

**PRINCETON–NATURE
CONFERENCE: 2nd
FRONTIERS IN
ELECTRON
MICROSCOPY FOR THE
PHYSICAL AND LIFE
SCIENCES**

**Princeton University
Maeder Hall, Andlinger Center
86 Olden Street
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September 28-30, 2022

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*Cover image: Electron diffraction pattern of first nature
Quasicrystal identified at Princeton University.*

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General Information

Conference Badges

Please wear your name badge at all times.

Location

The conference will take place on the Princeton University campus. Scientific sessions and Vendor displays will take place in the A Level of Maeder Hall on the corner of Olden Street and Prospect Avenue. The Welcome Reception, Poster Session and lunches will take place in the Friend Center Convocation Room, corner of Olden and Williams Street.

Scientific Session Protocol

Photography, audio or video recording of the scientific sessions are *not permitted*. Our Twitter, blogging and social media policy: publicly sharing information presented in any of our talks is permitted unless a speaker opts out. They will be asked to do this at the start of their talk or in advance of segments of their talk that they wish to remain confidential.

Abstracts may not be re-printed without permission of the presenter. Poster presentations are considered personal communications, and information in these may not be publicly shared without prior permission of the presenter.

Remember to turn off all cell phones or switch to vibrate during the sessions and step outside if you need to take calls.

Internet Access

Free Wifi is available across the Princeton University Campus. Select **puvisitor** in your network settings. A password is not required.

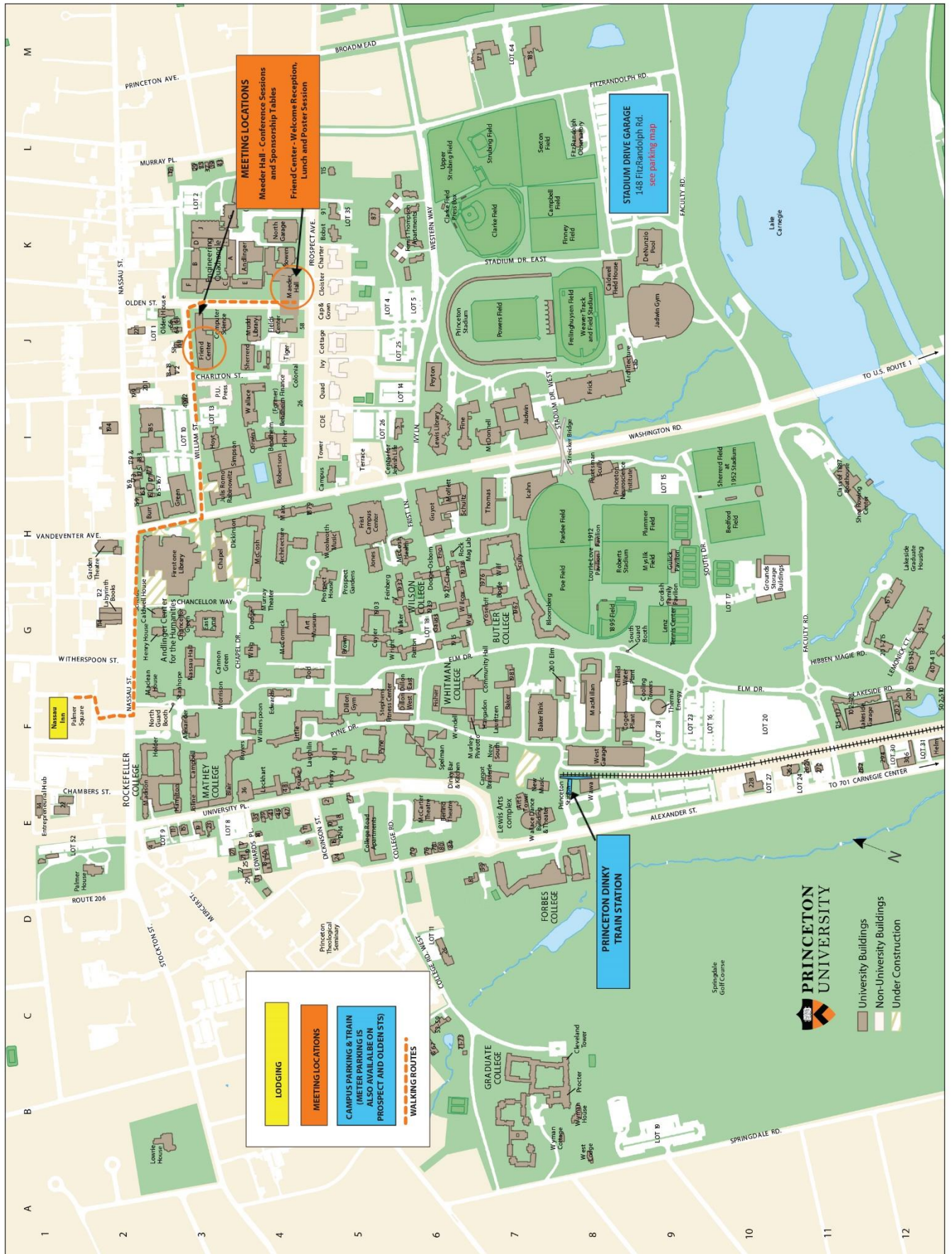
Conference Meals and Social Events

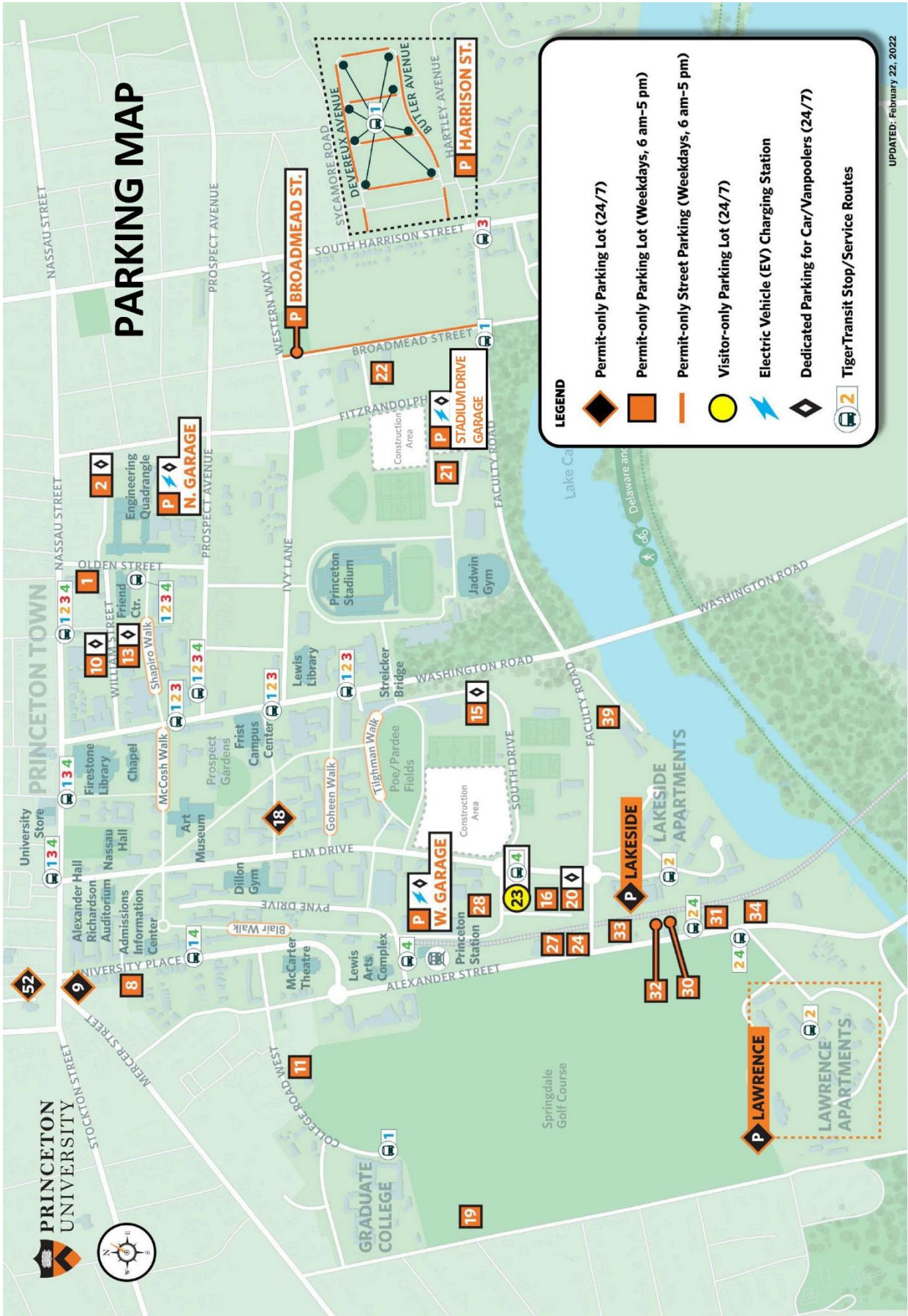
The conference will serve continental breakfast, coffee breaks and lunches. The welcome reception on September 28th and the Poster Session with refreshments on September 29th will take place in the Friend Center Convocation Room.

Poster Session

The Poster Session will take place on September 29th in the Friend Center Convocation Room. Board number assignments appear in the Poster Abstract List at the end of this book.

Posters can be displayed at the Friend Center Convocation Room from 3 PM Thursday, September 29th until 6:30 PM. We are not responsible for any posters left in the space after this time.





Princeton-Nature Conference: 2nd Frontiers in Electron Microscopy for the Physical and Life Sciences

Princeton University

September 28-30, 2022

Conference Program

*Speaker bios listed on the conference website (<https://conferences.nature.com/>)

September 28, 2022

- 8:00 a.m. **Registration check-in and breakfast, Maeder Hall**
- 8:50 a.m. **Welcome remarks**
Pablo G. Debenedetti, Dean for Research at Princeton University
- 9:00 a.m. **Keynote:** *Cryogenic electron imaging of macromolecules and cells beyond expectations*
Wah Chiu (Stanford University, USA)

Session IA **Instrumentation and sample preparation (mixed session)** Session Moderator: Ahne Myklatun (Nature Communications, Germany)

- 10:00 a.m. *Laser phase plate in cryogenic electron microscopy*
Holger Mueller (University of California-Berkeley, USA)
- 10:30 a.m. **Coffee break**
- 11:00 a.m. *From atomic-resolution imaging of inorganic two-dimensional materials towards imaging of organic two-dimensional materials: challenges and solutions*
Ute Kaiser (Ulm University, Germany)
- 11:30 a.m. *Operando, multimodal characterization of bimetallic catalysts with electrons and x-rays*
Eric Stach (University of Pennsylvania, USA)
- 12:00 p.m. **Lunch, Friend Center Convocation Room**

Session IB **Instrumentation and sample preparation (mixed session)** Session Moderator: Daniel McNally (Nature Materials, USA)

- 1:30 p.m. *3D characterization of nanoparticle transformations*
Sara Bals (University of Antwerp, Belgium)
- 2:00 p.m. *Nanoscale imaging of interfacial phonon modes and dynamics by electron microscopy*
Xiaoqing Pan (University of California-Irvine, USA)
- 2:30 p.m. *Towards high-resolution and high-throughput structural biology with electron cryo-tomography*
Abhay Kotecha (ThermoFisher, representing Life Sciences (Bio), The Netherlands)

- 3:00 p.m. **Coffee break**
- 3:30 p.m. *The breakthroughs of emerging electron diffraction techniques crystallography – from structure determination to high-throughput phase analysis*
Xiaodong Zou (Stockholm University, Sweden)
- 4:00 p.m. *Deciphering complex interfaces of energy materials in a stem*
Miaofang Chi (Oak Ridge National Laboratory, USA)
- 4:30 p.m. *Flash Talks Session A*
Session moderator: Daniel McNally (Nature Materials, USA)
- 5:00 p.m. **Welcome reception**
Friend Center Convocation Room

September 29, 2022

- 8:00 a.m. **Registration check-in and breakfast, Maeder Hall**
- 9:00 a.m. **Keynote:** *Using every electron: structure and properties from ptychography*
David Muller (Cornell University, USA)

Session II 2D materials and catalysis Session moderator: Bruno Castro (Nature Materials, UK)

