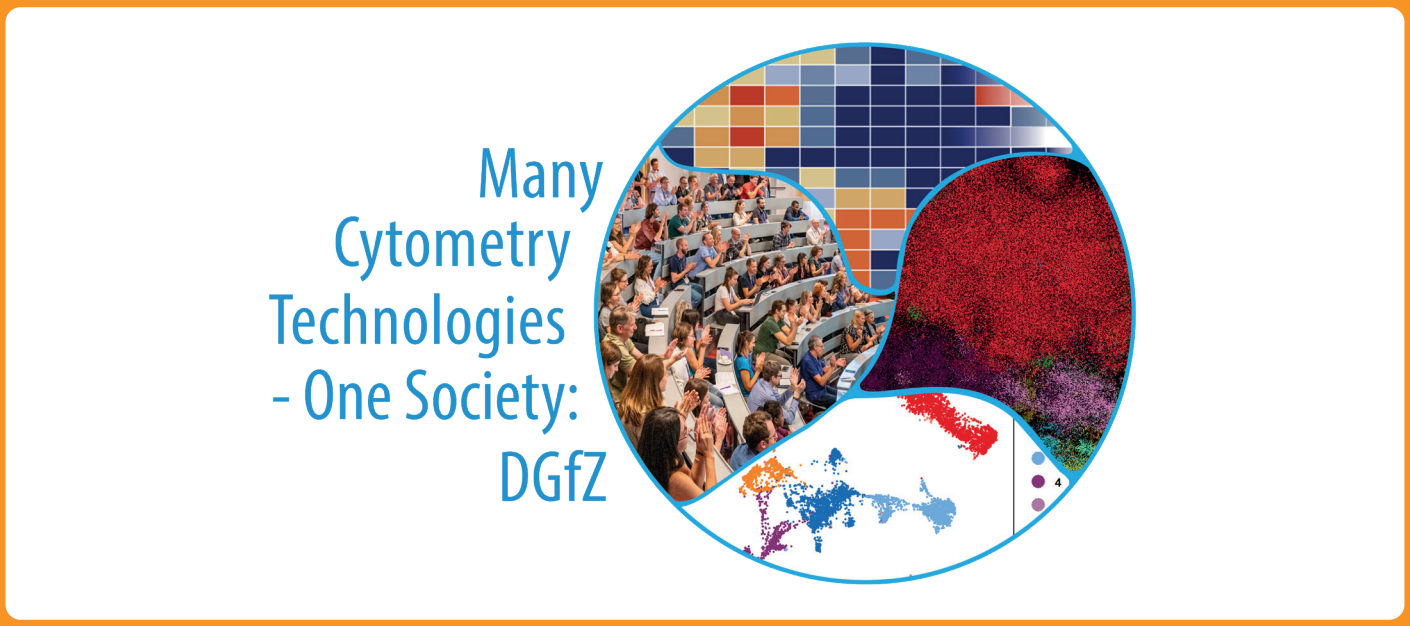
Abstract book

36th Annual Conference of the

German Society for Cytometry



Pre-Program

September 22 - 23, 2026

Tutorials and Welcome to the DGfZ - Young Cytometry

Conference

September 23 - 25, 2026

including Welcome Reception, Core Facility Networking Event
and Meet the Speaker

Venue

CRTD, Center for Regenerative Therapies Dresden
Fetscherstrasse 105, 01307 Dresden

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German Society for Cytometry
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Welcome to the 36th Annual Meeting of the German Society for Cytometry

It is our great pleasure to welcome you to the 2026 Annual Meeting of the German Society for Cytometry, taking place from September 22 to 25 at the Center for Regenerative Therapies Dresden (CRTD) under this year's theme:

"Many Cytometry Technologies – One Society."

This theme reflects what makes our community special: the diversity of cytometric technologies, expertise, and applications united by a shared commitment to quantitative cell science. By bringing together researchers, clinicians, core facility professionals, technology developers, industry partners, and early-career scientists, we hope to create a meeting that encourages exchange across methodological and disciplinary boundaries.

The 2026 program embraces this breadth from the very beginning. **Tutorials** and **hands-on activities** address topics ranging from FlowJo™ v11 and the measurement of submicron particles to Flow Vibe Coding, the financial management of core facilities, and a direct comparison of mass cytometry and spectral flow cytometry. The **"Welcome to the DGfZ – Young Cytometry"** meeting provides a dedicated opportunity for young colleagues to connect with our community.

The scientific sessions continue this broad perspective, with dedicated sessions on **Computational Cytometry**, **Mass Cytometry**, **Imaging and Spatial Transcriptomics**, **Clinical Applications**, and **Technical Developments** and **Emerging Technologies**. A **Core Facility Session** and the **Product Slam** further strengthen the exchange between scientific practice, technology development, and industry.

We are especially pleased to welcome the **Iberian Society for Cytometry** for this year's **European Guest Session**, continuing our tradition of strengthening international links within the cytometry community. Another highlight will be the **Klaus Goerttler Keynote** by Prof. Ulrich Keyser from the University of Cambridge, followed by the award ceremony for the this years **Klaus Goerttler prize winner** Dr. Tobias Walther. Beyond the lectures and sessions, we warmly encourage you to make full use of the **poster session**, the **comprehensive industry showcase**, and our **social activities** that promote informal exchange and collaboration.

On behalf of the German Society for Cytometry and the organizing committee, we thank you for joining us again in Dresden.

We wish you an inspiring, stimulating, and memorable meeting.

Best regards,

Claudia (Vice President) and
Oliver (President)
German Society for Cytometry

Tuesday 22.09.2026 - Pre program

12:00 pm – 2:30 pm

Hands-on Workshop: FlowJo™ v11 Software

This hands-on workshop introduces participants to the next generation of cytometric data analysis using FlowJo™ v11 Software. Through guided, practical exercises, attendees will analyze a shared high-dimensional data set while exploring the new immersive workbench and its core concepts.

3:00 pm – 5:30 pm

Tutorial 1 – Measuring Submicron Particles: Challenges, Theory, and Practical Solutions

By Jochen Behrends, Borstel and Anne Gompf, Technische Universität Dresden

5:30 pm – 6:30 pm

Welcome to the DGfZ – Young Cytometry

Whether you are new to the DGfZ or have been with us for years: come by, meet us and each other, and start the meeting together!
Backyard by the tent

5:30 pm – 6:30 pm

DGfZ Working Group "Mass Cytometry" – Members Meeting

Meeting Room (2.238), 2nd Floor

6:30 pm – 8:00 pm

Tutorial 2 – Calculating finances in Core Facilities

Chair: Claudia Giesecke-Thiel, Berlin
Speaker: Juliane Hoth, Dresden – Advisor Technology Platforms & Co-Speaker Financial & Legal Framework of Core Facilities, Elmar Endl, Bonn

As the focus here is on DFG-specific guidelines and technical terminology, the session will be conducted in German.

Wednesday 23.09.2026 - Pre program

9:00 am- 11:00 am

Tutorial 3 – Flow Vibe Coding

Tobias Kamman, PhD, Postdoctoral Fellowship Scientist, Basel, Switzerland

Learn how to use AI to “vibe code” custom R Shiny apps that automate the tedious parts of your flow cytometry analysis.

Requirement: please bring your laptop with R and RStudio pre-installed.

9:00 am - 11:00 am

Tutorial 4 – Mass Cytometry vs Spectral Flow Cytometry

Linda Hammerich[1], Axel R Schulz[2]
[1]Charité – Universitätsmedizin Berlin, Germany
[2]Mass Cytometry Core Facility, German Rheumatology Research Center (DRFZ), a Leibniz Institute, Berlin

Panel Design for Spectral Flow and Mass Cytometry: From Principles to Practice

Wednesday 23.09.2026 - Conference

11:00am - 11:45 am Registration & light lunch & Poster set up

11:45 am - 12:00 pm

Welcome by Oliver Otto, President DGfZ

12:00 pm - 1:00 pm

Core Facility Session

Chairs: Anne Gompf & Jochen Behrends

> Jochen Lamote, Leuven, Belgium

> Helena Jambo, Chur, Switzerland and Dresden, Germany

1:00 pm - 2:pm

European Guest Session: Sociedad Ibérica de Citometría (SIC)

Chairs: Oliver Otto, Greifswald, Jochen Behrends, Borstel

> Catarina Martins, PhD, Professora Auxiliar – Imunologia NOVA Medical School, Lisboa, Portugal

> Rebeca Sànchez Dominguez, PhD – Biomedical Sciences | Head of LACISEP – CIEMAT, Madrid, Spain

2:00 - 2:45 pm Coffee Break & Industry exhibition

2:45 pm – 4:00 pm

Session 1: Computational Cytometry

Chair: Bastian Höchst, München

> Lorenzo Bonaguro, Bonn, Germany

> Short talk: Maria Valeska Bianchi-Martin, Curiox

> Short talk: Marvin Weis, Tübingen, Germany

3:35 pm - 4:15 pm Coffee Break & Industry exhibition

4:00 pm - 6:00 pm

Klaus Goerttler Session

Chair: Oliver Otto

Klaus Goerttler Prize Winner

> Tobias Walther

Klaus Goerttler Lecture

> Ulrich F. Keyser, Cavendish Laboratory, University of Cambridge, UK

6:00 pm - 10:00 pm, Welcome Reception in the Exhibition area, sponsored by

Including time to view the posters until 10:00 pm.

7:30 pm - 10:00 pm

Core Facility Networking Event

Chairs: Désireé Kunkel, Sarah Warth

This Core Facility Networking Event is an opportunity to meet colleagues and share your experiences and challenges working in a core facility. We will have short presentations and hands-on (or rather brain-on) time on a selected topic for you, and lots of time for discussions afterwards.

We want to spend a wonderful evening with you at the DGfZ meeting 2026 in Dresden, with food & beverages & YOU!

Thursday, 24.09. 2026

8:30 am – 10:00 am

DGfZ Members Assembly

10:00 am – 10:30 am Coffee Break & Industry exhibition

10:30 am – 11:30 am

Session 2 – Mass Cytometry Session

Chairs: Axel R Schulz, Claudia Peitzsch

> **Ezgi Senoglu, CRTD Dresden**

> **Short Talk: Mehmet Serdar Kocag, Berlin**

> **Short Talk: Niclas Schierloh, Freiburg**

11:30 am – 11:45 pm Coffee Break & Industry exhibition

11:45 pm - 1:00 pm

Product Slam

Chairs: Elmar Endl, Tom Bauer & Lisa Budzinski

> **The industry partners will each present their latest innovative technological developments and products in three minutes**.

1:00 pm – 2:00 pm Lunch & Industry exhibition

2:00 pm – 4:15 pm

Poster Session & Industry exhibition

Chairs: Oliver Otto, Claudia Giesecke-Thiel

4:15 pm – 5:30 pm

Session 3 – Imaging and Spatial Transcriptomics

Chairs: Anja Hauser, Raluca Niesner, Berlin

> **Adrien Hallou, Kennedy Institute, Oxford, UK**

> **Short Talk: Deniz Tasgin**

> **Short Talk: Leonard Fiebig, Berlin, Germany**

We change the venue for the "Meet the Speaker" Event

5:45 pm - Departure by bus from the CRTD to the “Meet the Speaker” event.
Venue: Elbterasse Wachwitz, Altwachwitz 14, 01326 Dresden

6:00 pm – 11:00 pm

Meet the Speakers - sponsored by Stratocore S.A.S. & award ceremony - all participants are invited to join

At 11:00 pm, there will be a shuttle bus back to Hotel Terrassenufer and to Postplatz.

Friday, 25.09. 2026

9:00 am – 10:15 am

Session 4 – Clinical Applications

Chairs: Hyun-Dong Chang, Berlin, Axel Schulz, Berlin

> Birgit Sawitzki, BIH/Charité – Universitätsmedizin Berlin

> Short Talk: Adrián Barreno Sánchez, Berlin, Germany

> Short Talk: Bernadette L. Bramreiter Graz, Austria

10:15 am – 10:45 am Coffee Break & Industry exhibition

10:45 am – 12:00 pm

Session 5 – Technical Development/Emerging Technologies

Chairs: Michael Kirschbaum, Potsdam Toralf Kaiser, Berlin

> Marco Di Berardino, Fa. Amphasys, Zurich, Switzerland

> Short Talk: Bob Fregin, Greifswald, Germany

> Short Talk: Daniel Kage, Berlin, Germany

12:00 pm - 12:15 pm
Farewell

12:15 pm - 1:00 pm
Lunch (take away)

1:15 pm - 4:00 pm
DGfZ Board meeting & Program Committee

Tuesday 22.09.2026 - Pre program

12:00 pm – 2:30 pm

Hands-on Workshop: FlowJo™ v11 Software

Waters™ Biosciences Formerly BD Biosciences

This hands-on workshop introduces participants to the next generation of cytometric data analysis using FlowJo™ v11 Software. Through guided, practical exercises, attendees will analyze a shared high-dimensional data set while exploring the new immersive workbench and its core concepts. The session will highlight key functionalities including metadata-driven analysis, the unmixing platform, the graph gallery, virtual concatenation, chart generation, and integrated analysis algorithms, with a strong emphasis on efficient and reproducible workflows.

The workshop is designed to be interactive and accessible to participants with varying levels of experience in flow cytometry data analysis. Participants are expected to bring their own laptop with FlowJo™ v11 installed. Links to datasets and portal licenses will be shared and activated one week prior to the workshop, ensuring all attendees are prepared in advance.

Agenda / Topics covered

- Introduction to FlowJo™ v11 and the immersive workbench
- Metadata manager and metadata-driven workflows
- Quality control approaches in FlowJo™
- Keywords and parameter sets
- Compensation/unmixing and editing
- High-dimensional algorithmic analysis
- Statistical tables and data summaries
- Chart generation and visualization
- Report Editor and exporting results

3:00 pm – 5:00 pm

Tutorial 1 – Measuring Submicron Particles: Challenges, Theory, and Practical Solutions

Chairs: Jochen Behrends, Head Fluorescence Cytometry, Research Center Borstel, Evelyn Hammer, München and Anne Gompf, Head of Flow Cytometry Core Facility, Technische Universität Dresden

The analysis of small particles – such as extracellular vesicles (EVs), viruses, and nanoparticles – by flow cytometry remains technically challenging due to their small size, low refractive index, and limited signal generation. However, advances in instrumentation, calibration strategies, and reference materials have improved the detection and characterization of particles in the submicron range.

This tutorial provides an overview of the theoretical and practical aspects of measuring small particles by flow cytometry. The first part introduces key theoretical foundations, including light scattering, fluorescence detection, instrument sensitivity, and methodological limitations. It summarizes insights from state-of-the-art literature and current recommendations or guidelines in the field. Practical experience from our own work with small particle analysis will also be shared, highlighting common pitfalls, controls, and calibration approaches.

In the second part, practical strategies for optimizing small particle measurements will be presented, including instrument setup, thresholding strategies, sample preparation, and the use of appropriate reference materials.

The theoretical session is open to all participants. Due to technical limitations, the subsequent hands-on training at the flow cytometers (e.g. ImageStream, Discover S8, …) is limited to 18 participants. During this session, attendees will gain practical experience in instrument setup, optimization, and data acquisition for small particle analysis.

