

ZEISS

LIGHT
MICROSCOPY
AUSTRALIA

4th National Meeting
March 5 - 8, 2024
Melbourne
www.lma2024.org

Conference Booklet

Image credit: Claire Richards, UTS





About LMA2024

The LMA is a national organisation holding meetings, workshops, seminars or key note lecture programs independently or in conjunction with the our parent organisation, AMMS. Our objective is the advancement of the science and techniques of light microscopy including:

- Specimen preparation
 - Image formation
- Image analysis and recording
- Photography and equipment care / selection

LMA2024 is the 4th National Light Microscopy Australia meeting which is being held in Melbourne, Victoria from March 5th - 8th 2024 at Melbourne Connect. This 4-day conference includes oral and poster presentation sessions, pre-conference workshops, special interest group-focused sessions, trade displays, and social events.

Diversity and Inclusion Statement

The open exchange of ideas and the freedom of thought and expression are central to this symposium. Our aim is to create an inclusive, respectful environment that invited participation from people of all races, ethnicities, genders, ages, abilities, religions, and sexual orientations. We actively seek to increase the diversity of our presenters, delegates, and sponsors. We do not tolerate harassment of symposium participants. All interactions are to be respectful and constructive, including interactions during the review process, at the symposium itself, and on social media.

Acknowledgment of Country

In the spirit of reconciliation, Light Microscopy Australia acknowledges the Traditional Custodians of country throughout Australia and their connections to land, sea, and community. We pay our respect to their Elders past and present and extend that respect to all Aboriginal and Torres Strait Islander peoples today.

Venue Information

MELBOURNE CONNECT

Melbourne Connect is located on the doorstep of the University of Melbourne and the amenity rich community of Carlton provides co-located partners with easy commuting options, diverse spaces to work and socialise.

We pay our respects to Elders past and present of the Wurundjeri and Boon Wurrung peoples of the Kulin Nation. We extend that respect to all First Nations people as our first artists and scientists.

Melbourne Connect is located at 700 Swanson Street Carlton, on the edge of Melbourne’s CBD. Well serviced by key transport links, bike ways and close to green open spaces and a range of local dining, café and retail offerings.



PARKING AND TRANSPORT INFORMATION

Melbourne Connect is easily accessible through a variety of transport options. The precinct is located on the junction of Swanston Street and Grattan Street and bounded by Cardigan Street to the east.

Public transport is the most convenient option. Plan your trip by using the [PTV journey planner](#).



PEDESTRIANS

Pedestrian access is available from Swanston Street and Cardigan Street



TRAMS

Trams run along Swanston Street and stop at Melbourne Central Station and Flinders Street Station, which provide links to the outer suburbs of Melbourne.

Swanston Street tram numbers are 1, 3, 3A, 5, 6, 16, 64, 67, 72 & 19



TRAIN

The closest train station is Melbourne Central



BUS ROUTES

200, 207, 250, 251, 401, 402, 403, 505, 546



BICYCLE

Public Bike Parking Spaces along Grattan and Swanston Street



TAXI

Pick up and drop off point are located on Grattan Street, Taxi Rank located on Grattan Street



PARKING

The precinct has one reserved parking space available for VIPs and dignitaries. Please liaise with your Event Coordinator for availability if this is a requirement for your event.

Melbourne Connect does not have onsite public parking, however, the closest parking locations to the building are Cardigan House Parking and Eastern Precinct Carpark

Car Park: Cardigan House Parking
Location: 96 Grattan St, Carlton VIC 3053

[For more information and prices.](#)

Car Park: University of Melbourne, Parkville Campus - Eastern Precinct Car Park
Location: 375 Cardigan St, Carlton VIC 3053

[For more information and prices.](#)

INTERNET

Complimentary internet is available within the precinct.

Public WIFI Network: MelbConnectGuest - no password required

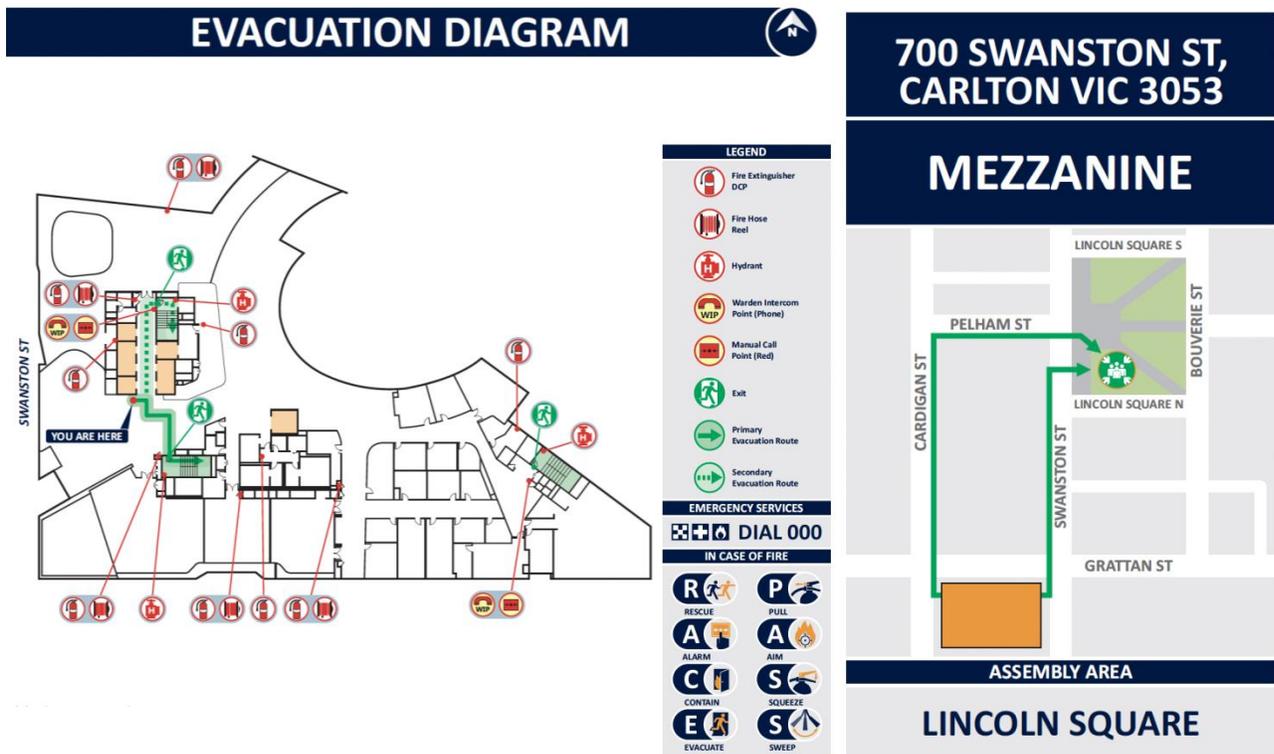
EMERGENCY EVACUATION PROCEDURE

ON SOUNDING OF THE ALERT TONE: “BEEP...BEEP”

- Be aware of possible emergency situations occurring
- Continually assess the situation and don't put yourself or others at risk
- Prepare to evacuate, follow work shut-down procedures. Secure your area immediately
- Wardens report to the Warden intercom Point (WIP), check for signs of an incident and account for occupants with a disability
- If some/fire/incident is discovered, commence evacuation and report this immediately to a Warden. Prevent others from entering the hazardous zone and alert surrounding people
- If safe and trained to do so, attempt to extinguish the fire/contain the emergency incident

ON SOUNDING OF THE ALERT TONE: “WHOOOP...WHOOOP”

- Evacuate via the nearest safe exit and proceed to the ASSEMBLY AREA (refer to plan site)
- Wardens will conduct a sweep to ensure occupants are clear and report to the Chief Warden
- When the area has been evacuated, all doors and windows should be closed to contain fire. Do not obstruct access to Exits.
- Wardens evacuate and remain with occupants at the ASSEMBLY AREA. Await further instructions from the responding Emergency Services.



Our Sponsors

LMA2024 would not be possible without the generous support of an amazing group of sponsors. Please visit their booths and thank them throughout the conference.

(P.S There are prizes for collecting stamps from all the sponsors in your Exhibit Hall Stamp Card!)



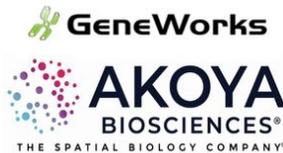
Australian Microscopy and
Microanalysis Society



MICROSYSTEMS



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Conference Schedule



4th National Meeting
March 5 – 8, 2024
Melbourne Connect



CONFERENCE GUIDE

TUES 5TH MARCH

Satellite Workshops

Parkville Precinct
9:00am – 5:00 pm

Student Panel Session

Bio21 Auditorium
12:30 pm – 2:30 pm

Registration Opens

Melbourne Connect
4:00 pm – 8:00pm

Welcome Reception

Melbourne Connect
5:00 pm – 8:00 pm

Workshops: Open-source AI image analysis, Aivia Masterclass, Qupath Masterclass, Miltenyi UltraMicroscope Blaze, ATA Scientific LiveCyte, CrestOptics Spinning Disk, Akoya Phenolmager

WEDS 6TH MARCH

Scientific Program: Day 1

Melbourne Connect
8:30 am – 5:30pm

LMA AGM 2024

Melbourne Connect
1:20 pm – 2:20pm

Conference Dinner

Cargo Hall, Southwharf
7:00 pm – 11:00pm

Plenary: Prof. Gail McConnell

Sessions: Live Cell Imaging, Imaging in 5D, Quantitative Microscopy, Tissue Imaging & Analysis

Guest Speaker: Drew Berry

THURS 7TH MARCH

Scientific Program: Day 2

Melbourne Connect
8:30 am – 5:30pm

Poster Session

Melbourne Connect
1:00 pm – 2:30 pm

Plenary: Dr. Siân Culley

Sessions: Advanced Technologies, Image Analysis, Super Resolution Microscopy, Microscopy Debates

FRI 8TH MARCH

Satellite Workshops

Parkville Precinct
9:00am – 5:00 pm

Facility Managers' Workshop

DLH, Uni Melb
9:30am – 5:00pm

Workshops: Stellaris 8 Falcon, Zeiss Lattice-SIM, Zeiss Arivis Pro Image Analysis, Andor/SciTech Imaris Masterclass, 3i AxL-CTLs

VENUE INFORMATION

WIFI Network: MelbConnectGuest – no password required

In case of emergency: Dial 000

Scientific Program



4th National Meeting
March 5 – 8, 2024
Melbourne Connect



SCIENTIFIC PROGRAM

WEDS 6TH MARCH

8:30 AM	Registration
9:00 AM	Welcome
9:20AM	Plenary Session 1: Prof. Gail McConnell <i>University of Strathclyde, Scotland UK</i>
10:00 AM	Session 1: Live Cell Imaging sponsored by Zeiss Dr Samantha Stehbens <i>University of QLD</i> Prof Lucy Palmer <i>The Florey Institute</i> Dr Jessica Greaney <i>ARMI; Monash University</i> Mr Jesse Rudd-Schmidt <i>Peter Mac</i> Dr Franciós Olivier <i>Monash University</i>
11:20 AM	Morning Tea
11:40 AM	Session 2: Imaging in 5D Prof Scott Mueller <i>University of Melbourne</i>  A. Prof Kevin Dean <i>University of Texas, USA</i> Mr Raymond Qin <i>Walter & Eliza Hall Institute</i> Miss Azadeh Anbarlou <i>ARMI; Monash University</i> Dr Xichun Li <i>IMB; University of QLD</i>
1:00 PM	Sponsor Lightning Sessions
1:20 PM	Lunch LMA 2024 AGM
2:20 PM	Session 3: Quantitative Microscopy sponsored by Nikon Dr John Lock <i>University of NSW</i> Dr Noa Lamm-Shalem <i>Children's Medical Research Inst.</i> Dr Azelle Hawdon <i>Monash IVF & ARMI; Monash University</i> Dr Amy Bottomley <i>University of Technology Sydney</i> Mr Matthew Rowe <i>MIPS; Monash University</i>
3:50 PM	Afternoon Tea
4:10 PM	Session 4: Tissue Imaging & Analysis sponsored by TissueGnostics Dr Raymond Yip <i>Walter & Eliza Hall Institute</i> Dr Jurgen Kriel <i>Walter & Eliza Hall Institute</i> Dr Bianca Capaldo <i>Walter & Eliza Hall Institute</i> Dr Gavin Tjin <i>St Vincent's Inst. Med Research</i> Miss Annabella Thomas <i>Walter & Eliza Hall Inst.</i>
5:30 PM	Depart

THURS 7TH MARCH

8:30 AM	Registration
9:00 AM	Plenary Session 2: Dr Siân Culley <i>Kings College London, UK</i>
9:40 AM	Session 5: Advanced Technologies sponsored by Miltenyi Biotec Dr Avinash Upadhyia <i>University of Adelaide</i> Dr Kylie Dunning <i>University of Adelaide</i> Miss Emma Gill <i>Swinburne University</i> Dr Scott Page <i>Victor Chang Cardiac Research Inst.</i> Ms Kylie Luong <i>Walter & Eliza Hall Institute</i>
11:00 AM	Morning Tea
11:20 AM	Session 6: Image Analysis sponsored by Leica Microsystems Dr Elvis Pandzic <i>University of NSW</i> Mr Sebastian Amos <i>University of WA</i> Dr Juan Nunez-Iglesias <i>Monash University</i> Dr Sonja Frölich <i>University of Adelaide</i> Ms Thanushi Peiris <i>Murdoch Children's Research Inst.</i>
12:40 PM	NewSpec Outreach Presentation
12:50 PM	Lunch Poster Session (1:00PM – 2:30PM)
2:40 PM	Session 7: Super Resolution Microscopy sponsored by Evident Dr Scott Berry <i>University of NSW</i> A. Prof Izzy Jayasinghe <i>University of NSW</i> Dr Ashley Rozario <i>La Trobe University</i> Dr Francesca Cavalieri <i>RMIT University</i> Mr Imala Alwis <i>Heart Research Institute</i>
4:00 PM	Afternoon Tea
4:20 PM	Session 8: Microscopy Debates #1: Image Analysts are indispensable #2: Microscopy Muses #3: The debate of dynamics
5:00 PM	Awards & Closing
5:30 PM	Depart

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PLENARY SPEAKERS

Gail McConnell – University of Strathclyde, Scotland UK

Gail McConnell is Professor of Biophotonics at the Strathclyde Institute of Pharmacy and Biomedical Sciences at the University of Strathclyde, Glasgow, UK. Following a first degree in Laser Physics and Optoelectronics (1998) and PhD in Physics from the University of Strathclyde (2002), she obtained a Personal Research Fellowship from the Royal Society of Edinburgh (2003) and a Research Councils UK Academic Fellowship (2005), securing a readership in 2008 and professorship in 2012. The work in Gail's multidisciplinary research group involves the design, development and application of



linear and nonlinear optical instrumentation and new methods for biomedical imaging, from the nanoscale to the whole organism. She is a Fellow of the Royal Society of Edinburgh, a Fellow of the Institute of Physics, and a Fellow of the Royal Microscopical Society, where she is the current Vice Chair of the Light Microscopy Committee.

WEDNESDAY 6th MARCH 9:20AM Optical Imaging with the Mesolens

Since the invention of the microscope, optics have been optimized to match the performance of the human eye. However, since the advent of sensitive and advanced photodetectors the human eye is no longer a limitation. This has created exciting possibilities for new instrumentation in biomedical imaging.

We have developed an objective we call the Mesolens that can study large unusually large objects with sub-cellular resolution. The pupil size of the lens is so great that it cannot be used with a conventional microscope frame, so we have built the imaging system around the giant lens. Like the original optical microscope, we have found that the Mesolens has a wide range of applications in biomedical research. I will present an overview of the Mesolens imaging technology, and I will show how we are using it to reveal new information from large biological and clinical specimens.

Siân Culley – Kings College, London UK

Siân is a Royal Society University Research Fellow at King's College London applying novel image analysis techniques to fluorescence microscopy data. During her postdoc, she developed analytical methods for achieving super-resolution in data from conventional microscopes (SRRF) and for assessing the quality of super-resolution images (SQUIRREL). Since starting her own group, she is focusing more on image quality assessment in microscopy. She is particularly interested in trying to discover how image quality relates to quantitative biological information within images, and under what conditions image processing methods can enhance accurate retrieval of this information.



THURSDAY 7th MARCH 9:00AM

The Good, The Bad and The Ugly in Fluorescence Microscopy

Fluorescence microscopy is one of the most important methods in modern cell biology. However, despite its widespread use, there is little guidance about what actually defines a good image that can support reliable biological measurements. This can have significant implications not just for choosing the correct imaging parameters when setting up an experiment, but also for applying cutting edge microscopy methods such as super-resolution and deep learning-based image processing. In this talk, I will explore the relationships between imaging conditions, image quality, and the amount of biological information that can be extracted from images via manual or automated analysis.

INVITED SPEAKERS

Samantha Stehbens – University of Queensland

Dr Stehbens is a cell biologist with an interest in understanding the fundamental mechanisms that regulate cell adhesion and the cytoskeleton. They have made key contributions to the fields of quantitative microscopy, cell motility, adhesion and the cytoskeleton. This work has been published in a series of Nature Cell Biology, Nature Neuroscience, and Journal of Cell Biology papers spanning multiple fields from ion channels in brain cancer, to growth factor signalling and autophagy in endometrial and breast cancer. They completed their PhD in the laboratory of Prof. Alpha Yap, before relocating to UCSF to work with Prof. Torsten Wittmann. They returned to Australia, to train in oncology focused



laboratories with the goal of applying their skill set to understanding cancer survival and metastasis. Their research group aims to understand how cells integrate secreted and biomechanical signals from their local microenvironment to facilitate movement and survival. They have uncovered a novel role for the microtubule cytoskeleton in protecting cells from rupture during 3D cell migration and invasion. Using patient-derived tumour cells, coupled to genetic alteration and substrate microfabrication, they use state-of-the-art microscopy to understand the fundamental mechanisms of cell migration with the aim of understanding how to better prevent cancer metastasis.

WEDNESDAY 6th MARCH 10:00AM

The Mechanobiology of Microtubules: Understanding How Cells Survive Confined Spaces

Cells in living organisms navigate highly crowded three-dimensional environments, where their coordinated migration provides the driving force behind developmental and homeostatic tissue maintenance. Mechanical forces are important for cells to move and function in normal and diseased contexts. Most work focuses the role of the actin cytoskeleton. We now reveal that microtubules also play key roles in sensing and responding to mechanical cues. Microtubules are damaged by mechanical force, and in response they are actively repaired and become more flexible and resistant to breakage.

Mechanical forces are felt across the lattice, effectively communicating the biophysical forces of the surroundings to intercellular structures mechanically linked to microtubules. In addition, microtubules sequester key upstream actin-regulatory factors, to ensure appropriate timing of release and activation of contractile forces to facilitate cell shape changes and movement. Thus, localized mechanochemical tuning mechanisms are necessary to ensure that microtubules are locally reinforced in areas of high mechanical load in both space and time. Our understanding of the mechanisms that govern the organization and mechanochemical tuning of microtubule properties in 3D in vivo systems, is not well

understood. We explore the adaptive role that the microtubule cytoskeleton plays in facilitating cell shape plasticity, matrix remodelling and resistance to compression during migration in 3D hydrogels. We apply these findings to understand how cancer cells exploit this to spread to distal tissues and the role of the mechano-environment.

Lucy Palmer – The Florey Institute

Professor Lucy Palmer is head of the Neural Network Laboratory at the Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia. She completed her Master of Science at the University of Minnesota, Ph.D at the Australian National University, and postdoctoral research at the University of Bern, Switzerland and Charite University, Berlin. Her research uses two-photon calcium imaging during behaviour to investigate how dendritic activity and neural networks contribute to learning and memory in health and disease.



WEDNESDAY 6th MARCH 10:20AM

Using two-photon calcium imaging to probe neural encoding during behaviour

Learning can result in memories which can last a lifetime and involve significant changes to the structure and function of cortical neurons. Although it defines us, our knowledge about how, and where, learning occurs is still in its infancy. Since they are the site of synaptic input on neurons, dendrites provide an ideal substrate for the dynamic encoding of information required during learning. However, due to their small size, measuring the dynamics of dendritic processes during learning is only possible using advanced imaging techniques. Here, I will discuss recent results from my laboratory which investigates the changes in dendritic activity that occur in cortical neurons during learning of a sensory-association task. Using two-photon calcium imaging, we recorded the calcium activity in tracked dendrites from layer 2/3 pyramidal neurons throughout learning. The results from this study illustrate individual dendrites are dynamic, and undergo significant changes in sensory and behavioural-encoding throughout learning.

Scott Mueller – University of Melbourne

Scott is an NHMRC Leadership Fellow and a Dame Kate Campbell Fellow, in the Department of Microbiology and Immunology, at the Peter Doherty Institute for Infection and Immunity, at The University of Melbourne. Research his laboratory is focused on dissecting the fundamental cellular processes involved in immune responses to viruses and cancer in order to identify new targets for vaccine design and therapeutics. The laboratory is interrogating neuroimmune interactions and stromal cell functions during infection and cancer, using tools including intravital imaging and transcriptomics.



WEDNESDAY 6th MARCH 11:40AM

Intravital 3-photon microscopy for deep tissue imaging of immune responses

To achieve a detailed understanding of how immune responses are choreographed we are interrogating cell dynamics and cell-cell interactions in vivo from the perspective of the immune cells (lymphocytes, dendritic cells) and cross-talk with the tissue microenvironment (nerves and structural cells). We have developed a unique 3-photon imaging system for improved imaging deep into tissues. To accompany this, we have developed a new open-source image analysis software tools to improve unbiased quantitative analysis of cell behaviours from live imaging data.

Kevin Dean – University of Texas, USA

Kevin Dean, a Northern California native, earned his B.A. in Chemistry at Willamette University, Oregon, where he was twice named an ESPN Regional Academic All-American in Football. After college, he cycled across the U.S. to raise awareness and funds for ALS, in memory of a friend affected by the disease. He completed his Ph.D. in Biochemistry at the University of Colorado, focusing on spectroscopy, protein engineering, and microfluidic analyses. Thereafter, Kevin established the first campus-wide light microscopy facility at the University of Colorado's BioFrontiers Institute. He then conducted postdoctoral research at the University of Texas Southwestern Medical Center, receiving several accolades including the Ruth L. Kirschstein Postdoctoral Fellowship. Currently, he leads a lab at UT Southwestern, developing advanced imaging and computational techniques to study cell biology in health and disease.



WEDNESDAY 6th MARCH 12:00PM

Imaging the Metastatic Cascade with Axially Swept Light-Sheet Microscopy

In melanoma, metastasizing cells are distributed broadly throughout the host via the general circulatory system. Yet, in a process known as organotropism, the colonization and formation of metastases at a particular site in a distant tissue is non-random and occurs in a patient-specific format. Why this is true remains poorly understood but is likely to involve a combination of cell intrinsic (e.g., the ability of a cell to survive differences in mitogenic factors, nutrient availability, and/or context-specific stressors) and extrinsic factors (tissue-specific mechanical and biochemical cues). Gaining molecular insight into the events involved in metastatic colonization is challenging, in part because such events are rare, pioneer colonies are small, and potential sites of colonization are widely distributed throughout large tissues. To identify the earliest events in metastasis, including the colonization of a tissue by a single metastatic cell, we are developing multiple self-driving Multiscale Cleared Tissue Axially Swept Light-Sheet Microscopes (MCT-ASLM). Together, these systems enable rapid imaging of large cleared or chemically expanded tissues and automatic identification and interrogation of biological features of interest. Microscope operation is achieved with our highly reconfigurable software package, navigate, which permits the integration of advanced robotics and creation of acquisition “recipes” that vary according to the specimen and biological feature of interest. Beyond metastasis, such an approach greatly accelerates the evaluation of sub-cellular features in select cells within the greater context of whole, intact tissues.

John Lock – University of New South Wales

John completed his PhD at the Institute for Molecular Bioscience, University of Queensland, and undertook a post-doc and Assistant Professorship at the Karolinska Institute, Stockholm, there pioneering the multidisciplinary field of Systems Microscopy, i.e. imaging-based single-cell systems biology. Systems Microscopy aims to fuse the spatial and temporal resolution of imaging with the quantitative scale and rigour of single cell omics.

John now leads the Cancer Systems Microscopy Lab at UNSW to address translational projects targeting liquid biopsy analyses of advanced prostate (CRPC) and lung (NSCLC) cancers, as well as fundamental analyses of cancer cell plasticity in the epithelial-mesenchymal spectrum. The team are also driving development of a novel drug-lead discovery platform to accelerate targeted therapy development. These diverse efforts are aligned around integrated development of experimental design and methods, particularly involving scalable automation, along with development and application of computational and statistical methods, currently emphasising deep learning tools to enrich and interpret image data.

John was recently awarded a UNSW Scientia Research Fellowship, is an affiliate researcher of the Ingham Institute for Applied Medical Research, and is a founding member of UNSW's Artificial Intelligence Institute.



WEDNESDAY 6th MARCH 2:20PM

Towards Precision Medicine with Systems Microscopy: an imaging- based framework to understand cell heterogeneity in space and time

Imaging-based single cell systems biology analyses, i.e. Systems Microscopy, enable detailed mapping of cellular heterogeneity with spatial resolution spanning cell populations, single cells and subcellular localisation. A key recent focus in the Cancer Systems Microscopy lab has been to overcome traditional limits on the number of molecular species observable with fluorescence microscopy. Thus, our evolving systems microscopy platform now combines several complimentary strategies for multiplexed molecular analysis of single cell populations, including experimental multiplexing of 20-30+ markers per cell, and computational multiplexing (via deep learning or machine learning strategies) of theoretically unlimited marker numbers.

By thus enriching the sampling of cellular information, we next utilize a variety of statistical, machine learning and deep learning-based methods for single cell data analysis, mapping cell states, state plasticity, and molecular dynamics, as well as the interdependency between these and other parameters. In this way, we seek to understand the cell a complex and contingent computational system. This strategy underpins our fundamental research into cancer cell plasticity in the EMT and stemness spectra. We are

also now deploying these methods to develop next-generation liquid biopsy-based precision diagnostics to support clinical decision-making in cancer treatment.

Noa Lamm-Shalem – Children’s Medical Research Institute

Noa Lamm is a group leader at the Children’s Medical Research Institute (CMRI), a conjoint Senior Lecturer at the Faculty of Medicine and Health at the University of Sydney, and a Col Reynolds Fellow. In her postdoctoral training period, she specialized in investigating nuclear dynamics following threats to genome integrity. Specifically, she focused on the role of nuclear actin filaments in promoting DNA movement and structural alternations using live cell microscopy and innovative image analysis tools.



In 2023, she started the Nuclear Dynamics group at CMRI. Her lab is driven by a profound interest in unravelling the intricacies of nuclear function as a dynamic entity essential for maintaining genome integrity by facilitating genetic material repair when necessary. Leveraging advanced techniques such as live-cell imaging, super-resolution microscopy, and customized image analysis tools, her research group is dedicated to pinpointing the structural, architectural, and physical alterations that transpire within the nucleus in response to stress. Their goal is to comprehend how these alterations regulate DNA repair both temporally and spatially.

WEDNESDAY 6th MARCH 2:40PM

Shedding Light on Genome Integrity: Exploring the Vital Role of Nuclear Filamentous Actin (F-actin) with light microscopy

Nuclear filamentous actin (F-actin) has rapidly emerged as a pivotal player in the intricate landscape of genome integrity, DNA repair, and the response to replication stress^{1,2}. While traditionally known for its roles in cytoskeletal functions, the revelation of nuclear actin polymerization, made possible by advancements in light microscopy, has revolutionized our understanding of cellular dynamics. F-actin now stands as a crucial determinant of nuclear architecture, profoundly influencing the cellular response to various genomic challenges^{1,2}. In particular, its involvement in the replication stress response in human cells has garnered significant attention.

