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ABSTRACTS

AM1: Recent advances in scanning electron microscopy and complementing techniques for life science research

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Scanning Electron microscopy technique is at the forefront of advances in life science research.

AM2: Advances in electron microscopy instrumentation

van't Schip, K

Scitek Australia Pty Ltd

Gatan, Inc., a global leader focused on enhancing and extending the operation and productivity of electron microscopes, today announced the launch of the Alpine™ as the first direct detection camera to democratize cryo-electron microscopy (cryo-EM) imaging.

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AM3: Correlative Microscopy in the Life Sciences - 3D CLEM to CryoEM

Bee Yi Tan

Zeiss

Correlative microscopy can combine a large variety of different imaging techniques such as confocal microscopy, X-ray microscopy and different volume electron microscopy methods. Recent developments in cryogenic preservation and imaging technologies now allow us to combine cryogenic close-to-native-state preservation of whole cells and tissues, and structural analysis of macromolecules in their cellular context. Cryogenic microscopy is an emerging technique for the structural analysis of macromolecules in a close to native state. However, cryogenic microscopy presents users with complex challenges, such as time-consuming preparation and imaging procedures, devitrification, ice contamination or loss of samples – and even more challenges if the user wants to correlate data across imaging modalities. ZEISS Correlative Cryo Workflow overcomes these challenges by seamlessly connecting widefield, laser scanning, and focused ion beam scanning electron microscopy. The Correlative Cryo Workflow is fully optimized for the needs of correlative cryogenic workflows, from localization of fluorescent macromolecules to high-contrast volume imaging and on-grid lamella thinning for cryo electron tomography.

AM4: MICA – The world's first Microhub

Riethmann, C

Bio-Strategy

A Microhub is a new type of wholly integrated imaging solution that leverages machine learning software, automation tools and unique fluorescence unmixing techniques to automate the imaging workflow for researchers, regardless of their microscopy experience levels. The Microhub intelligently automates sample-finding, parameter-setting and focus constancy, replacing manual setup with just one push of a button.

New FluoSync technology enables a User to visualize four labels simultaneously in widefield, offering four times more data with 100% correlation compared to traditional fluorescence imaging methods. It is a fast and gentle method for unmixing multicolour widefield fluorescence images.

This technology allows researchers to focus more on their biology than the specialism of microscopy.

AM5: Cryo Electron Microscopy at the Bio21 Ian Holmes Imaging Center

Hanssen, E[1]

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In the last 5 years the Commonwealth of Australia and its scientific community have invested very heavily in electron cryo microscopy (cryoEM) to enhance or develop nodes of knowledge around the country. Most of these facilities are open access and allow the wide structural and cell biology community to access techniques that are rapidly becoming vital in many research project but too costly to implement in individual laboratory in the context of the Australian funding landscape. Here we will present an example of one of the newly re-developed facility at the University of Melbourne, the Bio21 Ian Holmes Imaging Center (IHIC).

The IHIC has been redeveloped over the last five years from a local high end "old school" microscopy unit to one of the Australian flagship 3D and cryoEM center to cater for a growing number of interested cell and structural biologists in the 10,000 scientists strong Parkville Biomedical precinct. The development of a brand new building to house the IHIC has allowed for investment in otherwise impossible to house equipment and delivered a building that is far from the historical basement location of electron microscopy facilities. The IHIC now houses five cryoTEMs, three of which are equipped with direct detectors (Glacios, Arctica G2, Krios G4), as well as all the sample preparation equipment. Data is acquired either by a facility staff or an experienced user and processes on-the-fly using cryoSPARC or Relion depending on user request.

AM6: Microbioarchaeology and the role of microscopy

Tromp, M.[1]

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Over the past decade microarchaeological methods have advanced our knowledge of how people lived and interacted with each other and their environments in the past. In this presentation I will give examples of projects examining geological microparticle and plant microparticle analysis. I will discuss how these methods have helped to better understand the lives of the first people to colonise Remote Oceania (3000-2750 BP)[1], a medieval German artist and nun (953–788 BP)[2], and residents of an Irish

workhouse during the Great Famine (99-103 BP)[3]. Finally, I will discuss the kinds of advances I am hoping to see in microscopy to answer even more questions about the past.

1. Tromp, M., Matisoo-Smith, E., Kinaston, R., Bedford, S., Spriggs, M., & Buckley, H. (2020). *Exploitation and utilization of tropical rainforests indicated in dental calculus of ancient Oceanic Lapita culture colonists*. Nature human behaviour, 4(5), 489–495.

2. Radini, A., Tromp, M., Beach, A., Tong, E., Speller, C., McCormick, M., Dudgeon, J. V., et al. (2019). *Medieval women's early involvement in manuscript production suggested by lapis lazuli identification in dental calculus*. Science advances, *5*(1), eaau7126.

3. Geber, J., Tromp, M., Scott, A., Bouwman, A., Nanni, P., Grossmann, J., Hendy, J., et al. (2019). *Relief food subsistence revealed by microparticle and proteomic analyses of dental calculus from victims of the Great Irish Famine.* PNAS, 116(39), 19380–19385. National Academy of Sciences.

AM7: A curly question about hair answered using confocal microscopy

Duane P. Harland[1], James A. Vernon[1], Joy L. Woods[1], Shinobu Nagase[2], Takashi Itou[2], Kenzo Koike[2], David A. Scobie[1], Anita J. Grosvenor[1], Jolon M. Dyer[1], and Stefan Clerens[1]

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[2] Kao Corporation, Tokyo, Japan

Hair curvature is a key element of structural diversity and function in mammalian coats and is an important component of appearance in human hair [1], but what within the hair causes curl? We used a careful sample preparation process and confocal microscopy to collect *in situ* measurements of cortical cell types and cortical cell lengths in short snippets of merino wool fibres [2]. This allowed a direct test of two long-standing theories of the mechanism of curvature in mammalian hairs. We found evidence contradicting one theory of how curvature forms and revised our thinking on another. This narrowing has advanced our understanding of how keratins structures work and guided us toward further volume imaging to finally unravel the mysteries of hair, nature's most complexly organised protein fibre.

1. Richena, M. and D.P. Harland, *What causes curly hair?* Journal of Cosmetic Science, 2021. **72** (November/December): p. 643-654.

2. Harland, D.P., et al., *Intrinsic curvature in wool fibres is determined by the relative length of orthocortical and paracortical cells.* Journal of Experimental Biology, 2018. **221**: p. jeb172312.

AM8: Ready, STEDy, go – first steps into STED microscopy

Ross, J.M.

Biomedical Imaging Research Unit, School of Medical Sciences, The University of Auckland, New Zealand.

Researchers worldwide continue to demand improvements in technology in order to answer complex biological questions including in the field of microscopy. Increasingly, higher level technology is expected to be used. For example, where previously confocal microscopy would have been sufficient

to investigate colocalization of receptor subunits, now it is required that some form of super-resolution microscopy should be employed.

Stimulated emission depletion (STED) microscopy is able to achieve lateral resolution of 20 - 50nm. An Abberior Facility Line STED microscope was recently installed in the Biomedical Imaging Research Unit and is now operational and available for use.

Although researchers who have experience with immunofluorescence labelling and other fluorescence labelling for widefield and confocal microscopy will have an advantage in using the system, there are some changes required to specimen preparation such as fluorophore and mountant choice. An understanding of what kind of questions are best presented to the microscope is imperative. Learnings from the experience to date will be reported, which should provide useful guidance to those who are interested in using the technology.

Preliminary data from the system will be presented including some comparison data that illustrate the differences in resolution and application of confocal microscopy, Zeiss Airyscan and STED microscopy.

AM9: New on the Physics Menu: Superconducting Sandwiches!

Chan, A.[1,2,4,5], Simpson, M.C. [1,2,3,4,5], Söhnel, T.[2,4], Fürsich, K.[6], Minola, M.[6], Khmaladze, J.[7], Bernhard, C.[7], Mallett, B[1,2,3,4,5],

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Superconductors carry electrical current with zero resistance (i.e., no energy loss) when cooled below their critical temperature (Tc). Generally, however, superconductivity is degraded by large magnetic fields or electric currents – a key performance limitation that creates a bottleneck in emerging superconductor technologies.

Recently, we discovered surprising emergent properties in thin-film multilayers of a cuprate high-temperature superconductor (YBa2Cu3O7, YBCO) and magnetic manganite (Pr0.5La0.2Ca0.3MnO3, PLCMO). At low temperatures, this 'superconductor sandwich' hosts an exotic granular superconducting state characterized by an unusually high resistance at zero-field [2]. Surprisingly, the customary superconducting state is recovered in a large magnetic field and/or current! This phenomenon is hypothesized to originate at the YBCO-PLCMO interfaces that have been well characterized by STEM/HAADF/EELS, X-ray reflectivity, X-ray absorption spectroscopy, and Raman microspectroscopy.

This talk will give a brief introduction into how superconducting sandwiches are made by pulsed laser deposition followed by polarized Raman microspectroscopy as a new probe to extract layer thickness and lateral uniformity in superlattice films. Data shown here corroborate with synchrotron and neutron scattering results to gain a better understanding of their novel physics as they may be destined as components in tomorrow's electronic devices e.g., as superconducting spin-valves or as components for quantum computing.

Malik *et al*. Pulsed laser deposition growth of heteroepitaxial $YBa_2Cu_3O_7/La_{0.67}Ca_{0.33}MnO_3$ superlattices on $NdGaO_3$ and $Sr_{0.7}La_{0.3}Al_{0.65}Ta_{0.35}O_3$ substrates. *Phys. Rev. B* (2012), 85, 054514.

Mallett *et al*. Granular superconductivity and magnetic-field-driven recovery of macroscopic coherence in a cuprate/ manganite multilayer. *Phys. Rev. B* (2016), 94, 180503(R).

AM10: Is seeing believing: a brief overview of artificial intelligent processing tools for improving microscopy images

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Optical microscopy has always existed within the resolution limitations of the optics and the artefacts of the light path and detectors. Evolution of imaging techniques and processing tools consistently breaks the previous generations limits, providing researchers ever greater clarity of features and resolving power to fuel scientific discoveries. One such example is the increasing use within commercial systems to employ sophisticated algorithms and in particular artificial intelligence routines (Ai) to manage the detection of artefacts and improve image quality (and thereby resolving power) of images, often referred to as Automated Denoising. These routines promise a simplified automated method to improve your data so why not use them?

This short presentation aims to review the current Ai image processing tools sets available, frame this against the alternative and more commonly used image processing techniques and hopefully provides some food for thought on how we advise and offer advanced image processing for scientific data and can or should we believe what we see?

AM11: Role of host exocytosis in cell-to-cell spread of *Shigella flexneri*

Thilina U.B. Herath[1], Arpita Roy[1], Antonella Gianfelice[1], Keith Ireton[1]

[1] Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

Shigella flexneri is a gram-negative enteric pathogen that causes dysentery. Critical for pathogenesis is the ability of the pathogen to spread within the human colonic epithelium without rupturing the host plasma membrane. To perform cell-to-cell spread, *Shigella* acquires actin-based motility (ABM), which delivers bacteria to the plasma membrane. Bacteria then deform this membrane into protrusions, which are engulfed by adjacent human cells. How *Shigella* protrusions form is not well understood. Here we demonstrate that *Shigella* subverts a host process called 'polarized exocytosis' in order to efficiently generate protrusions that facilitate cell-to-cell spread. Polarized exocytosis is known to be mediated by an octameric human complex known as the exocyst, the exocyst regulator RalA, and the membrane fusion 'SNARE' protein VAMP3. Using an exocytic probe, we found that polarized exocytosis is upregulated in *Shigella* protrusions in a manner dependent on the host exocyst complex. Furthermore, inhibition of expression of exocyst proteins, VAMP3, or RalA using RNA interference (RNAi) reduced protrusion formation and cell-to-cell spread of *Shigella*. Moreover, we found that the exocyst component Exo70 and exocyst regulator RalA are recruited to *Shigella* protrusions, suggesting a direct role of these proteins in protrusion formation. Interestingly, RNAi-mediated depletion of the exocyst proteins or RalA reduced the length of protrusions, suggesting that the exocyst complex is needed for the elongation, as well as the formation, of *Shigella* protrusions. Finally, we demonstrate that stimulation of host exocytosis by *Shigella* requires the bacterium's type three secretion system (T3SS), which is known to stimulate infection by injecting ~ 25 effector proteins into host cells. Collectively, this study provides strong evidence that *Shigella* manipulates and uses one or more bacterial effector proteins to stimulate host exocytosis and resulting cell-to-cell spread.

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Seeing beyond

AM12: Critiquing starch and raphides as evidence of cultigens from wetland archaeological Māori ditch systems using light microscopy and SEM imaging

Benham, R.W.

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The northwestern wetland margins of Motutangi swamp, located on the Aupouri Peninsula in far-northern New Zealand, incorporate several former wetland ditch systems below high dunes. Māori ancestors used these ditch systems during the early phase of human occupation in Aotearoa. Radiocarbon dates suggest an occupational period from (if not before) c. 1450 – c. 1700 CE.

