Session 1: Flow cytometry – a powerful tool for Cytomics Research

Lectures

L.01.1

Cytometry or Cytomics – "To Be or Not To Be" – The Journey of Single Cells in the Era of Omics

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Flow cytometry has revolutionised single-cell research by integrating imaging, mass spectrometry, spectral technology, or genomics into cytomics. This approach sheds light on intricate cellular heterogeneity and provides a holistic view of cellular systems. Recent advancements in spectral cytometry have revolutionised single-cell research, enabling simultaneous measurement of multiple parameters with multiplexed dyes. Cytomics finds diverse uses in environmental, marine, microbiology, mycology, and other bioscience fields, offering valuable insights into various biological systems and ecosystems. The future of single-cell analysis in the omics era holds great promise. For example, the growing popularity of spatial transcriptomics and proteomics explores cellular organisation within tissue microenvironments, illuminating disease mechanisms and ecological interactions. Leveraging the full potential of these advancements requires integrating advanced data modelling with AI. AI-driven approaches extract meaningful patterns from vast datasets, enhancing cytomics' applications in bioscience research. In this talk, we explore the cutting-edge field of marine cytomics using spectral flow and AI analysis tools as an example, showcasing the transformative potential of cytomics in biomedical research, marine ecosystems, and cellular diversity.

L.01.2

Cytometric evaluation of the active DNA demethylation pathway

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Cellular DNA is exposed to various endogenous and exogenous factors. Exposure to them results in DNA damage and the formation of modified DNA bases. The 5-hydroxymethyluracil was originally identified as an oxidatively modified DNA base derivative. Recent evidence suggests its formation from thymine in the reaction catalyzed by TET proteins, the family of dioxygenases involved in an active DNA demethylation process. Another proposed mechanism includes the deamination of 5-hydroxymethylcytosine (intermediate of active DNA demethylation) by AID or another enzyme of the APOBEC family. The currently recommended method of evaluating 5-hydroxymethyluracil content is the highly-sensitive and highly-specific isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS). Despite many advantages, it has one great limitation–it is not able to measure compounds at a single-cell level. Our goal was to develop and optimize the method for the evaluation of 5-hydroxymethyluracil content at a single-cell level in peripheral leukocytes by means of the flow cytometry technique. Based on both the available literature data and our own experience, as part of the development and optimization of the technical protocol we initially tested three procedures. The best results were obtained after using high temperature (99ºC) and this protocol was the starting point for further standardization.

L.01.3

Flow and mass cytometry – tools for deciphering the immune system in all its complexity

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Single-cell technologies have now reached a level that allows entire biological systems to be deciphered in all their complexity. In this lecture, I will compare current single-cell technologies, with an emphasis on how they are applied in practice. As a real-world example, I will present data from our study in which we used mass cytometry (CyTOF technology) to deeply profile peripheral blood leukocytes in cohorts of older (>80 years) and younger adults (20-53 years) before they received at least two doses of BNT162b2 mRNA vaccine and correlated the data with SARS-CoV-2-specific response data. I will illustrate how we analyzed and combined classical flow cytometric data, i.e. intracellular cytokine levels after SARS-CoV-2-specific stimulation and 50-plex CyTOF immune phenotyping data, in order to predict vaccination response outcomes from baseline data.

Oral presentations

O.01.1

Multiparameter spectral flow cytometry with advanced unsupervised analysis to identify immune signature of neurological post-COVID Syndrome after mild SARS-CoV-2 infection

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Many COVID-19 convalescents experience severe symptoms, which include pulmonary fibrosis, chronic fatigue as well as neurological/cognitive dysfunctions ("brain fog"), lasting even longer than 3 months. Broad-spectrum of clinical manifestations indicate post-COVID Syndrome (PCS) as systemic illness, possibly driven by immune system deregulation. Here we investigated dynamics of T cell landscape and immune signatures, which can be predictors of PCS. For this, 28-parameter spectral flow cytometry followed by unsupervised clustering was used, to identify immune signatures specific for convalescents after either severe or mild infection.

Neurological symptoms of PCS have been observed in 42% of patients after mild form of COVID-19. Early after infection they showed elevated levels of naïve CD8+ T cells. At the same time we observed a decreased population of CM (central memory) CD8+ T cells and terminally differentiated effector CD8+ T cells expressing CD57. Moreover, we found lower production of granzyme B in combination with IFN-□ in CD8+ T cells after anti-CD3/CD28 stimulation. In the blood plasma of those patients, elevated concentrations of VCAM-1, OPN and cystatin C possibly involved in local neuroinflammation were found.

Altogether, the multiparameter spectral flow cytometry results suggest, that elevated population of naïve CD8+ T cells and inhibited production of granzyme B may be an immune signature of neurological post-COVID Syndrome after mild SARS-CoV-2 infection.