- 10:00 a.m. *Visualizing chemical processes at the atomic-scale*
Stig Helveg (Technical University of Denmark, Denmark)
- 10:30 a.m. **Coffee break**
- 11:00 a.m. *Advanced imaging of low-dimensional nanostructures: the essential step to go from processing to applications*
Valeria Nicolosi (Trinity College Dublin, Ireland)
- 11:30 a.m. *Do more with less: advanced electron microscope solutions for sample integrity preservation*
Paolo Longo (Thermo Fisher, USA)
- 12:00 p.m. **Lunch, Friend Center Convocation Room**

Session III Cryo-EM and beyond (combining cryo-ET and other developments) Session moderator: Allison Doerr (Nature Methods, USA)

- 1:30 p.m. *Visualizing molecular structure in situ by cryo-electron tomography*
Peijun Zhang (Oxford University, UK)
- 2:00 p.m. *New cryo-em methods for studying native biological complexes, in situ and in action*
Hong Zhou (University of California-Los Angeles, USA)

- 2:30 p.m. *Electron cryotomography of cells*
Grant Jensen (Caltech, USA)
- 3:00 p.m. **Coffee break**
- 3:30 p.m. *Structural titrations of human inositol trisphosphate receptors reveals mechanisms of activation and inhibition*
Richard Hite (Memorial Sloan Kettering Cancer Center, USA)
- 4:00 p.m. *Quo vadis MicroEd?*
Tamir Gonen (University of California-Los Angeles, USA)
- 4:30 p.m. *Flash Talks Session B*
Session moderator: Allison Doerr (Nature Methods, USA)
- 5:00 p.m. **Poster Session & refreshments**
Friend Center Convocation Room

September 30, 2022

- 8:00 a.m. **Registration check-in and breakfast, Maeder Hall**

SESSION IV: **Big data and machine learning**
Session moderator: Bryden Le Bailly (Nature, UK)

- 9:00 a.m. *TBD*
Ellen Zhong (Princeton University, USA)
- 9:30 a.m. *Machine learning for scanning probe and electron microscopy: from learning physics to atom-by-atom construction*
Sergei Kalinin (University of Tennessee, USA)
- 10:00 a.m. **Coffee break**
- 10:30 a.m. *Molecular modeling and simulation of the human nuclear pore complex*
Gerhard Hummer (Max Planck Institute, Germany)
- 11:00 a.m. *Building and validating protein structure models for cryo-EM maps using deep learning*
Daisuke Kihara (Purdue University, USA)
- 11:30 a.m. **Keynote:** *Developments in correlative cryo-microscopy applied to structural studies of viruses and virus assembly*
Elizabeth Wright (University of Wisconsin-Madison, USA)
- 12:30 p.m. **Meet the Editors Session, with Lunch**
- 1:15 p.m. **Closing remarks**
Richard A. Register, Director of the Princeton Institute of Materials, Princeton University, USA

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Speaker Abstracts

(in order of presentation)

CRYOGENIC ELECTRON IMAGING OF MACROMOLECULES AND CELLS BEYOND EXPECTATIONS

Wah Chiu¹

Department of Bioengineering and Department of Microbiology and Immunology, Stanford University, Stanford; SLAC National Accelerator Laboratory, Menlo Park, CA, USA

Cryogenic electron microscopy (cryoEM) has been advanced to resolve atomic structures of biochemically purified macromolecules with details equivalent to X-ray crystal structures. A unique aspect of cryoEM is to use image processing methods to sort out images of particles with heterogeneous compositions and conformations. This allows us to visualize structures of macromolecules that exist in an ensemble of biochemical states, which are interpretable in their functions. These have been demonstrated across a spectrum of macromolecules including membrane channels, protein folding machines with and without substrates, and RNAs. The reliability of these structures can be assured by using rigorous criteria that the atomic model obeys expected stereochemistry of the molecules and simultaneously matches well with the experimentally observed density maps. The resolvability of the cryoEM structures have been shown in many cases to be sufficiently good to resolve ligands, ions and water molecules that are critical to understand the chemical basis of their tertiary structures and to inform therapeutic developments.

A relatively recent development in electron imaging is cryogenic electron tomography (cryoET) of cells such as neurons, virus infected cells, pathogens, bacteria, and algae. The 3D tomograms can be visualized directly with artificial intelligence-based annotation of subcellular features (e.g., organelles) or processed further by subvolumes of repeating features of molecular assemblies. The latter approach has now reached the capability of resolving molecular structures at subnanometer resolution to visualize different structural intermediates *in situ*. Tomographic observations can reveal unknown mechanisms of various biological processes *in situ* which add to our fundamental knowledge in cell biology as well as serve as biomarkers of pathogenic cells.

LASER PHASE PLATE IN CRYOGENIC ELECTRON MICROSCOPY

Holger Mueller¹, J. J. Axelrod¹, P. Petrov¹, J. Remis¹, S. Sandhaus¹, R. M. Glaeser^{1,2}

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²Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

A phase plate can provide optimum image contrast for weak-phase object in cryogenic single-particle electron microscopy (cryo-EM) and tomography (cryo-ET). The laser phase plate (LPP) is based on coherently phase-shifting the electron wave function by using a continuous-wave laser beam, which is built up to a record intensity of $\sim 400 \text{ GW/cm}^2$ by resonance in a Fabry-Perot cavity. In our initial work, we demonstrated generating a high-resolution map of 20S proteasome particles. This work demonstrated the long-term stability of the phase plate and the contrast enhancement clearly. However, it was still limited by thermal magnetic-field fluctuations (Johnson noise) and ice buildup in our relatively old low-base Titan test microscope, both of which cause a drop of the contrast transfer function at high spatial frequencies.

The talk will focus on our recent work to eliminate these limitations and move beyond the proof-of-concept. Specifically, we constructed a new LPP with a larger (8-mm) hole to pass the electron beam, which we expect to make Johnson noise caused by the LPP negligible; we have reduced the ice buildup rate by an order of magnitude by installing an additional vacuum pump and using an objective aperture; and we have installed a gun monochromator to reduce the electron beam energy spread, which we and hope will overcome the current limit from chromatic aberration.

To fully demonstrate the benefits of the LPP to cryo-EM, we will soon begin working with a microscope equipped with a Cs corrector, in addition to a gun monochromator, as well as a post-column energy filter and an improved camera. It will make use of a newly designed dual LPP with crossed laser beams, which we expect to suppress the weak ghost images observed with a single LPP. This instrument is projected for delivery in Q2, 2023. Looking even further ahead, we are exploring whether the LPP can be combined with high-resolution, 2-D template matching and whether miniaturization would allow placing the LPP directly into the back focal plane of the objective lens, to considerably simplify the electron microscopy with a laser phase plate.

From Atomic-Resolution Imaging of Inorganic Two-Dimensional Materials towards Imaging of Organic Two-Dimensional Materials: Challenges and Solutions

Ute Kaiser

Ulm University, Central Facility Electron Microscopy, Materials Science Electron Microscopy, D-89081 Ulm, Albert Einstein Allee 11, Germany

In this study, we show that a detailed understanding of beam electron-sample interactions is required to achieve high-resolution structural imaging of two-dimensional materials. We start to derive basic understanding from atomically-resolved, time-dependent in-situ TEM imaging of inorganic two-dimensional (2D) transition metal dichalcogenides using the chromatic- and spherical-aberration-corrected low-voltage SALVE instrument operating in the voltage range between 80kV and 20kV [1-3]. We experimentally determine the accelerating-voltage-dependent formation of defects in transition metal dichalcogenides (TMDs), which surprisingly did not differ for TMDs with very different knock-on damage thresholds. Density functional theory molecular dynamics shows that excitations in the electronic system can form vacancies through ballistic energy transfer at electron energies, which are much lower than the knock-on threshold for the ground state, and thus we propose a two-step vacancy formation process as combination of elastic and inelastic events [4]. If the material under electron irradiation lost its ordered structure, the evaluation of the unordered structure is performed after developing an U-net-based fully convolution neural network (FCN). We further discuss the formation of defects in two-dimensional inorganic crystals and also their twisted heterostructures. We analyse in-situ structural and chemical transformations of different freestanding TMDs and of rarely reported TMPTs (TM phosphorus tri-chalcogenides). Complementary ab-initio calculations prove the stability of the newly formed phases and predict their properties [5]. Furthermore, we present in-situ studies of a miniaturized electrochemical cell, where reversibly single-crystalline bilayer graphene is lithiated and delithiated in controlled manner using an electrochemical gate confined to a device protrusion [6] with special emphasis on the Li crystal nucleation mechanism.

The knowledge gained for the study of 2D inorganic materials we apply to the study of 2D polymers [7] and 2D metal-organic frameworks (MOFs) [8], where however atomically-resolved imaging is hindered due to much lower resilience against electron irradiation. We present key strategies to achieve higher resolution in high-resolution TEM images of imine-based 2D polymer films [9], which include the selection of the appropriate electron accelerating voltage [10]. When comparing the achievable resolution at 300kV, 200kV, 120kV and 80kV, we found that imaging at 120kV offers the highest resolution (1.9Å). This resolution allowed even imaging the molecular nature of interstitial defects, which could be identified by means of quantum mechanical calculations [10]. After further electron irradiation, the crystalline PI film loses the well-ordered structure and an unordered porous polyimine film develops. The U-net-based FCN developed for the analysis of inorganic amorphous 2D structures was also successfully applied to the evaluation of amorphous polyimine structures to understand the pore size distributions [10]. In addition, we show that even Sub-Angstrom resolution can be achieved for hydrogen-free 2D BHT-Cu (BHT = benzenehexathiol) MOFs using the Cc/Cs-corrected SALVE microscope operating at 80 kV, as here the stability against electron irradiation is strongly increased, resulting in imaging single atoms with high contrast.

Further, we study experimentally and computationally the role of different organometallic bonds and hydrogen content on electron radiation stability, using a group of four structurally similar Cu-based 2D MOFs with well-defined differences to allow for a direct comparison of hydrogen-containing and

hydrogen-free MOFs, and of the presence of Cu - N, Cu - O and Cu - S chemical bonds. Trends in e-beam resilience among the 2D-MOFs found experimentally showed good agreement with *ab initio* molecular dynamics simulations.

- [1] U. Kaiser et al., Ultramicroscopy, 111, 8, (2011) 1239
- [2] M. Linck, et al. PRL 117, (2016) 076101.
- [3] F. Börrnert and U. Kaiser Physical Review A 98 (2), (2018) 023861
- [4] S. Kretschmer, et al, Nano Lett. 20, (2020) 2865.
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- [7] K. Liu et al., Nature Chemistry 11 (2019) 994
- [8] Z. Wang et al. J. Am. Chem. Soc. (2021) (doi.org/10.1021/jacs.1c05051)
- [9] H. Qi et al., Science Advances 6, (2020) eabb5976
- [10] B. Liang et al. Nature Communication 13 (2022) 3948.

OPERANDO, MULTIMODAL CHARACTERIZATION OF BIMETALLIC CATALYSTS WITH ELECTRONS AND X-RAYS

Eric A. Stach^{1,2}, Alexandre C. Foucher¹, Nicholas Marcella³, Jennifer D. Lee^{4,5}, Daniel J. Rosen³, Ryan Toppero⁴, Christopher B. Murray^{1,3}, Anatoly I. Frenkel^{2,5}

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Alloyed nanoparticles are of increasing interest in many applications, most notably as heterogeneous catalysts. Alloying allows the tuning of composition and structure to increase functionality, most specifically reactivity and selectivity. However, harsh, reactive environments can induce changes in the structure and composition of these materials in unexpected ways, which can inhibit their performance. These materials also present a significant characterization challenge: they are tiny (from single atoms to particles of 10 nm) and can also be heterogeneous in size, composition, and structure.

I will describe how we have developed a new approach to characterize catalysts using so-called 'operando' methods to take measurements. At the same time, the materials are 'in a working condition': i.e., in a reactive environment performing their function. We use a microreactor system compatible with imaging, diffraction, spectroscopy, using electron, photon, and x-ray probes. The presentation will describe how this multimodal approach can provide unique insights into the dynamic changes in these complex systems as they function.

