Abstract Booklet

33rd Annual Conference of the German Society for Cytometry

Pre-Program
September 18, 2023: Meeting Point Cytometry and Tutorials

Conference
September 19-21, 2023

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

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dgfz2023.de

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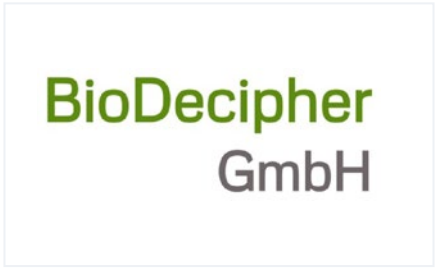
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Dear friends of cytometry,

on behalf of the German Society for Cytometry I have the pleasure to announce the
33rd Annual Meeting of the German Society for Cytometry in Berlin on September 19-21, 2023 to get together & discuss cytometry and the biology of cells in all its wonderful dimensions.

We have designed an exciting scientific programme for you, representing cytometric imaging, cutting-edge technologies, mass cytometry, nanotechnology, microbiology, advanced data analysis techniques, and mechanocytometry. We warmly welcome the Danish Society for Cytometry as hosts of this years´ Guest Session.

Get in touch during the poster session and industry exhibition while coffee breaks, the welcome reception and the networking event will provide plenty opportunity to expand your cytometry network.

Last but not least tutorials and our famous Product Slam featuring cutting-edge news on instruments, assays and services from the wide range of industry partners will round up the meeting.

What`s new this year?

For the very first time we will offer a Welcome session termed „Meeting Point Cytometry – First Time Attendee and New Member Welcome Orientation“. We are warmly welcoming scientists of all levels and core facility managers that are new to the community or a first time participant at our annual meeting.

In line with this years´ focus on technology transfer in cytometry, we introduce the new Entrepreneur networking event, providing a forum for exchange between everyone interested in securing IP and building a business around their innovation in cytometry, or discoveries made with the help of cytometry. We have invited role models for successful technology transfer to discuss and share their ways of making it happen.

The many facets of cytometry, its applications and the variety of career tracks it offers inspired to headline this edition of our annual meeting with „DIMENSIONS“, to represent the science of cytometry, the science enabled by cytometry, and to celebrate our vivid community carried by so many unique personalities.

I am looking forward to welcoming you!

Henrik Mei
President of the DGfZ

18.09.2023

Monday

Meeting Point Cytometry

Starting 1:30 pm with Soup - 3:00 pm, DRFZ Foyer

First Time Attendee and New Member Welcome Orientation session

Tutorial 1
Cycle Analysis by Flow Cytometry

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

Chairs: Toralf Kaiser, Claudia Giesecke-Thiel

Tutorial 3
Mass Cytometry Getting Started

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

Chair/Moderator: Sarah Warth

Speakers: Axel Schulz, Anika Rettig, Desiree Kunkel

19.09.2023

Tuesday

Tutorial 4
Mechanocytometry for label-free sorting

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

Chair: Oliver Otto

Tutorial 5
Data annotation, quality and sharing under FAIR principles

9:00 am - 11:00 am, PEH

Chairs: Christian Busse, Sebastian Ferrara & Amro Abbas

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

19.09.2023

Tuesday

8:30 - 11:45 CCO	*Registration & Soup*

Welcome

11:45 am - 12.00 pm, PEH — **Henrik Mei**

Imaging

12:00 pm - 1:30 pm, PEH — Chairs: Anja Hauser, Raluca Niesner

Marco Fritzsche — **Mechanobiology of T-cell activation in numbers**
Oxford, United Kingdom

Nicole Strittmatter — **Spatially resolved metabolomics using mass spectrometry imaging of tissues**
Munich, Germany

Laurenz Krimmel (Short Talk) — **The spatial and unique composition of infiltrating immune cells defines autoimmune- and checkpoint-therapy associated hepatitis**
Freiburg, Germany

Katja Sallinger (Short Talk) — **Imaging-based transcriptomics technology identifying predictive biomarkers for relapse in colon cancer stage II**
Graz, Austria

1:30 - 2:00 CCO	*Coffee Break/Industry Exhibition*

Product Slam

2:00 pm – 3:00 pm, PEH — Chairs: Elmar Endl, Tom Bauer

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

AHF, APE, Beckman Coulter, BD, BioDecipher, BIO RAD, BioLegend, Blue Cat Bio, Canopy, Curiox, Cytek, Cytolytics, Berlin Partner, Mileny Biotec, OLS, Particle Metrix, Sony, Standard Biotools, ThermoFischer, Zelmechanik Dresden

3:00 - 3:30 CCO	*Coffee Break/Industry Exhibition*

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Cutting Edge

3:30 pm -5:00 pm, PEH — Chairs: Bertram Bengsch & Henrik Mei

Ermelinda Porpiglia — **Through the lens of CyTOF: resolving signatures of muscle stem cell aging one cell at the time**
Aarhus, Denmark and Stanford, USA

André Rendeiro — **Spatially and temporally resolved pathology of COVID-19 in the lung**
Vienna, Austria

Alexandra E. Schlaak (Short Talk) — **Development of a competitive tetramer assay that allows mass-cytometry profiling of epitope-specific CD8+ T Cells after bead enrichment**
Freiburg, Germany

Axel Ronald Schulz (Short Talk) — **Vaccinology meets mass cytometry – Identifying baseline predictors of vaccination outcome**
Berlin, Germany

Toni Sempert (Short Talk) — **Single-cell microbiota phenotypes for classification of chronic-inflammatory diseases and functional characterization to decipher their role in pathophysiology**
Berlin, Germany

5:00 pm - 5:30 pm — ***Coffee Break/Industry Exhibition***

Keynote

5:30 pm - 6:30 pm, PEH — Chair: Henrik Mei, Betram Bengsch

Sean Bendall — **Bottom-Up Organization of Single Cell Human Systems**
Stanford, USA

Welcome reception

6:30 pm - 10:00 pm, CCO — Exhibition area

Core Facility Networking Event

8:00 pm - 10:00 pm, DRFZ — Chairs: Désireé Kunkel, Sarah Warth, Christian Kukat

Gert van Isterdael — **A new view on cytometry with the BD CellView™ Image technology**
Ghent, Belgium

Entrepreneur Networking Event

8:00 pm - 10:00 pm, CCO — Chairs: Lisa Budzinski, Henrik Mei

Kathrin Brenker, opto biolabs GmbH, Aida Meghraoui, AMKbiotech, Tyler Burns, Burns Life Sciences Consulting GmbH

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20.09. 2023

Wednesday

Rare cells Session

9:00 am - 10:30 am, PEH — Chairs: Thomas Kroneis & Frank Schildberg

Michael Eikmans — **Rare cells in pregnancy: the role of regulatory T cells and microchimeric cells in placental and fetal development**
Leiden, The Netherlands.

Udo Markert — **Transplacental migration of maternal natural killer and T cells assessed by ex vivo human placenta perfusion – evidence for microchimerism?**
Jena, Germany

Leonard Fiebig (Short Talk) — **Characterization of Antigen-Specific B Cells and Plasma Cells Using a Tetramer-based Detection Method by Flow and Mass Cytometry**
Berlin, Germany

Emiel Slaats (Short Talk) — **Image-based SNP detection for discriminating haploidentical microchimeric cells**
Graz, Austria

10:30 am - 11:00 am — *Coffee Break/Industry Exhibition*

European Guest Session: Austrian Society

11:00 am - 12:00 pm, PEH — Chair: Anja Bille Bohn (Ahrhus, Denmark)

Anja Bille Bohn — **Introduction**

Jesper Geert Pedersen — **Investigating host immune function in metastatic melanoma patients treated with immunotherapy**
Aarhus, Denmark

Carina Agerbo Rosenberg — **Rethinking diagnostics in myelodysplastic syndrome and unexplained cytopenia: employing morphometric evaluation of dysplasia by imaging flow cytometry**
Aarhus, Denmark

12:00 pm - 1:00 pm — *Lunch (Postersession starts at: 12.30 pm)*

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Poster Session

12:30 pm - 2:30 pm, CCO — Chairs: Henrik Mei, Oliver Otto

2:30 pm - 3:00 pm — *Coffee Break/Industry Exhibition*

Klaus-Goerttler-Session incl. award ceremony

3:00pm - 4:30pm, PEH — Chair: Elisa Rosati, Klaus Goerttler Awardee 2022

Giulia Pasqual
Enzymatic labeling of cell cell interactions in vivo
Padova, Italy

Bram Slütter
Single cell approaches to dissect antigen specific T-cell interactions in atherosclerosis
Leiden, The Netherlands

Annika Betzler
(Klaus Goerttler Awardee)
Molecular characterization of the transcriptional co-activator BOB.1/OBF.1 in B versus T lymphocytes and its specific contribution to the Germinal Center Reaction
Ulm, Germany

4:30 pm - 5:00pm — *Coffee Break/Industry Exhibition*

Guest Lecture

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

Eicke Latz
Inflammasome function in health and disease
Berlin, Germany

DGfZ-Members Assembly

6:00 pm - 7:00 pm, PEH — Chair: Henrik Mei

Meet the Speaker & Poster Prize Ceremony & Dinner

7:00 pm - 11:00 pm, DRFZ — for all speakers, participants and industry partners

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21.09. 2023

Thursday

Mechanocytometry

9:00 am - 10:30 am — Chairs: Marta Urbanska and Oliver Otto

Franziska Lautenschläger — **Cytoskeletal fibres as building blocks for life**
Saarbrücken, Germany

Salvatore Girardo — **Harnessing Microfluidic Technologies for Advancing Mechanobiology**
Erlangen, Germany

Marta Urbanska (Short Talk) — **De novo identification of universal cell mechanics gene signatures**
Cambridge, UK

Stefan Simm (Short Talk) — **Explainable artificial intelligence image analysis for blood cell discrimination**
Greifswald, Germany

10:30 am - 11:00 am — *Coffee Break/Industry Exhibition*

Microbiology

11:00 am - 12:30 pm — Chairs: Lisa Budzinski & Christin Koch

Fatima C. Pereira — **Probing microbiome function using single-cell chemical imaging**
Southampton, United Kingdom

Sabina Leanti La Rosa — **Hunting systems for typical and atypical glycan processing in gut microbiomes, one cell at a time**
Ås, Norway

Doreen Reichert (Short Talk) — **Impact of long term 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment on gut microbiome composition and glycosylation patterns in male and female C57BL/6 mice analyzed by flowcytometry**
Düsseldorf, Germany

Juan Lopez Galvez (Short Talk) — **Development of an Automated Online Flow Cytometry Method to Quantify Cell Density and Fingerprint Bacterial Communities**
Leipzig, Germany

Daniel Kage (Short Talk) — **Label-free flow-cytometric bacteria sorting with angle-resolved scattered light signals**
Berlin, Germany

12:30 pm - 1:30 pm — *Lunch*

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Nanotechnology

1:30 pm - 3:00 pm — Chairs: Ulrike Taylor and Wolfgang Fritzsche

Melanie Schürz
Overview of current methods for the characterization of extracellular vesicles with a focus on single vesicle imaging and quantification
Salzburg, Austria

André Görgens
Comparing Apples to Apples: Analysis of Extracellular Vesicles by Flow Cytometry
Stockholm, Sweden

Martin Hussels
(Short Talk)
Imaging of Scattered Light in a Flow Cytometer to Analyse Size, Shape and Internal Structures of Particles and Cells
Berlin, Germany

Michael Kirschbaum
(Short Talk)
High-resolution image-activated cell sorting at low shear stress
Potsdam, Germany

3:00 pm - 3:15 pm — ***Farewell***

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Program and Abstracts

Tuesday, 19.09.2023

Imaging Session

12:00 pm, PEH

Chairs: Raluca Niesner and Anja Hauser

Within tissues, cells are constantly exposed to a wide variety of stimuli which impact on their phenotype and function. In addition to "classical" receptor-ligand interactions, these stimuli include a whole range of other cues, for example mechanic and metabolic ones. In this session, we will focus on the analysis of these stimuli, whose importance is increasingly recognized. At the same time, the range of ways to analyze these stimuli continues to expand. The invited speakers in this session will focus on a variety of imaging methods, which enable the quantification of those stimuli in cells and tissues.
An expert in mechanobiology, Prof. Marco Fritzsche applies sensitive quantitative super-resolution imaging and probing methodologies to analyze the forces occurring during T cell receptor activation at a single cell level, and to understand these processes at a molecular scale. Prof. Nicole Strittmatter´s interest is to combine various imaging technologies in two- and three dimensions. Thus, she focuses on the generation and integration of spatial metabolomics in multi-modal workflows in order to decipher the processes occurring in tissues.

Mechanobiology of T-cell activation in numbers

Marco Fritzsche
University of Oxford, Oxford, United Kingdom

Mechanobiology is emerging in the biomedical sciences. Recent evidence indicates that immune cells regulate their cell mechanics not only downstream of signalling events triggered by ligand–receptor binding, but that cells dynamically adapt their mechanics in response to external mechanical signals. Quantifying cellular forces has therefore become an contentious challenge across multiple disciplines at the interface of biophysics and immunology. Applying sensitive quantitative super-resolution imaging and force probing methodologies to analyse resting and activated T cells, we demonstrate that the kinetics of the antigen engaging the T-cell receptor controls the nanoscale actin organisation and mechanics of the IS. Using an engineered T-cell system expressing a specific T-cell receptor and stimulated by a range of antigens, force measurements revealed that the peak force experienced by the T- cell receptor during activation was independent of the kinetics of the stimulating antigen. Conversely, quantification of the actin retrograde flow velocity at the IS revealed a striking dependence on the antigen kinetics. Novel ultra-thin superextended lightsheet technology allowed to correlate early calcium activation signalling, IS formation, and effector function. Taken together, these findings suggest that the dynamics of the actin cytoskeleton actively adjusted to normalise the force experienced by the T-cell receptor in antigen specific manner. Consequently, tuning actin dynamics in response to antigen kinetics may thus be a mechanism that allows T cells to adjust the length- and time-scale of T-cell receptor signalling.

Spatially resolved metabolomics using mass spectrometry imaging of tissues

Nicole Strittmatter

School of Natural Sciences – Department of Biosciences, Technische Universität München, Garching/Munich, Germany

Small metabolites are the building blocks of life and their sum constitute a chemical fingerprint of the cellular processes at play. Spatial metabolomics using mass spectrometry imaging is performed ex vivo, usually on fresh frozen tissue sections, and enables the localisation of small molecule metabolites, lipids and xenobiotics in two- and three-dimensional context in situ. In multimodal imaging workflows, this additional layer of information can be a crucial piece to generate a holistic view of the processes occurring in complex biological samples such as tissues, enabling the association of metabolites with certain cell types, tissue subregions or phenotypes. We are primarily deploying Desorption Electrospray Ionisation Mass Spectrometry (DESI-MS), an ambient MS technique that is ideally suited for multimodal imaging studies as it requires no sample preparation and leaves the tissue section intact for subsequent imaging-based analysis on the same cell layer. This presentation will showcase the main principles of spatial metabolomics and some applications from our group focussing on tumour metabolism and small molecule drug delivery and efficacy.