Previous archaeobotanical research at Motutangi has identified starch comparable to kūmara (*Ipomoea batatas*), taro (*Colocasia esculenta*) and uwhi/yam (*Dioscorea alata*)[1]. These studies argue that both crops were productive in the marginally subtropical to warm-temperate climate of the Aupouri Peninsula tombolo. Succeeding publications have disputed the identification of taro remains at Motutangi[2]. Calcium oxalate raphides, another type of taro evidence, have also been critiqued because previous studies did not apply detailed morphometric analysis[3] or utilize scanning electron microscopy (SEM)[2].

My research examines starch and raphides extracted from soil deposits at Motutangi. By employing a combination of light microscopy and SEM analysis, this study critiques starch and raphides as diagnostic microbotanical elements at Motutangi. From the samples analysed, I have identified starch granules that are consistent with reference samples of *D. alata* and *I. batatas*, and starch amyloplasts and raphides comparable to Araceae, of which *C. esculenta* was the only Polynesian crop in precontact Aotearoa. SEM examination of archaeological soil samples shows raphides with diagnostic Araceae features including a long thin morphology, asymmetric terminations, and a median groove along two of the raphide's opposite faces. Collectively and within the context of the variable microbotanical remains at Motutangi, this evidence supports the thesis that early Māori ancestors modified natural wetland environments during the early stages of Polynesian settlement to establish the crop staples of tropical Polynesia, semiaquatic taro and dry soil uwhi, alongside kūmara, in the sub-tropical climate and fields of far-northern Aotearoa.

Horrocks, M & Barber, I. (2005) *Microfossils of introduced starch cultigens from an early wetlands ditch in New Zealand.* Archaeology and physical anthropology in Oceania. 40: 106-114.

Horrocks, M., Nichol, S. L., Augustinus, P. C., Barber, I. G. (2007) *Late Quaternary environments, vegetation and agriculture in northern New Zealand*. Journal of Quaternary Science. 22(3): 267-279

Prebble, M., Anderson, A., Augustinus, P., Emmitt, J., Fallon, S. J., Furey, L. L., Holdaway, S. J., Jorgensen, A., Ladefoged, T. N., Matthews, P. J., Meyer, J., Phillipps, R., Wallace, R., Porch, N. (2019) *Early tropical crop production in marginal subtropical and temperate Polynesia.* Proceedings of the National Academy of Sciences. 116(18): 8824.

Prebble, M., Anderson, A., Augustinus, P., Emmitt, J., Fallon, S. J., Furey, L. L., Holdaway, S. J., Jorgensen, A., Ladefoged, T. N., Matthews, P. J., Meyer, J., Phillipps, R., Wallace, R., Porch, N. (2020) *Reply to Barber: Marginal evidence for taro production in north New Zealand between 1200 and 1500 CE*. Proceedings of the National Academy of Sciences. 117(3): 1259-1260.

Crowther, A (2009) *Re-viewing raphides: Issues with identification and interpretation of calcium oxalate crystals in microfossil assemblages.* In Fairbairn, A., O'connor, S. & Marwick, B., (eds) *New Directions in Archaeological Science*, ANU Press. p. 105 – 118.

AM13: Ultrastructure, function and relationships of Oocyte mitochondria

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The oocyte is crucial for the viability of an embryo. Therefore, the quality of the oocyte is essential for development. Oocyte quality or competency relies on two interdependent aspects of maturation, nuclear and cytoplasmic. My research focuses on understanding the cytoplasmic aspect of maturation. Specifically, the ultrastructure, function, and relationship to other organelles of the mitochondria. Past literature established that during in-vitro maturation, the morphology of the mitochondria changes from being round to hooded. Concomitantly, the proportion of hooded mitochondria and distribution is modified. It is thought that this morphological change is non-pathogenic, required, and reversible, however, the function of this change in oocyte mitochondrial morphology is unknown. One study in embryonic fibroblast cells showed that hooded mitochondria can be induced when exposed to mitochondrial inhibitors that alter the electron transport chain, however, the true effect of these inhibitors on oocyte mitochondria is undetermined. My research this year aims to fill this gap by treating cohorts of sheep oocytes with electron transport chain inhibitors FCCP, and Oligomycin in the final two hours of maturation to determine the effects on mitochondrial ultrastructure. The effects of inhibitors on mitochondrial ultrastructure and activity were examined using transmission electron microscopy (TEM) and confocal microscopy respectively. The confocal microscopy experiment used a TMRM fluorescent stain as a marker of mitochondrial membrane potential or mitochondrial activity. Oocytes were exposed to the inhibitors at the beginning and end of maturation and compared. Finally, serial block-face scanning electron microscopy was used to view the 3D interactions of the mitochondria with other organelles to understand the functionality of the hooded mitochondria. The images will be presented and discussed. Determining the effects of these inhibitors on mitochondrial function and ultrastructure could suggest avenues to improve assisted reproductive technologies such as in-vitro maturation.

AM14: Cryo-EM structure of the oversized bacteriophage FTE capsid

James Hodgkinson-Bean[1], Nadishka Jayawardena[2], Peter Fineran[3], Matthias Wolf[4], Mihnea Bostina[5]

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In recent years, head tailed bacteriophages of the order Caudovirales are increasingly explored for their use in vaccines and nanomedicines. As such, there is increased interest in exploring mechanisms that govern Caudovirale capsid architecture. We used cryo-EM to investigate a novel, large and highly decorated Pectobacterium phage.

FTE bacteriophage particles were purified and submitted to cryo-EM at 300kV. Manual and automatic particle picking of FTE capsid heads yielded 5590 particles which were submitted to 3D reconstruction with icosahedral symmetry imposed. The resulting capsid electrostatic-potential map was calculated as 3.4 Å by 0.1439, cut-off FSC. A molecular model was constructed using the ISOLDE and PHENIX software packages. The refined FTE asymmetric-unit (ASU) model was validated by MolProbity, CaBLAM, EMRinger and Q-Score prior to structural analysis.

The FTE ASU is composed of 13 major capsid proteins (MCP), 13 trimeric decoration proteins (DEC), and two protruding “Pagoda-like” proteins. The MCP shows the canonical HK97 fold, forming the hexamers and pentamers that compose the FTE icosahedral capsid head. The DEC proteins form a continuous network of trimers that stabilize the capsid. The DEC model shows structural homology to the b-tulip decoration family. Interestingly, FTE-DEC is very large relative to other b-tulip decorations and possesses novel motifs. The PAGODA protein was poorly resolved but can be observed as extended densities with variable occupancy between the two hexamers depending on pentameric proximity. Finally, the FTE capsid measures ~1000Å, making it the largest of the T = 13 phage reported structures.

The increased diameter of the FTE capsid results in a >20% increase in internal volume relative to other T = 13 phages.

In summary, FTE is the largest and most decorated T = 13 phage categorised to date and represents an exciting model phage for the study of capsid size determination mechanisms.

AM15: Visualising pancreatic islets; a method for multiplex immunohistochemistry staining, whole-slide imaging, and a Fiji-based image analysis workflow

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Quantification of the various pancreatic endocrine cell populations, such as β-cells, is frequently examined in experimental studies of diabetes. Traditional manual imaging of pancreatic sections stained with immunohistochemistry (IHC) is laborious and time-consuming and often assesses fields of view rather than the whole tissue section. Once images are captured, analysis is often also manual or utilises expensive proprietary image analysis platforms.

Whole-slide imaging is a powerful technique that allows rapid automated visualisation of entire tissue sections. Whole-slide imaging increases the quantum of data generated per slide, decreases user time compared to manual microscopy, and reduces selection bias. However, such large data sets mean that manual image analysis is no longer practicable, and a more automated process is required.

Therefore, we developed a three-part method for analysis of ovine pancreatic endocrine cell mass, describing staining of β-, α-, and δ-cells using IHC in paraffin-embedded sections, imaging using an automated slide-scanner, and the analysis of these large image data sets using the open-source software, Fiji, which is freely available to all researchers.

This protocol is highly versatile and may be applied either in full or in part to suit tissues generated from other species, or images created using other whole-slide imaging platforms.

AM16: Understanding the Role of Plasminogen Receptors in Lipoprotein(a) Uptake via Confocal Microscopy

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High levels of lipoprotein(a) (Lp(a)) are associated with cardiovascular diseases. Lp(a) is a complex lipoprotein consisting of a low-density lipoprotein (LDL) and an additional protein called apolipoprotein(a) (apo(a)). A recent study discovered a novel plasminogen receptor (PlgRKT) to be responsible for a significant proportion of Lp(a) uptake in liver cells. Based on this finding we aimed to investigate the involvement of other plasminogen receptors in the catabolism of Lp(a).
Human hepatoma (HepG2) and haploid human fibroblast-like (HAP1) cells were treated with Lp(a), apo(a) and fluorescent dextran after overexpressing plasminogen receptors: PlgRKT, Annexin A2 and S100A10.

Lp(a) and apo(a) uptake was detected and quantified by confocal microscopy and western blot using an anti-Lp (a) antibody. Conversely, Lp(a), apo(a) and dextran uptake was detected and quantified in HAP1 cells in which PlgRKT and S100A10 were knocked out.

Lp(a) and apo(a) uptake were significantly increased (2.7 and 3.2 fold, respectively) in HepG2 and both by 2.1 in HAP1 cells in all of which PlgRKT was overexpressed. Lp(a) and apo(a) uptake were also significantly increased in HepG2 cells overexpressing either AnnexinA2 (1.9 and 2.8 fold, respectively) or S100A10 (2.3 and 2.7 fold, respectively). Uptake of dextran was significantly increased only in cells overexpressing AnnexinA2 or S100A10. Interestingly, Lp(a) surface binding was significantly increased only in HepG2 cells overexpressing PlgRKT. Lp(a) internalisation was significantly reduced by 0.5 and 0.4 fold in HAP1 cells in which PlgRKT and S100A10 were knocked out, respectively.

Our results show that plasminogen receptors: PlgRKT, Annexin A2 and S100A10 are involved in Lp(a) internalisation. PlgRKT seems to bind to Lp(a) on the cell surface whereas AnnexinA2 and S100A10 look to be involved in Lp(a) macropinocytosis. Further studies using live cell imaging will aim to better understand the role of plasminogen receptors in Lp(a) uptake.

AM17: Cryo-EM Structural Investigation of Human Norovirus Protease-Polymerase Precursor

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Precursor proteins are incompletely cleaved protein products produced during polyprotein processing. These proteins are often multifunctional and perform important roles during viral replication. One such precursor protein, the protease-polymerase (ProPol), is an active 3C protease, RNA polymerase and nucleotidyl transferase. Despite being the primary form of nucleotidyl-transferase used by noroviruses during replication, structural studies on ProPol are lacking.

Negative-stain electron microscopic analysis of the ProPol dimer revealed useful experimental information about the behaviour of the specimen on carbon grids and the size and appearance of the particles, ultimately yielding a low resolution Coloumb map of the homodimer. This map indicated that the protein forms only one dimer interface (likely between the protease domains). Moving forward, cryo-EM was performed at Molecular Horizons in Wollongong, Australia, yielding a dataset of 9000+ images from which a structure was solved in cryoSPARC to an atomic resolution of 2.61 Å. This represents the first structure of a precursor protein to be solved in the *Caliciviridae* family. The structure reveals the conserved right-handed architecture of the RNA polymerase domain, and the metal cation coordinated between the catalytic aspartate triad, but lacks density for modelling the N-terminal protease domain. Further work is underway to resolve the protease domain of ProPol so that a complete model can be made.

AM18: Defining the structure and assembly of the antifeeding prophage

Hurst MRH[1], Pushpanjali A, [1] Rybakova D[1], Desfosses A[2], Venugopal H[2], Joshi T[2], Felix J[3], Jessop M[2], Jeong H[4], Hyun J[4], Heymann JB[5], Gutsch I[3], Beattie A[1], Mitra A[2].

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[4] Electron Microscopy Research Center, Korea Basic Science Institute, Cheongju-si, Republic of Korea.

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The Anti-feeding prophage (Afp) produced by the bacterium *Serratia entomophila* is the archetype, external contractile injection system that causes larvae on the New Zealand grass grub *Costelytra giveni* to cease feeding. The Afp is encoded by a cluster of 18 genes, 15 gene products of which combine to form a virus like particle of 110nm in length. The Afp is similar in appearance to bacteria derived R-pyocins, the sheathed tail of bacteriophages such as T4 and type VI secretion systems [1,2,3,4]. Observations through electron microscopy revealed that the Afp1-15 proteins combine to form an unsheathed Tube Baseplate Complex (TBC), whereas Afp1-16 form a sheathed Afp particle [4,5]. Unlike R-pyocins, or the T4 bacteriophage the Afp has three sheath proteins - Afp2, Afp3 and Afp4, the latter of which resides above the baseplate of the TBC complex [4]. Through the construction and *trans* complementation of several Afp variants, different assemblies were observed enabling us to define the function of Afp16 and Afp14 as a sheath maturation protein and a length determining protein respectively[5,6]. Further to this the requirement for three Afp sheath proteins is now understood[7]. This information, combined with bioinformatic analysis and the recently resolved, near-atomic 3-dimensional cryo-EM structure of extended Afp (2.8-3.3Å resolution)[8] has enabled us to propose a model on the maturation of the Afp assembly.