3D CHARACTERIZATION OF NANOPARTICLE TRANSFORMATIONS

Sara Bals¹, Wiebke Albrecht^{1,2}, Mikhail Mychinko¹, Ece Arslan Irmak¹, Adrian Perazo-Tardajos, Kellie Jenkison¹, Thomas Altantzis^{1,3}, Sandra Van Aert¹

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Electron tomography enables one to measure the morphology and composition of nanostructures in three dimensions (3D), even at atomic resolution. However, an emerging challenge is to fully understand the connection between the 3D structure and properties under realistic conditions, including high temperatures as well as in the presence of liquids and gases. Under such conditions, rapid reshaping of nanoparticles can be expected. Although *in situ* transmission electron microscopy provides an elegant platform to directly visualize nanoparticles changes down to the atomic scale, it is challenging to investigate nanoparticle transformations in 3D. In this presentation, I will discuss existing possibilities to obtain 3D information using either tomographic methods or the so-called atom counting technique, which utilizes single projection images. Next, I will show how these techniques can be combined with *in situ* holders to quantify structural and chemical transformations on a single nanoparticle level. By combining fast tomography with *in situ* heating, we were able to perform a dynamic characterization of shape changes of metal nanoparticles at high temperatures. Moreover, we measured the elemental diffusion dynamics of individual anisotropic bimetallic nanoparticles in 3D and determined the effect of parameters such as type of interfacial facets, aspect ratio, shape and presence of defects. By atom counting, it has furthermore become possible to monitor the evolution of crystalline facets of metal nanoparticles under gas and heat treatments, a change that influences catalytic properties. Next to *in situ* processes, we also demonstrate the value of electron tomography to assess external laser-induced NP transformations, making it viable to detect structural changes with atomic resolution.

NANOSCALE IMAGING OF INTERFACIAL PHONON MODES AND DYNAMICS BY ELECTRON MICROSCOPY

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Crystal defects and interfaces affect the thermal and heat-transport properties of materials by scattering phonons and modifying phonon spectra. Spatially resolved vibrational mapping of nanostructures and defects is indispensable to the development and understanding of thermal nanodevices, modulation of thermal transport and novel nanostructured thermoelectric materials. Through the engineering of complex structures, such as alloys, nanostructures and superlattice interfaces, one can significantly alter the propagation of phonons and suppress material thermal conductivity while maintaining electrical conductivity. There have been no correlative experiments that spatially track the modulation of phonon properties in and around individual defects and nanostructures due to spatial resolution limitations of conventional optical phonon detection techniques. In this talk, we demonstrate that space- and angle-resolved vibrational spectroscopy in a transmission electron microscope makes it possible to map the vibrational spectra of a single interface and a quantum dot. We detect a red shift of several millielectronvolts in the energy of acoustic vibration modes near a single stacking fault in cubic silicon carbide, together with substantial changes in their intensity, and find that these changes are confined to within a few nanometres of the stacking fault.[1] At a high-quality epitaxial Si-Ge interface, localized interfacial phonon modes at ~48 meV.[2] Simulations show that the interfacial phonon modes have an obvious contribution to the total thermal interface conductance. By tracking the variation of the Si optical mode in a phonon map from a single SiGe quantum dot, the nanoscale modification of the composition-induced red shift is observed.[3] We also develop a novel technique to differentially map phonon momenta, providing direct evidence that the interplay between diffuse and specular reflection largely depends on the detailed atomistic structure. Our work unveils the non-equilibrium phonon dynamics at nanoscale interfaces and can be used to study actual nanodevices and aid in the understanding of heat dissipation near nanoscale hotspots, which is crucial for future high-performance nanoelectronics.

References:

- [1] X. Yan *et al.*, *Nature* **589** (2021), p. 65–69.
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- [3] Z. Cheng, R. Li, X. Yan *et al.*, *Nat. Commun.* **12** (2021), p. 6901.
- [4] This work was supported by the Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering (DE-SC0014430), and partially by the NSF under grant number DMR-2034738. The authors acknowledge the use of facilities and instrumentation at the UC Irvine Materials Research Institute (IMRI) supported in part by the National Science Foundation through the Materials Research Science and Engineering Center program (DMR-2011967).

TOWARDS HIGH-RESOLUTION AND HIGH-THROUGHPUT STRUCTURAL BIOLOGY WITH ELECTRON CRYO-TOMOGRAPHY

Abhay Kotecha¹, Ron Kelly¹, Adrian Koh¹, Wen Yang¹, Xianjun Zhang¹, Jay Rai¹, Martin Obr¹

¹ Materials and Structural Analysis Division, Thermo Fisher Scientific, Eindhoven, The Netherlands.

Electron cryo-tomography (cryo-ET) is evolving into the method of choice for elucidating biological structures in their native environment. Together with sub-tomogram averaging (STA), it offers a unique way of imaging biological complexes both *in vitro* from purified proteins and *in vivo* directly inside the cells in a near to native state. Through STA method, the recurring structures in cryo-ET data can be resolved to sub-nanometer resolutions. In recent years, advances in microscope hardware, data collection, and computational algorithms have facilitated an increasing number of sub-5 Å maps achieved by STA demonstrating that the high-resolution information in cryo-ET data can be harvested for *in situ* structural biology.

Here, I will show that the high quality cryo-ET data acquired by Krios G4 equipped with Selectris energy filter and Falcon 4i detector enabled us to resolve apoferritin structure at 1.6 Å with STA from 100 tilt series and a ribosome structure from yeast lamella, prepared by cryo-Focused Ion beam (cryo-FIB) milling, at 5 Å resolution, with large subunit resolved to 3.5 Å. Furthermore, I will also show the results from whole cell volume cryo-slice and view using plasma-FIB milling and a high throughput cryo-FIB milling workflow with our new Arctis plasma-FIB system.

THE BREAKTHROUGHS OF EMERGING ELECTRON DIFFRACTION TECHNIQUES IN CRYSTALLOGRAPHY - FROM STRUCTURE DETERMINATION TO HIGH-THROUGHPUT PHASE ANALYSIS

Xiaodong Zou

Department of Materials & Environmental Chemistry, Stockholm University, Sweden

Knowing the 3D arrangement of atoms in a molecule or a material is essential for understanding its functions and properties. X-ray crystallography is currently the most important technique for determination of 3D crystal structures, but requires large single crystals. Electron crystallography has unique advantages in studying nano- and micrometer-sized crystals that are too small for X-ray diffraction. Although *Ab initio* structure solution by electron diffraction has already been demonstrated on inorganic and organic crystals since 1990s, it has not been widely used because of the concern about dynamical effects.

Since 2007, new techniques have been developed for collecting integrated 3D electron diffraction (3D ED) data; from step-wise rotation electron diffraction to continuous rotation electron diffraction. We also developed serial (rotation) electron diffraction (SerialED and SerialRED) where data from thousands of particles can be collected automatically in less than one hour! In this talk, I will show the impacts of electron diffraction, especially 3D ED, in studying various types of crystals, from inorganic porous materials such as zeolites and metal-organic frameworks, to organic pharmaceuticals and proteins.

Today, a complete 3D ED dataset can be obtained in less than a minute on a standard TEM. The data processing, structure solution and refinement are performed using standard X-ray crystallography software. The data can be treated as kinematical data, and the refined structural models can reach better than 0.05 Å in accuracy for all non-H atoms. Using dynamical refinement, more accurate structures and finer structural details can be obtained, including hydrogen location and absolute structures. Serial electron diffraction (SerialED) makes it possible not only for studying extremely beam-sensitive crystals, but also for phase analysis and for detection of minor phases invisible by X-ray diffraction. The developments of new 3D ED and SerialED techniques have revolutionized crystallography, and provided new opportunities for discovering novel structures and materials, and exploring their properties and applications.

DECIPHERING COMPLEX INTERFACES of ENERGY MATERIALS IN A STEM

Miaofang Chi, Michael Zachman, Xiaoming Liu, Kartik Venkatraman

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Designing next-generation energy conversion and storage systems, such as solid-state batteries and fuel cells, faces numerous challenges, many of which are related to interfaces. Elucidating interfacial phenomena in energy systems requires knowledge, not only of atomic-scale structure and chemistry but also of correlated local charge distribution and ion diffusion that are difficult to probe with conventional techniques. Several new scanning transmission electron microscopy (STEM) techniques, such as four-dimensional (4D)-STEM, monochromated EELS (electron energy loss spectroscopy), atomic-scale cryogenic and *in situ* environmental microscopy, allow the behavior of electrons, ions, and atoms to be probed, opening opportunities to tackle complex dynamic interfacial questions. In this talk, I will demonstrate how we combine these techniques to reveal the origin of unexpected dendrite growth within all-solid-state batteries, probe local ion transport behavior at interfaces and grain boundaries, and map charge density in electrides and interfaces in heterogeneous catalysts. Perspectives for the future advancements of STEM techniques for energy materials research will also be provided.

Acknowledgement: This research was supported by the U.S. DOE Office of Science Early Career project ERKCZ55 and were performed at the Center for Nanophase Materials Sciences (CNMS), which is a U.S. DOE Office of Science User Facility.

USING EVERY ELECTRON: STRUCTURE AND PROPERTIES FROM ELECTRON PTYCHOGRAPHY

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Electron microscopes use electrons with wavelengths of a few picometers, and are potentially capable of imaging individual atoms in solids at a resolution ultimately set by the intrinsic size of an atom. Until very recently, the best resolution was more than an order magnitude worse than this limit. This was caused by two things – first the intrinsic aberrations in electron lenses are much worse than for optical lenses – comparable to using a beer bottle as a magnifying glass. Second, electrons are multiply-scattered inside the sample – a process described by Hans Bethe over 90 years ago. It’s been a headache for electron microscopists ever since, but with recent advances in momentum-resolved detector technology [1] and reconstruction algorithms such as ptychography, the resolution of the electron microscope is now limited only by the dose to the sample, and thermal vibrations of the atoms themselves [2]. These approaches have allowed us to image, in a highly-dose-efficient manner, the detailed vibrational envelopes of individual atom columns. Solving the multiple scattering problem also recovers three-dimensional information about the sample, including surface relaxations and revealing interstitial dopant atoms that would be hidden by channeling of the probe with conventional imaging modes. The reduced sensitivity to chromatic aberrations also makes these ptychographic approaches of interest for thick biological samples.

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VISUALIZING CATALYTIC PROCESSES AT THE ATOMIC-SCALE

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The current quest for sustainable energy technologies creates an urgent need to improve our fundamental understanding of catalysis. Today, catalysis of chemical reactions is commonly perceived as a complex surface phenomenon that inescapably links structural dynamics and functionality of the catalyst surface. Insight into this intricate relation between catalytic active surface sites and their mechanistic actions at the atomic-scale has, however, remained challenging to retrieve due to the lack of suitable visualization competences. Here, I will outline recent breakthroughs in atomic-resolution electron microscopy building an exciting foundation for *operando* observations of the spatiotemporal behavior of catalysts and in turn, bridging the so-called *pressure and materials gaps* in surface and catalysis sciences.

ADVANCED IMAGING OF LOW-DIMENSIONAL NANOSTRUCTURES: THE ESSENTIAL STEP TO GO FROM PROCESSING TO APPLICATIONS

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Low-dimensional nanostructured materials such as organic and inorganic nanotubes, nanowires and platelets are potentially useful in a number of areas of nanoscience and nanotechnology due to their remarkable mechanical, electrical and thermal properties. However, difficulties associated with their lack of processability have seriously hampered both. In the last few years dispersion and exfoliation methods have been developed and demonstrated to apply universally to 1D and 2D nanostructures of very diverse nature, offering a practical means of processing the nanostructures for a wide range of innovative technologies. To make real applications truly feasible, however, it is crucial to fully characterize the nanostructures on the atomic scale and correlate this information with their physical and chemical properties. Advances in aberration-corrected optics in electron microscopy have revolutionised the way to characterise nano-materials, opening new frontiers for materials science. With the recent advances in nanostructure processability, electron microscopes are now revealing the structure of the individual components of nanomaterials, atom by atom. Here we will present an overview of very different low-dimensional materials issues, showing what aberration-corrected electron microscopy can do for materials scientists.

DO MORE WITH LESS: ADVANCED ELECTRON MICROSCOPE SOLUTIONS FOR SAMPLE INTEGRITY PRESERVATION

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Over the last decades we have witnessed a tremendous development in advanced transmission electron microscopy. To name a few, probe correctors have become more powerful and now in combination with ultra-brightness electron sources 60 – 70pm spatial resolution in STEM can be routinely reached with nearly 1nA probe current, improvements in energy resolution have led to phonon spectroscopy, EDS detector systems have become 6x more efficient and sensitive with the latest generation leading to low-dose data collection and in-situ analytical studies, detectors are now capable to count electrons dramatically reducing the contribution of instrumental noise and that has led to the revolution in cryo-EM.

However, the quest for high beam current electron probes has been over the last few years partly countered by the steady growing interest towards energy based and organic materials for advanced applications. The behaviour of this new class of materials can be dominated by processes occurring at defects, interfaces, and grain or phase boundaries and Electron Microscopy (EM) has long been a technique of choice to analyze these complex heterogeneous systems at the atomic level. However, these materials are often prone to changes upon exposure to electron beam, creating challenging conditions for imaging and analytical data collection and reliability. Here not only the best most sensitive hardware is important to successfully extract sub-Angstrom level information but also the best approaches in terms of electron dose optimization.

