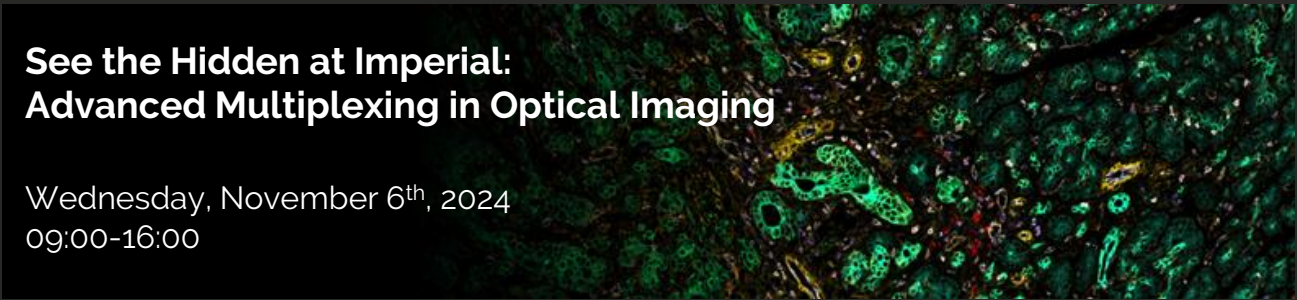


A high-resolution microscopy image showing a dense field of cells with various organelles and structures highlighted in green and yellow, set against a dark background.

See the Hidden at Imperial: Advanced Multiplexing in Optical Imaging

Wednesday, November 6th, 2024
09:00-16:00

Unveiling the Cellular Landscape in Health and Disease

Through microscopy showcases and scientific talks, we'll explore how the latest multiplex imaging technologies can provide new insights into your biomedical research. Proven applications will be shared by leading experts at Imperial College London and across the UK.

In this virtual event, you will discover:

- Insights into healthy and malignant haematopoiesis with multiplex imaging in murine and human bone marrow
- How 25-colour multiplex immunofluorescence sheds light on immune surveillance in skin cancer
- How multiplexed super-resolution single-molecule FISH is unveiling synaptic plasticity at the molecular level
- Methods to achieve 3D spatial imaging with at least 15 markers in a single pass, overcoming the high multiplexing barrier

Multiplex imaging offers unparalleled insights into complex cellular environments by enabling researchers to explore tissue architecture, cellular interactions, and molecular dynamics with unprecedented precision.

In this collaborative event, renowned speakers will showcase their research using cutting-edge multiplex imaging techniques across diverse applications. These include haematopoiesis, cancer research and neurodevelopment, offering fresh insights into synaptic plasticity, stem cell dynamics, tumour microenvironments, and the role of the immune system in tissue homeostasis.

You will learn how to take advantage of advanced multiplexing to get fresh insights into cellular heterogeneity and disease mechanisms at a spatial and molecular level.

Technologies that will be discussed include:

- Multiplex immunofluorescence imaging
- High-parameter fluorescence microscopy
- Multiplexing in confocal microscopy

We are pleased to partner with the **Royal Microscopical Society (RMS)** and **abcam** for this event.

AGENDA

ARRIVAL AND WELCOME

09:00 – 09:30 | Imaging Hub - Bessemer Building, level 1, labs B117 and B118/B119

WORKSHOP: HOW TO OVERCOME THE HIGH MULTIPLEXING BARRIER WITH STELLARIS

09:30 – 10:00 | Imaging Hub - Bessemer Building, level 1, labs B117 and B118/B119

Dr Emmanuelle Steib

WORKSHOP: HOW TO OVERCOME THE HIGH MULTIPLEXING BARRIER WITH STELLARIS

10:00 – 10:30 | Imaging Hub - Bessemer Building, level 1, labs B117 and B118/B119

Dr Emmanuelle Steib

WORKSHOP: MULTICOLOUR LIVE CELL IMAGING WITH MICA

10:35 – 11:05 | Imaging Hub - Bessemer Building, level 1, labs B117 and B118/B119

Dr Annalisa Rizza

WORKSHOP: MULTICOLOUR LIVE CELL IMAGING WITH MICA

11:05 – 11:35 | Imaging Hub - Bessemer Building, level 1, labs B117 and B118/B119

Dr Annalisa Rizza

NETWORKING LUNCH – WITH THE ROYAL MICROSCOPICAL SOCIETY (RMS)

11:35 – 12:45 | Royal School of Mines, Room 301C/D/E

WELCOME TO AFTERNOON SESSION

13:00 – 13:05 | Royal School of Mines, Room 131

Dr Periklis (Laki) Pantazis, Imperial College London & Colin Park, Leica Microsystems