Replication stress, a key instigator of genome instability and a hallmark of cancer, underscores the clinical importance of unravelling the mechanisms by which nuclear F-actin orchestrates nuclear

reorganization. Through the utilization of cutting-edge imaging techniques such as live-cell, super-resolution, and intravital imaging, coupled with bespoke image analysis tools, our research discovered the role of nuclear F-actin in reshaping the nuclear environment in response to replication stress. Our findings illuminate the dynamic nature of the F-actin network, demonstrating its role in driving architectural modifications within the nucleus and facilitating enhanced DNA mobility to expedite the repair of DNA lesions and replication fork impediments. These insights reveal how cancer cells exploit the functions of nuclear F-actin to gain resistance against replication stress-inducing chemotherapies, underscoring the urgent need for targeted therapeutic interventions.

Recently, we found that the nuclear phase-condensates Promyelocytic leukemia nuclear bodies (PML-NBs) act as nucleation hubs, initiating the formation of nuclear F-actin in response to replication stress. As these actin filaments grow, they exert mechanical pressure on the PML-NBs, thereby inducing their shape and composition alterations. Crucially, we have uncovered that this F-actin-driven structural transformation results in the release of the AKT oncogene from its sequestration within PML-NBs, leading to its phosphorylation and subsequent activation. Collectively, our findings converge to delineate a novel F-actin/PML-NBs/AKT pathway emerging in response to replication stress, influencing DNA repair choices, and promoting cell survival.

1. Lamm, N., Rogers, S. & Cesare, A. J. Chromatin mobility and relocation in DNA repair. *Trends Cell Biol* 31, 843-855, doi:10.1016/j.tcb.2021.06.002 (2021).
2. Lamm, N. et al. Nuclear F-actin counteracts nuclear deformation and promotes fork repair during replication stress. *Nat Cell Biol* 22, 1460-1470, doi:10.1038/s41556-020-00605-6 (2020).

Azelle Hawdon – Monash IVF, ARMI Monash University

Embryologist, Adjunct Research Associate (Australian Regenerative Medicine Institute, Monash University)

Azelle recently completed her PhD at the Australian Regenerative Medicine Institute, Monash University under the supervision of Dr Jennifer Zenker. Where her research used advanced live imaging to decipher the spatiotemporal dynamics underlying early mouse embryo development.

Azelle is now works as a Clinical Embryologist at Monash IVF. She is passionate about uncovering the mysteries of early embryo development to make reproductive technologies more effective.



WEDNESDAY 6th MARCH 3:00PM

Apicobasal RNA Asymmetries Regulate Cell Fate In The Early Mouse Embryo

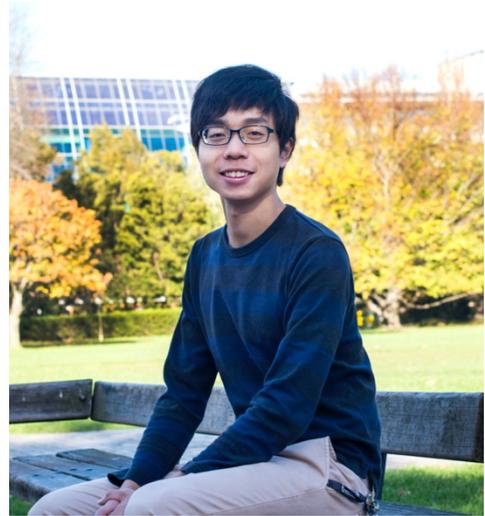
RNA localisation has indispensable roles for establishing asymmetries and coordinating cell fate decisions during early embryogenesis across a plethora of non-mammalian species. To direct the spatiotemporal distribution of RNA within the cells of an embryo, the microtubule cytoskeleton provides highly sophisticated trafficking pathways. Yet, the mechanisms of RNA localisation during early mouse embryogenesis and how it contributes to pluripotency remains unknown.

Using advanced live imaging we visualise the inner workings of cells of the living early mouse embryo at high spatiotemporal resolution from fertilisation to the blastocyst stage. In a non-invasive manner, we analyse the real-time movements of RNA, microtubules and organelles inside cells of the embryo. For the first time, we discover apicobasal subcellular asymmetries specific to outer trophectoderm cells of the 16-cell stage mouse embryo. Basally directed RNA transport is facilitated in a microtubule- and lysosome-mediated manner. Yet, despite an increased accumulation of RNA transcripts in basal regions, higher translation activity occurs at the more dispersed apical RNA foci, demonstrated by spatial heterogeneities in RNA subtypes, organelles and translation events. During the transition to the 32-cell stage, the biased inheritance of RNA transcripts, coupled with differential translation capacity, regulates cell fate allocation of trophectoderm and cells destined to form the pluripotent inner cell mass.

These findings offer insights into potential structural determinants for cell fate, closely associated with gene regulation. They also establish a framework illustrating how subcellular asymmetries and translation capacity synergistically contribute to cell plasticity during the development of the mouse embryo.

Raymond Yip – Walter & Eliza Hall Institute

Dr. Raymond Yip is a Senior Research Officer at WEHI with joint appointment across Imaging, Genomics, and Hawkins laboratories. His research focuses on studying myeloma bone marrow microenvironment using spatial omics technologies. He leads the implementation of Institute's spatial multi-omics initiative and supervises the operation of Australia's first MERSCOPE and Xenium. He is heavily involved in technology benchmarking activities and has extensive collaborations with biotechnology companies.



WEDNESDAY 6th MARCH 4:10PM

In situ single cell analysis of tissue microenvironment in blood cancer

The recent advent of spatial omics platforms has enabled high-plex imaging of proteins and RNA within tissues, offering unprecedented insights in multiple fields of life sciences. This presentation will discuss the underlying chemistry, hands-on experience, and data analysis techniques of spatial omics platforms. We will also demonstrate the utility of spatial omics in dissecting tissue microenvironment across diverse pathological contexts.

Avinash Upadhyaya – University of Adelaide

Avinash completed his PhD at the John Curtin School of Medical Research, Australian National University, where he investigated novel optical strategies to structure light for single particle tracking and live cell imaging. He is now a post-doctoral researcher at the Centre of Light for Life, University of Adelaide, where his work involves the development of gentle optical imaging techniques for long-term imaging of embryo development and health. This involves cutting edge techniques such as Bessel beam two photon light sheet microscopy, hyperspectral imaging, as well as novel acquisition strategies such as compressed sensing.



THURSDAY 7th MARCH 9:40AM

Gently Does it: Reducing photodamage in long term volumetric imaging

Recent developments in advanced optical microscopy have pushed high speed and resolution for volumetric imaging. However, photodamage can limit the biological relevance of data collected by these techniques especially over long imaging durations and at high resolutions. This restriction is even more pronounced when looking at samples such as embryos, where photodamage can initially be undetectable but may manifest itself in developmental outcomes. In this talk I will discuss the use of Light Sheet Fluorescence Microscopy (LSFM) to reduce photodamage in biological imaging. I discuss our work evaluating photodamage in LSFM against confocal microscopy for volumetric imaging of murine embryos, focusing on photodose and resultant image quality. I also introduce extension of LSFM with two photon excitation and Bessel beams for improved resolution and penetration in murine and bovine embryo samples with optimal use of the photon budget.

Kylie Dunning – University of Adelaide

Dr Kylie Dunning heads the Reproductive Success Group within the Robinson Research Institute at the University of Adelaide, Australia. Her transdisciplinary research uses microfabrication, biophotonics and imaging to better understand the mechanisms underpinning healthy oocyte and embryo development. Dr Dunning's interdisciplinary expertise is best illustrated by her multi-faceted studies of embryo development and metabolism, in which she uniquely capitalised on the use of advanced optical analyses to develop a non-invasive technology to diagnose both the presence and location of aneuploid cells within the developing embryo. Her interdisciplinary research has received international recognition, winning the Basic Science Award at the European Society of Human Reproduction and Embryology (2021) and highlighted in TIME magazine (USA, Sept 2022).



THURSDAY 7th MARCH 10:00AM

Improving IVF success with advanced photonics

Embryo quality is a key factor in determining whether an IVF cycle will result in a live birth. Current approaches for assessing embryo quality are subjective (morphology) or invasive (biopsy) and are not always predictive of live birth. Development of an accurate and non-invasive method to assess embryo quality would likely improve IVF success.

We have taken a different approach towards developing a non-invasive diagnostic for embryo quality: optical imaging. We use low levels of light to illuminate the embryo and obtain spatial information on cell metabolism as well as biophysical properties of the embryo. Here I will present our work showing that hyperspectral imaging can be used to accurately discriminate between euploid and aneuploid mouse embryos. Importantly, we show that this form of imaging is safe: compared to embryos that were not imaged, hyperspectral imaged embryos had similar, and not significantly different, pregnancy and implantation rates, with offspring weight at weaning also comparable.

Another potential optical approach to assess embryo quality is digital holographic imaging. To date, little attention has been paid to the physical parameters of an embryo and whether these could be markers of embryo quality. One such physical parameter is refractive index. We have shown that digital holographic microscopy is able to measure very small changes in refractive index between good and poor-quality mouse embryos.

Importantly, these optical approaches occur in the absence of stains or exogenous tags. Used alone, or in combination, such optical approaches may prove beneficial in developing to an accurate, non-invasive diagnostic for embryo quality.

Elvis Pandzic – University of New South Wales

Dr Elvis Pandzic is a Senior Lecturer and Advanced Fluorescence Microscopy Specialist at UNSW, working at Katharina Gaus Light Microscopy Facility at UNSW's Mark Wainwright Analytical Centre (MWAC).

He received his PhD in Biological physics in 2013 at the McGill University (Canada) during which he developed novel image fluctuation correlation analysis tool allowing the study of membrane protein confinement.

During his career he worked on development of several extension of these bespoke image analysis tools, by which one can quantify fluorescence microscopy images to measure protein density, oligomerization, co-localization and dynamics in live cells. His current work at KGLMF involves applying those methodologies and developing and adapting the new ones tailored to answer the biomedical problems for the researchers at UNSW and beyond.



<https://research.unsw.edu.au/people/dr-elvis-pandzic>

THURSDAY 7th MARCH 11:20AM

Image correlation spectroscopy approaches for the quantification of molecular and organellar dynamics in situ.

Image correlation spectroscopy (ICS) has been around for over 25 years and is applied to fluorescence microscopy data to quantify molecular concentration, dynamics, (diffusion, flow), chemical reactions and co-localisation. Over that time period there has been an emergence of ICS derived analysis approaches taking advantage of unique spatial and temporal capabilities of different microscopy modalities (Confocal, TIRF, Lightsheet, etc), providing a unique set of measurable parameters about biological systems.

In this talk, I will introduce you to ICS derived approaches that rely on Spatio-Temporal ICS (STICS) analysis resolving molecular and organellar flow in 2D and 3D. Furthermore, we will explore the benefits of its Fourier space counterpart, k-space ICS (kICS) in investigating dynamics of proteins in confined environments.

In the last part of this talk, I will demonstrate how these two approaches can be used to quantify the ciliary beating frequency (CBF) and ciliary spatial coordination length in the context of diagnosis of respiratory diseases.

Sebastian Amos – University of Western Australia

Sebastian is a PhD candidate working with the Choi Cell Mechanobiology lab and BRITelab at the University of Western Australia and Harry Perkins Institute of Medical Research in Perth, Western Australia. His research focuses on the changes to cell adhesion and behaviour that allow cancer cells to negotiate the mechanical challenges of the metastatic cascade in spheroids, single cells and subnuclear condensates. As an interdisciplinary scientist, he utilises and develops new ways to study these processes in biomimetic conditions using 3D biomaterials, soft lithography, microfluidics and conventional and novel correlative imaging techniques. Sebastian also develops data and image analysis tools for 3D cell biology to improve the accessibility of high-quality, open-source, and reproducible 3D bioimage analysis. He was recently awarded the John Farrant Prize for the best talk in biological/medical sciences at ACMM27 for his talk on 3D bioimage analysis. Sebastian is supported by a prestigious Hackett Postgraduate Research Scholarship and the interdisciplinary BioZone PhD Program.



THURSDAY 7th MARCH 11:40AM **An Open-Source Analysis Tool for 3D Cell Biology**

While 3D cell culture and fluorescence microscopy have improved the translatability of in-vitro research, there is a pressing need for accessible, user-friendly and open-source image analysis tools that can efficiently process 3D datasets and generate insightful outputs. Following image acquisition, researchers often seek to generate 3D regions of interest, measure those volumes to obtain complex shape data and quantify region-restricted pixel intensities. Also relevant is the ability to relate primary and secondary objects within 3D space (i.e. a cell and its nucleus) to resolve patterns of staining intensity or volumetric data within a single biological entity. However, many existing tools lack internal support for batch processing, advanced 3D-specific analysis or require substantial coding for optimal use, which can present a barrier or a steep, time-consuming learning curve. Here, we present the development of FOCUST, an accessible, batch-processing-enabled and GPU-accelerated 3D analysis tool distributed as an ImageJ/FIJI plugin. FOCUST empowers users to optimise, segment, relate, measure and fluorescently quantify 3D cellular datasets. The optimise mode enables users to configure and save pre-processing and segmentation parameters across multiple datasets, while three dedicated analysis modules are available for batch processing, offering analyses tailored to spheroid, single cell and speckle sample types. Under the hood, our plugin is designed with a modified model-view-controller architecture that enables easy parallelisation and future extension. FOCUST, the Fluorescent Object and Cell GPU-accelerated Segmentation Toolbox, streamlines 3D analysis, making it more accessible, reproducible and robust.

Scott Berry – University of New South Wales

Scott has a background in Theoretical Physics and Molecular Biology. He studied a PhD at the John Innes Centre in Norwich, UK, on mechanisms of epigenetic memory in plants, before moving to the University of Zurich in Switzerland as an HFSP and EMBO postdoctoral fellow. In Zurich, Scott worked on mechanisms of mRNA concentration homeostasis in mammalian cells. In 2021, Scott started his group at Single Molecule Science, the EMBL Australia node at the University of New South Wales, and is now also part of UNSW's RNA Institute. His group works on quantitative regulation of gene expression at the single-cell level, primarily employing microscopy and systems biology approaches – including mathematical modelling.



THURSDAY 7th MARCH 2:40PM

Highly multiplexed protein imaging from the subcellular to the cell-population scale.

Highly multiplexed protein imaging using iterative immunofluorescence enables localisation and quantification of tens to hundreds of different molecular species in the same sample, with a spatial resolution approaching the diffraction limit of light. As an image-based method, it scales in a cost-effective manner to a scale of millimetres, which allows the study of subcellular, cellular, and population-scale phenotypes in the same assay. I will discuss how we are using this technology in a high-throughput screening format to investigate mechanisms of cellular mRNA concentration homeostasis. I will also present our recently developed computational framework which uses deep learning to identify and quantify changes in subcellular organisation across perturbation conditions, directly from highly multiplexed image data.

Izzy Jayasinghe – University of New South Wales

Izzy is an Associate Professor at Single Molecule Science, UNSW, and a UKRI Future Leader Fellow. She was awarded her PhD in 2011 at the University of Auckland for the early work applying localisation microscopy (STORM) to visualise the cardiac ryanodine receptor. Izzy established her independent research group in the University of Leeds in 2015 where developed adaptations of optical imaging methods such as DNA-PAINT and Expansion Microscopy (ExM) to study pathological nanoscale remodelling in the failing heart. Her current research focuses on developing more accessible, faster and higher resolution imaging methods for imaging optically-thick (and biologically more complex) samples. Izzy is a Fellow of the Royal Microscopical Society and advocates



THURSDAY 7th MARCH 2:40PM

Optical microscopy tools for decoding nanoscale cellular signalling and broadening uptake of super-resolution

I will outline how the continued improvement and strategic adaptation of super-resolution microscopy tools have enhanced our understanding of the nanoscale structures underpinning life. In particular, I will illustrate some of these advances in visualising the ryanodine receptor (RyR) nanodomains in tissues like the heart, muscle, and neurones. Correlative structure-function imaging has allowed us to resolve, observe and identify the RyR clustering patterns which spatially-encode the fast calcium signals within the cells. The recently invented Expansion Microscopy technique has been particularly useful in imaging the three-dimensionally complex RyR nanodomains. I will outline some of the more recent developments of tools and experimental strategies developed in my team to advance the reproducibility, versatility, and accessibility of ExM as a bio-imaging tool.

SELECTED FROM ABSTRACT PRESENTATIONS

A multimodal light microscopy approach to visualise microtubule-organelle interactions regulating early embryogenesis

Jessica Greaney¹, Harald Janovjak², Jennifer Zenker¹

¹Australian Regenerative Medicine Institute (ARMI), Monash University, Melbourne, VIC

²Flinders Health and Medical Research Institute (FHMRI), Flinders University, Adelaide SA

Early mammalian embryogenesis presents a remarkable opportunity to visualise the inner secrets of a cell. The preimplantation mouse embryo is relatively transparent, allowing visual acumen of its interior as cells transition from totipotency to acquire a functional role in the embryo. The microtubule cytoskeleton is particularly critical in this transition as it forms a filamentous network used for intracellular transport, establishing polarity, cell division, and shaping intracellular structures. A unique, embryo-specific microtubule signature is organised in part by the microtubule nucleator, calmodulin-regulated spectrin-associated protein 3 (CAMSAP3). Visualising CAMSAP3 alongside the highly dynamic microtubule cytoskeleton during embryo development can help us broaden our understanding of how the subcellular architecture of a cell confers its identity in the growing organism. Due to their sensitivity to light and environmental disturbances, cutting-edge live imaging technologies must be adapted to overcome these limitations.

In order to address these challenges, we have employed a multi-modal imaging approach. To examine the real-time dynamics of a CAMSAP3-dependent microtubule network in embryos, we employed a laser scanning confocal microscope fitted with avalanche photodiode detectors. This enabled high-throughput imaging while limiting phototoxicity. Using this approach, we monitored CAMSAP3 during the first stages of embryonic growth whereby the embryo transitions of out totipotency and into a pluripotent state. To unravel this in higher spatiotemporal resolution, we optimised the application of lattice light sheet microscopy on the living mouse embryo, which provides detailed structural information on microtubule-organelle interactions as these adapt during embryogenesis. In addition, to manipulate these interactions, we developed a light-activated, optogenetic CAMSAP3, to couple with phasor illumination and spinning disk confocal microscopy to disrupt the microtubule cytoskeleton with subcellular precision. This allows us tight spatiotemporal control over the microtubule network as well as high temporal resolution imaging to better understand its roles in acquisition of cellular identity during embryogenesis.

This adaptive approach allows us the flexibility to switch between imaging modalities to best suit our needs while working within the challenges this unique model system presents. With this strategy, paired with optogenetics, we can manipulate and explore the inner world of living embryonic cells.

Visualizing the Immune Synapse to understand disease

Jesse Rudd-Schmidt¹, Daniela Castiblanco¹, Adrian Hodel¹, Tahereh Noori¹, Joe Trapani^{2,3},
Ilia Voskoboinik^{1,2}

¹ Killer Cell Biology Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia

² Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia

³ Cancer Cell Death Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia

Cytotoxic T Lymphocytes (CTL) are a critical part of the adaptive immune system. Their predominant form of killing is through the granule exocytosis pathway, whereby CTLs release key effector molecules into the immune synapse, perforin (PRF) and granzymes, to initiate target cell killing [1]. The pore-forming perforin is essential for the function of cytotoxic lymphocytes, as its pores disrupt the target cell membrane and allow diffusion of pro-apoptotic serine proteases, granzyme, into the target cell, where they initiate various cell death cascades [2].

By way of multiple live cell imaging techniques, our laboratory has been able to decipher many aspects of what does (and does not) occur within the immune synapse, both in healthy and disease settings, including primary immunodeficiencies [3]. From uncovering mechanisms by which healthy CTL protect themselves from their own lethal cargo [4], to determining the mechanism by which a lysosomal storage disorder impairs the function of patients CTLs [5], live cell microscopy has played a pivotal role in our studies. Here, we describe the detection of target cell membrane permeabilization by perforin ('PI blush'), and the recently developed 'ALFA-PRF' technique for visualizing murine perforin release by TIRF microscopy [6] and detail their applications in these studies.

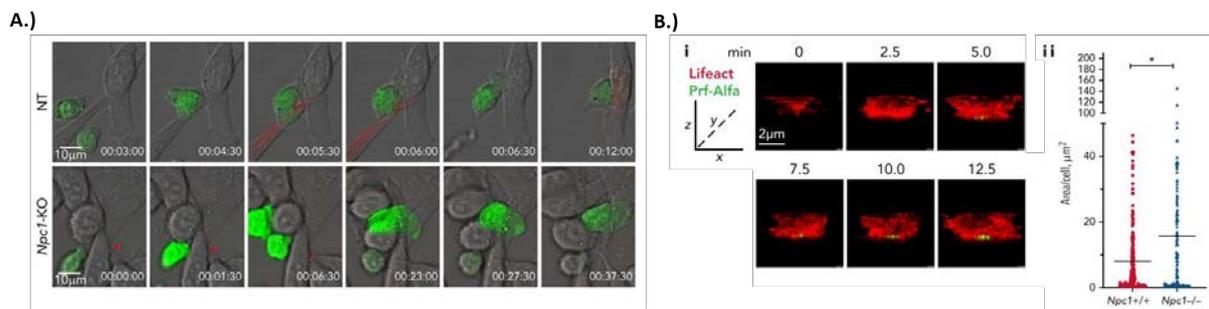


Figure 1: A.) Top Row: Upon synapse formation, CTL transfected with a Non-Targeting control guide (NT) flux calcium (indicated by increase in green fluorescence of Fluo-4 AM) just prior to a distinctive PI blush (red) occurring in target cell indicating perforin pore formation. Bottom Row: CTL transfected with guides to knock out NPC1, show calcium flux but no PI blush, indicating that there are no pores being formed on the target cell membrane.

B.) i.) ALFA-PRF (green) is detected by highly specific fluorescent nanobodies when released from a CTL expressing Lifeact Scarlet (red). **ii.)** Quantification of the amount of perforin released shows that NPC1^{-/-} cells release at least as much perforin as Npc1^{+/+} cells, demonstrating that the lack of PI blush in 1A is not due to failed release of perforin from the T cell.

*Figure adapted from [5].

References:

- [1] Barry and Bleackley, Nature Reviews Immunology **2** (2002) p. 401.
- [2] Lopez et al., The Journal of Immunology **191** (2013) p.2328.
- [3] Noori et al., Blood **141** (19) (2023) 2330-2342
- [4] Rudd-Schmidt et al., Nature Communications **10**, (2019) 5396
- [5] Castiblanco et al., Blood **139** (12) (2022) 1833–1849
- [6] Rudd-Schmidt et al., Front. Immunol. **13** (2022) doi: 10.3389/fimmu.2022.931820

Quantitative live-cell imaging of *Candida albicans* escape from immune phagocytes

Francios A.B. Olivier^{1,2}, Ana Traven^{1,2}.

¹ Infection Program, Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

² Centre to Impact AMR, Monash University, Clayton, VIC 3800, Australia

The innate immune response plays a crucial role in the clearance of bacterial and fungal pathogens, with phagocytes serving as key effectors. However, pathogens like *Candida albicans* employ strategies to survive within phagocytes, causing damage, lysis, and inflammation. With the rise of antimicrobial resistance, understanding these immune evasion pathways becomes essential for developing alternative therapeutic strategies.

Quantitative live cell imaging provides a powerful tool to dissect host-pathogen interactions. We developed a live cell imaging protocol to quantify the outcomes of an *in vitro* macrophage infection assay at population-level ¹. We distinguished between intracellular and extracellular fungi using a dual-labelling strategy: a fungal cell wall stain and endogenously expressed dTomato. A fluorescent DNA-binding dye (DRAQ7) allowed us to monitor macrophage permeabilization during fungal hyphae escape. Using the software CellProfiler, we quantified host cell numbers, host cell permeabilization, phagocytosed pathogen amounts, total viable pathogen counts, and kinetics of pathogen escape.

This method was applied to uncover mechanisms of *C. albicans* escape from macrophages. Our analyses, published in Cell Reports in 2022 ², showed that the pathogen engages both secreted toxins and host cell death pathways to escape. While limited by Gasdermin D deletion, *C. albicans* hyphal escape is not impacted by genetic or pharmacological inhibition of NLRP3 ². Instead, a toxin made by fungal hyphae (candidalysin) is a key contributor to *C. albicans* escape from macrophages ². We also show that hyphal escape is associated with the formation of macrophage extracellular traps, identifying an additional cell death pathway that facilitates fungal immune escape ². A detailed protocol outlining the infection assay, imaging and image analysis pipelines was published in STAR Protocols in 2023 ¹.

References:

1. Olivier, F. A., & Traven, A. (2023). Quantitative live-cell imaging of *Candida albicans* escape from immune phagocytes. STAR protocols, 4(4), 102737.
2. Olivier, F. A., Hilsenstein, V., Weerasinghe, H., Weir, A., Hughes, S., Crawford, S., ... & Traven, A. (2022). The escape of *Candida albicans* from macrophages is enabled by the fungal toxin candidalysin and two host cell death pathways. Cell Reports, 40(12).

**How T cells interpret complex migration signals:
From tissue to microfluidics**

Raymond Z. Qin^{1,2}, David Bryan^{2,3}, Crispin Szydzik⁴, Carolina Alvarado¹, Arnan Mitchell⁴, Niall D. Geoghegan^{2,3}, Kelly L. Rogers^{2,3}, Joanna R. Groom^{1,2}.

¹*Division of Immunology, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.*

²*Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia.*

³*Centre for Dynamic Imaging, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.*

⁴*School of Engineering, RMIT University, Melbourne, Victoria, Australia.*

Cell migration is a fundamental process for immune defence. Chemokines are key signaling molecules that guide migration direction by forming concentration gradients, enabling immune cells to efficiently survey tissues and strategically relocate. Within a lymph node, two major families of chemokines direct T cell migration, namely CCR7 and CXCR3 receptor families. Here, we use quantitative confocal imaging to characterise CXCR3 and CCR7 chemokine production and distribution in mouse lymph nodes during viral infection. The CXCR3 ligand CXCL10 is produced by cells in the interfollicular region at the lymph node periphery, while CCL21 is deposited in the paracortex at the lymph node center. By gradient quantification, we show these chemokines form opposing gradients along the interfollicular region-paracortex axis. T cells that express both receptors, such as early-activated or memory precursor T cells, are positioned to integrate these opposing signals. However, the mechanisms that determine migration directionality in these settings are largely unclear. Further, we extend these observations into the solid tumour microenvironment. We identified distinct chemokine niches at the tumour periphery and center. These niches are associated with T and B cell tumour infiltration and aggregate formation, which assist tumour control. These findings suggest chemokine niches within the lymph node and tumour microenvironments underpins T cell migration, differentiation and function.

To deconstruct complex chemokine environments, we developed a novel microfluidic “Lymph Node on a Chip” device. This allows precise control of fluid flow to generate stable, reproducible, and tunable chemokine environments. Here, we recapitulate two opposing chemokine gradients across the microfluidic chamber to study the migration of activated T cells suspended within a three-dimensional collagen environment. Combining this device with high-resolution Lattice Lightsheet Microscopy, we investigate T cell morphology at subcellular resolution. Quantifying dynamic T cell migration on this platform will reveal how T cells orientate the nucleus and cytoskeleton in a controlled but complex chemokine environment in 3D. Together, this work will provide new insights in T cell migration and differentiation during health and disease and highlight new avenues to target cell migration for immunotherapy.

