Abstract Booklet

34th Annual Conference of the German Society for Cytometry

Pre-Program
September 10, 2024: Meeting Point Cytometry and Tutorials

Conference
September 11-13, 2024

Location
Charité – Universitätsmedizin Berlin &
Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz Institut
Charitéplatz 1
10117 Berlin

E-mail
dgfz-meeting@drfz.de

Conference: dgfz2024.de
DGfZ: dgfz.org

Our conference sponsors

Dear friends of cytometry,

Join the CYTometry community in the heart of Berlin

We kindly welcome you to the Annual Meeting of the German Society for Cytometry in Berlin!
At DGfZ and our annual meetings, we cultivate a highly interactive, interdisciplinary and cooperative spirit to advance collaboration, communicate cutting edge approaches in cytometry and promote excellence in the application of cytometric technologies. We are proud to provide a national hub for the cytometry community in Germany, linking technology developers, engineers, physicists, biologists, chemists, and clinicians engaged in service facilities and research labs!

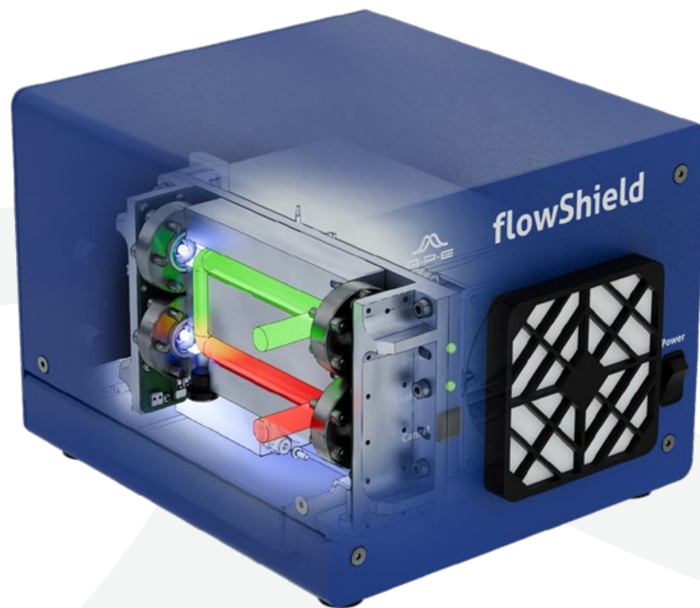
Specials: We kindly welcome the Irish Society for Cytometry (https://cytometry.wixsite.com/ireland) hosting this year´s guest session!
Also note the Keynote Lecture and Guest Lecture as additional highlights in our scientific program.

Welcome to Berlin!

Henrik Mei
President of the DGfZ

flowShield

UV-C decontamination
for germ-free cell sorting



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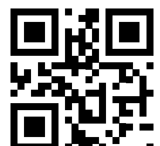

10.09.2024

Tuesday

Meeting Point Cytometry

Starting 14:00 pm - 3:00 pm, DRFZ Foyer

First Time Attendee and New Member Welcome Orientation Session

Tutorial 1
Securing Intellectual property

3:00 pm – 5:00 pm, DRFZ - Seminar room 1+2

Dr. Martin Behrndt, European Patent Attorney Hertin & Partner, Berlin

Dr. Konrad von Volkmann, CEO APE, Berlin

Dr. Christin Koch, Director IP Strategy Symrise AG, Holzminden

Tutorial 2
Deep Learning in Image Analysis

3:00 pm - 5:00 pm, DRFZ - Seminar room, first floor
Anja Hauser, Raluca Niesner & Pendar Alirezazadeh

Tutorial 3
Data annotation, quality, sharing, and FAIR principles

3:00 pm - 5:00 pm, DRFZ - Seminar room 3

Sebastian Ferrara & Christian Busse

Entrepreneur Networking Event

5:30 pm at DRFZ

Lisa Budzinski & Henrik Mei

11.09.2024

Wednesday

Tutorial 4
Core Facility / Core Manager Tutorial

9:00 am - 11:00 am, DRFZ Seminar room 3

Jochen Behrends & Elmar Endl

Tutorial 5
Flow Cytometry

9:00 am - 11:00 am, Seminar room 1+2

Claudia Giesecke-Thiel, Andreas Dolf, Daniel Kage,

Toralf Kaiser

PEH = Paul Ehrlich Hörsaal, Virchowweg 4 CCO = Cross-Over-Gebäude, Virchowweg 6 DRFZ = Virchowweg 12

11.09.2024

Wednesday

Registration & Soup

8:30 am - 11:45 am, CCO

Welcome by Henrik Mei, President DGfZ

11:45 am - 12:00 pm, PEH

Core Facility Session

12:00 pm - 1:00 pm, PEH, Chairs: Anne Gompf & Jochen Behrends

Malte Paulsen

Novo Nordisk, Måløv, Denmark

Enabling great Science everyday – staying innovative and selfcritica

Florian Mair

Flow Cytometry Core Facility, Institute of Molecular Health Sciences, ETH Zurich, Switzerland

High-dimensional cytometry in the spectral era: new metrics to achieve a 50-color pane

Short talk: Susanne Strahlendorff-Herppich

Precision for Medicine, Cell Biology, Berlin Germany

High-dimensional cytometry in the spectral era: new metrics to achieve a 50-color pane

High Dimensional Cytometry Session

1:00 pm - 2:pm, PEH, Chairs: Henrik Mei & Bertram Bengsch

Chotima Böttcher

Experimental and Clinical Research Center (ECRC), Charité – Universitätsmedizin Berlin and the MDC

Proteo-metabolomic features of multiple sclerosis

Nils Blüthgen

Institute of Pathology, Charite Universitätsmedizin Berlin & Institute of Biology, Humboldt University of Berlin

Using CyTOF and single cell RNA-sequencing to dissect oncogenic signalling in colon cancer

PEH = Paul Ehrlich Hörsaal, Virchowweg 4 CCO = Cross-Over-Gebäude, Virchowweg 6 DRFZ = Virchowweg 12

Short talk: Yaroslava Shevchenko

Department of Hepatology and Gastroenterology, Charité Universitätsmedizin Berlin, Berlin, Germany

A comprehensive assessment of compensation particles for generation of optimized reference controls for full spectrum flow cytometry

2:00 pm - 3:00 pm, CCO Coffee Break/Industry Exhibition

Product Slam

3:00 pm - 4:00 pm, PEH, Chairs: Elmar Endl & Tom Bauer

Industrial partners will present their newest innovative technological developments and products:

AHF, APE, Beckman Coulter, BD, BioLegend, Cytek, Cytolytics, Berlin Partner, Milteny Biotec, OLS, Particle Metrix, Sony, Standard Biotools, ThermoFischer

4:00 pm - 5:00pm, CCO Coffee Break/Industry Exhibition

Keynote Lecture

5:00 pm - 6:pm, PEH, Chair: Henrik Mei

Yvan Saeys

Ghent University

AI's Odyssey in cytometry wonderland: challenges and prospects of emerging foundation models for single-cell analysis

Welcome Reception

6:00 pm - 10:00 pm, CCO, Exhibition area

Core Facility Networking Event

7:30 pm - 10:00 pm, DRFZ Chairs: Désireé Kunkel, Sarah Warth

This event is an opportunity to meet colleagues and share your experiences and challenges working in a core facility. We will have short presentations and lots of time for formal and informal discussions

PEH = Paul Ehrlich Hörsaal, Virchowweg 4 CCO = Cross-Over-Gebäude, Virchowweg 6 DRFZ = Virchowweg 12

12.09. 2024

Thursday

Endosymbiosis to Nanobiotechnology Session

9:00 am - 11:00 am, PEH Chairs: Christin Koch, Lisa Budzinski, Wolfgang Fritzsche

Andrey Turchanin

Institut für Physikalische Chemie, Friedrich-Schiller-Universität Jena

Two-dimensional (2D) carbon materials for ultrasensitive detections of biomarkers

Thomas Gassler

Institute of Microbiology, ETH Zurich

Inducing Novel Endosymbioses by Bacterial Implantation into Fungi

Short talk: Simone de Carli

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany

X-ray Compatible Flow Cell for Dielectrophoretic Manipulation and Trapping of Cells and Microparticles

Short talk: Carrie Maynard

Lightcast Discovery Ltd, Cambridge, United Kingdom

A programmable and automated microfluidic platform for massively parallel and sequential processing of single cell assay operations

Short talk: Peter Rubbens

Kytos BV, Ghent, Belgium

Cytometric indicators quantify microbiome health in aquaculture systems

Short talk: Toni Sempert

German Rheumatology Research Center Berlin, a Leibniz Institute, Berlin, Germany

The stratification by age is critical for microbiome analyses of children with juvenile idiopathic arthritis

11:00 am - 11:30 am, CCO Coffee Break/Industry Exhibition

European Guest Session: Ireland

11:30 am - 12:30 pm, PEH, Chair: Barry Moran

Barry Moran

Trinity Biomedical Sciences Institute, Trinity College Dublin

Complementary cell analysis technologies to elucidate skin disease – No Kuddelmuddel!

David Finlay
Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

Using Click-chemistry to study nutrient uptake in single cells

Ella Fouhy
School of Biomolecular and Biomedical Science, University College Dublin

Uncovering the early circulating environment that precedes a diagnosis of Pre-eclampisa

12:30 pm - 1:30 pm, CCO, Lunch

Poster Session

1:30 pm - 3:00 pm, CCO, Chairs: Thomas Kroneis & Frank Schildberg

3:00 pm - 3:30 pm, CCO, Coffee Break/Industry Exhibition

Guest Lecture

3:30 pm- 4:30 pm, PEH, Chair: Henrik Mei

Lena Kaufmann
Bernstein Center for Computational Neuroscience, Humboldt Universität zu Berlin, Berlin, Germany

Berlin School of Mind and Brain, Humboldt Universität zu Berlin, Berlin, Germany

Elephant neurobiology and behavior: from trunks to brains

DGfZ - Members Assembly

4:30 pm – 6:00 pm, PEH, Chair: Henrik Mei

Meet the Speaker & Poster Prize Ceremony & Dinner

6:00 pm – 10:00 pm, DRFZ, for all speakers, participants and industry partners

13.09. 2024

Friday

9:30 am - 10:00 am, CCO Coffee Break/Industry Exhibition

Imaging & Cytometry Session

10:00 am - 12:00 pm, PEH, Chairs: Anja Hauser, Raluca Niesner & Oliver Otto

Fabian Coscia

Max Delbrück Center for Molecular Medicine

Spatial tissue proteomics to assess health and disease

Kerstin Göpfrich

Center for Molecular Biology of Heidelberg University

Engineering and sorting of synthetic cells

Esther Wehrle

AO Research Institute Davos, Switzerland

Spatial transcriptomics of bone healing

Short talk: Alexander Fiedler

Biophysical Analytics, German Rheumatology Research Center (DRFZ), Berlin, Germany

In vivo fluorescence lifetime micro-endoscopy creates new perspectives on immunometabolic heterogeneity in the bone

Short talk: Michael Kirschbaum

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses, Potsdam, Germany

On-chip integrated re-sorting enhances sort performance in microfluidic flow cell sorting in one single step

12:00 pm - 1:00 pm, CCO, Lunch/Industry Exhibition

Klaus Goerttler Session

1:00 pm - 2pm, PEH, Chair: Annika Betzler, Klaus Goerttler Awardee 2023

Marta Rizzi

Dept of Rheumatology and Clinical immunology and CCI – University Medical Center Freiburg and Center for Pathophysiology, Infectiology and Immunology; Institute of Immunology – Medical University of Vienna

