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Deutsche Gesellschaft
für Zytometrie

Abstract Booklet

Location: Charité - Universitätsmedizin Berlin, CCO & DRFZ & Paul Ehrlich Lecture Hal

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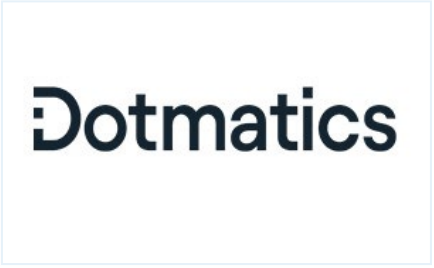
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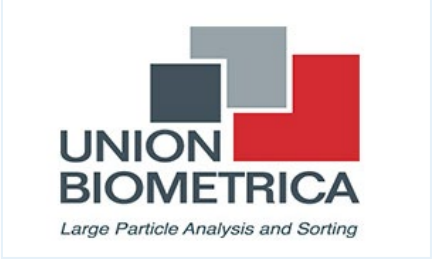
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Dear friends of cytometry,

After two challenging years due to the pandemic, it is my greatest pleasure to welcome you to the 32nd Meeting of the German Society for Cytometry (DGfZ), from Sept, 28th to 30th, in Berlin, at a historical location: Charité Campus Mitte.

Following this year's motto "Spotlight on cells: life style and environment", we strive to bring together experts from various disciplines for a lively discussion about game-changing technologies and their newest applications. A number of invited speakers have already confirmed their attendance, and we will have exciting sessions on microscopy/metabolic imaging, cutting-edge technologies, nanotechnology, microbiology, mass-cytometry as well as mechano-cytometry. We are sure, this inspiring program will spark vivid discussions between scientists, technologists and industrial partners, having the potential to give unique impulses to the cytometry science.

In addition to the well-established industry exhibition, where you will have the opportunity to directly get informed about recently launched products, we will again have the popular "Product Slam" format, where our industrial sponsors will present their newest innovations within three minutes. Learning from the pandemic, we will offer you the chance to get informed virtually about new commercial technologies during the industry webinars, on Sept. 26th and 27th.

I wish you all an exciting DGfZ 2022!

Raluca Niesner
President of the DGfZ

Monday, September 26th, 2022

Industry Webinars online in Zoom

09:30 am - 10:15 am Cyteck

10:30 am - 11:15 am OMNI Life Sciences

11:30 am - 12:15 pm Biolegend

Tuesday, September 27th, 2022

Industry Webinars online in Zoom

9:30 am - 10:15 am Fast Forward Discoveries GmbH

10:30 am- 11:15 am Particle Metrix GmbH

Tutorial 1: FACS - in presence

11:30 am - 2:30 pm Flow, FACS, usual fallacies – limited for 20 participants
DRFZ - Seminar room 1+2 Chairs: Toralf Kaiser, Claudia Giesecke-Thiel

Tutorials 2 and 3 - in presence

03:00 pm - 05:00 pm Image Analysis
DRFZ - Seminar room 1st floor Chair: Ralf Köhler

03:00 pm - 05:00 pm Mass Cytometry
DRFZ - Seminar room 3 Moderator: Sarah Warth

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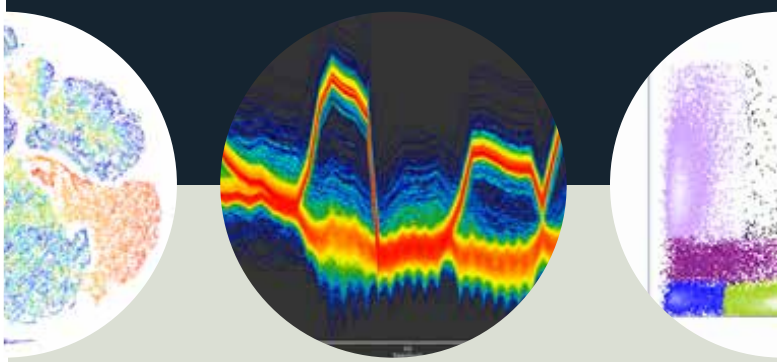

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Wednesday, September 28th, 2022

Tutorial 4 - in presence

9:00 am - 11:00 am Mechanocytometry
DRFZ Seminar room 3 Chair: Oliver Otto

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

11:45 am - 12.00 pm, PEH **Welcome**

Raluca Niesner

12:00 pm - 1:30 pm, PEH **Session 1: Imaging**

Chairs: Raluca Niesner, Anja Hauser

Ruslan Dmitriev, Gent	Probing intestinal organoids oxygenation and metabolism using phosphorescence and fluorescence lifetime imaging microscopies
Anna Pascual-Reguant, Berlin	Activated fibrovascular niches orchestrate immunopathology in severe COVID-19 disease
Alexander Fiedler (ST)	Development of functional in vivo fluorescence lifetime microendoscopy of the femoral bone marrow
Eric Sündermann (ST)	Cytometric single-cell analysis of membrane tension

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

2:00 pm - 3:00 pm, PEH **Session 2: Product Slam**

Chairs: Elmar Endl, Tom Bauer

Selected industrial partners will present their newest innovative technological developments and products

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

(ST) = short talk

3:30 pm -5:00 pm, PEH **Session 3: Cutting Edge**

Chairs: Henrik Mei, Asylkhan Rakhymzhan

Emily Stephenson, Newcastle	TBA
Rafael Jose Arguello, Marseille	Metabolism in the single cell era: approaches to sharpen the cutting edge of the metabolism field
Marie Burns, Berlin (ST)	Single cell phospho-signatures for precision medicine in SLE and other chronic inflammatory diseases
Daniel Kage, Berlin (ST)	Cell sorting based on angle-resolved pulse shapes

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

5:30 pm - 6:30 pm, PEH **Keynote (open to public)**

Chair: Marta Ferreira Gomes

Simon Haas, Berlin	Exploiting the power of single-cell genomic technologies to enable data-informed cytometry

6:30 pm - 10:00 pm, CCO **Welcome reception**

Exhibition area

*8:00 pm - 10:00 pm, DRFZ** **Core Facility Networking Event**

Chairs: Désireé Kunkel, Sarah Warth

Jochen Behrends, Borstel, Anne Gompf, Dresden, Marie Follo, Freiburg, Desiree Kunkel, Berlin, Sarah Warth, Ulm

**Seminar room 1+2, DRFZ*

Thursday, September 29th, 2022

9:00 am - 10:30 am, PEH **Session 4: Microbiology**

Chairs: Christin Koch and Hyun-Dong Chang

Naama Geva-Zatorsky, Haifa	Dynamics of gut microbe-host interactions
Jakob Zimmermann, Bern	Noninvasive assessment of gut function using transcriptional recording sentinel cells
Lisa Budzinski, Berlin (ST)	Single-cell bacterial phenotyping for disease classification and identification of disease-relevant microbiota populations
Shuang Li, Leipzig (ST)	Stabilization of complex microbial communities revealed by single-cell analysis

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

11:00 am - 12:00 pm, PEH **Session 5: European Guest Session: Austrian Society**

Chairs: Raluca Niesner, Henrik Mei

Andreas Spittler, Vienna	Introduction: The Austrian Society for Cytometry
Sieghart Sopper, Innsbruck	One tube to find them all
Beate Rinner, Graz	Thinking outside the box in cell culture
Lena Müller, Vienna	Mapping the cellular landscape of human airways by using mass cytometry

12:00 pm - 1:00 pm **Lunch**

1:00 pm - 2:30 pm, PEH **Speed Talk Session**

Chairs: Henrik Mei, Oliver Otto

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

3:00pm - 4:30pm **Session 6: Klaus-Goerttler-Session incl. award ceremony**

Chair: Janine Kemming (last year awardee)

Bertram Bengsch, Freiburg	Enolase represents a metabolic checkpoint controlling the differential exhaustion of virus-specific CD8+ T cells in HBV versus HCV
Asbjørn Christophersen, Oslo	A similar T-cell subset is increased across multiple autoimmune conditions and contains the antigen-specific cells in celiac disease

Klaus Goerttler-Awardee:

(ST) = short talk

Elisa Rosati, Kiel Profiling the SARS-CoV-2-reactive CD4+ T cell repertoire

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

5:00 pm - 6:00 pm, PEH **Guest Lecture**

Chair: Raluca Niesner

Sebastian Meller, Hannover Diagnoses by Dog Noses?

6:00 pm - 7:30 pm **DGfZ-Members Assembly**

Chairs: Raluca Niesner, Henrik Mei

7:30pm - 11:00pm, DRFZ **Meet the Speaker & Dinner**

For all speakers, participants and industry partners

Friday, September 30th, 2022

9:00 am - 10:30 am Session 7: Mechanocytometry

Chairs: Marta Urbanska and Oliver Otto

Chii Jou Chan, Singapore	Mechanical control of mammalian ovarian folliculogenesis
Anna Taubenberger, Dresden	Studying the mechanical and morphological phenotype of Cancer-associated fibroblasts of the prostate
Bob Fregin, Greifswald (ST)	Dynamic real-time deformability cytometry to decipher the response of bats to heterothermy
Benedikt Hartmann, Erlangen	Linking mechanical properties of cells with their ability to circulate using real-time deformability cytometry
Martin Kräter, Erlangen (ST)	Clinical application of physical characterization of major blood cell types during COVID-19 and beyond

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

11:00 am - 12:30 pm Session 8: Core Facility Session

Chairs: Alina Liebheit and Frank Schildberg

Harsharan Bhatia, Munich	Spatial molecular profiling of 3D imaged whole organs and organisms
Anika Grüneboom, Dortmund	From tissue clearing to cleared immunological processes
Till Mertens, Berlin	MarShie – a novel tissue clearing pipeline to comprehensively reveal myeloid-vasculature interactions throughout the murine bone marrow

12:30 pm - 1:00 pm Lunch

1:00 pm - 2:30 pm Session 9: Nanotechnology

Chairs: Wolfgang Fritzsche and Julia Böke

Tom Robinson, Berlin	High-throughput microfluidic production of multi-compartment synthetic eukaryotic cells
Claudia Lorenz, Aalborg, DK	Microplastics – Small particles, big challenges
Julia Böke, Jena	Imaging Flow Cytometry – from 3D tomographic over multispectral imaging to nanoparticle tracking
Martin Hussels, Berlin (ST)	Integration and Control of Low-Cost Industrial Cameras in a Home-Built Flow Cytometer

2:30 pm - 2:45 pm Farewell/Speed talk Award

(ST) = short talk

Wednesday, 28.09.2022

Program and Abstracts

12:00 pm - 1:30 pm Imaging Session

Chairs: Raluca Niesner and Anja Hauser

In line with the theme of the conference "Spotlight on cells-lifestyle and environment", the imaging session is focusing on various ways to analyze the interaction of cells with their microenvironment and cellular responses to various stimuli coming from their surroundings. The session will cover a variety of experimental imaging systems, ranging from single cells in microfluidic systems via tissue sections and organoids, to intravital microscopy. We will start with a talk by Prof. Dmitriev, who is using fluorescence and phosphorescence lifetime imaging to analyze tissue oxygenation and metabolism. Our second speaker is Dr. Anna Pascual-Reguant who is combining various spatial technologies, at the protein and transcriptomic level, to analyze COVID-19 lung pathology. Alexander Fiedler will tell us about a fluorescence lifetime (FLIM) based metabolic analyses *in vivo*, which he longitudinally performs in the context of bone regeneration. Our fourth speaker, Eric Sündermann, is reporting on the possibility to use FLIM to analyze the mechanical properties of cells. We are looking forward to an exciting session about cytometry in context.

Probing intestinal organoids oxygenation and metabolism using phosphorescence and fluorescence lifetime imaging microscopies

Ruslan Dimitriev

Tissue Engineering and Biomaterials Research Group, Dept. Human Structure and Repair, Ghent University, Ghent, Belgium

Success in studies of 3D (micro) tissues and organoids is often hampered by their intrinsic heterogeneity and dynamic gradients of biomolecules. Dynamics of cell metabolism, excretion products and O2 are hard to predict and are typically controlled by western blotting, next generation genome, RNA sequencing and traditional antibody-based microscopy methods. My team addresses the challenge of non-destructive quantitative multi-parametric imaging of 3D tissue models by using high-performance nanoparticles, new sensor chemistries and live fluorescence (FLIM) and phosphorescence (PLIM) lifetime imaging microscopies. In my talk I will briefly introduce the methodology of O2 sensing by PLIM and how this methodology can help visualizing live oxygenation of mouse adult stem cell derived intestinal organoids. Using this approach, complemented by the two-photon excited NAD(P)H autofluorescence imaging and other FLIM methods, we found that stem cell metabolism strongly depends on the nutrient availability in the growth medium. Collectively, presented methodology is highly useful for studies of the stem cell niche metabolism in the organoid cultures.

Activated fibrovascular niches orchestrate immunopathology in severe COVID-19 disease

Anna Pascual-Reguant

AG Hauser, Immune Dynamics, Deutsches Rheuma-Forschungszentrum & Charité-Universitätsmedizin Berlin

Post-acute lung sequelae of COVID-19 are challenging many survivors across the world, yet the mechanisms behind are poorly understood. We have analyzed post-mortem lung and lymph node tissue of COVID-19 cases and non-COVID-related pneumonia controls, by using several state-of-the-art imaging techniques, snRNAseq and spatial transcriptomics. We have stratified the COVID-19 donors based on disease duration and included a prolonged group (7 – 15 weeks of disease duration post-infection), where viral infection had been resolved. Nevertheless, prolonged COVID-19 lungs showed persistent tissue damage along with an increase in lymphocyte infiltration, features that recapitulate those previously reported for Long-COVID.

Our unique spatial approaches reveal that, upon endothelial dysfunction, activated fibrovascular niches emerge as crucial sites, where the molecular mechanisms driving a dysregulated interaction between immune cells and the surrounding tissue occur. We pinpoint two main factors, CCL18 and CCL21, which modulate the local stromal composition and favor endothelial to mesenchymal transition, resulting in the expansion of fibrovascular niches. This, in turn, results in an excessive accumulation of CCR7+ T cells, which become locally imprinted with an exhausted, T follicular helper-like phenotype, as opposed to the sustained T cell activation found in draining lymph nodes. The perpetuation of local chronic inflammation, which is independent of viral persistence, results in the formation of tertiary lymphoid structures as the ultimate manifestation of tissue repurposing.

