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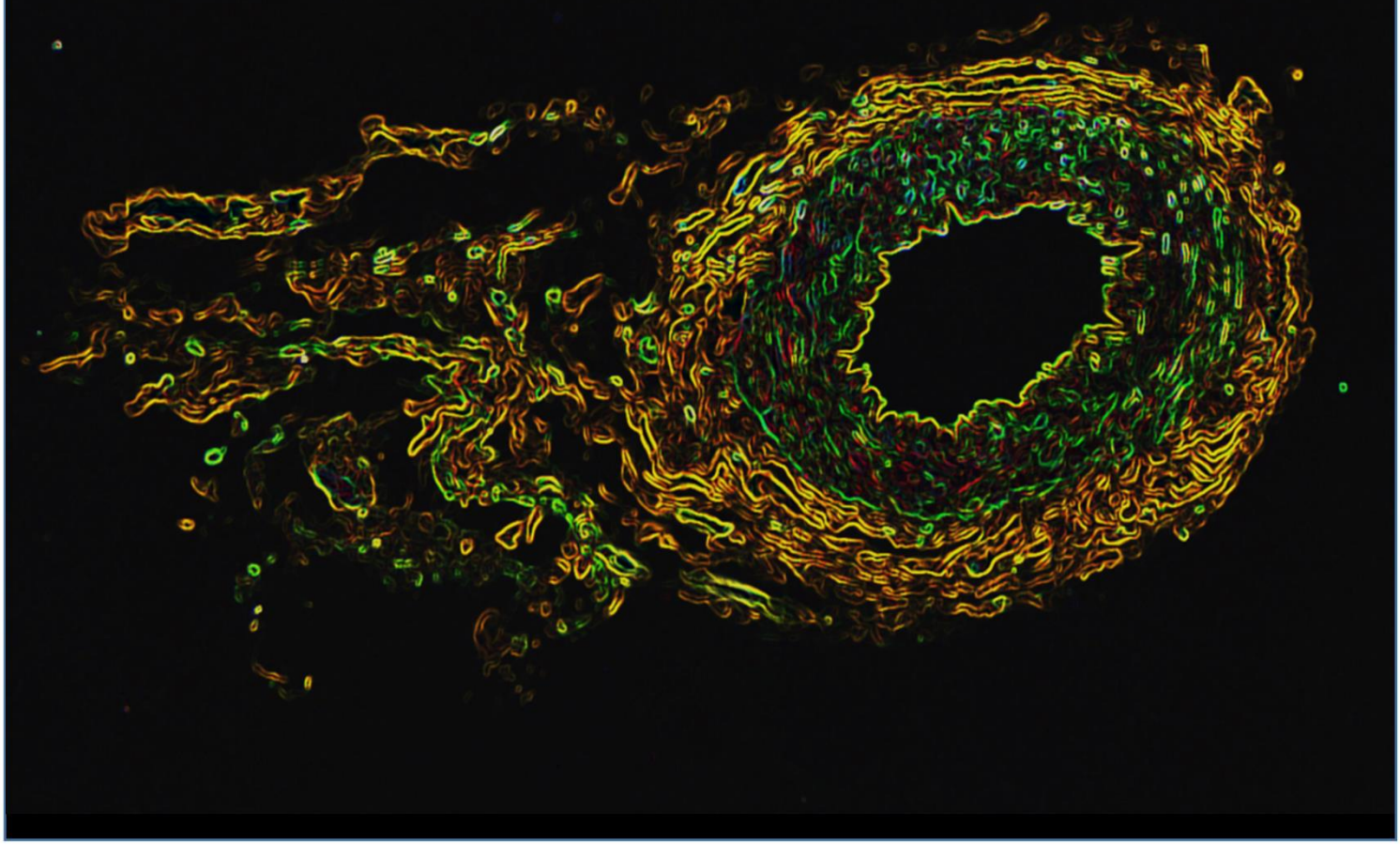

SCOTTISH MICROSCOPY SOCIETY

School of Life Sciences

University of Dundee

7th November 2023

University of Dundee

Abstracts – Posters

The £50 Poster Prize is kindly sponsored by Bruker

Please note, facility posters will not be considered for the poster prize

1. Ultrafast Microphotography at sub-microsecond framing rates

Professor Paul Campbell,
University of Dundee

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The concept for high-speed imaging using a rotating mirror was first mooted by CV Boys in the late 1800's and finessed in style by CD Miller in preparation for observing the first atomic weapon tests at Los Alamos during World War II. At Dundee, we exploit a camera operating on the 'Miller Principle' (a Cordin 550-62), which is capable of an upper framing rate of 4 million frames per second. Moreover, we have combined that instrument with microscope optics and optical trapping for target stabilisation [within fluid ambients] purposes. We have used this somewhat unique apparatus extensively for examination of dynamic microscopic cavitation processes driven by 1MHz [diagnostic] ultrasound exposure and the findings of that research programme, presented in the context of informing drug delivery processes, will be highlighted as a means of both introducing the technology to the masses and to stimulate the imagination of those who may desire full scene framing at reasonably high [diffraction limited] spatial resolutions and in the sub-microsecond per frame temporal regime. We will also demonstrate how this ultrafast imaging facility can be further modified and exploited to achieve in-frame nanosecond resolutions, and how multiple microscopic targets can be preferentially placed in scene using real-time-controlled holography-based optical tweezing.

2. FISHtoFigure: An easy-to-use tool for the analysis of sub-cellular mRNA expression in smFISH data

Calum Bentley-Abbot,
University of Glasgow

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Single molecule fluorescence in situ hybridisation (smFISH) has become a valuable tool in cell biology to investigate the mRNA expression of single cells while preserving the spatial context of the tissue. This technique produces highly information-rich confocal microscopy datasets. However, due to the computational complexity of existing open-source analytical software packages, many researchers resort to qualitative analysis of these data. Quantitative analysis of these data is paramount to answering key questions in cell biology regarding cell type identification, cell-to-cell signaling, and regulation of mRNAs in biological processes such as response to infection.

