# Visions
in Cytometry

Microscopy ◀▶ Multiplexing Analysis ◀▶ Flow-/Mass-Cytometry

29th Annual Conference of the German Society for Cytometry

# Abstract booklet

Charité Universitätsmedizin
Berlin,
Campus Mitte, CCO
Charitéplatz 1 - Virchowweg 6 -
10117 Berlin

**September, 25-27, 2019**

DGfZ
Deutsche Gesellschaft
für Zytometrie

**www.dgfz.org**

# Visions in Cytometry

Microscopy ◀▶ Multiplexing Analysis ◀▶ Flow-/Mass-Cytometry

29th Annual Conference of the German Society for Cytometry

**Dear friends of cytometry,**

With great pleasure I announce the 29th Meeting of the German Society for Cytometry (DGfZ), to be held from Sept, 25th-27th in Berlin.

It is a privilege for me to host this great meeting at the historic campus Charité Mitte, right in the center of Berlin. In keeping with this year´s motto "Visions in cytometry", we hope to bring together experts from various disciplines for an exciting exchange of new ideas.

As the venue is conveniently located in walking distance from the main station, we will for sure attract a large crowd of cytometry enthusiasts. A number of exciting invited speakers have already confirmed their attendance, and we will have sessions on attractive topics such as microscopy and image analysis, nanotechnology, microbiology, all focusing on the application of novel technologies. This inspiring program will for sure spark interesting discussion between scientists, technologists and industrial partners.

In addition to the scientific sessions, we will again have the popular "product slam" format, where our industrial sponsors will present their newest innovations within three minutes. There will be plenty of opportunities to meet and become informed about new products during the industrial exhibition. At this point I would like to thank the industrial sponsors for their generous financial support, which makes this meeting possible.

I am looking forward to welcoming you here in Berlin,

Anja Hauser

Anja Hauser
President of the DGfZ

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# Wednesday, September, 25th, 2019

| 9:00 am - 11:00 pm | Tutorials (parallel working group sessions) |
|---|---|
| *DRFZ*<br>*Seminar room 1+2* | 1. Flow Cytometry<br>Chairs: Toralf Kaiser, Claudia Giesecke-Thiel |
| *DRFZ*<br>*Seminar room 1st floor* | 2. Image Analysis<br>Chair: Ralf Köhler |
| *DRFZ*<br>*Seminar room 3* | 3. Publication in Cytometry A<br>Chair: Attila Tarnok |

| 11:15 am - 12:15 pm | Fluidigm-Satellite Symposium |
|---|---|
| *LH IM* | High-Dimensional Immune Monitoring Simplified<br>Easily quantify 37 immune subsets from PBMC and whole blood using mass cytometry |
| 12:00pm - 1:00pm | Registration, Lunch (Soup) |
| *CCO* | |

| 1:00 pm - 1:05pm | Welcome |
|---|---|
| *LH IM* | Anja Hauser |

| 1:00 pm - 2:30 pm | Session 1: Imaging |
|---|---|
| *LH IM* | Chairs: Anja Hauser, Raluca Niesner |
| David Entenberg | **ISAC-Lecture:** Surgical Engineering Enables Intravital Imaging of Mechanisms of Metastasis in Primary and Secondary Sites |
| Thomas Tull | Imaging mass cytometry as a tool to delineate B cell subsets in human gut associated lymphoid tissue |
| Anna Pascual-Reguant | Characterization of Innate Lymphoid Cells and their microenvironment by multiplexed histology in human tissue |
| Bob Fregin | Dynamic real-time deformability cytometry: High-throughput multiparametric mechanical single cell analysis |

| **2:30 pm - 3:30 pm** | **Session 2: Product Slam** |
|---|---|
| *LH IM* | Chairs: Elmar Endl, Thomas Bauer |
| | Selected industrial partners will present their newest innovative technological developments and products |
| 3:30 pm - 4:30 pm | Coffee Break/Industry Exhibition |
| *CCO* | |

| **4:30 pm -6:00 pm** | **Session 3: Cutting Edge** |
|---|---|
| *LH IM* | Chair: Henrik Mei, Asylkhan Rakhymzhan |
| Yvan Saeys | Machine learning challenges for single-cell biology |
| Raluca Niesner | Ultra-deep tissue imaging by three-photon laser-scanning microscopy |
| Asylkhan Rakhymzhan | Co-registered spectral optical coherence tomography and two-photon microscopy for multimodal deep-tissue imaging in adult mice |
| Axel Ronald Schulz | Mass cytometry combined with computational data mining reveals a multifactorial immune cell signature of active rheumatoid arthritis |

| **6:00 pm - 7:00 pm** | **Keynote (open to public)** |
|---|---|
| *LH IM* | Chair: Andreas Radbruch |
| Petter Brodin | Systems-level analysis of immune development early in life |
| 7:00 pm - 10:30 pm | Welcome reception (in exhibition area) |
| *CCO* | |

| **8:00 pm - 10:00 pm** | **Core Facility Networking Event** |
|---|---|
| *DRFZ, Seminar Room 1* | Chair: Desiree Kunkel, Steffen Schmitt |
| Steffen Schmitt | Modified collection devices facilitate sample handling on BD FACSAria cell sorter |
| Vladimir Benes | Many shades of single-cell sequencing - a guide to implement the technology in Flow Cores |

# Thursday, September, 26th, 2019

| **9:00 am - 10:30 am** | **Session 4: Core Facility Session: The many faces of single cell analysis** |
|---|---|
| *LH IM* | Chairs: Desiree Kunkel, Christian Kukat |
| Nina Cabezas-Wallscheid | Regulation of single dormant hematopoietic stem cells |
| Roman Sankowski | Integrating single-cell RNA sequencing and mass cytometry to profile human microglia heterogeneity |
| Lisa Budzinski | Multi-parameter Flow Cytometry of Human Gut Microbiota |

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

| **11:00am - 12:30pm** | **Session 5: European Guest Session: Sweden** |
|---|---|
| *LH IM* | Chairs: Anja Hauser, Raluca Niesner |
| Peter Thul | The Science for Life Laboratory in Sweden |
| Peter Thul | The HPA Cell Atlas: An image-based subcellular map of the human proteome |
| Julia Fernandez-Rodrigues | Swedish National Microscopy Infrastructure – Centre for Cellular Imaging, a Correlative Multimodal Imaging Facility |

| 12:30pm - 2:30pm | Lunchbreak / **Poster Session A** |
|---|---|
| *CCO* | Chairs: Torsten Viergutz, Raluca Niesner |

| **2:30pm - 4:00pm** | **Session 6: Klaus-Goerttler-Session incl. award ceremeony** |
|---|---|
| *LH IM* | Chair: Philipp Rosendahl (last year awardee) |
| Goerttler-Awardee Zishu Liu | Neutral mechanisms and niche differentiation in steady-state insular microbial communities revealed by single cell analysis |

*CCO: Atrium Cross Over, Virchowweg 6 LH IM: Lecture Hall "Innere Medizin", Sauerbruchweg 2*

| | |
|---|---|
| Oliver Otto | Label-free multiparametric flow cytometry in virtual fluidic channels: Single cell rheology and tissue mechanicsd |
| Angela Jacobi | Analysis of biomechanical properties of hematopoietic stem and progenitor cells using real-time fluorescence and deformability cytometry |

| | |
|---|---|
| 4:00 pm - 5:00pm | Coffee Break/**Poster Session B** |
| *CCO* | Chairs: Torsten Viergutz, Raluca Niesner |

## 5:00 pm - 6:00 pm Guest Lecture

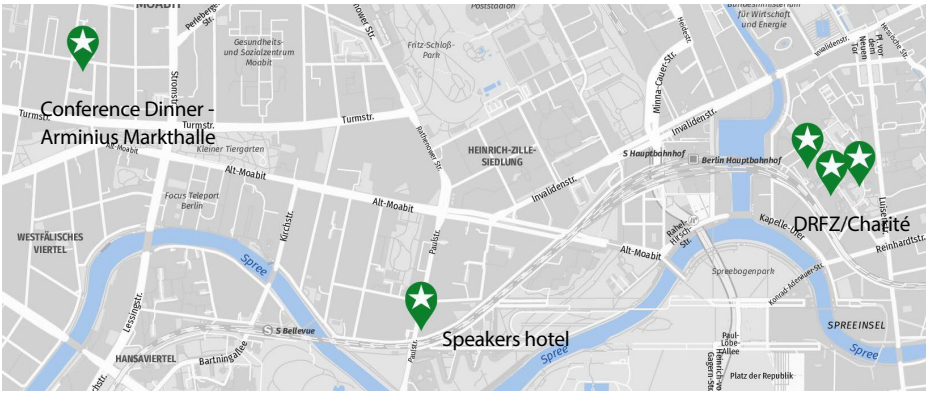
| | |
|---|---|
| *LH IM* | Chairs: Anja Hauser, Raluca Niesner |
| Nadja Froebisch | Fossils, bones and genes – the evolution of limb development and regeneration |

## 6:00 pm - 7:30 pm Members Assembly

| | |
|---|---|
| *LH IM* | Chairs: Anja Hauser, Raluca Niesner |

| | |
|---|---|
| 7:30pm - 11:00pm | Conference Dinner |

*Arminius Markthalle, Berlin-Moabit*






*CCO: Atrium Cross Over, Virchowweg 6 LH IM: Lecture Hall "Innere Medizin", Sauerbruchweg 2*

# Friday, September, 27th, 2019

| 9:30 am - 11:00 am | Session 7: Microbiology |
|---|---|
| *LH IM* | Chairs: Christin Koch, Alexander Grünberger |
| Anne-Kristin Kaster | Revealing Microbial Dark Matter by Targeted Cell Sorting Combined with Single Cell Genomics |
| Frank Delvigne | Outer membrane remodeling dynamics at a single cell resolution revealed by segregostat analysis |
| Jannike Lea Krause | Community diversity affects the MAIT cell response to intestinal microbiota in vitro |
| Julia Vierheilig | Flow cytometric analysis of microbial growth dynamics to determine the biostability of drinking water resources |

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

*CCO*

| 11:30am - 1:00pm | Session 8: Nanotechnology |
|---|---|
| *LH IM* | Chairs: Wolfgang Fritzsche, Ulrike Taylor |
| Katharina Landfester | Targeting of Nanocarriers |
| Maartje Bastings | The challenges and promises of DNA as programmable biomaterial |
| Sabine Baumgart | Mass cytometric application in nanotoxicology |
| Oskar Hübner | Luminescence lifetime encoding for flow cytometry with quantum-dot-encoded beads |

| | |
|---|---|
| 1:00 pm - 1:30 pm | Farewell/Snacks |

*CCO*

*CCO: Atrium Cross Over, Virchowweg 6 LH IM: Lecture Hall "Innere Medizin", Sauerbruchweg 2*

# German Society for Cytometry (DGfZ)

DGfZ
Deutsche Gesellschaft für Zytometrie

The Society of Cytometry (Gesellschaft für Zytometrie, GZ) was founded in 1989 in Heidelberg (Germany) by the Foundation Council represented by Ceses Cornelisse, Georg Feichter, Wolfgang Goehde, Klaus Goerttler, Holger Hoehn, Andreas Radbruch, Peter Schwarzmann, and Günter Valet. An association was born dedicated to provide an interdisciplinary platform for interested scientists in the field of flow and image cytometry. Founding and current members are scientists whose personal scientific development was and is still closely interlinked with the development of cytometric technologies in Europe.



**www.dgfz.org**

# Organizers Annual Meeting 2019, Berlin

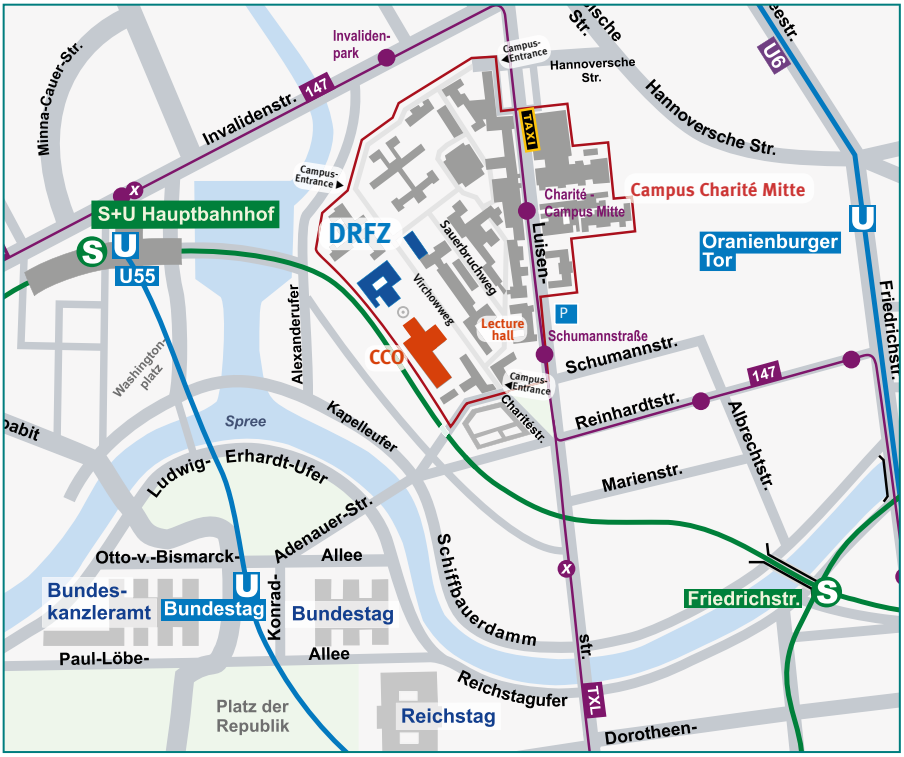
**Program Chairs**
Anja Hauser
Raluca Niesner

