LIFE SCIENCE

NEWSLETTER OF THE UNIVERSITY RESEARCH FACILITY IN LIFE SCIENCES, THE HONG KONG POLYTECHNIC UNIVERSITY / IS12 / FALL 2024

Another 10 Times Finer

Nobel Laureate Stefan W. Hell Shares His Latest Recipe for Doughnut-making with PolyU

ANOTHER 10 TIMES FINER

The ULS has been offering researchers access to structured illumination microscopy (SIM), stochastic optical reconstruction microscopy (STORM), and stimulated emission depletion (STED) microscopy. Although each of these super-resolution fluorescence microscopy (SRM) techniques has its own advantages and shortcomings, together they allow our users to image a wide range of biological samples at resolution surpassing the diffraction limit.

To further promote the use of SRM, the ULS has invited Prof. Stefan Hell, Nobel Laureate in Chemistry and inventor of STED microscopy to visit PolyU in November 2024, and to share his latest innovations in SRM for biological applications **(Fig. 1 and cover image)**. In STED microscopy, a doughnut-shaped depletion laser beam is used to "switch off" fluorophores everywhere, except at the centre, allowing molecules there to be excited by an additional excitation beam. Although remarkable resolution (~20 nm) can be achieved with STED, localisation precision is still hindered by the rather limited "emission budget".

Figure 1. Prof. Stefan Hell visits the ULS Microscopy Suite housing the first Abberior FACILITY STED Nanoscope in Hong Kong.

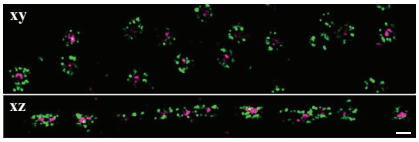
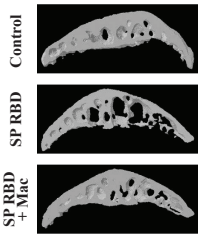


Figure 2. Dual-channel MINFLUX imaging of nuclear pore complexes in lateral (xy) and axial (xz) axes. Adapted from *Nat. Methods* **17**, 217 (2020). Scale bar is 100 nm.

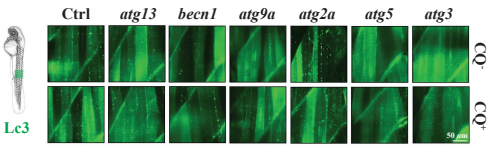
With the MINFLUX technique recently developed by Prof. Hell's research team, a doughnut-shaped beam is now used to scan across the focal plane to trace a single photon by targeting the latter with its centre (or "zero"). Thus, the location of the fluorophore would perfectly coincide with the excitation zero. Since the position of the beam is known, any resulting fluorescence would indicate the distance from the molecule to the zero. Localisation can therefore be precisely achieved without eliciting much emission. In principle, MINFLUX is not limited by such constraints as excitation/emission wavelengths, numerical aperture of the objective and orientation of the fluorophore. It has been demonstrated that true nanometer resolution (~2 to 3 nm) can be achieved in both the lateral and axial axes in living cells **(Fig. 2)**.

POLYU RESEARCH

1. Using rodent models infected with variants of SARS-CoV-2, Dr Chunyi Wen's group (BME) demonstrated that the endothelin receptor antagonist macitentan could alleviate chondrocyte senescence and subchondral bone loss, offering insights into treatment for post-COVID arthritis. Our Bruker Preclinical Micro-CT and MRI Scanners were used in this study.



Above: Micro-CT scans showing the rescue of SP RBD-induced bone loss in mice by macitentan. *Nat. Microbiol.* **9**, 2538 (2024). *Below*: Autophagosomes (Lc3$^+$ puncta) in the muscle of various *atg* mutant zebrafish embryos with (+) or without (-) chloroquine (CQ) treatment. *Autophagy* **20(4)**, 830 (2024).



2. Dr Alvin Ma's group (HTI) employed the zebrafish model to investigate the distinct functions of and intricate interplay amongst 6 core *atg* (macroautophagy/autophagy-related) genes in the regulation of autophagy and definitive haematopoiesis using both CRISPR-Cas9 and morpholino targeting. The results highlight the importance of *atg* genes in vertebrate definitive haematopoiesis. Our Zeiss Lightsheet 7 Microscope, Leica SPE Confocal Microscope and BD FACSAria III Cell Sorter were used in this study.