Modulation of peripheral B cell maturation and generation of autoantibodies: lesson from rare autoimmune diseases

Christian Busse

DKFZ Heidelberg

Farewell

2:00 pm - 2:15, PEH

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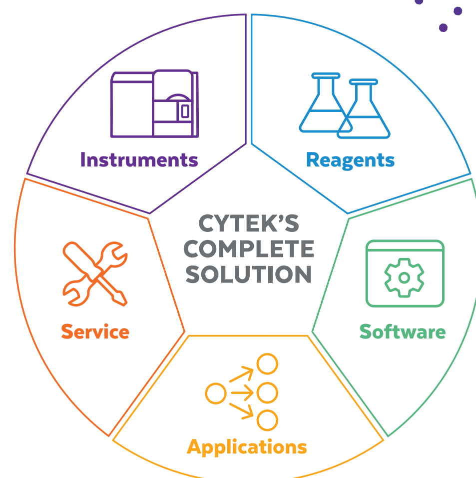

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Abstracts

Wednesday, 11.09.2024

Core Facility Session

12:00 pm, PEH

Chairs: Anne Gompf & Jochen Behrends

Core Facilities are shared resource laboratories that provide scientific and clinical researchers with access to instruments, services and expert consulting. These laboratories are set up with the aim of utilizing financial and intellectual resources more efficiently and generating high-quality data.
As different as the number of users and the orientation of core facilities at universities and companies can be, the demands on personnel in core facilities are just as extensive. The career paths of core facility team members are not predetermined and the output that may develop in addition to the daily core facility business is correspondingly diverse and will be the focus of this session.
Susanne Strahlendorff-Herppich from Precision for Medicine in Berlin tells us how to develop a complex detection method for analyzing the efficacy of drugs.
Florian Mair from ETH Zurich explains how to push the limits of what is possible and exploit the potential of fluorochrome combinations for comprehensive tissue characterization.
Malte Paulsen's example during his time at EMBL in Heidelberg shows how you can successfully develop complex technology that forms the basis for the development of novel instruments.

Malte Paulsen

Director of Strategy and Operations R&ED Greater Copenhagen – Novo Nordisk, Måløv, Denmark

Enabling great Science everyday – staying innovative and selfcritical

Being a core facility scientist can be a challenge towards staying innovative and avoiding to catch dust. Science around a core facility is moving forward and very often at a much different pace than the technology we are providing to our institutions. Throughout my career, I'm trying to drive innovation within my technology and also within myself. Coming from a basic science degree, I had a very interesting and challenging career path that I will present to highlight ups and downs I experienced – and I will draw some conclusions on why especially core facility personnel have a huge potential in exploring a broad career path if they dare to make some changes.

Florian Mair

Flow Cytometry Core Facility, Institute of Molecular Health Sciences, ETH Zurich, Switzerland

High-dimensional cytometry in the spectral era: new metrics to achieve a 50-color panel

To understand the function of the immune system in human samples it is imperative to capture as much information as possible from often size-limited biopsies.

Here, we report the first 50-color spectral flow cytometry panel to comprehensively study the functional state

of the human immune system in PBMCs and tissue samples. The panel contains lineage markers for all major immune cell subsets, and an extensive set of phenotyping markers focused on the activation and differentiation status of the T cell and dendritic cell (DC) compartment. Of note, the panel has also been tested to be suitable for cell sorting, which allows very fine-grained isolation of immune subsets utilizing 50 markers at the same time.

To establish such a complex panel, we developed novel approaches to systematically select fluorochromes. Furthermore, we utilized a new metric termed unmixing spreading error, that evaluates the fluorochrome-specific increase in background that is inherently generated when unmixing highly complex panels. In this presentation we show the systematic workflow we used to create the panel and how it can be used to obtain news insights into human immune cell function.

Short talk: Susanne Strahlendorff-Herppich

Precision for Medicine, Cell Biology, Berlin Germany

Optimization of a flow cytometry-based Receptor Occupancy (RO) assay for the pharmacodynamic analysis of a bispecific antibody biotherapeutic

Precision for Medicine (PfM) developed and validated a 10-color Receptor Occupancy (RO) assay to support a sponsor’s clinical trial focusing on a first-in-class bispecific antibody targeting two immune checkpoints.

RO assays aim at quantifying the binding of biotherapeutics to their specific target on the surface of cells. RO is a combination of three basic formats: free receptor measurement, total receptor measurement, or direct assessment of bound receptor. Since the staining is at single cell level, the RO is key to determine the saturation and EC50 concentration of the drug on the target cell population.

In this panel, free receptors are evaluated by using the fluorescent conjugated parental drugs while the total drug bound is detected using an anti-idiotype antibody recognizing the unconjugated drug. The present assay was optimized to accurately quantify receptor occupancy of two differentially and independently expressed immune checkpoints targeted by the bispecific drug on diverse cell populations.

Verified transfer of the RO assay from fresh to cryopreserved mononuclear cell samples allows to test samples in batch, analyzing e.g. several visits of the same patient simultaneously during the course of the clinical study. Moreover, the use of molecules of equivalent soluble fluorochrome (MESF) beads enables the standardized quantitation of the free and total receptors on the cell surfaces.

This study described PfM approaches to support sponsor with novel compounds to achieve clinical trial success and, ultimately, to more quickly bring effective therapies to the market.

High Dimensional Cytometry Session

1:00 pm, PEH

Chairs: Henrik Mei & Bertram Bengsch

This session delves into the expanding realm of high-dimensional cytometry, a pivotal area in clinical immunology with rapid technological advancements and increasing data output. Data robustness, integrative data analysis and mathematical modeling help to transition high-dimensional data into scientific advance, and are at the forefront of today´s research. By addressing this topics, our speakers feature exciting ways of single-cell-based precision medicine strategies in cancer and inflammation.

Chotima Böttcher

Experimental and Clinical Research Center (ECRC), Charité – Universitätsmedizin Berlin and the MDC

Proteo-metabolomic features of multiple sclerosis

Multiple sclerosis (MS) exhibits a heterogeneity of clinical features, genetic and environmental risk factors, and mechanisms of tissue damage. One of the fundamental pathomechanisms involves cell and humoral mediated immune dysregulation. Our study aims to investigate the association of diverse myeloid and B cells as well as molecular profiles with neuroinflammation in MS. We use a streamlined mass cytometry workflow to perform characterization of multiple immune cell types in CSF and peripheral blood of patients with MS as compared with other neuroinflammatory conditions. In addition, metabolomics and targeted proteomics were also performed. Multi-modal datasets on single-cell and molecular profiles reveal proteo-metabolomic features associated to neuroinflammation in MS.

Nils Blüthgen

Institute of Pathology, Charite Universitätsmedizin Berlin & Institute of Biology, Humboldt University of Berlin

Using CyTOF and single cell RNA-sequencing to dissect oncogenic signalling in colon cancer

We use mass-cytometry and single cell RNA-sequencing to study the role of different oncogenes on the intrinsic differentiation pathways in the intestinal epithelium. I will discuss different experimental and computational strategies that allow for high-throughput analysis of the Impact of oncogenes and drugs.

Short talk: Yaroslava Shevchenko

Department of Hepatology and Gastroenterology, Charité Universitätsmedizin Berlin, Berlin, Germany

A comprehensive assessment of compensation particles for generation of optimized reference controls for full spectrum flow cytometry

Full spectrum flow cytometry enables the analysis of 50 or more parameters in a single sample. Due to the

complexity of such large panels the correct and reliable unmixing of fluorochromes requires identical spectra in both reference controls and fully stained samples to prevent unmixing errors. It is considered optimal to use the cells of interest to create single stain controls, but this is often impractical due to low expression of certain markers or limited availability of rare cell types. Compensation beads provide a commercially available and inexpensive alternative, binding antibodies consistently irrespective of specificity and expression level. Here, we evaluated 30 commonly used and commercially available fluorochromes on various compensation beads and human and murine primary leukocytes to establish optimal reference controls for full spectrum flow cytometry. Five types of compensation beads from different vendors were used to generate single stain controls and spectral signatures were recorded on 3-laser and 5-laser instruments. Our findings demonstrate significant differences in the spectral profiles of certain fluorochromes and their influence on spectral unmixing depending on the type of compensation particles used, including some fluorochromes that should be used on cells exclusively. This study underscores the critical importance of selecting appropriate reference controls to prevent unmixing errors and ensure the generation of reliable flow cytometry data.

Product Slam

3:00 pm, PEH

Chairs: Elmar Endl & Tom Bauer

In this session the industry partners have the opportunity to present the latest, innovative technical developments and products in 3 minutes.

Keynote Lecture

5:00 pm, PEH

Chair: Henrik Mei

Yvan Saeys

Ghent University

AI's Odyssey in cytometry wonderland: challenges and prospects of emerging foundation models for single-cell analysis

Recent advances in artificial intelligence are quickly finding their way in scientific research, and applications of large language models, foundation models and other large-scale modelling efforts are leaving scientists bedazzled, wondering whether they should embrace these novel advances, or remain more cautious and rely on simpler models they can understand more easily. In this talk I will first introduce some basic building blocks of these novel AI models, and show some examples of how they can be useful to construct next-generation representations of single-cell data. Subsequently I will give a high-level overview of what might be future applications of all these models in the cytometry field, and which novel questions and challenges they raise.

Welcome Reception

6:00 pm – 10:00 pm, CCO

All participants are invited.

Core Facility Networking Event

7:30 - 10 pm, DRFZ

Chairs: Sarah Warth & Desiree Kunkel

This event is an opportunity to meet colleagues and share your experiences and challenges working in a core facility. We will have short presentations and lots of time for formal and informal discussions.

We want to spend a wonderful evening with you at the DGfZ meeting 2024 in Berlin, with food & beverages & YOU!

Thursday, 12.09.2024

Endosymbiosis to Nanobiotechnology Session

9:00 am

Chairs: Christin Koch, Lisa Budzinski, Wolfgang Fritzsche

The session will highlight innovative approaches and applications contributing to biosensor development and the understanding of single cell interactions.
Biosensors provide cost-effective, easy-to-use, sensitive and highly accurate detection devices for biomolecules of interest in a variety of research and commercial applications such as clinical research, diagnostics or bioprocess monitoring. The session will present the potential, developments and implementation approaches of carbon 2D materials e. g. graphen.

Further, this session will introduce Fluidic Force Microscopy which enables to manipulate cells with single cell resolution and has already successfully been applied to biological systems. As an example, it will be demonstrated how artificial endosymbiosis can be established in fungal cells based on a fluidic force microscope followed by characterisation of the fungal cells on single cell level.
The session will be complemented by short talks in the field of microbiology and nanotechnology, to explore the different facets of cutting edge technologies and developments in the nano- and micrometer range.

Andrey Turchanin

Institut für Physikalische Chemie, Friedrich-Schiller-Universität Jena

Two-dimensional (2D) carbon materials for ultrasensitive detections of biomarkers

Carbon based 2D materials like graphene – hexagonally ordered monolayer of carbon atoms – or molecular carbon nanomembranes (CNMs) – 1 nm thick molecular nanosheets – open broad avenues for applications in nanobiotechnology including the assembly of protein biochips, structural studies of biomolecules and ultrasensitive detection of biomarkers. In this talk, I will give an overview of the most relevant properties of these materials for highly sensitive, rapid and selective detection of biomarkers as well as challenges and prospects with their integration into clinical research and diagnostics. I will present our resent progress on implementation of 2D carbon materials in sensors functioning either on optical readout, related to the surface plasmon resonance (SPR) phenomena, or on electrical readout, based on measurements of the field-effect transistors (FETs). By studying such pathogens like RSV and SARS-CoV-2, it will be demonstrated that 2D carbon materials enable to achieve very high sensitivity in detection of the related biomarkers. In case of the SPR-based sensors the limit of detection is found to be in the range ~1 pM, and in case of the FET-based sensors it is even 5 orders of magnitude better ~10 aM. Moreover, I will discuss development of the microscopic sensor arrays capable to simultaneous and rapid (~1-10 min) screening of more than 100 biomarkers using small sample volumes and without any need for bioamplification.

