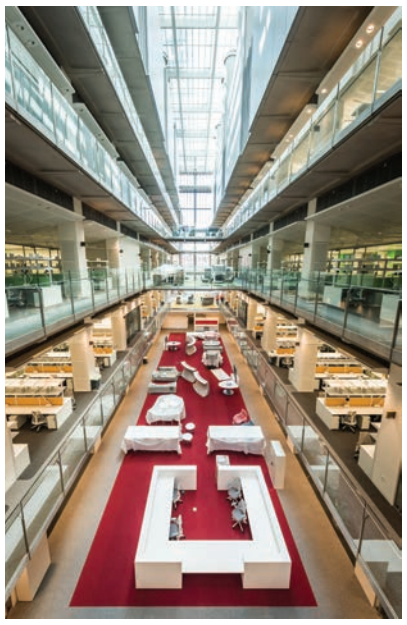
Electron Microscopy; A Platform Advancing Science at the Crick

Lucy Collinson, Lucy.Collinson@crick.ac.uk

The long awaited opening of the new Francis Crick Institute, an iconic building close to St Pancras Station in London, finally arrived in August, with the first of its 1250 researchers currently being brought together from a range of disciplines to discover the basic biology underlying human health. One of the world's largest dedicated biomedical research centres, it is a landmark partnership between the UK's three largest funders of biomedical research, the Medical Research Council's National Institute for Medical Research, Cancer UK's London Research Institute (Lincoln's Inn Fields and Clare Hall) and The Wellcome Trust, along with the three founding Universities, University College London (UCL) Imperial College London (ICL) and Kings College London.

Credit: Welcome images

A Multi and Interdisciplinary Approach



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The integration of the three university founders, UCL, Imperial and King's has brought to the Crick a huge volume and range of expertise and facilities, in particular from the physical, engineering and clinical sciences and it is increased interaction between biological and physical sciences that will pay dividends to both sides, introducing new data, methodologies, concepts and perspectives and stimulating the development of novel approaches to problems.

The internal structure at the Crick is therefore arranged to encourage the bottom-up development of 'interest groups' that bring together researchers from across the organisation to share insights and plan activities in areas of common scientific interest. With the whole building designed to encourage mixing, scientists will be drawn together at interaction and collaboration facilities located at the centre of each floor and at the institute-wide facilities on the ground floor. The laboratories, arranged in quadrants on four of the floors have also been constructed for high visibility, adding to opportunities for interaction and knowledge sharing.

The support offered to research groups, either in-house or external to the Crick is through the advanced technology and expertise of the Science Technology Platforms, including advanced DNA sequencing; the latest mass spectrometry equipment enabling gene expression, proteins and metabolic pathway characterisation; bioinformatics support for studies involving very large datasets and the high-throughput screening facility. Electron microscopy, X-ray crystallography and nuclear magnetic resonance suites allow biological structures to be studied in fantastic detail.

The Electron Microscopy (EM) STP

Because every imaging experiment is different, the EM team, made up of experienced post doctoral electron microscopists and scientists with image analysis and laser physics expertise, collaborates with the research scientists to design workflows unique to each research project, covering sample preparation protocols, microscope type, imaging strategy and data analysis.

The electron microscopy STP houses a range of high-end electron microscopes enabling imaging of samples across scales, from individual protein molecules to whole organisms such as fruit flies and zebrafish. Different light, electron and X-ray microscopes are often combined to image different information from the same sample - a process called correlative microscopy, one of the specialities of the lab.

"We work with about two-thirds of the labs at the Crick, across disciplines as diverse as structural biology, developmental biology, neurobiology, infection, immunity, cancer biology and more. The priority of the electron microscopy team is to collaborate with these Crick research groups to produce images that help them to understand their scientific questions," explained Lucy Collinson, who heads the electron microscopy suite.

Where established techniques and technology cannot answer the scientific question, the EM STP works with research scientists to design and develop new methods; these are then communicated on to the wider microscopy community through publications, workshops and conferences.

Lucy Collinson, Head of Electron Microscopy

Advice in big data handling and analysis, from alignment and reconstruction to 3D model generation and display is also offered. The group have ongoing collaborations with Computer Vision scientists to develop algorithms for automatic segmentation of electron microscopy images and correlation of data from different imaging modalities.

"In addition, the STP environment provides a hotbed for imaging research and innovation, which in recent years has delivered automated 3D EM of cells and tissues, maintenance of fluorophores in EM samples for simultaneous functional and structural imaging in integrated super-resolution light and electron microscopes; the implementation of super-quick protocols that take us from live samples to imaging in the electron microscope in just one day," Lucy added.

Building from the base up

Just a few weeks after moving into the Crick, Lucy also talked to ILM about some of the planning and vision behind the EM STP and how its strong capability can help researchers with various projects at the Crick and also through collaboration with UK and international partners.