Short Talk:
The spatial and unique composition of infiltrating immune cells defines autoimmune- and checkpoint-therapy associated hepatitis

Laurenz Krimmel

Freiburg University Medical Center, Department of Internal Medicine II, Freiburg, Germany

Background and Aims: Targeting of Immune-checkpoints is commonly used in cancer therapy, but often causes immune-related adverse events. These frequently occur in the form of Immune-checkpoint-blockade associated hepatitis (ICB-Hepatitis).
As the detailed composition and interaction of immune cells in ICB-Hepatitis is unknown and the distinction to autoimmune hepatitis (AIH) remains unclear, our aim was to perform a deep spatial analysis.

Method: We performed a high dimensional, spatially resolved, analysis of immune cell populations in liver biopsies of patients with ICB-Hepatitis (n=15), AIH (n=22) or control tissue (n=10), using a 40-marker Imaging Mass Cytometry (IMC) panel with single-cell resolution (1 µm2). Single cells were segmented utilizing a machine learning pipeline and classified high dimensionally.

Results: Analysis of the immune infiltrate revealed major differences between ICB-Hepatitis and AIH. An unbiased clustering identified disease-specific, phenotypically well defined, immune cell cluster, with unique spatial interactions.
CD8 T cell clustering resulted in enrichment of Ki-67+ Granzyme B+ Clusters in ICB-Hepatitis. AIH, on the contrary, was defined by an exhausted T cell phenotype, as well as tissue resident memory features (Trm). Interestingly, proliferative, and cytotoxic CD8 T cell clusters and the Trm cluster correlated with ALT levels in ICB-Hepatitis and AIH, respectively.
Classification of tissue phenotypes into spatial zones of the liver revealed an enrichment of CD8 T cells around the central vein in ICB-Hepatitis, whereas in AIH a closer interaction with the periportal triad was observed. In ICB-Hepatitis, we observed spatial co-localization of various myeloid clusters and CD8 T cells, especially of proliferative/cytotoxic CD8 clusters and pS6+ macrophages. In AIH, a more prominent involvement of B cells and CD4 T cells became visible.

Conclusion: The composition of the immune infiltrate and its distinct spatial distribution and interaction pattern points towards major differences in the immune mechanisms and pathogenesis driving ICB-Hepatitis and AIH. Our results may have implications

for the choice of immunosuppressive approaches in these diseases.

Short Talk:
Imaging-based transcriptomics technology identifying predictive biomarkers for relapse in colon cancer stage II

Katja Sallinger

Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Centre, Medical University of Graz & Center for Biomarker Research in Medicine (CBmed), Graz, Austria

Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Centre, Medical University of Graz, Graz, Austria and Center for Biomarker Research in Medicine (CBmed), Graz, Austria

Background Therapeutic management of stage II colon cancer remains difficult regarding the decision whether adjuvant chemotherapy should be administered or not. Low rates of recurrence are opposed to chemotherapy induced toxicity and current clinical features are limited in predicting relapse. Predictive biomarkers are urgently needed and therefore we hypothesise that the spatial tissue composition of relapsed and non-relapsed colon cancer stage II patients reveals relevant biomarkers.

Methods The spatial tissue composition of stage II colon cancer patients was examined by a novel spatial transcriptomics technology with sub-cellular resolution, namely in situ sequencing. A panel of 175 genes was designed investigating specific cancer-associated processes such as apoptosis, proliferation, angiogenesis, stemness, oxidative stress, invasion and components of the tumour microenvironment to examine differentially expressed genes in relapsed versus non-relapsed patient samples. Therefore, formalin-fixed paraffin-embedded slides of 5 relapsed and 5 non-relapsed patients were in situ-hybridized and computationally analysed. We identified a tumour gene signature to subclassify tissue into neoplastic and non-neoplastic tissue compartments based on spatial expression patterns generated by in situ sequencing (GTC-tool – Genes-To-Count).

Results The GTC-tool automatically identified tissue compartments that were used to quantify gene expression of biological processes upregulated within the neoplastic vs. non-neoplastic tissue and within relapsed vs. non-relapsed stage II colon patients. Three differentially expressed genes (FGFR2, MMP11 and OTOP2) in the neoplastic tissue compartments of relapsed patients in comparison to non-relapsed patients were identified predicting recurrence in stage II colon cancer.

Conclusions In depth spatial in situ sequencing showed potential to provide a deeper understanding of the underlying mechanisms that are involved in the recurrence of disease and revealed novel potential predictive biomarkers for disease relapse in colon cancer stage II patients. Our developed open-access GTC-tool allowed to accurately capture the tumour compartment and quantified spatial gene expression in colon cancer tissue.

Product Slam

2:00 pm, PEH

Chair: Elmar Endl, Thomas Bauer

What is the product slam and what is good for, well… On Tuesday, the first day of the conference, you have the opportunity to present the latest, innovative technical developments and products.

You can juggle, throw sweets at the audience or try to tell your 20-year company story in 3 minutes or wrap it up in a vision of the future.

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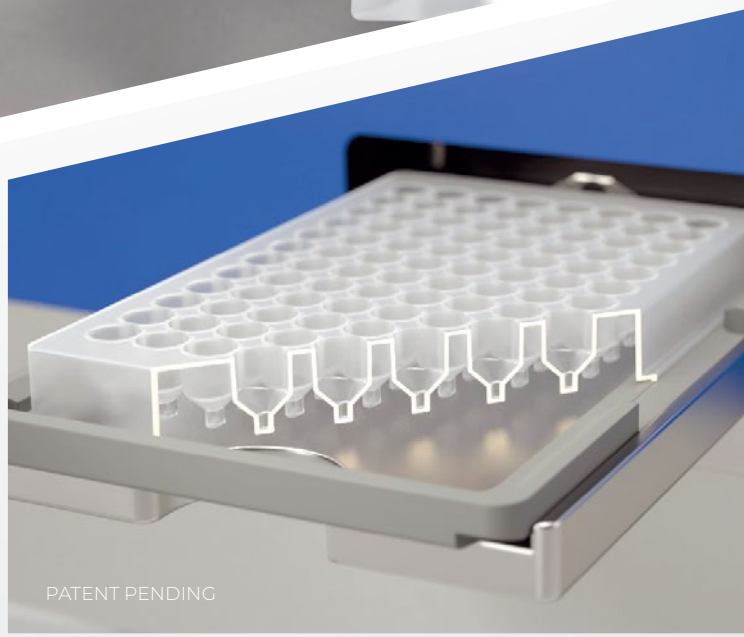

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Cutting Edge Session

3:30 pm, PEH

Chairs: Bertram Bengsch & Henrik Mei

Cutting edge cytometric technologies are at the forefront of technological R&D in single cell sciences. New concepts continuously emerge, and many of these will shape the way of future routines in cell analysis. This years programme features mass cytometry is a relatively new hybrid technology for generating high-dimensional single cell data. Emerging from single-cell analysis of cell suspensions with the help of metal isotope conjugated antibodies and a mass spectrometric readout adopted from trace element analysis, it today is often used for creating high resolution multiplexed imaging data of tissue sections, giving rise to multiple partly commercially available imaging platforms undergoing further development. In hindsight, mass cytometry is the disruptive technology that not only pioneered highly multiplexed single cell analysis but also stimulated the further advancement of fluorescence-based technologies, and the adoption of bioinformatics tools to analyze the new wealth of data. Mass cytometry remains a thrilling field of active interdisciplinary innovation with involvement of chemistry, mass spectrometry, flow cytometry, imaging and bioinformartics. The cutting edge: mass cytometry sessions features speakers with excellent contributions to wet lab and data analysis workflows in mass cytometry and imaging mass cytometry, and showcases exemplary applications in stem cell research and translational immunology.

Through the lens of CyTOF: resolving signatures of muscle stem cell aging one cell at the time

Ermelinda Porpiglia

Aarhus University, Department of Biomedicine, Aarhus, Denmark

Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine; Stanford, USA

Skeletal muscle mass, strength and regenerative capacity progressively decline with aging. This is partly due to functional impairment of muscle stem cells (MuSCs), the key players in muscle regeneration. However, the mechanisms responsible for age associated MuSC dysfunction remain elusive. A major barrier to gaining mechanistic insights into MuSC aging is the increased functional heterogeneity of the aged MuSC population, underscoring the need for single-cell studies. Here we capitalized on single cell mass cytometry to resolve MuSC heterogeneity during aging and identified a dysfunctional MuSC subset, marked by high CD47 surface expression (CD47hi). Mechanistically, increased expression of U1 snRNA in aged MuSCs shifted the balance of CD47 mRNA isoforms, leading to increased levels of CD47 protein on the cell surface. Aged CD47hi MuSCs act via paracrine signaling, through secretion of thrombospondin-1, a ligand for CD47, to suppress the regenerative capacity of CD47lo MuSCs. Strikingly, *in vivo* thrombospondin-1 blockade restored the proliferative potential of aged MuSCs and enhanced muscle regeneration and strength in aged mice. These findings uncover an unexpected role for thrombospondin-1/CD47 signaling in aged MuSCs and suggest a novel therapeutic approach to improve muscle regenerative function in the elderly.

Spatially and temporally resolved pathology of COVID-19 in the lung

André Rendeiro

AffiliCeMM Research Center for Molecular Medicine of the Austrian Academy of Sciencesation, Vienna, Austria

In this talk I will present how high-parameter flow cytometry, metabolomics, imaging mass cytometry, single-cell RNA-seq, and spatial transcriptomics can be used to explore unique facets of COVID-19 pathophysiology. These methods enabled us to dissect the intricate host response at systemic and tissue level during severe and fatal SARS-CoV-2 infection. We unravel substantial alterations in cellular composition and transcriptional cell states, as well as the intricate interplay between infected cells and the immune system at sites of infection in acute lung injury caused by COVID-19. In particular, multiplexed imaging with imaging mass cytometry offered us crucial understanding of the disordered lung structure and extensive immune infiltration, and by employing a comparative approach we were able to identify unique features of SARS-CoV-2 infection compared to other respiratory pathogens. Finally, I will highlight our most recent results on the long term, post-acute effects of SARS-CoV-2 infection in the lung by studying a cohort of patients followed for up to 359 days after infection. Altogether, these methods offer highly complementary insights into a landscape of lung pathology during COVID-19 and lung diseases in general.

Short Talk

Development of a competitive tetramer assay that allows mass-cytometry profiling of epitope-specific CD8+ T Cells after bead enrichment

Alexandra Emilia Schlaak

Clinic for Internal Medicine II, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Background The characterization and phenotyping of epitope-specific CD8+ T cells represent a significant area of research. Existing methods, such as tetramer enrichment using magnetic beads against fluorophore-tagged tetramers, enable the enrichment and detection of low frequencies of epitope-specific cells. However, a crucial limitation has been the inability to detect these cells by mass cytometry after tetramer enrichment.

Methods CMV-tetramers were generated from monomers which were tetramerized using either a) fluorophore-conjugated streptavidin, b) metal-conjugated streptavidin or c) streptavidin conjugated to both a fluorophore and a metal. PBMCs with a known CMV response were incubated with either fluorophore-tagged tetramer and metal-tagged tetramer simultaneously
("competitive tetramer assay"), or a fluorophore and metal double-tagged tetramer ("double labelling assay"). Then, tetramer-based enrichment of epitope-specific T cells and CyTOF staining was performed and compared to flow cytometry analysis. The strategy was further evaluated for additional epitope specificities in PBMCs or antigen-specific CD8+ T cell lines
(FLU, EBV, HBV, HCV).

Results Both the competitive tetramer assay and the double labelling assay were able to successfull enrich epitope-specific CD8+ T cells and simultaneously allow the detection via mass cytometry, enabling further multiparametric characterization. We observed a reduced tetramer signal intensity in the competitive tetramer assay compared to common tetramer staining, but this did not impact the ability to identify similar frequencies of epitope-specific CD8+ T cells as in control stainings.

Conclusions Our study introduces two innovative strategies to bridge the gap between tetramer enrichment and CyTOF staining. These approaches provide researchers with novel tools to further explore the phenotypic and functional characteristics of epitope-specific CD8+ T cells.

Short Talk
Vaccinology meets mass cytometry – Identifying baseline predictors of vaccination outcome

Axel Ronald Schulz
German Rheumatism Research Center, Berlin, a Leibniz-Institute, Berlin, Germany

Vaccination effectively protects from severe consequences of infection. However, little is known about immunological determinants of vaccination success in humans, specifically in groups with variable/poor vaccination response. We deeply profiled peripheral blood leukocytes by mass cytometry in cohorts of older (>80 years, n=55) and younger adults (20–53 years, n=44) before receiving at least two doses of BNT162b2 mRNA vaccine and correlated the data with the SARS-CoV-2-specific response data. Vaccination responses expectedly varied stronger among older compared to younger individuals, including older individuals with nearly no detectable T- and B-cell responses (Romero-Olmedo & Schulz et al., Nat Microbiol 2022, Lancet Infect Dis 2022).

While our mass cytometry data reproduced known features of immune ageing in senior adults, such as decreased frequencies of naive CD4+ CD31+ recent thymic emigrants, gamma-delta T cells, and plasmacytoid dendritic cells, we additionally identified clear signatures of high and low responsiveness among senior vaccinees. Older individuals with high antibody responses were characterized, by increased frequencies of proinflammatory, intermediate CD16+CD14++ and non-classical CD16+CD14+/- monocytes as well as proinflammatory CD38+CD11c+ NK cells. This suggests an unexpected beneficial role of these immune subsets for the vaccination response in elderly vaccinees, whereas they are usually considered detrimental to vaccine responsiveness in young individuals. In contrast, older subjects with low antibody response showed fewer transitional CD38+ naive B cells and more early immature neutrophils in the blood, most likely reflecting an imbalance in lymphopoiesis vs. neutropoiesis, which was confirmed by an increased Neutrophil-Lymphocyte-Ratio (NLR) in poor responders.

Altogether, we here report immune signatures associated with the success of mRNA vaccination in senior adults that can be of relevance in the clinical practice, i.e. to prospectively identify individuals that may require additional doses or differently formulated vaccination to achieve protection. In light of the development of a range of new mRNA-based vaccines, our results provide important clues to immune mechanisms underlying the magnitude of the vaccine response in the elderly, who represent a large at-risk population that needs to be protected by vaccination.