**Program Committee**
Thomas Bauer
Elmar Endl
Wolfgang Fritzsche
Alexander Grünberger
Anja Hauser
Christin Koch
Thomas Kroneis
Christian Kukat
Desiree Kunkel
Henrik Mei
Raluca Niesner
Philipp Rosendahl
Frank A. Schildberg
Stephan Schmid
Steffen Schmitt
Frank Schmidt
Ulrike Taylor
Gergely Toldi
Torsten Viergutz

**Local Organizing Committee**
Anja Hauser
Raluca Niesner
Ute Hoffmann, DRFZ
Jacqueline Hirscher, ScienceEvents/DRFZ

**Local Assistance**
Sandy Bauherr
Alexander Fiedler
Robert Günther
Ralf Köhler
Ruth Leben
Peggy Mex
Anna Pascual Reguant
Asylkhan Rakhymzhan
Toni Sempert
Ralf Uecker
Carolin Ulbricht

# Sitemap meeting venue




Charité – Universitätsmedizin Berlin
Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany

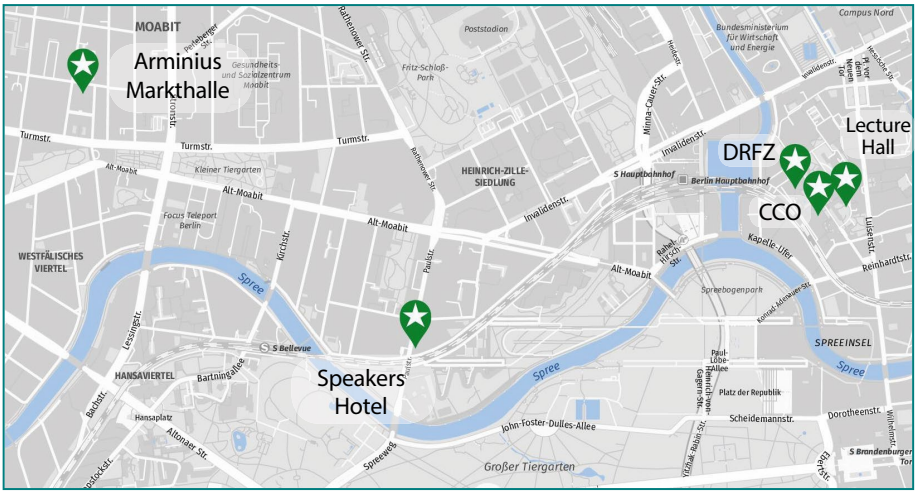
Campus addresses:

| | |
|---|---|
| CCO: | (Charité CrossOver) Virchowweg 6 |
| DRFZ: | (Deutsches Rheuma-Forschungszentrum Berlin) Virchowweg 12 |
| LH IM | Lecture Hall "Innere Medizin", Sauerbruchweg 2 |

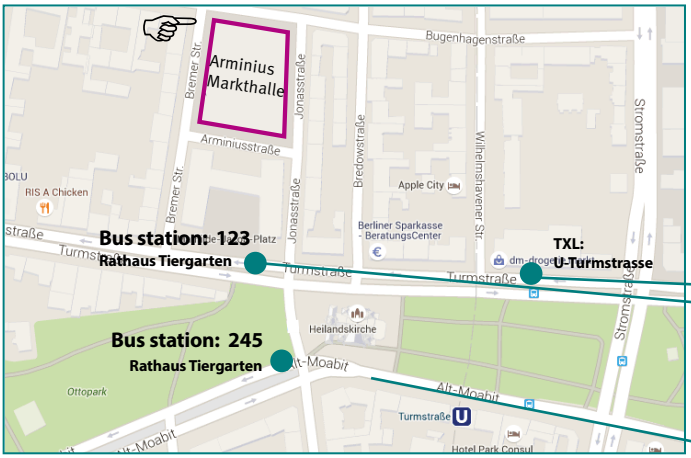
# Conference dinner

**Date: on Thursday, September 26 , 2019**

To get to the conference dinner venue, please take the bus line “123” from “Hauptbahnhof” to “Rathaus Tiergarten” or the TXL to U-Turmstraße or the Bus 245 to Rathaus Tiergarten.
Then it is just a short walk to the Arminius Markthalle. Entry is on the back side of the Markthalle.




Entrance Arminius Markthalle Bugenhagenstraße 18-34, backside of the Markthalle!

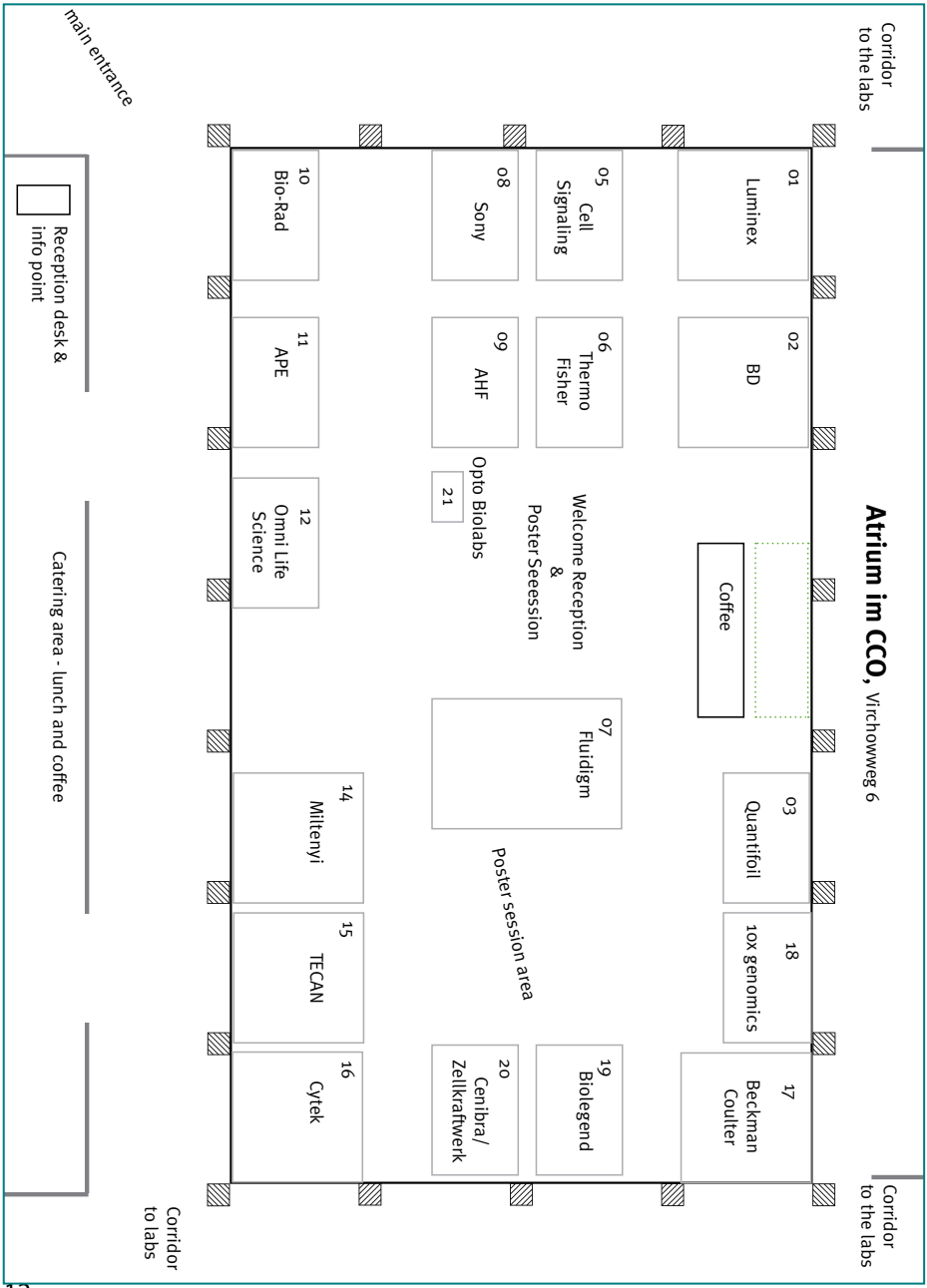



All public transport from / to main station Hauptbahnhof

**Bus: TXL**
**Bus: 123**

**Bus: 245**

# Industry exhibition



# Wednesday, September 25th, 2019

**9:00am - 11:00am**

## Tutorials

*Location: DRFZ*

### Tutorial 1: Flow Cytometry

*Location: DRFZ Seminar room 1+2*

Chair: Toralf Kaiser, Claudia Giesecke-Thiel

Co-Chair: Konrad von Volkmann, Sjoerd Visser

**Flow Cytometry -Instrument Characterization using an LED pulser**

The aim of this tutorial is to learn the practical use of an LED pulser (quantiFlash) in combination with the measurement of beads to perform flow cytometer characterization. The tutorial will cover the following topics:

**1. Presentation (9:00 – 9:30)**

- theoretical background of the LED pulser method for instrument characterization (for further information please see doi.org/10.1002/cyto.a.23250)
- data interpretation

**2. Hands-On (9:30-10:30)**

- How to use the quantiFlash
- Measurements of instrument background, SNR / DNR, and peak2 beads
- Data analysis, determination of operation point PMT voltages

**3. Data evaluation/ interpretation (10:30-11:00)**

- Discussion on the interpretation of the results (e.g. how to find the optimal PMT voltage, how to use the results in context to an experiment, limitations, problem fixing e.g. how to find a faulty PMT)
- Available software tools

### Tutorial 2: Image Analysis

*Location: DRFZ Seminar room 1st floor*

Chair: Ralf Köhler

### Tutorial 3: Publication in Cytometry A

*Location: DRFZ Seminar room 3*

Chair: Attila Tarnok

11:15am - 12:15pm

Open to public

# Fluidigm-Satellite Symposium

*Location: LM IM*

**High-Dimensional Immune Monitoring Simplified**
Easily quantify 37 immune subsets from PBMC or whole blood using mass cytometry

**Speaker 1: Jennifer Frahm**
*PhD (Field Application Specialist, Fluidigm)*

## Introduction to CyTOF technology

**Speaker 2: Lorinda Turner**

*PhD (Research Associate, Department of Medicine, University of Cambridge)*

## Using Deep Immunophenotyping in a proof-of-concept trial of a novel immunotherapy in depression

**Abstract:**
The Maxpar® Direct™ Immune Profiling System is the first complete sample-to-answer solution for high dimensional immune profiling of human PBMC and whole blood. Using only a single assay tube, you can easily quantify 37 immune subsets in every precious sample. In this presentation, Dr. Lorinda Turner from the University of Cambridge will explain how she has implemented high-dimensional mass cytometry to characterize the peripheral immune landscape of patients with depression. Together with gene expression and C-reactive protein measurements, these data have informed the design of the first immune-stratified clinical trial of a novel centrally-acting immunotherapy for major depressive disorder. She will discuss the performance of the Maxpar Direct Immune Profiling kit and how it will be used to assess the immune phenotype of depressed individuals in a multi-site clinical trial.

1:00-1:05 pm

### Welcome: Anja Hauser

*Location: LM IM*

## Session 1: Imaging

*Location: LM IM*

Chair: Anja Hauser
Chair: Raluca Niesner

The imaging session gives insight into cutting-edge developments and their application in the field of microscopy and imaging. In the first talk, the ISAC lecture by Prof. D. Entenberg, we will learn how technical limitations concerning intravital microscopy can be overcome by surgical engineering, and how this led to novel discoveries about the mechanisms of tumor metastasis. The second talk, given by Dr. T. Tull, will deal with the growing field of imaging mass cytometry, and how it is used to dissect B cell populations in human gut-associated lymphoid tissue in health and disease.
The third presentation, given by Dr. A. Pascual-Reguant, will feature another method of tissue multiplexing, MELC, and its application in order to analyze rare immune subsets such as innate lymphoid cells in human tissue. Finally, we will learn about real-time deformability cytometry, a tool for mechanical single cell analysis, during the talk by Dr. B. Fregin.

## ISAC-Lecture: Surgical Engineering Enables Intravital Imaging of Mechanisms of Metastasis in Primary and Secondary Sites

**David Entenberg**

*Albert Einstein College of Medicine, United States of Americat*

Metastasis is responsible for the vast majority of cancer mortality. While standard methods for studying metastasis are powerful, they have limitations in that they either do not capture the dynamics of the process, or are non-physiological and disconnected from rest of the living organism. Multiphoton microscopy of living animals (intravital imaging) can study cancer live and in real-time. However, technical challenges have limited the duration, the amount, and the locations of tissue that can be measured.

To overcome these challenges, we have employed Surgical Engineering, where we bring the skills of the surgeon into the optics laboratory, to develop Large-Volume High-Resolution Intravital Imaging (LVHR-IVI), and a new Window for High-Resolution Imaging of the murine Lung (WHRIL). These technologies expand the utility of mouse models by allowing: 1) imaging of more tissue (and in new locations) than previously possible, and 2) direct visualization of the process of metastasis in primary and secondary sites, *in vivo*, longitudinally, and at single-cell resolution.

We present these new technologies, as well as how we are using these tools to make several surprising discoveries about the process of metastasis. Namely: 1) how tumor cells access the bloodstream through doorways in the vasculature called Tumor Microenvironment of Metastasis (TMEM), 2) that metastatic dissemination is a much more efficient process than previously thought, and 3) that the process of dissemination from a primary tumor induces tumor cell stemness and dormancy. We additionally identify a small molecule inhibitor able to abrogate TMEM mediated dissemination, and conclude with published and new efforts to bring these insights directly into the clinic.