In vivo imaging of macrophage retraction fibres and migrasomes in zebrafish

Azadeh Anbarlou¹, Hao-Ruei Hsu¹, Nicholas D. Condon², Oleksandr Chernyavskiy³,
Stephen Firth³, Graham J. Lieschke¹

¹ Australian Regenerative Medicine Institute (ARMI), Monash University

² Institute for Molecular Bioscience (IMB), The University of Queensland

³ Monash Micro Imaging (MMI), Monash University

Macrophages are a widely-dispersed innate immune cell. Resident macrophages maintain structural homeostasis while infiltrating macrophages assume pro-inflammatory and pro-regenerative roles after tissue injury. Like other migrating cells, migrating macrophages form trails of retraction fibres (RFs) and migrasomes, a recently described form of extracellular vesicle (1). While these structures are readily demonstrated in 2D *in vitro* systems, their demonstration *in vivo* has been much more challenging, because of their size (RFs are 0.2 μm diam.), complex twisted shape within 3D volumes, and evanescent nature. We are using zebrafish models to image RFs and migrasomes by intravital imaging. Transgenic lines mark macrophage cytoplasm or cytoplasmic membrane to reveal cellular form or outline respectively (Figure 1). Macrophages migrating towards a tail-transection injury are imaged *in vivo* at high spatiotemporal resolution by time-lapse confocal, Airyscan super-resolution and lattice light sheet microscopy. Mounting techniques have been optimised. *In vitro* studies complement the *in vivo* analysis. Our initial work has confirmed that migrating zebrafish cells, including macrophages, form RFs and migrasomes *in vivo*, albeit much less profusely than *in vitro*, at least at currently-achievable resolution. We have captured sequences showing phases of RF elongation, vesiculation, migrasome budding and shedding. New mutant and transgenic lines have been made with the purpose of enhancing or reducing RF and migrasome formation and to functionally evaluate their impact on migrating macrophages. These ongoing studies are placing this novel capability of migratory macrophages into a physiological context and will enable the role and importance of retraction fibres for macrophage function to be determined.

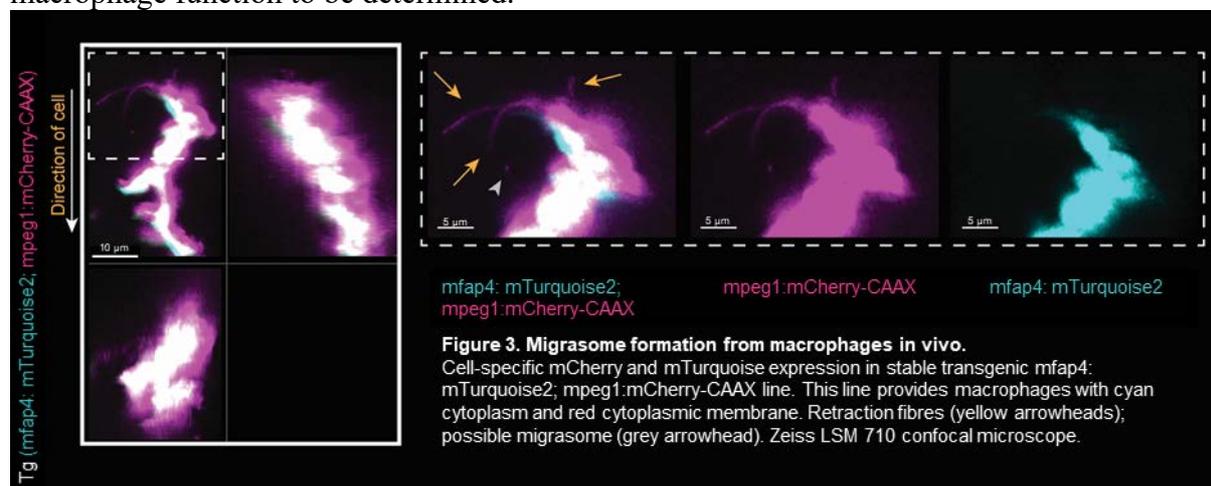


Figure 3. Migrasome formation from macrophages in vivo.

Cell-specific mCherry and mTurquoise expression in stable transgenic mfap4: mTurquoise2; mpeg1: mCherry-CAAX line. This line provides macrophages with cyan cytoplasm and red cytoplasmic membrane. Retraction fibres (yellow arrowheads); possible migrasome (grey arrowhead). Zeiss LSM 710 confocal microscope.

Live cell imaging to characterise migration-dependent membrane domains in macrophages.

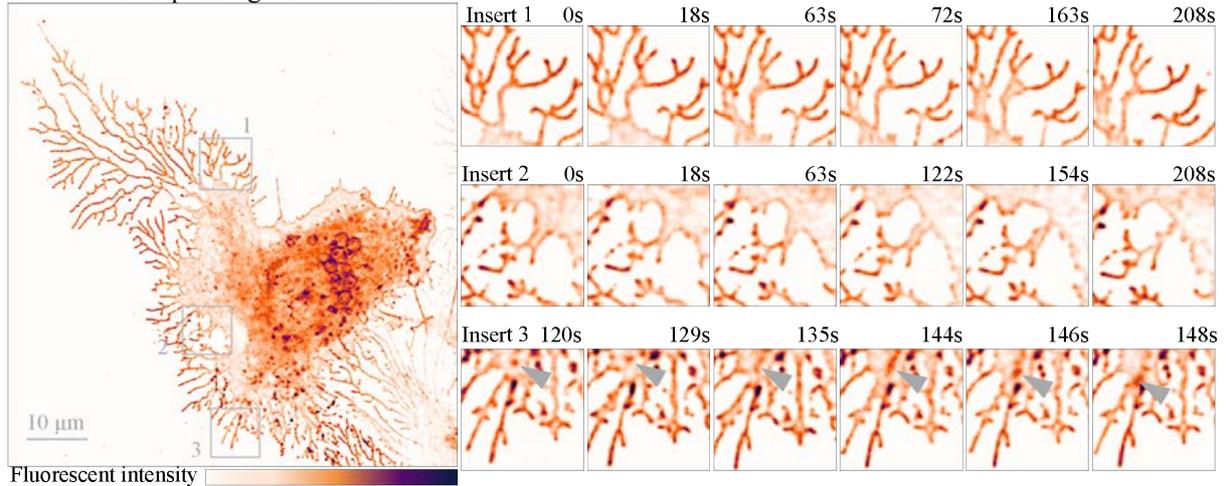
Xichun Li¹, Vrushali Maste¹, Nicholas D Condon¹, Darren Brown¹, Hongyu Shen¹, Amina Ashraf², Sylvia Jin Hui Tan¹, Rachael Z. Murray², and Jennifer L Stow¹.

¹ Institute for Molecular Bioscience (IMB), The University of Queensland and

² Faculty of Health, School of Biomedical Sciences, Queensland University of Technology, Brisbane QLD, Australia.

In the immune system, macrophages provide a first line response to infections and inflammation. Activated macrophages migrate to sites of injury or inflammation, where they engulf pathogens and release cytokines and other mediators to recruit cells of the adaptive immune system and fight infection. These inflammatory responses have been studied largely in activated, stationary immune cells, but how protective responses are executed in migrating cells is less well understood. To investigate, we employed multiple time-lapse live cell imaging techniques across varying scales (widefield, confocal, lattice light-sheet and Airyscan superresolution) to record activated cellular migration across different substrates. Three patterns of macrophage migration have been classified, including long distance and oscillatory trajectories with cell displacements of $>70 \mu\text{m}$. Live imaging of these cells at higher resolution revealed the production of migration-dependent retraction fibres (RFs) ($\sim 100 \text{ nm}$ diam) and migrasomes ($< 2 \mu\text{m}$ diam), which form trails of matrix-adherent membranes left behind by moving cells (Yu et al, 2021). We have identified a number of RFs and migrasomes labels and have begun dissecting different components and possible functions of these structures. Highly temporal but also long duration lattice light sheet microscopy acquisitions documented the dynamic growth and movements of RFs and the trafficking of vesicles and cytoplasm into RFs. Additionally, high resolution, quantitative confocal live imaging documented the formation and varied fates of large vesicular migrasomes on RFs. Overall, these imaging studies characterise the dynamic nature of adherent membrane trails in migrating macrophages and cast these structures as vital matrix-defined platforms for the spatio-temporal regulation of inflammatory responses in tissue environments.

RAW 264.7 expressing TSPAN4-GFP



Lattice light sheet live imaging of RAW 264.7 macrophages expressing TSPAN4-GFP. At the rear of this migrating macrophage, sections of cytoplasm were pumped into nearby proximal RFs (inset 1 and 2). TSPAN4-GFP decorated recycling endosomes were observed actively moving towards RF formatting points and trafficked into RFs (Inset 3, indicated by grey arrows). An inverted gem look-up table was applied to display the fluorescent intensity of TSPAN4-GFP.

Reference: Yu, FEBS J, doi:10.1111/febs.16183.

The role of bacterial size and shape in macrophage engulfment of uropathogenic *E. coli*

Elizabeth Peterson¹, Christian Evenhuis², Bill Söderström¹, Iain Duggin¹, Liz Harry¹, Bernadette Saunders³, Amy Bottomley^{1,2,*}.

¹ Australian Institute for Microbiology and Infection, Faculty of Science, University of Technology Sydney.

² Microbial Imaging Facility, Faculty of Science, University of Technology Sydney.

³ School of Life Sciences, Faculty of Science, University of Technology Sydney.

* Presenting author.

Bacteria undergo a variety of shape and size changes to survive different environmental niches. Filamentation occurs when bacteria transiently stop dividing and grow into long “spaghetti-like” cells with multiple chromosomes, resulting in cells that can be up to several hundred micrometers in length. Uropathogenic *Escherichia coli* (UPEC) are the major causative agent of urinary tract infections (UTIs), and filamentation is an essential part of the infection cycle of bladder cells. It has been hypothesised that this morphological plasticity allows UPEC to avoid and survive host immune responses. However, it is still unclear what properties of filaments are important in macrophage-bacteria interactions.

Viable populations of filamentous UPEC were isolated from antibiotic exposure, or an *in vitro* flow human bladder cell model. Quantification of the engulfment ability of human macrophages using gentamicin-protection assays and wide-field fluorescence microscopy demonstrated that the ability of filaments to avoid macrophage engulfment depends on a combination of size (length and volume), shape, surface and external environmental factors. Analysis of time-course microscopy infection assays revealed that the effectiveness of internalisation by macrophages remains stable over the course of infection independent of bacterial length, but filaments were less effectively fully engulfed overall. However, nearest neighbour distance calculated showed that filaments were more frequently partially engulfed compared to short rod cell counterparts, suggesting a difference in macrophage engulfment dynamics that is dependent on bacterial length. This mechanism of engulfment appears to be similar for both rods and filaments, as time-lapse and 3D-structured illumination microscopy showed an actin-dependent length-independent macrophage blebbing response during initial engulfment (Figure 1). Immunofluorescence microscopy and co-localisation analysis revealed that once internalized, filaments may have an improved survival ability in macrophages due to a delay or inactivation in the phagocytic pathway.

In summary, filamentation allows avoidance and survival of UPEC from the immune system by affecting the effectiveness of macrophage engulfment, the dynamics of internalization and maturation of the phagocytosis pathway. This work demonstrates the impact of bacterial morphological plasticity during infections and is an important consideration when developing effective treatment strategies.

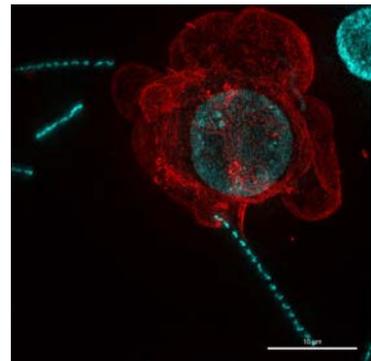


Figure 1. 3D-SIM MIP of macrophage engulfing UPEC filament. Cyan: DAPI; Red: Phalloidin AF568.

New Approaches to Characterise Goblet Cells and Mucus Properties in the Human Colon

Matthew C Rowe¹, Cameron J Nowell¹, Narges Mahdavian¹, Sebastian K King^{2,3,4}, Pradeep Rajasekhar⁵, Simona E Carbone¹, Daniel P Poole¹

¹ Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Parkville, VIC

² Department of Paediatric Surgery, Royal Children's Hospital, Parkville, VIC

³ Department of Paediatrics, The University of Melbourne, Parkville, VIC

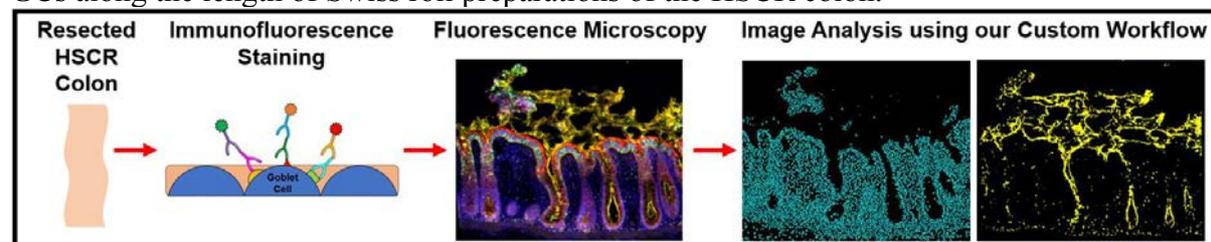
⁴ Surgical Research, Murdoch Children's Research Institute, Parkville, VIC

⁵ WEHI, Parkville, VIC

Goblet cells (GCs) line the gastrointestinal tract and are critical for the production and secretion of mucus. The mucus they secrete forms a physical barrier to protect the epithelium from pathogens and other sources of damage. A functional mucus layer is essential for intestinal homeostasis. Disruption of this layer leaves the epithelium vulnerable, increasing the susceptibility to bacterial infection and inflammation.

The enteric nervous system innervates the intestines and coordinates important functions including mucus and fluid secretion. Hirschsprung disease (HSCR) is a congenital enteric neuropathy where enteric neurons fail to colonise the distal end of the colon, resulting in severe constipation. Up to 50% of patients will also develop Hirschsprung associated enterocolitis (HAEC) which is a life-threatening comorbidity of HSCR that causes extensive mucosal inflammation. Neural signalling is an important regulator of mucus secretion, and the absence of enteric innervation suggests that mucosal barrier defence mechanisms may be compromised in HSCR. Although the pathogenesis of HAEC is poorly understood, disruption of mucosal barrier function may be a key contributor.

We hypothesise that GCs and the mucus layer are dysfunctional in HSCR patients and are predictive of HAEC susceptibility. We optimised six independent markers of GCs and mucus for immunofluorescence staining and multiplexing on both cryosections and formalin-fixed paraffin embedded colon tissue. We applied imaging techniques, including optical clearing and confocal microscopy, super resolution microscopy, and widefield microscopy, to characterise the goblet cell profile along the length of the resected colon (10-30cm of tissue) from 10 HSCR patients and 5 age-matched controls. To analyse the acquired images, we developed a novel analysis pipeline using ImageJ, Huygens deconvolution, Ilastik and StarDist to investigate the functional capacity of GCs to secrete mucus in response to bacterial products, and pharmacological, and electrical stimuli. We also assessed the location and distribution of GCs along the length of Swiss roll preparations of the HSCR colon.



We have developed custom methods to investigate the function and distribution of GCs in the human colon. Applying these methods to archival tissue samples from HSCR patients will allow us to assess the relationship between GCs and the incidence of HAEC. Furthermore, understanding the differences in mucus barrier function may provide a link between HSCR and HAEC susceptibility, which can be used to inform and guide treatment approaches for patients.

Developing a 3D multi-omic map of low grade glioma

Jurgen Kriel^{1,2}, Joel Moffet^{1,2}, Tianyao Lu^{1,2}, Oluwaseun Fatunla^{1,2}, Jim Wittle^{1,2,3}, Sarah Best^{1,2}, Saskia Freytag^{1,2}

¹ Personalised Oncology Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne Australia

² Department of Medical Biology, University of Melbourne, Australia

³ Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia

In order to accurately dissect tumour heterogeneity, information at both the genetic and tissue level is required. Current spatial biology technologies allow for combination of these datasets, however analysis focusses on single tissue sections only. Here, we present a preliminary workflow to produce a three-dimensional (3D) spatial transcriptomic and metabolomic map of low grade glioma (LGG).

LGG is the second most common form of brain cancer, with more than 50% of patients experiencing recurrence within five years of surgery. Recurrence is largely due to incomplete resectioning, mainly attributed to the ill-defined boundaries between LGGs and healthy tissue and therefore presents a significant challenge for surgeons. By generating a 3D multi-omic map, we aimed to develop a coherent understanding of glioma biology at the brain-tumour interface that can inform pro-drug development in the future.

Frozen patient samples were CT scanned to map the surface area of the tissue. A depth of 500um was serial sectioned at 10um thickness, allowing for conventional H&E staining, spatial transcriptomics comparing three commercial technologies (10x Xenium, Nanostring CosMx and Vizgen MERSCOPE) and imaging mass spectrometry (IMS) to measure metabolite abundance. The multi-omic modalities were registered using manual alignment of morphological landmarks in serial H&E sections using TrakEM (FIJI). Cell segmentation of immuno-fluorescence (IF) data was conducted on spatial transcriptomic datasets using custom trained models in Cellpose. Transcripts could then be assigned to the aligned datasets for cell type identification and niche analysis. IMS metabolite information were registered to the image data (STalign). For accurate depiction to the true 3D space, aligned datasets were spaced according to 3D voxel information from the initial micro-CT scan.

Preliminary findings include the identification of significant changes in cell type identification as a result of different segmentation approaches between the manufacturer and our custom pipeline. We also showcase the necessity of high-quality fluorescence microscopy data in spatial biology workflows, as image quality is a determining factor in segmentation efficiency. Our novel 3D-omic approach therefore provides a detailed view of the complex interplay between tumour, immune and healthy cells in LGG at the transcriptional and metabolite level.

Spatial irregularities in estrogen receptor staining: combined imaging approaches to identify regions of contiguous staining in preneoplastic breast tissue from BRCA2 mutation carriers.

Capaldo BD^{1,2}, Christie M³, Rajasekhar P⁴, Joyce R^{1,2*}, Pascual R^{1,2*}, Lindeman GJ^{1,5,6} and Visvader JE^{1,2}

1 ACRF Cancer Biology and Stem Cells Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia.

2 Department of Medical Biology, The University of Melbourne, Parkville, VIC 3010, Australia.

3 Department of Anatomical Pathology, Royal Melbourne Hospital, Parkville, VIC 3050, Australia

4 Advanced Technology and Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia.

5 Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, Parkville, VIC 3010, Australia.

6 Parkville Familial Cancer Centre and Department of Medical Oncology, The Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Parkville, VIC 3050, Australia.

*These authors contributed equally to this work

Inherited mutations or other pathogenic variants in the BRCA2 tumour suppressor gene. occur in about 1 in 225 individuals and the lifetime risk of developing breast cancer increases from ~11.7% to 69% for female carriers. We have applied large-scale confocal microscopy together with conventional microscopy and pathological examination to provide important spatial insights into the cellular changes that may contribute to breast cancer susceptibility in BRCA2 mutation carriers.

In our study examining preneoplastic specimens from age-matched, pre-menopausal patients, we detected the presence of atypical estrogen receptor (ER)-positive lesions. Using large-scale single-cell resolution 3D imaging (LSR-3D) techniques pioneered in our laboratory we observed these lesions as clusters of estrogen receptor positive cells in the lobules and ducts within the terminal ductal lobular units (TDLUs) of BRCA2 mutation carriers. We are interested in changes in the TDLU's of preneoplastic tissue as the TDLUs are known to represent the putative site of breast cancer formation.

To further examine this, we turned to a large-cohort (n= 76; 38 BRCA2^{mut/+} and 38 controls) and immunoassayed 2D FFPE sections for the estrogen receptor. We again observed large areas of contiguous estrogen receptor-stained cells in tissue sections that often occurred together with mild columnar cell change. As part of this work, we developed a quantification pipeline in Qu-Path to digitally examine the staining and intensity of estrogen receptor staining. However, results revealed no difference in the number ER+ cells or the overall intensity of staining (H-score). When stained tissue sections were scored blindly by a pathologist, we interestingly found a significant increase in the proportion of these contiguous areas of estrogen receptor expression in carriers compared to controls (unpaired t test; p=0.0239).

In conclusion, our tissue imaging approaches allowed us to identify spatial differences in estrogen receptor expression in pre-neoplastic tissue from BRCA2 mutation carriers. Future studies will examine if these estrogen receptor positive regions in breast tissue could represent potential precursor lesions in BRCA2 mutation carriers. This is particularly interesting as the breast tumours that arise in BRCA2 mutation carriers are commonly estrogen receptor positive.

This work forms part of a larger study that has profiled and examined other detectable molecular changes in preneoplastic breast tissue from BRCA2 mutation carriers identifying an aberrant luminal progenitor and found that mTORC1 inhibition has potential in chemoprevention. This work also shows proof-of-concept that heterozygous loss of BRCA2 function is sufficient to induce phenotypic and functional consequences in preneoplastic and pathologically 'normal' tissue.

Cancer treatments cause a permanent reduction in blood multipotent progenitor cells which is accompanied by alterations in bone marrow niche cells that support these progenitors.

Gavin Tjin^{1,2}, Kelli Schleibs¹, Lynette Mirembe¹ and Louise E Purton^{1,2}.

¹ Stem Cell Regulation Unit, St. Vincent's Institute of Medical Research, Victoria, Australia.

² Department of Medicine, The University of Melbourne, Victoria, Australia.

The haematopoietic stem and progenitor cell (HSPC) microenvironments (niches) are important for regulating haematopoiesis, but little is known on the identity of the niche for lymphoid-biased multipotent progenitors (MPP4). Furthermore, little is known about how HSPC niches and their interactions with the HSPCs are altered by cancer treatments.

We used mouse models treated with chemotherapy (5-FU) or a bone marrow transplant (BMT) to determine how HSPCs and their corresponding niches are impacted. There was a persistent long-term reduction in MPP4 with slight reductions in short-term and long-term repopulating HSCs (ST-HSCs, LT-HSCs), but not MPP2 or MPP3, post-cancer therapies. Fluorescent reporter mice which label HSPCs (hScl-CreER Confetti and Flk2Cre mTmG) treated with 5-FU or a BMT also provided additional information on the behaviour of HSPCs post-cancer treatment. To further identify the niches for the HSPCs and how they are impacted by cancer treatments we performed *in situ* single cell analysis of the HSPCs in the bone marrow (BM). We used fluorescence multiplexing and image analysis to identify and quantify the interactions of the HSPCs and niche cells *in situ*. Sinusoids were identified using VEGFR3, arteries by Sca-1 and mesenchymal-derived cells were identified using Col2a1Cre and Prrx1Cre R26EYFP niche reporter mice.

At baseline, CD34-CD135- LT-HSC, CD34+CD135- ST-HSC and MPP4 reside close to perivascular sinusoidal niches throughout the BM (all are 1-4 cells away), and MPP4 were also close to EYFP+ trabecular niche cells (~3 cells away), with no notable interaction with other niches. Treatment with 5-FU or a BMT caused loss of trabecular bone, and a BMT also caused reduced arteries and sinusoids and increased adipocytes. MPP4 are closer to EYFP+ perivascular cells post-5-FU or BMT. More ST-HSC and MPP4 were found in the metaphysis region post-BMT and both ST-HSC and MPP4 remained close to the EYFP+ trabecular niche cells.

Combined, our results show that perivascular cells, sinusoids, and mesenchymal trabecular niche cells are important niches for the HSPCs. The molecular changes in the BM niche cells that regulate MPP4 production are currently being investigated. Some niches, such as arteries, are rare and difficult to identify on 2D sections. Therefore, we modified the fluorescence multiplexing protocol to be used on 100µm thick bone marrow sections to visualise the HSPCs and BM niches in 3D. Imaging is performed on a confocal microscope using spectral unmixing and it is currently in progress.

Intravital imaging to investigate fluorescent reporter mice for p53 target genes involved in tumour suppression

Annabella Thomas^{1,2}, Elizabeth Lieschke¹, Lin Tai¹, Andrew Kueh¹, Edwin Hawkins^{1,2}, Marco Herold^{1,2}, Georgia Atkin-Smith^{1,2}, Gemma Kelly^{1,2}, Andreas Strasser^{1,2}.

¹Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

²The Department of Medical Biology, The University of Melbourne, Melbourne, Victoria, Australia

The *p53* gene is mutated in ~50% of human cancers and these mutations often contribute to poor responses to cancer therapy. The p53 protein is a master regulator of several cellular processes. It functions as a homo-tetrameric transcription factor, which directly transcriptionally regulates ~500 genes that suppress tumorigenesis, including the expression of *p21*, which is critical for the induction of cell cycle arrest/cell senescence, and *Puma* gene, which is a critical initiator of apoptotic cell death.

To investigate p53-mediated activation and immunological response of these pathways, we have created two reporter mouse lines, in which, GFP is knocked into the *p21* locus behind an IRES, or in which, the *Puma* coding region was replaced with the sequence for tdTomato. We have also inter-crossed p21 and Puma reporter mice to gain information on why certain cells undergo cell cycle arrest/senescence after p53 activation, while others undergo apoptotic cell death, and these have been validated via FACS analysis. Activity of both reporters were observed in a wide variety of immune cell types, including T and B cell subsets, following staggered irradiation, helping to reveal which immune cells die or senesce in response to treatment. The reporters have also been validated and investigated *in vivo* using intravital imaging of live mice, allowing reporter activity to be detected in diverse immune cell types in specific organs, including the bone marrow calvarium.

Intravital imaging enables us to investigate whether individual immune cells only activate the transcription of the critical initiator of cell cycle arrest/senescence, apoptotic cell death, or both. The added dynamics of live tracking in live mice will provide valuable new insight into the mechanisms by which p53 prevents tumour development and immune cell responses to therapeutics.

3D optical sectioning with vibrational infrared photothermal and phase signals (VIPPS)

E.T. Gill¹, P. Samolis², S.L. McArthur^{1,3}, P.R. Stoddart¹, M.L. Sander².

¹ Swinburne University of Technology, Melbourne, Australia

² Boston University, Massachusetts, USA

³ Deakin University, Melbourne, Australia

Photothermal imaging (PTI) is an innovative tool that can probe a sample's chemical and mechanical information. Vibrational infrared photothermal and phase signals (VIPPS) is a dual-beam pump-probe imaging modality that uses absorption of a mid-IR pump beam to induce a localised thermal lens, and a near-IR probe beam to measure the fluctuation in refractive index due to thermal relaxation. The advantages of VIPPS in studying 3D cell culture are the increased depth, optical sectioning, chemical targeting, and the new information that can be gained about structural thermal relaxation [1]. The aim of this research was to evaluate the performance of VIPPS for imaging 3D cell culture models, targeting the amide I absorption. A 3D cell culture model was created using human fibroblasts embedded in collagen hydrogel. The PTI absorption (lock-in amplifier amplitude signals) highlighted the collagen fibril texture. The PTI thermal transport images (lock-in amplifier phase signals) provide greater contrast on the intracellular vs extracellular environment. The textural features of the VIPPS images were verified by comparing them to SHG images of collagen's non-centrosymmetric helical structure. In conclusion, VIPPS demonstrated label-free 3D optical sectioning of the mechanical structures of collagen ECM in a fibroblast 3D cell culture model. Future research will explore increasing the pump beam range to target other cellular structures, such as DNA and lipids.

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Developing a Very Large Field-of-View Imaging System for Multiplexed High-Speed Optical Mapping of Action Potentials and Calcium Transients in Live Cells and Tissues

Scott L. Page¹, Monique J. Windley², Jordan Thorpe² and Adam P. Hill².

¹ Micro Imaging Facility, Innovation Centre, Victor Chang Cardiac Research Institute

² Computational Cardiology Laboratory, Victor Chang Cardiac Research Institute

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia and is associated with serious health complications and mortality. The underlying arrhythmia occurs in tissue across a multiple square centimetre (cm) area and is characterized by localized dynamic spirals of electrical activity called rotors. Studying the electrical phenomena involved in arrhythmia at clinically relevant spatial scales requires detection of small changes in voltage and/or calcium ion concentration at high sampling rates across a large area [1]. Currently, approaches such as multielectrode array (MEA) recordings are limited to small areas and low electrode numbers. Optical mapping approaches, in contrast, use microscopy to detect action potentials (APs) by recording fluorescent dye intensity changes at high frame rates but are limited by the field of view (FOV).

