch setup at ID10C in ESRF.
MaxiPix pixel array detector: 1400 Hz frame rate (512x512 pix), 290 microseconds readout delay, single photon-counting detection, and a pixel size of 55x55 μm^2



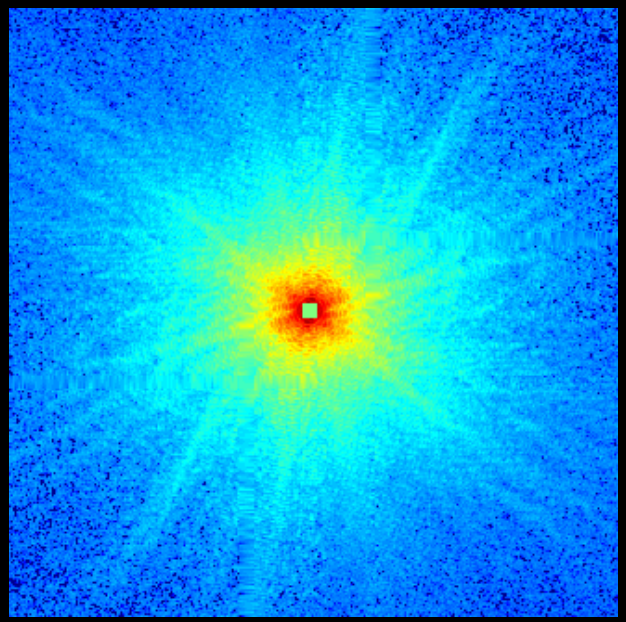
Toward a 3D structure of Frozen-Hydrated N. caninum



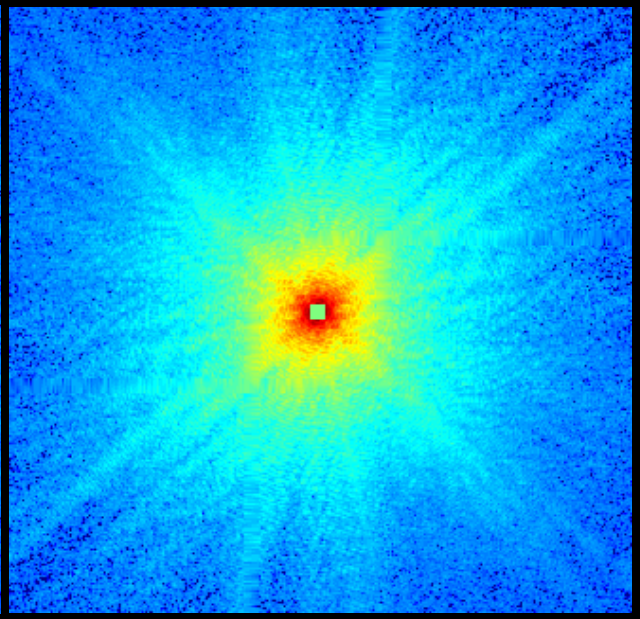
Diffraction pattern at 0 degrees

Diffraction pattern shown prior to background subtraction and symmetrization, taken before the start of the tomographic data acquisition scheme.

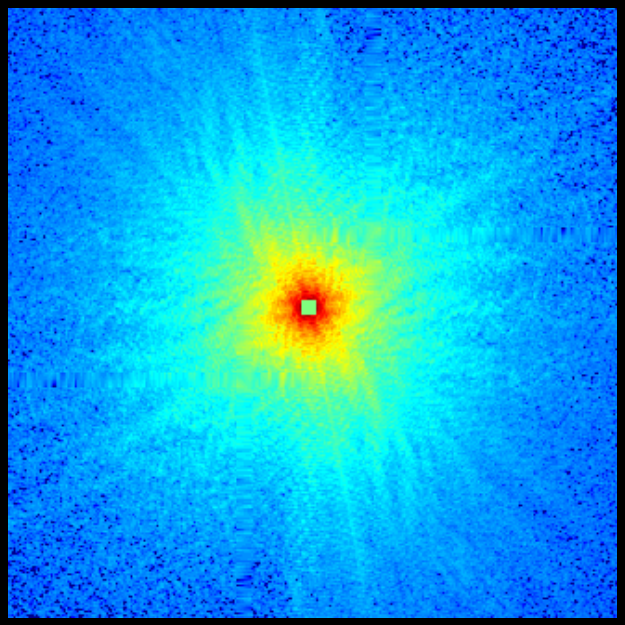
Toward a 3D structure of Frozen-Hydrated N. caninum



Diffraction at 0 deg.



Diffraction at 14 deg.



Diffraction at 29 deg.

Diffraction patterns shown after air background subtraction and symmetrization.

23 angles collected total:
Ranging from -36 to 29 degrees, in approximately 3 degree increments according to an equal sloped tomography collection scheme.

Summary

The three-dimensional reconstruction of a dry yeast spore cell, and the diffraction patterns obtained of frozen-hydrated Neospora caninum cells demonstrate the feasibility of 3D-CDI of whole cells.

We expect cryogenic techniques to facilitate the reconstruction of a 3D structure of intact Neospora caninum cells in the near future.

Perspectives I

- Three dimensional structural determination of whole cells using CDI is attractive, but poses a number of challenges.

- The total radiation dose is at least an order of magnitude higher than the doses that samples were exposed to for 2d.

- Frozen-hydrated samples are more resistant to radiation damage, but are embedded in a layer of ice which is a challenge for background subtraction and can reduce signal to noise ratio of collected diffraction patterns.

Perspectives II

- Improvements in instrumentation at ESRF are facilitating the routine acquisition of CDI data from frozen-hydrated cells:

- The use of a pixel-array detector allows for a single pattern to be acquired per angle (given the wide dynamic range and photon counting properties of the detector). This reduces radiation damage on the sample and the overall time required for the experiment.

- Currently, a full 3D data set can take several days to obtain

The stability of the sample and the beam must be guaranteed.

- Automated approaches to data acquisition, and sample manipulation should lessen the burden on the users and beamline scientists during 3D data collection schemes.

Thank You.

UCLA Coherent Imaging Group

Group leader: Jianwei Miao

Group members: Jose Rodriguez, Mary Scott, Rui Xu, Janice Zhao, Zhifeng Huang, Lu Rong, Chun Zhu, Chien-Chun Chen, Sara Salha, Thomas Darlington, James Kelvin.

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Riken Spring-8 Collaborators:

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Argonne National Laboratory:

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