# Cryogenic Electron Microscopy in Structural Biology

16th – 19th August 2022

In-person / hybrid event, signup on Eventbrite required

*Organisers: Kyle Morris (eBIC) & Anna Rackley (eBIC) & Emily Baird (DLS)*

## Day 1: Tuesday

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| 09:30–09:45 | Arrival at **RAL reception (R75)** |
| 09:45–10.00 | Introductions and welcome in **G59** – **Martin Walsh (Diamond Light Source)** |
| 10:00–10:30 | **Lecture 1:** Why cryogenic electron microscopy (cryo-EM)? – **Kyle Morris (eBIC)** |
| 10:30–11:15 | **Lecture 2**: The electron microscope – **Karen Davies (eBIC)** |
| 11:15–11:30 | **Coffee break (G59)** |
| 11:30–12:15 | **Lecture 3**: Sample preparation for cryo-EM – **Dimple Karia (Thermo Fisher Scientific)** |
| 12:15–12:45 | **Lunch break (R22)** |
| 12:45–13:00 | Health and safety talk |
| 13:00–16:00 | **Practical 1**: Cryogenic sample preparation for cryo-EM – **Peter Harrison (eBIC)** |

## Day 2: Wednesday

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|---|---|
| 09:30–10:15 | **Lecture 4**: Image formation in the electron microscope – **Dan Clare (eBIC)** |
| 10:15–11:00 | **Lecture 5**: Image processing theoretical introduction – **Colin Palmer (CCP-EM)** |
| 11:00–11:15 | **Coffee break (G59)** |
| 11:15–12:00 | **Lecture 6:** From 2D images to 3D density maps in SPA – **Yuriy Chaban (eBIC)** |
| 12:00–13:00 | **Lunch break (R22)** |
| 13:00–16:00 | **Practical 2**: Screening samples in cryo-EM **– Peter Harrison (eBIC)** |

## Day 3: Thursday

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|---|---|
| 09:30–10:15 | **Lecture 7**: From 2D images to 3D density maps in cryo-ET – **Lindsay Baker (Oxford)** |
| 10:15–11:00 | **Lecture 8**: Averaging and reconstructing 3D maps in cryo-ET – **Pranav Shah (Oxford)** |
| 11:00–11:15 | **Coffee break (G59)** |
| 11:15–12:00 | **Lecture 9**: Fitting and building of atomic models – **Agnel-Praveen Joseph (CCP-EM)** |
| 12:00–13:00 | **Lunch break (R22)** |
| 13:00–16:00 | **Practical 3**: Single-Particle Averaging data collection demonstration – **Vinod Vogirala (eBIC)** |

## Day 4: Friday

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|---|---|
| 09:30–12:30 | **Practical 4**: Cryo-ET data collection demonstration – **Davide Zabeo (eBIC)** |
| 12:30–13:30 | **Lunch break (R22)** |
| 13:30–14:10 | Research talks by eBIC PDRA & PhD students **(I14 1-10)** |
| 14:10–14:30 | **Coffee break (I14 1-10)** |
| 14:30–15:30 | DTP student presentations: Student learning & feedback talks **(I14 1-10)** |
| 15:30–16:00 | Wrap-up Q&A |

## End of course

**Lectures will be held in Diamond House G59 & Zoom. Research talks will be held on Zoom only.**

**Practical sessions held in I14 are for the 1st year WT DTP students only.**
**DTP student presentations are for the 1st year WT DTP students, lecturers, instructors and organisers.**

# Practical Curriculum

**7 students – suggested groups of 2, 2 & 3: One hour per group in person, other groups in I14 1-10 watching via zoom, groupings may be subject to change at you eBIC instructor's discretion.**

The curriculum below places emphasis on teaching over training. Thus, microscopes will be loaded and already prepared for imaging. Alignment and camera configuration outside of TFS software will not be covered. Pre-prepared standard specimens will be loaded for microscopy.

## Day 1: Tuesday – G27 and I14 1-10

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|---|---|
| 12:45–13:00 | Health and safety talk – Kyle Morris (eBIC) |
| 13:00–16:00 | **Practical 1**: Cryogenic sample preparation for cryo-EM – **Peter Harrison (eBIC)** |
| | *Discuss:* ***Do*** |
| | *Cryogen properties and their role:* ***cooling ethane*** |
| | *Grid surface structure:* ***grid inspection by eye or under light microscope*** |
| | *Grid surface properties:* ***glow discharge*** |
| | *Blotting process:* ***dry run*** |
| | *Blotting parameters:* ***wet run*** |
| | *Contamination free best practice:* ***grid transfer and storage*** |
| | *Discuss clipping, autoloader and loading:* ***demonstration of loading with prepared grids (3.30)*** |

## Day 2: Wednesday – Krios IV – Control room (+big screen) and I14 1-10

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|---|---|
| 13:00–16:00 | **Practical 2**: Screening samples in cryo-EM **– Peter Harrison (eBIC)** |
| | *Discuss:* ***Do*** |
| | *Grids are loaded from the cassette onto the microscope column:* ***load a grid*** |
| | *What we expect to see at low-medium magnifications:* ***acquire images using presets*** |
| | *Grid quality:* ***collect an atlas*** |
| | *Ice quality, thickness, homogeneity:* ***acquire hole eucentric images using presets*** |
| | *Particle distribution, aggregation, carbon adherence:* ***acquire data acquisition images using presets*** |
| | *How much data will a grid produce:* ***calculate how many squares are needed for 16 hrs collection*** |