## Imaging mass cytometry as a tool to delineate B cell subsets in human gut associated lymphoid tissue

**Thomas Tull**

*Guy's Hospital, United Kingdom*

Human gut-associated lymphoid tissue (GALT) is important for host immune defence and for maintenance of homeostatic equilibrium throughout the gastrointestinal tract that is rich in antigens from the microbiota. Here we describe the use of imaging mass cytometry to delineate the microanatomical location

of B cell subsets within GALT from healthy individuals and those with inflammatory bowel disease. Marginal Zone B cells in GALT (CD27+IgM+IgD+) were localized on the mucosal aspect of the germinal centre co-aligned with their CD27-IgD+IgM+CD45RB+ precursor population and sparse naïve B cells. Class switched memory B cells formed a separate zone from the marginal zone B cells on the periphery of the lymphoid tissue extending around the T cell zone and up to the follicle associated epithelium (FAE). These data show that marginal zone are present in the gut as in the spleen and that they are microanatomically separate from class switched memory B cells. IgM only B cells coaligned with both marginal zone and memory B cells consistent with data showing that they can be part of both populations. Furthermore, imaging mass cytometry allows for accurate visualisation of germinal centre structures and indicates these are aberrant in patients with inflammatory bowel disease.
This study demonstrates the power of imaging mass cytometry for accurate tissue mapping.

## Characterization of Innate Lymphoid Cells and their microenvironment by multiplexed histology in human tissue

**Anna Pascual-Reguant[1,2]**

*Ronja Mothes[1,2], Ralf Köhler[1], Sandy Bauherr[1], Ralf Uecker[1,2], Peggy Mex[1], Daniela Hernández[1], Chiara Romagnani[1,2], Raluca A. Niesner[1,3], Anja E. Hauser[1,2]*

*[1]DRFZ, Germany; [2]Charité-Universitätsmedizin Berlin; [3]Freie Universität Berlin*

Innate lymphoid cells (ILCs) are a rare and heterogeneous group of tissue-resident immune cells lacking antigen-specific receptors. They are tissue sensors that help maintain homeostasis, mediate inflammation and promote tissue regeneration, but also fibrosis. In line with these diverse functions, recent publications point to a high degree of plasticity in the ILC population.
Little is known about ILCs' precise localization within tissues and requirements in terms of their microenvironment. A high number of markers are required for this type of analysis, but histology has been limited due to spectral resolution. To overcome this, we have established MELC (Multi epitope ligand cartography), a pipetting robot coupled to a fluorescence microscope that allows to stain more than 80 markers on a single tissue section. We are developing an analysis pipeline aiming to extract, interpret and comprehensively display and quantify multiplexed histology data. Thereby, we are characterizing the micro-anatomical localization of ILCs in human tissues to identify putative tissue niches. We hypothesize that, although ILC niches may be diverse in their cell composition in a tissue-dependent manner, they share molecular patterns that ultimately shape ILC phenotype/function.
We could identify and characterize ILCs in tonsils clustering in certain regions of interfollicular areas. These regions were enriched in activated cytotoxic T cells. Although we found a bias towards a type I phenotype, an important number of ILCs co-

expressed ILC2 and/or ILC3 markers. ILCs in the tonsil expressed high levels of IRF4. However, its expression was restricted only to those cells localized closer to the B cell follicle and T-B border. We are extending this analysis to other healthy and inflamed tissues with MELC, as a tool to identify spatial fingerprints within tissues by delivering quantitative topographic information on single cells.

## Dynamic real-time deformability cytometry: High-throughput multiparametric mechanical single cell analysis

**Bob Fregin[1,4]**

*Fabian Czerwinski[1], Doreen Biedenweg[2], Salvatore Girardo[3], Stefan Groß[2,4], Konstanze Aurich[2], Oliver Otto[1,4]*

*[1]University of Greifswald, ZIK HIKE, Greifswald, Germany; [2]University Medicine Greifswald, Greifswald, Germany; [3]Technical University of Dresden, Biotechnology Center, Center for Molecular and Cellular Bioengineering, Dresden, Germany; [4]University Medicine Greifswald, Deutsches Zentrum für Herz-Kreislauferkrankungen (DZHK), Greifswald, Germany*

In life sciences, the material properties of suspended cells have attained significance close to that of fluorescent markers but with the advantage of label-free and unbiased sample characterization. Until recently, cell rheological measurements were either limited by acquisition throughput, excessive post processing, or low-throughput real-time analysis. Real-time deformability cytometry, a label-free technique for single cell mechanical analysis with high-throughput of up to 1,000 cells / second, expanded the application of mechanical cell assays to fast on-the-fly phenotyping of large sample sizes, but has been restricted to single material parameters as the Young's modulus.

Dynamic RT-DC (dRT-DC) overcomes this limitation and is capable to capture full viscoelastic properties of up to 100 suspended cells / 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 to disentangle 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.

Dynamic RT-DC was performed on a precursor myeloid cell line HL60 as well as on primary blood cells. We show for the cell line that our method is capable to monitor changes in the apparent Young's modulus and viscosity after actin depolymerization. We use these findings for a first rheological comparison between erythrocytes, granulocytes and peripheral blood mononucleated cells (PBMCs) in a single experimental assay. We demonstrate that granulocytes and PBMCs have a significantly increased Young's modulus as well as viscosity compared to red blood cells and that our method is capable of a label-free discrimination of B- and CD4+ T-lymphocytes.

# Session 2: Product Slam

*Location: LH IM*

Chair: Elmar Endl
Chair: Thomas Bauer

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

# Session 3: Cutting Edge

*Location: LH IM*

Chair: Henrik Mei
Chair: Asylkhan Rakhymzhan

Cutting-edge technologies provide ever growing insight into biology of cellular systems and help making important discoveries. The last years have not only witnessed stunning developments in laboratory techniques that permit studies of single cells - in suspension or in their native tissue environments - at unprecedented dimensionality and accuracy, but likewise the establishment of computational methods for analyzing complex data as parts of routine workflows in growing number of labs. This year's cutting-edge session focuses on ultra-deep, 3-dimensional tissue imaging, and on computational mining of cytometric data, and their application in life sciences.

## Machine learning challenges for single-cell biology

**Yvan Saeys**

*VIB - Ghent University, Department of Applied Mathematics, Computer Science and Statistics, Belgium*

Technological advances in single-cell research are creating bigger, more diverse, and more complex datasets that shed light on diverse aspects of cells, tissues and organisms. To make sense of these increasingly complex datasets, machine learning techniques have been developed to tackle a wide range of tasks that can be performed using single-cell data, including visualization techniques to study cellular heterogeneity, identifying cell types and states and their transitions (trajectories), and modeling intercellular communication.
In this talk I will present some recent work to better study the underlying dynamics of cellular processes. In the first part of the talk I will present some recent research in the area of modeling cell developmental dynamics, using trajectory inference techniques. We recently performed a large-scale benchmarking study that provides novel guidelines on how to optimally use trajectory inference methods for single-cell data. In the second part of the talk I will present NicheNet, a new algorithm to study intercellular communication between neighbouring cells in a niche enivornment (e.g. a tissue) from single-cell data. For both parts of the talk I will present ample case studies, mostly in the field of immunology, that show how these methods can be used in practice.

## Ultra-deep tissue imaging by three-photon laser-scanning microscopy

**Raluca Niesner**

*FU Berlin and DRFZ, Berlin, Germany*

Developed three decades ago, two-photon laser-scanning microscopy led to a breakthrough in imaging cells in living organisms due to the advantages provided by the non-linear nature of two-photon excitation of fluorescence markers combined with the application of far-red and near infrared lasers. In order to achieve effective two-photon excitation, we routinely use a combination of Ti:Sa and OPO laser sources for *in vivo* imaging of different cells of organs of the immune system, expressing various fluorescent markers from blue to far-red and infrared fluorescence proteins. Although two-photon microscopy allows deep tissue imaging, it yields relatively poor resolution and low penetration depth when used to image through optically dense tissues such as the bone. For this reason, three-photon microscopy represents a better approach that increases the imaging depth in such tissues because of weaker scattering at higher excitation wavelengths and better contrast due to higher-order of nonlinear excitation.

Here, we demonstrate three-photon imaging of different transgenic mouse organs, focusing on bone and bone marrow, and compare these results with the two-photon microscopy approach. We also focus on the great potential of label-free imaging techniques by detecting SHG and THG of different tissue structures. To enable three-photon excitation of green and red fluorescent proteins along with third-harmonic generation, a new optical parametric amplifier (OPA) at 2MHz or 1 MHz repetition rates is used. Particular interest was invested to characterize OPA laser pulses by measuring pulse widths, lateral and axial resolution. The demonstrated technique will open a new horizon in intravital deep imaging of highly scattering tissues.

## Co-registered spectral optical coherence tomography and two-photon microscopy for multimodal deep-tissue imaging in adult mice

**Asylkhan Rakhymzhan**[1]

*Lucie Reuter[1], Raphael Raspe[2,4], Daniel Bremer[1], Robert Günther[1,2], Ruth Leben[1], Judith Heidelin[3], Volker Andresen[3], Heinrich Spiecker[3], Anja Hauser[2,4], Helena Radbruch[5], Gereon Hüttmann[6], Hinnerk Schulz-Hildebrandt[6], Raluca Niesner[1,7]*

*[1]Biophysical Analytics, DRFZ, Berlin, Germany; [2]Immundynamics, DRFZ, Berlin, Germany; [3]LaVision BioTec, a Miltenyi Biotec Company, Bielefeld, Germany; [4]Immundynamics and Intravital Microscopy, Charité Berlin, Germany; [5]Institute for Neuropathology, Charité Berlin, Germany; [6]Medical Laser Center, University of Lübeck, Germany; [7]Dynamic and Functional in vivo Imaging, Veterinary medicine, Freie Universität Berlin, Germany*

Intravital two-photon microscopy (2PM) brought in the last decades unique insight into the mechanisms of immune system dynamics since it enables monitoring of cellular motility and communication of complex systems, in genuine environment. Optical coherence tomography (OCT), with broad application both in research and clinics, is a non-invasive diagnostic method which allows monitoring morphologic changes over large tissue regions. Hence, OCT represents a bridge from bench to bed-side. Here we developed a combined multimodal technology to achieve for the first time co-registered OCT, 2PM and second harmonic generation (SHG) imaging over large volumes (1x1x0.3 $mm^3$) of the paw, spleen, lymph node and femur in mice. We found that processes of resident macrophages in the tendons of the hind paw continuously probe the tissue following the extracellular matrix structures revealed by OCT and SHG. The new design of the combined OCT and 2PM approach opens new roads for multimodal *in vivo* imaging correlated with dynamic information of various, hardly accessible organs in adult mice.

# Mass cytometry combined with computational data mining reveals a multifactorial immune cell signature of active rheumatoid arthritis

**Axel Ronald Schulz**[1]

*Tyler Burns[1], Silke Stanislawiak[1], Sabine Baumgart[1], Vera Bockhorn[1], Julia Patermann[2], Sandra Burger[2], Andreas Krause[2], Andreas Grützkau[1], Henrik E. Mei[1]*
*[1]Mass Cytometry Lab, DRFZ, Germany; Immanuel Krankenhaus Berlin, Klinik für Innere Medizin, Abteilung Rheumatologie und Klinische Immunologie, Berlin, Germany*

Innate and adaptive immune mechanisms drive the pathogenesis of rheumatoid arthritis (RA) and are targets of approved therapies. However, not all patients can be appropriately treated, which defines the need for additional therapeutic concepts combined with personalized treatment. A systematic assessment of immune cell dysregulation in the patients` blood that may provide insight into common and individual immune pathology features of active RA and reveal treatment options.

We here employed 44-dimensional mass cytometry to deeply profile PBMC in 35 patients with active RA vs. 31 age/gender-matched controls, permitting the automated identification of 60 global PBMC, 80 T cell, and 50 B cell populations by a nested FlowSOM clustering approach.

Active RA was characterized by diminished frequencies of MAIT and gd T cell, IgA+ and IgM+ memory B cell and plasmablast clusters, while the frequency of classical CD14highCD16low monocytes were significantly increased (MWU test, BH-adjusted p-values, $p<0.05$). While MAIT and gd T cells frequencies were inversely correlated with serum CRP ($r=-0.55$ and $-0.56$, $p<0.001$), IgA+ memory B cells inversely correlated with DAS28 values ($r=-0.34$, $p=0.04$), suggesting that at least some components of the RA signature are associated with disease activity. Notably, the vast majority of differentially abundant T and B cell clusters were memory or effector cells, underpinning the impact of antigen-dependent lymphocyte differentiation for the immunological fingerprint of active RA. Furthermore, computational data mining by Citrus and CellCNN consistently revealed significantly lower detection of the inflammatory chemokine receptor CXCR3 in RA patients across different T, B and NK cell subsets.

In this study, we established a multi-component immune cell fingerprint of active RA featuring aberrations of innate and adaptive immune cells. This immune cell reference map of RA will serve for comparison with data from other autoimmune diseases and longitudinal profiling of patients during therapy.

Open to public

6:00 pm - 7:00 pm

## Keynote Lecture

*Location: LH IM*

Chair: Andreas Radbruch

Prof. Petter Brodin is the director of the Swedish National Mass Cytometry Facility and heads Clinical Pediatrics, at the Dept. of Women's and Children's Health and is Associated Professor of Immunology at the Karolinska Institutet, Stockholm, Sweden. He investigates the variability of the human immune system employing various cutting-edge OMICS and single-cell technologies including mass cytometry and computational data analysis tools. In particular his research is devoted to the development and the shaping of the immune system in newborns. He advocates for monitoring the immune system in clinical states for improved understanding, diagnostics and treatments of immune-mediated diseases.

### Systems-level analysis of immune development early in life

**Petter Brodin**

*Science for Life Lab, Sweden*

Epidemiological data suggests that early life exposures are key determinants of immune mediated disease later in life. Young children are also susceptible to infections, warranting more analyses of immune system development early in life. Such analyses have mostly been performed in mouse models or human cord blood samples, but these cannot account for the complex environmental exposures influencing human newborns after birth. We have performed a systems-level analysis of newborn immune system development and uncovered drastic developmental changes, triggered by environmental exposures, and following a shared stereotypic pattern. Here I will describe our latest results in our pursuit of understanding how early-life environmental exposures shape human immune systems, how tolerance to colonizing microbes is established and the functional capacity and infectious disease susceptibility is determined in human newborns.

