BioImagingUK

What is BioImagingUK and what is its current status?

To the readership of In**Focus,** it will come as no surprise that imaging is one of the key technologies in current life science research. Over the last few decades, developments in the field have been enormous and the number of microscopy techniques and associated tools that have emerged is astounding. This has gradually led to the realisation that any given lab or bioimaging facility in the UK will not have the capability or resources to cover the whole spectrum of imaging technologies. In order to remain one of the leading research countries in the world, we need to ensure that researchers can access any imaging technology, irrespective of the location of that researcher.

The framework of BioImagingUK.

In an effort to analyse and organise the existing imaging expertise and capabilities in the UK and define future needs, a large number of bioimaging specialists from the light and electron microscopy fields have organised themselves into a grouping called BioImagingUK (http://www.bioimaginguk.org). This is a community-driven effort, and anyone can be a member. We have organised a number of meetings and discussions over the last couple of years to formulate a framework that is capable of providing an effective bioimaging infrastructure to the UK scientific community. We are very happy to report that this initiative is supported as a UK Network by many of the major biomedical research councils in the UK (BBSRC, MRC and Wellcome Trust), allowing us to organise essential meetings to finalise the structure of the network and define the technical and strategic imaging technology priorities required by UK scientists.

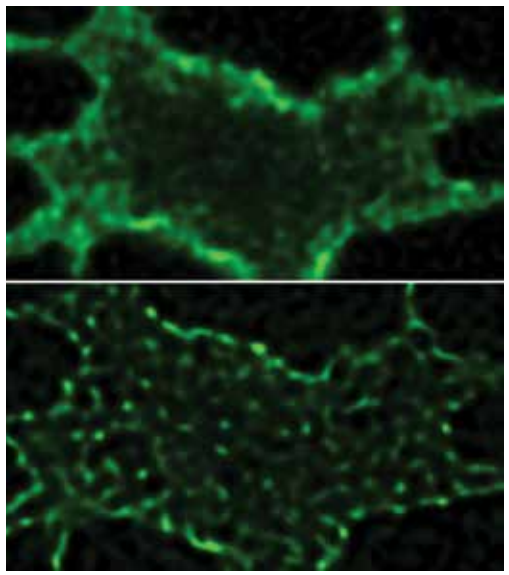
Figure 2 Example of the development of new imaging techniques: **Super resolution light microscopy**. *Immuno labelling of SNAP29 in the small blood platelets clearly shows the benefit of super resolution light microscopy (in this case STED). Unlike conventional confocal scanning light microscopy, STED clearly shows the surface-connected cannalicular membrane system running through the platelate. Courtesy of Prof. Alastair Poole, University of Bristol.*

What does BioImagingUK want to achieve?

BioImagingUK proposes to organise the UK bioimaging infrastructure into a 3-tiered pyramid. The base of the pyramid (Tier 1) would be formed by the local imaging labs and facilities. This is the most critical layer, producing 90% of all the bioimaging output in the UK, and forms the foundations of the initiative. However, as discussed above, local facilities cannot cover every microscopy technique required for modern day life science research. In order to provide access to more technically challenging or expensive techniques, a number of "Centres of Excellence" (CoEs; Tier 2) would be created. These CoEs will usually be based within general bioimaging facilities that have one or more specialised imaging technologies not available to the majority of other UK facilities. CoEs should be willing and able to open their doors to outside users and share their knowledge and instruments with them. The funding mechanism for access to and maintenance of COEs is still under discussion, and must certainly take into account the extra pressure on staff and machines. In a very few cases, there will be a need for a "National Facility" (Tier 3). Usually the resources needed to run such a facility are very high and the demand is very specialised. One example of a National Facility is the high-resolution electron microscopy facility for structural biology, now under construction at the Harwell site in Oxfordshire.

As imaging developments are continuing apace, we must bear in mind that techniques currently considered suitable for a CoE may in the (near) future be considered standard tools for a bioimaging facility. For example, it is possible that super resolution light microscopy will mirror confocal scanning light microscopy, which 20 years ago was state-of-the-art but is now a standard asset of most bioimaging facilities. As such, the pyramid is a fluid model, and as new techniques emerge so others will trickle down and be integrated into Tier 1 of the pyramid.

