THE WOLFSON FOUNDATION GRANT REFERENCE NUMBER: 18272

The Weatherall Institute of Molecular Medicine: The Wolfson Imaging Laboratory

Progress Report for The Wolfson Foundation

University of Oxford
Medical Sciences Division
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Zebrafish 48 hours post fertilisation
The University of Oxford is extremely grateful to the Wolfson Foundation for its support of the Wolfson Imaging Laboratory at The Weatherall Institute of Molecular Medicine, and is pleased to present this progress report to the Trustees.

The Weatherall Institute of Molecular Medicine (WIMM) was founded in 1989 to foster research in the field of molecular and cell biology with direct application to the study of human disease. It is now recognised as a leading international centre for basic and translational medical research, hosting 40 independent research groups, all of which are attached to a clinical department. It was awarded the prestigious Queen’s Anniversary Prize for Higher and Further Education by Her Majesty the Queen in February 1997, and the leading role of the WIMM in translational medical research in the UK was highlighted in the Cooksey Report into Medical Research in 2006.

The imaging of cells within living organisms and the visualisation of single molecules during cellular contacts, using techniques such as 2-photon microscopy and total internal fluorescence microscopy (TIRF), are vital for current biomedical research. The most generous grant of £1.5 million from the Wolfson Foundation has enabled major strides to be taken in 2012 to establish the Wolfson Imaging Laboratory.

It will be a core facility for all scientists at the WIMM; providing state-of-the-art imaging equipment and ensuring access to the most advanced technologies. Four of the microscopes within the Laboratory, crucial to the underpinning of future research and continuing the outstanding scientific development at the WIMM, have been ordered and will be installed at the beginning of November 2012.

All imaging activities in the WIMM will be integrated within a central structure with clear scientific leadership, bringing together groups which will benefit immensely from an interdisciplinary approach to their research. The key roles of Scientific Director and Manager, which will provide this leadership, have now been filled.
Project Progress

Staff
The new Scientific Director of the facility, Dr Christian Eggeling, will take up his position from 1 October 2012. His research interests were highlighted in the previous report. Dr Christoffer Lagerholm has now been appointed as Manager of the Imaging Facility. Dr Lagerholm is currently at the University of Southern Denmark and will be taking up his WIMM appointment on 1 November 2012. An introduction to Dr Lagerholm’s career and research is given on page 6.

Imaging Equipment
Four systems have now been ordered as follows:

1. **Leica SP8X inverted confocal microscope**
   The SP5X was demonstrated at the WIMM in 2011, and the SP8X is a new model. The SP8X is being upgraded concurrently for GSTED super-resolution imaging. The cost for the GSTED upgrade will be borne by Dr Christian Eggeling.

2. **Zeiss 780 upright multi-photon system with single photon capability**
   A multi-photon system has been ordered from Zeiss to sit on an upright 780 confocal microscope for *in vivo* imaging. The multi-photon laser, a Mai Tai Ti:Sapphire, has been purchased from Newport Spectra-Physics, together with an antivibration table. The multi-photon system will sit in a dedicated room with sinks, flow cabinet and procedure equipment.

3. **Zeiss Inverted 780 confocal system**
   This can be upgraded to multi-photon at a later date if desired. An antivibration table for the system has also been ordered from Newport Spectra-Physics.

4. **Zeiss spinning disc confocal**
   This system is based on the Yokagawa disc principle. It was originally proposed that a TIRF super-resolution microscope be purchased, however Dr Christian Eggeling has agreed to purchase the TIRF super-resolution microscope from his own funds.

All three Zeiss systems will run on Zen software. This has proved popular with users who have tested the Zeiss systems and there are obvious advantages to having the same software across several platforms in terms of ease of use. To that end, the WIMM has also negotiated an upgrade to Zen software for the two Zeiss 510 systems already present in the Institute.