Short Talk
Single-cell microbiota phenotypes for classification of chronic-inflammatory diseases and functional characterization to decipher their role in pathophysiology

Toni Sempert
German Rheumatism Research Center, Berlin, a Leibniz Institute and Department for Cytometry, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

Chronic-inflammatory diseases (CID) show alterations in mucosal immune response and composition of intestinal microbiota, referred to as dysbiosis. We determine cellular properties of human gut microbiota from stool samples on single cell-level with multi-parameter microbiota flow cytometry (mMFC). We capture the mucosal immune response of the host by isotype-specific stainings of bacterial coating with endogenous host immunoglobulins and the expression of specific sugar moieties on the bacterial surface by staining with plant-derived lectins, potentially reflecting adaption of the bacteria to altered micro-environmental conditions in the intestine. By using machine-learning, we identified disease-specific phenotypic signatures for different CIDs such as Crohn's Disease (CD), Ulcerative Colitis, Rheumatoid

Arthritis and IgG4-related diseases. To understand the link between single-cell bacterial phenotypes and its function in disease pathophysiology, we combine cell sorting of bacteria with particular phenotypes with a deeper molecular and functional characterization. Preliminary data show an enrichment of disease-specific pathobionts for host immunoglobulins across patients, suggesting that the immune system recognizes distinct species in the context of intestinal inflammation. This is not reflected in the lectin staining of the bacteria, which may rather reflect a response of the bacteria to an altered, inflammatory environment. We will aim to link the phenotypic characterization of the microbiota to the phenotypic and functional analysis of the host's immune system. By this, we hope to unravel the communication between bacteria of the intestinal microbiota and our immune system and determine its role in health and disease.

Keynote Lecture

5:30 pm, PEH

Chairs: Henrik Mei, Bertram Bengsch

Sean Bendall

Associate Professor of Pathology and Immunology, Stanford University School of Medicine

Single cell analysis, starting with the earliest low parameter fluorescent cytometry and microscopy experiments, helped define the major cell subsets of human cellular systems as we understand them today. Now, a novel combination of elemental mass spectrometry with single cell analysis (mass cytometry – CyTOF, Science 2011) and imaging (multiplexed ion beam imaging – MIBI, Nature Med. 2014, Cell 2018) offers routine examination of >50 parameters without fluorescent agents or interference from spectral overlap using heavy metal isotopes as reporters. With these platforms, we have reached new levels of organizational understanding in human pathobiology – especially when combined with novel single-cell visualization and analysis methods. Here, we will present our latest efforts to create single cell assays in order to answer new questions in human pathobiology by structuring human hematopoietic immune function and dysfunction. We will also highlight early applications of this in next generation imaging and single cell platforms to resolve human cellular function and dysfunction. Overall, these new approaches reveal there are unappreciated layers of human cellular organization and structure in human systems that can be exploited to understand and perturb dysfunction.

And then...

6:30 pm CCO	**Welcome Reception**
8:00 pm CCO	**Entrepreneur Networking Event**
8: 00 pm DRFZ	**Core Facility Networking Event**

Entrepreneur Networking Event

8:00 pm, CCO

Chairs: Lisa Budzinski & Henrik Mei

At DGfZ we bring together technical developments and innovative cytometry applications. This event will give inspiration on how to translate your advancements into a business – learning directly from founders inside our community. Get inspired, get some background information, get in touch at our interactive entrepreneur evening event

Katrin Brenker – opto biolabs GmbH

Dr. Kathrin Brenker studied Molecular Medicine at the Georg August University in Göttingen. During her PhD at the Max Planck Institute in Freiburg, Kathrin worked at the interface between immunology and optogenetics. The lack of efficient analysis tools for optogenetic research, forced her (like many others) to solder her own illumination devices to perform experiments. As most people in the field struggle with the same challenge, she decided to start to commercialize the prototypes from her PhD and start a company: opto biolabs GmbH. Although Opto Biolabs has won numerous prices in the past years (1st place in the Cyberone HighTech Award 2018, 3rd place in the SPIE startup challenge 2019, Luminate Accelerator 2022, German Innovation Award 2023), building a profitable company is a true rollercoaster experience. Kathrin will share her experiences and challenges finding the perfect team, perfect funding and perfect produc

Aida Meghraoui – AMKbiotech

AMKbiotech is a Contract Research Organization, providing the biomedical actors with a specialized expertise and tailored services in high-plex spatial biology and cytometry for biomarker identification and treatment efficacy validation.
AMKbiotech was founded by Aïda Meghraoui PharmD. PhD. based on her research expertise in immunology, immuno-oncology and computation data analysis. During her academic research experience, she optimized the use of high-plex approaches to address clinical issues. She used mass cytometry to identify an early diagnosis biomarker candidate of sepsis (Meghraoui-Kheddar et al, 2021) and used spatial biology to characterize the immune response during skin and oral cavity squamous cell carcinomas (Elaldi et al, 2021). With here pluri-disciplinary team, she is using now this expertise combined with up-to-date technologies to potentiate clinical and pre-clinical research project for new treatment validation.

Tyler Burns – Burns Life Sciences Consulting GmbH

Tykler Burns a biologist from California who moved to Berlin after he got his PhD. He has been an independent full-time remote consultant for just under five years. He specializes in data analysis, technical and scientific advising, and biosecurity.

Core Facility Networking Event

8:00 pm at DRFZ

Chairs: Sarah Warth & Desiree Kunkel & Christian Kukat

This event is an opportunity to meet and share your experiences and challenges working in a Core facility. We will have short presentations on latest technologies and an update on recent plans for extended networking opportunities. We hope for lots of ideas to discuss afterwards in an informal atmosphere among colleagues.
We want to spend a wonderful evening at the DGfZ meeting 2023 in Berlin, with food & beverages & YOU!

A new view on cytometry with the BD CellView™ Image technology

Gert van Isterdael
VIB Flow Core Ghent
VIB-UGent Center for Inflammation Research
Ghent, Belgium

The VIB Flow Core had the unique opportunity to test and evaluate BD's prototype of their image enabled cell sorter equipped with the BD CellViewTM Image technology. In my talk I will highlight some of the applications we have been working on over the last years and show the potential of real-time image enabled FACS allowing researchers to make faster and more confident sorting decisions. Imaging based sorting will lead to improved quality of sort outcomes and its connected downstream processes. I will take the opportunity to present some preliminary results from the newly launched BD FACSDiscoverTM S8 Cell sorter.

Wednesday, 20.09.23

Rare Cells Session

9:00 pm, PEH

Chairs: Thomas Kroneis & Frank Schildberg

Continuous technical improvements in the field of single-cell analysis and its data interpretation causes cytometry to find its way into ever more complex problems. This includes, among others, spatial analyzes and analyzes of rare cells or cell populations. In this session we will focus on such analyses, spanning the spectrum from FACS analysis to slide-based spatial biology. In addition to biological questions in connection with rare cells or cell populations, in particular with a focus on microchimerism, there should also be space for contributions dedicated to solving technical difficulties in the analysis of rare events.

Rare cells in pregnancy: the role of regulatory T cells and microchimeric cells in placental and fetal development

Michael Eikmans

Department of Immunology, Leiden University Medical Center, Leiden, The Netherlands.

In pregnancies, the mother and the baby are genetically semi-allogeneic to each other, which includes the HLA genotype. This represents an interesting immunologic situation at places where maternal cells have contact with fetal cells. Appropriate development of the placenta is required for healthy pregnancy and delivery of a healthy baby. For this, it is relevant that maternal immunologic tolerance toward fetal cells is maintained. Regulatory T cells (Tregs) constitute up to 5% of the total T cell population and are involved in dampening immune cell activation. Animal models have shown that maternal Tregs are essential for a successful allogeneic pregnancy. Furthermore, women with complicated pregnancy demonstrate decreased Treg numbers at the maternal-fetal interface. In our group, we have been analyzing Treg subsets and effector molecules by spectral flow cytometry both in healthy and complicated human pregnancies. Detailed results are shown and discussed during the lecture.
During gestation, it also has turned out that Tregs from the fetus are relevant in tolerizing maternal allo-antigens. Homing of maternal microchimeric cells in the fetus' lymphoid organs is a major driver of the development of such tolerogenic fetal Tregs, which persist years after birth. Maternal microchimerism means that cells from the mother transfer over the placenta and end up in the offspring, and it represents a major topic by our Microchimerism, Health and Evolution Consortium formed in 2021. The lecture comprises results concerning frequency and characteristics of these rare microchimeric cells, as obtained by flow cytometric analyses and isolation strategies using HLA monoclonal antibodies.

Transplacental migration of maternal natural killer and T cells assessed by ex vivo human placenta perfusion – evidence for microchimerism?

Udo Markert

Placenta Laboratory at the University Hospital Jena, Germany

Introduction:
The transplacental passage of cells between a mother and her fetus, known as microchimerism, is a less studied process during pregnancy. The frequency of maternal microchimeric cells in fetal tissues in physiological pregnancies and mechanisms responsible for transplacental cell trafficking are poorly understood.

This study aimed to evaluate the placental trafficking of maternal peripheral blood mononuclear cells (PBMC) using human ex vivo placenta perfusion.

Methods:
Ten placentas and maternal PBMC were obtained after healthy pregnancies. Flow cytometry was used to characterize PBMC subtypes. The isolated PBMC were stained with a fluorescent dye and perfused through the maternal circuit of the placenta in an ex vivo perfusion system. Subsequent immunofluorescence staining for CD3+ T cells and CD56+ NK cells was performed on placental tissue sections, and the number of detectable PBMC in different tissue areas was counted using fluorescence microscopy.

Results:
Peripheral blood showed a higher percentage of CD3+ T cells compared to CD56+ NK cells. Perfused PBMC were detected in all analyzed placentas, with higher numbers in contact with fetal tissue compared to cells without contact. CD3+ T cells were identified more frequently than CD56+ NK cells, and some CD3+ T cells were found inside fetal tissues and vessels. The study also indicates a step-wise mechanism for cell trafficking across the placenta.

Discussion:
Maternal PBMC are capable of transmigrating through the syncytiotrophoblast layer into fetal placental tissue and vessels. The findings demonstrate that human placenta perfusion is a suitable method for investigating microchimerism during pregnancy.

Short Talk

Characterization of Antigen-Specific B Cells and Plasma Cells Using a Tetramer-based Detection Method by Flow and Mass Cytometry

Leonard Fiebig

German Rheumatism Research Cente, Berlin, a Leibniz Institute, Berlin, Germany

Numerous studies have demonstrated that antigen-specific serum antibody titers are maintained for many years. Fractions of plasma cells (PC) of the bone marrow persist for very long time and are thus implicated in long-term antibody production. Studying them at the level of antigen-specificity is inevitable to understand the principles of memory induction and maintenance in the B-cell lineage.

We here present an approach for the high-avidity detection of antigen-specific human B cells and PC by flow and mass cytometry based on the combination of biotin-labeled antigens with fluorochrome-/isotope-labelled streptavidin, commonly known as tetramers. The method was validated using human blood and BM cells, and with different protein and viral antigens, including tetanus toxoid, SARS-CoV-2 spike (S1) and receptor binding domain (RBD), epstein barr virus EBNA1, and monkeypox virus antigens. Assay specificity was validated using biological controls, e.g., by reproducing expected cellular dynamics in response to vaccination. The approach was compatible with live and fixed cells, cell-surface and intracellular staining. In multiplexed analyses, up to four antigen specificities were assessed in the same assay, using combinatorial tetramer labeling.

Using SARS-CoV-2 RBD- and S1- tetramers, we identified phenotypically distinct subsets of SARS-CoV-2-specific PC within the human bone marrow of 16 donors after basic mRNA immunization. We found SARS-CoV-2-S1-specific PC, representing 0.22% of total BMPC, the majority expressing IgG, which indicates their emergence from a systemic vaccination response. Notably, one-fifth of SARS-CoV-2-specific PC showed the phenotype of long-lived memory plasma cells characterized by downregulated CD19 and present or absent CD45 expression.

Taken together, we established multiplexed detection of various antigen-specific B cell subsets in a single assay, providing analytical access to B cell responses at single-cell levels from limited sample size in infection, vaccination, and autoimmunity.

Short Talk
Image-based SNP detection for discriminating haploidentical microchimeric cells

Emiel Slaats
Medical University of Graz; Gottfried Schatz Research Center for Cell Signalling, Metabolism and Aging; Division of Cell Biology, Histology and Embryology, Graz, Austria

Pregnancy associated microchimerism research in humans is historically dominated by PCR based approaches targeting polymorphisms in the DNA. However, while those methods allow for sensitive detection of microchimeric sequences in various DNA extracts, most information on cell type, spatial localization and cellular activity is lost. It would be fair to say that the lack of a suitable method to identify and characterize maternal and fetal microchimeric cells within their spatial context, has obstructed significant progress over the last decade. Here we present or ongoing work on developing an imaging based, in situ approach to discriminate between haploidentical cells. We employ rolling circle amplification of padlock probes targeting InDels and SNPs present in the mRNA, to generate discrete point signals within a cell. This will allow us to visualize, in a sex-unbiased manner, microchimeric cells within their native tissues.

European Guest Session: Danish Society

11:00 am, PEH

Chair: Anja Bille Bohn, Aarhus, Denmark

Introduction

Danish Society for Flow Cytometry (DSFCM) was founded in 1988 with the purpose of uniting Danish researchers and clinicians working with flow cytometry. Initially, the society was highly involved in standardization for clinicians using flow cytometry. During the years, the research aspect and new applications of flow cytometry have become more central.

DSFCM organizes two annual meetings with varying themes and relevant national or international invited speakers. The purpose is to connect the Danish researchers who are particularly interested in flow cytometry, to facilitate the communication and stimulate the research amongst members. Networking and time to talk to company members is always prioritized as part of the meeting program. The two annual meetings are free to attend for everyone interested and are often combined with social events, such as dinners or city/museum tours, for members of the society only.
About every 3 years, DSFCM is part of a joint Nordic meeting with the Norwegian and Swedish flow cytometry societies.

The Danish Society for Flow Cytometry contributes to the European guest session with two speakers representing two different topics within dimensions of cytometry:Jesper Geert Pedersen, M.Sc, PhD, has established a 38-color full spectrum flow cytometry panel for in-depth immune characterization of metastatic melanoma patients. The aim is to monitor immunological changes during immunotherapy and potentially gain knowledge about treatment resistance.

Carina Rosenberg M.Sc, PhD, has implemented Imaging Flow Cytometry with the aim of quantifying rare dysplastic changes in bone marrow cells in patients with suspected myelodysplastic syndrome. This technique allows morphometric evaluation and can differentiate healthy hematopoietic stem cells from leukemic cells in bone marrow samples from patients with acute myeloid leukemia.

Investigating host immune function in metastatic melanoma patients treated with immunotherapy

Jesper Geert Pedersen
Department of Biomedicine, Aarhus University, Aarhus, Denmark

Immunotherapy has revolutionized the treatment of metastatic melanoma leading to long-term survival in a subgroup of patients. Yet, 40-60% of the patients do not respond to immunotherapy treatment. While several mechanisms for treatment resistance have been proposed, clinical biomarkers for predicting treatment response are still lacking, emphasizing the need for further investigations. Since immunotherapy directly targets and modulates the immune system to re-invigorate the anti-tumor immune response it is pivotal to understand the interplay between therapy, cancer, and the immune system to improve biomarker research and cancer treatment.