The tutorial aims to bridge theory and practice and provide guidance to improve the reliability and reproducibility of small particle measurements by flow cytometry.

5:30 pm – 6:30 pm

Welcome to the DGfZ – Young Cytometry

Our community is at the heart of the DGfZ, and the Meeting Point is a chance to come together and welcome everyone – with a special focus on our young scientists and new members of the society. In this informal setting, we will briefly introduce the DGfZ, our goals, motivation and vision, as well as the people behind the society and our Board. Most importantly, we would like to show you how you can become an active part of the DGfZ, contribute your ideas, connect with others and help shape our community. Whether you are new to the DGfZ or have been with us for years: come by, meet us and each other, and start the meeting together!

5:30 pm – 6:30 pm

DGfZ Working Group "Mass Cytometry" – Members Meeting

Chair: Axel Schulz
Meeting Room (2.238), 2nd Floor

We warmly invite all mass cytometry enthusiasts to join the new DGfZ Working Group "Mass Cytometry" Members Meeting. Whether you are an experienced user, technology developer, facility staff member, or newcomer to the field, this meeting offers an excellent opportunity to network with colleagues and contribute to the activities of the German Mass Cytometry Network (GerMaNet).

The newly established Working Group "Mass Cytometry" has recently been integrated into the German Society for Cytometry (DGfZ), providing a legal framework and organizational structure that will strengthen the visibility, collaboration, and long-term sustainability of the German mass cytometry community.

During this members' meeting, the different GerMaNet task forces will introduce themselves and provide brief updates on their ongoing activities, including:

- Public outreach and communication
- Membership and community development
- Mass Cytometry Club: regular networking meetings
- Organization of the annual German Mass Cytometry User Forum (GMCUF)
- Industry and sponsor relations

We welcome your ideas, feedback, and active participation. Join us to help shape the future of mass cytometry in Germany and expand our growing network.

6:30 pm – 8:00 pm

Tutorial 2 – Calculating finances in Core Facilities

Chair: Claudia Giesecke-Thiel

Juliane Hoth, Dresden – Advisor Technology Platforms & Co-Speaker Financial & Legal Framework of Core Facilities, Elmar Endl, Bonn

Preiskalkulation gemäß DFG Richtlinien

As the focus here is on DFG-specific guidelines and technical terminology, the session will be conducted in German.

Nutzungskosten sind wichtiger Bestandteil zur Refinanzierung von Core Facilities. Bei der konkreten Preiskalkulation stellen sich viele Fragen, z.B. welche Kostenbestandteile dort einbezogen werden und in welcher Höhe Personalkosten oder auch Geräteverschleiß angesetzt werden können.

Die DFG macht in ihrem Merkblatt 55.04 Vorgaben wie in ihren Förderprogrammen Nutzungskosten beantragt und kalkuliert werden können. Wir stellen an konkreten Beispielen vor, wie Preiskalkulation erstellt und welche Bausteine davon in DFG-Förderungen einbezogen werden können. Die Ergebnisse werden diskutiert im Zusammenhang mit dem Ziel der Anpassung der Nutzungskostenpauschalen bei der DFG für Durchflusszytometer und Zellsortierer (FACS).

Da hier ein Fokus auf DFG-spezifischen Richtlinien und Fachtermini liegt, wird der Workshop in deutscher Sprache durchgeführt.

Usage fees are an important component for the refinancing of core facilities. In the context of specific pricing calculations, a number of questions arise—for example, which cost components should be included and to what extent personnel costs or equipment depreciation can be accounted for.

In its guideline 55.04, the Deutsche Forschungsgemeinschaft (DFG) specifies how usage fees can be requested and calculated within its funding programs. Using concrete examples, we will present how pricing calculations are developed and which components can be included in DFG funding. The results will be discussed in the context of the goal of adjusting the DFG's usage fee lump sums for flow cytometers and cell sorters (FACS).

Wednesday 23.09.2026 - Pre program

9:00 am - 11:00 am

Tutorial 3 – Flow Vibe Coding

Tobias Kamman

PhD, Postdoctoral Fellowship Scientist, pharma Research and Early Development (pRED), F. Hoffmann-La Roche Ltd., Basel, Switzerland

Learn how to use AI to "vibe code" custom R Shiny apps that automate the tedious parts of your flow cytometry analysis. In this hands-on code-along session, we will build a functional, time-saving research tool from scratch to transform the way you analyze and visualize your everyday FlowJo results.

Requirement: please bring your laptop with R and RStudio pre-installed.

9:00 am - 11:00 am

Tutorial 4 – Mass Cytometry vs Spectral Flow Cytometry

Linda Hammerich[1], Axel R Schulz[2]

[1]Charité Universitätsmedizin Berlin, Germany

[2]Mass Cytometry Core Facility, German Rheumatology Research Center (DRFZ), a Leibniz Institute, Berlin, Germany

Panel Design for Spectral Flow and Mass Cytometry: From Principles to Practice

High-parameter cytometry has profoundly transformed our ability to resolve complex immune cell landscapes in a single experiment. Getting the most out of spectral flow and mass cytometry experiments starts at the panel design stage; long before the sample enters the instrument. Poor panel design remains one of the most common sources of data quality issues, from unresolved spectral overlap and autofluorescence interference in spectral flow cytometry, to isotope impurities, oxide formation, and mass spillover in mass cytometry.

This tutorial provides an introduction to panel design principles for both platforms side by side. We will cover the physical and biological sources of signal interference specific to each technology, discuss marker prioritization strategies based on antigen expression levels, and highlight how reagent choice impacts downstream data quality. A particular focus will be placed on the rapidly evolving landscape of AI-assisted panel design tools for spectral flow cytometry, including automated spectral unmixing simulation and fluorochrome compatibility scoring, which are beginning to reshape how panels are built in practice. We will also discuss the importance of selecting appropriate control samples, which are critical to evaluate the performance of a multi-parametric panel.

In the second half of the tutorial, participants will form two groups, one tackling a spectral flow cytometry panel design challenge, the other a mass cytometry challenge, and compete to design an optimized panel under realistic constraints. Results will be discussed and evaluated together, offering insight into the decision-making process and common pitfalls.

Wednesday, 23.09.2026 - Conference

12:00 pm

Core Facility Session

Chairs: Jochen Behrends, Borstel, Anne Gompf, Dresden

Core facilities are increasingly becoming innovation hubs that drive interdisciplinary research through the adoption and integration of emerging technologies. This session will explore how next-generation cytometry platforms, image-enabled cell sorting, AI-supported workflows, and digital communication strategies are reshaping the way core facilities operate, collaborate, and support scientific discovery.The session brings together perspectives from technology implementation and scientific outreach. Jochen Lamote will discuss the integration of advanced flow cytometry and image-based sorting technologies into modern research infrastructures, highlighting practical experiences from the VIB Flow Core Leuven and the adoption of next-generation instrumentation.Helena Jambor is a scientific information designer and data visualization expert. She combines art and science to create clear and engaging visuals that help communicate complex biomedical research. In our session she will share insights into how effective illustrations can transform scientific findings into accessible and comprehensible stories.Together, the speakers will provide insights into how technological innovation, data-driven workflows, and modern communication strategies can strengthen the role of core facilities as future-oriented partners in research and beyond. The session aims to stimulate discussion on user-centered service development, sustainable implementation, and the evolving role of core facilities in an increasingly digital and interdisciplinary research landscape.

Jochen Lamote

VIB Flow Core, VIB Technologies, and VIB Center for Cancer Biology, Department of Oncology, University of Leuven, Leuven, Belgium
https://vibneuroscienceleuven.be/en/flow-cytometry

Biography

Jochen Lamote is Head of the VIB Flow Core Leuven within VIB Technologies. He trained as a bioscience engineer at Ghent University and completed a PhD at the Faculty of Veterinary Medicine, developing flow cytometry protocols for porcine plasmacytoid dendritic cells. During his postdoctoral work at Johnson & Johnson, he advanced complex cell sorting and panel design workflows, combining intracellular staining with transcriptomics, and developed a strong interest in imaging cytometry through the Amnis ImageStream.

He founded a local FACS Expertise Center at the VIB-KU Leuven Center for Cancer Biology in 2020, which evolved into the institutional VIB Flow Core Leuven in 2023, supporting research in cancer biology, neuroscience, and microbiology.

As an early adopter of emerging technologies—including the BD FACSDiscover S8—Jochen combines innovation with a strong application-driven approach, aligning panel design and sorting strategies with what the biology and downstream workflows actually require. Within VIB Technologies, he works across core pipelines and collaborates with TechWatch, the VIB Flow Core Ghent, and the Flow Satellite in Antwerp to bring in new technologies when they are ready—and when they serve the purpose.

Helena Jambor

(https://www.fhgr.ch/en/persons/person/jambor)
Institute for Data Analysis, Artificial Intelligence, Visualization and Simulation, University of Applied Sciences of the Grisons, Chur, Switzerland andNational Center for Tumor Diseases, University Cancer Center, NCT-UCC, Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Dresden, Germany.

1:00 pm

European Guest Session – Sociedad Ibérica de Citometría (SIC)

Chairs: Oliver Otto, Greifswald, Jochen Behrends, Borstel

We are delighted to welcome the Sociedad Ibérica de Citometría (SIC). This special conference event celebrates collaboration, the exchange of experiences and organizational approaches among cytometry societies, and the strengthening of connections across the European cytometry community.

Representing SIC, Rebeca Sanchez Dominguez and Catarina Gregório Martins will share insights into the society's activities, achievements, and future perspectives, offering attendees a valuable overview of the Iberian cytometry community.

This session provides an opportunity to learn how SIC supports cytometry professionals throughout Spain and Portugal through education, training, networking, and the promotion of best practices. It also highlights the importance of cooperation among national and regional cytometry societies in advancing research, innovation, and professional development across Europe.

We are grateful to our guests for their participation, and we look forward to an engaging discussion that reflects the collaborative spirit of the cytometry community. Together, we continue to strengthen and expand the European cytometry network.

Catarina Martins

Catarina Martins, PhD, Professora Auxiliar – Imunologia
NOVA Medical School, Lisboa, Portugal

Biosketch

Catarina Martins is a Biologist with a PhD in Immunology and serves as Assistant Professor of Immunology at NOVA Medical School (NMS), Lisbon, Portugal. In addition to her teaching responsibilities, she coordinates the Biosafety Committee and is the Technical Director of the Immunology Laboratory. In her lab, she studies patients with primary immunodeficiencies (inborn errors of immunity) and other immune-mediated conditions, with extensive use of Flow Cytometry applications. Her work has contributed to more than 80 peer-reviewed publications in high-impact scientific journals.

Catarina has presented her research at numerous national and international conferences and has participated in workshops and training activities covering Immunology, Immunodeficiency, Flow Cytometry, and Quality in Clinical Laboratories. Since 2005, she has collaborated with National Accreditation Bodies in Portugal, Ireland, Slovenia, and the UAE as a quality assessor for standards such as ISO 15189, supporting quality management in clinical laboratories. She is also a CLSI advisor and actively collaborates with the NOVA Greenlabs task force, which achieved myGreenLab certification for NMS in 2025.

Within the Iberian Flow Cytometry Society (SIC), Catarina coordinates the Accreditation Working Group. After serving two terms as Board Member, she was elected Vice-President in 2025. In this role, she is committed to strengthening the Society's visibility, fostering national and international collaborations, and expanding its recognition while promoting excellence, quality standards, and accessible, high-quality training in Flow Cytometry.

Rebeca Sànchez Dominguez

PhD – Biomedical Sciences | Head of LACISEP – CIEMAT, Madrid, Spain

Biosketch

Rebeca Sánchez Domínguez is a biomedical researcher and specialist in Flow Cytometry and cellular analysis, currently Head of the Cytometry and Cell Separation Laboratory (LACISEP) at the Center for Energy, Environment and Technology Research (CIEMAT), Madrid, Spain. Her scientific activity focuses on hematopoietic stem and progenitor cells, the bone marrow microenvironment, hematopoietic regeneration, and the development and application of advanced Flow Cytometry approaches for biomedical research and translational studies.

She has contributed to more than 30 peer-reviewed scientific publications and has participated in national and international research projects in the fields of hematology, advanced therapies, stem cell biology and cellular analysis. Her research includes the characterization of hematopoietic stem and progenitor cells, analysis of the bone marrow niche and its regeneration following conditioning therapies, and the development of innovative approaches for non-genotoxic conditioning and hematopoietic transplantation.

Flamenco, Fado and Flow: - three decades crossing the borders of Cytometry with SIC

The Iberian Society of Cytometry (SIC) was established in 1992 with a clear mission: to promote the development of cytometry in Spain and Portugal, bringing together laboratories, researchers, and healthcare professionals while creating opportunities for education, collaboration, and knowledge exchange. Over more than three decades, SIC has accompanied the remarkable evolution of flow cytometry, becoming a reference partner for the Iberian cytometry community across both basic research and clinical practice. Its activities and collaborations have increasingly crossed national borders, establishing strong connections with cytometrists and scientific societies across Europe and Latin America and contributing to a broader international cytometry network. Currently, the Society counts 211 active members from 13 different countries.

From its early years, SIC recognized the importance of quality assurance to promote the development, particularly of clinical approaches. This has been a pilar of SIC's activities, and the external quality assessment program, established in 1994, currently involves more than 50 clinical laboratories in Spain, Portugal, and other European countries. The program is continually expanding and currently covers leukemia and lymphoma immunophenotyping, paroxysmal nocturnal hemoglobinuria, body fluid analysis, measurable residual disease in Multiple myeloma and Acute Lymphoblastic Leukemic (all the above co-organized with the Spanish Society of Hematology and Hemotherapy), with the recent addition of cutaneous lymphoma analysis. Furthermore, in collaboration with the Spanish Immunology Society, SIC also co-coordinates programs for quality assurance allied to the GECLID platform, that has brough responses to assays in Immunology, besides lymphocyte subsets (i.e., functional assays for phagocytes, T-cell proliferative responses, anti-CD20 and CAR-T cells monitoring, or enlarged lymphocyte immunophenotyping for immune monitoring).

In parallel, SIC congresses, courses, workshops, webinars, and specialized working groups have contributed to continuing education, harmonization of practices, and the development of collaborative networks across the different fields of cytometry. Currently, two SIC groups have continuous activities, i.e., group for Hematological Diagnosis by Cytometry (Diagnóstico Hematológico por Citometría de Flujo, DHC) and the group Accreditation in Flow Cytometry. The DHC group, established in 2016 and formally incorporated as a SIC working group in 2017, focuses on continuing education in the use of flow cytometry for hematological diagnosis. Its meetings are deliberately multidisciplinary and "open-door," bringing together hematologists, immunologists, pathologists, biologists, laboratory professionals, trainees and industry colleagues. Sessions typically use practical clinical cases, literature updates and discussion to share diagnostic experience. Additionally, the group organizes an annual clinical case competition for professionals in training, offering winners the opportunity to participate in cytometry-related training activities—such as receiving a mobility grant to support training at a recognized laboratory (preferably on the Iberian Peninsula) or attending courses or conferences. The Accreditation Working Group is centred on quality management and laboratory accreditation, particularly for clinical flow cytometry. Its activity became more regular from 2022 in response to members' increasing need to discuss practical accreditation challenges. The group addresses issues such as method validation, equipment and reagent management, personnel qualification and the application of standards such as ISO 15189. An important aspect is the exchange of experiences among laboratories in Spain and Portugal and the aim of strengthening dialogue with the respective accreditation bodies, ENAC in Spain and IPAC in Portugal.