AN INTRODUCTION TO MULTIPLEX IMAGING AT IMPERIAL

13:05 – 13:15 | Royal School of Mines, Room 131

Dr Miguel Ángel Hermida Ayala, Imperial College London

MAPPING NERVE REGENERATION INTO FREE MUSCLE GRAFTS

13:15 – 13:25 | Royal School of Mines, Room 131

Dr Aaron Lee, Imperial College London

Limb loss is a condition often marked by challenges in performing routine tasks and neuropathic pain due to disorganized regrowth of transected nerves and neuroma formation. A recent surgical approach, the formation of a regenerative peripheral nerve interface, involves implanting transected nerves into a denervated muscle graft. This method offers several advantages, including trophic support for the implanted nerve and encouragement of axonal branching for improved bioelectronic interfacing. To better understand nerve regeneration in these constructs, different imaging modalities such as x-ray microtomography, fluorescence imaging, and histological sectioning are being explored to determine the extent of axonal outgrowth and nerve identity.

IMAGING BLOOD-BRAIN BARRIER PERMEABILITY FOR NEURO-THERAPEUTIC DELIVERY

13:25 – 13:35 | Royal School of Mines, Room 131

Afraa Alzoubi, Imperial College London

We use focused ultrasound and microbubbles to facilitate the passage of drugs across the blood-brain barrier. In this short case study, I will present how we use fluorescent imaging to confirm drug delivery into brain parenchyma and colour imaging of stained sections to look at tissue morphology. I will also show how we can switch quickly between these different imaging modes using the Mica system.

USING MULTIPLEXED SUPER-RESOLUTION SINGLE MOLECULE FISH TO ELUCIDATE THE ROLE OF GLIAL MRNA LOCALISATION IN SYNAPTIC PLASTICITY

13:35 – 14:05 | Royal School of Mines, Room 131

Professor Ilan Davis, University of Glasgow

In neurons, the translation of localized mRNAs is thought to underpin synaptic plasticity by enabling rapid and distinct responses in different distal synapses. Glia have recently emerged as similar to neurons in structure and importance for nervous system function. Glial cells, like neurons, have long cytoplasmic projections that contact many other glial cells and multiple different neurons. These projections are known to regulate the plasticity of synapses they contact, which are now thought of as tri-partite, consisting of dendritic, axonal and glial projections. However, the mechanisms of such regulation are still poorly understood, as is the role of localized translation in glia. I will present our emerging evidence supporting the hypothesis that localized translation of glial mRNAs encoding a wide range of cellular functions in distal cytoplasmic projections are essential for the plasticity of their adjacent neuronal synapses. We have already discovered 1700 transcripts, enriched in neurological disease associations, that are predicted to localize at distal glial cytoplasmic projections. Using single molecule FISH (smFISH) has shown experimentally an enrichment in glial localized transcripts.

Some of the mRNAs are specifically required in the glia, not the neurons, for forming normal actin-rich cytoplasmic projections, neuronal plasticity in adjacent synapses and correct larval crawling behaviour. This work expands the known frontier of localized mRNA in distal glial projections, their interactions with neurons and mechanisms and function in neuronal plasticity. I will discuss in the talk the implications of our data for the regulation of distal cellular functions by localized translation in other cellular contexts, as well as potential novel mechanistic explanations of diseases of the nervous system. In the second half of my talk, I will discuss the prospects for increasing the coverage of detection of many transcripts at high plex making use of modern confocal instruments with spectral separation and super-resolution. I will describe our own efforts to develop approaches that democratize spatial biology and will discuss our aspirations in the context of the current landscape of commercial spatial biology approaches.

OVERCOMING THE HIGH MULTIPLEXING BARRIER: ACCESS 3D SPATIAL INFORMATION WITH AT LEAST 15 MARKERS IN ONE PASS

14:05 – 14:15 | Royal School of Mines, Room 131

Dr Emmanuelle Steib, Leica Microsystems

COFFEE BREAK

14:15 – 14:35 | Royal School of Mines, Room 131

SEEING IS BELIEVING HEALTHY AND MALIGNANT HAEMATOPOIESIS IN MURINE AND HUMAN BONE MARROW

14:35 – 15:05 | Royal School of Mines, Room 131

Professor Cristina Lo Celso, Imperial College London

Haematopoietic stem cells (HSCs) maintain the turnover of all blood cells, involving the generation of billions and trillions of new cells every day in mice and humans. While mostly quiescent, various stresses induce HSCs to increase proliferation and differentiation to cope with increased demand for differentiated progeny. These responses have vastly been associated with loss of functional HSCs. Over the last few years, my group has focused on understanding the mechanisms leading to HSC damage during acute, severe infection, using natural rodent malaria models. In particular we have focused on the role of the bone marrow microenvironment in mediating such damage and as a potential therapeutic target to safeguard HSC numbers and function. More recently, we started exploring mechanisms of regeneration.