In this project, we developed a 37-parameter spectral flow panel for deep single cell immunophenotyping of peripheral blood mononuclear cells collected before and during the first year of immunotherapy treatment of metastatic melanoma patients. Using this panel, we will perform thorough enumeration and characterization of T cells, B cells, NK cells, monocytes, dendritic cells, and plasmacytoid dendritic cells. By monitoring changes in the immune cells compartment during treatment, we can understand how the peripheral immune system is modulated by checkpoint inhibitor therapy and thereby identify potential resistance mechanisms. Furthermore, this enables identification of new biomarkers for predicting treatment response in melanoma patients. Overall, this will expand our knowledge of the interplay between the immune system and checkpoint inhibitor therapy and pave the way for identification of new biomarkers and thereby improve treatment of metastatic melanoma.

Rethinking diagnostics in myelodysplastic syndrome and unexplained cytopenia: employing morphometric evaluation of dysplasia by imaging flow cytometry

Carina Agerbo Rosenberg
Department of Hematology, Aarhus University Hospital, Aaarhus, Denmark

Myelodysplastic syndrome (MDS) is a group of malignant blood disorders marked by dysplastic myeloid cells in the bone marrow (BM) causing varying degrees of BM failure with peripheral blood cytopenia(s) resulting in symptoms of anemia, recurrent infections and bleeding. MDS arises in hematopoietic stem cells and has a propensity to progress into acute myeloid leukemia (AML). Even though cytogenetic and molecular analyses are important diagnostic tools in MDS, significant morphologic BM dysplasia remains the diagnostic hallmark of most MDS subtypes. However, accurate diagnosis of cases in which only subtle dysplastic changes are present are very difficult, and inter-scorer variability and subjectivity may be present, even among experienced hematopathologists – thus, novel techniques to assist the morphologic evaluation of BM cells are warranted.

In our laboratory, we have studied imaging flow cytometry to explore its diagnostic potential in the complex field of MDS diagnostics. The technique combines the high-throughput and multiplexing capabilities of conventional flow cytometry with the high-resolution imagery obtainable through fluorescence microscopy. This facilitates the identification of rare cell populations through flow cytometric gating of immunophenotypically defined subsets, and subsequent retrieval and quantification of image-based parameters such as size, shape, texture, and signal location. Specifically, we investigate how imaging flow cytometry can be used to quantify dysplastic changes in BM cells in patients with suspected MDS, and if morphometric evaluation can differentiate healthy hematopoietic stem cells from leukemic cells in BM samples from patients with AML.

Postersession

12:30 pm - 2:30 pm, CCO

Chairs: Henrik Mei, Oliver Otto

1.) Shared Resource Laboratory collaboration: the story of upholding the 3Rs

Ana Agua-Doce, Emma Russell, Clive Da Costa, Andy Riddell

The Francis Crick Institute, London

Communication between the different research facilities within an institute can be a powerful tool to advance projects and enhancing science. The flow cytometry laboratory at The Francis Crick Institute has been involved in a number of productive collaborations with other shared resource laboratories (SRL). These partnerships have brought forth new technologies, synergised technical skills and knowledge leading to innovative solutions and efficient communication. The latest collaboration between the Flow Cytometry core and the Biological Research Facility (BRF) at The Francis Crick Institute has demonstrated the benefit of such collaborations.
In this presentation we will discuss how the combined expertise of the BRF and Flow Cytometry facility has successfully generated a portfolio of experimental protocols. How we designed, tested and produced a validation tool for genotype / phenotype correlation of the BRF's mouse strains. This has allowed us to strengthen our quality assurance of mouse strains across The Crick, which leads to improved reproducibility of scientific data by providing quick, sensitive and transferable results.

2.) Spatial Mapping of the Heterogenic Sup-populations in the Mono-species Biofilms

Sania Arif, Kathrin Stückrath

Department of Environmental Microbiology, Helmholtz Centre for Environmental Research–UFZ, Leipzig, Germany

In a mono-species biofilm, individual cells may contribute to many phenotypically and genetically diverse sub-populations in response to their local microenvironment stimuli, stochastic gene expression, and genetic mutations. Resolving the heterogeneity among closely assembled sub-populations in a biofilm requires precise spatial sampling and segregation procedures. The punch biopsy method for sampling the ≈ 800-200µm micro-spaces of the biofilms was combined with the single cell flow cytometry to segregate multiple sub-populations based on their scatter and fluorescence properties. Five different single-species biofilms were punched with the different-sized pipette tips (1000µl, 200µl, 10µl) and 27G syringe needle, followed by staining with the fluorescent dyes, Syto9 and DAPI for live and fixed cells imaging, respectively. The spatial distribution of phenotypically distinct sub-populations in the biofilm was mapped based on the size (forward scatter), density (side scatter), type (cells/spores), DNA content (DAPI), and live cells (Syto9) properties. The spatial distribution of different subpopulations varied drastically within a single-species biofilm and these spatial maps were unique fingerprints of each mono-species biofilm. The sampled heterogenic sub-populations were of smaller magnitude 103-5 cells which cannot be otherwise differentiated from the 109 cells of the whole colony. The punch biopsy-flow cytometry method maps the heterogenicity across the spatial and temporal scales of the biofilms, which could extend to the downstream single-cell omics and growth studies through direct sorting of distinct subpopulations.

3.) A clustering-based machine learning approach for automated debarcoding of mass cytometry data

Vera Bockhorn[1], Axel Schulz[1], Lena Teichert[2], Tobias Alexander[2], Henrik Mei[1]

[1]Deutsches Rheuma-Forschungszentrum Berlin, a Leibniz Institute, Berlin, Germany

[2]Department of Rheumatology and Clinical Immunology, Charité - Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

Mass cytometry offers the advantage to measure multiple samples from different sources at once, using sample-specific metal isotope labels, i.e. barcode tags. The debarcoding of the measured sample convolute back into separate samples is an essential step when doing multi-sample CyTOF measurements. Traditionally, debarcoding is done by manual gating, but also algorithmic solutions to solve this problem have been developed. Here, we propose a new computational debarcoding method based on the FlowSOM algorithm, which is a widely used clustering method for cytometry data.
Our method correctly assigned 75% of the events of a 20-plex 6-choose-3 barcoded sample convolute of human B cells, closely resembling a manual debarcoding approach (73%). In contrast, the debarcoding algorithm implemented in the CATALYST package assigned 80% of the cells to one of the input samples, but at least 7% of the cells were considered misclassified after manual inspection. Hence, our FlowSOM debarcoder showed a better accuracy than existing solutions, by taking into account the entire information of neighboring cells with similar barcode signatures and not only the individual location of a cell within the barcode space for the sample assignment decision. This, together with the ability to set many parametric options to control quality and quantity of sample assignments, renders the new FlowSOM debarcoding method particularly suitable for barcoding situations with low and heterogeneous barcode separations such as surface barcoding of living cells.

4.) Characterizing cells isolated from amniotic fluid with respect to microchimerism

Bernadette Bramreiter[1], Katja Sallinger[1], Michael C. Gruber[1], Emiel Slaats[1], Julia Schönberger[1], Katharina Schuch[1], Bettina Amtmann[2], Una Kurtovic[2], Herbert Fluhr[2], Philipp Klaritsch[2], Frank A. Schildberg3, Thomas Kroneis[1]

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

[2]Department of Obstetrics and Gynaecology, Medical University of Graz, Graz, Austria

[3]Department of Orthopedics and Trauma Surgery, University Hospital Bonn, Bonn, Germany

Microchimerism can be defined as the presence of a small population of genetically distinct cells from another individual in a host. During pregnancy, a bi-directional exchange of maternal and fetal cells occurs and may lead to the maintenance of these cells in host tissues, known as maternal (mMC) and fetal microchimerism (fMC), respectively. We hypothesize that maternal-fetal cell trafficking occurs via ingestion of amniotic fluid (AF) and transmigration of maternal cells into fetal tissue, i.e., via the gastro-intestinal tract. We further hypothesize that these trafficking maternal cells represent a subpopulation of so-called amniotic fluid stem cells (AFSCs), which have been shown to differentiate into cell lineages of all three germ layers, supporting the known phenotypes of maternal microchimeric cells.
Therefore, we aim to (i) identify maternal cells in AF, (ii) characterize maternal cells with respect to cell (sub)type and (iii) functionally characterize maternal cells with respect to their differentiation potential.
We present current data from patient samples (n=9; gestational age: 16-35; volume: 1-4 l) after CD117+ cell isolation followed by expansion, characterization, and immunophenotyping of AFSCs.

5.) Citizen science vs. scientist science for flow cytometry cell population identification and machine learning

Benedikt G Brink[1,2], Daniel Yokosawa[2,3], Leon Li[3], Alexander Butyaev[4], Sebastiano Montante[3], Mehrnoush Malek[3], Quentin Michalchuk[3], Alan Milligan[3], Razzi Movasaghi[3], Li Xie[2],Albina Rahim[3], Sameer Shankar[3], Justin Meskas[2], Julia Boira Esteban[5], Kornél Erhart[5], Bergur Finnbogason[5], Alexandra Winther Geirsdóttir[5], Sæmundur Hermannsson[5], George Kelion[5], Hjalti Leifsson[5], Þórður Matthíasson[5], Haukur Pálsson[5], Asimakis Reppas[5] , Josh Rivers[5], Nelle Stahl[5], Andrea Cossarizza[6], Zsolt Bányai[6], Gabriel Daza[6], Bernard Revaz[6,] David Ecker[5], Jérôme Waldispühl,[4] Attila Szantner[7] and Ryan R Brinkman[2,3,8]

1 OMIQ, 2 Dotmatics, 3 BC Cancer, 4 McGill University, 5 CCP Games, 6 University of Modena and Reggio Emilia, 7 MMOS, 8 University of British Columbia

Published research (Grant 2021 PDA J Pharm Sci Technol) has shown the subjectivity of manual gating can have a profound effect on analysis and consequent decisions, with a between-analyst range of percentage counts of up to 31.8%. Various automated approaches have been developed to accelerate and standardize flow cytometry cell population identification and have shown success in many applications. However, none have the performance or capabilities to lead to widespread adoption. Machine learning approaches hold the most promise to advance performance but the lack of sufficient training data remains a major bottleneck. In response to this need, we developed a citizen science infrastructure for the analysis of flow data within Eve Online, a massively multiplayer online game gathering 300k players monthly. The same research team as above has shown that participants with significant experience using flow cytometry analysis software have a range of uncertainty of their results of 8% compared to those with little experience at 12%, however this was not significantly different. We built upon this research by examining differences between citizen scientists (Eve Online gamers) and professional flow cytometrists (attendees of CYTO 2022 who visited the Dotmatics booth and participated in a gating challenge) in the identification of cell populations of potential interest in bivariate plots, within the Eve Online gating interface.

6.) Simplifying High-parameters Phenotypicandfunctional Characterization of CancerImmune Cells

Shaghayegh Derakhshani, Melissa Klug

Standard BioTools

Interrogating immune cell composition and function in patients with cancer is critical for making disease prognoses, monitoring clinical efficacy of tumor immunotherapies, identifying novel therapeutic targets, and discovering predictive biomarkers of disease. Both the adaptive and innate arms of the immune system play important roles in generating pro- or anti-tumor milieus.

In multiple myeloma, malignant plasma cells accumulate in the bone marrow through clonal expansion, crowding out other cells and leading to anemia, renal insufficiency, immunosuppression, and increasing risk of multisystem organ damage if untreated. Cellular and antibody-mediated immunotherapeutic approaches, including CAR T cells and monoclonal antibodies targeting CD38, have been developed to treat multiple myeloma. Since NK cells can also indirectly impact CAR T cell or antibody-based immuno-therapies, characterizing these cells using optimized and reproducible assays is critical.

CyTOF® is a high-plex flow cytometry technology that uses metal-isotope-tagged antibodies to probe cellular phenotypes and functions. In contrast to fluorescence-based conventional and spectral flow cytometry, CyTOF experimental workflows are streamlined because autofluorescence is not an issue and signal spillover is minimal, allowing rapid design and application of 40-plus-marker panels. To expand on the increasing clinical and preclinical utility of the 30-marker Maxpar® Direct™ Immune Profiling Assay™ (Maxpar Direct Assay), we developed 9 add-on Expansion Panels for deeper phenotyping of specific

cell types and activation states, including panels designed to characterize ex vivo and activated myeloid cells, T cells, and NK cells. Here we demonstrate combining the Maxpar Direct Immune Profiling Assay with the NK Cell Expansion Panel (CD181, NKp30, NKp46, PD-1, NKG2A, ICOS, and TIGIT) or the T Cell Expansion Panel 3 (OX40, TIGIT, CD69, PD-1, Tim-3, ICOS, and 4-1BB) with the Basic Activation Expansion Panel (IL-2, TNFα, IFNγ, CD107a, perforin, granzyme B) to enable deep immunoprofiling of multiple myeloma PBMC.

7.) Classification of Different Cell Types Based on Pulse Shapes using Deep Neural Networks

Vinod Devaraj[1], Daniel Kage[1], Alexander Wolf[1], Kerstin Heinrich[1], Jenny Kirsch[1], Hyun-Dong Chang[1,2], Toralf Kaiser[1]

[1]German Rheumatism Research Centre Berlin (DRFZ) – Flow Cytometry Core Facility, Berlin, Germany

[2]Department of Cytometry, Institute for Biotechnology, Technische Universität Berlin, Berlin, Germany

Traditionally, flow cytometers have been used for analyzing and sorting cell populations and other particles using laser-based fluorescence detection methods. However, the use of fluorescent staining presents certain drawbacks. Labelling cells with specific reagents may affect cell viability and functionality, or fluorescent labels for cells or particular properties are lacking. Additionally, the assessment of morphological cell parameters without direct imaging is limited in scope. To address these challenges, we recently introduced a novel technique called multi-angle pulse shape flow cytometry, which enables comprehensive analysis of scattered light of unlabelled cells. This technique allows us to characterize cells based on their scattered light properties alone, employing unsupervised clustering methods.

Here, we present a supervised deep learning model designed for the classification of different cells types solely based on the light scatter signal pulse. The model comprises an encoder, a decoder, and a classifier that are trained simultaneously. The encoder employs a convolutional neural network to learn a representation from the input raw pulse shapes. The output of the encoder is then fed into both the decoder and the classifier. The decoder reconstructs the original pulse shapes, while the classifier, implemented as a fully-connected network, assigns labels to the input data. The model is trained iteratively with the objective of improving the classification accuracy and the quality of pulse shape reconstruction simultaneously. By using this deep learning technique, our approach captures intricate hidden patterns within the pulse shapes, thereby enabling a deeper understanding of the morphological properties of cells.