To achieve a large FOV up to several cm in the X and Y dimensions, we developed a low/no magnification instrument based on a BrainVision THT Mesoscope with a MiCAM-03-N256 camera, 465nm LED epifluorescence light source, filter set for green fluorophore emission, and 1.0x, 1.6x and 0.63x Leica M-series lenses. Maintenance of live cells and tissues is supported by an Okolab stage incubator with customized electrode access ports, supplemental heated base and a CO₂/O₂ gas mixer. In an upright configuration, combinations of lenses in objective and condenser positions allow XY FOV sizes from 0.7-3.0cm to be achieved, with larger FOVs (3.1-10.4cm) possible with lower fluorescence detection capability. The system can acquire up to 1,818 frames per second across multiple cm at 256x256 pixels.

Testing the system with iPSC-derived cardiomyocyte monolayers loaded with FluoVolt voltage-sensitive fluorescent dye demonstrated that the system can detect APs with as low as a 1ms sampling frequency in a 3.0cm FOV. Measurement of APs and rotors is possible across entire 6- or 12-well plate wells (3.5 and 2.2cm diameters, respectively) followed by semi-automated whole well data analysis using BV Workbench software. The system can also record from multiple wells simultaneously for comparative experiments and higher throughput, with comparable AP duration (APD₆₅) and conductance velocity measurements as smaller FOVs, but with 5 ms sampling frequency, as demonstrated in a 6.2cm FOV (six wells/24-well plate). The system is expected to be capable of recording dynamic events at high temporal resolution in a range of specimens, including cardiac tissue slices, murine heart, organoids and engineered tissues, neuronal cultures and brain slices.

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The power of intravital two-photon imaging of Chronic Cranial Windows: insights into T cell dynamics and the influence of the tumour microenvironment in mouse models of glioma.

Matthias Mulazzani¹, Kylie Luong¹, Verena C Wimmer²³, Valeria Arcucci¹, Melinda Iliopoulos¹, Katherine A Watson¹, Kelly L Rogers²³ and Misty R Jenkins¹³⁴

1. Immunology Division, Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia
2. Centre for Dynamic Imaging, Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia
3. Department of Medical Biology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, Victoria, Australia
4. Department of Biochemistry, La Trobe Institute for Molecular Science, Bundoora, Victoria, Australia

Chimeric Antigen Receptor (CAR) T cell therapy involves re-engineering patient-derived T cells to redirect T cell cytotoxicity against tumour cells. Whilst CAR T cell therapy has demonstrated remarkable success in treating haematological malignancies, the same success has not yet been recapitulated in solid tumours, including brain tumours. To enhance CAR T therapy strategies, it is crucial to understand the dynamics of CAR T cells within the tumour microenvironment and their interactions with other cellular players.

We have established a chronic cranial window implant, which provides a window into the brain of immunocompetent mouse model of glioma (Mulazzani et al, 2019, PNAS). In combination with two-photon microscopy, this allows us to visualize tumour cell growth intravitaly, longitudinally over the entire tumour course. We can examine the extent of T cell infiltration, accumulation, directionality, velocity, and persistence across time in the same mouse.

We found that antigen-specific CAR T cells can infiltrate and accumulate in the brain as early as two days after infusion, but the CAR T cells did not show migration towards or away from tumour; instead, they travelled along tumour border. Despite our in vitro T cell killing assays demonstrating CAR T cell effectiveness, they were unable to clear the tumour in vivo and the mice all eventually succumbed to disease. This suggests a possibility of other cellular players within the tumour microenvironment hindering T cells cytotoxicity, emphasising the need for combination therapy approaches.

This cutting-edge in vivo intravital imaging technique allows us to gain new insights into the complex interactions between CAR T cells and other immune cells and tumour cells within the brain microenvironment, which can ultimately inform the development of more effective immunotherapies for brain tumours.

Explore, annotate, and analyse multi-dimensional images in Python with napari

Nicholas Sofroniew*¹, Talley Lambert*², napari core developers³, napari contributors⁴, & **Juan Nunez-Iglesias⁵**

¹ Chan Zuckerberg Initiative

² Harvard Medical School

³ <https://napari.org/stable/community/team.html#current-core-developers>

⁴ <https://github.com/napari/napari/graphs/contributors>

⁵ Monash eResearch Centre

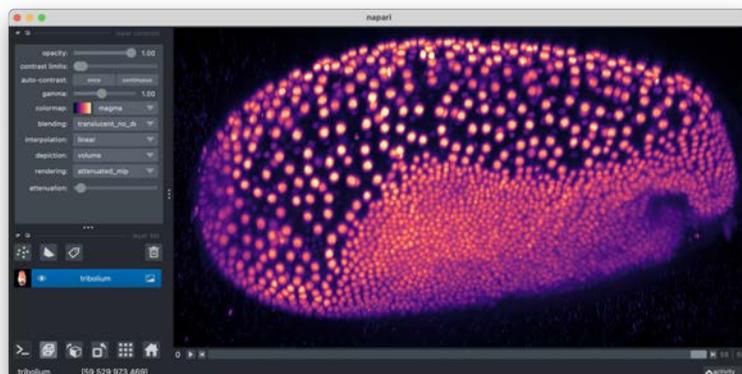
* co-first authors

A growing number of scientists analyse their data using the Python programming language and its numerical computing libraries — particularly when using the latest deep learning advances. Until recently, though, to look at high-dimensional images, those scientists had to rely on either manually subsetting their data or working with complicated cross-language solutions. We created the open source multidimensional image viewer *napari* to reduce the friction between scientific Python users and their image data. Napari provides a canvas that can be 2D or 3D. When the displayed data has more dimensions than the canvas, napari automatically creates sliders for those additional dimensions, allowing users to rapidly explore the full data. Further, in addition to image data, napari can display and overlay derived data such as segmentations, point annotations, vector fields, and more. Finally, our graphical user interface also allows developers to extend napari via a plugin system and provide point-and-click access to the scientific Python ecosystem for researchers who may not yet be comfortable with programming.

I'll cover napari's history, design principles, capabilities, and long-term vision: that napari will become a standard tool to work with scientific image data, whether as a scientific programmer or as a non-coding scientist. I'll explain our open source governance and community — key strengths that have helped napari grow at an incredible pace, and that we hope others emulate. We continue to develop napari openly and invite newcomers to help improve it for their specific needs.



```
Python data/vis/020-001
In [2]: tribolium = ds.from_xz('01_000_001/0')
In [3]: tribolium.shape
Out[3]: (40, 900, 1000, 964)
In [4]: viewer, layer = napari.imshow(tribolium, colormap='magma')
In [5]:
```



Development of machine learning based image analysis pipeline for accurate analysis of Malaria parasite proliferation and invasion

Authors:

Sonja Frölich^{1,2}, Benjamin Liffner¹, Meghan Zadow¹, Maxim Buckley¹, Shamit Singla¹ and Danny Wilson^{1,2}

Affiliations:

¹Department of Molecular and Biomedical Science, School of Biological Sciences, Research Centre for Infectious Diseases, University of Adelaide, Adelaide, Australia.

²Institute for Photonics and Advanced Sensing (IPAS), University of Adelaide, Adelaide, Australia.

Abstract:

Malaria, a persistent global health crisis, annually inflicts over 200 million cases and claims approximately 600,000 lives. The complex life cycle of *Plasmodium falciparum* includes a critical 48-hour blood stage characterised by merozoite invasion into a red blood cell (RBCs). The physical interactions between the erythrocyte surface and invasion ligands from merozoite rhoptries are indispensable for RBC invasion and the formation of a parasitophorous vacuole (PV). Within this PV, the intracellular parasite undergoes extensive genome remodelling via “closed” mitosis and unique cytokinesis, culminating in the emergence of a multinucleated schizont, with each daughter merozoite inheriting the cellular machinery to function independently.

To resolve the dynamics of rhoptry-associated proteins during erythrocyte invasion and subsequent genome remodelling, our study introduces an innovative machine learning (ML)-driven image analysis pipeline. Leveraging the robustness of random forest algorithms, we automated the detection and segmentation of parasite nuclei and rhoptries in super-resolved three-dimensional volumes of immune-labelled infected erythrocytes. In contrast to traditional threshold-based methods, our ML approach excels in accurately identifying nuclei in distinct stages of mitosis and quantifying structural features of rhoptries, providing a better understanding of schizont proliferation and maturation.

The training phase of our pipeline involved exposing the random forest model to a diverse dataset containing schizonts in various stages of development, resulting in a robust classifier capable of differentiating pre-, post- and replicating nuclei and structural rhoptry features associated with schizont maturation. Rigorous validation against ground truth and manual quantification methods demonstrated the superior accuracy and efficiency of our ML pipeline. Notably, our pipeline outperformed traditional methods in protein knockdown studies, specifically with the rhoptry-associated protein PFCERLI1. The incorporation of seed-point-diameter and region-growing algorithms into the pipeline further enhanced its performance, enabling the identification of more rhoptry organelles in both untreated and PFCERLI1-depleted samples that were otherwise missed by the traditional approach.

This novel ML random forest-based image analysis pipeline not only streamlines quantification but also enables a deeper exploration of nuclear replication dynamics within infected erythrocyte and functional characterisation of rhoptry-associated proteins during erythrocyte invasion. Our innovative methodology not only surpasses traditional threshold-based segmentation of schizonts but also holds promise for advancing microscopy-based analysis of malaria parasites in other stages of the life cycle, demonstrating the potential of machine learning as a valuable asset in current and future investigations concerning antimalarial drug development and functional genomics, offering speed and enhanced accuracy while minimising subjectivity and bias.

***Segment Anything Model* based pipeline for annotating and analysing microscopy images of kidney organoids**

Thanushi Peiris^{1,2}, Michelle Scurr^{1,2}, Pei Xuan Er^{1,2}, Emma Scully^{1,2}, Allara Zylberberg^{1,2,3}, Melissa H. Little^{1,2,3} & Kynan T. Lawlor^{1,2,3}

¹ Murdoch Children's Research Institute, Flemington Rd, Parkville, VIC, Australia

² Novo Nordisk Foundation Centre for Stem Cell Medicine, University of Copenhagen, Copenhagen, Denmark

³ Department of Paediatrics, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, Victoria, Australia

Organoids are 3D tissues grown *in vitro* that model the development and disease of organs better than 2D cell cultures and have the potential to provide a source of tissue for regenerative medicine. However, current quality assessment of organoids predominantly focuses on RNA sequencing of individual cells instead of how they form intricate 3D structures essential to their function. We have developed a pipeline that integrates open-source annotation tools to rapidly segment key features of multidimensional immunofluorescent organoid images. This method is based on the Segment Anything Model (SAM), a foundational image segmentation model trained on over 11 million natural photos (Kirillov et al., 2023). It demonstrates impressive one-shot performance on a variety of segmentation tasks but has so far had limited application to immunofluorescent microscopy. We benchmark SAM on a dataset of immunofluorescent confocal images of kidney organoid tissue, investigating the speed and performance of different modes of SAM. The resulting pipeline can be used to assess morphology and nephron patterning more rapidly across different organoid culture conditions. We have developed a napari plugin to enable easy application of the pipeline to similar segmentation tasks. It builds on the existing napari-sam plugin (Gotkowski et al., 2023) with added features to support faster, more user-friendly annotation of 3D images and generation of metrics and graphs after annotating. This method contributes to enabling an inexpensive high-throughput assessment of factors affecting the quality of stem cell-derived kidney tissue by providing spatial information about tissue morphology.

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Application of single molecule imaging and expansion microscopy to study genomic structures

Ashley Rozario¹, Alison Morey², Sarah Hilet³, Cathryn Hogarth³, Stephen Turner², Toby Bell⁴, and Donna Whelan¹.

¹ La Trobe Institute for Molecular Science, La Trobe University Bendigo, Vic, Australia

² Biomedicine Discovery Institute, Monash University, Clayton, Vic, Australia

³ Department of Pharmacy and Biomedical Sciences, La Trobe University, Wodonga, Vic, Australia

⁴ School of Chemistry, Monash University, Clayton, Vic, Australia

Super-resolution microscopies overcome the diffraction limit using specialized microscope hardware or fluorescent labels. Single molecule localization microscopy (SMLM) uses reversibly photoswitching fluorophores to achieve ~20 nm imaging resolution. Expansion microscopy (ExM) improves visible detail from tissue and cell structures by physically enlarging the sample. For ExM, cellular structures of interest are fluorescently labelled and embedded into a hydrogel that expands ~4X in one dimension (ie ~64X volumetric expansion), thus enlarging previously unresolved detail that is visible using conventional fluorescence microscopes. The orthogonal principles of ExM and SMLM allow them to be combined with a cumulative resolution gain.

Adaptive immunity involves the differentiation of naïve T cells, upon interaction with a viral challenge, into effector T cells that help clear the invading pathogen. Following clearance, a subset of effector T cells become memory T cells that are more responsive to subsequent viral challenges. As such, the genomic landscape at each stage of cell differentiation is unique to elicit the required function. We have applied a combined ExM-SMLM imaging assay to interrogate the spatial distribution of chromatin in primary mouse T cells. Specifically, we imaged histone modifications that are associated with either transcriptionally active or suppressed regions of the genome. With radial analysis, we revealed significant shifts in chromatin distribution between the center and periphery of the nucleus through T cell differentiation.

We have also applied SMLM and ExM to image the synaptonemal complex of meiotic mouse spermatocytes and visualize genomic recombination events via DNA damage and repair markers γ -H2Ax, MLH1 and RAD51. Characterizing the molecular events of meiosis will provide novel impact in understanding reproductive health and genomic variability.

Tracking the Endosomal Escape of Nanoparticles in Live Cells Using a Triplex-Forming Oligonucleotide

Sukhvir Kaur Bhangu,^{1,2‡} Liviana Mummolo,^{2‡} Soraia Fernandes², Alessia Amodio,² Agata Radziwon,² Brendan Dyett,¹ Marco Savioli,^{2,3} Nitin Mantri,¹ Christina Cortez-Jugo,² Frank Caruso,² and Francesca Cavalieri^{1,3}

¹School of Science, RMIT University, Victoria 3000, Australia

²Department of Chemical Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

³Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma “Tor Vergata”, Via della Ricerca Scientifica 1, 00133 Rome, Italy

Nanoparticle-mediated intracellular delivery of oligonucleotides is a complex phenomenon that depends on the architecture and the intracellular trafficking of the engineered nanoparticles. Unravelling the molecular arrangements of oligonucleotides within the nanoparticles as well as their intracellular behavior are essential for designing effective nucleic acid delivery systems. Herein, a simple and general strategy for probing the endosomal escape of nanoparticles carrying oligonucleotides in live cells is reported. A triplex-forming oligonucleotide probe is designed to target the transcription factor, kappa-light-chain-enhancer of activated B cells, in the cytosol of cells and to transduce the binding into a fluorescent Förster resonance energy transfer (FRET) signal. The combined use of the triplex-forming oligonucleotide probe and super-resolution microscopy enables the elucidation of the morphology, intracellular localization, and endosomal escape of the oligonucleotide-loaded nanoparticles on a molecular level and with nanoscale resolution. The co-delivery of the FRET probe and mRNA in cells via lipid- and polymer- based nanoparticles allowed us to simultaneously correlate the endosomal escape properties of nanoparticles and gene expression efficiency.

A 3D STED microscopy approach to investigating megakaryocyte buds.

Imala Alwis^{1,2}, Marc L Ellis^{1,2,3}, Rhyll Smythe^{1,2}, Yuping Yuan^{1,2}, Shaun P Jackson^{1,2,4}

¹Thrombosis Group, The Heart Research Institute, Newtown, NSW, Australia; ²The Charles Perkins Centre, The University of Sydney, Camperdown, NSW, Australia; ³Haematology Department, Concord Repatriation General Hospital, NSW, Australia; and ⁴Department of Molecular Medicine, MERU-Roon Research Center on Vascular Biology, Scripps Research, La Jolla, CA

It is generally assumed that the physiological release of platelets from megakaryocytes (MKs) occurs primarily by the release of platelet-sized swellings from the tips of long, branching membrane structures (proplatelets) that extend from the MK surface into sinusoidal blood vessels in the bone marrow. They are also prominent in the lungs, and both MK and proplatelet fragmentation are thought to make a major contribution to *in vivo* platelet production. Much of the current understanding of proplatelet formation has been based on the characterization of cultured MKs, however it is notable that the structure and molecular mechanisms regulating *in vitro* proplatelet formation can differ significantly from what occurs *in vivo*.

A recent study from Samir Taoudi's laboratory has shown that most platelet-sized particles are produced from unique membrane structures called MK buds, and that proplatelet creation in the bone marrow is rare¹. This work has challenged the long-held notion that proplatelets are the major MK membrane structures that generate platelets *in vivo* by directly measuring bud release at the whole-organ level. The MK budding theory, however, remains controversial. Italiano et al. have legitimately expressed concerns with the presence of distinct structural characteristics of mature platelets—in buds². Italiano et al. have proposed that buds may not be platelet precursors but rather MK-derived microvesicles (MVs), which are free of organelles, platelet granules, and microtubule coils, based on transmission electron microscopy of bone marrow MKs.

While platelet generation from proplatelets has been extensively researched for decades, this mechanism of MK budding has not been widely recognized. One of the main reasons for this is that buds are very difficult to visualize microscopically. Additionally, buds can only truly be appreciated using 3D imaging modalities as the examination of their full structure is necessary to ensure that these structures are truly platelet precursors. Until recently, most studies have relied on 2D evaluation of MKs. We therefore examined C57BL/6 bone marrow cryosections using both super-resolution confocal and 3D stimulated emission depletion (3D STED) microscopy. We chose to use STED microscopy to provide sufficient resolution to evaluate subcellular structures of MKs and platelets. Our studies have confirmed the existence of MK membrane buds and demonstrated that they contain ultrastructural features consistent with those of platelets. They highlight the importance of using multiple platelet markers and imaging modalities to classify the diverse membrane structures derived from MKs.

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POSTERS

An Optimised Protocol for Collagen Quantification in the Metastatic Mouse Lung

Alisha Dabb^{1,2}, Alfonso Schmidt^{1,2}, Regan Fu², and Kylie Price^{1,2}.

¹ Hugh Green Cytometry Centre at the Malaghan Institute of Medical Research, Wellington, NZ

² Malaghan Institute of Medical Research, Wellington, NZ.

Type I and III collagen deposition within the metastatic lung are common complications of cancer and metastasis, often exacerbated by treatment with chemotherapies. Collagen deposition and subsequent fibrosis formation within the lung has been consistently associated with chronic lung injury and metastasis relapse, and has been identified as a novel predictive biomarker for poor cancer prognosis. The quantification of collagen in metastatic lung tissue is therefore key to understanding disease progression and treatment efficacy.

Current gold-standard collagen quantification assays are unsuitable for lung tissue due to the inability to decipher conflating tracheal collagen, and the loss of structural and spatial data due to sample destruction prevents further analysis. While histological staining overcomes these issues, it is easily biased by region of interest (ROI) selection during imaging, and by the subjective and often manual assessment of collagen staining during analysis. Similarly, the lack of consistency in staining quantification methods prevents replicability between experiments. Here, we aimed to develop a semi-automated method for collagen quantification in metastatic mouse lung tissue. Briefly, 24 whole C57BL/6 mouse lung sections with MB49 bladder cancer metastasis were stained in picosirius red collagen stain. Whole-lung imaging was performed under brightfield and circular polarized light, and whole-image analysis was conducted using the freely available QuPath¹ analysis software following the pipeline outlined in **Figure 1**.

Imaging of whole-lung sections under both brightfield and polarised light eliminated the limitation of subjective ROI selection, and allowed accurate identification and exclusion of tracheal collagen within each section. Similarly, the implementation of a machine-learning based QuPath analysis pipeline trained using randomly selected regions from each sample removed positive collagen detection bias. The present protocol therefore presents a novel, accessible, and robust method for quantifying collagen in metastatic whole lung samples.

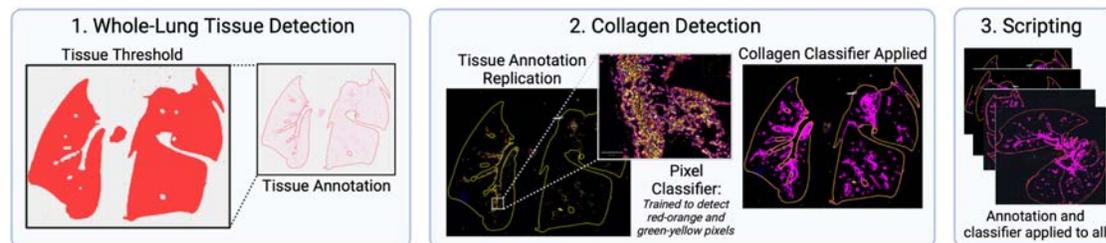


Figure 1. QuPath collagen analysis pipeline. Brightfield images were used to generate tissue masks. A pixel classifier was trained with randomly selected 2 mm² regions from each image to objectively detect collagen (red-orange and yellow-green birefringence). The algorithm was applied to all polarized images, and the collagen % (of total lung area) was measured.

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Application of diffusible iodine-based contrast-enhanced computed tomography (diceCT) to study the development of the zika virus infected mouse brain

Amy L. Green¹, Evangeline Cowell¹ and Jillian M. Carr¹.

¹ Department of Microbiology and Infectious Diseases, College of Medicine and Public Health, and Flinders Health and Medical Research Institute (FHMRI) Flinders University, Adelaide, South Australia

Diffusible iodine-based contrast-enhanced computed tomography (diceCT) refers to the process of enhancing contrast of a specimen's soft tissues via submersion or injection of an iodine-based solution prior to CT scanning (Metscher, 2009; Gignac and Kley, 2014). DiceCT allows for the acquisition of high-contrast soft tissue scans and three-dimensional (3D) visualisation and analysis of anatomical structures at relatively lower overall cost and efficiency in comparison to methods such as magnetic resonance imaging (MRI) or light-sheet microscopy. Here we have used diceCT to study the impact of zika virus (ZIKV) on development of the brain.

Zika virus (ZIKV) is a positive-sense single-stranded RNA virus in the Flaviviridae family. Vertical transmission of the virus, i.e. from mother to child in utero, has shown a multitude of adverse consequences in the developing foetus, including but not limited to effects in the brain, including microcephaly, and eyes, and termed congenital zika syndrome (CZS) (Freitas et al. 2020). Whilst animal models including mice, have defined the biology of ZIKV infection of the brain such as regions of infection and loss of neurons, we aimed to link molecular changes with gross morphological changes in the 3-dimensional (3D) structure of the ZIKV-affected brain.

1-day post-natal mice were infected with ZIKV. At day 3 and 6 – time points where we know the virus is replicating and an innate immune response is induced, and 10 post-infection (pi) – where the mice begin to show signs of neurological deficits, whole heads were subjected to iodine staining and microCT analysis (diceCT). Scans were reconstructed and resulting images segmented and analysed using the free, open-source program 3D Slicer (Fedorov *et al.* 2012). Qualitative analysis demonstrated clear visualisation of regions of the developing brain with diverse defects visualised in the ZIKV-infected brain at day 6 pi. Quantitative analysis of segmented brain regions demonstrated an ability to quantify the olfactory bulb, hippocampus, cerebrum, cerebellum, midbrain, and hindbrain, with increased brain volume over developmental time. Further, the brains of ZIKV-infected mice were clearly smaller in numerous regions at day 10 pi, but specifically in the cerebrum at day 6 pi. No significant differences were observed at day 3 pi.

Thus, we have developed a system to quantify brain region volume from CT scans of the developing mouse using free, open-source programs and applied this to study the impact of ZIKV on the developing brain. Currently we are assessing the ability to extend this to analyse the retina and if tissue can be utilised for histological and immunofluorescent staining post diceCT analysis.

Updating a 19th century staining method to optimize imaging of infection threads in *Medicago truncatula*

Angus Rae¹, Ulrike Mathesius², David Collings², David McCurdy³, Daryl Webb¹

¹ Centre for Advanced Microscopy, Australian National University

² Research School of Biology, Australian National University

³ School of Environmental and Life Sciences, University of Newcastle

Infection threads are tubes of plant cell wall that legume root cells produce to transport symbiotic nitrogen fixing rhizobacteria into growing root nodule organs. Infection threads as complex structures often deep within root tissue have been historically difficult to image, and a solution to this has been whole tissue clearing, coupled with fluorescent cell wall staining and confocal microscopy.

Plant cell walls are often fluorescently labelled for confocal imaging with the dye propidium iodide, using a periodic acid-Schiff (PAS) reaction that was originally developed in 1866 using the Schiff reagent, basic fuchsin. This reaction binds free amine groups on dye molecules to aldehyde groups on carbohydrates such as cellulose that result from oxidation with periodic acid. Although effective, propidium iodide as a pseudo-Schiff reagent is suboptimal due to its hazardous nature; particularly given that any dye with free amine groups should behave as a pseudo-Schiff reagent, meaning many more alternatives should be available. To identify preferable alternatives, we tested a range of applicable fluorescent dyes for their ability to act as pseudo-Schiff reagents. Using the low-pH Schiff solution historically used for the Schiff reaction, these alternative dyes failed to label cell walls as strongly as propidium iodide; but, replacing the acidic Schiff solution with water greatly improved fluorescence labelling. Under these conditions, rhodamine-123 provided improved staining of plant cell walls compared to propidium iodide.

Utilising rhodamine-123 as a new pseudo-Schiff reagent, coupled with another cell wall dye, calcofluor white, allowed stable and semi-specific labelling of infection threads within whole cleared roots of the model legume *Medicago truncatula*. Confocal imaging of samples stained in this way has made possible accurate quantification of infection thread numbers per cell, and detailed analysis of infection thread phenotypes through volume imaging and 3D projection.

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Investigation of TRIM55-associated hypertrophic cardiomyopathy in hiPSC-CMs

Antonia T. L. Zech^{1,2,3,4}, James W McNamara^{1,2,3,5,6}, Hayley L. Pointer^{1,2,4}, Rachel Morris^{1,2,4}, Ellen Keen^{1,2,4}, Benjamin L. Parker^{5,6}, Enzo R. Porrello^{1,2,3,5} & David A. Elliott^{1,2,3,4}

¹Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Victoria, Australia

²Melbourne Centre for Cardiovascular Genomics and Regenerative Medicine, Royal Children's Hospital, Melbourne, Victoria, Australia

³ School of Biomedical Sciences and Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

⁴Novo Nordisk Foundation Centre for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Melbourne, Victoria, Australia

⁵ Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia

⁶ Centre for Muscle Research, University of Melbourne, Melbourne, Victoria, Australia

Hypertrophic cardiomyopathy (HCM) is the most common human inherited cardiomyopathy with no curative treatment available. HCM is commonly associated with variants in genes encoding sarcomeric proteins, either altering protein function or leading to accumulation of aggregates and/or toxic proteins. Thus, cellular homeostasis is compromised, highlighting the crucial role of the protein degradation machinery in the disease development and progression of HCM. The ubiquitin-proteasome system (UPS) is the major proteolytic system within cells, that has been shown to be altered in HCM. Next to an impaired protein quality control, also impaired contractility and the disarray of the cardiac sarcomere are known disease hallmarks of HCM. The cardiac sarcomere is the smallest functional unit of heart muscle cells, and its formation requires coordinated assembly and disassembly of multiple sarcomeric proteins and is mandatory for proper cardiac function.