The paper will discuss many of the strategies that can be employed to preserve sample integrity from both the hardware and software integration perspectives. Given the need to accommodate the characterization of many different materials in the same transmission electron microscope, not one single approach but a toolbox of many strategies will benefit most multi-user facilities and lead to higher success in pushing the boundaries of advanced atomic level investigation.

VISUALIZING MACROMOLECULAR STRUCTURES IN SITU BY CRYO-ELECTRON TOMOGRAPHY

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Structures of purified proteins and protein complexes are now routinely determined to atomic or near-atomic resolutions using single particle cryoEM. Structures of macromolecular assemblies that are intrinsically flexible, dynamic, heterogeneous, and often function in higher-order assemblies, have recently been analysed to near-atomic resolutions using cryo-electron tomography (cryoET) and subtomogram averaging (STA). The study of native complexes in their cellular context using cryoET STA, coupled with cryoFIB/SEM and correlative and multiscale imaging, opens a new frontier in structural cell biology. Here I present our recent multi-modal, multi-scale imaging of virus infection in cells and *in situ* structural determination of bacterial enzymes functioning in carbon fixation to demonstrate the power of *in situ* structural biology using cell lamellae-based cryoET STA.

NEW CRYOEM METHODS FOR STUDYING NATIVE BIOLOGICAL COMPLEXES, *IN SITU* AND IN ACTION

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The last decade has witnessed advances in high-resolution cryoEM “revolutionizing” structural biology; as such, cryoEM has become a tool of choice for determining atomic structures of macromolecular complexes, within and outside the cell. In an effort to capture functional complexes *in situ* and *in action*, we have developed an integrative proteomics cryoEM methods to determine atomic structures of native cellular complexes, sub-particle refinement and nucleic acid modeling methods to model genomic RNA and DNA *in action*. Of particular note, our *cryoID* method allows determination of atomic structures of native complexes in cellular milieu, capturing their multiple states, including those *in act* of carrying out their functions.

While the single-particle cryoEM methods have made it routine to determine structures of isolated macromolecular complexes at atomic resolution by averaging hundreds of thousands of particles. The biological functions of these complexes, however, are carried out through their interactions and often depend on their spatial arrangements within cells or sub-cellular organelles. Such molecular sociology information requires the use of cryogenic electron tomography (cryoET), which has another limitation, known as the “missing-wedge” problem. Anisotropic resolution arising from the intrinsic “missing-wedge” problem has presented major challenges in visualization and interpretation of tomograms. We have developed *IsoNet*, a deep learning-based software package that iteratively reconstructs the missing-wedge information and increases signal-to-noise ratio, using the knowledge learned from raw tomograms.

Applications of *cryoID* have led to atomic structure determination of previously intractable biological systems from mosquito saliva and cellular milieu. *IsoNet* has enabled direct interpretation of molecular sociology of native complexes in cells. Integration of these new computational methods now open the door to *in situ* atomic resolution structures of cellular complexes in their native cellular environment.

ELECTRON CRYOTOMOGRAPHY OF CELLS

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In the last several years electron cryo-tomography (cryo-ET) has made it possible to see inside intact cells in a near-native, "frozen-hydrated" state in 3-D to a few nanometers resolution. Increasingly, atomic models of individual proteins and smaller complexes obtained by X-ray crystallography, NMR spectroscopy, or other methods can be fit into the resulting 3D images ("cryotomograms") to reveal how the various pieces work together inside cells. A few good pictures sometimes give incredible new insight into structure and function. To illustrate these points, I will present examples of current results from our recent work on bacterial microcompartments and eukaryotic stress responses. I will also report on our efforts to accelerate data collection and disseminate results.

STRUCTURAL TITRATION OF HUMAN INOSITOL TRISPHOSPHATE RECEPTORS REVEALS MECHANISMS OF ACTIVATION AND INHIBITION

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Inositol 1,4,5-trisphosphate receptors (IP₃R) are intracellular Ca²⁺-permeable cation channels that mediate Ca²⁺ release from endoplasmic reticulum stores in response to extracellular signals. IP₃Rs are activated by inositol 1,4,5-trisphosphate (IP₃) and low concentrations of its permeant ion Ca²⁺, yet are inhibited by high Ca²⁺ concentrations, giving rise to cytosolic Ca²⁺ oscillations. To elucidate how Ca²⁺ both activates and inhibits IP₃Rs, we determined cryo-EM structures of human type 3 IP₃R over a range of physiological Ca²⁺ concentrations. This structural Ca²⁺ titration revealed an ensemble of distinct structural states including resting, preactivated, activated and inhibited whose relative abundances are Ca²⁺-dependent. Comparisons of these states revealed how Ca²⁺ binding at a high-affinity site in the presence of IP₃ leads to channel activation while Ca²⁺ binding at a second lower affinity site leads to inhibition. Mutagenesis demonstrated the critical roles of these sites in Ca²⁺ oscillations with disruption of the activating site ablating Ca²⁺ oscillations and disruption of the inhibitory site leading to increased oscillatory frequency. Together, these studies provide a mechanistic basis for the biphasic Ca²⁺-dependence of IP₃R channel activity.

QUO VADIS MICROED?

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My laboratory studies the structures of membrane proteins that are important in maintaining homeostasis in the brain. Understanding structure (and hence function) requires scientists to build an atomic resolution map of every atom in the protein of interest, that is, an atomic structural model of the protein of interest captured in various functional states. In 2013 we unveiled the method Microcrystal Electron Diffraction (MicroED) and demonstrated that it is feasible to determine high-resolution protein structures by electron crystallography of three-dimensional crystals in an electron cryo-microscope (CryoEM). The CryoEM is used in diffraction mode for structural analysis of proteins of interest using vanishingly small crystals. The crystals are often a billion times smaller in volume than what is normally used for other structural biology methods like x-ray crystallography. In this seminar I will describe the basics of this method, from concept to data collection, analysis and structure determination, and illustrate how samples that were previously unattainable can now be studied by MicroED. I will conclude by highlighting how this new method is helping us discover and design new drugs; shedding new light on chemical synthesis and small molecule chemistry; and showing us unprecedented level of details with important membrane proteins such as ion channels and G-protein coupled receptors (GPCRs) at atomic resolutions.

MACHINE LEARNING FOR SCANNING PROBE AND ELECTRON MICROSCOPY: FROM LEARNING PHYSICS TO ATOM-BY-ATOM CONSTRUCTION

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Machine learning and artificial intelligence (ML/AI) are rapidly becoming an indispensable part of physics research, with domain applications ranging from theory and materials prediction to high-throughput data analysis. However, the constantly emerging question is how to match the correlative nature of classical ML with hypothesis-driven causal nature of physical sciences. In parallel, the recent successes in applying ML/AI methods for autonomous systems from robotics through self-driving cars to organic and inorganic synthesis are generating enthusiasm for the potential of these techniques to enable automated and autonomous experiment (AE) in imaging.

In this presentation, I will discuss recent progress in automated experiment in electron and scanning probe microscopy, ranging from feature to physics discovery via active learning. The applications of classical deep learning methods in streaming image analysis are strongly affected by the out of distribution drift effects, and the approaches to minimize though are discussed. I will further illustrate transition from post-experiment data analysis to active learning process, including learning structure-property relationships and materials discovery in composition spread libraries. Here, the strategies based on simple Gaussian Processes often tend to produce sub-optimal results due to the lack of prior knowledge and very simplified (via learned kernel function) representation of spatial complexity of the system. Comparatively, deep kernel learning (DKL) and structured Gaussian Processes methods allow to realize both the exploration of complex systems towards the discovery of structure-property relationship, and enable automated experiment targeting physics (rather than simple spatial feature) discovery. The latter is illustrated via experimental discovery of the edge plasmons in STEM/EELS and ferroelectric domain dynamics in PFM. For probing physical mechanisms of tip-induced modifications, I will demonstrate the combination of the structured Gaussian process and reinforcement learning, the approach we refer to as hypothesis learning. Finally, I will discuss the strategies for direct atomic fabrication via electron beams, targeting desired structures and desired functionalities.

MOLECULAR MODELING AND SIMULATION OF THE HUMAN NUCLEAR PORE COMPLEX

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The nuclear pore complex provides the gate for the molecular traffic between the cell nucleus and the cytosol. Assembled from ~1000 proteins, it forms a ring-shaped structure that stabilizes the half-toroidal pore connecting the inner and outer nuclear membranes of the nuclear envelope. Advances in cryo-electron tomography and AI-based structural modeling have made it possible to assemble a nearly complete structure of the scaffold of the human nuclear pore complex at near-atomic detail. Complementing the information on the scaffold, advances in in-situ single-molecule fluorescence imaging give us a molecular view also of the network of disordered FG-nucleoporins filling the pore interior. Building on this detailed structural information, we use molecular dynamics simulations to gain a dynamic view of the nuclear pore complex. In particular, we look at the response of the nuclear pore complex to tension in the nuclear envelope and at the organization of the FG-nucleoporins mediating the nucleocytoplasmic transport through the pore.

BUILDING AND VALIDATING PROTEIN STRUCTURE MODELS FOR CRYO-EM MAPS USING DEEP LEARNING

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Cryo-electron microscopy (cryo-EM) has become one of the main experimental methods for determining protein structures. protein structure modeling from cryo-EM is in general more difficult than X-ray crystallography since the resolution of maps is often not high enough to specify atom positions. We have been developing a series of computational methods for modeling protein structures from cryo-EM maps. For maps at medium resolution, deep learning can provide useful structure information for modeling. Captured features from density maps can be used for modeling as well as validating existing structure models. We present tools for structure modeling, fitting, and validation for cryo-EM. All the tools we developed are available at <https://kiharalab.org/emsuites/>.

DEVELOPMENTS IN CORRELATIVE CRYO-MICROSCOPY APPLIED TO STRUCTURAL STUDIES OF VIRUSES AND VIRUS ASSEMBLY

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Respiratory Syncytial Virus (RSV) is a pleomorphic, enveloped, negative-sense, single-stranded RNA virus. The surface exposed attachment glycoprotein (G), fusion glycoprotein (F), and small hydrophobic protein (SH) are embedded in the viral membrane. The matrix protein (M), which drives virion formation and elongation, lines the interior of the viral membrane. The genomic RNA is encapsidated in the ribonucleoprotein complex (RNP) that is present in the interior of virions. Cryo-ET studies have shown that the morphology of individual virions varies, with diameters of ~130 nm and lengths of ~500 nm to over 10 μm [1, 2]. The general arrangement of structural proteins within the virion is known, however, the molecular organization of M and other structural proteins has remained elusive. We will discuss developments with correlative microscopy, whole-cell cryo-electron tomography (cryo-ET), multi-shot montage tomography [3], and sub-tomogram averaging and their application to structural studies of RSV (Fig. 1). We will show that RSV M is arranged in a helical-like lattice of M-dimers ordered preferentially to the viral long axis (Fig. 2). Sub-tomogram averages that include both F and M suggest a co-organized arrangement.

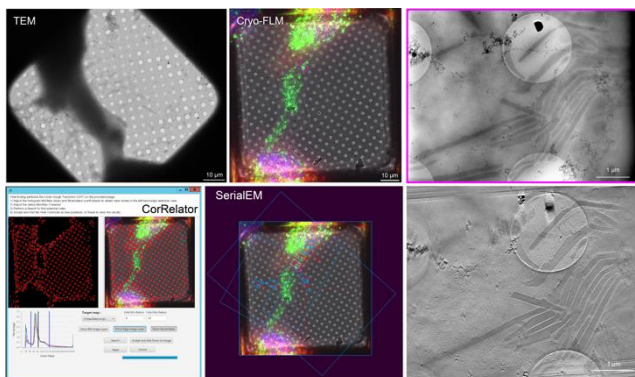


Fig. 1. Correlative cryo-microscopy strategy for studying RSV assembly.

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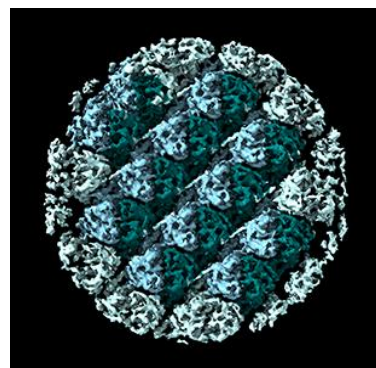


Fig. 2. Structure of the RSV matrix (M) protein lattice at 4.6 Å.