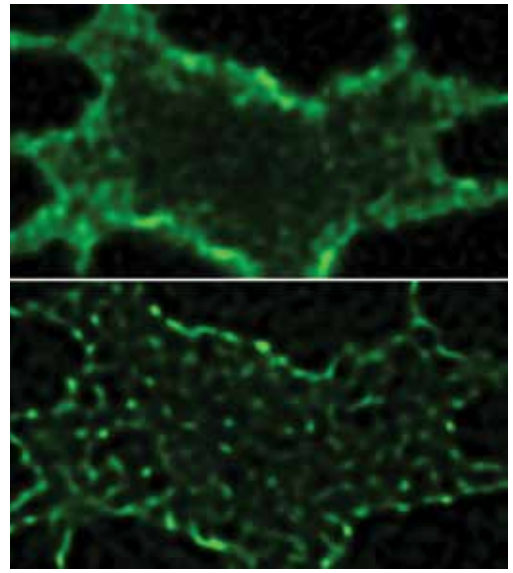
Figure 3 Electron Microscopy of Islet of Langerhans as a high-resolution means of studying Diabetes

It must also be stressed here that "just" providing the equipment for such an initiative (as unfortunately is currently the case in a lot of the funding initiatives) will not work. Provision of adequate levels of skilled staff is absolutely key, which will be fundamentally linked to training and career structures at all levels from undergraduate to postgraduate to lab leader. We may be able to buy or build a space rocket, but without the astronaut it isn't going anywhere!

Further information can be found in the strategy meeting report http://www.bioimaginguk.org/images/0/04/BioImagingUK_Meeting_Summary_v5.pdf

How will BioImagingUK achieve this?

In order to achieve the creation of a successful BioImagingUK infrastructure we have formulated five aims.

Aim 1. Strategic Technology: Continue to **define strategic priorities** for imaging technology, data management and analysis, training, and career development via meetings of the community of scientists who use and develop imaging tools and resources.

Aim 2. Training: Extend existing resources for **training in biological imaging**, covering imaging technology, data processing, management and analysis. Develop a catalogue of training courses available in the UK and contribute to existing public on-line training resources so that all UK imaging facilities and scientists can access world-class, up-to-date training materials.

Aim 3. Careers: As highlighted above, qualified staff are essential for running a successful facility. We aim to develop quantified measures of **investment into the personnel** that develop and deliver imaging technology and resources. We will provide a direct and quantified link between investment and scientific impact (using citations and other metrics) to enable evidence-based (rather than anecdotal) discussion on the critical role of imaging technology staff. Thus, we aim to drive the creation of sustainable career structures for the community.

Aim 4: Outreach: **Extend the range and domains of BioImagingUK's community** to include more scientists from medical imaging, digital pathology, materials sciences and any other fields that complement this activity. For example, we have recently made successful links with the Materials Science (EM) community that has lead to the organisation of a joint meeting at the Microscience and Microscopy Congress (MMC 2014) in Manchester.

Aim 5. Communication: **Maintain the BioImagingUK Wiki site and other web resources.** The resources BioImagingUK uses to share its activity and content must be maintained


Figure 4 text: **Training** *is a critical aspect of ensuring the future of BioImaging in the UK.*

and current. While this is an accessory activity, maintaining and supporting these resources is critical to increase the reach and maximise the impact of BioImagingUK.

Now that the initial framework is set up, let's hope that we can convince the funding bodies, universities and research institutions to further invest in this initiative, to create a solid and sustainable bioimaging infrastructure for the UK scientific community that will deliver the necessary support for the highest quality scientific research and the best possible return on investment for the UK.

More details of BioImagingUK and a full record of its activities can be found at (http://www.bioimaginguk.org). Article written by Paul Verkade and Lucy Collinson as members of the BioImagingUK Organising Committee.

A Correlative Light Electron Microscopy Centre of Excellence for BioImagingUK

Why a CoE for CLEM?