Hurst MRH, Glare TR, Jackson TA, Ronson CW. 2000. *Plasmid-Located Pathogenicity Determinants of* Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of* Photorhabdus luminescens. J Bacteriol 182:5127-5138.

Hurst MR, Glare TR, Jackson TA. 2004. *Cloning* Serratia entomophila *antifeeding genes--a putative defective prophage active against the grass grub* Costelytra zealandica. J Bacteriol 186:5116-28.

Hurst MR, Beard SS, Jackson TA, Jones SM. 2007. *Isolation and characterization of the Serratia entomophila antifeeding prophage*. FEMS Microbiol Lett 270:42-48.\ Heymann JB, Bartho JD, Rybakova D, Venugopal HP, Winkler DC, Sen A, Hurst MR, Mitra AK. 2013. *Three-dimensional structure of the toxin-delivery particle antifeeding prophage of* Serratia entomophila. J Biol Chem 288:25276-84.

Heymann JB, Bartho JD, Rybakova D, Venugopal HP, Winkler DC, Sen A, Hurst MR, Mitra AK. 2013. *Three-dimensional structure of the toxin-delivery particle antifeeding prophage of* Serratia entomophila. J Biol Chem 288:25276-84.

Rybakova D, Radjainia M, Turner A, Sen A, Mitra AK, Hurst MRH. 2013. *Role of antifeeding prophage (Afp) protein Afp16 in terminating the length of the Afp tailocin and stabilizing its sheath*. Mol Microbiol 89:702-714.

Rybakova D, Schramm P, Mitra AK, Hurst MR. 2015. Afp14 is involved in regulating the length of Anti-feeding prophage (Afp). Mol Microbiol 96:815-26.

Pushpanjali B, Mitra AK, and Hurst M. 2021. Investigating the process of sheath maturation in Anti-feeding prophage- a phage tail-like protein translocation structure. Journal Bacteriol *In press*

Desfosses A, Venugopal H, Joshi T, Felix J, Jessop M, Jeong H, Hyun J, Heymann JB, Hurst MRH, Gutsche I, Mitra AK. 2019. *Atomic structures of an entire contractile injection system in both the extended and contracted states*. Nat Microbiol 4:1885 *-1894.*

AM19: Visualising transport in neurons

Gumy, L.F.[1]

[1]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, Aotearoa New Zealand.

Intracellular trafficking of cellular material is essential to maintain cell structure and function. This involves the movement of cellular cargoes such as proteins, vesicles and organelles, by motor proteins that move along cytoskeletal microtubules. Such trafficking is especially critical for neurons because the extreme length of axons (up to 1 metre in humans) demands that cargoes originating in the cell body travel very long distances to reach their target destinations. Defects in the distribution and delivery of cargoes in axons can lead to neuronal dysfunction. Despite the importance of long-range transport to proper cell functioning, knowledge on the basic mechanisms regulating the distribution of cargoes in axons over long distances are poorly understood. By using high-resolution live-microscopy, genetic and biochemical approaches we show that **M**icrotubule **A**ssociated **P**roteins (MAPs) are critical regulators of axonal trafficking. Given the ubiquitous presence of MAPs in neurons, these proteins are likely candidates for providing signals for a "MAP code" to coordinate specific trafficking routes in neurons.

AM20: Unravelling the mechanisms of bacterial gene regulation

Dobson, R.C.J.

Biomolecular Interaction Centre and School of Biological Sciences, University of Canterbury, Christchurch, New Zealand.

Bacteria rapidly adapt to changes in nutrient availability. The physiological response to these changes is multi-layered, but a key element is gene regulation: Genes that encode the appropriate metabolic machinery are induced, while those that are unnecessary are repressed. In this talk, I will summarise our work to reveal the molecular basis by which bacteria regulate the expression of genes necessary for amino-sugar uptake and metabolism.[1]

1. Horne, C. R. *et al. Mechanism of NanR gene repression and allosteric induction of bacterial sialic acid metabolism*. *Nat Commun* **12**, 1988 (2021)

AM21: Visualizing viral replication and assembly using volume electron microscopy

Velamoor, S.[1], Mitchell, A.[2], Humbel, B.[3], Bostina, M.[1,2]

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ, [2.] Otago Micro and Nano Imaging, University of Otago, Dunedin, NZ, [3.] Okinawa Institute of Science and Technology, Japan

A key goal of electron microscopy is to link cellular structure and function. However, obtaining high resolution spatial and temporal information from the same specimen is very challenging. Volume electron microscopy (VEM) has provided a snapshot of high-resolution structural information on the shape and organization of multiple subcellular structures simultaneously. In recent years, serial block-face scanning electron microscopy (SBF-SEM) and focused ion beam scanning electron microscopy (FIB-SEM) are widely used to achieve this goal.

This study emphasizes the power of these two techniques in obtaining detailed structural information on viral replication using Oryctes rhinoceros nudivirus (OrNV) as a model.

OrNV is a dsDNA virus belonging to the nudiviridae family that replicates in the nucleus. Although the previous electron microscopic studies have established the timeline for virus entry, replication and budding, little is known regarding the mechanism of viral assembly and egress. The three-dimensional reconstruction from SBF-SEM and FIB-SEM showed that OrNV assembles into mature particle inside the nuclear compartment. Mature particles were observed to cross the nuclear envelope using a series of membrane vesicles and transit the cytoplasm through some multivesicular bodies, before being released into the extracellular space either as single virions or as virions contained inside vesicles. We were also able to precisely characterise the membranous and cellular changes incurred during nucleocytoplasmic transport and viral egress following OrNV infection.

AM22: Designer skins - features contributing to lowered transpiration rates in fresh produce

Rebstock, R.S.[1], Shaw, N.M.[1], Chan, A.[1], Schon, B.[2], Plowman-Holmes, M.[2], Brown, M.[2], Schwendel, H.[3], Hunt, M.[3], Tattersall, A.[4], Lloyd, H.[4], Hallett, I.C.[1], Johnston, J.[4]

[1]Microscopy and Imaging, The New Zealand Institute for Plant and Food Research Limited, Auckland, NZ,
[2]Materials Science, The New Zealand Institute for Plant and Food Research Limited, Christchurch, NZ,
[3]Metabolite Chemistry, The New Zealand Institute for Plant and Food Research Limited, Palmerston North, NZ,
[4]Postharvest, The New Zealand Institute for Plant and Food Research Limited, Hawke's Bay, NZ.

To preserve the quality of fresh produce, many commodities are refrigerated and then shipped to overseas markets. However, refrigeration requires high energy inputs and creates carbon emissions, both of which raise sustainability questions. As part of a wider programme focused on delivering high-value, fresh produce offshore without the need for refrigeration, this project investigates increased transpiration rates and water loss in produce stored at ambient temperatures, which is expected to shorten shelf life and reduce quality.

This research has used a population of apples that segregates into fruit with high and low transpiration fruit to better understand which biological features enable low permeance to water. Light and electron microscopy are being used to identify key structural features of the skin (e.g., cuticle, epidermis) that work as major transpiration barriers, along with 3-dimensional imaging using micro-computed tomography. Preliminary research found that fruit with low transpiration had a thick and continuous cuticle layer and an expanded hypodermis, in comparison to fruit with high transpiration. In addition, differences were also found in the internal structure of apple flesh between low and high transpiration fruit that could influence movement of water.

Along with genetic mapping of the apple population, this microstructural investigation will identify novel targets for more detailed molecular and biochemical approaches. The ultimate goals of this research are to provide breeding targets for new cultivars, or to provide information that can be used to engineer bio-barrier based materials with the right combination of structural features that can be applied to fresh produce to complement the natural features of the skin, to further enhance its function as a transpiration barrier.

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AM23: Confocal SRRF microscopy of plant cell walls

Donaldson, L.A.

Molecular Physiology, Scion, Rotorua, NZ

The nanostructure of plant cell walls is of interest for food and biomaterials applications but high-resolution techniques such as electron microscopy require the sample to be dry or frozen and hence changed from the natural state. Light microscopy allows examination of cell walls in the wet state but with limited resolution. We developed a protocol for high-resolution imaging of wet cell walls using confocal microscopy and SRRF [super-resolution radial fluctuations] based super-resolution. We compared autofluorescence with acriflavin staining for lignin and rhodamine B staining for lignin/porosity visualisation.

SRRF microscopy offers advantages over other super-resolution techniques in that no specialised hardware is needed, and the technique can be used with any fluorescent stain as well as with autofluorescence which is particularly important for plant tissue imaging. Images are acquired as a sequence of 100 images of the same location. These data are then processed with ImageJ software using a computer with graphics acceleration. Under the conditions we used, image acquisition took 4 minutes and 15 seconds with a further 15 seconds processing time. The 'temporal radiality pairwise product mean' algorithm gave the best results with our samples giving a final pixel size of 24 nm and a maximum resolution of approximately 70 nm based on measurement of pit membrane nanofibrils. The three fluorescent signals gave variable results related mainly to the amount of noise which was highest in autofluorescence. The amount of fading measured across the 4-minute exposure was comparable among autofluorescence, acriflavin, and rhodamine but with rhodamine showing minimal amounts of noise and hence the greatest improvement in resolution.

We demonstrate for the first time that radial and concentric lamellae co-exist in wood cell walls in the native state.

POSTERS

AM24: Obtaining Seneca valley virus variants with increased capsid stability under evolutionary pressure

Shakeel Waqqar[1], Nadishka Jayawardena[1], Cormac McCarthy[1], Blair Lawley[1], Ivy Wang[2], Timothy Bilton[3], Laura N. Burga[1], Miguel Quinones Mateu [1], Mike Strauss[2], Mihnea Bostina[1,4]

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[2]Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada
[3]Invermay Agriculture Centre, Ag Research limited, Mosgiel, New Zealand
[1,4]Otago Micro and Nano Imaging, University of Otago, Dunedin, New Zealand

Oncolytic viruses are a novel and promising therapeutic approach for treatment of tumors. The prime notion of oncolytic virotherapy is to treat malignancies using viruses that can selectively replicate, cause apoptosis in tumor cells and improve anticancer immune responses, without infecting normal cells.

Seneca valley virus (SVV) is a novel oncolytic virus that was shown in early phase clinical trials to be both effective and safe in humans. During SVV replication, a mixture of full and empty capsids is produced, both capsids having the capacity to bind cellular receptors. Therefore, empty capsids have the potential to be used as vehicles for tumor targeting. However, they are less stable under natural pressures like high temperature and low pH.

We have analyzed the behavior of SVV quasispecies under directed evolutionary pressure and used next generation sequencing to explore the complete SVV genome. We have identified several non-synonymous mutations both in capsid proteins and non-structural proteins that confer significantly improved capsid stability.

To further validate the contributions of capsid mutations for structural stability, we have performed cryo-EM studies on a thermal variant that demonstrated improved thermal resistance compared to wild type SVV. The cryo-EM map at 2.5 Å resolution showed confirmational changes at the inter-pentameric surfaces and formation of additional hydrogen bonds, suggestive of stabilizing factors for capsid under thermal stress.

AM25: 3D Melt extrusion of PCL/PEG/curcumin-loaded PLA microspheres for suture applications

Deng, X., Gould, M., Ali A.

Centre for Bioengineering & Nanomedicine, Department of Oral Rehabilitation, Faculty of Dentistry, University of Otago, Dunedin, NZ.

Wound healing is a complex process critical in restoring the skin's barrier function. This process can be interrupted by numerous diseases resulting in chronic wounds that represent a major medical burden [1]. Available clinical methods have not produced the desired results so far. Additionally, antibiotic resistance has become more common within pathogens, which accelerates the search for alternative strategies. Therefore, studies on medical devices to facilitate chronic wound healing are essential. The use of natural products as part of a suture drug delivery system is an exciting new field [2]. In this study, curcumin with its anti-inflammatory, antimicrobial and antioxidant properties was used as an alternative, to develop a microsphere-loaded bioabsorbable surgical suture via solid-in-oil-in-water emulsion [3] and melt extrusion techniques [4] for facilitating chronic wound healing. During microsphere production, it was found that the higher the concentration of the water phase, the smaller particle size became (Avg. 40 µm). However, utilizing 4% w/v and above concentrations of the water phase resulted in the formation of aggregates after lyophilizing. Furthermore, the suture sample displayed a smooth surface, which ascribed to the miscibility of PCL/PEG with curcumin-PLA microspheres. We hypothesize that our product will be biocompatible and possess excellent mechanical properties to accelerate chronic wound healing.

1. Deng X, Gould M, Ali MA, *A review of current advancements for wound healing: Biomaterial applications and medical devices.* Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2022

2. Deng X, Qasim M, Ali A, *Engineering and polymeric composition of drug-eluting suture: A review.* J Biomed Mater Res A, 2021. **109**(10): p. 2065-2081 DOI: 10.1002/jbm.a.37194.