Poster Abstracts

(in alphabetical order by presenting author)

AUTOMATIC 4D-STEM STRAIN MAPPING USING CYCLE-CONSISTENT SPATIAL TRANSFORMING AUTOENCODERS

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Atomic-scale imaging of 2D and quantum materials benefits from precisely extracting crystallographic strain, shear, and rotation to understand their mechanical, optical and electronic properties. One powerful technique is 4D-STEM (4-dimensional scanning transmission electron microscopy), where a convergent electron beam is scanned across a sample while measuring the resulting diffraction pattern with a direct electron detector. Extracting the crystallographic strain, shear, and rotation from this data relies either on the correlation strain measurement method (e.g., implemented in py4DSTEM) or determining the center of mass (CoM) of the diffraction peaks. These algorithms have limitations. They require manual preprocessing and hyperparameter tuning, are sensitive to signal-to-noise, and generally are difficult to automate. There is no one-size-fits-all algorithm.

Recently, machine learning techniques have been used to assist in analyzing 4D-STEM data, however, these models do not possess the capacity to learn the strain, rotation, or translation instead they just learn a good approximation if the test examples are within the training dataset distribution. We developed a novel neural network structure – Cycle Consistent Spatial Transforming Autoencoder (CC-ST-AE). This model takes a set of diffraction images and trains a sparse autoencoder to classify an observed diffraction pattern to a dictionary of learned “averaged” diffraction patterns. Secondly, it learns the affine transformation matrix parameters that minimizes the reconstruction error between the dictionary and the input diffraction pattern. Since the affine transformation includes translation, strain, shear, and rotation, we can parsimoniously learn the strain

tensor. To ensure the model is physics conforming, we train the model cycle consistently, by ensuring the inverse affine transformation from the dictionary results in the original diffraction pattern.

We validated this model on several benchmark tasks including: A simulated 4D STEM dataset with known strain, rotation and shear. Secondly, we test this model experimental 4D STEM on 2D-heterostructures of tungsten disulfide (WS₂) and tungsten diselenide (WSe₂).

This model shows several significant improvements including: 1. When tested on simulated data, the model can recover the ground truth with minimal error. 2. The model can learn the rotation and strain on noisy diffraction patterns, it performs equivalently or better than CoM and correlation strain measurement methods. 3. Our model can accommodate large and continuous rotations. 4. Our model is more robust to noisy data. 5. Our model can map the strain, shear and rotation; identify dislocation and ripples; and distinguish background and sample area automatically. 6. Our model can be distilled and deployed on field-programmable gate arrays with inference latencies <100 μ s.

Ultimately, this work demonstrates how embedding physical concepts into unsupervised neural networks can simplify, automate, and accelerate analysis pipelines while simultaneously leveraging stochastic averaging that improves robustness on noisy data. This algorithmic concept can be extended to include other physical phenomena (e.g., polarization, sample tilt), can be used in automated experiments, and can be applied to other applications in materials characterization.

RECENT DEVELOPMENT IN DIRECT DETECTION TECHNOLOGY - COUNTING LOW ENERGY ELECTRONS (60 – 200 kV)

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Direct detection and electron counting represent one of the most impactful technology developments in transmission electron microscopy (TEM) in the last decade. This technology advancement has enabled new scientific discoveries and driven a rapid growth of TEM based characterization techniques in both academic research and industrial applications.

Success at detecting electrons with energies of 300 or 200 kV with high spatial and temporal resolution has been demonstrated by the large number of scientific breakthroughs in structural biology, soft and porous materials, etc. However, designing large format direct detection electron counting cameras for low kV applications with performance equivalent to 300 kV has remained a challenging endeavor.

Electrons with a lower acceleration voltage are challenging to detect because of competing constraints in sensor design: the pixel size needed to contain the electron interaction volume is quite large, but the sensor thickness required to detect only the incoming electron and not the sideways-scattered and back-scattered electrons must be very thin. When these parameters are not simultaneously optimized, the detective quantum efficiency (DQE) of the sensor drops significantly as the accelerating voltage is decreased. While hybrid pixel sensors have been shown to perform well at lower kV, the large pixel size of these devices makes them unsuitable for creating cameras larger than 1k x 1k in size. To increase the number of pixels (> 2k x 2k) and also achieve DQE, it is necessary to develop sensors with smaller pixel size (< 20 μm).

In this presentation, we will show how a combination of theoretical and experimental methods allowed us to rapidly evaluate a range of different pixel designs and identify the conditions that enable a “small” pixel with a high DQE at 60 - 200 kV similar to that of traditional direct detectors operating at 300 kV. Furthermore, we will show how this design has already been incorporated into cameras suitable for practical applications. These new cameras allow data to be collected that was not possible only months ago.

IN SITU ARCHITECTURE OF THE LIPID TRANSPORT PROTEIN VPS13C AT ER-LYSOSOME MEMBRANE CONTACTS

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VPS13 is a eukaryotic lipid transport protein localized at membrane contact sites. Previous studies suggested that it may transfer lipids between adjacent bilayers by a bridge-like mechanism. Direct evidence for this hypothesis from a full-length structure and from electron microscopy (EM) studies in situ is still missing, however. Here, we have capitalized on AlphaFold predictions to complement the structural information already available about VPS13 and to generate a full-length model of human VPS13C, the Parkinson's disease-linked VPS13 paralog localized at contacts between the endoplasmic reticulum (ER) and endo/lysosomes. Such a model predicts an ~30-nm rod with a hydrophobic groove that extends throughout its length. We further investigated whether such a structure can be observed in situ at ER-endo/lysosome contacts. To this aim, we combined genetic approaches with cryo-focused ion beam (cryo-FIB) milling and cryo-electron tomography (cryo-ET) to examine HeLa cells overexpressing this protein (either full length or with an internal truncation) along with VAP, its anchoring binding partner at the ER. Using these methods, we identified rod-like densities that span the space separating the two adjacent membranes and that match the predicted structures of either full-length VPS13C or its shorter truncated mutant. One intriguing finding from subtomogram averaging analysis is that the density is less continuous with the ER membrane than with the endo/lysosome membrane. Ongoing work further investigates transport mechanism of VPS13C by examining its conformational heterogeneity in situ. Taken together, our study provides in situ evidence for a bridge model of VPS13 in lipid transport. (This work was supported in part by a postdoctoral fellowship from the Parkinson Foundation to SC)

ASPIRE: AN OPEN-SOURCE SOFTWARE PACKAGE FOR 3D MOLECULAR STRUCTURE DETERMINATION USING CRYO ELECTRON MICROSCOPY

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ASPIRE (Algorithms for **S**ingle **P**article **R**econstruction) is an open-source Python package, installable from the Python Package Index (PyPI), which aims to implement an end-to-end single particle reconstruction pipeline. It provides many novel algorithmic solutions to the cryo-EM data processing pipeline including particle picking, CTF correction, orientation estimation for symmetric and non-symmetric molecules, 2-D classification and averaging, and 3-D ab-initio modelling.

ASPIRE is designed using a modular framework which allows users the flexibility to tailor the computational workflow of the reconstruction pipeline to the specific needs of their experiment. This modular design gives researchers the ability to incorporate newly developed algorithms and features by swapping out existing methods with their own. This extensibility is further supported by ASPIRE's homegrown tools for generating realistic synthetic datasets which are highly customizable, providing a sandbox for researchers to develop and refine new cryo-EM image processing techniques.

We demonstrate ASPIRE's functionality by running through a typical reconstruction pipeline on a synthetic dataset of projection images. We generate this dataset using ASPIRE's Simulation module, into which we feed a molecular volume map and apply custom noise and CTF filters. This results in a set of noisy, CTF corrupted, projection images taken from random viewing angles. Using these images as our experimental base, we apply CTF correction, 2-D classification, and class averaging to prepare the images for orientation estimation. We then apply a common-lines algorithm to estimate the relative orientations of our images. Finally, using ASPIRE's unique 3-D Fourier basis representation we perform a mean volume reconstruction of the molecule.

REVEALING THE EXOTICS IN COMPLEX MATERIALS WITH ATOMIC RESOLUTION

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With the advancement of technology, researchers keep interests in exploring novel materials (such as topological magnets and topological insulators) with advanced physical properties for specific applications (spintronics, quantum computation, and etc.). Investigation of the atomic structure of these materials is not only to identify or verify their atomic configurations but also provide further understanding of their atomic bonding, so as to elucidate the underlying mechanism in the material system. In this poster, we will demonstrate the imaging and analysis of atomic structure in several complex materials by using atomic-resolution scanning transmission electron microscopy (STEM) and energy dispersive X-ray spectroscopy (EDS). Four examples will be presented. First, we report a perfect 12-fold atomic arrangement in a novel quasicrystal synthesized in a nature lightning strike. It allows us to further understand the formation mechanism of such complex material. Second, we reveal a unique topological distribution of surface magnetic domains in a Weyl line ferromagnet by STEM and magnetic force microscopy. This is for the first time to observe non-trivial topological distribution of magnetic domains in a room temperature ferromagnet. Third, we explore the detailed atomic structure of a topological Kagome magnet and a topological insulator by STEM imaging and atomic-level elemental mapping. We finally discuss the interfacial structure between Ta film and Al₂O₃ substrate which was designed for quantum computing. The authors acknowledge the use of Princeton's Imaging and Analysis Center (IAC), which is partially supported by the Princeton Center for Complex Materials (PCCM), a National Science Foundation (NSF) Materials Research Science and Engineering Center (MRSEC; DMR-2011750).

FACILE METALLIC BACKFILLING METHODS GREATLY ENHANCES 3D NANOSCALE IMAGING WITH ELECTRONS AND X-RAYS

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With recent advances in electronic microscopy, 3D imaging is now possible for a wide range of samples and materials, from biological to self-assembled polymeric systems and across lengthscale spanning from hundreds of nanometers to tens of nanometers. Factors such as lengthscale, porosity, as well as lack of contrast usually make these systems challenging to image. Here we present sample preparation strategies to enhance and facilitate the acquisition and analysis of 3D datasets obtained via focused ion beam-scanning electron microscope (FIB-SEM) slice-and-view tomography and x-ray ptychography. These include ex-situ metallic electrodeposition in polymeric systems (unit cell ~50-100 nm) as well as in-situ platinum electron-beam induced deposition (Pt-EBID) in porous biological systems (unit cell ~ 500 nm). Backfilling with a metal also preserves structural integrity by improving mechanical robustness and beam energy dissipation. Using such high-contrast backfilling materials greatly facilitates the automation of both image acquisition and post-processing, which enable precise structural analysis at different length-scales from large statistically significant volumes down to single repeating units, giving information not only on the general space-group and structural parameters but also quantifying distortions and defects, thus shedding light on the underlying assembly processes.

PROBING MOLECULES AT ATOMIC LEVEL AND BEYOND

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Resolving and manipulating the electronic structure of a single atom is of fundamental importance for understanding and predicting the chemical and physical properties of functional molecules. However, atomic level engineering on electronic structures has been challenging. Here, we report (1) a direct identification of two adjacent transition-metal atoms, Fe and Co, within phthalocyanine (FePc and CoPc) molecules; (2) the details of a single dative bond (between CO and FePc) breaking process using different atomic force microscope (AFM) tips mounted on a qPlus sensor.

Our combined experimental and theoretical results demonstrate that, for (1), AFM imaging reveals that the Co atom is brighter and presents four distinct lobes on the horizontal plane, whereas the Fe atom displays a “square” morphology. Pico-force spectroscopy measurements show a larger repulsion force of about 5 pN on the tip exerted by Co in comparison to Fe. We attribute the distinguishable features in AFM images and the variation in the measured forces to Co’s higher electron orbital occupation above the molecular plane (dz^2 , dxz and dyz); for (2), our results show that the C-Fe bond can be ruptured either by applying an attractive force of ~150 pN (Cu tip) or by a repulsive force of ~220 pN (CO tip) with a significant contribution of shear forces, accompanied by changes of the spin state of the system. Our work could provide a promising approach to characterizing and manipulating electronic structure of individual atoms within molecules using AFM.