7:00pm - 10:30pm

## Welcome Reception at Industry Exhibition

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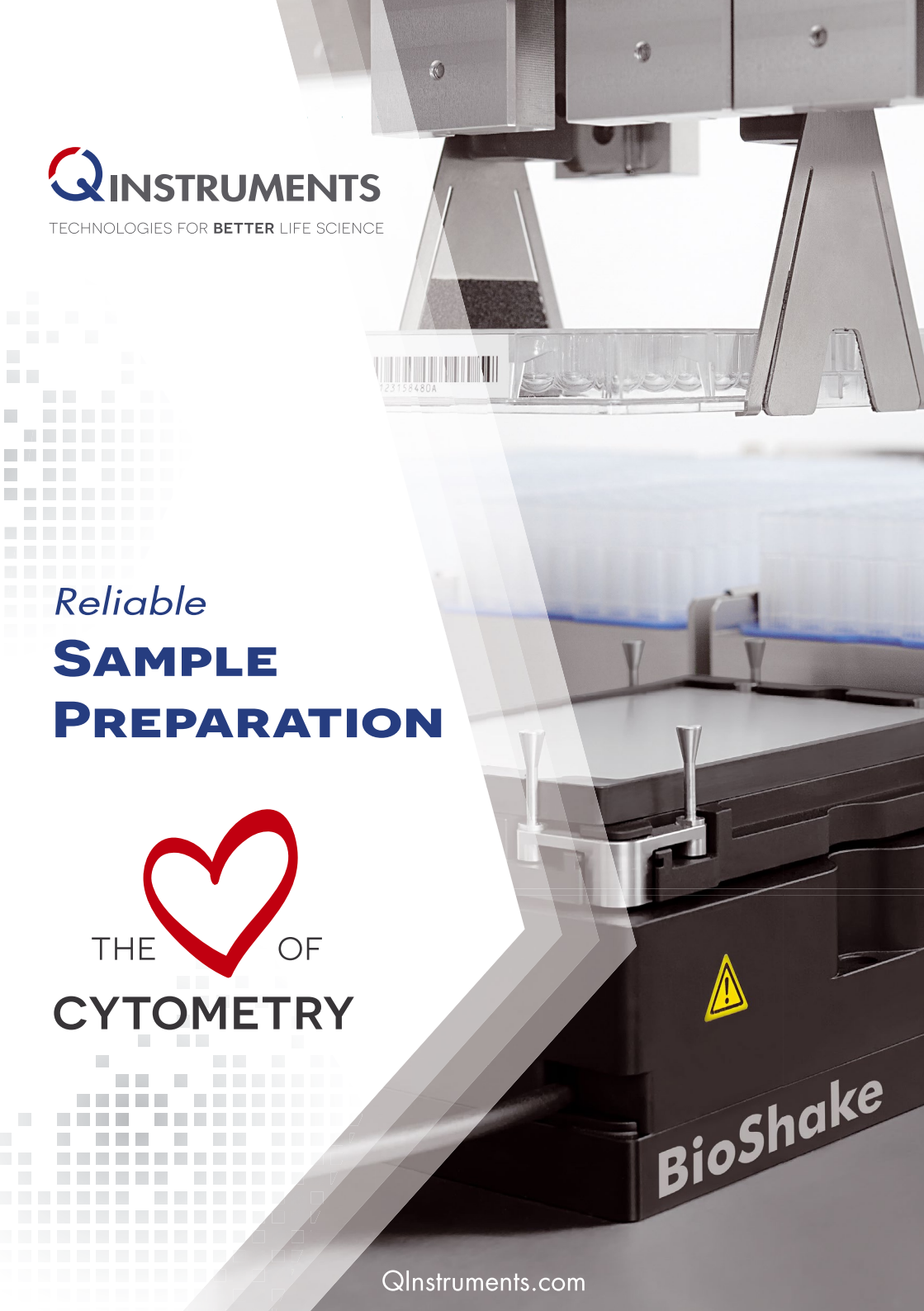


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8:00 pm - 10:00 pm

# Core Facility Networking Event

*Location: DRFZ Seminar room 1*

Chair: Desiree Kunkel
Chair: Steffen Schmitt

This event should be an opportunity to meet and share your experiences and challenges working in a core facility. We will have some short presentations and hopefully lots of ideas to discuss afterwards in an informal atmosphere among colleagues. We hope to spend a wonderful evening with you at the DGfZ meeting 2019 in Berlin.

## Modified collection devices facilitate sample handling on BD FACSAria cell sorter

**Steffen Schmitt**

*German Cancer Research Center (DKFZ)*

Dealing with small and precious cell material is a frequent requirement in cell sorting experiments. Some instrument modifications will be discussed to improve the handling of collection devices of FACSAria cell sorters, especially regarding single cell analysis in plates.

## Many shades of single-cell sequencing - a guide to implement the technology in Flow Cores

**Vladimir Benes**

*EMBL, Germany*

Single-cell sequencing applications are a hot topic for researchers and a big challenge to traditional Flow Core Facilities. Single-cell sequencing is still an art and comes with unique requirements towards downstream handling and sequencing limitations. At EMBL, we have supported single-cell sequencing approaches for years and established a solid and yet flexible platform utilising tailored approaches based on the scientific background and technical limitations to the samples. This platform entails detailed consultations from a Flow and Genomics point of view prior to any start of wet lab work and dedicated support to select the correct platform to use to generate single-cell datasets. With each additional experiment that we support we constantly learn and try to improve our guidelines and technical solutions that we make available to all our users.

# Thursday, September 26, 2019

## Session 4: Core Facility Session: The many faces of single cell analysis

*Location: LH IM*

Chair: Desiree Kunkel
Chair: Christian Kukat

Cutting edge research often depends on high-end technologies which are more and more often placed in core facilities to be shared by researchers for their various research topics. Core facilities aim to facilitate the use of these technologies by providing expert knowledge and by keeping the necessary instrumentation in good shape. The members of flow cytometry and imaging core facilities form a considerate part of the society and aim to contribute to the annual conference by presenting topics that are relevant for both, core facilities and researchers.
This year's topic will be centered around single cell analysis, different technologies that can be used and ways to combine those for even more information output.

## Regulation of single dormant hematopoietic stem cells

**Nina Cabezas Wallscheid**

*Max Planck Institute of Immunobiology and Epigenetics, Germany*

Hematopoietic stem cells (HSCs) harbor the capacity to generate a series of multipotent progenitors (MPPs) that differentiate into lineage-committed progenitors and subsequently mature cells. To explore essential HSC features, we integrated quantitative proteome, transcriptome, and methylome analyses of five FACS-sorted HSCs and MPP populations (MPP1-4) and combined these OMICs analyses to their functional potential. We have recently expanded this analysis to dormant HSCs (dHSCs) identified by label-retaining assays (Wilson et al., 2008). Rare dHSCs reside at the top of the blood hierarchy harboring the highest long-term reconstitution capacity. However, till the date the molecular identity of dHSCs, as well as the mechanism regulating maintenance and the transition out of dormancy remain unknown. We now show by single-cell RNA-seq analysis that the transition from dormancy towards cell cycle entry is achieved by a continuous and coordinated up-regulation of all major biosynthetic processes rather than a switch on/off mechanism. Although HSCs are quiescent we show that cell cycle-related genes get gradually upregulated in aHSCs indicating that aHSCs are stem cells primed towards cell cycle. Indeed, dHSCs have a delayed entry into cell cycle when compared to aHSCs as shown by single-cell tracking analysis. In this study we describe a unique G0 molecular signature that define dHSCs. Finally, we show that retinoic acid (RA) is molecularly enriched in dHSCs and is responsible for *in vivo* and *in vitro* maintenance of HSCs quiescence by sustaining low levels of ROS, translation and Myc expression. These findings have broader implications since RA is used as standard treatment in several types of cancer.

## Integrating single-cell RNA sequencing and mass cytometry to profile human microglia heterogeneity

**Roman Sankowski**

*Universitätsklinikum Freiburg, Germany*

Microglia are the resident macrophages of the mammalian brain. Having populated the brain in utero before the formation of the blood-brain-barrier these cells represent a long living self-sustained myeloid population with diverse functions. These functions comprise, among others, critical involvement in brain development, homeostasis and immune defense. It is conceivable that these functions may require specialized microglia subpopulations, especially if they have to occur simultaneously. With the recent development of single-cell based

high-throughput techniques it has become possible to profile microglia in an unbiased way. Here, we have integrated single-cell RNA sequencing and mass cytometry to acquire transcriptomic and proteomic profiles of human microglia from control brains and Glioblastoma multiforme (GBM). In control brains we found an astonishing heterogeneity that showed regional and aging-dependent phenotypes. Moreover, in GBM-affected brains we observed the occurrence of distinct context-dependent microglia subpopulations. Our findings pave the way to a cell atlas of the human brain and may contribute to novel therapeutic approaches to GBM.

## Multi-parameter Flow Cytometry of Human Gut Microbiota

**Lisa Budzinski**

*Rene Maier, Toni Sempert, Tanisha Momtaz, Pawel Durek, Gitta Anne Heinz, Toralf Kaiser, Mir-Farzin Mashreghi, Andreas Radbruch, Hyun-Dong Chang*
*German Rheumatism Research Centre (DRFZ), A Leibniz Institute, Berlin, Germany*

The human gut microbiota impacts human health. Composition and dynamics can change drastically in the context of inflammatory diseases, cancer or neurodegenerative diseases. However, it remains unclear which changes are causative for the disease or whether it could be regulated by the individual immune response. The investigation of the complex interactions and influencing factors between bacteria and the individual human requires analysis tools capable of elucidating multiple parameters at the same time. We are applying flow cytometry for the characterization of the human gut microbiota to rapidly access complexity and dynamics of bacterial populations. We aim to establish a multi-parameter panel that allows for a phenotypical discrimination of bacterial populations in order to define their crosstalk with the human immune system in greater detail. We have evaluated coating by different immunoglobulin isotypes, membrane-associated sugar moieties, quantitative DNA, RNA and protein staining as well as light scatter for the flow cytometric assessment of human stool samples of different donors singly and in combination, and determined the specificity of these stainings. From such flow cytometric data and concomitant 16S rDNA sequencing we can highlight the individual differences of bacteria-host interaction in certain donors or elucidate commonalities between multiple individuals. The correlation of these data with clinical data of the given donor can pave the way towards personalized microbiota-based medicine.

# Session 5: European Guest Session: Sweden

*Location: LH IM*

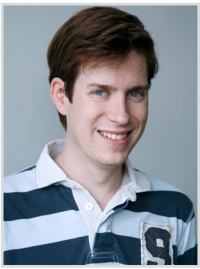
Chair: Anja Hauser
Chair: Raluca Niesner

The Science for Life Laboratory (SciLifeLab) was created in 2010 as joint research center of four host universities and collaborators around Sweden with two main nodes in Stockholm and Uppsala. Within less than a decade, it has become one of the largest molecular biology labs in Europe with more than one publication a day.
SciLifeLab forms a facility on a national level providing the infrastructure and knowledge of advanced technologies to users from any university in Sweden as well as from industry or healthcare. Collaborations with healthcare are of particular significance. SciLifeLab has a platform for clinical research aimed at developing diagnostic methodology based on DNA-sequencing and biomarkers that can be effectively translated into applied healthcare.
The research at SciLifeLab is performed within two main focus areas: health and environmental research with a strong focus on genomics, comparative genetics, proteomics, functional biology, and bioimaging. SciLifeLab has two fellowship programs for recruiting young and promising PIs on a national and international level, supporting the development and research of new technologies.
Part of the SciLifeLab mission is the education of PhD students, postdocs, investigators and other employees by offering educational courses and workshops within all Swedish universities. The courses and workshops cover knowledge and understanding about the advanced techniques and data analysis methods available at the SciLifeLab technical platforms to educate the science of the future.
All these aspects have the potency to translate research into a lasting impact on society.

# The Science for Life Laboratory in Sweden

**Peter Thul**

Introduction of the Science for Life Laboratory (SciLifeLab) in Sweden.



## The HPA Cell Atlas: An image-based subcellular map of the human proteome

**Peter Thul**

*Science for Life Laboratory Stockholm, Sweden*

The Human Protein Atlas (HPA) is an ambitious project aiming to characterize the complete human proteome using the integration of various omics-technologies, such as antibody-based imaging and transcriptomics. The HPA itself consists of six separate parts, each focusing on a particular aspect of analysis of the human proteins on different levels.
The HPA Cell Atlas shows the subcellular distribution of proteins in single cells. Subcellular distribution is an essential step in protein characterization, as it is linked to the protein's function and availability of possible interaction partners. In the Cell Atlas, we detected the subcellular distribution of proteins in their cellular context by indirect immunofluorescence (IF) using more than 14,000 antibodies from the unique antibody collection of the HPA. 12,390 human proteins were mapped to 32 subcellular structures defining the proteome of 13 major organelles. The high spatial resolution of the IF images allowed the identification of novel protein components of fine structures such as the midbody, nuclear bodies or rods and rings.
An intriguing observation was the level of multilocalization (MLP) among the proteins. MLPs are proteins, which are detected at more than one organelle, either simultaneously or at different cell states. Approximately half of the analyzed proteins were MLPs (6647), with 29% of them (1960) at three or more locations, confirming that multilocalization is a common feature of the human proteome. MLPs play a major role in increasing the functionality of the proteome and the complexity of the cell from a systems perspective.
Another key feature of the HPA Cell Atlas is the possibility to look at single cell variations. About a fifth of the analyzed proteins showed spatial or quantitative variations in the protein expression. Ongoing research demonstrates that only a subset of these variations can be explained by the cell cycle.

Furthermore, we implemented the localization data from the HPA Cell Atlas to a protein-protein interaction network and thereby improved its quality. This indicates that other network analyses could benefit comparably. Finally, the IF images have been successfully used in the training of various models for image classification, underlining the high quality of images.
The data from HPA Cell Atlas, providing the most comprehensive map of spatial proteomics, can be used to as discovery tool to unravel novel mechanisms and connections within the human proteome in health and disease.