3. Sawant A, Kamath S, Kg H, Kulyadi GP, *Solid-in-oil-in-water emulsion: an innovative paradigm to improve drug stability and biological activity.* AAPS PharmSciTech, 2021. **22**(5): p. 1-14

4. Deng X, Gould M, Ali MA, *Fabrication and characterisation of melt-extruded chitosan/keratin/PCL/PEG drug-eluting sutures designed for wound healing.* Mater Sci Eng C Mater Biol Appl, 2021. **120**: p. 111696 DOI: 10.1016/j.msec.2020.111696.

AM26: Skin characteristics influencing post-harvest damage in fruit

Shaw, N.M.[1], Chan, A.[1], Hallett, I.C.[1], Rebstock, R.S.[1]

[1]The New Zealand Institute for Plant and Food Research Ltd, Auckland, NZ

A major function of fruit skins is to provide protection for the internal flesh and seeds. The robustness of the skin has important implications for handling and storage of fruit within the supply chain. Delicate skins are more likely to become damaged both on the plant and post-harvest and will therefore be more susceptible to pathogen infection and rots, leading to fruit loss. A number of factors contribute to the level of protection provided by the skin, including its overall thickness, the nature of the cells comprising the skin layers (for example whether the skin is comprised of living or dead cells) and the nature of the surface that faces the environment (for example roughness, waxiness and presence of hairs and glands).

This research has used microscopy techniques to identify characteristics of the skin and underlying flesh of genetically similar fruit that exhibit noticeably different rates of damage to identify those that could contribute to susceptibility. Histology was used to characterise tissue structure, while immunolabelling was used to investigate cell wall composition. The use of micro-computed tomography (microCT) was also trialled as a tool for understanding ripening changes in dense tissues with little contrast.

AM27: Effects of Seneca Valley virus with clinically available chemotherapeutic drugs on 3D tumour-like cell cultures of triple negative breast cancer cells

En Watanabe[1,2], Laura Burga[2], Sai Velamoor[2], Mhairi Nimick[3], Richard Easingwood[4], Mihnea Bostina[2,4].

[1]Department of Medicine, [2]Department of Microbiology and Immunology, [3]Department of Pharmacology and Toxicology, [4]Otago Micro and Nano Imaging, University of Otago, Dunedin.

Triple negative breast cancer (TNBC) makes up approximately 15%–20% of all breast cancers. There is currently no targeted therapy against TNBC. Oncolytic virotherapy, the use of viruses to eliminate cancer cells, is a promising strategy for targeted cancer therapy. Seneca Valley virus (SVV), a novel oncolytic virus, shows high affinity for tumours and no affinity for healthy tissue. The cellular receptor responsible for this affinity is anthrax toxin receptor 1 (ANTXR1) also known as tumour endothelial marker 8 (TEM8). TEM8 is present in over 60% of human solid cancers including TNBC and absent in healthy tissue. Clinical trials of SVV as a monotherapy proved the safety of the treatment but yielded inconclusive results of therapeutic efficacy.

This study explored combination therapies of SVV and clinically approved drugs on tumour-like 3D cell cultures (tumourspheres) of TNBC models. The efficacy of combination therapies was determined by measuring the cell viability using an ATP bioluminescence assay. Qualitative observational studies of the tumourspheres were also carried out through various microscopic imaging modalities.

Results of the ATP bioluminescence assay were analysed to predict for the presence and degree of synergy between SVV and drugs of interest, from which we hope to confidently proceed onto in vivo studies. Microscopic investigations were also analysed to better our understanding of how SVV affects triple negative breast cancer cells.

AM28: Comparison of the structural and phylogenetic evolution of the *Coronaviridae* spike protein

Hills, F.R.[1], Burga, L.[1], Watanabe, E[1], Bostina, M [1].

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), responsible for the COVID19 pandemic, has caused global disruption as well as the deaths of over six million people as of July 2022[1]. The main focus for therapeutics and vaccines has been the spike protein, a key immunogenic site and mediator of viral entry[2]. While many human targeted spike proteins have been characterized, far less is known about the structure and evolution of spike proteins originating from other species[3]. This gap in research knowledge is troubling as cross -species transmission of coronaviruses, which is mediated by the spike protein, have been frequently documented, with five of the seven human coronaviruses having emerged from non-human hosts within the last 20 years[2]. In light of this, we are undertaking phylogenetic and structural analysis of spike proteins from a range of viruses within the *Coronaviridae* family. With this we aim to reduce the gap in research by producing novel Cryo-Electron Microscopy structures of *Coronaviridae* spike proteins originating from animal hosts. This will be combined with structural, and sequence based phylogenetic analysis to fill in the gap in evolutionary knowledge within the *Coronaviridae* family[3]. This research hopes to identify broadly conserved sites for long-lasting, broad-spectrum treatments as well as predict the potential for future cross-species transmission from animal hosts[2]. With cross-species transmission of animal viruses increasing, research into the structure and evolution of viral spike proteins from a range of host species essential to global health[2,4].

1. COVID-19 Map - Johns Hopkins Coronavirus Resource Center. (2022). Retrieved 29 June 2022, from https://coronavirus.jhu.edu/map.html

2. Sauer, M., Tortorici, M., Park, Y., Walls, A., Homad, L., & Acton, O. et al. (2021). Structural basis for broad coronavirus neutralization. *Nature Structural &Amp; Molecular Biology*, *28*(6), 478-486. doi: 10.1038/s41594-021-00596-4

3. Zhang, S., Qiao, S., Yu, J., Zeng, J., Shan, S., & Tian, L. et al. (2021). Bat and pangolin coronavirus spike glycoprotein structures provide insights into SARS-CoV-2 evolution. *Nature Communications*, *12*(1). doi: 10.1038/s41467-021-21767-3

4. Geoghegan, J., Duchêne, S., & Holmes, E. (2017). Comparative analysis estimates the relative frequencies of co-divergence and cross-species transmission within viral families. *PLOS Pathogens*, *13*(2), e1006215. doi: 10.1371/journal.ppat.1006215

AM29: Chitooligosaccharide-based inks for 3D printing of hard tissue scaffolds

Rajabi, M.[1], Ali, A.[1], Cabral, J.[2], Saunderson, S.[3]

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Bone defects caused by congenital and genetic abnormalities, trauma, and infection place an enormous burden on global healthcare systems [1]. The limitations associated with current treatments, such as lack of bone grafts, have made tissue engineering an influential alternative for bone regeneration [2]. 3D printing is an advanced manufacturing technique, providing precise control over a scaffold's geometry as well as biomolecule distribution within a complex 3D construct [3].

Nonetheless, developing a printable ink with good fidelity and cellular support presents great challenges. In this study, for the first time, a photo-curable ink was formulated based on chitooligosaccharide (COS), which is a low molecular weight chitosan with higher water solubility. The COS-based ink precursors were synthesized by grafting amino groups of COS (13 kDa) with acrylate groups of polyethylene glycol (PEGDA, 700 Da) via Michael reaction. To improve the rheological properties of the ink, pluronic F127 was incorporated into the COS-PEGDA solutions. The hydrogel inks showed shear-thinning behaviour required for extrusion 3D printing and excellent printability by forming straight and uniform filaments as confirmed by light microscopy. UV photo-polymerization of each layer enhanced shape fidelity (resolution≈ 200 µm) and prevented the collapse of the subsequent printed layers. By increasing COS concentration up to 2 wt%, the swelling ratio decreased, the mechanical strength improved, and the degradation rate increased. MTT assay showed high cell viability of immortalized human bone mesenchymal stem cells (ihBMSCs) on these scaffolds. The 3D printed scaffolds containing 2 wt% COS improved osteogenic differentiation of ihBMSCs measured by alkaline phosphatase (ALP) activity and Alizarin Red S (ARS) staining, and also increased mineral formation after immersion in simulated body fluid (SBF) confirmed by scanning electron microscopy (SEM). We envision the developed COS-based inks can be used to 3D print complex tissue engineered scaffolds with high shape fidelity for hard tissue regeneration.

1. Gómez, S., et al., *Design and properties of 3D scaffolds for bone tissue engineering.* Acta biomaterialia, 2016. **42**: p. 341-350.
2. Wubneh, A., et al., *Current state of fabrication technologies and materials for bone tissue engineering.* Acta Biomaterialia, 2018. **80**: p. 1-30.
3. Rajabi, M., et al., *Chitosan hydrogels in 3D printing for biomedical applications.* Carbohydrate Polymers, 2021: p. 117768.

AM30: Simple methods to assess collagen self-assembly structure

Parker, K., Richena, M., Plowman, J., Middlewood, P., Harland, D.P., Deb-Choudhury, S.

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Collagen is a biologically derived, mechanically functional material with a multi-scalar organization. In nature, collagen is abundant, and it forms high-performance materials such as skin, bone or tendon. Collagen's ability to form networks; its inherent mechanical strength and biocompatibility, make engineered collagen materials attractive for applications in biomedicine and therapeutics. Harnessing the full potential of collagen into functional materials requires knowledge of how structured materials can be generated from collagen monomers by controlled fibril self-assembly. In this study, collagen monomers were isolated from bovine tendons using salt solubilisation [1]. Collagen monomers were allowed to self-assemble on the surface of epoxy resin, TEM grid and in Eppendorf tubes. A range of temperatures, relative humidity and incubation times were tested for the efficiency of collagen monomer association which were then prepared for TEM analysis to assess the nano-scale integrity of collagen fibril assembly. Inconsistent fibril formations were noticed on the epoxy surface and TEM grids, possibly due to the inefficient control of the humidity and temperature. The most successful monomeric associations were achieved in the Eppendorf tubes as the critical conditions required for fibrillogenesis were met.

1. Deb-Choudhury, S., et al., Effect of oxazolidine E on collagen fibril formation and stabilization of the collagen matrix. Journal of Agricultural and Food Chemistry, 2007. 55: p. 6813.

AM31: Novel thermoresponsive hydrogels (NTH) to prevent reactive gliosis after stroke

Mozammel Haque Bhuiyan[1,2], Azam Ali[1], Andrew Clarkson[2]

[1]Center for Bioengineering and Nanomedicine, Faculty of Dentistry, University of Otago, Dunedin, New Zealand, [2]Department of Anatomy, Brain Health Research Centre and Brain Research New Zealand, University of Otago, Dunedin, New Zealand

Stroke is the leading cause of adult disability worldwide. The primary reason for the lasting functional impairment following stroke is the brain's limited ability to regenerate after an injury. Treatment measures such as systemic delivery of drugs and growth factors is limited by blood brain barrier (BBB) and off-target effects. Recently, localized delivery of therapeutic molecules by biopolymer hydrogels have gained significant attention for the treatment of stroke.

In the present study, silk fibroin was combined with chitosan to prepare a novel injectable thermoresponsive hydrogel (NTH).

The NTH was physically, mechanically and chemically characterised to evaluate the suitability for injection into the brain. MTT assay and live-dead assay were performed to assess *in vitro* cytocompatibility using PC12 cells. Finally, NTH was injected into the infarct cavity of an ischemic stroke model to investigate *in vivo* biocompatibility and effects on reactive astrogliosis. Rheological analysis demonstrated that NTH retains a similar mechanics to that of brain tissue (~300 Pa) with an osmolality equivalent to that of cerebrospinal fluid (~290 mmol/kg). The *in vitro* cytocompatibility testing showed that NTH is nontoxic to PC12 cells with ~99% cell viability. The *in vivo* assessment revealed a smaller infarct cavity and decreased reactive astrogliosis as shown by the expression of glial fibrillary acidic protein (GFAP) and Iba1 in the peri-infarct region. These results suggest that NTH can be combined with drugs or growth factors as a potential therapeutic approach for stroke recovery.