CRYO-EM ANALYSIS OF A MEMBRANE PROTEIN EMBEDDED IN LIPSOME

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Membrane proteins (MPs) used to be the most difficult targets for X-ray crystallography. With the revolution of single-particle cryo-EM, rapid progress has been made for structural elucidation of isolated MPs. The next challenge is to preserve the electrochemical gradients and membrane curvature for a comprehensive structural elucidation of MPs that rely on these chemical and physical properties for their biological functions. Toward this goal, we present a convenient workflow for cryo-EM analysis of MPs embedded in liposomes. Combining optimized proteoliposome isolation, cryo-sample preparation on graphene grids, and an efficient particle selection strategy, the 3D reconstruction of AcrB embedded in liposomes was obtained at near-atomic resolution. Our approach, which can be widely applied to cryo-EM analysis of MPs with distinctive soluble domains, lays out the foundation for structural analysis of integral or peripheral MPs whose functions are affected by transmembrane electrochemical gradients or/and membrane curvatures.

SINGLE PARTICLE CRYO ELECTRON MICROSCOPY TO ELUCIDATE COLICIN-TOLC INTERACTION

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Bacteriocins are short proteins that bacteria excrete to kill closely-related strains. To learn more about how bacteriocins translocate through the outer membrane to kill target cells, we studied colicin E1 of *E. coli*, which engages the multidrug efflux pump TolC. We reconstituted the complex in nanodiscs using in-vitro-refolded TolC and determined the high-resolution cryoEM structure. To overcome throughput challenges associated with the standard optics of a 200 kV Talos Arctica cryoTEM, we retrofitted the instrument with a 20 μ m condenser aperture. This enabled us to acquire multiple images per hole despite the lack of a third condenser lens. Although aperture fringing is observable in micrographs, no defects are present in the reconstruction. The atomic model was refined in AMBER using the experimental density map as a force field, which yields a better model than traditional refinement protocols. We found that colicin E1 hinges open to plug the TolC channel, and we pinpointed intermolecular interactions (including key hydrogen bonds) between the channel and the colicin plug.

DESIGN OF AN ULTRAFAST PULSED PONDEROMOTIVE PHASE PLATE FOR CRYO-ELECTRON TOMOGRAPHY

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Ponderomotive phase plates have shown temporally consistent phase contrast is possible within electron microscopes via high fluence static laser modes resonating in Fabry-Perot cavities. Here, we explore using pulsed laser beams as an alternative method of generating high fluences. We find through forward-stepping finite element models that picosecond-or-less interactions are required for meaningful fluences and phase shifts, with higher pulse energies and smaller beam waists leading to the predicted higher fluences. An additional model based on quasiclassical assumptions is used to discover the shape of the phase plate by incorporating the oscillatory nature of the electric field. From these results, we find the transient nature of the laser pulses removes the influence of Kapitza-Dirac diffraction patterns that appear in the static resonator cases. The addition of a second laser aligned 90° to the first induces anisotropy to the shape of the phase plate. By incorporating a shifting-electron-beam algorithm, the effects of a finite electron beam crossover are also simulated. We conclude by predicting that a total laser pulse energy of 8.7 μJ is enough to induce the required $\pi/2$ phase shift for Zernike-like phase microscopy.

UNDERSTANDING STRAIN AND DEFORMATION IN 2D MATERIALS VIA 4D-STEM AND CRYO-EM

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Strain and deformations play a critical role in the mechanical, electrical, and chemical properties of two-dimensional (2D) materials due to their atomic thinness. This poster presentation will discuss using the state-of-the-art transmission electron microscopy (TEM) techniques to study the strain and deformations in 2D materials. First, the speaker will introduce the study of strain relaxation in epitaxial lateral heterojunctions of 2D transition metal dichalcogenides (TMD) using nano-beam four-dimensional scanning TEM (4D-STEM)¹. By developing an unsupervised learning approach, we were able to accelerate and automate the method for more general users (**Figure 1**)². In addition, the speaker will also present the direct visualization of the floppiness of 2D DNA origami using cryo-EM (**Figure 2**). The work uncovers flexible arms in 2D cross tiles, as well as clusters and stacks, which may potentially affect the assembly of DNA origami³.

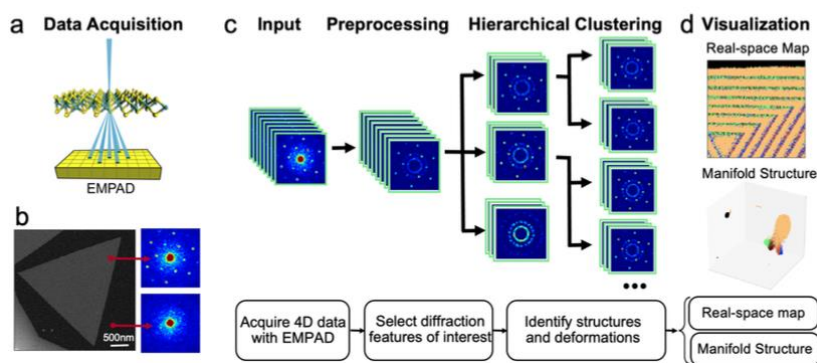


Figure 1. Uncovering strain and deformations in 2D TMDs using divisive hierarchical clustering.

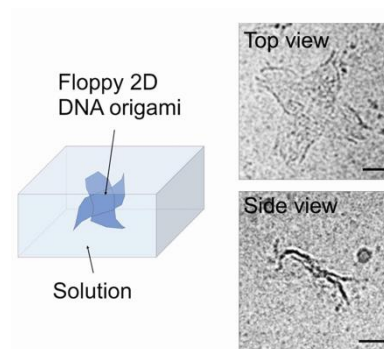


Figure 2. Visualizing floppy 2D DNA origami using cryo-EM.

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ISONET AND QUANTIFYING THE RELIABILITY OF RECOVERED INFORMATION

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Cryogenic electron tomography (cryoET) allows visualization of cellular structures in situ. However, anisotropic resolution arising from the intrinsic “missing-wedge” problem has presented major challenges in visualization and interpretation of tomograms. We developed IsoNet (Isotropic Reconstruction of Electron Tomography), a deep learning-based software package that iteratively reconstructs missing-wedge information and increases signal-to-noise ratio, using knowledge learned from raw tomograms. Without sub-tomogram averaging, IsoNet generates tomograms with significantly reduced resolution anisotropy and enables functional interpretation. Applications of IsoNet to cryoET data demonstrate greatly improved structural interpretability and identification of differently oriented complexes, as tested on tomograms of *Saccharomyces cerevisiae* 80S ribosomes. We also devise a novel mathematical method to quantify the reliability of recovered missing-wedge information. Such quantification provides a measure of the recovery quality of IsoNet and informs optimization of parameters.

STRUCTURAL STUDY ON BACTERIAL TRANSCRIPTION USING TIME-RESOLVED CRYO-EM

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Transcription is an RNA synthesis process that occurs in cells to express genes. Multi-subunit RNA polymerase (RNAP) is the main player of transcription and is highly conserved in all domains of life – prokaryotes, archaea, and eukaryotes. The 'Resolution Revolution' – technical advances in cryo-EM that greatly improved the EM map resolution – remarkably advanced the structural study of the RNAP complex because its crystallization is very challenging and limits the structural study by X-ray crystallography. Since the high-resolution cryo-EM became available to structural biologists, tens of the structures of the RNAP complexes in diverse contexts have been reported revealing crucial information on how RNAPs are regulated to fine-tune gene expression.

In particular, cryo-EM enables us to observe multiple states of RNAP structures in the same batch condition, revealing the complexes' dynamic properties. Often, these different structures are intermediates during the enzymatic reaction. Although those intermediates could be ordered from their structural features, capturing the complexes at specific time points would provide more accurate and detailed information on the structural changes occurring in the reaction process. Therefore, we aim to develop time-resolved cryo-EM using the 'mixing-spraying method' by microfluidic devices.

To produce more reproducible ice and consume less protein, we developed a thin-film microfluidic device that enables faster stabilization of spray and uniform droplet sizes, reducing the sample consumption significantly. Using this microchip, we obtained optimal droplet size distribution and ice thickness on the cryo-EM sample, which allowed precise imaging of the prokaryotic RNA polymerase and transient transcription elongation complexes.

STRUCTURAL BASIS OF TRANSCRIPTIONAL REGULATION BY A NASCENT RNA ELEMENT, HK022 *PUT*RNA

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Transcription, in which RNA polymerases (RNAPs) produce RNA from DNA, is the first step of gene expression. As such, it is highly regulated either by trans-elements like protein factors and/or by cis-elements like specific sequences on the DNA. Lambdoid phage HK022 contains a cis-element, *put*, which suppresses pausing and termination during transcription of the early phage genes. The *put*RNA transcript solely performs the anti-pausing/termination activities by interacting directly with the *E.coli* RNAP elongation complex (EC) by an unknown structural mechanism. In this study, we reconstituted *put*RNA-associated ECs and determined the structures using cryo-electron microscopy. The determined structures of *put*RNA-associated EC, *put*RNA-absent EC, and σ^{70} -bound EC suggest that the *put*RNA interaction with the EC counteracts swiveling, a conformational change previously identified to promote pausing and σ^{70} might modulate *put*RNA folding via σ^{70} -dependent pausing during elongation.

TWO-DIMENSIONAL (2D) NANOPORE ENGINEERING USING ABERRATION-CORRECTED SCANNING TRANSMISSION ELECTRON MICROSCOPY (AC-STEM)

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(Nanopores in thin membranes have facilitated a wide range of fundamental studies and applications related to DNA sequencing, protein sensing, water desalination, ion transport, and others. The ability to create small, sub-nanometer pores is crucial to increasing sensitivity in DNA sequencing devices and for engineering membranes for ion transport applications. In this work, we present detailed methods to controllably produce nanopores and vacancies down to the sub-nanometer scale in two-dimensional (2D) materials through aberration-corrected scanning transmission electron microscopy (AC-STEM) drilling. Through the proper choice of parameters, such as camera length and probe size, we optimize working conditions to efficiently create sub-nanometer pores in 2D MoS₂. We discuss the use of rapid thermal annealing (RTA) and in situ beam shower parameters used to mitigate contamination build-up at the site of the nanopore, allowing for the creation of cleaner nanopores. This work also details the use of atomic resolution electron energy loss spectroscopy (EELS) to monitor nanopore creation and size in real-time. Through these methods, we show that we can controllably create arrays of nanopores in MoS₂ of desired size, shape, and location. This work provides in detail the steps necessary to cleanly and controllably generate sub-nanometer pores in 2D MoS₂, laying the groundwork for the controllable engineering of sub-nanometer pores in this and other 2D materials.

KEEPING COOL DURING LIFT-OUT — AN ELEGANT SOLUTION FOR PREPARING SAMPLES IN CRYO-FIB

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Current advances in cryo-electron microscopy (cryo-EM) workflows are accelerating progress in many research fields: in materials investigations, cooling specimens halts aging, allows studying phase transitions, and enables new methods like cryo atom probe tomography. In life sciences, cryo-EM allows unprecedented structural investigation without denaturation. Some nanomaterials and biological molecules can be prepared for cryo-EM with minimal effort. Single cells can also be plunge frozen directly on TEM grids. However, bulk materials and high-pressure frozen samples require additional sample preparation. Lift-out preparation with cryo focused ion beam (cryo-FIB) is the method of choice because of its site-specificity. A lamella containing the region of interest is excavated, removed from the bulk with a manipulator, and then attached to a TEM grid for further thinning and observation.

In conventional FIB preparation, ion beam induced deposition of precursor gases is used to attach the lamella to the manipulator probe and the TEM grid. This process is slow, error-prone, and does not work at low temperatures. Instead, cryo-FIB uses a similar approach where gas is introduced into the microscope chamber to freeze the lamella to the probe and the TEM grid. In addition to poor reliability, both methods require frequent probe replacement since the probe tip has to be cut with the focused ion beam to release the lamella after lift-out. An alternative to IBID deposition is an actuated gripper that reversibly grabs the lamella for lift-out.

This work presents a novel piezo-actuated micro gripper that operates at cryogenic temperatures and the streamlined sample preparation workflow it enables.

DISCOVERY, STRUCTURE, AND FUNCTION OF FILAMENTOUS 3-METHYLCROTONYL-COA CARBOXYLASE

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3-methylcrotonyl-CoA carboxylase (MCC) is a biotin-dependent enzyme necessary for leucine catabolism in most organisms. While the crystal structure of recombinant bacterial MCC has been characterized, the structure and potential polymerization of native MCC remain elusive. Here, we used a bottom-up structural proteomics cryoEM approach (Ho et al., 2020; Pfab et al., 2021) to identify and determine the near-atomic resolution structure of native filamentous *L. tarentolae* MCC (LtMCC) in mitochondria enriched through streptavidin-coated magnetic beads. Using single-particle reconstruction, we obtained two differently centered cryoEM maps of the filament middle segments, one at 3.4 Å and another at 3.9 Å resolution. Additionally, we processed the termini particles and found that the two termini have the same structure, which was resolved at 7.3 Å resolution. Through direct structural comparison of the filament middle and termini maps, we discovered that LtMCC assemble into filaments by stacking LtMCC $\alpha_6\beta_6$ dodecamers. This stacking immobilizes the biotin carboxylase domains, sequestering the enzyme in an inactive state within the mitochondrial matrix. Our results support a new model for MCC catalysis, termed the dual-swinging-domains model, and cast new light on the functional significance of diverse modes of polymerization in the carboxylase superfamily and beyond.

