OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership

**Industrial CASE Studentship Advertisement – 2021-22**


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| **Project Title:** | Charting Cytoskeleton-Organelle Interplay in Living Cells Through High Resolution 3D Correlative Cryo-Imaging |

**Brief description of project:**

Key to the development of a eukaryotic cell is the constant and balanced production, upkeep and distribution of organelles. This is achieved through interactions between the cytoskeleton and ubiquitous substructures such as the endoplasmic reticulum, Golgi and endosomes. To date, the transient nature of such interactions (seconds to minutes) along with difficulties in imaging simultaneously both cytoskeletal (easy to immobilize but near invisible to high resolution imaging) and membranous structures (visible in electron imaging but easily disrupted by requisite treatment) deep inside living cells, have made the study of these systems in 3D difficult.

We have now at our disposal a new cryo-imaging platform for 3D correlative cryo-imaging that allows us to image the intricate interplay of components in the cytoplasmic universe without compromising in detail, resolution or physiological relevance. This involves the combination of soft X-ray tomography with super resolution fluorescence imaging at the UK synchrotron biological cryoimaging beamline B24 and delivers (a) 3D imaging of cryo-preserved samples (b) correlative microscopy using X-ray and laser light and (c) nanometer resolution of features inside fully hydrated cells at near physiological conditions.

Work will be undertaken primarily at beamline B24 at Diamond Light source with extended visits to the department of Biochemistry at Oxford University for biochemical and immunology work and two extended placements (up to three months each) at Linkam scientific premises (in the UK and the Netherlands respectively) for product development and testing.

The student will learn cell and tissue culture techniques; immunolabelling; *Drosophila* culture and genetics; sample preparation; data collection, processing and analysis for nanometre imaging using X-rays and laser light; cryogenic preparations; commercial product development; python programming.

The specific aims of this project are:
1) To use correlative light and X-ray cryo-microscopy to answer the following biological questions:
- How do organelles bud at the ER/Golgi interface and how are they recruited by the cytoskeleton to shuttle to areas of activity where they are needed?
- How does the cytoskeleton change organelle distribution under stress conditions such as a

pathogenic challenge?
- What is the extend of clathrin-mediated cell membrane remodelling events during pathogen engulfment and endosome recruitment (clathrin is the key player in endocytosis and vesicle budding intracellularly).

We will use established cell lines and *Drosophila* primary haemocytes (the insect equivalent of the mammalian circulating macrophage) to investigate structures and interactions between the cytoskeleton and ER at points of anchorage and budding. All cell populations will also be exposed to common bacterial opportunistic pathogens such as Staphylococci. As pathogen sensing and clearance effects massive cytoplasmic rearrangements, recruitment of organelles and mitochondrial shuttling it is a perfect backdrop to apply our refined protocols to and extract essential knowledge for cell mechanisms.

2) To further develop and streamline the correlative 3D imaging technology available to the scientific community with special emphasis on sample handling and cryo-preservation; both steps are crucial for the success of any cryo-imaging experience and the ones that would benefit the most from optimised kit and rigid protocols to ensure minimal user-to-user variation.

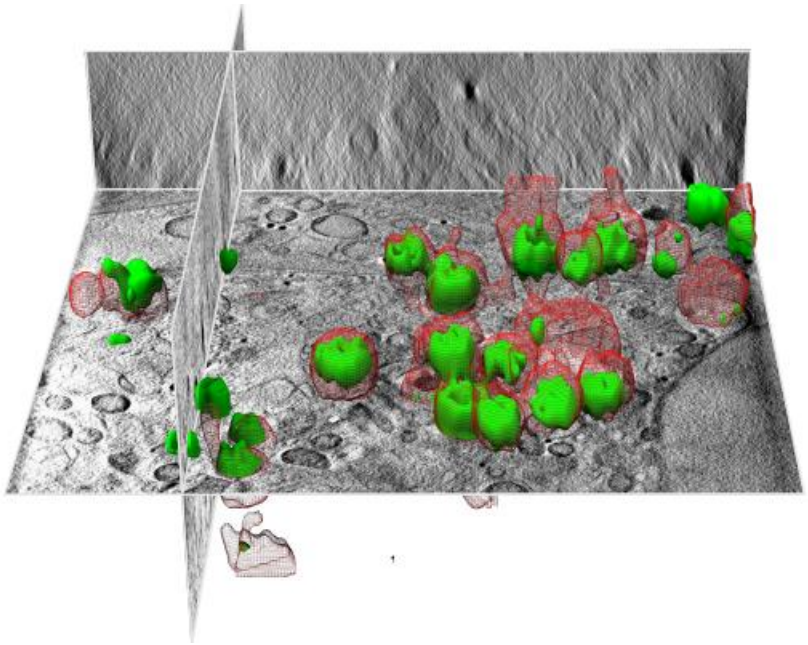

Figure 1. B24 3D imaging of endosomes (red/hashed) involved in the clearance of a viral pathogen (green) within a mammalian cell ultrastructure (grayscale tomogram)

References:

Kounatidis, I., *et al.* 3D Correlative Cryo-Structured Illumination Fluorescence and Soft X-ray Microscopy Elucidates Reovirus Intracellular Release Pathway. *Cell* **182**, 1–16. [Includes details on the imaging platform that will be used].

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Vaz, F., *et al*. Accessibility to peptidoglycan is Important for the recognition of Gram-positive bacteria in *Drosophila. Cell Reports* **27,** 2480-2492 (2019). [Includes the use of *Drosophila* primary macrophages to study *Staphylococcus aureus* infection.]

**Attributes of suitable applicants:**

The candidate should have a keen interest in microscopy, cellular stress responses and- host pathogen interaction.

Past experience in biomedical, advanced microscopy methods, laboratory techniques and pathogen handling is desirable. IT and coding skills would also be welcome.

Good time management and communication skills are essential.

Candidates should have a first or upper second class degree in a relevant field of science.

**Funding notes:**

This project is funded for four years by the Biotechnology and Biological Sciences Research Council UKRI-BBSRC and Diamond Light Source. UKRI-BBSRC eligibility criteria apply (https://www.ukri.org/files/funding/ukri-training-grant-terms-and-conditions-guidance-pdf/). Successful students will receive a stipend of no less than the standard UKRI stipend rate, currently set at £15,285 per year, which will usually be supplemented by the industrial partner

*This project is supported through the Oxford Interdisciplinary Bioscience Doctoral Training Partnership (DTP) studentship programme. The student recruited to this project will join a cohort of students enrolled in the DTP's interdisciplinary training programme, and will participate in the training and networking opportunities available through the DTP. For further details, please visit www.biodtp.ox.ac.uk. The DTP and its associated partner organisations aim to create a community that is innovative, inclusive and collaborative, in which everyone feels valued, respected, and supported, and we encourage applications from a diverse range of qualified applicants.*