Thomas Gassler

Institute of Microbiology, ETH Zurich

Inducing Novel Endosymbioses by Bacterial Implantation into Fungi

Microbial endosymbioses have had a profound impact on the evolution of life. The endosymbiosis-mediated acquisition of aerobic respiration and photosynthesis, resulting in mitochondria and chloroplasts, respectively, are prominent examples. However, understanding the requirements and mechanisms for endosymbiogenesis is challenging. By combining atomic force microscopy, optical microscopy, and nanofluidics, we developed a FluidFM-based method to extract, inject, and transplant organelles and bacteria directly into living cells. This method enables us to track artificially induced endosymbiosis in the widespread filamentous fungus Rhizopus microsporus. FluidFM-based injections of bacteria into R. microsporus, followed by FACS-mediated positive selection, allowed to introduce endosymbionts into this novel fungal host. We showed that the transplanted bacterium can be vertically transmitted across host generations and can be selected for. Adaptive laboratory evolution mitigated initially observed compromised host fitness and stabilized the endosymbiosis. Genetic and transcriptomic analyses shed light on the dynamics and fitness constraints during early endosymbiogenesis. Overall, our findings could improve the understanding of the balance between mutualism and antagonism in early endosymbiogenesis and help to study cost-benefit trade-offs.

Short talk: Simone de Carli

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany

X-ray Compatible Flow Cell for Dielectrophoretic Manipulation and Trapping of Cells and Microparticles

In this work we present a soft X-ray microscopy compatible microfluidic device for single-cell and microparticle studies. In a single-cell study, efficient dielectrophoretic (DEP) manipulation of cells and stable trapping are crucial for accurate microscopy and image acquisition. We present an approach that enables robust trapping of micro-objects in an X-ray transparent flow cell.
By photolitographically patterned gold electrodes on a silicon nitride (Si3N4) membrane and using negative dielectrophoresis (n-DEP), the system allows micrometer-scale manipulation of cells in a microfluidic channel and a downstream single-cell trapping for soft X-ray inspection. Our approach handles, traps and rotates the object of interest, ensuring its stable position within a field-cage in the laser focus, for *in vivo* high-resolution cell imaging. Additionally, the system offers easy sample replacement for sequential inspection without the need for full vacuum decompression, allowing for the inspection of a wide variety of samples, including bacteria, algae, yeasts and microparticles.

X-ray microscopy represents a powerful label-free technology for cellular analysis of intrinsic biophysical properties with nanometric resolution of the three-dimensional structure of intact cells. Soft X-rays ranging from 284eV to 543eV ("water window") are particularly suitable for high-resolution analysis, where the absorption of photons by functional groups, especially carbon and nitrogen, prevails over the transparency of water. Therefore, Soft X-rays have a large penetration depth in water and still enable highresolution imaging of biological specimens in their natural environment.
Our chip consists of two commercial Si3N4 multi-windows frames, XUV and soft X-rays transparent, bonded using a patterned pressure-sensitive adhesive with a thickness of just 10µm. Each substrate contains custommade gold planar microelectrodes to perform different DEP operations on microparticles (focus-deflectiontrapping).

Short talk: Carrie Maynard

Lightcast Discovery Ltd, Cambridge, United Kingdom

A programmable and automated microfluidic platform for massively parallel and sequential processing of single cell assay operations

Cancer immunotherapy has seen significant advancements, revolutionising treatment and improving patient outcomes. Single cell profiling has been instrumental in this development. However, traditional single cell technologies, while providing extensive 'omic datasets, have not facilitated direct functional analysis at the single cell level. We propose that such analysis is crucial for understanding the complex cellular interactions within the tissue microenvironment.
We present a platform that utilises droplet microfluidics and optical electrowetting-on-dielectric (oEWOD) to conduct controlled, sequential, and multiplexed single cell assays in massively parallel workflows. This enables complex cell profiling during screening. Soluble reagents, cells, or assay beads are encapsulated into droplets in fluorous oil. These droplets are actively filtered based on size and content, ensuring only desirable droplets (e.g., single cell droplets) are retained for analysis, thereby overcoming the Poisson distribution. The droplets are stored in a temperature-controlled chip array, and each droplet's history is tracked from filtering to workflow completion.
On the chip, droplets undergo an automated suite of operations, including merging sample droplets and acquiring fluorescent assay readouts. This enables complex sequential assay workflows. To illustrate the platform's utility in immuno-oncology, we present single-cell functional workflows for antibody discovery and cell and gene therapy. For instance, droplets containing single immune effector cells, such as T-cells, merge twice with droplets containing single target cells. This workflow can be used to investigate

T-cell exhaustion or test effector cell killing specificity. Our platform provides a powerful tool for single cell functional analysis, offering potential advancements in cancer immunotherapy. By enabling complex cell profiling and overcoming limitations of traditional single cell technologies, it opens new avenues for understanding cellular interactions and functional profiling of individual cells.

Short talk: Peter Rubbens

Kytos BV, Ghent, Belgium

Cytometric indicators quantify microbiome health in aquaculture systems

Aquaculture farmers breed fish, crustaceans and aquatic plants in a (semi-)controlled way. Variations in the microbiome can have a large impact on these cultured organisms, and as such, may result in considerable production losses. For example, it is estimated that 10% of all cultured fish die from infectious diseases. Early mortality syndrome, caused by several species of Vibrio, has been responsible for large losses in cultured shrimp. At Kytos, we analyze the microbiome in aquaculture systems using flow cytometry. By now, we have analyzed about 40,000 samples coming from 125 unique farms and companies. Backed by previous research in microbial ecology, we have developed multiple cytometric indicators to quantitatively characterize changes in the aquaculture microbiome.
For example, a sudden decline in microbial diversity increases the risk for the outgrowth of (opportunistic) pathogens. A sudden increase in microbial productivity can be related to overfeeding by the farmer. We provide an overview of these cytometric indicators, which include microbial load, diversity and productivity, and discuss how they should be interpreted with regards to microbiome health in aquaculture systems. Additionally, we show how cytometric measurements can be integrated with other culture-independent technologies in order to predict potential risks for specific pathogens, such as Vibrio spp. Context is provided through examples from shrimp aquaculture based in the Mekong Delta.

Short talk: Toni Sempert

German Rheumatology Research Center Berlin, a Leibniz Institute

The stratification by age is critical for microbiome analyses of children with juvenile idiopathic arthritis

Juvenile idiopathic arthritis (JIA) is the most common autoimmune disease affecting the joints in children and teenagers. The etiology of JIA is unclear, but genetic and environmental factors, such as the intestinal microbiome, have been implicated. We have analyzed the intestinal microbiota from JIA patients and sex- and age-matched healthy individuals using single-cell analysis by multi-parameter microbiota flow cytometry and 16S rRNA gene amplicon sequencing. For microbiota flow cytometry, we assess coating of bacteria with different host immunoglobulin isotypes and the expression of specific sugar moieties on the bacterial surface. We then use a self-organizing map algorithm for dimensionality reduction and clustering combined with machine-learning to identify disease-specific microbial phenotypic signatures. We can show that overall the JIA microbiome significantly differs from that of healthy controls on the taxonomic level. For the identification of a JIA-specific microbial phenotypic signature by flow cytometry, a further stratification of the JIA patients according to age was critical. This suggests that age-dependent, perhaps physiological, alterations additionally shape the JIA microbiota. The stratification by age also revealed that different bacterial taxa were decisively differentially abundant in the microbiomes of JIA patients and healthy donors. Thus, our data indicate that a stratification by age could be particularly important for the specific identification of alterations of the microbiome in JIA and in chronic inflammatory diseases affecting children in general.

European Guest Session: Ireland

11:30 am, PEH

Chairs: Alfonso Blanco

Barry Moran

Trinity Biomedical Sciences Institute, Trinity College Dublin

Complementary cell analysis technologies to elucidate skin disease – No Kuddelmuddel!

Background: Cell analysis technologies can add important information to under-recognized and understudied skin diseases such as chronic spontaneous urticaria (CSU) and hidradenitis suppurativa (HS). CSU is a skin condition characterized by recurrent itchy hives that has a major impact upon quality of life, while HS is a chronic skin disease with painful, oozing lesions and abscesses. Treatment to date for both conditions is inadequate in many patients.
Objectives: Characterising the skin and blood immune cells, their transcriptome and secretome, in these diseases will lead to a better understanding of their pathogenesis, providing a rationale for new or stratified therapeutic strategies.
Methods: In these studies we employed flow and imaging cytometry, microscopy, single cell RNA sequencing, multiplex assays and ELISA to characterise immune cells isolated from patients and healthy donors.
Results: This presentation will discuss the findings of both studies. The CSU study identified an increase in mast cell precursors in patient blood, with high expression of these cells a strong predictor of better clinical response. The HS study identified distinct immune cell subsets and an enhancement of specific genes and pathways associated with Th17 cells, IL-17, IL-1β, and the NLRP3 inflammasome (an inflammatory protein complex) in patient skin. Inhibiting this NLRP3 inflammasome in HS patient skin explants significantly reduced inflammatory markers, providing a rationale for a potential new therapy.
Conclusion: Complementary single cell analysis technologies can provide invaluable insights into skin disease pathology and treatments.

David Finlay

Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

Using Click-chemistry to study nutrient uptake in single cells

It is now clear that some of the metabolic discoveries made using *in vitro* cultured immune cells do not hold true for the metabolic features of these cells *in vivo*. The *in vivo* environment is not homogenous and there are differences in metabolic conditions at the sites of diseases including cancer and infection. The next great frontier for the immunometabolism field is to measure single immune cell metabolism *in vivo* and at the site of disease. Working towards this goal, we have developed a new technology to accurately measure nutrient uptake, the first rate limiting step for cellular metabolic pathways, using a biorthogonal chemistry-based (CLICK-chemistry) approach. This involves using a minimally modified nutrient, containing a small CLICK handle, that is taken up by the cell via the normal physiological route. A fluorophore is attached to the CLICK-nutrient after transport has occurred, when inside cell. Using multiple CLICK configurations it is possible to perform 3 sequential CLICK reactions in each immune cell. In this way we have simultaneously measured amino acid uptake, fatty acid uptake and rates of protein synthesis, providing multiple dimensions of metabolic flux analysis in individual cells. When combined with other single cell metabolic stains and assays it is now possible to generate an

ex vivo metabolic footprint of each individual immune cell taken from complex immune populations from diverse tissues and disease sites. This approach, what we have called 4D+MetaFlux, is revealing new insights into the heterogeneity of immune cells metabolism within and between tissues.