Here, we present FISHtoFigure, a new software tool developed specifically for the analysis of mRNA abundance and co-expression in QuPath-quantified, multi-labelled smFISH data. FISHtoFigure facilitates the automated spatial analysis of transcripts of interest, allowing users to quickly and easily analyse populations of cells expressing specific combinations of mRNA species without the need for bioinformatics expertise. As a proof of concept and to demonstrate the capabilities of this new research tool, we have validated FISHtoFigure in multiple biological systems in health and disease. These analyses demonstrate the ease of analysing cell expression profiles using FISHtoFigure and the value of this new tool in the field of confocal microscopy data analysis.

3. Sensing Across Scales: Quantifying the Oxygen Microenvironment in Biofilm Transport Channels to Inform Better Therapeutics

Dr Liam Rooney,
University of Strathclyde

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Understanding the chemical microenvironment in biological systems informs the development of therapeutic applications. Biofilms are dense, complex microbial communities; they are drivers of antimicrobial resistance, contribute over $4 trillion in global economic impact, and are associated with over 80% of chronic infections. New treatments are urgently required. We discovered an intricate network of channel structures which transport nutrients around the biofilm using the Mesolens; a giant microscope capable of imaging multi-millimetre-sized biofilms while resolving every single cell. We hypothesised that the transport function could be exploited for targeted antibiotic delivery but first, we must understand the channel microenvironment to ensure antibiotic molecules are not destroyed by oxidation.

We developed a dual-pronged approach to measure the local oxygen concentration in intact mature biofilms, using both electrophysiology and imaging. We quantified the oxygen concentration inside channels and in the rest of the biofilm using the Unisense Microsensor platform. In parallel, we delivered fluorescent oxygen-sensing nanoparticles directly into biofilm channels, where they were imaged using confocal laser scanning microscopy to provide a ratiometric measure of oxygen contraction along the channels. Using both methods found that the oxygen concentration in channels did not significantly differ from the rest of the biofilm, but that it decreased proportionally along the length of the channels towards the core. These findings indicate that no chemical protection is required to tailor antibiotics for targeted channel delivery, and that we can repurpose existing antimicrobials to develop new and impactful delivery methods and therapeutics.

4. Printing, Characterising and Assessing Transparent 3D Printed Lenses for Optical Imaging

Dr Liam Rooney,
University of Strathclyde

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High-quality glass bulk optics are commonplace in the design of optical instrumentation. However, research-grade glass lenses are often costly, delicate and, depending on the prescription, can involve intricate and lengthy manufacturing. We present 3D printing as a viable alternative for the manufacture of glass-like optical elements. We demonstrate a method to print custom lens designs using an off-the-shelf consumer-grade 3D printer and consumables. Lenses were printed using a Digital Light Processing-style 3D printer with transparent photopolymerising resins. Printed surfaces were post-processed using an optimised spin coating method, resulting in a thin layer of transparent resin that mitigated micron-scale surface imperfections and layering effects from the printing process, and improved optical transmission and performance. We measured the optical performance of 3D printed lenses using classic optical methods and we profiled the curvature using a high-resolution interference reflection microscopy method. The prescription and curvature of 3D printed lenses was commensurate with their glass counterparts. We also performed optical throughput measurements and we observed that the optical loss of 3D printed optics was comparable to that of commercial N-BK7 glass across the visible spectrum. Our findings showed that 3D printed lenses are a viable substitute for commercial glass lenses, with the advantage of being relatively low-cost, accessible, and suitable for use in optical instruments.

5. Understanding the Role of Biofilms in the Failure of Voice Prosthesis Implants

Louise Bolton,
University of Strathclyde

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(1) University of Strathclyde, Glasgow

(2) Queen Elizabeth University Hospital, Glasgow

Silicone tracheoesophageal valves are routinely fitted in patients following surgical removal of advanced head and neck cancers; however, they are routinely colonized by biofilms in vivo which leads to high failure rates. The failure rate of voice prosthetics (VPs) leads to the patient's dependency on regular clinical intervention to replace failed VPs, and almost daily use of high-dose antimicrobials to control the colonization of the implant. Despite these burdens, the composition and impact of colonizing pathogens on VP integrity are poorly understood. We present a multi-faceted workflow to determine the bioburden on explanted VPs to inform better clinical practice and manufacture of tracheoesophageal implants. We used a combination of reflection and fluorescence confocal microscopy to map the surface of the VP and reveal the presence of bacterial and fungal biofilms. The bioburden was determined by quantifying the levels of different biofilms on the surface, and deterioration of the VP surface was monitored by reflection imaging. We show that VPs were colonized by both fungal and bacterial communities, which form discreet biofilms over the surface. The spatial organization is also linked to the degradation of the VP surface, where we show that fungal biofilms invade and perforate the surface of VPs. The new insights show that complex polymicrobial biofilms are responsible for implant failure. Furthermore, our findings indicate that more tailored antimicrobial therapies and higher standards of implant manufacturing are required to lower the bioburden of VPs.

6. Obtaining super-resolved images at the mesoscale through Super-Resolution Radial Fluctuations

Mollie Brown,
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Widefield fluorescence microscopy is used for diffraction limited imaging of fluorescently labelled cellular structures. Super-resolution methods have been able to overcome this diffraction limit to achieve significantly higher spatial resolutions, which has led to a greater understanding of cellular structures. However, these methods often have a restricted field of view, limiting the understanding of behaviours and interactions on a larger scale. Here, we have applied Super-Resolution Radial Fluctuations (SRRF)[1,2] with image data from the Mesolens[3] to obtain super-resolution images over a field of view of 4.4mm x 3.0mm. We demonstrate that SRRF processed images display a notable improvement in resolution and contrast over Mesolens images obtained with diffraction limited illumination. We assessed the accuracy of SRRF images using a secondary analysis method, super-resolution quantitative image rating and reporting of error locations (SQUIRREL)[4.] Our data shows consistent structural agreement between the original raw image data and the SRRF processed images, demonstrating a cost-efficient and accurate method of obtaining super-resolved images over a large field of view.

1. Gustafsson, N. et al. Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. Nat. Commun. 7, 12471 (2016).

2. Culley, S. et al. SRRF: Universal live-cell super-resolution microscopy. Int. J. Biochem. Cell Biol. 101, 74-79 (2018).

3. McConnell, G. et al. A novel optical microscope for imaging large embryos and tissue volumes with sub-cellular resolution throughout. eLife 5, e18659 (2016).