Leukaemia is cancer of the blood, developing in the bone marrow and overtaking healthy haematopoiesis. Acute myeloid leukaemia (AML) is the prime example of a stem cell-based cancer and is particularly difficult to treat due to the ability of leukaemia stem cells (LSCs) to evade therapeutic interventions. Similarly to healthy HSCs, LSCs are affected by inflammatory stimuli, and we have recently focused on understanding how heterogeneity in the response to interferon may provide mechanisms of LSC survival and, eventually, AML relapse following inflammation-inducing therapies such as chemotherapy and immunotherapy.

SET YOURSELF UP FOR SUCCESS: ACHIEVE MORE WITH BETTER ANTIBODIES

15:05 – 15:25 | Royal School of Mines, Room 131

Dr Elena Loche, abcam

USING CELL DIVE MULTIPLEX IMAGING TO INVESTIGATE THE ROLE OF TERTIARY LYMPHOID STRUCTURES FOR IMMUNE SURVEILLANCE IN CUTANEOUS SQUAMOUS CELL CARCINOMA

15:25 – 15:55 | Royal School of Mines, Room 131

Dr Jennifer Shelley, Imperial College London

Cutaneous squamous cell carcinoma (cSCC) is the second most common form of skin cancer, accounting for 5% of all cancers and 20% of skin cancers in the UK. B and T cell clusters resembling tertiary lymphoid structures (TLSs), which are associated with positive prognosis in some solid cancers, have recently been identified in cSCC. However, whether these aggregates contain germinal centre (GC)-like reactions and are contributing to anti-tumour immunity has not been clear. Our group has used high-parameter spectral unmixing flow cytometry to identify GC-like B cells from human cSCC samples, as well as the vast array of other cell types which are contributing to the tumour immune microenvironment. To put these cells into spatial context, we have now applied multiplex 25 colour Cell DIVE immunofluorescence imaging to our human samples. With this platform, we have uncovered GC-like structures and created immunological maps of TLS-containing tumour tissue, up to 1 cm². These dermal TLSs exhibit incredible degrees of variability, ranging from loose B cell aggregates through to mature, complex B cell foci. Unpicking the variation in the immunological output of these structures, we can identify those with a greater enrichment of cytotoxic T cells, versus fully differentiated plasmacytes. Together, this data offers insight into the immunological dynamics occurring within a human cSCC, and the role TLSs play in dictating these responses.

CLOSING WORDS

15:55 – 16:00 | Royal School of Mines, Room 131

Dr Periklis (Laki) Pantazis, Imperial College London & Colin Park, Leica Microsystems

SPEAKERS



Professor Cristina Lo Celso

Professor, Imperial College London

Cristina Lo Celso pioneered intravital microscopy of the haematopoietic stem cell (HSC) niche during her postdoctoral training at Harvard University. In 2009 she started her independent research group at Imperial College London, where she is now Professor of Stem Cell Biology in the Department of Life Sciences, and co-director of the Centre for Haematology in the Department of Immunology and Inflammation. Cristina also established a satellite laboratory at the Sir Francis Crick Institute. Her research aims to understand the mechanisms regulating HSC function during steady state and during stresses such as infections, leukaemia and transplantation. Her interdisciplinary approach combines mouse bone marrow intravital microscopy techniques, computational image analysis, molecular profiling and mathematical modelling of the HSC niche. Cristina's publications have been cited over 7,000 times. She is the first woman to have received the Foulkes Medal award (2017); she received the ISEH New Investigator award in 2017, presented the DGZ Carl Zeiss Lecture 2018 and received the Royal Microscopical Society Life Sciences Medal 2019. She also received the Australian Intravital Microscopy Symposium Goppert-Mayer medal in 2023. She regularly engages in outreach activities and is keen to interact with the public, patients and industry.



Dr Jennifer Shelley

Research Associate, Imperial College London

Jennifer Shelley is a member of the Strid group at Imperial College London, researching how local immune structures contribute to anti-cancer responses in the skin. Having previously worked on various immunological and dermatological research projects, including those regarding atopic dermatitis and aging in human skin equivalent models, Jennifer has extensive experience and high-level expertise regarding microscopy, disease modelling in vivo and in vitro, and ex-vivo human disease research. She is now applying these skills and principles to skin cancer research, as well as additional projects in the wider Imperial research community, which has included aiding in the set up and use of the Cell DIVE multiplex imaging platform.