## Day 3: Thursday – Krios IV – Control room (+big screen) and I14 1-10

| | |
|---|---|
| 13:00–16:00 | **Practical 3**: Single-Particle Averaging data collection demonstration – **Vinod Vogirala (eBIC)** |
| | *What is eucentric height:* ***calibrating eucentric*** |
| | *Effect of defocus:* ***acquisition of manual exposure at -1 and -4 microns*** |
| | *Low dose imaging/radiation damage:* ***acquisition of double manual exposure (before and after)*** |
| | *Automated imaging pt I:* ***set up a square, find holes, adjust ice filter*** |
| | *Automated imaging pt II:* ***set up the template, enter defocus, enter exposure time, test template*** |
| | *Automated imaging pt III:* ***start data collection, note stage behaviour, discuss pipeline*** |

## Day 4: Friday – Krios IV – Control room (+big screen) and I14 MR

| | |
|---|---|
| 09:30–12:00 | **Practical 4**: Cryo-ET data collection demonstration – **Davide Zabeo (eBIC)** |
| | *Discuss:* ***Do*** |
| | *Tomo needs more advanced collection strategy:* ***target area setup, exposure, focusing and tracking*** |
| | *Tilt schemes:* ***set range, tilt increment, dose symmetric*** |
| | *Radiation damage in tomo requires lower dose:* ***use eBIC website calculator*** |
| | *How much tomography data:* ***calculate number of tomograms needed for 16hrs, start tilt series*** |

## End of course

**Background:**

This course will provide fundamental lectures and practical demonstrations in cryo-EM, from sample preparation and the electron microscope, through to image formation and processing, reconstruction in SPA and cryo-ET, and model building. This is a hybrid event targeted primarily at Oxford Wellcome Trust Doctoral Training Program (WT DTP) students, but lectures will be made open to the wider community via Zoom and a limited number of in-person places for the lectures are also available. Practical sessions covering sample preparation, cryo-EM screening, SPA data collection and cryo-ET are in-person and limited to first year Oxford Wellcome Trust Doctoral Training Program (WT DTP) students.

**Notice:**

We look forward to welcoming you all in the Lectures either in-person or online. Those who have site access may join in G59 on the first day, those coming from outside Diamond, please report to **RAL reception (R75)**. Lectures will be held in **Diamond House G59** & Zoom. Please note that the Research talks by eBIC PDRA & PhD students will be held on Zoom only, due to limited capacity in the **I14 meeting room**. The practical sessions and Student presentations held in I14 are for the 1st year WT DTP students only.

**Eventbrite registration page:**

https://www.eventbrite.co.uk/e/cryo-electron-microscopy-for-structural-biology-dtp-workshop-tickets-374171054697

**eBIC webpage for materials post course:**

https://www.diamond.ac.uk/Instruments/Biological-Cryo-Imaging/eBIC/Training/Course-pages/Cryo-EM-course/2022.html

**Diamond on site attendance:**

Directions to Diamond: https://www.diamond.ac.uk/Home/Directions.html

Registration for students and external speakers will be at **RAL reception (R75)**. Lectures will be held in **G59 in Diamond House**. Lunch will be available from the **RAL R22 restaurant**. Practicals will be held in **I14 (eBIC)**.


**COVID-19:**

Please do not attend site if you have tested positive for COVID-19. If you have recently tested positive, you must receive a negative LFD test result before attending site. Please contact Kyle if you have test positive (kyle.morris@diamond.ac.uk).

**Virtual attendance:**

**Lecture zoom link:**

https://diamondlight.zoom.us/j/97759410285?pwd=Mlg3cmVFRElXb3JiZ2t4L1Q4eXBOdz09

Webinar ID: 977 5941 0285 Passcode: 565950

**Research talks by eBIC PDRA and PhD students:**

**Instructions for PhD's and PDRA's giving short talks** Why did you choose the particular 'flavour' of EM. What was the type of sample (cell, big virus, small virus, large protein complex, small protein). How was the sample optimized for making cryo-EM grids. How were the grids made? Are the imaging conditions (dose, fractionation, movies, mag, defocus) special for the project to work. Accessories you needed (phase plate? GIF?). What software? How much data? Feel free to focus on methods, challenges/problems, solutions, context is important but the goal is to convey the EM side of your project and not primarily the biology.

**PhD/PDRA talks - 15 mins talk + 5 mins questions**

13:30 – 13:50

13:50 – 14:10

**14:10 – 14:30**

**Coffee break**

14:30 – 14:50

14:50 – 15:10

15:10 – 15:30

Name: Miriam (Confirmed)

*Talk title: Chameleon next-generation freezing*

Name: Pete Harrison (Confirmed)

*Talk title: Small membrane protein lab structures by cryo-EM*

Name: Emily Lacey (TBC)

*Talk title: Multimodel bioimaging of viral inclusion bodies and replication organelles*

Name: Ishika Kumar (TBC)

*Talk title: Structural analysis of dynamin-like rings interconnecting synaptic vesicles by cryo-electron tomography*

Name: Angela Kirykowicz (TBC)

*Talk title: Structure and Function of Bacterial Transport Machines in their Cellular Context*

**DTP student presentations: Student learning & feedback talks:**

The team at eBIC hope you'll find this course useful, helpful to your research, and we would value your feedback. Please be prepared to present a few powerpoint slides, up to 10 minutes, to summarise what you have learnt from the course, what you liked, what could be improved and how you see this helping your research. This session will be attended only by eBIC members and members involved in delivering the course.