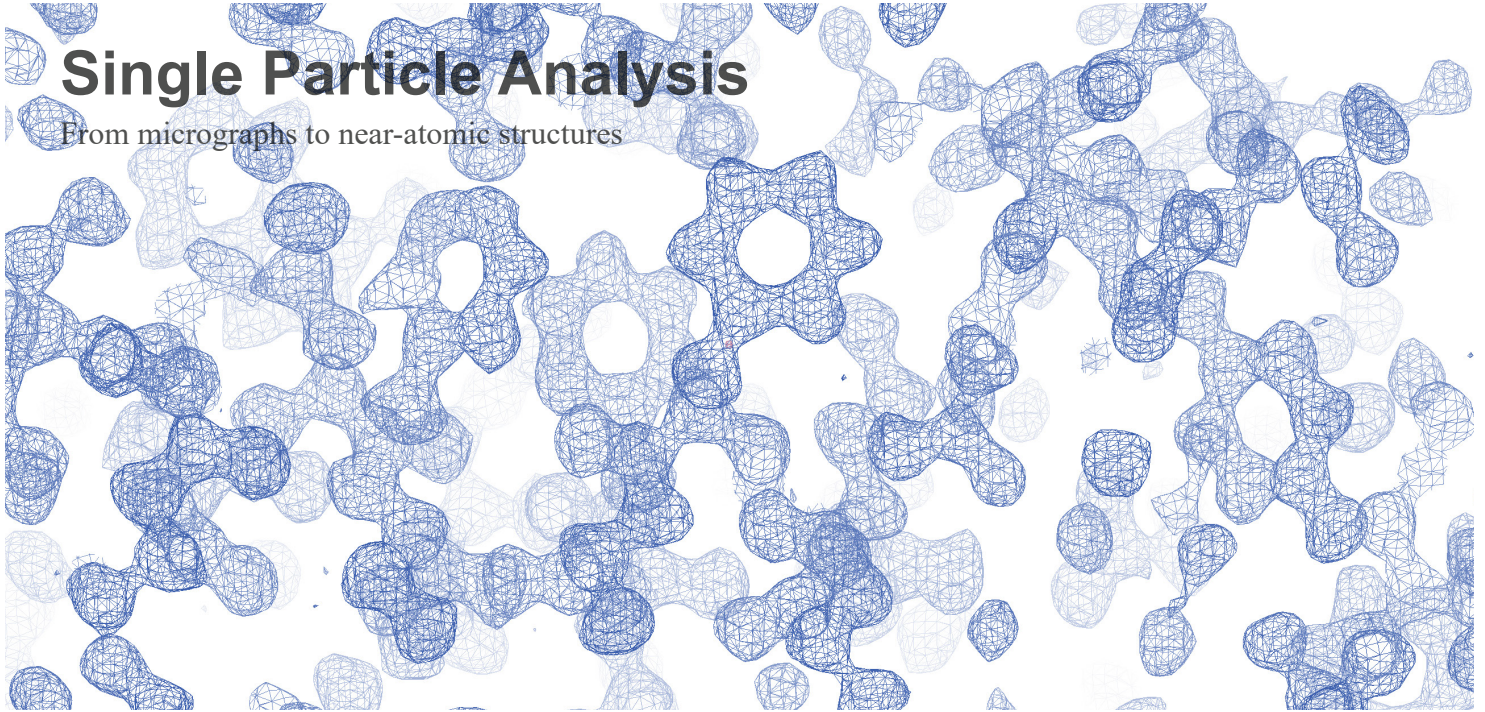


# LIFE SCIENCE

NEWSLETTER OF THE UNIVERSITY RESEARCH FACILITY IN LIFE SCIENCES, THE HONG KONG POLYTECHNIC UNIVERSITY | IS11 | SPRING 2024

## Single Particle Analysis

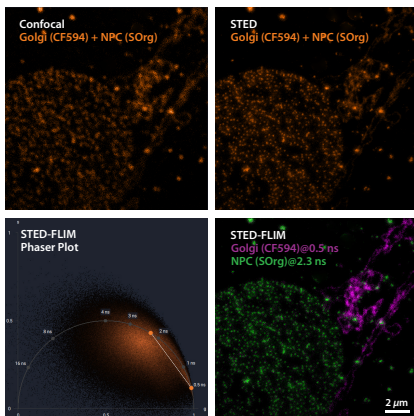
From micrographs to near-atomic structures



### NEW AT THE ULS

#### MATRIX Detector on the Abberior STED SR Microscope

The ULS has recently installed a new MATRIX detector on our Abberior STED Super-resolution Microscope. The new detector is an array-type avalanche photodiode detector (APD) consisting of more than 20 single-photon-counting APDs in a hexagonal arrangement. Background signals can be suppressed using physical principles instead of image postprocessing. In addition, the new detector enables researchers to perform fluorescence lifetime imaging microscopy (FLIM) in combination with STED (*i.e.*, STED-FLIM) or confocal microscopy.



STED-FLIM imaging using the new MATRIX detector.

