Winter School

25th July - 29th July, 2022

Day 1 - Foundations: *Monday 25th July 2022*

Time	Session	
10:00 am	**Why Cytometry**	Associate Professor Laurence Macia and Dr Adrian Smith *The applications that drove the development of "Cytometry" in its traditional format, what "Cytometry" means and the modalities that are captured in this definition.*
10:30 am	**The physics and foundations of fluorescence**	Steven Allen *Fundamentals and physics of fluorescence, implications for cytometry.*
11:00 am	**What's in the box?**	Steven Allen *A look inside the flow cytometer. Giving you a technical perspective on flow cytometers as a basis for a deeper understanding of how to develop your flow cytometry experiments.*
12:00 pm	**Panel Discussion: Uses of flow**	Facilitator: Dr Thomas Ashhurst *TBA*
1:00 pm	***Break***	
2:00 pm	**Cell sorting**	Steven Allen *Building on the session "What's in the box?", Steven will move from the technical underpinnings of cell sorting through to applications of this technology and along the way provide key considerations for a successful experiment.*
2:30 pm	**Anatomy of flow data**	Steven Allen *After seeing how flow cytometry data are generated in the previous sessions, this session will offer a look at the structure of the data and the basics of how we view and make sense of it.*
3:15 pm	**Multicolour flow cytometry**	Dr Kathy On *The importance of flow cytometry experimental design and the flow on implications for data interpretation.*
4:00 pm	***Close***	

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Day 2 - Next Steps: *Tuesday 26th July 2022*

Time	Session	
10:00 am	**Sample preparation considerations, complexities and troubleshooting**	Dr Mahmoud Azar and Dr Thomas Ashhurst *In this session, we will review methodologies for extracting single cells from tissues, as well as common protocols for labelling cells with antibodies for flow cytometry.*
12:00 pm	**Panel Design**	Dr Thomas Ashhurst *In this session, we will consider best practices in modern panel design for flow cytometry.*
1:00 pm	***Break***	
2:00 pm	**Multicolour flow cytometry, the nuance of voltage setting, spill over and compensation**	Dr Thomas Ashhurst *In this session, we will consider how fluorescent signals are processed on modern flow cytometers. We will explore how voltage setting on photomultiplier (PMT) detectors relates to fluorescence spillover, spreading error, and signal resolution.* *This session will provide attendees with the technological concepts to understand and identify:* • *When and how to adjust instrumentation settings* • *The consequences of poorly acquired data* • *Techniques to avoid introducing unnecessary errors into experimental outcomes*
3:00 pm	**Have you tried turning it off and on again?**	Dr Adrian Smith *Instrumentation troubleshooting for the technologically aware.*
4:00 pm	***Close***	

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Day 3 - Beyond Theory: *Wednesday 27th July 2022*

Time	Session	
10:00 am	**High dimensional and other technologies - how do they compare?**	Dr Adrian Smith and Dr Thomas Ashhurst *In this session we will discuss a variety of cytometry technologies, drilling into 'high-dimensional' platforms, and exploring their strengths, weakness and use cases in a variety of settings.*

Concurrent Sessions

	Image Cytometry	***Mass Cytometry (CyTOF)***	***Full Spectrum Cytometry***	***Single Cell (Genomic Cytometry)***
11:30 am	Dr Kathy On and Dr Kristina Jahn *We will discuss the field of image cytrometry, reviewing the existing and emerging technologies. From there we will move to look at key applications and how this technology can expand your research outcomes.*	Jayden O'Brien *Together we will review the fundamentals of the technology, and explore how expanded antibody panels in mass cytometry can enable deep profiling of immune cells.*	Alanna Spiteri and Dr Mahmoud Azar *In this session we will review the basis of the technology, and implications for panel design and applications, including usage in highly autofluorescent cells.*	Dr Thomas Ashhurst *We will detail the fundamentals of single-cell RNA sequencing technologies, including novel assays that allow for the simultaneous profiling of RNA, protein, and chromatin accessibility from single cells.*
12:30 pm	***Break***			
1:30 pm	Dr Kristina Jahn and Dr Kathy On ***Laboratory Practical*** *Amnis ImageStream*	Jayden O'Brien ***Laboratory Practical*** *Standard BioTools Helios*	Alanna Spiteri and Dr Mahmoud Azar ***Laboratory Practical*** *Cytek Aurora*	Dr Thomas Ashhurst ***Laboratory Practical*** *BD Rhapsody Single Cell Analysis System*
4:00 pm	***Close***			