Short talk:

Development of functional in vivo fluorescence lifetime microendoscopy of the femoral bone marrow

Alexander Fiedler

Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz-Institut, Biophysical Analytics, Berlin, Germany

Immune cell (dys)function is closely related to their metabolism. However, the particular cause and mechanisms remain elusive, mainly due to the lack of technologies to visualize local metabolism *in vivo* over long periods.
My project is about the development of Fluorescence-lifetime Longitudinal Intravital Microendoscopy of the Bone marrow (FLIMB). This novel method will combine fluorescence lifetime imaging and longitudinal micro-endoscopy of the bone marrow (LIMB) in live mice: Multi-photon microscopy and implant-based endoscopic gradient optics allow very deep and dynamic fluorescence imaging of the bone marrow tissue and the finely orchestrated self-organization of (immune) cells (e.g. re-vascularization after injury). Additionally, the fluorescence lifetime imaging of the autofluorescent metabolic molecules NADH and NADPH will allow the simultaneous analysis of the local cellular (immuno) metabolism and function, respectively.
With FLIMB, we found dynamic metabolic and functional heterogeneity of myeloid cells during bone regeneration after osteotomy. We further aim to gain deeper insights into spatiotemporal and functional aspects of bone marrow biology during health and disease and expect that environmental factors (like nutrient and oxygen availability and the inflammatory milieu) determine metabolic activity, which impacts on the diverse functions myeloid cells fulfil.

Short talk:

Cytometric single-cell analysis of membrane tension

Eric Sündermann

ZIK HIKE, Institute of Physics, University of Greifswald, Greifswald, Germany

Real-time deformability cytometry (RT-DC) is a biomechanical method which is able to characterise the physical properties of cells. To do so, cells travel through a microfluidic chip assembled on an inverted microscope. Every cell is imaged by a high-speed camera, and a convex contour is fitted to its shape to calculate the corresponding deformation. While mechanical properties of cells can be derived from analytical models and knowing the deformation as well as the hydrodynamic stress distribution, this approach is not suitable to discriminate between membrane and cytoskeletal contributions toward cell mechanics.
Here, we aim to shed light on dynamic alterations in membrane tension of cells passing a microfluidic constriction by introducing a new method that combines cytometry with fluorescence lifetime imaging microscopy (FLIM). As a fluorescent probe we applied Flipper-TR that is incorporated in the lipid-bilayer membrane of cells. In preliminary experiments, we measured the membrane tension of myeloid precursor cells (HL60 cells) at various hydrodynamic and osmotic stresses. We are able to observe a stress-dependent membrane tension response. We also found a change in the membrane tension for dimethyl sulfoxide (DMSO), which influences the membrane fluidity, but not for the exposure with cytochalasin D, which alters the level of polymerization of the underlying actin cortex. In future studies, we want to complement FLIM with RT-DC to simultaneously measure the contribution of membrane tension and the cytoskeleton to the mechanical properties of cells.

2:00 pm - 3:00 pm Product Slam Session

Chairs: Elmar Endl, Thomas Bauer

What is the product slam and what is good for, well…

for company representives, you definitely won't be tired to stare into a monitor on a Zoom meeting this year and hope that people will see your yellow raised hand. We are back live, in 3D and Dolby X Surround Sound.
Back from the pandemic it might be even more important for companies to look for opportunities to present yourself .
We're giving you 3 minutes to do that.

We call it the Product Slam.

3:30 pm - 5:00 pm Cutting Edge Session

Chairs: Henrik Mei and Asylkhan Rakhymzhan

Cytometric technologies ranging from instruments and assays to data analysis are an important pillar of scientific advance in the study of life, with applications in basic and medical research, and diagnostics. The cutting-edge session aims to present the cytometry of tomorrow and features a variety of topics and speakers. This years` focus is the intersection of high-dimensional and functional single-cell cytometry. By capturing functional states of many cell types and differentiation states at a time, at single-cell levels and high throughput, these approaches promise to aid the understanding of human health and disease.

N.N.

Emily Stephenson

Haniffa Lab, Newcastle University and Visiting Scientist, Wellcome Sanger Institute, Newcastle, UK

The COVID-19 pandemic has shown to be a complicated disease that continues to evolve as more variants of SARS-CoV-2 emerge. In the spring of 2020, very little was known about how the virus entered the body and how an individual's immune system responded to the infection. The Human Cell Atlas (HCA) database enabled the identification of the specific cell types that were permissive to the virus and the particular entry receptors. We generated further HCA datasets using single cell RNA sequencing combined with surface proteome and T and B lymphocyte antigen receptor analyses from the blood of patients infected with COVID-19. This data revealed a coordinated immune response that contributed both to the resolution and the pathogenesis of the disease. We also generated reference maps that could be utilised in the generation of therapies and prophylaxes.
In 2022, the COVID-19 situation is different across the globe. With the rollout of many vaccination programmes and better therapies, the risk to the general population is much lower. However, for some patient cohorts, COVID-19 still poses a major threat. End stage renal failure has shown to have the highest mortality rate out of these groups. We have collected longitudinal data from ESRF patients with COVID-19 to study the reasons behind why the disease is worse for these individuals. The dataset has also given us the chance to study the kinetics behind the disease and the effect of treatments.

Metabolism in the single cell era: approaches to sharpen the cutting edge of the metabolism field

Rafael Jose Argüello

Metabolic regulation of immunity team
Centre d'Immunologie de Marseille-Luminy (CIML), Marseille, France.

Personalized medicine requires methods that capture biological markers that can predict cell function and response to treatment. There is compelling evidence that the response to treatments in the context of cancer and infection correlate with the metabolic state of cells. Indeed, the metabolic profile of immune cells, infected cells and cancer cell subsets is a universal hallmark of their functional state. Current methods to profile energy metabolism require large number of cells, cell culture media and are not adapted to analyze patient samples. We have recently developed SCENITH, a method to functionally profile energetic metabolism with single cell resolution by FACS. Here, we present a SCENITH based approach that allows to functionally determine the metabolic profile in micro-samples of whole blood (i.e. <500 ul) compatible with non-invasive blood extraction systems. Our approach is

fixation and shipping compatible and compatible with epigenetic analysis. We present here a proof of concept using a very robust, 25 colors spectral flow cytometry panel that allows to determine the metabolic profile of all immune in the blood. This revolutionary approach has the potential to be used at home by end users to link their health status and response to treatment with their immune phenotype and functional immunometabolic profile. We envision that by using our SCENITH-based functional profiling as a personalised medicine approach. We predict that these high dimensional functional information of immune cells will contribute to predict disease development and response to treatment, one of the biggest challenges of modern medicine.

Short Talk:

Single cell phospho-signatures for precision medicine in SLE and other chronic inflammatory diseases

Marie Burns

Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz-Institut, Immune Monitoring & Mass Cytometry, Berlin, Germany

Chronic inflammatory diseases are complex and multifactorial, and often comprise clinically unapparent endotypes, differing in immunopathogenesis and pathology. Such heterogeneity is a major challenge for treatment-decision making and prognosis. We developed a novel pipeline for the analysis of the ex vivo phosphorylation states of nine important signal transduction proteins in patients´ deeply resolved blood leukocytes by highly multiplexed mass cytometry to capture cell type and -state-specific activation patterns in chronic inflammatory diseases.

Phosphorylation patterns differed most between haematopoietically and functionally distinct cell types. Also, profiles of naïve and memory B and CD4, and CD8 T cells differed by both lineage and differentiation state, showcasing highly polarized phospho-signatures across the immune system. The cellular fingerprint of 20 patients with active Systemic Lupus Erythematosus (SLE) was characterized by selective leukopenia affecting myeloid populations and B cells, an induction of plasmablasts and activated CD8 memory T cells, and a prominent and selective increase in ex vivo STAT phosphorylation. The latter was limited to specific cell subsets and highlighted an important role for monocytes, NK cells, PB/PC, as well as CD8 T cells in SLE.

Phospho-readouts improved the classification of healthy individuals vs. SLE, the representation of patient individuality and were associated with distinct immuno(patho)logical features of SLE. In line with the expectation that specific immune cell overactivity is associated with maintenance of disease, responsiveness to the JAK inhibitor Baricitinib was associated with high baseline levels of pSTAT3 in monocytes and T cells in distinguished one responder patient from three patients with poor to intermediate response.

Notably, cross-disease comparisons with patients suffering from rheumatoid arthritis and spondyloarthritis yielded disease-specific phosphorylation patterns when considering averages over patient groups, while single RA patients grouped with SLE patients in multidimensional clustering, identifying shared cross-disease patterns of immune activation, potentially requiring clinical consideration in individual patients.

By integrating ex vivo immune cell activation cues into cytometric immune profiling, functional phospho-mass cytometry provides a new platform to capture patient heterogeneity and disease endotypes, expected to contribute to precision medicine in chronic inflammation.

Short Talk:

Cell sorting based on angle-resolved pulse shapes

Daniel Kage

Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz-Institut, Flow Cytometry Core Facility, Berlin, Germany

Light scattering or imaging technologies can be used to characterize cells. However, in standard flow cytometers, information from scattered light is limited by the lack of angular resolution. Also, only integrated or peak intensities from the cell transit through the laser beam are provided. Although imaging flow cytometry techniques are advancing, they require high computational power and sorting is limited to certain simple metrics calculated from the images.
We recently developed a technique that enables the acquisition of full intensity pulse shapes during cell transit through the laser beam and provides angular resolution in the forward scatter direction. The technique was combined with clustering-based data analysis and has proven to be useful for labelfree cell cycle analysis. This method is now extended for cell sorting based on pulse shape features. First results were obtained with this custom-built setup in sorting different cell types only based on scattered light pulse shapes.

5:30 pm – 6:30 pm **Keynote Lecture**

Chair: Marta Ferreira Gomes

Exploiting the power of single-cell genomic technologies to enable data-informed cytometry

Simon Haas, BIH

- *Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, Berlin, Germany*
- *Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany*
- *Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Department of Hematology, Oncology and Cancer Immunology, Berlin, Germany*
- *Division of Stem Cells and Cancer, Deutsches Krebsforschungszentrum (DKFZ), and DKFZ-ZMBH Alliance, Heidelberg, Germany*
- *Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Heidelberg, Germany*
- *German Cancer Consortium (DKTK*), Heidelberg, Germany

Single-cell genomic technologies have transformed our understanding of complex cellular systems. However, excessive cost, a low cellular throughput and a lack of strategies for the purification of newly identified cell types impede their functional characterization and large-scale profiling. Unlike single-cell transcriptomics, flow cytometry offers a massive throughput in terms of samples and cells, is commonly used in the clinicsand remains unrivaled in the ability to prospectively isolate live populations of interest for downstream applications. However, flow cytometry provides low-dimensional measurements and relies on predefined sets of surface markers and gating strategies that have evolved historically in a process of trial and error. In this talk, I will provide insights into how to most efficiently use both technologies and how to empower integrated single-cell transcriptomic and surface-proteogenomic assays to rationally design cost-effective high-throughput cytometry schemes that outperform state-of-the-art approaches, accurately reflect complex topologies of cellular systems and permit the purification of precisely defined cell states.

6:30 pm –10:00 pm Welcome Reception

for all participants in the industry area, CCO

08:00 pm Core Facility Networking Event

Chairs: Desiree Kunkel and Sarah Warth

This event is an opportunity to meet and share your experiences and challenges working in a core facility. We will have short presentations on latest technologies and an inside into some of germany's core facilities. We hope for lots of ideas to discuss afterwards in an informal atmosphere among colleagues.
We want to spend a wonderful evening with you at the DGfZ meeting 2022.
In Berlin!
In person!
With food and beverages!

Speakers:

- Jochen Behrends: Core Facility Fluorescence Cytometry, Research Center Borstel, Leibniz Lung Center, Borstel
- Anne Gompf: Head of Flow Cytometry Core Facility, Center for Molecular and Cellular Bioengineering (CMCB), TU Dresden
- Marie Follo: Lighthouse Core Facility, Dept. of Medicine I, Medical Center – University of Freiburg
- Desiree Kunkel: BIH Cytometry Core Facility, BIH at Charite – Universitätsmedizin Berlin
- Sarah Warth: Core Facility Cytometry, Medical Faculty – Ulm University

Thursday, 29.09.2022

9:00 am – 10:30 am Microbiology

Chairs: Christin Koch and Hyun-Dong Chang

Second only to plants, bacteria make up a large part of earth's biomass, critical for most biogeochemical cycles, turnover of matter, nutrition and health of all other living organisms. While molecular biological tools, such as high throughput sequencing, have helped us understand the vast genetic diversity of the microbiome, we know very little about function and phenotype of bacterial cells. Single cell-based analysis tools for the microbiota are still in their infancy but have gained more and more importance for the understanding of microbial interactions within microbial communities, with their environment and with their hosts in health and disease. This years' microbiology session will highlight how novel approaches at single cell-based microbiota analyses can be leveraged for the analysis of microbial dynamics, interaction and function both in human health and environmental ecology.

Dynamics of gut microbe-host interactions

Naama Geva-Zatorsky

Rappaport Faculty of Medicine, Technion, Rappaport Technion Integrated Cancer Center, Haifa, Israel

Biosketch

Naama Geva-Zatorsky is an Assistant Professor at the Rappaport Technion Integrated Cancer Center. She completed her Ph.D., with honors in Systems-Biology, and her postdoctoral fellowship at Harvard, with Prof. Kasper, for which she received the Human Frontiers and EMBO fellowships, and the UNESCO-L'Oréal awards. In her lab, with her team, she is applying Systems-Biology, Microbiology and Immunology to study the functional interactions of the microbiota with our immune system. She is focused on mechanistic studies, including the analyzing the molecules involved and the dynamics of the gut microbiota as well as in gut microbe-host interactions. She recently was elected to the National Academy of Science, received the Johnson&Johnson WISTEM2D and Alon awards and was appointed as a CIFAR Humans&Microbiome fellow.