# Swedish National Microscopy Infrastructure – Centre for Cellular Imaging, a Correlative Multimodal Imaging Facility

**Julia Fernandez-Rodriguez**

*University of Gothenburg, Sweden*

The National Microscopy Infrastructure (NMI) in Sweden has been established as a multi-location infrastructure with specialized imaging facilities (nodes) at Stockholm, Göteborg, and Umeå, with the mission to provide open access to state-of-the-art microscopy. Each NMI node has a defined operation and specialty: Super-resolution, FCS, Light-sheet and High content microscopy; Correlative Multimodal Imaging; Intravital microscopy and Correlative microscopy and Electron cryo-tomography. NMI is open to all researchers that has a need for advanced imaging in fundamental and translational biomedical research projects. NMI is also coordinating national and international knowledge exchange in microscopy.

The Centre for Cellular Imaging (CCI) – The CCI is an open-access facility into the NMI that integrates light and electron microscopy in one single multi-user facility. Our vision is that the CCI will allow users to think broadly and select the best possible instrument(s), light and/or electron microscope(s), to provide detailed answers to their scientific questions at the highest resolution. The CCI specializes in three-dimensional correlative multimodal imaging (CMI). CMI describes the sequential combination of two or more imaging techniques to gain holistic and complementary knowledge about the same sample across multiple scales, including spatial, structural, biochemical and biophysical information. It is hence particularly suited to tackle the intricate questions arising in biological and biomedical research. CMI integrates the best features of the combined imaging techniques and overcomes limitations that would be faced when applying single modalities independently. Any single microscopy modality is not sufficient to comprehensively illustrate the inner working of a cell, cellular network and organisms. Biological processes can be studied within their overall spatio-temporal context, and pathologies and diseases can be targeted down to an individual cell and underlying molecular events. Additionally, the sequential combination and application of techniques to the same specimen and region of interest also allows to validate and evaluate single-modality conclusions since each technique can provide unique information based on fundamentally different contrast mechanisms. We are committed to developing a functional systems microscopy approach, which can address new scientific questions with a multi-modal imaging strategy using only a few or many samples. Using modern cell biological tools, expertise in live cell imaging and state-of-the-art equipment and image analysis we will continue to help researchers relate structure to function and morphology to mechanism.

## Lunch & Poster Session A

# Poster Abstracts Session A

Chair: Torsten Viergutz
Chair: Raluca Niesner

### Systematic enzyme mapping of cellular metabolism by phasor-analyzed label-free NAD(P)H fluorescence lifetime imaging

**Ruth Leben[1],**

*Markus Köhler[1], Helena Radbruch[2], Anja E. Hauser[3], Raluca Niesner[1,4]*
*[1]Biophysical Analytics, German Rheumatism Research Center (DRFZ), Berlin; [2]Institute of Neuropathology, Charité – Universitätsmedizin Berlin, Berlin; [3]Immune Dynamics, German Rheumatism Research Center (DRFZ); [4]Dynamic and Functional in vivo Imaging, Free University, Berlin*

Label-free fluorescence lifetime imaging of the ubiquitous coenzymes NADH and NADPH (NAD(P)H-FLIM) is able to monitor cellular metabolism in living cells and tissues, since the fluorescence lifetime elongates significantly by several nanoseconds when the coenzyme ($\tau$free = 450 ps) binds to an metabolic enzyme ( $\tau$enzyme ≈2000 ps). This has been already applied to study metabolic changes both under physiologic and pathologic conditions [1].

However, due to the complex distribution of NAD(P)H-dependent enzymes in cells, which composition continuously changes over time, a thorough interpretation of NAD(P)H-FLIM results, resolving the contribution of various enzymes to the overall metabolic activity, remains difficult.

To facilitate the interpretation of FLIM data we transferred the time-domain raw data into the virtual phase domain by calculating the discrete Fourier Transformation numerically (phasor approach [2]) and developed a systematic framework to map both the general cellular activity and the distribution of highly abundant NAD(P)H-dependent enzymes in cells.

We benchmarked our framework by means of homogeneous mixtures of NAD(P)H and pure enzymes. These data revealed the fact that the SNR value has a strong impact on the quality of the phasor data and their interpretation. Whereas the reference enzyme system cannot be changed, the SNR values in an image can be increased by increasing NAD(P)H and/or enzyme concentration or the excitation power, by smoothing the raw data in time or in space or by increasing the image acquisition time.

Considering that we are able to identify those active metabolic enzymes with subcellular resolution at a certain time point. In order to exemplary verify the performance of our approach we applied it on a stromal-like cell line and identified a different group of enzymes being active in the cell nuclei as compared to the cytoplasm.

## Lens characterization and image correction for intravital micro-endoscopy

**Alexander Ferdinand Fiedler**

*Raluca Niesner, DRFZ, Germany*

BACKGROUND

With our own intravital two-photon fluorescence micro-endoscopy based imaging approach, we are able to investigate complex physiological and pathological cell processes inside the bone marrow over months. The integrated Gradient Refractive Index lenses have intrinsic optical aberrations, which have to be corrected.

AIM

We aim to develop an algorithm to correct for optical confounding factors, especially chromatic and wave-front aberrations.

METHODS

The optical properties of the lenses and optical aberrations were quantified by imaging a reference grid with known features and agarose-gel containing fluorescing (200 nm) beads. In order to correct for the chromatic and wave-front aberrations an image post-processing algorithm was designed consisting of three main steps. The first step corrects for the axial image field curvature by cropping the image slices of a z-stack with specific concentric masks and shifting them to the correct z-position. Sequentially, the second step corrects for image distortions in the xy-plane by performing a barrel-shaped deformation based on cubic b-splines. The third step corrects for inhomogeneous illumination, back-ground signal, and smoothes the fluorescence signal by using a morphological white top-hat filter.

CONCLUSIONS

An assessment of image quality was done by comparing the feature extraction from the raw and corrected images. The extraction of cellular and nuclear structures of cells involved in the immune response to an injury (drill hole) as well as newly forming vessel structures (neoangiogenesis) inside the femoral bone marrow exhibited better results in the corrected images. Consequently, due to increased contrast as well as decreased noise and distortions, image quality was significantly increased.

## Calibration Beads for Characterization of the Performance of Fluorescence-based High-Throughput and Imaging devices

**Katrin Hoffmann[1]**

*Nithiya Nirmalananthan-Budau[1], Marc Wegmann[1,2], Ute Resch-Genger[1]*
*[1]Bundesanstalt für Materialforschung und -prüfung (BAM), Germany; [2]Medipan GmbH*

In all fluorescence-based techniques, the measured signals contain not only sample-related but also instrument-specific contributions, which limit the direct comparison of fluorescence data obtained e.g. on different devices or at different times and often hamper quantification. To rule out instrumentation as major source of variability

of emission data, accepted fluorescence standards and procedures for the control of instrument specifications and long-term performance are required. For flow cytometry (FCM), a broad variety of fluorophore-stained polymer beads differing in emission wavelength and intensity is available for the testing of the alignment, sensitivity, and other parameters of FCM. These calibration tools are intended to facilitate the assessment of instrument performance to ensure reliable measurements and to improve the comparability of FCM experiments.

As a step towards an improved comparability of fluorescence data, with special emphasis on spectroscopic methods measuring nano- and micrometer-sized fluorescent objects, we are currently developing a set of fluorescent polystyrene (PS) beads loaded with luminophores from the certified BAM-Kit "Spectral fluorescent standards", initially developed for the calibration of fluorescence spectrometers. Here, we present first results from studies of these fluorophore-loaded polymer beads. Moreover, new beads are made to supplement this kit by encapsulating near-infrared (NIR)-emissive luminophores in PS beads to cover the UV/VIS, and NIR wavelength range.

These beads are designed for calibration of flow cytometers and other fluorescence imaging systems to meet the increasing demand for reliable and comparable fluorescence data especially in strongly regulated areas like e.g. medical diagnostics.

## In situ-characterization of ILC niches during homeostasis and tissue regeneration by using multiplexed quantitative histology

**Sandy Bauherr[1,2]**

*Anna Pascual-Reguant[1,2], Ralf Köhler[1], Ronja Mothes[2], Robert Günther[1], Ralf Uecker[1,2], Gino Gulamhussene[4], Karolin Holzwarth[1], Raluca A. Niesner[1,3], Anja E. Hauser[1,2]*

*[1]DRFZ, Germany; [2]Charité – Universitätsmedizin Berlin, Germany; [3]Freie Universität Berlin; [4]Otto-von-Guericke-Universität Magdeburg, Germany*

Functionality of the BM is based on its complex microstructure, also known as niches. Different cell types and cellular factors form niches and, thus, provide perfect ancillary conditions for the development, survival and functions of respective cell populations. Homeostasis can be affected by mechanical stress that leads to destroyed tissue integrity and vascularization. The forced destruction of cells results in the release of damage-associated factors (DAMPS), provoking local inflammation. Hence, local stromal network undergoes strong rearrangement, resident immune cells are activated and migrating immune cells are triggered to join the injury site. All components of this machinery aim to restore homeostasis.

By using a bone injury model, where mechanical stress provoked through a drill hole (DH) in the cortex of the murine femur, we aim to investigate regenerative processes taking place at the injury site after different time points and characterize the interplay of cell types involved in healing. The robot-based multiplex microscopy technique MELC perfectly suits multiparameter analysis with spatial information. This allows the characterization of the cell types interacting in homeostatic tissue, during acute local inflammation and during regeneration.

We detected clustered ST2+/IL-33R+ cells near

the DH area after 72 h. Partly, those cells could be identified as ILCs, fitting to publications describing the presence (especially ST2-expressing ILC2s) after injury. During automated analysis, we have repeatedly detected CD49a+ ST2+ NKp46+ ILCs at 72 h p. DH, a phenotype comprising markers of the three lineages ILC1, ILC2, and ILC3, respectively, and to our knowledge, has not been described until now. Interestingly, some of those ILCs showing a mixed phenotype also expressed MHCII and localized together with CD3+ CD90.2+ T cells. It seems as ILCs possess cellular plasticity, and might not be strictly separated into three clear subgroups, but might rather react and adapt to their environmental milieu.

## Development of a commercially available flow cytometric Diagnostic Kit to test for latent Tuberculosis

**Annett Howe**[1]

*John Zaunders*[2], *Jose Mario Morgado Tenera*[3], *Anthony Dominic Kelleher*[1,2]

[1]*The Kirby Institute, UNSW, Sydney, Australia;* [2]*St Vincent's Hospital Sydney;* [3]*Cytognos, Spain*

**Background:**

Tuberculosis (TB) is a massive global health problem, the leading cause of death from infection worldwide. Currently, the gold standard for detection of latent TB is the Tuberculin Skin Test (TST/Mantoux test) and detection of interferon-gamma produced by CD4+ T lymphocytes exposed to TB antigens *in vitro*. Both have limitations.

We are developing an In Vitro Diagnostic (IVD) certified, TB-specific version of the Act-T4 Cell™ kit, that robustly gives clear cut positive and negative discrimination of latent TB infection via Flow Cytometry, decreasing the need for repeat testing and subjective clinical interpretation.

**Methods:**

Sodium heparin anti-coagulated whole blood from known positive TB patients was incubated with pools of peptides (15mer) representing TB-specific antigens (ESAT-6, CFP-10) for 44-48hours and measured co-expression of CD25 and CD134 (OX40) on CD4+ T cells via 4-colour flow cytometry. The formulation of the TB peptides was optimized to maximize their ease of use, especially with respect to peptide solubility into whole blood. Incubation time, transport conditions and peptide stability were optimized.

**Results:**

Our data shows that pooled peptides can be directly diluted into culture media to give clear positive results on the OX40 assay, equivalent to those dissolved in minimal concentrations of DMSO, with optimal concentration of 10ug per peptide/test. Assay set up can be delayed up to 24hrs (4.91% CD25+/CD134+) while yielding a similar result to that set up ≤ 6hrs after taking blood (4.82% CD25+/CD134+). Incubation in a water bath or oven with CO2 Independent Media instead of Iscove's Modified Dulbecco's Medium in a standard 37°C CO2 Incubator does not diminish results: 5.9% versus 5.84% CD25+/CD134+ respectively.

**Conclusion:**

We show that our flow-based kit is a simple robust assay that may facilitate the diagnosis of TB in developing countries. This potentially has a global impact especially in countries where HIV/TB co-infection is prevalent.

## Quantifying Minimal Residual Disease in Flow Cytometry Measurements of Acute Lymphoblastic Leukemia Patients Automatically

**Markus Diem**[1]

*Michael Reiter*[1]*, Florian Kleber*[1]*, Michael Dworzak*[2]
*[1]TU Wien, Austria; [2]Children's Cancer Research Institute, Austria*

Determining the Minimal Residual Disease (MRD) during therapy of leukemia patients allows for monitoring the curing process and adjusting treatment if needed. The MRD is quantified three times alongside therapy of Acute Lymphoblastic Leukemia patients using Flow Cytometry measurements of the bone marrow. In order to quantify the MRD, blasts have to be found in the Flow Cytometry Measurements (FCM) and compared to all viable cells. This process is manually performed by gating different cell populations with flow analysis software. We propose a fully automated gating procedure that uses machine learning to identify blast populations in FCM. This allows for an objective MRD quantification which is not dependent on operator skill level and expertise.

For each labelled population in a training set, we compute Gaussian Mixture Models (GMM) using Expectation Maximization. In order to label blasts in an unknown sample, a GMM is estimated for all cells in the sample. We then find samples with similar phenotypes in the training set by comparing the GMMs. Finally, the best matching samples from the training set are combined to automatically determine the blast population of an unseen sample.
For testing, FCM from three different labs containing data of more than 300 patients under treatment were collected. The data is recorded with different Flow Cytometers and different antibody panels, which allows for testing cross-system performance. Tests show that the best performance is gained if the same system is used (minimal median F-Score is 0.92) but that cross-system performance remains high with an F-score of 0.85.