Looking ahead, SIC is strengthening its international dimension and creating new opportunities for collaboration across the Mediterranean region. Recent collaborations and joint meetings with the Spanish and Portuguese Societies of Immunology, as well as with ESCCA, are helping to build stronger connections across the cytometry and immunology

communities. In 2026, this internationalisation will reach another milestone with the first Mediterranean Congress, jointly organised by SIC and the Association Française de Cytométrie in Granada, Spain. Furthermore, SIC seeks to address the challenges of a rapidly evolving discipline by promoting education in emerging technologies, interdisciplinary and international collaboration, and the involvement of the next generation of cytometrists. Initiatives such as the Margarida Lima Mobility Grant, interaction with effervescent local networks such as FLxFlow, and dedicated educational activities sponsored by the society in several collaborating centres of Spain and Portugal, reflect this commitment to capacity building and community renewal. Furthermore, SIC has a professional website, social media channels, and a quarterly newsletter that ensure continuous, high-quality communication, providing members with up-to-date information, useful resources, and direct access to the Society's scientific and educational activities.

In brief, more than a scientific society, SIC is a community built around knowledge sharing, quality, and collaboration. Its future challenge is to continue anticipating the evolution of cytometry, bringing science, technology, and clinical practice closer together while ensuring that Spain and Portugal maintain an active voice in shaping the future of the field.

Short abstract

Flamenco, Fado and Flow: - three decades crossing the borders of Cytometry with SIC

Founded in 1992, the Iberian Society of Cytometry (SIC) has become a reference community for cytometry in Spain and Portugal, bringing together 211 members from 13 countries. For more than three decades, SIC has promoted education, collaboration, quality assurance and knowledge exchange, supporting both clinical practice and research.

A strong commitment to quality and harmonization is reflected in its external quality assessment programs, currently involving more than 50 clinical laboratories, while its working groups, courses, congresses and educational initiatives foster continuous professional development.

Looking ahead, SIC is strengthening its international dimension, supporting the next generation of cytometrists and building new collaborations across Europe, Latin America and the Mediterranean.

More than a scientific society, SIC is a community built around knowledge, quality and collaboration, aiming to ensure Spain and Portugal maintain an active voice in shaping the future of the field.

2:45 pm

Session 1 – Computational Cytometry

Chair: Bastian Höchst, München

Recent advancements in high-dimensional cytometry have revolutionised our understanding of cellular heterogeneity, particularly within complex immune landscapes. However, the exponentialincrease in data dimensionality presents significantchallenges for traditional manual gating strategies, which are prone to bias and lack scalability. To address this, novel computational frameworks integrating machine learning and automated clustering algorithms are essential to extractrobust, unbiased biological insights. This session highlights the development and application of cutting-edge bioinformatics tools designed to streamline data preprocessing, batch-effectcorrection, and single-cell phenotyping.

Lorenzo Bonaguro

Group Leader Molecular and Translational ImmunomicsGerman Center for Neurodegenerative Diseases (DZNE), Bonn& University Hospital Bonn | Institute for Clinical Chemistry and Clinical PharmacologyDeep immunophenotyping of PBMCs from lung cancer patients to explore responses to immunotherapy combinations

cyCONDOR: end-to-end solution for high-dimensional cytometry data analysis

cyCONDOR provides a unified and open-source framework for the analysis of data from high-dimensional flow cytometry, spectral flow cytometry, mass cytometry (CyTOF), and proteogenomics (CITE-seq/Ab-seq) technologies. It accepts commonly used input formats (e.g. FCS files, including those exported from FlowJo) and emphasizes reproducibility, interpretability, and ease of use, making advanced cytometry analysis accessible not only to computational experts but also to wet-lab scientists with little or no prior bioinformatics experience.

Short talk: Maria Valeska Bianchi-Martin

Curiox

Software-Driven Standardization of Flow Cytometry Workflows: The Antibody Cocktailing Wizard and C-FREE™ Sample Preparation

Reproducible flow cytometry immunophenotyping requires consistency at two critical steps: antibody cocktail preparation and sample preparation. Both remain largely manual in most laboratories, introducing independent sources of operator-driven variability. The Pluto platform addresses both through integrated software and hardware solutions.

The Antibody Cocktailing Wizard (ACW) automates cocktail calculation, volume dispensing, and protocol tracking — eliminating manual pipetting errors and ensuring consistent reagent preparation across operators and runs. ACW-prepared cocktails were benchmarked against manually prepared cocktails across NK-cell and memory T-cell panels using stain index data from the Stanford University Human Immune Monitoring Center (HIMC). Panel performance was consistent regardless of cocktailing method, confirming that software-guided

preparation maintains staining quality equivalent to manual methods.

Complementing the ACW, the Pluto C-FREE™ automated sample preparation workflow was evaluated against conventional centrifuge-based methods using PBMCs from healthy donors. C-FREE™ maintained NK-cell population discrimination (R^2 1) and CD45 cell retention within ±20% of manual. CD4 and CD8 memory T-cell subset distributions were comparably resolved, with stain index differences that were not statistically significant.

Together, these results demonstrate that software-driven automation of both cocktailing and sample preparation reduces operator variability, providing a reproducible foundation for standardized immunophenotyping in research and clinical laboratory settings.

Short talk: Marvin Weis

Marvin Weis1, 2, 3, Claudia Giesecke-Thiel2, 4, 5
1 Interfakultäres Institut für Biochemie, Eberhard Karls Universität Tübingen
2 formerly: Max-Planck-Institut für Molekulare Genetik, Berlin
3 Institut für Medizinische Virologie und Epidemiologie der Viruskrankheiten, Universitätsklinikum Tübingen
4 Klinik für Infektiologie und Intensivmedizin, Charité – Universitätsmedizin Berlin
5 Der Simulierte Mensch, Charité – Universitätsmedizin Berlin

Cluster diffusion reveals, visualizes, and quantifies the complex transitions and trajectories of the human B cell manifold

Peripheral B cell subsets, including transitional and naïve, various activated and memory stages, as well as plasmablasts, are frequently characterized by flow cytometry to understand immunological health and disease states. However, different phenotyping approaches, panels, and analysis strategies make cross-study comparisons difficult and often fail to reflect the phenotypic B cell manifold with its stable populations and the interconnections between them.

We integrated multiple B cell phenotyping approaches into a unifying backbone staining. Dissatisfied by existing bioinformatics tools, we developed trajectory preserving unbiased and custom dimensionality reductions, a novel pseudotime algorithm capable of branching and merging trajectories, and novel differential abundance testing at quasi-single cell level to compare abundance of rare transitioning cells.

Starting with a clustering, we diffuse one-hot encoded cluster labels along a transition matrix and obtain individual cluster proximity values for each cell. Intuitively, rare cluster-transitioning cells stand out by their mixed cluster proximities. Forwarding cluster proximities into UMAP, we obtain CLUMAP, an unbiased dimensionality reduction with improved trajectory preservation. Alternatively, diffusing user-determined cluster coordinates, we obtain USERMAP, to our knowledge the first truly custom dimensionality reduction, conveniently enabling reproducible single cell plot layouts. Finally, where common pseudotime tools fail due to branching and merging trajectories, our USERPATH aligns a selection of connected clusters and shows marker trends, cell state density and differential abundance along the trajectory, even at rare transitional stages.

In our data, these tools reflect known and unknown paths of class switching and verify CD45RB acquisition as a major early milestone towards memory B cells.

Our algorithms mostly use matrix multiplications and are therefore fast, deterministic, and mathematically simple. Given their robustness, versatility, and novel capabilities, we anticipate they will make bioinformatic analyses of flow cytometry data more rational, scalable, and accessible – for B cells and beyond..

4:45 pm – 6:00 pm

Klaus Goerttler Session

Chairs: Oliver Otto, Greifswald, Claudia Giesecke, Berlin

Klaus Goerttler Prize winner 2026

Tobias Walther

Institute for Bioengineering of Catalonia (IBEC), Spain

DNA hydrogel microparticles as phantom cells for tissue engineering

Abstract

Hydrogel microparticles (HMPs) are powerful tools for the study and manipulation of 3D cellular systems. Engineered to allow physical interactions with living cells and chemical signalling through external triggers, they can be directly interfaced with growing tissues and act as functional cell mimics: phantom cells. Most polymeric HMPs, however, lack versatility in terms of their capacity for targeted chemical functionalization combined with fine-scale mechanical programmability, limiting their complexity. DNA-based materials offer promising properties in this regard due to their sequence-defined self-assembly, precise mechanical tuning, and straightforward chemical modification. Here, we introduce fully DNA-based hydrogel microparticles (DNA-HMPs) as a new class of multifunctional phantom cells for tissue engineering1,2,3. DNA-HMP size can be controlled via microfluidics to span the size range of human tissue cells. Making use of DNA sequence design, DNA-HMPs demonstrate finely programmable mechanics with viscoelastic behaviour and Young's Moduli between 30 Pa – 6 kPa, as validated by real-time deformability cytometry and indentation. This way, their mechanical properties can be tailored to mimic specific cell types of target tissues. DNA-HMP modification based on click chemistry further enables controlled physical interactions with living cells for force sensing in 3D1, as well as binding of morphogenic compounds. Through light-based stimuli-response, these morphogens can be released from the DNA-HMPs to spatiotemporally control stem cell differentiation in organoids, increasing cell type diversity2. Combining programmable material properties with straightforward functionalization and stimuli-responsiveness, DNA-HMPs represent a versatile new tool to probe and manipulate tissue behaviour in 3D cell culture, advancing tissue engineering through DNA nanotechnology.

1: Walther T et al. (2026) Advanced Materials 38, no. 37: e14218, doi.org/10.1002/adma.202514218

2:Afting C*, Walther T* et al. (2024) Nature Nanotechnology 19, 1849–1857, doi.org/10.1038/s41565-024-01779-y

3: Brauburger S*, Kraus BK*, Walther T* et al. (2026) Soft Matter, 22 (30): 4978–4993, doi.org/10.1039/d6sm00242k

*: Authors contributed equally

Klaus Goerttler Lecture

Chairs: Oliver Otto, Greifswald, Claudia Giesecke, Berlin

Ulrich F. Keyser

Professor of Applied Physics, Cavendish Laboratory, University of Cambridge, UK

Nanopore microscopy: Towards single-cell readout of RNA with single-molecule sensitivity

Biosensing with nanopores is developing rapidly with commercial DNA and RNA sequencing platforms challenging established next generation sequencing technologies. Nanopores offer the unique ability to translate molecular structure directly into electrical signals without the need of fluorescent labels. Beyond sequencing of nucleic acids and proteins, many other applications are enabled by counting single molecules with nanopores.

First, I will describe the identification of long transcript isoforms at the single-molecule level using solid-state nanopore microscopy. We refold target RNA into RNA identifiers (IDs) with designed sets of complementary DNA strands. Each reshaped molecule carries a unique sequence of structural (pseudo)colours. The sequence of structural colours of RNA identifiers enables simultaneous identification and relative quantification of multiple RNA targets without prior amplification. RNA IDs discriminate circular and linear transcript isoforms in a one-step, enzyme-free reaction in a complex human transcriptome with single-molecule resolution [1]. We will show recent results on detection of RNA modifications like 5mC, Inosine and MeC using RNA nanotechnology in ribosomal RNA (rRNA) from pathogenic bacteria like A. baumannii [2]. Finally, I will discuss how the technique may enable single-cell RNA detection by adapting the process for integration into droplet microfluidics.

References

Bošković and U. F. Keyser. Nanopore microscope identifies RNA isoforms with structural colors. Nature Chemistry, 14:1258-1264, 2022.

Li, S. C. Meng, Y. Wang, C. M. Platnich, M. K. Earle, E. Mylona, P. Naydenova, S. Baker, J. Zhu and U. F. Keyser. Nanopore detection of single-nucleotide RNA mutations and modifications with programmable nanolatches. Nature Nanotechnology, published online, 2025.

6:00 pm – 10:00 pm, CRTD Industry Area

Welcome Reception sponsored by Waters

Including time to view the posters

7:30 pm, Seminar room 1

Core Facility Networking Event

Chairs: Désireé Kunkel, BIH Charité Universitätsmedizin Berlin, Sarah Warth, UlmTec Core Facility Cytometry, Medical Faculty, Ulm University

This event is an opportunity to meet colleagues and share your experiences and challenges working in a core facility. We will have short presentations and hands-on (or rather brain-on) time on a selected topic for you, and lots of time for discussions afterwards.

We want to spend a wonderful evening with you at the DGfZ meeting 2026 in Dresden, with food & beverages & YOU!

Thursday, 24.09.2026

8:30 am – 10:00 am

Members Assembly DGfZ

Board of the DGfZ. All members have received the program.

10:30 am - 11:45

Session 2 – Mass Cytometry Session

Chairs: Axel R Schulz, Claudia Peitzsch

Mass cytometry (Cytometry by time-of-flight, CyTOF) is one of the most powerful tools for high-dimensional single-cell analysis. Due to the lack of signal overlap and cellular autofluorescenceit enables simultaneous measurement of more than 50 parameters in cell suspensions and for spatial analysis of tissue sections. By conjugating antibodies to stable heavy metal isotopes and detecting them by time-of-flight mass spectrometry, the method offers excellent signal resolution, an extensive and expandable marker space, and a comparatively straightforward and rapid panel setup.

In this session, we will briefly introduce the newly formed Mass Cytometry Working Group, which became part of the DGfZ in January 2026. Our invited speaker Ezgi Senoglu from the CRTD in Dresden will present her work on Epi-CyTOF. She used this technique to study epigenetic regulation of human neocortical development in cortical organoids to gain a deeper understanding of the epigenetic deregulation in neuropathologies. In addition, we look forward to contributions from the mass cytometry community that will be selected for oral presentations.

Ezgi Senoglu

CRTD Dresden

Biosketch

The neocortex is the brain structure attributed to higher cognitive functions in humans. Its development is governed by spatiotemporal gene expression programs regulated by epigenetic mechanisms, such as post-translational modifications of histone proteins. Specific histone modifications act on genomic loci to repress or promote gene expression, thereby contributing to the regulation of proliferation and differentiation of neural progenitor cells. Given the large number of histone modifications, we currently lack an understanding of the epigenetic state of neural cell populations beyond a few well-studied histone modificaitons. Moreover, the interplay of these modifications and their temporal changes in the developing human neocortex remain largely uncharacterized. To unravel this combinatorial crosstalk and complexity of the epigenome during neurogenesis at single-cell resolution, we employ cytometry by time of flight with a comprehensive epigenetic panel spanning over 30 different epigenetic markers, referred to as Epi-CyTOF, and cell type markers covering different neural cell types. Our data proves that Epi-CyTOF can detect and distinguish distinct neural cell populations in human foetal tissue and human cortical organoids.