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7. Investigating the Bacterial Burden of Chronic Oral infections

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Paediatric recurrent tonsilitis is one of the most common issues that GP practices manage, accounting for 1.3% of outpatient visits within the UK. The associated pain can cause significant impact and distress on a child's life with children receiving multiple rounds of antibiotic treatment and potentially undergoing high-risk surgery. Chronic tonsil infections are commonly linked to colonisation and invasion by Gram-positive Group A Streptococcus bacteria. Gram-negative bacteria, such as Klebsiella pneumoniae, are reported to be involved in tonsillar disease[1], but the bacterial burden has not yet been quantified. It is necessary to understand the significance of Gram-negative and Gram-positive pathogens, as coincidence may contribute to synergistic drug resistance, cross-feeding, and increased chronicity.

Here we report an optimised staining method to robustly and repeatedly distinguish Gram-positive and Gram-negative bacteria using fluorescent antibiotic conjugates. We will show preliminary data using cultures of laboratory Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive), before discussing applications to clinical diagnostics. Using these optimised staining methods, in future work we will stain post-operative ex vivo tonsil tissue and, using open-access image analysis methods determine the bacterial burden, thereby providing information to improve primary care and minimise long term clinical intervention.

1. Klagisa, Renata et al. Analysis of Microorganism Colonization, Biofilm Production, and Antibacterial Susceptibility in Recurrent Tonsillitis and Peritonsillar Abscess Patients. International journal of molecular sciences vol. 23,18 10273. 7 Sep. 2022, doi:10.3390/ijms231810273.

8. Whole brain optical tissue clearing and light sheet microscopy to study autism spectrum disorders

Kirsty Craigie,
The University of Edinburgh

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(1) Simons Initiative for the Developing Brain, The University of Edinburgh, Scotland.

(2) Centre for Discovery Brain Sciences, The University of Edinburgh, Scotland.

Recent advances in optical tissue clearing and light sheet microscopy have enabled whole-brain, 3D imaging of the intact mouse brain. Optical clearing allows the visualisation of entire structures without compromising spatial information and introducing slicing artefacts. Despite these advances, optical clearing has not been optimised for use in rat brain tissue. Although there are over one hundred clearing methods available, the larger size of rat brain tissue and its higher lipid concentration, renders most of these methods unsuitable. As such, we can experience poor antibody penetration to deeper tissue structures. In addition, the lack of adequate rat brain atlas and analysis tools makes registering and quantifying our data difficult.

Rats are the preferred model for studying neurodevelopmental disorders such as Autism Spectrum Disorders (ASD) due to their closer evolutionary relation to humans. In addition, rats possess more complex behaviour in comparison to mice giving us a deeper insight into the emotional complexities of ASD. Whole-brain approaches allow us to explore the neuropathology underlying ASD, and identify “bigger picture” anomalies. To aid with this, we have developed a novel optical clearing method called RatDISCO that allows us to successfully render our rat brain tissue transparent, and immunolabel deep tissue structures. We have also developed 3D rat brain atlases for specific structures of interest and corresponding analysis pipelines with which we can register and quantify our light sheet microscopy data. Furthermore, we have test our resources against a variety of neuronal and glial markers, and are actively developing this work further by investigating the compatibility of a new whole-brain analysis software platform with our rat brain data.

Facility Poster

9. Phenotypic Screening at NPSC for Discovery and Understanding

Alistair Langlands,
NPSC, University of Dundee

A.J. Langlands, L. Sesma Sanz, S. Kane, M. McIntyre, J.R.Swedlow

University of Dundee

NPSC provides phenotypic screening services for the School of Life Sciences and external academic and commercial partners. High throughput phenotypic screening (often referred to as “high content screening” or HCS) uses quantitative image-based readouts of cell and tissue models to measure the effects of molecular perturbations on cell and/or tissue models of biological systems and functions. The NPSC has run assays examining a broad range of processes, including nuclear translocation assays; live cell assays quantitatively assessing cell dynamics in response to drug treatments; pathway reporters (antibody and fluorescence-based reporters); and Cell Painting utilising organelle labelling to reveal mechanism of action. These have involved multiple cell types including iPSCs, human-derived primary keratinocytes, immune cells, and germ cells, and standard laboratory immortalised cell lines.

NPSC’s facilities include all necessary cell and tissue culture, automated liquid and compound handling, plate handling and data acquisition for running large-scale screening assays. Advanced computational resources (compute cluster, fully backed up data storage, OMERO Plus data management, CellProfiler and R workflows, Jupyter Hub server) are available for data reduction and analysis.

We have a proven record of working with Academics from within the University of Dundee, other universities in the UK and EU and biotech and pharma companies around the world. Our assays have identified tool compounds to aid understanding of cellular processes and driven drug discovery programmes from initial hit identification through hit-to-lead development.

10. The Cellular Role of the Group A Carbohydrate in Streptococcus pyogenes

Dr Saria Mansoor,
University of Dundee

S. Mansoor (1), Paul Appleton (1), Graeme Ball (1), Helge Dorfmueller (1)

(1) University of Dundee

Streptococcus pyogenes (Group A Streptococcus or GAS), a bacterium capable of causing a broad spectrum of clinical manifestations once it breaches the host's natural barriers. From mild streptococcal pharyngitis to severe conditions like necrotizing fasciitis and toxic shock syndrome, GAS infections pose significant health risks. Tragically, individuals who survive these infections may develop long-term autoimmune post-streptococcal disorders, which claim the lives of half a million people annually worldwide. The clinical management of GAS infections relies heavily on antibiotics, but the rise of antibiotic resistance in clinical GAS isolates is a concerning trend that necessitates the development of new antibiotics and a protective vaccine.

The Group A Carbohydrate (GAC), a rhamnose polysaccharide (RhaPS) specific to GAS, has emerged as a virulence factor implicated in GAS's ability to evade the host immune system. GAS exhibits ovococcal characteristics, incorporating septal and peripheral peptidoglycan from the septum outward and producing a cell wall rhamnose polysaccharide like its ovococcal homologues. Notably, RhaPS has been found to play a crucial role in cell division, ensuring correct septal placement in related bacteria such as Streptococcus mutans. This highlights the potential significance of GAC in GAS cell division processes. Using fluorescently labelled D-amino acids (FDAA) in a chase experiment, different stages of the GAS cell cycle were precisely identified and quantified through meticulous microscopy analysis. The cell division pattern of GAS closely resembled that of ovococcal bacteria such as Streptococcus pneumoniae. Staining for GAC along with FDAA-labelled cells revealed its crucial involvement in cell division by marking the correct site for septal formation. Cells lacking fully formed GAC, expressing only the polyrhamnose backbone, exhibited deformities and improper septal placement, emphasizing the critical role of GAC in maintaining proper cellular morphology. These findings contribute to the understanding of GAS biology and provide valuable insights for potential therapeutic strategies targeting GAS infections.

