
Australasian
Cytometry
Society
ACS2024
CONFERENCE
HOBART TASMANIA
20–23 OCTOBER 2024
CONFERENCE HANDBOOK

CONTENTS

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CONFERENCE ORGANISING COMMITTEE

Convenor and Clinical Program Lead

- Susan Wright

Research Program Lead

- Eva Orlowski-Oliver

ACS Executive Committee

- Maggie Wang, President
- Robert Salomon, President Elect
- Joanna Roberts, Treasurer
- Henry Hui, Secretary

ACS 2024 HOBART ORGANISING COMMITTEE

Local Organising Committee

- Chrissie Ong
- Deni Cupit
- Oliver Eltherington
- Shayli Harris
- Emily Hinds
- Deirdre Tuck

ACS 2024 CONFERENCE MANAGERS

Leishman Associates

Morgan Flockhart

227 Collins Street

Hobart TAS

morgan@laevents.com.au

Leishman
it's all about people

WELCOME FROM THE CONVENOR

On behalf of the ACS 2024 Organising Committee, welcome to Hobart!

We are delighted to host you for the in-person meeting here in Hobart and we thank you for joining us. We are fortunate enough to be hosting the meeting at Hotel Grand Chancellor Hobart, which is located at Hobart's most premium and much-loved area - Hobart waterfront.

Our committee has worked hard on delivering a robust and interesting program, and we thank our invited speakers, some of whom have travelled long distances to be here. Additionally, we thank all delegates who submitted abstracts for assisting us deliver quality content in clinical, research, and translational scientific endeavours.

A huge thank you goes to our industry partners who continue to support the ACS and its Meeting. The in-person meeting would be impossible without their contribution, and we encourage you to visit their booths, engage with industry delegates, and of course get your mobile app passport stamped for a chance to win some fantastic prizes.

In 2024 our committee has been fervently working away to deliver an always popular social program. This year delegates can soak up the Hobart weather whilst gazing out to beautiful Hobart waterfront from Hotel Grand Chancellor, and also enjoy a dinner with some of your peers at Waterline Berthing Deck, located at Brooke Street Pier.

Thank you to ACS council and committee members for dedicating your time and efforts into making this conference a successful vehicle to disseminate knowledge and to promote research, development and applications in flow cytometry.

Enjoy,

Susan Wright
ACS2024 Convenor

ACS 2024 PRESIDENT'S WELCOME

Dear ACS members,

With immense pleasure and honour, I extend a warm and heartfelt welcome to all of you gathered here in the picturesque and vibrant city of Hobart for the 2024 Australasian Cytometry Society Annual Conference. As the President of this esteemed society, I am delighted to see so many brilliant minds come together to share knowledge, expertise, and ideas in the ever-evolving field of cytometry.

ACS has continuously strived to push the boundaries of science and technology, paving the way for ground-breaking advancements in cytometry. This conference serves as a testament to the dedication and passion of each member of ACS, whose efforts contribute to promote development of cytometry applications in research and clinical field and the dissemination of cytometry knowledge.

Over the course of this conference, we have an exceptional lineup of renowned speakers who will share how cytometry enhanced/enabled their cutting-edge research findings and innovative applications. We are confident that the knowledge exchanged during this event will undoubtedly drive progress in our field and bring us closer to transformative breakthroughs.

Our annual conference presents a unique opportunity to strengthen existing professional bonds and build new connections with other colleagues. As we embrace diversity in perspectives and experiences, let us foster an inclusive and supportive environment where all participants feel valued and heard.
I must extend my heartfelt appreciation to the organising committee, led by Susan Wright, as well as to the sponsors and volunteers whose tireless efforts have brought this event to fruition. Your dedication has made this gathering possible, and I am certain that it will be a resounding success.

I encourage all attendees to take full advantage of this enriching experience and actively engage in discussions, ask questions, and share your own expertise. By actively participating, each one of you contributes to the collective knowledge and creates an environment conducive to innovation and progress.

Hobart, with its stunning landscapes, warm hospitality, and rich cultural heritage, provides the perfect place for this special occasion. I wish while you engage in insightful discussions, attend enlightening workshops, and forge new collaborations, you could take a moment to revel in the natural beauty that surrounds us, as inspiration often arises from the most unexpected places.

Let us embrace this unique opportunity to network, learn, and collaborate with our peers, leaving Hobart with newfound insights and lasting connections. May our time together in Hobart be filled with academic excellence, cultural enrichment, and memories that will endure for a lifetime.

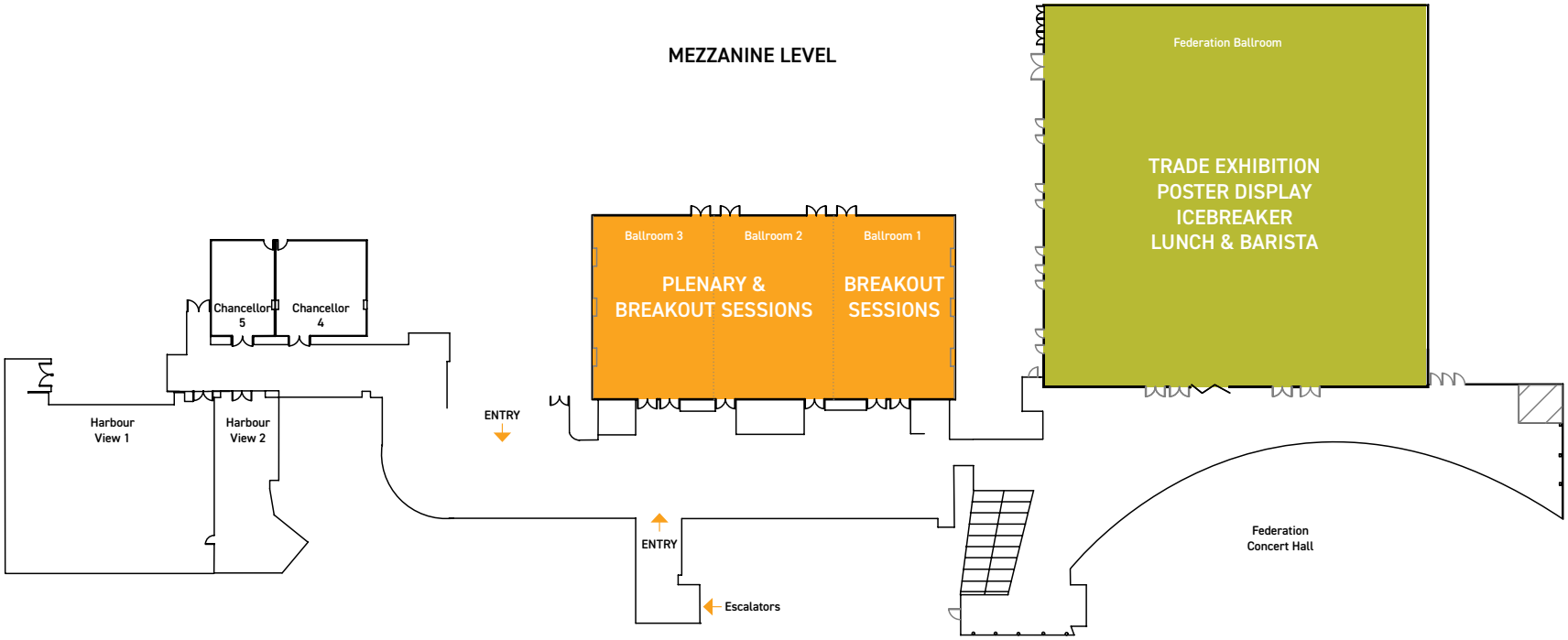
Thank you for being a part of this extraordinary event, and I look forward to meeting each one of you and learning from your exceptional contributions.

With warm regards,

Dr Xin Maggie Wang
President, Australasian Cytometry Society

VENUE FLOOR PLAN

HOTEL GRAND CHANCELLOR



GENERAL INFORMATION

CONFERENCE SECRETARIAT

Leishman Associates

227 Collins Street
Hobart 7000

Ph: 03 6234 7844

Email: paula@laevents.com.au

VENUE DETAILS

Hotel Grand Chancellor

1 Davey Street, Hobart, TAS 7000

+61 (3) 6235 4535

REGISTRATION DESK LOCATION AND OPENING HOURS

Sunday 20th October

Menzies Medical Research Centre Entrance Level Foyer

17 Liverpool St, Hobart
Registration opens at 8:30 am - 4:00 pm

Hotel Grand Chancellor, Mezzanine Level

1 Davey Street, Hobart
Registration opens at 4.30 pm

Monday 21st October

Mezzanine Foyer, Hotel Grand Chancellor

8:00 am - 5:00 pm

Tuesday 22nd October

8:00 am - 5:00 pm

Wednesday 23rd October

8:30 am - 1:00 pm

EMERGENCY CONTACT DETAILS

If you need to contact the organisers during the conference, please call 0419 824 844.

AUSTRALIAN CYTOMETRY SOCIETY 2024 ANNUAL GENERAL MEETING

The Australian Cytometry Society Annual General Meeting will be held from 1:15 pm - 2:30 pm on Wednesday 23 October.

CONCURRENT SESSION SELECTIONS

You may attend the concurrent session of your choice.

INFORMATION FOR SPEAKERS

There will be a dedicated AV tech available during set times of the conference. Speakers should present themselves to the Speakers Preparation Room, at least two hours prior to their scheduled presentation time, to upload their presentation.

Where possible, speakers are requested to assemble in their session room 5 minutes before the commencement of the session to meet with the session chair and familiarise themselves with the room and the AV equipment. There will be facility to test and modify your presentation if required within the Speakers Preparation Room. All presentations should be prepared in 16:9 ratio.

GENERAL INFORMATION

INFORMATION FOR POSTER PRESENTERS

Presenters need to adhere the poster to the poster board by 10am on Monday, 21st October and retrieve their poster by 1pm on Wednesday, 23rd October. Uncollected posters will be disposed of.

Poster Size

All poster authors are requested to prepare their posters in an A0 format (1189mm high x 841mm wide).

Please prepare your poster in PORTRAIT FORMAT.

Affixing Your Poster

We will be using display boards that will require Velcro adhesive. Please see the team at the registration desk, we will have supplies available.

CATERING

Pre-Conference Workshop

All catering during the workshop will be served in the Room 113 -Kitchen/Foyer Area. Refreshments will be served throughout the day.

During the conference

All catering during the conference will be served in the Federation Ballroom, Exhibition Trade Area. If you have indicated that you have a dietary requirement in your registration, please speak with the friendly staff from the venue who will direct you to the dietaries table.

SPECIAL REQUIREMENTS

If you have special dietary or mobility requirements that you have not previously advised, please see the registration team at your earliest convenience. For pre-arranged special dietary requirements please make yourself known to the waiting staff at all functions.

NETWORKING AND SOCIAL EVENTS

ICE BREAKER

DATE **Sunday 20 October 2024**

TIME **5:30 pm - 7:00 pm**

VENUE **Mezzanine Level, Hotel Grand Chancellor**

DRESS CODE **Smart Casual**

Inclusive in all Full Registrations, RSVP required

Guest/Day Delegate Ticket Price | **$80**

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POSTER NIGHT

DATE **Monday 21 October 2024**

TIME **5:30 pm - 6:30 pm**

VENUE **Exhibition Hall, Hotel Grand Chancellor**

DRESS CODE **Smart Casual**

Inclusive in all Full Registrations, RSVP required

Guest/Day Delegate Ticket Price | **$75**

GALA DINNER

DATE **Tuesday 22 October 2024**

TIME **6:30 pm - 10:30 pm**

VENUE **Waterline, Brooke Street Pier, Lower Level 12, Franklin Wharf**

DRESS CODE **Cocktail**

8-minute walk from the Hotel Grand Chancellor.

The theme for the ACS2024 dinner is Aurora Australis, otherwise known as the Southern Lights, with Tasmania being one of the most fortunate vantage points in the world to view this phenomenon.

We encourage you to start planning your attire – Cocktail Dress, according to the spectrum of colours inherent to the aurora - pinks, purples, reds and greens, aquas, and everything in between!

Inclusive in all Full Registrations, RSVP required

Guest/Day Delegate Ticket Price | **$140**

AFTER PARTY

DATE **Tuesday 22 October 2024**

TIME **Following the Gala Dinner**

VENUE **Twisted Lime, 1/112 Liverpool Street, Hobart**

After an unforgettable gala dinner, let's extend the night into a night of lively music at Twisted Lime.

PROGRAM

SUNDAY 20 OCTOBER 2024

Australasian Cytometry Society

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Pre-conference Workshops				Menzies Institute for Medical Research
0830	Registration			Foyer, 17 Liverpool Street Hobart
Room	Lecture Theatre		Room 205	
0900-1030	**How to go with the flow: an introduction to flow cytometry** **Kate Pilkington, Malaghan Institute of Medical Research**		**Science Communication** **Olly Dove** **That's What I call Science** Limited to 30 people	
1030-1100	**Morning Refreshments**			Room 113 -Kitchen/Foyer Area
1100-1230	**CLINICAL WORKSHOP 2A**	**RESEARCH WORKSHOP 2B**	**SHARED RESOURCE LAB WORKSHOP 2C**	**BUILD YOUR OWN CYTOMETER**
Room	Lecture Theatre	Room 207/208	Room 205	Room 206
1100-1215	**Technical challenges in flow cytometry** **Dr. Ben Hedley, Victoria Hospital, Canada**	**Interrogating immune cells from human tissue, including skin, type II mucosae and intestine: Considerations and consequences of isolation protocols to develop a high-parameter Optimized Multicolor Immunofluorescence Panels (OMIP)** **Dr. Kirstie Bertram, The Westmead Institute for Medical Research**	**Cell sorting best practice, tips and recommendations for maximum recovery of the populations that you want** **Dr. Vanta Jameson, University of Melbourne**	**Build your own cytometry** **Suat Dervish** Limited to 24 people
1215-1230	**Viable CD34 QAP** **Scott Ragg, Tasmanian Health Service**			
1230-1300	**Lunch and Learn – Join one of these sessions; and lunch is on our sponsors**			
	LL1 – SYSMEX	**LL2 – SARTORIUS**	**LL3- MILLENNIUM SCIENCE**	
Room	Lecture Theatre	Room 205	Room 207/208	
	Integration of high-parameter analysis and multi-autofluorescence on the FP7000 Spectral Cell Sorter **Dr. Makoto Ishihara, Sony Biotechnology**	**iQue® High-Throughput Screening by Cytometry** **Nabiha Elias, PhD**	**Unleashing Advanced Multicolor Analysis with NovoCyte Opteon: Full Spectrum Flow Cytometry at Its Best** **Federica Tomay, PhD, Agilent**	
1300-1330	**Lunch Break** Thanks to our sponsors for a delicious lunch. Available for those who attend a lunch and learn session.			Room 113 - Kitchen/Foyer Area

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1330-1500	CLINICAL WORKSHOP 3A	RESEARCH WORKSHOP 3B	SHARED RESOURCE LAB WORKSHOP 3C	BUILD YOUR OWN CYTOMETER
Room	Lecture Theatre	Room 207/208	Room 205	Room 206
1330-1405	**Introduction to the Concepts of Computational High-Dimensional Flow Analysis and Practical** **Dr. Paul D. Simonson, Weill Cornell Medicine**	**Making Sense of Cytometry data in R** **Dr. Felix Marsh-Wakefield, Centenary Institute**	**Biosafety: What is it to you?** **Avrill Aspland, University of Sydney**	(continued from morning session) **Suat Dervish**
1405-1425	**Update on the National Certification Scheme for the Medical Laboratory Scientific Workforce** **Tina Pham, St Vincent's Hospital**			
1425-1500	**RCPAQAP – challenges in Immunophenotyping EQA** **Loriza Khan, RCPAQAP**			
1500-1530	**Afternoon Refreshments**			Room 113 - Kitchen/Foyer Area
1630	Registration Desk open			Federation Foyer, Hotel Grand Chancellor
1530-1700	**CLINICAL WORKSHOP 4A**	**RESEARCH WORKSHOP 4B**	**SHARED RESOURCE LAB WORKSHOP 4C**	**THANK YOU TO OUR PLATINUM SPONSORS** BECKMAN COULTER Life Sciences CYTEK® TRANSCEND THE CONVENTIONAL
Room	Lecture Theatre	Room 207/208	Room 205	
	ASK THE EXPERTS **Panel Discussion**	**Introduction to Dimensionality Reduction Algorithms for Flow Cytometry** **Dr David Novo-Lake, Denovo Research**	**Incorporating R&D in a Shared Resource Laboratory** **Dr. Rui Gardner, Memorial Sloan Kettering Cancer Center**	
1730-1900	**Icebreaker Event**			Federation Ballroom, Hotel Grand Chancellor
1730-1800	Arrival Drinks *Proudly sponsored by our Platinum Sponsors CYTEK & BECKMAN COULTER*			

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SUPPORTER: UNIVERSITY of TASMANIA

MONDAY 21 OCTOBER 2024

Plenary Session 1		Hotel Grand Chancellor, 1 Davey Street Hobart, Ballroom 2&3
Chair	Dr. Maggie Wang	
0845-0900	**Conference Opening** **Welcome to Country** – presented by Iris Wright **President's Welcome** – Dr. Maggie Wang	DAVID COLLINS Leukaemia FOUNDATION OF TASMANIA INC.
0900-0945	**Flow Cytometry – From origins to twenty-second century perspectives** **Prof Marie C. Bene, Nantes University**	
0945-1030	**Furthering Avian Conservation Strategies: Generating Tools for Biobanking and Genetic Rescue in Birds** **Dr. Matthew Biegler, Rockefeller University**	
1030-1100	**Morning Refreshments** *Proudly sponsored by BD*	Federation Ballroom, Exhibition Trade Area
Plenary Session 2		Ballroom 2&3
Chair	Scott Ragg	
1100-1145	**Stem cell transplantation in the age of CAR T-Cell therapy** **Dr. Ben Hedley, Victoria Hospital**	
1145-1230	**High-throughput screening coupled with Imaging Transcriptomics in 2D and 3D cell models** **Prof Kaylene Simpson, Peter MacCallum Cancer Centre**	
1230 – 1300	**Platinum sponsor presentation** **Harnessing the power of imaging flow cytometry to assess cytogenetics in leukaemias** **Dr Kathy Fuller (Heel)**	CYTEK® TRANSCEND THE CONVENTIONAL
1300-1400	**Lunch Break** – Check out what our exhibitors have got to offer. Don't forget to scan to win some great prizes. *Proudly sponsored by CYTEK*	Federation Ballroom, Exhibition Trade Area

Breakout Session		
1400-1530	**RESEARCH BREAKOUT SESSION 1** **SRL INSTRUMENTATION**	**CLINICAL BREAKOUT SESSION 1** **ONCOLOGY**
Room	Grand Ballroom 2&3	Grand Ballroom 1
Chair	Rob Salomon	Dr. Archna Sharma
	Contributions of Melbourne Cytometry Platform to the enhancement of scientific discovery **Dr. Vanta Jameson, University of Melbourne**	**Development of a highly sensitive multiple myeloma minimal residual disease spectral flow cytometry assay** **Dr. Edward Abadir, Royal Prince Alfred Hospital**

MONDAY 21 OCTOBER 2024

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	Using Spectral Cytometry to Investigate Novel Pre-conditioning Regimens in a Congenic Adoptive Immunotherapy Mouse Model **Dr. Joanne Davis, Royal Melbourne Hospital**	Blastic plasmacytoid dendritic cell neoplasms: BPDCN **Prof Marie C Bene, Nantes University**
	Spatial mapping of the hepatocellular carcinoma landscape identifies unique intratumoral perivascular-immune neighborhoods **Dr. Felix Marsh-Wakefield, Centenary Institute**	Flow cytometry in the diagnosis of vitreoretinal lymphoma **Prof William Sewell, St Vincent's Hospital Sydney**
1530-1600	**Afternoon Refreshments**	Federation Ballroom, Exhibition Trade Area
1600-1700	**RESEARCH BREAKOUT SESSION 2**	**CLINICAL BREAKOUT SESSION 2** **AUTOMATION**
Room	Grand Ballroom 2&3	Grand Ballroom 1
Chair	Chrissie Ong	Deni Cupit
	Human Intestinal Dendritic Cells Are Dysregulated in Crohn's Disease **Dr. Kirstie Bertram, The Westmead Institute for Medical Research**	Automating Lymphoma Diagnosis with a 13-Colour Multiparametric Flow Cytometry Panel **Tina Pham, St Vincent's Hospital Melbourne**
	Single-cell RNASeq of Cerebral Spinal Fluid in Children with Brain Cancer **Rob Salomon, CCI**	LabPlus Networking the Flow Lab **Danny Lim, LabPlus**
		The introduction of spectral flow cytometry into a diagnostic laboratory **A/Prof Elizabeth Tegg, NSW Health Pathology**
1730-1830	**Poster Session** – Light refreshments provided	Federation Ballroom, Exhibition Trade Area

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TUESDAY 22 OCTOBER 2024

Plenary Session 3		Grand Ballroom 2&3
Chair	Joanna Roberts	
	Acknowledgement of Country	
0845-0930	**Harnessing AI for Spatial Omics Analysis** **Dr. Jinmiao Chen, Singapore Immunology Network**	
0930-1000	**Applications of Computational and Artificial Intelligence in Clinical Cytometry** **Dr. Paul Simonson, Weill Cornell Medicine**	
1000-1030	**Platinum sponsor presentation** **Rarity SuperRCA, An Innovative mutation Detection technology : Bridging Molecular Biology and Flow Cytometry** **Dr. Shankar Pattebhiraman, Beckman Coulter**	BECKMAN COULTER Life Sciences
1030-1100	**Morning Refreshments** *Proudly sponsored by THERMOFISHER*	Federation Ballroom, Exhibition Trade Area
Plenary Session 4		Grand Ballroom 2&3
Chair	Eva Orlowski-Oliver	
1100-1145	**A customizable murine spectral backbone panel for immune surveillance in complex tissues** **Dr. Rui Gardner, Memorial Sloan Kettering Cancer Center**	
1145-1200	**Gold sponsor presentation** **Harnessing imaging and spectral potential in flow cytometry with Attune™ flow cytometers** **Dr. Sai Praneeth Narla, Thermo Fisher Scientific Australia and New Zealand**	ThermoFisher SCIENTIFIC
1200-1215	**Gold sponsor presentation** **Unlock new discoveries using real-time imaging and spectral flow cytometry (RTI-SFC)** **Chia How Sim, BD Biosciences, Central South Asia and Japan**	BD
1215-1315	**Lunch Break –** Time to visit the exhibitors and check out what's new! *Proudly sponsored by BECKMAN COULTER*	Federation Ballroom, Exhibition Trade Area

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TUESDAY 22 OCTOBER 2024

Plenary Session 5		Grand Ballroom 2&3
Chair	Dr. Helen McGuire	
1315 – 1430	**High performing teams and psychological safety, fireside chat about opportunities, logistics and benefits of mentor/menteeship and facilitated networking session** Broken up into three sections, this session will cover psychological safety and the correlation with high performing teams, what is it, why it is important and how we can increase it in the workplace. Mentor and mentee roles within SRL's, Q & A discussion with Eva Orlowski-Oliver and Prof. Kaylene Simpson about the benefits, logistics and opportunities that come with mentorship. Finishing with small group mentor/mentee opportunities from different areas including research, SRL, clinical, commercial and industry. TAKE THE QUIZ	
1430-1500	**Afternoon Refreshments**	Federation Ballroom, Exhibition Trade Area
1500-1700	**RESEARCH BREAKOUT SESSION 3**	**CLINICAL BREAKOUT SESSION 3** **NON ONCOLOGY**
Room	Grand Ballroom 2&3	Grand Ballroom 1
Chair	Oliver Eltherington	Susan Wright
	A Workflow for Longitudinal Biomarker Discovery Studies in Human Disease **Dr. Kathryn Hally, University of Otago**	**Utility B cell subset analysis by Flow cytometry – Beyond B cell malignancies** **A/Prof Pravin Hissaria, Royal Adelaide Hospital**
	Environmental sustainability in STEMM and a role for shared resourced laboratories **Dr. Darryl Johnson, University of Melbourne**	**The other side to flow cytometry** **Dr. Ben Hedley, Victoria Hospital**
	Enabling imaging features in single cell RNA sequencing analysis **Dr. Wenyan Li, Children's Cancer Institute Australia**	
	Comprehensive Immunophenotyping Reveals Immune Hallmarks of Psoriatic Multiple Sclerosis Sub-type **Dr. Varitsara Mangkorntongsakul, University of Sydney**	
	Understanding Inflammatory Mononuclear Phagocyte Heterogeneity in Human Anogenital Tissue **Freja Warner Van Dijk, The Westmead Institute for Medical Research**	**Preservation of Functionality, Immunophenotype and Recovery of HIV RNA from PBMC's Cryopreserved for more than 20 years** **Prof John Zaunders, St Vincent's Hospital Sydney**
1830-2230	**Gala Dinner** Theme – Aurora Australis After Party 2230-Late	Waterline Brooke St Pier Lower Level 12, Franklin Wharf

WEDNESDAY 23 OCTOBER 2024

Australasian Cytometry Society

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Plenary Session 6		Grand Ballroom 2&3
Chair	Chrissie Ong	
	Acknowledgement of Country	
0845-1015	**TASMANIAN MADE**	
	Ribonucleoprotein delivery to Salmonid sperm for high-throughput gene editing **Amanda Patchett, CSIRO**	**Reduction in the threshold of basophil activation testing by flow cytometry is a useful biomarker to predict outcomes after immunotherapy in patients with anaphylaxis to Jack Jumper ant venom** **Dr. Emily Mulcahy, Royal Hobart Hospital**
	Characterizing Plankton Communities in Oceans Using Flow Cytometry **Lennart Bach, Institute of Marine & Antarctic Science**	**Using high dimensionality flow to assess models of bone marrow failure syndromes** **Kirsten Fairfax, University of Tasmania**
	Cytometric methods for developing and validating a Tasmanian devil facial tumour vaccine **Andy Flies, University of Tasmania**	
1015-1045	Morning Refreshments	Federation Ballroom, Exhibition Trade Area
Plenary Session 7		Grand Ballroom 2&3
Chair	Dr. Matthew Linden	
1045-1230	**EARLY/MID CAREER CYTOMETRISTS**	
	Imaging Flow Cytometric Detection of amp(1q21) and del(17p) "Double-Hit" Abnormalities in Myeloma Plasma Cells **Thomas Mincherton, The University of Western Australia**	**The Art of Juggling: Internal users versus External Clients in a NATA Accredited SRL** **Dr. Elizabeth McDonald, QIMR Berghofer**
	Lessons Learnt: Establishment of a benchmark cytometric immune phenotyping workflow for multi-center clinical trials **Natalie Smith, University of Sydney**	**An in situ quantitative map of mononuclear phagocytes across human mucosal tissue using high parameter imaging platforms** **Dr. Thomas O'Neil, The Westmead Institute for Medical Research**
	The application of deep learning for better batch effect removal allows detection of subtle cellular phenotypes in very large flow datasets **Dr. Ben Mashford, John Curtin School of Medical Research, ANU**	**Blood Biomarker Discovery: High-Dimensional Blood Immune-Profiling in Children with Different Disease Settings Showed Major Age-Related Changes in Proportion of Immune Cells** **Dr Sedi Jalali, Murdoch Children's Research Institute**
	High-throughput cultivation of the faecal microbiome using flow cytometry **Dr. Allison McInnes, Queensland University of Technology**	
1230-1330	Lunch Last chance to connect with our sponsors and exhibitors.	Federation Ballroom, Exhibition Trade Area
1330 - 1445	**ACS 2024 AGM AND CONFERENCE AWARDS**	Grand Ballroom 2&3
Chair	Dr. Maggie Wang	
	Conference close	

POSTERS

CHECK OUT THE POSTER DISPLAY FROM MONDAY TO WEDNESDAY.

Poster authors are reminded to collect their poster by close of lunch on Wednesday, otherwise it may be disposed.

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#	AUTHOR	ORGANIZATION	PAPER TITLE
1	Claudia Bramley	Flowjoanna	Optimising genome size measurement of kuku/green-lipped mussel/Perna canaliculus by flow cytometry
2	Nancy Cai	NSW Health Pathology	Acute Megakaryoblastic Leukaemia Associated with Mediastinal Germ Cell Tumours
3	Dr. Ya-Ting Chuang	National Taiwan University Hospital	GATA-3 regulates the effector lineage differentiation of iNKT cells through ICOS
4	Dr. Joanne Davis	The Royal Melbourne Hospital	Ibrutinib Protects T cells in Patients with CLL from Proliferation-induced Senescence
5	Dr. Melinda Dean	University of the Sunshine Coast	Analysis of soluble inflammatory mediators in human breast milk by flow cytometry
6	Suat Dervish	The Westmead Institute for Medical Research	A Novel Cytometry Reference Control Bead With Advantages
7	Oliver Eltherington	The University of Melbourne	Sorter PDR: Quantitatively evaluating a new sorter for the SRL
8	Jennifer Gleadhill	Queensland Public Health and Scientific Services	Mast Cell Leukaemia - A rare and aggressive entity not to be missed in the flow cytometry laboratory
9	Shayli Harris	Royal Hobart Hospital	A Mysterious Case of Burkitt Lymphoma
10	Dr Anis Larbi	Beckman Coulter Life Sciences	A flow cytometry approach for the characterization and isolation of extracellular vesicles
11	Kelly Andrews	Beckman Coulter Life Sciences	A Prototype Approach for the Detection of Spectral Data Using 88-Channel Detection System and a CytoFLEX LX Flow Cytometer
12	Dr Anis Larbi	Beckman Coulter Life Sciences	Fostering Consistency in EV-Based Vaccine Development and Clinical Trials: Advancing Towards Standardization
13	Dr Anis Larbi	Beckman Coulter Life Sciences	MISEV 2023 : Isolation and characterization of extracellular particles
14	Dr Anis Larbi	Beckman Coulter Life Sciences	Optimizing a workflow for the analysis of extracellular vesicles
15	Na Kang	Pathology Queensland	Distinguishing immature and mature monocytes by Flow Cytometry – An Old Topic Revisited
16	Prof Roslyn Kemp	University of Otago, Otakou Whakaihu Waka	Integrating Complex Immunology into Clinical Cancer Trials
17	Dr. Edwin Lau	The Westmead Institute for Medical Research	Utilising TauSense separation to enhance signal to noise ratio in the microsphere-based aerosol containment assay
18	Mandy Li	Peter MacCallum Cancer Centre	Flow Cytometric Quantification and Absolute Counting between Neat and Washed Samples in Lithium Heparin and EDTA

POSTERS

#	AUTHOR	ORGANIZATION	PAPER TITLE
19	Dr. Matthew Linden	The University of Western Australia	Application and adaptation of an experiential, learning-by-doing approach to clinical and research flow cytometry training
20	Dr. Kevin Lo	Cellcarta	Multi-Site Instrument Alignment Using Lyophilised BD™ CompBeads
21	Sean Macdonald	Peter MacCallum Cancer Centre	Leveraging mass cytometry to identify prognostic immune subsets in paediatric B-ALL patients undergoing CD19 CAR-T cell therapy
22	Eva Orlowski-oliver	Peter MacCallum Cancer Centre	Psychological Safety in a Shared Resource Laboratory (SRL)
23	Fatlma Panetta	PETERMAC	Investigation of Fluorescent activated cell sorting (FACS) purity using varied sort masks
24	Geza Paukovics	Flowlogic Software	Are Manual Gating Approaches to Complex Data Analysis still the best way to go? FlowLogic provides Solution!
25	Geza Paukovics	Flowlogic Software	Still Fixing Spectral Unmixing Errors Manually? FlowLogic provides Solution!
26	Jack Pepper	Telethon Kids Institute	Characterising The Immune Response To A Novel Intranasal Vaccine For Otitis Media Prevention using Spectral Flow Cytometry
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29	Joanna Roberts	Flowjoanna	Examining cells in cow milk using natural autofluorescence
30	Christina Swart	Te Whatu Ora - Canterbury	A case of myeloid neoplasm post cytotoxic therapy, subtype acute erythroid leukaemia
31	Sarah Tan	NSW Health Pathology	Systematic Troubleshooting Of Antibody Aggregates In Diagnostic Flow Cytometry
32	Kristie Theodore	Pathology Queensland	CD4+ recent thymic emigrant enumeration is a useful tool in differentiating causes of T-cell lymphopenia detected by newborn screening
33	Dr. Margaret Veale	La Trobe University	Estimating Genome Size and Ploidy of Plant Species using Flow Cytometry
34	Susan Wright	Royal Hobart Hospital	Flow cytometry and coeliac disease - A retrospective review of testing at the Royal Hobart Hospital

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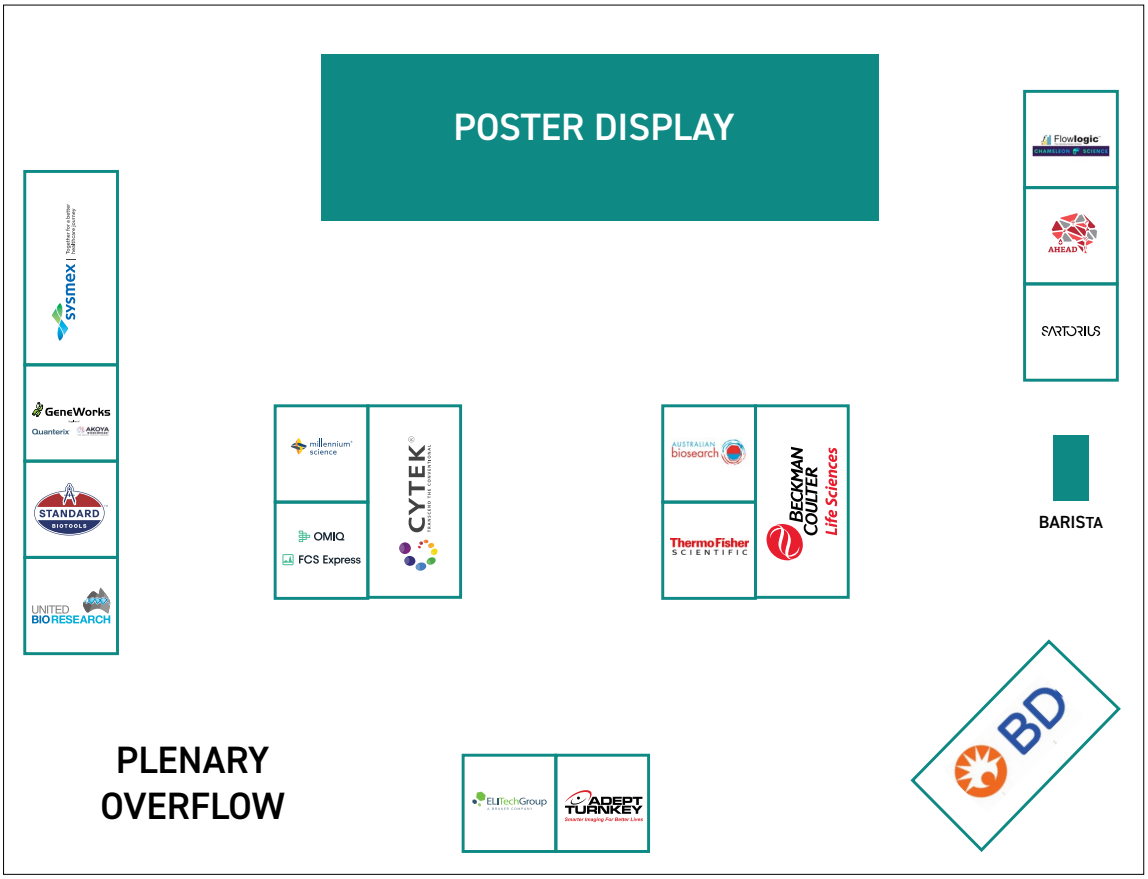

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INVITED SPEAKERS

DR BEN HEDLEY

Victoria Hospital

Ben Hedley was born in the Wales and educated in Belgium, the United Kingdom and Canada. At Imperial College he read chemical engineering and graduate with masters of engineering. His post graduate studies were in molecular biology and cancer research at Western University in Canada. His research allowed him to develop into different areas and started me on the path toward my current position as Medical Scientist within the teaching hospital in London Ontario. The hospital is the largest tertiary health center in the region and a referral site and covers approximately 2.5 million people. In his role he oversee multiple laboratories in the immediate for hematology and coagulation and the only flow cytometry laboratory one for the region. Ben's laboratory is also a Canadian reference site for measurable residual disease (MRD) testing for B and T cell acute lymphoblastic leukemia. Currently they do referral work for 14 centers across Canada and see approximately 500 cases a year. Ben currently sit of the College of American Pathologists Diagnostic Immunology and Flow Cytometry Committee, actively involved with the Clinical and Laboratory Standards Institute and also a board member for the International Council on Standardization of Hematology. His interests have been primarily on furthering the standardization of flow cytometry, through guidelines or products with the goal of elevating our practices. He has had the opportunity to published a number of scientific articles on standardization and was part of the latest Clinical and Laboratory Standards Institute guideline for flow cytometry testing. With current regulations evolving he believe this will form the basis for some of the regulations that will come. Most recently Ben has started a collaboration with a group that has artificial intelligent software for perform analysis of clinical flow cytometry which he holds to bring to their laboratory as a Canadian first.