O.01.2

Flow cytometry in testing bacterial physiology after exposure to nanomaterials

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Bacterial physiology can specifically respond to various environmental stressors. Physical structures such as nanomaterials can interact with cells causing numerous negative effects, including membrane disruption, cytoplasm leakage, cell lysis, cell agglomeration, biofilm induction, or stimulation of the production of virulence factors. Flow cytometry is a powerful technique that was successfully used in microbiology, e.g., in the enumeration of bacteria in water samples. Theoretically, flow cytometry can be used to determine the physiological state of the population.

Therefore, the aim of the study was to test the possibility of using flow cytometry in evaluating selected physiological measures in bacterial populations exposed to nanomaterials.

Two reference strains, i.e., *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were contacted with carbon nanomaterials and metal oxides. Afterward, cells were tested on the flow cytometer (Accuri C6 Plus) and the spectral flow cytometer (Aurora) without staining (for fingerprinting and autofluorescence) and stained with selected fluorochromes to determine their integrity, viability, membrane potential, and aggregation.

The results revealed that flow cytometry can be used to study samples simultaneously containing bacteria and nanomaterials. However, the most important factor for the analysis was the selection of the bacteria/nanomaterial ratio for the analyses. It means that the method is limited to low nanomaterial concentrations.

Posters

P.01.1

The use of phosflow cytometry assay to assess the impact of physical effort on T cells' activation

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Th1 cell subset is involved in the immunological response triggered by physical exercise. The aim of this work was to evaluate the post-effort activation of Ras/MAPK and JAK/STAT signaling pathways in T cells of young, physically active men. Seventy-six physically active, healthy men between 15 and 21 years old performed standard physical exercise protocol (Beep test). Phosphorylation levels of Ras/MAPK- (p38 MAPK, ERK1/2) and JAK/STAT-related (STAT1, STAT3, STAT5, and STAT6) proteins were evaluated by flow cytometry in Th and Tc cells post-effort and during the lactate recovery period. The performed physical effort was not strong enough physiological stimulant to provoke the phosphorylation of ERK1/2, p38 MAPK, STAT1, STAT3, STAT5, and STAT6 proteins in T cells, at least for the duration of our study (the end of the lactate recovery period). We conclude that more observation time-points, including shorter and longer times after the exercise are required to determine if the Ras/MAPK signaling pathway is involved in modulating the post-effort immunological response.

Reference

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P.01.2

Autofluorescence extraction as a powerful tool for adequate gating and pure sorting of rat neural system isolates

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Autofluorescence extraction is an advantage key step in spectral flow cytometry, aimed at removing background fluorescence caused by cellular components such as lipofuscin, flavins, porphyrins, as well as extracellular matrix components. These endogenous fluorochromes overlap with the emission spectra of specific labeling fluorochromes, hindering color separation. Complex samples, like tissues, tumors, nervous systems isolates, and hematopoietic organs with varying autofluorescence patterns require autofluorescence extraction.

We present successful autofluorescence extraction examples that yield accurate measurements and improved resolution. Using newborn rat sciatic nerve isolates, we identified three autofluorescence patterns specific for fibroblast, macrophages and Schwann cells. Full spectrum profiling aided cell types annotation and removal of highly autofluorescent populations. This approach ensures a reliable gating strategy and pure sorting using Cytek Aurora CS. Other examples include mice cord blood, mice spleen and bone marrow, human fibroblasts, aging cells and leukemia cell lines.

We propose that full spectrum profiling of unstained tissues may be relevant in panel design and optimization to avoid using fluorochromes emitting in the range of highest autofluorescence. Additionally, careful autofluorescence extraction before making a final gating strategy for sorting should be taken into account, especially in the case of heterogeneous samples and cell mixtures.

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P.01.3

Studies on the immunomodulatory effects of bacteriophages on functions of immune cells – a preliminary report

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The main purpose of this project is to extend our original observations suggesting that aside from antibacterial action phage therapy may have also anti-inflammatory and/or immunomodulatory functions. We have been conducting a wild basic study on the influence of two model phages (*E. coli* T4 phage and staphylococcal A5 phage) on the function of different subpopulations of human peripheral blood mononuclear cells (PBMC).

The populations of interest (neutrophils, monocytes, B cells, NK cells, T lymphocytes, helper T cells, and cytotoxic T cells) were isolated from PBMCs of healthy donors using MojoSort Isolation Kits and incubated at 37°C for 24 hours with a high or low dose of the phage. After 24 hours concentration of TNF-alpha, IL-2, IFN-gamma, IL-4, IL-5, IL-13, IL-10, IL-17, IL-6, Il-1 beta, IL-21 in cell supernatants was determined using a Luminex method.

Our preliminary observations showed that both phages may exert an immunomodulating effect on the immune cells. For example, they were able to stimulate specific secretion of both pro- (TNF-alpha, IL-1 beta, and IL-6) and anti-inflammatory (IL-10) cytokines in monocytes. Interestingly high dose of T4 phage tended to diminish the production of pro-inflammatory cytokines by PBMCs when compared to the control stimulated with lipopolysaccharide in concentration relevant to that in tested phage preparations.

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