OPERANDO TWO-TERMINAL DEVICES INSIDE A TRANSMISSION ELECTRON MICROSCOPE

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Advanced nanoelectronics materials are guided by their electric performance under operative conditions. A detailed understanding of the correlation between the electrical properties and the material structure at the nano and sub-nano meter scale is nowadays crucial in electronic industries. In this context, electrical biasing in situ transmission electron microscopy (TEM) using MEMS-based platforms is a promising technique for nano-characterization under operative conditions. However, electrical biasing experiments using MEMS-based platforms have been to date overshadowed due to stray leakage current paths formed during focused ion beam (FIB)-based sample preparation routines, leading to over-estimated electric responses and therefore, ambiguous in situ observations. Here, we undertook this problem by establishing a short-circuit-free FIB methodology that shows an improvement of at least five orders of magnitude in leakage current in comparison with the conventional-FIB approach and the reported values found in the literature. Our methodology enables current measurements as low as 10 pA, allowing realistic and reproducible biasing operations of various TEM lamella devices of oxide nanoelectronics.

STRATEGIES FOR LIMITING CONFORMATIONAL HETEROGENEITY IN SAMPLE PREPARATIONS OF AN INWARDLY RECTIFYING POTASSIUM CHANNEL FOR CRYO-EM.

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As automation of high throughput image acquisition and data processing routines have become standard it is recognized that sample preparation for cryo-EM can be the limiting factor for structure determination. Conformational variability poses a difficult challenge to overcome for high throughput structure determination by cryo-EM, severely limiting the attainable resolution. Regions of the biological molecule that display heterogeneous features are incorrectly aligned and averaged with other images causing the loss of coherent signal with regions showing the highest heterogeneity become blurred in the reconstruction, leading to less detailed maps. In apo preparations of the inwardly rectifying K⁺ (Kir) channel Kir7.1 we observed a continuum of conformations between open and closed states of the channel leading to low resolution reconstructions. Kir channels are an important class of K⁺ channels responsible for maintaining membrane potential and extracellular K⁺ concentrations. Kir channels conduct K⁺ ions on hyperpolarization, rather than on depolarization as in other K⁺ channels. Kir channels are embedded in the plasma membrane as a tetrameric assembly surrounding a central pore. Each subunit of the tetrameric Kir channel consists of two trans-membrane domains, a pore-forming loop that contains the selectivity filter and two cytoplasmic polar tails. K⁺ channels usually have three states: resting, activated, and inactivated. The channels are usually closed in the resting state, and opened after stimuli activation, followed by turning to the nonconductive states. Gating of the channel occurs at both intracellular and extracellular locations. Since the cytosolic domain is relatively independent from the pore-forming domain, it may adopt various conformations in the resting, activated, and inactivated states. We describe here biochemical approaches taken to limit conformational heterogeneity including targeted mutagenesis of key residues involved in gating of the channel and the use of channel activators and blockers to stabilize various conformations of the channel. We present here the high resolution cryo-EM structures determined using these approaches.

FAST CONTRAST ESTIMATION AND DENOISING OF CRYO-EM IMAGES

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The contrast of cryo-EM images varies from one to another, primarily due to the uneven thickness of ice layers. The variation of contrast can affect the quality of 2-D class averaging, 3-D ab-initio modeling, and 3-D heterogeneity analysis. Contrast estimation is currently performed during 3-D iterative refinement. As a result, the estimates are not available for class averaging and ab-initio modeling. However, these methods require good initial estimates of 3-D volumes and 3-D rotations of molecules. This work aims to jointly estimate the contrast, denoise the images, and correct the CTFs in the ab-initio stage, without estimating the 3-D volume. The key observation underlying our analysis is that the 2-D covariance matrix of the raw images is related to the covariance of the underlying clean images, the noise variance, and the contrast variability between images. We show that the contrast variability can be derived from the 2-D covariance matrix and use the existing Covariance Wiener Filtering (CWF) framework to estimate it. We also demonstrate a modification of CWF to estimate the contrast and approximately restore the clean images. Our method improves the contrast estimation by a large margin, compared to the previous CWF method. The more accurate contrast estimation also improves the quality of image denoising as demonstrated in both synthetic and experimental datasets. Moreover, we improve the speed of our algorithm by applying a recent method for Fourier-Bessel transform of images. As a result, our covariance estimation is several orders of magnitude faster than the previous CWF method, which breaks its computational bottleneck.

ACCELERATED CRYO-EM HETEROGENEITY ANALYSIS BY LOW RANK COVARIANCE ESTIMATION

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Covariance estimation is one of the earliest methods proposed for heterogeneity analysis in cryo-EM. It relies on computing the covariance of the conformations directly from projection images and extracting the optimal linear subspace of conformations through an eigendecomposition.

Unfortunately, the standard formulation is plagued by the exorbitant cost of computing the $N^3 \times N^3$ covariance matrix. We present a new low-rank estimation method that requires computing only a small subset of the columns of the covariance while still providing an approximation for the entire matrix. This scheme allows us to estimate tens of principal components of real datasets in a few minutes at medium resolutions and under 30 minutes at high resolutions. Furthermore, the method is automatically regularized and requires minimal user interaction. After the initial covariance computation, we can reconstruct conformational states from the latent space in real-time. We demonstrate this with a ChimeraX binding allowing users to interactively explore the latent space by generating and rendering volumes with a single click.

DEVELOPING INTERCONNECTED GRAPHENE-CARBON FIBER AEROGEL AND ITS POTENTIAL FOR WATER PURIFICATION

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Interconnected graphene aerogels have emerged as a new class of highly porous and ultra-lightweight materials exhibiting exceptional structural, electrical, electronic, and electrochemical properties offering a broad scope for applications such as energy storage, catalysis, water desalination/filtration and more. However, the conventional methods of synthesizing these materials are complicated and non-sustainable. We have developed a scalable and green approach to synthesizing a uniquely structured hierarchical graphene aerogel composed of 2D graphene sheets stretched across an underlying carbon fiber network. We have established insights into the surface structure and chemistry of the developed material and how it depends on the process conditions such as temperature, time, and the precursor protein. We have performed structural and chemical analyses of the developed materials and explored them for energy storage and water desalination/purification applications. Specifically, our studies have shown this material's ability to remove 99.99% of nano/microplastic contamination and reduce ionic levels by over 98% in seawater using gravity-based filtration, making it promising for water filtration/desalination applications.

MicroED FOR MACROMOLECULAR CRYSTAL STRUCTURE DETERMINATION

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Microcrystal electron diffraction (MicroED) has recently emerged as a promising method for macromolecular structure determination. The method complements existing structural biology methods by providing the opportunities to study small macromolecular structures (i.e. <50 kDa) from crystals too small for conventional single crystal X-ray diffraction. Several protein structures have been determined and various studies indicate that MicroED is capable of (i) revealing atomic structures with charges, (ii) solving new protein structures by molecular replacement, (iii) visualizing ligand-binding interactions and (iv) solving membrane protein structures from microcrystals embedded in lipidic mesophases. Recently, we solved two novel protein structures by MicroED and showed that it is feasible to use MicroED for structure-based drug discovery. However, comparing to X-ray diffraction, MicroED is still in its infancy. Further optimization and innovations in new software and hardware are required to make MicroED more robust and more accessible to the structural biology community. Here, I would like to present our latest development in specimen preparation, data collection and processing routine, as well as examples of macromolecular crystal structure determination by MicroED in our lab.

VISUALIZING VOLTAGE-INDUCED HALIDE REDISTRIBUTION IN MIXED-HALIDE PEROVSKITE DEVICES

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Metal halide perovskites are an emerging class of semiconductors with promising optoelectronic device prospects. One feature of these materials is that the optical bandgap can be easily tuned via halide ratio. However, some mixed-halide perovskites are not stable under operational conditions, including light illumination and voltage bias, and will undergo detrimental halide segregation that undermines the bandgap tunability. Voltage-induced halide segregation, which restricts applications of mixed-halide perovskites, is less investigated and the underlying mechanism has remained unclear. Herein, we conduct a systematic study on voltage-induced halide segregation using different metal electrodes and different voltage bias polarities, visualize the halide distribution after segregation by cross-sectional scanning transmission electron microscopy–energy dispersive X-ray spectroscopy (STEM-EDX), and reveal the electrochemical origin of halide segregation. Finally, we propose that a halide perovskite device can be considered as an electrochemical cell in terms of the stability behavior under voltage bias.

IN SITU STRUCTURAL BIOLOGY - OPTIMIZING CRYO-FIB LAMELLAS FOR SUB-5Å RESOLUTION

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In recent years, advances in the field have enabled exponential growth of determining macromolecular structures with cryoEM and many institutions are routinely employing single particle analysis (SPA) for structural biology. Cryo-electron tomography (CryoET), on the other hand, is less developed but has been recently increasing in popularity. Together with subtomogram averaging (STA) it offers a unique way of imaging biological complexes in a native environment, complementing SPA with missing contextual information. Sub-tomogram averages reaching sub-5 Å resolution are still an exception and confined to high symmetry proteins or viral particles, although several high-resolution structures do suggest that there is no fundamental barrier for employing cryoET for high resolution imaging.

Here we present a method based on metallic platinum sputtering on prepared lamella that can substantially enhance the quality of subtomogram averages. Using yeast ribosome as a target, we demonstrate the eukaryotic ribosome structure can be resolved to 5 Å and the large subunit can be further resolved to 3.5 Å with STA. We assess the effect of sputtering on achievable resolution by STA in FIB-milled samples by comparing lamellas with and without metallic platinum coating. Data from platinum-coated lamellas show significant reduction of the B-factor of sputter-coated lamellae by 42% in a sample with the same preparation method, data collection and processing, and of comparable data size.

We show that post-sputtering lamella samples reduces intrinsic charging and hence beam induced motion that is not easy to correct with motion correction algorithms. This approach allows reduction of the number of particles needed for high-resolution subtomogram averaging, which can position cryoET as a method of choice for elucidating biological structures in their native environment

Flash Talk Session A Time Slots

Wednesday, September 28

4:30 - 5:00 p.m.

Name	Title	Talk Slot
Agar, Joshua	AUTOMATIC 4D-STEM STRAIN MAPPING USING CYCLE-CONSISTENT SPATIAL TRANSFORMING AUTOENCODERS	4:30 - 4:32 p.m.
Cai, Shujun	IN SITU ARCHITECTURE OF THE LIPID TRANSPORT PROTEIN VPS13C AT ER-LYSOSOME MEMBRANE CONTACTS	4:32- 4:34 p.m.
Carmichael, Josh	ASPIRE: AN OPEN-SOURCE SOFTWARE PACKAGE FOR 3D MOLECULAR STRUCTURE DETERMINATION USING CRYO ELECTRON MICROSCOPY	4:34- 4:36 p.m.
Cheng, Guangming	REVEALING THE EXOTICS IN COMPLEX MATERIALS WITH ATOMIC RESOLUTION	4:36- 4:38 p.m.
Djehdi, Kenza	FACILE METALLIC BACKFILLING METHODS GREATLY ENHANCES 3D NANOSCALE IMAGING WITH ELECTRONS AND X-RAYS	4:38- 4:40 p.m.
Fan, Dingxin	PROBING MOLECULES AT ATOMIC LEVEL AND BEYOND	4:40- 4:42 p.m.
Fan, Xiao	CRYO-EM ANALYSIS OF A MEMBRANE PROTEIN EMBEDDED IN LIPOSOME	4:42- 4:44 p.m.
Firlar, Emre	SINGLE PARTICLE CRYO ELECTRON MICROSCOPY TO ELUCIDATE COLICIN-TOLC INTERACTION	4:44- 4:46 p.m.
Fitzpatrick, Anthony W.P.	DESIGN OF AN ULTRAFAST PULSED PONDEROMOTIVE PHASE PLATE FOR CRYO-ELECTRON TOMOGRAPHY	4:46- 4:48 p.m.
Han, Yimo	UNDERSTANDING STRAIN AND DEFORMATION IN 2D MATERIALS VIA 4D-STEM AND CRYO-EM	4:48- 4:50 p.m.
Hu, Jason J.	ISONET AND QUANTIFYING THE RELIABILITY OF RECOVERED INFORMATION	4:50- 4:52 p.m.