Ella Fouhy

School of Biomolecular and Biomedical Science, University College Dublin

Uncovering the early circulating environment that precedes a diagnosis of Pre-eclampisa

Pre-eclampsia (PE) is a serious complication affecting 1 in 12 pregnancies, defined by new-onset hypertension and proteinuria after 20 weeks of gestation. Early detection of PE is key for appropriate clinical decision-making throughout pregnancy. Extracellular vesicles (EVs) are important circulating messengers in the blood regulating a myriad of biological and pathological processes. EVs are highly implicated in pro-inflammatory diseases and their levels are significantly increased following a clinical diagnosis of PE. We hypothesise that EVs circulating in the first trimester are altered in those that subsequently develop PE and may be an early indication of impending disease. Ethical approval was gained to access the Biological Resource Bank in the Coombe Women and Infants University Hospital Dublin for plasma samples taken in early pregnancy (11-14+6 weeks' gestation) for healthy women, and women who had a confirmed diagnosis of preterm PE or term PE, after 20 weeks of gestation. Multicolour flow cytometry was employed to quantify circulating platelet-derived EVs in plasma and significant alterations in concentration and cell of origin was observed between the groups. Strikingly, we identified distinct circulating EV populations that could differentiate between future incidence of either preterm or term PE. These FCM findings provide novel insights into the underlying aetiology of PE and its distinct associated phenotypes.

Poster Session

Thursday, 12.09.2024, 1:30 pm

Chairs: Thomas Kroneis & Frank Schildberg

Amro Abbas

DRFZ, Berlin

Enhancing Immunological Research through Data Harmonization and Integration: The NFDI4Immuno Consortium's Role in Standardizing Flow Cytometry and Experimental Data

The National Research Data Infrastructure for Immunology (NFDI4Immuno) consortium aims to enhance immunological research by providing an integrated platform for the harmonization and utilization of data and metadata across diverse experimental technologies. This initiative addresses the critical need to combine complementary observations from cytometry, sequencing, immunoassay, and imaging to improve our understanding of immune processes.

A key focus of NFDI4Immuno is the standardization and harmonization of flow cytometry data and metadata, a cornerstone technology in immunological studies. By aligning data representations, metadata standards, and ontologies with those used by other NFDI consortia, NFDI4Immuno will facilitate queries and cross-referencing across different research domains, ensuring that flow cytometry data can be integrated and compared with other types of experimental data, enhancing the depth and breadth of immunological analyses.

NFDI4Immuno will develop and implement standardized metadata annotations and ontologies for flow cytometry to ensure compatibility with existing frameworks and facilitate cross-consortium interoperability. The consortium will provide consistent metadata annotations for both existing and new flow cytometry datasets, making them accessible through comprehensive repositories. NFDI4Immuno will actively support users in leveraging the consortium's resources and promote the adoption of FAIR (Findable, Accessible, Interoperable, and Reusable) data practices within the immunological community, fostering a culture of Open Science.

Furthermore, the consortium will build a robust network of federated repositories specifically designed for immunological data, enabling efficient data sharing and retrieval. Additionally, NFDI4Immuno will develop advanced tools and services that enable standardized, reproducible, and high-quality data analyses for flow cytometry and other immunological data types.

By addressing the complexities of data integration and standardization, NFDI4Immuno will enhance the utility of flow cytometry data, supporting the advancement of immunological research and discovery. These efforts will ensure that researchers can efficiently access, share, and analyze high-quality, standardized data, ultimately accelerating scientific progress and fostering innovative breakthroughs in immunology.

Gorkhmaz Abbaszade,

Department of Applied Microbial Ecology, Helmholtz-Centre for Environmental Research - UFZ, Leipzig, Germany

Optimizing Biopsy and Flow Cytometric Techniques for Analyzing Spatial Heterogeneity in Bacterial Colonies

Department of Applied Microbial Ecology, Helmholtz-Centre for Environmental Research - UFZ, Permoserstr. 15, 04318 Leipzig, Germany

Understanding the spatial heterogeneity of bacterial colonies is crucial for insights into their physiology and behavior. This study focuses on optimizing biopsy and flow cytometric techniques for analyzing bacterial colony heterogeneity. We investigated the effects of biopsy tool size and sampling location on colonies of five bacterial strains: Paenibacillus polymyxa, Stenotrophomonas rhizophila, Kocuria rhizophila, Pseudomonas citronellolis, and Bacillus subtilis. Samples were collected using 27G needles and varying sizes of pipette tips (10 µL, 200 µL, 1000 µL) from different colony positions, covering from the center to the edge.

By employing a newly developed rapid DAPI staining method and the conventional Syto9/PI staining techniques, cell cycles, differentiation of live and dead cells, as well as spores were identified, respectively. Our results demonstrated significant spatial heterogeneity within colonies, e.g. with B. subtilis showing increased spore counts in the inner part of the colony (ranges from ~31-65%) compared to the edges (~9-27%). The analysis indicated that the biopsies had the greatest similarities obtained from the same colony sites, with tool size being a secondary factor. For B. subtilis, the 200 µL tip was found to be the most effective, ensuring cell similarity across all colony positions. Whereas, the 27G needle and 10 µL tip were suitable for close-range biopsies but often insufficient for solid colonies.

This study highlights the importance of precise biopsy technique combined with flow cytometry to uncover bacterial colony heterogeneity. The current methodological framework provides a robust approach for studying spatial heterogeneity and thus overarching functions within bacterial colonies which can be adapted to various bacterial strains.

Marina Aparicio-Soto

Dermatotoxicology Study Centre, Department of Chemical and Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany

Improving the in vitro assessment of sensitizing chemicals by an activation-induced marker (AIM) assay: a study on p-phenylenediamine and Bandrowski's base

Dermatotoxicology Study Centre, Department of Chemical and Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany
The hair dye p-phenylenediamine (PPD) and its oxidation product Bandrowski´s base (BB) can trigger allergic contact dermatitis (ACD), a T cell-mediated skin disease, in susceptible individuals. Currently there is no validated *in vitro* test for predicting chemical-specific T cell activation. Therefore, we aim to establish a short-term activation-induced marker (AIM) assay to detect chemical-specific T cells in human peripheral blood mononuclear cells (PBMC). Using flow cytometry, we performed PPD and BB concentration series to control for potential fluorescence interference, chemical toxicity, as well as monocyte (antigen-presenting cells) and T cell function.
We determined an optimal working concentration for both substances as 6 µM, considering the absence of flow cytometry interferences and toxic effects in T cells. Both chemicals induced an upregulation of T cell receptor (TCR) specific surface activation markers (CD154 and CD137) on CD4+ and CD8+ memory T cells, respectively, with higher frequencies of chemical-specific T cells among allergic individuals. Pilot data on TCR sequencing revealed extensive T cell cross-reactivity between PPD and BB, indicating that PPD and BB-induced epitopes appear structurally related.
Collectively, AIM assays can be used to identify and quantify PPD and BB-specific T cells with high sensitivity. AIM assays may overcome current limitations of *in vitro* chemical allergy diagnosis and predictive sensitizer testing, also contributing to the risk assessment of chemical sensitizers.

Borros Arneth

Institute of Laboratory Medicine and Pathobiochemistry, Justus Liebig University Giessen, Giessen Germany

Institute of Laboratory Medicine and Pathobiochemistry, Philipps University Marburg, Marburg Germany

Use of Myeloid Activation Markers with or without a lymphocyte subset test for the management of acute unclear fever

Fever and clinical signs of infection can be caused by a variety of pathogens and are often difficult to differentiate clinically. The early detection and differentiation of infection origin in emergency patients is a daily and ever-present challenge for clinicians. Established laboratory markers, e.g., CRP and PCT, are often not sufficient to determine a definitive diagnosis. So far there has been a lack of reliable, sensitive, and fast turnaround time point-of-care- diagnostics to precisely distinguish between viral and bacterial cause of infection.
In this multicenter study we aim to determine the expression of the three surface markers neutrophil CD64, monocyte CD169 and HLA-DR in a total of 160 patients presenting to the emergency department of university hospitals Giessen and Marburg (UKGM) with fever or clinical suspicion of infection. Therefore, a fast flow cytometric approach using the Beckman Coulter Myeloid activation antibody cocktail is performed and expression levels are compared to standard laboratory parameters, e.g., CRP and PCT as well as microbiology work up. Independently, lymphocyte subset analysis with adjunct activation markers CD25 and HLA-DR are analyzed for each sample.
Preliminary results suggest significant differences in myeloid CD64 ($p<0.0001$) and CD169 ($p< 0.0001$) expression between virally and bacterially infected patient groups and not infected controls respectively and a promising diagnostic potential for neutrophil CD64 in bacterial infection (AUC= 0.8955 and $p<0.0001$) and for monocyte CD169 expression in viral infection (AUC= 0.9358 and $p<0.0001$) is shown. Objectives are to assess the diagnostic use of myeloid

and lymphocyte activation markers in acutely infected patients and thus improve patient care, point of care diagnostics and the reduction of antibiotic overuse.

Adrian Barreno-Sanchez

German Rheumatology Research Center, a Leibniz Institute, Berlin, Germany

Identifying multi-omic baseline predictors of vaccination outcome in peripheral blood and gut microbiota

Biomarkers supporting precision medicine are urgently needed. While recent technological advances permit the swift generation of enormous data, efficient strategies to compare and integrate this data are still being developed. Here we approach the comparison and integration of two data sets from the same cohort of senior adults (>80 years, n=26) aiming at identifying baseline features predicting serological outcome of a dual-dose mRNA vaccination against COVID-19.
A 50-marker mass cytometry (CyTOF) panel was employed to deeply profile peripheral blood leukocytes, and novel multi-parametric bacterial flow cytometry was used to analyze gut microbiota phenotype from baseline stool samples based on the binding of different host immunoglobulins and the presence of sugar moieties on the bacterial surface. Both datasets were integrated and correlated with serological vaccination outcome using a partial least squares discriminant analysis (PLS-DA)-based method, DIABLO.
We identified signatures of high and low vaccination response among senior vaccinees. High antibody responders were characterized by increased frequencies of pro-inflammatory CD38+ HLA-DR+ effector memory (TEM) and terminally differentiated memory (TEMRA) CD4 and CD8 T cells. Moreover, antibody response correlated positively with increased frequencies of pro-inflammatory CD14+CD16+ and non-classical CD14-CD16+ monocytes, and CD38+ IgD+ transitional B cells. Furthermore, strong antibody response in elderly donors correlates with a particular microbial phenotype characterized by low DNA content and a high extent of host immunoglobulin coating, mainly by IgA2.
Interestingly, integration of high-resolution microbiota cytometry and blood immune cell data revealed an unexpected linkage between the setup of the gut microbiota, and an activated, pro-inflammatory immune state that correlates with mRNA vaccination response in the elderly. While understanding causalities requires further work, DIABLO enabled efficient integration and joint analysis of the two omic datasets. Present data indicate a gut microbiota - immune cell axis evident in a real-world cohort of senior human adults. Non-invasive analysis of stool samples may be explored as a potential source of predictive biomarkers.

Poster #1 by Beckman Coulter

Authors: Fanuel Messaggio[1], Katily Ramirez[1], Jen Bon Lui[1], Milan Popovick[1], James Tung[1], Anne-Claire Giai[1], Alfonso Blanco[2], Irene Castrosin[3], Margaret McGee[3], Anis Larbi4

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

Introduction: The world of extracellular vesicles (EVs) is highly diverse, with variations in size, internal composition, and membrane-bound proteins. Understanding this diversity and its significance is crucial for unraveling the physiological role of EVs. In this study, we explore the use of flow cytometry to characterize EVs and enhance our comprehension of their functions.
Methods: 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.