There are two outstanding items yet to be purchased as follows:

1. **Deltavision widefield system**
   This has deconvolution software (for fast time-lapse fluorescence imaging with a highly accurate stage for revisiting multiple locations).

2. **TIRF super-resolution system**
   This system is used for imaging at the cell surface. As mentioned above it will be purchased using Dr Eggeling’s funding, however it will be available for general use. It is anticipated that this microscope will have the additional capacity for PALM/STORM super-resolution and single molecule/particle tracking analysis. It is expected that these two items will be ordered during October.

Costs of the Imaging Equipment
To date, £1,284,876 of the generous pledge of £1,500,000 from the Wolfson Foundation towards imaging equipment for the Wolfson Imaging Laboratory has been committed. The four systems ordered are due to be delivered very soon under an agreement for 80% to be payable on delivery and 20% after acceptance testing. Acceptance testing is expected to take two to three weeks and will be supervised by Dr Chris Lagerholm, Dr Veronica Buckle and Dr Christian Eggeling.

The cost of the antivibration tables, and the laser, as detailed above, have been included in these costs, as they are essential for the microscope systems to function. The outstanding balance of £215,124 will be used to purchase the Deltavision widefield system.
The costs of the microscopes, together with their dates of delivery are given in the table.

<table>
<thead>
<tr>
<th>Microscope</th>
<th>Date of Delivery</th>
<th>Cost</th>
<th>Funds payable on delivery (80%)</th>
<th>Funds payable after acceptance testing (20 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leica SP8X</td>
<td>Week beginning 5 November 2012</td>
<td>£300,000</td>
<td>£240,000</td>
<td>£60,000</td>
</tr>
<tr>
<td>Zeiss 780 inverted confocal (multi-photon ready)</td>
<td>Week beginning 29 October 2012</td>
<td>£295,430</td>
<td>(Including antivibration table costing £2297)</td>
<td>£236,344                         (Including antivibration table costing £2297)</td>
</tr>
<tr>
<td>Zeiss 780 upright multiphoton system with single-photon capability</td>
<td>Week beginning 29 October 2012</td>
<td>£419,446</td>
<td>(including antivibration table costing £5649 and Mai Tai Ti:Sapphire laser costing £82,000)</td>
<td>£335,557                         (including antivibration table costing £5649 and Mai Tai Ti:Sapphire laser costing £82,000)</td>
</tr>
<tr>
<td>Zeiss Spinning disc</td>
<td>November 2012</td>
<td>£270,000</td>
<td>£216,000</td>
<td>£54,000</td>
</tr>
</tbody>
</table>

The total cost of the equipment is £1,284,876.

**Building Work**

The first large imaging room has been prepared to take four imaging systems, with four independent power circuits, four separate lighting controls, and a central ceiling-mounted air conditioning unit. The Leica SP8X GSTED confocal, the TIRF system and the Deltavision widefield deconvolution system will be there. The room will also house a Zeiss 510 inverted confocal microscope which is already in use. The second imaging room is currently being split into two rooms to house the Zeiss multiphoton upright confocal for *in vivo* work, the inverted Zeiss 780 confocal and the Zeiss spinning disc microscope. The building work is complete and the antivibration tables were installed on Friday 26 October.

**Image Storage and Analysis**

Details of the centralised image storage system, using the OMERO database, were given in the previous report. OMERO is client-server software for visualisation, management and analysis of biological microscope images. The system was purchased as part of a grant to the Computational Biology Research Group (CBRG) within the WIMM. The imaging facility now has one high-specification workstation set up in the CBRG office in the WIMM. This is available for image analysis and is equipped with Imaris, Matlab and ImageJ/Fiji analysis software. Douglas Russell, OME Software Developer for Oxford Micron has been appointed by Micron to implement an OMERO image storage system in the Biochemistry Department, and the WIMM has continued to hold discussions with him. The introduction of OMERO in the WIMM will be taken forward once the new Imaging Facility manager is in place.