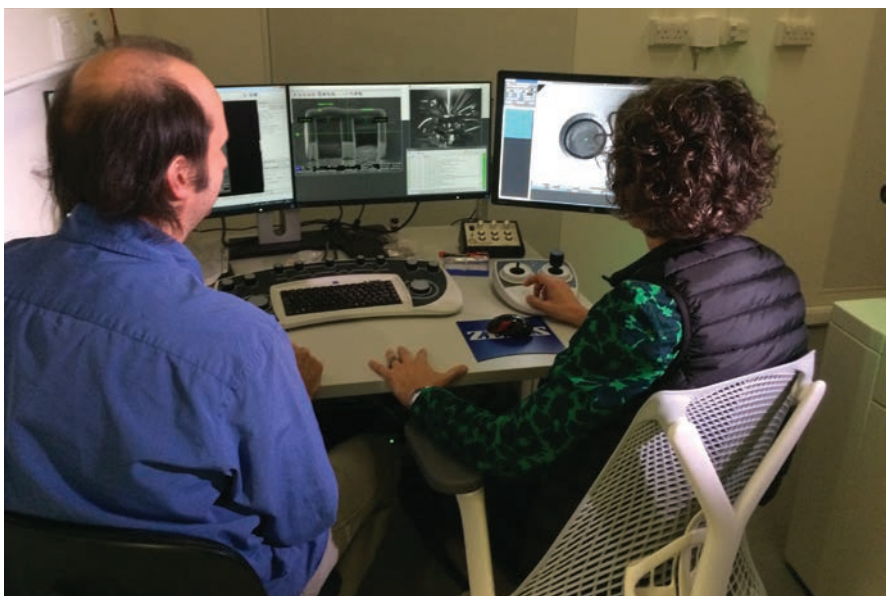
"The design process for the Electron Microscopy suite at the Francis Crick Institute started almost ten years ago. Working with the architects and engineers, the vibration and electro-magnetic field profiles of the site were mapped, to understand how the tube lines and trains at St. Pancras would affect the microscopes. A 'sweet spot' was identified at the south west corner of the site, which was least affected. The eight electron microscope rooms were then designed and built with advanced protective systems to shield them

from fields and vibration, as well as introducing tight control of the room air flow, air temperature and humidity. These many years of work involving a broad and highly-skilled team have delivered extremely 'quiet' rooms that enable the most advanced microscopy in an extremely challenging site in central London.

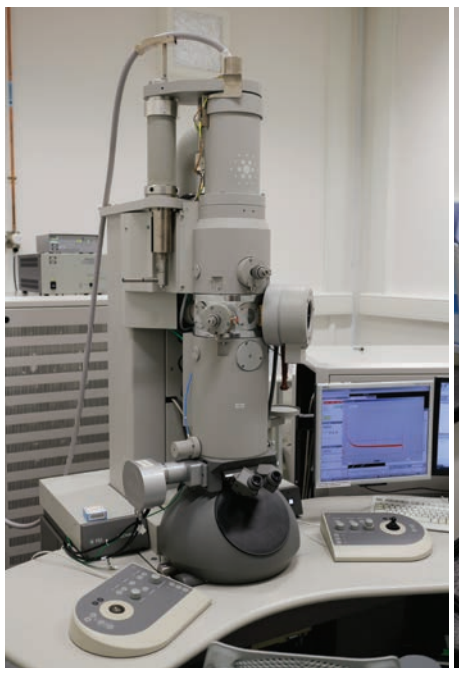
"The Electron Microscopy Science Technology Platform (EM STP) is now operational, containing an impressive suite of microscopes, from confocal and cryo-fluorescence light microscopes, to benchtop SEMs, an integrated light and SEM, several Serial Block Face SEMs, a Focused Ion Beam SEM, two 120 kV TEMs and a micro CT system. Alongside the microscopes sits a full suite of sample preparation equipment, including coaters, plunge freezers, a high pressure freezer, freeze substitution units, ultramicrotomes and cryo-ultramicrotomes. This means that we can pick and choose the best tool for answering a particular biological question, often combining microscopes into correlative workflows, which enable us to image different types of information from a single sample across scales.

"The primary focus of the EM STP is to collaborate with Crick scientists to image their samples and shed light on their biological questions. Recent collaborations have seen us study proteins binding to DNA in a study of DNA repair mechanisms relevant to both breast and ovarian cancer with Simon Boulton's lab; a study of Mycobacterium tuberculosis infection in human lymphatic endothelial cells with Max Gutierrez's lab; and a study of HIV assembly within human macrophages with Mark Marsh at one of our partner universities, UCL. These are just three of 40-60 projects that we have running at any one time. At steady state, the Crick will have 120 research groups, so it is likely this workload could double or even triple in the near future.