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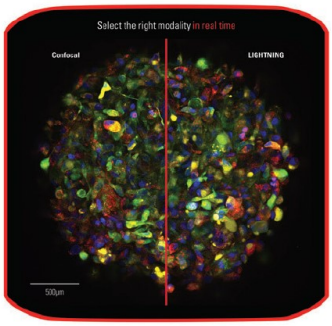

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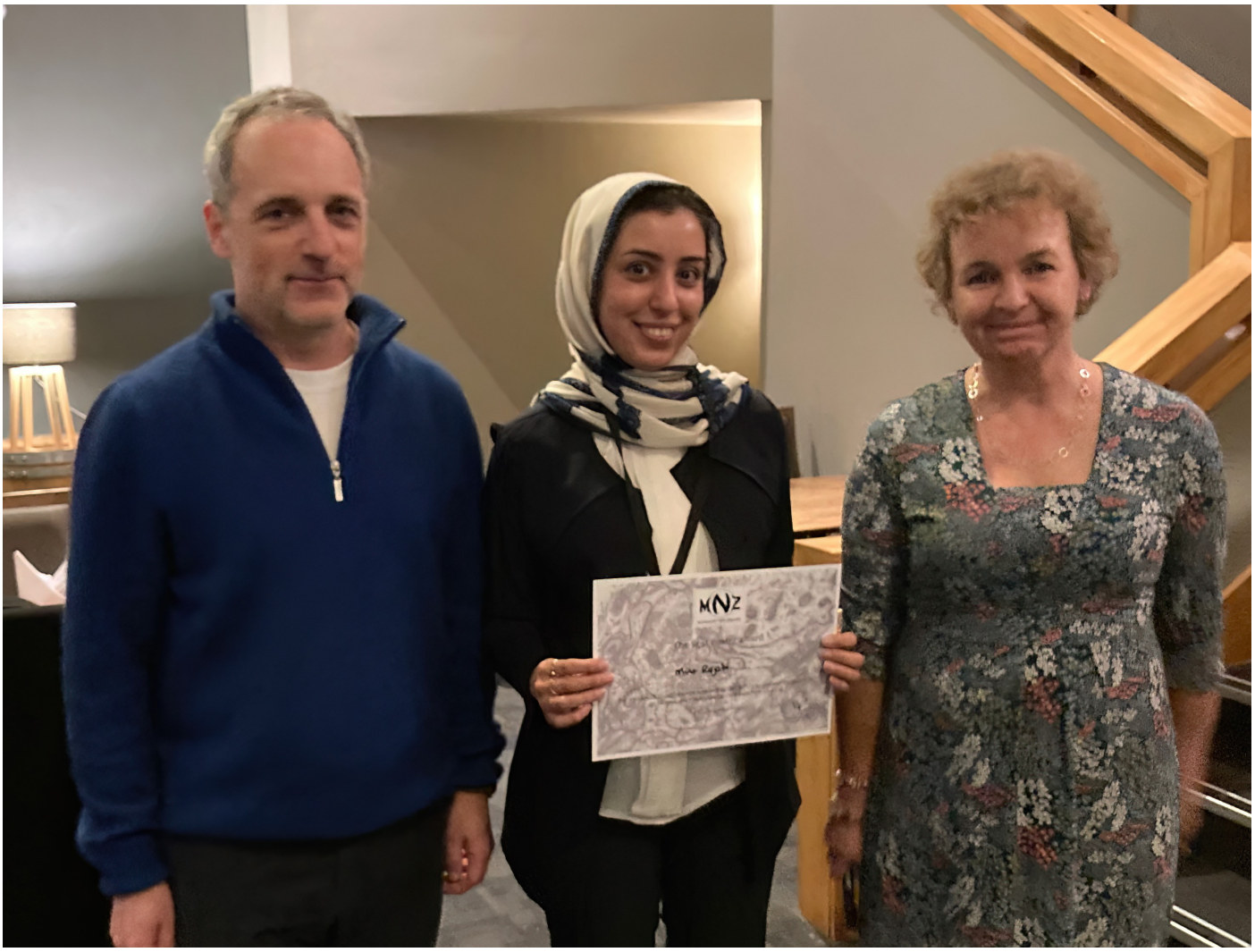
The Keith Williamson medal was awarded to Alice Eruera, University of Otago. Ka pai Alice.



The best student presentation was awarded to Mina Rajabi, University of Otago. Congratulations Mina.

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Contact Michael Loh Michael.Loh@fonterra.com

Microscopy Knowledge Exchange Visits

It our great pleasure to announce a new microscopy exchange scheme between Australia and New Zealand established in partnership with the Australian Microscopy and Microanalysis Society (AMMS) and Microscopy New Zealand (Microscopy NZ).

Facility staff will get the opportunity to participate in a visit to another facility to exchange training, expertise and mentoring skills to benefit their career, their colleagues and the host facility. The research experience and collaborative opportunity will benefit the successful applicants and enhance the support provided by the host facility to their users.

The funding will allow up to 9 exchange visits (up to $2,500ea) over the next 2 years. The applications will be assessed against the need of both the applicant and the institution, and not against the applicant's Curriculum Vitae. Both professional and academic staff are eligible.

https://micro.org.au/news/microscopy-knowledge-exchange-visits/

Microscopy Knowledge Exchange Visits

Anatomical Pathology, LabPlus, Auckland City Hospital, Te Toka Tumai, Auckland, Te Whatu Ora, Health New Zealand have the only laboratory team in New Zealand who are accredited by IANZ to perform diagnostic electron microscopy (EM).

The LabPlus EM team use the facilities provided by the Biomedical Imaging Research Unit (BIRU) at the Faculty of Medicine and Health Sciences, University of Auckland.

Earlier this year (2022) Anatomical Pathology, LabPlus was approached by Professor Murray Killingsworth of the University of New South Wales and New South Wales Health Pathology to ask if we would host a visit by Tzipi Cohen-Hyams of The Ingham Institute of Applied Medical Research, under the Knowledge Exchange programme sponsored by the Microscopy Societies of NZ and Australia.

Prof. Killingsworth and Tzipi Cohen Hyams are working on validating the application of High Resolution Scanning Electron Microscopy for use in diagnostic pathology (see paper below). Tzipi wished to visit a laboratory currently using TEM for diagnostic EM. Prof Killingsworth had previously visited the LabPlus EM team and recommended our laboratory for the visit.

Cohen-Hyams T; Mam K; Killingsworth MC, 2020, 'Scanning electron microscopy as a new tool for diagnostic pathology and cell biology', Micron, vol. 130, pp. 102797 - 102797, http://dx.doi.org/10.1016/j.micron.2019.102797

LabPlus agreed to host the visit, and Tzipi's application to Microscopy Australia/ Australian Microscopy and Microanalysis Society for this visit was approved. The visit took place during the week of 7-11 November.

Which was very good timing as this coincided with presentations on Super Resolution Microscopy hosted by BIRU on 7th of November.

Unfortunately the timing was not so good for the Team leader of the LabPlus EM team, Tamaki Inoue or the LabPlus EM Technical Specialist, Linda Graham, as unforeseen events prevented either from acting in person, as hosts to Tzipi. Hosting activities were carried out by the rest of the EM team and in particular by Catherine Gray, Grace Casabuena, and by Jacqui Ross of the BIRU.

Under their guidance the visit was very successful and culminated with a presentation on Friday 11th November by Tzipi Cohen-Hyams, presented to the Anatomical Pathology Registrars and Pathologists, plus the LabPlus EM team and BIRU. This was followed by a visit by many of the registrars to the EM facility in the BIRU.

Title of Tzipis Presentation below

Linda Graham

Novel translational applications of Electron microscopy in research and diagnostics

Tzipi Cohen Hyams[1-3] and Murray Killingsworth[1-4]

[1]Research scientific manager (CMF), Ingham Institute of Applied Medical Research

[2]Western Sydney University, School of Medicine

[3]UNSW Sydney, SWSCS Medicine

[4]NSW Health Pathology

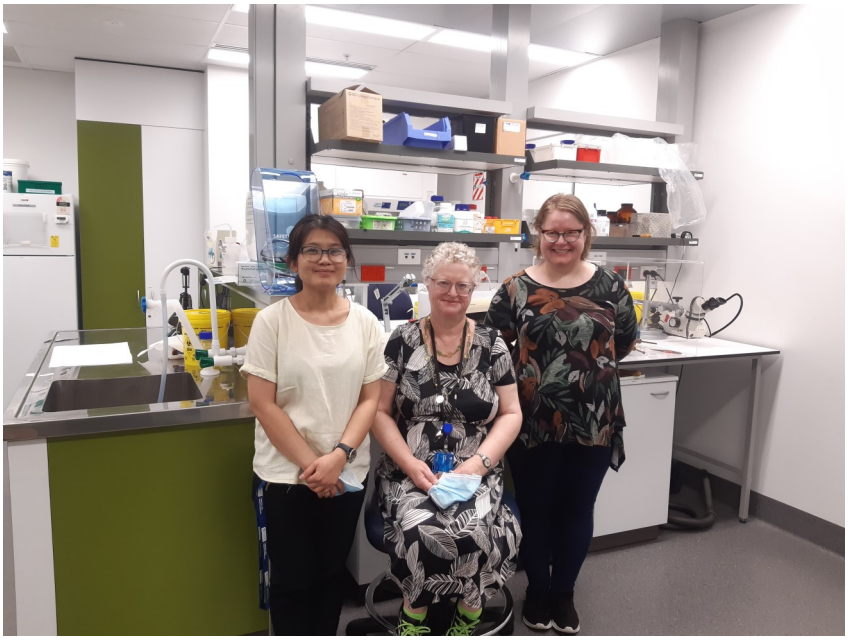
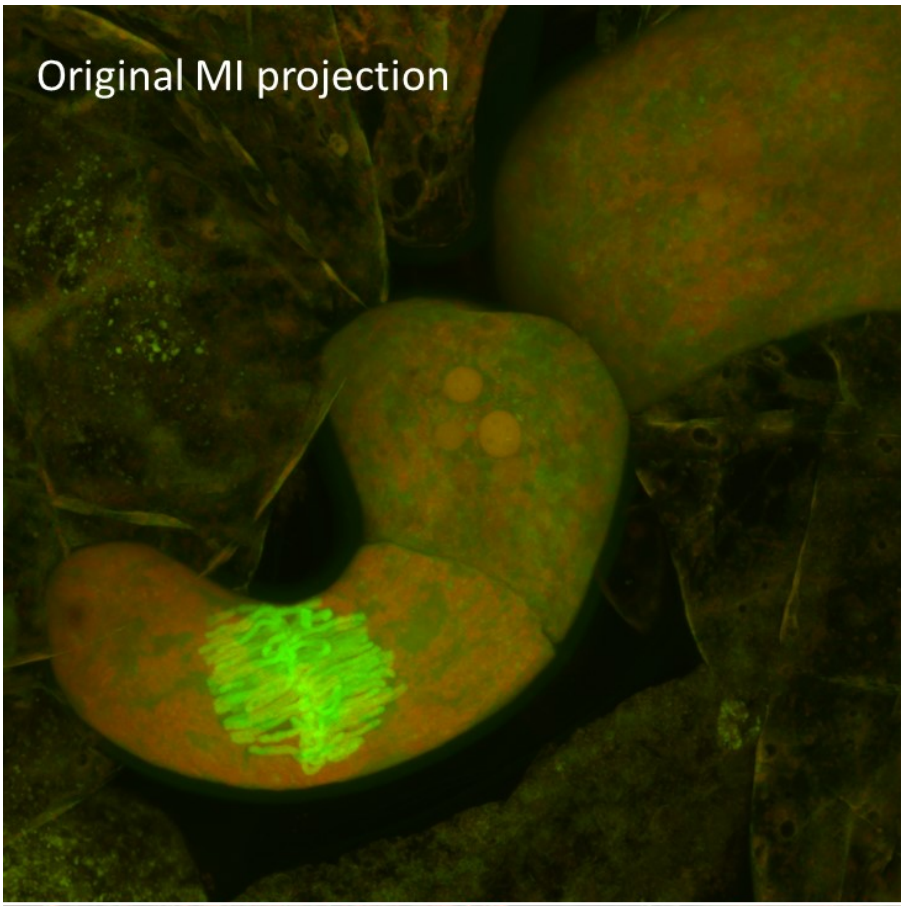
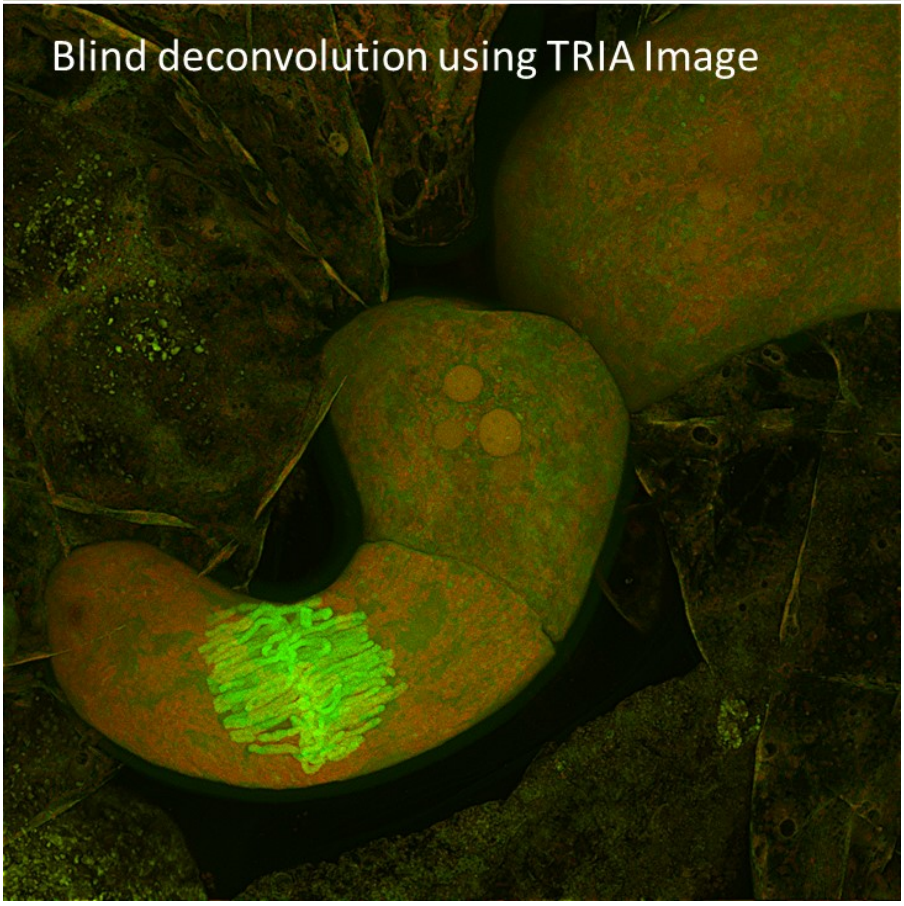


Image of EM team in Biru Electron Microscopy Laboratory. Grace Casabuena on left, Linda Graham seated in the middle and Catherine Gray on the right.