HCM has also been associated with variants in non-sarcomeric genes including in TRIM55, an E3 ubiquitin ligase, that is encoded by Tripartite motif-containing protein 55. TRIM55 is a crucial part of the UPS, as it exerts the transfer of ubiquitin to the degradation-targeted protein, thereby ensuring the proper removal of short-lived or misfolded proteins. Further, TRIM55 has been shown to localize to the cardiac sarcomere, to interact with crucial sarcomeric proteins and it is assumed to play a regulatory role in sarcomere formation. Furthermore, overexpression studies suggested that *TRIM55* variants reduce contractility and may lead to heart failure.

To date, various *TRIM55* variants have been associated with HCM but the underlying disease mechanism is still unknown. To unravel how *TRIM55* variants lead to development and progression of HCM, I have generated a *TRIM55* Knockout (KO) hiPSC line by using CRISPR/Cas9 gene editing. Our preliminary data revealed an HCM-like phenotype in 3D cardiac organoids with a higher force, lower beating rate, a longer activation and relaxation time for *TRIM55_KO* compared to healthy control organoids, indicating an alteration of contractile function. We are performing high-content image-based analysis to unravel disease hallmarks of *TRIM55* related HCM by confocal imaging, including cell size, sarcomere organisation and aggregation of sarcomeric proteins. Further, we are generating a suite of cell lines that help us to study patient-derived *TRIM55* variants, as well as to perform live-cell imaging of TRIM55 and the turnover of the cardiac sarcomere. The project goal is to better understand and visualise the role of TRIM55 in cardiac function and sarcomere turnover, ultimately aiming to develop of novel therapeutic approaches.

FILAMENTOUS ACTIN FACILITATES AKT RELEASE FROM PROMYELOCYTIC LEUKEMIA NUCLEAR BODIES TO PROMOTE DNA REPAIR AND SURVIVAL IN RESPONSE TO REPLICATION STRESS

Blake Lane¹, Vicki Willett¹, Hannah W. Loh^{1,2}, Christopher B. Nelson³, Maté Biro², Anthony J. Cesare⁴ and Noa Lamm⁵

¹ Nuclear Dynamics Group, Children's Medical Research Institute, University of Sydney, Westmead, New South Wales 2145, Australia.

² EMBL Australia, Single Molecule Science Node, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia.

³ Telomere Length Regulation Unit, Children's Medical Research Institute, University of Sydney, Westmead, New South Wales 2145, Australia.

⁴ Genome Integrity Unit, Children's Medical Research Institute, University of Sydney, Westmead, New South Wales 2145, Australia.

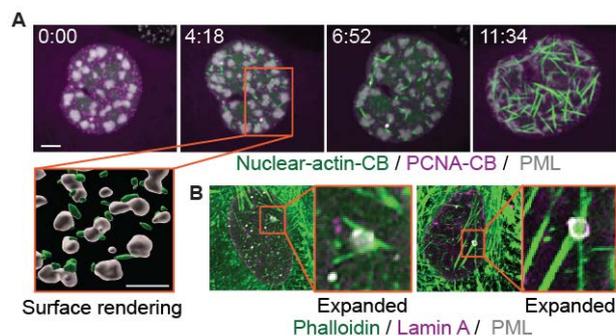
⁵ Nuclear Dynamics Group, Children's Medical Research Institute, University of Sydney, Westmead, New South Wales 2145, Australia. Correspondence: nlamm-shalem@cmri.org.au

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DNA replication stress triggers reorganization of the nuclear environment, leading to alterations in nuclear morphology and DNA dynamics. A compelling emerging factor behind these adaptive changes prompted by stress is the presence of nuclear filamentous actin (F-actin). This dynamic F-actin network plays a pivotal role in orchestrating architectural modifications within the nucleus while also facilitating enhanced DNA mobility to expedite the repair of DNA lesions and replication fork impediments. Notably, these functions of nuclear F-actin bestow upon cancer cells a resistance against replication stress-inducing chemotherapies. Consequently, understanding the mechanisms through which nuclear F-actin instigates nuclear reorganization is of paramount clinical significance.

We found that Promyelocytic leukemia nuclear bodies (PML-NBs) act as nucleation hubs, initiating the formation of nuclear F-actin in response to replication stress. As these actin filaments grow, they exert mechanical pressure on the PML-NBs, thereby inducing their shape and composition alterations. Crucially, we have uncovered that this F-actin-driven structural transformation results in the release of the AKT oncogene from its sequestration within PML-NBs, leading to its phosphorylation and subsequent activation. Collectively, our findings converge to delineate a novel F-actin/PML-NBs/AKT pathway emerging in response to replication stress, influencing DNA repair choices, and promoting cell survival.



Nuclear F-actin initiates from- and associates with PML NBs in response to replication stress. A) Nuclear F-actin polymerises from PML-NBs in APH treated cell cultures. Surface rendering of nuclear F-actin extending from PML-NBs is shown. B) Super-resolution images of endogenous nuclear F-actin (Phalloidin) and PML (immunofluorescence) co-localising in APH treated cells. Scale bar = 5µm, time (hr:min).

Studying the immune microenvironment in lung cancer patients using spatial multi-omic approaches

Claire Marceaux^{1,2,4}, Kenta Yokote¹, Velimir Gayevskiy¹, Daniel Batey¹, Lucy Riley¹, Laurie Choux¹, Nina Tubau Ribera², Kelly Rogers^{2,4}, Michael Christie⁷, Phillip Antippa^{6,7}, Terence P Speed^{3,5}, Belinda Phipson^{3,5}, and Marie-Liesse Asselin-Labat^{1,4*}.¹

¹Personalised Oncology Division, ²Advanced Technology and Biology Division, ³Bioinformatics Division; The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

⁴Department of Medical Biology, ⁵School of Mathematics and Statistics, ⁶Department of Surgery, The University of Melbourne, Parkville, Australia

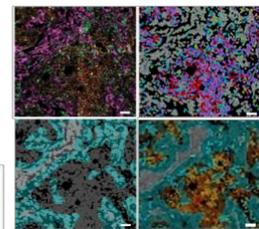
⁷The Royal Melbourne Hospital, Parkville, Australia

Lung cancer is the leading cause of cancer death in the world. Currently, surgical resection is the most suitable treatment for patients with early-stage non-small cell lung cancer (NSCLC). However, recurrence is very frequent. Tumour immune infiltration has been correlated with patient outcome; therefore, a better understanding of the tumour immune microenvironment would provide critical information for the development of novel therapeutic approaches.

We aim to correlate spatial characteristics of the immune microenvironment with patient outcome and study how the microenvironment influences the evolution of the disease to identify new predictive biomarkers and propose therapeutic strategies for NSCLC.

This project is using emerging methodologies on paraffin-embedded tissue sections: the MIBIScope that enables multiplex imaging at single cell resolution using heavy metal-conjugated antibodies bound to the tissue and detected by mass spectrometry-time-of-flight; and the GeoMX that allows quantification of the entire transcriptome in selected areas of interest for in situ spatial transcriptomic analysis.

tissue imaging and single-cell spatial analysis with MIBI



We have developed a 38-antibody panel to study the immune cell infiltration with MIBI. We have performed spatial proteomics and transcriptomics analysis of 94 tissues of NSCLC patients for which we possess survival and smoking status. Our first results confirm the importance of the spatial context with specific lymphoid cells infiltrating the tumour of patients with long survival post-surgery (>6yrs) and specific myeloid cells inside the tumour of short survival post-surgery patients (<3yrs). We are also exploring how transcriptional differences between the survival outcomes can relate to the immune infiltrate obtained from the spatial proteomic analysis.

Combined, these technologies will provide in depth analysis of the activation status of immune cell types and their local impact on tumour cell characteristics and evolution.

Quantifying the morphology of gangue minerals with advanced SEM technique: A talc case study

Daniel Dodoo^{1,2}, Nathan A.S. Webster^{2,3}, Matthew P. Glenn³, Nick Owen³, Liza Forbes^{2,4},
Shane P. Usher^{1,2}, Peter J. Scales^{1,2}, Anthony D. Stickland^{1,2}

1. Department of Chemical Engineering, The University of Melbourne, Victoria 3010, Australia
2. ARC Centre of Excellence on Eco-Efficient Beneficiation of Minerals, Australia
3. CSIRO Mineral Resources, Victoria 3168, Australia
4. Julius Kruttschnitt Mineral Research Centre, Sustainable Minerals Institute, The University of Queensland, Queensland, Australia

Particle morphology can have a significant impact on many mining operation processes. Plate-like shapes are often found in gangue clay minerals like mica, talc, kaolin, and chlorite, and their platy morphology significantly impacts the recovery of high-grade minerals as well as the management of tailings [1]. Talc is a highly problematic clay since its platy morphology imparts a certain level of hydrophobicity. The face surfaces account for about 90% of its total surface area, meaning talc can be easily floated, which decreases concentrate grade and increases the energy required for smelting. Hence, quantifying the aspect ratio or platiness of talc in feed, product, and waste streams of key mineral processing operations is crucial to understand its impacts and designing equipment and circuits to optimize recovery of valuable minerals. Several attempts have been made to quantify the platy-shape of gangue minerals, but talc research is limited, and most studies have been done on pure talc. This is due in part to the fact that talc in a mineral ore body is rarely pure in nature consisting of other minerals, making it difficult to characterise and quantify its platy shape.

This study, use advance scanning electron microscopy (SEM) technique to quantify the platy morphology of talc in a real ore obtained from different geological locations. New sample preparation techniques were developed that involved suspending and orienting talc suspensions for easy and accurate quantification of their platy shape before SEM characterisation. Advanced image analysis with ilastik and FIJI software enabled quantitative results from the SEM. In all, the SEM quantification was successful and showed that the particle morphology of talc ranged from platy to blocky, depending on the geological location of the talc. Researchers interested in particle morphology can benefit from this technique because it can be extended to measure colloidal particle shapes, including disc-shaped, flaky, and platy particles.

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***In situ* multi-element analysis of splenocytes using laser ablation imaging mass spectrometry**

Darryl Johnson¹, Lukas Schlatt², and Phil Shaw².

¹ Materials Characterisation & Fabrication Platform, The University of Melbourne

² Nu Instruments, Wrexham, UK

The regulation, or dysregulation, of metals and other elements has important implications for health and disease. Mass cytometry (MC) and Imaging Mass Cytometry/Spectrometry has been widely used to investigate complex population heterogeneity owing to its ability to detect up to 40 different parameters using isotope labelled reagents. However, current technologies are limited in their analysis endogenous biologically relevant elements. The aim of this work was to utilise the sensitivity and full mass range isotopic detection of a novel TOF-ICP-MS coupled with laser ablation to determine the elemental content within various splenocyte populations and image these cells *in situ*.

Both suspension of single cells and tissues sections from mouse spleens were stained with various population defining metal labelled markers as per normal MC protocols. Individual splenocytes were introduced into the Vitesse ToF-ICP-MS to detect the various markers along with other biologically relevant elements. A range of major immune cell types from red and white pulp of mouse spleen could be determined based on their expression of various cellular markers. Most subsets contained similar levels of K, P and Ca. However, analysis indicated a clear difference in the Fe content of cells primarily located in the two major histological zones of the spleen. Neutrophils and macrophages, cell types that both reside in the Fe rich red pulp, contained a greater amount of Fe than the white pulp resident T and B cells. Analysis of the elemental distribution within the tissue *in situ* with laser ablation not only confirmed the Fe content of these Macrophages, but also their specific location within the splenic red pulp. Given a major function of red pulp macrophages is to clear expended eosinophils from circulation, it is not unexpected that these cells would contain a higher level of Fe. Unexpectedly though, the red pulp macrophages were found to contain less Mg, Ca, Mn, Ni, Zn, and Sr than other splenocytes.

These data indicated hereto unknown elemental composition of splenocytes. This highlights the ability of Vitesse TOF-ICP-MS to analyse cellular heterogeneity and metallomics at the single cell and tissue level and opens intriguing new avenues of research.

Can you SRRF on an ONI?

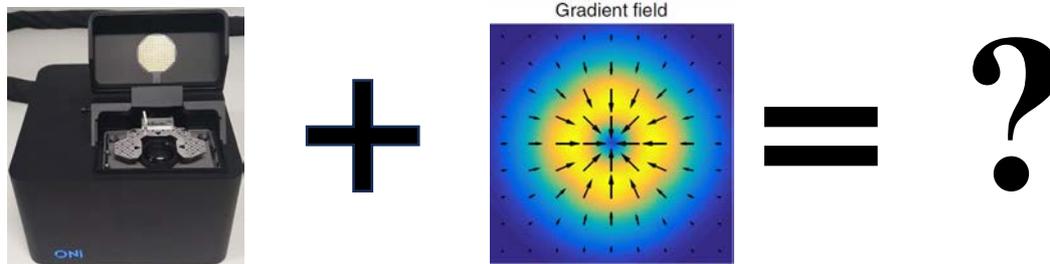
Daryl Webb and Angus Rae.

Centre for Advanced Microscopy, The Australian National University, 131 Garran Road, Acton, ACT 2601

The ONI Nanoimager is marketed as the world's first desktop super resolution microscope, offering multi-channel Epifluorescence, dSTORM, PALM, PAINT, SPT, and smFRET¹. Illumination angles can be adjusted incrementally from 0° Epi, through HiLo and ultimately TIRF. Additional imaging modalities include RGB circular oblique brightfield and SIM, through the use of a DMD device. Couple that with high performance online data analysis and file sharing via CODI and it is no wonder that they are becoming popular with Australian researchers.² All of this and up to 100 full frames per second.

100fps... Super-resolution radial fluctuation (SRRF) microscopy requires around 100 frames to generate a super-resolution image with high density labelling, more with low signal. A two-to-six-fold improvement in resolution, free ImageJ plugin³. A new and improved eSRRF algorithm promising “user friendly” live cell super resolution⁴. I wonder...

Can you really use an ONI Nanoimager for SRRFing, or is that just a pipe dream?



Here we aim to provide a definitive answer to that pressing question, expand on the acronyms, drop into the pipeline, and offer tips and tricks for the Groms.⁵

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5. shortened form of Grommet; a young or inexperienced SRRFer

Australian Community for Intravital Microscopy

Deborah Barkauskas¹, Angela Fontaine¹, Astrid Magenau², Paul Timpson², Tri Phan³.

¹ INCITe Centre, Garvan Institute of Medical Research

² Tumour Ecosystems, Garvan Institute of Medical Research

³ Precision Immunology, Garvan Institute of Medical Research

Intravital imaging generates real-time insights on cellular dynamics and interactions, and it enables researchers to interrogate biological processes within physiological context. Traditional imaging often involves fixed tissues or cell cultures, whereas intravital imaging requires surgical techniques to expose the organ of interest. For example, acute imaging of lymph nodes or longitudinal animal studies (by way of abdominal or trans-cranial window implants) are possible. Although widefield and confocal microscopes are compatible, intravital imaging researchers now employ two- or three-photon excitation to explore biological processes at depth.

The INCITe Centre at the Garvan Institute of Medical Research was funded by the Australian Cancer Research Foundation to democratize deep tissue imaging by developing new technologies and techniques and supporting an Australian community. Scientists are continually working to enhance the techniques and address existing challenges. This requires interdisciplinary collaboration among physicists, engineers, biologists, and computational experts to acquire data. In addition, extracting ground-breaking insights from dynamic imaging needs sophisticated computational analysis. With the support of Light Microscopy Australia, there are plans to provide accessible resources to the Australian community from animal ethics to surgical protocols, to microscope development, to image analysis tools. As technology continues to advance, intravital imaging will become more accessible for answering critical biological questions.

Single-molecule super-resolution and particle tracking reveal the role of lipid droplets in the innate immune response.

Abbey J Milligan¹, Ashley M Rozario¹, Ebony A Monson², Jay L Laws², Karla J Helbig² and Donna R Whelan¹

¹ La Trobe Institute for Molecular Science, La Trobe University, Bendigo, Victoria, 3552

² La Trobe School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, Victoria, 3086

Lipid droplets (LDs) are cellular organelles comprising a phospholipid monolayer and a large neutral lipid core. Ubiquitous across cell types, these structures have historically been considered principally involved in energy storage and metabolism. More recently, potential signaling and trafficking roles for LDs have been identified, including in pathogen response pathways¹. We have deployed fluorescence microscopy approaches to probe the role of LDs in the innate immune response. We were able to show that LDs increased in size and number across several cell types in both cell culture and animal models in response to viral infection, as well as direct interferon stimulation². Using particle tracking, we next demonstrated that LDs move further and faster during the innate immune response. Importantly, the ability to clear the virus was downstream of the LDs' ability to increase in number, size, and motility³.

To further investigate the mechanism by which LDs protect against viral infection, we developed a super-resolution assay for visualizing LDs using the dye BODIPY 493/503 and antibody-labelled proteins of interest. Namely, we have investigated LD resident proteins and key STAT (signal transducer and activator of transcription) proteins. Activation of STATs via phosphorylation is crucial for expression of the interferon stimulated genes that enable viral clearance. However, the mechanism by which these modifications are achieved within the cell in a spatially and temporally controlled manner, remains poorly understood. With single-molecule visualisation of STATs and LDs, we have uncovered a significant increase in colocalization upon viral infection, pointing towards a novel anti-viral signalling role for LDs.

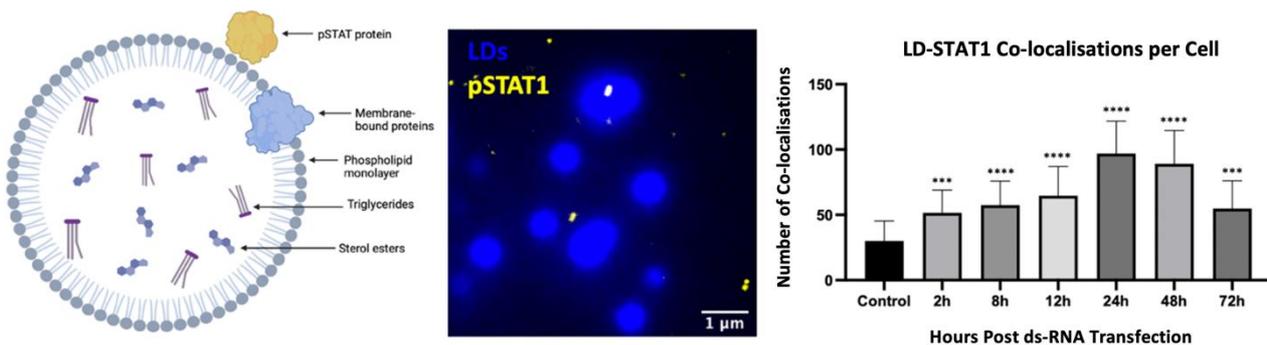


Figure 1. (Left) Schematic of a lipid droplet (LD). (Middle) Two-colour fluorescence image of LDs labelled with BODIPY imaged with conventional widefield overlaid with immunolabelled pSTAT1 imaged using single molecule super-resolution. (Right) Quantification of LD-STAT1 colocalizations during a time course following transfection of cells with a viral RNA mimic.

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Versatile and bespoke bioimage analysis support in a core facility

Ellie Cho¹ and Paul McMillan¹

¹ Biological Optical Microscopy Platform, The University of Melbourne, Parkville, Australia

Biological image analysis is one of the rapidly growing fields of science. The analysis approaches are highly complicated and diverse that there are currently more than 1300 types of software/workflows available¹.

At the Biological Optical Microscopy Platform (BOMP) at the University of Melbourne, we provide expert advice in data analyses to researchers from multiple fields including biomedical and biomedical engineering research, including immunology, ophthalmology, otolaryngology, neurobiology, muscle physiology, environmental microbiology, molecular biology, regenerative medicine, parasitology, cancer cell biology.

We provide one-on-one consultations on image analysis to more than 100 researchers and provide more than 10 bioimage analysis workshops annually. Consultations are designed to, where possible, help direct researchers to perform their own analysis, but more complicated cases develop into formal collaborations. Here we show examples of collaborative image analysis projects to highlight the versatility of the analysis support available at BOMP.

The common analysis workflow includes the measurement of fluorescence intensity², morphology (e.g. diameter, length, thickness³), co-occurrence (e.g. counting positivity of multiple staining^{4,5}, colocalisation coefficient^{6,7}), spatial relationship⁸ in 2D and 3D images as well as in time lapse. These measurements can be performed in open-source applications such as FIJI/ImageJ, Cell Profiler, QuPath or licensed applications such as Imaris, Volocity, Amira that are available at BOMP. In addition, we provide support for pre-processing the raw microscopy images which is the first step of bioimage analysis in most cases. This may include restructuring of file type, enhancing the signal-to-noise ratio using noise reducing filters or background correction, or improving resolution with deconvolution using licensed software such as Huygens. Lastly, most of analysis can be performed as automated or semi-automated manner for high- throughput and unbiased quantification.

Our research support on bioimage analysis is highly versatile, so it has been applied to the multiple fields of biomedical and biomedical engineering research, including immunology, ophthalmology, otolaryngology, neurobiology, muscle physiology, environmental microbiology, molecular biology, regenerative medicine, parasitology, cancer cell biology. Our support is open to all researchers and therefore provides great benefits to academic community.

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High throughput screening to identify compounds for heart regeneration

Frankie Butera¹, Rachel Morris¹, Henry Beetham¹, Alejandro Hidalgo-Gonzalez¹, Kevin Watt¹, David Elliott¹ & Enzo Porrello¹.

¹ Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Victoria, Australia

Dilated cardiomyopathy (DCM) involves enlargement of one/both heart ventricles and is associated with loss of cardiomyocytes (heart muscle cells). DCM is a life-threatening disease due to inability of the heart to repair and limited proliferative capacity of postnatal cardiomyocytes. Therefore, we aim to identify compounds that enhance cardiomyocyte proliferation as a potential treatment for DCM.

Using a 15 day differentiation protocol, iPSCs can form 2D heart models with a mix of beating cardiomyocytes, fibroblasts and endothelial cells. We screened the response to >700 compounds of differentiated iPSCs stemming from a DCM patient, staining for a cardiomyocyte marker and a cell cycle marker by immunofluorescence. We imaged cells using high throughput confocal microscopy and analysed images using CellPathFinder software to identify compounds enhancing the proportion of post-G1 cardiomyocytes (Fig 1A-C).

Currently, we are validating hits from the primary screen by using timelapse microscopy and automated image analysis to assess cell cycle and cell number changes with each compound, using fluorescently endogenously tagged PCNA as a cell cycle and nuclear marker (Fig 1D).

Overall, our data show that iPSC-derived 2D heart models can be used for high throughput screening with high reproducibility. Moreover, we have developed experimental and image analysis pipelines that enable assessment of drug-induced cell cycle changes in heart cells.

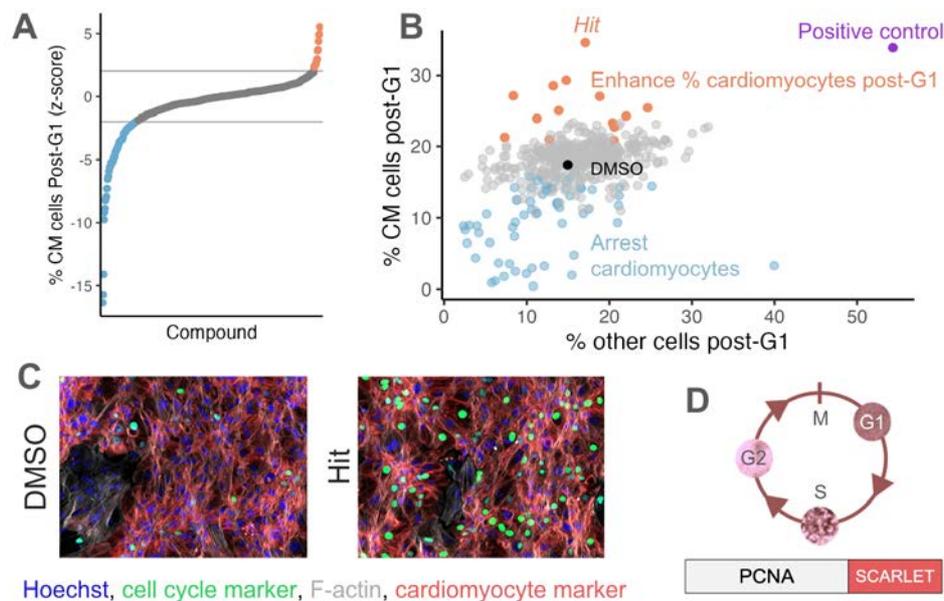


Figure 1: (A) Compounds ranked by % cardiomyocytes post-G1. (B) % cardiomyocytes post-G1 against % other cell types post-G1 per compound. Compounds in orange increased % post-G1 cardiomyocytes without enhancing the post-G1 population of other cell types (including fibroblasts). (C) Immunofluorescence stains to quantify cell cycle progression of cardiomyocytes versus other heart cells. (D) iPSCs with PCNA-SCARLET are used to enable cell cycle tracking of differentiated heart cells. In the nucleus, PCNA has uniform low intensity in G1, foci during S phase, and uniform high intensity in G2.

The caveats of sample mounting for lightsheet comparing Ultramicroscope II and Ultramicroscope Blaze

Gabriela Segal¹ and Paul McMillan¹

¹ *Biological optical microscopy platform (BOMP), School of Biomedical Sciences, Faculty of Medicine, Dentistry & Health Sciences, The University of Melbourne*

Lightsheet microscopy has become a key technology for investigating biological process with spatial resolution. There has been a lot of developments on the field with lots of commercially available system. The Ultramicroscope originally designed by Richard Zsigmondy has evolved and been redesign into a user friendly, low bleaching technique that can accommodate large samples and image them at cellular resolution. This abstract explores the crucial aspect of sample mounting within the context of the Ultramicroscope II system and compares to the newer model Ultramicroscope Blaze.

One of the most important considerations for lightsheet microscopy is sample mounting, this is a critical step that directly impacts the imaging quality and experimental results. A few factors that are critical in lightsheet sample mounting are the orientation respect to the lightsheet and the camera, stability of the sample and possible vibrations.

The Ultramicroscopes presents both opportunities and challenges for sample mounting as its architecture allows for re-designs and 3D printing holders. Researchers can exploit various mounting approaches tailored to different specimen types, such as tissue slices, organoids, and cleared whole organs. This abstract delves into the methodologies and adaptations required for effective sample mounting, addressing issues such as potential motion artifacts, light penetration, distortion, and deformation.

In conclusion, this abstract underscore the pivotal role of sample mounting in maximizing the capabilities of the Ultramicroscope II and compares with the capabilities of Ultramicroscope Blaze. This work will provide guidelines for users interested in different scale imaging and speed alternative to other microscopy techniques and will help in decision making for light sheet microscopy users.

Feedback and Crosstalk in Signal Transduction: Insights into Akt/PI3K Signaling

Jonathan Scavuzzo¹, Dougall Norris¹, Alison Kearney¹, Sean Humphrey¹, David James¹ and James Burchfield¹.

¹ School of Life and Environmental Sciences, The University of Sydney, NSW, Australia

Insulin orchestrates key cellular processes via the activation of numerous signalling pathways, including Akt (Protein Kinase B) and ERK (Extracellular-signal Regulated Kinase). While individual network components are well-understood, the complex interconnectivity shaped by feedback and crosstalk between distinct pathways is less established. We have recently described novel Akt-mediated negative feedback onto IRS1 and 2, which limits both the production of PIP3 (Phosphatidylinositol (3,4,5)-trisphosphate) synthesis by PI3K (Phosphoinositide 3-Kinase) and downstream signalling such as Akt directed phosphorylation. Given the role of PIP3 as a potent signalling molecule, we hypothesized that disrupting this feedback would lead to aberrant signal transduction and the identification of novel, non-canonical regulatory mechanisms within the insulin signalling nexus.