8.) Diffcyt-based extraction of early immune signature associated with severe courses of COVID-19

Sebastian Ferrara[1], Axel Ronald Schulz[1], Vera Bockhorn[1], Amro Abbas[1], Leif Erik Sander[2], Birgit Sawitzki[2], Henrik E. Mei[1]

[1]DRFZ Berlin

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Considering that COVID-19 can become life-threatening or even fatal, early diagnosis and prediction of severe courses requiring special clinical care are needed. We here used mass cytometry data of blood leukocytes obtained after hospital admission of COVID-19 patients (Schulte-Schrepping et al., Cell, 2020; Georg et al., Cell, 2022), to determine early features associated with severe courses of COVID-19. For this, we implemented a workflow starting with FlowSOM clustering and extraction of cell frequencies and signal intensities in OMIQ.ai, followed by a modified diffcyt protocol in R (Nowicka et al., F1000res 2017) permitting the analysis of the same cytometric markers for clustering and per-cluster expression analysis. Using linear and generalized linear mixed models, we extracted a signature of severe disease comprising expansions of immature neutrophils, plasmablasts, activated T cells, besides diminished numbers of $KLRG1^{+}CD27^{+}$ NK

and naïve B cells, and enhanced LAG3 expression by activated and terminally differentiated T cell subsets, consistent with inhibited T cell reactivity in immune synapses. Hierarchical clustering revealed two groups of patients with poor outcome, one characterized by a combined plasmablast / $CD16^+$ T cell signature most strikingly detectable in early diseasehood, and another group with persistently expanded, terminally differentiated $CD27^-$ CD8 T cells, altogether suggesting the presence of distinct immunopathological axes involved in severe COVID-19. Based on our findings yet pending confirmation in validation cohorts, we envisage the development of a diagnostic cytometric test for the prediction of severe COVID-19 to enable timely treatment and clinical care.

9.) Dynamic real-time deformability cytometry – a tool for erythrocyte thermomechanics

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10.) Verifying Feasibility of Looped Mass Transfer Design Basedon Single Cell Analyses and Ecological Theory

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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).

Here we replicated and confirmed the repeatability and feasibility of the looped mass transfer design setup. Using the same original environmental sample and same setup, five local microbial communities were continuously grown in parallel and connected by a regional pool with constant mass transfer rate. The dynamics of microbial community were monitored by using quantitative high-throughput flow cytometry while the structure variation was detected and evaluated by cytometric fingerprinting method.

Comparing our results with the preliminary study of the looped mass transfer design setup, we observed a repeatedly similar trend attributed to the rescue effect. The rescue effect was interrupted several times by experimental design. In this study we want to explore to which degree the rescue effect can be re-established. This 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.

Reference

Li, S., Abdulkadir, N., Schattenberg, F., Nunes da Rocha, U., Grimm, V., Muller, S., & Liu, Z. (2022). Stabilizing microbial communities by looped mass transfer. Proc Natl Acad Sci U S A, 119(17), e2117814119. https://doi.org/10.1073/pnas.2117814119

11.) Vio®Dyes in Spectral Flow Cytometry

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High levels of irreproducibility in pre-clinical research cause high economic damage and lead to longer drug development times and in turn, to higher costs for approved drugs and treatments. The irreproducibility is caused by variations in reagents, data analysis and instrument performance. Minimizing these variations improves data quality for basic research as well as clinical studies. Miltenyi is known for offering a package of instruments, consumables and reagents that harmonize work flows and offer reliable and reproducible research. The MACSQuant® Analyzer flow cytometer family ensures equal data acquisition across sites, users and time via smart gain technology. A variety of express modes for data analysis add the next level of standardization by automation of compensation and gating. However, a reliable automation of data analysis is only possible on the basis of reagents that provide precise signals with minimal unspecific background staining. Miltenyi's Vio® Bright Dyes are combined in highly potent conjugates with the recombinant REAfinity antibodies, delivering a superior lot-to-lot consistency and purity and diminishing the need for FcR blocking or multiple isotype controls. Herein we characterized these highly reliable Vio® and Vio® Bright Dyes in spectral flow cytometry and show remarkably bright signals on cells as well as beads using Cytek Aurora and Sony ID7000 spectral instruments. The results show that that Vio® and Vio® Bright reagents are powerful tools enabling spectral flow cytometry.

12.) Microchimerism Literature Atlas

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The "Microchimerism, Human Health & Evolution Project" seeks to provide fundamental information about the phenomenon of microchimerism (MC) at the cellular and molecular level, and to establish web-based tools which assist the microchimerism community in their daily scientific life. Our first tool, that can be accessed online via the link: https://literature-atlas.microchimerism.info/, is the "MC Literature Atlas" - a literature database containing the metadata of more than 12 thousand entries from the field of microchimerism, covering peer-reviewed articles and reviews from 1970 to the present. The MC Literature Atlas makes it easy for users to search, find, access and export their desired literature metadata, i.e., by offering an intuitive multidimensional search possibility and by highlighting keywords. The MC Literature Atlas offers the possibility to investigate the embedding of an article in the field of MC literature via a graphical citation network. Additional figures provide useful information about the literature of interest and will be the starting point for in-depth semantic MC data analysis. The poster provides an overview of the features and QR codes to directly visit and explore the MC Literature Atlas.

13.) Detection of HER2-positive extracellular vesicles in plasma from breast cancer patients using spectral flow-cytometry

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Pastucha[13], Mathias Reisbeck[6], Lukas Rief[2], Holger Bronger[2], Andreas R. Bausch[13,14,15,16], Andreas Pichlmair[12], Thomas Brocker[9], Gabrielle Schricker[3], Oliver Haydn[6], Reinhard Zeidler[4,5,17], Wolfgang Hammerschmidt[4], Carmen López-Iglesias[6], Ron Heeren[7], Marion Kiechle[2], Sabine Grill[2], Olaf Wilhelm[3], Percy Knolle[1,18], Bastian Höchst[1]

Breast cancer is classified based on the expression of estrogen/progesterone receptors and the human epidermal growth factor receptor HER2, which is the basis for the decision on personalized therapy e.g. endocrine and anti-HER2 therapy, respectively. This classification requires tissue obtained through biopsies for immunohistochemistry and molecular analysis to determine the hormone receptor and HER2 expression status. HER2 targeting therapies are important instruments in treating breast cancer patients and have been demonstrated to significantly improve survival. However, only 20-25 % of tumors do express HER2. In the course of cancer diagnosis to metastatic disease, the tumor biology and the mutational profile might change. Therefore, to verify that the correct treatment is indicated in the metastatic disease, a tissue biopsy of the metastatic lesion is required but in the case of brain metastasis not feasible. Analysis of the tumor biology and current phenotype through exosome diagnostics from blood („liquid biopsy“) could solve the problem of this high unmet medical need to determine the HER2 status. Here, we demonstrate that spectral flow cytometric (SFC) analysis of individual extracellular vesicles from the peripheral blood of patients can be used to detect HER2 expression by breast cancer and allowed us to sensitively determine HER2 expression on single EVs from breast cancer cells under defined conditions *in vitro* and to determine their absolute numbers. In a proof-of-concept clinical study in breast cancer patients, SFC analysis of patient plasma samples at the time of cancer diagnosis detected and quantified the frequencies of circulating $HER2^{+}$EVs with high sensitivity.

This new method bears the promise to provide important information on breast cancer expression of hormone receptors and HER2, especially in situations where tissue is not readily available, and could emerge as a novel approach to monitor breast cancer evolution over time and to tailor personalized molecular therapies.

14.) Autoantigen-specific CD8+ T-cell signature in Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a common chronic autoimmune disease with a prevalence of 0.5- to 1% worldwide and is characterized primarily by inflammation and progressive destruction of bone, cartilage and soft tissues. One hallmark of RA disease is the infiltration of various immune cells with recent evidence specifically suggesting a role for CD8+ T-cells, yet limited knowledge exists on their frequency, epitope specificity and role in disease development.

Material and Methods: Here, we comprehensively analyzed CD8+ T-cell recognition towards six proteins of relevance for disease development and immunological response in RA; aggrecan, alpha-enolase, collagen type II, GRP78, fibrinogen and vimentin. From these proteins, we predicted 538 major histocompatibility complex class I (MHC-I) binding peptides in both native and citrullinated forms and evaluated CD8+ T-cell recognition in peripheral blood using state-of-the-art cytometry-based DNA-barcoded peptide-MHC (pMHC) multimer techniques.

Results: We assessed the prevalence of auto-reactive T-cells in a cohort of 45 RA patients in comparison to 28 healthy donors (HDs) while additional examination of 20 patients with early RA vs long-standing RA each, to decipher the mechanisms of disease development and progression, is ongoing. Interestingly, significantly higher levels of activated autoreactive T-cells were found in the peripheral blood of RA patients, but, albeit to a lesser extent, inactive autoreactive CD8+ T-cells could also be detected in HDs. Collagen type II and aggrecan were the most frequently recognized proteins. Moreover, autoreactive CD8+ T- cells in RA patients displayed a phenotype consistent with chronic antigen exposure – further differentiating these from rare autoantigen-reactive CD8+ T-cells observed in HDs.

Conclusions: Here we show that auto-reactive CD8+ T-cells are enriched in the blood of RA patients and display a distinct phenotype compared to HDs. These

findings suggest a potential role of auto-reactive CD8+ T-cells in RA disease, which can serve as new targets for therapeutic interventions.

15.) Impact of the microbiome on the mucosal immune response and the production of pathogenic antibodies in patients with IgA nephropathy

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Immunoglobulin A (IgA) nephropathy (IgAN) is the most common primary glomerulonephritis worldwide caused by deposition of immune complexes in the kidney and subsequent inflammation. The pathophysiology is often summarized with a 4-hit hypothesis: (1) An increased expression of IgA1 deficient for galactose in the hinge region (gd-IgA1) and (2) the formation of autoantibodies directed against exposed N-Acetylgalactosamine (GalNAc) in the hinge region of gd-IgA1 resulting in (3) the formation of immune complexes. (4) Glomerular deposition of these immune complexes eventually leads to fibrosis and chronic inflammation in the kidneys. What triggers the formation of the pathogenic antibodies and where they are formed is still unknown. It has been hypothesized that the microbiome is involved in the etiopathogenesis of IgAN, because bacteria have an impact on the mucosal IgA production and provide a potential source of carbohydrates triggering the production of carbohydrate-specific autoantibodies. In this project, we aim to elucidate whether and how the microbiota modulates B and plasma cells leading to the production of gd-IgA1 antibodies and pathogenic autoantibodies. We have shown that despite significantly elevated serum levels of IgA1 and gd-IgA1 patients do not have increased antibody levels in the fecal and salivary supernatant. Accordingly patients do not show more bacterial coating by IgA1 or gd-IgA1 but an increased staining with a GalNAc-binding lectin – representing a possible epitope for the production of the immune complex forming autoantibodies. By sorting and sequencing gd-IgA1-, IgA1- and GalNAc-positive bacteria we will further clarify if patients show a different taxonomic composition promoting the production of the antibodies. Additionally we will analyze (gd)-IgA1+ B cells to elucidate their potential mucosal origin and tissue homing behaviour possibly explaining the discrepancy of serum and mucosal antibody levels in patients with IgAN.

16.) Training a customized segmentation model for improved detection of Liver-Cells in high-dimensional spatial profiling data generated by imaging mass cytometry

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Background
Segmentating cell masks from high-dimensional imaging data is required for downstream single-cell analysis. Current strategies include supervised machine learning pipelines that are however time-consuming and resource-intensive. Alternatively, deep learning models for automation of cell segmentation are being developed, such as Mesmer[1] used in DeepCell, and Cellpose[2]. However, the training data used for these models does not include a significant amount of liver cells. We thus set out to understand if the deep learning based models can reliably identify liver parenchymal and immune cell types in imaging mass cytometry data. We further set out to compare the performance to supervised machine learning-based segmentation and train a custom model using Cellpose for improved liver tissue cell segmentation.

Methods
We used 3 datasets from imaging mass cytometry experiments analyzing liver tissue from diseased (infection, cancer, autoimmune) tissue states (n=282) and the supervised machine learning – based segmentation masks. We performed a 80/20 Training-Test-Split and trained both a model based on the TN2-Model provided by Cellpose and a new model that also included the segmentation data. We then compared the performance of our new model against the Test split. We also compared Models trained on only one liver-segmented dataset against the ground-truth on others to exclude over- fitting

Results
We found that our model based on TN2 and additional liver-specific training masks outperforms the standard TN2 and Mesmer Model using standard segmentation quality metrics. We also investigated whether the Single-Cell-Data quality downstream is improved (e.g. less overlap between T- and B- Cells resulting in cleaner clusters).

Conclusions
Training a custom model for tissue types which were not included in the datasets used for training existing networks improves segmentation quality and allows improved automation of tissue-specific segmentation.

References
Noah F. Greenwald et al. “Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning”. eng. In: Nature Biotechnology
40.4 (Apr. 2022), pp. 555–565. issn: 1546-1696. doi: 10.1038/s41587-021-01094-0.

Marius Pachitariu and Carsen Stringer. “Cellpose 2.0: how to train your own model”. en. In: Nature Methods 19.12 (Dec. 2022). Number: 12 Publisher: Nature Publishing Group, pp. 1634– 1641. issn: 1548-7105. doi: 10.1038/s41592-022-01663-4. url: https://www.nature.com/ articles/s41592-022-01663-4 (visited on 06/28/2023).

17.) Digital twin-assisted process design for NK-cell therapies

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Human natural killer (NK) cells represent a promising leukocyte population for use in immune cell therapies. Solid as well as hematologic tumors have been shown to respond to treatment with autologous or allogeneic NK cells. Providing NK cells of sufficient quantity and quality, i.e. efficacy, safety, identity, purity and yield, remains a challenge. Deeper insight into the critical process parameters (CPP) and critical quality attributes (CQA) of primary NK cell culture is needed. This information is essential for building a digital twin-assisted process design for NK-cell therapies. Here, we report the first data set of CPPs and CQAs using the human NK cell line NK92.
NK92 cells were cultivated under batch conditions with varying CPP (IL2, glucose, glutamine, pH, O2) over a time course of seven days. Cell culture parameters were measured daily, e.g. viable cell count (VCC), glucose, lactate concentration, pH. CQAs like viability, lineage markers, activating receptors and death ligands were investigated using flow cytometry at multiple time points.
IL2 concentration between 10 to 500 IU/ml had no distinctive influence on viability or growth rate. Low glucose levels of 5.55 mmol/L led to a high consumption of glutamine but had no effect on VCC or viability, even in combination with low glutamine levels. Low glucose levels were associated with a higher stability of the CD56 marker. Neither initial pH values between 7.2 and 7.6 nor low oxygen concentration of 10% had an influence on the CQAs.
The investigated CPPs had no or only slight influence on the CQAs of NK92 cells. Testing a wider dynamic range of CPPs for NK92 cells seems mandatory. Future tests will include primary human NK cells from various donors.