Tzipi Cohen Hyams, Western Sydney University Sydeny

Cool software—https://www.quarktet.com/joom/index.php/tria-image-pro





Lloyd Donaldson

New FESEM for Waikato

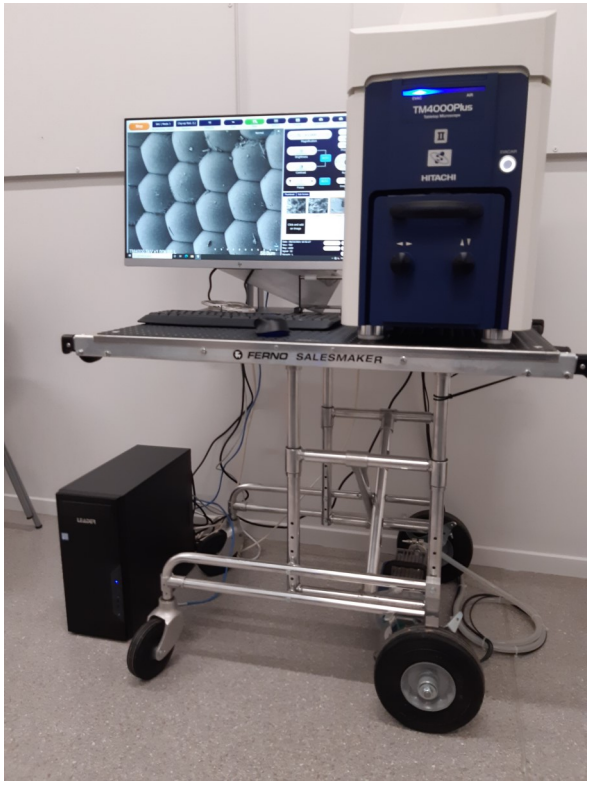
Hitachi Regulus SU8230 at University of Waikato

Well it has been a long road, courtesy of covid, but our new Hitachi Regulus SU8230 FE-SEM with Oxford Instruments AZtec Synergy EDS and EBSD System is now finally complete and running!

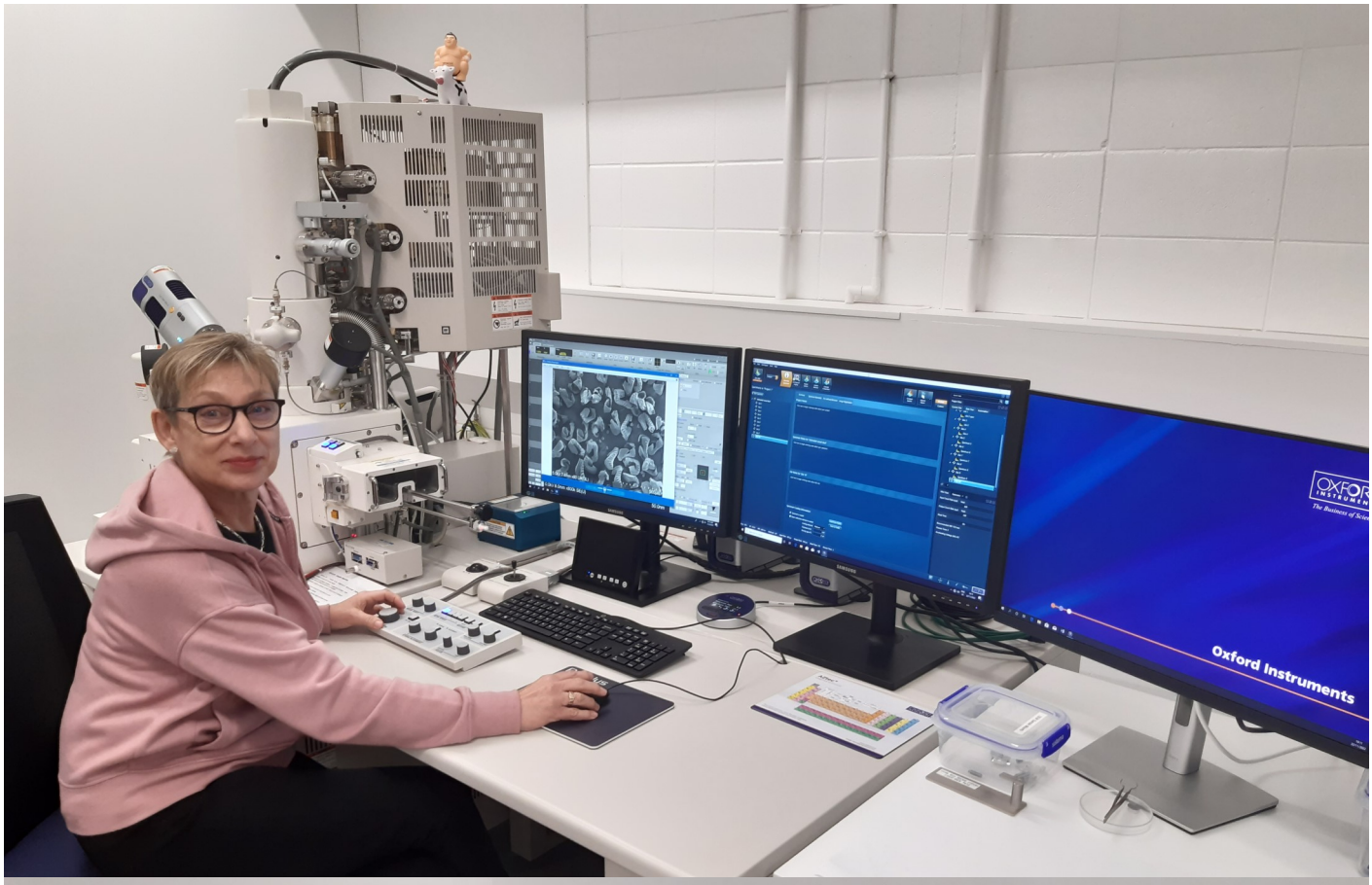
Installation began with the opening of travel between NZ and Australia in July 2021. That travel window then abruptly closed before the Oxford detectors could be installed. Nearly a full year went by before the Oxford installation was possible.

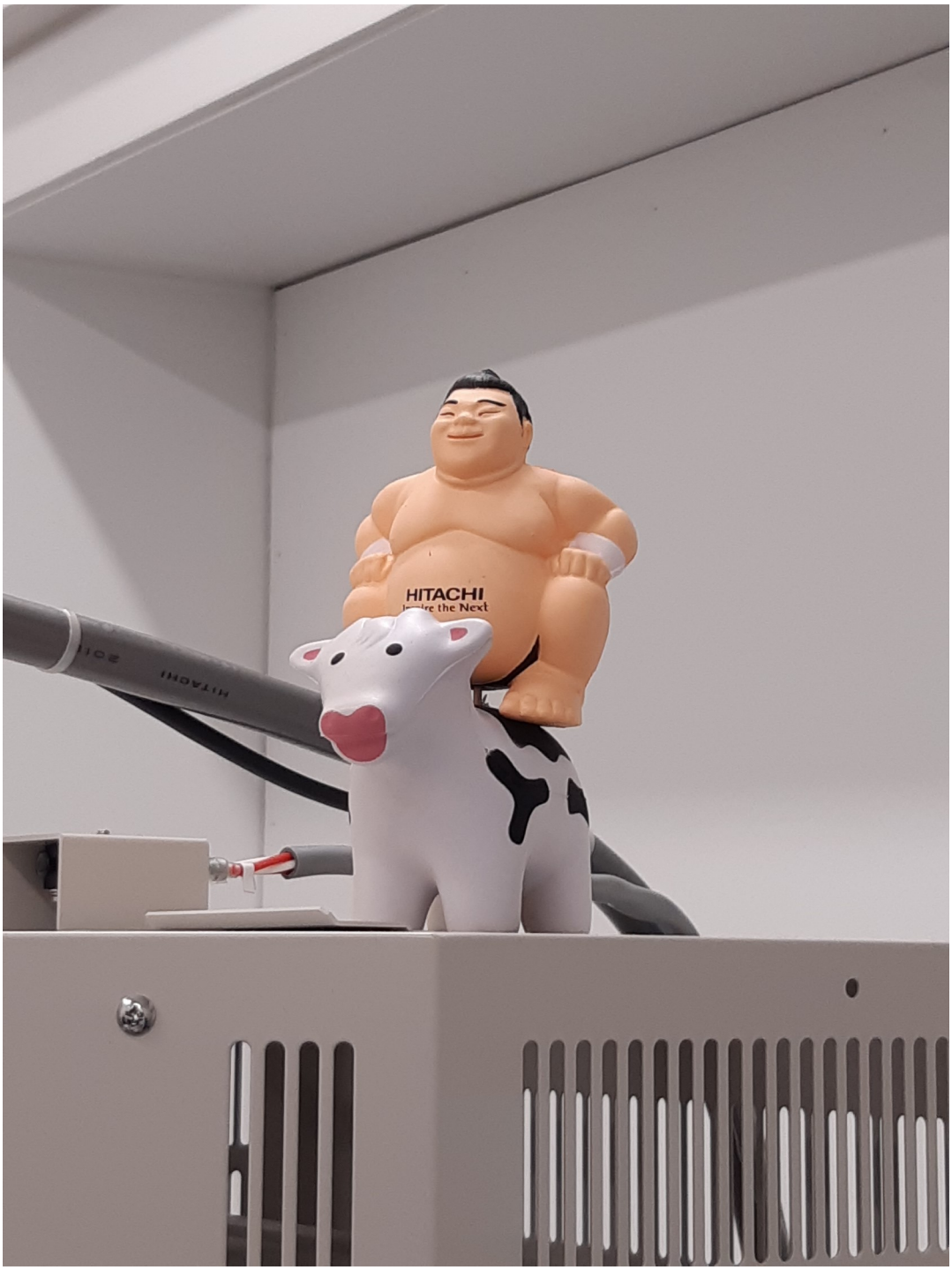
Along with the SEM and detectors we have an Hitachi IM4000 Ion Milling System, a ZoneUV Benchtop anti contaminator and a Quorum Q 150V ES Plus Ion Sputter Coater.

We have also taken possession of an Hitachi TM4000 Tabletop SEM. For those of you who heard Graeme Jones of NewSpec give a talk at our 2019 conference you may remember the drive and excitement around STEM outreach to schools. I am happy to report that we have a programme underway here at Waikato to do just that.



Helen Turner


Oxford Instruments


HITACHI

BIRU SEMINAR

Monday 7 November
1pm -2pm
FMHS Room 503-028

Super-resolution microscopy

Confocal SRRF microscopy of plant cell walls
Dr Lloyd Donaldson, Forest Genetics & Biotechnology, Scion

The nanostructure of plant cell walls is of interest for food and biomaterials applications but high-resolution techniques such as electron microscopy require the sample to be dry or frozen and hence changed from the natural state. Light microscopy allows examination of cell walls in the wet state but with limited resolution. We developed a protocol for high-resolution imaging of wet cell walls using confocal microscopy and SRRF [super-resolution radial fluctuations] based super-resolution. We compared autofluorescence with acriflavin staining for lignin and rhodamine B staining for lignin/porosity visualisation.

