BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Enju Lima

eRA COMMONS USER NAME (credential, e.g., agency login): ENJU_LIMA

POSITION TITLE: Cryo-EM Facility Manager

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Kyungpook National University, South Korea	BS	02/1990	Nursing
City University of New York	BA	05/1999	Physics
Stony Brook University, New York	PhD	08/2006	Physics
European Synchrotron Radiation Facility, France	Postdoc	08/2008	Physics

A. Personal Statement

Prior to becoming the Cryo-EM facility manager at Stony Brook University, my research focused on extending x-ray crystallography to non-crystalline biological samples, such as whole cells or tissue sections [1 - 3]. The research was driven by the need for a new imaging methodology to probe in high resolution, thick samples as a whole that are not currently accessible by electron microscopy or light microscopy [4]. My graduate work was part of a group developing cryo X-ray Diffraction Microscopy (XDM) with soft x-rays at the water window. As a postdoctoral fellow, I had applied the method to hard x-rays and demonstrated its first feasibility [1]. Later at Brookhaven National Lab, I investigated high-pressure cryopreservation to overcome the phasing difficulty in reaching high-throughput capability [2]. To expand its applicability to larger samples, I demonstrated, through collaboration, the feasibility of cryo-XDM in scanning mode to image cryopreserved specimens of tens-of-microns in size [3].

At my current institution as a Cryo-EM facility manager, I bring my extensive research experience in instrumentation, cryo-sample preparation and computational image analysis to assist facility users with their projects of single particle cryo-EM reconstruction of proteins and macromolecules.

1. Lima E, Wiegart L, Pernot P, Howells M, Timmins J, Zontone F, Madsen A. (2009) Cryogenic X-ray diffraction microscopy for biological samples. *Phys Rev Lett.* 103, 198102.
2. Lima E, Diaz A, Guizar-Sicairos M, Gorelick S, Pernot P, Schleier T, Menzel A. (2013) Cryo-scanning x-ray diffraction microscopy of frozen-hydrated yeast. *J Microscopy*. 249, 1-7.
3. Lima E, Chushkin Y, van der Linden P, Kim CU, Zontone F, Carpentier P, Gruner SM, Pernot P. Cryogenic x-ray diffraction microscopy utilizing high-pressure cryopreservation. *(*2014) *Phys Rev E* 90, 042713.
4. Shapiro D, Thibault P, Beetz T, Elser V, Howells M, Jacobsen C, Kirz J, Lima E, Miao H, Neiman AM, Sayre D. (2005) Biological imaging by soft x-ray diffraction microscopy. *Proc Natl Acad Sci* 102, 15343-6.

B. Positions, Scientific Appointments, and Honors

Employment

1990 – 1991	Registered Nurse, Kyungpook National University Hospital, South Korea
1992 – 1999	Registered Nurse, Mount Sinai Hospital, New York
1999 – 2006	Teaching/Research Assistant, Department of Physics, Stony Brook University, New York
2006 – 2008	Post-doctoral Scientist, European Synchrotron Radiation Facility, France
2008 – 2010	Assistant Physicist, Brookhaven National Laboratory, New York
2010 – 2013	Associate Physicist, Brookhaven National Laboratory, New York
2022 – current	Cryo-EM Facility Manager, Stony Brook University, New York

Honors

1999	Thomas W. Smith Fellowship, CUNY
2006	Gertrude Scharff-Goldhaber Prize, Brookhaven National Laboratory

C. Contributions to Science

1. Demonstration of cryo XDM at hard x-rays: At ESRF, we studied the feasibility of cryo XDM at hard x-rays and showed the first demonstration by imaging *D. radiodurans* bacteria using 8 keV x-rays. Cryo sample handling can be cumbersome and carries the risk of crystalline ice contamination. To overcome this challenge, we developed a non-vacuum cryo sample stage utilizing a commercial cryogenic gas stream to measure coherent x-ray diffraction from cryo specimens. Employing conventional protein crystallography sample holders, this system allowed x-ray powder diffraction measurement to ensure vitreous ice conditions prior to imaging and facilitated cryo sample transfers with fast turnovers in the search for quality diffraction data. Together this led to the first frozen-hydrated image at hard x-rays of unstained *D. radiodurans* bacteria reaching a spatial resolution of 30 to 50 nm.

a) Lima E, Wiegart L, Pernot P, Howells M, Timmins J, Zontone F, Madsen A. Cryogenic X-ray diffraction microscopy for biological samples. (2009) *Phys Rev Lett*. 103, 198102.

2. Cryo XDM utilizing high-pressure cryopreservation: Although the feasibility of cryo XDM was demonstrated, the low phasing convergence with cryo specimens persisted, even as numerous diffraction data from the specimens were obtained. This brought into question the initial specimen condition derived from the ambient pressure cryopreservation, a routine procedure in cryo EM with sub-micrometer-size specimens. With LDRD funding from Brookhaven National Laboratory and through collaborations, we employed high-pressure cryopreservation at around 200 MPa to improve the specimen condition. In our study, we compared high-pressure (HP) and ambient-pressure (AP) cryo specimens, across both reconstructions and small angle x-ray scatterings. Although specimens from both methods produced diffraction data of similar quality in both speckle visibility and general ice condition from x-ray powder diffraction, the final results showed a correlation between local ice conditions and imaging convergence. HP cryo specimens yielded three reconstructions including a 15 degree rotation image of a sample and, at the same time, x-ray scatterings from the local ice substrate around the samples were measured low. However, AP cryo specimens continued to produce no images while presenting higher ice signals. Although statistics on phasing performance could not be obtained with the limited beamtime, our finding suggests that the poor quality of the local ice substrate is the possible cause of the earlier phasing difficulty. This is partly due to the fact that oversampling of diffraction data cannot be sufficiently met if the presence of ice crystals in the substrate is not negligible, even when a large portion of the ice substrate measures as vitreous.

a) Lima E, Chushkin Y, van der Linden P, Kim CU, Zontone F, Carpentier P, Gruner SM, Pernot P. (2014) Cryogenic x-ray diffraction microscopy utilizing high-pressure cryopreservation. *Phys Rev E*. 4, 042713.

3. Extension of cryo XDM to extended specimens: The field-of-view set by currently available x-ray detectors allows small cells of eukaryotes or bacteria to be applicable in XDM. Ptychography, a scanning mode in XDM,

provides an extended field-of-view for larger specimens, while presenting improved phasing convergence from overlapped illumination. In collaboration with Swiss Light Source, we combined cryo XDM at hard x-rays with ptychography and demonstrated cryo-scanning XDM for imaging wet, extended biological samples. To overcome the instability of commercial cryogenic gas streams in this feasibility experiment, we averaged reconstructions from multiple short-term measurements and achieved a spatial resolution of 85 nm with a phase sensitivity of 0.0053 radians in imaging a cluster of yeast cells. Compared to earlier cryo XDM, cryo-scanning XDM showed markedly improved phasing convergence, while the observed instability of the cryogenic gas stream during scanning limited the current resolution. A significant improvement is expected with an in-vacuum cryo-scanning system.

a) Lima E, Diaz A, Guizar-Sicairos M, Gorelick S, Pernot P, Schleier T, Menzel A. (2013) Cryo-scanning x-ray diffraction microscopy of frozen-hydrated yeast. *J Microscopy* 249, 1-7.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1HCf6YPdG8iwz-/bibliography/public/