Besides super resolution light microscopy, one of the specialised techniques being considered for a Centre of Excellence is Correlative Microscopy. In Correlative Microscopy, the same sample is analysed using a variety of imaging modalities, with the combination of technologies providing more information than the sum of the parts (1 + 1 = 3). The most established of the correlative imaging techniques is Correlative Light Electron Microscopy (CLEM), a catch-all term that encompasses a wide variety of workflows. For light microscopy (LM), one can image fluorescent probes in fixed samples or use live imaging to follow a specific biological event. The sample is then prepared for electron microscopy (EM), and can be imaged using a variety of different EMs, from transmission electron microscopes (TEM) and scanning electron microscopes (SEM) to 3DEM in the Serial Block Face SEM (SBF SEM) or Focused Ion Beam SEM (FIB SEM). Images from the same region of the sample give both functional (LM) and structural (EM) information that can be used to answer biological questions in a range of samples from single cells to tissues and model organisms. Most recently, sample preparation workflows have been developed that preserve fluorescent probes within samples prepared for EM, which can then be imaged within the vacuum chamber of the latest integrated light and electron microscopes (ILEM).

To provide access to such a wide variety of advanced CLEM workflows, a distributed network needs to be created, as no single site covers all of these techniques and technologies. This network is an obvious candidate for a BioImagingUK CoE, which would act as a resource of knowledge and training as well as providing access to advanced technology. To achieve this, specialist CLEM bioimaging facilities from the University of Bristol, the University of York and the London Research Institute have teamed up with the aim of functioning as a distributed "CoE for Correlative and Integrated Microscopy" within the BioImagingUK framework.

Over the next few pages we will introduce the three different imaging facilities and describe their capabilities in the field of Correlative Microscopy. As a group, we are very excited by the recent developments in the CLEM field (see e.g. Müller-Reichert and Verkade, 2012 and 2014), and would like to share these with you (as we are sure other potential CoEs will for their respective specialisms). In the future, as BioImagingUK progresses, we hope that together we will be able to provide expertise, training and access to these CLEM technologies for the UK scientific community.

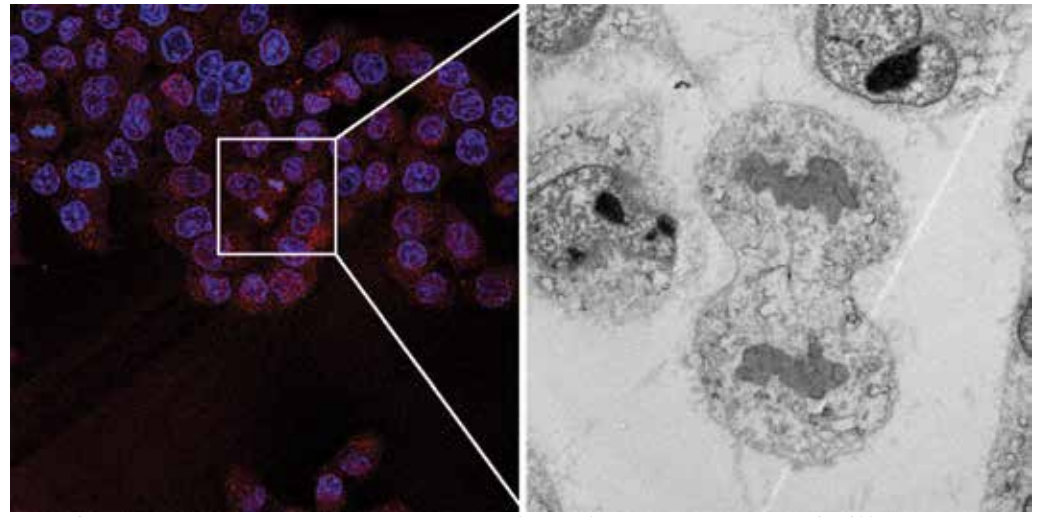

Figure 6 CLEM: Finding the needle in the haystack. Example shown, specific stage of cell division shown by less than 1 in 100 cells