Furthermore, it reveals cell-type specific distribution of histone modifications. Epi-CyTOF presents a powerful technology to decipher the complexity and dynamics of histone modifications during brain development and can in the future be applied to elucidate epigenetic changes in human neurodevelopmental disorders caused by mutations in epigenetic modifiers.

Short Talk: Mehmet Serdar Koca

Mehmet Serdar Koca[1], Katrien L.A. Quintelier[2,3], Lucía Rodríguez-Doña[1], Adrián Barreno[4], Axel Schulz[4], Sonia Gavasso[5,6], Patrice Hemon[7], Divi Cornec[7], Sofie Van Gassen[2,3], Sarah Bonte[2,3], Yvan Saeys[2,3], Marta E Alarcón-Riquelme[1,8], Concepción Marañón[1], Paulina Rybakowska*[1]*

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[4] Mass Cytometry Lab, German Rheumatism Research Center (DRFZ), A Leibniz Institute, Berlin, Germany
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[8] Institute for Environmental Medicine, Karolinska Institute, Stockholm, Sweden
**Equal contributions*
Keywords: CyTOF, Multicenter study, Harmonizations, Immune monitoring

Multisite Harmonization of Mass Cytometry Workflows for Translational Immune Profiling

Background: Harmonized workflows are essential for reproducible mass cytometry (CyTOF) data in multicenter clinical trials. This study evaluates optimization strategies for sample preservation, acquisition conditions, and compensation to ensure inter-center comparability.

Methods: Three barcoded samples were stained with 25- or 46-plex antibody panels. Signal stability during long acquisition was assessed across different acquisition solutions (CAS, CASPlus, Water) and post-thaw storage conditions (4°C/−80°C for up to 1 month). Compensation robustness was tested using single-stained compensation beads stored frozen for up to 1 year or subjected to freeze-thaw cycles. Stained samples and compensation beads were distributed across four acquisition centers over three batches to evaluate inter-center reproducibility. Cell frequencies (CF) and median signal intensity (MSI) values were analyzed.

Results: CASPlus provided the most stable signal intensities during long acquisition compared with CAS and Water. CSB and CASPlus at 4°C caused solution-dependent cell depletion and marker redistribution. In contrast, stained samples frozen at −80°C remained stable for up to 2 months, maintaining low coefficients of variation (<15% for CF and <20% for MSI). Compensation beads remained highly stable for up to 1 year, and a centralized compensation matrix successfully corrected spillover across all sites. Inter-center analyses of CF and MSI consistently distinguished donor samples, while intra-sample variability was further reduced by separate analysis of PBMC and granulocyte.

Conclusions: Optimization of acquisition solutions, sample and compensation bead preservation, and centralized compensation matrix calculation enables robust harmonization of CYTOF data across centers, supporting reliable immune monitoring in multi-center clinical studies.

IMI: PRECISESADS(GA#115565), 3TR(GA#831434), SIGNATURE(GA#101072891)

Short Talk: Niclas Schierloh

Niclas Schierloh[1]/Laura Riechert[2,3*]/Anna-Lena Schäfer[2,3*], Emilia Schlaak[1], Ke Meng[1], Reinhard E. Voll[2,3], Nina Chevalier[2,3*]/Bertram Bengsch[1,4*]*

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SceniTOF - Functional single-cell multiplexed metabolic profiling to map bioenergetics of heterogeneous immune cells by mass cytometry in mice and men

Immune cell activation, differentiation, and effector function are tightly coupled to cellular metabolism, but many functional metabolic assays lack single-cell resolution. Single-cell metabolic regulome profiling (scMEP) by mass cytometry quantifies metabolic proteins alongside immunophenotypes, but protein levels do not necessarily reflect metabolic activity or flux. The method SCENITH (Single Cell ENergetIc metabolism by profilIng Translation inHibition), developed by flow cytometry, may overcome this limitation by quantifying pathway-specific effects on protein synthesis after brief ex vivo inhibitor treatment.

Here we established a mass cytometry-compatible version of the SCENITH assay using a secondary metal-coupled antibody to detect the translation inhibitor puromycin as an integral part of the SCENITH assay and combine it with the scMEP approach. Briefly, human or murine immune cells are treated ex vivo or after *in vitro* stimulation with inhibitors of glycolysis and oxidative phosphorylation, pulsed with puromycin, barcoded, and stained with a multiplexed CyTOF panel targeting metabolic regulators, lineage markers, and activation states. This combined SCENITH–CyTOF approach enables scalable mapping of energetic pathway dependence while concurrently resolving phenotypic and metabolic profiles. We demonstrate the applicability of the SceniTOF assay to identify the differences in metabolic state and flux across human T cell differentiation states and the murine B cell compartment.

Together, this integrated approach links multiplexed single-cell phenotyping with functional metabolic readouts, allowing SceniTOF to relate metabolic potential to actual metabolic flux by mass cytometry..

11:45 am – 1:00 pm

Product Slam

Chairs: Elmar Endl, Tom Bauer & Lisa Budzinski

Participation in the Product Slam is included in the booking for all industry exhibitors. You can use these three minutes alone or with support to present your company's position to an interested audience. Three minutes for your product – convince us!

2:00 pm – 4:15 pm

Poster Session

Chairs: Oliver Otto, Greifswald, Claudia Giesecke, Berlin

Gorkhmaz Abbaszade

01

Spatial phenotypic heterogeneity in bacterial colonies reveals emergent multicellular organization

Gorkhmaz Abbaszade, Kathrin Stückrath and Susann Müller
Department of Applied Microbial Ecology, Helmholtz-Centre for Environmental Research - UFZ, Permoserstr. 15, 04318 Leipzig, Germany

Bacterial colonies are highly organized multicellular systems in which local microenvironments shape cellular differentiation, growth, and survival strategies. Despite their ecological importance, the spatial distribution of physiological cell states within colonies remains poorly understood at single-cell resolution. Here, we investigated the biogeography of five bacterial species - Bacillus subtilis, Paenibacillus polymyxa, Kocuria rhizophila, Stenotrophomonas rhizophila, and Pseudomonas citronellolis - to determine how localized physiological specialization contributes to emergent colony organization. Localized sampling of defined colony regions combined with high-resolution flow cytometric fingerprinting based on DAPI and SYTO9/PI staining.

The analyses resolved diverse physiological subpopulations and thus spatial heterogeneity in cell-cycle activity, viability, and sporulation dynamics. Analysis revealed that distinct colony regions contained characteristic physiological subpopulations. For e.g., in B. subtilis, sporulation was strongly localized to inner colony regions, where spores accounted for ~31–65% of cells, whereas outer regions exhibited vegetative populations with elevated proliferative activity, reduced cell death, substantially lower sporulation frequencies (~9–27%). Across all strains, colonies displayed highly differentiated multicellular organization, whereas some maintained relatively uniform physiological distributions which reflects their adaptation to spatially distinct microenvironments and resource availability.

These findings demonstrate that bacterial colonies function as locally specialized and spatially structured ecosystems rather than homogeneous cell assemblies to coordinate growth, stress responses, and survival at the microscale. By uncovering unresolved patterns of multicellular organization, this work advances our understanding of microbial ecology, collective behavior, and adaptive survival strategies within bacterial populations.

Jochen Behrends

02

Image-enabled flow cytometry enables characterization and high-purity sorting of nanoscale particles, EVs and synaptosomes

Jochen Behrends1, Isabel Graf2,3, Amanda Salviano-Silva4, Anne Rissiek5, Santra Brenna3,6, Hela Uplegger3,6, Bente Siebels7, Christopher Urbschat2,3, Cecile L. Maire4, Kristoffer Riecken8, Katrin Lamszus4, Anke Diemert9, Franz Ricklefs4, Tim Magnus3,6, Petra Arck2,3, Berta Puig3,6, Ada Nowosad10,11, Artemis Koumoundourou12,13, Joris de Wit12,13, Pedro Santana Ribeiro Magalhães14,15, Bart Ghesquière16,17, Dharshini Raju18,19, Tharshana Stephen20,21, Jochen Lamote18,19.
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8 Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany.
9 Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
10 Laboratory for Molecular Cancer Biology, CCB, VIB, Leuven, Belgium.
11 Laboratory for Molecular Cancer Biology, Department of Oncology, KU Leuven, Leuven, Belgium.
12 Laboratory of Synapse Biology, CNL, VIB, Leuven, Belgium.
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19 VIB-KU Leuven Center for Cancer Biology, KU Leuven, Leuven, Belgium.
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21 Inserm, CEA/IBFJ/DRCM/CyACT, Cytometry facility, Université Paris-Saclay, Stabilité Génétique Cellules Souches et Radiations, Fontenay-aux-Roses, France.

Introduction and Objectives: Analysis of submicron particles remains challenging in flow cytometry due to limited scatter sensitivity, background noise, and lack of orthogonal quality control parameters. We evaluated imaging-enabled flow cytometry (BD FACSDiscover S8 with CellView™ technology) for detection, characterization, and sorting of nanoscale particles, including extracellular vesicles (EVs) and synaptosomes.

Materials and Methods: NIST-traceable size standards (50-90 nm), Megamix-Plus beads (100–900 nm), EVs (80–120 nm), and synaptosome preparations were analysed and sorted. Imaging-derived parameters from side scatter reconstruction were used. Sorting experiments were performed with imaging-based gating strategies. EV and synaptosome populations were validated using orthogonal methods, including Mass Spectrometry and TEM.

Results, Discussion and Conclusion: Image-derived parameters enabled detection and discrimination of nanoscale particles from buffer background. ‘Diffusivity’ and ‘Radial moment’ provided information on signal distribution and allowed separation of size standards and bead populations beyond conventional intensity-based metrics. Sorting of nanoparticle standards achieved purities exceeding 98% for 100 nm particles and >99% for larger populations. In EV samples, ‘Eccentricity’ and ‘Radial moment’ enabled discrimination of heterogeneous populations and supported identification of swarm-related artefacts. Orthogonal measurements confirmed the presence and integrity of sorted EV populations. Synaptosomes were identified as single-particle populations based on imaging features, enabling discrimination from debris and aggregates. These findings demonstrate that image-derived morphological parameters provide complementary information to conventional scatter and fluorescence signals, enabling improved

resolution, quality control, and high-purity sorting of nanoscale particles and biologically complex subcellular structures.

Jochen Behrends / Jochen Lamote

03

Image-derived parameters enable discrimination, quantification, and isolation of cellular uptake

Jochen Behrends1, Ioannis Panetas2, Laura Ferrer-Font3, Nadine Aboutara4,5, Gareth Prosser6, Manuel Hein1, Martina Hein1, Thomas Scholzen1, Norbert Reiling5,6, Dominik Schwudke4,5,7, Dharshini Raju8,9, Ada Nowosad10,11, Carolina Ruivo10,11, Julie Bonnereau12,13, Apostolos Nikolakopoulos14,15, Patrizia Agostinis12,13, Colinda Scheele14,15, Massimiliano Mazzone16,17, Tharshana Stephen18,19, Jochen Lamote8,9.

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15 Laboratory of Intravital Microscopy and Dynamics of Tumor Progression, Oncology, KU Leuven, Leuven,.Belgium.
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Introduction and Objectives: Conventional flow cytometry relies on fluorescence intensity, which limits the ability to distinguish between surface-associated and internalized signals and does not capture spatial distribution within cells. We evaluated image-enabled flow cytometry for the analysis of cellular uptake processes across multiple biological systems, including extracellular vesicles (EVs), phagocytosis, efferocytosis, and infection models.

Materials and Methods: Uptake processes were analysed using image-derived parameters extracted from reconstructed images (BD FACSDiscover S8), including signal ‘diffusivity’ and displacement between fluorescence and scatter centroids (‘center-of-mass shift’). EV uptake assays, phagocytosis

experiments using fluorescent particles, efferocytosis assays combining spectral-enabled multi-parameter fluorescence panels, and bacterial infection models were investigated. Control conditions, including pharmacological inhibition of phagocytosis, were used to support interpretation of uptake readouts.

Results, Discussion and Conclusion: In EV uptake experiments, fluorescence positive events exhibited heterogeneous spatial patterns, indicating the presence of distinct states including potential internalization and surface-associated signal that could not be resolved using intensity-based gating alone. Image-derived parameters distinguished these states by quantifying signal distribution within cells. In phagocytosis assays, 'diffusivity' and 'center-of-mass' shift enabled discrimination between internalized and surface-bound particles and allowed quantification of uptake, including identification of cells containing multiple particles. Control conditions confirmed specificity of these measurements. In efferocytosis experiments, imaging-derived spatial information was combined with multi-parameter fluorescence readouts, enabling characterization of uptake events within heterogeneous cell populations. In infection models, imaging parameters provided additional insight into intracellular signal localisation. Across all systems, spatial metrics provided consistent and interpretable information on signal distribution, enabling discrimination between uptake states and quantitative assessment of internalization processes. These features can be directly used as sorting criteria, enabling isolation of defined uptake states for downstream omics applications. These findings demonstrate that imaging flow cytometry extends conventional cytometry by incorporating spatial resolution, thereby enabling more accurate interpretation and functional exploitation of uptake assays across diverse biological contexts.

04 Jochen Behrends

Mind the Gap: Identity, Recognition, and Sustainability in the Global Cytometry Specialist Workforce

Eva Orlowski-Oliver1, Jochen Behrends2, Pat Metharom3.
1 Peter MacCallum Cancer Centre Research Flow Core, Melbourne, Victoria, Australia.
2 Science and Technology Unit Fluorescence Cytometry, Research Center Borstel, Leibniz Lung Center, Borstel, Germany.
3 Curtin Medical Research Institute Flow Cytometry Facility, Curtin University, Perth, WA, Australia.

Background: Cytometry specialists provide critical scientific, technical, and educational expertise that supports research quality and reproducibility. However, institutional job titles often fail to reflect their expertise, responsibilities, and leadership contributions. This mismatch between professional identity and formal recognition, termed the 'Identity Gap', may contribute to reduced job satisfaction, limited career progression, and workforce attrition. This study investigated the prevalence and impact of the Identity Gap within the global cytometry specialist workforce.

Methods: An anonymous 29-question international survey was conducted between March and April 2026. The survey examined professional background, role identity, career development, visibility, recognition, barriers to progression, and sustainability. The primary measure compared respondents' official institutional job titles with their preferred professional titles. Mismatches were classified as either discipline identity-seeking or leadership recognition-seeking.

Results: A total of 175 responses from 24 countries were analysed. Respondents represented a highly experienced workforce, with most reporting more than 10 years of experience; nevertheless, 30% remained on fixed-term contracts. Institutions assigned 51 different official job titles, whereas preferred titles clustered into five distinct professional categories, demonstrating substantial inconsistency in workforce classification. Over one-third of respondents reported performing uncompensated responsibilities beyond their formal roles, including student supervision (35%), research leadership (34%), and experimental research (29%). Experimental design,

troubleshooting, and intellectual input were identified as the most overlooked contributions. When asked about barriers to career progression, 80% attributed challenges to institutional structures rather than demographic factors.