Poster #2 by Beckman Coulter

Authors: Kelly Andrews, Omayra Mendez-Solis, Milan Popovic, Marcus Zhao, Ellison Han, Josen Ren, Leon Huang, James Tung

Affiliation: Research and Development, Beckman Coulter Life Sciences, Miami, FL 33196

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

Introduction: CytoFLEX LX is a conventional cytometer with up to 6 lasers (Ultraviolet (U): 355nm, Violet (V): 405nm, Blue (B): 488nm, Yellow (Y): 561nm, Red (R): 638nm, and Infrared (IR): 808nm) and 21 color parameters (U3, V5, B3, Y5, R3, IR2). By attaching a novel 88-channel spectral detection module (U20, V20, B16, Y12, R10, IR3) along with 6 side scatter detectors (USSC, VSSC, BSSC, YSSC, RSSC, and IRSSC) and 1 forward scatter detector to the conventional cytometer, we were able to expand the detection capabilities to at least 40 color parameters .

Methods: Whole blood was obtained from normal donors. Red blood cells (RBC) were lysed using VersaLyse and the remaining white blood cells (WBC) were washed with PBS. ViaKrome 808 was used for live/dead discrimination to take advantage of the CytoFLEX 808nm laser. The white blood cells were then stained based on a 40-color panel, washed and fixed with IO Test 3 solution. Target value settings for the 88 channels were established using CytoFLEX Ready to Use Daily QC Fluorospheres and CytoFLEX Daily IR QC Fluorospheres. Data was collected using a prototype software. Single color-stained controls were made using WBCs and VersaComp beads. Multicolor data were unmixed using a modified Poisson algorithm.

Conclusion: At least 40 unique spectral signatures can be detected with the prototype detection module. Using the modified Poisson algorithm for unmixing resulted in reduced spreading compared to least squares method (LSM). The software allows for multiple unstained cell samples for unmixing of autofluorescence. Similar to previously published data, we were able to identify CD4+ T cells, CD8+ T cells, Regulatory T cells, gamma delta T cells, NKT-Like cells, Innate Lymphocyte Cells, B cells, Basophils, Dendritic Cells, and Monocytes.

Vera Bockhorn

German Rheumatology Research Center, a Leibniz Institute, Berlin, Germany

SplitSOM automates debarcoding of mass cytometry data

Sample barcoding offers the advantage of measuring multiple samples from different sources simultaneously, thereby effectively harmonizing assay preparation and sample acquisition conditions. Debarcoding, i.e. the extraction of single sample data from barcoded data convolutes is a crucial step in typical mass cytometry data curation workflows. Traditionally, debarcoding is performed by manual gating, although algorithmic solutions have also been developed for this task.

Here, we introduce SplitSOM, a novel computational debarcoding method utilizing FlowSOM, a widely accepted clustering method for cytometry data.

In contrast to existing debarcoding solutions, SplitSOM considers the entire information of cellular neighborhood based on overall barcode stainings to group cells with similar barcode signatures, rather than assessing and assigning each cell individually. Additionally, SplitSOM can automatically resolve different barcode configurations, including examples using varying numbers of barcode markers.

We evaluated the recovery and assignment accuracy of SplitSOM. In a 20-sample data set with a 6-choose-3 B2M-based barcode configuration, the events-to-sample assignment rate was 92%, closely matching the results of manual gating. We further investigated the rate of cell misclassification after successful SplitSOM debarcoding by analyzing a secondary CD45 barcode not used for debarcoding. We assessed misclassification for three samples. Less than 0.3% of cells were misclassified, indicating minimal cross-contamination between the debarcoded sample data.

In summary, our newly developed SplitSOM debarcoder permits quick, flexible, accurate and efficient debarcoding of mass cytometry data, facilitating swift and automated data curation in high throughput facilities.

Bernadette Bramreiter

Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria

Characterization of amniotic fluid derived stem cells: The origin of maternal microchimerism?

Microchimerism (MC) is defined as the presence of a small population of genetically distinct cells from another individual in a host. Throughout pregnancy maternal and fetal cells are known to traffic across the feto-maternal interface and result in maternal MC and fetal MC, respectively. Since microchimeric cells represent cell types derived from all three germ layers, we assume cells with stem cell-like properties to be responsible for the establishment of lifelong microchimerism. However, the type of cell trafficking and the routes remain unclear. We hypothesize, among others, maternal-fetal cell-trafficking via ingestion of amniotic fluid (AF) and transmigration of maternal cells into fetal tissue, i.e. via the gastro-intestinal tract. We propose these trafficking maternal cells to represent a subpopulation of so-called amniotic fluid stem cells (AFSCs). We present current data from patient AF samples (n=20; gestational age: 16-35; volume: 1-4 l) after CD117+ cell isolation followed by expansion and characterization of AFSCs.

Danja Brandt

German Rheumatology Research Center, a Leibniz Institute, Berlin, Germany

Freie Universität Berlin, Fachbereich Veterinärmedizin, Berlin, Germany

Radon Transform based method to analyse in vivo images of mice femurs

Image analysis methods are fundamental for understanding interactions within cells and tissues. Depending on the imaging techniques and the type of structures analysed, various tools and software are available. Despite being well-established, image analysis methods are continually evolving due to the introduction of new imaging techniques and technological advancements. One such advancement is the intravital limbostomy technique introduced by Stefanowski and colleagues [1], which allows the *in vivo* observation of the progressive healing process in the femur of a mouse following bone injury. This technique provides a better understanding of the healing process, which involves multiple factors. In particular, we focus on the interaction between blood vessels, which supply the bone with essential nutrients [2], and collagen, whose organization and presence during the early stages of ossification are crucial for bone properties [3]. However, *in vivo* imaging has the disadvantage of a low signal-to-noise ratio compared to ex vivo images, making automated structural analysis challenging.
Here, we present an image analysis method based on Radon transform (RT) to quantify the orientation and position of newly sprouted vessels and new collagen fiber bundles. RT, typically used for tomographic reconstruction, can also be applied in image processing to recognize edges. It is an integral transformation of a function into two variables. The line integral of image intensity along all straight lines of the x-y plane is determined and analysed to identify the orientation and position of elongated structures. Compared to the Fast Fourier Transform (FFT), RT is less sensitive to noise and retains spatial information lost in the frequency domain, allowing a coordinate to be stored for each detected structure. Additionally, RT can identify multiple orientations in an input image, whereas differently oriented structures in the input image result in an average orientation in the Fourier frequency domain.

Vinod Devaraj

German Rheumatology Research Center, a Leibniz Institute, Berlin, Germany – Flow Cytometry Core Facility

Enhanced Classification Using Hierarchical LSTM Networks on Multi-Angle Scattered Light Pulse Shapes in Flow Cytometry

Flow cytometer is a powerful tool applied in various scientific fields for performing single-cell or particle analysis and sorting. In general it has relied on fluorescent markers, with emerging imaging techniques for sorting purposes. As an alternative, we have developed a flow cytometer setup that utilizes scattered light pulse shapes from multiple scattering angles extending beyond the commonly used forward and side scatter measurements. This technique enables the identification of particles based on the light scattering properties, reflecting their morphology and optical characteristics.
In this work, we propose a hierarchical Long Short-Term Memory (LSTM) network for analysing data from our custom flow cytometer. The network has a dual branch architecture, where one branch processes the raw pulse shapes, and the other processes their first-order derivatives or alternative features, thus capturing and interpreting the sequential nature of these signals. Each branch has bidirectional LSTM layers for learning long-range dependencies in both directions and uses dropout techniques to prevent overfitting. The combination of the outputs from these two branches by our network enables comprehensive temporal dynamics analysis of pulse shapes for label-free classification. This method identifies fine differences in morphology and optical properties that may not be detected with the raw pulse shape.
As an application of high relevance, we used this method to identify Plasmodium falciparum sporozoites, the infective stage of the human malaria parasite, in the complex mixture of mosquito cell debris. These sporozoites reside in the mosquito salivary glands and are transmitted to humans together with saliva during mosquito bites. To study potentially infective sporozoites in the lab, they are

dissected from the mosquito thorax. Obtaining large quantities involves dissection of the salivary gland area, leading to contamination with other mosquito materials. Consequently, fluorescent labelling has been required for accurate identification during flow cytometry. As a novel approach, our model demonstrates the potential to create a robust and accurate classification model without the need for fluorescent markers. By combining deep learning with a custom flow cytometry setup, our research provides advanced data analysis capabilities and opens new possibilities in the analysis of particles across various scientific fields. Moreover, future implementation of AI-based sorting on pulse shapes will enable label-free isolation of relevant subsets.

Leonard Fiebig

German Rheumatology Research Center, a Leibniz Institute, Berlin, Germany

Exploring Antigen-Specific Plasma Cell Phenotypes in the Human Bone Marrow

Long-lived plasma cells (LLPCs) in the bone marrow (BM) maintain specific serum antibody titers with varying long-term half-lives. The underlying mechanism for this differential regulation remains unknown, and ongoing research aims to elucidate the dynamics of the BMPC pool during immune responses. According to current a model, LLPCs are characterized by the CD19-negative phenotype, while a dynamic subset represented by CD19+ plasma cells may allow for adaptation of the BMPC population during immune responses.
Using SARS-CoV-2 immunization as a model, we demonstrate that approximately 20% of SARS-CoV-2-specific BMPCs exhibit the phenotype of long-lived plasma cells, on average 8 months post-vaccination or infection, while the majority display the CD19+ phenotype. To compare this data to BMPC formed earlier than SARS-CoV-2-specific BMPC, we developed a 21-marker spectral flow cytometry assay capable of multiplexed detection of five antigen specificities, including SARS-CoV-2, tetanus, EBNA-1 (EBV), and monkeypox. We analyzed BM samples from 31 donors and observed antigen-specific BMPCs at frequencies of approximately one cell per 1,000-10,000 BMPCs, predominantly expressing IgG. Total and antigen-specific BMPCs exhibited considerable phenotypic diversity, characterized by differential expression of CD19, CD56, CD45, CCR2, CD44, and CD9, i.e. markers of PC differentiation, cell adhesion and migration, with distinct phenotypical setups observed across specificities.
Our data indicate that LLPC phenotypes can be induced in humans already upon basic immunization with an mRNA vaccine, suggesting that antigen-specific humoral immunity relies on a spectrum of PC phenotypes distinguished by varying expression of PC adhesion and differentiation markers. This aligns with the notion that PC populate distinct molecular niches in the BM, where they may differentially resist cell death or mobilization i.e. mechanisms proposed to regulate the BMPC pool during adaptation in ongoing immune responses.

Christoph Freier

Metafora Biosystems, Paris, France

Subject: Rare cell detection: AI-Driven Detection of Rare Cells in Flow Cytometry Data for Clinical Applications

Identifying and characterizing rare cells from heterogeneous mixtures is crucial for disease diagnosis, understanding tissue regenerative capacity, and elucidating key biological phenomena. Flow cytometry facilitates rapid acquisition of large cell quantities by analyzing the proteomic profile of heterogeneous mixtures. However, detecting rare events is hampered by complex panel designs, instrumentation, data volume, and manual analysis.
To overcome these challenges, various computer-driven analysis methods have been proposed. Despite numerous algorithms aimed at reducing the analysis bottleneck while reliably detecting rare cells, no single solution has been widely adopted for routine tasks.

Key obstacles include the need to input the expected number of populations or to fine-tune algorithms for specific data sets due to panel complexity and non-standardization.
The recent success of artificial intelligence (AI) models in distilling complex information into actionable insights, suggests that AI could similarly transform flow cytometry data analysis. AI has already proven effective in processing diverse data types at industrial scales. However, flow cytometry data's inherent noise and variability in marker expressions present significant challenges.
Here, we describe the application of a proprietary AI model to analyze a large clinical data set for detecting minimal residual disease (MRD) in B cell acute lymphoblastic leukemia patients. Despite variable marker expression and sample heterogeneity, the AI detected blast populations with frequencies ranging from 0.03% to 34%, demonstrating robust learning and application capabilities. In MRD-negative samples, the AI accurately reported the absence of MRD, with high concordance to published results. Notably, defining the blast population in a single representative file sufficed for model application to the entire data set. Our AI model presents a promising approach for rapid, robust rare cell detection in diverse data sets with minimal fine-tuning.