11. Are model-based tools still relevant for bioimage analysis? New methods for background estimation and denoising fight back

Mauro Silberberg,
Universidad de Buenos Aires

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Artificial Intelligence (AI) methods have taken the field of bioimage analysis by storm, eclipsing model-based methods with their promises of *learning* the *model* from the data. However, model-based methods offer several advantages: from avoiding the *training* process to better generalization when facing out-of-distribution samples. More importantly, they are *understandable*, based on hypotheses that let us know *a priori* if they are well-suited for the task.

Here, we demonstrate two of our recently published model-based methods and compare them against AI ones. The first method, SMO, is based on robust directional statistics to recover an image's background intensity distribution. Leveraging the lack of spatial correlation in flat background regions, it allows to robustly select a fair sample of those regions, providing an unbiased estimation of the distribution. The second method, binlets, is an adaptive binning algorithm based on the Haar wavelet, which shines when handling (transformations of) multichannel data. It puts a spin on standard wavelet denoising by considering the trade-off between accuracy and resolution on all channels simultaneously. Its only hypothesis is that the underlying signal is smooth such that neighboring pixels may be averaged, and just requires a statistical test to compare them.

As a community, it would be pertinent to revise this bewitchment with AI/ML. To publish new methods, it is becoming a requirement to compare against them, which might not be already pre-trained for the same task. AI can be a tool to explore data and generate new hypotheses, but not the end goal of science. After all, to overcome the diffraction limit it was necessary to model the whole measurement process, and could not have been done from a purely data-driven perspective.

Facility Poster

12. 3D volume electron microscopy to reveal ultrastructural changes in a gene-edited sheep model of CLN1 disease

Jon Moss,
Roslin Institute (University of Edinburgh)

J. Moss (1), S.L. Eaton (1), J.D. Cooper (2), T.M. Wishart (1)

(1) Roslin Institute, University of Edinburgh

(2) Washington University in St. Louis, School of Medicine

The Roslin Institute 3D Electron Microscopy facility was founded in August 2022 to provide 3D ultrastructural solutions to research projects. Over the last fifteen months we have developed workflows to satisfy these aims and this year the first projects are receiving their first data. The facility principally uses serial block-face scanning electron microscopy (SBF-SEM) and correlative array tomography, but also has the capacity for serial section transmission electron microscopy for smaller 3D volumes, and SEM for topological data.

Here, we present two SBF-SEM 3D image stacks collected for a sheep model of CLN1 Batten disease. The image stacks of this tissue allow us to screen the cells of grey and white matter regions of the frontal cortex for the lysosomal abnormalities observed in the disease. These stacks consist of electron micrographs captured from the block-face after every 50 nm cut of the tissue. Two stacks are presented; one, with 15 micron square images through 300 (50 nm) planes (XY resolution: 5 nm), to capture an individual cell, and another, with 80 micron square images through 300 (50 nm) planes (XY resolution: 10 nm), to capture multiple cells, within a region of white matter. We are currently working on developing an analysis pipeline for this tissue, which can then also be used for other projects that come through the facility.

We make an effort to collaborate with other microscopists through different national and international networks and would like to do the same on a local level in Scotland.

13. Electro-Optical Coherence Tomography

Dorian R. Urban,
University of Dundee

D. Urban (1, 2), T. Vettenburg (1), M. Preciado (2), P. Novak (2)

(1) University of Dundee, (2) Optos plc

Optical Coherence Tomography (OCT) is an interferometric imaging technique that bridges the broad area between high-resolution of microscopy with deep-tissue ultrasound. This often makes it the tool-of-choice for material inspection, dentistry, and in particular, ophthalmology. Here we show, without the need for mechanical repositioning of the reference arm, how harmonic images can be dynamically synthesized via opto-electronic frequency shifting in OCT imaging. The extreme coherence lengths of Micro-Electromechanical Vertical Cavity Surface Emitting Lasers (MEMS-VCSEL) have opened the door to high-speed OCT without the roll-off that restricts its imaging capability. We showcase the opto-digital reference arm with an experimental demonstration on a realistic model eye. Without loss in speed or resolution, this technique has the potential to enable OCT imaging without the limitations associated with a mechanical delay line.

Facility Poster

14. Edinburgh Super-Resolution Imaging Consortium

Dr Jessica Valli,
Heriot-Watt University

J. Valli (1), A. Wheeler (2), M. Pearson (2), C. Rickman (1), W. Bickmore (2)

(1) Heriot-Watt University

(2) University of Edinburgh

ESRIC is an open access advanced imaging facility specialising in super-resolution microscopy with a focus on interdisciplinary collaboration and teaching. We offer multiple super resolution imaging modalities, pushing the limits of resolution to meet your scientific needs. We can help you to identify the most suitable super resolution technique for your scientific question, and we can provide support from project planning through to image analysis.

15. Utilising 3D Printed Modular Devices to Improve the Versatility of Expansion Microscopy

Rajpinder Singh Seehra,
University of Sheffield

R.S. Seehra (1), B.H.K. Allouis (1), T.M.D. Sheard (1), M.E. Spencer (1), T. Shakespeare (1), A. Cadby (1), I. Jayasinghe (1)

(1) University of Sheffield

Expansion microscopy (ExM) is a novel technique which allows cellular structures to be physically expanded in a uniform and calculable way. This allows us to image structures below the diffraction limit without super resolution techniques, which are not always accessible and often require a high skill level to produce usable results. Innovation into the technique has provided a robust methodology to the chemical process present, allowing current opportunities to focus on improving the toolkit surrounding it. A focus on reducing the manual handling of a gel and increasing the consistency of experiments will lead to an increase in throughput and the robustness of the data. Whilst the use of 3D printing and rapid prototyping allows the creation of a bespoke pathway to increase the consistency of pre- and post-expansion imaging. By preserving gel geometry and orientation through the use of 3D printed microplates wells we can enable pre- and post-ExM image acquisition, and distortion mapping of cells and regions of interest.