The Wolfson Bioimaging Facility

University of Bristol

Introduction

The Wolfson Bioimaging Facility (http://www.bristol.ac.uk/biochemistry/wbif/), located in the Medical Sciences Building at the University of Bristol, was founded in 2008 with the integration of the EM unit into the MRC cell imaging facility. With support from the Wolfson Foundation, the MRC and the University of Bristol, the decision was made to add EM to the excellent existing live cell imaging capabilities to create one integrated facility ideally suited for performing CLEM experiments. As a central resource for the Faculty of Medical and Veterinary Sciences, the facility mainly serves researchers in the basic biomedical sciences. But, especially through the newly emerging themes of synthetic biology and regenerative medicine, it also has a substantial user base from other sciences such as Chemistry, Engineering, Physics, and Clinical Medicine. As multi-technique approaches are becoming increasingly important, the future of the Wolfson Bioimaging Facility will also see stronger links with our Proteomics, Flow cytometry, and Histology facilities.

The integrated nature of the Wolfson Bioimaging Facility does not mean that every microscopy experiment is based around CLEM, indeed most experiments will only require standard LM or EM techniques. Indeed, our effort in placing light and electron microscopes side-by-side has not only paid off for CLEM workflows but has also generated better awareness of the individual capabailities of light and electron microscopy. It is through discussions with the scientists that a decision is made on the most appropriate workflow.

Expertise

Our current expertise in CLEM is mainly focused on the combination of LM and TEM (http://www.bris.ac.uk/biochemistry/wbif/em/). We have a variety of widefield light microscopes and confocal scanning microscopes (including a spinning disk) available (http://www.bris.ac.uk/biochemistry/mrccif/index.html). These can be used for either live or fixed cell experiments. Our main CLEM workflows include pre-embedment CLEM (Benito-Alfonso et al., 2013), live cell imaging in combination with chemical or cryo-fixation (Verkade, 2008, Brown et al., 20012), and CLEM based on the Tokuyasu cryo-sectioning and immunolabelling technique (Hodgson et al., 2014).

Through a recent award made by the BBSRC we

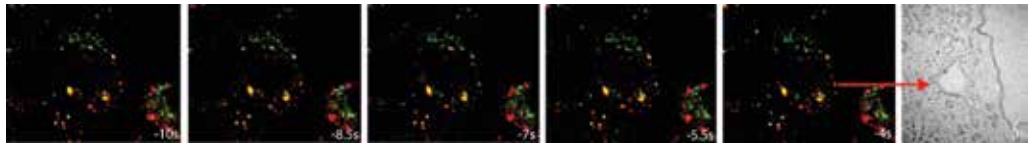

Figure 7: CLEM: High-resolution analysis of dynamic events

are now expanding our capabilities to include cryo-fluorescence for a complete cryo-CLEM workflow, and introducing the capability to (photo)convert fluorescence into an electron dense precipitate for high resolution protein localisation in the EM. These processing techniques will open up completely new avenues for researchers wanting to use the facility.

Technology development

One of the crucial factors to be able to provide state-of-the-art technology is its further development. Besides developing new strategies and processing techniques for the combination of light and electron microscopes, we currently focus on strengthening the other two pillars of a CLEM experiment, namely probes and analysis, through collaborations with the Schools of Chemistry and Engineering, respectively. Also, through collaborations with our industrial partners we can test and use the latest equipment and analyse their capability for integration into the CLEM workflow.

For some more advanced LM technologies such as Total Internal Reflection (TIRF) microscopy and the recently acquired multi-photon microscope, we have not yet developed CLEM strategies, but as technologies evolve they could well be integrated in the future.

Training

One of the major objectives within BioimagingUK is to provide training in specific technologies and other aspects of the bioimaging world e.g. facility management. The Wolfson Bioimaging Facility is the current home of the EMBO practical course on Correlative Light Electron Microscopy. Whereas running this prestigious course as an EMBO course initiates alot of international contacts, it can only offer a very limited number of spaces to UK scientists. As part of BioimagingUK however it is our intention to run a practical course on CLEM together with York and London, based on the EMBO model, dedicated to UK scientists and alternating with the existing EMBO course.