Here, using Mass Spectrometry-based phosphoproteomics and live-cell imaging, we demonstrate that acute loss of Akt feedback causes PI3K-dependent ERK hyperactivation, emphasizing the importance of feedback and crosstalk in shaping the signalling landscape. We validate this finding by demonstrating a sustained and robust potentiation of the MEK/ERK/RSK pathway with Akt inhibition in the presence of a physiological dose of insulin. Further, we leverage Immunoprecipitation-Mass Spectrometry (IP-MS) and Total Internal Reflection Fluorescence Live-Cell Imaging (TIRFM) to reveal extensive Akt feedback onto upstream adaptors, such as SHP2, Grb2, and Gab2, consistent with a major role for adaptors as hubs for signal integration and pathway regulation.

This research highlights a novel mechanism of crosstalk between two strongly defined and highly studied signalling pathways. Due to the numerous pathologies associated with the hyperactivation of the ERK signalling pathway, these data additionally suggest an aberrant signalling phenotype within the context of diminished Akt activity, lending to the studies potential clinical relevance. Additionally, this study sheds light on the regulatory landscape sculpted by Akt feedback within insulin signalling and provides valuable insights into inhibitor directed network rewiring and a mechanistic basis for the development of targeted therapies in diabetes and cancer.

Pair correlation microscopy of intracellular protein transport

Julissa Sanchez-Velasquez¹, Ashleigh Solano¹, Michelle A. Digman², Enrico Gratton², Francesco Cardarelli³, Elizabeth Hinde¹.

¹ School of Physics, Faculty of Science, University of Melbourne, Melbourne, Australia, 3010.

² Laboratory of Fluorescence Dynamics, The Henry Samueli School of Engineering, University of California, Irvine, CA, USA

³ Scuola Normale Superiore, Laboratorio NEST, Piazza San Silvestro 12, 56127 Pisa, Italy.

The intricate functionalities of a cell rely on the dynamic interplay and transport of biomolecules. Employing minimally invasive techniques is essential for exploring the underlying molecular processes within the native cellular environment. Among them, pair correlation microscopy, centered around a spatial pair correlation function (pCF), has the unique capacity to quantitatively map the diffusive route a population of fluorescent proteins adopts with respect to live cell architecture with single-molecule resolution. Yet, a broader use of this method is inherently limited by the complexity of data analysis. Here, we provide a protocol for pair correlation microscopy that details an accurate, step-by-step workflow for performing and analyzing the pCF. We show how to quantitatively analyze the corresponding pCF data to characterize the directionality of intracellular transport, such as nucleocytoplasmic transport, and the diffusion law of a protein's trafficking event, such as DNA target search. This protocol also shows for the first time, to our knowledge, a strategy to fit pCF profiles in a model-free manner, further minimizing the complexity of data analysis. The intent is that our protocol simplifies the analysis and guides the interpretation of pCF-derived data. Moreover, from application of this protocol to the study of nucleocytoplasmic shuttling, we show how pair correlation microscopy can be used as a tool to decipher how the selective filter inside the nuclear pore complex (NPC) relates to function and gives rise to a transport mechanism. By providing spatiotemporal information of single pore components, we propose that the molecular transport through the NPC can be powered, at least in part, by the interaction of the transported molecules with specific nucleoporins. We anticipate that the protocol presented here will make the pCF method more widely accessible to help increase our understanding of how intracellular diffusion regulates biological function at the single-molecule level.

Semi-Automated Confocal Laser Scanning Approach For Phenotypic Screening

Kalyan Shobhana^{1*}, Jenna Hall², Grace E. Lidgerwood², Alice Pébay^{2,3,4} and Ellie Cho¹.

¹. Biological Optical Microscopy Platform, University of Melbourne, Parkville, Australia.

². Department of Anatomy and Physiology, The University of Melbourne, VIC, Australia

³. Phenomics Australia, The University of Melbourne, VIC, Australia

⁴. Department of Surgery, Royal Melbourne Hospital, The University of Melbourne, VIC, Australia

Laser scanning confocal microscopy stands out from traditional widefield microscopy by offering superior optical sectioning properties, enabling enhanced 3D imaging with improved lateral and axial resolution. Despite its widespread popularity, its adoption for screening is often hindered by the slow acquisition speed.

In our research, we devised a semi-automated method for screening and quantifying various retinal pigment epithelial (RPE) cell lines, derived from induced pluripotent stem cells (iPSCs) sourced from patients with Age-Related Macular Degeneration (AMD) and age-matched controls. Employing an inverted LSM900 point scanning confocal system, we captured images and Z-stacks for a 96-well Cell Carrier Ultra Plate (Perkin Elmer #6055300) using a 20x/0.8 NA Air Objective. The Zen Blue 3.2 software facilitated the setup of the semi-automated acquisition.

Upon instrument initialization, the plate is calibrated in the software and three random points for unbiased imaging are distributed across each well. During the study, the thickness of the samples varied significantly between cell lines, prompting the establishment of a large Z stack based on manual verification to capture the phenotype of interest across all samples. Although the manual setup could consume 1-2 hours, the saved coordinates (x, y, and z) in the metadata allowed for subsequent use in longitudinal studies. Once the imaging parameters were confirmed, the stage automatically acquired the Z stack for multiple positions. A scan of 16 wells with three channels and a thickness range of 60 μm required approximately 8-9 hours, making it feasible to run overnight.

This workflow enhanced data collection efficiency by eliminating the need for manual well-by-well scanning. The outcome was a consolidated file in .czi format, annotated for different wells and positions, which could be exported as individual files for subsequent image analysis. Integration into an image analysis batch workflow using software like Imaris enabled 3D reconstruction and quantification. The approach is customizable for various acquisition setups, such as altering laser intensity, channel configuration, larger imaging, or time-lapse imaging.

This intermediate throughput workflow is particularly recommended for researchers requiring confocal resolution in their screening processes, especially for those lacking access to dedicated high-content 3D confocal imaging instruments. Thus, this adaptable workflow proves valuable for phenotyping different disease models.

Peptide-MHC-I-targeting chimeric antigen receptor (CAR) and T cell fusion constructs (TRuC) T cells form T cell receptor-like immune synapse morphology and cytotoxicity.

Shiqi Wang^{1,2}, **Kylie Luong**¹, Hannah Huckstep², Katherine A Watson¹, Jian Ding³, Fiona M Gracey⁴, Shereen Jabar⁴, Brad McColl⁴, Robert Tighe³, Alexandra Garnham², Robert Hofmeister³, Ryan Cross¹, Misty Jenkins^{1,5,6}

1. Walter Eliza Hall Institute of Medical Research, Parkville, VIC, Australia
2. Murdoch Childrens' Research Institute, Parkville, VIC, Australia
3. TCR2 Therapeutics Inc, Cambridge, Massachusetts, 02142. United States of America
4. Myrio Therapeutics, Scoresby, VIC, Australia
5. Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia
6. La Trobe Institute for Molecular Science, Bundoora, VIC, Australia

Chimeric antigen receptors (CARs) and T cell fusion constructs (TRuCs) are synthetic receptors, engineered into T cells to harness the cytotoxic functions of T cells towards to any tumour antigen of choice. There has been increasing interest in understanding how the receptor design could influence killing kinetics, persistence, and efficacy to improve and develop novel immunotherapies.

To evaluate the effectiveness our CARs and TRuCs, we studied the morphology of the immune synapse, the junction between the T cell and tumour cell, which consists of highly organised positioning of cytoskeletal and signalling components required for effective T cell cytotoxicity. Using laser scanning confocal microscopy and lattice lightsheet microscopy, we were able to visualise and interrogate the morphology of the T cell immune synapse and perform time lapse studies into T cell killing kinetics, measuring the time between T cell contact, signalling, degranulation, and target cell death.

We generated synthetic receptors against known tumour antigens in various formats, including surface antigen-targeting TRuCs and second-generation peptide-MHC-I (pMHC)-targeting and surface antigen-targeting CARs, to compare the morphology and functionality of CARs and TRuCs against the native T cell receptor (TCR).

We found that both pMHC-targeting CARs (Wang et al, 2021, Biomedicines) and TRuCs form a morphologically TCR-like immune synapse and TCR-like cytotoxicity, contrasting with CARs targeting surface antigen HER2 (Davenport et al, 2016, PNAS). Interestingly, despite other groups reporting transcriptional and functional differences between receptor design and choice of co-stimulation (Boroughs et al, 2020), mesothelin-specific CAR T cells with either 41-BB or CD28 co-stimulation domains both showed similar TCR-like immune synapse morphology and TCR-like killing kinetics, both effectively killing antigen-expressing tumour cells.

Our findings imply that both the synthetic receptor design and the tumour antigen play a role in the formation of the immune synapse, which directly contributes to T cell cytotoxicity. Further work is required to understand how the combination of the receptor and tumour antigen influences immune synapse formation and T cell killing kinetics, so that we could further optimise and improve our synthetic receptor designs to create more effective immunotherapies.

Validation of the HALO system as a reliable tool for assessing microglia

Laura Carr¹, Bianca Guglietti¹, Ing Chee Wee¹, Sanam Mustafa^{1,2}, Lyndsey E Collins-Praino¹

¹ School of Biomedicine, University of Adelaide, Adelaide, Australia

² Davies Livestock Research Centre, The University of Adelaide, Roseworthy, SA, Australia

In the immune privileged central nervous system, microglia act as the resident immune cells to fight injury and infection. Phenotypic changes in these cells have strong links with neurodegenerative diseases such as Parkinson's Disease (PD). Identifying and characterising microglial phenotypes in a consistent manner is essential in gaining further understanding of the link between microglia and disease. Currently there are several ways by which microglial population and morphology are assessed including manually or using open-source image analysis platforms such as ImageJ. Indica Labs have developed an image analysis software called Halo which has been used in high impact publications for cytonuclear and multiplex

immunohistochemistry (IHC) analysis, and *In Situ* hybridisation (ISH) analysis. A module for analysis and quantitation of activated microglia has been developed however this is not as widely cited or validated. The current study aimed compare the performance of the Halo microglial analysis module to manual counting, and automated counting using ImageJ.

Two datasets with tissue of varying thickness were utilised for analysis. For dataset 1 (D1), 5um thick human brain tissue from brain regions of interest in a PD cohort was used. For dataset 2 (D2) 20um thick rat brain tissue from a traumatic brain injury cohort was used. In both datasets tissue was stained for IBA1 using immunohistochemistry, and images were analysed manually, using ImageJ, and using Halo. Cell counts/mm² were calculated and compared within regions.

In D1, none of the methods used were able to identify any differences in total microglial number between control and PD brains with the exception of the striatum, where Halo identified an increased number of microglia ($p < 0.01$). No differences in number of activated microglia were identified by any method in the regions analysed. In D2, total number of microglia differed with injury in the striatum when analysed using ImageJ ($p < 0.05$), but no differences were identified in other regions of interest. Number of activated microglia in D2 did not differ when analysed using any method.

The above results suggest that the Halo microglial module is as accurate in identifying and classifying microglia, as manual analysis. When compared to other automated methods such as ImageJ, Halo is more accurate. Thus, this data suggest that Halo is a reliable method to count microglia and to assess microglial activation state in tissue of varying thickness and can be broadly utilised in future studies assessing microglial dynamics.

Using high-fidelity tumoroids and 3D imaging to decipher the pathways of therapy response.

Laura Rangel¹, Holly Pearson¹, Emma Ronca¹, Veronika Valova¹, Denise Attwater^{2,3}, Elgene Lim^{2,3}, Louise Cole¹, David Gallego-Ortega¹.

¹ School of Biomedical Engineering, Faculty of Engineering and IT, University of Technology Sydney (UTS)

² The Kinghorn Cancer Centre

³ Garvan Institute of Medical Research

Breast cancer (BCa) tumours are inherently heterogeneous and evolve upon treatment. Novel drugs reach clinical trials based on their efficacy in preclinical models, however, due to this tumour heterogeneity, it is estimated that most anti-cancer drugs are ineffective in 75% of patients. This highlights the necessity to 1) shift conventional therapies into a personalised approach, 2) establish reproducible, cost-effective and high-throughput preclinical models that recapitulate the complexity of human cancers and 3) develop novel visualisation tools to gain a deeper understanding of the cellular responses in their original niche during therapeutic intervention.

Here we use ALTEN, a high-throughput tissue-engineering platform that allows the *ex vivo* culture of tumour tissues for long periods of time (Fig. 1A). ALTEN preserves cell viability and diversity as well as the original tumour microenvironment and architecture. We have previously combined ALTEN with scRNAseq to produce high-resolution transcriptional landscapes and identify the molecular drivers of therapy response in breast cancer patient-derived xenografts (PDXs). However, sequencing-based technologies do not capture the 3D architecture of the tumour, lacking information about cellular distribution and interactions.

To address this question, we performed a high-resolution 3D imaging of BCa ALTEN tumoroids using the large-scale single-cell resolution 3D (LSR-3D) clearing method to validate and define the 3D spatial distribution of therapy response markers. LSR-3D is a single-step clearing technique enabling the visualization of breast tumours and soft tissues with high-fat content at cellular resolution. Notably, LSR-3D imaging demonstrated ALTEN's capability to preserve the main cellular lineages and their spatial architecture (Fig. 1B). Furthermore, we also used this technology to validate the 3D organisation of potential molecular markers of therapy response.

This study demonstrates that ALTEN serves as a reliable model for drug screening in BCa tumours. Additionally, the combination of scRNAseq and 3D imaging to identify molecular drivers of therapy response provides a comprehensive understanding of tumour architecture and the mechanisms underlying therapeutic response, facilitating the discovery of novel potential therapeutic targets.

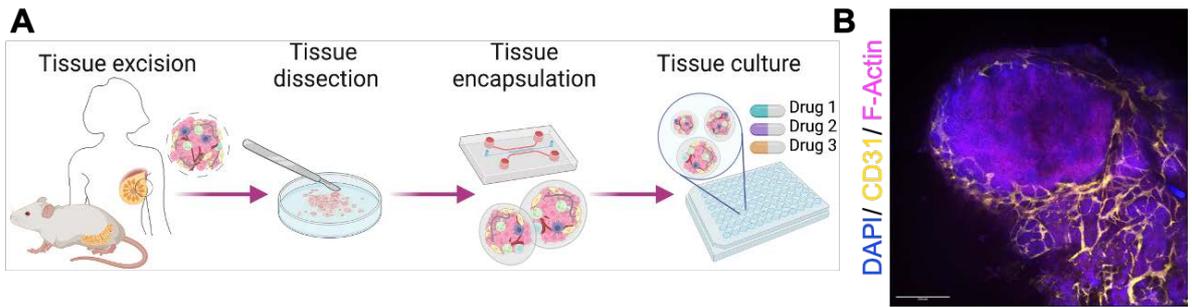


Fig 1. A) ALTEN tumoroids preparation workflow. **B)** 3D projection of a cleared BCa ALTEN tumoroid. Nuclei (blue), actin cytoskeleton (magenta), vasculature (yellow). Scale bar (200um).

AI-powered Live-Cell Imaging Reveals Mitochondrial Remodeling and Multi-organelles Interaction Induced by Mechanical Stimuli

Leo Zhang^{1,2}, Yang Guo², Yunpeng Guan¹, Charles Cox², Dan Shao¹, Boris Martinac², and Dayong Jin^{1,*}

¹ Institute for Biomedical Materials and Devices (IBMD), University of Technology Sydney, Australia

² Mechanosensory Biophysics Laboratory, Victor Chang Cardiac Research Institute, Australia

Abstract

Cells maintain their normal function and survival through the interdependence and coordinated work of multiple organelles, including material transfer, energy transference, and signal transduction. Increasing evidence suggests that cells can alter the network of interactions between organelles by mechanical force signal transduction, thereby influencing cell function. However, the molecular mechanisms underlying this process remain unclear. Piezo-1 is an ion channel protein present on the cell membrane. Although studies indicate that Piezo-1 can sense mechanical pressure or stretch and convert this mechanical signal into intracellular electrophysiological signals, whether Piezo-1 mediates the alteration of the organelle network by mechanical force signals remains a puzzle. In this study, we employed our recently developed AI-powered multi-organelle segmentation technique and utilized a variety of newly developed polarized fluorescent molecular probes to dynamically observe organelle interaction spectra in live cells. We demonstrated the crucial role of Piezo-1 in regulating mitochondrial remodeling and the interaction between mitochondria and the endoplasmic reticulum (ER). This leads to Piezo-1-regulated mitochondrial reshaping induced by chemical and mechanical stimuli, stimulating mitochondrial division while increasing interaction with the ER (Fig.1). Furthermore, we found that Piezo-1-regulated mitochondrial remodeling depends on Piezo-1-specific elevation of mitochondrial calcium levels; conversely, Piezo-1 knockout or non-Piezo-1-induced elevation of calcium levels did not cause changes in mitochondrial morphology. This suggests that Piezo-1 transmits specific calcium signals to the mitochondria, inducing unique signaling pathways and thereby regulating mitochondrial remodeling. We further studied the mechanisms of Piezo-1 activation in influencing mitochondrial remodeling, primarily focusing on Piezo-1 distribution on the mitochondrial surface. Through transcriptomic analysis, we unveiled the molecular mechanisms of Piezo-1 activation specifically inducing mitochondrial calcium overload. Additionally, we studied alterations in the membrane contact sites between mitochondria and the ER. This study, for the first time, investigated the mechanisms by which Piezo-1 mediates organelle interactions, holding significant importance in understanding the organelle interaction landscape under mechanical stimuli. Further research will focus on the physiological significance of Piezo-1-regulated dynamic morphology changes of mitochondrial, and its interaction with other organelles.

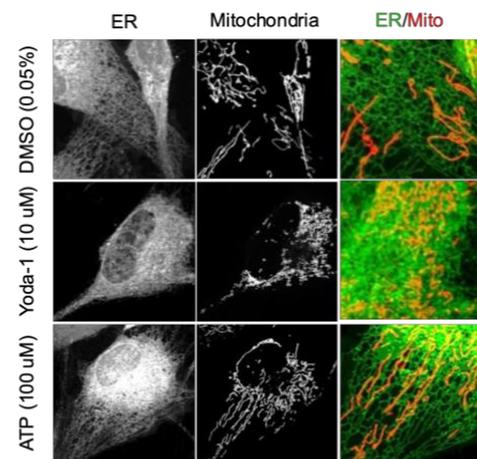


Figure 1. Mitochondria remodeling induced by Piezo-1 activation in human dermal fibroblast cells

Investigating cytotome formation in artemisinin resistant *Plasmodium* parasites by expansion microscopy

Long Huynh¹, Stuart Ralph¹

¹ Department of Biochemistry and Pharmacology, The University of Melbourne

Resistance against the frontline antimalarial, artemisinin, is mediated by mutations of the Kelch 13 (K13) gene. K13 is involved in the formation of the cytotome; the apparatus which brings haemoglobin from the host red blood cell into the *Plasmodium* parasite. Haemoglobin digestion supplies essential amino acids for parasite growth and releases haem which is required for the activation of artemisinin. Parasites expressing mutant K13 have a slowed rate of parasite feeding which is characterised by a reduction in haem biosynthesis and delayed growth. This reduced level of haem leads to less artemisinin activation, resulting in less parasite death. However, the mechanisms as to how mutation of K13 causes this slowed feeding phenotype remains unclear. We hypothesise that mutation of K13 reduces its stability and abundance, affecting the rate at which new cytotomes are formed and thus parasite feeding. Using expansion microscopy, we have resolved K13 as ring-shaped structures that localise to the periphery of the parasite. We performed expansion microscopy coupled with super-resolution microscopy at various timepoints throughout the asexual life cycle and compared the morphology and number of K13 rings present in the mutant vs WT. We found that K13 mutant parasites formed new K13 rings at a slower rate than the wild type. Some K13 mutants also appeared to form cytotomes that were not regulated by a cytotomal ring. These data provide a potential mechanism linking the artemisinin resistance mutations to reduced rate of haemoglobin uptake.

Alteration of the amyloid precursor protein (APP) trafficking by familial Alzheimer's disease mutation featured by quantitative live-cell microscopy

Lou Fourriere, Jingqi Wang, and Paul A. Gleeson

The Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia.

Alteration of the membrane trafficking can lead to neurodegenerative diseases. Accumulation of amyloid plaques in the brain is a hallmark of Alzheimer's disease and are formed by the aggregation of amyloid β peptide ($A\beta$) generated after a proteolytic processing of the amyloid precursor protein (APP) by the protease β -secretase (BACE1). Defining the intracellular trafficking of newly synthesized BACE1 and APP is required to understand the regulation of $A\beta$ production, the development of Alzheimer's disease and the design novel therapeutics. By combining cell biological techniques and high-resolution imaging, we observed that APP and BACE1 are sorted into different post-Golgi transport pathways in HeLa cells and primary mouse neurons. Moreover, using super-resolution imaging and live-cell imaging, we have shown that the partitioning of APP and its secretase BACE1 early in the secretory pathway is critical to regulate APP processing¹. We used the Retention Using Selective Hooks (RUSH) system to synchronise APP trafficking. By combining live-cells and immunoblot analysis, our temporal-spatial analysis of APP anterograde trafficking and processing has revealed different intracellular locations for the preferential secretase cleavage of wild-type APP and familial APP mutants, and $A\beta$ production, with the Golgi as the major processing site for the pathogenic Swedish APP mutation^{2,3}. We are now extending our techniques to explore BACE1 and APP trafficking in primary human neurons following our recent organelle mapping in human iPSC-derived neurons⁴.

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Using unbiased quantitative ptychography imaging to understand human olfactory ectomesenchymal cells for neural cell therapy manufacturing.

Mariyam Murtaza¹, Megha Mohan¹, and James St John^{1,2}.

¹ Griffith Institute for Drug Discovery, Griffith University

² Menzies Health Institute Queensland, Griffith University

Human olfactory ectomesenchymal stem cells are a unique source of cells obtained from an intranasal biopsy with the potential for neural repair in traumatic injuries of the nervous system. We are on the verge of a phase I/IIa clinical trial for treating spinal cord injuries using olfactory cellular nerve bridges. The efficacy of the cell therapy is reliant on the characteristics and function of the therapeutic cells, and safety is reliant on the cell population being free of unwanted cell types. Current process analytical technologies for primary cells require a large number of cells and are challenging for autologous cell therapies wherein each manufacturing process results in a therapeutic product for a single patient. There is a need to better understand variation within the cells and to address normal human variation across different samples. This will help us understand why some patients respond to the olfactory transplantation therapy and why some others may not. In addition, we need to determine if undesirable cells which may increase risk of adverse events are present within the population.

Objective: To develop an unbiased live cell imaging method to examine the intrinsic characteristics of primary human olfactory mesenchymal cells.

Results: We have used quantitative ptychography using the Liveocyte microscope to profile human olfactory ectomesenchymal cells. The Liveocyte ptychography provides high resolution live imaging and tracking of unlabelled cells and can track all cells within the field of view.

We initially imaged cells from a single donor and four different regions of the olfactory system to focus on their cell proliferation, growth and migration. We then expanded the analysis to human cells from different donors to compare their profiles. We also applied this imaging technology to characterise the intermediate cell population and the nerve bridge derived final cellular populations. Finally, we used immunostaining to confirm the marker expression profile of the olfactory cells.

Conclusion: The use of ptychography imaging analysis provides a powerful label-free imaging tool which is suitable for cell manufacturing. This systematic quantitative cell profiling approach will facilitate the development of standardised and translationally relevant assays for olfactory glial cells and facilitate personalised treatment strategies.

Shining a Light on the Bacterial Division Complex

Matthew Pittorino¹, Daniel Daley², Iain Duggin¹ and Bill Söderström¹.

¹ Australian Institute for Microbiology and Infection, University of Technology Sydney, Sydney, NSW, 2007

² Department of Biochemistry and Biophysics, Stockholm University, Stockholm, 106 91, Sweden

Cell division in Gram-negative bacterium, *Escherichia coli* is mediated by the collective action of approximately 40 proteins with over a dozen specialised functions, termed the divisome. These different processes of the divisome are coordinated at the division site to remodel all three layers of the cell envelope containing the inner membrane, outer membrane and cell wall. Previously, it was believed the divisome was one large, singular complex, however, advances in super-resolution microscopy approaches have suggested it is rather composed of multiple separate subcomplexes with transiently interconnected parts.

In this study, I sought to understand the weather proteins in the division complex responsible for cell wall synthesis (DamX) and also division protein recruitment (FtsZ) are separated. PhotoActivated Localisation Microscopy (PALM) revealed that recruitment (FtsZ) and cell wall synthesis (DamX) proteins contain separate assembly patterns to each other, with FtsZ assembling and disassembling before DamX. DamX was also observed to be retained at the division site following inner membrane closure, indicating the cell wall synthesis complex could have unknown functions passed inner membrane constriction. The differences between the cell wall synthesis and recruitment apparatuses were also reflected in their structures as DamX possessed a highly homogenous structure, compared to the FtsZ, which has previously been shown to be blotchy and heterogenous. Using two-colour PALM, I observed FtsZ and DamX together and discovered these proteins present with dynamic localisation patterns. During early stages of constriction, FtsZ and DamX colocalise, however, during later stages, the two proteins became spatially separated up to 100 nm, perhaps indicative of migrating functions between the cell wall synthesis and recruitment complexes.

Overall, my results using PALM show that proteins involved in division protein recruitment and cell wall synthesis contain different assembly, localisation and structural characteristics. These data provide strong support for the 'multiple subcomplex' model of the divisome, presenting new, exciting research avenues to gain a more in-depth understanding of bacterial cell proliferation.

New Approaches to Characterise Goblet Cells and Mucus Properties in the Human Colon

Matthew C Rowe¹, Cameron J Nowell¹, Narges Mahdavian¹, Sebastian K King^{2,3,4}, Pradeep Rajasekhar⁵, Simona E Carbone¹, Daniel P Poole¹

¹ Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Parkville, VIC

² Department of Paediatric Surgery, Royal Children's Hospital, Parkville, VIC

³ Department of Paediatrics, The University of Melbourne, Parkville, VIC

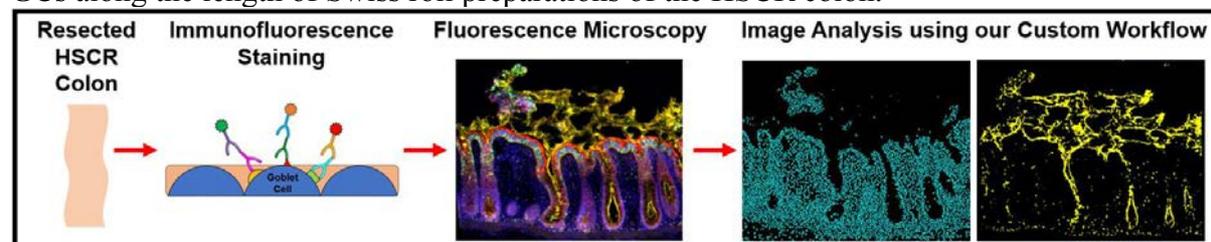
⁴ Surgical Research, Murdoch Children's Research Institute, Parkville, VIC

⁵ WEHI, Parkville, VIC

Goblet cells (GCs) line the gastrointestinal tract and are critical for the production and secretion of mucus. The mucus they secrete forms a physical barrier to protect the epithelium from pathogens and other sources of damage. A functional mucus layer is essential for intestinal homeostasis. Disruption of this layer leaves the epithelium vulnerable, increasing the susceptibility to bacterial infection and inflammation.

The enteric nervous system innervates the intestines and coordinates important functions including mucus and fluid secretion. Hirschsprung disease (HSCR) is a congenital enteric neuropathy where enteric neurons fail to colonise the distal end of the colon, resulting in severe constipation. Up to 50% of patients will also develop Hirschsprung associated enterocolitis (HAEC) which is a life-threatening comorbidity of HSCR that causes extensive mucosal inflammation. Neural signalling is an important regulator of mucus secretion, and the absence of enteric innervation suggests that mucosal barrier defence mechanisms may be compromised in HSCR. Although the pathogenesis of HAEC is poorly understood, disruption of mucosal barrier function may be a key contributor.