Professor Ilan Davis

MVLS chair of Spatial Biomedicine, University of Glasgow

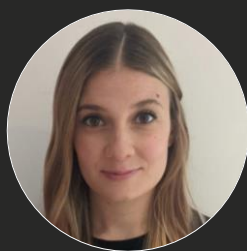
Professor Ilan Davis is the Medical, Veterinary & Life Sciences (MVLS) chair of Spatial Biomedicine at the University of Glasgow. His research is focused on the role of RNA in the *Drosophila* nervous system. From 2007 to 2023 he was a Professor of Cell Biology and Wellcome Investigator at the Department of Biochemistry, University of Oxford. Previously (1996-2007) he was a Professor and Wellcome Trust Senior Fellow (2002-2007) at the Wellcome Trust Centre for Cell Biology, University of Edinburgh. In 2011, he was elected as an EMBO fellow. Ilan studied Natural Sciences (Genetics) at King's College, Cambridge, earning a BA in 1986. He then went on to complete a DPhil in Developmental Biology in 1990 under the supervision of David Ish-Horowicz at the Department of Zoology at University of Oxford. He completed postdoctoral research from 1992 to 1995 at the Department of Biochemistry and Biophysics, UCSF, in Patrick H. O'Farrell's laboratory, with a Nato SERC postdoctoral fellowship followed by a Boyer postdoctoral Fellowship. Davis previously held, as the principal applicant, a Wellcome Career Development Fellowship, Lister Senior Fellowship (2000-2002), three Wellcome Trust Senior Research Fellowships (2002-2017), two Wellcome Trust Strategic Awards (2010-2021), MRC grant for Nanoscopy Oxford (2015-2020) and a Wellcome Investigator Award (2017-2023).



Dr Elena Loche

Senior Product Manager, Oncology & Immuno-oncology, Abcam

Elena is a Senior Product Manager at Abcam, focusing on multiplex IHC/spatial biology solutions for oncology and immuno-oncology. Elena holds a PhD in Metabolic and Cardiovascular Disease from the University of Cambridge, and a MSc in Molecular Biotechnology from the University of Bologna.



Dr Emmanuelle Steib

Advanced Workflow Specialist, Confocal Microscopy, Leica Microsystems

Emmanuelle currently works as an Advanced Workflow Specialist in Confocal Microscopy at Leica Microsystems. She was formerly an MSCA fellow at Imperial College London (UK) and has a PhD in Life Science from the University of Geneva (CH) and PharmD from the University of Strasbourg (FR).



Dr Miguel Ángel Hermida Ayala

Deputy Technical Operations Manager, Imperial College London

Miguel serves as the Facility Manager at the Imperial College London and Leica Microsystems Imaging Hub and has held the position of Deputy Technical Operations Manager for the Department of Bioengineering since 2023. Additionally, he leads the Undergraduate Teaching Technical team. He completed his Master's Degree at Hospital Universitario de la Princesa in Madrid and earned his PhD in Bioengineering and Cancer Biology from Heriot-Watt University in the UK in 2017. From 2017 to 2019, Miguel worked as a Postdoctoral Research Assistant at Barts Cancer Institute. He then joined Imperial College London as a Core Laboratory Technician in the Bioengineering Department. In 2020, he became the CRUK Microfabrication and Prototyping Facility Manager and subsequently took on the role of Manager at the Biosciences Core Facility in 2021.



Dr Periklis (Laki) Pantazis

Reader in Advanced Optical Precision Imaging, Imperial College London

Dr Periklis (Laki) Pantazis is a Reader in Advanced Optical Precision Imaging at the Department of Bioengineering at Imperial College London (ICL). He is also Director of the Leica Microsystems and ICL Imaging Hub. Dr. Pantazis obtained his PhD in Biology and Bioengineering at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden. As a Royal Society Merit Award recipient, he established the Laboratory of Advanced Optical Precision Imaging at ICL in 2018/19.



Colin Park

Product Specialist, Leica Microsystems

Colin is a Product Sales Specialist at Leica Microsystems, specializing in Confocal Microscopy solutions. With a background in Applied Physics and Materials Science, he brings around 30 years of valuable expertise to the Life Science field.

Afraa Alzoubi

PhD Student, Imperial College London

Afraa is a second-year PhD. student at the bioengineering department of Imperial College London. She did her undergraduate studies in pharmaceutical sciences, and her Masters in gene, drugs, and stem cells at the faculty of medicine. Her main research interest is molecular signaling pathways in brain endothelial cells. She is currently working on blood-brain barrier opening using focused ultrasound and microbubbles for neurotherapeutic delivery. She is supervised by Dr. James Choi.

Dr Aaron Lee

Postdoctoral Research Associate, Imperial College London