18.) High-plex spatial imaging to decipher skin microenvironment complexity

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OBJECTIVE: Unravelling tissue contexture is emerging as a key step to understand mechanisms of tissue homeostasis and its imbalance in order to develop adapted preventive and more effective products. Addressing the skin as a heterogeneous complex system allows the analysis of its cellular and acellular component phenotypes, functions and interactions and obtain a complete snapshot of the skin at each investigated state.
METHODS: Imaging Mass Cytometry (IMC) is a High-Plex single-cell spatial imaging system which allows the simultaneous visualization and quantitative analysis of 40 markers and 200 targets in one skin section after a unique staining step and image acquisition. Combined to a tailored computational image analysis based on machine learning algorithms, this approach allows a quantitative evaluation of skin components' variations and treatments' effects.
RESULTS: The main challenge for the application of IMC is constructing the best antibody clone combination suitable for formalin-fixed and paraffin embedded (FFPE) or frozen tissue. Therefore, we developed a process to efficiently test cross-reactivity of anti-human FFPE and frozen antibodies in human tissues to incorporate the efficient ones in an IMC panel suitable for skin sections. We developed an innovative IMC panel allowing the analysis of key structural skin components, main dermis and epidermis resident cells and infiltrating immune cells, at once. This panel allows the characterisation of human skin sections. The computational analysis of the obtained high-plex images gives access to skin inflammation mechanisms, skin structure integrity and breakdown and skin regeneration from one image.
CONCLUSION: Such a comprehensive and integrative analysis drives to a global scheme that depict skin balance breakdown and allows deeper investigation of products' effects.

Keywords: Imaging technologies, OMICs, high-plex spatial biology, new generation tissue imaging, biomarker identification.

19.) Calibrating liquid tweezers for mechanocytometry

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With a throughput of 1000 cells per second, Real-Time Deformability Cytometry (RT-DC) is a fast method for studying the mechanical properties of cells or other micron-sized objects. The objects are deformed in a microfluidic channel due to hydrodynamic stresses. However, for a sensitive readout, the channel dimensions need to be chosen to be on the same length scale as the objects of interest.
Virtual fluidic channels as an implementation of a pair of liquid tweezers overcome this limitation since they can be adapted to the object size within seconds. This flexible liquid-based microfluidic system can be achieved by two different polymer solutions in the sample and sheath flow of a cytometric setup, creating a co-flowing liquid-liquid interface. The surface tension of the interface creates a normal force superpositioned to the hydrodynamic forces, which can be utilized to mechanically probe cells as well as other objects. Here, we would like to investigate the forces exerted by the interface onto the deforming objects. For that, we study oil droplets with known mechanical properties utilized as test particles deforming in interaction with the virtual fluidic channel walls.
I established oil droplets as single and double emulsions in a size range of 1 µm to 35 µm. In the virtual fluidic channel, the droplets deform while surrounded by a solution of methylcellulose. Additionally, the microfluidic system and procedure were optimized for hydrophilic solutions. With the system established, the surface tension of the liquid-liquid interface can be approximated depending on the flow velocity, polymer type, polymer chain length, and polymer concentration. Once calibrated, the mechanical properties of cells and other small objects can be determined from an indentation test by the liquid tweezers.

20.) Spatial immunological analysis of novel immune-mediated liver diseases during the pandemic

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Background
During the pandemic, a rise in cases of severe hepatitis in children of unknown origin has been noted. Several hypotheses are discussed, such a connection to HAdV

infections in combination with a second viral infection or exuberant post-COVID immunity.

Methods

Immune and non-immune cell phenotyping of 13 formalin-embedded liver tissue sections obtained from pediatric patients presenting with acute hepatitis (≤16 years old, ALT (U/L) >500) was performed using imaging mass cytometry (IMC). The 40+ marker panel focused on liver parenchyma and immune markers related to proliferation, activation, and T cell exhaustion. SARS-CoV detection was performed using PCR, serology, immunofluorescence, and immunohistochemistry.

Results

7/13 patients displayed pronounced periportal immune cell infiltrations accompanied by elevated liver enzyme levels. Notably, CD8 T cells were found to be the predominant immune cell population, displaying varying degrees of exhaustion. 2 patients tested positive for HAdV^{+}. In 11 out of the 13 biopsies, SARS-CoV was detected. Additionally, ACE2^{+}/CX3CR1^{+} macrophages were observed to aggregate around regions exhibiting SARS-CoV^{+} signal.

Conclusions

Our findings highlight the presence of SARS-CoV antigen in the liver tissue of pediatric patients with acute hepatitis of unknown etiology that occurs after resolution of clinically manifest Covid19, suggesting a potential association between SARS-CoV infection and the observed hepatic manifestations.

21.) Comparison of open-source data batch normalization tools for the use on imaging mass cytometry data

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22.) Tackling the question of cellular heterogeneity: Microfluidicsingle-cellcultivation formammalianbioprocesses

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Cellular heterogeneity in mammalian bioproduction processes is under the suspicion to be responsible for unexpected process outcomes and impaired process robustness [1]. Yet, this assumption has not been investigated systematically in terms of bioprocess development and optimization.

Tackling this lack of knowledge, we developed a PDMS-glass based microfluidic single-cell cultivation (MSCC) platform for the analysis of heterogeneity over the last years. By successfully benchmarking our setup against conventional cultivation systems (shake flask, lab-scale bioreactor) we lay the foundation for subsequential systematic investigation of bioprocess related key performance indicators [2]. Applying industrially relevant Chinese hamster ovary (CHO) cells, first results already showed heterogenous growth behavior [3], heterogeneity in single- cell productivity [2], cell-to-cell differences concerning cell cycle progression, and cellular heterogeneity related to osmotic stress response [4] – all of it detected in isogenic populations. Perspectively, MSCC might also be applied for the characterization of high producing clones during cell line development or the development/optimization of cultivation media.

Our results show that cellular heterogeneity is omnipresent in CHO cell lines and can have a distinct effect on bioprocess key performance indicators. Additionally, MSCC holds a huge potential for systematic heterogeneity studies and other fields of application. For future implementation in bioprocess development discussion of chances, upcoming challenges, and required improvements is indispensable.

23.) Prediction of chemical sensitizing hazards by activation-induced marker (AIM) assays and T cell receptor repertoire analysis

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Many chemicals exhibit sensitizing properties and may induce allergic contact dermatitis, a T cell-mediated skin disease. However, none of the OECD-validated *in vitro* test for the risk assessment of skin sensitization addresses chemical-specific T cell activation.
We establish activation-induced marker (AIM) assays to detect chemical-specific T cells in human PBMCs. Using concentration series, we carefully control for interferences with flow cytometry, toxicity as well as monocyte (antigen-presenting cell) and T cell function. As examples, the hair dye *p*-phenylenediamine (PPD) and a sensitizing plant protection product were analyzed. Both chemicals induced upregulation of CD154 and CD137 on CD4+ and CD8+ memory T cells, respectively. For PPD, higher frequencies in allergic individuals were identified (e.g., 0.54% vs. 0.01% CD154+CD4+, 16 h, n = 7 - 14). TCR sequencing revealed extensive cross-reactivity to Bandrowski's base (BB), an oxidation product of PPD. Thus, the PPD and BB-induced epitopes seem structurally related. Regarding the plant protection product, a sensitizing co-formulant containing lignosulfonic acid (>90%), tested positive in the AIM assay (~0.02%, 5 h, n = 4).
In conclusion, AIM assays represent an efficient approach to detect chemical-specific T cells and may thus close current gaps in chemical allergy diagnosis and predictive sensitizer testing.

24.) Fluorescence lifetime flow cytometry as a membrane tension probe

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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 combined deformability cytometry with fluorescence lifetime flow cytometry 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. 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. 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.
In summary, our results emphasize the importance of the cellular membrane for the mechanical stability of cells. Given the fact that the dynamics of membrane-bound organelles play a major role in cellular homeostasis, we strongly believe that this approach allows for answering questions that have not been accessible earlier, e.g., the role of membrane tension for mitochondrial fission and fusion.

25.) Enolase represents a metabolic checkpoint controlling the differential exhaustion programs of hepatitis virus-specific CD8⁺ T cells

Frances Winkler[1,2], Anna V. Hipp[1], Carlos Ramirez[3], Bianca Martin[1], Matteo Villa[4], Emilia Neuwirt[2,5,6], Oliver Gorka[5,6], Jeroen Aerssens[7], Susanne E. Johansson[7], Nisha Rana[1], Sian Llewellyn-Lacey[8], David A. Price[8,9], Marcus Panning[10], Olaf Groß[5,6], Erika L. Pearce[4,11], Carl M. Herrmann[3], Kathrin Schumann[12], Luciana Hannibal[13], Christoph Neumann-Haefelin[1], Tobias Boettler[1], Percy Knolle[14,15], Maike Hofmann[1], Dirk Wohlleber[14], Robert Thimme[1], Bertram Bengsch[1,6,16]

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[15] German Center for Infection Research (DZIF), Munich Partner Site, Munich, Germany

[16] German Cancer Consortium (DKTK), partner site Freiburg, Freiburg, Germany

Background

Exhausted CD8⁺ T cells with impaired function are enriched in chronic HBV- and HCV-infected patients. Metabolic regulation is a contributing mechanism to CD8⁺ T cell dysfunction. However, it is unclear how metabolic programs relate to different exhaustion states, and whether metabolic checkpoints regulate severe dysfunction.

Methods

The exhaustion and metabolic state of virus-specific CD8⁺ T cells from chronic HBV- (n=31) and HCV-infected patients (n=52) were determined *ex vivo* by metabolism-directed high-parametric flow cytometry. Metabolic pathways were assessed by transcriptional profiling and metabolic checkpoints were tested *in vitro*.

Results

HBV- and HCV-specific CD8⁺ T cells exhibit different metabolic profiles associated with their exhaustion states. Mitochondrial impairment despite intact glucose uptake was prominent in severely exhausted HBV polymerase- and HCV-specific CD8⁺ T cell responses. In contrast, relative metabolic fitness was observed in HBV core-specific responses. We identified enolase 1 as a metabolic checkpoint in exhausted CD8⁺ T cells. Metabolic bypassing improved glycolysis and CD8⁺ T cell effector function.

Conclusions

Metabolic programs of hepatitis virus-specific CD8⁺ T cells are strongly connected to their exhaustion severities. Our results highlight enolase as a metabolic regulator of severely exhausted CD8⁺ T cells and link differential bioenergetic fitness with different exhaustion subtypes, with implications for therapeutic intervention.

Klaus Goerttler Session

3:00 pm, PEH

Chair: Elisa Rosati, Klaus Goerttler Awardee 2022

Directly exploiting the immune system to fight disease is considered key for next generation precision medicine. Accordingly, recent years have seen the exponential arise of personalized immunotherapies and related research. In particular, T cell immunotherapies have started to draw attention not only in the context of cancer therapies but also in the context of infection, chronic inflammation and autoimmunity. Moreover, the concept of tolerogenic immunotherapy, aiming to induce regulatory T cell responses against for example autoimmune antigens, increased even more the possible range of applications of such therapeutic approaches. Therefore, innovative technologies are necessary to deeply investigate the different aspects of T cell immunity including (i) mechanisms of interaction with antigen presenting cells, B cells and others as well as (ii) potentially exploitable pathways of differentiation into regulatory or conventional T cell phenotypes in conjunction with (iii) the investigation of possible therapy delivery systems. This year Klaus Goerttler Session will provide conceptual and technological insights in some of such key topics in order to be able to answer the many open questions in the field and support the development of new research and therapeutic strategies.

Enzymatic labeling of cell cell interactions in vivo

Giulia Pasqual

Laboratory of Synthetic Immunology, Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy, Veneto Institute of Oncology IOV-IRCCS, Padova, Italy

Interactions between different cell types are key for immune function. By coupling chemical and synthetic biology with mouse genetics, we developed innovative technologies to study cell-cell communication in the immune system and beyond. We will show how these approaches can be exploited to dissect interactions between dendritic cells and T cells in the context of T cells activation.

Single cell approaches to dissect antigen specific T-cell interactions in atherosclerosis

Bram Slütter

Div. of BioTherapeutic, LACDR, University of Leiden, Leiden, The Netherlands

Atherosclerosis is a lipid-driven chronic inflammatory disease and the primary cause of cardiovascular diseases, including heart attack and stroke. Despite the successful introduction of lipid-lowering drugs, atherosclerosis is still a leading cause of death and there is an urgent need for novel therapeutic options targeting the inflammatory arm of this disease. We hypothesize that inflammation is sustained by self-reactive T-cells and this “autoimmune-like” component provides new therapeutic targets. Using single cell RNA sequencing we have mapped the major T-cell subsets in human atherosclerotic lesions. To identify whether these T-cell subsets are drivers of disease we have combined single cell RNA sequencing with TCR sequencing and identified distinct CD4+ T-cell subsets that show clonal expansion, plaque specific accumulation and recent antigen engagement. We compared these CD4+ T-cell subsets with RNA seq data from bonafide autoimmune disease such as psoriatic arthritis and showed clonally expanded CD4+ T-cells from atherosclerotic plaque greatly resemble expanded CD4+ T-cells from synovial fluid of PSA patients. Thus our work strongly suggests self-reactive CD4+ T-cells accumulate in

atherosclerotic lesions and undergo antigen specific activation. To understand what drives this process we applied immunopeptidomics and screened epitopes presented by HLA molecules. We observed a large number of ApoB100 derived peptides presented in the plaque and selected they highest affinity binders to generate a peptide pool capable of detecting self-reactive CD4+ T-cells in CVD patients. We show that the size of the circulating ApoB100 specific CD4+ T-cell pool positively correlates with disease stage, suggesting these T-cells are associated with disease progression.

Klaus Goerttler Awardee
Molecular characterization of the transcriptional co-activator BOB.1/OBF.1 in B versus T lymphocytes and its specific contribution to the Germinal Center Reaction

Annika Betzler

Department of Oto-Rhino-Laryngology, Ulm University Medical Center, Ulm, Germany & Core Facility Immune Monitoring, Ulm University, Ulm, Germany

BOB.1/OBF.1 is a lymphocyte-specific transcriptional co-activator involved in octamer-dependent transcription. BOB.1/OBF.1 activates octamer-dependent transcription by its synergistic interaction with Oct proteins, thereby regulating the expression of genes essential for lymphocyte physiology.

We detected a novel previously unknown function of BOB.1/OBF.1, namely its involvement in Peyer's patch (PP) development by affecting lymphotoxin signaling during embryonic PP organogenesis and postnatal lymphocyte homing by affecting the CXCL13-CXCR5 signaling axis. The most striking characteristic of BOB.1/OBF.1-deficient mice is the complete absence of germinal centers (GCs). To further elucidate the precise contribution of BOB.1/OBF.1 to the GC reaction, a mouse system, which allows the conditional deletion of BOB.1/OBF.1 at different stages of B cell development in the presence of immunocompetent T cells and vice versa, was established and analyzed. Thereby, a requirement of BOB.1/OBF.1 during complete B cell ontogeny for efficient GC formation was revealed. Our mouse model showed that the failure to form GCs is a GC B cell autonomous defect and not exclusively a consequence of defective early B cell maturation. Hitherto, the GC defect was mainly attributed to the defective B cell compartment, while the contribution of BOB.1/OBF.1 expression in T cells for the GC reaction was unknown. Analysis of our conditional mouse system proved that BOB.1/OBF.1 expression in T cells is also required for efficient GC formation. Consequently, the contribution of BOB.1/OBF.1 to the GC reaction cannot be exclusively attributed to its expression in B cells.