We hypothesise that GCs and the mucus layer are dysfunctional in HSCR patients and are predictive of HAEC susceptibility. We optimised six independent markers of GCs and mucus for immunofluorescence staining and multiplexing on both cryosections and formalin-fixed paraffin embedded colon tissue. We applied imaging techniques, including optical clearing and confocal microscopy, super resolution microscopy, and widefield microscopy, to characterise the goblet cell profile along the length of the resected colon (10-30cm of tissue) from 10 HSCR patients and 5 age-matched controls. To analyse the acquired images, we developed a novel analysis pipeline using ImageJ, Huygens deconvolution, Ilastik and StarDist to investigate the functional capacity of GCs to secrete mucus in response to bacterial products, and pharmacological, and electrical stimuli. We also assessed the location and distribution of GCs along the length of Swiss roll preparations of the HSCR colon.



We have developed custom methods to investigate the function and distribution of GCs in the human colon. Applying these methods to archival tissue samples from HSCR patients will allow us to assess the relationship between GCs and the incidence of HAEC. Furthermore, understanding the differences in mucus barrier function may provide a link between HSCR and HAEC susceptibility, which can be used to inform and guide treatment approaches for patients.

High Content Immunofluorescence Imaging and Quantitation of Mouse and Rat Cerebrovascular Dysfunction and Neuroinflammation

Michael Nesbit^{1,2}, Virginie Lam^{1,2}, Ryu Takechi^{1,2}, John Mamo^{1,2}

¹ Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University, Bentley, Australia,

² School of Population Health, Faculty of Health Sciences, Curtin University, Bentley, Australia

Our group uses rodent models to investigate neurodegenerative phenotypes in conditions including Alzheimer's, Parkinsons, Multiple Sclerosis, Sub-Concussion and 'Chemo-Brain', with a focus on blood brain barrier (BBB) dysfunction. Large study cohort numbers (100-300) are necessitated by experimental variables including age, genotype, and dietary intervention, which provide challenges in workflow time and consistence in quality from the cryostat, to bench, to microscope, to analysis and quality control.

Recent advances at our institute including multi-colour immunofluorescence staining optimisation and rapid acquisition microscopy (fluorescent tissue scanning and multi-point confocal) have allowed us to expand the number of targets we investigate while significantly reducing the time required to complete each measure. Method development for large area batch analysis using commercial software (Zeiss ZEN Blue) allows us to quantitate multiple measures in short time frames (days per experiment). Artificial analysis tools and hierarchal multi-channel segmentation improves the specificity and robustness of segmentation over large cohorts compared to 'traditional' thresholding.

This poster will provide an overview of the neurodegeneration and cerebrovascular dysfunction markers, their optimised staining conditions and tailored analysis workflows over large cohort, large area datasets.

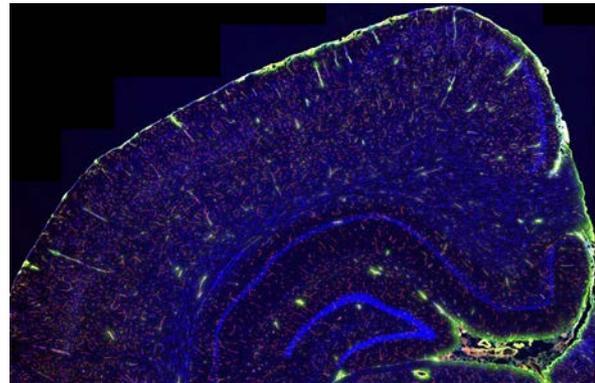


Figure 1. Large area fluorescent scanning of cerebrovasculature (red) and IgG leakage (green) in coronal mouse brain tissue sections.

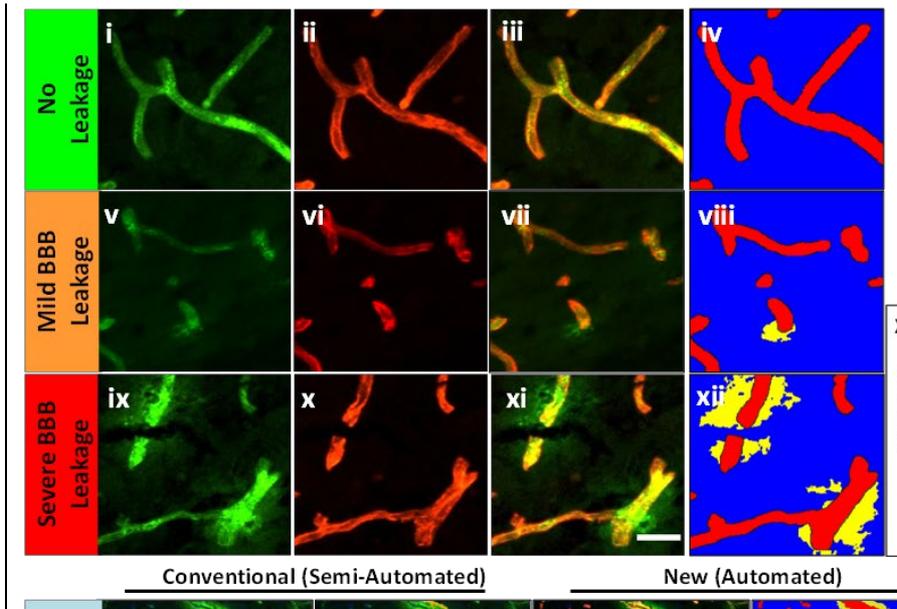


Figure 2. Method to automatically detect and measure mild BBB leakage events using ZEN software, compared to semi-automated methods without counterstains.

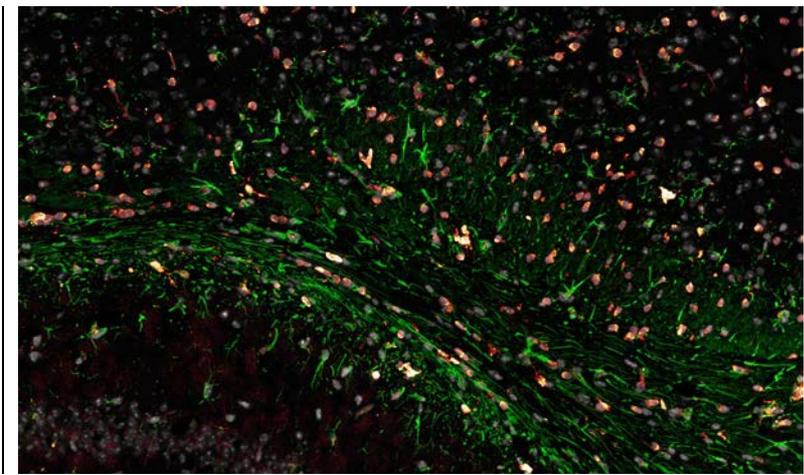


Figure 3. Optimised method for imaging and analysing oligodendrocyte cells in the corpus callosum after demyelination insult. (Oligodendrocytes - Orange;

Empowering Microscopy Research: From Tools to Trends

Nicholas D Condon¹, Nishanthi Dasanayaka Mudiyansele², Mark Endrei², Deborah Barkauskas^{1,3}, Kestutis Barkauskas⁴, Calvin Evans¹, James Springfield¹

¹ Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia

² Research Computing Centre, The University of Queensland, Brisbane, Australia

³ Garvan Institute of Medical Research, Sydney, Australia

⁴ Independent Consultant, Sydney, Australia

At the IMB Microscopy Core Facility, our commitment lies in providing practical solutions that significantly enhance the research experience and outcomes for our users. Our toolset spans from on-device scripts and programs to a sophisticated front end for High-Performance Computing (HPC). In tackling the challenges posed by big data in microscopy, we're excited to share our strategies for handling extensive datasets generated by advanced microscopy technologies, including lattice light-sheet, spinning disc confocal, & timelapse-widefield microscopy.

Between the microscope and HPC, we've implemented virtual desktops, enabling users to seamlessly access microscopy data and analysis tools remotely. Our custom software package provides tracking mechanisms monitor usage, ability to remotely send notifications and monitor hardware utilization, as well as ensuring consistency and version control, for the reliability and reproducibility of research outcomes.

Our HPC front end, the Image Processing Portal (IPP), has evolved significantly in 2023, incorporating new features such as open-source deconvolution, custom metadata scraping tools, and the ability to execute image analysis scripts, directly from a web browser. The user-friendly, wizard-like workflows now can auto-populate complex metadata fields, simplifying the process for users who may not easily recall specific experimental setup details. Moreover, the IPP democratizes access to extensive computing resources without the typical challenges associated with classical HPC, eliminating the need for command-line interfaces.

For a comprehensive overview of trends, statistics, and reporting, we have integrated diverse data streams using Microsoft Power BI for visualization and analysis. This integration facilitates real-time, unified visualization of facility operations, benchmarking, software usage metrics, system performance, and workshop/training conversion rates to actual equipment & software usage. Additionally, our system allows for the automatic creation of training records and certificates for students and users.

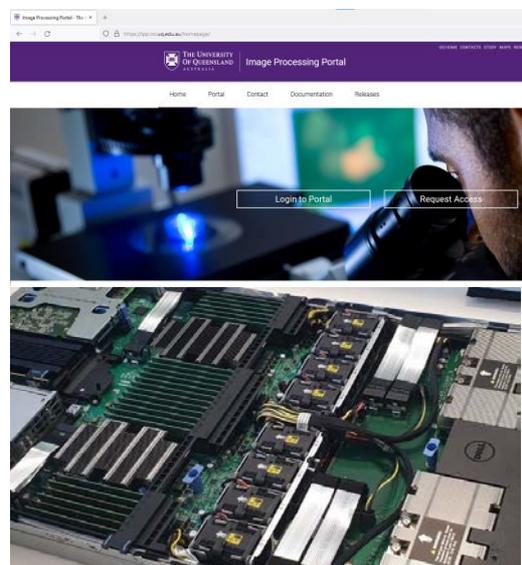


Image Processing Portal Landing page (ipp.rcc.uq.edu.au) and one of 34 nodes in the HPC system

Understanding Spatial Cell Biology in Histology Tissue using Immunohistochemistry and Transcriptomic Platforms

Tam Nguyen, Ashwini Potadar, and **Nigel Waterhouse**

QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, Qld 4006

Immunotherapy has fundamentally changed the treatment of cancer. Nevertheless many patients do not respond and improvement in immune-oncology treatments are still needed. Understanding how immune cells influence cancer progression and understanding immune cell responses to therapy is essential for developing new and better treatments. Diverse immune cells and cancer cells can now be identified in a single slice of histology tissue using complex expression of proteins (100s) and transcripts (1000s), which can also be used to identify potential biomarkers that could be used to guide treatment of patients with immunotherapy. QIMR Berghofer has installed various instruments for acquiring complex spatial information for protein expression and expressed transcripts including the CODEX, CosMx, and Xenium. We will discuss various learned parameters for acquiring and analysing datasets from these platforms.

Microtubule-dependent pluripotent cell plasticity orchestrated by centrosomal and non-centrosomal switching

Oliver Anderson¹, Jessica Greaney¹, Israa Hameed¹, Asma Aberkane¹, Sue Mei Lim^{1,2,3,4}, Jose M Polo^{1,2,3,4}, Jennifer Zenker¹.

¹Australian Regenerative Medicine Institute, Monash University, Clayton

²Adelaide Centre for Epigenetics, University of Adelaide

³Department of Anatomy and Developmental Biology, Development and Stem Cells Program, Monash Biomedicine Discovery Institute

⁴South Australian immunoGENomics Cancer Institute, University of Adelaide

300-400 word abstract, Times New Roman, with figures allowed, and references could be useful, but must fit to single A4 page.

Pluripotency is the extraordinary ability of cells to adopt any cellular identity of the adult organism. To reach their final identity as a differentiated cell, bespoke genetic, epigenetic, and metabolic regulations are essential. However, significantly less is understood of the role of cell biology in the establishment and execution of pluripotency.

The internal structure of a cell is directed by its microtubule cytoskeleton, a framework that assists in signalling and organelle trafficking. The typical centrosome-dependent microtubule organisation is not seen in the naïve pluripotent cells of the preimplantation mouse embryo, which instead uses a non-centrosomal microtubule arrangement anchored and nucleated by the microtubule minus end protein CAMSAP3. CAMSAP3-depleted cells of the early embryo are unable to contribute to the pluripotent inner cell mass. We sought to further explore this link between the microtubule cytoskeleton and the pluripotency continuum using cutting-edge live imaging technologies on human induced pluripotent stem cells (hiPSCs).

Following the transition to primed pluripotency, pluripotent cells in vitro maintain a CAMSAP3-dependent microtubule organisation. High temporal resolution imaging demonstrated the directional growth of microtubules from an apically located CAMSAP3 cap to the basal region of the cell. Despite the similarity to naïve pluripotent cells in utilising CAMSAP3, the microtubule organisation of primed hiPSCs differs in its widespread apical localisation and molecular composition, suggesting a signature of a more differentiated state. Trilineage differentiation of hiPSCs induced a switch from non-centrosomal to centrosomal microtubule organisation. When comparing genetic lineage markers, CAMSAP3-depleted hiPSCs showed altered expression profiles during differentiation, indicating an essential role for the microtubule cytoskeleton in executing pluripotency.

Uncovering the dynamics of cellular architecture during pluripotency entry and exit using live imaging may allow us to better manipulate hiPSCs, with anticipated applications in regenerative and reproductive biology, and shedding light on the fundamental cell biological differences between pluripotent stages.

Optimization of Mitochondrial Staining in Retinal Explants

Pattamatta Ushasree^{1,2}, Yu Hong⁴, Neugen Tasha, White Andrew^{1,3}

1. University of Sydney, Sydney, NSW, Australia
2. Centre for Vision Research, The Westmead Institute for Medical Research, Westmead, NSW, Australia
3. Department of Ophthalmology, Westmead Hospital, Westmead, NSW, Australia
4. Westmead Imaging Facility, The Westmead Institute for Medical Research, Westmead, NSW, Australia
5. University of California, San Diego, California, USA

Aim: Glaucoma, a neurodegenerative eye disease, involves the progressive death of retinal ganglion cells (RGC) resulting in irreversible visual loss. The aim is to optimize the mitochondrial staining in ex-vivo retinal explants to understand its role in RGC survival.

Methods: For *ex-vivo* model, eyes from C57BL/6 mice were enucleated immediately post-mortem to make two retinal explants per eye. Explants were cultured in a chamber slide or a 24 well plate for 1 or 2 days in culture medium at 37C and 5% CO₂. At the end of the experiment, retinal explants were stained with 100nM mitotracker red CMXRos dye for 30 minutes at 37C and 5% CO₂ and then the tissue was fixed with 4% PFA and RGC was visualized using BIII tubulin antibody. The tissue was then cleared using clearing solution (RapiClear 1.52 solution) for 72hr and the tissue was then imaged using Olympus BX53 fluorescence microscope.

Results: Axonal mitochondrial staining was observed in the tissue on both day 1 and day 2 of the culture. This staining was not very evident before clearing. RGC staining was also improved after using clearing the tissue.

Conclusions: Mitochondrial staining was observed in axons in the retinal tissue which is necessary to protect the RGC survival. We could successfully improve the staining protocol for mitochondrial imaging for our future study on investigating role of mitochondria in RGC survival.

Multiplication and division: Calculating growth and proliferation at population and single cell level with Livecyte.

Peter Davis¹, Meetal Jotangia², and Martin Humphrey².

¹ ATA Scientific, Sydney, NSW, Australia

² Phasefocus, Sheffield, United Kingdom

Growth and proliferation may be the bread and butter of cell-based assays but are the inaccuracies of your results proving hard to digest? An imbalance of these crucial pathways is observed in several disease states including cancer and cellular hypertrophy [1], however confluence-based proliferation outputs only tell half the story. Using our unique dry mass metric, a quantitative measure of the cellular biomass, Livecyte can calculate both individual cell growth and proliferation independently. What does this mean for you? You can finally gain a complete picture of cell behaviour and truly investigate the disparity in these fundamental cellular mechanisms. If that wasn't enough, dry mass also has the added advantage of explicitly identifying cell mitosis events allowing users to investigate aberrant and non-symmetrical cell division events in more detail giving Livecyte the most in-depth cell proliferation offering on the market.

The various dashboards generated from Livecyte's Analyse software paint a full picture of cell behaviour by providing metrics at both a population and single-cell level. The Proliferation, Mitosis and Morphology Dashboards can be utilized to understand the affect cytochalasin D has on cell proliferation and cell growth. A dose-dependant reduction in cell proliferation and mitotic events with cytochalasin D treatment was observed but an increase in cell dry mass values indicating cell growth.

In addition to the dashboard outputs, Livecyte allows insight in to cell cycle changes at a single cell level through the explore results page. Through changes in growth characteristics of cells such as dry mass, Livecyte is able to distinguish heterogeneity in cell division times in a seemingly homogenous population of cells. This can be exhibited as a lineage tree comparing individual cells and their progeny over multiple generations.

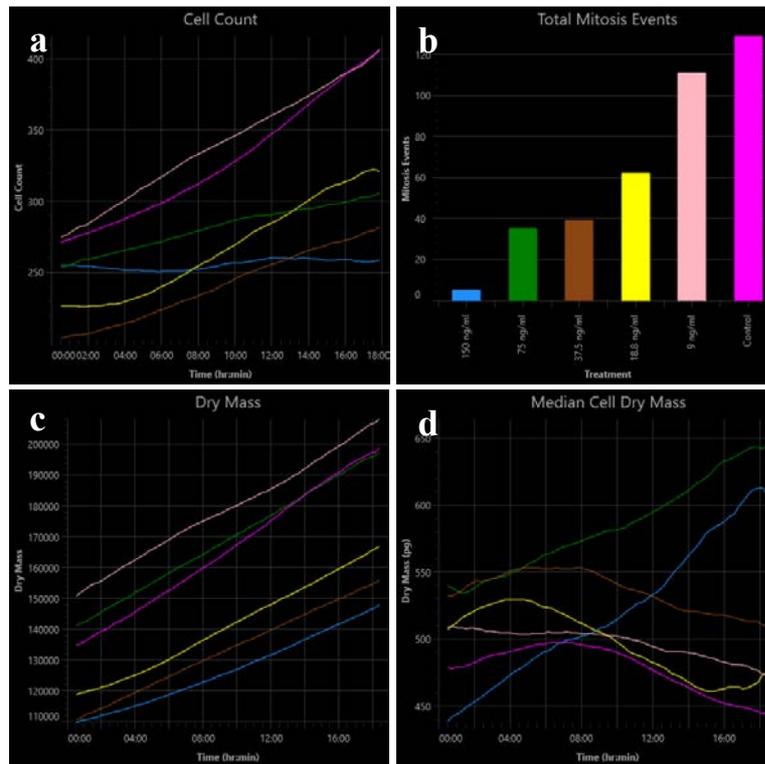


Figure 1: Illustrative plots from the Proliferation, Morphology and Mitosis Dashboard. Both the cell count (a) and mitotic events (b) are good indicators of proliferation and show a decrease in the number of cell divisions with higher concentrations of cytochalasin D. Growth can be measured independently of this as a total dry mass plot (c) and individual median cell dry mass (d) showing that higher concentrations of cytochalasin D increase the cell dry mass and thus growth of cells.

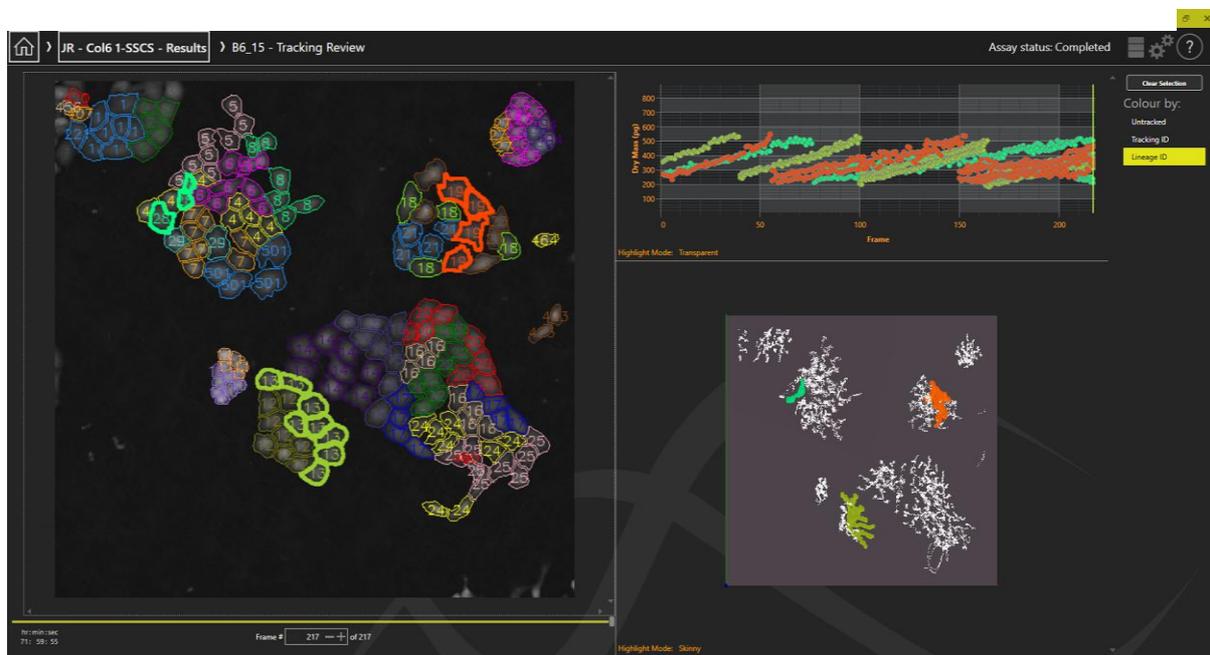


Figure 2: Cell cycle and lineage analysis of individual cells over time using Livecyte's Analyse Software. Using a unique lineage ID, multiple cell lineages can be viewed and compared in the form of a lineage tree. By looking at dry mass changes over time it's also possible to investigate cell cycle changes.

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Probing Phase Behaviour of Complex Bio-Composites Using 3D Confocal Laser Scanning Microscopy

Pranita Mhaske¹, Asgar Farahnaky¹, Stefan Kasapis¹, Mina Dokouhaki¹

¹ School of Science, RMIT University, Bundoora West Campus, Plenty Road, Melbourne, VIC 3083, Australia

Complex biomaterial mixtures are used increasingly in added value food, nutraceutical and pharmaceutical industries to design materials of enhanced techno-functionality. A thorough understanding of the structure–function relationship of components in these composites is crucial for the successful fabrication of materials with multifaceted properties to suit a variety of industrial applications. Of the many techniques used for studying structure–function dynamics, rheology-based blending law analysis remains one of the most popular. Though the theoretical blending laws are robust, they are an indirect method of estimating phase volume and require considerable experimentation and modelling expertise. The present work examines the efficacy of confocal laser scanning microscopy (CLSM), paired with Imaris image analysis, in estimating the phase behaviour of tertiary systems comprising distinct hydrocolloids and a lipid phase. The combined protocol of microscopy/image analysis was able to provide phase volumes of cold-setting agarose and gelatin in a binary gel with canola oil as the lipid phase. Similarly, reliable predictions were obtained for the phase volumes of thermally treated whey protein isolate in mixture with gelatin and canola oil. The rapid microscopic approach may serve as an alternative to the rather demanding protocol of rheological experimentation with theoretical blending law analysis that is heavily relied upon to provide predictions of phase volume in phase separated biopolymer networks. Given the increasing need to design complex materials with advanced techno-functionality, the microscopic approach advocated presently might deliver a rapid and accurate methodology to probe and quantify phase morphology in tertiary (and even quaternary) gelling bio-composites.

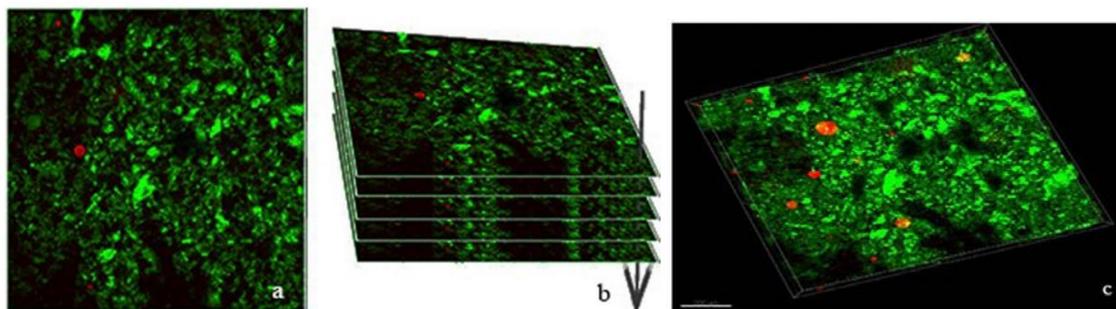


Fig. 1 (a) 2D, (b) Z-stack, and (c) 3D CLSM images of 3% (w/w) agarose plus 2% (w/w) gelatin and 2% (w/w) canola oil. The agarose, gelatin and oil phases are denoted in green, black, and red, respectively.

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Live imaging of mitochondrial membrane lipid order during apoptosis

Uren R.T.^{1,2}, Mlodzianoski M.^{1,2}, Geoghegan N.D.^{1,2}, Whitehead L.^{1,2}, Lou J.³, Wong A.W.^{1,2}, Shi M.X.^{1,2}, Iyer S.^{1,2}, Hinde E.³, Rogers K.L.^{1,2} and Kluck R.M.^{1,2}

¹Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria, 3052, Australia.

²Department of Medical Biology, University of Melbourne, 1G Royal Parade, Parkville, Victoria, 3052, Australia.

³School of Physics, University of Melbourne, Parkville, Victoria, 3010, Australia.

Background A key decision point during apoptotic cell death is the rupture of the mitochondrial outer membrane by effector proteins of the BCL-2 family such as BAK and BAX. During apoptosis membrane-embedded BAK dimers cluster on the mitochondrial outer membrane via an unknown mechanism before rupturing the membrane. We hypothesize that clustering of BAK or BAX dimers is driven by long-range forces arising from the membrane lipid disorder that accompanies embedded BAK or BAX dimers.

Results To explore the forces governing clustering of BAK dimers, we have established two imaging approaches that measure lipid order in live isolated mitochondria. Both approaches use the environment-sensitive lipid dye NR4A (a derivative of Nile Red) that localises to the outer mitochondrial membrane where BAK functions. Our first approach of PAINT utilises the fact that NR4A binds membranes reversibly, and involved optimising multi-color single molecule localization microscopy (SMLM²) with NR4A to achieve exceptional ~10 nm spatial resolution (note bilayers are ~4 nm thick). Our preliminary data reveal that triggering apoptosis induced membrane disorder. Our second complementary technique measures changes in NR4A lifetime and involves optimising Fluorescence Lifetime Imaging Microscopy (FLIM) on the Leica Stellaris platform. We are implementing FLIM-phasor⁴ analysis of lifetime shifts for enhanced spatial and temporal resolution of membrane heterogeneity in isolated mitochondria.

Conclusion Our preliminary data support membrane-intrinsic forces as essential drivers of BAK dimer clustering and formation of the apoptotic pore.

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**Tau biomolecular condensates induce nanoscale sequestering:
A novel mechanism to control Fyn activity and synaptic function.**

Ramón Martínez-Mármol¹, Shanley F. Longfield¹, Mahdie Mollazade¹, Tristan P. Wallis¹,
Rachel S. Gormal¹, Merja Joensuu¹, Jesse R. Wark², Ashley J. van Waardenberg^{3,4},
Christopher Small¹, Mark E. Graham², Frédéric A. Meunier¹.