## High-throughput Mechanophenotyping of Single Platelet Activation Induced by Bacterial Proteins

**Lea Lenkeit**

*Oliver Otto, Raghavendra Palankar*
*Universitätsmedizin Greifswald, Germany*

**Background**
Gram-negative and Gram-positive bacteria and their secreted proteins are capable of interacting directly and indirectly (through serum proteins) with human platelets. Previously, we have identified several proteins from Staphylococcus aureus capable of activating platelets.1 These proteins belong to both microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) as well as secretable expanded repertoire adhesive molecules (SERAMs).

**Aim**
Here we aimed to quantify changes in viscoelastic properties of single platelets induced by S.aureus proteins using real-time 1D-imaging fluorescence and deformability cytometry (RT-FDC) which is capable of screening > 1000 cells/ second.2

**Methods**
50 µL of washed platelets (300,000/µL) labelled with anti-CD41-FITC incubated for 10 minutes with S.aureus proteins: phospholipase C (Plc, 4 µM), major autolysin (AtlA-1, 2 µM), extracellular adherence protein (EapD3D4 domain, 4 µM), chemotaxis inhibitory protein of S. aureus (CHIPS,4 µM) and formyl peptide receptor-like 1 inhibitory protein (FLIPr, 4 µM). Using RT-FDC2, platelet activation was detected by anti-CD62P-AlexaFluor 647 in combination with platelet deformation in microfluidic chips with a cross-section of 15µm (Flic15).

**Summary**
RT-FDC allows for high-throughput biomechancial fingerprinting of changes in platelet viscoelastic properties based on their deformation and activation status.
Differences in viscoelastic property of platelets in the presence of bacterial proteins reveal heterogenity in platelet activation response in individial donors.

## Investigating the human intestinal microbiota by flow cytometry

**Tanisha Momtaz[1]**

*Toni Sempert[1], Lisa Budzinski[1], René Maier[1], Gitta Anne Heinz[2], Pawel Durek[1], Mir-Farzin Mashreghi[2], Hyun-Dong Chang[1]*
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The bacterial community colonizing the human gastrointestinal tract has become of major scientific interest as an important factor for human health and disease. Drastic changes in the microbiota composition, termed dysbiosis, determined by 16 SrDNA sequencing have been associated with cancer, inflammatory, metabolic and neurodegenerative diseases. Here, we use flow cytometry to investigate and characterize the human intestinal microbiota on the single-cell level. For logistic purposes, we have developed a storage and processing protocol that maintains essential features of the intestinal microbiota, i.e. composition and endogenous antibody-coating, is suitable for the development of additional microbiota markers and for accompanying clinical studies. We are currently establishing baseline data by characterizing the intestinal microbiota of a large cohort of healthy humans using flow cytometric fingerprinting based on light scatter, DNA and protein content, surface immunoglobulin coating and sugar moiety-dependent binding of different lectins, in conjunction with 16S rDNA profiling. The cohort data will contribute to our understanding of individual and common single-cell features of the intestinal microbiota of healthy individuals. This will facilitate the identification of disease-related changes in the microbiota and to isolate by preparative cell sorting bacterial populations of interest.

# Developing a CAR-cell efficacy test using chip-cytometry

**Yvonne Wolff**

*Zellkraftwerk GmbH, Germany*

In the fight against cancer, the use of immune effector cells that are genetically engineered to express chimeric antigen receptors (CAR) becomes increasingly important. While good results have already been obtained in the treatment of leukemias, the effects on solid tumours are less satisfactory. A reproducible and easy to use assay would be of great help to determine the efficacy of the CAR-construct and of the CAR-cell itself.

The here presented project intends to develop such an assay based on a chip-cytometry platform provided by Zellkraftwerk GmbH. This method allows almost unlimited marker analysis of cell surface antigens as well as intracellular molecules due to multiple bleaching and staining cycles. In addition, cell fixation allows long-term storage to retain and re-analyse the probes.

The assay will be based on a co-culture of CAR-cells with corresponding target cells on a cytometry chip. After fixation surface antigens of both the CAR cells and target cells will be identified. Furthermore, activation markers of the CAR cells as well as caspase-3 activity of the target cells will be determined to demonstrate the efficacy of CAR-cells in killing their targets.

In summary, we intend to develop a CAR-cell efficacy test based on chip-cytometry that is reproducible, easy to handle and useful for research and clinic application.

# Session 6: Klaus-Goerttler-Session incl. award ceremony

Session Chair: Philipp Rosendahl

**Klaus-Goerttler-Prize**
Since 1996 the DGfZ awards a younger scientist with the so called Klaus-Goerttler-Prize, which goes along with prize money of 1000 €.
The award is named after Prof. Klaus Goerttler, he was a pathologist and trend-setting member of the foundation board of our society.
The Prize is dedicated to award a younger scientist for a scientific work out of the wide field of Cytometry which appears outstanding both in scientific quality and innovation as well as presentation and layout. The scientific work reflects a (almost) finished scientific graduation (Diploma, Bachelor, Ph.D. or an equivalent work) and was selected out of several submissions by the council board of the society.

# Goerttler-Awardee: Neutral mechanisms and niche differentiation in steady-state insular microbial communities revealed by single cell analysis

**Zishu Liu[1]**

*Nicolas Cichocki[1], Thomas Hübschmann[1], Christine Süring[1], Irina Dana Ofiţeru[2], William T. Sloan[3], Volker Grimm[4], Susann Müller[1]*

*[1]Department of Environmental Microbiology, Helmholtz Centre for Environmental Research GmbH - UFZ, Permoserstr. 15, 04318 Leipzig, Germany; [2]School of Engineering, Newcastle University, Newcastle upon Tyne NE1 7RU, United Kingdom; [3]Department of Civil Engineering, University of Glasgow, Glasgow G12 8LT, United Kingdom; [4]Department of Ecosystems Analysis, Helmholtz Centre for Environmental Research GmbH - UFZ, Permoserstr. 15, 04318 Leipzig, Germany*

In completely insular microbial communities, evolution of community structure cannot be shaped by the immigration of new members. Also, when those communities are run in steady-state, the influence of environmental factors on their assembly is reduced. Therefore, one would expect similar community structures under steady-state conditions. Yet, in parallel setups variability does occur. To reveal ecological mechanisms behind this phenomenon, five parallel reactors were studied at the single-cell level for about 100 generations and community structure variations were quantified by ecological measures. Whether community variability can be controlled was tested by implementing soft temperature stressors as potential synchronizers. The low slope of the log-normal rank-order abundance curves indicated a predominance of neutral mechanisms, i.e., where species identity plays no role. Variations in abundance ranks of subcommunities and increase in inter-community pairwise β-diversity over time support this. Niche differentiation was also observed, as indicated by steeper geometric-like rank-order abundance curves and increased numbers of correlations between abiotic and biotic parameters during initial adaptation and after disturbances. Still, neutral forces dominated community assembly. Our findings suggest that complex microbial communities in insular steady-state environments can be difficult to synchronize and maintained in their original or desired structure as they are non-equilibrium systems.

## Label-free multiparametric flow cytometry in virtual fluidic channels: Single cell rheology and tissue mechanics

**Oliver Otto**

*University of Greifswald, Germany*

Microfluidic chip fabrication and utilization in flow cytometry applications have made great promise of flexible measurements reducing experimental bias due limited crosscontamination. However, designing the master mold as well as replica production can be considered the time-limiting step and requires sophisticated clean-room equipment. Here, we introduce the concept of virtual fluidic channels to dynamically tune the hydrodynamic dimensions of the cuvette within seconds. Virtual channels are formed by coflowing aqueous polymer solutions at low Reynolds numbers where a stabilizing sheath confines a sample flow between immiscible liquid phases. We demonstrate that virtual channel dimensions follow a simple scaling law and can be tailored towards nearly arbitrary hydrodynamic stress distributions in real-time.

As proof-of-principle experiments we combine our technology with real-time deformability cytometry (RT-DC) as a high-throughput method for label-free analysis of single cell mechanical properties. While RT-DC has already demonstrated its relevance in basic life science research, e.g. by observing the activation of immune cells, linking immune response to underlying tissue alterations has not been possible so far. Using spheroids as a tissue model system, we show that virtual fluidic channels can be used to bridge the gap between cellular rheology and tissue mechanics.

## Analysis of biomechanical properties of hematopoietic stem and progenitor cells using real-time fluorescence and deformability cytometry

**Angela Jacobi**

*Max Planck Institute for the Science of Light and Technische Universität Dresden, Germany*

Stem cell mechanics, determined predominantly by the cell's cytoskeleton, plays an important role in different biological processes such as stem cell differentiation or migration. Several methods to measure mechanical properties of cells are currently available, but most of them are limited in the ability to screen large heterogeneous populations in a robust and efficient manner—a feature required for successful translational applications. With real-time fluorescence and deformability cytometry (RT-FDC) mechanical properties of cells in

suspension can be screened continuously at rates of up to 1,000 cells/s—similar to conventional flow cytometers—which makes it a suitable method not only for basic research but also for a clinical setting. In parallel to mechanical characterization, RT-FDC allows to measure specific molecular markers using standard fluorescence labeling. With this technology, we provide a way to characterize hematopoietic stem and progenitor cells (HSPCs) from bone marrow using RT-FDC and present a specific morpho-rheological fingerprint of HSPCs that allows to distinguish them from all other blood cell types.
4:00pm - 5:00pm

# Poster Abstracts Session B

Chair: Torsten Viergutz
Chair: Raluca Niesner

*CCO*

## Analysis of NK cell populations in human blood and bone marrow by multicolor flow cytometry

**Kristin Bieber[1]**

*Manina Günter[2], Stella E. Autenrieth[1,2]*
*[1]Core Facility Flow Cytometry (FCF), Medical Faculty, University of Tübingen; [2]Department of Internal Medicine II, University of Tübingen*

Clear identification of specific cell populations by flow cytometry is important to define the immune status of patients. The objective of this study was to develop a multi-parametric flow cytometry panel for prospective immune-profiling of high risk multiple myeloma patients in a phase II multicenter trial. We performed 12-color immunofluorescence flow cytometry on frozen human blood and bone marrow samples. We defined a multicolor flow panel that identifies major NK cell populations. These include the three NK cell subsets: CD57-CD56hi NK cells, CD57-CD56dim NK cells and CD57+CD56dim NK cells. Additionally, the expression of the checkpoint-markers Tim-3, PD-1 and Lag-3 as well as the inhibitory and the activating NK cell receptors NKG2A and NKG2D were analyzed for each population. In conclusion, we defined a multi-parametric flow cytometry panel in human blood and bone marrow that allows identification of multiple immune cell types at once. This study has important implications for defining NK cells in high risk multiple myeloma patients.

## Analysis of Dendritic Cell populations in human blood by multicolor flow cytometry

**Stella Autenrieth**

*Dominik Treier, Jennifer Richardson, Manina Günter, Kristin Bieber,*
*Universität Tübingen, Germany*

Dendritic cells (DC) are key players in initiating adaptive immune responses as they show unique ability to prime T cells. In the context of the B cell malignancy Multiple Myeloma their role seems to be even more important, yet not completely understood: A reduced number of DCs in general is well documented while their functional status as well as the relationships between DCs, their progenitors and the tumor cells are widely unclear. To address DC-functioning in this disease, a 13-color flow cytometry panel was used. It allows to determine the three different major human DC-subsets (defined as classical DCs (cDCs) Typ 1: lin(CD3,CD19,CD20,CD56)-HLA-DR+CD141+CD1c-, cDCs Typ 2: lin(CD3,CD19,CD20, CD56)-HLA-DR+CD1c+CD141-CD11c+ and plasmacytoid DCs (pDCs): lin(CD3,CD19,CD20, CD56)-HLA-DR+CD141-CD1c-CD303+) in mononuclear cells purified from peripheral blood samples. Furthermore, the expression of different surface molecules giving insights in functional status and the ability to prime T cells can be measured for each subset: the immunomodulatory molecules CD40, CD80, CD86 and CD274 as well as the migration marker CD197. Therefore this panel can be used as a powerful tool to examine the state of DCs in patients suffering from Multiple Myeloma and healthy controls to contribute to elucidation of the role of DCs in this disease. Moreover the panel can be a useful tool for characterization of DCs also in other types of diseases.

## Analysis of platelet functions in patients with degenerative aortic valve disease using multicolor flow cytometry

**Stella Autenrieth**

*Carolin Langnau, Manina Günther, Karin Müller,*
*Universität Tübingen, Germany*

Degenerative aortic valve disease remains the most prevalent valvular disease in western countries characterized by calcification, lipid accumulation and inflammation. Platelets accumulate at the side of injured valves and advance the local and systemic thrombo-inflammatory response. Platelets and platelet-mediated leucocyte accumulation initiate atherogenetic processes and accelerate the degeneration of the aortic valve. Activated platelets release pro-inflammatory cytokines and chemokines, which promote the recruitment of monocytes, osteoblastic transformation and connective tissue remodeling in inflamed valves. The platelet-derived mediators CXCL12, CXCL14 and macrophage migration inhibitory factor (MIF) regulate the function and activation of circulatory inflammatory cells including monocytes, macrophages and progenitor cells. It is known that these chemokines have pro-inflammatory and pro-atherosclerotic

effects in coronary artery disease and myocardial infarction and therefore might also play a key role for thrombo-inflammation during valve calcification. This study aimed to establish a multicolor flow cytometry panel for platelets using freshly isolated human blood of patients suffering from a high degree of aortic valve stenosis. We identify platelets due to the staining of CD42b, CD61 and CD41 while CD31 was used to study platelet-endothelial interactions. The activation of platelets was assessed due to the expression of P-Selectin (CD62P). Additionally, the panel includes an intracellular staining of the platelets-induced chemokines CXCL12, CXCL14 and MIF. To summarize, we established a 9-color flow cytometry panel analyzing the function of platelets and its expression of CXCL12, CXCL14 and MIF in patients with a severe, calcified aortic valve stenosis.