Noninvasive assessment of gut function using transcriptional recording sentinel cells

Jakob Zimmermann

Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland

Background: The mammalian immune system is heavily influenced by environmental factors such as diet and the intestinal microbiota. The latter provides key stimuli for proper immune system functioning and is a potent biomarker for diseases and treatment decisions. While commonly sampled from the stool, such measurements are poorly reflective of the luminal conditions at the site of host engagement. Noninvasive tools are required to report the environmental cues encountered through the gut that shape the immune system.

Methods: Here we used transcriptional recording sentinel cells that through a reverse transcriptase-Cas1–Cas2 complex record their own short-lived mRNA expression into long-lived DNA-based CRISPR arrays to report on gut function. We mono-colonized germ-free mice with E. coli sentinel cells and performed Record-seq on fecal samples to reconstruct their cellular histories as they passed along the gastrointestinal tract.

Results: Upon mono-colonization of mice, sentinel cells reported on diet, inflammation, and microbial interactions. Through transcriptome-scale information, Record-seq elucidated E. coli's adaptations to intraluminal conditions including pH, oxygen levels, and ion availability. Unlike RNA-seq, Record-seq performed on stool samples retained information from proximal gut sections to non-invasively report on the luminal conditions *in vivo*. Using barcoded CRISPR arrays enabled multiplexed Record-seq in two isogenic E. coli strains coinhabiting the intestine to reveal compensatory responses of a single-gene mutant to competition with the WT.

Conclusions: Transcriptional recording sentinel cells noninvasively report on gut function and reveal environmental cues such as diet, inflammation, and microbial interactions.

Short Talk:

Single-cell bacterial phenotyping for disease classification and identification of disease-relevant microbiota populations

Lisa Budzinski

Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz-Institut, Schwiete Laboratory for Microbiota and Inflammation, Berlin, Germany

The intestinal microbiota is implicated in many chronic inflammatory diseases, however our understanding of its role for disease pathology and course is limited. To date, alterations of the microbial community, a hallmark for disease, is mainly described by 16S rRNA gene sequencing of bulk samples, which does not lead to conclusive identification of disease-relevant bacterial candidates. We developed an approach to analyze the human intestinal microbiota from stool samples on the single cell level and assess phenotypic properties of the bacteria by multi-parameter microbiota flow cytometry. We interrogate the coating of the intestinal microbiota with host immunoglobulins by isotype-specific staining to capture the immunological context of their recognition by the host. In addition, we characterize microbial surface sugars with specific plant agglutinins, potentially indicating metabolic conditions, adhesive ability and bacteria-host-crosstalk. The single-cell bacterial phenotyping allowed us to obtain distinct microbial signatures in patients with different chronic inflammatory diseases (Crohn´s disease, ulcerative colitis, IgG4-related disease, juvenile idiopathic arthritis, rheumatoid arthritis). Applying machine-learning, we can delineate phenotypic clusters that allow the robust classification of disease entities. We demonstrate that multi-parametric microbiota flow cytometry of stool samples can be used for disease diagnosis and could have potential use for disease-monitoring but also to identify intestinal microbial communities specific for certain diseases and likely to play a role in disease pathogenesis.

Short Talk:

Stabilization of complex microbial communities revealed by single-cell analysis

Shuang Li

Helmholtz Centre for Environmental Research, Leipzig, Germany

Stabilization of complex microbial communities revealed by single-cell analysis
Building and changing a microbiome at will and maintaining it over hundreds of generations has so far proven challenging. Despite best efforts, complex microbiomes appear to be susceptible to large stochastic fluctuations. Current capabilities to assemble and control stable complex microbiomes are limited. Here, we propose a looped mass transfer design that stabilizes microbiomes over long periods of time. Five local microbiomes were continuously grown in parallel for over 114 generations and connected by a loop to a regional pool. Mass transfer rates were altered and microbiome dynamics were monitored using quantitative high-throughput flow cytometry and taxonomic sequencing of whole communities and sorted subcommunities. Increased mass transfer rates reduced local and temporal variation in microbiome assembly, did not affect functions, and overcame stochasticity, with all microbiomes exhibiting high constancy and increasing resistance. Mass transfer synchronized the structures of the five local microbiomes and nestedness of certain cell types was eminent. Mass transfer increased cell number and thus decreased net growth rates μ'. Subsets of cells that did not show net growth μ'SCx were rescued by the regional pool R and thus remained part of the microbiome. The loop in mass transfer ensured the survival of cells that would otherwise go extinct, even if they did not grow in all local microbiomes or grew more slowly than the actual dilution rate D would allow. The rescue effect, known from metacommunity theory, was the main stabilizing mechanism leading to synchrony and survival of subcommunities, despite differences in cell physiological properties, including growth rates.

11:00 am – 12:00 pm European Guest Session: Austrian Society

The Austrian Society for Cytometry offers a platform for everyone interested in flow cytometry and related fields such as fluorescence microscopy. The society sees itself as an interdisciplinary, integrative competence center in which information is developed, collected and passed on to the members in the most efficient way possible. This should promote a broader application of the knowledge and methods of this scientific area and guarantee an optimal use of the diverse possibilities. It is therefore a declared goal of the OEGfZ to build a bridge beyond the boundaries of existing specialist groups – between research, teaching and (routine) application.

The founding of the society in 2001 goes back to a series of activities that already took place in Austria in the 1990s. Ring-tests (inter-laboratory tests) have been carried out since 1992, the Vienna Flow Cytometry course (No Magic course) has been held since 1998, the magazine FlowMania appeared irregularly around 1999, and in April 2000 a regular cytometry meeting was initiated with the Flow Time event. Aware that there are several other dedicated cytometry users in Austria, the organizers of these events have decided to establish a society to create a common platform for such activities. The OEGfZ currently organizes cytometry congresses, leukemia-lymphoma workshops, cell culture days as well as basic and advanced courses in flow cytometry on a regular basis.

The Society's goals should therefore appeal to all those working in the field of cellular characterization. This includes students as well as representatives of technical professions, natural scientists and physicians of all disciplines.

In such a technology-intensive area, in which the availability and quality of the appropriate devices and reagents play an essential role in successful work, close cooperation with the manufacturers and producers is of great importance. The OEGfZ understands the exchange with the companies, some of which are the main sponsors of the society, as a very fruitful cooperation on a very friendly basis.

Introduction: The Austrian Society for Cytometry

Andreas Spittler

Core Facility Flow Cytometry & Surgical Research Laboratories, Medical University of Vienna, Austr

Biosketch

Andreas Spittler received his diploma in medicine and his habilitation in Pathophysiology at the Medical University of Vienna. He started his research career in 1991 at the Department of Pathophysiology and was appointed Associate Scientist at the Surgical Research Laboratories in 1998. At that time, he investigated the influence of amino acids on monocyte immune regulation in both cell culture and patient-oriented studies. Since 2008 he has headed the Core Facility Flow Cytometry. Andreas Spittler was a founding member of the Austrian Society for Cytometry (current President) and of the Austrian Society for Extracellular Vesicles. His main scientific interests are in the characterization of extracellular vesicles using flow cytometry and imaging flow cytometry, as well as in the characterization of the neonatal immune system and the functional characterization of monocytes in inflammation and sepsis.

One tube to find them all

Sieghart Sopper

Internal Medicine V, Hematology and Oncology, Medical University Innsbruck and Tyrolean Cancer Research Institute, Innsbruck, Austria

High-dimensional single cell analysis has provided unprecedented insights into the cellular makeup of heterogeneous samples. However, especially in clinical and translational applications, where robust assessment of a high number of samples even in multicenter settings is necessary, information content and practicability have to be carefully weighted.

Using a carefully selected combination of 14 surface markers, we designed a multicolor panel to identify and quantify all principal leukocyte populations, compatible with standard flow cytometric instruments and thus accessible to a particularly large research community. Optimized for use in whole blood, this panel is well-suited for identification and absolute cell counting of neutrophils, eosinophils, basophils, T cells, natural killer cells, B cells, plasma cells, monocytes, myeloid and plasmacytoid dendritic cell populations as well as progenitor cells. Separation of populations is high and virtually no cells remain undefined after gating. In addition, promiscuous expression of some antigens allows determination of subpopulations and activation status.

For the analysis of tissue derived cells, we have expanded the panel to include live/dead discriminators and markers for epithelial, endothelial and mesenchymal cells. Using this panel for orthogonal validation of single cell sequencing data, we demonstrate that not all cell populations are captured equally well by different platforms.

In summary, we present a versatile panel for immune profiling on broadly available instrumentation, which can also be used as a backbone for more sophisticated investigations.

Thinking outside the box in cell culture

Beate Rinner

Core Facility Alternative Biomodels & Preclinical Imaging, Medical University of Graz, Graz, Austria

Sarcomas originate from a diversity of mesenchymal tissue lineages including muscle, cartilage, bone or fibrous and can therefore occur in almost all organs. Most common histological subtypes include liposarcoma, chondrosarcoma, osteosarcoma and Ewing sarcoma, with the latter two being primarily pediatric tumors. A major obstacle to drug development and subsequent preclinical studies is the lack of suitable *in vitro* models due to the rare incidence of each sarcoma subtype. In this study post-surgical tumor tissue from a multitude of sarcoma entities was enzymatically and mechanically dissociated. In addition to the tumor tissue, surrounding tumor tissue and/or healthy skin tissue was collected and immortalized. The obtained patient-derived primary cells underwent extensive quality control and detailed characterization by using various technologies. Specifically, we would like to present the clear cell sarcoma lines MUG Lucifer prim and MUG Lucifer met, which allow translational research in terms of innovative treatment strategies and the study of the metastatic process. Particularly exciting is the release of extracellular vesicles from the established sarcoma cell lines, where we have been able to achieve robust reproducible results using the ExoView technology. With recent advances in the field of cancer research, the use of complex patient derived models has become increasingly popular and important. The biology of tumor diseases, the communication of tumor cells with their environment, tumor progression and especially metastasis behavior can only be successfully studied and captured with adequate models.

Mapping the cellular landscape of human airways by using mass cytometry

Lena Müller

Core Facility Flow Cytometry, Medical University of Vienna, Austria

Biosketch

Lena Müller obtained her PhD from the Medical University of Vienna, Austria. Her research was on understanding the molecular mechanisms of T cell development under the supervision of Prof. Willfried Ellmeier (Institute of Immunology). She then joined the laboratory of Dr. Michael Bonelli (Department of Rheumatology/ General Hospital of Vienna) to investigate T cells in the context of autoimmunity. During her research projects she gained an extensive expertise in assay development focusing on phenotyping of immune cells. In 2021 she joined the Core Facilities at the Medical University of Vienna and started to work with mass cytometry. She is coordinating the application of mass cytometry on various projects and operates the Helios/ Hyperion instrument.

1:00 pm – 2:30 pm Speed talks

Chairs: Oliver Otto and Henrik Mei

Flow cytometric quantification of Hsp70 on the surface of circulating exosomes by applying the unique cmHsp70.1 monoclonal antibody

Ali Bashiri Dezfouli[1,*], Mina Yazdi 2, Gabriele Multhoff[1]

[1]Central Institute for Translational Cancer Research Technische Universität München (TranslaTUM), Department of Radiation Oncology, Klinikum Rechts der Isar, Einstein Str. 25, 81675 Munich, Germany

[2] Pharmaceutical Biotechnology, Department of Pharmacy, Ludwig-Maximilians-Universität (LMU), 81377 Munich, Germany

[*] Correspondence to: Ali Bashiri Dezfouli, PhD; E-mail: ali.bashiri@tum.de

Exosomes are membrane-enclosed lipid vesicles which are actively released by nearly all living cells into the extracellular milieu. These microvesicles are considered novel biomarkers in liquid biopsies, reflecting the molecular profile of the cells of their origin. Extracellular vesicles including exosomes play a role in cell-to-cell communications. The lumen of exosomes consists of a mixture of cytosolic proteins, DNA, RNAs, and micro RNAs, which are crucial in regulating communications between parent (i.e., tumor cells) and recipient cells (i.e., other tumor cells, immune cells, stem cells).
Exosomes derived from tumor cells contain large amounts of cytosolic heat shock proteins (HSPs), especially Hsp70 (the major stress-inducible Heat shock protein 70), which is highly overexpressed in tumor cells and is also presented on their plasma membrane. It has been reported that exosomal Hsp70 is a prominent molecule triggering inflammation and immunity-associated processes. Using the compHsp70 ELISA, we could demonstrate that elevated levels of exosomal Hsp70 in the circulation reflect a membrane Hsp70 positive status in tumor patients. Moreover, patients with high exosomal Hsp70 levels in the blood end up with a poor prognosis. Hence, measuring the protein content inside and outside tumor-derived exosomes can help predict tumor response and clinical outcomes.
For a more detailed characterization of tumor-derived exosomes, we have established a method that enables the quantification of surface-bound Hsp70 on exosomes by applying the unique cmHsp70.1 mAb using multiparameter flow cytometry (MACSQuant) together with exosome-specific markers such as tetraspanins (i.e., CD9, CD63, and CD81).

Innovative robust high-throughput basophil activation test (BAT) to reliably diagnose allergy

Jochen Behrends[1], Christian Schwager[2], Martina Hein[1], Thomas Scholzen[1], Skadi Kull[2], Theresa Walsemann[2], and Uta Jappe[2, 3]

[1]Core Facility Fluorescence Cytometry, Research Center Borstel, Leibniz Lung Center, Borstel, Germany

[2] Division of Clinical and Molecular Allergology, Priority Area Asthma and Allergy, Airway Research Center North (ARCN), German Center for Lung Research (DZL), Germany

[3] Interdisciplinary Allergy Outpatient Clinic, Department of Pneumology, University of Lübeck, Lübeck, Germany

Allergic disorders have become the most common chronic disease in industrialized countries. Therefore, an increased need exists to establish highly specific and sensitive high-throughput diagnostic tests that not just detect sensitizations but prove the existence of an allergy with the utmost probability. The routine application of the basophil activation test (BAT), which mimics the *in vivo* situation of an allergic reaction, is still hampered by multiple factors such as complexity, costs, and lack of automation of the operation procedure. In this study, a novel gating strategy, including FCεRIα, CD203c and CD63 for a robust high-throughput analysis of basophils with an excellent performance has been established using over 1,300 samples. No significant differences were found between automated and manually analyzed samples. In addition, automated analysis saved up to 90% of working time. Furthermore, the time frame for basophil activation measurement after blood donation has been extended considerably. Respective storage conditions were optimized, which was confirmed by a nationwide ring trial showing the robustness and the applicability of our BAT on a wide variety of flow cytometers. All in all,

our optimizations overcame the hurdles that prevented the application of the BAT as high-throughput allergy diagnostic test in routine diagnostic laboratories, so far.