QUALITY IN, QUALITY OUT

Mastering macromolecule purification with gradient fixation

Previously, we introduced the comprehensive support provided by the ULS Cryo-EM Centre on single-particle analysis (SPA) for solving protein structures at near-atomic resolution. Since SPA relies primarily on computational averaging of hundreds of thousands of identical particles, having a homogenous sample is crucial for simplifying the workflow and enhancing the final resolution. Although classical purification methods like gel filtration chromatography can separate macromolecules based on their sizes, in practice, separating more complex proteins with multiple subunits poses challenges to these methods. For example, macromolecules are often exposed to various surfaces, disrupting their 3-dimensional structures or even leading to partial degradation. The resulting structural heterogeneity often has a negative effect on the subsequent determination of high-resolution structure by SPA.

The gradient fixation (GraFix) method was introduced in the late 2000s for the purification of homogenous, unbound macromolecules in their native states **(Fig. 1)**. Chemical crosslinking is a way to stabilise large and/or fragile protein complexes. However, chemical crosslinkers, such as glutaraldehyde or formaldehyde, often promote intermolecular, rather than intramolecular crosslinking, resulting in aggregation, sample heterogeneity

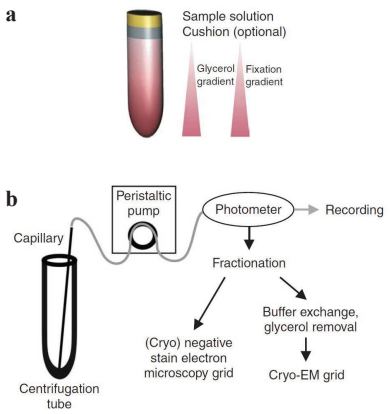


Figure 1. Overview of the GraFix method. **(a)** The centrifuge tube contains gradients of the glycerol (creating the density) and chemical crosslinker. Sample solution and an optional cusion buffer are added over the gradient. **(b)** Gradients are fractionated from the bottom to the top following ultracentrifugation. Adapted from *Nat. Methods* **5(1)**, 53 (2008).

or even sample loss due to precipitation. The GraFix method combines the use of a weak crosslinking agent and centrifugal force acting on the macromolecules. The former promotes intramolecular crosslinking, and latter disrupts weak intermolecular crosslinking. It has been demonstrated that the vast majority of macromolecules would preferentially undergo intramolecular crosslinking if the sample concentration is not exceedingly high.

Another important advantage of using the GraFix method is that the chemical crosslinker is added to a density gradient, instead of the sample directly. As such, the buffer environment for the macromolecules is replaced before the latter come into contact with the crosslinker. This prevents components in the sample buffer from reacting with the crosslinker, as well as potential sample loss due to an extra buffer exchange step.

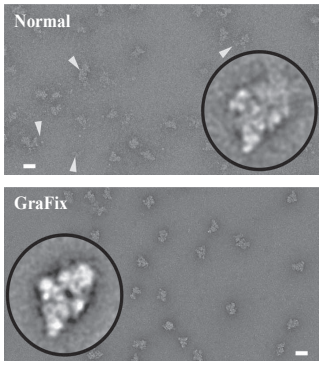


Figure 2. Negative-stained electron images of spliceosomes purified by conventional and the GraFix methods. Scale bars are 40 nm. Adapted from *Nat. Methods* **5(1)**, 53 (2008).

GraFix-purified samples may directly undergo negative staining and then observation *via* transmission electron microscopy (TEM). Due to a significantly reduced amount of fragmented particles, these samples typically show better contrast and particle dispersion, and cleaner background in the TEM image **(Fig. 2)**. For SPA, an extra step is needed to remove glycerol in the buffer using a buffer exchange column, since the presence of a high concentration of glycerol is known to deteriorate image contrast following sample vitrification. The benefits of adopting GraFix have been widely demonstrated. The ULS offers full support on this workflow with our new Biocomp Gradient Master 108 Gradient Mixer and other equipment. Mastering macromolecule purification has never been easier.

COMING SOON

With the new ThermoFisher Orbitrap QE Plus LC/MS, an MS platform will be established and operated complementarily with our cryo-EM platform to facilitate structural biology-related research at PolyU.

GET IN TOUCH

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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
- HPF, Micropatterning & Live-CLEM Workstation

Fluorescence Microscopy
- Abberior STED Super-resolution Microscope
- Leica SPE Confocal Microscope
- Leica SP8 Multiphoton/Confocal Microscope
- Nikon T*i*2-E Live-cell Imaging System
- Nikon SIM/STORM/A1 SR/Confocal Microscope
- Nikon SMZ1270i Fluorescence Stereomicroscope
- Nikon AX R MP Upright Microscope w/ NSPARC
- 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 XFe24 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 TwoMP 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