**Further Development of the Facility: The Next Generation Optical Microscopy Initiative**

In July 2012, an application was submitted to the Next Generation Optical Microscopy Initiative led by the Medical Research Council. Over the past decade entirely new technologies have been developed that bypass the diffraction limits of light microscopy and this has created new possibilities to investigate, in super-resolution at the nanometre scale, normal cell structure and function and how these are perturbed in inherited and acquired diseases. The principal aim of the WIMM’s application, submitted jointly with Micron Oxford, is to coordinate multidisciplinary groups (biologists, physicists, engineers and chemists) on the Oxford campus, to develop different approaches to super-resolution microscopy and to make this available to a broad biomedical community. The principal applicants are Professor Ilan Davies (Micron), Dr Christian Eggeling (WIMM), Professor Vincenzo Cerundolo (WIMM), Professor Douglas Higgs (WIMM) and Dr Martin Booth (Engineering Science and the Centre for Neural Circuits and Behaviour). The WIMM will already have access to GSTED super-resolution imaging via the Leica SP8X system arriving at the end of October. The Next Generation grant would allow the purchase of open STED-like super-resolution systems in the laboratory of Dr Christian Eggeling for further technological development. The WIMM would also have access in Micron to a PALM/STORM system and to new developments in OMX structured illumination microscopy where they will be developing a further increase
Introducing the new Imaging Facility Manager

Dr Christoffer Lagerholm
Dr Christoffer Lagerholm is a native of Sturkö, Sweden. He obtained his PhD in Chemistry from the University of North Carolina (UNC) at Chapel Hill. Following the completion of his PhD, he joined the current Molecular Biosensor and Imaging Center (MBIC) at Carnegie Mellon University as a post-doctoral researcher; a time during which he was introduced to fluorescent semi-conductor nanoparticles or quantum dots (QDs).

He has since focused most of his work on exploiting the optical properties of QDs for biological applications both in vitro and in vivo. In recent years, the focus has been on applying QDs for single particle tracking (SPT) in order to investigate the nano-organisation of the mammalian plasma membrane. This latter work was initiated while Dr Lagerholm was a post-doctoral researcher at the Department of Cell and Developmental Biology at UNC – Chapel Hill and was subsequently continued at MEMPHYS – the Center for Biomembrane Physics at the University of Southern Denmark in Odense, Denmark. This work has included the development of simultaneous orthogonal multi-colour SPT of up to four different molecular species (example given at Figure 1 opposite) and high-speed SPT at image acquisitions rates up to about 1700 Hz (example given at Figure 2 opposite).

Importantly, Dr Lagerholm spearheaded the establishment of a core imaging centre, the Danish Molecular Biomedical Imaging Center (DaMBIC), which spans the Natural and Health Science faculties. Dr Lagerholm is the Centre Coordinator at the Natural Science Faculty. This entails day-to-day management of equipment, budgeting and user training. During the course of his career, Dr Lagerholm has gained extensive experience with a wide assortment of light microscopy configurations, techniques and molecular labelling strategies.

Strategic planning for high-end imaging within Oxford University
Increasingly research agencies expect institutions to justify and coordinate their funding applications. This has led to discussions about developing an efficient strategy for assessing and coordinating current and future imaging needs for biological imaging across Oxford. Furthermore Oxford has great strengths in the Maths and Physical Life Sciences Division (MPLS) in the development of new imaging technologies that can be applied to biological questions. Historically, it has often been difficult to forge effective links between these technology developers and technology users. This is an area of huge potential, however, and one that the University must encourage and coordinate with the assessment of future imaging requirements. The WIMM has been represented in these discussions and proposals for addressing these issues are currently being formulated.