Automatic and non-overlapping elliptical gating of flow cytometric data with flowEMMi v2

Carmen Bruckmann

Helmholtz-Centre for Environmental Research, Leipzig, Germany

Helmholtz-Centre for Environmental Research
Flow cytometry has become a powerful technology for studying microbial community dynamics and ecology. These dynamics are tracked over long periods of time based on multiple community fingerprints consisting of subsets of cell distributions with similar cell properties. These subsets are highlighted by cytometric gates all of which assembling to a gate template when samples are compared over time or between sites. The template is usually created manually by the operator which is time consuming, prone to human error and dependent on human expertise. Manual gating thus
lacks reproducibility, which in turn might impact ecological downstream analyses such as various diversity parameters, turnover and nestedness or stability measures. We present a new version of our flowEMMi algorithm – originally designed for an automated construction of a gate template, which now generates non-overlapping elliptical gates
within a few minutes. Gate templates can be created for both single measurements and time-series measurements, allowing immediate downstream data analyses and on-line evaluation. Furthermore, it is possible to adjust gate sizes to Gaussian distribution heights. This automatic approach makes the gate template creation objective and reproducible. Moreover, it can generate hierarchies of gates. flowEMMi v2 is essential not only for exploratory studies, but also for routine monitoring and control of biotechnological processes. Therefore flowEMMi v2 bridges a crucial bottleneck between automated cell sample collection and processing, automated flow cytometric measurement and automated downstream statistical analysis.

Activation-induced marker T cell assay for the in vitro assessment of sensitizing chemicals: a p-phenylene diamine and Brandrowski´s base study

Marina Aparicio-Soto[*1], Caterina Curato[*1], Melanie Leddermann[1], Niko Himmeldirk[1], Maximilian Schäfer[1], Johanna Bachmeier[4], Wolfgang Bäumler[4], Andreas Luch[1,2,3], Katherina Siewert[1]

[1]*Dermatotoxicology Study Centre,*

[2]*Department of Chemical and Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany*

[3]*Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany*

[4]*Department of Dermatology and Allergology, University of Regensburg, Regensburg, Germany*

[*]*These authors share first authorship*

Allergic contact dermatitis (ACD) is a T cell-mediated disease, but the development of T cell-based *in vitro* assays remains challenging. Here, we introduce activation-induced marker (AIM) T cell assays by applying p-phenylene diamine (PPD), a strong chemical sensitizer, and Bandrowski's Base (BB), an oxidation product of PPD. For both chemicals, the induced frequencies of allergen-specific T cells and the extent of TCR cross-reactivity remain elusive.
Using peripheral mononuclear blood cells (PBMC), we determined a concentration of 6 µM PPD or BB (dissolved in DMSO ≤0.5%) as non-toxic and non-interfering with multi parameter flow cytometry for 5 and 16 h incubation times. We then assessed frequencies of PPD- and BB-specific CD4+ and CD8+ memory T cells from allergic (n=7) and non-allergic (n=8-11) individuals using the activation markers CD154, CD137, OX40, and CD69, respectively.
As prior proliferation assays, we detected BB-specific CD154+CD4+ memory T cells in all donors (means allergic/non-allergic: 0.060%/0.014%, 16 h). In addition, we reached detection limits for PPD-specific T cells (0.010%/0.004%). Thus, both BB- and PPD-specific T cells may serve as allergy indicator, although PPD-induced epitope formation is rather slow and requires longer incubation time. We also detected increased PPD/BB-specific CD154+CD8+ memory T cell frequencies (16 h) in allergic donors. Increased cutaneous lymphocyte-associated antigen (CLA) co-expression indicated the involvement of PPD/BB-specific T cells in allergic immune responses, thus showing that

these activation markers combined giving reliable allergy detection rates. In a preliminary TCR repertoires analysis, we did not detect overrepresentation of gene segments or CDR3 amino acids but considerable clonotype overlap in line with common PPD/BB epitope formation or TCR cross-reactivity.
In conclusion, this work represents the first application of AIM T cell assays to detect human-relevant organic chemical sensitizers. Being fast, sensitive and quantitative, AIM assays enable efficient optimization of assay conditions including epitope generation. Thus, they can serve to characterize chemical-mediated T cell activation and cross-reactivity for regulatory and diagnostic purposes.

A comparison of cytometric and substrate-based methods to understand the role of surface contacts for the biophysical properties of HEK293T cells

Venkata Dabbiru

Universität Greifswald, Germany

Cell physiological processes are accompanied by integral changes in their biophysical properties, e.g., during the gradual loss of cell-surface interactions and the resulting transition from adherent to suspended state. While an increasing number of reports are available investigating cells in the presence or absence of surface contacts, fewer studies focus on the transition itself. The reason is found in challenges mimicking this semi-adherent state and in the availability of corresponding methods to characterize the physical properties of cells like size, volume, deformation and elasticity. Here, we employed atomic force microscopy and real-time deformability cytometry to study human embryonic kidney 293T (HEK293T) cells attached to a surface, in suspension as well as passively adhered to a substrate using surface-tethering molecules. Our results demonstrate that seeding density alters cell size but does not impact on their stiffness. Furthermore, the loss of cell-surface interactions is linked to an increase in the cellular elasticity. Interestingly, we observe that the contribution of F-actin density to the cellular elasticity is minimal. Instead, geometrical features including circularity, volume and morphology are predominantly important for the biophysical properties of cells.

Construction of Functional Active Artificial Communities Based on Individual Cell Analyses and Ecological Theory

Ruyu Gao[1], Shuang Li[1], Susann Müller[1]

[1]Department of Environmental Microbiology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

Microbial communities are prone to varying and even stochastic changes in composition and function. Natural microbial communities are important parts of ecosystems and human life, and show high complexity of microbial interactions and environmental dependencies. In contrast, using constructed artificial microbial communities might provide a chance to create communities more easily with desired functions when known strains with known functions are exploited. Designing functionally synthetic microbial communities requires two strategies, which is either a top-down design or bottom-up design. Here, we will use a design to construct microbial communities based on a looped mass transfer setup (Li et al., 2022) . As before, we will use individual-based single-cell analysis tools such as microbial community flow cytometry as a standard method: Cytometric fingerprinting will detect and evaluate variation in community structure, flow cytometry will identify the dynamics of microbial community, track labelled fluorescent strains and their abundance when grown together. Furthermore, bioinformatics tools will analyze the resulting high-throughput data and proteomics technology will be used to determine the desired function of the microbial community. We want to establish microbial ecology theory as a tool to control these types of processes such as keep proportions of organisms' constant in microbial community set ups. This study will contribute to a grand perspective of microbial ecology that meets an acute need in the health care and biotechnology industries.

Cell volume changes in confined environments on short timescales

Felix Graf

Universität Greifswald, Germany

Implementing spectral cytometry in phenotyping of human early B cell progenitors

Iga Janowska[1], Jakov Korzhenevich[1], Julian Staniek[1], Miriam Erlacher[2], Marta Rizzi[1]

[1]Department of Rheumatology and Clinical Immunology, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[2]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Our fundamental understanding of immunological processes originates from mouse models. However, due to significant differences between mice and humans the discoveries acquired by murine studies cannot be directly translated to humans. One of these processes is early B cell development, which has been thoroughly investigated in mice but is less well understood in humans. Therefore, we used a feeder-free *in vitro* system to develop human early B cells from CD34+ hematopoietic stem cells (HSC) (Kraus et al, 2014) that can reproduce all differentiation steps of early B cell lymphopoiesis. Progressive development of lymphoid progenitors and committed early B cell precursors was monitored by spectral flow cytometry. We established a 17-color panel for the spectra analyzer Cytek Aurora including 10 surface and 7 intracellular/ nuclear markers, 4 of which were transcription factors. In the *in vitro* system cord blood-derived CD34+ HSCs cells were expanded in presence of SCF, Flt3-L, and IL-6 for 7 days. Then, the medium was supplemented with SCF, Flt3-L, and IL-7 for additional 7 days. From day 14 on, cells were cultured in medium without addition of cytokines. Between day 14 and 49 of the culture, we find all early B cell developmental stages starting with common lymphoid progenitors (CLP) expressing CD10 and CD38 on the surface and the transcription factor (TF) Ikaros. CLPs differentiate into pro-B cells by upregulating CD79a expression. At the pre-BI stage, cells start to express the TF Pax5 and on the surface CD19. Subsequently, pre-BII cells that already express the rearranged immunoglobulin heavy chain (cytoplasmic Igμ) can be divided in proliferating pre-BII large cells - expressing Ki67 and CD179a (surrogate light chain of the pre-BCR) - and pre-BII small cells, which downregulate CD179a, exit the cell cycle and start to rearrange the immunoglobulin light chain. During the last differentiation step, immature B cells induce expression of the complete IgM BCR on the surface.

By implementing spectral flow cytometry in the characterization of *in vitro* human early B cell development we could extend the stratification of B cell progenitors and optimize usage of scarce human samples. This powerful system enables us to study the capacity of human HSCs to differentiate into B cells in various settings, e.g. the addition of drugs, cytokines, and genetic manipulations. Finally, we can apply the *in vitro* system to bone marrow derived HSCs from patients to investigate the dynamics and dysfunction of human early human B lymphopoiesis in diseases.

A flow-through UV-C decontamination unit for sheath fluid or waste of flow cytometers

J. Kirsch[1], K. Heinrich[1], D. Kage[1], J. Glaab[2], B. Grothe[3], K. v. Volkmann[3], T. Kaiser[1]

[1]German Rheumatism Research Centre Berlin (DRFZ) - Flow Cytometry Core Facility, Charitéplatz 1 (Virchowweg 12), 10117 Berlin, Germany

[2] Ferdinand-Braun-Institut gGmbH, Leibniz-Institut für Höchstfrequenztechnik, Gustav-Kirchhoff-Str. 4, 12489 Berlin, Germany

[3] *APE Angewandte Physik und Elektronik GmbH, Plauener Straße 163-165 / Haus N, 13053 Berlin, Germany*

Flow-cytometry and cell sorting are indispensable techniques in bio-medical research. To enable subsequent cell cultivation or to prevent misleading downstream experimental results, any contamination of the sorted cells must be avoided. However, since cell sorters typically do not operate in a sterile environment, contamination with microorganisms is not uncommon.
A chemical cleaning procedure can be applied to reduce the risk of contamination, but such a process is time-consuming, and its success not guaranteed. Furthermore, toxic residues of cleaning reagents may be critical to cell viability. Also, antibiotics are applied to the sorted cells prevent the proliferation of bacteria entering through the sheath fluid. This, however, can cause an unwanted change in gene expression and regulatory level of the cells and the widespread use of antibiotics aggravates the existing resistance issues.
As ultraviolet light is well known for inactivating microorganisms by inhibiting DNA replication, it is very interesting for applications in decontamination. Thus, decontamination of sheath fluids in flow-cytometric cell sorters by UVC irradiation is a promising alternative to chemical cleaning procedures.
Here we present a UVC unit for flow-through irradiation of sheath fluid to enable aseptic cell sorting. In a proof-of-principle study, the decontamination efficiency of the unit was tested on bacteria obtained from the laboratory's room air. A 5 log10 reduction of the contamination level was obtained.

Impact of reactive oxygen species on the mechanical properties of myeloid precursor cells

Yesaswini Komaragiri[1,2], Ricardo Hugo Pires[1,2], Stefanie Spiegler[1,2], Huy Tung Dau[1], Doreen Biedenweg[1], Clara Órtegon Salas[3], Md Faruq Hossain[1], Bob Fregin[1,2], Stefan Gross[2,3], Manuela Gellert[3], Uwe Lendeckel[3], Christopher Lillig[3], and Oliver Otto[1,2]

[1] *ZIK HIKE, University of Greifswald, Greifswald, Germany*

[2] *DZHK, University Medicine Greifswald, Greifswald, Germany*

[3] *University Medicine Greifswald, Greifswald, Germany*

Reactive oxygen species (ROS) are crucial in redox equilibrium by acting as secondary messengers in a variety of cellular processes such as activation, proliferation, and differentiation. Hydrogen peroxide (H2O2) is a major ROS present intracellularly at nanomolar levels that, when elevated to supraphysiological levels, promotes cell and tissue damage. While various ROS have been extensively studied at the molecular level, the precise mechanism by which they modify cell mechanics remains unknown. By employing varying H2O2 concentrations to stimulate ROS in human myeloid precursor cells (HL60), we observed a semi-quantitative relationship between ROS generation and an increase in cellular elasticity. The observed cell mechanical changes were not accompanied by changes in F-actin and microtubules levels but rather caused by lysosome disruption. Excess proton leakage into the cytosol due to lysosomal damage caused intracellular acidification, which lead to increased cell stiffness, establishing a molecular mechanism that connects redox homeostasis and cell mechanics as key mediators of cell function.

Two-photon excitation spectra of various fluorescent proteins within a broad excitation range

Ruth Leben, Randall Lindquist, Asylkhan Rakhymzhan, Raluca Niesner

Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz-Institut, Berlin, Germany

Two-photon excitation fluorescence laser-scanning microscopy is the preferred method for studying dynamic processes in living organ models or even in living organisms. Thanks to near-infrared and infrared excitation, it is possible to penetrate deep into tissue, reaching areas of interest for life sciences and biomedicine. In those imaging experiments, two-photon excitation spectra are needed to select the optimal laser wavelength to excite as many fluorophores as possible simultaneously in the sample under consideration. The more fluorophores that can be excited, the more cell populations that can be studied, and the better the access to their arrangement and interaction in complex systems such as lymphoid organs (Rakhymzhan et al. 2017). However, the two-

photon excitation properties are poorly predicted by the single-photon spectra and are not yet available in literature or in databases, for many fluorophores (Ricard et al. 2018). Here we present the broad excitation range (760 nm to 1300 nm) photon flux-normalized two-photon spectra of several fluorescent proteins in their cellular environment. This includes following fluorescent proteins: mCerulean3, mTurquoise2, mT-Sapphire, Clover, mKusabiraOrange2, mOrange2, LSS-mOrange, mRuby2, mBeRFP, mCardinal, iRFP670, NirFP, iRFP720, ranging from blue to red and even infrared fluorescence.