"All of this work, as well as our own activity in designing and building new light microscopes to integrate with electron microscopes for advanced correlative microscopy, is handled by a team of ten post-doctoral research scientists. With PhDs in either the biological or physical sciences, each member of the team is capable of working across a



Research Scientists Chris Peddie and Rafaella Carzaniga looking at images from the FIB SEM



FEI BioTwin T12 12-kV TEM

Zeiss Sigma FEG SEM with Gatan 3View

broad range of techniques and technology applied to the Crick research projects, whilst developing new techniques and technologies, also keeping the complex and demanding equipment running smoothly. Meanwhile, we also collaborate with leading imaging labs across the world to develop and apply new imaging techniques to Crick science. A major ongoing collaboration sees us working with Liz Duke at the Diamond Light Source in Oxfordshire, Eva Pereiro at the ALBA synchrotron in Barcelona and Gerd Schneider at the BESSY II synchrotron in Berlin, to image whole frozen cells at high resolution and as close to their living state as possible.

"Perhaps the most exciting and impressive part of the new Crick building, apart from its scale and position, is the provision of countless collaboration spaces, beautifully designed to provide areas for scientists to come together and relax with each other and with external academics and commercial collaborators, creating the space and opportunity to explore the most exciting interdisciplinary ideas. Comfortable sofas and booths with AV equipment hang on bridges across the enormous atrium, and coffee stations come equipped with glass walls that can be moved to create an impromptu meeting area, written on and then photographed and emailed as a record of the brain-storming session. A 400-seat auditorium nestles in the atrium space alongside multiple breakout areas and a public exhibition space, bringing our science to the local area and an international audience travelling through St. Pancras station next door.

"After a decade of planning and building, we are extremely excited about the advances that will be enabled by this unique building and it's occupants, over the next ten years and beyond. I don't think there is a better place to discover how life works at the smallest of scales."

Cryomicroscopy shows how flu virus fuses with host cell

Scientists at the Francis Crick Institute have visualised how the influenza virus fuses with the membrane of a host cell. This is an essential step in the virus's lifecycle and the findings could lead to new approaches to prevent infection.

Peter Rosenthal of the Crick said: "The influenza virus is an important human pathogen and understanding all the steps in its lifecycle are important for understanding virus infection. Entry into the host cell is a key step in virus infection.

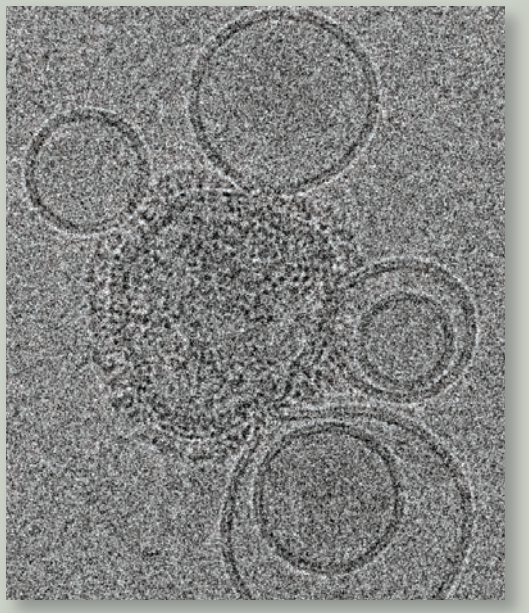
"During infection, the virus delivers its genome into the host cell by fusing its own lipid membrane with that of the host cell membrane. This process is achieved by structural changes in the hemagglutinin, which is one of two protein spikes on the virus surface. The hemagglutinin spikes insert into host cell target membrane and mediate the close approach and fusion of membranes."

Lesley Calder, the first author of the study, used cryomicroscopy, a high-resolution imaging method, to observe how the virus fuses with a target membranes in the laboratory.

Cryomicroscopy images biological structures in a frozen-hydrated state that preserves high-resolution features. Images are then acquired via an electron microscope using minimal electron exposure so that the electrons do not damage the structures of interest. This technique allowed the scientists to record images from many angles and to calculate 3D maps of the virus fusing with the target membranes. The 3D maps, combined with information from previous biochemical and structural studies, provide an explanation of how membrane fusion occurs.

Dr Rosenthal said: "By imaging at high-resolution the way the virus fuses with a membrane, we can learn how the viral proteins and their structural changes bring about membrane fusion. The more detailed understanding of this process identifies steps that could be blocked and may therefore provide new targets for drugs designed to inhibit these steps in the future."

The paper, 'Cryomicroscopy provides structural snapshots of influenza virus membrane fusion', is published in *Nature Structural and Molecular Biology*.



Influenza virus fusing with target membrane called a liposome.

- Francis Crick Institute scientists have used cryomicroscopy to visualise how the influenza virus fuses with the membrane of a host cell.
- Flu viruses are categorised into types A, B and C. Type A viruses are the source of seasonal and pandemic flu outbreaks and are categorised depending on two proteins on their surface - called haemagglutinin and neuraminidase. The haemagglutinin is responsible for the flu virus binding to host cells, followed by the delivery of the virus's genome to the inside of the cell where it multiplies. Our main antibody response is directed against haemagglutinin.

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