16. Mesoscale imaging of cm-sized cleared rabbit heart tissue slices

Sharika Mohanan,
University of Glasgow

Sharika Mohanan (1), Steven M. Moreno (1), Callie Lorimer (1), Camilla Olianti (2), Eline Huethorst (3), Erin Boland (3), Leonardo Sacconi (2), Godfrey Smith (3), Caroline Müllenbroich (1)

(1) School of Physics and Astronomy, University of Glasgow, UK

(2) European Laboratory for Non-Linear Spectroscopy, Florence, Italy

(3) School of Cardiovascular and Metabolic Health, University of Glasgow, UK

Tissue scattering limits the depth into which a sample can be imaged using optical methods. This effect can be mitigated by rendering the tissue transparent using optical clearing techniques that allow for imaging of cubic cm-sized samples. Light sheet microscopy is especially suited to image these samples as it can provide isotropic resolution across a large field of view whilst imaging at a high speed. By employing mesoscale light-sheet microscopy and optical tissue clearing protocols, we plan to image tissue slices excised from the left ventricle of the heart to assess structural remodeling in a rabbit model due to myocardial infarction.

Here, we present the initial results when applying the CLARITY tissue clearing protocol on healthy cardiac tissue slices from New Zealand white rabbit with respect to tissue transparency and structural preservation [1,2]. Further, we present first imaging performance results from our light-sheet microscope constructed following the MesoSPIM (Mesoscale Selective Plane Illumination Microscopy) project [3]. The mesoSPIM is designed to accommodate cleared samples to perform structural imaging studies over a large field of view (14 mm). We have built the microscope using a 100 mW 488 nm diode laser for dual-sided excitation. A combination of digitally and axially scanned light sheet provides uniform illumination and resolution across the large field of view [4]. Furthermore, zoom control on the Olympus MVX10 macroscope is implemented in the detection optics which can provide high-resolution imaging for a zoomed-in region of interest. We present the spatial resolution characterization across the large field of view and the suitability of the microscope for high resolution mesoscale imaging of cleared samples. With these results, we plan to apply the system to conduct quantitative studies of structural remodeling post myocardial infarction in the rabbit heart.

References:

[1] Chung, Kwanghun, and Karl Deisseroth. "CLARITY for mapping the nervous system." Nature methods 10.6 (2013): 508-513.

[2] Olianti, Camilla, et al. "Optical clearing in cardiac imaging: A comparative study." Progress in Biophysics and Molecular Biology 168 (2022): 10-17.

[3] Voigt, Fabian F., et al. "The mesoSPIM initiative: open-source light-sheet microscopes for imaging cleared tissue." Nature methods 16.11 (2019): 1105-1108.

[4] Dean, Kevin M., et al. "Isotropic imaging across spatial scales with axially swept light-sheet microscopy." Nature protocols 17.9 (2022): 2025-2053.

17. Protein aggregate size and structure-dependent clearance from the brain extracellular space

Pietro Esposito,
University of St Andrews

Pietro Esposito (1), Vanya Metodieva (1), Frank Gunn-Moore (1), Mathew Horrocks (2), Juan A. Varela (3)

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Misfolded protein aggregates in the extracellular space of the brain (ECS) play a key role in neurodegeneration. Extracellular aggregates (together with many other solutes) are largely cleared to the cerebrospinal fluid and lymphatic system. Fluid extracellular trafficking in the brain parenchyma remains poorly understood and core open questions in the way interstitial fluid circulates and washes out solutes remain to be answered. To evaluate how the size and shape or structure of solutes influence their clearance (or accumulation in the brain) we developed protocols to quantitatively compare cleared vs accumulated nano-objects in the brain of rodents at the single-molecule level. To understand how solute size affects clearance from the ECS in vivo we performed stereotaxic injections of fluorescently labelled nanoparticles of different colours and sizes in the hippocampus of living mice and analysed particle distribution in brain tissue and CSF. Control 3-, 7- and 10-month-old mice were used to investigate the effects of ageing on the accumulation and clearance of the injected solutes. We carried out similar experiments with labelled Ab42, choosing the labelling strategy in such a way that half of the monomer can act as a FRET donor and the other half can act as FRET acceptor. Measuring the FRET signal of individual aggregates in CSF in a bespoke microfluidic chamber integrated with a single-molecule detection setup we could quantitatively compare injected vs cleared aggregates, using the signal magnitude to infer size and the FRET efficiency to infer structural features of each aggregates. This quantitative study will help characterising clearance mechanisms in the living brain.

Facility Poster

18. Beatson Advanced Imaging Resource (BAIR)

Dr Nikki R. Paul,
CRUK Scotland Institute

Nikki R. Paul, Peter Thomason, Claire Mitchell, Lynn McGarry, Ryan Corbyn, Leo Carlin

CRUK Scotland Institute

Facility poster for the Beatson Advanced Imaging Resource (BAIR) at the CRUK Scotland Institute.

BAIR have a wide range of specialised microscopes that provide users with the flexibility to approach their research using a diverse range of applications. Microscopes include a variety of confocal and widefield systems, super-resolution systems, high-content screening, in vivo imaging systems, and equipment for advanced techniques such as TIRF, FLIM/FRET and Multiphoton imaging.

Facility Poster

19. Microscopy and Histology Core Facility

Dr Debbie Wilkinson,
University of Aberdeen

Debbie Wilkinson, Gillian Milne

University of Aberdeen

The Microscopy and Histology facility has many years' experience in Histology (wax, cryostat and resin) and Light, Fluorescence, Confocal along with MicroCT and also Electron Microscopy. Light microscopes include upright and inverted confocal, spinning disk confocal, widefield, phase holographic and slide scanner system with timelapse, multiposition, Class II live pathogen capabilities. Electron microscopes include SEM and TEM with tomographic capabilities, plus high pressure freezer and freeze substitution and microwave processor.