Figure 8: The Wolfson Bioimaging Facility is the home of the EMBO practical course on Correlative Light Electron Microscopy

These are thus exciting times, when new technologies and workflows emerge and are integrated into the facility. We believe that through the BioimagingUK initiative we will be able to interact with a large number of scientists across the UK, either to train them into CLEM technology to export back to their lab, or to support and work with them on site in Bristol to generate some very exciting and outstanding science.

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Imaging and Cytometry Laboratory

University of York

Introduction

The Imaging and Cytometry Lab (www.york.ac.uk/biology/tf) is part of the Bioscience Technology Facility (www.york.ac.uk/biology/tf) in the multidisciplinary Department of Biology at the University of York. Its purpose is to provide expertise, access and services for Confocal Microscopy, high-end Fluorescence Microscopy, Electron Microscopy and Flow Cytometry. The imaging laboratory houses around £4.5 million of top-end instrumentation plus associated peripheral equipment. Housing the various forms of microscopy and flow cytometry in a single lab provides a unique and powerful mechanism to deliver fully integrated and correlative imaging.

Figure 9: The Technology Facility at the University of York

Expertise

The lab has two distinct roles. First and foremost is offering access, training, service and maintaining a range of high end off-the-shelf systems, which

Figure 10: The team at York

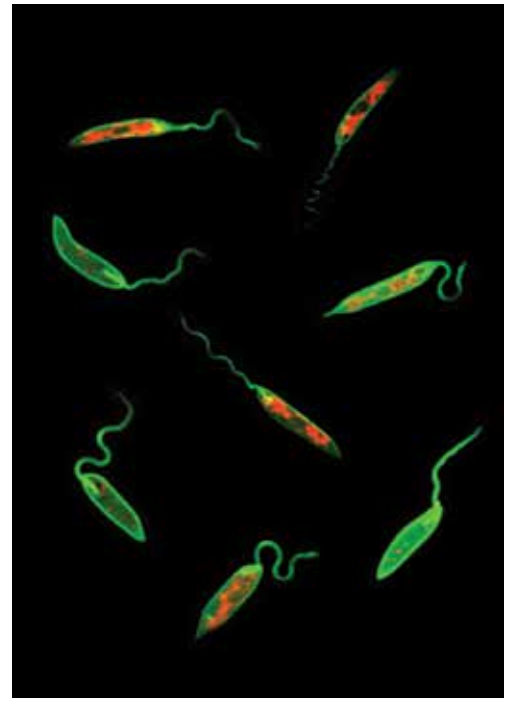

Figure 11: a confocal composite image of live leish which are then studied later on the electron microscopes to give more detailed, diverse information. Image in collaboration with D Smith H Price and L MacLean at the University of York.

is facilitated by the six dedicated Experimental Officers and expert Technical team who not only cover the breadth of instrument types, but also a breadth of scientific specialisms. This supports around 180 internal users and 30 external users per annum. The second element is the labs own driven R&D programmes with five staff including postdoctoral researchers, PhD and MSc students all feeding into MRC/BBSRC/EPSRC/TSB funded projects. The two roles of the facility work in tandem, fusing expertise and technologies in LM and EM to best exploit CLEM approaches in a multidisciplinary environment.

Technology Development

The Research side of the laboratory is currently focused on two different technologies.

The first, ptychography, is a quantitative, label-free, high-contrast, live-cell imaging technique (Marrison et al., 2013) that can be used to study cell cycle, apoptosis and differentiation and is now being applied to cancer, immunology, stem cell and