Ruyu Gao

Department of Environmental Microbiology, Helmholtz Centre for Environmental Research - UFZ, 04318 Leipzig, Germany.

How to Use Ecological Tools to Synchronize Microbiomes Based on Single Cell Analyses

Ubiquitous in ecosystems and human life, natural microbial communities play significant roles and exhibit high complexity in terms of microbial interactions and environmental dependencies. However, complex microbiomes are prone to varying and stochastic fluctuations in composition and function. Few works have been done on assembling and controlling stable complex natural microbial communities with the exception of a looped mass transfer design that shown the ability to stabilize microbiomes over long periods of time (Li et al., 2022).
In this study we want to explore to which degree the rescue effect can be re-established. The looped mass transfer design setup was replicated using the same original environmental sample. Five local microbial communities were continuously grown in parallel, connected by a regional pool with a constant mass transfer rate. The dynamics of these communities were monitored using quantitative high-throughput flow cytometry. Complex microbiome structure variations were evaluated through automatic gating, with the resulting data analyzed using bioinformatic pipelines based on microbial ecology theory.
Results showed that we confirmed the repeatability and feasibility of the looped mass transfer design setup. We observed a repeatedly similar trend attributed to the rescue effect. To explore the ability to synchronize the assembly of microbial communities, the rescue effect was interrupted two times by experimental design. Each time the rescue effect was re-established within the same round but rebuilt to a different structure among rounds. Ecological analysis revealed that both stochastic and deterministic processes play significant roles in our microbial community assembly.
This study is connected to our further aim to gain deeper insights into stabilization and controllability of microbial communities based on this looped mass transfer design and ecological theory, such as constituting artificial microbial communities by bottom-up approaches.

Neus Godino

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany

Centrifugation-free elimination of bacteria or debris from cell samples

One of the main priorities when working with cell samples is to minimize the risk of contamination. Unfortunately, there are situations where the sample is already contaminated or accidentally becomes contaminated during sample preparation. Thus, the elimination of bacteria and debris from contaminated cell samples is a critical sample preparation step in many biological and medical applications, as contaminants can interfere with experimental results and compromise the integrity of the samples. Obtaining the necessary degree of decontamination required for medical or cell culture applications is still nowadays a technical challenge1. Moreover, traditional methods, such as centrifugation-based cell washing, can damage delicate cell structures and often result in the loss of target cells. For those purposes, we present a novel centrifugation-free technique for the efficient removal of bacterial contaminants and debris through dielectrophoresis (DEP), offering a high degree of decontamination level and a gentle processing of the cells.

In the present approach, the target cells are separated from the contaminants in a microfluidic chip based on a the different dielectrophoretic behaviors associated to the different sizes. The cells are considerable larger than most of the contaminants (bacteria and debris like cell proteins or parts of the cells). The balance between hydrodynamic forces with DEP forces in the microchannels allows the precise handling of the target cells modifying their trajectories and their subsequent separation from the initial contaminated sample2. On the other hand, the trajectories of the contaminants are exclusively controlled by the movement of the fluid. Having two co-flow inlets in the microfluidic chip is possible to separate the cells extracting them from the contaminated flow and collect a completely decontaminated sample at the outlet of the chip.
Another important feature is to ensure sterile conditions after use. In this work, we present as well sterilization protocols for the chip.

Collin Hucklesby

Hellma GmbH & Co. KG. Müllheim, Germany

Innovation in Flow Channel Cuvettes

Hellma is a provider and manufacturer of solutions and products that are used in modern process and laboratory spectroscopy. The product portfolio contains ranges from micro to macro cuvettes and high-end quartz glass flow channel cuvettes (flow cells) & HPLC cuvettes as well as certified reference materials and optical probes or ready for use PAT systems. With the manufacturing site based in the very heart of Europe, Hellma builds on over a century of experience in glass manufacturing, delivering highest quality products, made in Germany.
In this lecture you will be introduced to Hellma´s manufacturing method for quartz glass flow channel cuvettes in conjunction with innovative glass processing techniques, which are unique and yet unknown to most of the stakeholders in this field. These enable us, for example, to design the cuvettes of your cytometry cell sorters in a way, that the viability of the cells or organisms remains at an above-average level, even at very high flow rates.
In addition to cell viability, you can also increase the stability of cell alignment using hydrodynamic focusing, through another revolutionary connection manufacturing technology. The Hellma connections of your flow channel cuvettes to the sheath and sample tubes are not made from plastic, but also from quartz glass, which can be bonded directly to the flow channel cuvette using a sophisticated thermal process without any additional connecting media.

Martin Hussels

Physikalisch-Technische Bundesanstalt (PTB), Berlin, Germany

Measuring Real-Time Antibody Binding Kinetics for Quantification of Antibody-Binding Capacity

Quantification of antibody binding capacity (ABC) allows the determination of antigen expression, which can be important in the diagnosis and monitoring of disease. Traditionally, the number of expressed antigens on the cell surface is estimated by measuring the fluorescence intensity of labeled antibodies bound to cells under equilibrium conditions. The amount of bound antibodies is then determined using calibration beads such as Sphero™ Rainbow Calibration Particles or Quantum™ Simply Cellular® Beads. However, these quantification methods can be confounded by several factors, including differences in fluorescence emission spectra and buffer/matrix effects that affect fluorescence yield. An alternative quantification method is based on antibody binding kinetics first demonstrated by Moskalensky et al. 2015 (DOI: 10.1016/j.jim.2015.11.002) and Khalo et al. 2018 (DOI: 10.1002/cyto.a.23494).
The kinetics-based method is independent of absolute fluorescence emission intensity and may be more robust than the calibration-based approaches. However, the protocols proposed in these publications involve several different preparations and measurements for 3 different antibody concentrations and 6 different time points for each antibody concentration. We have developed a method that measures antibody binding kinetics in real time, requiring only one measurement for each of the antibody concentrations. We present CD4 expression data of T lymphocytes for CD-Chex Plus® control samples in order to demonstrate the efficacy of our real-time kinetic measurement method and data analysis. Furthermore, we compare the determined ABC with the ABC determined by calibration with Quantum™ Simply Cellular® beads at equilibrium conditions.

Edit Kotogány

Laboratory of Functional Genomics, Core Facility, HUN-REN Biological Research Centre, Szeged, Hungary

Multiplex immunophenotyping of patients with antiphospholipid syndrome using the Cytek Aurora spectral flow cytometer

The antiphospholipid syndrome (APS) is an acquired autoimmune disorder characterized by thrombotic events, obstetric morbidity and a myriad of systemic manifestations induced by the persistent presence of autoantibodies directed at phospholipids or phospholipid- binding proteins (aPLs).
The following patient's groups of APS were enrolled a) Primary APS, b) Secondary APS (related to other autoimmune disorder, frequently SLE), c) Immunoserological positivity for aPLs without clinical manifestation. Controls were age and gender matched healthy controls. PBMCs were purified by density gradient centrifugation using Leucosep tubes.
For the understanding of single cell heterogeneity in APS flow cytometric immunophenotyping of peripheral blood was performed using the Cytek Aurora full spectrum profiling system. The optimized multicolor immunofluorescence panel consisted of 25 markers, such as: CD45RA, CD20, CD141, CD8, CD14, HLA-DR, CD25, CD4, CD16, IgD, TCRγδ, CD11c, CD127, CD1c, CD19, CD123, CD45, CD27, CD197, IgM, CD3, CD28, CD38, CD56, CD279.
Unsupervised clustering algorithms such as UMAP (Uniform Manifold Approximation and Projection for Dimension Reduction) and FlowSOM (flow cytometry data that builds self-organizing maps) were used to reveal the subpopulations of human APS related PBMCs.
The applicability of spectral FACS for the monitoring of the APS associated immunophenotype was shown in our study.

David Lallinger

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany

Towards an image-based analysis and separation of neutrophils from whole blood

In life science, the processing and analysis of cells in microfluidic systems is a well-established field. Nevertheless, sensitive or precious cell samples suffer serious drawbacks when sorted by standard high throughput cell sorting techniques associated with high shear stresses, biochemical perturbations or low recovery rates.
Of importance is the case of neutrophiles, which represent a heterogeneous cell population that is inherently unstable ex vivo and is rapidly activated by many common methods of sample preparation and sorting. These factors hamper the progress in understanding this subset of circulating leukocytes1,3. Here we present a microfluidic system for a gentle, image- based processing of neutrophiles of human origin based on dielectrophoresis (DEP) 2,4 from a single human drop of blood as loading sample. The aim of this work is to maximize two key parameters: the neutrophil viability after sorting from a heterogenous leukocyte population in comparison with unprocessed cells, and the final total target cell recovery. To achieve this, we abstain from labeling the neutrophiles with fluorescent labeled antibodies to mitigate neutrophile activation1,3. Instead we use sorting protocols based on negative selection and – in future - also morphological patterns. Furthermore, we optimize sheath flow velocity, exposure time, DEP-electrode controls and voltage and use a mild sample preparation to decrease neutrophil perturbation. In future, this will lead to a simple and robust system that enables analysis and sorting of this cell population comparable to other commercial techniques.

Juan López-Gálvez

Department of Applied Microbiology Ecology, Helmholtz-Centre for Environmental Research, Leipzug, Germany

Development of an Automated Flow Cytometry Method to Combine a Double Staining with DAPI and Alexa-488 to Quantify Bacterial Synthesis.

In the past a novel automated method was developed for the cell concentration quantification and fingerprinting of bacterial populations using flow cytometry (López-Gálvez et al., 2023, Cells) and the OC-300 automation unit (onCyt Microbiology, Switzerland). Based on this, a new method that expands on the capabilities of the previous procedure was developed. The objective of this new procedure was not only to use DAPI as a DNA stain to detect the presence of a particular bacterial strain, but also the Alexa-488 stain that is specifically designed to quantify active DNA synthesis. The Alexa 488 stain is a Click-iTTM based technology that uses EdU (5-Ethinyl-2’-Desoxyuridin) as a thymine analogous that is incorporated into the newly synthesized DNA strain. This EdU residue then is covalently bound to the Alexa-488 fluorophore to be detected by flow cytometry. Thanks to this double staining, DAPI and Alexa-488, not only we can quantify the cell concentration of a bacterial strain, but also its proportion that is actively making new DNA and thus proliferating. This new method was tested on three different bacterial strains: E. coli, S. rhizophila and Bradyrhizobium sp.

Felix Pfisterer

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany.

Continuous Microfluidic flow-through protocol for selective and image-activated electroporation of single cells

The permeabilization of the cell membrane by electroporation is an important tool in biotechnology, basic research and medicine for introducing molecules into cells and extracting cell components.
If certain subpopulations of heterogeneous cell samples are to be selectively porated, pre-sorting or processing at single cell level is required. Current approaches for this are complex or can lead to cell damage and cell loss.
In this work, a flow-through method is presented for selective poration of specific target cells in a heterogeneous cell sample. Cell alignment was precisely controlled with dielectrophoretic forces. The target cells were porated in real time with miniaturized pulse electrodes after microscopic image analysis. An important aspect was the synchronization of image analysis and pulse application, which was realized with a self-developed optical feedback system. With this continuous-flow electroporation system, we show the selective poration of differently colored cells. The poration efficiency increased with the pulse voltage and duration and achieved poration rates of up to >90%, depending on the desired cell vitality.
It is a low-cost and low-complexity method for basic research, e.g. for manipulating and analyzing single cells, as well as for potential therapeutic applications.