Conclusions: The global cytometry specialist workforce performs advanced scientific, technical, intellectual, and leadership functions that remain insufficiently recognised within current institutional frameworks. The Identity Gap represents a systemic mismatch between role expectations and professional recognition. Addressing this gap through clearer career pathways, equitable recognition, and sustained investment in specialist expertise will be essential for workforce retention, innovation, and the long-term sustainability of cytometry research infrastructure.

Kristin Bieber

05

IMMUNOMAP-Mouse: A 40-color Comprehensive Immune and Hematopoietic Profiling Platform for Systems-Level Mapping of Murine Immunity

Bieber K., Stanger A.M.
Core Facility Flow Cytometry, University Hospital Tübingen

Developing high-dimensional flow cytometry panels for murine systems remains challenging, particularly when integrating rare markers while maintaining compatibility with existing datasets. In contrast to human immunophenotyping, robust and comprehensive murine panels – especially those combining surface and intracellular markers within a single assay – are still limited.

To address these challenges and reduce sample requirements in line with the 3R principles of animal welfare, we developed IMMUNOMAP-Mouse, a standardized and systematic workflow for a robust 40-color spectral flow cytometry panel. This approach maximizes data output per specimen, minimizes batch effects, and enables the simultaneous assessment of lineage identity, differentiation states, and functional activation markers across co-expressed pathways.

Our panel supports in-depth characterization of hematopoietic and immune compartments, including progenitor populations such as hematopoietic stem cells (HSCs), LSK cells, multipotent progenitors (MPP1–6), as well as mature myeloid subsets (e.g., conventional dendritic cells, macrophages, monocytes), and lymphoid populations (e.g., T, B, and NK cells). By incorporating various transcription factors and cytokines, IMMUNOMAP-Mouse allows simultaneous evaluation of cellular function, activation status, and developmental progression. In addition, it enables longitudinal monitoring of hematopoietic reconstitution following stem cell transplantation. Its applicability across spleen, blood, and bone marrow makes it a versatile tool for system-level analysis of murine immunity under physiological and pathological conditions as e.g. cancer, infection or auto-immunity.

IMMUNOMAP-Mouse provides a validated, high-dimensional framework for comprehensive immunophenotyping, supporting reproducible and integrative murine immune profiling in basic and translational research.

06 Vinod Devaraj

Automatic label-free hierarchical immunophenotyping

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Conventional flow cytometry-based immunophenotyping relies on fluorescent antibody labelling followed by manual gating. Such procedures are reagent intensive, user-variable and impractical for large-scale studies where repeated staining is not feasible and expensive.

We present a label-free hierarchical phenotyper which works directly on time and angle resolved pulse data acquired using MAPS-FC, a custom multi-angle pulse-shape flow cytometer. Phenotypic information is inherently encoded in the morphological and structural properties of cells. MAPS-FC exploits this information by capturing the spatio-temporal light-scattering signature of each cell across multiple detector angles, accessing the cell identity information that has always been present but previously unexploited.

The acquired pulse-shape data is further processed using a two stage AI pipeline. In the first stage, a variational autoencoder maps the raw pulse-shape data into a compact 16-dimensional latent representation in a fully unsupervised manner across all cell types simultaneously, capturing biological variation inherent to each cell type. Second, a set of MLP classifiers are trained on these latent embeddings using conventional antibody-gated populations as ground truth. Each MLP classifier is arranged hierarchically, with each node in the tree responsible for discriminating between cell subtypes. Once the model is trained, it operates on unlabelled samples completely. Raw pulse acquisitions are standardised, encoded, and classified in a single automated procedure with no staining or manual intervention required. This pipeline demonstrates that light-scattering signatures alone contain information for immunophenotyping, enabling a scalable and reagent-free alternative to conventional staining-based approaches. Our investigations improved imaging quality and the resolution of mechanical analysis of platelets in RT-DC, paving the way towards improved functional and medical assays on fundamental as well as applied research on platelets.

07 Johannes Droste

Multi-focus imaging in flow cytometry for microplastic detection

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The rapidly increasing contents of microplastics (MPs) in ecosystems and the food chain have become a serious environmental problem with far-reaching consequences for wildlife and human well-being. Current MP detection methods have limited throughput or rely on extracting the MPs from tissue samples. We use flow cytometry to visually detect MPs label-free in living human cells with high throughput.

With the current flow cytometry setup, MPs of a few hundred nanometers in size can be quantified, provided they lie within the focal plane, which on average encompasses less than 10% of the cells volume.

To overcome the limited depth of field we use multi-focus imaging. By tilting the micro-channel, cells traverse the focal plane. The acquisition of multiple images at different points in the channel creates a multi-focus image stack, in which every part of the cells volume is in focus.

Leonard Fiebig

08

Spatial and Functional Insights into Plasma Cell Longevity and Immune Memory Maintenance

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Mature antibody-secreting plasma cells (PCs) in human the bone marrow (BM) are critical for long-term humoral immunity and immunological memory, yet regulatory cues and tissue niches that support their persistence remain poorly understood. Here, we investigate the spatial, phenotypic, and functional heterogeneity of mature human BM PCs using an integrated approach combining spatial proteomics with primary BM culture systems.

We established a platform using fresh human femur heads that enables tissue sectioning and highly multiplexed spatial proteomic imaging of up to 55 markers without decalcification, which preserves sensitive epitopes that are often difficult to detect in conventional FFPE bone sections. In parallel, flow cytometric and secretome profiling of matched primary whole BM cultures from the same donors offers a complementary functional systeme to define survival requirements across the major BM memory cell compartments

Using this integrated workflow, we aim to localize plasma cell subsets within the BM microenvironment, characterize their interactions with stromal and immune cell populations associated with PC longevity, and functionally assess their dependence on key survival pathways.

Together, these analyses provide new insight into the cellular organization of the human BM niche and the mechanisms that sustain durable antibody and immune memory. Ultimately, we aim to discover new targets for therapies that selectively disrupt pathogenic plasma cells or immune memory compartments while sparing protective immune memory in autoimmune disease.

Tristan Franke

09

Enhancing optical parameters of platelets in real-time deformability cytometry

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Real-time deformability cytometry (RT-DC) is a high-speed, label-free cytometric method for imaging and analyzing cell mechanical properties. Cells are flushed through a narrow microfluidic channel and deform by hydrodynamic stresses, while being illuminated by a stroboscopic high-power LED and imaged by a high-speed CMOS camera mounted on an inverted microscope.

Using RT-DC allows for investigating platelet mechanics, which are key to blood coagulation and may help to explain underlying causes of, e.g., coagulopathy and thrombosis. However, with a cell size of only a few micrometers, platelets appear to be difficult to analyze optically, resulting in low image quality, often characterized by motion blurring, low brightness, and a low level of morphological detail.

We investigated the interplay between system parameters, including LED exposure time (brightness), sample illumination area, hydrodynamic flow rate, objective magnification, and numerical

aperture as well as camera settings, to identify optimal imaging parameters. To further enhance image quality, we inserted a 2.18x intermediate lens in our inverted microscope setup to increase image magnification, previously not possible to achieve, while preserving the depth of focus. In combination with an extended exposure time of 9.3 µs, and a 2x digital gain our results demonstrate a balance between image brightness and motion blurring while achieving improved contrast and resolution in RT-DC. In summary, these results might pave the way towards improved functional and medical assays in fundamental as well as applied research on platelets.

10 Johannes Groffmann

High-Throughput Mass Cytometry Phospho-Profiling Reveals Immune Signaling Signatures in Inflammatory Rheumatic Diseases

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Background: Inflammatory rheumatic diseases may share clinical manifestations, but are biologically heterogeneous. Despite therapeutic advances, treatment responses remain hard to predict. PRISM (Precision Medicine in Rheumatology through Molecular Stratification) is a prospective study aiming to identify molecular and functional signatures of disease mechanisms, patient stratification, and therapy outcome.

Methods: PRISM includes newly diagnosed, treatment-naïve patients sampled at diagnosis and after 3 months of therapy. Whole blood is stimulated with 20 ligands targeting key innate and adaptive pathways, then barcoded, stained for 18 phospho-proteins and 34 surface markers, and analyzed by mass cytometry. A robotics-based workflow enables standardized high-throughput processing.

Results: We established a semiautomated, robotics-assisted mass cytometry phospho-profiling workflow, combining whole blood stimulation with high-plex-barcoding of >100 samples per acquisition. All phospho-antibody stainings performed robustly and reproducibly, generating biologically meaningful, cell-type-specific signaling responses across major immune cell-subsets. The platform detected disease-relevant signaling differences between healthy controls and individuals at risk of or affected by autoimmunity. Preliminary analysis of IRF5 risk variant carriers and SLE patients revealed stronger pathway activation than controls, with increased pIRF3 after IFNβ stimulation in monocytes, indicating enhanced responsiveness of the TBK1–IRF inflammatory axis. These findings demonstrate the ability of the platform to identify functionally distinct immune phenotypes.

Conclusion: Automated mass cytometry phospho-profiling enables scalable and reproducible functional immune-profiling in rheumatic disease. It captures disease-relevant signaling differences at single-cell resolution and may support molecular stratification, and identification of novel therapeutic targets. Integration with longitudinal clinical, proteomic, and transcriptomic data may support stratification and treatment response prediction.

Gishnu Harikumar Parvathy 11

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B cells are increasingly recognized as key regulators of the immune landscape within the tissues they inhabit. However, most current knowledge is derived from studies of lymphoid organs, and a comprehensive characterization of B cell populations across human solid organs and their relationship to disease states remains limited. Here, we employed high-parameter mass cytometry to profile B cells across human peripheral blood, spleen, and bone marrow (lymphoid tissues), as well as tonsil, liver, kidney, and lung (non-lymphoid tissues). Using unbiased unsupervised PhenoGraph clustering and visualization by Uniform Manifold Approximation and Projection (UMAP), we identified distinct B cell populations across these anatomical sites. Furthermore, applying a computational framework for differential analysis of high-dimensional cytometry data, we detected numerous PhenoGraph clusters that differed significantly between lymphoid and non-lymphoid organs. Notably, one cluster exhibiting a highly significant enrichment in the kidney (peri-tumoral region) compared with the spleen was found to be exclusively present in renal tissue. Phenotypically, these cells were characterized as CD19□Syk□CD20□CD32B□CD45RA□IgD□CD27□IgG□ with partial IgM expression, suggesting a chronically stimulated B cell population with inhibitory features. The unique tissue distribution and phenotype of this subset warrant further investigation, particularly in the context of pathological conditions such as transplant rejection and tumor-associated immune responses.

Marcel Henkelmann 12

Tunable multi fluorescent polymer particles for flow cytometry applications

Dr. Marcel Henkelmann, Applied Microspheres GmbH

Applied Microspheres is a leading manufacturer of particle standards and related products. Over the past decade, we have focused our efforts on identifying and developing a range of essential applications in the field of flow cytometry. This has involved precise adjustments to the spectral properties and particle surface chemistry, with the objective of aligning our products with the evolving demands of the market.

In conventional flow cytometry applications, predominantly predicated on a solitary spectral channel or multiple spectral channels, salient characteristics for reliable instrument control are predominantly attributed to monodisperse particle size, minimal standard deviations for colloidal and spectral properties, and high reproducibility.

Recent approaches are presented for instrument control in terms of market developments. The primary objective of these approaches is to reduce the size of objects detected in flow cytometry while maintaining the given precision. Furthermore, the full optical spectrum of the analyte can be observed to gain valuable information on the sample, which represents an even more challenging objective. Consequently, a novel platform for polymer particles has been recently developed. This platform can be excited by most common illumination sources and exhibits broadband emission of light over the entire spectrum, ranging from UV to near-IR wavelengths. It has been demonstrated that the particle species in question exhibit a high degree of flexibility with respect to their optical properties, the diameter distribution of the particles, and the chemistry of their surfaces.

The following presentation outlines the present investigation on capture beads. The beads have been modified with a super adsorbent surface, which enables their capacity to bind a broad range of

proteins and other molecules. These beads can be customised for specific single-channel applications or integrated with our innovative full optical spectrum bead platform.

13 Martin Hussels

High sensitivity flow cytometry for direct counting of viral particles

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PTB and KRISS both developed sensitive flow cytometers based on single photon counting of fluorescence signals to measure concentrations of very small particles such as nucleic acid fragments and viral particles. Originally, these devices were used for the direct counting and concentration determination of DNA fragments. The aim was to determine reference measurement values for potential reference materials [1,2]. In addition, the instruments were also successfully used to determine the concentration of RNA in an international comparative study [3]. Detection is based on labeling of target particles with a fluorescent dye and the very sensitive detection of the fluorescence signals using single photon counting APDs. Due to the low signal intensity, it is necessary to accumulate the signals for a 100 to 1000 times longer period than is usual in conventional flow cytometers. As a result, the sample volumes and volume flow rates used are also correspondingly much smaller, which places high demands on the stability of the fluidic system and the measurement uncertainty of the sample volume measurement. KRISS and PTB are currently working on the direct counting of viral particles using these instruments to characterize potential reference materials for viral vector concentration. The concentration of such materials is commonly measured by PCR based methods. However, PCR requires processes such as extraction, free DNA removal, assay design and optimization which cannot guarantee 100% efficiency. Furthermore, this indirect detection of viral particles via their DNA inevitably introduces systematic deviations and increases measurement uncertainty even if digital PCR is used. Direct counting of viral particles may overcome this as no extraction is needed and the viral particles are detected directly. KRISS has developed suitable materials and a labeling protocol based on click chemistry. We present data on the performance of the highly sensitive flow cytometer developed at PTB and first data of direct counting of viral particles.

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14 Maria Kuzminskaya

A 41-Color Spectral Flow Cytometry Panel for High-Dimensional Profiling of Hepatic Dendritic Cells in Murine MASLD

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Metabolic dysfunction-associated steatotic liver disease (MASLD) and its progression to the more severe inflammatory stage, metabolic dysfunction-associated steatohepatitis (MASH), are driven by complex immune-metabolic interactions. Dendritic cells (DCs), as professional antigen-presenting cells,

play key roles in inflammatory regulation and have previously been shown to exhibit lipid-associated functional alterations during disease progression. However, high-dimensional characterization of hepatic DC subsets remains challenging due to high tissue autofluorescence, cellular heterogeneity, and dynamic activation states.

We developed and optimized a 41-color full-spectrum flow cytometry panel for comprehensive profiling of hepatic immune cell populations with a particular focus on resolving DC heterogeneity on a 5-laser Cytek Aurora system. The panel enables simultaneous assessment of phenotypic subsets, activation markers, scavenger receptors implicated in MASLD progression, and intracellular neutral lipid accumulation across all immune cell subpopulations. Iterative panel optimization included refinement of marker-fluorochrome combinations, extensive antibody titration, and selection of high-quality spectral reference controls to ensure robust unmixing and reliable resolution of closely related immune cell populations. Furthermore, a systematic unmixing strategy incorporating multiple autofluorescence signatures was established to improve signal discrimination and maximize accuracy in cells derived from highly autofluorescent liver tissue.