INVITED SPEAKERS

DR KIRSTIE BERTRAM

Westmead Institute for Medical Research

Dr Kirstie Bertram has specialised for over a decade in isolating immune cells from human tissue and interrogating them by high-parameter flow cytometry. In 2015, as a post-doctoral scientist, she optimised protocols for isolating myeloid cells (particularly dendritic cells and macrophages) from all human tissues physiologically relevant to HIV transmission, spanning skin, type I and type II mucosae (foreskin, labia, vagina, cervix, penile tissue, perineum, rectum). With the aim to keep them as immature and functionally intact as possible to investigate how they interact with HIV and HSV. Her work is located at the Westmead Institute for Medical Research, part of the Westmead Health precinct, which is the largest health precinct in Australia enabling close ties between clinicians and scientists to access a vast array of human tissue. Now Dr Bertram has optimised two OMIPS to study Innate Lymphoid Cells, NK cells, MAIT cells and γδ T cells, as well as resident memory T cells from human mucosal tissue. She's also involved in projects to study immune cells from a huge range of human tissue and diseases ranging across skin, type II mucosae, intestine, cornea, and lymph nodes, studying how these cells interact with HIV, Herpes Simplex virus, and inflammatory bowel disease and vaccine adjuvants. Her work in high-parameter flow cytometry is complemented by work with Prof Andrew Harmans lab working on high-parameter imaging mass cytometry and spatial transcriptomics to thoroughly interrogate the immune environment of human mucosal tissues.

INVITED SPEAKERS

DR VANTA JAMESON

Peter Doherty Institute

Vanta is a cell biologist and cytometrist, managing the Peter Doherty Institute (PDI) cytometry SRL, the largest of four cytometry nodes at the University of Melbourne's Cytometry Platform (MCP). She was granted her BSc (Hons) (1999) and PhD (2006) from the University of Melbourne (Peter MacCallum Cancer Institute) and continued her scientific career as a postdoctoral researcher (Monash University, 2007-2012). Vanta's research spanning adult haemopoietic and then embryonic stem cell biology commenced in the early 2000's and pushed the limits of flow cytometry instrumentation and immunophenotyping. Her daily exposure to cytometry contributed to her deep appreciation of the crucial nature of sample and experimental optimisation and a deep curiosity for the technology. During her postdoctoral appointment, she became the resident educator of newcomers to cytometry. Her love of cytometry prompted a move into cytometry SRL management, where, starting in 2012 she oversaw the inception and growth of the University of Melbourne Brain Centre's cytometry SRL for 10 years. She took on management of the PDI cytometry SRL in 2022 where she now oversees a team of cytometrist and technicians and 16 cell sorters/ analysers. With over a decade's first-hand experience as a medical research scientist and in her 13th year managing a SRL, she is passionate about cytometry and teaching to achieve robust, reproducible and publishable data.

SUAT DERVISH

Westmead Institute for Medical Research

Suat Dervish focuses are on quality data generation and translational medical research in collaboration with researchers utilising advanced instrumentation. Studying at the University of Sydney he began his cytometry career as a Cytometry Development Specialist and now manages Westmead's Cytometry & Imaging facilities, where he drives innovation and technological advancements. Dedicated to education, Suat teaches cytometry to University of Sydney students and has hosted the "Build Your Own Cytometer" workshops at the Australian Cytometry Society Conferences. His primary interests lie in educating, inspiring, and fostering developments in cytometry.

INVITED SPEAKERS

DR PAUL D. SIMONSON

Weill Cornell Medicine

Dr. Paul D. Simonson is a practicing hematopathologist and faculty member at Weill Cornell Medicine, Cornell University's medical school located in Manhattan, New York, associated with NewYork-Presbyterian Hospital. He completed his medical and graduate school degrees at the University of Illinois at Urbana-Champaign where his graduate research work under the direction of Drs. Enrico Gratton and Paul Selvin focused on single-molecule fluorescence analysis and biophysics. Following medical school, Dr. Simonson completed his residency in anatomic and clinical pathology and his hematopathology fellowship training at the University of Washington in Seattle, under the mentorship of Dr. Brent Wood, a world-renowned expert in clinical flow cytometry. During training, Dr. Simonson developed computational and machine learning approaches for interpretation of clinical flow cytometry data, which he continues to expand upon as a member of the hematopathology faculty at Weill Cornell. Dr. Simonson also serves as a member of the College of American Pathologists Artificial Intelligence Committee, a member of the AIDS Malignancy Consortium's Emerging Technologies Committee, and co-director of Weill Cornell Pathology's Multiparametric In Situ Imaging lab.

DR FELIX MARSH-WAKEFIELD

University of Sydney

Dr. Felix Marsh-Wakefield is a post-doctoral researcher in the Liver Injury & Cancer Program at the Centenary Institute and the Human Cancer & Viral Immunology Laboratory at the University of Sydney. As an immunologist, he focuses on investigating the role of various immune cells in diseases such as hepatocellular carcinoma and multiple sclerosis. His work primarily involves using bioinformatics to analyse high-dimensional data, including imaging mass cytometry. Dr. Marsh-Wakefield is also a participant in the Marylou Ingram Scholarship Program, run by the International Society for the Advancement of Cytometry (ISAC).

INVITED SPEAKERS

AVRILL ASPLAND

University of Sydney

Avrill is a Technical Manager on the Sydney Biomedical Accelerator project at the University of Sydney. She has spent many years in roles providing technical expertise in the application of biosafety control measures, achieving success through close collaboration with safety officers and researchers. Avrill is an active member of the International Society for the Advancement of Cytometry's Biosafety Committee, working alongside world-leading experts in Shared Resource Laboratory biosafety to deliver improved safety outcomes to the global cytometry community. Avrill translates biosafety principles and practices into practical applications for anyone working within a laboratory setting.

TINA PHAM

St Vincent's Hospital Melbourne

Tina Pham is a Senior Scientist at St Vincent's Hospital Melbourne, Australia, where she manages the Special Haematology laboratory which includes Flow Cytometry and Cellular Therapy. Her flow cytometry experience spans over 10 years. She is the Vic Branch chair of the Australian Institute of Medical Scientists (AIMS), board director of the Australian Council for Certification of Medical Scientific Workforce (CMLS) and guest lectures at RMIT. She is passionate about education for future medical scientists in flow and cell therapy.

LORIZA KHAN

Royal College of Pathologists of Australian Quality Assurance Programs

Loriza Khan is a Senior Scientist at the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) and is the technical head of the Haematology and Transfusion Discipline. She has over 25 years of experience in various areas of haematology while working in Australia and New Zealand and has been with the RCPAQAP for 6 years. Her area of expertise is in external quality assessment programs.

INVITED SPEAKERS

DR JINMIAO CHEN

Singapore Immunology Network, A STAR

Jinmiao Chen got her Bachelor in Computer Science, PhD in machine learning and artificial neural networks. After her PhD, she joined the Bioinformatics service core of SIgN, A * STAR Singapore in 2009. She has worked closely with bench scientists from SIgN and other research institutes to analyze microarray, next generation sequencing and microbiome data. In 2014 when high-dimensional mass cytometry and single-cell RNA-sequencing become available in Singapore, she developed a series of new computational methodologies for analyzing high-dimensional single-cell data. Since 2014, she started her own research lab, single-cell computational/system immunology (SCCI) lab. Her lab currently focuses on integrated analysis of high-dimensional flow/mass cytometry and single-cell RNA-sequencing data, with the aim of studying cellular diversity, cell lineage, cell-cell interaction, cell movement and rare cell populations. Her lab carries out independent research to develop new data analysis methods, and meanwhile massively involves in data analysis through collaboration. In addition, the lab also initiates and runs biological research projects together with clinicians and biologists.

DR RUI GARDNER

Memorial Sloan Kettering Cancer Centre

Rui Gardner, PhD, is currently Head of Flow Cytometry Core Facility at Memorial Sloan Kettering Cancer Center.With a background in Biochemistry, Rui Gardner started his career in science as a mathematical biologist. In 2007, Rui became Head of the Flow Cytometry Core Facility at Instituto Gulbenkian de Ciência. Serving more than 40 research groups in many diverse fields including Immunology, Inflammation, Stem-Cell Biology, Microbiology, Virology, Plant Biology, among others, has given Rui a comprehensive experience in many Flow Cytometry techniques. Passionate about Science and Technology, Rui's background in Mathematics, Physics, and Biology allows bridging the gap between the operational and technical details in flow cytometry and the science within flow applications. Rui is a wonderful speaker and has lectured many courses on Flow Cytometry and Cell Sorting, being continuously involved in organizing various workshops and meetings on Flow Cytometry and Core Management in Europe.

INVITED SPEAKERS

DR MARIE C. BENE

Nantes University

Initially a professor of Immunology in the French Nancy University Hospital Immunology Laboratory, Dr. Marie C. Bene developed a deep interest in flow cytometry immunophenotyping when monoclonal antibodies developed as laboratory reagents. This led her to co-found the European Group for Immunophenotyping of Leukemias (EGIL) then become head of the European LeukmiaNet WP10 on morphological and cytometric diagnosis of leukemias. She then applied and was retained as head of the EHA (European Hematology Association) Scientific Working Group on Haematological Diagnosis. Meanwhile, from September 2012 to September 2022, Marie became head of Nantes University Hospital Hematology Laboratory (routine CBC and haemostasis assays, morphology, flow cytometry, cytogenetic and molecular analyses as well as cell-bank). More recently she developed interest in IA-assisted unsupervised FCM analyses.

DR MATTHEW BIEGLER

The Rockefeller University

Dr. Matthew Biegler is a postdoctoral researcher at The Rockefeller University in New York City, working in the Laboratory of Neurogenetics of Language headed by Dr. Erich Jarvis. He received his PhD from the Department of Neurobiology at Duke University, developing molecular tools to enhance the study of vocal learning neurogenetics in the zebra finch song system. Dr. Biegler's current research focuses on enhancing reproductive stem cell culture methods and assisted reproductive technologies to facilitate biobanking, surrogacy, and genetic rescue efforts across avian species. With a particular focus on songbirds, representing nearly two-thirds of all avian species, his innovative approaches combine single cell sequencing of developing reproductive tissues with novel techniques in the isolation, culture, and reintroduction of primordial germ cells (PGCs). These endeavors have been advanced by the creative utilization of flow cytometry and cell sorting methodologies, bridging the gap between traditional practices and cutting-edge reproductive technology. Beyond his primary research focus, he has a broad interest in species conservation in the midst of a sixth mass extinction, particularly in how biomedical advances in cellular, molecular, and genomic biotechnologies can be translated into cataloguing and preserving the rich biodiversity of the planet's species.

INVITED SPEAKERS

PROFESSOR KAYLENE SIMPSON

Victorian Centre for Functional Genomics

Professor Kaylene Simpson heads the Victorian Centre for Functional Genomics at Peter MacCallum Cancer Centre in Melbourne Australia and holds a joint appointment with the Department of Biochemistry and Pharmacology, University of Melbourne. She completed her BSc (Hons) at Monash University in plant science (1992) and spent 3 years as a research assistant at Florigene Pty Ltd (blue rose company) before undertaking a PhD in lactation and mammary gland biology at the Victorian Institute of Animal Science (1998). Her first postdoc was a shared appointment with Prof Melissa Brown (Uni Melb) and Profs Jane Visvader and Geoff Lindeman (WEHI) where she studied BRCA1 dependent breast cancer and developed the methodology that led to the identification of mammary gland stem cells. In 2002 she moved to Boston as a senior postdoc and then Instructor in the Dept Cell Biology, Harvard Medical School in the lab of Prof Joan Brugge. Returning to Melbourne in 2008 to head the VCFG, she has built a team of highly skilled research assistants and postdocs who enable researchers to perform unbiased target discovery using high throughput approaches including CRISPR, RNAi and compound screening in both 2D and 3D underpinned by sophisticated cell phenotyping using high content imaging. The VCFG team customise analysis for each specific project. Kaylene is a strong advocate for alternate career paths and is a formal and informal mentor to many researchers.

DR EDWARD ABADIR

Royal Prince Alfred Hospital

Dr. Edward Abadir is a clinical and laboratory Haematologist at Royal Prince Alfred Hospital. He oversees the Royal Prince Alfred flow cytometry service. He has an interest in flow cytometry of rare events and minimal residual disease detection. His clinical duties include participating in the haematopoietic stem cell transplant and CAR T services.

INVITED SPEAKERS

DANNY LIM

LabPlus

Danny Lim is a technical specialist of immune deficiency studies at LabPlus, New Zealand.

He was born in Malaysia and after spending some years in Singapore, migrated to New Zealand to enjoy the tranquil lifestyle. He joined the Haematology team at Labplus in December 2001 and made his way to the flow cytometry section in 2004 and never left the section. It was something new, exciting and challenging to him. he likes learning new tricks and embrace new technology with open arms.

In his spare time he rides bikes. He rides bikes a lot although not as much as he used to. In winter he likes a little bit of time in the mountains snowboarding and partake in a bit of apres ski.

ASSOCIATE PROFESSOR ELIZABETH TEGG

NSW Health Pathology

Associate Professor Elizabeth Tegg is a dual trained pathologist in Haematology and Genetics. She is currently based at The Institute of Clinical Pathology and Medical Research, which is part of New South Wales Health Pathology at Westmead Hospital. She is head of department of both Hematology and Genetics where she oversees a busy tertiary referral laboratory as well as a network of Branch laboratories across NSW.
She has undertaken a PhD in the genetic predisposition of Haematological malignancies and continues an active research interest in this field.

INVITED SPEAKERS

EVA ORLOWSKI-OLIVER

Peter McCallum Cancer Centre

Eva Orlowski-Oliver, SCYM(ASCP), is the Research Flow Core Manager at the Peter McCallum Cancer Centre in Melbourne, Australia. Her role involves educating, training, and supporting research staff who utilise flow cytometry technology. Her passion for research science started during her honours year through the University of Melbourne, and was followed by 7 years working as an RA under Associate Professor Mark Hogarth at the Burnet Institute. Eva found her love of flow cytometry through experiments at the bench and soon moved to working in the AMREP Flow core facility under the guidance of Geza Paukovics. Over 5 years Eva became the Assistant Manager of the core and most recently became the Research Flow Core Manager at the PeterMac Cancer Centre where she is supported by an excellent team of experts, highlighted by achieving the ISAC SRL Recognition Award. Eva is deeply committed to the continued education and training of researchers and core staff. She is an active member of ISAC and has contributed to publications in Cytometry A. She actively takes part in the SRL Recognition Program task force and is the SRL Committee Chair. Additionally, she contributes to ACS workshops, virtual presentations, and conference committees. Eva believes in providing young students with opportunities to explore careers in science, often hosting them for site visits or work experience. Eva is always happy to engage in conversations about flow cytometry, SRL management/education, or the A-League.

SUSAN WRIGHT

Royal Hobart Hospital

Susan Wright is a Senior Scientist in the Flow Cytometry Laboratory and Stem Cell Transplant Laboratory at the Royal Hobart Hospital. Committed to furthering her own knowledge and supporting the growth of others she has been an active member of ACS guidelines committees, as well as volunteering as convenor of ACS2024. In her spare time she fosters cats and boards Guide Dogs in training, along with her husband and 2 kids.

INVITED SPEAKERS

ASSOCIATE PROFESSOR PRAVIN HISSARIA

Royal Adelaide Hospital

A/Prof Pravin Hissaria is a Senior Clinical Immunologist at the Royal Adelaide Hospital, Immunopathologist at SA Pathology and Clinical Associate Professor in the Adelaide University Department of Medicine. Immunology Lab in SA pathology offers a comprehensive diagnostic Flow cytometry services to help diagnose Immunodeficiency, benign and malignant haematological conditions to all SA public hospitals. Dr Hissaria has a strong interest in all aspects of Clinical and Lab Immunology and is currently a member of the Royal College of Pathologists Australia Immunopathology Advisory committee and QAP committee. His main clinical interests are in setting up registries of rare autoimmune and auto-inflammatory diseases. He has received grants, authored 98 publications, and has given invited talks at national and international meetings.

AMANDA PATCHETT

CSIRO

Amanda is an immunologist with a keen interest in understanding the complex interactions between immune systems and infectious diseases at the molecular level. Having trained in medical research at the University of Tasmania, Amanda was motivated by the remarkable diversity in immune systems across the animal kingdom to apply her expertise in animal health. She completed a PhD and postdoctoral fellowship at the Menzies Institute for Medical Research studying Tasmanian devil immunology from 2014 to 2021. By combining immunological and molecular techniques, Amanda's PhD and postdoctoral research investigated the molecular nature of devil facial tumour disease and contributed to the design of candidate vaccines in devils. In 2022, Amanda joined the CSIRO in Hobart as a research scientist in aquaculture where she leads projects developing vaccines against fish pathogens and uses gene editing and molecular technologies to understand mucosal immunity.

DR EMILY MULCAHY

Royal Hobart Hospital/University of Tasmania

Dr Emily Mulcahy is the Senior Medical Scientist for the Allergy and Immunology Department at the Royal Hobart Hospital. She completed her PhD at the University of Tasmania utilizing flow cytometry to investigate the immunological aspects of Cystic Fibrosis. Her research interests now focus on venom allergy and the use of flow cytometry as a diagnostic tool for immunotherapy.

INVITED SPEAKERS

ASSOCIATE PROFESSOR LENNART BACH

University of Tasmania

Lennart Bach is an Associate Professor at the Institute for Marine and Antarctic Studies. His research focuses on the effects of climate change on plankton communities. From 2012 to 2019, he was a postdoctoral researcher at the GEOMAR Helmholtz Centre for Ocean Research Kiel, where he learnt utilizing flow cytometry to characterize and quantify plankton community structure. Lennart's work is relevant to the understanding the intricate dynamics of marine ecosystems and their responses to environmental changes.

DR KIRSTEN FAIRFAX

University of Tasmania

Dr Kirsten Fairfax is the Alex Gadomski Fellow.She leads a small team working on the immunology and genetics underpinning Bone Marrow Failure and haematopoiesis within the School of Medicine. She also teaches into first, second and third year research methodology, molecular biology and biochemistry subjects in the College of Health and Medicine. Her research work focusses on using molecular biology techniques such as single-cell RNA sequencing and CRISPR to understand more about the development of blood cells, and to generate new potential therapeutics.

ASSOCIATE PROFESSOR ANDREW FLIES

Menzies Institute for Medical Research, University of Tasmania

Andrew Flies completed a BSc in Computer Science at Minnesota State University, Mankato in 2002, with minors in Chemistry and Math. He then transitioned to immunology and worked as a research assistant at The Mayo Clinic (2003-2004) and Johns Hopkins University (2004-2006). He earned his PhD from Michigan State University in Dec 2012 studying the immune system of spotted hyenas, spending a year in the field in Kenya. His focus since 2014 has been on the development of vaccines and immunology tools for Tasmanian devils. He leads the Wild Immunology Group as an ARC Future Fellow and Associate Professor at the Menzies Institute for Medical Research at the University of Tasmania.

ABSTRACTS

Development of a highly sensitive multiple myeloma minimal residual disease spectral flow cytometry assay

Grand Ballroom 1, October 21, 2024

Dr Edward Abadir[1],
[1]Royal Prince Alfred Hospital

Biography
Dr. Edward Abadir is a clinical and laboratory Haematologist at Royal Prince Alfred Hospital. He oversees the Royal Prince Alfred flow cytometry service. He has an interest in flow cytometry of rare events and minimal residual disease detection. His clinical duties include participating in the haematopoietic stem cell transplant and CAR T services.

Abstract
Minimal residual disease testing in multiple myeloma is an accepted surrogate endpoint for patient outcomes in multiple myeloma. Current established methods utilise multicolour flow cytometry or next generation sequencing to identify malignant plasma cells. Spectral flow cytometry allows for improvement over assays but assessing additional markers to distinguish malignant plasma cells from normal populations. We have developed a spectral flow cytometry assay that can reliably detect circulating malignant plasma cells at least at an the level of next generation flow cytometry. Spectral flow cytometry has the ability to eclipse standard methods in terms of specificity and sensitivity to detect malignant plasma cells.

POSTER

A Prototype Approach for the Detection of Spectral Data Using 88-Channel Detection System and a CytoFLEX LX Flow Cytometer

Ms Kelly Andrews[1]
[1]Beckman Coulter Australia, Lane Cove West, Australia

Biography
Kelly Andrews gained her undergraduate studies at Western Michigan University with a major in Biomedical Sciences with a minor in Chemistry. Her PhD in Immunology was from the University of Tennessee Health Sciences Center in Memphis, TN researching Hypersensitivity Pneumonitis in a mouse model. She then continued with postdoctoral research at St. Jude Children's Research Hospital investigating methods of expanding human invariant natural killer T cells for potential cellular therapy applications. The laboratory moved to the University of Miami, where she continued her research as a Laboratory Manager, and finally an Assistant Scientist. She joined Beckman Coulter Life Science in 2019 where she supports flow

Abstract
Aim:
Commentary on MISEV2023, guidelines and recommendations for the analysis and reporting of extracellular vesicle studies

Method:
The recent release of MISEV2023 marks a significant milestone for the EV research community, aiming to establish standardized guidelines and recommendations for the analysis and reporting of extracellular

vesicle studies. The pursuit of knowledge in this field involves utilizing various solutions to generate data, while ensuring quality and reproducibility. This report focuses on how current practices in centrifugation and flow cytometry align with MISEV2023, along with potential gaps. Detailed protocols are provided for centrifugation methods like differential ultracentrifugation and analytical ultracentrifugation, as well as flow cytometry protocols for particle counting, characterization, and sorting. Specifically, the ability to detect and characterize small EVs by flow cytometry is highlighted for its exceptional fluorescence sensitivity (scatters and fluorescences). We also delved into the analysis and reporting aspects of the different techniques, offering a critical review of the current status quo. By examining the needs for proper analysis and reporting of EV-derived data scientists can gain valuable insights to enhance their EV research endeavors.

Results:
N/A

Conclusion:
N/A

Table/Figure:
The poster contains Diagrams

References:
"This research was supported by Beckman Coulter Life Sciences."

Biosafety: What is it to you?

Room 205, October 20, 2024

Avrill Aspland[1]
[1] *The University of Sydney*

Biography
Avrill is a Technical Manager on the Sydney Biomedical Accelerator project at the University of Sydney. She has spent many years in roles providing technical expertise in the application of biosafety control measures, achieving success through close collaboration with safety officers and researchers. Avrill is an active member of the International Society for the Advancement of Cytometry's Biosafety Committee, working alongside world-leading experts in Shared Resource Laboratory biosafety to deliver improved safety outcomes to the global cytometry community. Avrill translates biosafety principles and practices into practical applications for anyone working within a laboratory setting.

Abstract
The workshop "Biosafety: What is it to you?" is designed to engage cytometry professionals in a comprehensive discussion on biosafety practices specific to their field. Participants will explore the core principles of biosafety, share experiences, and discuss personal responsibility. Through an interactive case study and discussion, this session will foster a deeper understanding of the critical role biosafety plays in ensuring the safety of everyone who works with and around us. This workshop will provide practical guidance and encourage personalised reflections on the importance of biosafety in the cytometry community.

Characterizing Plankton Communities in Oceans Using Flow Cytometry

Grand Ballroom 2&3, October 22

Lennart Bach1
[1] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Australia

Biography

Lennart Bach is an Associate Professor at the Institute for Marine and Antarctic Studies. His research focuses on the effects of climate change on plankton communities. From 2012 to 2019, he was a postdoctoral researcher at the GEOMAR Helmholtz Centre for Ocean Research Kiel, where he learnt utilizing flow cytometry to characterize and quantify plankton community structure. Lennart's work is relevant to the understanding the intricate dynamics of marine ecosystems and their responses to environmental changes.

Abstract

Aim:

This presentation explores and showcases the use of flow cytometry in characterizing plankton communities in biological oceanography.

Method:

Oceanographic flow cytometry utilizes off-the-shelf medical flow cytometry, but also specialised plankton flow cytometry, including in situ and submersible methods. These specialized plankton methods have, however, no widespread application due to their high cost.

Results:

I will show case studes where flow cytometry was used in biological oceanography to make important findings. Currently, flow cytometry is becoming an increasingly important tool, widely used in plankton physiology, ecology, and biogeochemistry.

Conclusion:

Flow cytometry has demonstrated its versatility and effectiveness in advancing our understanding of marine ecosystems, aiding in environmental management and conservation strategies. It is likely that flow cytometry will become increasingly important in global observation networks and become even more widely used than today.

Flow Cytometry - from origins to twenty second century perspectives

Grand Ballroom 2&3, October 21, 2024

Prof Marie Bene[1]
[1]Nante University, Paris, France

Biography

Initially a professor of Immunology in the French Nancy University Hospital Immunology Laboratory, Marie developed a deep interest in flow cytometry immunophenotyping when monoclonal antibodies developed as laboratory reagents. This led her to co-found the European Group for Immunophenotyping of Leukemias (EGIL) then become head of the European LeukmiaNet WP10 on morphological and cytometric diagnosis of leukemias. She then applied and was retained as head of the EHA (European Hematology Association) Scientific Working Group on Haematological Diagnosis. Meanwhile, from September 2012 to September

2022, Marie became head of Nantes University Hospital Hematology Laboratory (routine CBC and haemostasis assays, morphology, flow cytometry, cytogenetic and molecular analyses as well as cell-bank). More recently She developed interest in IA-assisted unsupervised FCM analyses.

Abstract
The name « flow cytometry » was agreed upon in 1976 at the Conference of the American Engineering Foundation in Pensacola, Florida. At that time, a series of instruments had been devised to count cells, progressively incorporating laser beams, assessing forward and side scatter, cell sorting, and fluorochrome(s). A lot of early work was devoted to DNA staining and analysis of the cell cycle. Yet, at the time of the Pensacola meeting, Cesar Milstein and George Köhler had just discovered how to produce monoclonal antibodies and rapidly grasped their huge potential as reagents. As they had generously shared their know- how, the production of monoclonal antibodies all over the world flourished, allowing for the discovery of unsuspected antigens on leukocytes and other tissues. The enthusiasm was so great that some order had to be brought, which came in the early 1980's with the first human leukocyte differentiation antigens (HLDA) workshop and the CD nomenclature. Concomitantly to the use of these reagents in immunology and hematology research, flow cytometry entered routine laboratories with increasingly sophisticated instruments. Accompanying software also blossomed proposing an array of strategies to identify and count cell subsets. Nowadays, fully automated compact instruments are applied, for instance, for the enumeration of lymphocyte subsets. In hematology, flow cytometry is fully integrated to the first diagnostic steps of hematological malignancies and some non-malignant conditions. The technology has improved, and current flow cytometers are getting smaller and smaller yet more performing than ever. This rich history keeps moving forward with sister methods such as CyTOF, combining flow cytometry and mass spectrometry. The increasing availability of conjugates and lasers leads to complex data sets, and artificial intelligence is becoming a new companion of flow cytometrists in a continuing adventure.

Blastic plasmacytoid dendritic cell neoplasms: BPDCN

Grand Ballroom 1, October 21

Prof Marie Bene[1]
[1]Nante University

Biography
Initially a professor of Immunology in the French Nancy University Hospital Immunology Laboratory, Marie developed a deep interest in flow cytometry immunophenotyping when monoclonal antibodies developed as laboratory reagents. This led her to co-found the European Group for Immunophenotyping of Leukemias (EGIL) then become head of the European LeukmiaNet WP10 on morphological and cytometric diagnosis of leukemias. She then applied and was retained as head of the EHA (European Hematology Association) Scientific Working Group on Haematological Diagnosis. Meanwhile, from September 2012 to September 2022, Marie became head of Nantes University Hospital Hematology Laboratory (routine CBC and haemostasis assays, morphology, flow cytometry, cytogenetic and molecular analyses as well as cell-bank). More recently She developed interest in IA-assisted unsupervised FCM analyses.

Abstract
BPDCN is a rather rare but severe condition characterized by the proliferation of a specific cell type involved physiologically in immune responses to viral infections. More a leukemia than a lymphoma, this neoplasm however is associated with cutaneous lesions of highly variable characteristics. Although some morphological specificities can lead to suspect BPDCN, its diagnosis relies on a very atypical immunophenotype that can be the sole expression of CD4 and CD56 with no lineage marker. Various types

have been described associated to the expression of sometimes to myeloid marker. The full diagnosis however requires the exploration of BDCA antigens and CD123, since BPDCN rely on interleukin 3 for their growth. Therapeutic approaches are evolving , allogeneic hematopoietic stem cell transplantation remaining a potential curative strategy.

Interrogating immune cells from human tissue, including skin, type II mucosae and intestine: Considerations and consequences of isolation protocols to develop a high-parameter Optimized Multicolor Immunofluorescence Panels (OMIP).

Room 207/208, October 20, 2024

Erica Vine[1,2], Freja Warner van Dijk[1,2], Chloe Doyle[1,2], Thomas O'Neil[1,2], Sana Arshad, Andrew Harman [2] **Kirstie M Bertram**[1,2],

1 The Westmead Institute for Medical Research, Centre for Virus Research, NSW, Australia
2 The University of Sydney, School of Medical Sciences, Faculty of Medicine and Health, NSW, Australia

Biography
Dr Kirstie Bertram has specialised for over a decade in isolating immune cells from human tissue and interrogating them by high-parameter flow cytometry. In 2015, as a post-doctoral scientist, she optimised protocols for isolating myeloid cells (particularly dendritic cells and macrophages) from all human tissues physiologically relevant to HIV transmission, spanning skin, type I and type II mucosae (foreskin, labia, vagina, cervix, penile tissue, perineum, rectum). With the aim to keep them as immature and functionally intact as possible to investigate how they interact with HIV and HSV. Her work is located at the Westmead Institute for Medical Research, part of the Westmead Health precinct, which is the largest health precinct in Australia enabling close ties between clinicians and scientists to access a vast array of human tissue. Now Dr Bertram has optimised two OMIPS to study Innate Lymphoid Cells, NK cells, MAIT cells and γδ T cells, as well as resident memory T cells from human mucosal tissue. She's also involved in projects to study immune cells from a huge range of human tissue and diseases ranging across skin, type II mucosae, intestine, cornea, and lymph nodes, studying how these cells interact with HIV, Herpes Simplex virus, and inflammatory bowel disease and vaccine adjuvants. Her work in high-parameter flow cytometry is complemented by work with Prof Andrew Harmans lab working on high-parameter imaging mass cytometry and spatial transcriptomics to thoroughly interrogate the immune environment of human mucosal tissues.

Abstract
Myeloid cells, particularly dendritic cells (DCs) and macrophages are professional antigen presenting cells that represent one of the first lines of defence against invading pathogens within human mucosal tissues. However, their extraction from tissue is difficult, and the isolation technique used has phenotypic and functional consequences. Dendritic cells from tissue are quickly activated and induced to mature, and liberation by enzymes results in cleavage of surface markers.

Our lab has spent over a decade refining techniques to isolate myeloid cells from human skin (body skin, labia, foreskin, perineum), type II mucosa (vagina, anal canal, ectocervix) and type I mucosa (intestine, urethra), including isolating compartments of each tissue type, including the stratified squamous epithelium, the underlying dermis, lamina propria and isolation of the submucosa and lymphoid follicles, as well as the more readily available biopsy samples. Through careful selection of enzymes, antibody clones and isolation techniques, we have previously developed high-parameter immunofluorescence panels for

tissue for tissue resident T cells (OMIP-096), innate lymphocytes (OMIP-082). We have also profiled immune cells from human cornea and lymph node.

Now, we have developed a 27-parameter immunofluorescence panel for the BD Symphony to define the increasingly heterogenous myeloid compartment, including Langerhans Cells, DC1, DC2, DC3, monocyte derived macrophages (Mf1, Mf2), tissue resident macrophages (Mf3), plasmacytoid DC, $Axl^{+}siglec6^{+}$ DC, monocyte-derived DC as well as granulocyte populations (neutrophils, mast cells, basophils, eosinophils) across tissue compartments (skin, type II mucosa and intestine). We have also defined pattern-recognition receptors on these cells (including CD169, CD206, CD207, CD209, CD327) and activation/ functional receptors (CD86, CCR7, Ki67).

Here we present the careful consideration, design, and testing of high parameter flow cytometry panel from human tissue to uncover heterogeneity of myeloid cells and other immune cells within those tissues and avoid the pit falls rife in the literature which have mis-identified cells due to cleave of surface markers, maturation of liberated cells, or non-specific binding by of dyes to these cells.

Human Intestinal Dendritic Cells Are Dysregulated in Crohn's Disease

Grand Ballroom 2&3, October 21

Kirstie M Bertram[1,2], Chloe Doyle[1,2], Erica Vine[1,2], Thomas O'Neil[1,2], Freja Warner van Dijk[1,2], Anja Skilton[1,2], Scott Byrne[1,2], Andrew Harman[1,2]

[1] *The Westmead Institute for Medical Research, Centre for Virus Research, NSW, Australia*
[2] *The University of Sydney, School of Medical Sciences, Faculty of Medicine and Health, NSW, Australia*

Biography
Dr Kirstie Bertram has specialised for over a decade in isolating immune cells from human tissue and interrogating them by high-parameter flow cytometry. In 2015, as a post-doctoral scientist, she optimised protocols for isolating myeloid cells (particularly dendritic cells and macrophages) from all human tissues physiologically relevant to HIV transmission, spanning skin, type I and type II mucosae (foreskin, labia, vagina, cervix, penile tissue, perineum, rectum). With the aim to keep them as immature and functionally intact as possible to investigate how they interact with HIV and HSV. Her work is located at the Westmead Institute for Medical Research, part of the Westmead Health precinct, which is the largest health precinct in Australia enabling close ties between clinicians and scientists to access a vast array of human tissue. Now Dr Bertram has optimised two OMIPS to study Innate Lymphoid Cells, NK cells, MAIT cells and γδ T cells, as well as resident memory T cells from human mucosal tissue. She's also involved in projects to study immune cells from a huge range of human tissue and diseases ranging across skin, type II mucosae, intestine, cornea, and lymph nodes, studying how these cells interact with HIV, Herpes Simplex virus, and inflammatory bowel disease and vaccine adjuvants. Her work in high-parameter flow cytometry is complemented by work with Prof Andrew Harmans lab working on high-parameter imaging mass cytometry and spatial transcriptomics to thoroughly interrogate the immune environment of human mucosal tissues.

Abstract
Aim:
Myeloid cells, particularly dendritic cells (DCs) and macrophages are professional antigen presenting cells that represent one of the first lines of defence against invading pathogens within human mucosal tissues and orchestrate the adaptive immune response. However, their extraction from tissue is difficult, and the isolation technique used has phenotypic and functional consequences. Dendritic cells from tissue are

quickly activated and induced to mature, and liberation by enzymes results in cleavage of surface markers. Our lab has spent over a decade refining techniques to isolate myeloid cells from human tissue compartments with the aim of understanding the role dendritic cells in human intestine and Crohn's Disease.

Method:
Using high-parameter flow cytometry, we profiled myeloid cells including DC1, DC2, DC3, pDCs and macrophages from fresh human intestinal tissue in collaboration with colorectal surgeons across Western Sydney. We have used our techniques to isolate intestinal compartments, including lymphoid follicles, mucosae, sub-mucosae and matched blood to understand the antigen presenting cell populations within these tissues. We have also FACS sorted DCs from tissue to understand their function in driving T cell responses. Additionally, we have used transcriptomics to further define these cells.

Results:
We have identified that CD207+ DC2s are depleted in ileum from Crohns Disease patients. Additionally we have found that CD207+ DC2s from control human intestine drive a similar level of proliferation of naïve CD4+ T cells, but induce differentiation of CD4+ T cells that produce higher levels of Th2, Th17, and Th22 related cytokines than CD207- DC2s. Through transcriptomic analysis, we have shown that DC2s from control intestine express more CD207 and are more tolerogenic.