## "Smells Like Goat Spirit" - Key sub-community dynamics of medium-chain carboxylate production

**Johannes Lambrecht**

*Nicolas Cichocki, Florian Schattenberg, Sabine Kleinsteuber, Hauke Harms, Susann Müller, Heike Sträuber*
*Helmholtz Centre for Environmental Research - UFZ, Germany*

**Background**
The carboxylate platform is a promising technology for substituting petrochemicals in the provision of specific platform chemicals and liquid fuels. It includes the chain elongation process that exploits reverse β-oxidation to elongate short-chain fatty acids and forms the more valuable medium-chain variants. The pH value influences this process through multiple mechanisms and is central to effective product formation. Its influence on the microbiome dynamics was investigated during anaerobic fermentation of maize silage by combining flow cytometric short interval monitoring, cell sorting and 16S rRNA gene amplicon sequencing.

**Results**
Caproate and caprylate titres of up to 6.12 g L-1 and 1.83 g L-1, respectively, were achieved in a continuous stirred-tank reactor operated for 241 days. Caproate production was optimal at pH 5.5 and connected to lactate-based chain elongation, while caprylate production was optimal at pH 6.25 and linked to ethanol utilisation. Flow cytometry recorded 31 sub-communities with cell abundances varying over 89 time points. It revealed a highly dynamic community, whereas the sequencing analysis displayed a mostly unchanged core community. Eight key sub-communities were linked to caproate or caprylate production ($rS > |\pm 0.7|$). Amongst other insights, sorting and subsequently sequencing these sub-communities revealed the central role of Bifidobacterium and Olsenella, two genera of lactic acid bacteria that drove chain elongation by providing additional lactate, serving as electron donor.

**Conclusions**
High-titre medium-chain fatty acid production in a well-established reactor design is possible using complex substrate without the addition of external electron donors. This will greatly ease scaling and profitable implementation

of the process. The pH value influenced the substrate utilisation and product spectrum by shaping the microbial community. Flow cytometric single cell analysis enabled fast, short interval analysis of this community and was coupled with 16S rRNA gene amplicon sequencing to reveal the major role of lactate-producing bacteria.

## Can microbial community assembly be controlled?

**Shuang Li**

*Zishu Liu, Susann Müller*
*Helmholtz Centre for Environmental Research, Leipzig*

Bacteria grow as community entities in nature. This is the form where they provide their functions driving biochemical processes such as compound production and waste treatment in bio-economy. However, unlike pure strains, knowledge on how microbial communities assemble and vary is still in its infancy. Both stochastic and deterministic processes have been frequently observed in our previous studies. Simple assembly and succession processes of microbial communities or classical niche-based theory cannot explain most of the phenomena observed. The idea of the neutral theory, which regards all organisms in a system equal, could provide an alternative view on processes of community assembly. However, neutral processes might not allow a control of microbial community structures or even functions. Therefore, our aim is to increase deterministic over stochastic processes through manipulated niche selection to strengthen the controllability of microbial community.

One of the strategies which need to be followed will be a testing of environmental disturbance, which selects for certain species combinations and favors deterministic process. By a well-developed workflow based on flow cytometry, the real-time monitoring of the microbial community composition can be realized. Whether the community assembly is predominated by deterministic or stochastic processes can be determined, for instance, by the rank order of abundance distribution of the subcommunities, where a steeper geometric-like slope indicates deterministic processes and a lower lognormal-like slope indicates stochastic processes. This effort to investigate and control the microbial community assembly gives a clue to manageable microbe-driven processes concerning bio-economy.

## Quantitative Image Analysis and Visualization of Microfluidic Obtained Microbial Single-Cell Data

**Yannic Kerkhoff[1,2,]**

*Alexander Grünberger[2]*
*[1]Institut für Chemie und Biochemie, Freie Universität Berlin, Takustraße 3, 14195 Berlin, Germany; [2]Multiscale Bioengineering, Bielefeld*

*University, Universitätsstr. 25, 33615 Bielefeld, Germany*

Microorganisms and their communities are one of the most important systems on this planet. They can be causes of diseases, interact symbiotically with other living beings and be used biotechnologically for analysis and production purposes . Cultivating and studying them has been a common practice for many years. Despite years of experience in this field, there are still many questions and ambiguities, e.g. regarding the development of heterogeneity in isogenic cell cultures or the reaction to changing environmental conditions. Here, novel microfluidic single-cell cultivation methods allow the investigation of individual cells, cell clusters, and different strains which offers many new possibilities to clarify old questions. Microfluidic single-cell cultivation devices offer the advantage of high degree of automation, the large number of biological replicates, and image-based analysis with high spatio-temporal resolution. Combined with live-cell imaging, large number of datasets are obtained, which are currently hard to analyse in an automated manner and represent a bottleneck in the experimental flow.

In this work, we developed an automated quantitative image analysis workflow to tackle these challenges and offer solutions for the processing of large amounts of data from microfluidic single-cell cultivations. We present a cluster-based (and thus phenotypic independent) workflow as well as special visualization techniques. As proof of concept, we demonstrate the functionality and versatility of the workflow on selected microbial data sets of E.coli and C.glutamicum. Different cellular parameters such as cell growth, division, gene expression and morphologies were analysed, enabling the visualization of different sub-populations from isogenic cultures. This lays the foundation for systematic analysis of dynamic microfluidic single-cell data, currently hardly assessable with existing software-solutions.

## Applications of optogenetic flow cytometry

**Kathrin Brenker**[1,2]

*[1]Opto Biolabs, Freiburg, Germany; [2]Centre for Biological Signaling Studies, BIOSS, Albert-Ludwigs-Universität Freiburg, Germany*

Optogenetic tools allow the remote control of cellular pathways using light. This enables the isolated, functional investigation of almost any signaling molecule within complex signaling pathways. More interestingly, the remote control of cellular pathways has major implications for therapeutic tools, including targeted immunotherapy.

A major obstacle for optogenetic researchers is the controlled delivery of light to the cell sample and hence the most popular tools for optogenetic studies are microscopy-based cell analyses and *in vitro* experiments. The flow cytometer has major advantages over a microscope, including the ability to rapidly measure thousands of cells at single cell resolution. However, it is not yet widely used in optogenetics. Opto Biolabs, a spin-off from the University of Freiburg, develops specialized illumination devices that enable the combination of optogenetics with flow cytometry.

Optogenetic flow cytometry can be applied for any optically controlled substance including cage compounds or photoconvertible fluorescent proteins and substantially expands the set of possible experiments. Here,

we present first results from our collaborative research labs using this novel technology.
Our future work will focus on the expansion of our technology to optogenetic cell sorting, which will greatly simplify the discovery and development of novel, cell-based optogenetic therapies.

## Preparation and characterization of reference particles for the calibration of flow cytometry for the detection of extracellular vesicles within the EMPIR project 18HLT01 MetVes II

**Daniel Geißler**

*Ute Resch-Genger*
*Bundesanstalt für Materialforschung und -prüfung (BAM), Fachbereich 1.2 Biophotonik, Berlin, Germany*

Extracellular vesicles (EV) are cell-derived particles in body fluids, which have excellent potential as next-generation biomarkers. The exploitation of EV requires reliable measurements, which is currently very difficult, as most EV are smaller than 200 nm. At present, flow cytometry (FCM) is the most appropriate technique for EV analysis in biological samples, as FCM is readily available in many clinical laboratories and allows to identify cell-specific EV at high throughput. However, due to technical variations between different FCM instruments, EV concentration measurements are currently not well comparable between most laboratories. Therefore, EV reference materials and standardized reference methods are urgently needed to calibrate flow rate, light scattering intensity, and fluorescence intensity of FCM in the sub-micrometer size range. This requires a better matching of the optical properties of calibration beads and EV as can be realized with current polystyrene calibration beads.
The EMPIR project 18HLT01 "MetVes II" aims to develop synthetic reference materials and traceable measurement methods to standardize EV measurements. The reference materials should resemble EV properties, so that calibrations are reliable and do not require a change of acquisition settings. Hence, the reference materials should contain particles with a traceable number concentration in the range of 109–1012 particles/mL to calibrate flow rate, a traceable size with discrete diameters between 50–1000 nm and a refractive index (RI) in the range of 1.37–1.42 to calibrate scattering intensity, and a traceable fluorescence intensity between 100–100,000 molecules of equivalent soluble fluorochromes (MESF). At BAM, various approaches to prepare such low-RI nanometer-sized reference materials will be studied, preliminary results of the primary characterization of these candidate reference particles will be presented, and possible applications besides FCM-based EV detection will be outlined.

## Side scatter module for enhanced detection of Extracellular Vesicles by flow cytometry.

**Tina Van Den Broeck**[1]

*Ludovic Monheim*[1], *Ihor Berezhnyy*[2], *Oleg Guryev*[2], *Tatyana Chernenko*[2], *Marybeth Sharkey*[2], *Geoffrey W. Osborne*[2]
[1]*BD Biosciences, Belgium;* [2]*BD Biosciences, San Jose, CA*

EVs are nanosized (20~5000 nm) membrane vesicles released from cells that can transport cargo - including mRNA and proteins - between cells as a powerful way of intercellular communication. Currently, flow cytometry is the only high throughput technique capable of single particle cell surface phenotyping and sorting with the possibility of concentration determination. Unfortunately, the drawback of standard flow cytometry is lack of sensitivity to detect smallest particles, especially for those with a size less than or equal to the dimensions of the excitation laser wavelength.BD has developed an accessory side scatter (SSC) module for enhanced scatter detection of small particles by flow cytometry: the SP SSC module. The SP SSC module should be used in combination with a laser power of at least 100 mW. Small particle detection enhancement is achieved by significantly increasing the signal-to-noise ratio of the SSC. The SP SSC module can be installed on most commercially available BD flow cytometers, which have sufficient laser power, as an additional option. The normal SSC detector remains in place and the SP SSC module has minimal impact on regular SSC and fluorescent performance therefore use of the system for cell analysis applications is still possible. Initialresults using the SP SSC modulewere obtained using a BD FACSCelesta™ SORP and a BD FACSAria™ Fusion, respectively having a 100 and 200 mW 488 laser. Side-by-side comparison of the regular SSC detection vs SP SSC detection was done using polystyrene beads, silica beads, EV reference material and antibody-stained EV material.Utilization of the SP SSC module for sorting of natural (plasma EVs) and artificial (liposomes) membrane particles is currently being undertaken.

## Quantification of Aldehydes Functional Groups on Polymeric Microbeads via Catch and Release of Reporters

**Alexander Roloff**[1]

*Nithiya Nirmalananthan-Budau*[1], *Bastian Rühle*[1], *Heike Borcherding*[2], *Thomas Thiele*[2], *Uwe Schedler*[2], *Ute Resch-Genger*[1]
[1]*Bundesanstalt für Materialforschung und -prüfung, Germany;* [2]*PolyAn GmbH, Germany*

Aldehyde-functionalized materials have found broad use in bioconjugation applications. For example, coupling of aldehyde surface groups to proteins or amine-functionalized oligonucleotides can readily produce biomolecule-covered microarray and bead surfaces for multiplex analyses. Additionally, aldehyde-modified nanoparticles can possess bioadhesive properties that can extend their retention time in biological compartments. These emerging novel bioanalytical

applications call for reliable tools and methods to detect and quantify accessible aldehyde functionalities.
We present here a straightforward concept to quantify the amount of accessible aldehyde moieties on the surface of polymethylmethacrylate (PMMA) particles through the specific binding and subsequent release of small reporter molecules such as absorbing and fluorescent dyes utilizing hydrazone formation as a reversible covalent labeling strategy. Unbound reporter molecules can be easily removed by washing steps, eliminating inaccuracies caused by unspecific adsorption to hydrophobic surfaces. Cleavage of the hydrazones at acidic pH assisted by a carbonyl trap releases the optical reporters rapidly and quantitatively and allows for their optical detection at low concentration. Importantly, this strategy separates the signal-generating molecules from the bead surface, thereby circumventing light scattering and signal distortions due to binding-induced changes in reporter fluorescence and quenching dye-dye interactions on crowded material surfaces. The potential of this catch-and-release strategy for surface group quantification is representatively demonstrated for a set of microparticles functionalized with different aldehyde densities. This concept is validated by a colorimetric assay with a different optical probe, which contains a reductively cleavable disulfide bond and a reporter that can be quantified photometrically in solution after its cleavage. The excellent match of the results of both optical assays confirms their suitability for the rapid and sensitive quantification of aldehydes on microbead surfaces. These simple catch-and-release assays are excellent tools for process control during bead fabrication and the comparison of different bead batches.

# Guest Lecture

Session Chair: Anja Hauser
Session Chair: Raluca Niesner

## Fossils, bones and genes – the evolution of limb development and regeneration

**Nadja Fröbisch**

*Museum für Naturkunde, Leibniz Institut für Evolutions- und Biodiversitätsforschung, Insitut für Biologie, Humboldt Universität zu Berlin, Germany*

When it comes to the evolution and development of the vertebrate body plan, the limb is one of the best examples of how data from different fields, including morphology, paleontology and developmental biology, have come together to gain a broad understanding of the complex connections between the development and evolution of an organ. Both fossils and genes have provided important insights into the initial evolution of the limb of four limbed-vertebrates (tetrapods) from finned-ancestors and the subsequent diversification of tetrapods.
Despite the great diversity in the form and function of tetrapod limbs, their skeletal development follows a very conservative

sequence with a so-called postaxial dominance. Salamanders are the only living tetrapods that deviate from this pattern by showing a reversed, pre-axial polarity in limb development and are also the only tetrapods capable of full limb regeneration. Both features were assumed to be highly derived for salamanders, while it remained largely unknown how preaxial polarity is established developmentally, when it evolved in salamander evolution, and if and how it may be connected to the regenerative capacities of the limbs. New developmental data shows expression patterns of genes with well-known roles in limb development seem to be canonical in early stages of salamander limb development, but very different from other tetrapods in late stages of limb development. Interestingly, data from fossil amphibians and lungfish indicates that both preaxial polarity in limb development and salamander-like regenerative capacities are not derived for modern salamanders, but are much more ancient features that may even be ancestral for all tetrapods.