in spatial resolution by a factor greater than two. Importantly the WIMM would be able to appoint an image analyst on site. The outcome of this application is expected by late November this year.
Figure 1. Example of multi-colour SPT of the sphingolipid GM1 labelled with cholera toxin subunit B conjugated QDs emitting at 705 nm (QD705, red), acyl carrier protein (ACP) epitope tagged lipid anchored protein (CD59) labelled with CoA conjugated QD655 (green), and biotin ligase acceptor peptide (BLAP) epitope tagged epidermal growth factor receptor (EGFR) labelled with streptavidin (sAv) conjugated QD605 (blue). (Top left) DIC image of labelled Mouse embryo fibroblast (MEF). (Bottom left) Wide-field fluorescence image of yellow fluorescent protein fusion protein that localises to the plasma membrane with high specificity. (Right) Re-constructed single particle trajectories from a time-lapse image sequence acquired on a conventional wide-field microscope at 25 Hz for a duration of 600 image frames (Arnspang et al. (2012) PLoS One, in press; Clausen et al. (2012) manuscript submitted).

Figure 2. Example of high-speed single particle tracking in the plasma membrane of a MEF of an artificial lipid, biotin-cap-DPPE, that was labelled with sAv-QD655. Representative examples of re-constructed single particle trajectories from a time-lapse image sequence acquired on a conventional wide-field microscope at 1700 Hz for a duration of 9000 image frames. These examples show trajectories that were statistically best described as free diffusion (top left), transient diffusion (top right), and confined (or immobilized) diffusion (bottom left) as was determined by analysing the shape of the mean squared displacement curves (bottom right) (Clausen and Lagerholm (2012) manuscript in revision).
Recent Images from the Weatherall Institute of Molecular Medicine

It is vital to the WIMM’s purpose of applying the techniques of molecular and cell biology to the study of human disease, to use the most recent technological advances to improve our understanding of the mechanisms underlying human genetic diseases. The development of the Wolfson Imaging Laboratory has already enthused many of the research groups within WIMM to add a strong component of state-of-the-art microscopy to their research programmes.

Figures 1 and 2 show images of cells of the immune system. Figure 1 was taken in collaboration with Professor David Klenerman in the Department of Chemistry at Cambridge, using a non-commercially built TIRF system. Figure 4 shows aspects of zebrafish development. Figures 2 and 4 were imaged on confocal systems already sited in the WIMM.

Figures 1, 3 and 5 are all examples of super-resolution imaging. Figure 3 was obtained using a structured illumination system in Micron, Dept of Biochemistry. Figure 5 is an example of RESOLFT-STED microscopy imaged on a system at the Max Planck Institute, Göttingen, Germany.

Figure 1. Total internal reflection fluorescence (TIRF) microscopy is being used to track the movements of signalling proteins in T cells, during receptor triggering with antibodies. The T-cell outline is marked with a white dashed line. It was found that the large phosphatase CD45, labelled with Alexa-488 tagged anti-CD45 Gap8.3 Fab fragments, segregates from contacts, forming ring-like structures (a). A HaloTag®-labelled short form of the kinase Lck (Lck-short; green), whose extracellular domain is only a 9-residue antibody tag, is not excluded (b), while in a separate cell, a large form of the kinase whose extracellular domain is the same size as that of CD45 (Lck-long; green) segregates from the contact zone (c). Fluorescence scans across the contact zones are shown in panels d and e. These observations support the idea that receptor triggering in T cells might be triggered by the passive re-organization of signalling molecules. This work was done by Veronica Chang (T-cell Biology Lab, WIMM) and Steve Lee (Chemistry, Cambridge).
Figure 2. Human dermis explants, resected from healthy skin of a patient undergoing elective surgery, then stimulated with TNFalpha for 24h before frozen-sectioning and immunostaining for podoplanin (red), a lymphatic marker, and fractalkine (green), an inflammatory chemokine, with nuclei counterstained with TOPRO (blue). A) Area surrounding a hair follicle, bar = 50 microns. B) Lymphatic vessel, with podoplanin on the cell surface and intracellular fractalkine, bar = 10 microns. Both imaged with a 60x water immersion objective on a BioRad Radiance 2000 confocal. (Louise Johnson)