Alexander Putz

Physikalisch-Technische Bundesanstalt, Berlin, Germany

Glare Points in Flow Cytometry

In recent years, imaging of single cells or particles, particularly in the sideward direction, has gained popularity in flow cytometry. This technique typically relies on fluorescence staining. However, it is not always feasible to stain cells without causing damage to them.
In contrast to fluorescence imaging, when imaging the elastically side-scattered light of a spherical microparticle, one often observes two or more bright spots rather than an image resembling the outline of the particle. This phenomenon has already been observed in flow cytometry, but its cause remains unclear within the flow cytometry community.
Through a comparison of simulations and experiments, we demonstrate that this effect is indeed due to elastically side-scattered light and not other factors such as entry-exit points, surface roughness, or fluorescence.
Additionally, we discuss glare points for elongated or stretched particles, showing that a break in symmetry leads already to “chaotic” glare points. This is particularly relevant for cells, which are usually asymmetric and significantly more complex than polystyrene spheres. We will explain and discuss whether the insights gained through particles can be transferred to the characterization of cells in the context of imaging flow-cytometry.

Benedek Rónaszéki

Department of Internal Medicine, Hematology Centre, Faculty of Medicine, University of Szeged, Szeged, Hungary

Immunophenotyping of human acute myeloid leukemia patients revealed single cell heterogeneity with special attention on therapy sensitive and therapy resistant subpopulations

Acute myeloid leukemia (AML) is the most common acute leukemia form in adults, and it represents a biologically complex and clinically heterogeneous disease. The estimated 5-year overall survival of AML remained only 30% despite the revolution of targeted therapies.
For the understanding of single cell heterogeneity in therapeutic resistance flow cytometric immunophenotyping of peripheral blood of AML patients (n=14) versus age and gender matched healthy controls (HCs) was performed using Cytoflex S system. The FACS panel consisted of Viobility 405/520 Fixable Dye, Anti-Human CD45, CD19, CD3, CD7, CD33, CD34, CD38, CD64, CD117, CD135, HLA-DR.
Unsupervised clustering algorithms such as UMAP (Uniform Manifold Approximation and Projection for Dimension Reduction) and FlowSOM (flow cytometry data that builds self-organizing maps) were used to reveal the subpopulations of human AML cells. Four metaclusters (MCs) represented the HCs, and eleven MCs were associated with AML samples. Both UMAP and FlowSOM showed the normalization of leukemia associated immunophenotype (LAIP) similar to the HCs immune landscape by the 3rd sampling following therapy. However, the follow-up of AML samples revealed 4 MCs of sensitive cells and 1 MC composed of therapeutic resistant AML cells (CD7- CD33+ CD38+ CD64+ HLA-DR+ CD117+ CD135+).
The applicability of FACS for the monitoring of the LAIP was shown in our study.

Steffen Schmitt

DKFZ, Heidelberg

Using UV-C Light to decontaminate liquid Flow Cytometry Waste A proof of principle study across German core facilites

In almost every flow cytometry laboratory a significant amount of liquid waste containing potential biohazardous materials, including cells, bacteria, and viruses, is produced and pose a significant risk to humans and the environment. The conventional methods of chemical or physical inactivation relay on specialized infrastructure and/ or are complicated, leading to time-consuming processes for core facilities. This study addresses this challenge by presenting a new approach to flow cytometer liquid waste management.
Our study focuses on a multi-site investigation involving several core facilities across Germany. These facilities are using a flow-through device producing UV-C light to inactivate the waste fluid. The reactor is equipped with four UV-C LEDs emitting at a wavelength of 265 nm and was utilized across various types of cell sorters and analyzers to deactivate biological specimens of different origins.
The results demonstrate the efficacy of the UV-C reactor in decontaminating waste fluids, eliminating the need for prolonged and resource-intensive decontamination procedures. By leveraging this technology, core facilities can streamline their waste management processes, reducing the environmental impact associated with biohazardous waste and chemical decontaminants. This proof-of-concept study provides a practical solution for enhancing the efficiency and sustainability of core facility operations.

Axel Ronald Schulz

German Rheumatology Research Center, a Leibniz Institute, Berlin, Germany

Allelic variants of CD16a predict SARS-CoV2 mRNA vaccination response in senior adults

Vaccination effectively protects against severe consequences of infection. However, little is known about immunological determinants of variable or poor vaccination outcomes observed in elderly individuals. Using 50-parameter mass cytometry to investigate a cohort of senior BNT162b2 mRNA vaccine recipients (>80 years, n=55), we identified a multifactorial baseline immune signature predictive of SARS-CoV-2-specific antibody titers two weeks post-second vaccination dose. A pivotal component of this signature was the mean signal intensity (MSI) of the Fc receptor CD16a on nonclassical and transitional monocytes, as well as NK cells, which exhibited a clearly dichotomous expression pattern across individuals. Genotyping revealed that the observed variability in cytometric CD16a staining was linked to an allelic dimorphism of FCGR3A, resulting in either valine (V) or phenylalanine (F) at position 158 of the CD16a molecule. Strikingly, elderly individuals carrying two copies of the "F" variant of CD16a, which is associated with reduced IgG affinity, had an 83% chance of being excluded from the group of high vaccination responders. Conversely, the high-affinity "V" variant of CD16a was a crucial prerequisite of our multifactorial signature for elderly vaccinees to belong to the high responding group.
Our findings establish CD16a as a previously unrecognized regulator of antibody responses in elderly individuals, possibly by tuning the activation capacity of innate immune cells such as antigen-presenting cells. Furthermore, our discovery can help identify elderly individuals (i.e., carriers of two copies of the "F" variant) who are at risk of mounting inadequate immune responses to SARS-CoV-2 mRNA vaccination and who may therefore benefit from adapted vaccination strategies.

Juliane Schulze

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany.

Characterizing the performance of a microfluidic cell cell sorting process in terms of throughput, purity and yield of the sorted cells

The classification and isolation of specific cell types is a crucial process in biomedical research, such as in cancer research1-2. For example, pure isolation of specific cell types from heterogeneous cell populations is often of utmost importance in single-cell genomics3. A novel system for flow- and electrode-controlled cell sorting has been developed at Fraunhofer IZI-BB 4. It includes a flow-through microfluidic chip that enables high-precision live cell sorting based on the stepwise arrangement of dielectrophoresis electrodes. By switching these electrodes on and off individually according to the cell movement, the trajectories of a cell in the channel can be precisely defined. As only depending on the size of the small microelectrodes, the size of the sorting window can be thus reduced to the size of a single cell, supporting high purity and high yield sorting, even when particle densities are high. In this study, we have optimized the timing of the electrode switching with cell movement and have characterized the sorting process of a new chip generation with a sorting window of only 20 µm. By sorting differently stained live T cells at a mean cell spacing of 500 µm, we achieved a yield and purity above 95% at throughputs of up to 3 cells per second and 60 µl/h.

Yaroslava Shevchenko

Department of Hepatology and Gastroenterology, Charité Universitätsmedizin Berlin, Berlin, Germany

A comprehensive assessment of compensation particles for generation of optimized reference controls for full spectrum flow cytometry

Full spectrum flow cytometry enables the analysis of 50 or more parameters in a single sample. Due to the complexity of such large panels the correct and reliable unmixing of fluorochromes requires identical spectra in both reference controls and fully stained samples to prevent unmixing errors. It is considered optimal to use the cells of interest to create single stain controls, but this is often impractical due to low expression of certain markers or limited availability of rare cell types. Compensation beads provide a commercially available and inexpensive alternative, binding antibodies consistently irrespective of specificity and expression level. Here, we evaluated 30 commonly used and commercially available fluorochromes on various compensation beads and human and murine primary leukocytes to establish optimal reference controls for full spectrum flow cytometry. Five types of compensation beads from different vendors were used to generate single stain controls and spectral signatures were recorded on 3-laser and 5-laser instruments. Our findings demonstrate significant differences in the spectral profiles of certain fluorochromes and their influence on spectral unmixing depending on the type of compensation particles used, including some fluorochromes that should be used on cells exclusively. This study underscores the critical importance of selecting appropriate reference controls to prevent unmixing errors and ensure the generation of reliable flow cytometry data.

Eric Sündermann

Institute of Physics, University of Greifswald, Greifswald, Germany
DZHK Partner Site Greifswald, Greifswald, Germany

Membrane tension cytometry - A fast microfluidic technique to assess the membrane mechanics of cells

The development of high-throughput methods for cell mechanical research is becoming increasingly important in biology, medicine and physics as the analysis of large samples opens up possibilities for basic science and clinical use. Various mechano-cytometric techniques are available, but hardly any can discriminate between membrane and bulk contributions to the mechanical properties of a cell.
Here, we use a novel technique combining fluorescence lifetime with flow cytometry of mechanically-stressed cells to study the response of membrane tension to hydrodynamic stress. HL60 cells, a human myeloid precursor cell line, were first stained with Flipper-TR, a fluorescent dye with a lifetime proportional to the membrane tension, and then flushed through the constriction of a microfluidic chip, where they deform under shear stress. We first used an osmotic shock to characterize the changes in membrane tension. Under steady-state conditions, our data shows that the membrane tension of HL60 cells increases with increasing hydrodynamic stress.
Based on these initial results we exposed HL60 cells to methyl-ß-cyclodextrin to reduce the amount of cholesterol in the cell membrane. While we observe alterations in the membrane tension, a comparative study using real-time deformability cytometry (RT-DC) reveals no impact on the bulk mechanics. We investigated different concentrations of dimethyl sulfoxide (DMSO) to probe the cell membrane after exposure to a common cryoprotective agent. The results show, that even small concentrations of DMSO influence the membrane tension. Finally, we interfered with the cytoskeleton and inhibited actin polymerization using Cytochalasin D. Here, we observed a reduced Young's modulus while membrane tension remained unaffected.

Jan Maurice Wilder

Institute of Physics, University of Greifswald, Greifswald, Germany
DZHK Partner Site Greifswald, Greifswald, Germany

Membrane tension cytometry for in situ investigation of mitochondrial membrane tension in response to mechanical and chemical stress

Mechanical properties of cells and their reaction to mechanical forces have been recognized to play a pivotal role in cell function, migration, and differentiation. This principle applies not only to the cell as a whole but also to its individual organelles. Mitochondria as a central organelle in metabolism are often referred to as the powerhouse of the cell due to their important role in ATP production. A fundamental mechanical property of mitochondria is their membrane tension, which has been shown to play a key role in mitochondrial function and dysfunction, e.g., fission and fusion.
Here, we extend membrane tension cytometry (MTC), a novel high-throughput method for analysing the tension of lipid bilayer membranes, onto mitochondria. Using HL60 cells as a model system and the mechanosensitive fluorescent probe Mito Flipper-TR, we were able to adapt MTC to measure mitochondrial membrane tension in situ. Preliminary results indicate a correlation between hydrodynamic stress and mitochondrial membrane tension suggesting a mechanical stress propagation inside the cytosol. Furthermore, by incubating HL60 cells with hydrogen peroxide to generate mitochondrial superoxide, the response of mitochondria to oxidative stress has been investigated, revealing an impact on mitochondrial membrane tension.
In summary, our preliminary data show that membrane tension of individual organelles can be studied inside cells and under pathophysiological conditions. Future research could focus on the question how the structure of the cytoskeleton impacts on cytosolic stress propagation while investigating the relevant timescales or if our results could be generalized for other cellular model systems.