Conclusion:
CD207+ DC2s are depleted in Crohns Disease, through functional and transcriptomic profiling we have found CD207+ DC2s are a more tolerogenic dendritic cell that may promote better intestinal barrier function. Understanding DC2s with in the intestinal mucosae to promote a tolerogenic phenotype may serve as a therapeutic target for Crohns Disease.

Furthering Avian Conservation Strategies: Generating Tools for Biobanking and Genetic Rescue in Birds

Grand Ballroom 2&3, October 21, 2024

Dr Matthew Biegler[1], Dr Anna Keyte[1,2], Prof. Erich Jarvis[1,3]
[1]The Rockefeller University, New York, United States, [2]Colossal Biosciences, Dallas, United States, [3]Howard Hughes Medical Institute, Chevy Chase, United States

Biography
Dr. Matthew Biegler is a postdoctoral researcher at THE ROCKEFELLER UNIVERSITY in New York City, working in the Laboratory of Neurogenetics of Language headed by Dr. Erich Jarvis. He received his PhD from the Department of Neurobiology at Duke University, developing molecular tools to enhance the study of vocal learning neurogenetics in the zebra finch song system. Dr. Biegler's current research focuses on enhancing reproductive stem cell culture methods and assisted reproductive technologies to facilitate biobanking, surrogacy, and genetic rescue efforts across avian species. With a particular focus on songbirds, representing nearly two-thirds of all avian species, his innovative approaches combine single cell sequencing of developing reproductive tissues with novel techniques in the isolation, culture, and reintroduction of primordial germ cells (PGCs). These endeavors have been advanced by the creative utilization of flow cytometry and cell sorting methodologies, bridging the gap between traditional practices and cutting-edge reproductive technology. Beyond his primary research focus, he has a broad interest in species conservation in the midst of a sixth mass extinction, particularly in how biomedical advances in

cellular, molecular, and genomic biotechnologies can be translated into cataloguing and preserving the rich biodiversity of the planet's species.

Abstract
Biobanking and genetic rescue methods are increasingly used for mammalian conservation, but these techniques lag behind in other clades, particularly in birds. Advances in understanding chicken primordial germ cell (PGC) development have yielded breakthroughs in poultry germline cell culture, genome editing, and biobanking. However, insights taken from identified gene regulatory networks in chicken gametes have not led to successful development of these techniques in other bird species. The challenges in sustaining PGCs *in vitro* across the avian clade necessitate in-depth comparative studies to characterize interspecies differences. We have applied developmental analyses over multiple species using single-cell RNA and ATAC sequencing to identify and distinguish the conditions and signaling pathways involved in avian germ cell development, uncovering a previously unidentified and unexpectedly rich diversity in their developmental trajectories. Subsequent work in comparative gene network analysis has informed our understanding of the biological pathways and conditions essential for isolating, maintaining, and preserving these cell types *in vitro*.

Applying insights from the broader phylogeny to the zebra finch songbird model, we have begun to optimize *in vitro* conditions that maintain a self-renewing population of reproductively competent primordial germ cells through high-throughput growth factor screens and cell cultures assays. Our work has enabled the successful germline transmission of cultured gonadal germ cells, providing a valuable substrate for biobanking and genetic rescue in songbirds. From these successes, our ongoing studies aim to characterize how the development and survival of songbird germ cells are affected by an enigmatic germline restricted chromosome, which disqualifies the use of somatic cell reprogramming and other assisted reproductive technologies in this clade.

This work provides a glimpse into the burgeoning frontier of conservation biotechnology, which has and will continue to benefit from biomedical advances in genomics and cytometry to address and mitigate our current biodiversity crisis.

POSTER

Optimising genome size measurement of kuku/green-lipped mussel/Perna canaliculus by flow cytometry

Miss Claudia Bramley[1,3], Dr Nathan Kenny[2,5], Miss Jorgia Knight[1,4], Ms Joanna Roberts[1]
[1]Flowjoanna, , New Zealand, [2]Te Tari Matū Koiora/Dept of Biochemistry, University of Otago, Dunedin, New Zealand, [3]Ngāpuhi, Ngāti Kahu ki Whaingaroa, , New Zealand, [4]Rangitāne, , New Zealand, [5]Ngāi Tahu, Te Ātiawa, , New Zealand

Biography
I am working as a Cell Science Laboratory Technician for Flowjoanna while simultaneously completing my Masters in Animal Science at Massey University. LinkedIn: @ClaudiaBramley

Abstract
Aim:
Kuku/green lipped mussels (*Perna canaliculus*) are a taonga (treasured) species and Māori are recognised to hold rangatiratanga (sovereignty) over them under Te Tiriti o Waitangi, on 3 of New Zealand's founding documents. Bivalve (such as kuku) genome sizes can be variable; they can exhibit profound gene

presence/absence variation, with a pan-genome (the entire set of genes within a species) whose representation can vary greatly between individuals. Using flow cytometry, we hope to understand how widely kuku genome size varies. Our aim was to optimise a method for this. To give effect to Te Tiriti we need to take into consideration mātauranga māori, tikanga, kaitiakitanga, and rangatiratanga (somewhat captured by English concepts of knowledge, customs, guardianship and sovereignty) and we aim to incorporate these approaches in our work.

Method:
We have previously assayed molluscs using a method by Lamatsch, University of Innsbruck (unpublished) and this method was attempted. We also developed a protocol based on Roebuck, 2017. Briefly, samples were finely chopped in Galbraith's buffer, filtered, and successively centrifuged at 300g. Once a visible pellet was confirmed, supernatants were stained using propidium iodide with RNase, incubated in the dark, on ice, for 30 minutes, then measured on the FACSCalibur.

Results:
Unexpectedly, methods for determining genome size in other molluscs were not successful in kuku, however, we have shown successful recovery of stained nuclei from kuku using the modified Roebuck method. Tissue types will be compared along with other sample preparation methods to determine the optimal assay for genome size measurement in kuku.

Conclusion:
Determining the genome size of kuku by flow cytometry will provide insight about genome size variability in this species. Bivalve genome sizes are known to vary between individuals, and noting the variability in this species is of interest to kaitiaki as it may inform future genomic analysis.

References:

Roebuck, K. (2017). *Nuclear Genome Size Diversity Of Marine Invertebrate Taxa Using Flow Cytometric Analysis* [Master's thesis, Nova Southeastern University]. Retrieved from NSUWorks. https://nsuworks.nova.edu/occ_stuetd/462

This research was conducted by Flowjoanna Tāpui as part of a project lead by Dr Nathan Kenny, (Ngāi Tahu and Te Ātiawa) in conjunction with Te Ātiawa o te Waka-a-Māui, funded by a Rutherford Discovery Fellowship.

POSTER

Acute Megakaryoblastic Leukaemia Associated with Mediastinal Germ Cell Tumours

Miss Nancy Cai[1], Ms Esther Aklilu[1], Dr Edward Abadir[1,2]
[1]Royal Prince Alfred Hospital, Camperdown, Australia, [2]University of Sydney, Sydney, Australia

Biography
Nancy is a Scientific Officer at New South Wales Health Pathology, based at Royal Prince Alfred Hospital (RPAH). With seven years of experience working in the Haematology Laboratory, she has spent the last two years working in the highly specialised clinical flow cytometry laboratory at RPAH.

Abstract
Aim:
Extragonadal germ cell tumours (EGGCTs) are a rare form of neoplasms that can localise and develop in

other areas of the body, most commonly in the mediastinum, retroperitoneum, and brain.[1] While rare, there have been reports of an association between mediastinal germ cell tumours (MGCTs) and haematological malignancies, especially acute megakaryoblastic leukaemia's (AMKL). [2,3] These cases are very aggressive in nature and are known to have high mortality and poor prognosis.[4] This case study highlights the importance of flow cytometric immunophenotyping as a sensitive diagnostic tool for rapid detection and diagnosis.

Method:
A 22-year-old male presented with high grade fevers, significant lower back and bilateral thigh pain with a background of mixed EGGCTs, specifically, teratoma and yolk sac carcinoma (YSC). 9 months prior, the patient had his mediastinal mass resected, and was disease free for 6 months after completing chemotherapy (Bleomycin, etoposide and cisplatin (BEP)).
Results:
Initial peripheral blood examination showed a haemoglobin: 84g/L, red cell count: $3.07x10^{12}$/L, platelets: $51x\ 10^{9}$/L with a leukoerythroblastic blood picture comprising of approximately 6% of circulating blasts. His lactate dehydrogenase (LDH): 8981u/L was markedly elevated.

Morphological findings on the bone marrow (BM) aspirate and trephine revealed an increased blast population medium to large in size, consisting of a fine nuclear chromatin with 2-3 prominent nucleoli, a small proportion of deeply basophilic cytoplasm, and increased cytoplasmic blebbing and hypergranulation. Scattered granulocytic and erythroid precursors were also noted.

Immunophenotyping by flow cytometry on his BM showed a blast population which was CD2+(aberrant), CD13+, CD33+, CD34+, CD41a+(cytoplasmic), CD61+(cytoplasmic), and CD71+, consistent with AMKL.

Conclusion:
This is a rare case of AMKL arising from EGGCT and supports the suspected association of MGCT with AMKL. This case emphasizes the importance of flow cytometry analysis in supporting the diagnosis.

References:
No conflict of interest to disclose.
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Mosaic integration of spatial multi-omics with SpaMosaic

Room 207/208, October 20

Dr. Jinmiao Chen[1]
[1]Singapore Immunology Network, A STAR

Biography

*Dr. Jinmiao Chen is an Associate Professor at Duke-NUS Medical School and a Senior Principal Investigator at A*STAR, Singapore. Her research lab specializes in AI-powered single-cell and spatial omics analysis for precision medicine, with a particular focus on developing AI algorithms and omics databases. Recognized as a Highly Cited Researcher from 2020 to 2023 and selected as an EMBO Global Investigator in 2023, Dr. Chen holds a bachelor's degree in computer science from Sun Yat-sen University, China, and a PhD in AI and Computational Biology from Nanyang Technological University, Singapore. Following her PhD, she joined A*STAR as a postdoctoral researcher before establishing her own research lab.*

Abstract

With the advent of spatial multi-omics, we can mosaic integrate such datasets with partially overlapping modalities to construct higher dimensional views of the source tissue. SpaMosaic is a spatial multi-omics mosaic integration tool that employs contrastive learning and graph neural networks to construct a modality-agnostic and batch-corrected latent space suited for analyses like spatial domain identification and imputing missing omes. Using simulated and experimentally acquired datasets, we benchmarked SpaMosaic against single-cell multi-omics mosaic integration methods. The experimental spatial omics data encompassed RNA and protein abundance, chromatin accessibility or histone modifications, acquired from brain, embryo, tonsil, and lymph node tissues. SpaMosaic achieved superior performance over existing methods in identifying known spatial domains while reducing noise and batch effects. We also integrated a set of five mouse brain datasets of RNA and different epigenomic modalities, and imputed the missing omes. We found the genes in the imputed omes enriched in the correct tissue specific biological processes, supporting the imputation accuracy.

Harnessing AI for Spatial Omics Analysis

Grand Ballroom 2&3 , October 21, 2024

Dr. Jinmiao Chen[1]
[1]Singapore Immunology Network, A STAR

Biography

*Dr. Jinmiao Chen is an Associate Professor at Duke-NUS Medical School and a Senior Principal Investigator at A*STAR, Singapore. Her research lab specializes in AI-powered single-cell and spatial omics analysis for precision medicine, with a particular focus on developing AI algorithms and omics databases. Recognized as a Highly Cited Researcher from 2020 to 2023 and selected as an EMBO Global Investigator in 2023, Dr. Chen holds a bachelor's degree in computer science from Sun Yat-sen University, China, and a PhD in AI and Computational Biology from Nanyang Technological University, Singapore. Following her PhD, she joined A*STAR as a postdoctoral researcher before establishing her own research lab.*

Abstract

Spatial transcriptomics technologies generate gene expression profiles while retaining spatial context. These technologies offer unprecedented opportunities in dissecting tissue heterogeneity but also present significant challenges in data analysis, necessitating the development of spatially informed tools for a myriad of analytical tasks. Spatial transcriptomics often suffers from measurement noise including

dropouts. To address this challenge, we developed SEDR, a masked self-supervised graph learning method for constructing denoised and imputed spatial gene expression matrix. In addition, spatial transcriptomics yields high-dimensional gene expression measurements, making it crucial to obtain a biologically meaningful low-dimensional representation for interpretation and downstream analysis. In response, we developed STAMP, a deep generative model that returns biologically relevant, low-dimensional spatial topics and associated gene modules. Furthermore, we introduced GraphST to enable spatially informed clustering, multi-sample integration, and cell type deconvolution. With the latest advancements in spatial omics technologies, multiple omics modalities can now be acquired from the same tissue slice. To harness the full potential of such data and decipher spatial domains at a higher resolution, we developed SpatialGlue. This tool employs graph neural networks with a dual-attention mechanism to achieve within-omics integration of measured features and spatial information, followed by cross-omics integration.

POSTER

GATA-3 regulates the effector lineage differentiation of iNKT cells through ICOS

Dr Ya-Ting Chuang[1], Tzong-Shyuan Tai[2]
[1]National Taiwan University Hospital, , Taiwan, [2]Chang Gung Memorial Hospital, , Taiwan

Biography

Ya-Ting Chuang obtained her PhD in Immunology from National Taiwan University, and finished her first postdoctoral training in the Institute of Molecular Biology, Academia Sinica, Taiwan, with Dr. Ming-Zong Lai for 3 years. She then moved to the Department of Immunology, Boston Children's Hospital, Harvard Medical School, USA, for her second postdoctoral fellowship with Dr. Dale Umetsu in 2011 to 2013. Ya-Ting is currently an associated researcher in the Department of Medicine Research at National Taiwan University Hospital and supervise the core facility of flow cytometry. Her most recent works has focused on the T cell and NKT cell biology and investigate their heterogeneous role in human disease.

Abstract

Aim:

Invariant Natural Killer T (iNKT) cells are implicated in the regulation of various pathologic conditions such as infection, allergy, autoimmune disease, maintenance of transplantation tolerance, and cancer. This striking multifaceted role of iNKT cell in immune regulation is correlated with the presence of multiple functionally distinct effector lineages with unique transcriptional and cytokine profiles. However, it remains largely unresolved how this puzzling diversity of iNKT cell functional subsets emerges and what factors dictate the type of effector cell differentiation during the thymic differentiation. GATA-3 is a T cell-specific transcription factor that is also expressed in iNKT cells. Its significance in thymocyte development and its crucial role in the differentiation of peripheral Th2 cells have been thoroughly characterized, but its specific influence on iNKT cells remains relatively unexplored. In the present study, we aimed to investigate the role of the transcription factor GATA-3 in the differentiation and development of iNKT cell subsets.

Method:

We used multicolor flow cytometry and single-cell RNA sequencing (scRNA-seq) to analyze the effects of GATA-3 deficiency on the distribution and phenotype of iNKT cell subsets in GATA-3fl/fl Cd4-cre mice. The expression of key transcription factors and effector molecules involved in iNKT cell differentiation was examined. Additionally, we further investigated the potential regulatory mechanisms by which GATA-3 controls the development of specific iNKT cell subsets, focusing on the expression of genes such as Icos, Cd127, and Eomes.

Results:
We demonstrated that GATA-3 deficiency in mice led to the absence of iNKT2 and iNKT17 cell subsets, as well as an altered distribution of iNKT1 cells. Thymic iNKT cells lacking GATA-3 exhibited diminished expression of the key transcription factors PLZF and T-bet, and reduced production of Th2, Th17, and cytotoxic effector molecules. The scRNA-seq analysis revealed a comprehensive absence of the iNKT17 cell cluster and a substantial reduction in the iNKT2 cell population in GATA-3-deficient mice. Differential expression analysis identified several genes, including Icos, Cd127, and Eomes, that were differentially expressed in GATA-3-deficient iNKT cells, suggesting their potential involvement in the GATA-3-mediated regulation of iNKT cell effector lineage differentiation. Notably, the restoration of Icos, but not Cd127, expression could rescue iNKT cell development in GATA-3-deficient mice.

Conclusion:
The findings of this study demonstrate the pivotal role of the transcription factor GATA-3 in orchestrating the differentiation of iNKT cell effector lineages. GATA-3 appears to regulate the development of distinct iNKT cell subsets, including iNKT2 and iNKT17 cells, through the modulation of T cell activation pathways and the expression of genes critical for iNKT cell differentiation, such as Icos. These insights contribute to a better understanding of the molecular mechanisms governing iNKT cell development and function, which may have implications for the regulation of immune responses and the potential therapeutic targeting of iNKT cells in various disease contexts.

References:
No conflict of interest to disclose.

Using Spectral Cytometry to Investigate Novel Pre-conditioning Regimens in a Congenic Adoptive Immunotherapy Mouse Model

Grand Ballroom 2&3, October 21, 2024

Dr Joanne Davis[1,2], Ms Mandy Ludford-Menting[1,2], A/Prof Rachel Koldej[1,2], Professor David Ritchie[1,2,3]
[1]ACRF Translational Research Laboratory, The Royal Melbourne Hospital, Melbourne, Australia, [2]Department of Medicine, The University of Melbourne, Melbourne, Australia, [3]Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital , Melbourne, Australia

Biography
Dr Joanne Davis is a senior research officer at the ACRF Translational Research Laboratory, Royal Melbourne Hospital, Melbourne. Dr Davis has had a productive scientific career in which she has helped to develop immunotherapies for cancer, with a focus on understanding the immune responses surrounding human bone marrow transplantation. She has developed a number of mouse models of BMT to investigate graft rejection and GVHD. Dr Davis has a strong publication record having co-authored more than 25 primary papers and reviews and contributed as an investigator on 6 grants. She was the recipient of a Peter Doherty Postgraduate Training Fellowship soon after completing her PhD and has also held an Australian Academy of Science NH&MRC Early Career Award. More recently, she has held the Rabinowicz and Amarant Family Cancer Research Fellowship. Joanne has published 25 papers, with several as first author, and has a H index of 11 and approximately 900 career citations (Scopus).

Abstract
Aim:
We modified the method of cell trace violet (CTV) labelling that can be applied directly to either conventional or spectral flow cytometry, that maintained lymphocyte viability and function, yet minimised dye spill-over into other fluorochrome channels.

Method:
C57BL/6 (CD45.2+) mice (n=5/group) were pre-treated with the oral Bcl-2 inhibitor Venetoclax (100 mg/kg) for 2 days, then given split-dose total body irradiation (2 × 400 cGy). $5x10^6$ CTV-labelled T cells from B6.SJL-Ptprc<a>Pepc<b>/Boy (CD45.1+) donors were injected into the lateral tail vein. After 7-14 days, mice were killed and donor T cell proliferation within the inguinal lymph nodes (LN) and the spleen was analysed by flow cytometry. Samples were acquired at the Melbourne Cytometry platform, The University of Melbourne, using a 5 laser Becton Dickinson LSR Fortessa or a 5 laser Cytek Aurora, and analysed using FlowJo software. Statistical analysis was performed using Prism V9.0 (GraphPad). Comparisons between unpaired groups were performed using the non-parametric Mann-Whitney test (1).

Results:
The modified CTV-labelling method allowed us to identify congenic donor T cell proliferation and replication index in LN and spleen. In combination with congenic and memory T cell markers, this provided a reliable method to identify proliferating T cells using spectral cytometry. Pre-conditioning recipients with venetoclax, in combination with reduced intensity conditioning (RIC) irradiation, allowed donor T cells to expand and localise to LN, compared to mice treated with irradiation alone. Furthermore, donor T cells had increased division but lower replication index, and retained a central memory phenotype.

Conclusion:
Our modified CTV-labelling method provides a robust platform for investigating adoptive immunotherapy models in mice, and is suitable for multiparameter spectral cytometry analysis. Pre-conditioning recipients with venetoclax and RIC increased congenic CD8+ T cell expansion in vivo, whilst maintaining a central memory phenotype. Further application to CAR T, antigen or tumour-specific T cell models is warranted.

References:
(1) Davis JE et al,. Modified Cell Trace Violet proliferation assay preserves lymphocyte viability and allows spectral flow cytometry analysis. Cytometry A. 2024 May;105(5):394-403

POSTER

Analysis of soluble inflammatory mediators in human breast milk by flow cytometry

Dr Melinda Dean, Courtney Slegers[1], Mark Holmes[1, 2], Isabelle Lightbody[1]
[1]School of Health, University of the Sunshine Coast , Sunshine Coast, Australia, [2]2Centre for Bioinnovation, University of the Sunshine Coast, Sunshine Coast, Australia

Biography
Dr Melinda Dean is a Senior Lecturer in Biomedical Science at the University of the Sunshine Coast. She joined UniSC in December 2019 and is based at the Moreton Bay campus. Melinda is a biomedical scientist with expertise in the fields of innate immunology, inflammation, blood transfusion and erythrocyte structure and function. Her research is focused at the interface of immunology and haematology and she is passionate about advancing our knowledge and understanding in these fields in order to improve the quality and safety of blood products and improving transfusion outcomes. Melinda also has a particular interest in innate immune protein and pattern recognition molecule mannose binding lectin.

Melinda is an advocate for science, education and research in Australia. She is dedicated to providing the highest level of education and training to the next generation of scientists. She is an active member of professional societies including the Australian Society of Medical Research, Australian and New Zealand Society of Blood Transfusion, the International Society of Blood Transfusion, Women in Technology (WiT) and Professionals Australia. Melinda has received a number of awards including Professionals Australia

Science in Parliament Scholarship (2019) and Women in Science and Engineering professional development scholarship (2018). She holds honorary appointments at Australian Red Cross Lifeblood, Queensland University of Technology and the Critical Care Research Group at the Prince Charles Hospital. Melinda enjoys mentoring and supervising undergraduate and post graduate students and is interested in developing new research collaborations domestically and abroad.

Abstract

Background and Aim: Breastmilk is a complex bioactive fluid that plays a critical role in infant health. The composition of inflammatory markers in breastmilk during different stages of lactation, or, in response to infection, involution and maternal dietary status is not well understood. To advance our understanding of inflammatory markers in human breast milk, we optimised a cytometric bead array to enable multiplex quantification of cytokines by flow cytometry.

Methods: Human inflammatory cytokines cytometric bead array (BC Biosciences, IL-6, IL-8, IL-10, IL-1b, IL-12, TNFa) was selected and a human plasma protocol was followed. Parameters assessed were: whole milk and skimmed milk tested neat, 1:2 and 1:4 at 1µl and 0.5µl bead per test. A spike recovery protocol with recombinant standards was also performed. A three-laser FACSCANTO II flow cytometer was used for acquisition and FCAP array for data analysis.

Results: Cytokines were quantified in whole milk at all concentrations tested, but automated analysis wasn't possible due to inaccurate gating, poor identification of bead populations, erroneous events and many error results. Cytokines were detected in neat, 1:2 and 1:4 skimmed milk at similar levels using either 1µl or 0.5µl bead per test. Automated gating and analyses were successful with skimmed milk.
Conclusions: We developed a protocol for quantification of inflammatory mediators in human breast milk by flow cytometry and recommend neat, skimmed milk for analysis. This protocol will be used to investigate whether the inflammatory status of maternal diet correlates with breastmilk inflammatory mediators.

Build Your Own Cytometry

Lecture Theatre, October 20, 2024

Suat Dervish[1]

[1] The Westmead Institute for Medical Research

Biography

Suat Dervish focuses are on quality data generation and translational medical research in collaboration with researchers utilising advanced instrumentation. Studying at the University of Sydney he began his cytometry career as a Cytometry Development Specialist and now manages Westmead's Cytometry & Imaging facilities, where he drives innovation and technological advancements. Dedicated to education, Suat teaches cytometry to University of Sydney students and has hosted the "Build Your Own Cytometer" workshops at the Australian Cytometry Society Conferences. His primary interests lie in educating, inspiring, and fostering developments in cytometry.

Abstract

This workshop will enable attendee to grasp a deeper understanding of the core components of cytometers using 3D printed parts in an interactive manner. The presentation will include information on individual components, and an interactive step by step on how the system functions. Attendees will be

exposed cytometer building in an interactive manner in the hope that attendees will walk away with a better understanding of how the four major components - optics, fluidics, electronics and data processing work in modern cytometers.

POSTER

A Novel Cytometry Reference Control Bead With Advantages

Mr Suat Dervish[1], Dr Edwin Lau, Erica Vine, Dr Maggie Wang
[1]Westmead Institute For Medical Research, Westmead, Australia

Biography
Suat Dervish obtained his B.MedSci(Hons) focusing on T cell immunology from the University of Sydney and subsequently worked as a Cytometry Development Specialist at the Advanced Cytometry Facility. Now he manages Westmead Cytometry driving quality data generation and technological developments to facilitate translational medical research.

Abstract:
Aim:
Determine whether commercially available Pierce™ Protein L Magnetic Beads (ProL_beads) could be used as reference controls and subsequently characterise technical advantages and disadvantages.

Method:
ProL_beads were acquired and characterised for binding capacity, spectral fingerprint, autofluorescence, size, value, amenability to high throughput filter plate preparation and applicability as reference controls.

Results:
ProL_beads bound commercially available fluorescently conjugated antibodies, emitted decreased autofluorescence, and displayed improved spectral matching to cell reference spectra than certain commercially available cytometry reference beads. ProL_beads have a nominal size of 1um but did vary in size creating minor thresholding challenges. Routinely, only 0.5ul of ProL_beads were used to generate an excess of required events (25k) rapidly indicating significant value. The resultant spectral signatures generated could be used to spectrally unmix flow cytometry data.

Conclusion:
ProL_beads can bind multiple species (human, murine, rat & pig), multiple immunoglobulin classes (IgG, IgM, IgA, IgE and IgD), as well as single chain variable and Fab fragments. Our work indicates that ProL_beads may provide particular advantages when generating reference controls. The total binding capacity (absolute brightness), applicability to multiple light chain subunits and spectral impact is yet to be completely determined.

Science Communication with That's What I Call Science

Room 205, October 20, 2024

Olly Dove[1]
[1]That's What I call Science

Biography

Living up to her bird name, Olly Dove has recently completed a PhD on the foraging behaviour of little penguins and short-tailed shearwaters. Olly's favourite part of working in zoology is undoubtedly the adventures of fieldwork it leads her to, and she loves sharing stories about the natural world with others. When not hanging out with critters, Olly spends her free time by running around footy ovals and escaping into creative writing. Within the TWICS team, Olly is the weekly host of the show, as well as the content manager and one of the team's editor.

Abstract

That's What I Call Science (TWICS) is a Eureka award-winning nipaluna/Hobart-based national public radio show and internationally-distributed podcast.

This workshop will provide an introduction to sharing work and research with the wider public. The session will consist of a facilitated discussion followed by practicing in small groups.

The content and topics covered in the session will include:

- What scicomm is, and the opportunities that are out there to get involved in it.
- Introducing yourself and your work in a succinct manner.
- Identifying jargon and learning to break down what you're trying to communicate.
- The difference between written and spoken scicomm, with tips and tricks for each.
- Tactics for and embracing any nerves caused by being interviewed about your work.
- Overcoming the imposter syndrome of "But I'm not an expert".
- The use of social media for sharing information safely in a scary online world.

This session will be limited to 30 attendees

Lunch & Learn:

iQue® High-Throughput Screening by Cytometry

Room 205, October 20, 2024, 12:30 PM - 1:00 PM

Dr Nabiha Elias[1]
[1]Sartorius

Biography

Dr Nabiha Elias is a Field Application Specialist (FAS) based in Sydney, Australia, specializing in advanced bioanalytical systems. With over three years of experience at Sartorius, she oversees the Incucyte® Live-Cell Analysis and iQue® High Throughput Screener by Cytometry platforms across Australia and New Zealand. Her role involves providing application and workflow support for the company's technologies. As FAS, Nabiha conducts technical seminars and product demonstrations, leveraging her expertise to assist sales and facilitate customer engagement. Previously, Nabiha worked as a Scientific Officer, Laboratory

Technician, and Consultant. Her experience also includes roles in clinical trial research, quality control, and laboratory management. Nabiha holds a PhD in Chemistry from the University of Sydney, with a focus on the total synthesis and assessment of therapeutic natural products and analogues. Her academic and professional journey reflects her passion for research and development in the life sciences, making her a valuable asset in the biotech field.

Abstract
The iQue® High-Throughput Screening (HTS) Cytometry Platform re-imagines the standard flow cytometry workflow through combined innovations in both the platform and software. The result is an equally powerful but intuitive and versatile alternative, designed to offer high-throughput assay capabilities with the ease-of-use, high multiplexing, cost effectiveness and speed desired in modern workflows.

During this lunch and Learn presentation, we will explore the iQue®'s capacity to perform high-throughput suspension cell and bead-based assays from the same sample, in the same well, to provide a complete understanding of the biology of your sample. Requiring as little as one microliter of sample with fast and easy acquisition, the iQue® is suitable for conserving precious samples and saving money on reagents.

And with the Forecyt® Software, which is designed to process whole plates of data, the iQue® technology offers a simplified, interactive workflow where all the analysis, visualization and interpretation tools are integrated together. Using unique plate-level analytic and visualization tools, the iQue® can truly deliver on its purpose of delivering actionable answers faster and with confidence to empower and advance your research goals!

POSTER

Sorter PDR: Quantitatively evaluating a new sorter for the SRL

Mr Oliver Eltherington[1], Dr Alexis Perez-Gonzalez[1], Dr Alison Morey[1], Dr Vanta Jameson[1]
[1]Melbourne Cytometry Platform, Parkville, Australia

Biography
Flow Cytometry enthusiast working as a Senior Tech in the Melbourne Cytometry Platform. Previous flow cytometry experience in the University of Otago, NZ and Newcastle University, UK.

Abstract
Aim:
To quantitatively evaluate a new sorter in terms of sensitivity and recovery of various particle sizes.

Method:
Data was acquired on the Cytek Aurora CS, BD ARIA III and the BC SRT as well as the BD Fortessa and Cytek Aurora. Instruments were set-up according to manufacturer's recommendations. Sensitivity testing used a modified stain-index between negative and positive populations (Hoffman & Wood, 2007; Jameson et al, 2022). Sort recovery was calculated via Rmax (Perez-Gonzalez, et al, 2024) and absolute counts. Methods follow Mazel, et al, 2017 when looking into laser delays.

Results:
The sensitivity of the Aurora CS matched or exceeded that of the platform analysers. An issue with an analyser was observed only during sensitivity testing. Sizing rainbow beads were sorted on the CS, SRT and ARIA using manufacturer recommend drop charge delay (DCD) procedures. The instruments had similar 6µm bead recoveries; the CS had the highest (~98%) consistently for the 100µm nozzle. Results show a particle size dependency of sort recoveries. Recovery of particles ranging from 3 to 10µm is high (96%-98%)

when using the manufacturers drop delay calculation. Particles >10um suffer reduced recoveries (90%-50%). Inter laser delay changes did not improve signal for larger particles. Different sized rainbow beads can be used to set DCD on the ARIA and Aurora. The auto DCD generated by these larger particles falls close to the optimal off-set when compared to a DCD scan. This was tested by sorting 35µm Nanovials. Recoveries were improved compared to manufacturer auto-drop delay and reported recoveries (90% vs 60%).

Conclusion:
The Aurora CS has sensitivity equal to or greater than the SRT or ARIAIII. Manufacturer QC procedures cannot capture all issues thus low-end signal sensitivity monitoring is prudent. Recovery falls as the particle size increases past 10µm but can be rescued by adjusting DCD. It is possible to use sizing particles to improve automatic DCD accuracy. Changing laser delays did not improve signals for different sized beads indicating consistent velocities for different sized particles at the point of interrogation. By using 32µm rainbow Spherotech beads we improved the recovery of a 35µm Nanovials from reported recoveries without wasting actual samples on a DCD scan.

References:
Mazel, S, Fang, F., Semova, S. Semova, Georgala, P., and Gardner, R. "Size Matters": On the Challenges of Sorting Large Cells with Conventional Droplet Cell Sorters. Poster, Cyto Asia, 2017 https://cdn.fccf.aws.mskcc.org/CYTO_2017_B44_size_matters_final_201707116_6871f9a5e0.png

Perez-Gonzalez, A., Lopes, T., Martinez, L., Bispo, C., Gardner, R., & Riddell, A. (2024). Evaluation of sort recovery via *Rmax*. *Current Protocols*, 4, e986. doi: 10.1002/cpz1.986

Hoffman, R.A. and Wood, J.C.S. (2007), Characterization of Flow Cytometer Instrument Sensitivity. Current Protocols in Cytometry, 40: 1.20.1-1.20.18. https://doi.org/10.1002/0471142956.cy0120s4

Jameson VJ, Luke T, Yan Y, Hind A, Evrard M, Man K, et al. Unlocking autofluorescence in the era of full spectrum analysis: Implications for immunophenotype discovery projects. Cytometry. 2022; 101(11): 922–941. https://doi.org/10.1002/cyto.a.24555

Using high dimensionality flow to assess models of bone marrow failure syndromes

Grand Ballroom 2&3, October 22

Fairfax, KA[1], Glaser, A [2] Deans, A [2] D'Angelo, R [2] Simpson, A [3]

[1]*UTAS School of Medicine, TAS, Australia*
[2]*St Vincent's, Victoria, Australia*
[3]*The Menzies Institute, TAS, Australia*

Biography
Dr Kirsten Fairfax is the Alex Gadomski Fellow. She leads a small team working on the immunology and genetics underpinning Bone Marrow Failure and haematopoiesis within the School of Medicine. She also teaches into first, second and third year research methodology, molecular biology and biochemistry subjects in the College of Health and Medicine. Her research work focusses on using molecular biology techniques such as single-cell RNA sequencing and CRISPR to understand more about the development of blood cells, and to generate new potential therapeutics.

Abstract

Aim:

To assess the haematopoietic development in a mouse model of bone marrow failure syndrome

Method:

High dimensional flow was used to probe bone marrow development in a mouse model of bone marrow failure. One-way ANOVA was performed, followed by student's t-test with correction for multiple testing.

Results:

Bone Marrow Failure Syndromes are a collection of disorders in which the stem cells that reside in the bone marrow are no longer capable of generating the range of cells that are required in the blood to keep us healthy. These bone marrow stem cells generate red blood cells, platelets and white blood cells, and in bone marrow failure syndrome any one of these cell types can go awry. We have been generating novel models of bone marrow failure syndromes, modelling human patient mutations in mice. One example of this is the FANCL^{TATdel} mouse in which there is a 3bp deletion that results in FANCL dysfunction. Using flow cytometry we can observe a statistically significant decrease in the bone marrow hematopoietic stem and progenitor cells, or LSK cells in these mice.

Our lab has a major focus on CRISPR gene editing technologies to correct mutations such as the TATdel mutation, and to probe cellular function. Flow cytometry allows us to sort for different cell types in which we have introduced CRISPR pools to assess which genetic perturbations are altering cellular outputs.

Conclusion:

This mouse model was shown to have a hematopoietic stem cell defect. We are working to correct the defect in this mouse using novel CRISPR gene technologies.

Cytometric methods for developing and validating a Tasmanian devil facial tumour vaccine

Grand Ballroom 2&3, October 22

Andrew S. Flies[1]

[1]Menzies Institute for Medical Research, College of Health and Medicine, University of Tasmania, Hobart, TAS, Australia

Biography

Andrew Flies completed a BSc in Computer Science at Minnesota State University, Mankato in 2002, with minors in Chemistry and Math. He then transitioned to immunology and worked as a research assistant at The Mayo Clinic (2003-2004) and Johns Hopkins University (2004-2006). He earned his PhD from Michigan State University in Dec 2012 studying the immune system of spotted hyenas, spending a year in the field in Kenya. His focus since 2014 has been on the development of vaccines and immunology tools for Tasmanian devils. He leads the Wild Immunology Group as an ARC Future Fellow and Associate Professor at the Menzies Institute for Medical Research at the University of Tasmania.

Relevant social media details
UTAS: https://www.utas.edu.au/profiles/staff/menzies/andrew-flies
Website: https://wildimmunity.com/
Twitter: @WildImmunity https://twitter.com/WildImmunity

Facebook: https://www.facebook.com/WildImmunity/

Contact details
Andy.flies@utas.edu.au.