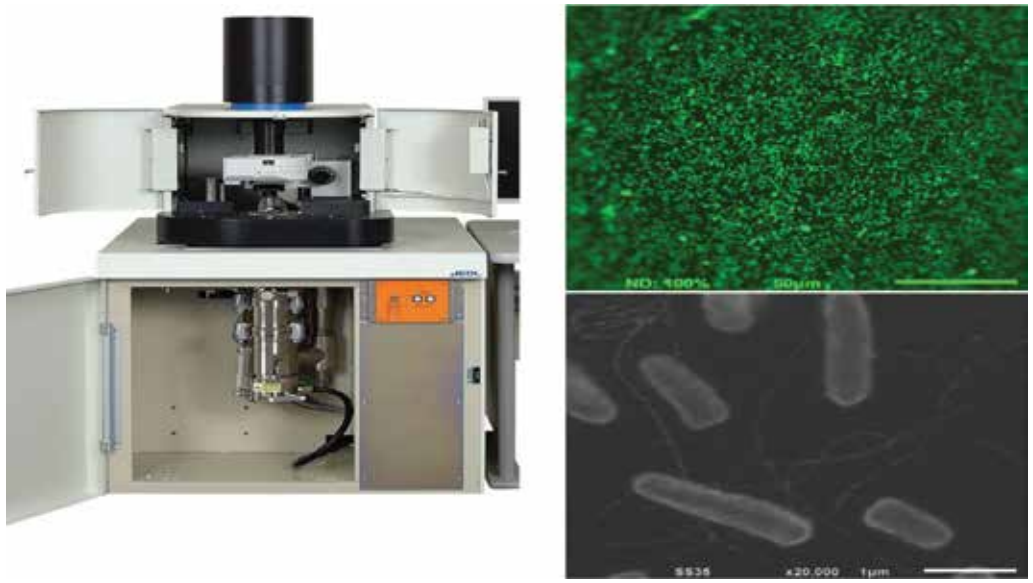

Figure 12: Opened up image of the novel JEOL ClairScope JASM6200 (left). The top right image shows the fluorescence of GFP in GFP-transfected and Nanogold labelled salmonella (curvature of SiNx window under vacuum causes loss of focus at edges). The bottom right shows the flagella on the bacteria in the corresponding SEM image of the wet sample after in-situ gold enhancement (thanks to Dr. Erica Kintz for providing the bacteria).

neurobiology research. This work is in collaboration with Phase Focus and supported by the TSB and EPSRC.

The second element is focused on developing novel integrated light and electron microscopy techniques. Our approaches use a novel electron-excited Super Resolution Microscopy (eSRM) technique, which integrate super resolution LM into the EM using newly-developed electron-excited multicolour probes. Bringing an even higher degree of novelty is our work on electron imaging outside the vacuum chamber in an atmospheric electron microscope (JEOL ClairScope), where samples remain hydrated in a specialised, open, accessible petridish (Morrison et al., 2012), and are imaged using an integrated workflow for structural and functional information. This work is part performed in collaboration with Brunel University for novel probes, and is part of a larger Next Generation Optical Microscopy award from the MRC/BBSRC/EPSRC to develop new super resolution integrated systems in collaboration with Lucy Collinson at the CRUK London Research Institute. It is not only biologists that use such a novel set-up; chemists, physicists (Verch et al., 2013) and food scientists (Luo et al., 2013) are all now coming to York to exploit these novel features.

Training

The Imaging and Cytometry Lab organizes and hosts a range of training courses for the imaging community, including the RMS Light Microscopy Summer School, the 2-Day Hands-on Confocal Microscopy and 4-Day Hands-on Advanced Confocal Microscopy Course. All of these microscopy courses help train the users on the LM elements necessary for successful CLEM experiments.

It is never easy to balance a Core Service lab with a Research lab and provide a professional point of access, but we are fortunate to have developed a lab that is sufficiently large to maintain these different roles. Excitement comes from all aspects of the job, from visiting students leaving with primary research data that will contribute to solving complex research driven questions, to the longer collaborative programmes that see real technological developments.