Guest Lecture

3:30 pm, PEH

Chair: Henrik Mei

Lena Kaufmann

Bernstein Center for Computational Neuroscience, Humboldt Universität zu Berlin, Berlin, Germany
Berlin School of Mind and Brain, Humboldt Universität zu Berlin, Berlin, Germany

Elephant neurobiology and behavior: from trunks to brains

My current research is focused on finding out how the elephant brain has adapted to the unique specialization the elephant's trunk represents. Elephants are not only a flagship species and ecosystem engineers and as such of special importance for conservation biology and the preservation of whole ecosystems but for us neuroscientists they are also specifically interesting animals for several reasons. Their big brains with extensive gyrification and big but sparse cells are in some regards on the other extreme commonly studied species such as mice or rats represent one side of. They are highly specialized in morphology and behavior, depending on their trunks, a fusion organ of nose and upper lip, for food and water uptake, body care, communication, and manipulation of their environment. Even though elephants are of great public and scientific interest we still don't know much about their brains and nothing about actual *in vivo* neuronal activity. My research aims to better understand the morphology and physiology of the trunk, the importance of the trunk for social and non-social behaviors, how the brain and peripheral nervous system of the elephant have adapted to this specialization and how the brain responds to stimuli in actual living elephants. Here, my focus lies on African (Loxodonta africana) and Asian (Elephas maximus) elephants and specifically also on how differences in their bodies and behavior go along with differences in

neuroanatomy. These two elephant species diverged 5-7 million years ago and pose a great model to study different adaptations in relatively similar species.

DGfZ Members Assembly

4:30 pm – 6:00 pm, PEH

Meet the Speaker event

6:00 pm – 10:00 pm, DRFZ

All participants are invited.

Friday, 13.09.2024

Imaging & Cytometry Session

10:00 am - 12 pm, PEH

Chairs: Anja Hauser, Raluca Niesner & Oliver Otto

For a comprehensive understanding of molecular mechanisms of cellular functions and dysfunction, in health and disease, information on the effects of environmental and microenvironmental cues in the tissue is needed. With the emergence of spatial multi-omics technologies and analysis, we recently experienced a leap towards achieving this aim. In this session, Fabian Coscia (Max Delbrück Center for Molecular Medicine, Berlin) will give us an overview about image-guided tissue proteomics, involving deep-learning-based analysis, and its value for deciphering disease mechanisms. Esther Wehrle (AO Research Institute Davos) will focus in her talk on spatial transcriptomics in bone healing, addressing next to molecular, also mechanical cues in tissue environment impacting on cellular functions. But can we understand this emergence of complexity also from bottom-up approaches? The biophysicist Kerstin Göpfrich will introduce us to the world of synthetic cells. She tackles the problem of complexity by designing lipid vesicles that include molecular hardware made from RNA origami to mimick cell function. The session will be rounded up by short talks addressing metabolic cues in bone regeneration by Alexander Fiedler (German Rheumatology Research Center, Berlin) and the introduction of a high-throughput image-based microfluidic cell sorting system by Michael Kirchbaum (Fraunhofer Institute Potsdam).

Fabian Coscia

Max Delbrück Center for Molecular Medicine

Spatial tissue proteomics to assess health and disease

Formalin-fixed and paraffin-embedded (FFPE) tissues represent an invaluable resource for studying molecular mechanisms underlying diseases. The long archival times of FFPE tissue and the fact that proteins are largely

stable therein make them ideal analytes for global mass spectrometry-based proteomics approaches for biomarker and drug target discovery. Classical approaches have provided “averaged” descriptions of the disease related proteome and fail to characterize the critical disease promoting cell populations within the complex tissue environment. To address this, we have recently co-developed Deep Visual Proteomics (DVP) for image-guided tissue proteomics. DVP leverages high resolution microscopy and machine learning based image analysis to identify phenotypically distinct cell populations, while preserving the spatial context. Cells of interest are isolated in situ by automated laser microdissection and analyzed by ultrasensitive mass spectrometry. In my presentation, I will provide an overview of our spatial tissue proteomics pipelines, present recent developments to profile few and even single cells and give an outlook on spatial proteo-transcriptomics workflows for the profiling of solid tumors at unprecedented resolution.

Kerstin Göpfrich

Center for Molecular Biology of Heidelberg University

Engineering and sorting of synthetic cells

Today’s living cells emerge from the complex interplay of thousands of molecular constituents. Our vision is to create a simpler model of a cell that consists of a lipid vesicle and operates based on our own custom-engineered molecular hardware made from highly functional and folded RNA realized using the co-transcriptional folding of RNA origami. Previously, we demonstrated DNA-based mimics of cytoskeletons [1] and DNA microbeads characterized by real-time deformability cytometry and capable of targeted morphogen-release in organoids [2]. We now demonstrated that similar functions can be genetically encoded with RNA origami and expressed inside of vesicles. We developed a high-throughput image-based sorting technology based on photopolymerization, to select for highly functional variants of the initially rationally engineered synthetic cells. Beyond synthetic cells, we apply our sorting technology for functional selection of immune cells [3], exemplifying how initially blue skies research on engineering life can provide technological solutions to other fields.

Esther Wehrle

AO Research Institute Davos, Switzerland

Spatial transcriptomics of bone healing

Bone healing is a spatially and mechanically controlled process involving crosstalk of multiple tissues. Despite the major advances in osteosynthesis after trauma, impaired healing and non-union bone fractures remain a challenging clinical problem. While known risk factors exist, e.g. advanced age or diabetes, the exact molecular mechanism underlying the impaired healing is largely unknown and identifying which specific patient will develop healing complications is still not possible in clinical practice. We combine novel omics technologies with mechanically-controlled and individualized preclinical animal models to capture, understand and target underlying biological changes during fracture healing. To facilitate molecular analyses, we have established a protocol for preparing formalin-fixed paraffin-embedded (FFPE) musculoskeletal tissue samples from mice for spatial transcriptomics. To capture the mechanical conditions in the bone defect region, we have developed an *in vivo* stiffness

measurement approach allowing for monitoring of healing progression in individual animals. In my presentation, I will provide an overview of our multimodal preclinical set-up including mechanically-controlled fracture healing models, individualized *in vivo* monitoring approaches with integration of spatial and systemic omics. Subsequently, I will present recent spatial transcriptomics data showing distinct spatiotemporal gene expression patterns in non-union and union fractures with translational relevance for individualized treatments to improve impaired bone healing.

Short talk: Alexander Fiedler

Biophysical Analytics, German Rheumatology Research Center (DRFZ), Berlin, Germany

In vivo fluorescence lifetime micro-endoscopy creates new perspectives on immunometabolic heterogeneity in the bone

Immune cell (dys)function is linked to their metabolism. However, the mechanisms remain elusive, mainly due to the lack of technologies to visualize local metabolism *in vivo* over longer timescales.
Our new method combines metabolic fluorescence lifetime imaging (FLIM) and longitudinal micro-endoscopy of the bone marrow (LIMB). This allows dynamic imaging of the bone marrow tissue and the finely orchestrated self-organization of (immune) cells (e.g. re-vascularization after injury). Additionally, the NAD(P)H-fluorescence lifetime provides simultaneous info about the local cellular (immuno)metabolism. This can be done in one and the same animal e.g. in a bone injury model to observe the migration of LysM+ immune cells during inflammation and regeneration inside the fracture gap (till 28 days post osteotomy). The UMAP-clustering of segmented cells, based on the FLIM-derived metabolic features, showed high heterogeneity of myeliod cells *in vivo* (but not *in vitro*). Our method allows to study distinct niche environments and gain deeper insights into complex biological systems, that can only be investigated narrowly with *in vitro* culture systems that are limited to controlled environments.

Short talk: Michael Kirschbaum

Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses, Potsdam, Germany

On-chip integrated re-sorting enhances sort performance in microfluidic flow cell sorting in one single step

In flow cell sorting, cells are analyzed and classified before target cells are isolated from the bulk using different types of actuators. Depending on the desired throughput, technical limitations, like the simultaneous passage of two cells through the actuator, can lead to decreased purity or enforce the discarding of both cells and thus reduce the yield.
To enhance purity and yield, an additional sorting step can be added to remove erroneously captured non-target cells, but implementing re-analysis and re-sorting into a single sorting device can be costly, unfavorable or geometrically hardly possible, especially when imaging is used for cell analysis and -classification.
We introduce a novel microfluidic concept for two-step-, image-activated cell sorting using a single image acquisition unit and dielectrophoretic actuators for efficient sorting. The microchannel, designed in a hairpin configuration, redirects the particle stream back into the detection area for re-analysis and re-sorting of the initially sorted sample. Even when using high-resolution optics with a limited field of view, microchannels of several hundred micrometers width can be utilized, allowing throughputs of 100 µl/h or 10-100 cells per second with purity AND yields above 95%. This method improves throughput, purity and yield of microfluidic cell sorting at the same time without the need for a duplicate analysis unit or increased process duration, thereby reducing costs, effort and stress for the cells.

Klaus Goerttler Session

1:00 pm - 2 pm, PEH

Chair: Annika Betzler, Klaus Goerttler Awardee 2023

Germinal centers (GCs) are transient structures that form within B cell follicles of secondary lymphoid organs in response to T cell dependent antigens. Within GCs, B cells undergo intensive proliferation, somatic hypermutation, class-switch recombination and affinity maturation. As a result, high affinity antibody secreting plasma cells and memory B cells are generated. The GC is not the only environment in which B cells can participate in the immune response. B cells can also develop via the extrafollicular pathway into short-lived plasma cells to provide an early source of antibodies during infection. High-affinity antibodies are essential for combating of pathogens, but they can also trigger allergies and autoimmune disease, when directed toward self-antigens. In healthy individuals, self-reactive B cells are counter-selected or regulated. However, in some people, failure of B cell tolerance checkpoints leads to the formation of autoantibodies and the potential development of autoimmune diseases. Understanding the origin of self-reactivity, more specifically of autoreactive B cells, is of therapeutic importance. This year's Klaus Goerttler Session will give insights into methods and tools for the precise characterization of autoreactive B cells in B cell-mediated autoimmune diseases and how to close potential gaps in their treatment.

Marta Rizzi

Dept of Rheumatology and Clinical immunology and CCI – University Medical Center Freiburg and Center for Pathophysiology, Infectiology and Immunology; Institute of Immunology – Medical University of Vienna

Modulation of peripheral B cell maturation and generation of autoantibodies: lesson from rare autoimmune diseases

The presence of autoantibodies indicates a breach of B cell tolerance. Antibody diversity in the primary repertoire and the occurrence of somatic hypermutation in activated mature B cells carry the inherent risk of generating self-reactive specificities. Peripheral selection ensures the maintenance of tolerance. After initial activation, B cells can either develop via the extrafollicular pathway, resulting in the rapid generation of antibody-secreting cells, usually of low affinity but enriched in autoantibodies, or migrate to the germinal center, where they undergo affinity maturation, resulting in the generation of a long-lasting memory response. In several autoantibody-mediated diseases, extrafollicular maturation is associated with the generation of autoantibodies, such as in systemic lupus erythematosus (SLE) or multiple sclerosis. The persistence of autoantibodies after treatment with B cell depleting agents, on the other hand, may indicate a germinal center origin of autoreactive B cells. The study of the origin of tolerance breaches within the B cell compartment not only helps to understand the pathogenesis of the disease, but also to anticipate the benefit of targeted therapies in modulating disease-specific B cell function.

Christian Busse

DKFZ Heidelberg

Farewell

2 pm - 2:15 pm, PEH

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