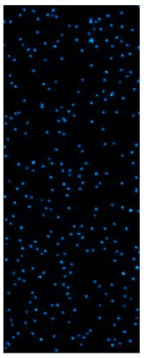
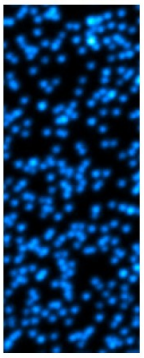
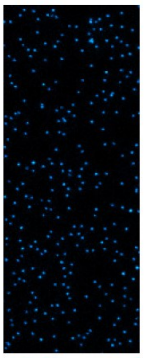
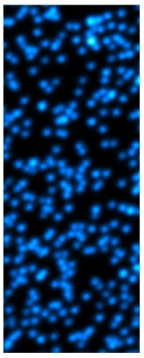
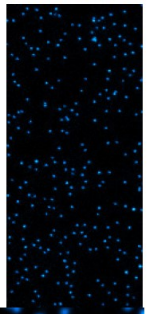
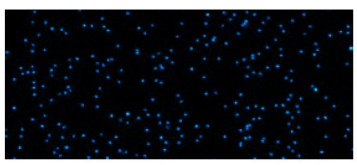
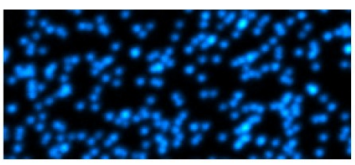
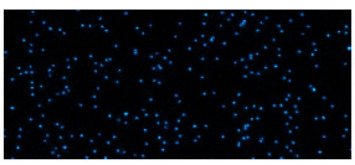
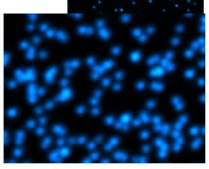
SRRF microscopy offers advantages over other super-resolution techniques in that no specialised hardware is needed and the technique can be used with any fluorescent stain as well as with autofluorescence which is particularly important for plant tissue imaging. SRRF microscopy offers advantages over other super-resolution techniques in that no specialised hardware is needed and the technique can be used with any fluorescent stain as well as with autofluorescence which is particularly important for plant tissue imaging. Images are acquired as a sequence of 100 images of the same location. These data are then processed with ImageJ software using a computer with graphics acceleration.

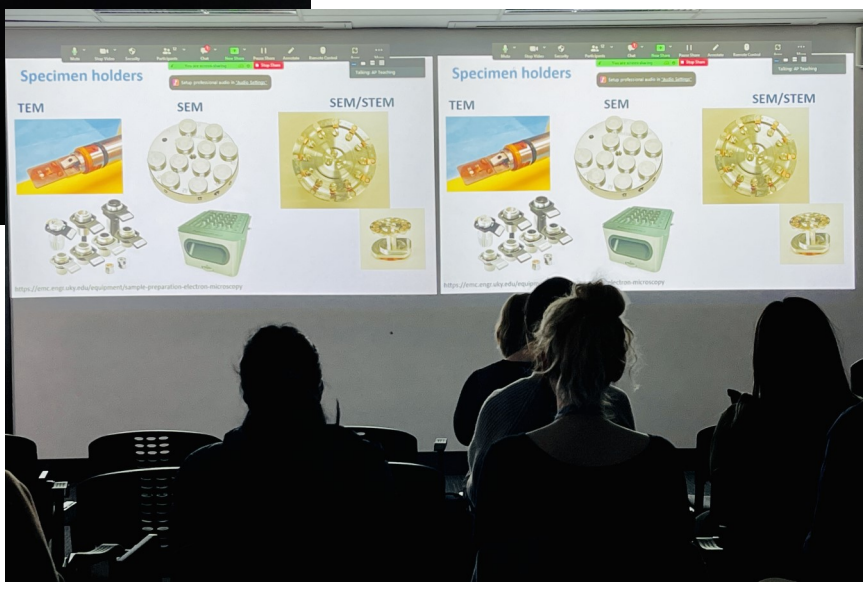
Ready, STEDy, go – first steps into STED microscopy
Jacqueline Ross, Biomedical Imaging Research Unit

Researchers worldwide continue to demand improvements in technology in order to answer complex biological questions including in the field of microscopy. Increasingly, higher level technology is expected to be used. Stimulated emission depletion (STED) microscopy is reported to be able to achieve lateral resolution of 20 - 50nm.

An Abberior Facility Line STED microscope, funded through The University of Auckland Shared Research Infrastructure Fund, was installed in the Biomedical Imaging Research Unit last year and is available for use.

Researchers who have experience with immunofluorescence labelling and other fluorescence labelling for widefield and confocal microscopy will have an advantage in using the system, however there are some changes required to specimen preparation such as fluorophore and mountant choice. Learnings from the experience to date will be reported, which should provide useful guidance to those who are interested in using the technology. Preliminary data from the system will be presented.


Specimen holders
TEM
SEM
SEM/STEM
Specimen holders
TEM
SEM
SEM/STEM

Post-conference workshop at Otago University sponsored by Zeiss

The Otago Micro and Nano Imaging Unit have recently installed a ZEISS Sigma 300VP with Serial Block Face (SBF). A one-day workshop was organized by the Microscopy New Zealand (MNZ) Society on 2nd September 2022 at the University of Otago, Dunedin. Approximately, 30 students, postgraduates and research investigators from various institutions across New Zealand participated in the workshop. Dr Philipp Bastians and Tan Bee Yi from ZEISS Research Microscopy Solutions conducted this workshop.

In the morning session, Dr Philipp Bastins, introduced us to the operational principles of SBF. This was followed by the multi-step workflow processes including a few tips on the sample preparation protocols. Dr Bastins also illustrated the power of SBF to understand and/or resolve interesting biological problems, especially of the central nervous system (reference given below). Towards the end, he outlined a few challenges associated with using the SBF. For instance, if samples are extremely conductive, it causes overcharging and image distortions. However, the nitrogen gas, used for focal charge compensation, helps to de-distort extremely conductive samples and keep the sample/stage stable. Next, Tan Bee Yi introduced us to the world of segmentation. The Zeiss offers Zen connect and Arivis for segmenting a range of data captured from confocal, electron and correlative microscopes. Later in the afternoon, she also showed the capability of both the software to segment large data sets and provided us with a one-month free trial for the Arivis software.

What fascinated me the most was the hands-on training for using the SBF. This was helpful to understand the capabilities and functions of this state-of-the-art equipment. Especially, the ability to capture different regions of interest within a given selection with varying resolutions will enable the collection of data to the fullest potential. In addition to thoroughly enjoying the workshop, this provided me with an opportunity to network with microscopy peers and experts across New Zealand. I wish to take this opportunity to thank the MNZ for organizing such an amazing workshop.

Few references mentioned

Boergens, K., Berning, M., Bocklisch, T. *et al.* webKnossos: efficient online 3D data annotation for connectomics. *Nat Methods* **14**, 691–694 (2017). https://doi.org/10.1038/nmeth.4331

Schmidt, H., *et al.* Axonal synapse sorting in medial entorhinal cortex. *Nature* **549**, 469–475 (2017). https://doi.org/10.1038/nature24005

Fabian N. et al. Volume EM Reconstruction of Spinal Cord Reveals Wiring Specificity in Speed-Related Motor Circuits. Cell Reports, 23(10),2942-2954 (2018). https://doi.org/10.1016/j.celrep.2018.05.023.

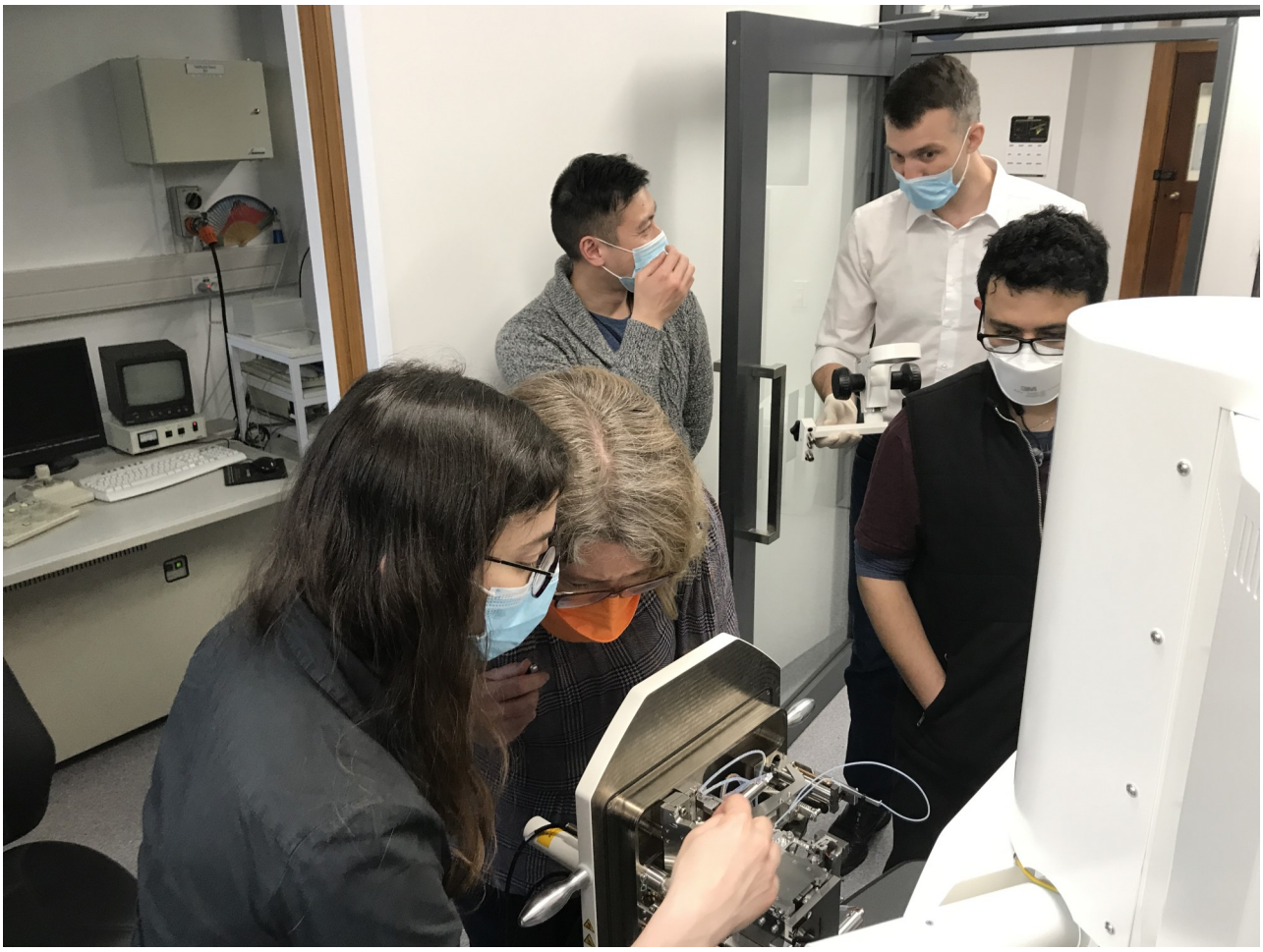
Deerinck, T. J., et al. High-performance serial block-face SEM of nonconductive biological samples enabled by focal gas injection-based charge compensation. J Microsc, 270, 142-149 (2018).

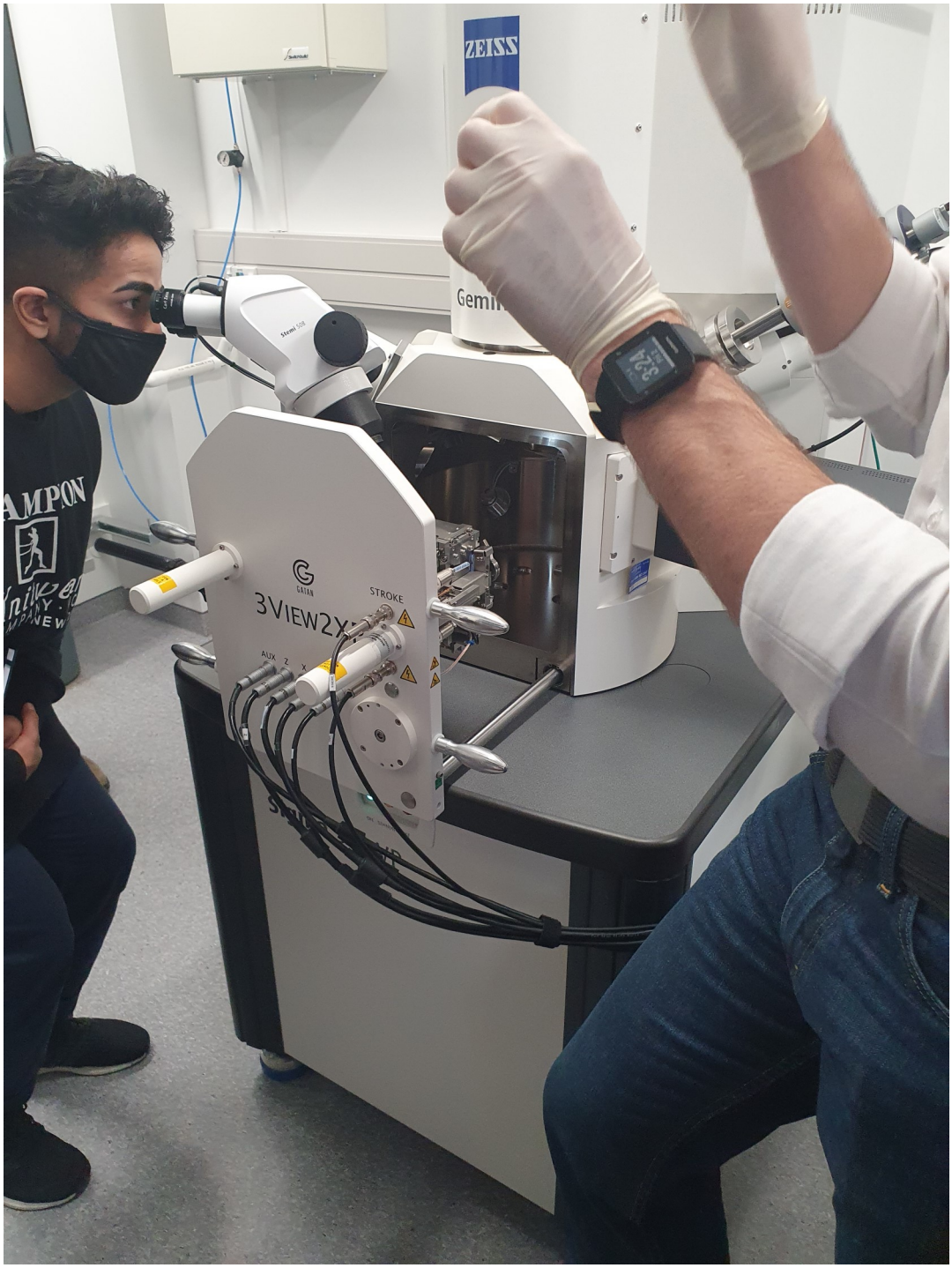
Susaki EA, Ueda HR. Whole-body and Whole-Organ Clearing and Imaging Techniques with Single-Cell Resolution: Toward Organism-Level Systems Biology in Mammals. Cell Chem Biol., 23(1):137-157 (2016). doi: 10.1016/j.chembiol.2015.11.009. PMID: 26933741.