#### Refeyn Two<sup>MP</sup> Mass Photometer

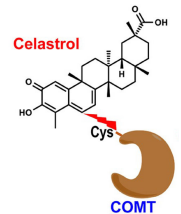
The ULS has acquired a Refeyn Two<sup>MP</sup> Mass Photometer that allows researchers to perform highly sensitive, rapid, and label-free molecular mass measurement of such biomolecules as proteins and DNAs in their native states. The equipment adopts a light scattering-based technique, and is able to detect mass that ranges from 30 kDa to 5 MDa at high resolution. The Refeyn system is suitable for a wide range of applications, such as the study of protein-protein interactions, determination of protein oligomerisation states, and assessment of sample integrity and homogeneity, *etc.* Only a few microliters of sample solution is required and the assay is compatible with a variety of buffers.

#### Zeiss V16 Zoom Microscope with Apotome 3

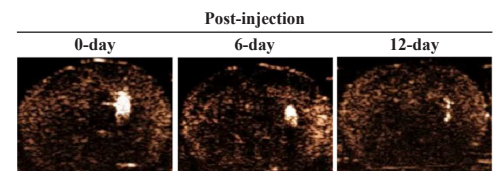
The new Zeiss V16 Zoom Microscope is equipped with multiple high-quality objective lenses for high-resolution fluorescence imaging of relatively large biological samples. In combination with the 16× internal zoom optics, the microscope is able to deliver a magnification range from 3.5× to 258× (when viewed through the eyepieces). The Apotome 3 module offers optical sectioning using structured illumination, which enhances signal-to-noise ratio and allows 3D rendering from thick specimens.

### POLYU RESEARCH

1. Using the Agilent 6540 Quadrupole-TOF LC/MS at the ULS, Dr Zhao Qian's group (ABCT) identified COMT as a major binding target of the natural product celastrol, which inhibits the enzymatic activity of the former and increases the dopamine level in neuroendocrine chromaffin cells. Their study sheds lights on the development of drugs to treat neurological disorders.



Above: Binding of celastrol to COMT via a cysteine residue inhibits enzymatic activities of the latter. *ACS Chem. Biol.* 17, 2003 (2024). Below: Injected PEGylated gas vesicles (nanobubbles) were shown to be stable in the mouse brain for several days, as revealed by ultrasound imaging. *Nat. Comm.* 15, 2253 (2024).



2. Prof. Sun Lei's group (BME) has developed a novel acoustic nanobubble-mediated ultrasound (US) stimulation approach to precisely stimulate specific regions of the mouse brain non-invasively. Modulation of various neural circuits has been demonstrated; the mice displayed distinct and predictable behaviour or attenuation of calcium signalling, *etc.*, in response to US stimulation. The Fujifilm US/PA Imaging System, IVIS *in vivo* Imaging System, Leica SP8 MP/Confocal and Nikon Ti2-E Microscopes were used in this study.

## SINGLE PARTICLE ANALYSIS

*From micrographs to near-atomic structures*

In the previous newsletter issue, we gave a brief introduction of the cryo-electron microscopy (cryo-EM) technique, and the support on single-particle analysis (SPA) provided by the newly established Cryo-EM Centre at the ULS. In this issue, we delve deeper into SPA and see how it has revolutionised and accelerated the understanding of protein structures in the recent decade.

Following plunge freezing, the vitrified sample would contain individual protein molecules trapped in a thin layer of amorphous ice in a sample grid. After loading into the microscope, the sample would be subjected to low-dose electron beam during imaging. Electron images are captured using a direct detection device (DDD) camera (Fig. 1a). In contrary to such conventional imaging media as photographic film or charge-coupled device (CCD) cameras, DDD cameras offer higher detective quantum efficiency (DQE) than CCD cameras, and much higher image throughput than using film. To reduce beam-induced motion, electron images are captured by the DDD camera as short movies to allow motion correction to be performed in order to form sharp images. The latest-generation Falcon 4i DDD cameras on our Krios G4 and Glacios Cryo-TEM Systems can deliver significantly higher DQE and frame rate, as well as shorter overhead acquisition time than the previous generation, resulting in a shorter data collection time and better resolution.

Since hundreds of particles are being captured in each electron micrograph, and each particle may have a different orientation, it is necessary to identify and extract images of individual particles for further classification in 2D. Particle picking may be performed

automatically or semi-automatically. The goal of 2D classification is to compare particles against each other and group similar particles into multiple classes (Fig. 1b) to facilitate stack cleaning and removal of the so-called junk particles. These are important steps in ensuring only particles of good quality are included for subsequent computational analysis and 3D reconstruction.

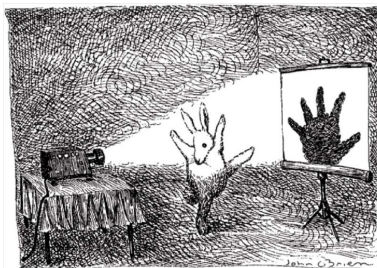


Figure 2. An illustration of the “projection problem” by John O’Brien in *The New Yorker* in 1991. A 3D object (a dancing rabbit in this example) may appear as something else (a hand) in the 2D projected image.