This panel provides a robust platform for high-dimensional immune phenotyping with particular applicability to DC-focused studies in metabolic liver disease. Its broad immune profiling capacity and high dimensionality additionally allow application to other tissues, such as spleen, bone marrow, or lymph nodes, and enable adaptation to other inflammatory disease settings.

Yifan Lu

15

Using MDR S-map to investigate the interaction based on flow cytometry —— Detect the asynchronous interaction in synchronous microbial communities

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Understanding how interactions response to community state remains a major challenge in microbial ecology. Long-term experimental microbial communities provide an ideal system for investigating ecological dynamics under controlled conditions, yet the interaction mechanisms underlying long-term coexistence are still poorly understood.

In this study, we analyzed time-series data from a wastewater-derived microbial community maintained in a looped mass-transfer system for over 300 days. The experiment was divided into insular and recycle phases to control the community state. Time-series data were generated using an automated flow-cytometry gating pipeline. Multiview Distance Regularized S-map (MDR S-map) applied to quantify time-varying interaction strengths without assuming predefined mathematical models.

The results indicate that subcommunity interactions are highly state-dependent and exhibit substantial temporal variation. Microbial interaction patterns of low-abundant subcommunities across reactors were not synchronized in the looped mass transfer system. The strongest interactions were concentrated among rare subcommunities and responded to community state change. Some specific subcommunities showed clear shifts before and after the change in community state, suggesting that these subcommunities responded strongly to the transition and may suggesting a potential role in community reorganization.

These findings demonstrate the value of integrating flow cytometry and MDR S-map approaches to uncover hidden interaction networks in microbial systems. The proposed framework provides new insights into the mechanisms governing community state and offers a foundation for predicting and designing resilient synthetic microbial ecosystems.

16 Stefan J. Maurer

Automated Image-Based Cell Sorting by Targeted Photopolymerization

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Image-based cell sorting on microscopy platforms enables the isolation of cells according to spatial, temporal, and morphological information that is inaccessible to conventional flow cytometry. However, existing image-based sorting methods often require specialized instrumentation or suffer from limited throughput.

Here, we present an automated microscopy-based cell sorting method capable of selectively depleting undesired cells at rates of hundreds of cells per second. Cells are resuspended in a photoresist and undesired cells are encased in hardened structures by spatially controlled photopolymerization, enabling their removal by filtration. Our microscopy platform integrates image acquisition, machine-learning based classification and subsequent depletion of up to 99.98% of negative cells, preserving target cells for collection. Applied to peripheral blood mononuclear cells, the method achieves the label-free, morphology-based enrichment of activated T cells for downstream applications.

The system is designed for implementation on microscopy platforms commonly available in life science laboratories, avoiding costly equipment or extensive training. It is controlled through an intuitive graphical user interface built on an extensible Python software package that can be readily adapted to specific research needs. The method provides a versatile, accessible and cost-effective sorting solution for applications where morphology and other microscopy-derived phenotypes cannot readily be exploited using conventional sorting technologies.

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Animation of the working principle:

https://youtu.be/NSBW8zB2q7w

Florian Mayerle

17

Automated Sample Inlet and Process Monitoring Platform for Cell Cytometry in Microfluidic Systems

Fraunhofer IPA, Stuttgart

Flow cytometric analysis of single cells on microfluidic chips remains complex and error-prone in both laboratory and professional applications. Automated sample loading is particularly challenging when sample volumes are low or when cells require gentle handling. Chip-to-fluidic-system connections often result in errors such as leakage, blocking or air entrapment. With single-use chips, manual replacement further increases process time. Process monitoring and control of the entire analytical chain is often critical for reliable and reproducible analytical results, however, it frequently remains inadequate, as many systems operate as "black boxes".

To address these issues, we developed a fully automated, “chip-open” platform. It enables flexible sample loading, automated chip replacement, and integrated process monitoring. Cross-contamination is prevented in reusable chip configurations. The system is optimized for small sample volumes (30–300 µL) and delivers the sample to the analytic region (e.g. optical or electrical detection modalities) within seconds.

We validated the platform using deformability cytometry, a method with high stability demands and limited throughput due to 20×20 µm analysis channels. In this use-case the quality of the analytical output is highly dependent on process parameters such as flow, pressure and temperature and require precise control of sample injections as well as automated cleaning cycles. Automation ensures stable operation and eliminates manual error sources that typically affect data consistency and throughput.

Our solution removes several bottlenecks and provides a practical approach for automated microfluidic cytometry in research and clinical settings. The modular design allows adaptation to various microfluidic assays beyond deformability cytometry.

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Inês Monteiro

18

Breaking the Rules: Voltage Variation on Spectral Unmixing

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The standardization mode on the SONY ID7000 Spectral analyzers sets the equipment to optimized specifications, enabling users to reuse the same spectral references between different experiments and across several machines. Suggesting that, when using the standardization mode, single colour controls can be acquired with different PMT settings without affecting the unmixing matrix as the analyzer’s response is normalized by this mode. Moreover, this clearly challenges the “golden rule” stating that single colour controls and samples must be acquired with the same settings in order to achieve a correct unmixing matrix.

This study was designed to test this concept, by acquiring the same single colour controls at different PMT settings, and then analyzing the resulting unmixing performance across different samples. Single colour controls for twelve different fluorochromes were acquired with five different sets of voltages, consequently creating five independent unmixing matrices that were latter applied to three different mouse tissues (spleen, liver and bone marrow), all stained with a twelve colour panel, to understand if altering the voltages on spectral references acquisition would create any changes on the unmixing data. Additionally, acquisition was performed on two SONY ID7000 instruments at the same time, to assess whether importing settings acquired on one analyzer to another equipment, with the same optical configuration, would create any significant differences in the resulting data.

19 Ralph Müller

Direct determination of absolute lymphocyte subpopulation concentrations in human blood

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Reference measurement procedures are indispensable to reliably determine blood cell concentrations, since medical diagnosis, initiation of therapy and control of blood products are often based on concentration limits of specific cell subpopulations. The standard procedure for measuring subpopulations of lymphocytes in e.g. testing the immune status comprises lysis of red blood cells followed by sample washing to exclude debris from the sample before flow cytometric investigation. However, these preparation steps hamper the absolute measurement of cell concentrations, and external quality assurance in related ring trials is based on consensus values so far.

To improve the accuracy in cell counting of lymphocyte subpopulations like CD4 or CD8 positive cells, we carry out the lysis of red blood cells without subsequent washing in order to ensure that the dilution is traceable and reproducible. In addition, the dilution is monitored gravimetrically. Flow cytometric measurements with our home-built instrument are immediately done after lysis incubation to avoid degradation of the samples. Our home-built flow cytometer uses direct injection of the cell suspension with gravimetrically calibrated syringes to ensure accurate sample volume determination. This enables direct determination of CD4 and CD8 cell concentrations avoiding uncertainties which may result from relative counting to all Leucocytes. This method was applied on blood samples of the Reference Institute for Bioanalytics (RfB) serving as test material for the ring trial immune status. We discuss the accuracy and stability of our results and compare our findings with the results of the other ring trial participants.

20 Anni Muñoz

Novel 3D tri-cell-type platform for live-cell monitoring of PDAC cell invasion in a heterologeous stromal cell environment with EC tubular structures

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Pancreatic ductal adenocarcinomas (PDAC) are characterized by a highly complex tumor microenvironment with extensive stromal involvement and dynamic interactions between tumor, mesenchymal, and vascular components that contribute to disease progression. However, conventional *in vitro* systems frequently fail to reproduce the cellular heterogeneity and structural organization observed *in vivo*, limiting the investigation of tumor microenvironment interactions. Therefore, more physiologically relevant 3D models are needed to better represent PDAC biology and the interactions between the tumor and stromal compartments. To address this challenge, we established a novel multilayer, spheroid-based 3D co-culture model for live monitoring of PDAC cell behavior in a complex, heterogeneous environment. We used human umbilical vein endothelial cells (HUVECs) and pancreatic stellate cells (PSCs) to present a multilayer co-culture system capable of forming EC tubular structures. Furthermore, both PDAC mono- and co-culture spheroids generated by combining PDAC cells with PSCs were incorporated into the assay for evaluating the impact of stromal components on tumor organization and cellular interactions. Fluorescence-based labeling

approaches were introduced for live-cell imaging and continuous monitoring of cellular dynamics within the system. For this purpose, we used mCherry-tagged tumor cells and tested various cell trackers for transient, ≥10-day stable labeling of EC. Analytical endpoints of interest were assessed by immunohistochemical staining with Histogreen and Fast Red as chromogens. Live-cell monitoring of the formation of endothelial tubular structures and visualization of the invasion of spheroid-originating cells within the system have been achieved. The novel 3D tri-cell-type platform thus enables reproducible generation of multicellular structures and spatiotemporal visualization of tumor, stromal, and endothelial compartments over time.

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Josias Neumüller

21

Biophysical Response of Artificial Cells to Osmotic Stress on the Millisecond Timescale

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Cells are continuously exposed to rapidly changing osmotic environments in the human body. These fluctuations induce immediate passive changes in cell volume, cell morphology, and cell mechanical properties, which act as initiating stimuli for downstream biological processes. While osmotic responses on long timescales are well studied, the initial response occurring within milliseconds remains poorly understood. In particular, it is still unclear if the limiting factor is found in cell membrane properties or properties of the cytosol.

Here, we investigate the contribution of the lipid membrane to the biophysical osmotic stress response. The use of giant unilamellar vesicles (GUVs), cell-sized vesicles consisting solely of a lipid bilayer, enables the measurement of osmotic responses independent of cytoskeletal or protein-mediated effects.

GUVs were generated via electroformation and gel swelling, characterized, and optimized for analysis using real time deformability cytometry (RT-DC), a label-free, high-throughput technique capable of probing biophysical properties of cells on millisecond timescales.

The vesicle response to osmotic stress was investigated on long (minutes) and short (milliseconds) timescales. Long-term measurements served as controls to confirm osmotic effects, while short-term experiments focused on the immediate membrane response. Initial results demonstrate characteristic osmotic swelling behaviour and time-dependent mechanical changes under anisotonic conditions.

Celina Owczarek

22

Real-Time Fluorescence and Deformability Cytometry identifies agonist-specific platelet signatures

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Background: Platelet activation involves cytoskeletal reorganization, membrane remodeling, degranulation, integrin activation, and changes in biomechanical properties such as stiffness and size. While biochemical and mechanical activation pathways are established, agonist-induced biomechanical alterations remain poorly characterized. We therefore applied real-time fluorescence and deformability cytometry (RT-FDC) to simultaneously assess platelet activation states and biomechanical signatures after agonist stimulation.

Aims: To investigate concentration-dependent, agonist-specific effects on platelet activation markers and biomechanics using RT-FDC.

Methods: Platelet-rich plasma from six healthy donors was stimulated with increasing concentrations of TRAP-6, CRP, ADP or epinephrine; phosphate-buffered saline served as control. RT-FDC was performed in a viscosity-adjusted, calcium-enriched buffer. Degranulation (CD62P), integrin activation (PAC1 binding), deformation, and size were analyzed simultaneously in CD61-positive platelets.

Results: All agonists except epinephrine induced dose-dependent increases in degranulation and integrin activation. Platelet deformation decreased after stimulation with all agonists, indicating increased stiffness and cytoskeletal remodeling. Notably, the decrease in deformation was not uniform with increasing agonist concentration. Maximal deformation changes were observed with TRAP-6 at 20 µM (Δ36.5%), CRP at 5 µM (Δ35%), ADP at 5 µM (Δ40%), and epinephrine at 5 µM (Δ38%). Platelet size changes were agonist-specific: increased stiffness was associated with increased size after TRAP-6 (Δ10.6%; p=0.0041) and CRP (Δ6.3%; p=0.9697), but decreased size after ADP (Δ10.2%; p=0.0264) and epinephrine (Δ2.5%; p=0.3571).

Conclusions: RT-FDC enables integrated assessment of platelet activation and biomechanics under flow conditions. Changes in deformation and size only partially correlated with classical activation markers and differed between agonists, suggesting the induction of biomechanically distinct platelet subpopulations.

23 Claudia Peitzsch

The Metabolic Environment in Type 1 Diabetes Shapes Phosphokinase Signaling and Neonatal HSPC Phenotype Revealed by Phospho- and Epi-CyTOF

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Hematopoietic stem and progenitor cells (HSPCs) are key regulators of immune system development and may be particularly susceptible to metabolic perturbations during early life. In this study, we used phospho-specific mass cytometry (phospho-CyTOF) to characterize signaling states and population dynamics of cord blood HSPCs from neonates born to parents with Type 1 Diabetes (T1D) and mothers with gestational diabetes mellitus (GDM), compared with healthy controls. To model diabetes-associated metabolic conditions, healthy cord blood-derived HSPCs were cultured ex vivo in the presence of serum from patients with T1D and under hyperglycemic conditions.

Phospho-CyTOF enabled high-dimensional, single-cell analysis of intracellular signaling networks across phenotypically defined HSPC subsets, revealing alterations not detectable using conventional approaches. We observed increased frequencies of CD34+ HSPCs in neonates born to mothers with T1D compared with offspring of fathers with T1D risk and healthy controls. Furthermore, cord blood HSPCs from neonates exposed to maternal T1D displayed altered lineage-associated marker expression suggestive of an embryonic-

like myeloid-biased differentiation program. Ex vivo exposure of healthy HSPCs to T1D-derived serum and hyperglycemic conditions recapitulated these phenotypic changes and confirmed altered activation of key phosphokinase signaling pathways associated with myeloid lineage bias within the HSPC compartment.

In parallel, we performed an exploratory application of Epi-CyTOF to investigate epigenetic alterations in HSPCs exposed to diabetic metabolic environments. Although preliminary, these findings demonstrate the feasibility of integrating epigenetic profiling with functional single-cell signaling analyses and establish a foundation for future studies investigating metabolic imprinting of hematopoiesis.

Together, our results support the concept that diabetes-associated metabolic conditions can reprogram early hematopoietic signaling and influence immune lineage specification. Furthermore, this study highlights the complementary potential of phospho- and Epi-CyTOF for comprehensive single-cell characterization of stem and progenitor cell states in health and disease.

Lea Rosenfeld

24

EdU labeling combined with multi-parameter microbiota flow cytometry for functional single-cell characterization of the intestinal microbiome

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2 Department for Cytometry, Institute of Biotechnology, Technische Universität Berlin, Berlin;

The human microbiome consists of diverse, highly individualized bacterial communities that are of great importance to human health, as they regulate the immune system, metabolism, and the gut barrier function. Previously, high-throughput sequencing methods have only captured the taxonomic composition, however the proliferative activity and functional phenotypic profile of individual bacteria *in vivo* remain largely unexplored. We present here a single-cell workflow that simultaneously quantifies DNA synthesis using the nucleoside analog 5-ethynyl-2′-deoxyuridine (EdU) and, via a 10-color panel (Hoechst DNA dye, various immunoglobulins, and four plant lectins) to evaluate multiple phenotypic parameters, such as DNA content, light scattering, surface immunoglobulin coating, and sugar-binding patterns, via high-throughput microbiota flow cytometry (mMFC).

