



Industrial CASE Studentship Advertisement – 2020-21

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Project Title: Tuneable multifocal plane microscopy for high frame rate three-dimensional fluorescence microscopy of cilia/flagella

Brief description of project:

Cilia and flagella are found across life with vital functions driving cell swimming or fluid movement. However, analysing their extremely fast three-dimensional movement poses challenges. This project will develop tuneable multifocal plane microscopy to address this, and is a project run in collaboration between the Wheeler Lab (a parasite flagellum research group in the University of Oxford) and Cairn Research Ltd. (a UK company which develops scientific instruments).

This project will develop new multifocal plane microscopy instruments, replacing fixed focal length lenses with electrotunable lenses and integrating them into new multi-way splitter designs currently in development. Multifocal plane microscopy uses a multi-way splitter with semi-silvered mirrors to separate light coming from a microscope into multiple light paths. These travel through different lenses to shift the focal planes of individual light paths. Combined with a high frame rate camera, this allows capture of three-dimensional movement at very high frame rates (typically 400 frames per second). Multifocal plane microscopy is currently inflexible, but replacing fixed focal length lenses with electrotunable lenses will greatly open up the potential of this approach in many areas of biology and microscopy. As part of the project, there will be a placement of at least 12 weeks with Cairn Research Ltd. in Faversham in Kent – an opportunity to be involved in scientific instrument product development and develop the optical system, computer control of the electrotunable lenses and image analysis tools for tuneable multifocal plane microscopy.

The instruments developed with Cairn Research Ltd. will be applied to biological questions about the control of flagellum beating and cell swimming in *Leishmania* and *Trypanosoma* unicellular eukaryotic parasites, working with the Wheeler Lab in the Medawar Building for Pathogen Research in the South Parks Road science area. Control of flagellum beating to achieve necessary swimming behaviours is vital across eukaryotic life, from human sperm, to marine microorganisms and human parasites. *Leishmania* and *Trypanosoma* are extremely genetically tractable and the project will include generation of new cell lines with tagged proteins and/or deletion mutants, then analyse their swimming to understand how intra-flagellum signalling and dynamics control three-dimensional flagellum movement. This will include producing cell lines expressing advanced reporters of intracellular state (e.g. FRET reporters of calcium and cAMP) and reporters of intra-flagellar dynamics (i.e. IFT).

This project provides opportunities for learning many scientific and non-scientific skills. This includes CRISPR/Cas9 genome engineering and in vitro work with pathogenic eukaryotic cells, incorporating molecular cell biology techniques like PCR, gene cloning, validation of cell lines by PCR and transcriptome/genome sequencing, analysis of mutants by Western Blot or mass spectrometry, etc.



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Microscopy skills will include high frame rate and high sensitivity fluorescence microscopy on widefield systems and design and modification of microscope and optical splitter light paths. There will be significant opportunities to learn computer control of hardware and image analysis programming. In addition to programming, other transferrable skills that will be learnt are data management and analysis, formal scientific writing and data presentation.

Attributes of suitable applicants:

This project requires a student with a strong academic background (preferably a 1st class Bachelor's degree in a relevant scientific subject) and the organisation, responsibility and personal drive to carry out a collaborative research project. A Master's degree or other research experience is beneficial, but candidates will be assessed relative to their career stage. They need to be able to follow existing protocols precisely but also innovate when needed, keep detailed records and have a close attention to detail. They need to work effectively to deadlines a collaborative project demands and communicate ideas and experimental data/results effectively.

This project is ideally suited to applicants with a molecular cell biology background. A background in *Leishmania* or *Trypanosoma* biology is not vital, the molecular cell biology techniques that will be involved are transferrable across cell biology systems and training in the specific details will be provided. A strong interest in microscopy and microphotography is vital as this is the main focus of the project and practical experience with microscopy would be valuable. A formal physics/optics background is not vital, however good mathematical competency is important. As MicroManager plugin development and automated image analysis will play an important part of the project programming experience is extremely beneficial, although training will be provided in the specific languages to be used.

Funding notes:

This project is funded for four years by the Biotechnology and Biological Sciences Research Council BBSRC. BBSRC eligibility criteria apply (<https://www.ukri.org/files/legacy/publications/rcuk-training-grant-guide-pdf/> Annexe 1). EU nationals who do not meet BBSRC residence criteria are encouraged to contact the programme administrator to check their eligibility for BBSRC funding before submitting a formal application. Successful students will receive a stipend of no less than the standard UKRI stipend rate, currently set at £15,009 per year.

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 be able to take full advantage of the training and networking opportunities available through the DTP. For further, details please visit www.biodtp.ox.ac.uk.