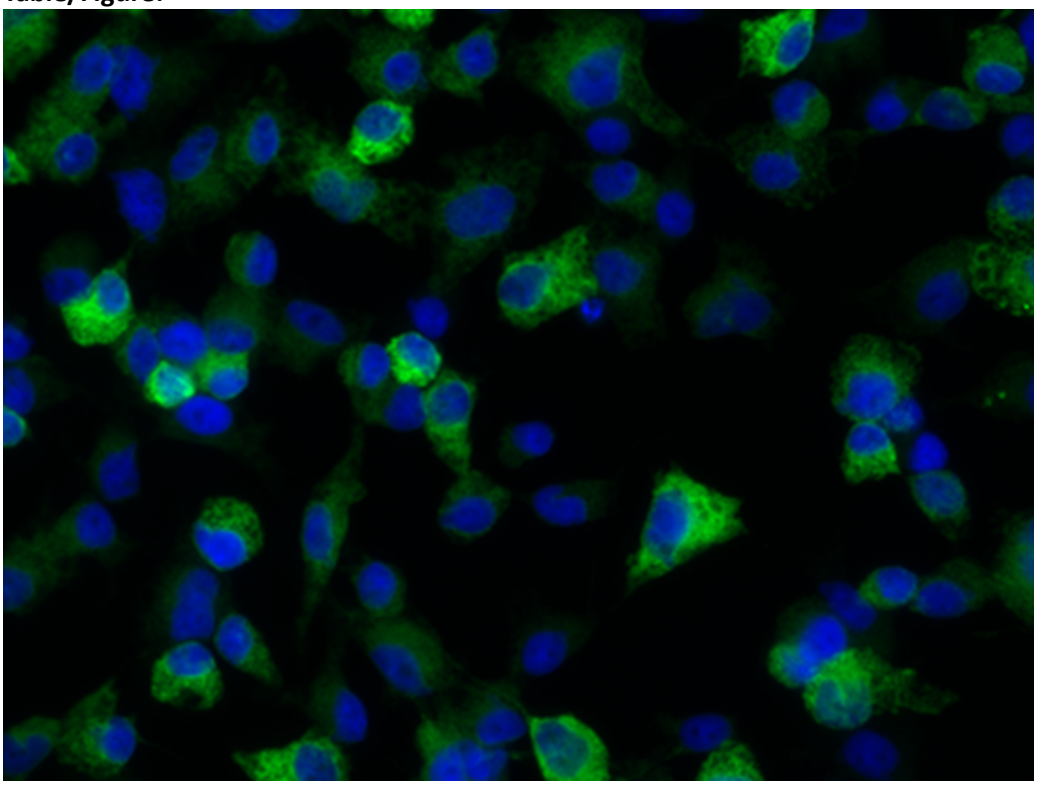
Abstract
Aim:
We aimed to develop a vaccine to protect Tasmanian devils from transmissible cancers.

Method:
An EVOS M5000 was used for day-to-day analysis of the virus transduction and subcellular location of fluorescent fusion proteins in the vaccine design. The titre of adenoviral vector-based vaccines was determined using a flow cytometry-based transducing unit (TU) assay.

Results:
We confirmed that recombinant Tasmanian devil MHC-I proteins can be expressed in multiple cell lines and encoded into an adenoviral vector vaccine. We showed that a fluorescently tagged vaccine cargo protein could be localised in the non-nuclear portion of devil facial tumour cells. Additionally, the fluorescent fusion protein allowed rapid titration through a flow cytometry-based transducing unit assay.

Conclusion:
Fluorescent fusion proteins have the drawback of introducing a non-target protein into a vaccine design and potentially altering protein function. However, in early stages of vaccine research it can allow small teams to more efficiently work towards in vivo vaccine testing than with untagged vaccine cargo proteins.

Table/Figure:



Devil facial tumour cells transduced with a replication-deficient human adenovirus encoding a vaccine cargo protein fused to mGreenLantern.

References:
"ASF has a patent application for vaccine related to this abstract". "This research was supported by

Australian Research Council and the University of Tasmania Foundation. The company had no role in analysing the data or preparing the abstract."

Platinum Sponsor Presentation

CYTEK Biosciences Inc.

Harnessing the power of imaging flow cytometry to assess cytogenetics in leukaemias

Grand Ballroom 2&3, October 21

Dr Kathy Fuller[1]
[1]Cytek Biosciences

Biography

Dr Kathy Fuller (Heel) earned her PhD from The University of Western Australia for research into the effects of post-surgical nutrition on gut immunity. She was awarded a Raine Medical Research Foundation grant to continue her research, received a Queen's Trust for Young Australians Award to travel to Oxford University and study advanced immunohistochemistry techniques and won the WA Young Australian of the Year Award (Science and Technology). Dr Fuller continued developing models of human disease as a postdoctoral researcher at Thrombogenix at Monash University designing and optimising in vivo models of thombogenesis, including real-time imaging of thrombus formation in rodent mesentery using fluorescence microscopy. Dr Fuller returned to UWA, becoming the lead academic of the flow cytometry and cell sorting facility located in the Centre for Microscopy Characterisation and Analysis where she gained extensive experience in flow cytometry including development and validation of the Sperm Chromatin Structure Assay (SCSA) for routine diagnostic use by Western Australian embryology clinics. In 2012 Dr Fuller joined Prof Wendy Erber as Scientific Lead in the Translational Cancer Pathology Laboratory where she is developing imaging cytometry protocols for the analysis of haematological malignancies and minimal residual disease. Dr Fuller is co-inventor of "immuno-flowFISH", a world-first automated flow cytometry method that can detect chromosome signals inside cells identified by their immunophenotype. Her development of this patented method was awarded the Australian Museum ANSTO 2018 Eureka Prize for Innovative Use of Technology. She lectures on standard flow and imaging flow cytometry techniques for research and diagnosis to third year biomedical science undergraduates and Masters postgraduate students.

Incorporating R&D in a Shared Resource Laboratory

Room 205, October 20, 2024, 3:30 PM - 5:00 PM

Dr Rui Gardner[1]
[1] *Memorial Sloan Kettering Cancer Center*

Biography

Rui Gardner, PhD, is currently Head of Flow Cytometry Core Facility at Memorial Sloan Kettering Cancer Center. With a background in Biochemistry, Rui Gardner started his career in science as a mathematical biologist. In 2007, Rui became Head of the Flow Cytometry Core Facility at Instituto Gulbenkian de Ciência. Serving more than 40 research groups in many diverse fields including Immunology, Inflammation, Stem-Cell Biology, Microbiology, Virology, Plant Biology, among others, has given Rui a comprehensive experience in many Flow Cytometry techniques. Passionate about Science and Technology, Rui's background in Mathematics, Physics, and Biology allows bridging the gap between the operational and

technical details in flow cytometry and the science within flow applications. Rui is a wonderful speaker and has lectured many courses on Flow Cytometry and Cell Sorting, being continuously involved in organizing various workshops and meetings on Flow Cytometry and Core Management in Europe.

Abstract
In this workshop, we will cover some of the several R&D projects being developed in the Flow Cytometry Core Facility at Memorial Sloan Kettering Cancer Center, NY. We will engage the audience to participate and share some of their own projects, and discuss ways to incorporate R&D in their own SRLs.

A customisable murine spectral backbone panel for immune surveillance in complex tissues

Grand Ballroom 2&3, October 20, 2024

Dr Rui Gardner[1]
[1] *Memorial Sloan Kettering Cancer Center*

Biography
Rui Gardner, PhD, is currently Head of Flow Cytometry Core Facility at Memorial Sloan Kettering Cancer Center.With a background in Biochemistry, Rui Gardner started his career in science as a mathematical biologist. In 2007, Rui became Head of the Flow Cytometry Core Facility at Instituto Gulbenkian de Ciência. Serving more than 40 research groups in many diverse fields including Immunology, Inflammation, Stem-Cell Biology, Microbiology, Virology, Plant Biology, among others, has given Rui a comprehensive experience in many Flow Cytometry techniques. Passionate about Science and Technology, Rui's background in Mathematics, Physics, and Biology allows bridging the gap between the operational and technical details in flow cytometry and the science within flow applications. Rui is a wonderful speaker and has lectured many courses on Flow Cytometry and Cell Sorting, being continuously involved in organizing various workshops and meetings on Flow Cytometry and Core Management in Europe.

Abstract
Even with the advent of AI-driven panel design tools, building a complex high-parameter flow cytometry panel is still considerably challenging, especially for non-experts. From design to troubleshooting and validating the panel, considerable expertise is required to handle the combinatorial and complex nature of building a reliable and reproducible panel. Though a significant number of panels have been published, their implementation is still difficult due the number of modifications needed to adapt these panels to the different research questions that require different subsets of specific markers. To address these challenges, we developed a 14-marker backbone spectral flow cytometry panel, designed to comprehensively profile immune populations across diverse murine tissues, including tumors. With the flexibility to incorporate 7 additional fluorochromes tailored to specific research needs, this panel offers a versatile solution for immunologists and immuno-oncology researchers. We will provide insights into how this customizable backbone panel not only enhances resolution and consistency across different cytometers and tissues but also serves as a practical guide for developing similar high-parameter panels in diverse research contexts.

POSTER

Mast Cell Leukaemia - A rare and aggressive entity not to be missed in the flow cytometry laboratory

Mrs Jennifer Gleadhill[1], Dr Sarah Thang[1], Dr Teng Fong Ng[2], Dr Kisoth Arasaratnam[2], Ms Kerri Prain[1], Dr David Gillis[1], Dr Aruna Kodituwakku[1]
[1]Division of Immunology, Central - Pathology Queensland, Queensland Public Health and Scientific Services, , Brisbane, Australia, [2]Haematology, Gold Coast University Hospital Laboratory, Queensland Public Health and Scientific Services, , Gold Coast, Australia

Biography

Since 2008, Jennie has been a scientist working in the Immunology Laboratory – Pathology Queensland, assisting in the diagnosis of leukemias and lymphomas by flow cytometry. She has been involved in a number of significant projects over the years such as the implementation of new flow cytometers and validating new antibodies for clinical use. She is also dedicated to staff training and prioritising professional growth in others, as well as fostering collaborative relationships. She is currently pursuing a post-Graduate research degree. On the weekend she is known to enjoy a glass of pinot grigio.

Abstract

Introduction

Mast Cell Leukaemia (MCL) is an extremely rare and aggressive subtype of systemic mastocytosis in which bone marrow aspirates contain ≥20% aberrant mast cells. Here we present a case of acute MCL in a 43-year-old male that presented with constitutional symptoms with night sweats, lethargy and weight loss and was found have elevated tryptase (>90.0 H ug/L), rare circulating blasts, anaemia, splenomegaly and lytic bony lesions.

Method:

Bone marrow aspirate specimen was received for flow cytometry, stained cells run on BD FACSCanto™ II and analysed using Kaluza C.

Results:

A significant population (28% of total cells) of mast cells were found demonstrating an aberrant immunophenotype of CD2, CD9, CD11c (weak), CD33 (strong), CD117 (strong) and were negative for CD34, CD16, CD25, CD30 as well as other lineage markers. This corresponded to morphology demonstrating a dense heterogenous infiltrate of immature appearing medium-large sized, variably granulated, mononucleated to multinucleated cells that on immunohistochemistry were strongly positive for CD117. Less common for MCL, a non-canonical *KIT* activated *V559D* variant was detected alongside *RUNX1* and *TP53* variants.

Discussion and Conclusion:

These results in addition to the clinical features and prior laboratory findings led to a diagnosis of de novo aleukaemia variant MCL with C findings. Unfortunately, he subsequently progressed despite trial of type-1 tyrosine kinase inhibitor therapy. This case demonstrated the importance of distinguishing this entity in the diagnostic flow cytometry laboratory given the implications for disease aggression and prognosis.

A Workflow for Longitudinal Biomarker Discovery Studies in Human Disease

Grand Ballroom 2&3, October 22, 2024, 3:00 PM - 3:24 PM

Ms Ceridwyn Jones[1], Miss Kate Pilkington[2], **Dr Kathryn Hally**[3]
[1]Te Herenga Waka (Victoria University of Wellington), Wellington, New Zealand, [2]Cytek Biosciences Inc., , United States, [3]Ōtākou Whakaihu Waka (University of Otago), Wellington, New Zealand

Biography
Dr Kathryn Hally is a research cytometrist at the University of Otago, Wellington campus. She has a special interest in characterizing the immune response to acute vascular conditions including myocardial infarction and peripheral vascular disease. Dr Hally is also interested in best practice for running longitudinal studies involving flow cytometry.

Abstract
Aim: Optimized and reproducible cytometry assays are key for running high-quality longitudinal biomarker discovery studies in human disease. These characteristics are essential for phenotyping cells or markers affected by storage, both of which necessitate real-time staining and acquisition. Here, we aim to develop a simplified workflow for real-time high-dimensional flow cytometry to facilitate biomarker discovery research.

Method: Peripheral blood mononuclear cells (PBMCs) or neutrophils were separated from EDTA-anticoagulated blood by negative magnetic bead isolation, washed and concentrated to 2 million (neutrophils) or 20 million (PBMCs) cells/mL. Three flow cytometry panels were optimized to comprehensively phenotype T, B and Natural Killer cells (28 markers), monocytes and dendritic cells (23 markers) and neutrophils (20 markers). Activation was defined broadly to capture quintessential immune functions like endothelial cell adhesion and antigen presentation. Staining procedures were rationalized to ensure clinical translatability and minimal experimental variability: cells were stained with antibodies in two stages (each 30 minutes), fixed, washed and acquired on a three-laser Aurora spectral cytometer (Cytek, California, USA).

Results: Isolation and concentration of cells was achieved within 45 minutes of venepuncture with a single centrifugation step to minimize platelet and erythrocyte aggregation. Staining was achieved within 2 hours of cell isolation, and immune cells were identified as $CD45^{+}CD42b^{-}CD235a^{-}$ to exclude residual platelet/erythrocyte co-expression. In totality, these panels identify ≥3 activation markers on the surface of 35 cell subsets. Concentrating cells post-isolation ensured acquisition of ≥400 cells/subset. Cryopreserved/thawed live ($Zombie\text{-}NIR^{-}$) PBMCs and granulocytes were effective batch controls for all markers except CD62L, which is shed from the surface of cryopreserved cells.

Conclusion: We present a streamlined workflow for characterizing immune cells, focusing on real-time phenotyping of cells (neutrophils, monocytes) and markers (CD62L) compromised by storage. Further, we identify appropriate batch controls that can assess, and account for, experimental and instrument variance in longitudinal studies.

POSTER

A Mysterious Case of Burkitt Lymphoma

Miss Shayli Harris[1]
[1]Royal Hobart Hospital, Hobart, Australia

Biography
Since completing her Bachelor of Biomedical Science at the University of Tasmania in 2019, Shayli Harris has worked for the Tasmanian Health Service at the Royal Hobart Hospital across the only clinical Flow Cytometry and Stem Cell Transplant Laboratories in the state.

Abstract

Burkitt Lymphoma (BL) is an aggressive mature B-Cell Lymphoma[1] which is closely associated with Epstein Barr Virus[2]. Clinical features often seen in BL are central nervous system (CNS) involvement, jaw/dental pain, kidney lesions and rapidly growing tumour masses in the abdomen and other organs[3].
Burkitt cells appear morphologically to be monomorphic, medium-sized cells with basophilic cytoplasm and small nucleoli[4]. The immunophenotype is generally CD10+, CD19+, CD20+ with surface kappa or lambda light chain expression, while an IG::*MYC* rearrangement is frequently demonstrated by cytogenetics[5].

This case looks at a patient presenting with extreme jaw pain and gum hypertrophy leading to complete teeth extraction, night sweats, weight loss and impaired eye movement. The patient was referred for urgent haematology review due to the severity of symptoms and abnormal full blood count.
All clinical features pointed towards a high-grade Lymphoma with Burkitt Lymphoma or Diffuse Large B Cell Lymphoma (DLBCL) being the most probable suspects. Due to the lack of CNS and peripheral blood involvement in initial samples, a diagnosis was unable to be obtained until a bone marrow procedure was performed almost a week after the patient's initial presentation, leading to a delayed diagnosis.

The bone marrow findings found a marked infiltrate by large mildly pleomorphic cells with high N/C ratio and scanty deeply basophilic cytoplasm and numerous vacuoles. This immunophenotype was found to be CD10+, CD19++, CD20+, Lambda+, HLA-DR++ with increased forward and side scatter properties. With morphological and flow cytometry findings highly suspicious for Burkitt Lymphoma, the final diagnosis was confirmed by abnormal conventional karyotyping and Fluorescent in Situ Hybridisation (FISH) results.
FISH detected the MYC gene rearrangement and IGH::MYC fusion in the absence of BCL2 and BCL6 rearrangement which strongly supported the diagnosis of Burkitt Lymphoma.

References:

"No conflict of interest to disclose".

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Technical Challenges in flow cytometry

Lecture Theatre, October 20

Dr Ben Hedley [1]

[1]Victoria Hospital, Canada

Biography

I was born in the Wales and educated in Belgium, the United Kingdom and Canada. At Imperial College I read chemical engineering and graduate with masters of engineering. My post graduate studies were in molecular biology and cancer research at Western University in Canada. My research allowed me to develop into different areas and started me on the path toward my current position as Medical Scientist within the teaching hospital in London Ontario. The hospital is the largest tertiary health center in the region and a referral site and covers approximately 2.5 million people. In my role I oversee multiple laboratories in the immediate for hematology and coagulation and the only flow cytometry laboratory one for the region. Our laboratory is also a Canadian reference site for measurable residual disease (MRD) testing for B and T cell acute lymphoblastic leukemia. Currently we do referral work for 14 centers across Canada and see approximately 500 cases a year. I currently sit of the College of American Pathologists Diagnostic Immunology and Flow Cytometry Committee, actively involved with the Clinical and Laboratory Standards Institute and also a board member for the International Council on Standardization of Hematology.

My interests have been primarily on furthering the standardization of flow cytometry, through guidelines or products with the goal of elevating our practices. I have had the opportunity to published a number of scientific articles on standardization and was part of the latest Clinical and Laboratory Standards Institute guideline for flow cytometry testing. With current regulations evolving I believe this will form the basis for some of the regulations that will come. Most recently I have started a collaboration with a group that has artificial intelligent software for perform analysis of clinical flow cytometry which I hold to bring to our laboratory as a Canadian first.

Abstract

Flow cytometry is widely used in both clinical and research diagnostics for the analysis of patient samples. Flow cytometry can aid in screening out suspected hematological neoplasms in patients suspected of malignancy. Despite its utility and speed, several technical challenges persist, impacting data accuracy, reproducibility, and overall effectiveness. Spillover and spread, together with signal sensitivity and resolution, often make subtle differences in fluorescence intensities difficult to resolve. These problems are especially complicated or low cellularity samples. Moreover, issues such as spectral overlap between fluorophores require sophisticated techniques to deconvolute the signals, which can introduce errors. Data analysis and interpretation may also pose challenges, particularly with increasing adoption of high complexity cytometry, as it demands expertise to manage, analyze, and interpret the data. Instrument variation and calibration inconsistency further complicate reproducibility across different laboratories or even across instruments in the same lab. These challenges require advanced hardware, robust software tools, and standardized protocols, alongside enhanced training. As flow cytometry continues to evolve, overcoming ever challenging technical hurdles will be essential to fully harness its potential for scientific and clinical advancements.

Stem Cell Transplantation in the Age of CAR T-Cell Therapy

Grand Ballroom 2&3, October 21

Dr Ben Hedley [1]

[1]Victoria Hospital, Canada

Abstract

Hematopoietic Stem cell transplantation (HSCT) has been a treatment option for decades, offering curative potential in diseases such as aplastic anemia, leukemia, lymphoma, multiple myeloma and sickle cell disease. However, the advent of chimeric antigen receptor T-cell (CAR T-cell) therapy has revolutionized the treatment of hematological malignancies by providing a novel, targeted approach to effectively manage these diseases. This talk will explore the how HSCT has evolved since its infancy and how it's current role in the context of CAR T-cell therapy. Can both exist and be complementary given the potentially competitive nature of these treatments. The benefits, limitations, and indications for each modality, and examine current evidence and future directions for integrating these therapies to optimize patient outcomes will be discussed. To clarify the roles of HSCT and CAR T-cell therapy in an era where personalized precision treatment is increasingly sort.

The other side to flow cytometry

Grand Ballroom 1, October 22, 2024

Dr Ben Hedley[1]

[1]Victoria Hospital, Canada

Abstract

Flow cytometry is a tool for characterizing hematologic diseases but is also valuable in identifying non-malignant hematologic disorders, where the goal is to detect and classify abnormal cellular populations without evidence of malignancy. Non-malignant flow cytometry spans a range of conditions, including immune cytopenia's, reactive lymphocytosis, and immunodeficiencies. Flow cytometry can assess immune cell subsets, detecting abnormal cell surface markers, and quantifying cell populations. In patients with immune cytopenia's, flow cytometry can identify autoantibodies or complement-bound erythrocytes. When reactive lymphocytosis is present the lymphocyte expansion may be polyclonal, suggesting a reactive process rather than a clonal expansion. Flow cytometry is critical in diagnosing and monitoring patients with primary immunodeficiency disorders by quantifying T, B, and NK cells. Other cell populations that can be investigated using flow cytometry are red cells and platelets aiding in the diagnosis of non-malignant disease when combined with other testing and clinical findings. The findings for patients with non-neoplastic disease and the exclusion of malignant hematologic disorders aids clinicians in providing accurate diagnoses and directing appropriate treatment strategies.

Utility of B cell subset analysis by Flow cytometry – Beyond B cell malignancies

Grand Ballroom 1, October 22, 2024

A/Prof Pravin Hissaria[1]
[1]Royal Adelaide Hospital

Biography
A/Prof Pravin Hissaria is a Senior Clinical Immunologist at the Royal Adelaide Hospital, Immunopathologist at SA Pathology and Clinical Associate Professor in the Adelaide University Department of Medicine. Immunology Lab in SA pathology offers a comprehensive diagnostic Flow cytometry services to help diagnose Immunodeficiency, benign and malignant haematological conditions to all SA public hospitals. Dr Hissaria has a strong interest in all aspects of Clinical and Lab Immunology and is currently a member of the Royal College of Pathologists Australia Immunopathology Advisory committee and QAP committee. His main clinical interests are in setting up registries of rare autoimmune and auto-inflammatory diseases. He has received grants, authored 98 publications, and has given invited talks at national and international meetings

Abstract
The rapid advances in flow cytometric techniques coupled with the in depth bioinformatic analysis has enabled precise quantitation and characterisation of various immune cells. This talk will focus of the use of B cell subset analysis in diagnosing and monitoring various diseases of Inborn Errors of Immunity and Autoimmune diseases as well as its use in guiding B cell Depletion therapies.

Lunch & Learn:

Integration of high-parameter analysis and multi-autofluorescence on the FP7000 Spectral Cell Sorter

Lecture Theatre 20 October 20, 2024, 12:30 PM - 1:00 PM

Dr Makoto Ishihara[1]
[1]Sartorius

Blood Biomarker Discovery: High-Dimensional Blood Immune-Profiling in Children with Different Disease Settings Showed Major Age-Related Changes in Proportion of Immune Cells

Grand Ballroom 2&3, October 23, 2024

Dr Sedi Jalali[1], Dr Adam Piers[1], Dr Chantal Coles[1], Dr Jeremy Anderson[1], Dr Peter Houweling[1], Dr Ian Woodcock[2], Dr Katy De Valle[2], Ms Marizyeh Taheri[1], A/Prof Paul Licciardi[1], Dr Thomas Ashhurst[3], Prof. Igor Konstantinov[1], A/Prof Daniel Pellicci[1]
[1]Murdoch Children's Research Institute, Melbourne , Australia, [2]Royal Children's Hospital , Melbourne , Australia , [3] The University of Sydney, Sydney, Australia

Biography

Sedi holds a Bachelor of Science in Biomedical Sciences and a Master of Science in Biotechnology. With over a decade of professional experience across various medical institutes, her journey has been marked by a persistent curiosity of understanding and advancing medical science.

In 2023, Sedi was conferred with a PhD from the University of Melbourne. Her doctoral research focused on the sophisticated profiling of the immune system employing high-dimensional flow cytometry, a cutting-edge technique at the immunology research.

Following her PhD, she transitioned into a Postdoctoral position at the Murdoch Children's Research Institute (MCRI) in Melbourne. Here, she continues her work in biomarker discovery in children's diseases using high-dimensional flow cytometry.

With a dedicated commitment to advancing medical knowledge and a proven track record of academic excellence, Sedi is graceful to make significant contributions to the field of immunology.

Abstract

Aim:

The immune response against disease requires cells from both the innate and adaptive arms of the human immune system. The impact of disease is often significantly influenced by age. In humans, newborns have immature immune system, which typically renders them more susceptible to some infectious diseases, whereas infants less than 14 months old are more likely to tolerate grafted heart transplants, even if ABO mismatched. However, it is still unclear that how immune cell populations play protective roles in infants who receive HTx or in contrast how these cells a role in severity of genetic muscle diseases, such as Facioscapulohumeral muscular dystrophy (FSHD) patients, where an immune cell infiltrate proceeds the replacement of muscle with fat. Therefore, understanding the composition of human immune system throughout life is crucial if we are to endeavour to find biomarkers of diseases and ultimately manipulate the immune response for treating disease.

Method:

We used high-dimensional flow-cytometry to analyse PBMC samples from healthy individuals ranging from cord blood samples to 75 years old which were collected at the Royal Children's Hospital, Melbourne. Next, we analysed two paediatric patient cohorts include heart transplant recipients (N=35) and FSHD patients (N=23). Finally, we compared the patients' samples with the healthy age-matched controls (N=40).

Results:

We found that the immune landscape in healthy controls undergoes major changes early on in life and this likely influences how the body responds to certain diseases. Our results showed that naïve T cells are replaced with different proportions of memory cell subsets. Moreover, while the proportion of MAIT cells and gd T cells were low during the first few years after birth, they were drastically increased in frequency in older children, before waning in number in older adults. The numbers of plasmablasts followed a similar trend. We also observed that the proportion of several immune cell subsets such as innate cells significantly changed in the blood of FSHD patients compared to healthy controls. Furthermore, we found that several subsets of cells from innate and adaptive immunity changed comparing pre- and post-HTx which were age-related changes.

Conclusion:

This work provides the first comprehensive study of the immune system throughout ontogeny and the foundation for understanding how the immune system changes in the context of HTx and FSHD muscular dystrophy.

Cell sorting best practice, tips and recommendations for maximum recovery of the populations that you want

Room 205, October 20, 2024

Dr Vanta Jameson[1,2], Mr Oliver Eltherington[1,2],
[1]Melbourne Cytometry Platform, Department of Microbiology and Immunolog
[2]The Peter Doherty Institute for Infection and Immunity, The University of Melbourne.

Biography

Vanta is a cell biologist and cytometrist, managing the Peter Doherty Institute (PDI) cytometry SRL, the largest of four cytometry nodes at the University of Melbourne's Cytometry Platform (MCP). She was granted her BSc (Hons) (1999) and PhD (2006) from the University of Melbourne (Peter MacCallum Cancer Institute) and continued her scientific career as a postdoctoral researcher (Monash University, 2007-2012). Vanta's research spanning adult haemopoietic and then embryonic stem cell biology commenced in the early 2000's and pushed the limits of flow cytometry instrumentation and immunophenotyping. Her daily exposure to cytometry contributed to her deep appreciation of the crucial nature of sample and experimental optimisation and a deep curiosity for the technology. During her postdoctoral appointment, she became the resident educator of newcomers to cytometry. Her love of cytometry prompted a move into cytometry SRL management, where, starting in 2012 she oversaw the inception and growth of the University of Melbourne Brain Centre's cytometry SRL for 10 years. She took on management of the PDI cytometry SRL in 2022 where she now oversees a team of cytometrist and technicians and 16 cell sorters/ analysers. With over a decade's first-hand experience as a medical research scientist and in her 13th year managing a SRL, she is passionate about cytometry and teaching to achieve robust, reproducible and publishable data.

Abstract

Cell sorters enable the high-speed analysis and isolation of particles with specific physical properties and fluorescence attributes from mixed suspensions. Recovered, pure populations can be applied to many downstream techniques including cell culture, functional studies, adoptive transfer, imaging, genomics, proteomics, etc. While many researchers may be experienced in the use of analysers, the translation of samples to sorters is not (in most cases) a simple 'plug and play'.

Annually, the Melbourne Cytometry Platform (MCP) perform thousands of hours of cell sorting experiments for University of Melbourne and external researchers on BD FACS AriaIII/ Fusion, Beckman Coulter CytoFLEX SRT (conventional) and Cytek Aurora CS (spectral) sorters. Samples handled include infectious and non-infectious human and animal primary cells and tissues and cell lines for immunology, neuroscience, drug discovery, developmental biology, small particles, marine biology, earth sciences and microbiology. Samples sorted include barely detectable fluorescent protein-expressing neurospheres, to label-free marine bacteria and algae, to antibody panels of 20+ colours to separate distinct immune subsets in influenza studies. Suffice to say, we've seen a lot of different things – from tissue diversity to experimental complexity and requirement for optimisation!

This workshop will take you through MCP's cell sorting best practices, including cell sorter setup, QC and operation for optimal sort recovery of desired populations for specific onward workflows, and considerations for experimental design and sample preparation. It will end with a real-life troubleshoot of a poorly prepared sample brought for sorting. Never say never!!

Contributions of Melbourne Cytometry Platform to the enhancement of scientific discovery

Grand Ballroom 2&3, 1, October 21

Dr Vanta Jameson[1], Dr Alexis Perez Gonzalez[1]
[1]Department of Microbiology and Immunology, Melbourne Cytometry Platform, The University Of Melbourne, Parkville, Australia

Abstract
The University of Melbourne Cytometry Platform (MCP) team guide over three hundred researchers and students annually in all aspects of cytometric experiments. MCP's clients come from across three University of Melbourne faculties, affiliated research institutes and industry with interest areas covering immunology, cell biology, vertebrate and invertebrate biology and embryology, physiology and anatomy, pharmacology, neuroscience, parasitology, bacteriology, nanoparticles, marine biology, bioengineering, and palaeontology, each of which bring unique challenges and requirements. For optimal particle and fluorescence resolution, the MCP team invest time in testing the performance of instrumentation - prospective purchases and existing cytometers- in their ability to resolve low-level fluorescence and scatter signal, cross-cytometer sensitivity analysis, fluorochrome comparisons, and sorter recovery. This information is used to assign the most appropriate instruments to samples and onward applications. The unique repertoire of samples run and tested on our stable of conventional and spectral cytometers has led to groundbreaking discoveries surrounding the effect of autofluorescence on signal resolution in biological samples. Our findings "Unlocking autofluorescence in the era of full spectrum analysis: Implications for immunophenotype discovery projects" were published in Cytometry Part A in 2022 and we were awarded publication of the year at CYTO Montreal in 2023. Our work has greatly improved the research outcomes of many of our scientists' cytometric experiments, and our learnings from the last 8 years leading to this publication and their impact on biological discovery will be presented here.

Environmental sustainability in STEMM and a role for shared resourced laboratories

Grand Ballroom 2&3, October 22, 2024, 3:24 PM - 3:48 PM

Dr Darryl Johnson[1], Dr Elena Taran[1]
[1]The Materials Characterisation & Fabrication Platform, The University of Melbourne, Australia

Biography
Darryl has long had a keen interest in the technical aspects of research. During his PhD studies and postdoc, his research heavily involved the development of novel cytometry and microscopy techniques. As an Academic Specialist at The University of Melbourne's Materials Characterization and Fabrication Platform, Darryl is deeply involved in researcher training, assisting with experimental design, and developing novel mass cytometry and imaging mass spectrometry techniques.

As well as his research interests, Darryl is an advocate for sustainability in science. He has led sustainability actions both as the MCFP's Sustainability Champion and team lead for the Engineering and Information Technology Faculty's Green Impact team "The Greengineers". Furthermore, he has completed accredited Carbon Literacy training facilitated through Australasian Campuses Towards Sustainability and the UK based Carbon Literacy Project and is a My Green Lab Ambassador.

Abstract
The impacts of climate change on humanity present many challenges as well as opportunities. There is a need for all areas of society to develop sustainable practices and behaviours to mitigate and adapt in the face of these challenges. The STEMM disciplines are not immune to these challengers and with higher energy and resource use then most other industries it can be argued that STEMM has a large part to play in working towards environmental sustainability. Excitingly, STEMM is in the privileged position to not only contribute operationally to this goal, but can offer research, education, and leadership to the challenges at hand.

Shared Resource Laboratories (SRLs) provide increased resource efficiency, are enablers of research, and are impactful in the research community. Indeed, SRLs play an important role in promoting safety, technological improvement, and research integratory. This role makes SRLs invaluable contributors, directly and indirectly, to sustainability in STEMM.

The Materials Characterisation and Fabrication (MCFP) is increasingly engaging in sustainable activities. As well as undertaking a range of direct actions do reduce resource use, the MCFP has begun to engage with partners, programs, and organisation working towards sustainability. Furthermore, the MCFP has recently begun promoting the sustainability research undertaken by the researchers that use the platform's instruments.

While not all actions undertaken by one SRL will be practical for all, it is hoped that the examples presented in this case study can assist in driving further discussion and actions from all those in STEMM.

POSTER

Distinguishing immature and mature monocytes by Flow Cytometry – An Old Topic Revisited

Ms Na Kang[1], Ms Donna Cross[1], Dr David Gillis[2]
[1]Special Investigations, Department of Haematology, Pathology Queensland, Princess Alexandra Hospital, Brisbane, Australia, [2]Department of Immunology, Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Australia

Biography
Senior flow cytometry scientist

Abstract
We present an objective approach to more precisely differentiate and quantitate mature and immature monocytes, particularly the blast equivalents, promonocytes in bone marrow aspirates, by utilising an integrative methodology that combines reference cell population, an overlay analysis composite, and an eight-colour monoclonal panel.

Methods:
Mature monocytes from peripheral blood of health donors without reactive monocytosis were defined as reference population. Both reference samples and bone marrow aspirates from patients referred to our institution for the investigation of myeloid neoplasms were stained with an eight-colour monoclonal panel. This panel was extracted from an in-house 4-tube panel and was primarily designed for the screening and diagnosis of acute myeloid leukaemia with monocytic differentiation. It includes CD11b APC, CD13 PC7, CD14 AA750, CD16 Pacific Blue, CD34 PC-5.5, CD45 Krome Orange, CD56 FITC, and CD64-PE. The FCS files were merged and were analysed using an overlay composite created on Kaluza 2.1.

Sequential gating strategy was employed with CD64 as the backbone marker which ensures only monocytic cells were interrogated for the antigen expression levels to identify the best combination of markers that can distinguish immature monocytes from their mature counterparts.

Results:

Comparison to reference monocytes of peripheral blood reveals that lower expression levels of CD11b and CD13 was the most striking feature to identify immature monocytes present in bone marrow (Fig1), correlating to antigen intensity of CD14. However, promonocytes of very late stage may show homogeneous expression of CD14 which overlaps with that of mature cells, making the quantification of promonocytes solely based on CD14 expression more subjective and less accurate. Promonocytes can be accurately quantified on the plot of CD11b vs CD13 by gating on cells with dim antigen intensities, when using reference populations as a template. (Fig2). The most immature form, monoblasts, were recognised by the absence of CD13 expression (Fig3). In contrast, mature monocytes in marrow samples exhibited homogeneous expression of CD11b, CD13, and CD14, equivalent to those on reference monocytes from peripheral blood.

Conclusion:

Accurate quantitative assessment of monocytic cells is essential for the correct diagnosis and classification of haematological neoplasms involving monocytic lineage. Analysis of superimposed antigen profiles of monocytes between patient and reference sample with focus on examining expression pattern of CD11b and CD13 provides a refined approach for distinguishing immature and mature monocytes by flow cytometry. It overcomes the subjectivity of using CD14 expression levels to define the maturational stages of monocytic cells, as neoplastic monoblasts and promonocytes may not always lack CD14 expression.