Suppression of specific cellular immune response after mRNA vaccination against COVID-19 in rheumatic patients receiving SARS-CoV2 neutralizing antibody Ronapreve as pre-exposure prophylaxis.

Henrik Mei[1], Axel Ronald Schulz[1], Heike Hirseland[1], Elisa Habermann[2], Alexander ten Hagen[2], Fredrik Albach[2], Gerd-Rüdiger Burmester[2], Robert Biesen[2]

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

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

Background: Active and passive immunizations against SARS-CoV-2 are used to protect against the development of severe COVID-19. However, the effect of passive immunization on the success of a subsequent active vaccination is unknown.
Objective: To investigate the cellular immune response to mRNA vaccination against SARS-CoV-2 in patients receiving preventive treatment with a SARS-CoV-2 neutralizing antibody cocktail, Ronapreve (Casirivimab/Imdevimab).
Methods: After poorly responding to multiple mRNA vaccinations against SARS-CoV-2, four patients with different autoimmune diseases treated with immunosuppressants including rituximab received 600 mg Ronapreve i.v. and were re-vaccinated 14-25 days later. Eleven clinically comparable patients served as controls. All participants were re-immunized with the mRNA-based Spikevax vaccine. Antigen-specific T- and B-cell responses were assessed by flow cytometry, after short-term stimulation with spike peptides and tetramer staining for spike-specific B cells before, 1 week, and 4 weeks after vaccination.
Results: As expected, most control patients showed a specific CD4 T- and B-cell response in the blood after vaccination, including induction of antigen-specific plasmablasts at week 1 and a significant increase of antigen-specific CD4 T cells producing IFNγ, IL-4, or IL-10 at week 4. In patients receiving Ronapreve, the induction of plasmablasts in the blood was effectively suppressed but not completely eliminated. In contrast to control patients, there was no consistent increase in SARS-CoV-2-specific CD4 T cells in Ronapreve-treated patients.
Our data indicate that passive immunization effectively inhibits the cellular immune response to active vaccination.
Conclusions: The timing of active and passive immunizations to homologous antigen in the same patient requires consideration. The benefit of pre-or post-exposure prophylaxis by neutralizing antibodies must be weighed against the risk of ineffective vaccination.

Hema.to

Karsten Miermans

hema.to GmbH, Munich, Germany

We've built hema.to to improve blood cancer diagnostics from flow cytometry data. hema.to is a CE-marked web-application that gives diagnostic recommendations in seconds from the raw .fcs or .lmd files, and can integrate into clinical workflows without SOP changes or disruptions. This is made possible by using modern machine learning technology that combines data from all laboratories. This way, smaller sites can learn from larger, more specialized hematological laboratories. hema.to grew out of a cooperation with the Münchner Leukämielabor (MLL) and has been shown to provide expert-level recommendations in a large prospective trial. Estimates indicate that the workflow can be sped up by ca. 5x, which would help clinical laboratories deal

with an increasing diagnostic volume even in the current lab staff shortage crisis. In our most recent (four-center) clinical trial, we demonstrated that hema.to accelerated the diagnostic analysis by >2x and reduced variability by 15%. We're currently commercially integrating hema.to into the first laboratories for routine use for B-cell Non-Hodgkin Lymphoma. Our final aim is twofold. First, we want to support existing flow cytometry workflows by reducing the need for subjective human input, which greatly accelerates and objectifies the analysis. Second, we want to use machine learning to extract novel insights from immunophenotyping data, for example to detect which cell populations have a strong impact on clinical endpoints. Taken together, we want to use our combined expertise in deep learning, flow cytometry and medical software development to improve patient care.

Multiparametric mass cytometry data analysis in the search for the chronic lymphocytic leukemia cell of origin

Mikulová, Antonia[1], Vondálová Blanářová, O.[1], Plešingerová, H.[1,2], Stančíková, J.[3], Stuchlý, J.[3], Kalina, T.[3], Kotašková, J.[4], Arpáš, T.[2], Bryja, V.[1] and Janovská, P.[1]

[1]Laboratory of Cell Communication, Department of Experimental Biology, Masaryk University, Brno, Czech Republic

[2]Centre of Molecular Biology and Genetics, IHOK, University Hospital Brno, Brno, Czech Republic

[3]Childhood Leukaemia Investigation Prague (CLIP), Department of Pediatric Hematology and Oncology, 2nd Medical School of Charles University, Prague, Czech Republic

[4]Medical Genomics, Central European Institute of Technology (CEITEC), Brno, Czech Republic

Chroniclymphocyticleukemia(CLL)isalymphoproliferative disease characterized by clonal expansion of CD5+ B cells which can be found in peripheral blood, lymph nodes and bone marrow. It is the most common leukemia in the western countries and despite the effort dedicated to the research of CLL, only little is still known about the origin of the disease. CLL cells almost uniformly express surface receptor tyrosine kinase-like orphan receptor (ROR1). It is also true for clonal B cell subsets in monoclonal B cell lymphocytosis (MBL), stage preceding CLL, where cells are also ROR1+. In contrast, only 1-5% of B cells in peripheral blood of healthy young individuals is ROR1-positive.
Our aim is to characterize ROR1+ subsets of B cells in peripheral blood of healthy donors and define their connection to MBL and CLL cells. To achieve that, we have used flow cytometric and mass cytometric approaches to measure expression of selected markers on B cells from peripheral blood of healthy donors (including multiple samples enriched for ROR1+ B cells), patients with MBL and CLL. Subsequent analysis of B cell subpopulations allowed us to characterize ROR1+ subsets and their similarities with MBL and CLL cells. Mass cytometric data using 30 markers were employed to reconstruct the CLL progression in pseudotime using topological data analysis tools available in tviblindi package, with the main aim to elucidate whether any ROR1+ population from healthy individuals B cells precedes acquisition of MBL and CLL cell phenotype.

High dimensional spectral flow cytometric analysis of human blood and tissue correlates

Michael Müller

Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Hans-Knöll-Institut, Jena, Germany

We use a 5L Cytek Aurora Analyzer and Aurora CS with a 40-colour surface marker panel to characterize the immune system in patient samples. Our focus lies on tissue resident T cell populations within different tissues and different diseases. The setup and application of this panel on several tissues is challenging because the different isolations techniques have an impact on marker expression. With the combination of spectral flow cytometry and single cell transcriptomics we want to identify mechanisms of tissue specific immune homeostasis and immune abrogation.

Viscoelastic characterization of biological cells in hyperbolic microfluidic channels

Felix Reichel

Max Planck Institute for the Science of Light, Erlangen, Germany

Research over the last decades revealed that single-cell mechanical properties can serve as label-free markers of cell state and function and that mechanical changes are a sign of alterations in the cell's molecular composition. This led to the development of a number of microfluidics tools to rapidly measure the deformability and also the viscoelastic properties of cells. The quantification of the stresses, that cause the deformation of the cells in these channels, is often challenging and with that the derivation of a stress-strain relation for such a system becomes complex. Here, we used hyperbolic channels to create an extensional flow field where the acting stresses can be measured using calibration particles and yield a simple relationship between acting stress and resulting cell strain. We then used the setup to measure the Young's modulus and bulk viscosity of HL60 cells over a wide range of time scales. Our simple setup offers a straightforward measurement of the viscoelastic properties of cells, soft particles and possible also liquid-liquid phase separated droplets.

Functional CyTOF profiling of SARS-CoV-2-specific T cells induced during B-cell depletion

Axel Ronald Schulz[1*], Addi J. Romero-Olmedo[2*], Svenja Hochstätter[3], Sebastian Ferrara[1], Dennis Das Gupta[2], Iiris Virta[1], Heike Hirseland[1], Daniel Staudenraus[2], Bärbel Camara[2], Carina Münch[3], Véronique Hefter[3], Siddhesh Sapre3, Christian Keller[3#], Michael Lohoff[2#] and Henrik E. Mei[1#]

[1]German Rheumatism Research Center, Berlin, Germany

[2] Institute of Medical Microbiology and Hospital Hygiene, Philipps-University Marburg, Marburg, Germany

[3] Institute of Virology, Philipps-University, Marburg, Germany

[, #] equal contribution of first and senior authors*

T-helper cells are known regulators of B-cell responses, but whether and how B cells influence T cells is less clear. Here we longitudinally investigated spike-specific CD4 T cells in a B-cell-depleted unvaccinated survivor of severe COVID-19, using single-cell flow and 48-plex mass cytometry, to determine the quantity, durability, and quality of the CD4 T-cell response under conditions of induced B-cell deficiency. Specific CD4 T cells were identified as CD40L+ CD137+ T cells after overnight stimulation with a SARS-CoV-2 spike peptide pool. Four age-, gender- and infection-severity-matched controls and one age-matched vaccinated control were analyzed for comparison.

Our results show remarkably high frequencies of approximately 1% of spike-specific CD4 T cells in the B-cell-depleted COVID-19 survivor, which were exceptionally stable over the 5-month observation period. B cells returned seven months after recovery, and the patient was vaccinated with BNT162b2, which further increased specific T-cell frequencies. Mass cytometry of spike-specific CD4 T cells revealed combinatorial expression of 14 cytokines. Boolean analysis in recovered patients identified a predominant Th1 response characterized by combinations of TNF, IFNγ, GM-CSF and/or IL-21. Notably, the BNT162b2 vaccination induced additional Th2 cells and hybrid Th1/2 cells, expressing combinations of IL-4, IL-5, and IL-13, along with a combination of Th1 cytokines, respectively. This indicates a multifaceted T-cell response with a broadened effector range after BNT162b2 vaccination in a previously recovered individual. While the T-cell-cytokine combinome revealed rather minor differences between the B-cell depleted and the control subjects, the high frequency and persistence of peripheral spike-specific CD4 T cells was unmatched versus controls and published cohorts.

Within the limitations of a single-patient analysis, our results indicate that relatively normal spike-specific CD4 T cells develop when B cells are depleted in a primary infection, although the absence of B cells appears to be compensated by a massive expansion of the spike-specific CD4 T-cell compartment. In summary, we here combined sensitive antigen-specific T-cell detection with high dimensional mass cytometry to provide insight in the quantitative and qualitative features of antigen specific T-cell responses, serving a blueprint for functional T cell studies in infection, vaccination and autoimmunity.

IgA-Seq enhances discrimination between Crohn's Disease patients and healthy controls, but IgA1- and IgA2-bound microbiota do not differ significantly

Toni Sempert[1,2], Lisa Budzinski[1], René Maier[1], Victoria von Goetze[1], Amro Abbas[1], Gi-Ung Kang[1], Leonie Lietz[1,2], Robin Kempkens[1], Janine Büttner[3], Bettina Bochow[3], Anja Schirbel[3], Benjamin Moser[3], Hyun-Dong Chang[1,2]

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

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

[3]Medizinische Klinik mit Schwerpunkt Hepatologie und Gastroenterologie, Charité-Universitätsmedizin Berlin, Berlin, Germany

Crohn's Disease (CD) shows alterations in immune response and composition of intestinal microbiota. Previous studies reported different effector functions of IgA1 and IgA2 on immune tolerance or inflammation (Steffen et al. 2020, Michaud et al. 2022). Here, we apply multi-parameter microbiota flow cytometry (mMFC) to identify and isolate bacterial populations coated by host IgA1 and IgA2 on single-cell level comparing Crohn's Disease patients to healthy controls. We can show that the majority of bacteria coated with IgA are coated by both IgA1 and IgA2. CD patients have a higher frequency of IgA1- and IgA2-bound bacteria compared to healthy controls, while the amount of IgA per cell is not significantly different. Full-length 16S rRNA gene sequencing shows that bacterial composition differs between CD patients and controls (Bray-Curtis beta diversity). The distinction between CD patients and controls is enhanced when analyzing IgA-coated bacterial only. Our data indicate that (1) there is little difference between the specificity of intestinal IgA1 and IgA2 regardless of disease state and that (2) CD patients show a pattern of IgA-coated bacteria which differs significantly from healthy controls. Thus, multi-parameter microbiota flow cytometer combined with 16S rRNA gene sequencing is as a powerful tool to obtain disease-specific signatures and to better describe microbial composition regarding host-microbiota-crosstalk.

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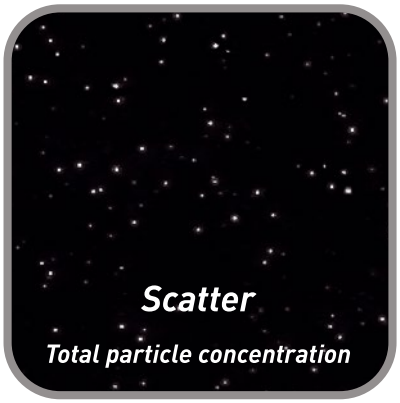

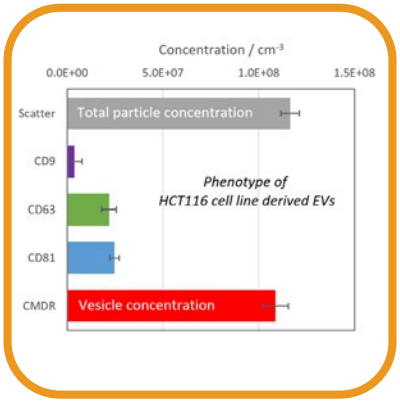

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3:00 pm – 4:30 pm Goerttler Session

Chair: Janine Kemming

In this year's Klaus-Goerttler session Bertram Bengsch from Freiburg and Asbjørn Christophersen from Oslo will share some of their exciting data on CD8+ T cell phenotyping in the context of autoimmunity and infectious diseases using diverse flow and mass cytometry techniques. Also this year's winner of the Klaus-Goerttler award, Elisa Rosati, will be revealed! Make sure to join for this session full of cutting-edge research carried out using state-of-the-art cytometry techniques!