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The Electron Microscopy Core Technology Facility

Cancer Research UK London Research Institute

Introduction

The Cancer Research UK London Research Institute (CRUK LRI) is a core-funded institute located in Lincoln's Inn Fields near Holborn in central London. The Institute hosts 38 research groups and 15 Core Technology Facilities (CTFs). The Electron Microscopy CTF (http://www.london-research-institute.org.uk/technologies/electron-microscopy) provides the equipment and expertise necessary to image the structure of molecules, cells and tissues at high resolution. With a team of six postdoctoral researchers, we process up to 40 projects with 25 research groups at any one time, across disciplines as diverse as genomic integrity and cell cycle, cell biology, immunology, neurobiology, cancer biology, vascular biology and developmental biology. The EM CTF mainly serves the researchers at the CRUK LRI, but with recent RCUK funding, open access is now available to external users for both 3D CLEM and Integrated Light and Electron Microscopy (ILEM). In 2015, the LRI will move to the new Francis Crick Institute next to St Pancras Station in central London. This exciting move will see an expansion of the EM facility to provide capacity and cutting edge imaging capability for 1250 Crick scientists (http://www.crick.ac.uk/).

Expertise

Our expertise lies mainly in the electron imaging domain, but we also use light and X-rays to probe biological samples. We have experience of samples as diverse as proteins, DNA, standard cell lines, primary cell lines, virus-infected cells, fungi, yeast, Drosophila melanogaster, Caenorhabditis elegans, zebrafish, 3D matrix models and tissues. We have a wide range of sample preparation expertise and equipment, covering room temperature embedding,

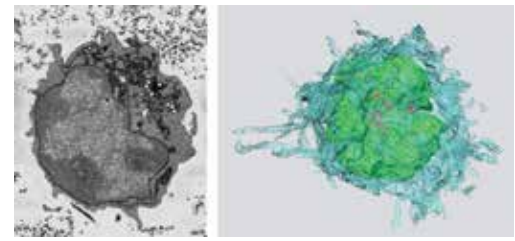

Figure 13: Immune cell containing 200 nm fluorescent beads, imaged in the SBF SEM (left) with a 3D model created in Amira (right) (Thaunat, 2012)

ultrathin sectioning, serial sectioning, electron tomography, cryosectioning, immunolabelling, high pressure freezing, freeze substitution, volume EM and correlative imaging. In terms of imaging technology, we have the instrumentation and/or expertise for widefield and confocal light microscopy, cryo-fluorescence light microscopy, SEM, TEM, electron

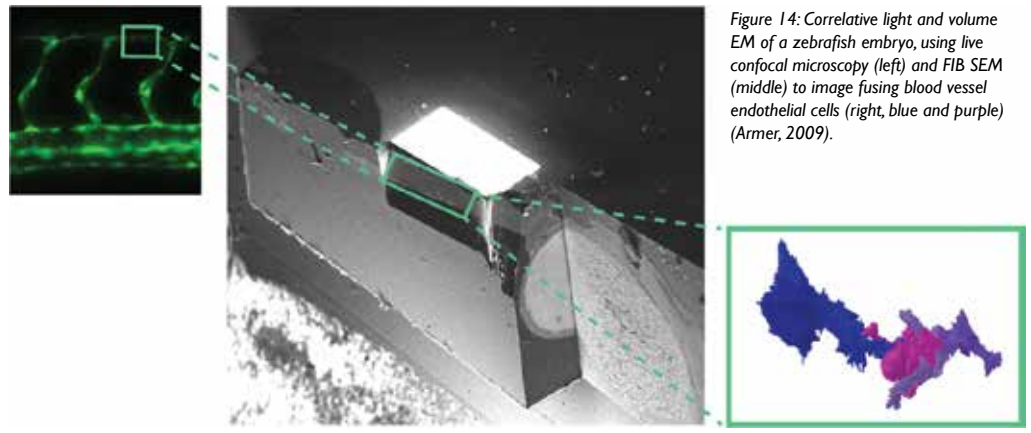

Figure 14: Correlative light and volume EM of a zebrafish embryo, using live confocal microscopy (left) and FIB SEM (middle) to image fusing blood vessel endothelial cells (right, blue and purple) (Armer, 2009).