Figures

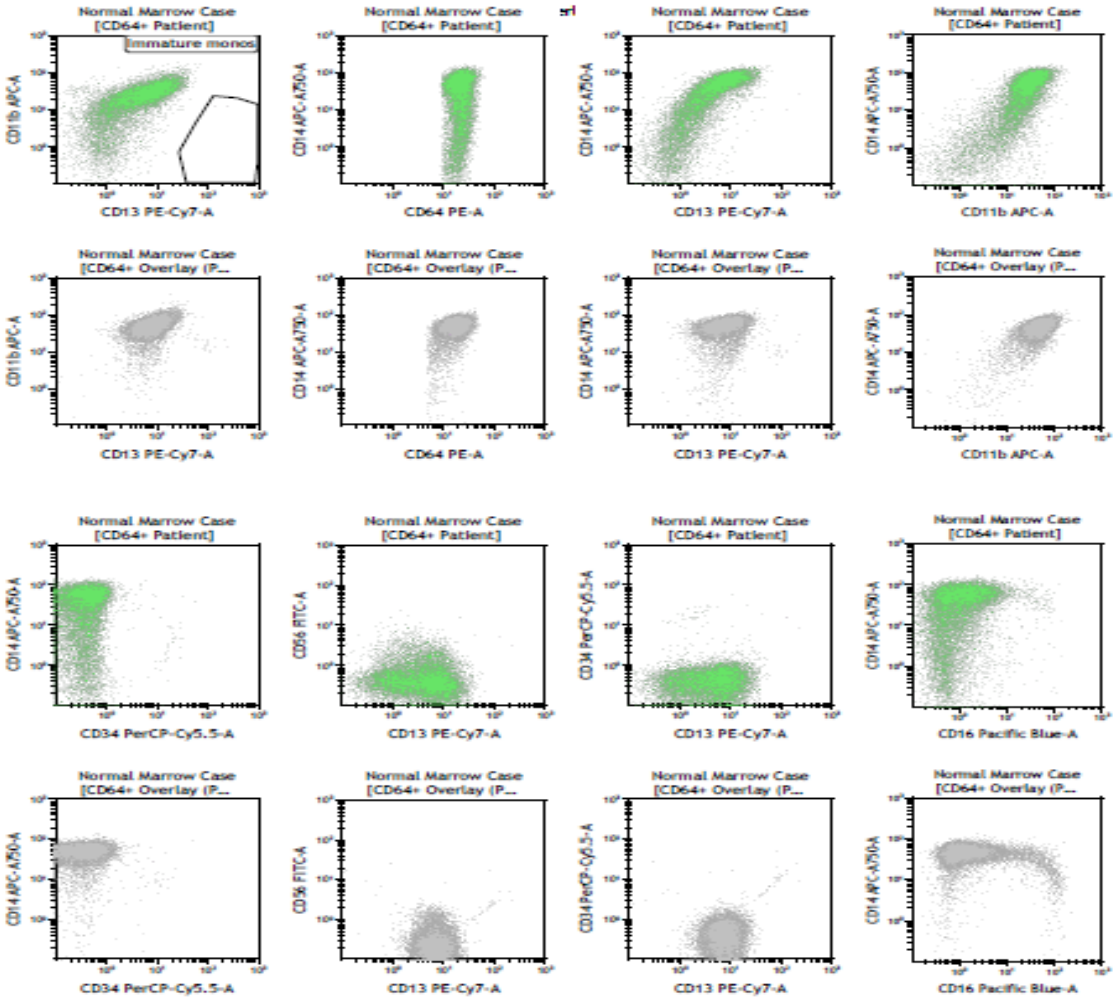

Fig 1. Overlay analysis shows the difference in the antigen expression levels between reference monocytes (grey colour), and monocytes from a normal marrow aspirate (green colour).

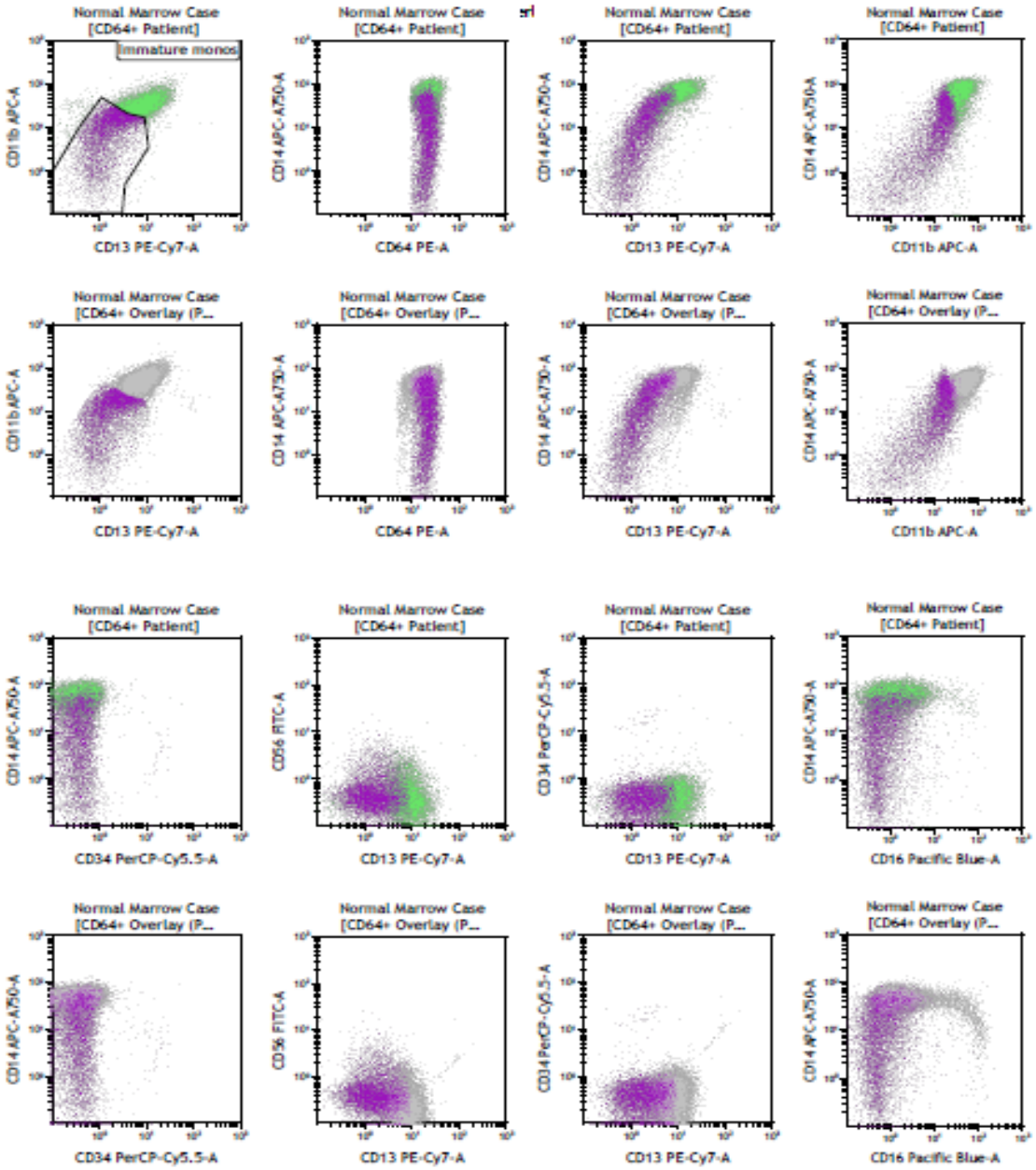

Fig 2. Using reference monocytes as template, promonocytes and monoblasts (purple colour) in a normal marrow were demarcated on CD11b vs CD13 plot. These immature monocytes exhibit gradually increased antigen intensity of CD 13 and CD11b. The mature monocytes in the marrow and peripheral blood show equivalent antigen expression levels of CD11b, CD13, CD14.

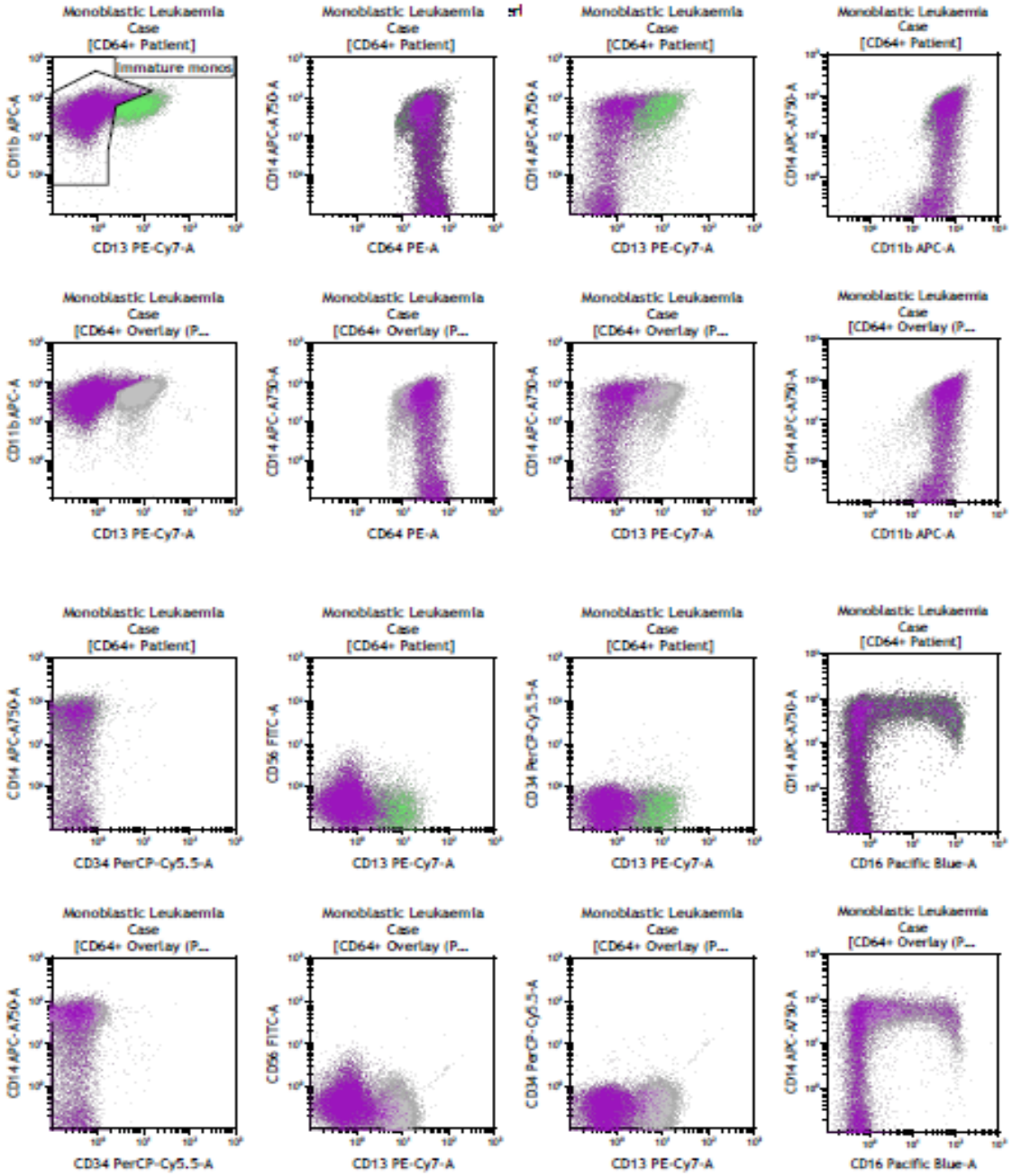

Fig 3. Neoplastic monoblasts and promonocytes are defined on CD11b vs CD13 plot using reference monocytes as template. In contrast to Fig 2, these abnormal cells demonstrate asynchrony in the myelomonocytic antigen expression.

References:

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POSTER

Integrating Complex Immunology into Clinical Cancer Trials

Prof Roslyn Kemp[1], Brad Devery[1], Naomi Grambin[1], Rory Costello[1], Dr Kirsten Ward-Hartstonge[1]
[1]University Of Otago, Dunedin, New Zealand

Biography

RK leads a research lab at the University of Otago, Dunedin, NZ, focussed on the study of immune responses in people with colorectal cancer and inflammatory bowel diseases. Her lab aims to identify ways to target treatments to individual patients using analysis of their individual immune responses. She has been in leadership of the International Union of Immunological Societies since 2016, and has established gender equity policies at national and international levels.

Abstract

Aim:

Cancer treatments based on patient immune responses, such as immune checkpoint inhibition (ICI), have had significant positive impact for many cancer patients. The success of ICIs is not consistent, especially for solid tumours. The majority of clinical cancer trials use progression-free survival and other clinical factors as primary endpoints. However, rarely is the baseline or responding immune response of cancer patients an endpoint in clinical trials nor is it monitored during the trial, despite the fact that the immune response is the target of the therapy. Importantly, the underlying immune response is not considered when determining appropriateness of treatment; unlike genetic mutations or overall health. This partly stems from a lack of training in fundamental immunology at medical schools, but also an understandable inability for clinical trial researchers to interpret complex immunology. The cytometry community has both the knowledge to perform immune profiling in clinical trials and the technical skills to present complex data in an interpretable way.

Method:

Our laboratory has designed several techniques to incorporate complex immunology into clinical trials, including logistics of sample handling and processing. We have designed ways to visualise and interpret the data for non-specialist researchers.

Results:

We are currently testing these techniques and approaches in existing clinical cancer trials. We have been advocates for the oncology community to incorporate immunology as an essential part of cancer clinical trials.

Conclusion:

A collaborative and standardised approach is needed for the uptake of immunology data in clinical cancer trials for the oncology community. The role for cytometrists is this approach is essential and a broader discussion of how best to ingrate immunology in these sometimes very rigid protocols is required.

RCPAQAP – challenges in Immunophenotyping EQA

Lecture Theatre, October 20

Loriza Khan[1]
[1] RCPAQAP

Biography

Loriza Khan is a Senior Scientist at the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) and is the technical head of the Haematology and Transfusion Discipline. She has over 25 years of experience in various areas of haematology while working in Australia and New Zealand and has been with the RCPAQAP for 6 years. Her area of expertise is in external quality assessment programs.

Abstract

An overview RCPAQAP Immunophenotyping program, review of survey performance and challenges.

POSTER

Fostering Consistency in EV-Based Vaccine Development and Clinical Trials: Advancing Towards Standardization

Dr Anis Larbi[1]
[1]Beckman Coulter Australia, Lane Cove West, Australia

Biography

*Dr. Anis Larbi graduated in 2005 from the Immunology Program at the University of Sherbrooke (Canada) and has developed thereafter his own research program in immunology at the Singapore Immunology Network institute at A*STAR. He was the A*STAR Flow Cytometry platform Director (2010-2020) and an ISAC Scholar (2011-2016). During his scientific career he published >200 peer-reviewers articles and is a 2020-2023 Highly Cited Researcher. He joined Beckman Coulter Life Sciences in 2020 and is now a Senior Manager in the Medical and Scientific Affairs Department*

Abstract

Aim:

Making the case for standardisdation in EV preparation and characterisation

Method:

A standardized protocols was established for the isolation, characterization, and functional analysis of EVs. We used automation methods for these processes and compared it with the manual processing. Comparison of experienced versus naïve users was also performed. We also measured immune responses by flow cytometry in the context of infectious disease. We compared the performance of manual, liquid antibody processing versus the use of dry-reagents.

Results:

Automated density-based separation EVs (from plasma and urine) reduces variability their recovery when using orthogonal methods. Reproducibility, recovery, and specificity is the highest with automation. Analysis by mass-spectrometry revealed the higher variability in the content of EV linked to manual processing. Not only automation increases reproducibility but reduces errors. Immune monitoring to follow treatment efficacy is limited by the variability of multi-center studies. Standardizing the process with dry reagents enable to include immune profiling in the One Study. The data demonstrates the robustness of the method and the significantly reduced variability of flow cytometry-based immune monitoring.

Conclusion:
By establishing standardized EV preparation, counting, characterization, and production methods, as well as standardized approaches to assess their biological effects, we can significantly increase the reproducibility of EV-based vaccines. Integrating these processes from the early phases of R&D to the immune profiling in research clinical trials would improve their generalizability and enhance the overall advancement of EV-based vaccine research.

Table/Figure:
The poster contains Diagrams
References:
"This research was supported by Beckman Coulter Life Sciences."

POSTER

A flow cytometry approach for the characterization and isolation of extracellular vesicles

Dr Anis Larbi[1]
[1]Beckman Coulter Australia, Lane Cove West, Australia

Abstract
Aim:
Use of flow cytometry to characterize EVs and enhance our comprehension of their functions.

Method:
To assess the potential of flow cytometry in detecting, characterizing, and isolating EVs, we employed nano flow cytometry for analysis and sorting. Engineered EV (GFP), EVs isolated from biological samples and beads of various sizes were used in this study. EVs isolation was performed using ultracentrifugation or SEC. EVs were analyzed for their size and for the fluorescent reporter to test the sensitivity of the flow cytometer. FCM PASS software was used for light scatter standardization.

Results:
By utilizing flow cytometry, we compared the quality of EV samples obtained through ultracentrifugation. Through side scatter analysis in the violet channel (V-SSC), we successfully identified the isolated EVs. Furthermore, we were able to resolve particles as small as 40 nm, highlighting its effectiveness in characterizing small EVs. To confirm the heterogeneity of the EV preparation, EV preparations were sorted. Post-sort data revealed distinct profiles based on scatter and fluorescence characteristics. Finally, we highlight the superior sensitivity of the last generation of flow cytometer dedicated for nanoparticles. EV-related data was further analysed using the FCM PASS method. Overall, these findings strongly support the use of flow cytometry for counting, characterizing, and sorting EVs.

Conclusion:
The field of EVs is rapidly advancing, necessitating a deeper understanding of their heterogeneity. This knowledge is crucial for comprehending their physiological roles and involvement in diseases. In this study, we successfully applied flow cytometry to analyze EVs, employing various flow cytometry tools. Further research will enable the development of tailored flow cytometry protocols for EV characterization based on specific requirements

Table/Figure:
The poster contains Diagrams

References:
"This research was supported by Beckman Coulter Life Sciences."

POSTER
Optimizing a workflow for the analysis of extracellular vesicles

Dr Anis Larbi[1]
[1]Beckman Coulter Australia, Lane Cove West, Australia

Abstract
Aim:
EVs Preparation workflow to reduce variability.

Method:
A comprehensive workflow for the characterization of EVs is proposed, with two distinct models depending on the purpose: biomarker/heterogeneity analysis (commonly used in R&D) and specific EV production (for manufacturing purposes). The workflow includes several key steps such as EV preparation, purification, quality control, and characterization. Flow cytometry is a widely used method in this process and is often complemented with orthogonal methods for comparison.

Results:
We highlight the significant benefits of automation in EV preparation, which effectively reduces variability introduced by human operators. This automation ensures consistent and reliable results throughout the process. Additionally, flow cytometry stands out as a superior method for EV characterization due to its ability to sensitively detect and count single EVs using fluorescence. The ability to analyze EVs individually is particularly important in biomarker/heterogeneity analysis. Moreover, the user-friendly nature and robustness of the tested cytometer offer significant advantages, especially in a manufacturing setting. Lastly, the seamless integration of the flow cytometry into an EV workflow analysis further proves its advantages.

Conclusion:
In this work, we demonstrate the seamless integration of flow cytometry into EV research. The utilization of the flow cytometry allows for the acquisition of high-quality and reproducible data, owing to its exceptional sensitivity. Collectively, these findings strongly suggest the advantages of incorporating flow cytometry as a complement to, or even a replacement for, orthogonal methods in EV analysis.

Table/Figure:
The poster contains Diagrams

References:
"This research was supported by Beckman Coulter Life Sciences."

POSTER

MISEV 2023 : Isolation and characterization of extracellular particles

Dr Anis Larbi[1]
[1]Beckman Coulter Australia, Lane Cove West, Australia

Abstract

Aim:
Commentary on MISEV2023, guidelines and recommendations for the analysis and reporting of extracellular vesicle studies

Method:
The recent release of MISEV2023 marks a significant milestone for the EV research community, aiming to establish standardized guidelines and recommendations for the analysis and reporting of extracellular vesicle studies. The pursuit of knowledge in this field involves utilizing various solutions to generate data, while ensuring quality and reproducibility. This report focuses on how current practices in centrifugation and flow cytometry align with MISEV2023, along with potential gaps. Detailed protocols are provided for centrifugation methods like differential ultracentrifugation and analytical ultracentrifugation, as well as flow cytometry protocols for particle counting, characterization, and sorting. Specifically, the ability to detect and characterize small EVs by flow cytometry is highlighted for its exceptional fluorescence sensitivity (scatters and fluorescences). We also delved into the analysis and reporting aspects of the different techniques, offering a critical review of the current status quo. By examining the needs for proper analysis and reporting of EV-derived data scientists can gain valuable insights to enhance their EV research endeavors.

Results:
N/A

Conclusion:
N/A

Table/Figure:
The poster contains Diagrams

References:
"This research was supported by Beckman Coulter Life Sciences."

POSTER

Utilising TauSense separation to enhance signal to noise ratio in the microsphere-based aerosol containment assay

Dr Edwin Lau[1]
[1]Wimr, , Australia

Biography
Edwin is the advanced cytometry specialist at Westmead Cytometry, Sydney, Australia. He has over 10 years of experience in Cytometry.

Abstract
Background:
Perfetto and colleagues have previously described a microsphere-based assay measuring containment of aerosols generated in cell sorters (1). Challenges in the practical implementation at Westmead Cytometry has included:
A) The presence of debris with autofluorescence that is unavoidable during sample collection, presenting a challenge in microsphere identification. B) Achieving accurate focusing on the test slide due to the absence of particles in a typical sample (zero bead to pass).

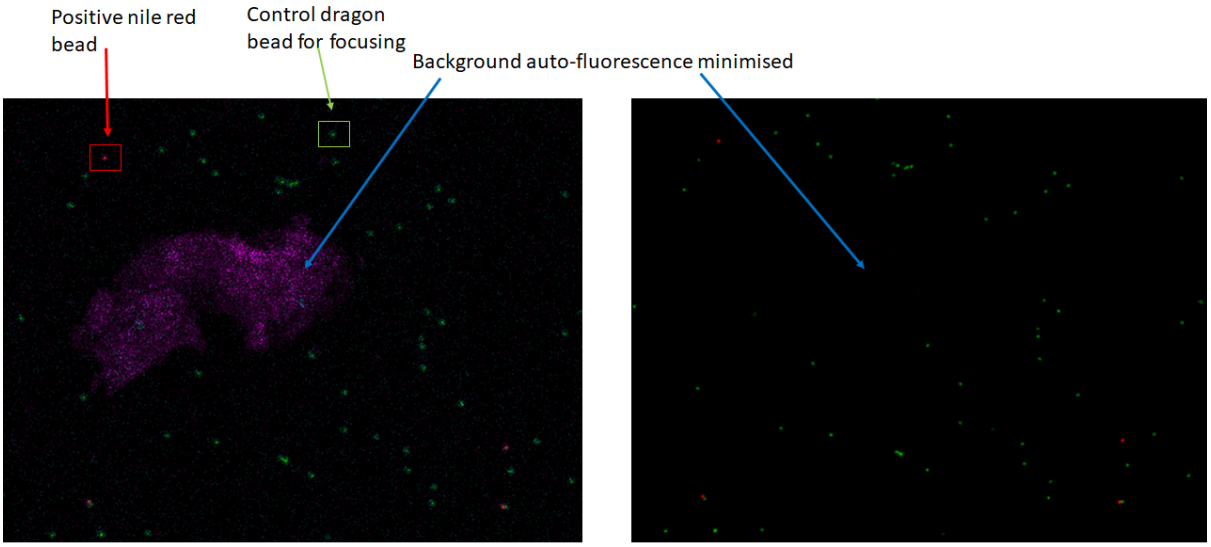
Aim:
In this study, we propose the introduction of control beads to enhance focusing and explore the application of the TauSense technology to reduce background fluorescence.

Method:
Samples were collected as described by Perfetto and colleagues (1), with the Dragon green beads replaced by 1.0-1.4µm Nile Red beads (Excitation 555nm, Emission 638nm; Spherotech Inc.). 1-2 spray of control beads were then applied at the end of the collection. Control beads were prepared by adding 10µl of 1.0µm Dragon green beads (Excitation 488nm, Emission 520nm; Bangs Laboratories) to 2ml of saline. Diluted beads were then transferred to an atomising bottle.

Alternatively, a mock positive sample was prepared mock aerosol collection for 10 minutes, followed by 1-2 sprays of a mixed bead solution. The mixed bead solution is prepared by adding 10 µl of Dragon green beads & 10ul of 1:50 diluted Nile red beads to 2ml of saline.

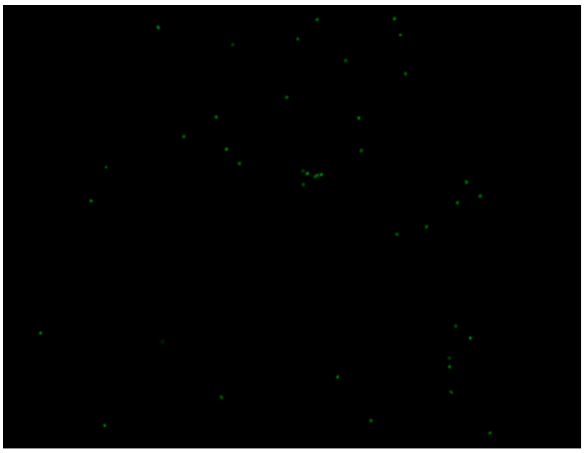
Images of coverslips were acquired on the STELLARIS 5 confocal platform (Leica) with a 10x objective. TauSense Separation was applied to remove background using fluorescence lifetime-based information (2).

Results:

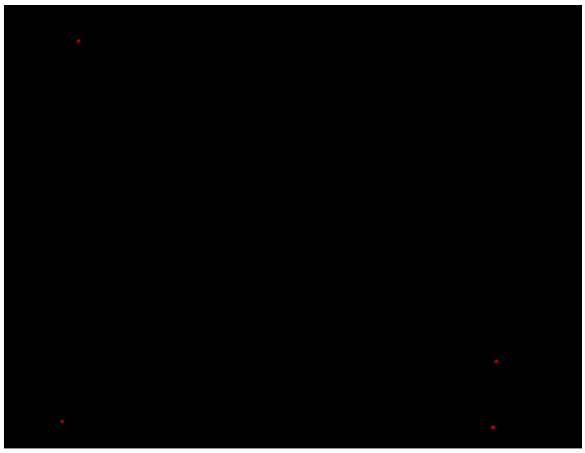


raw image merged

TauSense separation applied merged



TauSense separation applied 500-573nm (Dragon green beads only)



TauSense separation applied 630-750nm (Nile red beads only)

Conclusion:

We demonstrated that TauSense separation can be used to assist in distinguishing background autofluorescence and therefore identify genuinely positive beads. This method will improve the efficiency of our routine aerosols containment monitoring and further address biosafety during cell sorting.

References:

1): Perfetto *et al* (2019) Novel Impactor and Microsphere-based Assay used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter. Cytometry A 2019 Feb; 95(2):173-182.

2): Roberti et al (2000) Application Note: TauSense: the potential of STELLARIS. *Nat. Methods*https://www.nature.com/articles/d42473-020-00364-w

No conflict of interest to disclose.

POSTER

Flow Cytometric Quantification and Absolute Counting between Neat and Washed Samples in Lithium Heparin and EDTA

Ms Mandy Li[1], Tracy Pham[1], Kylie Baldwin[1], David Westerman[1,2], Vuong Nguyen[1]
[1]Peter MacCallum Cancer Centre, Parkville, Australia, [2]University of Melbourne, Parkville, Australia

Biography

Mandy Li joined the core laboratory team in 2009 at Peter MacCallum Cancer Centre Melbourne, Australia after completing her undergraduate degree at Monash University and postgraduate diploma at RMIT. She is a multidisciplinary scientist trained in Flow Cytometry, Haematology, Morphology, Blood bank, and Biochemistry. She has over 10 years' experience in the Flow Cytometry laboratory and is currently the Deputy Senior Scientist where she educates and mentors junior staff and assists in providing a high quality, effective and innovative clinical flow cytometry service.

Abstract

Aim:

Currently, absolute lymphocyte quantitation uses an EDTA tube lymphocyte count from the haematology analyser. This is discordant with the routine preference utilising a lithium heparin tube and a stain/lyse/wash procedure. This raises uncertainty regarding the accuracy of using the absolute count.

Our aim was to prove absolute counting is accurate and sample manipulation washing/reconstitution does not affect white cell (WC) population composition or lymphocyte subset enumeration.

Method:

Lithium heparin (LH) and EDTA tubes were collected from normal donors and patients. One normal and one patient (MBL) were re-tested at Day 3.
The WCC and differential was performed on an Abbott Alinity haematology analyser. Lymphocyte screening tube (LST Cytognos kit) was prepared using a single platform (SP) method and a stain/lyse/wash (DP) method on a BD FACSLyric. Analysis utilized Kaluza software (V2.1.2.1). Percentage positive and absolute counting of 5-part WC differential and lymphocyte subsets were calculated.

Results:

7 PB samples (3-normal donors, 1-Burkitt's lymphoma, 2-CLL, 1-MBL) were tested. The percentage positive and absolute values in normal donors showed no significant differences between LH and EDTA tubes at day 0 and 3. Table 1 shows increased percentage differences in abnormal patients due to the complexity of the patients' disease, however, population composition was consistent. Absolute values calculated with washed WCC were markedly decreased (>40%) comparing to SP and DP method. All EDTA samples showed a lower white cell viability fraction (WVF) at day 3, 0.482 (EDTA median) vs 0.898 (LH median).

Conclusion:

Concordant results were demonstrated between haematology analyser and flow methods using neat and washed samples using either LH or EDTA at day 0 which supports current practice. LH may be preferred over EDTA given their lower VWF at day 3. Washed WCC however, should not be used for absolute calculations due to cell loss.

Table 1: Observed highest percentage difference of absolute counts and respective range ($x10^9$/L) in abnormal patients. SP = Single Platform, DP = Dual Platform, N/A = Not Applicable

	EDTA		Lithium Heparin	
	Haem Analyser vs Flow (SP)	**Flow (SP) vs Flow (DP)**	**Haem Analyser vs Flow (SP)**	**Flow (SP) vs Flow (DP)**
Neutrophils	25.4 (1.650-2.130)	56.2 (4.353-7.761)	16.4 (1.747-2.060)	47.6 (4.806-7.812)
Lymphocytes	68.5 (4.500-9.191)	12.2 (144.588-148.396)	53.4 (4.300-7.434)	11.9 (1.286-1.466)
Monocytes	139.8 (0.514-2.900)	46.9 (0.514-0.829)	158.2 (0.423-3.630)	72.9 (0.256-0.550)
Eosinophils	101.8 (0.027-0.083)	115.3 (0.027-0.101)	126.2 (0.084-0.019)	166.6 (0.019-0.209)
Basophils	135.2 (0.017-0.300)	200.0 (0.000-0.026)	84.2 (0.022-0.054)	200.0 (0.000-0.022)
CD3+ T-cells	N/A	44.4 (1.615-2.538)	N/A	43.5 (1.634-2.543)
CD4+ T-cells	N/A	40.4 (0.872-1.315)	N/A	61.6 (0.121-0.228)
CD8+ T-cells	N/A	78.2 (0.974-2.227)	N/A	95.1 (0.899-2.531)
CD19+ B-cells	N/A	34.7 (1.094-1.597)	N/A	51.2 (3.304-5.580)
CD3-, 16+ 56+ NK-cells	N/A	178.2 (0.001-0.017)	N/A	183.5 (0.001-0.023)

References:

"No conflict of interest to disclose".

1. Wood B, Jevremovic D, Béné MC et al. ICSH/ICCS Working Group. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part V - assay performance criteria. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):315-23.

Enabling imaging features in single cell RNA sequencing analysis

Grand Ballroom 2&3, October 22, 2024, 3:48 PM - 4:12 PM

Dr Wenyan Li[1], Dr. Sajad Razavi Bazaz[1], Mr Rob Salomon[1]
[1]Children's Cancer Institute Australia, Randwick, Australia

Biography

Wenyan Li is a Cytometry Innovation Specialist in the ACRF Liquid Biopsy Program at Children's Cancer Institute Australia with his work focused on advanced single-cell multi-omics and flow Cytometry.

Abstract

Aim: The recent obsession with single-cell multiomics data has led to the development of approaches that can allow the simultaneous analyse the transcriptome alongside a variety of other modalities including proteomics, epigenetics and mutation detection. While these tools are transforming our ability to decipher biology, scientists often do this blind, trusting that what was loaded into the scRNAseq platform is what made it to the capture. Here, using the images generated by the BD Rhapsody system, we have developed a method that utilises imaging of the cells immediately prior to capture to assist with run QC and to provide insight into the types of cells loaded even before sequencing libraries are made.

Method: First, we develop a R script to automatically identify and crop image regions that contain fluorescence information from the images in the "Cell Load" folder generated by the BD Rhapsody Scanner. The cropped images are then ordered by cell size and stitched together as one image. In addition, we also build a QC metrics based on fluorescence patterns to categorise images into noise, viable cell singlets and viable cell multiplets.
This script was then tested in samples into which relatively larger size cancer cells were spiked at a known ratio and in primary patients samples of cerebrospinal fluid (CSF) from patients with brain cancer.

Results: Our method can remove image regions of empty wells and tile all the cropped images into one large image for the fluorescence and brightfield channels, respectively. Noise or non-cell events are identified if fluorescence signal is low, or fluorescence area is too small or too large.
When applied to biological samples, we show that the script can identify the spiked in large cancer cells (based on the cell size), which are also identified through gene expression. Importantly we now regularly use the script can be used to assess whether there are large cancer cells in clinical samples (circulating tumour cells for the CSF of children with brain cancer) and if the sample is in good condition for scRNAseq.

Conclusion: We demonstrate that rapid imaging of the cells, immediately prior to introduction of beads and capture for scRNASeq, has value in the early stage of scRNAseq as it can provide insight into quality of the loaded sample and can provide a rapid glimpse into the biology of a patient and may provide evidence of cancer spread (via identification of a large CTC population of cells) even before sequencing.

LabPlus Networking the Flow Lab

Grand Ballroom 1, October 21, 2024, 4:20 PM - 4:40 PM

Danny Lim[1],
[1]LabPlus, New Zealand

Biography

Danny Lim is a technical specialist of immune deficiency studies at LabPlus, New Zealand.

He was born in Malaysia and after spending some years in Singapore, migrated to New Zealand to enjoy the tranquil lifestyle. He joined the Haematology team at Labplus in December 2001 and made his way to the flow cytometry section in 2004 and never left the section. It was something new, exciting and challenging to him. he likes learning new tricks and embrace new technology with open arms.
In his spare time he rides bikes. He rides bikes a lot although not as much as he used to. In winter he likes a little bit of time in the mountains snowboarding and partake in a bit of apres ski.

Abstract

Automation and Information Technology (IT) have pivotal roles in the modern diagnostic laboratory both in capability and capacity; enhancing our ability to deliver an efficient and safe service. At the same time, it changes the way we think and the way we operate.

At LabPlus, we had the opportunity to automate workflows with the introduction of the Becton Dickinson (BD) Sample Prep Assistant (SPA) in 2005, which was supplanted by FACSDuet Premium in 2024. The FACSduet Premium is promoted to be much more capable instrument with the inclusion of a wash station and centrifuge giving it the capability to automate entire sample preparation processes and even cocktailing. What's more is the inclusion of the BD FACSLink interface that came part and parcel of our FACSLyric/FACSDuet package. For the first time since the establishment of the flow cytometry laboratory at LabPlus, the BD FACSLink interface would make the probable possible. Getting there though, there was a little bit of luck but it was fun.

POSTER

Application and adaptation of an experiential, learning-by-doing approach to clinical and research flow cytometry training

Dr Matthew Linden[1], Dr Henry Hui[1], Dr Calila Santos[1], Dr Sonia Fernandez[1], A/Prof Kathy Fuller[1]
[1]University of Western Australia, Perth, Australia

Biography

Dr Matthew Linden leads the Discipline of Pathology & Laboratory Science at the University of Western Australia. A teaching and research academic, his research focus is the development of cytometric tools to better diagnose, understand, monitor and treat haematological disorders. Matthew trains medical scientists with a bespoke accredited curriculum.

Abstract

Aim:

Adaptation of a pedagogical approach that uses experiential, problem-based learning to enhance skills in flow cytometry in clinical and research applications

Method:
We adapted our previously published pedagogical approach for flow cytometry education and skill building for a range of applications in clinical and research training. Students engaged in various tasks across undergraduate and postgraduate biomedical science curriculums, including antibody titration, compensation, staining, instrument operation, gating strategies, and analysis of cell viability, phenotype and function. Innovative hands-on activities to iteratively build student understanding, such as "gating" felt pieces for shape/colour before applying this logic to cytometry analysis software, have been developed. Innovative approaches were called for during the pandemic to accommodate remote learning. Further adaptations based on evolving clinical guidelines and technological shifts to bring-your-own-device (BYOD) learning practices at UWA have occurred.

Results:
Specific learning-by-doing classroom applications include measurement of viability and apoptosis in cancer cell lines treated with herbal medicine, staining and analysis of lymphocyte subsets in immunodeficiency syndromes, analysis of macrophage phagocytosis of fluorescently labelled microorganisms, establishing an intracellular staining protocol using to measure cytokine synthesis, measurement of cell proliferation in response to mitogens, design of gating strategies to identify clonal expansion, aberrant expression and leukaemia associated immunophenotypes in haematological malignancies, and analysis of apoptosis or protein phosphorylation in different haematological cancers.