Enolase represents a metabolic checkpoint controlling the differential exhaustion of virus-specific CD8+ T cells in HBV versus HCV

Bertram Bengsch

Bengsch Lab, Clinic for Internal Medicine II, University Medical Center Freiburg

Exhausted T cells with limited effector function accumulate in patients with chronic infections and cancer. Metabolic regulation is a contributing mechanism to dysfunction. In Hepatitis -B and -C virus infection (cHBV/cHCV), different subtypes of exhausted T cells have been described. To understand the metabolic programs tied into the regulation of exhausted T cells, we used metabolism-directed flow cytometry, transcriptome analysis and metabolic flux analysis to understand whether similar metabolic programs and exhaustion states are induced in virus-specific T cells in both infections, how they are affected by antiviral therapy, and if specific metabolic checkpoints regulate severe dysfunction.

Interestingly, we find that HBV- and HCV-specific T cells exhibit strikingly distinct metabolic profiles. HCV-specific CD8+ T cells had severe mitochondrial impairment despite enhanced glucose uptake whereas HBV-specific CD8+ T cells displayed intact metabolism. These metabolic differences were connected to severe exhaustion of HCV-specific T cells and, clinically, more severe hepatitis in our cHCV cohort. DAA therapy partially improved mitochondrial programs in severely exhausted HCV-specific CD8+ T cells. We identified enolase 1 (ENO1) as metabolic checkpoint that limits glycolytic flux and mitochondrial function in HCV-specific CD8+ T cells. Bypassing this checkpoint improved HCV-specific T cell metabolism and effector function.

Our analysis indicates that metabolic programs of HBV- and HCV-specific CD8+ T cells are linked to exhaustion severity, subset differentiation and liver inflammation. They connect differential bioenergetic fitness with distinct exhaustion subtypes and varying liver disease.

A similar T-cell subset is increased across multiple autoimmune conditions and contains the antigen-specific cells in celiac disease

Asbjørn Christophersen

Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway. Lovisenberg Diakonale Hospital and Oslo University Hospital, Oslo, Norway.

Celiac disease (CeD) is a chronic disorder with autoimmune properties that is driven by oral gluten exposure. HLA-restricted T cells specific to gluten are key players in the pathogenesis and can be found in gut and blood of CeD patients, but not in controls. We have studied gluten-specific T cells under different clinical conditions aiming to find novel diagnostic tools and therapeutic targets. For this purpose, we have combined RNA sequencing, T-cell receptor sequencing and mass and multi-parameter flow cytometry with HLA-class II tetramer technology.

We find that gluten-specific T cells have a distinct phenotype and metabolic characteristics in gut and blood in untreated disease. However, when removing or later re-introducing gluten in the diet, we detect instant phenotypic changes in these disease-specific cells. Importantly, detection of T cells with this distinct phenotype accurately identifies patients with CeD and healthy controls. Furthermore, their metabolic and phenotypic properties may be targeted for therapeutic purposes.

Interestingly, we and other research groups find T cells phenotypically similar to gluten-specific cells increased also in autoimmune conditions such as in rheumatoid arthritis, type-1 diabetes, systemic lupus erythematosus and systemic sclerosis. Thus, T cells with this phenotype may be targeted for better treatment and diagnosis of CeD and potentially other autoimmune conditions alike. As the disease-driving antigen recognized by CD4+ T cells for most autoimmune diseases remains elusive, the T-cell receptor repertoire of cells with this phenotype may be used to reveal epitope specificity.

Klaus Goerttler Awardee

Profiling the SARS-CoV-2-reactive CD4+ T cell repertoire

Elisa Rosati

Previously: University of Kiel, Germany.
Currently: Research Scientist in Bioinformatics at Evotec SE.

The unprecedented scientific efforts put in place as a consequence of the COVID-19 pandemic allow for the development and application of new research approaches to better understand immune responses to SARS-CoV-2. One of the major technical hurdles in T cell immunology is the characterization of specific HLA-restricted peptide-T cell receptor (TCR) interactions.
Combining ultra-sensitive approaches for the investigation of antigen-reactive T cells with cutting edge sequencing and bioinformatics methods allows for the characterization of immunodominant T cell responses and epitopes.
We employed antigen-reactive T cell enrichment (ARTE) in combination with bulk and single-cell T-cell receptor (TCR) repertoire profiling to analyze CD4+ T cell response to SARS-CoV-2 infection and vaccination. Additionally, an integrative meta-analysis of multiple datasets allowed for a detailed characterization of public T cell epitopes.
We identified thousands of TCRs reactive to SARS-CoV-2, of which over a thousand highly public and forming clusters against shared immunodominant epitopes. We followed TCR clonal behavior before and after vaccination and observed different expansion levels of pre-existing and newly arised TCRs in different individuals.
Our findings both improve our understanding of CD4+ T cell response and, at the same time, provide new methodological frameworks for application to other diseases and conditions.

Biosketch
Elisa Rosati obtained a BSc and MSc in Molecular Biology at the University of Padua, Italy. During her PhD and Postdoc at the University of Kiel, Germany in the groups of Prof. Andre Franke and Prof. Petra Bacher, she focused on the analysis of the T-cell receptor (TCR) repertoire in the context of inflammatory bowel diseases (IBD) and identified a novel unconventional T cell population associated with Crohn's disease. In recent years she contributed to the identification and characterization of T cells reactive to SARS-CoV-2.

5:00 pm – 6:00 pm **Guest Lecture**

Chair: Raluca Niesner

Diagnoses by Dog Noses? Real-time Detection of SARS-CoV-2 Infections by Trained Dogs

Sebastian Meller

Department of Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover

COVID-19 developed into a pandemic within months. Polymerase chain reaction (PCR), antigen tests, and countermeasures such as vaccines against the causative SARS-CoV-2 have consequently become the state of the art tool box for pandemic control. However, frequent pre-symptomatic, asymptomatic and, recently, post-symptomatic transmissions continue to pose a major challenge. To overcome some of the limitations of the current test strategies, scientists around the world have investigated dogs' olfactory capability as a real-time detection method. Several diseases induce "smellprints" in affected individuals (volatile organic compounds (VOCs)), which can be easily detected by dogs.

Initially, a case-controlled pilot-study was conducted with samples from RT-qPCR-SARS-CoV-2 positive and negative individuals. Individuals with severe COVID-19 were identified with 83% sensitivity and 96% specificity. A follow-up study addressing further emerging questions demonstrated that there exists a universal COVID-19 smell across different body-fluids in individuals with symptomatic as well as asymptomatic infection. In a third step, it was shown that dogs were able to distinguish SARS-CoV-2 from other viral pathogens and that they also can identify the post-COVID-condition, highlighting that specific SARS-CoV-2-induced VOCs exhibit prolonged temporal dynamics. Finally, a real-life and real-time mass screening event was conducted. Dogs were able to detect sweat samples from concertgoers with 82% sensitivity and 100% specificity.

Dogs are able to detect samples from SARS-CoV-2-infected individuals and can be deployed in real-time mass screenings as a supplement to conventional detection methods against pandemics. Current studies address standardization measures (e.g., training with viral proteins) to reinforce infrastructures for comprehensive canine training and deployments.

And then...

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

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Friday, 30.09.2022

9:00 am – 10:30 am Mechanocytometry

Chairs: Marta Urbanska and Oliver Otto

Integrating biophysical perspective into the description of cellular behaviors — typically studied from a biochemical angle — fosters comprehensive understanding of health and disease. In its broad understanding, mechanocytometry encompasses all methods that measure mechanical properties of the cells, such as stiffness or deformability. Classically, methods such as micropipette aspiration or atomic force microscopy-based indentation are used to characterize such properties. More recent developments aim at addressing challenges such as measuring of cell mechanical properties in situ in developing tissues (for example using Brillouin microscopy or intratissue bead sensors), or characterizing the mechanical properties of singles cells at high throughput in a flow cytometry-like manner (using a class of methods called deformability cytometry). The goal of measuring the mechanical properties of cells is to unravel their role in physiological and pathological processes such as tissue development and cancer metastasis, or to harness their potential as a diagnostic marker in various diseases. Our session will showcase new developments in the field of mechanocytometry, and present how cell mechanics can play a role in mammalian development and in breast cancer progression as ßstudied in 3D model systems with adaptable environment stiffness.

Mechanical control of mammalian ovarian folliculogenesis

Chii Jou Chan

Principal Investigator, Mechanobiology Institute, National University of Singapore, Singapor
Assistant Professor, Department of Biological Sciences, National University of Singapore, Singapore

The formation of ovarian follicles that house the functional eggs (oocytes) is a critical process in mammalian development as it ensures successful reproduction and species propagation. While past molecular genetics studies have revealed genes that are critical for regulating the bidirectional communication between the oocyte and the somatic cells, the underlying mechanisms driving follicular growth remain enigmatic. Recent work suggests that follicle growth is sensitive to surrounding matrix stiffness, which calls for a need to understand cell-cell interactions within the follicle. Here, we investigate the mechanical functions of theca cells (TCs), which are the outer cells encapsulating the follicles. Using both *in vivo* staining and *in vitro* reconstitution assay, we demonstrate that the TCs are highly contractile and generate significant compressive stress to modulate follicle growth. TCs are also mechanosensitive and can modulate their proliferation rate and YAP signalling in response to cell stretch and substrate stiffness, suggesting that follicle growth or basement membrane stiffness may regulate TC functions *in vivo*. Finally, we found that the follicles are relatively compressible, and perturbing mechanical stress can influence somatic cell proliferation, oocyte signalling and growth. Overall, our data suggest that the interplay between tissue pressure and cell mechanics may control robust follicle morphogenesis and oocyte maturation through mechanotransduction pathways. These findings will have profound implications for future understanding and treatment of ovarian diseases and infertility.

Studying the mechanical and morphological phenotype of Cancer-associated fibroblasts of the prostate

Anna Taubenberger

Biotechnology Center, Technische Universität Dresden, Germany

Reciprocal interactions between prostate epithelial cells and their adjacent stromal microenvironment not only are essential for tissue homeostasis but also play a key role in tumor development and progression. Malignant transformation is associated with the formation of a reactive stroma where cancer-associated fibroblasts (CAFs) induce matrix remodeling and thereby provide atypical biochemical and biomechanical signals to epithelial cells. Previous work has been focused on the cellular and molecular phenotype as well as on matrix stiffness and remodeling, providing potential targets for cancer therapeutics. So far, biomechanical changes in CAFs and adjacent epithelial cells of the prostate have not been explored. Here, we compared the mechanical properties of primary prostatic CAFs and patient-matched non-malignant prostate tissue fibroblasts (NPFs) using atomic force microscopy (AFM) and real-time deformability cytometry (RT-FDC). We found that CAFs exhibit an increased apparent Young's modulus, coinciding with an altered architecture of the cytoskeleton compared with NPFs. In contrast, co-cultures of benign prostate epithelial (BPH-1) cells with CAFs resulted in a decreased stiffness of the epithelial cells, as well as an elongated morphological phenotype, when compared with co-cultures with NPFs. Moreover, the presence of CAFs increased proliferation and invasion of epithelial cells, features typically associated with tumor progression. Altogether, this study provides novel insights into the mechanical interactions between epithelial cells with the malignant prostate microenvironment, which could potentially be explored for new diagnostic approaches.

Short Talk

Dynamic real-time deformability cytometry to decipher the response of bats to heterothermy

Bob Fregin

ZIK HIKE, Institute of Physics, University of Greifswald, Greifswald, Germany

Dynamic real-time deformability cytometry (dRT-DC) is a label-free method to capture the full viscoelastic properties of suspended cells at a throughput of up to 100 per second. Cellular shape-changes along the entire length of the microfluidic channel are tracked in real-time and are subsequently analyzed by a Fourier decomposition. We demonstrate that this approach allows disentangling the cell response to the complex hydrodynamic environment at the inlet from the steady-state stress distribution inside the channel. A superposition of both effects is present in almost all microfluidic systems and potentially biases label-free cytometric measurements relying on steady-state flow conditions. We show that dRT-DC allows for cell mechanical assays at the millisecond time scale fully independent of cell shape.

Using dRT-DC we address for the first time the role of red blood cell viscoelasticity in temperature control in hibernating animals. We measured blood from the bat species Nyctalus Noctula over 3 years to understand how hibernating animals can maintain red blood cell function at low body temperatures during torpor. For that purpose, we analyzed the mechanical properties at three different temperature levels, ranging from approximately 10 °C to 37 °C. We also acquired data from a non-hibernating bat species, Rousettus Aegyptiacus, and humans to compare red blood cell mechanics among these 3 different species and corresponding regulatory effects. While between the bat species, only minor variations exist, we observe a clear difference in comparison to humans.

Short Talk

Linking mechanical properties of cells with their ability to circulate using real-time deformability cytometry

Benedikt Hartmann

Max Planck Institute for the Science of Light & Max Planck Zentrum für Physik und Medizin, Erlangen, Germany

Blood cells are sequentially squeezed in many constrictions during their round trip through the body. Especially in the capillary bed in organs, such as the lung, their deformability determines how quickly they pass through these constrictions. Yet the deformability is not just a matter of a cell's elasticity, but also their viscosity. Real-time deformability cytometry (RT-DC) is an established technique to quantify mechanical properties of cells and offers a way to measure viscoelastic properties of cells as they are exposed to shear stress in a microfluidic channel. From the steady state deformation at the end of the channel, the elasticity can be calculated, while from the time course of the deformation over the length of the channel one can estimate the viscosity. Using custom channel designs, including constrictions of various width and length, the influence of viscoelastic properties as well as other parameters, like cell size, can be linked with passage time through a constriction in order to provide inside into the question "What determines how good a cell can squeeze through a constriction?".

11:00 am – 12:30 pm Core Facility Session: Light Sheet

Chairs: Alina Liebheit and Frank Schildberg

This year's topic will spotlight the field of Light Sheet Fluorescence Microscopy (LSFM), a rapidly developing 3D imaging technique that is shared in core facility approaches around the globe. This technique provides a three-dimensional view into the inner working of the biological system. A prerequisite for this imaging method is a process called clearing, which renders the tissue transparent. Recent developments have produced a growing toolbox of tissue clearing, whole-mount immunostaining, and image analysis methods enabling the 3D exploration of almost every organ. The session will discuss how Light Sheet Fluorescence Microscopy can be used in human and mouse tissues when analyzing a biological question and will cover the strengths and limitations of the different methods.