Flash Talk Session B Time Slots

Thursday, September 29

4:30 - 5:00 p.m.

Name	Title	Talk Slot
Booth, Chris	RECENT DEVELOPMENT IN DIRECT DETECTION TECHNOLOGY - COUNTING LOW ENERGY ELECTRONS (60 – 200 kV)	4:30 - 4:32 p.m.
Hwang, Seungha	STRUCTURAL BASIS OF TRANSCRIPTIONAL REGULATION BY A NASCENT RNA ELEMENT, HK022 PUTRNA	4:32- 4:34 p.m.
Lechner, Lorenz	KEEPING COOL DURING LIFT-OUT - AN ELEGANT SOLUTION FOR PREPARING SAMPLES IN CRYO-FIB	4:34- 4:36 p.m.
Lee, Jane K.J.	DISCOVERY, STRUCTURE, AND FUNCTION OF FILAMENTOUS 3-METHYLCROTONYL-COA CARBOXYLASE	4:36- 4:38 p.m.
Molina-Luna, Leopoldo	OPERANDO TWO-TERMINAL DEVICES INSIDE A TRANSMISSION ELECTRON MICROSCOPE	4:38- 4:40 p.m.
Peisley, Alys	STRATEGIES FOR LIMITING CONFORMATIONAL HETEROGENEITY IN SAMPLE PREPARATIONS OF AN INWARDLY RECTIFYING POTASSIUM CHANNEL FOR CRYO-EM.	4:40- 4:42 p.m.
Shi, Yunpeng	FAST CONTRAST ESTIMATION AND DENOISING OF CRYO-EM IMAGES	4:42- 4:44 p.m.
Singer, Amit	ACCELERATED CRYO-EM HETEROGENETIY ANALYSIS BY LOW RANK COVARIANCE ESTIMATION	4:44- 4:46 p.m.
Wani, M. Shaharyar	DEVELOPING INTERCONNECTED GRAPHENE-CARBON FIBER AEROGEL AND ITS POTENTIAL FOR WATER PURIFICATION	4:46- 4:48 p.m.
Xu, Hongyi	MICROED FOR MACROMOLECULAR CRYSTAL STRUCTURE DETERMINATION	4:48- 4:50 p.m.
Xu, Zhaojian	VISUALIZING VOLTAGE-INDUCED HALIDE REDISTRIBUTION IN MIXED-HALIDE PEROVSKITE DEVICES	4:50- 4:52 p.m.
Zaoralová, Magda	IN SITU STRUCTURAL BIOLOGY - OPTIMIZING CRYO-FIB LAMELLAS FOR SUB-5Å RESOLUTION	4:52- 4:54 p.m.

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Xu, Hongyi	23
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Delegate List

(Registrants up to September 16, 2022)

*The delegates below opted-in to be included on this list

Adawi, Hayat
Princeton University

Agar, Joshua
Drexel University

Aggarwal, Shradha
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Bals, Sara
EMAT-University of Antwerp

Batson, Philip
Rutgers University

Bazazi, Parisa
Princeton University

Booth, Christopher
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Princeton University

Cai, Shujun
Yale School of Medicine

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Princeton University

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Chen, Pengcheng
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Chen, Xiaobing
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Damico, Kevin
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Djehdi, Kenza
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Princeton University

Eatmon, Yannick
Princeton University

Ergun, Sabrina
Princeton University

Fan, Dingxin
Princeton University

Fan, Xiao
Princeton University

Firlar, Emre
Rutgers University

Fitzpatrick, Anthony
Columbia University in New York City

Franklin, Eric
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Fu, Hao
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Hughson, Fred
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Hummer, Gerhard
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Jaber, Nora
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Kalra, Aarat
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Keneipp, Rachael
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Kihara, Daisuke
Purdue University

Kotecha, Abhay
Thermo Fischer Scientific

Kulczyk, Arek
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Langfield, Christopher
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Muller, David
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Myers, Benjamin
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Olsen, Gregory
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Park, Jong-Duk
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IAC, Princeton University

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Schreiber, John
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Seyedsayamdost, Mo
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Shao, Paul
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Shi, Yunpeng
Princeton University

Shirkey, Jaden
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Zeiss Group

Wan, Alan
Eurofins | EAG Laboratories

Wani, Mohd Shaharyar
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Xie, Saien
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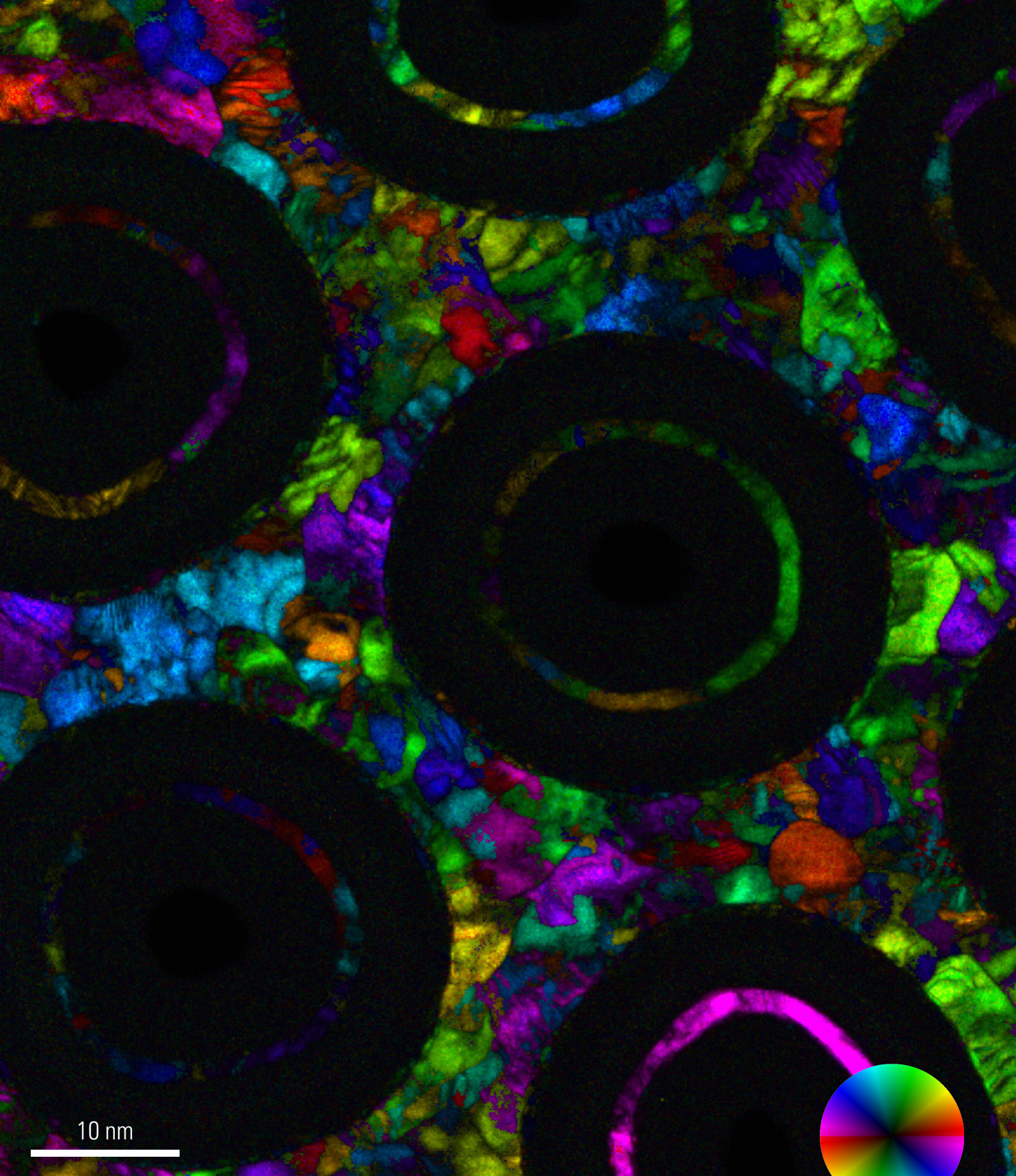
Zhang, Peijun
University of Oxford and Diamond Light
Source

Zhao, Kewei
Princeton University

Zhong, Ellen
Princeton University

Zhou, Hong
University of California, Los Angeles

Zou, Xiaodong
Department of Materials and
Environmental Chemistry,
Stockholm University



10 nm

Capture 4D STEM data with sharper detail and less background noise

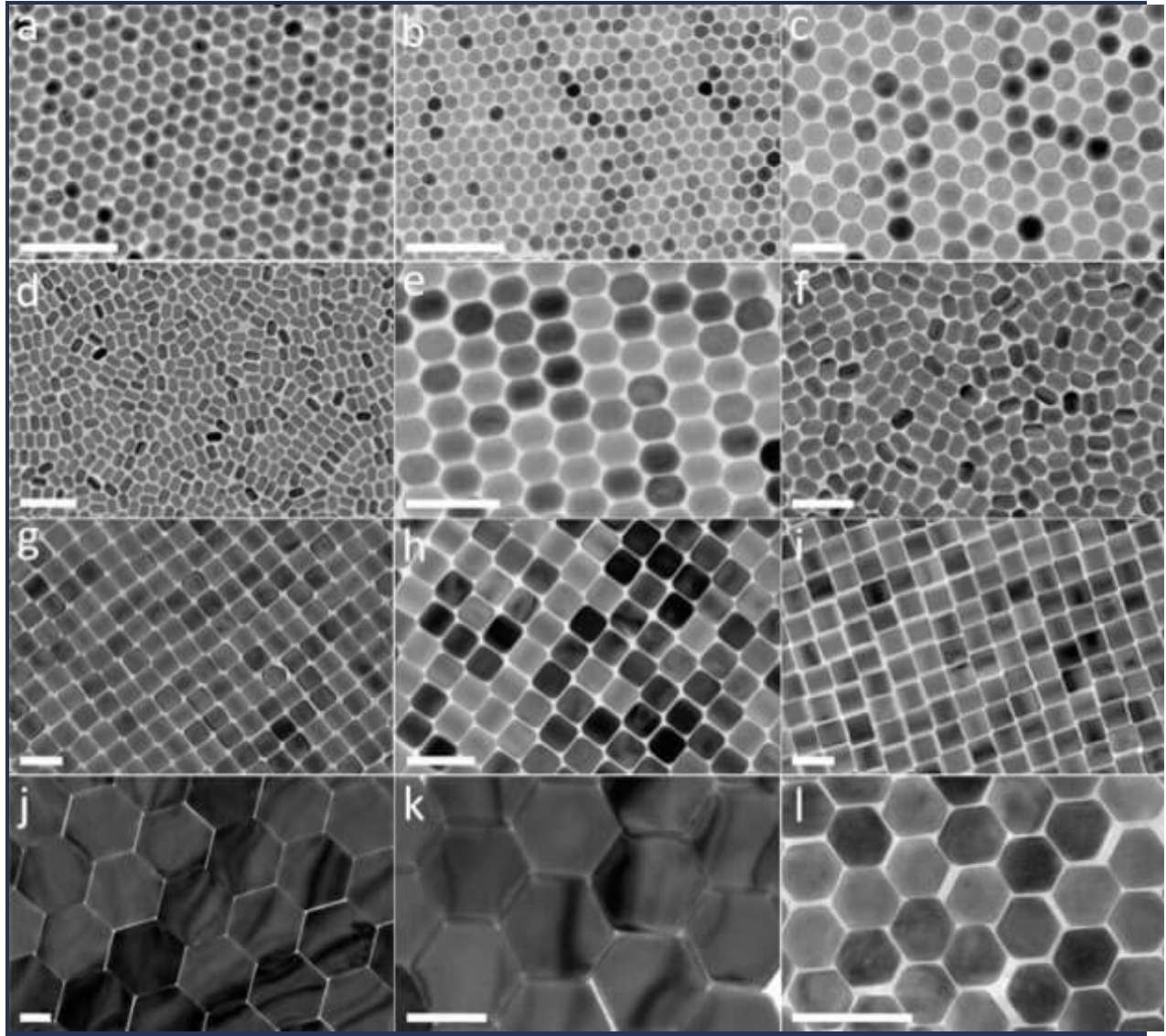
4D STEM collected with the Metro™ counting camera and STEMx® from a 3D NAND sample. Data was processed via a Python script in DigitalMicrograph® to produce a map of crystalline regions.

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