We will present data outlining our updated approaches, with reflections on the effectiveness in improving student understanding and engagement in the different teaching activities, as well as how our approach has been adapted in the changing landscape of medical and research scientist training in the pandemic and post-pandemic setting.

Conclusion:
Adaptations of our experiential, problem-based, iterative learning strategy provide opportunities to effectively engage students and improve student confidence and competence in the analysis and interpretation of clinical cytometry data in research and clinical training.

References:
Adv. Physiol. Educ. 2016;40(2):176-85
"No conflict of interest to disclose".

POSTER

Multi-Site Instrument Alignment Using Lyophilised BD™ CompBeads.

Dr Kevin Lo[1], Mr Dominic Gagnon[2]
[1]CellCarta, Sydney, Australia, [2]CellCarta, Montreal, Canada

Biography
Kevin Lo is a flow cytometry specialist at CellCarta, where he manages the Sydney Flow Cytometry Unit. He completed his PhD with the Dendritic Cell Research group at the University of Sydney in 2018 and stayed with the group as a post-doctoral scientist until 2019. Kevin began his specialisation in clinical flow cytometry when he spent 3 years as a hospital scientist at SydPath, St. Vincent's Hospital, Sydney. During his time at SydPath, Kevin completed the ASCP Specialist in Cytometry exam and was awarded the certification in 2022. After joining CellCarta, Kevin continues his passion for flow cytometry by providing cytometry services for clinical trial studies.

Abstract

Aim:

Flow cytometry has become an integral component of clinical trials, particularly for immunotherapies. Since clinical trials are often conducted at multiple locations, flow cytometric measurements must be consistent across all sites. In the current study, we aimed to develop a reliable and cost-efficient method to transfer flow cytometry setup to all testing laboratories.

Method:

A set of CD4 antibodies covering all parameters of BD LSR Fortessa™ (5-laser/18-color) were stained on BD™ CompBeads and lyophilised by the BD Biosciences Custom Technology Team. The product, LyoBeads, was then shipped to all sites for matching target values. FlowCytes® beads (SlingShot Biosciences) were used to align FSC and SSC parameters. The precisions of the settings were then tested by comparing the readouts of reference samples between sites. The stability of the LyoBeads was also tested at two different sites by measuring the MFI of each LyoBead between 15 minutes and 48 hours post-reconstitution.

Results:

We have successfully transferred 10 flow cytometric assays from reference laboratories to testing sites. The logistic cost and time have significantly reduced compared to conventional BD™ CompBeads, which require shipping of reagents of the same lot each time an assay is shared. The consistency of the readout has also greatly improved.

Among the LyoBeads, APC rapidly declined in MFI, followed by APC-R700, PE-CF594, and PE. To address these observations, other alternatives were tested for better stability. Alexa Fluor®-647 and Alexa Fluor®-700 are more stable than APC and APC-R700. BD Horizon RY586 and BD Horizon RY610 were also better than PE and PE-CF594, respectively.

Conclusion:

Having consistent measurement is one of the major elements of conducting successful global clinical trials. The reduction in cost and time is also crucial in maintaining cost-efficient studies. The LyoBeads are evidently one of the reliable tools available for generating consistent data that are independent of location and instruments.

Comprehensive Immunophenotyping Reveals Immune Hallmarks of Psoriatic Multiple Sclerosis Sub-type

Grand Ballroom 2&3, October 22, 2024, 4:12 PM - 4:36 PM

Dr Varitsara Mangkorntongsakul[1]

[1]The University Of Sydney, Sydney, Australia

Biography

Varitsara is a Higher Degree Research (HDR) student at the University of Sydney, investigating the association between psoriasis and multiple sclerosis. She is passionate about skin health and immunology.

Abstract

Aim:

Multiple Sclerosis (MS) and psoriasis are both autoimmune disorders whereby T lymphocytes, in particular Th1 and Th17, play a significant role in the pathogenesis. We hypothesized that concurrent diagnosis of psoriasis (PsoMS) is a variant of MS which mediated by specific T lymphocytes. This study aims to determine T cell subsets that are involved in both MS and psoriasis.

Method:
Mass cytometry was performed to identify and quantify T cells subsets (T con, T reg, Th1, Th2, Th17 and Th17.1) in MS patients (n=25), MS with psoriasis (PsoMS, n=12) and healthy control (HC, n=10). Flow data were analyzed using FlowJo X 10.0.7r2 Cytometry Analysis Software (FlowJo, LLC, Ashland, OR, USA). GraphPad Prism Version 6 software (GraphPad Software Inc., San Diego, CA, USA) was selected for statistical analysis. One-way ANOVA was conducted for three independent groups comparison.

Results:
A significant depletion of Th1 ($CXCR3^+$ $CCR4^-$ $CCR6^-$) and Th17.1 ($CXCR3^+$ $CCR4^-$ $CCR6^+$) subsets were evident in MS [p= 0.0027 for Th1 and p= 0.0485 for Th17.1] and PsoMS groups [p= 0.0130 for Th1 and p= 0.0272 for Th17.1] when compared to HC. The significant reduction was demonstrated in all MS study groups regardless of whether they have psoriasis, and irrespective of which therapy they were on. In contrast, further phenotyping of these subsets revealed a PsoMS specific deficit in CCR5 expression across all Thelper subsets, which strikingly was restricted to patients being treated with non-B cell targeted therapies, suggesting a psoriatic-MS sub-type therapeutic effect.

Conclusion:
We report the interesting association between Th1/Th17.1 and MS subtypes, using comprehensive immunophenotyping. The role of Th17.1 lymphocytes in MS pathogenesis is becomingly evident and this study through its utilisation of high dimensional cytometry to characterise cellular phenotypes highlights them as a promising therapeutic target.

Making Sense of Cytometry data in R

Room 207/208, October 20, 2024

Dr. Felix Marsh-Wakefield[1]
[1]Centenary Institute

Biography
Dr. Felix Marsh-Wakefield is a post-doctoral researcher in the Liver Injury & Cancer Program at the Centenary Institute and the Human Cancer & Viral Immunology Laboratory at the University of Sydney. As an immunologist, he focuses on investigating the role of various immune cells in diseases such as hepatocellular carcinoma and multiple sclerosis. His work primarily involves using bioinformatics to analyse high-dimensional data, including imaging mass cytometry. Dr. Marsh-Wakefield is also a participant in the Marylou Ingram Scholarship Program, run by the International Society for the Advancement of Cytometry (ISAC).

Abstract
As high-dimensional cytometry data becomes increasingly accessible and continues to grow in complexity, the ability to efficiently analyse it has become a significant challenge for researchers. Fortunately, several robust methods in R can help streamline cytometry data analysis. In this workshop, participants will be introduced to a straightforward discovery workflow, covering key techniques such as clustering, dimensionality reduction, and visualization of results. No previous coding skills required!

Spatial mapping of the hepatocellular carcinoma landscape identifies unique intratumoural perivascular-immune neighbourhoods

Grand Ballroom 2&3, October 21, 2024

Dr Felix Marsh-wakefield[1,2], Cosi Santhakumar[1,2,3], Angela Ferguson[1], Joo-Shik Shin[3], Ken Liu[1,2,3], Geoff McCaughan[1,2,3], Umaimainthan Palendira[2]
[1]Centenary Institute, , Australia, [2]University of Sydney, , Australia, [3]RPA Hospital, , Australia

Biography
Felix Marsh-Wakefield is a post-doctoral researcher with the Liver Injury & Cancer Program (Centenary Institute) and Human Cancer & Viral Immunology Laboratory (University of Sydney). He is an immunologist interested in investigating the role of various immune cells in a range of diseases, including hepatocellular carcinoma and multiple sclerosis. This primarily involves bioinformatics to assist in the analysis of high-dimensional data, including single-cell and spatial data. His expertise in high-dimensional analysis was recognised in 2020, when he was one of four international emerging leaders selected to join the International Society for the Advancement of Cytometry Marylou Ingram Scholars Program. Felix was awarded his Immunology PhD in 2018 at The University of Sydney, for his work investigating the ability of mast cells to activate regulatory B cells. This project involved the use of high dimensional cytometry, particularly mass cytometry. These skills were then used during his first post-doc, where he interrogated circulating immune cells in multiple sclerosis patients after disease-modifying therapeutics, including alemtuzumab and cladribine. During this time, he became experienced in coding and bioinformatics, which he has since applied in a range of disease contexts. This provided him the opportunity present his work at multiple international and national conferences.

Abstract
Aim:
Hepatocellular carcinoma (HCC) develops in the context of chronic inflammation, however, the opposing roles the immune system plays in both the development and control of tumours is not fully understood. Mapping immune cell interactions across the distinct tissue regions could provide greater insight into the role individual immune populations have within tumours.

Method:
A 39-parameter imaging mass cytometry panel was optimised with markers targeting immune cells, stromal cells, endothelial cells, hepatocytes, and tumour cells. We mapped the immune landscape of tumour, invasive margin, and adjacent non-tumour regions across sixteen resected tumours comprising of 144 regions of interest. X-shift clustering and manual gating were used to characterise cell subsets, and Spectre quantified the spatial environment to identify cellular neighbourhoods. Ligand-receptor communication was quantified on two single-cell RNA-sequencing datasets and one spatial transcriptomic dataset.

Results:
We show immune cell densities remain largely consistent across these three regions, except for subsets of monocyte-derived macrophages which are enriched within the tumours. Mapping cellular interactions across these regions in an unbiased manner identifies immune neighbourhoods comprised of tissue-resident T cells, dendritic cells, and various macrophage populations around perivascular spaces. Importantly, we identify multiple immune cells within these neighbourhoods interacting with VEGFA+ perivascular macrophages. VEGFA was further identified as a ligand for communication between perivascular macrophages and CD34+ endothelial cells.

Conclusion:

Immune cell neighbourhood interactions, but not cell densities, differ between intratumoural and adjacent non-tumour regions in HCC. Unique intratumoural immune neighbourhoods around the perivascular space points to an altered landscape within tumours. Enrichment of VEGFA+ perivascular macrophages within these tumours could play a key role in angiogenesis and vascular permeability.

Application of deep learning for better batch effect removal allows detection of subtle cellular phenotypes in very large flow datasets

Grand Ballroom 2&3, October 23, 2024

A/Prof Dan Andrews[1], **Dr Ben Mashford**[1]
[1]John Curtin School of Medical Research, Australian Natrional University, Canberra, Australia

Biography
Dr. Benjamin Mashford is a research scientist at John Curtin School of Medical Research & School of Computing, Australian National University. He has applied machine learning methods to a range of problems in the biomedical domain, including epilepsy seizure analysis and prediction. His current research focuses on the application of neural network models to the analysis of flow cytometry data, as well as developing a deep-learning based image analysis tool to assist in kidney disease diagnosis.
Abstract

Aim:
To allow detection of subtle disease-associated cellular phenotypes from longitudinal and multi-centre flow cytometry datasets, though improved methodology for normalisation of batch-effects in samples lacking consistent controls.

Method:
We have used existing, large datasets from both inbred mice and human clinical samples to train neural networks to predict batch effects and then remove this confounding signal from raw fcs datasets. We have applied unsupervised deep learning that can be used to learn a latent-space representation of a dataset. We have built a new type of autoencoder deep learning model, which we have named *FlowCoder*.

Results:
After training the FlowCoder deep learning model on a batch of fcs files (training set), the trained model is used to reconstruct the signals in other (test set) fcs samples. The output cell signal intensities of the reconstructed samples are shifted in accordance with the range of cell intensities observed in the training batch (Figure 1). This data-driven approach does not rely on explicit identification of cell clusters or any other features and *FlowCoder* is shown to provide outputs with fewer artifacts than other batch normalisation methods.

Conclusion:
Adversarial autoencoder models normalise batch effects from flow data better than existing tools. This advance provides the ability to detect subtle disease phenotypes from very large data collected over time and/or between multiple centres. The *FlowCoder* tool does not require controls with each batch.

Table/Figure:

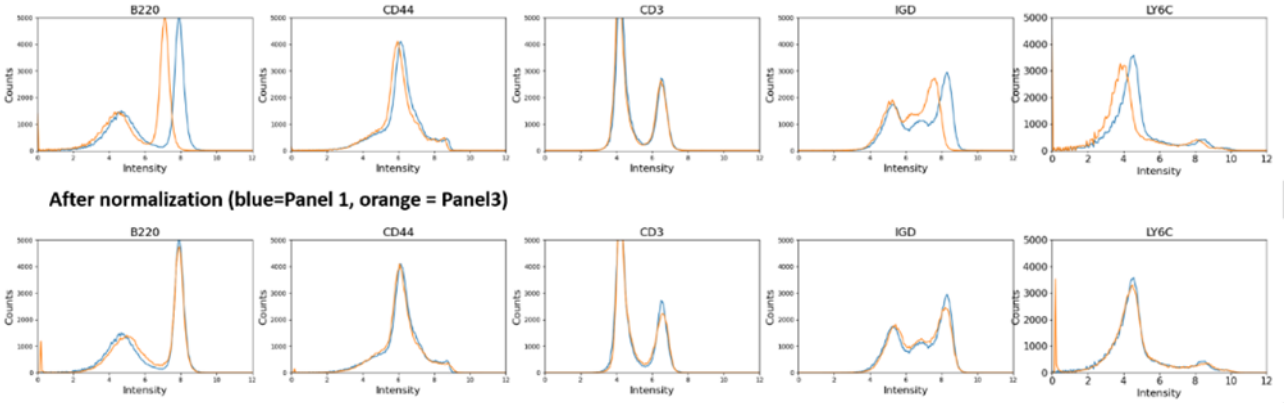


Figure 1. *Histogram plots of intensity measurements over two batches (blue/orange). Top- before normalisation, Lower- after FlowCoder normalisation.*

High-throughput cultivation of the faecal microbiome using flow cytometry

The Art of Juggling: Internal users versus External Clients in a NATA Accredited SRL

Grand Ballroom 2&3, October 23, 2024

Dr Elizabeth McDonald[1]
[1]Qimr Berghofer, Australia

Biography

Elizabeth has worked in the Flow Cytometry and Imaging (F&I) Facility at QIMR Berghofer for nearly 4 years, where she has been instrumental in developing and implementing the quality management system that is now used in multiple departments of Scientific Services. She is the quality co-ordinator within the F&I Facility, and was therefore crucial for the acquisition of NATA accreditation within the Facility - including leading the Facility audits during the assessments. Prior to her work in core facilities, Elizabeth worked as a research scientist, first gaining her PhD at the University of Liverpool in 2016, working on the cell signaling pathways involved in chronic myeloid leukaemia, before moving to the University of Glasgow for her first post-doctoral position studying immunological changes of inflammatory bowel diseases. In 2018, Elizabeth moved to sunny Brisbane to take up a position in the cancer immunology department of QIMR Berghofer, working on various immunotherapy studies before stepping into a core facilities role in 2021.

Abstract

Aim: To provide high quality results to contract clinical trial clients, without sacrificing our ongoing commitments to our internal researchers.

Results and Discussion:

The Flow Cytometry and Imaging (F&I) Facility at QIMR Berghofer is a shared resource laboratory (SRL) that primarily focuses on supporting the internal research labs within the Institute. In September 2022 the F&I laboratory acquired ISO17025 Accreditation through NATA (National Association of Testing Authorities in Australia), with the idea of expanding our client base to include external contract clinical trial work. We are accredited for the provision of immunophenotyping analysis in support of clinical trials and pre-clinical studies, which uniquely positions our lab to offer bespoke immunophenotyping experimental design and analysis that, importantly, can be offered under an accredited framework.

Working under our NATA accreditation framework requires a coordinated approach from the whole flow cytometry team as ISO 17025 standards require meticulous record keeping, performance tracking, equipment monitoring, assay validation procedures, and participation in external Quality Assurance Programmes. Balancing these requirements with the normal operation of a shared SRL is challenging in a variety of ways and demands a focussed and experienced team.

Undertaking contract clinical trial work to the high standards demanded by our accreditation, with the tight deadlines so often involved in clinical trial work, while still supporting the internal researchers of the Institute has posed some unique challenges, which will be discussed. However, the quality management system that was developed and implemented as a part of our NATA accreditation journey has been crucial in overcoming many of the challenges we have faced and continues to benefit both internal researchers and external clients alike.

POSTER

Leveraging mass cytometry to identify prognostic immune subsets in paediatric B-ALL patients undergoing CD19 CAR-T cell therapy

Mr Sean Macdonald[1]
[1]Peter Maccallum Cancer Centre, Melbourne, Australia

Biography
Sean is a Research Assistant from the Peter MacCallum Cancer Centre working under Paul Neeson in cancer immunology and clinical trials. Sean's work is mainly using flow or mass cytometry, using the latter

Abstract
Aim:
CD19 Chimeric Antigen Receptor (CAR)-T cells (CART19) show great therapeutic success in Paediatric B Cell-Acute Lymphoblastic Leukaemia (B-ALL). However, 50% of patients will lose CART19 persistence and relapse. Previous studies linked enhanced memory T-cell compartments in apheresis and CAR-T products to better CART19 persistence and clinical outcomes. Here, we aim to investigate the memory compartments in the initial apheresis product and asses CART19 persistence in patients' peripheral blood (PB). To address the challenge of high-dimensional analysis on limited cells, we developed two novel mass cytometry panels and an in-house analysis pipeline.

Method:
Paired apheresis product (n=27) and Day 30 PB mononuclear cells were obtained from children with B-ALL (Royal Children's Hospital, Melbourne). We developed two 40-parameter mass cytometry panels: 1) a Pan-Immune panel covering lymphoid and myeloid lineage subsets, and 2) a T-cell panel with comprehensive markers for T-cell differentiation. Cells were stained with rare-earth metal-tagged antibodies. Data were collected via the Helios (Standard Biotools Inc) mass cytometer. After gating single live cells in FlowJo (V10.8.1), data were exported to R for CATALYST clustering (Figure 1). Clusters were annotated by cell type, including progenitor memory T cells, and cell frequencies were plotted against patient outcome, such as B cell aplasia loss, or relapse.

Results:
Each panel was resolved into 26 clusters, visualized with UMAP for cluster relationships, and heatmaps for marker expression level. We identified dendritic cells, monocytes, $CD4^+$ and $CD8^+$ T-cells at various differentiation stages including CAR+ T-cells (e.g., transitional memory, stem-cell-memory-like, and

progenitor memory T cells). Re-clustering on these cells will help us to further determine their phenotypes and correlate these to our study's original aims and hypotheses.

Conclusion:
Mass cytometry enables the collection of high-dimensional datasets from paediatric patient samples, providing a comprehensive overview while allowing specific inquiries. In this dataset, our findings will help stratify patients for CD19 CAR-T therapy.

Table/Figure:

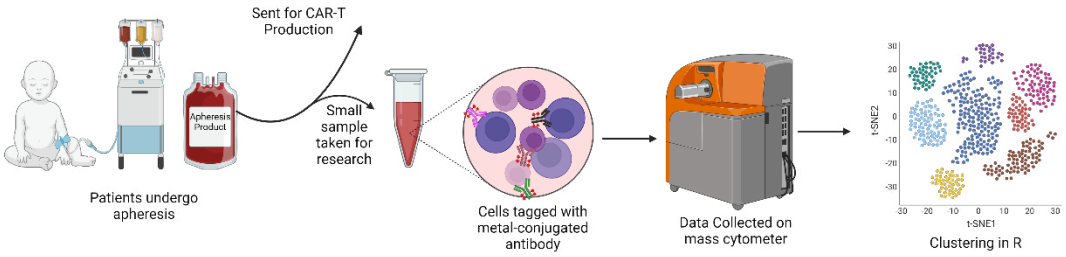


Figure 1:
Methodology pipeline. A small apheresis sample was taken prior to CAR-T production and incubated with metal-tagged antibodies to characterise the immune system. The tagged cells were analysed using a mass cytometer, with data processed for live cells and clustered into R to identify immune populations (generated using BioRender).

References:
No conflict of interest to disclose.

High-throughput cultivation of the faecal microbiome using flow cytometry

Grand Ballroom 2&3, October 23, 2024
Dr Allison McInnes[1]
[1]Queensland University Of Technology, Brisbane, Australia

Biography
As the Senior Flow Cytometry Scientist, Dr Allison McInnes oversees the flow sorting facility for the Center of Microbiome Research. Allison's professional goal is to develop and optimise methodologies that will allow scientists to conduct microbial ecology on ecologically relevant time and space scales. With a PhD in Biological Oceanography Dr. McInnes has a strong background in microbial ecology. She has over 10 years in flow cytometry and cell sorting and has led the development of the high-throughput single-cell identification and isolation platform that feeds into the biobank.

Abstract
Aim:
The human gut is remarkably diverse, encompassing more than 4,500 identified microbial species. However, ~70% of these species lack cultured representatives, limiting our functional understanding to predictions based on bioinformatic analyses. Here, we developed an innovative platform for the high-throughput cultivation of microorganisms designed to bring the uncultured majority of the human gut microbiome into culture for the first time.

Method:
The novel platform uses a BD FACS Aria Fusion modified for microoxic sort conditions, for the high-throughput isolation and axenic culture of microorganisms from human fecal samples. To increase the

diversity of isolates we have utilised various commercially available dyes. Different DNA dyes stain different proportions of the microbial population, combining these to target specific groups has proven useful in selectively sorting portions of the community. Activity dyes allowed deposition of fractions with similar metabolic activity to prevent overgrowth of faster "weeds".

Results:
Our current biobank consists of >12,000 isolates, spans both prokaryote domains, including at least 525 distinct species 51% of which represent previously uncultured lineages.

Conclusion:
With our ongoing isolation efforts and increased precision in excluding common species, we endeavour to provide cultured representatives for >50% of the human gut microbiome. This method and our ever-growing biobank have expanded the toolbox and knowledge for gut microbiome studies.

Imaging Flow Cytometric Detection of amp(1q21) and del(17p) "Double-Hit" Abnormalities in Myeloma Plasma Cells

Grand Ballroom 2&3, October 23, 2024

Mr Thomas Mincherton[1], Dr Stephanie Lam[2,3], Dr Hun Chuah[2,4], A/Prof Kathy Fuller[1], Professor Wendy Erber[1,2]
[1]University of Western Australia, Nedlands, Australia, [2]PathWest Laboratory Medicine, Perth, Australia, [3]Haematology, Fiona Stanley Hospital , Murdoch, Australia, [4]Haematology, Royal Perth Hospital, Perth, Australia

Biography
Thomas Mincherton is a 4th year PhD student in the school of biomedical sciences, at the University of Western Australia (UWA). He is completing his PhD in the Translational Cancer Pathology Laboratory (TCPL), with supervisors A/Prof. Kathy Fuller and Prof. Wendy Erber. His Work is focused on the detection of chromosomal abnormalities in multiple myeloma patients, using imaging flow cytometry. This work includes the development of methodology and analysis strategies to develop an high-throughput, automated technique for accurate and sensitive monitoring of patients.

Abstract
Aims: "Double hit" myeloma is the presence of two high-risk genetic abnormalities in myeloma with amp(1q21) and del(17p) being of the most prognostic significance.[1, 2] Our aim was to determine whether amp(1q21) and del(17p) are colocalise or are in discrete cells using an imaging flow cytometric approach ("immuno-flowFISH").[3]

Methods: Samples (blood and marrow) from 8 myeloma patients known to have abnormalities of both chromosomes 1 and 17 were analysed. Following density gradient centrifugation or red cell lysis, cells were incubated with CD38/CD138/CD319-AF647, CD19-BV480 and CD3-BV510 conjugated antibodies. dsDNA was denatured and cells hybridised with FISH probes to 1q21 and 17p13, and the centromere of chromosome 17 (C17). 50,000 to 500,000 cells were acquired on the Amnis® ImageStream®^{X}Mk II imaging flow cytometer. Digital images and quantitative data (IDEAS software) were used to assess FISH signals overlying the counterstained nuclei.

Results. There were 0.01- 43.7% cells with a plasma cell phenotype detected in the samples. In 6 cases one 17p13 and three 1q21 FISH signals were seen in the same cell, indicating colocalised "double hit" abnormalities. Serial analysis of one of these showed clonal evolution with acquisition of an additional

del(17p) only clone. In another patient the double hit only appeared after disease relapse. At initial testing there has been two separate plasma cell populations, one with del(17p) and the other with amp(1q21). Following therapy, the amp(1q21) clone was eradicated but with subsequent emergence of the "double hit" +1q/del17p cells at relapse. The remaining 2/8 cases had 3 FISH signals for all probes, indicating trisomy 1 and trisomy 17.
Conclusion: The amp(1q21) and del(17p) abnormalities were detected in the same plasma cells (colocalised) in 6 cases indicating true "double hit" disease. Sequential time-course monitoring showed these genetic abnormalities can evolve over time, with both eradication of clones and emergence of the "double hit" clone at relapse.

Table/Figure:
Neither

References:

1. Rajkumar SV. Am J Hematol. 2022;97(8):1086-107.
2. Walker BA, Mavrommatis K, Wardell CP, et al. Leukemia. 2019;33(1):159-70.
3. Hui HYL, Clarke KM, Fuller KA, et al. Cytomketry A. 2019;95(5):521-533.

Reduction in the threshold of basophil activation testing by flow cytometry is a useful biomarker to predict outcomes after immunotherapy in patients with anaphylaxis to Jack Jumper ant venom

Grand Ballroom 2&3, October 22

Emily M Mulcahy[1,2], Thanh-Thao Adriana Le[1], Griffith B Perkins[3,4,5,6], Susan Lester[7] Wun Y Lau[1], Malcolm Turner[1,2], Richard J Woodman[8], Sherin J Vareeckal[9], Shweta Mhatre[7,9], Troy Wanandy[1,2], Nikolai Petrovsky[10,11], Simon GA Brown[12,13], Michael D Wiese[14], Robert J Heddle[15], Pravin Hissaria[3,4,15]

[1]Department of Clinical Immunology and Allergy, incorporating the Jack Jumper Allergy Program, Royal Hobart Hospital, Hobart, Tasmania, Australia
[2]College of Health and Medicine, University of Tasmania, Hobart, Tasmania, Australia
[3]Immunology Directorate, SA Pathology, Adelaide, Australia
[4]Central and Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia
[5]Adelaide Medical School, University of Adelaide, Adelaide, Australia
[6]Department of Haematology, Flinders University and Medical Centre, Adelaide, Australia
[7]Rheumatology Unit, QEH; Discipline of Medicine, University of Adelaide
[8]Flinders Centre for Epidemiology and Biostatistics, Flinders University, Bedford Park, South Australia, Australia
[9]Department of Immunology, Royal Melbourne Hospital and North Western Mental Health, Melbourne, Victoria, Australia
[10]Flinders University, Bedford Park, South Australia, Australia
[11]Vaxine Pty Ltd, Bedford Park, South Australia, Australia
[12]Division of Emergency Medicine, Medical School, University of Western Australia, Perth, Western Australia, Australia
[13]Aeromedical and Medical Retrieval Division, Ambulance Tasmania, Hobart, Tasmania, Australia
[14]Clinical and Health Sciences, University of South Australia, Adelaide, South Australia, Australia
[15]Department of Immunology, Royal Adelaide Hospital, Adelaide, South Australia, Australia

Biography
Dr Emily Mulcahy is the Senior Medical Scientist for the Allergy and Immunology Department at the Royal Hobart Hospital. She completed her PhD at the University of Tasmania utilizing flow cytometry to investigate the immunological aspects of Cystic Fibrosis. Her research interests now focus on venom allergy and the use of flow cytometry as a diagnostic tool for immunotherapy.

Abstract

Aim:

Jack Jumper ant (*Myrmecia pilosula*; JJA) stings are a primary cause of insect sting anaphylaxis in Australia. Venom immunotherapy (VIT) has shown to be highly effective, however its applicability is limited by venom availability and systemic reactions are still relatively common. There are no useful biomarkers available to assess the efficacy of VIT. The only current method to confirm VIT efficacy is a live sting challenge which is a cumbersome and potentially dangerous procedure requiring complex logistics. Our aim was to assess the usefulness of various biomarkers to predict the outcome of VIT by comparing with a sting challenge as the gold standard test in a clinical trial situation.

Method:

We conducted a single-blind, randomised, phase 1/2 trial over two sites (Royal Hobart Hospital and Royal Adelaide Hospital) to assess the efficacy and safety of JJA VIT at a maintenance venom dose of 25 mcg or 50 mcg ± Advax adjuvant (i.e., four treatment groups) in 44 JJA allergic subjects. Response to treatment over a 14-month period was assessed using a combination of live sting challenges after 12 months of maintenance VIT and JJA venom-specific sIgE, sIgG4 and basophil activation testing (BAT) at multiple time points. BAT was done using upregulation of CD63 as an activation marker on basophils after incubation of whole blood with various concentrations of JJA venom. Anti-IgE was used as a positive control to identify the non-responders.

Results:

There was no statistically significant difference between the four treatment groups for outcome in terms of a failed sting challenge or systemic reactions (SR) to treatment. Subjects with a high baseline BAT venom sensitivity which markedly reduced in response to treatment were less likely to have a positive sting challenge (p=0.005) while subjects with a lower BAT venom sensitivity at baseline had proportionately less improvement and were more likely to experience a failed sting challenge. BAT sensitivity alone at 12 months was not a predictor of sting challenge outcome (p=0.65). There was a trend for higher sIgG4 in the 50 mcg with Advax treatment group compared with the other 3 groups (p=0.047), and a higher sIgG4/sIgE ratio with addition of Advax (p=0.029) although this was not associated with superior clinical outcomes. There were no associations identified between venom skin testing, sIgE, sIgG4, sIgE/IgG4 ratio and a positive sting challenge outcome.

Conclusion:

Change in threshold of BAT over time was shown to correlate with sting challenge outcome. However, individual BAT testing at 12 months was not an equivalent surrogate for the gold standard sting challenge. The addition of Advax was associated with a greater sIgG4/sIgE ratio and the 50 mcg with Advax group had a greater rise in sIgG4, but the sting challenge results, and SR rates were not significantly different between groups indicating that a larger/longer duration study may be required to determine if Advax confers any clinical benefit.

No conflicts of interest to disclose.

The research was supported by research grants from the Australian Respiratory and Sleep Medicine Institute, Health Services Charitable Gifts Board, Allergy and Immunology Foundation of Australasia, and the Royal Hobart Hospital Research Foundation. These organisations had no role in analysing the data or preparing the abstract. Development of the Advax adjuvant was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and US Department of Health and Human Services.

Gold Sponsor Presentation – Thermo Sisher

Harnessing imaging and spectral potential in flow cytometry with Attune™ flow cytometers

Grand Ballroom 2&3, October 22, 2024

Dr. Sai Praneeth Narla[1]
[1]Thermo Fisher, New Zealand

Biography

Sai Praneeth Narla graduated with Master in Immunology and Allergy with the Developing Solutions Masters Scholarship from The University of Nottingham, UK. Sai was a PhD scholar under the supervision of Prof. John Upham and Prof. Peter Wark. His research project focuses on anti-viral immunity and the regulation of macrophages and dendritic cells, in relation to severe asthma.

Dr Sai Praneeth Narla is currently a Field Application Scientist in Thermo Fisher Scientific.

Abstract

The Invitrogen™ Attune™ CytPix Flow Cytometer combines proven Attune technology with groundbreaking automated image analysis, delivering exceptional precision and efficiency in cellular imaging. It seamlessly integrates with high-throughput workflows, allowing researchers to capture and analyze detailed cellular images and obtain two datasets in one step with minimal manual effort. We are also excited to introduce the new Attune Xenith™ Spectral Flow Cytometer, which offers high-resolution spectral analysis and provides deeper insights into cellular diversity and complex biological systems. Discover how together, the Attune CytPix and Attune Xenith flow cytometers enable your lab to embark on a new wave of scientific discoveries with unprecedented clarity and efficiency.

An in situ quantitative map of mononuclear phagocytes across human mucosal tissue using high parameter imaging platforms

Grand Ballroom 2&3, October 23, 2024

Dr Thomas O'Neil[1,2], Dr Heeva Baharlou[1,2], Dr Kevin Hu[1,2], Dr Nicolas Canete[1,2], Dr Kirstie Bertram[1,2], Professor Andrew Harman[1,2]
[1]Centre for Virus Research, Westmead Institute For Medical Research, Westmead, Australia, [2]School of Medical Sciences, University of Sydney, , Australia

Biography

I am a postdoctoral researcher at the Westmead Institute for Medical Research under the supervision of Professor Andrew Harman. My PhD thesis focused on investigating CD4+ resident T cell subsets in human genital mucosa using high parameter flow cytometry. After isolating CD4 T cells from human tissue, we were also able to expose them to HIV to determine the subsets and characteristics of preferentially infected. During the pandemic lockdowns, I was able to upskill in analytic programming and bioinformatics, taking advantage of publicly available single cell RNA datasets. Since graduating, I have been applying my analytic skills to interrogating high parameter spatial modalities, including CosMx, Visium and imaging mass cytometry.

Abstract

Aim:

Tissue resident antigen presenting cells (APC), especially dendritic cells (DC) play critical roles in pathogen recognition and antigen presentation to T cells. Most of our knowledge of APCs at the initial host-pathogen interface is derived from studies of *ex vivo* isolated cells. Most published *in situ* studies use animal tissues, and the few human studies are highly qualitative in nature. Furthermore, detection of most pathogens can only be achieved after multiple rounds of replication long after initial exposure. Therefore, we aim to develop a comparative map of initial MNP-pathogen responses in intact human tissues, especially in the context of HIV viral transmission.

Method:

we have developed several analysis pipelines across three high parameter imaging platforms - cyclic immunofluorescent imaging, Imaging Mass Cytometry (38 proteins) and CosMx spatial transcriptomics (up to 1000 genes). We have also developed a model for investigating initial responses to transmission by topically applying HIV and HSV to mucosal surfaces and integrating virus-specific probes into existing imaging workflows, to further characterise host-viral interactions in initial transmission events.

Results:

we have constructed a comprehensive proteomic and transcriptomic spatial atlas of inflamed and uninflamed colorectal tissues, including the composition of the lymphoid aggregate. We found that within just 2 hours of exposure, HIV was enriched in DCs, and not its primary target (CD4 T cells). DCs rapidly carried out several functions in response, including clustering with T cells at the mucosal surface and viral trafficking to lymphoid aggregates.

Conclusion:

These findings significantly advance our understanding of the early dynamics of HIV transmission in a physiologically relevant human setting and provides a framework for *in situ* disease studies in human mucosal tissues.

Panel Discussion :

High performing teams and Psychological safety, Fireside chat about opportunities, logistics and benefits of Mentor/Menteeship and facilitated networking session.

Grand Ballroom 2&3, October 22, 2024

Mrs Eva Orlowski-Oliver, Prof Kaylene Simpson

Biography

Eva Orlowski-Oliver, SCYM(ASCP), is the Research Flow Core Manager at the Peter McCallum Cancer Centre in Melbourne, Australia. Her role involves educating, training, and supporting research staff who utilise flow cytometry technology.

Her passion for research science started during her honours year through the University of Melbourne, and was followed by 7 years working as an RA under Associate Professor Mark Hogarth at the Burnet Institute. Eva found her love of flow cytometry through experiments at the bench and soon moved to working in the AMREP Flow core facility under the guidance of Geza Paukovics. Over 5 years Eva became the Assistant Manager of the core and most recently became the Research Flow Core Manager at the PeterMac Cancer

Centre where she is supported by an excellent team of experts, highlighted by achieving the ISAC SRL Recognition Award.

Eva is deeply committed to the continued education and training of researchers and core staff. She is an active member of ISAC and has contributed to publications in Cytometry A. She actively takes part in the SRL Recognition Program task force and is the SRL Committee Chair. Additionally, she contributes to ACS workshops, virtual presentations, and conference committees. Eva believes in providing young students with opportunities to explore careers in science, often hosting them for site visits or work experience.

Eva is always happy to engage in conversations about flow cytometry, SRL management/education, or the A-League.

Abstract
Broken up into three sections, this session will cover psychological safety and the correlation with high-performing teams, what is it, why it is important and how we can increase it in the workplace. Mentor and mentee roles within shared resource laboratories (SRL's), Q and A discussion with Eva Orlowski-Oliver and Prof. Kaylene Simpson about the benefits, logistics and opportunities that come with mentorship. Finishing with small group mentor/mentee opportunities from different areas including research, SRL, clinical, commercial and industry.