20. Measuring and Managing Dispersion in Multiphoton Microscopy at the Sample

Lewis Williamson,
University of Glasgow

Lewis Williamson (1), Giedre Astrauskaite (1), Caroline Muellenbroich (1)

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Two-photon microscopy (2PM) relies on nonlinear processes that scale quadratically with intensity and therefore require high photon density in spatial and temporal dimensions[1]. For this reason, femtosecond pulsed lasers are commonly employed, providing the necessary high instantaneous powers. However, most microscopes are comprised of optical elements inducing high levels of optical dispersion, more so when customisations such as remote focusing units are utilised. This can lead to a reduction in generated signal in 2PM as initially short laser pulses are progressively broadened before reaching the sample. Pulse compressors are commonly employed to compensate for pulse broadening by introducing negative dispersion.

Autocorrelators are commonly employed to measure the pulse width. However, these measurements often exclude the dispersion introduced by the microscope objective itself as its high numerical aperture (NA) causes a highly divergent beam that is difficult to input into the autocorrelator.

Here we present dispersion compensation in a 2PM with additional remote focusing with a custom-made single-prism pulse compressor. We measure the pulse duration with a bespoke ‘inline’ autocorrelator[2] that is capable of measuring laser pulses with a higher degree of divergence, post objective, directly in the sample plane. Exploiting the objective’s high NA, 2 photon absorption is generated within a photodiode[3], creating a gating mechanism that allows pulses to be temporally resolved. We present pre-compensation of pulse dispersion through direct measurement of pulse duration post microscope objective. This technique will now find application in our 3-photon microscope where dispersion must be managed even more carefully

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21. Developing 3D standards for super-resolution imaging

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Single molecule localisation microscopy, or SMLM, is a set of super-resolution imaging techniques that are based on separating individual point spread functions (PSF) in space. Most commonly SMLM is performed in 2D, however several 3D techniques have been developed recently, and most are based on manipulating PSFs to allow determination of molecular position in the Z-axis. All of these 3D techniques require calibration standards, which our research group has been working on. Specifically, we have focused on astigmatism and biplane imaging techniques.

Two general types of errors typically occur during 3D imaging. The first type is the localisation precision error, which depends on the signal-to-noise ratio and determines the accuracy of localising a single molecule. The second type is how accurate the localised molecular positions describe to the underlying structure. Localisation precision is empirically determined during the localisation process based on photon statistics. However, to determine the ability of SMLM to accurately reproduce a structure, standards that establish a ground truth for error calculation are required. We have developed a 3D structure of known dimensions using a chemically reactive bead with an impermeable surface decorated with fluorophores. This results in a thin, defined curved surface in three dimensions with a known shape over a large range of axial position. Because of this, such standards will be widely applicable to a range of super-resolution techniques, such as STED, PALM, D-STORM and others.

22. Construction and characterisation of high-throughput optical imaging system

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Current high-throughput optical imaging technologies involve mechanically translating and sequentially imaging each sample within a standard multiwell plate. This procedure is time-consuming and can result in gaps in data. The current project involves building a Random Access Parallel (RAP) microscope providing video-rate imaging across multiple wells [1]. The RAP microscope utilises a parabolic reflector to increase the field of view and allows near simultaneous brightfield imaging of multiple wells in a 96-well plate.

The current configuration uses two Neopixel LED arrays controlled by an Arduino UNO microcontroller to illuminate the sample. The LED light is collimated using aspheric lenses (f = 9mm) to provide uniform illumination. A single element plano-convex (f =72 mm) lens is placed above each well to collect the light after interacting with the sample. An image is formed on the CMOS camera (Basler acA1300-200um) using a parabolic reflector (f = 100 mm). This setup can rapidly switch to image any well under the parabolic reflector without moving the sample or camera. Here, we present step-by-step design and construction of the RAP system using custom made 3D printed optomechanical parts. Next, using resolution targets, we assess the deterioration of imaging quality of the microscope as we move away from the optical axis of the parabolic reflector. These preliminary results will allow us to ascertain the spatial and temporal characteristics of the microscope and its applicability for performing high-throughput functional imaging studies.

References:

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23. T cell priming niches are compromised in the tumour-draining lymph node

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Sarwah Al-Khalidi - SMOTY Runner-Up

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Dendritic cells (DC) play key roles in directing T cell immune responses both within tissues and lymph nodes (LN). Despite their key role in initiating T cell responses in the LN, we have a limited understanding as to how different lineages of DC cooperate to drive effective T cell immunity, and how this is dysregulated in cancer.

To thoroughly define the roles of DC subsets – probing for dozens of markers – requires a high-dimensional approach beyond the scope of conventional imaging techniques. Advanced technologies are emerging to overcome this, including the Co-Detection by indexing (CODEX) method, which uses primary antibodies conjugated to specific oligonucleotides to stain tissues. Iterative cycles of staining, imaging and washing yields a potentially unlimited number of markers. The main limitations of this powerful multiplex technology are the high cost of commercial equipment and reagents, and the limited number of conjugated antibodies available.

To increase the cost effectiveness and antigen flexibility I took a custom approach. Using this method I am now able to routinely probe more than 25 markers in a range of tissues from different biological settings, such as whole LN from lung cancer models. I have written an analysis pipeline for open-source software, with the main focus of deciphering unique DC and T cell niches that form in an infection-draining LN that may be missing in cancer.

One important discovery to date has been to show the relocation of a specific lymph node-resident DC subtype to the paracortex of the lymph node in a chemokine-dependent manner, where they provide activating signals to T cells by directly interacting with them, driving their acquisition of effector function. In the cancer setting these same DCs fail to upregulate chemokine-dependent signalling. As such, they remain outside of the T cell zone and do not provide the needed interactions to drive full T cell differentiation, with consequent implications for their effectiveness in cancer.

24. 10X Expansion Microscopy reveals the dynamics of endocytosis

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We are using 10X expansion microscopy to investigate the organisation of proteins around the endosomal membrane at different stages of endocytosis. We fluorescently tagged proteins that have key roles in endocytosis, namely Early Endosome Antigen-1 (EEA1), Rab5A GTPase, and ZFYVE16 (Endofin). We then used expansion microscopy to physically separate these targets by a factor of 10X in each direction, allowing their improved localisation outside of the diffraction-limited space.

We reveal, for the first time, the complex pattern of these proteins around the endosomal membrane. Our aim is to further investigate how these patterns vary throughout the different stages of endocytosis, as well as how endosomal proteins associate with different cargos during the sorting process.