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## Members Assembly

6:00pm - 7:30pm

*LH IM*

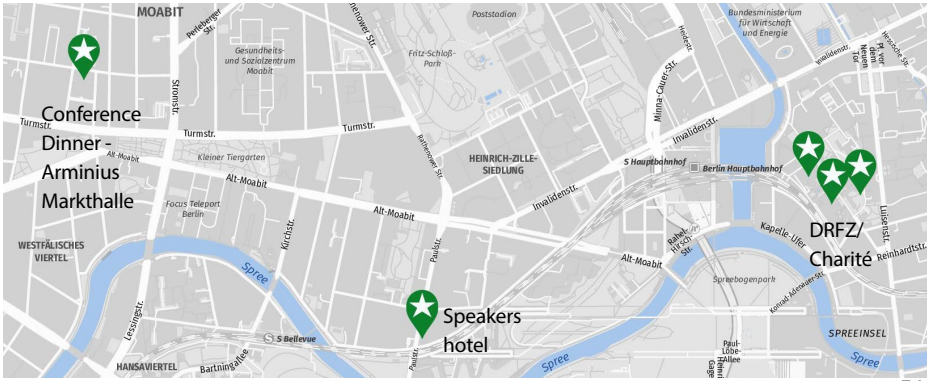
Chair: Anja Hauser

Chair: Raluca Niesner

## Social Event & Conference Dinner

8:00pm - 11:00pm

*Location: Arminius Markthalle*



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# Friday, September 27, 2019

## Session 7: Microbiology

Session Chair: Christin Koch
Session Chair: Alexander Grünberger

From single cell genomics to advanced control of microbial processes – this years microbiology session explores again the cutting edge technologies in microbial single-cell analysis. While single cell sequencing is, by now, well established for eukaryotic cells, its application for microbial cells is still more challenging. An excursion into the microbial dark matter will reveal the current understanding and recent methodological advances. While identifying the unknown dark matter is challenging, controlling the well-known microbial systems is not simpler. Microbial heterogeneity is a key principle underlying biotechnological processes. Here, novel concepts to control phenotypic diversification dynamics are available that lay the foundation for the development of novel bioprocesses control strategies. Further technological advances for microbial single cell analysis will complement this session.

## Revealing Microbial Dark Matter by Targeted Cell Sorting Combined with Single Cell Genomics

**Anne-Kristin Kaster**

*Karlsruhe Institute of Technology (KIT)*

Rare members of environmental microbial communities are often overlooked and unexplored, primarily due to the lack of techniques capable of acquiring their genomes. Chloroflexi belong to one of the most understudied phyla, even though many of its members are ubiquitous in the environment and some play important roles in biotechnological applications. We here used a targeted cell sorting approach via fluorescent activated cell sorting compatible with subsequent single cell genomics to enrich for rare Chloroflexi species from a wastewater treatment plant and obtain their genomes. The combined workflow was able to retrieve a substantially higher number of novel Chloroflexi draft genomes with greater phylogenetical diversity when compared to a metagenomics approach from the same sample. Our method offers an opportunity to access genetic information from rare biosphere members which would have otherwise stayed hidden as microbial dark matter. It can therefore serve as an essential complement to cultivation-based, metagenomics, and microbial community-focused research approaches.

## Outer membrane remodeling dynamics at a single cell resolution revealed by segregostat analysis

**Frank Delvigne**

*Université de Liège, Belgium*

Stochasticity in gene expression is known to affect the behavior of bacterial populations. Up to now, most of the efforts have been focused on mechanisms depending on the intrinsic source of noise, i.e. linked with bursting mechanisms described for transcription and translation. In this work, we describe a more complex phenomenon involving an intricated set of mechanisms, i.e. stochasticity in gene expression, sRNA regulation, segregation of outer-membrane (OM) proteins upon division and (potentially) formation of vesicles. This led to the appearance of two sub-populations when E. coli is cultivated under nutrient limiting conditions. Upon diversification, one of the subpopulation exhibited low OM permeability, whereas the other exhibited enhanced OM permeability (but is still viable), in a mechanism similar to bet-hedging. More interestingly, each subpopulation exhibited distinct metabolic properties.

Finally, the segregostat, i.e. a technology involving the use of on-line flow cytometry with feedback control, has been successfully

used for characterizing in real time the diversification dynamics and has shown that bursting is an important component of noise in this case.

## Community diversity affects the MAIT cell response to intestinal microbiota in vitro

**Jannike Lea Krause[1]**

*Stephanie Serena Schaepe[2], Florian Schattenberg[3], Arkadiusz Pierzchalski[1], Ulrike Rolle-Kampczyk[2], Nico Jehmlich[2], Susann Mueller[3], Martin von Bergen[2,4], Gunda Herberth[1]*
*[1]Helmholtz Centre for Environmental Research - UFZ, Germany - Dep. Environmental Immunology; [2]Helmholtz Centre for Environmental Research - UFZ, Germany - Dep. Molecular Systems Biology; [3]Helmholtz Centre for Environmental Research - UFZ, Germany - Dep. Environmental Microbiology; [4]Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, University of Leipzig, Germany*

**Introduction**
Mucosal-associated invariant T cells (MAIT cells) reside at barrier sites within the human body. They are activated by microbial riboflavin metabolites, whereas microbial folate metabolites can inhibit MAIT cell activation. Studies with single bacterial species showed that MAIT cells play a pivotal role in microbiota-immune homeostasis. However little is known about the response of MAIT cells to microbial communities.

**Objectives**
We investigated the MAIT cell response to microbial samples with different diversity *in vitro*.

**Materials & Methods**
Escherichia coli was grown in batch culture, whereas the extended simplified human intestinal microbiota (SIHUMIx) and colonic microbiota were continuously cultivated in *in vitro* bioreactors. Using microbial flow cytometry, we determined sample diversity by depicting the number of sub-populations within a bacterial sample. Furthermore, bacteria were used for *in vitro* MAIT cell stimulation (n=3) and their respective culture supernatants for riboflavin and folate concentration analysis.

**Results**
The colonic community comprised of 31 sub-populations, SIHUMIx of 19 sub-populations and E. coli of 5 sub-populations indicating high, medium and low diversity, respectively. Co-cultivation of peripheral blood mononuclear cells (PBMCs) with colonic bacteria, SIHUMIx, or E. coli showed 9.54%, 14.91%, and 25.57% of activated CD69+/TNF+ MAIT cells, respectively.
The riboflavin concentration in the colonic and the SIHUMIx culture supernatant decreased, whereas for E. coli the riboflavin concentration slightly increased. In contrast, the folate concentration increased in all microbial cultures, though the folate concentration in the supernatant of the colonic community and SIHUMIx were higher than in the supernatant of E. coli. The folate/riboflavin ratio was high in the colonic community and decreased with declining sample diversity being lower for SIHUMIx and very low for E.coli.

**Conclusion**
Our results suggest an impact of microbiota diversity on MAIT cell stimulation mediated by the availability of riboflavin and folate.

# Flow cytometric analysis of microbial growth dynamics to determine the biostability of drinking water resources

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The issue of biostability of drinking water has gained increasing attention. Excessive proliferation of naturally occurring water microorganisms may deteriorate its taste, odour and appearance; growth of facultative pathogens is of particular concern for public health. The aim of this project was to develop a standardized protocol for the determination of microbial growth dynamics in ground- and drinking water based on flow cytometry.

Ground- and drinking water samples from a riverbank filtration system were collected from sites in different distance to the river. Biostability was assessed by incubating them in batch at 10°C for three weeks, simulating water stagnation during storage and distribution in the supply network. At daily to weekly intervals, microbial cell numbers were measured by flow cytometry and compared to heterotrophic plate counts obtained by the standard cultivation method. Specific attention was paid on determining the reproducibility of the method and the influence of different incubation temperatures.

An innovative protocol for the flow cytometric determination of biostability was established. Samples from sites in close vicinity to the river showed much higher dynamics and inter-sample variability than those from remote sites. Surprisingly, water incubated at higher temperatures did not reach higher microbial cell concentrations compared to standard temperature incubations at 10°C.

Future work will focus on combining flow cytometric investigations with taxonomic community analyses and linking it to biogeochemical parameters for a comprehensive determination and interpretation of microbial growth dynamics. Additionally, specific bacterial populations will be sorted by FACS to foster in-depth analysis. Moreover, automated flow cytometry tools enabling a higher time resolution of microbial growth shall be applied, supporting a reproducible and straightforward determination of biostability and its prediction in ground- and drinking water.

# Session 8: Nanotechnology

Session Chair: Wolfgang Fritzsche
Session Chair: Ulrike Taylor

Nanotechnology and -materials enable innovative solutions in biomedicine, both in diagnostics as well as in therapy. So, a specific targeting of nanocarriers and controlled release is possible, as it will be discussed in the session. Novel nucleic-acid based nanostructures allow for a high degree of control about the steric arrangement also of bioactive ligands. This approach will be demonstrated for the study of the shape related cellular uptake mechanisms, as well as for the immune-pathway activation. Other themes will include the quantification of silver nanoparticle uptake by individual cells, and the latest improvements in life time flow cytometry using a quantum dot system.

## Targeting of Nanocarriers

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Our vision is to construct multipotent drug-loaded nanocapsules of high homogeneity in size and surface functionality, which find their target cells in the desired organs and release the drug in a controlled manner in the cytoplasm of these cells. For the delivery of bioactive compounds to a specific cell, it is not only vital to improve the stability of the therapeutic agent during passage through the blood stream, but also to extend the circulation time in the body.
Consequently many interactions to biological matter have to be considered and tuned: the interaction with blood components (proteins etc.) has to be controlled to limit aggregation processes. Furthermore, the interaction to cell membranes and uptake in blood cells like macrophages has to be minimized. Only then the drug can reach the target cells. The specific interaction to target cells have to be tuned.

## The challenges and promises of DNA as programmable biomaterial

**Maartje M.C. Bastings**

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DNA-nanotechnology offers unprecedented control over the precise structure and location in a nanostructure since each element has a unique sequence. In the DNA-origami method, a 7-kilobase "scaffold" strand is self-assembled with hundreds of shorter "staple" strands to form a parallel array of double helices. Using this method, one can approximate any desired three-dimensional shape up to the size of a small virus.The technology suffers however from inherent stability challenges when used in cellular environments.
In this talk, I will start with a brief overview of the DNA nanotechnology principles and will go into depth on stabilization solutions that allow for cellular manipulation using DNA architectures. We developed DNA-nanostructures with the aim to selectively target cells as well as study the shape related cellular uptake in various cell types.Finally, I will touch upon our activities to explore precise activation of the immune system through controlled maturation of dendritic cells. By systematic screening of immune-pathway activation of DNA-origamis combined with antigens and danger-signals, we are making small steps into a better understanding of the complex mechanisms of our immune system. This knowledge holds potential to be translated toward the development of vaccines for autoimmune diseases and cancer.

## Mass cytometric application in nanotoxicology

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Silver nanoparticles (AgNPs) have a high scientific and commercial impact due to their important antibacterial properties. However, there are serious concerns about their toxicological adverse effects as a consequence of their broad range of applications. Particularly, the impact of AgNPs on cells is not very well understood yet and there is a current demand to develop analytical methodologies providing information about the interaction and distribution of AgNPs at a single cell level. In this research, mass cytometry was used to introduce a new quantitative approach to study the uptake of AgNPs by individual THP-1 macrophages as a cell model system. Here, we show that this methodology provides not only multi-variate phenotypic information of individual cells but enables the quantitative analysis of AgNPs associated to cells in a single measurement by performing an external calibration using AgNPs suspension. Using differentiated THP-1 cells, we monitored and quantified the uptake of 50 nm AgNPs in a time and dose-dependent manner by mass cytometry. 7 to 120 AgNPs per cell (2 to 89 fg Ag/cell) were determined after exposure of differentiated THP-1 cells to low AgNPs concentrations of 0.1 and 1.0 mg L-1, at time points of 4 and 24 h. The results were validated by mass cytometric analysis of digested cells working as a conventional inductively coupled plasma mass spectrometry, ICP-MS. This study demonstrates the power of single cell analysis by mass cytometry even for low doses experiments as a new analytical tool for hitherto unaddressed questions in nanotoxicology.

## Luminescence lifetime encoding for flow cytometry with quantum-dot-encoded beads

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Spectral encoding of cells or particles and the discrimination of multiple spectral codes are a critical process in flow cytometry (FCM). Typical issues in spectral encoding are, e.g., the spectral overlap of codes, or the increasing complexity of instruments. The exploitation of the photoluminescence lifetime (LT) as an encoding parameter could be used to

circumvent both of these issues, as it adds another dimension to the parameter space, or, when used as a stand-alone parameter, requiring only one excitation light source and one detector. While LT encoding was considered already decades ago it is still not implemented as a routine technique in FCM yet, mainly due to the challenge of very few photons being available within the limited transition time of a cell or particle through the laser spot.

Recently, we demonstrated LT-FCM based on luminophores with ns LTs in a compact and low-cost flow cytometer. Measurements on polymer microbeads containing luminophores with distinctly different excited state LTs enabled the complete discrimination of three LT codes and five codes in total could be identified.

Now, we have extended our approach towards considerably longer LTs by custom-made polymer microbeads loaded with different ratios of InP/ZnS and AgInS2 quantum dots. The use of these materials significantly expands the usable time range for LT encoding to up to several hundred ns. Our studies demonstrate the possibility to further increase the number of viable LT codes for multiplexing in LT-FCM without the need for extensive hardware modifications.

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