POSTER

Psychological Safety in a Shared Resource Laboratory (SRL)

Mrs Eva Orlowski-oliver[1,3], Katy Betar[2]
[1]Research Flow Core, Peter McCallum Cancer Centre, Melbourne, Australia, [2]People and Culture, Peter McCallum Cancer Centre, Melbourne, Australia, [3]ISAC Emerging Leader Program 2018-2025, Melbourne, Australia

Abstract
Shared Resource Labs (SRL's) are unique work environments which require a specialised team of people to perform under pressure, and the ability to troubleshoot and adapt to unpredictable samples. There have been various sources (Don't walk on by how to confront bias and bigotry aimed at others, Nature 608, 633-635 (2022)), which shows there is still a high prevalence of toxic work culture, specifically in the STEM sector. There is clear evidence that shows the negative impacts of having a toxic work culture within a team and the impact lack of psychological safety has on productivity and team morale. We will illustrate the greater implications such as the organisation cost of a toxic culture within an SRL which include increased staff attrition, disengagement and decreased productivity, higher health care costs, reputation damage and legal liability "The High Cost of a Toxic Workplace Culture: How Culture Impacts the Workforce — and the Bottom Line," (Alexandria, Virginia: Society for Human Resource Management, September 2019). When a team is high performing, it has been shown to correlate to a high psychological safety, which refers to creating an environment where individuals feel comfortable, secure, and confident in expressing their thoughts, ideas, and concerns without fear of negative consequences (The Fearless Organization: Creating Psychological Safety in the Workplace for Learning, Innovation, and Growth, by Amy C. Edmondson, © 2018). This concept is particularly important in collaborative settings like shared resource laboratories, where researchers, scientists, and other stakeholders work together on various research projects to achieve expected outcomes. We will explore what elements are needed to build a good foundation for a high performing team; inclusion safety, learner safety, contributor safety, and challenger safety as well as some of the hurdles are that might be holding your team back from excelling; absence of trust, fear of conflict, lack of commitment, avoidance of accountability, and inattention to

results, and what can be done to help move your team past these points to build a psychologically safe, high performing SRL.

POSTER

Investigation of Fluorescent activated cell sorting (FACS) purity using varied sort masks.

Mrs Fatma Panetta[1]
[1]PETERMAC, Australia

Biography
Fatma Panetta has worked as a research assistant for 15 years primarily working with dendritic cells and investigating their role in cancer immunotherapy.

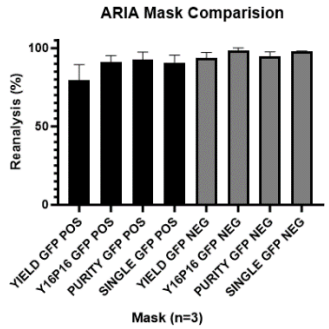
Fatma transferred over into a SRL facility in 2021 where she continues to expand her flow cytometry knowledge via the guidance of her manager and colleagues.

Abstract
Aim:
Compare the purity of sorted cells across various precision sort modes Yield, Purity, and Single cell using 3 BD FACS cell sorters.
Investigate a custom precision sort mask "Y16P16" purity outcomes in comparison to the existing default options (Yield, Purity and Single cell).

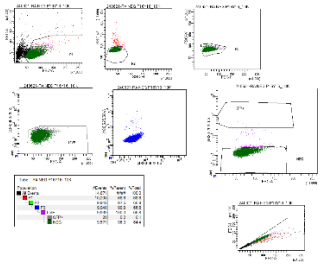
Method:
The work was performed within the Peter MacCallum Cancer Centre, Research Flow Core (RFC) facility, across 3 BD FACS instruments using DIVA software. A mouse sarcoma cell line, transduced with human Her-2 expressing GFP and a 243K parental cell line were used for all sorts, and performed in triplicate. The cell lines were grown in 10%CO2 complete DMEM.
Cells were harvested with Tryple, washed, filtered using 100um filter and incubated with 7AAD viability dye for 10mins.Two populations were sorted, GFP positive and GFP negative, at least 50,000 cells were sorted followed by re-analysis on 5000 cells. The re-analysis percentages were graphed using Graph Pad Prism.

Results:
There are 3 default precision modes, yield, purity and single cell, our facility has customized our own named Y16P16. Each mode has their benefits based on the researchers' required needs and outcomes. The yield mode is used to maximise recovery at the expense of purity.
The purity mode maximises purity and single cell is optimal for plate sorting and useful when precise counting is required. Our custom mode, Y16P16, is used to maximise both yield and purity.
The results show that Y16P16 precision mode produces comparable results to the default Purity mode, while being less stringent in droplet exclusions while sorting.

Figure: Purity percentage across masks



Representative Plot



Conclusion:

Precision modes are important to optimise the sorters priorities to sort only the cells of interest, each mode is useful and allow users to decide how stringent their sorting requirements are to be. We can conclude that there are differences between the precision modes and based on our data our custom Y16P16 mode produces high purity comparable to the Purity and Single cell modes which offers the potential for higher sort yields without compromising purity.

References:

This research was supported by Peter MacCallum Cancer centre and RFC SRL facility. The company had no role in analysing the data or preparing the abstract.

BD FACS ARIA users guide.

Ribonucleoprotein delivery to Salmonid sperm for high-throughput gene editing

Grand Ballroom 2&3, October 22

Amanda L. Patchett[1], Andrew Wood[1], Agus Sunarto[1], James W. Wynne[1]

[1]Agriculture & Food, CSIRO, Hobart, Tasmania

Biography

Amanda is an immunologist with a keen interest in understanding the complex interactions between immune systems and infectious diseases at the molecular level. Having trained in medical research at the University of Tasmania, Amanda was motivated by the remarkable diversity in immune systems across the animal kingdom to apply her expertise in animal health. She completed a PhD and postdoctoral fellowship at the Menzies Institute for Medical Research studying Tasmanian devil immunology from 2014 to 2021. By combining immunological and molecular techniques, Amanda's PhD and postdoctoral research investigated the molecular nature of devil facial tumour disease and contributed to the design of candidate vaccines in devils. In 2022, Amanda joined the CSIRO in Hobart as a research scientist in aquaculture where she leads projects developing vaccines against fish pathogens and uses gene editing and molecular technologies to understand mucosal immunity.

Abstract

Maintenance of animal health and well-being is an ongoing challenge in aquatic systems. Indeed, fish health is influenced by a number of external factors including climate and disease. Development of fish that are resilient to changing environments is an industry priority, however an improper understanding of traits that lead to resilience prevents the production of such fish. Gene edited fish provide a useful tool for studying resilient traits in the laboratory environment. Furthermore, increased interest in developing gene edited animals (non-GMO, STD1) for industry application overseas warrants development of capability in this field for potential health, welfare and sustainability purposes in Australia. Traditional methods for

generating gene edited animals involves delivery of the ribonucleoprotein DNA or complexes to singular fertilised eggs via microinjection. This labour and time-intensive process requires specialised equipment and a skilled user, and prevents large-scale production of gene edited animals. Previously, editing of chicken embryos was achieved using Sperm Transfection Assisted Gene Editing (STAGE), a technique whereby sperm are pre-loaded with gene editing reagents for delivery to eggs during fertilisation. This approach potentially allows for high-throughput editing of fertilised embryos, overcoming many limitations of microinjection-mediated delivery of gene editing reagents. Successful delivery of RNP complexes into sperm is a limiting factor for effective implementation of STAGE for gene editing. In this study, we tested various techniques for transfection of reagents into salmonid sperm including electroporation, chemical transfection (lipofectamine) and delivery by cell penetrating peptides (CPPs). Analysis of uptake and maintenance of sperm viability and motility was assessed by flow cytometry and microscopy. Our results demonstrate successful transfection of salmonid sperm, however reductions in sperm motility and viability prevented production of edited embryos. Ongoing efforts to optimise sperm transfections will aim to improve levels of uptake and sperm viability for successful implementation of the STAGE technology in Salmonid fish.

Platinum Sponsor Presentation - Beckman Coulter Life Sciences

Rarity SuperRCA, An Innovative Mutation Detection technology: Bridging Molecular Biology and Flow Cytometry

Grand Ballroom 2&3, October 22

Dr Shankar Pattabhiraman [1]
[1]BC

Biography
Shankar Pattabhiraman earned his Ph.D. in human medicine from the Charite Medical University Berlin, Germany. He spent a decade as a Research Scientist at INSERM, France, where he focused on the molecular basis of leukemia development, following a post-doctoral fellowship at UCLA, Los Angeles, CA. He is currently the Senior Product Manager for the Flow Cytometry Business Unit at Beckman Coulter Life Sciences, where he is responsible for the development of multicolor flow cytometry applications for research and clinical use. His primary research interests include the development of clinical flow cytometry techniques, molecular MRD, and liquid biopsy.

Abstract
Rarity superRCA is a highly sensitive and versatile test that can detect uncommon nucleic acid sequences in biological samples such as liquid biopsies obtained from blood, bone marrow, and plasma. The technology platform's format and flexibility, along with its read-out based on flow cytometry, make it well-suited for clinical research applications. The assay will enhance clinical research by offering greater sensitivity compared to current methods. We provide an extensive range of mutations, which have been enhanced and tailored mutations can also be obtained. This assay is simple to implement and can be used in both manual and automated workflows. Currently, probes are available for the standard mutations for Acute Myeloid Leukemia, other leukemias and lymphomas, colon and lung cancer.

POSTER

Still Fixing Spectral Unmixing Errors Manually? FlowLogic provides Solution!

Mr Geza Paukovics[2]
[1]Inivai Technologies, Mentone, Australia, [2]Chameleon Science, Sydney, Australia

Biography

Geza Paukovics Dip Med Cytopathol, B Med Lab Sci

ACS Career Recognition Award Recipient (Awarded 2019)

Geza was nominated by Eva Orlowski and Maggie Costa, with a letter of support from Suzanne Crowe AM. Geza was recognised for his pioneering work in establishing infectious PC3 sorting platforms in Australia, his leadership in changing the culture of recognition received by shared resource laboratory staff in research and research outputs, and his mentorship of many people who have gone on to become emerging national and international leaders of shared resource laboratories. This reflects not only an ability to impart technical knowledge and understanding of science, but an ability to foster a lifelong engagement with the field of cytometry. Geza has provided and facilitated service to the society over many years, including local organising committees, workshops and cytometry education.

Abstract

Aim:

Spectral Flow Cytometry (SFC) and the expansion of commercially available antibody bound fluorophores has enabled the flow cytometry community to delve into and develop large multicolour immunofluorescence panels (30-40+ colours). However, there is an epidemic of data being generated that requires additional manual and time-consuming attention by researchers to fix frequently occurring post-unmixing errors.

The aim of the project was to develop AI algorithms to assist with fixing unmixing/compensation errors (automatically or semi-automated) and to simplify the workflow associated with achieving correctly unmixed data for downstream analysis.

Method:

The main factors for an "ideal panel" include an optimal panel design minimising spectral overlaps and spreading errors, accounting for receptor co-expression on certain cells, and providing ideal compensation controls. Even with these taken into consideration, often researchers are grappling with post unmixing errors manually. Often NxN plots are generated and obvious unmixing errors are corrected by “eye” and only in a two-dimensional space, forgetting that adjustment of unmixing errors via compensation matrices have repercussions in other permutations. This approach leads to hours if not days being spent in fixing unmixing anomalies in a multidimensional space, resulting in perpetual checking whether one fix caused a problem somewhere else. We developed Comp-Check and Fix feature in FlowLogic Solution software to assist in correcting such anomalies and simplify workflows associated with such fixes.

Results:

Upon importing fully stained unmixed or compensated files into FlowLogic Solution software, researchers can holistically overview, evaluate and generate reports for unmixing/compensation anomalies. All permutations for all parameters are displayed and presented for evaluation by “eye” but importantly statistical information is provided. Negative and Positive populations are identified via autogates, excluding double positive populations. Delta median fluorescence intensity (dMFI) is calculated and subsequently adjusted automatically in a multidimensional space until the mismatched populations align to selected dMFI tolerances.

Conclusion:
Here we present an AI automated, semi-automated and an improved manual approach to rectifying unmixing and compensation anomalies in fully stained samples using FlowLogic Solution software. The algorithm works in a multidimensional space and uses statistics to:

1. Evaluate and Report on unmixing anomalies
2. Offer full panel visual evaluation and verification for all plots or chosen locked in X-parameters
3. Offer a fully or semi-automated approach in fixing anomalies in a multi-dimensional space.

References:
"No conflict of interest to disclose".

POSTER

Are Manual Gating Approaches to Complex Data Analysis still the best way to go? FlowLogic provides Solution!

Mr Geza Paukovics[2]
[1]Flowlogic Software, Mentone, Australia, [2]Chameleon Science, Sydney, Australia

Abstract
Aim:
To explore and further develop flow cytometry data analysis tools to aid and simply downstream analysis of complex multidimensional data.

Method:
Flow Cytometry panels are growing, with advancements in spectral flow cytometry and reagent developments, it is not unusual to encounter 20+ colour immunofluorescent panels. With the increase in fluorophores and parameters within panels, there are also increasing challenges to multidimensional data analysis when approached by traditional means. Historically, manual gating approaches have been employed to analyse flow cytometry data, ranging from simple to quite complex. For smaller panels (up to 15-20 parameters), the manual gating of data and data interpretations are fairly simple to follow and understand. However, as flow cytometry now regularly expands to 25-40+ colour panels, manual gating data analysis has become cumbersome, time consuming, confusing especially when considered all the analysis is happening on tens if not hundreds of 2-dimensional dot plots. Is there was a way to unravel all the complexity and display all the data in a meaningful way? Yes!

Results:
2D (dimensional) manually gated dot plot data analysis approaches are still widely used to analyse large multicolour panels. Cluster extraction algorithms and data reduction plots exists to visualise and aid in analyses processes (tSNE, UMAP etc). However, often clustered populations are non-phenotypically defined, plots are non-interactive, stochastically based, time consuming (multiple iterations are required to attain statistical significance). We have developed AI assisted Cluster extraction algorithms that phenotypically define cell populations without the need for complex manual gating approaches. The extracted populations can be holistically displayed in Global and Spatial Separation Plots, to give researchers a complete overview of their data, of phenotypic changes-immune functions of targeted cell populations within a sample or between multiple samples.

Conclusion:
We have developed AI assisted algorithms to simplify and deconvolute complex datasets (25-40+parameters).

Semi-Automated (AI) algorithms offer meaningful Phenotypically defined populations via Cluster extractions in a multidimensional space. The complexity of the data is simplified on our Global Displays and Spatial Separation Plots (SSP). Amazingly these display types can be easily applied to manually gated data as well.

References:
"No conflict of interest to disclose".

Update on the National Certification Scheme for the Medical Laboratory Scientific Workforce

Lecture Theatre, October 20

Tina Pham[1]
[1] St Vincent's Hospital, Melbourne

Biography
Tina Pham is a Senior Scientist at St Vincent's Hospital Melbourne, Australia, where she manages the Special Haematology laboratory which includes Flow Cytometry and Cellular Therapy. Her flow cytometry experience spans over 10 years. She is the Vic Branch chair of the Australian Institute of Medical Scientists (AIMS), board director of the Australian Council for Certification of Medical Scientific Workforce (CMLS) and guest lectures at RMIT. She is passionate about education for future medical scientists in flow and cell therapy.

Abstract
Launched in July 2020, the Australian Council for Certification of the Medical Laboratory Scientific Workforce (CMLS) was established as a not-for-profit entity to oversee the certification of the laboratory scientific workforce. The certification framework, developed with input from bodies such as AIMS, AACB, ASM, HGSA, ASC, ANZBT, ASCIA, FSA, THANZ and ACS, ensures professionals meet standards for qualifications, competence, and continuing professional development (CPD). A public register was also introduced to verify certified professionals.

Following the dissolution of CMLS in April 2023, professional bodies running CMLS-approved CPD schemes are now authorised to issue certification independently. Organisations such as AIMS, through its APACE scheme, continue to offer certification access to affiliated members like ACS. This transition allows members of participating associations to maintain industry-recognised credentials by fulfilling CPD and competency requirements through their respective professional bodies.

Automating Lymphoma Diagnosis with a 13-Colour Multiparametric Flow Cytometry Panel

Grand Ballroom 1, October 21

Tina Pham[1],
[1]St Vincent's Hospital, Melbourne, Australia

Abstract
The recent acquisition of a 13-colour flow cytometer created an opportunity to improve lymphoma diagnosis by upgrading from a 10-colour panel. This transition aimed to improve diagnostic precision by selecting markers to effectively differentiate between normal and abnormal T-cells, B-cells, and natural

killer (NK) cells, allowing more detailed and accurate identification of lymphoma subtypes. A key objective was to automate the workflow—from sample preparation through acquisition to analysis—ensuring greater efficiency, and reproducibility in laboratory operations.

However, automation in flow cytometry introduces challenges, such as optimising reagent combinations, managing spectral overlap and refining gating strategies to ensure precise identification of cell populations. Overcoming these complexities is essential to minimising manual intervention and building a robust, automated system that delivers enhanced diagnostic accuracy. A well-optimised system provides critical insights into lymphoma subtypes, enabling more effective patient care. This initiative aligns with advancements in flow cytometry technology that emphasise streamlining workflows to improve efficiency and reproducibility in clinical diagnostics.

POSTER

Characterising The Immune Response To A Novel Intranasal Vaccine For Otitis Media Prevention using Spectral Flow Cytometry

Mr Jack Pepper[1], Dr Christian Tjiam[1,2], Ms Caitlyn Granland[1], Ms Josephine Bayliss[1], Mr Edison Foo[1], Dr Naomi Scott[1], Dr Elke Seppanen[1], Professor Peter Richmond[1,3], Associate Professor Lea-Ann Kirkham[1]
[1]Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, Australia,
[2]Department of Clinical Immunology, PathWest Laboratory Medicine WA, Murdoch, Australia,
[3]Departments of Immunology and General Paediatrics, Perth Children's Hospital, Nedlands, Australia

Biography

Jack is a research assistant with the Bacterial Respiratory Infectious Diseases Group (BRIDG) at Telethon Kids Institute. He completed his Honours in Biomedical Science at the University of Western Australia in 2023 characterising the response of neutrophils in solid tumours to checkpoint blockade in the post-surgery tumour microenvironment. Jack is now researching the local and systemic immune effects for a novel intranasal vaccine for NTHi-induced otitis media, known as Sprtiz-OM, to reduce the development and recurrence of acute and chronic otitis media in children.

Abstract

Aim:

Nontypeable *Haemophilus influenzae* (NTHi) is the predominant pathogen causing recurrent/chronic otitis media (OM) with no preventative therapies available. We have demonstrated previously that intranasal administration of a related commensal bacteria prevents NTHi OM in mice. We aimed to enumerate the major immune cell populations induced by this novel intranasal vaccine (Spritz-OM) using spectral flow cytometry.

Method:

A 21-colour Spectral Flow Cytometry panel was designed to enumerate murine neutrophils, monocytes, macrophages, natural killer cells, T-cells, B-cells, and dendritic cell subsets on a 5-laser, 48-detector FACSymphonyÔ A5 SE (BD Biosciences). Adult BALB/cJ mice were intranasally administered Spritz-OM or placebo, then nasal tissue, lungs, and spleens were collected at 2-,6-,24-,48-,72-,96- and 144-hours post-treatment (n=6/timepoint/treatment). Tissues were homogenised and enzymatically digested into single cell suspensions for staining and analysis. Data were analysed using FlowJo(v10.10.0) and GraphPad(v10.1.0), with high dimensional clustering using R(v4.3.1), and CytofKit2(v0.99.80).

Results:
Spritz-OM induced neutrophil infiltration into lungs at 6h post-treatment (2.08 log_2-fold change, $p<0.0001$). Monocytes transitioned to an activated state with an increase in CD64hi-MHC-II+ monocytes in nasal tissue between 6-72h post-treatment (peak log_2-fold change of 1.56 at 24h post-treatment, $p<0.0001$), compared to placebo. CD8+ T-cells decreased in lung tissue at 24h (0.98 log_2-fold change, $p=0.01$) and B-cells in nasal tissue, between 24-48h (0.57 log_2-fold change at 48h, $p=0.005$). There were limited differences in splenic immune cell populations between groups.

Conclusion:
The spectral flow panel and single-cell processing method developed have enabled assessment of systemic and mucosal immune cell dynamics in mice. Spritz-OM induced a rapid and local innate immune response, characterised by monocyte activation in nasal tissue and recruitment of neutrophils into the lungs. We propose this to be a mechanism for the early non-specific protection afforded against NTHi OM in mouse models using Spritz-OM. Biomarkers/immune signatures identified in this study will inform suitable measurements of efficacy in upcoming clinical trials.

How to go with the flow: an introduction to flow cytometry

Lecture Theatre, October 20, 2024

Kate Pilkington[1]
[1]Malaghan Institute of Medical Research

Biography
Kate's enthusiasm for flow cytometry began in her undergraduate and post-graduate studies, and that passion quickly diverted her career from academia to that of a specialised and dedicated cytometrist. With diverse experience in research, clinical and commercial aspects of flow cytometry, Kate's proficiency as a spectral cytometry expert, her reputation as a skilled educator and her advocacy of quality cytometry ensures that scientists can achieve the greatest success with their research. After obtaining her degree from the University of Adelaide, Kate worked for 15 years at the Detmold Family Cytometry Facility as part of SA Pathology and the Centre for Cancer Biology. From there she embraced a move to Cytek where she became a spectral cytometry expert and a well-known cytometry educator. Now the Head of Cytometry and the Malaghan Institute for Medical Research, Kate's educational proficiency and eagerness to reinforce cytometric excellence in scientific research is perfectly aligned with the Institute's vision.

Abstract
Learning a new technique and technology can feel like a minefield of jargon and unfamiliar concepts. This introductory workshop is designed to help you get started along your flow cytometry journey with some fundamental concepts and an introduction to how cytometry data is generated and help you interpret and understand it. Come and enjoy a pun-filled, I mean fun-filled, workshop that unravels the mysteries and so-called voodoo of cytometry (spoiler alert: there is no voodoo!)

POSTER

Examining cells in cow milk using natural autofluorescence

Miss Kate Pilkington[3], Dr Christine Couldrey[2], Dr Andrew Wallace[2], Miss Claudia Bramley[1,4], Miss Jorgia Knight[1,5], Ms Joanna Roberts[1]
[1]Flowjoanna, , New Zealand, [2]Livestock Improvement Corporation, , New Zealand , [3]CytekBiosciences, , United States of America, [4]Ngāpuhi ki Whaingaroa, Ngāti Kahu ki Whaingaroa, , New Zealand, [5]Rangitāne o Manawatū, , New Zealand

Abstract

Aim:

Milk contains cells of host origin (somatic cells) and bacterial cells. Increased somatic cells in cattle milk is linked to mastitis and more than 400,000 somatic cells/ml in farm milk leads to penalties from milk processors. We aimed to use the natural auto-fluorescent profiles of somatic and bacterial milk cells to identify these using spectral cytometry.

Method:

Somatic cells in cow milk are comprised of various leukocyte subsets and mammary epithelial cells (MEC). We postulated that working with lysed cattle blood would provide material similar to immune cells in milk for a spectral control while cultured MEC may serve as a spectral control for milk MEC. We used bacterial cultures known to be found in milk as spectral controls for bacteria. We trialled enzymatic treatment protocols to aid in measuring bacteria in milk and centrifugation protocols to capture somatic cells in milk. Measurements were performed on a Cytek Aurora data was analysed in OMIQ and SpectroFlo.

Results:

Assessing bacterial cultures showed a variety of spectral profiles. Enzymatic treatment (e.g. proteinase k or savinase) of cultures modifies the spectral profile, generally decreasing intensity in the case of proteinase k, and increasing intensity with savinase, however strength of natural autofluorescent signals in bacteria is poor and insufficient for robust detection from milk.
Leukocytes and MEC prepared for reference controls for cattle milk cells were spectrally distinct from cattle cells present in milk rendering their use as reference controls ineffective. Never-the-less, milk particles have a distinct spectral profile to somatic cells and this may be able to be used to improve identification of somatic cells.

Conclusion:

Bacterial spectral profiles are too dim to distinguish bacteria in milk. Spectral profiles of cattle cells and milk particles are being further examined to understand how these may be used to aid identification of somatic cells.

Conflict of Interest declaration

"This research was conducted by Flowjoanna Tāpui as part of a project lead by Dr Christine Couldrey, LIC, supported by the New Zealand Ministry of Primary Industries SFF Futures Programme funding titled "Resilient Dairy – Innovative Breeding for a sustainable dairy future" (Grant Number: PGP06-17006),in collaboration with Katherine Pilkington, Cytek Biosciences. Cytek Biosciences is the manufacturer of Cytek Aurora.

POSTER

A new assay for B-cell development and MRD assessment in the immunotherapy era

Mrs Dina Ragab[1], Melinda Wu[1], Kylie Baldwin[1], Vuong Nguyen[1], David Westerman[1,2]
[1]Peter MacCallum Cancer Centre, , Australia, [2]University of Melbourne, , Australia

Biography

Dina Ragab is a flow cytometry scientist at Peter MacCallum Cancer Centre. She was granted her MBChB (2007) and her Master's degree in Clinical and Chemical Pathology (2012) at Tanta University where she also had good experience working in the Hematology and Flow Cytometry lab before moving to Australia in 2016. Since 2017, She has been working at the Flow Cytometry department at Peter MacCallum Cancer Centre. Dina is highly interested in the field of Hematology and Flow Cytometry Immunophenotyping.

Abstract

Aim:

Targeted immunotherapy is widely utilised for treating refractory/relapsed mature B-cell non-Hodgkin lymphomas. These therapies interfere with B cell flow population identification. With additional B-cell markers, we established a new 12-colour mature B-cell assay.

Method:

A single tube 12-colour assay comprised CD45/CD19/CD20/CD22/CD24/CD81/CD40/CD10/ CD5/CD2,CD7/surface Kappa & Lambda. Validation was performed against our 10-colour assay (CD45/CD19/CD20/CD22/CD24/CD79b/CD10/CD5,CD2,CD7/surface Kappa & Lambda) and our 8-colour B-cell tube (CD45/CD19/CD20/CD22/CD10/CD5/surface Kappa & Lambda). A stain/lyse/wash protocol was used for diagnostic samples and lyse/stain/wash prelysis-protocol for MRD assessment. Instrument: BD FACS-Lyric-Cytometer. Analysis: Kaluza software (Version 2.2.1). New antibodies underwent titration and voltration to determine optimal settings. 12 normal peripheral blood (PB) controls, and 9 normal bone marrow (BM) samples were pooled to enable population descriptions. All samples were interrogated using two different gating strategies (CD19, and CD22/CD24). Antibodies across all methodologies were compared, where applicable. Diagnostic and MRD samples were acquired. A dilution experiment is pending.

Results:

A total of 49 (12-colour) samples included 8 normal and 11 abnormal samples (CLL/SLL, SMZL, MCL) comprising 6 positive samples & 5 delineation positive samples were correlated with the 10-colour assay. A further 30 clinical trial samples with MCL were undertaken and compared to the 8-colour assay. The 12-colour assay demonstrated concordant results (R2=0.99) for all diseased samples ranging from (0.001% to 83.7%). Two samples were CD19 negative, with 1 also CD20 negative and 3 samples were CD19+dim and CD20 negative. Both old and new assays demonstrated disease, except for one 8-colour tube sample which was equivocal but positive in the 12-colour assay. Accuracy of definitive B cell population delineation was improved using CD24, 40 & 81 in the 12-colour assay.

Conclusion:

The new 12-colour assay has improved malignant and normal B cell population delineation in patients treated with various immunotherapies and will become a useful assay for MRD detection.

Viable CD34 QAP

Lecture Theatre, October 20

Scott Ragg[1]
[1]Tasmanian Health Service, Tasmania

Biography
Scott Ragg is a senior medical scientist in bone marrow transplantation and flow cytometry at the Royal Hobart Hospital. After completing a PhD in cancer immunology and a postdoctoral stint in Canada, he returned to Hobart to develop the fledgling flow and BMT labs into statewide services. Scott is a former president of both the AFCG and the Bone Marrow Transplantation Scientists Association of Australasia and is the chair of the NPAAC Haematopoietic Progenitor Cell Standards drafting committee.

Abstract
Flow cytometric enumeration of viable CD34+ haematopoietic progenitor cells is commonly used for post-cryopreservation process control in bone marrow transplantation. Presently, an external quality assurance program (EQAP) for this analyte does not exist in Australia, with the anecdotal observation of considerable intra-laboratory variation leading to the RCPAQAP Inc. funding a project to establish the feasibility and logistics of commencing a viable CD34+ HPC enumeration QAP in Australia.

This presentation will outline the progress to develop an analytically consistent cryopreserved HPC sample that can be economically transported to participant centres. Results from pilot studies where cryopreserved samples and list mode data files have been distributed, analysed and used to build the myQAP Results Entry portal will be discussed, together with identified trends and the plan for EQAP launch.

POSTER
Going with the Flow: Riding the Waves of Instrument Usage in a Western Australian Flow Cytometry Shared Resource Facility

Dr Catherine Rinaldi[1]
[1]University Of Western Australia, Perth, Australia

Biography
I graduated with a BSc(Hons) from the University of Western Australia. I completed my PhD at UWA where I used passive immunotherapy to prevent influenza infection. After completing my PhD I researched the role of the immune system in cancer treatment and gained extensive experience with high-parameter flow cytometry.

I joined the Centre for Microscopy, Characterisation and Analysis (CMCA) in 2019 as the platform leader for flow cytometry. I oversee the operation and development of research projects and train users on flow cytometry instruments including analysers (BD FACS Canto II and BD LSR Fortessa), cell sorters (BD FACSMelody and BD Influx), cell picking (ALS CellCelector), multiplexing assays (Luminex) and mass cytometry (CyTOF).

Abstract
Research infrastructure facilities offer researcher access to cutting-edge technology and expertise in highly specialised fields to support the highest levels of academic research. Often these facilities battle to secure

funding from central University hierarchy as defining the importance of these facilities is not always easy and it is difficult to quantify with hours of usage and publication outputs not outlining the full value of the facility. The model by which the facility is funded is often contentious with the question of whether researchers should be charged for access to the instrumentation and the model to use. Here, the long-term usage trends of a flow cytometry platform in Western Australia are presented. Researcher booked hours were extracted from the AC Lab Systems online booking system for 7 instruments for the period 2016 to 2023. Hourly usage decreased over the last 8 years, with the 3-year average usage decreasing from 2399 hours between 2016 and 2018 to 1781 hours between 2021 and 2023. The decrease usgae is not due to less individuals booking the instruments as this has remained relatively unchanged (71 vs 74 individuals) over the same assessed period. Reasons for this decline are multi-faceted and include a charging model change (subscription to hourly rate in 2020), reduction in core staff numbers, the COVID pandemic and related causes including; reduced international students, availability of consumables and other research funding pressures. Facility staff continue to investigate ways to increase usage and researcher engagement in flow cytometry techniques with efforts to reach out and engage with new and varied fields of research. In 2024, we installed a new spectral cytometer in the facility, and have already observed an increase in user booked hours. Investment in new flow technologies may be only one piece in the puzzle to continue to evolve the flow facility.

Single-cell RNASeq of Cerebral Spinal Fluid in Children with Brain Cancer

Grand Ballroom 2&3, October 21

Mr Rob Salomon[1], Dr Wenyan Li[1]
[1]CCI, , Australia

Biography

Rob Salomon is a single-cell specialist with deep technical expertise in cytometry, cellular genomics and the development of minimally invasive liquid biopsy techniques. He is a scientifically trained technologist with a track record in adopting, adapting, and developing novel technologies and using these to drive large projects and built effective teams to leverage cutting-edge technologies for the advancement of science.

Rob is internationally recognised for his innovative approach to building large-scale multidisciplinary research programs based around technologically advanced instrumentation and the broad incorporation of genomics and microfluidics to build high-sensitivity multi-omic approaches.

In addition to his work in developing new technologies, Rob is currently the Operations and Technology Manager in the ACRF Child Cancer Liquid Biopsy Program (ACRF CCLBP), The Technical Director of the ACRF Spatial Immune-oncology Research program for Childhood cancer (ASpIRe) and the Technology Innovation Lead for the Children's Cancer Institute. In theses role he leads the wet lab component and is responsible for developing and implementing novel cellular and molecular tools to advance our understanding of children's cancer.

Abstract

Aim:
To date, a largescale analysis of the cellular ecosystem in the CSF of children with brain cancer has never been performed. As such very little is known about the cellular makeup of the fluid in disease nor how this knowledge of the cell types found within the CSF may be leveraged to provide valuable clinical data. Here we set out develop the

method to allow high throughput single-cell RNASeq on CSF samples from children with brain cancer and to assess if the data could provide insight into disease progression.

Method:
Working closely with clinicians from Sydney Children hospital Network, we have implemented a novel liquid biopsy workflow for cellular profiling of the CSF using the BD rhapsody system. This process allows individual cells to be characterised by not only gene expression but, by using advanced informatic tools, we can assess copy number variation, single nucleotide variation and biological activities. These data were compared against bulk RNA and whole genome sequencing data of the matched tumours.

Results:
To date, we have performed scRNAseq on 92,965 cells isolated in CSF from >14 patients, including 4 patients with sequential samples. Cell types found within the CSF include an array of immune cells, fibroblasts, microglial and cancer cells. Of the 11 patients in which cancer cells were found in CSF (defined as having more than 10 putative cancer cells), we have shown that while these CTCs resemble the primary tumour cells obtained from surgical resection they can also exhibit heterogeneity which may provide a mechanism of treatment escape.

Conclusion:
We show that scRNAseq and CTC profiling is feasible in paediatric brain tumours and present, for the first time the cellular landscape of single cells in the CSF of children with brain cancer. Further, this provides a novel and powerful way to understand the biology of paediatric brain cancer and may have implications in disease monitoring, assessment of treatment efficacy, treatment selection and in prognosis.

Flow cytometry in the diagnosis of vitreoretinal lymphoma

Grand Ballroom 1, October 21

Prof William Sewell[1,2,3], Dr Nikita Garg[1], Ms Toni Saba[3], Ms Channelle Succar[3], Mr Steven Le[1], A/Prof Svetlana Cherepanoff[1,3]
[1]St Vincent's Hospital , Sydney, Australia, [2]Garvan Institute of Medical Research, Sydney, Australia, [3]Faculty of Medicine and Health, University of New South Wales, Sydney, Australia

Biography
Bill (William) Sewell is an immunopathologist with a strong interest in flow cytometry. He has been a long standing member of the Australasian Cytometry Society and its predecessor the AFCG. He has a strong commitment to flow cytometry research and education, and has received the Career Recognition Award from the ACS. He is an Expert Editor of the current edition of the WHO "Blue Book" of Classification of Tumours of Haematopoietic and Lymphoid Tissues.

Abstract
Aim:
Flow cytometry has become a useful ancillary test in paucicellular cytology samples such as fine needle aspirates and CSF. Vitreoretinal lymphoma, although rare, is a well-recognized entity. There is limited published information on flow cytometry of ocular samples. This study reviewed the performance of flow cytometry in conjunction with cytology on vitrectomy samples analyzed at SydPath, a referral centre for ocular oncology.

Method:
A retrospective review of vitrectomies performed at SydPath over an 8 year period was carried out. Samples were analyzed by cytology and where appropriate by flow cytometry, immunohistochemistry, microbiology and molecular tests. Two samples were normally submitted for flow cytometry: 0.5 mL of neat vitreous fluid, and washings from the vitrectomy cassette. The washings were delivered to the flow laboratory in RPMI medium.

Results:
Out of 176 vitrectomy episodes, flow cytometry was performed in 139 cases and yielded interpretable population(s) in 71 cases. Population sizes by flow cytometry were typically small, with fewer than 100 events in some cases. 21 cases were diagnosed as lymphoma, of which 20 were B-lineage and 1 was T-lineage. Flow cytometry contributed to the diagnosis in 16 of these cases. Demonstration of light chain restriction by flow cytometry was particularly useful in the diagnosis of B-cell lymphoma in cases with insufficient material for cell block immunohistochemistry. By cytology alone, some of these cases would have been classed as atypical or suspicious.

Conclusion:
Flow cytometry is a useful ancillary test for assessment of lymphoma in vitreous samples, particularly in view of their paucicellular nature. As with other paucicellular samples, care needs to be taken in sample handling, and in analyzing and interpreting low numbers of events. Diagnostic yields are improved with customised protocols for vitreous sample processing and analysis.

Gold Sponsor Presentation – BD

Unlock new discoveries using real-time imaging and spectral flow cytometry (RTI-SFC)

Grand Ballroom 2&3, October 22, 2024

Chia How Sim[1]
[1]BD

Biography
Sim Chia How
Product Marketing Manager, Flow Cytometry
BD Biosciences, Central South Asia and Japan
Chia How is the current Product Marketing Manager of the BD Biosciences (Flow Cytometry) for CASA-J. With more than 15 years of experience in flow cytometry and healthcare services, he contributes to the community in advancing the world of health and sciences through flow cytometry trainings and industry collaborations.