25. BUILDING AND OPTIMIZING LIGHT-SHEET MICROSCOPE TO ANALYSE THE COORDINATION AND CONTROL OF CRITICAL CELL BEHAVIOURS DRIVING GASTRULATION IN THE CHICK EMBRYO

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Gastrulation is a key stage in early embryonic development of vertebrate embryos, where the embryo reorganizes from a one-dimensional epithelial layer to a three-germ layered structure. This involves coordinated flows of hundreds of thousands of cells in the epiblast. During gastrulation, mesendoderm precursor cells move toward the midline of the epiblast to form a structure known as the primitive streak from which the cells ingress into the embryo and migrating out creating the inner germ layers. The tissue motions driving the formation of the primitive streak are caused by apical contraction and elongation along the opposite direction which leads to ingressions, and directional intercalations. Both ingressions and intercalation are controlled by the dynamic modulation of the actin-myosin cytoskeleton. To investigate these processes, we have built and optimized a dual-channel light-sheet microscope, which allows imaging in vivo for extended time periods with minimum light-induced damage and developed computational methods for image analysis. Along with these, two new transgenic chick lines were developed, 1), a strain labelling Myosin IIA and actin (ACTM1) to analyse epiblast cell behaviours (Intercalations) and 2), a strain labelling actin and nuclei (ACTN) to analyse the cells once they move deep into the tissue (Ingressions). Changing the pattern of cell intercalations in ACTM1 perturbation experiments has proven the driving force of myosin cables due to the increase in myosin activity and orientation during tissue motion. Also, perturbation experiments in ACTN have shown that cell divisions are not essential for tissue flow but are required for successful streak formation.

26. Application of 3D image analysis to facilitate the identification of antiviral inhibitors

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The global spread of SARS-CoV-2 has highlighted the necessity for more effective antiviral screening methods to facilitate the identification of inhibitors. Most small molecule inhibitors are initially identified using 2D immortalised cells that are amenable to high-throughput drug screening; however, these cells fail to recapitulate the complex lung microenvironment and tissue-specific architecture. Consequently, many inhibitors fail to show antiviral activity in animal models. Alternatively, 3D cell culture models contain multiple cell types, mimic spatial organisation of the lung, and express gene profiles comparable to in vivo. Here we create improved 2D and 3D cell culture systems to identify drugs exhibiting antiviral activity against influenza virus. First, we establish a 2D real-time assay to monitor virus replication kinetics using a fluorescently tagged influenza virus. We demonstrate this assay can be used to screen potential antiviral inhibitors as findings show influenza virus replication kinetics can be monitored in real-time over 48 hours and model drug favipiravir inhibits influenza virus replication with an IC50 value of ~25 μM. Second, we differentiate human airway cells into respiratory epithelium. Using confocal microscopy and image analysis to track influenza virus replication in 3D, we show virus migration through multiple cell layers. We analysed depth and volume of infection foci, tissue thickness, and cilia damage, to determine efficacy of antiviral inhibitors. Our data demonstrate the potential utility of using real-time imaging and 3D models of infection to monitor respiratory virus replication and identify antiviral inhibitors. These findings could be implemented for future inhibitor studies against viral pathogens.

27. Sub-cellular Imaging using 3D Printed Lenses

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We present our work on manufacturing low-cost optical quality 3D printed optics, as well as their application within a brightfield and epi-fluorescence biological imaging system. A variety of 3D printed lens prescriptions are shown to provide sub-cellular resolution with comparable contrast to their off-the-shelf glass counterparts. We depict the 3D printed optics manufacturing process using an affordable Elegoo Mars 2 3D printer and Formlabs Clear Resin for printing, with post processing resin spin-coating step to obtain optical quality surfaces. Also shown are the results of their transmission at 445, 520 and 638 nm, as well as the lens surface quality matching within 6% of the commercial lenses using stylus profilometry (KLA Tencor Alpha-Step). Finally, shown is the 3D printed lenses comparative sub-cellular imaging performance relative to their off-the-shelf equivalent lenses using biological samples. The manufactured and tested lenses include a 20 mm focal length, 12.7 mm diameter lens used as an 8X objective, as well as a combination of four 6 mm diameter lenses for 50X magnification. The 3D printed lenses and their commercial equivalents were tested in both brightfield and epi-fluorescence setups as microscope objectives using a chrome lithography resolution target (1951 USAF Target), as well as onion, cyanobacteria, and variegated Hosta as imaging samples.

28. Role of the Brain Microenvironment in driving enhanced GBM cell invasion in response to Radiotherapy

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Glioblastoma is a highly infiltrative primary brain tumour. The treatment by ionising radiation through radiotherapy has been shown to induce pro-invasive phenotypic characteristics in GBM cells, making it an important clinical challenge to target. The GBM tumour microenvironment is a heterogeneous niche of varying ECM components and different cell types that interact with the brain vasculature. Most research studies focus on the effect of radiation on GBM cells without incorporating the component of the brain microenvironment. In this study, we look at defining the role of the brain microenvironment in response to radiation in GBM, and postulated that IR influences the brain stroma to release

promigratory factors. We study the role of the secretome through conditioned media-based experiments to understand the effect of promigratory factors released by irradiated GBM cells, and elucidate the role of the brain microenvironment and GBM cells in response to radiation, through ex vivo brain slice assays. The results indicated that conditioned media from irradiated cells positively influenced the ability of naïve cells to migrate in a 2D assay. Additionally, the invasion of fluorescently labelled GBM cells was increased when seeded onto irradiated brain slices (p-value<0.005). Thus, showing that promigratory factor release is not exclusive to the irradiated GBM cells alone, but the brain microenvironment also contributes to the release of pro-invasive factors upon radiation. Analyses of the irradiated GBM secretome and the role of exosomes will be investigated in the future to identify their effects in radiation driven invasion in GBM.