Guest Lecture

5:00 pm, PEH

Chair: Henrik Mei

Inflammasome function in health and disease

Eicke Latz

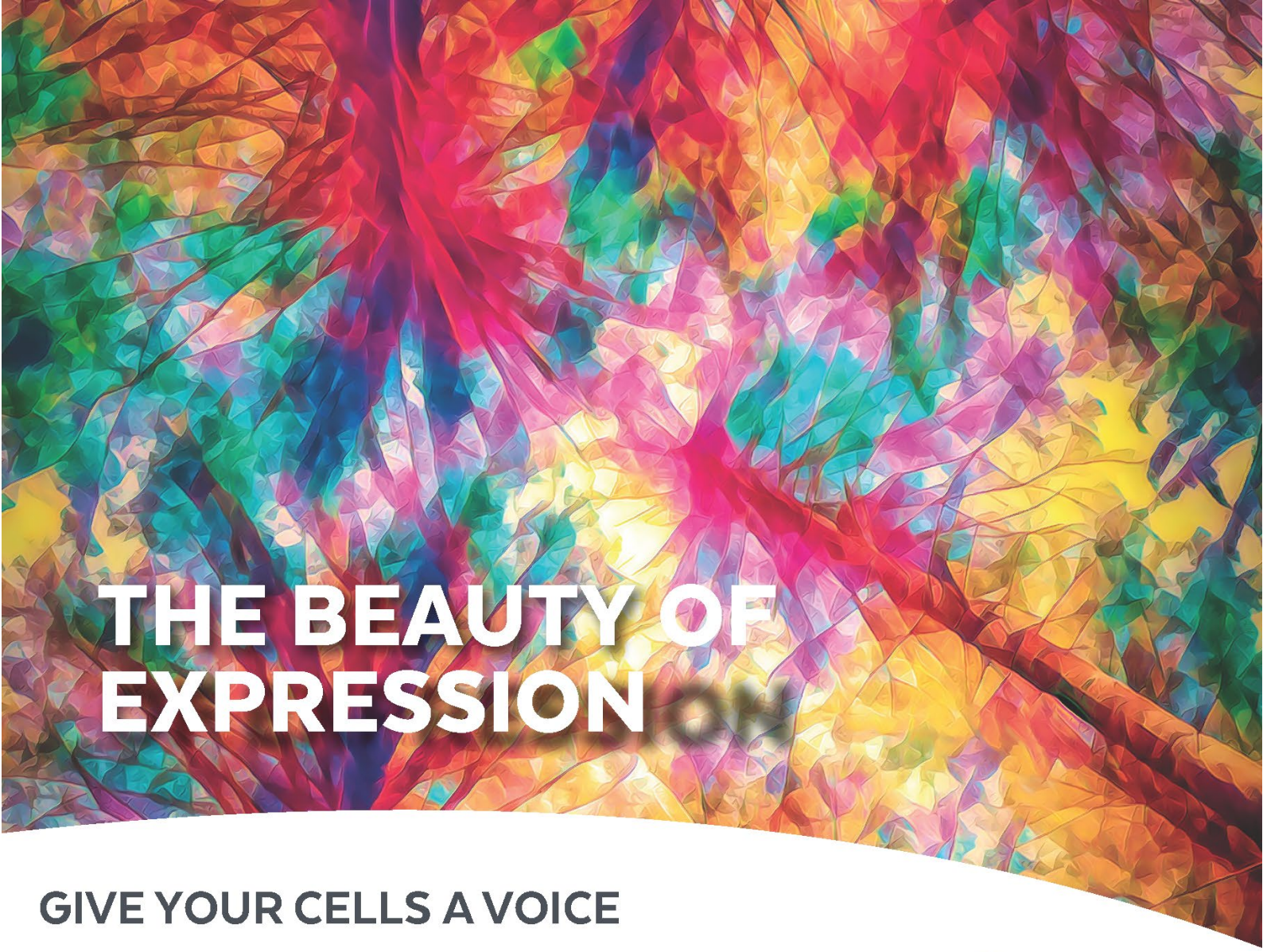
Deutsches Rheuma Forschungszentrum Berlin (DRFZ), a Leibniz-Institute | Charité -Universitätsmedizin Berlin, Berlin, Germany

Inflammasomes represent key components of the innate immune system that play a central role in maintaining immune homeostasis and orchestrating immune responses. These multiprotein complexes are responsible for the activation of caspase-1, leading to the maturation and release of pro-inflammatory cytokines, such as IL-1β and IL-18. In health, inflammasomes are involved in host defense against microbial infections, tissue repair, and maintenance of tissue integrity. However, dysregulation of inflammasome function can contribute to the pathogenesis of numerous diseases. Inflammasome dysfunction has been implicated in various inflammatory disorders, including autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, as well as chronic inflammatory conditions like atherosclerosis and inflammatory bowel disease. In addition, abnormal inflammasome activation has been linked to neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Furthermore, mounting evidence suggests a role for inflammasomes in the development of metabolic disorders, including obesity and type 2 diabetes and the NLRP10 inflammasome appears to be critical for skin and gut tissue homeostasis.

Understanding the intricate mechanisms underlying inflammasome activation and regulation is crucial for developing therapeutic strategies targeting these complexes. Several inflammasome inhibitors have shown promising results in preclinical and clinical studies, highlighting the therapeutic potential of modulating inflammasome function. Furthermore, the identification of specific inflammasome-associated biomarkers may aid in the diagnosis, prognosis, and monitoring of various diseases. Flow cytometric approaches have been developed that allow monitoring inflammasome activation by virtue of formation of the large inflammasome protein complexes. Monitoring inflammasome activation in patients holds promise for the development of novel treatments aimed at mitigating the detrimental effects of inflammasome-driven inflammation in various health conditions.

And then...

7: 00 pm Networking Event – Meet the speakers at the DRFZ



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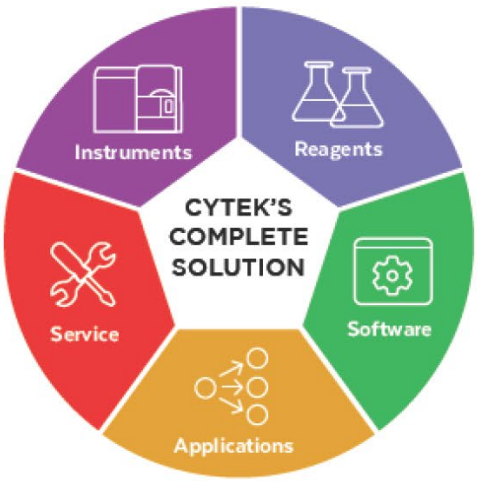

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Thursday, 21.09.23

Mechanocytometry Session

9:00 pm, PEH

Chairs: Marta Urbanska & Oliver Otto

Integrating a biophysical perspective into the description of cellular behaviors fosters a comprehensive understanding of health and disease complementing the biochemical approach often followed in biology. In its broad understanding, mechanocytometry encompasses all methods that measure mechanical properties of cells, such as stiffness or deformability. These properties reflect the state of the cytoskeleton, the cell membrane and the organelles and are thus considered a label-free biomarker for cell and tissue function. In this year's session we will focus first on the role of the cytoskeleton for cell migration and cell polarity (Franziska Lautenschläger, Universität des Saarlandes). Second, we will shed light on the question how smart materials and microfluidic technologies contribute to our understanding of mechanobiology (Salvatore Girardo, Max-Planck-Insitut für die Physik des Lichts, Erlangen).

Cytoskeletal fibres as building blocks for life

Franziska Lautenschläger

Universität des Saarlands, Saarbrücken, Germany

The cytoskeleton is a fibrous network of biopolymers with incredible functions in living cells. In my lab, we study the role of the cytoskeleton in the structure, properties, state, and movement of living cells. For example, we study how the cytoskeleton determines the shape of cells, their mechanical properties, and their ability to adhere or migrate. The cytoskeleton consists of three different subtypes, namely actin, microtubules and intermediate filaments. In this talk, I will specifically report on the role of microtubules in immune cell migration and their mechanical properties. I will also discuss the differences in mechanical properties between adherent and suspended cells.

Harnessing Microfluidic Technologies for Advancing Mechanobiology

Salvatore Girardo

Lab-on-a-chip Systems Technology Platform, Max-Planck-Insitut für die Physik des Lichts & Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany

Microfluidics is a field that involves the manipulation of small volumes of fluids on the microscale. In recent years, microfluidics has become increasingly relevant due to its ability to provide new insights into cellular and molecular processes, enabling the development of high-throughput and sensitive assays for disease diagnosis, drug discovery and personalized medicine. The flow of cells inside tailored design microfluidic chips can enable a high-throughput and sensitive analysis and sorting of cells based on their physical phenotype. Furthermore, droplet microfluidic technologies provide a unique tool for the development of tailored soft microgel

beads mimicking cell physical properties, such as size and elasticity. The development of reliable, simplified and standardized microfluidic-based technologies is essential to enable their broader use and facilitate the translation of research findings into practical applications. Here we illustrate a portfolio of microfluidic chips and standardized cell-mimicking microgel beads rationally designed for enabling the analysis, sorting, and mimics of cells based on their physical properties. Their development was carried out to improve the performance of microfluidic-based technologies by looking at their easy usage, improve performance and reliability. These techniques have the potential to impact various fields, including biophysics and medicine enabling a unique, faster, sensitive, and cost-effective analysis and manipulation of cells.

Short Talk
De novo identification of universal cell mechanics gene signatures

Marta Urbanska

Biotechnology Center, CMCB, TU Dresden, Dresden, Germany; Max Planck Institute for the Science of Light & Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany; Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

Cell mechanical properties determine many physiological functions, such as cell fate specification, migration, or circulation through vasculature. Identifying factors that govern the mechanical properties is therefore a subject of great interest. Here we present a mechanomics approach for establishing links between single-cell mechanical phenotype changes and the genes involved in driving them. We combine mechanical characterization of cells across a variety of mouse and human systems with machine learning-based discriminative network analysis of associated transcriptomic profiles to infer a conserved network module of five genes with putative roles in cell mechanics regulation. We validate in silico that the identified gene markers are universal, trustworthy and specific to the mechanical phenotype, and demonstrate experimentally that a selected target, CAV1, changes the mechanical phenotype of cells accordingly when silenced or overexpressed. Our data-driven approach paves the way towards engineering cell mechanical properties on demand to explore their impact on physiological and pathological cell functions.

Short Talk
Explainable artificial intelligence image analysis for blood cell discrimination

Stefan Simm

Institute of Bioinformatics, University Medicine Greifswald; Institute of Bioanalytics, Coburg University of Applied Science and Art, Greifswald and Coburg, Germany

In terms of decision support processes for high throughput methods it is of major importance to create explainable output for further analysis. In the field of artificial intelligence (AI) and Machine Learning (ML) the addition of explainable components to neural networks (NN) allow the interpretation of classification outputs by finding important features within the input for the decision. As high-throughput imaging approaches of single cells via real time deformability cytometry (RT-DC) create huge imaging datasets we wanted to create an explainable AI (XAI) to discriminate between different cell types within a PBMC blood sample to improve real time sorting without fluorescent labelling of the cell types for later single cell or bulk RNA-Seq analysis on pure cell populations.

For this reason, we used a cohort of ~100 probands with fluorescently labelled blood samples to discriminate between CD19 (B-cells), CD14 (Monocytes), CD3, CD4 or CD8 (T-cells) receptor protein concentrations. After assignment of ~1mio. cells based on an inter-individual 95% quantile threshold we divided in seven cell types and analyzed the possibility to classify them without fluorescence signal information on whether the raw input images via convolutional neutral networks (CNN) or extracted image features like the mechanoprofile via Support Vector Machine (SVM). By the images as well as the mechanoprofile most

B cells and Monocytes can be correctly predicted by the CNN and SVM. For the populations of T helper cells (CD3+, CD4+, CD8-) and T suppressor cells (CD3+, CD4-, CD8+), which share a comparable cell size, e-modulus was the most important feature to separate the groups as well as T and B cells differ in the feature: size_y. On the raw images we could observe differences in the importance at the different locations at the membrane to discriminate between the cell types.

Microbiology Session

11:00 am, PEH

Chairs: Lisa Budzinski & Christin Koch

Bacterial communities have a profound role in human health, environmental systems and industrial processes, which relies on their cellular function and properties – therefore this years' microbiology session again explores cutting edge technologies in microbial single-cell analysis.
The genetic diversity of microbiomes has been assessed extensively by sequencing technologies, but to elucidate the function of bacterial cells it requires different technological approaches. Single-cell analysis tools enable the investigation of bacterial metabolism such as adaption processes and are essential to contextualize microbial communities in their hosts or environments. Shifting from bulk to single-cell information brings potential of targeted control of microbial processes and allows to understand taxonomic shifts in bacterial communities functionally.
This session will highlight innovative approaches and applications contributing to the understanding of microbiomes in regards to composition, function and management

Probing microbiome function using single-cell chemical imaging

Fatima C. Pereira
University of Southhampton, Southhampton, United Kingdom

Humans and other animals host diverse communities of microorganisms that play fundamental roles in their physiology and health. In order to understand how microorganisms interact with and shape the environments that they inhabit, analysing the phenotype of cells in their native habitat is essential. Stable isotope probing (SIP) is a key tool for this purpose, as it enables tracking of isotopically-labelled atoms into microbial biomarkers and/or cells of interest. We have developed and applied new SIP techniques that exploit non-destructive Raman microspectroscopy to enable the detection of stable isotopes in single cells of bacteria. These techniques can be coupled with methods to specifically identify the labelled bacteria, i.e., to link identity to function and activity. By combining heavy water as a general tracer for metabolic activity, Raman-activated cell sorting and mini-metagenomics we were able to identify a consortium of gut commensal microbes able to reduce pathogen colonization. More recently, we developed a novel high-throughput approach to combine stimulated-Raman scattering (SRS) with fluorescence in situ hybridization (FISH), which is 10-100 times faster than previous approaches, thereby enabling high-throughput single-cell SIP for the first time. We exploited SRS-FISH to identify individual responses of the human gut microbiome to nutrients and pharmaceutical drugs, as well as to detect drug bioaccumulation with unprecedented resolution. Overall, we show that single-cell Raman-SIP approaches are powerful for directly demonstrating microbial functions within complex communities.

Hunting systems for typical and atypical glycan processing in gut microbiomes, one cell at a time

Sabina Leanti La Rosa

Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway

The community of microbes inhabiting the gastrointestinal tract includes a large variety of bacterial species that collectively influence numerous aspects of host health and nutrition. Firmicutes and Bacteroidetes phyla are typically dominant, with specific symbiotic members supplying an arsenal of carbohydrate-active enzymes for the depolymerization and fermentation of otherwise indigestible complex carbohydrates to short-chain fatty acids. This talk will present some of our recent research, which utilizes methodological toolsets that combine traditional culturing, meta-omics (including next-generation sequencing and functional multi-omics), biochemistry and enzymology, to fully elucidate enzymatic pathways that microbes employ for the utilization of nutrients consumed by the host. This includes typical plant-derived polysaccharides, such as β-mannans, and atypical glycans such as the food additive xanthan gum. In particular, coupling of detailed knowledge of microbial saccharolytic mechanisms to unique structural features of β-mannans has allowed us to design intervention strategies to selectively engage beneficial microbes at species level. Additional application of multi-omics has enabled visualization of the impact of β-mannans on the gut microbiota composition and functions, unveiling interactions between key players in degradation of this fiber directly in complex endogenous animal microbiomes and elucidating mechanisms by which these microorganisms affect host biology. We demonstrate that a multi-faceted approach is needed for deciphering and implementing efforts to enhance host health and minimize disease by manipulating gut microbial composition and metabolism.