Spatial molecular profiling of 3D imaged whole organs and organisms

Harsharan Bhatia

Institute for Tissue Engineering and Regenerative Medicine (iTERM), Helmholtz Zentrum München, Neuherberg, Germany.
Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians University (LMU), Munich, Germany.

While many diseases affect the entire body, research is usually conducted on the tissue of interest. To overcome potential bias, and discover previously unnoticed yet essential phenotypic changes in health and disease, we use a holistic approach. Towards this goal, we have developed novel tissue transparency methods- DISCO clearing, enabling in-depth antigen labeling, clearing and imaging of intact organs and organisms. DISCO clearing is fast (can be completed within a few hours) and versatile (applicable to various tissues including the brain, spinal cord, lung, heart, immune organs, and tumor bearing organs). We can now collect information at sub-cellular levels in whole organs and organisms without sectioning. Using DISCO clearing, we were able to characterize novel skull-dura connections and locate antibody-targeted vs. untargeted metastatic tumors in whole body of a mouse. Moreover, to unravel the underlying molecular basis for the morphological changes in intact organs, we recently developed DISCO-MS. It's a technology combining whole-organ/ism imaging, deep learning-based image analyses, micro tissue extraction and proteome analyses using ultra-high sensitivity mass spectrometry. DISCO-MS yielded qualitative and quantitative data indistinguishable from uncleared samples in both rodent and human tissues. We characterized microenvironment of early individual amyloid-beta plaques in Alzheimer's disease mouse model. Furthermore, we studied regional proteome heterogeneity of immune cells in intact mouse bodies and aortic plaques in whole human heart. Thus, DISCO-MS enables unbiased proteome analysis of pre-clinical and clinical tissues after unbiased imaging of entire specimens in 3D, providing new diagnostic and therapeutic opportunities for complex diseases.

From tissue clearing to cleared immunological processes

Anika Grüneboom

Leibniz-Institut für analytische Wissenschaften – ISAS – e.V., Dortmund
University Hospital Essen, University Duisburg-Essen, Germany

Fluorescence microscopic analyses of large biological samples are subject to technical limitations due to their opacity, which causes light absorption, reflection and scattering. These physical characteristics are particularly challenging for analyses of anatomy and physiology. However, a new technology that overcomes these limitations is light-sheet fluorescence microscopy, which in combination with optical tissue clearing enables 3-dimensional analyses of large tissue samples. By using fluorescently labelled antibodies and obtaining endogenous fluorescence proteins, optical clearing methods together with light-sheet fluorescence microscopy (LSFM) enable the specific identification of biological structures down to individual cells.
The combination of optical clearing and LSFM represents a promising technology in basic and translational biomedical research. Based on a self-invented clearing protocol, we were able to identify and characterize for the first time a previously unexplained vascular system in long bones, which represents a significant contribution to the anatomical and physiological understanding of this organ. We were also able to identify a previously unknown macrophage population in knee joints that forms a physical barrier to the joint and shields it from inflammatory immune cells. This discovery opens up completely new therapeutic approaches for joint-associated diseases such as rheumatoid arthritis. These examples illustrate the wide range of applications of this technology and its high potential for future studies in the field of biology, translational research and clinical application.

MarShie – a novel tissue clearing pipeline to comprehensively reveal myeloid-vasculature interactions throughout the murine bone marrow

Till Mertens

Charité – Universitätsmedizin Berlin, Department of Rheumatology and Clinical Immunology, Berlin, Germany.

Bone regeneration is a highly orchestrated process based on the micro-environmental interplay between the immune cell compartment, mesenchymal cells, and the vascular system. Around 5-10% of all fractures do not heal satisfactory and may result in mal- or nonunion. Refining our understanding of basic principles in the regeneration of bone potentially aids to the development of new regenerative strategies. Type H vessels, which are known to couple angiogenesis to osteogenesis during bone development are present in the fracture gap. The factors determining vessel growth under these conditions, as well as the complex spatial relationships between cellular compartments in the fracture gap have yet to be investigated in detail. We present here a novel tissue clearing pipeline termed MarShie (Marrow-Stabilization under harsh conditions via intramolecular epoxide linkage). This method enables us to analyze the three-dimensional interactions of myeloid cells with the vascular system, with cellular resolution. Our pipeline preserves endogenous fluorescence and diminishes light scattering and autofluorescence. Combined with light sheet fluorescence microscopy we used our pipeline to detect endogenous fluorescence of the myeloid compartment in CX3CR1-GFP+ cells, and the vascular network via Cdh5-tdTomato fluorescence. By using machine-learning based classifiers for cellular segmentation, we were able to quantitatively analyze these interactions in homeostatic state as well as in defect models for intramembranous and endochondral ossification. We observe that immediately after the tissue damage, myeloid cells sequester the area of bone injury. Later, they precede the growth of vessels and remain in close contact with endothelial cells throughout the regenerative process.

1:00 pm – 2:30 pm Nanotechnology

Chairs: Wolfgang Fritzsche and Julia Böke

The session deals with micro- and nanosized particles and their investigation. On one hand, microfluidic platforms can be used to produce artificial cells, studying the cellular phenomena of self-organisation and confinement of reactions.
On the other side, micro- and nanosized particles can have a negative impact on organisms: The last years witnessed a growing awareness of the presence and potential negative impact of small plastic particles. Methods are required in order to detect and characterize these particles, preferably in a higher throughput – here aspects of methods established in cytometry could play a role. An introduction into the field will be given, and analytical approaches discussed with a special focus on a combination of imaging and flow through characterization. The potential of additional information from (spectroscopic) imaging will be discussed.

High-throughput microfluidic production of multi-compartment synthetic eukaryotic cells

Tom Robinson

Max Planck Institute of Colloids and Interfaces, Berlin

Bottom-up synthetic biology aims at the de novo construction of synthetic cells using non-living components. Building biomimetic cells provides the opportunity to understand real cells and their origins, but also provides alternative routes to novel biotechnologies. Giant unilamellar vesicles (GUVs) are used extensively to construct synthetic cells but traditional methods to form them are limited. Microfluidic-based approaches for GUV production show great potential for encapsulating large biomolecules required for mimicking life-like functions (Yandrapalli et al. Micromachines, 2020; Love et al. Angew Chemie, 2020). First, we present a microfluidic design that is able to produce surfactant-free pure-lipid GUVs in high-throughput (Yandrapalli et al. Commun Chem, 2021). The major advancement is that the lipid membranes are produced in the absence of block co-polymers or surfactants that can affect their biocompatibility – which is commonly overlooked. The design can produce homogenously sized GUVs with tuneable diameters from 10-130 µm. Encapsulation is uniform and we show that the membranes are oil-free by measuring the diffusion of lipids via FRAP measurements. Next, we encapsulate two sub-populations of nano-sized vesicles to establish enzymatic cascade reactions across membrane-bound compartments, therefore mimicking eukaryotes (Shetty et al. ACS Nano, 2021). The final synthetic cell comprises three coupled enzymatic reactions, which propagate across three separate compartments in a specific direction due to size-selective membranes pores. Not only does microfluidics provide a high-degree of control over the intra-vesicular conditions such as enzyme concentrations, buffers, and the number of compartments, but the monodispersity allows us to directly compare the effects that compartmentalization has on the biochemical reaction rates.

Microplastics – Small particles, big challenges

Claudia Lorenz

Aalborg University, Department of the Built Environment, Denmark

With global plastic production inexorable on the rise, the occurrence of plastic pollution in the environment has been acknowledged as an emerging topic of international concern. This plastic litter is derived mainly from the inadequate disposal and management of plastic waste and will, due to physical and chemical weathering, get brittle and release a multitude of smaller and smaller plastic particles termed microplastics (1 µm – 5 mm) and nanoplastics (<1 µm).
In recent years, this microplastic pollution has received increasing attention not only from the scientific community but also from society and policymakers. However, its extent and the resulting impacts on the aquatic environment and human health remain largely unknown. This is especially true regarding the conclusive identification and characterization of "small" microplastics. The smaller the particles, the bigger the challenges related to extracting and identifying microplastics in complex environmental matrices. Furthermore, various methods for sampling, extraction, and analysis of microplastics are available, which complicate the establishment of standardized operational protocols (SOPs). So far, no single analytical method exists that can provide reliable identification and quantification of all microplastic polymers and simultaneously assess desired characteristics, e.g., size, shape, and mass.

Light and, more recently, fluorescence microscopy are widely used in microplastic research to visualize and characterize microplastics. Microscopy can be combined with spectroscopic techniques, like Fourier Transform Infrared (FT-IR) and Raman spectrometry to assess also the chemical composition. Thermoanalytical methods and mass spectrometry further provide relevant information regarding the polymers' mass and composition, including additives.
Historically, flow cytometry was used to enumerate and characterize cells but has long since broadened in scope and is now applied for all microparticles, including microplastics. Although only a few studies have explored the possibility of utilizing flow cytometry for the quantification and characterization of microplastics in environmental samples, flow cytometry has become a helpful tool in the field of microplastic research for, e.g., the validation and quality assurance of digestion protocols, aiding in recovery experiments, and setup of ecotoxicological experiments.
Some of these applications will be presented here with a focus on the use of the FlowCam® (Yogogawa Fluid Imaging Technologies), an imaging particle analyzer combining the benefits of flow cytometry, microscopy, and digital imaging. This instrument is used worldwide for quality control for (bio)pharmaceutical products, water quality monitoring, counting cells of cyanobacteria and other marine and freshwater plankton organisms, and microplastics research.

Imaging Flow Cytometry – from 3D tomographic over multispectral imaging to nanoparticle tracking

Julia Böke

Leibniz Institute of Photonic Technology, Jena

Particles constitute the basic building blocks of cells and tissues, which form living matter, organisms and biosystems. They are omnipresent in daily life and the environment spreading over fields like nutrition, agriculture, sustainability, waste and technology. The gold standard for analyzing large particle populations is flow cytometry (FC). It captures forward and side scattering or the fluorescent signal of each measured particle through a point detector, without resolving the spatial distribution of biomarkers or internal structures. Conventional imaging flow cytometry (IFC) bridges this gap by combing flow cytometry with an imaging setup. In our group, we combine fluid dynamics, microsystems technology, photonics and automation to develop microfluidic devices for applications ranging from microfluidic design automation over biotechnology process monitoring to motion analysis. These efforts let us develop a unique IFC platform (Figure 1) utilizing a smart combination of flow focusing and flow rotation (Henkel et al. 2020). The IFC platform enables applications in the field of tomographic IFC (tIFC) (Kleiber et al. 2020), volumetric image-based absorbance imaging, multispectral IFC (mIFC) (Kraus et al. 2022) and even Brownian motion imaging flow cytometry (bmIFC) for the analysis of nanoparticles and liposomes. Thus, it captures different particle properties, ranging from morphological to spectral characteristics, in high-throughput. Combined with a high-speed camera and suitable optics, the setup can be tailored to a variety of applications to address fundamental research questions on nanoparticles or enable continuous monitoring of cells or particles for different process parameters.

Short Talk

Physikalisch-Technische Bundesanstalt (PTB), Berlin, Germany

Martin Hussels

Physikalisch-Technische Bundesanstalt (PTB), Berlin, Germany

Integration and Control of Low-Cost Industrial Cameras in a Home-Built Flow Cytometer

Imaging flow cytometry combines the high information content of microscopic images with the high throughput of flow cytometry. Possible applications include the diagnosis of acute myeloid and lymphoid leukemia in immunophenotyping, detection of rare circulating tumor cells in liquid biopsy, or analysis of morphological cell changes like mitosis. In these applications, very small populations of target cells must be identified and quantified within huge numbers of other cells, coincidences, and agglomerates. Minor errors in counting those populations can have a major impact on the diagnosis.

As differentiation between single cells, coincidences, and cell agglomerates is challenging for common flow cytometers, we developed a flow cytometer with integrated multi-dimensional imaging. Our instrument combines the high signal detection rates of photomultiplier tubes (PMTs) with the ability to capture images of cells of interest.