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Day 4 - Data Analysis: *Thursday 28th July 2022*

Concurrent Sessions

	Flow Cytometry Data Analysis	*Mass Cytometry (Helios) Data Analysis*	*Full Spectrum Cytometry Data Analysis*
	Dr Kathy On and Steven Allen	Jayden O'Brien	Dr Thomas Ashhurst and Dr Mahmoud Azar
10:00 am	***FlowJo Practical - Basic Applications***	***FlowJo Practical - Basic Applications***	***FlowJo Practical - Basic Applications***
11:30 am	***FlowJo Practical - Compensation*** *Fundamental best practices in the analysis of flow cytometry data, including basic compensation, quality assessment, clean up gating, and population gating using fluorescence minus one (FMO) controls.*	*In this session we will explore the fundamentals of mass cytometry analysis, including basic cleanup and quality assessment steps, as well as tips and tricks for gating and visualising mass cytometry data.*	***FlowJo Practical - Spectral Unmixing*** *Consider key practices in the analysis of spectral cytometry data. While inherently similar to conventional flow cytometry data, we will cover essential tips and tricks for taking advantage of this unique data type for efficient analysis.*
1:00 pm	***Break***		
2:00 pm	**High dimensional data analysis: concepts, tools and applications**	Dr Thomas Ashhurst *Exploring the various forms of 'high-dimensional' analysis, including clustering and dimensionality reduction, including guidance on best practices for applying these approaches to flow, mass, or spectral cytometry data.*	
2:45 pm	**High dimensional data analysis: in practice**	Dr Thomas Ashhurst *In this interactive session, we will apply clustering (e.g. FlowSOM) and dimensionality reduction (e.g. UMAP) approaches to cytometry datasets in FlowJo, leveraging these computational approaches to reveal the cellular composition of cells between of mock- or viral-infected samples.*	
3:15 pm	**Single-cell (genomic cytometry) data analysis**	Dr Thomas Ashhurst *In this session we will explore the basics of single-cell genomics analysis, covering essential aspects including initial pre-processing and data filtering, followed by applications of clustering and dimensionality reduction using the Seurat package.*	
4:00 pm	***Close***		

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Day 5 - Advanced Topics: *Friday 29th July 2022*

Concurrent Sessions

	Spatial Tissue Analysis	*R and Spectre: End-to-end data analysis*	*Image Cytometry - High Content BioImaging*
	Dr Thomas Ashhurst	Dr Felix Marsh-Wakefield	Dr Kathy On and Avrill Aspland
10:00 am	***Context: High dimensional spatial tissue analysis technologies*** *Overview of imaging technologies that enable spatial analysis in tissues, such as Imaging Mass Cytometry.*	***Context: R - What is it good for? Absolutely everything!*** *We will discuss the uses of R for analysing cytometry data*	***Context: What is high content bioimaging?*** *We will discuss the concepts of high content bioimaging and what makes it different to microscopy.*
11:00 am	***Context: Spatial analysis approaches*** *We will consider various approaches for analysing spatial datasets, including visualisation, cell segmentation, and joint cellular-spatial analysis.*	***Practical: Setting up R to handle data*** *The basics of using R to read in cytometry data. This will include how to get data from software (such as FlowJo) into R, and how to handle the data once in R.*	***Practical: PerkinElmer Opera Phenix Plus*** *We will head into the laboratory to set up and run an experiment on the PerkinElmer Opera Phenix Plus. During this session we will discuss the capabilities of the system.*
12:00 pm	***Practical: Cell segmentation (multicut)*** *We will use the Ilastik program to perform cell segmentation using a machine learning approach based on the classification of cell boundaries.*		
1:00 pm	***Break***		
2:00 pm	***Practical: Spatial analysis using FlowJo*** *In this hands-on session, we will analyse segmentated spatial data using FlowJo.*	***Practical: Using R to analyse cytometry data*** *We will walk through an analysis pipeline in R using a demo dataset. This will include clustering, dimensionality reduction, PCA, statistics and plotting results. Get excited!*	***Practical: Image Analysis (PerkinElmer Harmony)*** *We will spend the afternoon reviewing a data set in Harmony and working through building an image analysis pipeline.*
4:00 pm	***Close***		