Short Talk

Impact of long term 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment on gut microbiome composition and glycosylation patterns in male and female C57BL/6 mice analyzed by flowcytometry

Doreen Reichert

IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

Toxic compounds can affect the microbiota of the gut directly or via liver toxicity. Microbiota changes can be studied by 16S sequencing, or by multi-parameter flow cytometry using, e.g., scatter characteristics and DNA-dyes. According to literature reports, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a compound notorious for its (immune-)toxicity, changes the microbial pattern in the gut. However, high TCDD doses were used, which might cause liver toxicity, resulting in bile acids changes. We asked here, if TCDD affects the gut microbiome also at very low doses in the absence of liver damage. Male and female C57BL/6 mice were orally administered 0.1µg/kg TCDD for 12 weeks (1000 times less than the LD50 in mice). This dosing was harmless for the gut and had only minimal impact on the liver in male mice. Next, we assessed the microbial patterns in fecal pellets and cecal samples of TCDD-exposed mice. 16S sequencing revealed no differences. Surprisingly however, analysis by flow cytometry identified significant differences in the microbiota community profile upon TCDD exposure (in female mice). While 16S identifies microbes by the genome, flow cytometry has the advantage that it might pick up in addition phenotypic changes such as glycosylation or a tendency of bacteria to stick together. We therefore stained the bacteria with a panel of fluorescent sugar-specific lectins. Indeed, this unveiled significant alterations in the glycosylation patterns of TCDD-treated mice versus controls. Again, this was more pronounced in female than in male mice. Dysregulation of glycosylation patterns by toxic compounds is a novel finding, which has implications for the health of the host because glycoconjugates govern biofilm forming, infectious behavior of bacteria,

and host immune responses. We posit that changes in glycosylation and bacterial community structure are relevant parameters in assessing the toxicity of chemical compounds in the gut in male and female mice.

Short Talk
Development of an Automated Online Flow Cytometry Method to Quantify Cell Density and Fingerprint Bacterial Communities

Juan Lopez Galvez
Helmholtz-Zentrum für Umweltforschung, Department of Environmental Microbiology, Leipzig, Germany

Cell density is an important factor in all microbiome research, where interactions are of interest. It is also the most important parameter for the operation and control of most biotechnological processes. In the past, cell density determination was often performed offline and manually, resulting in a delay between sampling and immediate data processing, preventing quick action. While there are now some online methods for rapid and automated cell density determination, they are unable to distinguish between the different cell types in bacterial communities. To address this gap, an online automated flow cytometry procedure is proposed for real-time high-resolution analysis of bacterial communities. On the one hand, it allows for the online automated calculation of cell concentrations and, on the other, for the differentiation between different cell subsets of a bacterial community. To achieve this, the OC-300 automation device (onCyt Microbiology, Zürich, Switzerland) was coupled with the flow cytometer CytoFLEX (Beckman Coulter, Brea, USA). The OC-300 performs the automatic sampling, dilution, fixation and 4',6-diamidino-2-phenylindole (DAPI) staining of a bacterial sample before sending it to the CytoFLEX for measurement. It is demonstrated that this method can reproducibly measure both cell density and fingerprint-like patterns of bacterial communities, generating suitable data for powerful automated data analysis and interpretation pipelines. In particular, the automated, high-resolution partitioning of clustered data into cell subsets opens the possibility of correlation analysis to identify the operational or abiotic/biotic causes of community disturbances or state changes, which can influence the interaction potential of organisms in microbiomes or even affect the performance of individual organisms.

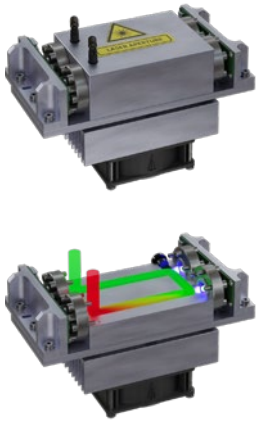
Label-free flow-cytometric bacteria sorting with angle-resolved scattered light signals

Daniel Kage
German Rheumatism Research Center, Berlin, a Leibniz-Institute – Flow Cytometry Core Facility, Berlin, Germany

The analysis and sorting of bacteria with conventional flow cytometers is challenging for two main reasons. First, few reagents for targeted staining of bacteria are available which makes it more difficult to specifically identify types and subsets as compared to eukaryotic cells. Second, with the small size of bacteria, most conventional flow cytometers operate at their detection limit since their optics are designed for larger cells.
To address these issues, we designed a flow cytometer comprising (1) signal processing electronics that acquire and store the whole time-resolved signal pulse shapes during particle transit through the laser beam, (2) optical components providing a reduced laser spot size for improved sensitivity, and (3) fiber arrays that allow for high-resolution scattered light detection at a multitude of scattering angles. Object classification and sorting is based on assigning the wavelet transform coefficients of the time- and angle-resolved signal pulses to clusters that were defined by the preceding analysis of defined cell populations. With this setup, we were able to distinguish and sort bacteria types solely based on time- and angle-resolved scattered light pulse shapes, without the need for fluorescent labels. Even bacteria with similar shapes, e.g. rod-shape (E. coli and Achromobacter spp.) that were indistinguishable in the scattered light signals obtained with conventional instruments could be clearly separated.

flowShield
UV-C decontamination for germ-free cell sorting

Introduction



Flow-through reactor equipped with 4 UV-C LEDs

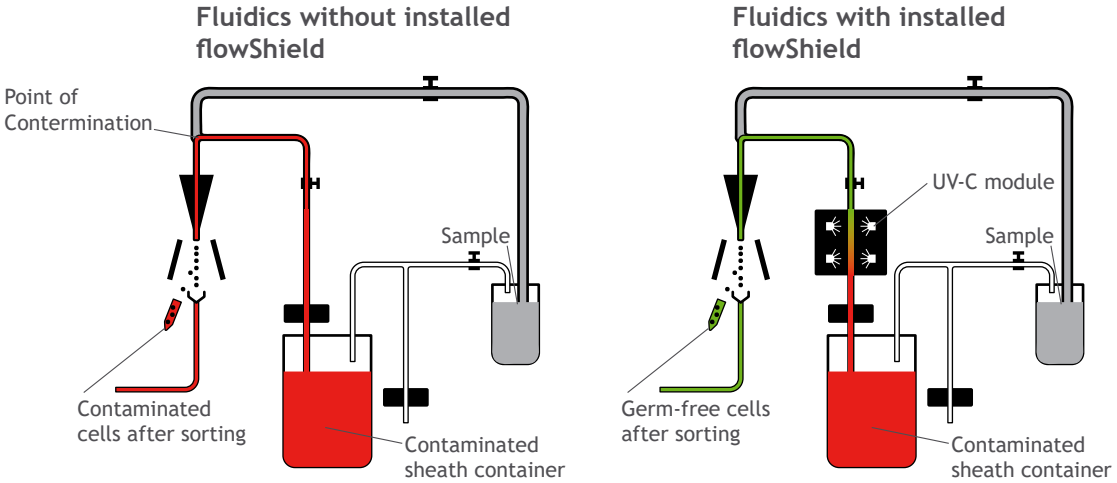
The contamination of the sheath fluid of a cell sorter by germs inhibits the cultivation of the sorted cells. Such contamination can happen, for example, when the sheath fluid reservoir is opened for refilling or through contamination by compressed air that is required for cell sorter operation. For this reason, decontamination is usually accomplished by regular flushing the fluidics with sodium hypochlorite or ethanol. However, such cleaning procedures are time consuming and residues of cleaning reagents in the fluidics are toxic for the cells of interest.

The lethal effect of UV-C light is well known for inactivating microorganisms without the side effects of a chemical treatment. The antimicrobial effect of UV-C light is based on the absorption of photons in the wavelength range of 200 – 280 nm by the DNA, resulting in the formation of pyrimidine dimers which inhibit DNA replication and consequently block transcription to RNA. This principle is used by the flowShield: UV-C emitting LEDs are combined in a flow reactor in such a way that the sheath fluid of a cell sorter is continuously irradiated. The resulting UV-C dose of four LEDs enables the reduction of P. aeruginosa by log 5.8.

Advantages

Using flow-through irradiation with UV-C LEDs to decontaminate the sheath fluid and liquid waste is an easy-to-use, robust, reliable and energy saving method.

Conventional inactivation of contaminated liquid waste (autoclaving, chemical inactivation) is energy and labor intensive. Waste inactivated using flowShield may save a lot of energy and precious time.



Nanotechnology

1:30 pm, PEH

Chairs: Ulrike Taylor and Wolfgang Fritzsche

The session deals with Extracellular Vesicles (EVs) and their characterization, with a special focus on exosomes. Exosomes are extracellular vesicles of 30-200 nm size generated by all cells, carrying nucleic acids, proteins, lipids, and metabolites. In the framework of liquid biopsy approaches, they are being investigated for their exploitation in early cancer detection, monitoring of disease progression and chemotherapeutic response, and development of novel targeted therapeutics.

Overview of current methods for the characterization of extracellular vesicles with a focus on single vesicle imaging and quantification

Melanie Schürz

Chemical Biology and Biological Therapeutics, Department of Biosciences and Medical Biology, University of Salzburg, Austria

Extracellular vesicles (EV) are nanosized biomolecules involved in cell- to – cell communication which are released by virtually all cells. Within the last decade they gained substantial interest due to their potential use as therapeutics, drug delivery system or biomarker. A big challenge in the field however is the intrinsic heterogeneity and complexity of EVs. To address this, different technologies including NTA (Nanoparticle tracking analysis), TRPS (tunable Resistive Pulse Sensing), DLS (Dynamic Light scattering), FCM (Flow cytometry measurement) and EM (electron microscopy) are commonly used to characterize extracellular vesicles. With the growth of the fields also more and more additional nano-specialized technologies including Nano-FCMs, FCS (fluorescence correlation spectroscopy) and imaging after capture on chips or on biofluids are emerging. All these technologies generally require dedicated instrumentation, expensive consumables and/or proprietary software not readily accessible to every lab, limiting their implementation for routine EV characterization in the rapidly growing EV field. In our group we focus on the development and establishment of robust, simple and low cost protocols for the capture, characterization (immune- labelling, validation of staining, validation and quantification of vesicle loading) and quantification of EVs at the single vesicle level. Additionally, we developed EVAnalyzer as an open source / open access plugin for easy, fast and automated quantification and gating of the vesicles from imaging data. EVAnalyzer can further be used for automated quantification of cell uptake at the single cell – single vesicle level, thereby enabling high content EV cell uptake assays and plate- based screens. Additionally, we will show how the program can be used for the in situ quantification of EVs in tissues as well as serum for applications investigating the biodistribution and pharmacokinetics of EVs in rodent or NHP *in vivo* models.

Comparing Apples to Apples: Analysis of Extracellular Vesicles by Flow Cytometry

André Görgens
Karolinska Institutet | Department of Laboratory Medicine
Division of Biomolecular and Cellular Medicine (BCM/MCG/BMM)
Stockholm, Sweden

Extracellular vesicles (EVs) including exosomes and microvesicles are submicrometer-sized biological vesicles released by all cells, and can be found in all body fluids or harvested from cell culture supernatants. It is nowadays widely accepted that EVs can serve as vesicular messengers in various physiological and pathophysiological contexts. Over the last 10–15 years, the EV research community has grown exponentially and the field has attracted a lot of attention following numerous studies connecting EVs to therapeutic approaches such as vaccination, antitumor therapy, immunomodulation and drug delivery. Since the protein composition and cargo of EVs is assumed to resemble the cell releasing them, EVs also have come into focus as potential diagnostic biomarkers.
Due to their heterogenous nature and their small diameter (<200 nm), it is challenging to accurately measure individual EVs, and to quantify basic parameters such as diameter, size, and concentration of EVs in a sample of interest. In this context, flow cytometry is increasingly used to detect, quantify and phenotype EVs, and newer high-sensitivity flow cytometers have become available and are explored to measure EVs. Current challenges, however, comprise sensitivity, standardization (despite general lack of appropriate standards) and comparability of obtained data. I will give an introduction into possibilities and share examples and experiences in context of EV analysis by flow cytometry in various contexts.

Short Talk
Imaging of Scattered Light in a Flow Cytometer to Analyse Size, Shape and Internal Structures of Particles and Cells

Martin Hussels
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Imaging flow cytometry combines the high information content of microscopic images with the high throughput of flow cytometry. In contrast to conventional imaging flow cytometers which – like a microscope – optically process the light coming from cells or particles into real-space images (brightfield or fluorescence), our home-built instrument records the angular intensity distribution of the scattered light of single cells and particles in the far field with fast cameras.
We investigated beads of different materials, synthetic dumbbell particles, as well as cells and bacteria from culture. We have compared measured scatter images with simulations of light scattering and have explored the possibilities for image analysis for the various cases. For spherical particles we used Lorenz-Mie theory to model the far-field intensity to determine the diameter and refractive index of single particles.
For more complex, non-spherical objects, we have performed light scattering calculations using the discrete dipole approximation (DDA). Since cells and bacteria are much more complex and variable than synthetic particles, we compared simulations by simple modelling approaches with measurements to understand the impact of shape and internal structure on light scattering.
In conclusion, the recorded angular intensity distributions contain substantial information about the size, shape, refractive index, and possible internal structures of the scatterers.

High-resolution image-activated cell sorting at low shear stress

Michael Kirschbaum

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Flow cell sorting based on image analysis is a powerful concept that exploits spatially-resolved features in cells to isolate previously inaccessible cell types.
Here, we present a low-complexity microfluidic approach that enables image-activated cell sorting based on high numerical aperture fluorescence microscopy, achieving an unprecedented level of image quality and resolution (i.e. 216 nm).
Unlike previously published systems, we do not constrict the sample flow to align the cells for imaging, but handle them directly via negative dielectrophoresis (DEP). Without the need to constrict (and thus accelerate) the sample flow, we achieve an acceptable volume throughput at significantly lower flow velocities, greatly facilitating image acquisition and data processing and reducing shear stress.
Cells are aligned and centered in the focal plane via DEP electrodes before images of the cells are taken and analyzed for the presence of target cells. DEP electrodes are also used to sort and guide targets to the sample outlet. Using our approach, we sorted thousands of live T cells at sample volume throughputs in the lower µl min-1 range based on subcellular localization of fluorescence signals (e.g., membrane vs. cytosol) and recovered >85% of the target cells analyzed.
Thanks to the simple and robust concept of our protocol, a standard Windows PC is sufficient to operate the whole system, even when more advanced artificial intelligent computing is used for cell classification.

Farewell

3:00 pm, CCO

Chair: Henrik Mei

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