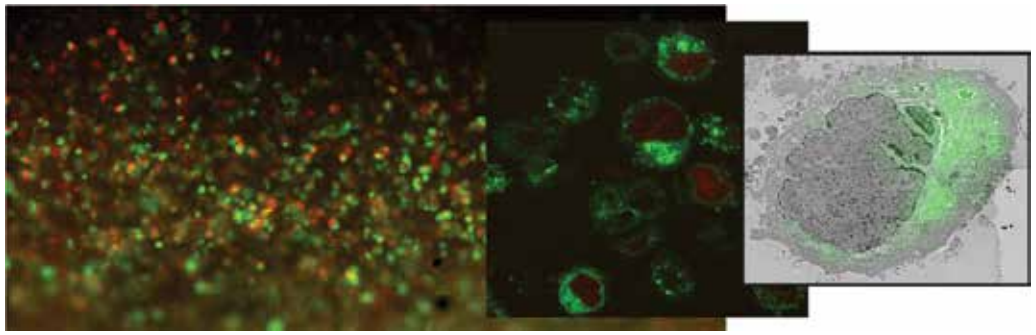

Figure 15: HeLa cells expressing GFP and mCherry and stained for EM, embedded in a resin block (left), in a 200 nm section (middle) and in an integrated light and SEM (right) (Peddie, 2014).

tomography, microCT, soft X-ray tomography, Serial Block Face SEM, Focused Ion Beam SEM, Integrated Light and SEM, and Integrated Light and TEM.

Development

Our technology development themes cover both multiscale correlative imaging and near-native state imaging.

Though we use pre-embedding CLEM in cells as a standard technique, the demands of the researchers at the LRI have driven us to adapt and develop our protocols and instrumentation to perform CLEM in much larger 3D specimens. For Correlative Light and Volume EM (CLVEM), we developed sample preparation protocols and imaging strategies to locate and image very small regions of interest within tissues and model organisms, using automated serial imaging with FIB SEM and SBF SEM (Armer et al., 2009; Bushby et al., 2012; Peddie and Collinson, 2014).

To streamline 3D CLEM and improve accuracy, we developed In-Resin Fluorescence sample processing for maintenance of GFP fluorescence in mammalian cells and tissues after preparation for EM. We have demonstrated that GFP fluoresces in resin-embedded cells inside the vacuum of an integrated light and scanning electron microscope (Peddie et al., 2014), enabling integrated imaging for numerous biological applications. With laser physicists at the LRI, we are now building our own integrated LM/EM systems for 3D structure/function studies. This work is part of a larger Next Generation Optical Microscopy award from the MRC/BBSRC/EPSRC to develop new super resolution integrated systems in collaboration with Peter O'Toole at the University of York.

In collaboration with Dr Liz Duke at the Diamond Light Source, we are localising proteins in cells preserved as close to their native state as possible, without the use of chemical fixatives, heavy metal stains or resins. Together, we have developed procedures for correlative imaging of intact plunge frozen cells using cryo-fluorescence and cryo-soft X-ray tomography at synchrotrons in Berlin and Barcelona (Carzaniga et al., 2013; Duke et al., 2013). Most excitingly, this technology will be available at the Diamond Light Source in Oxfordshire later in 2014.

Expertise in data alignment, reconstruction, rendering and analysis is a pre-requisite for handling 3D image datasets, and we are complementing this with collaborations to develop algorithms for automatic recognition and selection of structures within EM images.

Training

As well as training the LRI scientists in electron microscopy, we also run one-to-one hands-on training for imaging specialists in advanced EM and CLEM techniques. We host specialist meetings like the UK High Pressure Freezing meeting, and co-organise the EMBO CLEM course with Paul Verkade in Bristol. In addition, we frequently present lectures and workshops at national and international conferences, usually alongside Paul Verkade and Peter O'Toole, to deliver a complete picture of modern CLEM technology.

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Summary

The UK imaging community is already well networked and supportive, and by working together, it is a short leap to a position where non-specialists can access rapidly developing cutting edge technologies to help solve their specific scientific questions. The CLEM expertise in Bristol, York and London has already generated interest from a number of research groups in the UK and internationally. Where possible, we have started collaborations with these research groups and have tried to provide support to tackle their biological questions. However, as yet there is no formal scheme in place to provide access, training and support. In the future, we hope that BioImagingUK will help us to co-ordinate our efforts to make best use of our available resources across the three sites, which in turn will enable UK scientists to access expertise, training and equipment to drive discovery in their chosen disciplines.

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