¹ Queensland Brain Institute, The University of Queensland. QLD, Australia. ²Synapse Proteomics, Children's Medical Research Institute (CMRI), The University of Sydney. NSW, Australia. ³i-Synapse. QLD, Australia. ⁴Australian Institute of Tropical Health & Medicine (AITHM), James Cook University. QLD, Australia.

Liquid-liquid phase separation has emerged as a unique form of intracellular organization through membraneless gel-like structures known as biomolecular condensates (BMCs). The formation of BMCs represents a novel form of protein and nucleic acid compartmentalization that plays pivotal roles in human health and disease. Among other processes, BMCs are involved in neuronal mRNA trafficking and local protein synthesis during memory consolidation, and the stabilization of both pre- and postsynaptic components essential for neuronal communication. TDP-43, α -synuclein, and Tau undergo phase separation, forming toxic BMCs that have been found in the brains of patients with amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease (AD), respectively. Tau is a microtubule-associated protein expressed in the nervous system that works as a scaffold component with crucial roles in neuronal plasticity and migration. Unfortunately, hyperphosphorylation of Tau represents a pathological switch that promotes the formation of cytotoxic Tau aggregates commonly observed in AD. Tau also undergoes phase separation, but the precise physiological and pathological functions of Tau-BMCs still need to be fully understood. Electron microscopy (EM) and fluorescence recovery after photobleaching (FRAP) are among the most used imaging techniques to investigate the structure, composition and dynamics of BMCs. However, EM only provides static information, and FRAP does not achieve sufficient spatial resolution. The development of super-resolution imaging (SRI) has revolutionized neuroscience research, offering unprecedented insights into how the brain works¹, and providing exceptional levels of temporal and spatial resolution, necessary for the study of elusive structures such as BMCs.

Combining large-scale phosphoproteomic analysis with single-molecule SRI in live neurons, we revealed that Tau molecules undergo liquid-liquid phase separation, generating presynaptic nanoclusters whose density and number are regulated by synaptic activity. Tau translocates from the axon into the presynapse in an activity-dependent manner, forming sub-diffraction nano-BMCs that selectively control the mobility of the recycling pool of SVs². At the post-synapse, we also found that a mutant version of Tau associated with frontotemporal dementia (P301L-Tau) forms aberrant BMCs that sequester the Src kinase Fyn³, leading to enhanced clustering and accelerated synaptotoxicity. Moreover, our results showed that targeting the formation of these Tau gel-like aggregates prevents Fyn aberrant clustering⁴.

Overall, we use SRI to uncover a novel mechanism that controls neuronal communication based on the formation of Tau nano-BMCs. Dysregulation of these transient Tau condensates may produce "seeds" that sequester neuronal proteins, amplifying their toxic activity and leading to the development of neurological disorders.

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Resonance Imaging Microscopy: Breaking the diffraction limit with visible light

Raymond Dagastine^{1,2}, Christopher Bolton²

¹ Department of Chemical Engineering, University of Melbourne

² Tiny Bright Things

Motivated by the need to visualise anisotropic nanoparticles in the formation of engineered coatings and dynamic processes in life sciences, we have developed a new imaging modality not limited by the diffraction limit using visible light, called Resonance Imaging Microscopy (RIM). We describe this multi-source evanescent field scattering technique to enable high-speed, non-destructive, label and stain-free imaging of particulates with nearly any shape and composition, ranging in size from hundreds of microns to tens of nanometres. We simultaneously or sequentially record the spatially correlated scattering of multiple evanescent waves, generated via laser light sources, from individual particles or objects of arbitrary shape, and use this data to reconstruct size or shape of the particle. We discuss the physical principles behind this method as well as results of modelling the scattering behaviour using generalised Lorentz Mie theory. We then describe both direct and statistical results validating the fidelity of geometric measurements for a selection of spherical calibration particles with radii spanning four orders of magnitude. We outline a variety of different methods for implementing this technique with increasing ease of use for the users. We also briefly demonstrate imaging and measurement of a diverse range of particulates including nanospheres, nanotubes/rods, mineral powders and various biological materials. In addition to particle metrology applications, this technique will be especially useful in the life sciences for in situ imaging and measurement of delicate or transient biological systems under ambient conditions.

Quantitative Analysis of Human Colorectal Cancer Samples by Immunohistochemistry for Levels of Cancer Stem Cell Marker Expression

REEM ALI ALHULAIS¹, Stephen John Ralph¹

¹ School of Pharmacy and Medical Sciences, Gold Coast, Griffith University

Studies have shown that a subpopulation of cells known as cancer stem cells (CSCs) exist within tumours and are proposed to be the underlying cause of efficient tumour metastasis. Although this malignant colon cancer cell population is estimated to typically account for 9 percent or less of the cells in tumours, only some of these cells have the capacity to form new tumours. To advance therapeutic approaches targeting CSCs, it is important to study them using the cell surface markers that identify them. This study focused on identifying the relationship between the CSC markers ABGC2, Cripto-1, CD271, EPCAM, OCT4 or SSEA1 and pathology samples derived from human colorectal cancer (CRC) patients.

Surgically excised CRC pathology samples were categorised by immunohistochemistry as either negative, low positive, positive or high positive regions of staining within the defined tumour sections. The different types of colorectal cancers, including polyps (n = 10), primary adenocarcinoma (n = 20) or metastatic groups (n = 20), were categorised and immunostained and, in some cases, compared to the adjacent normal colon with antibodies against ABGC2, Cripto-1, CD271, EPCAM, OCT4 or SSEA1. The percentages of negative, weakly positive, positive and highly positive stained regions as a proportion of the total area from each of the patient's cancer samples were compared to each other and were then averaged across the same sample types using the methods for quantifying immunostaining.

In summary, the analysis of the putative CSC markers was identified to confirm the low level of expression of these markers within tumour sections from the primary tumour samples of human CRC patients. Interestingly, the levels of highly positive expression were obtained in the more advanced metastatic tumour samples, with coexpression as a common finding suggesting that they are involved in the CSC phenotype.

However, this study encountered several limitations. Data analysis proved challenging due to limited access to advanced histology software and funding restraints, which limited the ability to acquire more effective advanced histology software for automatically scoring positive cells stained with DAPI and other markers on the slides. Future studies would benefit from using Image-Pro, for example, to count and score positive cells as a more impartial approach than human-researcher-based estimations. It would also be interesting to compare the differences between automated counting/scoring and a pathologist's manual scoring methods.

Title: Fluorescence In vivo Confocal Laser Endomicroscopy analysis with Convolutional Neural Networks for the early detection oral potentially malignant disorders and oral cancer

Rishi S Ramani¹, Ivy Tan¹, Lachlan Whitehead^{2,3}, Michael McCullough¹, Tami Yap¹

1 Melbourne Dental School, University of Melbourne

2 Centre for Dynamic Imaging, Walter and Eliza Hall Institute

3 Department of Medical Biology, University of Melbourne

High resolution fluorescence in vivo confocal laser endomicroscopy is a powerful imaging technique that utilizes fluorescent agents such as Acriflavine and Fluorescein to enhance the visualization of cellular and molecular structures in living tissues. Quantitative analysis of these confocal micrographs with deep learning could help in the detection of disorders such as oral cancer which present with disordered epithelial cell architecture. Convolutional neural networks (CNNs) are deep learning algorithms that are exceptional at image identification tasks using a pixel-by-pixel analysis approach.

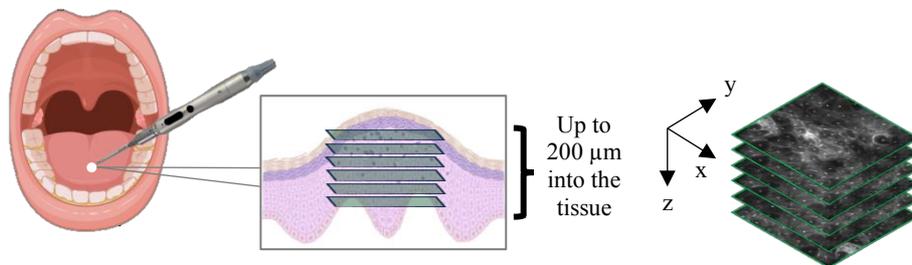


Figure 1: A stack of in vivo confocal laser micrographs captured in the *en face* orientation of oral epithelium tissue up to a depth of 200 μm .

Fluorescence in vivo confocal micrographs were captured using InVivage confocal laser endomicroscope (Optiscan Imaging, Australia) in 59 patients attending the Oral Medicine department at the Melbourne Dental School, who presented with oral mucosal lesions and needed to be diagnosed for oral potentially malignant disorders and oral cancer. Three distinct CNNs were developed, including a quality filtering network and diagnostic triage networks for fluorescence agents acriflavine and fluorescein. The quality filtering network underwent training and testing on 1200 images, while the diagnostic triage networks were trained and tested on 2403 images. Transfer learning was employed to modify the pre-trained CNN Inception-V3, adapting it to human oral mucosa in-vivo fluorescence confocal micrographs for both image quality control and diagnostic triage. Results showed that the quality filtering neural network (QMR) achieved an accuracy of 87.25%, processing images at a speed of 0.087 seconds per image. The diagnostic triage neural network for acriflavine (APMAC) exhibited a sensitivity and specificity of 0.84 and 0.92, respectively, with an image classification speed of 0.09 seconds per image. Similarly, the diagnostic triage neural network for fluorescein (FPMAC) demonstrated a sensitivity and specificity of 0.88 and 0.94, respectively, with an image classification speed of 0.14 seconds per image. Notably, FPMAC accurately recommended immediate biopsy in 100% of cases and advised against biopsy in 95.8% of cases. APMAC, on the other hand, correctly suggested immediate biopsy in 95.30% of cases. The integration of convolutional neural networks with in vivo fluorescence confocal laser endomicroscopy for image quality control and diagnostic triage yields highly accurate and rapid real-time analysis.

Investigating T Cell Development in the Thymic Microenvironment: An *In Situ* Study

Roshan Jalaldeen^{1,2}, Amr Allam³, Sarah Russell^{1,2}

¹Peter MacCallum Cancer Center

²Swinburne University of Technology

³Olivia Newton-John Cancer Research Institute

The investigation of developing T cells within the thymic environment is typically conducted through *in vitro* time-lapse experiments, *ex vivo* flow cytometry, and immunohistochemistry experiments. Our recent findings highlighted the formation of an immune synapse by developing T cells, resembling a mature immune synapse, to navigate the β -selection checkpoint [1]. Expanding on our existing knowledge, we aim to explore correlations between cellular events such as proliferation, differentiation, and apoptosis, and subcellular events such as immune synapse formation, asymmetric cell division, and phosphorylation in signalling pathways within the thymic microenvironment. This investigation will span different ages, providing a comprehensive understanding of T cell development dynamics.

Thymus tissues from C57BL/6 mice of different ages (fetal to 20 weeks). The Opal multiplex immunohistochemistry assay will be employed to identify cells and events of interest. The HALO software's region classifier module will help distinguish medullary, cortical, and subcapsular zones. DN3 cells will be identified through surface markers CD4, CD8, CD44, and CD25. Markers such as CXCR4, Notch1, pT α , and pLCK for β -selection and immune synapse, along with Ki67 for proliferation, mitochondrial markers for metabolic rate, and stage markers CD2, CD5, and E-cadherin, will be incorporated [2]. Quantitative grading of Ki67 and mitochondrial markers will be conducted to explore their association with cellular events in T cell development. The study will be progressively expanded by incorporating additional markers for both cellular and subcellular events.

Results of this research will enable cross-referencing of cellular events ascertained from *in vitro* single-cell longitudinal information with *ex vivo* information regarding the niche and rates of differentiation. The study will contribute to our group's integrated mathematical model of β -selection and lay the groundwork for future single-cell spatial -omics studies of T cell development within the thymic environment.

Reference:

1. Allam AH, Charnley M, Pham K, Russell SM. Developing T cells form an immunological synapse for passage through the β -selection checkpoint. *J Cell Biol.* 2021 Mar 1;220(3):e201908108. doi: 10.1083/jcb.201908108. PMID: 33464309; PMCID: PMC7814350.
2. Charnley M, Allam AH, Newton LM, Humbert PO, Russell SM. E-cadherin in developing murine T cells controls spindle alignment and progression through β -selection. *Sci Adv.* 2023 Jan 18;9(3):eade5348. doi: 10.1126/sciadv.ade5348. Epub 2023 Jan 18. PMID: 36652509.

Development of a tissue clearing protocol for the evaluation of cellular and molecular mechanisms following traumatic spinal cord injury

Ryan Dorrian¹, Jayden Christie¹, Srisankavi Sivasankar¹, Carolyn Berryman², Antonio Lauto³, Anna Leonard¹.

¹ School of Biomedicine, The University of Adelaide

² Impact in Health, University of South Australia

³ School of Science, Western Sydney University

Introduction

Spinal cord injury (SCI) causes devastating motor, sensory and autonomic deficits that often persist indefinitely, as there is a paucity of effective treatment options. Developing novel interventions that improve outcomes necessitates a concrete understanding of key pathophysiological processes (neuroinflammation, axonal injury). However, cellular and molecular analysis post-SCI has largely been restricted to 2D methodologies (immunohistology, immunofluorescence), flow cytometry, or blotting techniques. These techniques are limited, as they lack the spatial resolution necessary to understand the complicated cellular processes that occur at various locations throughout the injured spinal cord. Hence, utilising 3D analysis methods is pivotal to characterise cellular and molecular processes following SCI. Lightsheet microscopy (LSM) facilitates 3D imaging of cleared tissue samples, and may be applied post-SCI to ameliorate our understanding of SCI pathophysiology. However, few studies have utilised LSM post-SCI. Hence, this study investigated several tissue clearing protocols (CE3D, X-CLARITY, uDISCO) on injured spinal cord tissue to evaluate SCI pathophysiology.

Methods

Male Sprague Dawley rats (12 weeks old, n=5, Ethics: M-2021-038) underwent a T10 SCI (200kdyne via infinite horizon device), before being intracardially perfused (10% formalin) at 5 days (n=4) or 5 weeks (n=1) post-SCI. Spinal cord tissue was collected and allocated to a tissue clearing protocol (CE3D, X-CLARITY, or uDISCO), before being imaged on a ZEISS lightsheet 7 microscope. Images for each protocol were compared to determine the best performing protocol based on image quality and preservation of anatomical features. This protocol was then coupled with immunolabelling to evaluate neuroinflammation (Iba-1), axons (NF-L) and synapses (synaptophysin) following SCI

Results

uDISCO was the best performing tissue clearing protocol, preserving key anatomical features of the spinal cord. While uDISCO proved compatible with immunolabelling, crossover between antibody channels impeded analysis and necessitates further protocol development.

Conclusion

Despite current limitations in immunolabelling, uDISCO represented the preferred clearing method for both injured and uninjured spinal cord tissue. As such, future applications of this protocol or alternative DISCO-based clearing methods may be preferential for spinal cord tissue.

Imaging analysis of patient derived cortical organoids modelling neurodevelopmental disorders

Sarah Handcock¹, Timothy J. Karle, Pamela Kairath, Carolina Chavez, Alita Soch, Kay Richards, Steve Petrou, Snezana Maljevic

¹ Epilepsy Mission, Florey Institute of Neuroscience and Mental Health, Parkville, Victoria 3052, Australia

Conventional image analyses present significant challenges when used to classify cortical organoids, due to the presence of a necrotic core, a lack of reference atlas and the difficulty of identifying antibodies located outside of the cell nucleus. To address these, we developed a custom image analysis pipeline for assessing the viability and cytoarchitecture of cortical organoids (complex 3D structures derived from human induced pluripotent stem cells) which we used to model a loss-of-function SYNGAP1-DEE mutation.

These organoids emulate the cellular morphology of the human cortex during prenatal development, enabling the exploration of disease mechanisms in early life. Our primary focus was the morphological characterisation of the organoids and the establishment of imaging workflows to quantify measures of cellular composition, health and growth. Hundreds of organoid slices were immunolabelled, rapidly imaged using a wide field microscope and “computationally cleared”. Images were then segmented and fed into our analysis pipeline. Two novel methods were developed for identifying cell-state characteristics (including necrosis), cell density, maturation in astrocytes and neurons, and quantified fibrous proteins in the peri-nucleic region.

Our dataset was hierarchical in nature; with multiple sections sampled from each organoid and multiple organoids sampled from each cell line. To account for the heterogeneity of the organoids, a linear mixed effects model was applied to each study measure.

Within the control group, mirroring aspects of *in-vivo* corticogenesis, our analysis pipeline determined a significant decrease in cell density occurring at 6-months post-induction – indicative of a change in developmental progression. Astrocytic maturation remained stable, but the neuronal population appeared to shift towards favouring inhibitory neurons.

We examined differences in the development of cortical organoids with SYNGAP1-DEE mutations, when compared to controls. SYNGAP1-DEE cortical organoids displayed significantly higher cell density and an increased proportion of mature astrocytes. Aggregate measures of both astrocytic and neuronal maturation were found to be reduced in the SYNGAP1-DEE model, which might reflect the presence of larger populations of less mature cells. These results are consistent with previous findings in models of Autism Spectrum Disorder and underscore the potential of cortical organoids when used as models for complex neurodevelopmental disorders.

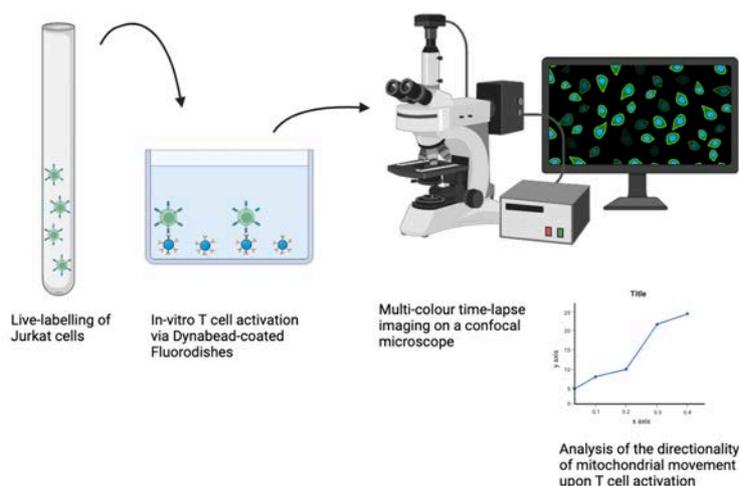
This study provides an important proof-of-principle that the cellular mechanisms of DEEs can be examined in biologically relevant systems, using automated image analysis protocols.

CONFOCAL MICROSCOPY REVEALS THE DIRECTIONAL MOVEMENT OF MITOCHONDRIA UPON T CELL ACTIVATION VIA ANTIBODY-COATED NANOBEADS.

Sofía Alfonso-Sánchez¹, Qian Su¹

¹ School of Biomedical Engineering, University of Technology Sydney

T cell lymphocytes are crucial players of the adaptive immune system as they are involved in both the cytotoxic and humoral responses. Several studies have uncovered that immune synapse formation and signal transduction are necessary for successful T cell lymphocyte function following activation via T cell receptor (TCR) triggering, and mitochondria seem to play a role in both of these areas. Specifically, mitochondria have been shown to play a role in T cell activation by providing a local pool of ATP and performing calcium intake buffering at the immune synapse. Furthermore, mitochondrial reactive oxygen species (ROS) are necessary for efficient T cell activation and for signal transduction events that lead to T cell proliferation. In the present research, we explore mitochondrial organisation upon T cell activation within the Jurkat cell line through the use of Dynabeads (nanobeads coated in anti-CD3 and anti-CD28). Our multi-colour confocal microscopy approach reveals mitochondrial enrichment with directionality towards the activation site in a time-dependent manner, supporting their role in early T cell activation.



Tiny Bright Things Halo Element: Label-free life science samples as you've never seen them before.

Tanweepriya Das¹, Andrew Glenn¹, Sudat Tuladhar¹, Christopher Bolton¹, Raymond Dagastine^{1,2}.

¹ Tiny Bright Things

² Department of Chemical Engineering, University of Melbourne

Since viewing cells and other biological materials with white light provides little useful information, proteins, structures, and key markers are commonly tagged with fluorescent proteins, dyes or conjugated antibodies, so that features, processes and dynamics can be observed. Such tags provide high sensitivity and specificity, and allow for real-time tracking of proteins and living cells, but they are expensive and time consuming to prepare, and can be degraded by photobleaching when examined using laser-based microscopy systems.

The Halo Element, which employs a RIM technology that has been patented by Tiny Bright Things, is a new analytical technique which overcomes these limitations by eliminating the need for sample tagging, yet allows the external and internal structure of biological samples to be accurately characterised – even at sub-diffraction limit spatial resolution.

In this presentation we will provide a brief overview of the Halo Element and present the results of a multi-modal characterisation studies that demonstrate how the Halo Element is able to elucidate the internal structure of, and relationship between, untagged cells and bacteria, and provide suggestions for additional exciting areas of future investigation.

Live cell imaging and super resolution imaging to identify cyclic peptide drug entry and mechanism in prostate cancer cells.

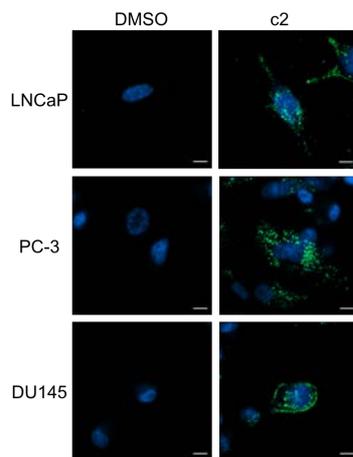
Timothy Mann¹, Mila Sajinovic¹, Monavvar Andarva², Paul de Souza¹ and Kieran Scott².

¹ Nepean Clinical School, The University of Sydney

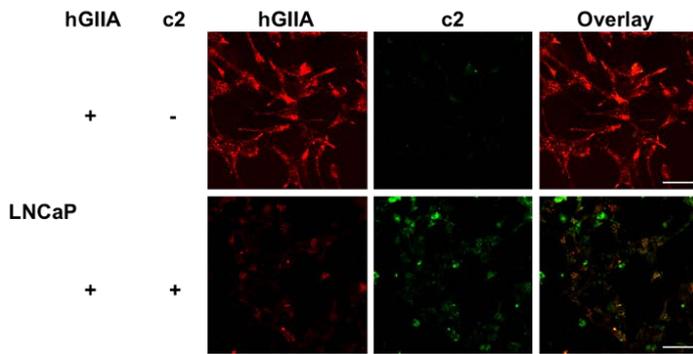
² School of Medicine, Western Sydney University

Prostate cancer (PCa) is the second leading cause of cancer death of men in the western world. A significant proportion of men develop metastatic disease, and eventually, androgen-independent disease, rendering them incurable. Human group IIA secreted phospholipase A₂ (hGIIA), an important enzyme in mediating innate immunity, is overexpressed in PCa, where its expression is closely aligned with malignancy, yet its specific interactions are unknown. Cyclic peptide c2, derived from the structure of hGIIA was developed as an inhibitor. A range of live cell, ratiometric and super resolution imaging techniques were employed to investigate novel interactions of hGIIA and how c2 inhibited these interactions.

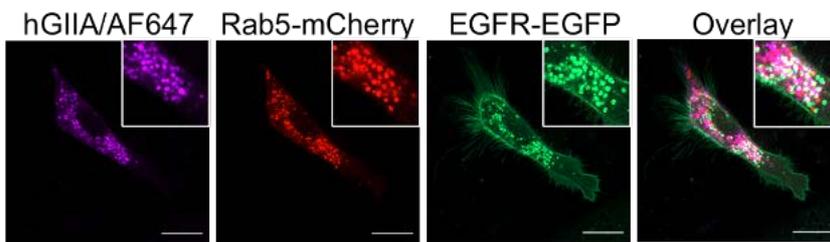
1) c2 was identified to be autofluorescent, likely due to the naphthyl rings in its structure. Allowing its entry and localization to be imaged confocally (green).



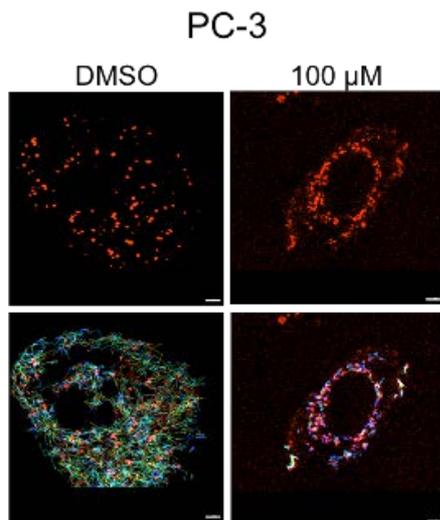
2) To investigate how hGIIA (a secreted protein) enters PCa cells, it was fluorescently tagged with Alexa Fluor 647, allowing hGIIA entry to be visualized. hGIIA entered both positive and negative hGIIA cell lines – highlighting it can follow autocrine and paracrine pathways. c2 also inhibited hGIIA and colocalized with hGIIA internally, indicating it functions as a competitive inhibitor.



3) A novel interaction of hGIIA with epidermal growth factor receptor was identified – triggering EGFR signaling pathways. This interaction provides a mechanism for how hGIIA contributes to tumorigenesis, which is inhibited by c2.



4) hGIIA was also identified to interact with vimentin, which contributes to hGIIA's trafficking around the cell, as identified through live cell imaging and Imaris analysis. c2 was also identified to bind to the second coil of vimentin, inhibiting intracellular trafficking of hGIIA and causing apoptosis. C2's targeting of vimentin was confirmed via loss of function in vimentin knockout cell line.



This research highlights that hGIIA's pro-tumorigenic role is a function primarily of its catalysis-independent mechanism. We have begun here to elucidate a novel function for vimentin in inflammation driven malignancy in PCa. C2, a novel first in class inhibitor of hGIIA's non catalytic function, was also shown to reduce tumour volume and cause apoptosis in androgen dependent and independent xenograft models of PCa at low as 0.1

mg/kg thrice weekly. C2 is also shown to be non-toxic and orally bioavailable and has since been progressed to clinical trial for the treatment of advanced prostate cancer.

Unraveling the Role of Microfold (M) Cells in Gut Immunity

Wang Cao^{1,2}, Yue You², Verena Wimmer², Stephen Nutt², and Gabrielle Belz¹.

¹ University of Queensland Frazer Institute, Brisbane, QLD, Australia

² Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

The intricate interplay between the microbiota and the immune system is paramount in safeguarding the gastrointestinal (GI) tract and ensuring optimal gut health. Central to this defense mechanism are Microfold (M) cells, specialized epithelial cells residing in the intestinal lining. These cells serve as vigilant guardians, constantly sampling luminal contents and swiftly triggering immune responses against potential threats by facilitating interactions with local immune cells. Yet, the precise mechanisms governing M cell behavior and their impact on mucosal immunity have remained mystery.

To bridge these knowledge gaps, our research focused on the development of novel gene-modified mouse strains aimed at unraveling the intricacies of M cell biology. Our findings challenged conventional notions, revealing that M cells are not limited to Peyer's patches but are distributed throughout the entire GI tract. This discovery hints at a broader role for M cells in fortifying gut immunity.

Our investigation delved deeper into M cells across various gut sites, uncovering surprising regional tissue-specific disparities. Through comprehensive analysis, we identified distinct signatures associated with M cells, unveiling their diverse nature influenced by factors such as the intestinal content composition, including the gut microbiota and ingested materials. Understanding these mechanisms provides insight into how M cells orchestrate site-specific immune responses within the GI tract.

By deciphering the regulatory mechanisms and maturation programs of M cells, our study offers insights into the dynamic immune responses that shape the gastrointestinal ecosystem. This approach, coupled with the development of innovative tools, opens new avenues for contemplating the design and targeted delivery of therapies aimed at modulating gut immunity. Our research contributes to comprehending the pivotal role played by M cells in fortifying gut immunity. The revelations concerning their distribution, regional variations, and the factors influencing their behavior allow a big step in a deeper understanding of GI immune responses.