Figure 3. Images of a lymphoblast nucleus collected on the OMX V3 structured illumination microscope sited in Micron, Dept Biochemistry. Lymphoblasts were hybridized with a pool of oligonucleotide probes covering a 2 megabase region around the human alpha globin genes. The inserts show an expanded view of the FISH signal detected in green plus a central portion in red. The image on the left is a pseudo widefield image to demonstrate the increased resolution achievable with structured illumination (right). (Jill Brown and Veronica Buckle)
Figure 4. Two-day old zebrafish. Nuclei are in blue (Hoechst), α-actinin (actin binding protein) in red, Phalloidin (F-actin) in white-blue and aldh1a2 (Retinoic acid pathway) in green. The “neck” is visible from a ventro-lateral view exposing the heart and jaw as well as the branchial arches where the gills develop. Imaged on a Zeiss 510 confocal microscope with a 40x/1.1 water immersion objective. (Jana Koth)

Figure 5. Super-resolution RESOLFT microscopy of living cells: E. coli bacterium expressing rsEGFP-MreB. Confocal (left) and corresponding RESOLFT (right) image. Scale bar: 1 µm. Adapted from T. Grotjohann et al. Diffraction-unlimited all-optical imaging and writing with a photochromic GFP. Nature 478, 204-208 (2011). (Christian Eggeling)
News from The Weatherall Institute of Molecular Medicine

Professor Sir Andrew McMichael retired as Director of the Institute on 30 September 2012 after a twelve-year tenure at the helm of the Institute. During this time he steered major developments to the building, infrastructure and research programmes of the Institute. A building programme begun in 2003 increased floor space by 30%. A strategic alliance between Oxford University and the Medical Research Council led to their joint governance of the WIMM and raised additional funding. This permitted the establishment of six WIMM Senior Fellowships and an accompanying major refurbishment. A critical development for the Institute during Andrew’s directorship has been the establishment of core facilities, of which the Imaging Facility will be the latest.

The first Institute-wide facility was for Fluorescent Activated Cell Sorting (FACS) analysis and this has proved a very successful model, with improved infrastructure and training. Subsequent facilities have all been built on the same management structure of Scientific Committee, Scientific Director and Manager. The WIMM now has core facilities for FACS analysis, Sequencing, Proteomics, Bioinformatics/CBRG and Imaging. Such continuing developments under Andrew’s directorship mean that the WIMM is an international leader in biomedical research and plays a major role in the newly established Radcliffe Department of Medicine. After retirement from the Directorship, Andrew is continuing his research and will be based in the new Target Discovery Institute on the Old Road Campus. Andrew is succeeded by Professor Doug Higgs, who will also continue as Director of the MRC Molecular Haematology Unit and Professor of Molecular Haematology.

Two new WIMM Senior Fellows have been appointed. Professor Ahmed Ahmed moved from the Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital to take up his appointment on 1 April 2012. Professor Ahmed works on ovarian cancer, focussing on personalisation of therapy to circumvent antimitotic drug resistance in cancer. Dr Wojciech Niedzwiedz’s appointment began on 1 October 2012. Dr Niedzwiedz’s area of interest is DNA replication and repair, particularly relating to Fanconi’s Anaemia (FA). His work focuses on elucidating how the repair of damaged DNA is executed in the context of the replication fork, and how fork stability is achieved under stressful conditions. He is particularly interested in the molecular function of the FA proteins in these processes.

Professor Claus Nerlov of the Edinburgh Cancer Research UK Centre, who is investigating the cellular mechanisms of haematopoietic lineage commitment, joined the MRC Molecular Haematology Unit on 1 October 2012. His goal is to understand the molecular basis for and spatial organisation of normal, ageing and malignant haematopoiesis, and to use this knowledge to devise cell based and molecular therapies that can be used to treat hematopoietic insufficiencies and malignancies.
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