Smith JJ, Timoshevskiy VA & Saraceno C. Programmed DNA Elimination in Vertebrates. Annu Rev Anim Biosci., 16;9:173-201 (2021). doi: 10.1146/annurev-animal-061220-023220.

Sai Velamoor

Post-conference workshop at Otago University sponsored by Zeiss

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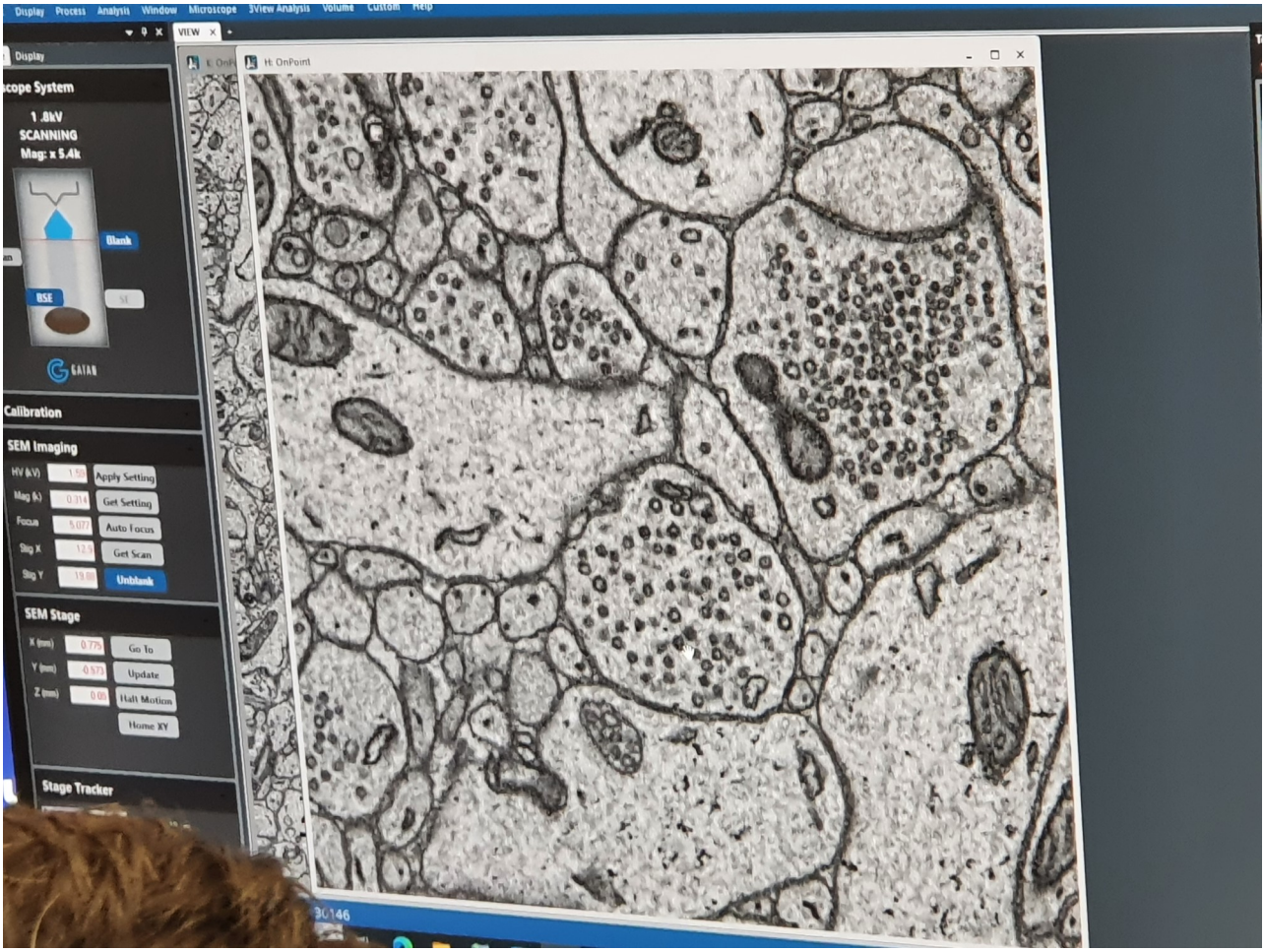
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Richard Easingwood & Duane Harland

Conferences in 2023

20th Advanced Imaging Methods (AIM) Workshop - 2023

We return to a live in-person event in Berkeley on January 24 - 26.

The Advanced Imaging Methods Workshop is a 3-day event focused on new and emerging optical microscopy techniques and their applications with a special focus on time-resolved techniques.

The *20th Annual Advanced Imaging Methods Workshop* is a 3-day event focused on new and emerging optical microscopy techniques and their applications with a special focus on time-resolved techniques hosted by the CRL Molecular Imaging Center at UC Berkeley.

This workshop brings together internationally-renowned researchers in a broad variety of fields including biology, chemistry, physics, engineering, probe design, cellular biology, neuroscience, optics, metabolism and more. Past speakers have included six Nobel Laureates: Roger Tsien, WE Moerner, Stephen Hell, Eric Betzig, Steven Chu, and Carolyn Bertozzi. This year we will have sessions on fluorescence lifetime imaging (FLIM), neuroscience, probe development, *in vivo* imaging, clearing and expansion, breakout workshops on data management, and more!

In addition to invited talks from leaders in the field, AIM2023 will include real lab demonstrations on campus, sponsor demonstrations, software tutorials, tours of UC Berkeley state-of-the-art imaging facilities, and a special reception at the outdoor lounge (weather permitting). This year will also bring back the popular poster session with prizes for student presenters. AIM includes gourmet lunch on-site each day, coffee breaks, and plenty of opportunities for interactions among participants.

The workshop is organized by UCal Berkeley's Cancer Research Laboratory, Becker & Hickl and Boston Electronics.

https://www.eventbrite.com/e/20th-annual-advanced-imaging-methods-workshop-tickets-438774776167

Conferences in 2023

The 27th Australian Conference on Microscopy and Microanalysis is committed to being a face-to-face event that will bring together colleagues across the life and physical sciences, to work towards new visions and goals in microscopy. We take pride in putting together a strong program for the conference based on advances in electron and light microscopy and microscopy-enabled research in the life sciences and physical sciences. The conference will include oral and poster presentation sessions, pre-conference workshops, Special Interest Group-focussed sessions, trade displays and social events.

https://acmm27.org/

Porto, Portugal, Sunday April 2 to Wednesday April 5, 2023

We would like to announce FOM2023, the next conference in the FOM series. FOM2023 will take place in Porto, Portugal, from Sunday April 2 to Wednesday April 5, 2023. Please note that this will be in the week before Easter 2023.

The FOM2023 conference will take place at Centro de Congressos da Alfândega do Porto, Rua Nova da Alfândega, Edifício da Alfândega, Porto, Portugal.

FOM2023 continues a long-standing (since 1988), yearly conference series on the latest innovations and developments in (optical) microscopy and their application in biology, medicine, and the material sciences.

https://www.focusonmicroscopy.org/

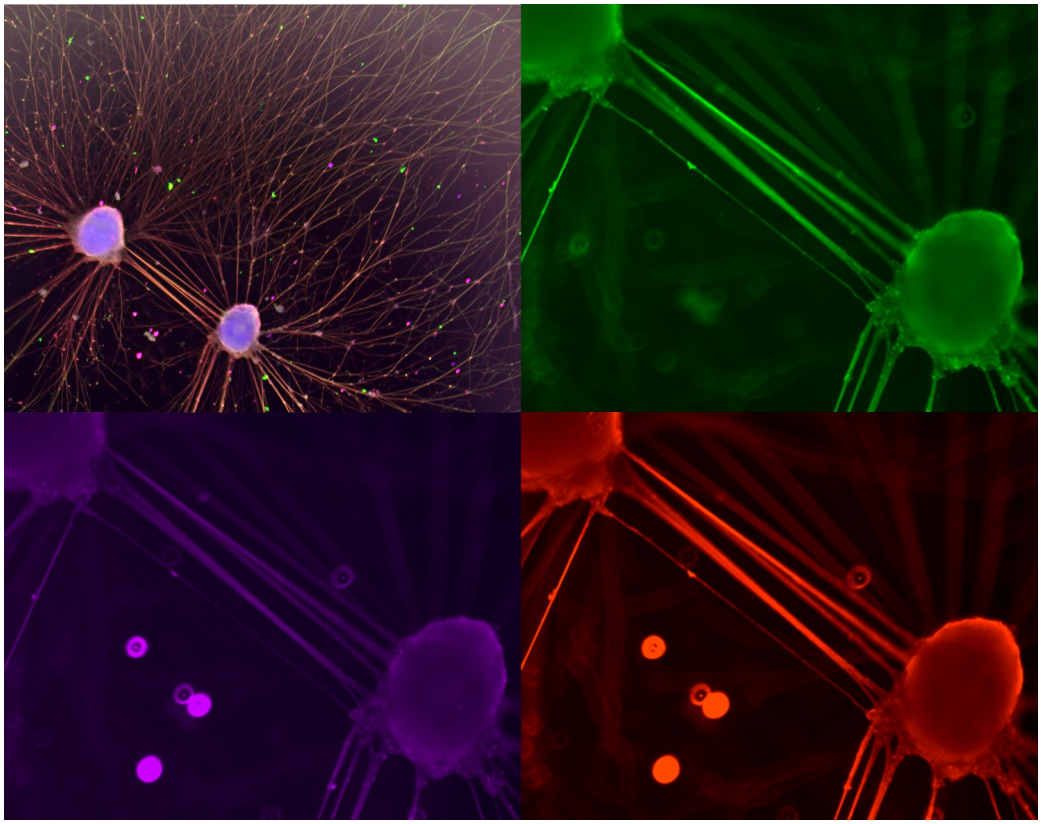
BIRU Imaging Awards

And the winner is…."Human Connection!"

The Biomedical Imaging Research Unit at The University of Auckland runs an annual image competition. There are 4 categories, Light microscopy (all types of transmitted light and wide field fluorescence), Confocal microscopy, Electron microscopy (SEM and TEM) and Visualisation and Analysis (processed images, time-lapse and medical imaging). This year, the Light Microscopy category winner and recipient of the Hilary Holloway Prize for best image was Kyrah Thumbadoo from the School of Biological Sciences. The prize is named after Hilary Holloway, who was a long serving BIRU staff member and member of Microscopy New Zealand Inc. who retired in 2019.

Her image was acquired using an EVOS FL microscope with 20x magnification.

The image shows IPSC-derived motor neurons that were successfully differentiated from fibroblasts of a person with Charcot-Marie Tooth disease, a peripheral neuropathy. Two neurospheres formed robust links to one another and outward, creating highly connective axonal branches as marked by neuron-specific cytoskeletal elements beta III tubulin (red) and neurofilaments (green, magenta). The fluorophores were Alexa Fluors 488, 594 and 647.



The judges were unanimous in their decision, and it seemed also appropriate given that the name and subject of the image fitted the current environment where people are returning to face-to-face contact and making connections. The results of the image competition were announced at the BIRU Research Celebration on Thursday 24 November.

Jacqui Ross

BIRU Imaging Awards

This year, the Light Microscopy category winner and recipient of the Hilary Holloway Prize for best image was Kyrah Thumbadoo from the School of Biological Sciences.

Enjoy reading this newsletter? Become an MNZ member—only $20. Join at the link below for 2023.

http://microscopynz.co.nz/membership.html