In an ex vivo proof-of-concept experiment using complex human stool samples, it was demonstrated that EdU labeling specifically detects actively dividing bacteria without relying on antibodies. By combining EdU staining with the 10-color panel described above, we were able to simultaneously characterize the proliferation activity, DNA concentration, and phenotypic profile (e.g., immunoglobulin coating and sugar expression) of individual bacteria.

The established method will next be applied *in vivo* to wild-type mice to map bacterial proliferation in different sections of the intestine. This combination of EdU labeling and phenotypic mMFC is expected to provide insights into segment-specific growth dynamics that may be relevant to health and disease.

25 Andreia Ramalho-dos-Santos

Optimizing a Panel Design Service in a Flow Cytometry Platform 1 year later – what improved?

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[1] Fundação GIMM - Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal

Over the past two years, after implementing a Panel Design Service, our Flow Cytometry Platform has continued to expand and refine this service to address the increasing complexity of spectral flow cytometry experiments.

One of the major developments was the creation of a standardized panel design workflow form that centralizes all relevant experimental information for panel building. This workflow begins with the evaluation of the sample autofluorescence to identify detectors receiving the highest autofluorescence signals, allowing these to be avoided for critical markers. In addition, the form collects more detailed biological information, including the specific biological question being addressed and the classification of markers according to function and relevance.

Our panel design strategy was also refined. The panel construction now prioritizes biological and technical relevance over similarity and complexity scores alone. Emphasis is placed on assigning low expression critical markers to bright fluorochromes associated with minimal spread acceptance, based on the Spread Scaling Matrix (SSM). Highly expressed lineage markers are preferentially assigned to dimmer fluorochromes with low spread donation.

To increase the support to our users, the Panel Design Service was made publicly accessible, providing a simple and straightforward entry point for researchers requesting support.

Additionally, we expanded user support and follow-up by assisting researchers through all stages of panel implementation: autofluorescence assessment, antibody titration, panel testing, optimization, and troubleshooting.

Through these improvements, our Panel Design Service has evolved from a support initiative into a comprehensive and structured framework for high-dimensional flow cytometry assay development.

26 Eric Schneider

Holographic vibration spectroscopy: Extracting the mechanical properties of adherent animal cells from their vibrational response

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The mechanical properties of biological cells are tightly linked to their pathophysiological state, and high-throughput quantification is essential for using them as biomarkers in basic and translational research. While microfluidic technologies can characterize suspended cells at rates above 1,000 cells per second, no comparable method currently exists for adherent cells or tissues. To address this gap our group recently developed holographic vibration spectroscopy (HVS), where adherent cells are harmonically vibrated at defined frequencies and amplitudes. Their vibrational response with respect to amplitude and phase encodes their mechanical properties.
Here, we present a theoretical framework based on finite-element simulations using the open-source library AMDIS to model cellular vibration across a broad parameter space. From these simulations, we derive a protocol to solve the inverse problem and show that the elasticity as well as the viscosity,

can be uniquely determined using only the amplitude response at two vibration frequencies. Combined with HVS, this framework will next be used to rapidly and non-invasively quantify the viscoelastic properties of adherent cells and tissues.

Toni Sempert

27

Distinct age- and therapy-specific single cell-signatures in the intestinal microbiota of juvenile idiopathic arthritis patients

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Juvenile Idiopathic Arthritis (JIA) comprises diverse chronic inflammatory conditions driven by malfunction of the immune system. The intestinal microbiota is considered a crucial environmental factor correlating with chronic inflammatory diseases. Here, we have characterized the JIA microbiota of 54 JIA patients and 38 age-matched healthy controls using 16S rRNA sequencing and multi-parameter microbiota flow cytometry (mMFC) in relation to age dynamics and therapy response to methotrexate (MTX). With mMFC, we interrogate bacterial coating with endogenous host immunoglobulins and expression of surface sugar moieties using sugar-specific lectins. The multivariate data are analyzed with machine learning algorithms to define disease- or therapy-specific microbial signatures. We found that age is a major confounder in the microbiota analyses of children. When stratifying patients and healthy controls according to age into three groups, we could show that each age group has a distinct, non-overlapping taxonomic and phenotypic signature (PERMANOVA, all $p < 0.05$). We also identified age group-specific phenotypic signatures differing between JIA patients and healthy controls based on immunoglobulin coating and lectin staining. Using mMFC and based on microbial signatures, we were able to discriminate responders and non-responders to MTX-therapy (PERMANOVA, $R2 = 0.28$, $p = 9.9 * 10^{-5}$) and also to predict therapy responsiveness (AUC = 0.873). Our results highlight the dynamic nature of the intestinal microbiota in pediatric patients and that age-stratification is crucial in microbiome analyses in children. The mMFC approach demonstrates that single-cell analysis of the intestinal microbiota from stool samples has the potential to impact not only therapy monitoring but also therapy prediction, a first step towards microbiota-guided personalization of medicine.

28 Eric Sündermann

A fast cytometric approach to assess the membrane tension of cells

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Current research emphasises the importance of cell mechanics to understand cell state and function. Recently, the focus shifted from mechanical bulk properties to membrane tension, which plays a key role in various pathophysiological processes, e.g., the host-pathogen interaction in immune response. However, traditional methods that measure membrane tension are not able to provide sufficient throughput to screen entire cell populations or resolve dynamic processes within a cell. Furthermore, direct measurements of membrane tension on a sub-second timescale are largely unknown.

Recently, we introduced membrane tension cytometry (MTC) for analysing the membrane tension of suspended cells on a millisecond timescale, resulting in a throughput of 100 cells per second. MTC integrates flow cytometry and fluorescence lifetime analysis, utilising a membrane tension-sensitive dye (Flipper-TR). We studied human leukaemia cells (HL60), aiming to disentangle membrane and bulk contributions to the mechanical properties of cells. We depleted cholesterol in the plasma membrane and inhibited actin polymerisation. While we observed a reduction in fluorescence lifetime but no change in bulk mechanics when cholesterol was depleted, interference with the cytoskeleton impacts bulk stiffness only. Furthermore, we investigated the cellular response to the solvent dimethyl sulfoxide (DMSO). By combining membrane and bulk mechanical measurements at DMSO concentrations reaching from 0.01 % to 10 %, we revealed two distinct realms. Low concentrations up to 1 % mainly affect membrane properties, i.e. increased membrane tension, while higher concentrations also affect bulk mechanical properties, such as cell stiffness and volume. This highlights the potential of MTC to rapidly identify cellular mechanisms in the membrane that would otherwise be difficult to access.

29 Lucia Wegbünder

The role of filamentous actin for the biophysical response of myeloid and lymphatic cells to a millisecond osmotic stress

Lucia Wegbünder1, Eric Sündermann1, Lea Graichen1, Doreen Biedenweg1, Marta Urbanska2, and Oliver Otto1
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Local differences in the concentration of osmolytes within the human body require cells to consistently regulate their water in- and efflux. While cells are known to change their volume and mechanical properties on the timescale of seconds to minutes, their millisecond response to an osmotic stress has been, so far, unexplored. Specifically, it is unknown if the poroelastic organization of the actin cortex limits the rate of the response.

Here, we leverage real-time deformability cytometry to apply osmotic challenges to cells and measure their physical properties including size and elastic modulus 0.5 to 10 milliseconds after exposure. We focus on HL60 cells (a myeloid precursor cell line) and Jurkat cells (a lymphatic cell line), which are flushed through a microfluidic channel, where they are in contact with a sheath fluid of varying osmolality. We observe swelling under hypoosmotic,

and shrinkage under hyperosmotic conditions. Independent of stress direction the Young´s modulus decreases in HL60 cells and increases in Jurkat cells. The measurements were repeated under hypotonic conditions after treating the cells with two different concentrations of Cytochalasin D (CytoD), an inhibitor of filamentous actin. In both cell lines the stiffness decreased with increasing concentration of CytoD. However, the observed differences in changes of cell size between HL60 and Jurkat cells suggest a cell type-specific contribution of filamentous actin to the short timescale osmotic stress response.

Florian Wegner

30

Impact of stromal pancreatic stellate cells on PDAC spheroid growth and curative radioresponse

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A common characteristic of pancreatic ductal adenocarcinomas (PDAC) is the presence of a dense desmoplastic stroma. Therefore, we aimed to assess the impact of stromal cells on PDAC cell growth and radioresponse in a 3D environment. We established spheroid co-culture models of two fluorescently labeled PDAC cell lines in combination with human pancreatic stellate cells (hPSC). Flow cytometric analyses upon spheroid dissociation and immunohistochemical stainings in spheroid median sections were performed to (i) quantify the different cell populations and (ii) visualize their local distribution in the co-cultures at defined time points during and after a 4-day initiation period. Volume growth kinetics were documented until a plateau was reached, while the radioresponse was assessed using a curatively-intended 60-day spheroid control probability assay. We found that hPSCs cluster in the spheroid core in both models. Despite identical seeding cell numbers, the higher-grade PDAC cell line PANC-1 showed a higher fraction of hPSCs (30%) after a 4-day initiation period than the intermediate-grade PDAC model PaTu 8902 (5%). Vice versa, the volume growth of PaTu 8902 spheroids was critically enhanced in the presence of hPSC during the initiation phase, whereas PANC-1 cultures were less affected. In addition, the latter showed similar spheroid control probabilities upon radiotherapy in the absence and presence of hPSCs. While the role of hPSCs in PDAC remains ambiguous, the model-specific responses well reflect the *in vivo* heterogeneity of tumor-stromal cell interactions. We will next study the radioresponse in PaTu 8902 mono- versus co-cultures to verify whether the stromal-related growth advantage translates to a change in therapeutic outcome.

31 Marvin Weis

One-hot diffusion reveals, visualizes, and quantifies the complex transitions and trajectories of the human B cell manifold

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Peripheral B cell subsets, including transitional and naïve, various activated and memory stages, as well as plasmablasts, are frequently characterized by flow cytometry to understand immunological health and disease states. However, different phenotyping approaches, panels, and analysis strategies make cross-study comparisons difficult and often fail to reflect the phenotypic B cell manifold with its stable populations and the interconnections between them.

We integrated multiple B cell phenotyping approaches into a unifying backbone staining. Dissatisfied by existing bioinformatics tools, we developed trajectory preserving unbiased and custom dimensionality reductions, a novel pseudotime algorithm capable of branching and merging trajectories, and novel differential abundance testing at quasi-single cell level to compare abundance of rare transitioning cells.

Starting with a clustering, we diffuse one-hot encoded cluster labels along a transition matrix and obtain individual cluster proximity values for each cell. Intuitively, rare cluster-transitioning cells stand out by their mixed cluster proximities. Forwarding cluster proximities into UMAP, we obtain CLUMAP, an unbiased dimensionality reduction with improved trajectory preservation. Alternatively, diffusing user-determined cluster coordinates, we obtain USERMAP, to our knowledge the first truly custom dimensionality reduction, conveniently enabling reproducible single cell plot layouts. Finally, where common pseudotime tools fail due to branching and merging trajectories, our USERPATH aligns a selection of connected clusters and shows marker trends, cell state density and differential abundance along the trajectory, even at rare transitional stages.

In our data, these tools reflect known and unknown paths of class switching and verify CD45RB acquisition as a major early milestone towards memory B cells.

Our algorithms mostly use matrix multiplications and are therefore fast, deterministic, and mathematically simple. Given their robustness, versatility, and novel capabilities, we anticipate they will make bioinformatic analyses of flow cytometry data more rational, scalable, and accessible – for B cells and beyond.

Session 3 – Imaging and Spatial Transcriptomics

4:15 pm – 5:30 pm

Chairs: Anja Hauser, Raluca Niesner, Berlin

Over the past decade, the technologies and analysis to study cells in tissue context have evolved significantly. They now allow to comprehensively understand molecular mechanisms in health and disease, providing information about the effect of environmental and microenvironmental cues in the tissue. This includes effects of mechanical stimuli on cells. In this session, Adrien Hallou will give us an overview about the novel field of mechano-transcriptomics, which investigates how mechanical cues shape transcriptional profiles within the tissue context.

Adrien Hallou, Kennedy Institute, Oxford, UK

Short Talk: Deniz Tasgin

Deniz Tasgin [1,2], Alex Fiedler [3], Ruth Leben [3], Robert Günther [2], Luise Asmussen [1,2], Sandy Kroh [1,2], Johanna Ehl [1,2], Ralf Uecker [2], Raluca Niesner [3], Anja E. Hauser [1,2]

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[2] Immune Dynamics, Deutsches Rheuma-Forschungszentrum Berlin, Germany.

[3] Biophysical Analytics, Deutsches Rheuma-Forschungszentrum Berlin, Germany.

Mechanical Control of Macrophage Immunometabolism: Piezo1–Calcium Axis in Fracture Healing

Mechanical cues are integral features of tissue microenvironments and become profoundly altered during chronic inflammation, fibrosis, and bone injury. While mechanotransduction is well characterized in neurons and stromal cells, emerging evidence suggests that immune cells also convert mechanical inputs into biochemical programs that shape inflammation and repair. Tissue-resident macrophages are prime candidates for such regulation because they adapt their polarization, cytokine output, and metabolism to local tissue demands. Mechanosensing may provide an additional regulatory layer, fine-tuning these cells to heterogeneous tissue contexts. Piezo1, a highly expressed mechanosensitive cation channel in macrophages, has been implicated in inflammatory signaling, yet how Piezo1-dependent calcium influx rewires macrophage metabolism *in vivo*, and how this impacts bone healing, remains unclear.

Our project will define Piezo1-mediated mechanotransduction in bone marrow macrophages and establish its relevance for fracture healing. Using intravital two-photon microscopy coupled to fluorescence lifetime imaging (FLIM), we will quantify macrophage metabolic states in their native niche via NAD(P)H lifetime signatures and link these readouts to Piezo1-associated calcium dynamics measured with genetically encoded calcium reporters such as GCaMP or TN-XXL in selected immune populations. In parallel, controlled *in vitro* perturbations of mechanical context and pharmacological Piezo1 modulation will causally map mechanical inputs to calcium signaling, metabolic switching, and polarization outputs. To test *in vivo* relevance, fracture healing after osteotomy will be evaluated in myeloid-specific Piezo1-deficient mice. Longitudinal outcomes will be assessed by micro-CT, histology, and multiepitope-ligand cartography to relate macrophage states to vascular, stromal, and inflammatory zones within the healing callus. By integrating intravital metabolic imaging, reporter mouse models, and spatial histology, this project will define how mechanical cues shape macrophage metabolism and test whether Piezo1 functions as a mechanometabolic regulator of bone repair.

Short Talk: Leonard Fiebig

Leonard Fiebig[1], Florian Kralik, Heike Hirseland[1], Axel R. Schulz[1], Sandy Kroh[1], Ralf Uecker[1], Lisa-Marie Diekmann[1], Antonia Niedobitek[1], Simon Reinke[2], Sebastian Hardt[3], Schayan Yousefian[4], Simon Haas[4], Anja Hauser[1], Henrik E. Mei[1], Hyun-Dong Chang[1]

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4 Max Delbrück Center - Berlin Institute for Medical Systems Biology

Spatial and Functional Insights into Plasma Cell Longevity and Immune Memory Maintenance

Mature antibody-secreting plasma cells (PCs) in human the bone marrow (BM) are critical for long-term humoral immunity and immunological memory, yet regulatory cues and tissue niches that support their persistence remain poorly understood. Here, we investigate the spatial, phenotypic, and functional heterogeneity of mature human BM PCs using an integrated approach combining spatial proteomics with primary BM culture systems.