2:30 pm – 2:45 pm **Farewell, Speed-talk award ceremony**

First name	Last name	Institute	City	E-Mail
AMANY	ABDALAZIZ	National Research Centre	Cairo	dr_amanyaziz@yahoo.com
Julia	Altmaier	Universitätsmedizin Mainz	Mainz	lenzj@uni-mainz.de
Ali	Bashiri Dezfouli	Technical University of Munich	Munich	ali.bashiri@tum.de
Tom	Bauer	evorion Biotechnologies	Muenster	thm.bauer@gmx.de; t.bauer@evorion.de
Sabine	Baumgart	Universitätsklinikum Jena	Jena	sabine.baumgart@med.uni-jena.de
Jochen	Behrends	Head Fluorescence Cytometry; Research Center Borstel	Borstel	jbehrends@fz-borstel.de
Jochen	Behrends	Research Center Borstel	Borstel	jbehrends@fz-borstel.de
Bertram	Bengsch	Universitätsklinikum Freiburg	Freiburg	bertram.bengsch@uniklinik-freiburg.de
Harsharan	Bhatia	Helmholtz Munich - German Research Center for Environmental Health	Munich	harsharan.bhatia@helmholtz-muenchen.de
Kristin	Bieber	UKT Tübingen	Tübingen	kristin.bieber@med.uni-tuebingen.de
Stefanie	Bock	Klinik für Neurologie, Universitätsklinikum Düsseldorf	Düsseldorf	stefanie.bock@med.uni-duesseldorf.de
Jan	Bodinek	Uniklinik Freiburg	Freiburg	jan.bodinek@uniklinik-freiburg.de
Julia	Böke	IPTH	Jena	JuliaSophie.Boeke@leibniz-ipht.de
Caroline	Braeuning	MDC	Berlin	caroline.braeuning@mdc-berlin.de
Ana Teresa	Branco	Charité - University Medical Centre Berlin (CBF)	Berlin	ana-teresa.branco@charite.de
Carmen	Bruckmann	Helmholtz-Zentrum für Umweltforschung GmbH - UFZ	Leipzig	carmen.bruckmann@ufz.de
Lisa	Budzinski	Deutsches Rheuma-Forschungszentrum Berlin (DRFZ) - Ein Leibniz Institut	Berlin	lisa.budzinski@drfz.de
Chii Jou	Chan	National University of Singapore	Singapore	dbschii@nus.edu.sg
Hyun-Dong	Chang	Schwiete-Labor for Microbiota and Inflammation, DRFZ	Berlin, Germany	chang@drfz.de
Asbjørn	Christophersen	Insitute of Clinical Medicine, University of Oslo, Norwey	Oslo, Norwey	a.o.christophersen@medisin.uio.no
Caterina	Curato	German Federal Institute for Risk Assessment	Berlin	caterina.curato@bfr.bund.de
Venkata Aditya Saimadhukiran	Dabbiru	Universität Greifswald	Greifswald	venkata.dabbiru@uni-greifswald.de
Lisa-Marie	Diekmann	DRFZ	Berlin	
Ruslan	Dmitriev	"Tissue engineering and Biomaterials Lab, Ghent University "	Ghent, Belgium	Ruslan.Dmitriev@UGent.be
Andreas	Dolf	Universitätsklinikum Bonn	Bonn	andreas.dolf@uni-bonn.de
Susann	Dr. Müller	Helmholtz Centre for Environmental Research - UFZ	Leipzig	susann.mueller@ufz.de
Elmar	Endl	University Bonn	Bonn, Germany	eendl@uni-bonn.de
Sebastian	Ferrara	DRFZ	Berlin	

First name	Last name	Institute	City	E-Mail
Marta	Ferreira Gomes	Therapeutic Immuneregulation	Berlin, DRFZ	marta.fgomes@drfz.de
Leo	Fiebig	DRFZ	Berlin	
Alexander	Fiedler	Deutsches Rheuma-Forschungszentrum	Berlin	fiedler@drfz.de
Marie	Follo	Universitätsklinikum Freiburg, Klinik für Innere Medizin I, Lighthouse Core Facility	Freiburg, Germany	marie.follo@uniklinik-freiburg.de
Bob	Fregin	ZIK HIKE, Universität Greifswald	Greifswald	bob.fregin@uni-greifswald.de
Wolfgang	Fritzsche	IPTH	Jena	wolfgang.fritzsche@leibniz-ipht.de
Daniela	Frölich	Ulm University /Core Facility Cytometry	Ulm	daniela-1.froelich@uni-ulm.de
Ruyu	Gao	Helmholtz-Zentrum für Umweltforschung	Leipzig	ruyu.gao@ufz.de
Naama	Geva-Zatorsky	Technion, Haifa, Israel	Haifa, Israel	naamagvz@gmail.com
Claudia	Giesecke	Max-Planck-Institut für molekulare Genetik	Berlin, Germany	giesecke@molgen.mpg.de
Jesus	Gil-Pulido	Cytek Biosciences	Mainz	jgilpulido@cytekbio.com
Christian	Goerke	Physikalisch-Technische Bundesanstalt	Berlin	Christian.Goerke@ptb.de
Anne	Gompf	CMCB der TU Dresden	Dresden, Germany	anne.gompf@tu-dresden.de
Anne	Gompf	TU Dresden Flow Core	Dresden	anne.gompf@tu-dresden.de
Christoph	Göttlinger	Institut für Genetik der Universität zu Köln	Köln	cg4711@t-online.de
Felix	Graf	ZIK HIKE, Universität Greifswald	Greifswald	s-fegraf@uni-greifswald.de
Lothar	Gröbe	Helmholtz Zentrum f. Infektionsforschung	Braunschweig	lothar.groebe@helmholtz-hzi.de
Anika	Grüneboom	"Anika Grüneboom. Bioimaging, ISAS e.V. "	Dortmund, Germany	anika.grueneboom@isas.de
Simon	Haas	Max-Delbrück-Zentrum für molekulare Medizin, Berlin, Deutschland	Hannover, Germany	simon.haas@bih-charite.de
Romy	Hackbusch	Universitätsklinikum Hamburg-Eppendorf	Hamburg	r.hackbusch@uke.de
Benedikt	Hartmann	Max Planck Institut für die Physik des Lichts	Erlangen	benedikt.hartmann@mpl.mpg.de
Anja	Hauser	Immune dynamics, DRFZ	Berlin, Germany	hauser@drfz.de
Yannik	Hein	PTB	Berlin	yannik.hein@ptb.de
Jacqueline	Hirscher	DRFZ	Berlin	hirscher@drfz.de
Heike	Hirseland	DRFZ	Berlin	
Ute	Hoffmann	DRFZ	Berlin	hoffmann@drfz.de
Henrik	Holm-Kjar	Slingshot Bio	Emeryville	henrik@slingshotbio.com
Adrian	Huck	Charite-Universitätsmedizin	Berlin	adrian.huck@charite.de
Martin	Hussels	Physikalisch-Technische Bundesanstalt	Berlin	martin.hussels@ptb.de

First name	Last name	Institute	City	E-Mail
Iga	Janowska	UNIVERSITÄTSKLINIKUM FREIBURG	Freiburg	iga.janowska@uniklinik-freiburg.de
Rafael	Jose Arguello	Centre d`Immunologie de Marseille-Luminy	Marseille, France	arguello@ciml.univ-mrs.fr
Daniel	Kage	Deutsches Rheuma-Forschungszentrum (DRFZ)	Berlin	daniel.kage@drfz.de
Toralf	Kaiser	Flow Cytometry Core Facility, DRFZ	Berlin, Germany	kaiser@drfz.de
Janine	Kemming	Technical University of Denmark	Kopenhagen, Danmark	jsoke@dtu.dk
Jacqueline	Keye	Charité - BIH	Berlin	jacqueline.keye@bih-charite.de
Christin	Koch	Microbiology Research, Symrise AG	Holzminden, Germany	christin.koch@symrise.com
Christin	Koch	Symrise AG	Holzminden	christin.koch@symrise.com
Ralf	Köhler	Immune Dynamics, DRFZ	Berlin, Germany	ralf.koehler@drfz.de
Ralf	Köhler	DRFZ	Berlin	
Yesaswini	Komaragiri	ZIK HIKE Universität Greifswald	Greifswald	yesaswini.komaragiri@uni-greifswald.de
Marcel	Kordt	Core Facility für Zellsortierung und Zellanalyse Universitätsmedizin Rostock	Rostock	marcel.kordt@med.uni-rostock.de
Martin	Kräter	Max Planck Institute for the Science of Light	Erlangen	Martin.kraeter@mpl.mpg.de
Felicia	Kruse	BIH at Charite	Berlin	simone-felicia.kruse@bih-charite.de
Désirée	Kunkel	BIH Cytometry Core, Charité - Universitätsmedizin Berlin	Berlin, Germany	desiree.kunkel@charite.de
Shuang	Li	Helmholtz Centre for Environmental Research	Leipzig	shuang.li@ufz.de
Alina	Liebheit	Immune Dynamics, DRFZ	Berlin, Germany	Alina.Liebheit@drfz.de
Wjatscheslaw	Liublin	DRFZ	Berlin	
Juan	Lopez Galvez	UFZ	Leipzig	juan.lopez-galvez@ufz.de
Claudia	Lorenz	Aalborg University, Department of the Built Environment, Thomas Manns Vej 23, 9220 Aalborg Ø, Denmark	Aalborg, Denmark	clo@build.aau.dk
Tamara	Manuelian	Visiopharm A/S	Hamburg	tma@visiopharm.com
Hanna	Mayer	Helmholtz-Cetre for Environmental Research	Leipzig	hanna.mayer@ufz.de
Henrik	Mei	Mass Cytometry Core Facility, DRFZ	Berlin, Germany	mei@drfz.de
Sebastian	Meller	Tierhochschule Hannover	Hannover, Germany	sebastian.meller@tiho-hannover.de
Till	Mertens	DRFZ	Berlin, Germany	Till.Mertens@drfz.de
Aleks	Mezydlo	hema.to GmbH	München	aleks@hema.to
Karsten	Miermans	hema.to GmbH	München	karsten@hema.to
Antonia	Mikulova	Masaryk University	Brno	antonia.mikulova@sci.muni.cz
Stefanie	Möckel	Institute of Molecular Biology	Mainz	s.moeckel@imb-mainz.de

First name	Last name	Institute	City	E-Mail
Lena	Müller	Core Facilities Flow Cytometry, Medical University of Vienna, Vienna, Austria	Vienna, Austria	lena.mueller@meduniwien.ac.at
Michael	Müller	Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e. V. - Hans Knöll Institut -	Jena	Michael.Mueller@leibniz-hki.de
Franziska	Münz	Uniklinik Ulm	Ulm	franziska.muenz@uni-ulm.de
Alexander	Navarrete Santos	martin Luther Universität Halle-Wittenberg	Halle/Saale	alexander.navarrete@uk-halle.de
Alexander	Navarrete Santos	Martin Luther Universität Halle-Wittenberg	Halle/Saale	alexander.navarrete@uk-halle.de
Alexander	Navarrete Santos	Martin Luther Universität Halle-Wittenberg	Halle/Saale	alexander.navarrete@uk-halle.de
Raluca	Niesner	Biophysical Analytics, DRFZ	Berlin, Germany	niesner@drfz.de
Ciro	Novaes Rosa Lino	Precision for Medicine	Berlin	ciro.novaes@ precisionformedicine.com
Franziska	Nowak	BIH @ Charité	Berlin	franziska.nowak@bih-charite.de
Nikos	Oikonomou	CECAD Research Center	50931, Cologne	noikonom@uni-koeln.de
Oliver	Otto	Uni-Greifswald	Greifswald, Germany	oliver.otto@uni-greifswald.de
Anna	Pascual Reguant	Immune dynamics, DRFZ	Berlin, Germany	Pascual@drfz.de
Claudia	Peitzsch	Center for Regenerative Therapies Dresden (CRTD)	Dresden	claudia.peitzsch@tu-dresden.de
Christian	Peters	UK-SH, Institut für Immunologie / Zytometrie Core Facility	Kiel	christian.peters@uksh.de
Nicole	Pochert	Helmholtz Zentrum München - Institut für Umweltmedizin	Augsburg	nicole.pochert@helmholtz-muenchen.de
Alexander	Putz	Physikalisch-Technische Bundesanstalt Berlin	Berlin	alexander.putz@ptb.de
Katharina	Raba	Universitätsklinikum Düsseldorf, ITZ	Düsseldorf	Katharina.Raba@med.uni-duesseldorf.de
"Asylkhan "	Rakhymzhan	Biophysical Analytics, DRFZ	Berlin, Germany	Asylkhan.Rakhymzhan@drfz.de
Felix	Reichel	Max-Planck-Institut für die Physik des Lichts	Erlangen	felix.reichel@mpl.mpg.de
Anika	Rettig			rettig@molgen.mpg.de
Beate	Rinner	"Core Facility Alternative Biomodels & Preclinical Imaging, "	Graz, Austria	Beate.Rinner@klinikum-graz.at
Tom	Robinson			Tom.Robinson@mpikg.mpg.de
Elisa	Rosati	Institute of Clinical Molecular Biology, University of Kiel	Kile, Germany	e.rosatiscalchi@gmail.com
Leben	Ruth	DRFZ	Berlin	
Florian	Schattenberg	Helmholtz-Centre for Environmental Research - UFZ	Leipzig	Florian.Schattenberg@ufz.de
Frank	Schildberg	"Clinic for Orthopedics and Trauma Surgery, University Hospital Bonn "	Bonn, Germany	Frank.Schildberg@ukbonn.de

First name	Last name	Institute	City	E-Mail
Johanna	Schleep	FLI Institut für Alterforschung Jena	Jena	simone.taenzer@leibniz-fli.de
Konrad	Schuldes	Max-Planck-Institut für Immunbiologie und Epigenetik	Freiburg	schuldes@ie-freiburg.mpg.de
Axel	Schulz	Mass Cytometry Core Facility, DRFZ	Berlin, Germany	axel.schulz@drfz.de
Toni	Sempert	German Rheumatism Research Center (DRFZ), Berlin, a Leibniz Institute	Berlin	toni.sempert@drfz.de
Sieghart	Sopper	FACS-Core-Unit & Tumor-Immunology, Medical University of Innsbruck, Innsbruck, Austria	Innsbruck, Austria	Sieghart.Sopper@i-med.ac.at
Till	Sörensen	Bioretis GmbH	Berlin	till.soerensen@bioretis.com
Andreas	Spittler	Medical University of Vienna	Vienna, Austria	andreas.spittler@meduniwien.ac.at
Bettina	Stahl	Uniklinik Ulm	Ulm	bettina.stahl@uniklinik-ulm.de
Stefan	Stein	Georg-Speyer-Haus	Frankfurt	s.stein@georg-speyer-haus.de
Emily	Stephenson	Haniffa Lab, Faculty of Medical Sciences, Newcastle University	Newcastle, UK	Emily.Stephenson2@newcastle.ac.uk
Eric	Sündermann	ZIK HIKE, Universität Greifswald	Greifswald	eric.suendermann@stud.uni-greifswald.de
Simone	Tänzer	FLI Institut für Alterforschung Jena	Jena	simone.taenzer@leibniz-fli.de
Anna	Taubenberger	TU Dresden (Group Leader of Oncomechanics group at Biotec)	Dresden, Germany	anna.taubenberger@tu-dresden.de
Marta	Urbanska	"University of Cambridge "	Cambridge, UK	marta.urbanska@mpl.mpg.de; mu272@cam.ac.uk
Marta	Urbanska	Max Planck Institute for the Science of Light	Erlangen, Germany	marta.urbanska@tu-dresden.de
Christian	von Rein		Dummerstorf	
Olga	Vondálová Blanář ová	Masaryk University Brno	Brno	olgavondalova@sci.muni.cz
Sarah	Warth	Sarah Warth, Core Facility Cytometry, Ulm University Medical Faculty	Ulm, Germany	sarah.warth@uni-ulm.de
Jakob	Zimmermann	Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern,	Bern, Switzerland	jakob.zimmermann@dbmr.unibe.ch