The selected particle classes are essentially 2D projections of the original protein molecule in 3D (Fig. 2). Since their orientations differ and are unknown, the determination of such is usually done by comparing with a reference 3D model of a similar protein. 3D reconstruction is an iterative process; each cycle serves as the reference for the subsequent one. The accuracy of the orientation and thus the resolution of the density map would improve when the cycle repeats, up to a point where further iterations do not enhance these further. This yields the final 3D reconstructed model at near-atomic resolution (Fig. 1c).

Single-particle cryo-EM is a game-changer in structural biology that allows researchers to examine some of the tiniest machines of life in action, in their native environments, and in great detail. It is without doubt a golden era to utilise such tool to make important discoveries that will help elucidate the molecular basis of life and disease.

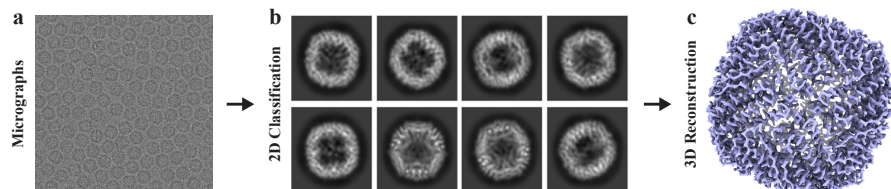


Figure 1. (a) In a typical single-particle cryo-EM experiment, hundreds of electron micrographs of the vitrified protein sample (apoferritin in this example) are captured by a DDD. (b) 2D classification is then performed to group similar particles into classes. (c) Particle orientation is determined by such algorithms as projection matching to reconstruct the final high-resolution 3D model.

## COMING SOON

An NVIDIA A800 GPU Cluster will be available at the ULS Cryo-EM Centre in Q3 2024 to support demanding image processing tasks following cryo-EM or cryo-tomography data collection.

## GET IN TOUCH

- Z215/Y208, PolyU
- polyu.edu.hk/uls
- uls.notice@polyu.edu.hk



## ULS EQUIPMENT AT A GLANCE

### Mass Spectrometry

- Bruker AmaZon Speed Ion Trap-ETD MS
- Bruker UltrafleXtreme MALDI-TOF/TOF MS
- Agilent 6460 Triple Quadrupole LC/MS
- Agilent 6540 Quadrupole-TOF LC/MS
- SCIEX 6500+ QTrap LC/MS
- ThermoFisher Orbitrap IQ-X LC/MS
- Waters UPLC with QDa Mass Detector

### Cryo-Electron Microscopy

- ThermoFisher Krios G4 300 kV Cryo-TEM System
- ThermoFisher Glacios 200 kV Cryo-TEM System
- ThermoFisher Talos L120C 120 kV TEM System
- ThermoFisher Aquilos 2 Cryo-FIB System
- ThermoFisher Vitrobot Mark IV

### Fluorescence Microscopy

- Abberior STED Super-resolution Microscope
- Leica SPE Confocal Microscope
- Leica SP8 Multiphoton/Confocal Microscope
- Nikon Ti2-E Live-cell Imaging System
- Nikon SIM/STORM/A1 SR/Confocal Microscope
- Nikon SMZ1270i Fluorescence Stereomicroscope
- Nikon AX R MP Upright Multiphoton Microscope
- Zeiss Lightsheet 7 Microscope
- Zeiss Lattice Lightsheet 7 Microscope
- Zeiss V16 Zoom Microscope with Apotome 3

### Preclinical Animal Research

- Bruker BioSpec 70/20 USR MRI System
- Bruker LF90II Body Composition Analyser
- Bruker SkyScan 1276 *in vivo* Micro-CT Scanner
- PerkinElmer IVIS *in vivo* Imaging Systems
- Fujifilm Vevo LARZ US/PA Imaging System
- Promethion Metabolic Cage System

### Cell and Molecular Biology

- BD FACSAria III Cell Sorter
- BD FACSymphony A3 Cell Analyser
- BD Accuri C6/FACSVia Cell Analysers
- Roche LightCycler II qPCR System
- Applied Biosystems QS 5/7 Flex qPCR Systems
- Seahorse XF<sup>24</sup> Extracellular Flux Analyser
- Logos X-CLARITY Tissue Clearing System
- Invitrogen Countess II FL Auto Cell Counter

### Biochemical Analysis

- Bio-Rad Bio-Plex 200 Suspension Array System
- Bruker Sierra SPR-32 Pro Analyser
- Jasco J-1500 Circular Dichroism Spectrometer
- Jasco CPL-300 CPL Spectrometer
- Malvern MicroCal Automatic ITC System
- Refeyn Two<sup>MP</sup> Mass Photometer
- Tecan Automatic Liquid Handling System

### Genomic Science

- Agena MassARRAY Analyser 4 System
  - Agilent 2100 Bioanalyser System
  - Covaris ME220 Focused-ultrasonicator
  - 10× Chromium iX Single Cell Analysis System
  - Illumina MiSeq NGS System
  - Illumina NextSeq 2000 NGS System
  - Nanopore GridION Mk1 Sequencing System
- New in 2024 • Upgraded in 2024