Prior to BD, he was employed in a clinical hematology laboratory specializing in immunophenotyping of leukemia and lymphoma and HIV monitoring amongst other hematological disorders.
Chia How graduated with a degree in Biomedical Sciences from the National University of Singapore and is an ASCP certified specialist in cytometry.

Abstract
The BD FACSDiscover™ S8 Cell Sorter revolutionises cell sorting by merging cutting-edge spectral flow cytometry with the innovative BD CellView™ Image Technology. This breakthrough system captures detailed images of individual cells in motion, enabling precise sorting based on spatial image analysis at

unmatched speeds. Scientists can now gain unprecedented insights into cell populations and characteristics, visually confirming data in real time. This game-changing combination allows for the interrogation and sorting of previously unidentifiable cells, all within a streamlined experimental workflow.

Introduction to Computational High-Dimensional Flow Analysis and Practical Considerations

Lecture Theatre, October 20

Dr Paul Simonson[1]
[1]Weil Cornell Medicine, , United States

Biography

Dr. Paul D. Simonson is a practicing hematopathologist and faculty member at Weill Cornell Medicine, Cornell University's medical school located in Manhattan, New York, associated with NewYork-Presbyterian Hospital. He completed his medical and graduate school degrees at the University of Illinois at Urbana-Champaign where his graduate research work under the direction of Drs. Enrico Gratton and Paul Selvin focused on single-molecule fluorescence analysis and biophysics. Following medical school, Dr. Simonson completed his residency in anatomic and clinical pathology and his hematopathology fellowship training at the University of Washington in Seattle, under the mentorship of Dr. Brent Wood, a world-renowned expert in clinical flow cytometry. During training, Dr. Simonson developed computational and machine learning approaches for interpretation of clinical flow cytometry data, which he continues to expand upon as a member of the hematopathology faculty at Weill Cornell. Dr. Simonson also serves as a member of the College of American Pathologists Artificial Intelligence Committee, a member of the AIDS Malignancy Consortium's Emerging Technologies Committee, and co-director of Weill Cornell Pathology's Multiparametric In Situ Imaging lab.

Abstract

Introduction to Computational High-Dimensional Flow Analysis and Practical Considerations
As the number of measurable markers increases with the introduction of new flow cytometers, which are becoming more commonplace, so too does the complexity of the resulting data. An increasing number of subpopulations can be identified; however, the use of traditional gating analysis will often fall short given problems with gating bias and challenges in reproducibility. In response to this need, many new computational approaches have been and are being developed to assist in the analysis. In this talk I will introduce some of the concepts associated with computational high-dimensional flow analysis, including clustering, dimensionality reduction, and visualization, and I will focus in particular on the use of FlowSOM, t-SNE, and UMAP algorithms in the discussion. I will show how this can be applied in the development of a T cell immunomonitoring panel.

Applications of Computation and Artificial Intelligence in Clinical Cytometry

Grand Ballroom 2&3, October 22, 2024

Dr Paul Simonson[1]
[1]Weill Cornell Medicine, United States

Abstract

Applications of Computation and Artificial Intelligence in Clinical Cytometry
Flow cytometry data are already digitized, and hundreds if not thousands of example cases of common diseases are found in the data repositories of most clinical flow cytometry laboratories. This provides a

wealth of information and opportunity for developing artificial intelligence and machine learning algorithms to assist in the evaluation of flow cytometry data for diagnosis and to create efficiencies in workflows. I will discuss example applications (primarily those developed by myself and group members) including use of supervised machine learning to identify diagnoses, supervised machine learning to automate flagging cases for adding antibody panels, and prediction of various associated disease features (e.g., mutation status). I will discuss using interpretability algorithms to better understand the functioning of the algorithms. I will also discuss the comparison of reported machine learning algorithms. Finally, I will end with some discussion of validation for clinical use.

High-throughput screening coupled with Imaging Transcriptomics in 2D and 3D cell models

Grand Ballroom 2&3, October 21, 2024

Prof Kaylene Simpson[1]
[1]Peter MacCallum Cancer Centre

Biography

Professor Kaylene Simpson heads the Victorian Centre for Functional Genomics at Peter MacCallum Cancer Centre in Melbourne Australia and holds a joint appointment with the Department of Biochemistry and Pharmacology, University of Melbourne. She completed her BSc (Hons) at Monash University in plant science (1992) and spent 3 years as a research assistant at Florigene Pty Ltd (blue rose company) before undertaking a PhD in lactation and mammary gland biology at the Victorian Institute of Animal Science (1998). Her first postdoc was a shared appointment with Prof Melissa Brown (Uni Melb) and Profs Jane Visvader and Geoff Lindeman (WEHI) where she studied BRCA1 dependent breast cancer and developed the methodology that led to the identification of mammary gland stem cells. In 2002 she moved to Boston as a senior postdoc and then Instructor in the Dept Cell Biology, Harvard Medical School in the lab of Prof Joan Brugge. Returning to Melbourne in 2008 to head the VCFG, she has built a team of highly skilled research assistants and postdocs who enable researchers to perform unbiased target discovery using high throughput approaches including CRISPR, RNAi and compound screening in both 2D and 3D underpinned by sophisticated cell phenotyping using high content imaging. The VCFG team customise analysis for each specific project. Kaylene is a strong advocate for alternate career paths and is a formal and informal mentor to many researchers.

Abstract

Image-based profiling in 2D and 3D cells is a well-established strategy at the Victorian Centre for Functional Genomics (VCFG). Using sophisticated, customised data analytics, this approach reduces the rich information present in biological images to a multidimensional profile of image-based features. These readouts help understand disease mechanisms and predict the functional consequences of gene perturbation using CRISPR, or a drug's activity, toxicity, or mechanism of action. However, mechanistic changes that do not reflect changes in cell morphology can be missed, especially in organoids and spheroids, and imaging features are often hard to interpret in isolation.

To address this, we have developed "Imaging Transcriptomics," which aims to integrate data from high-content imaging and high-throughput transcriptomics. Multiplexed Analysis of Cells (MAC-seq) allows for multiplexed sequencing of samples from 384-well plates at a fraction of the cost, and we have optimised this method for use with spheroids and organoids grown in Matrigel. This application enables us to characterise not only morphological changes in minimal amounts of patient-derived organoids over time, during drug treatment, and during the development of resistance but also their transcriptional changes in a high-throughput fashion.

Lessons Learnt: Establishment of a benchmark cytometric immune phenotyping workflow for multi-centre clinical trials.

Grand Ballroom 2&3, October 23, 2024

Ms Natalie Smith[1], Michael Cohen[2], Julie Alipaz[2], Christina Loh[2], David King[2], Barbara Fazekas de St Groth[1], Helen Mcguire[1]
[1]The University Of Sydney, , Australia, [2]Standard Biotools, , USA

Biography
Natalie is a PhD student in the Translational Immunology Laboratory at the University of Sydney. She is supervised by Dr Helen McGuire.

Abstract
Aim:
Comprehensive immune monitoring of clinical trials relies on the generation of high-quality data. However, the application of fluorescence flow cytometry to longitudinal clinical trials is complicated by logistical challenges in sample preparation, reagent stability, and compensation. This project aims to optimise and implement a benchmark mass cytometry workflow to improve data collection from multi-centre clinical trials.

Method:
Checkpoint therapies targeting the PD-1/PD-L1 pathway are currently the most effective treatment for late-stage lung cancer. Our group has developed a novel blood-based “immune signature” that robustly predicts failure to make a clinical response to this class of immunotherapy. These ground-breaking findings were achieved through applying a comprehensive 38-plex immunophenotyping CyTOF® panel to biobanked cryopreserved peripheral-blood mononuclear cells (PBMCs). Recent advances in CyTOF technology now give us the opportunity to expand our clinical implementation to remote settings, with a lyophilized panel further expanded to 50-plus markers to achieve comprehensive marker coverage while minimizing blood sample required. We present a workflow where sample preparation and surface staining with the lyophilised panel are performed in remote locations to facilitate best resolution of markers adversely affected by sample storage, such as chemokine receptors. Fixed and frozen samples are then shipped in batches to a central site for barcoding, intracellular staining, and acquisition.

Results:
Here we present the lessons learnt from the optimization of our workflow, including panel design and the best practices for sample fixation. We demonstrate that several ‘stopping points’ can be integrated into a CyTOF staining protocol to reduce up-front sample processing time while achieving ideal resolution.

Conclusion:
Overall, we demonstrate the clinical impact of the large panel size and utility of CyTOF technology for a multi-site clinical trials workflow. This highly simplified workflow will be easily implemented in current remote hospital laboratory environments

Conflict of interest statement:
Reagent development was supported by Standard Biotools. The company had no role in analysing the data or preparing the abstract.

POSTER

A case of myeloid neoplasm post cytotoxic therapy, subtype acute erythroid leukaemia

Ms Christina Swart[1], **Ms Nandini Ghosh**[1]
[1]Canterbury Health Laboratories, Health New Zealand, Christchurch, New Zealand

Biography
Christina is medical laboratory scientist at Canterbury Health Laboratories in Christchurch, New Zealand. She began there in the haematology core laboratory after graduating from the University of Otago, but quickly moved over to flow cytometry where she has been for six years. She enjoys all facets of clinical flow, particularly seeing how the development of new assays improves diagnostic capabilities.

Abstract
Aim: to present a case of a rare form of acute myeloid leukaemia. We will outline the history, summarise investigations, prognosis and management. We will also discuss the immunophenotypic features that are characteristic of this leukaemia subtype, and how they swayed clinical decision making in our case, particularly CD71.

Introduction: Myeloid neoplasm post cytotoxic therapy (MN-pCTs) accounts for 20% of all cases of acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and myelodysplastic /myeloproliferative neoplasm (MDS/MPN). However, the acute erythroid leukaemia (AEL) subtype is rare; representing fewer than 1% of all cases of AML. AEL may present de novo, but usually occurs after cytotoxic therapy or progression of a prior myeloid neoplasm.

Case Study: A woman in her mid-seventies with a history of multiple myeloma presented with worsening cytopenias in March 2024. Her previous treatment included melphalan and autologous stem cell transplant in 2016 followed by lenalidomide maintenance. Cytogenetics testing in 2023 showed a complex karyotype consistent with MDS. A bone marrow aspirate was performed in March 2024 to differentiate between recurrence of myeloma versus progressing MDS.

Flow cytometry analysis of a bone marrow aspirate showed an expanded population of abnormal cells positive for CD71 (moderate to bright), glycophorin A (weak), CD117, CD45 (moderate), CD13 and CD4, negative for CD34 and CD138.This phenotype confirmed erythroid lineage.

Conclusion: The patient was diagnosed with MN-pCTs subtype AEL. MN-pCTs has a poor prognosis, with median survival of less than one year.
The World Health Organisation (WHO) classification requires erythroid predominance by morphology as an essential diagnostic criterion. Although flow cytometry is not listed as essential or desirable diagnostic criteria for AEL, in this instance it was valuable in identifying the abnormal cells seen morphologically as being erythroid rather than plasmacytoid lineage.

End Note:
No conflict of interest to disclose.

POSTER

Systematic Troubleshooting Of Antibody Aggregates In Diagnostic Flow Cytometry

Miss Sarah Tan[1]
[1]Nsw Health Pathology, , Australia

Biography

Sarah Tan and Li (Cindy) Yang are Scientists at RPAH Immunology

Abstract

Aim:

This study highlights troubleshooting steps taken to decipher where the debris population in question was derived from and how to remove it.

Method:

Different lot numbers of BD Multitest™ 6-color TBNK reagents were used along with BDFACS lysing solution as per manufacturer's guidelines. Results were acquired using BDFACS Canto II flow cytometer and BDFACS Diva Software. Variables tested included using a new antibody reagent vial with the same lot number, centrifugation of the reagent, heating the reagent, different antibody titres, increasing parameter thresholds, analyser monthly maintenance, as well as washing patient samples post stain and lyse.

Results:

We have demonstrated that washing samples post stain and lyse removes the debris, whereas all other changes in variables had limited impact. As part of troubleshooting, BD was contacted to aid in our investigation and provided the following statement to confirm an issue with the reagent lot number due to the "stickiness of the APC-Cy7 tandem fluorophore used to conjugate to CD8 antibody".
No issues were observed on the softwares and hardware of the flow cytometer in use, as the debris population were displayed in both BDFACS Diva and BDFACSCanto softwares. There was no evidence of analyser issues by CS&T and 7 colour set up beads quality checks. Furthermore, we saw no debris populations in our other flow cytometry assays. These findings highlight the need for pre-lot testing for changes in reagent lot numbers before the new lot number is put into routine use for diagnostic testing.

Conclusion:

Tandem dye aggregates may occur in pre-mixed reagents. A systematic approach is needed to troubleshoot when non-specific debris is present. Pre-lot testing is helpful in identifying differences between reagent lot numbers to provide quality assurance in a diagnostic lab. Washing samples post stain and lyse was effective in removing the non-specific population.

References:

No conflict of interest to disclose.

The introduction of spectral flow cytometry into a diagnostic laboratory

Grand Ballroom 1, October 21, 2024

A/Prof Elizabeth Tegg[1],
[1] NSW Health Pathology

Biography

A/Prof Elizabeth Tegg is Chair, NSWHP Haematology Clinical Stream and Clinical Director, Haematology, NSWHP-Westmead, ICPMR. She is a joint trained pathologist in haematology and genetics. Her research interest is in the genetics of haematological malignancies.

Abstract

The introduction of spectral flow cytometry (SFC) into a diagnostic laboratory represents a significant advancement in the assessment of complex cellular populations. This technology allows for the simultaneous detection of multiple fluorescent markers, providing a more comprehensive analysis of cellular characteristics compared to traditional flow cytometry. In this talk I will outline our initial steps in this introduction, and I will assess it's impact on the accuracy and efficiency of immunophenotyping in haematological malignancies. I will present on our approach to validating the Sony ID7000 spectral cell analyzer. Additionally, the integration of SFC into routine workflows has the potential to streamline workflow in the lab and I will touch on the potential of this (data acquisition and analysis, reducing turnaround times and facilitating better patient management). Overall, the adoption of spectral flow cytometry is poised to enhance diagnostic capabilities and improve clinical outcomes in a diverse range of haematological conditions.

POSTER

CD4+ recent thymic emigrant enumeration is a useful tool in differentiating causes of T-cell lymphopenia detected by newborn screening

Ms Kristie Theodore[1], Dr Elizabeth Forbes[1], Dr Aruna Kodituwakku[1], Dr David Gillis[1], Ms Kerri Prain[1]
[1]Pathology Queensland, Queensland Public Health and Scientific Services, Brisbane, Australia

Biography

Kristie's scientific career began in 2014 in private pathology where she had her first encounter with flow cytometry.

When a position became available in 2015 in the Autoimmune-Immunobiology Section of Pathology Queensland, she jumped at the chance to further develop her keen interest in clinical flow cytometry.

Kristie has had the opportunity to work on various projects within the laboratory including the validation of instrumentation and the development of new methods with a special interest in primary immunodeficiency diagnosis.

Outside of work, Kristie loves travelling, listening to true crime podcasts and eating pasta.

Abstract

Introduction

Severe combined immunodeficiency (SCID) is a group of life-threatening immunodeficiencies characterised by impaired T lymphocyte development. SCID is typically fatal in the first year of life without intervention.

Since May 2023, the newborn screening (NBS) program in Queensland includes testing for SCID by enumeration of T-cell receptor excision circles (TRECs). Babies who are found to have a low copy number of TRECs require further testing to delineate the cause, as any cause of T-cell lymphopenia may result in a low TREC number. Aside from SCID, other causes of low TRECs include prematurity, syndromic inborn errors of immunity (IEI) and hydrops fetalis. Therefore, the introduction of SCID newborn screening in Queensland brought with it the need to perform second tier testing in order to evaluate patients with low TRECs. At the Immunology Laboratory, Pathology Queensland, lymphocyte subsets and $CD4^+$ recent thymic emigrants (RTEs) enumeration by flow cytometry was performed on all newborns in Queensland with less than 150 TREC copies/10^5 cells as detected by SCID NBS.

Results

Here we describe three select cases from the first 12 months of SCID NBS in Queensland. All three cases had extremely low TRECs (less than 20 copies/10^5 cells). While all three patients were shown to have T-cell lymphopenia, $CD4^+$ RTE enumeration was able to clearly delineate the patient with SCID from other causes of lymphopenia; in this case non-immune hydrops and syndromic IEI (22q11.2 deletion syndrome).

Conclusion

In our laboratory, $CD4^+$ RTE enumeration has been found to be a useful and robust method of distinguishing SCID from non-SCID T-cell lymphopenia.

Lunch & Learn:

Unleashing Advanced Multicolor Analysis with NovoCyte Opteon: Full Spectrum Flow Cytometry at Its Best

Room 207/208 October 20, 2024, 12:30 PM - 1:00 PM

Federica Tomay, PhD[1]
[1]Agilent

Biography

Federica is a dedicated Field Application Scientist with a passion for cutting-edge technologies, covering Agilent Cell Analysis products. She holds a Master in Genomics and a Ph.D. in Immunology and brings a wealth of academic and industry experience to her role. During her academic studies spanning across cancer immunology ,aging, and metabolism, she developed expertise in a wide range of techniques, including molecular analysis, flow cytometry, imaging, and high-content screening. In her role, Federica works closely with scientists in academia, industry and hospitals to provide technical expertise and support in utilizing Agilent's advanced cell analysis platforms. Her knowledge of assay design, experimental protocols, and data analysis enables her to guide customers through every step of their research projects.

Abstract

The Agilent NovoCyte Opteon Spectral Flow Cytometer is the latest addition to the Agilent flow cytometry family, offering advanced spectral flow cytometry technology with an easy-to-use platform. It allows for the simultaneous use of more markers, expanding research capabilities and enabling sophisticated experiments. The Opteon features stable fluidics and ultra-fast processing, handling up to 100,000 events per second, and is equipped with high-quality components, including up to five lasers and 73 detectors, to deliver reliable, high-quality data. Its free-space light collection and wavelength separation minimize signal loss, enabling 40 or more color combinations for complex analyses. With a wide dynamic range, intuitive NovoExpress software, live spectral unmixing, and autofluorescence subtraction, the Opteon ensures efficient, accurate data acquisition and analysis. With the Novo Sampler S, it also supports various plates and tube racks, integrating with lab automation systems to maximize efficiency. The NovoCyte Opteon

provides the sensitivity to detect dim signals and the resolution for closely related markers, allowing for more advanced experiments with minimal setup and analysis time

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POSTER

Estimating Genome Size and Ploidy of Plant Species Using Flow Cytometry

Dr Margaret Veale[1], Uyen Hong[2,3], Muluneh Tamiru-Oli[2,3], Mark Clifton[5], Susan Hoebee[5], Mathew Lewsey[2,3,4]

[1]Bioimaging Platform, La Trobe University, Bundoora 3086, Australia, [2]La Trobe Institute for Sustainable Agriculture and Food, AgriBio Building, La Trobe University , Bundoora, VIC 3086, Australia., Australia, [3]Australian Research Council Research Hub for Medicinal Agriculture, AgriBio Building, Bundoora, Australia, [4]Australian Research Council Centre of Excellence in Plants for Space, AgriBio Building, La Trobe University, Bundoora, Australia, [5]Department of Environment and Genetics, School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, Australia

Biography

Margaret Veale is a flow cytometry specialist who managers the shared flow cytometry facility that is included in the Bioimaging Platform at La Trobe University.

Abstract

Aim:

Genome size and ploidy can provide information about plant morphology, physiology and environment-related traits. Our aim was to optimise methods to estimate genome size and ploidy in nuclei isolated from a variety of plants using flow cytometry.

Method:

We used modified standard methods (1, 2) to isolate nuclei from leaf material from tomato**,** poppy, radish, and callistemon. Leaf tissue was chopped using razor blades into a petri dish on ice, containing lysis buffer. The homogenate was filtered using a 40 µm nylon filter and RNase was added. After incubating on ice, the homogenate was centrifuged, and the supernatant was removed. The pellet was resuspended in lysis buffer and incubated on ice followed by further filtration. For nuclei staining, Propidium Iodide (PI,1mg/mL) was added to the nuclei suspension for binding to the DNA and analyzed using a CytoFLEX S flow cytometer (Beckman Coulter, CA, USA), using the PerCP channel and processed using CytExpert software (Beckman Coulter, CA, USA). An internal standard of a plant species with known 2C DNA content was included with each sample acquired. The fluorescence intensity of the stained nuclei was shown as histograms of relative DNA content comprised of peaks representing different phases of the cell cycle. The PerCP-A fluorescence intensity of the G0/G1 and G2 phase peaks of the internal standard were used to determine the DNA content to estimate the genome size of the samples.

Results:

The isolation of nuclei from plant species using these modified methods provided high quality intact nuclei and high intensity staining with PI. Fresh plant material and young leaves resulted in improved isolation and staining of the nuclei. Some plants such as Australian natives required further modifications to the contents of the lysis buffers to overcome the inhibition of PI binding. Distinct, well resolved fluorescent peaks were obtained from isolated nuclei from all plants tested using the optimised methodologies. The nuclear DNA content (2C value in pg.) of the sample, was estimated using an internal standard with known 2C nuclear DNA content obtained from an existing Plant DNA C-values

Conclusion:
Comparisons between genome size and ploidy provide valuable information for correlations between different species of plants that may be influenced by variable environmental factors. The easy isolation of nuclei from plant tissue and the optimisation of staining and flow cytometry analysis provides a rapid tool for accurately estimating plant genome size and ploidy.

References:
1.Galbraith DW, Hawkins KR, Maddox JM *et al. (*1983) Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220(4601): 1049-1051.
2. Ruben Gutzat and Ortrun Mittelsten Scheid (2020) Preparing Chromatin and RNA from Rare Cell Types with Fluorescence-Activated Nuclear Sorting (FANS). Methods Mol Biol 2093:95-105.
No conflict of interest to disclose.

Understanding Inflammatory Mononuclear Phagocyte Heterogeneity in Human Anogenital Tissue

Grand Ballroom 2&3, October 22, 2024

Miss Freja Warner Van Dijk[1,2], Erica Vine[1,2], Prof Andrew Harman[1,2], Dr Kirstie Bertram[1,2]
[1]The Westmead Institute For Medical Research, Centre for Virus Research, , Australia, [2]The University of Sydney, School of Medical Sciences, Faculty of Medicine and Health, , Australia

Biography
Freja is a third year PhD student under the supervision of Dr Kirstie Bertram, Prof Andrew Harman and Dr Najla Nasr. Her research is focused on characterising the innate immune cells of anogenital tissues that first capture HIV in an inflammatory environment using high parameter flow cytometry.

Abstract
Aim:
Anogenital inflammation is undeniably a causative factor in HIV transmission. However, the key inflammatory HIV target cells in anogenital tissues are poorly understood. Mononuclear phagocytes (MNP) are innate immune cells and known HIV targets, comprising of Dendritic cells (DC), macrophages and Langerhans cells (LC). DCs and LCs are professional antigen presenting cells and responsible for sampling and delivering invading pathogens to CD4 T cells, subsequently causing systemic HIV infection. This study aims to identify and characterise all known mononuclear phagocytes in inflamed human anogenital tissues using high-parameter flow cytometry and investigate their role in HIV transmission.

Method:
Through extensive collaborations with surgeons across Western Sydney our lab has access to all human anogenital tissues including labia, vagina, cervix, foreskin, glans penis, fossa navicularis, anus and rectum. We process these tissues within 15 minutes of removal from the body utilising optimised tissue digestion protocols and perform flow cytometry with a 27-parameter panel or HIV uptake and infection assays.

Results:
We have demonstrated that inflammatory MNPs in human anogenital tissue have vastly different phenotypical expressions, functions and HIV binding capacity compared to healthy tissue. We have recently described an MNP population in the epidermis, epidermal $CD11c^+$ DCs, which not only preferentially bind and transport HIV to CD4 T cells but are enriched in inflamed anogenital mucosa and epithelium. Furthermore, we show for the first time that the novel inflammatory $Axl^+Siglec\text{-}6^+$ DCs (ASDCs)

are present in inflamed anogenital tissues where HIV transmission occurs and demonstrate blood-derived ASDCs are capable of both HIV uptake and infection.

Conclusion:
As anogenital inflammation is a prerequisite for HIV transmission, heightened understanding of MNPs in an inflammatory environment will allow for the development of targeted HIV therapeutic strategies and vaccine development.

Ask The Experts Panel

Lecture Theatre, October 20, 2024

Susan Wright[1]
[1]Royal Hobart Hospital

Biography
Susan Wright is a Senior Scientist in the Flow Cytometry Laboratory and Stem Cell Transplant Laboratory at the Royal Hobart Hospital. Committed to furthering her own knowledge and supporting the growth of others she has been an active member of ACS guidelines committees, as well as volunteering as convenor of ACS2024. In her spare time she fosters cats and boards Guide Dogs in training, along with her husband and 2 kids.

Abstract
Lead by our esteemed panel of speakers, come prepared to engage in a robust discussion about the issues affecting your peers and contemporaries.

Audience members will have the opportunity to engage directly with panellists, posing questions that address technical challenges or theoretical inquiries. This interactive format is designed to foster collaborative learning and encourage discussion amongst all delegates.

Come prepared with difficult cases, or technical and quality issues for assistance, through to training and education or general laboratory management questions.

If you have any plots for difficult cases please come with them prepared on a PowerPoint for sharing, or email them, through to susan.wright@cytometry.org.au

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POSTER

Flow cytometry and coeliac disease - A retrospective review of testing at the Royal Hobart Hospital.

Susan Wright[1], Dr Sam Hitchins[1], Dr Declan Cuschieri[1]
[1]Royal Hobart Hospital, Hobart, Australia

Abstract
Introduction:
Two subtypes of Refractory Coeliac Disease (RCD) are recognised based on the absence (RCD1) or presence (RCD2) of abnormal intraepithelial lymphocytes (IELs).

Flow cytometry is considered the best modality to assess IEL immunophenotype, with a cut-off of >20% of abnormal cells showing a high sensitivity in discriminating RCD1 and RCD2[1].

Aim:
To review the historical approach by the Royal Hobart Hospital Flow Cytometry Laboratory to testing of gastric biopsies, specifically regarding, RCD and EATL between January 2017 and December 2023.

Method:
A review was performed on samples received at the Royal Hobart Hospital for flow cytometry testing between 01/01/2017 and 31/12/2023, with an extraction for search terms “duodenum”, “duodenal”, “jejunum” and “jejunal”. All reports were reviewed, including clinical notes provided, requested testing, and testing performed, as well as assessed for the presence or absence of abnormal IELS.

Results:
Of the 22664 samples received in the laboratory over the review period

- 54 samples were biopsies from the duodenum or jejunum
    - 15 of which were excluded due to known B-cell lymphoma, or subsequently found B cell lymphoma with no evidence of aberrant T cell population.
- 25 samples were received with clinical notes mentioning coeliac disease
    - 15 of which specifying refractory coeliac.
- Testing performed on samples was inconsistent, particularly the inclusion of CD103, CD30 and cCD3.
- Reporting of result interpretation and significance of populations seen was varied, despite similar immunophenotype on populations seen.

Conclusion:
Inconsistent treatment of samples and reporting of results reflect a historic lack of understanding of the role of flow cytometry in the investigation of refractory coeliac disease EATL.

Recommendations:
A standardised approach to gastric biopsy testing by flow cytometry at the RHH to be devised, including a comprehensive panel of antibody testing, as well as education of staff to ensure consistency in reporting of results.

References:

1. WHO Classification of Tumours Editorial Board. Hematolymphoid tumors [Internet; beta version ahead of print (in progress)]. Lyon (France): International Agency for Research on Cancer; 2022. [cited 2022 Aug 29]. (WHO classification of tumors series, 5th ed.). Available from: https://tumourclassification.iarc.who.int.

Preservation of Functionality, Immunophenotype and Recovery of HIV RNA from PBMC's Cryopreserved for more than 20 years

Grand Ballroom 1, October 22, 2024

Prof John Zaunders[1], Dr Wayne B Dyer[2], Dr Kazuo Suzuki[1], Angelique Levert[1], Prof Andrew Lloyd[3]
[1]Centre for Applied Medical Research, St Vincent's Hospital, Sydney, Darlinghurst, Australia, [2]Australian Red Cross, Lifeblood, Alexandria, Australia, [3]The Kirby Institute, UNSW Sydney, Kensington, Australia

Biography

John Zaunders is a Senior Scientist at St Vincent's Hospital, Sydney, whose expertise is in the immunology of HIV and other infectious diseases, based on flow cytometry and immunological techniques. He has contributed to knowledge of human CD4 T cell subsets, including Tregs, CCR5+ cells, and viral antigen-specific cells.

Abstract

Aim:

Repositories of cryopreserved peripheral blood mononuclear cells (PBMC) are costly to maintain but are of uncertain utility for immunological studies after decades of storage. We studied preservation of cell surface phenotypes and in-vitro function of PBMC after >20 years cryopreservation of samples from HIV+ versus healthy control subjects.

Methods:

Thawed PBMC were tested for viability and 18-colour flow cytometry immunophenotyping for major lymphocyte, monocyte and dendritic cell subsets, which was compared with the 1996 immunophenotyping of freshly collected blood. T cell function in thawed PBMC was assessed by polyclonal activation (anti-CD3/CD28/CD2), or response to influenza antigen, by measuring CD25/CD134(OX40) upregulation on CD4 T cells at day 2 of culture, and proliferative CD25+ CD4 blasts at day 7. Intracellular HIV RNA in extracted RNA from proliferating CD4+ blast cells was measured using short amplicons for both the Double R and pol regions by respective pi code assays, while, separately, long 4Kbp amplicons were sequenced up to and including pol.

Results:

Major PBMC sub-populations were well conserved. Proportions of naïve, memory and effector subsets of T cells in cryopreserved PBMC correlated with old results from the fresh blood, except for a decrease in activated CD38+HLA-DR+ CD4 and CD8 T cells from HIV+ PBMC samples. Polyclonal and antigen-specific T cell OX40 and proliferation responses were readily detected in cryopreserved PBMC from both HIV+ patients and healthy control donors. We were also able to activate production of HIV RNA from cultured PBMC after 27 years cryopreservation, detecting intracellular HIV-1 RNA transcripts. We also found long unspliced transcripts in both cells and supernatants from 5/12 donors, which were used to generate 4kb amplicons that mostly had ≥80% wild-type sequences, consistent with replication competence.

Conclusion:

This unique study provides strong rationale and validity of using well-maintained biorepositories to support immunovirological research even decades after collection.

AUSTRALASIAN CYTOMETRY SOCIETY ANNUAL GENERAL MEETING AGENDA

AGM MEETING AGENDA 2024

1:15pm, Monday 23rd October, 2024

Hotel Grand Chancellor, Hobart, Australia

1. AGM opening & apologies (Maggie Wang)
2. Acceptance of 2023 AGM minutes (Henry Hui)
3. President's report (Maggie Wang)
4. Treasurer's report (Joanna Roberts)
    a. Report on 2023-24 budget
    b. Forecast of 2024-25 budget
5. Motion to determine annual membership fee (Maggie Wang)
    a. Proposal to retain annual membership at $50
6. 2024 ACS Clinical E-Show (Tina Pham)
7. 2025 Annual conference planning report (Matthew Linden)
8. 2026 Annual conference planning discussion (Wenyan Li)
9. CYTOAsia 2027 planning discussion and member vote (Maggie Wang)
10. Election of office bearers (Robert Salomon)
    a. Executive Secretory
    b. Councilors
11. Vote of thanks to outgoing committee members (Maggie Wang)
12. 2024 Annual conference brief report (Susan Wright)
    a. Travel scholarships
    b. Poster awards
    c. Trade passport prizes

AGM MEETING MINUTES 2023

Summary of the Australasian Cytometry Society Annual General Meeting 2023 Minutes

Date and Time
Date: Monday, 28th August 2023
Time: 12:45 PM
Venue: Heritage Hotel, Queenstown, New Zealand

AGM Opening & Apologies
Chair: Maggie Wang
Maggie acknowledged the traditional custodians of the land and paid respects to elders past, present, and emerging.
Apologies were received from members, including those who sent proxies (Rob Saloman).

2. Acceptance of 2022 AGM Minutes
Presenter: Henry Hui
Decision: The minutes of the 2022 AGM were unanimously accepted as a true and accurate record. Seconded by Eva Orlowski-Olivier.

3. President's Report
Presenter: Maggie Wang
Summary:

- The society remains aligned with its mission to promote research, development, and dissemination of cytometry knowledge.
- Membership has grown to over 150 members.
- The conference is internationally well-regarded, and the society is in a strong financial position.
- Active engagement with members through various communication channels, including Twitter, YouTube, Mailchimp, Linkin and WeChat.
- Highlights of the year included awards and recognitions for members (including Scholars and SRLs), significant presentations, and contributions to international cytometry events.
- examples of representation by members was ESTA, AIMS and Mexican Society of Immunology (Helen Mcguire).
- Professor Heddy Zola recognised for ACS career award.
- Helen McGuire – Homeward bound leadership development award.
- Vanta Jameson – Best publication award for CYTO A (The University of Melbourne and Peter Doherty Institute of Infection and Immunity).
- Westmead cytometry core – ISAC SRL recognition.
- Roadshow for research and clinical members – different formats proposed from survey.
- Taskforces and working groups proposed for members to join.
- Suat Dervish – spectral e-show announcement 3rd of November save the date.

4. Treasurer's Report
Presenter: Joanna Roberts
Summary:

- 2022-23 Budget Report: Detailed overview of financial performance, showing a stable financial status.
- A vote of thanks to the sponsors.
- 2023-24 Budget Forecast Projections and plans for the upcoming financial year.

- The society's total assets are at $228,000, with a net asset position of $230,309.88 as of June 30, 2023; Cash Balance (as of June 30, 2023) of $226,143.
- Major portion of income from sponsorships. Additional income from meeting registrations, membership dues, and other sources.
- Significant expenses for venue hire for conferences. Other notable expenses include consulting, accounting, and international conference setup costs.
- Decline in cash balance compared to previous years due to pandemic-related challenges. Peak funds were recorded on June 13, 2021.
- The forecast for the current meeting is a potential loss of $20,000 AUD, covered by seed payments.
- Smaller than anticipated conference with lower delegate numbers and sponsorship.
- **Proposed Budget for Upcoming Year:** Budget accounts for potential losses in future meetings. Key expenses include travel awards, career awards, consulting and accounting fees, insurance, and website maintenance. No allocation for international engagement and new initiatives for the last two years. Total operating activities cash flow projected at $57,822 AUD.
- Emphasis on determining a minimum reserve fund to safeguard against future financial challenges while supporting ongoing operations.
- Society is in a stable financial position post-pandemic.
- Encouragement to utilize existing funds strategically..

5. Motion to Determine Annual Membership Fee
Presenter: Maggie Wang
Proposal: To retain the annual membership fee at $50.
Decision: The motion was carried unanimously.

6. 2024 Annual Conference Planning Report
Presenter: Susan Wright
Summary:
- Venue booked for October 3, 2024, in Hobart.
- Planning includes social events and speaker sessions.
- Call for community input on session topics and speaker suggestions.

7. 2025 Annual Conference/CYTOAsia Planning Discussion
Presenter: Maggie Wang and Mathew Linden
Proposal: Hosting the 2025 Annual Conference/CYTOAsia in Perth, Western Australia.
Outcome: Proposal received positively, further planning to continue.

8. Election of Office Bearers
Facilitator: Maggie Wang
Positions Elected:
- President Elect: Robert Saloman
- Treasurer: Joanna Roberts
- Councillors: Tina Pham, Michael Thompson, Sam Small, Susan White, Helen Maguire, Michelle Burns, Eva Orlowski-Olivier.

9. Vote of Thanks to Outgoing Committee Members
Presenter: Maggie Wang
Acknowledgment: Maggie expressed gratitude to outgoing committee members Teresa and David.

10. 2023 Annual Conference Brief Report
Presenter: Anna Brooks
Summary:

- Attendance: 144 attendees.
- International Speakers: 7, with 2 virtual presentations.
- Awards:
- Travel scholarships awarded to nine members.
- Research and clinical poster prizes, early career cytometrist awards, and trade passport prizes were announced and distributed.

11. Urgent General Business
Discussion: No urgent matters raised.

12. Meeting Close
Conclusion: Maggie Wang formally closed the meeting, thanking all attendees and looking forward to the next year's conference.

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