We established a platform using fresh human femur heads that enables tissue sectioning and highly multiplexed spatial proteomic imaging of up to 55 markers without decalcification, which preserves sensitive epitopes that are often difficult to detect in conventional FFPE bone sections. In parallel, flow cytometric and secretome profiling of matched primary whole BM cultures from the same donors offers a complementary functional systeme to define survival requirements across the major BM memory cell compartments

Using this integrated workflow, we aim to localize plasma cell subsets within the BM microenvironment, characterize their interactions with stromal and immune cell populations associated with PC longevity, and functionally assess their dependence on key survival pathways.

Together, these analyses provide new insight into the cellular organization of the human BM niche and the mechanisms that sustain durable antibody and immune memory. Ultimately, we aim to discover new targets for therapies that selectively disrupt pathogenic plasma cells or immune memory compartments while sparing protective immune memory in autoimmune disease.

6:00 pm – 11:00 pm

Meet the Speaker - sponsored by Stratocore S.A.S. & Awards

Bus to Elbterasse Wachwitz leaves CRTD at 5:45pm

At 11:00 pm, there will be a shuttle bus back to Hotel Terrassenufer and to Postplatz.

Friday, 25.9.2026

Session 4 – Clinical Applications

9:00 am – 10:15am

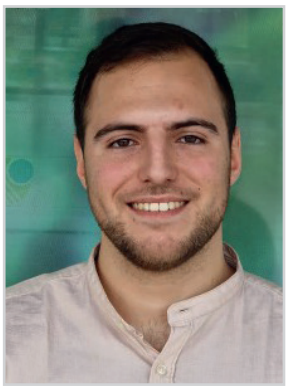
Chairs: Hyun-Dong Chang, Berlin, Axel Schulz, Berlin

Birgit Sawitzki

BIH/Charité – Universitätsmedizin Berlin

Cross-cohort immune profiling reveals treatment-relevant endotypes in Long Covid-ME/CFS

Post-acute infection syndromes (PAIS), including Long Covid (LC), can severely impair quality of life, yet no approved therapies exist. A subset of patients develops myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), a heterogeneous disorder characterized by post-exertional malaise, cognitive dysfunction, pain and functional impairment. Variable responses to experimental therapies suggest the existence of biologically distinct disease endotypes and highlight the need for predictive biomarkers.To identify treatment-relevant immune endotypes, we analysed two complementary LC-ME/CFS cohorts: a non-selected LC-ME/CFS cohort representing the broad disease population and an immunoadsorption (IA) cohort enriched for patients with elevated GPCR-directed autoantibodies and linked treatment response data.Using NULISA plasma proteomics, we constructed correlation networks of soluble mediators and identified an ME/CFS-specific molecular network. Unsupervised clustering based on mediators within this network defined three reproducible molecular clusters characterized by low, medium and high abundances of network-associated soluble mediators. Integration with CyTOF-based whole-blood immune profiling revealed distinct immunological phenotypes underlying these molecular states. The high NULISA cluster was characterized by expansion of $CXCR5^-$ non-germinal center (non-GCB) IgM^+ non-switched memory B cells, consistent with extrafollicular B-cell activation. In contrast, the medium cluster was enriched for germinal center-associated B-cell populations, whereas the low cluster exhibited expansion of $CD127^+CD161^+$ $CD4^+$ and $CD8^+$ T cells, suggesting a fundamentally distinct immunological state.Importantly, these molecular clusters were associated with clinical characteristics. IA non-responders were strongly enriched within the high NULISA cluster, whereas IA responders preferentially localized to the medium cluster. In contrast, the low cluster consisted almost exclusively of patients from the non-selected LC-ME/CFS cohort, suggesting a distinct disease endotype that was largely absent from the GPCR-directed autoantibody-enriched cohort.To further assess autoimmune features, we applied an autoimmunity score based on soluble mediators that distinguishes Sjögren's disease from acute SARS-CoV-2 infection. The high NULISA cluster exhibited the highest autoimmunity scores and showed the greatest similarity to Sjögren's disease, whereas the low cluster had the lowest autoimmunity scores and was most distinct from Sjögren's disease.Together, our findings identify three biologically distinct LC-ME/CFS endotypes that differ in soluble inflammatory networks, immune cell composition and treatment association. These data provide a framework for biomarker-guided patient stratification and support the development of precision therapies for LC-ME/CFS.



Short Talk: Adrián Barreno Sánchez

Adrián Barreno Sánchez[1,2], Alissa Karina Yudiputri[1], Heike Hirseland[1], Thuy-Vi Tran[1], Sebastian Ferrara[1], Anne Beenken[1,4], Paulina Rybakowska[3], Concepción Marañón Lizana[3], Marta E. Alarcon Riquelme[3,5], Tobias Alexander[1,4], Henrik E. Mei[1], Hyun-Dong Chang[1,2], Axel R. Schulz[1]

1.German Rheumatology Research Center Berlin – A Leibniz Institute, Berlin, Germany
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Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Keywords: Systemic Lupus Erythematosus, mucosal immunity, mass cytometryFlow cytometry for bacteria and microbial communities using advanced scattered light detection and specialized data analysis

Mass Cytometry Analysis Defines Clusters of SLE Patients with Differential Mucosal Phenotypes that Correlate with Immune Activation and Disease Severity

Systemic lupus erythematosus (SLE) is a heterogeneous systemic autoimmune disease driven by loss of immune tolerance and autoantibody production. Disease progression, clinical presentation, and treatment response vary widely across patients and remain poorly understood. Since emerging evidence implicates the gut-immune axis in SLE pathogenesis, we aim to examine mucosal immunity as a potential driver of this heterogeneity.

To do so, we developed a robust CyTOF-based workflow for ex-vivo phosphorylation profiling and mucosal immune phenotyping using fixed whole blood, designed to be scalable for large clinical cohorts. The pipeline uses a cell-sorting strategy to separate blood into lymphoid and myeloid fractions, each analyzed with optimized panels of more than 50 antibodies. These panels capture mucosal-associated populations, alongside phosphoprotein markers reflecting intracellular signaling activity.

Applying this workflow to 326 SLE samples reproduced known lymphoid and myeloid differences versus healthy controls, and additionally revealed significant reductions in mucosa-associated populations, particularly MAIT cells and αEβ7+ CD8+ T cells. Clustering patients by abundance of these mucosal-related lymphoid populations identified three subgroups with distinct phenotypic and clinical profiles. One cluster, marked by elevated MAIT cells and naive-like γδ T cells but reduced circulating IgA+ B cells, showed higher rates of lupus nephritis and disease activity, along with altered B cell, NK cell, and monocyte subsets and increased pZAP70/Syk and pCREB signaling in several immune populations.

The findings demonstrate this workflow's value for deep immune phenotyping focused on gut-related populations and signaling activity, and its potential to reveal clinically meaningful subtypes of SLE patients.

Short Talk: Bernadette L. Bramreiter

Bernadette L. Bramreiter[1], Rachel C. Quilang[2], Carin van der Keur[2], Anne Wagenmakers[2], Katja Sallinger[1], Emiel Slaats[1], Julia Schönberger[1], Katharina Schuch[1], Hyun-Dong Chang[3,4], Dave Roelen[2], Marie-Louise van der Hoorn[5] , Thomas van den Akker[5], Michael Eikmans[2], Thomas Kroneis[1]

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An optimized flow cytometry-based method for the isolation of foreign rare cell populations in human stem cells using monoclonal HLA class I specific antibodies.

Objectives

Microchimerism, (Mc) defined as the presence of genetically distinct cells from another individual, occurs during pregnancy through a bi-directional cell transfer yielding maternal and fetal Mc (mMc, fMc). Current hypothesis assign microchimeric cells stem and progenitor cell-like properties contributing to a lifelong establishment of Mc. This study aimed to develop and optimize a sex-unbiased flow cytometry-based method to isolate viable potential mMc cells from human fetal stem cell populations using monoclonal HLA class I-specific antibodies (HuMoAbs).

Methods

Hybridoma derived HuMoAbs of IgG and IgM isotypes were generated at LUMC. Eleven HuMoAbs targeting HLA-A and HLA-B were selected and validated for the separation of maternal and fetal cells. To improve population discrimination and detection sensitivity, maternal specific HuMoAbs were separately conjugated to PE and AF488, respectively, and used simultaneously for staining. Fetal specific HuMoAbs were labeled with AF647. PE/AF488 double-positive and AF647 negative cells were considered of maternal origin. This approach was optimized in spike-in experiments and subsequently applied to fetal stem cell populations derived from bone marrow (BM), liver (L), amniotic membrane (AM) and amniotic fluid (AF).

Results

The optimized method enabled reliable separation of maternal and fetal cells, with detection sensitivity down to 0.004% maternal cells. Dual fluorochrome labelling significantly improved population discrimination. Across multiple fetal tissue-derived stem cell samples, viable maternal cells were isolated at average frequencies of 0.04% in AM, 0.7% in BM, 0.1% in L and 0.03% in AF.

Conclusion

We established a robust, sex-unbiased flow cytometry-based method for the pre-enrichment and isolation of viable potential maternal Mc cells from human fetal stem cell populations. This approach provides a valuable tool for downstream functional and molecular analyses of mMC cells.

Session 5 – Technical Development/Emerging Technologies

10:45 am – 12:00 pm

Chairs: Michael Kirschbaum, Potsdam Toralf Kaiser, Berlin

In the session on "Technical Development / Emerging Technologies," innovative approaches to addressing cytometric challenges will be presented. This includes exceptional analytical techniques in sorting processes, as well as advancements in device development and technical improvements of cytometric instruments. Experts will cover the latest developments in the impedance-based analysis of cells and particles and showcase microfluidic techniques that enhance the precision and efficiency of cytometric analyses. We will learn about solutions for analyzing or sorting particles of extraordinary sizes, properties, or handling requirements. In summary, novel technical strategies will be highlighted that overcome existing limitations and open up new possibilities for research, therapy and diagnostics.

Marco Di Berardino

Fa. Amphasys

Impedance Flow Cytometry: From Fundamental Single-Cell Analysis to AI-Driven Bioprocess Intelligence

Impedance Flow Cytometry (IFC) is a label-free single-cell analysis technology that combines the Coulter Counter principle with modern microfluidics and semiconductor technologies. By measuring the electrical properties of individual cells at multiple frequencies, IFC reveals cell size, membrane integrity, and intracellular features without staining or labeling. This keynote introduces the fundamentals of IFC, traces its evolution from pollen analysis to biotechnology and bioprocessing applications, and presents selected case studies. It concludes with an outlook on AI-enabled data analysis and predictive bioprocess control.

Marco's bio:

Dr. Marco Di Berardino received his PhD in Microbiology from ETH Zurich. After a brief period in the pharmaceutical industry working on antibiotic development, he moved into the field of diagnostic instrumentation, developing automated liquid-handling platforms for medical applications. In 2004, he initiated the development of an impedance flow cytometer based on technology originating from a PhD thesis at the École Polytechnique Fédérale de Lausanne (Switzerland). When the original project was discontinued, he co-founded Amphasys to further develop and commercialize the technology. Under his technical leadership, Amphasys introduced the world's first commercial chip-based high-frequency impedance flow cytometer, establishing impedance flow cytometry as a practical tool for label-free single-cell analysis. Today, Dr. Di Berardino serves as Chief Technology Officer of Amphasys AG in Lucerne, Switzerland, where he continues to drive innovation in cell analysis for research, biotechnology, and bioprocessing applications.

Short Talk: Bob Fregin

Bob Fregin1,2, Stefanie Spiegler1,2, Eric Schneider1, and Oliver Otto1,2

1 Institute of Physics, University of Greifswald, Greifswald, Germany

2 DZHK (German Center for Cardiovascular Research), Partner Site North, Greifswald, GermanyA fast cytometric approach to assess the membrane tension of cells and mitochondria in situ

Holographic vibration spectroscopy: Probe- and contact-free viscoelastic analysis of adherent cells at high throughput

Cell mechanical properties can serve as inherent biomarkers of cell state, fate, and function, revealing comprehensive and fundamental information about a cell. Several high-throughput methods with analysis rates beyond 1,000 cells per second are available to characterize cells in suspension, e.g., peripheral blood cells, without any labeling.

However, fast and robust methods for adherent cells are lacking, even though the majority of cells (by mass), e.g., in the human body, are aggregated into tissues. Most existing techniques for measuring the mechanical properties of adherent cells suffer from low throughput, whereas some methods enable parallel measurement of many cells in a tissue at a time. However, often tracers need to be integrated into the tissue, such as magnetic particles, beads, fluorescently labeled beads, oil droplets, or ferrofluid microdroplets, which potentially influence tissue integrity.

Here, we are closing this methodological gap by introducing a new probe- and contact-free method for label-free mechanical phenotyping of adherent cells at high spatiotemporal resolution. Cells in an aqueous solution of high viscosity adhere to a surface and are excited mechanically by a vibration with varying frequencies. Their response is determined optically from cell height oscillations utilizing holographic laser Doppler interferometry. By analyzing vibrational frequency bands, vibrational amplitude and phase can be reconstructed for every single pixel of our camera sensor.

In proof-of-principle experiments, we demonstrate the applicability of our novel technique on first, a liquid-liquid interface, and second, a monolayer of adherent cells.

Short Talk: Daniel Kage

Kage[1], V. Devaraj[1], A. Eirich[2], J. Popien[2], B. Grothe[2], A. Wolf[1], J. Kirsch[1], K. Heinrich1, K. v. Volkmann[2], T. Kaiser[1]

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A custom-built cell sorter for label-free sorting with scattered light and AI

Label-free analysis and sorting of cells and other biological objects is gaining more and more relevance. At the forefront of this development are imaging technologies that aim to combine the advantages of microscopy with those of flow cytometry. While this is the intuitive way of looking at cells, other approaches for the measurement of intrinsic cell parameters are promising candidates

as well.

One of these alternative approaches is multi-angle pulse shape flow cytometry (MAPS-FC). It collects the light scattered by cells or particles at multiple angles and with sub-µs resolution during the cell transit. The resulting high-dimensional datasets contain valuable information about the particles analyzed. This technique has proven useful in various fields from cell proliferation analysis over automated label-free immunophenotyping, microbiome analysis, and parasite identification to functional cell analysis.

Here, we describe how we translated these capabilities from a custom-built analyzer into a high-speed cell sorter, which enables sorting decisions made by artificial intelligence for label-free cell sorting. We will report on the technical development process and (hopefully) present first outcomes of a completely new view on defining target populations for cell sorting.

12:00 pm – 12:15 pm

Farewell

12:15 pm - 1:00 pm

Lunch (take away)

1:15 pm - 4:00 pm

DGfZ Board meeting & Program Committee

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