29. Serotonin 1B receptor agonists inhibit hunger promoting neurons to reduce food intake

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Obesity has become a worldwide health challenge and commonly results from the intake of more calories than the body requires. The brain represents the master controller of food intake and as such has been the target of obesity medications. However, key mechanisms of druggable targets remain to be defined. Neurons within the arcuate nucleus of the hypothalamus co-expressing neuropeptide Y (NPY), agouti-related protein (AgRP) and GABA (NAG) are fundamental stimulators of hunger and food intake. Agonists of the 1B subtype of metabotropic serotonin receptor (5-HT1BR) reduce food intake in part through the inhibition of hunger-promoting NAG neurons. We first confirmed that 5-HT1BR activation suppressed intake of a palatable Western diet in a mouse model of common dietary-induced obesity and genetically prone obesity. Histochemical analysis using a Zeiss Axioscop 2 of NPY-green fluorescent protein (Npy-GFP) mice confirmed that GFP (rabbit anti-GFP primary antibody, 1:20,000) was co-expressed with endogenous Npy mRNA (35S labeled riboprobe to Npy mRNA) within NAG neurons. Next, we created a triple reporter line of mice allowing the visualisation of NAG neurons and 5-HT1BRs (5-HT1BR-Cre:Tomato::Npy-GFP). Immunohistochemical analysis revealed that approximately 20% of NAG neurons (endogenous GFP fluorescence) co-expressed 5-HT1BRs (rabbit anti-RFP antibody, 1:500). Finally, we performed electrophysiology to analyse the effect of a 5-HT1BR agonist on NAG neuron cell activity and found that this drug hyperpolarised NAG neurons. Our results provide a key mechanism through which 5-HT1BR drugs inhibit appetite-stimulating neurons within the brain to suppress food intake.

30. Nucleus of the solitary tract Neuropeptide Y neurons drive feeding behaviour

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Hunger is an important survival signal, but the neurobiology mediating its induction has not been fully defined. Neuropeptide Y (Npy) is a key hunger-promoting neuropeptide that is expressed in the nucleus of the solitary tract (NTS). The NTS is the principal gateway linking nutritional cues from the gut to the brain. Here we profiled the neurochemical phenotype and function of Npy cells within the NTS with the objective of defining a primary brainstem neurons that control hunger. First, we performed histochemistry on NPY-green fluorescent protein (Npy-GFP) mice to confirm that GFP was co-expressed with endogenous Npy mRNA within the NTS on a Zeiss Axioscop II. We found over 90% co-localisation illustrating that Npy-GFP mice may be used to examine the neurochemical identity of Npy NTS cells. Using dual-immunohistochemistry, we found Npy NTS cells were co-expressed with paired-like homeobox 2b (Phox2b), the transcription factor essential for the development of the autonomic nervous system, but not the neurotransmitter acetylcholine (using choline acetyltransferase), which is localised outside the NTS. Npy-GFP co-expression was also observed with the neurotransmitters GABA and the catecholamines. We then examined that the function of Npy NTS neurons by stereotaxic injection of the Cre-dependant chemogenetic viral constructs into the NTS of Npy-CRE mice. We observed that activating Npy NTS neurons significantly increased food intake whereas inhibiting Npy NTS neurons decreased food intake. These data provide the first detailed characterisation of Npy neurons within the NTS and reveal that this population of Npy neurons regulates appetite in mice. Acknowledgements: BB/v01/6849/1

Facility Poster

31. IMPACT Facility Poster

Dr Adrian Burgos Garcia,
University of Edinburgh

IMPACT provides high quality imaging equipment and image analysis support to life scientists. Microscopes include multiphoton, light-sheet, confocal, airyscan and widefield systems, FLIM and TIRF modules. A dedicated computer suite includes access to Huygens, Imaris, Volocity and Matlab.

Trade Poster

32. Correlating Nano-Mechanics, Nano-Electrical and Nano-Chemical ID at the Nanoscale: The new Seeing is Believing

P. Schön, M. Febvre, P. De Wolf

Bruker Nano Surfaces & Metrology,

Atomic Force Microscopy (AFM) has remained a key tool for metrology ever since it was first commercialised in the late 1980's. Since then, the technique has vastly evolved from simply measuring the morphology of a surface, to now being able to measure nano-mechanics, nano-electrical, nano-chemical, and much more. While these additional AFM based techniques reveal a wealth of information on their own, combining several of these techniques to do correlative studies has become increasing common. This type of advanced characterisation on the nanometre scale allows researchers at universities and industry a more complete understanding of their sample.

PeakForce Quantitative Nanomechanical Mapping (PF-QNM) is one of the most common nanomechanical characterisation techniques, providing the user not only topographic information, but also high-resolution maps of modulus, adhesion, deformation, and energy dissipation.

PeakForce Kelvin Probe Force Microscopy (PF-KPFM) is a technique that utilises PF-QNM as the base mode and in-situ maps out the surface potential. This is used to reveal information related to trapped charges, work function, doping, applied voltages and other electrical properties. Scanning Thermal Microscopy (SThM) is a nanometre scale thermometer that can map the local temperature and thermal conductivity.

AFM-IR is a technique that utilises pulsed IR lasers to photothermally excite the sample and AFM probe is used as a nanometre scale detector of the sample's IR absorption band. This technique reveals a true chemical-ID of the sample with sub-10 nm spatial resolution, by way of either imaging the surface at a given wavenumber or collecting an IR spectrum.

Facility Poster

33. Cryo FIB-SEM facility at the University of Edinburgh

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Cryo FIB-SEM microscopy is becoming an increasingly popular technique to study the 3D structure of cells and tissue but also non biological soft and liquid materials. Cryo FIB-SEM tomography can provide a complementary technique to Cryo EM microscopy, able to produce high resolution tomography datasets over larger volumes than TEM. A wide range of soft samples have been looked at in the past year with our Cryo FIB-SEM. Non-biological examples include investigating the interface of stimuli-responsive Pickering emulsions, while biological examples include carrying out FIB-SEM tomography on bacteria in water to investigate how they interact with each other. Here I will present some examples of the materials that have been imaged in our facility, and discuss the capabilities of the Cryo FIB-SEM.

Learning Opportunity

34. Scottish Optical Microscopy Course

Brad Amos, Gail McConnell, Liam Rooney,
University of Strathclyde

Details and apply: *centreforbiophotonics.com/somc24*

Contact: SOMC@strath.ac.uk

Application deadline: 19 January 2024

Lecturers: Brad Amos, Gail McConnell,

Bob Edkins, Susan Cox, Siân Culley,

Lucy Collinson, Ralf Bauer, Liam Rooney

Topics: Ray & wave optics, polarisation & interference, fluorescence & photoproteins, light sources & detectors, laser scanning microscopy, super-resolution microscopy, localisation microscopy, correlative & multimodal microscopy, lightsheet microscopy, mesoscopy, image analysis.