# Cytometry 2017
## Life in Focus.

## October 4-6 2017

**University of Applied Sciences**
Carl-Zeiss-Promenade 2
Jena // Germany

www.dgfz.org

# Program

## WEDNESDAY, OCTOBER 4ᵀᴴ, 2017

| | |
|---|---|
| **9:00-12:00** | **Institute of Immunology // Tutorial "Handling of Flow Cytometers"** |
| **9:30-11:30** | **Lecture Hall 6 // Core Facility Networking Event** |
| **10:30-12:00** | **Seminar Room 04.03.07 // Tutorial "Scientific Publication"** |
| **11:45-12:45** | **Foyer // Welcome & Registration** |
| **12:45-14:15** | **Lecture Hall 6 // Session 1: Nanotechnology** |
| **14:15-15:45** | **Lecture Hall 6 // Session 2: Product Slam** |
| 15:45-16:45 | FOYER // COFFEE BREAK / INDUSTRY SESSION |
| **16:45-18:15** | **Lecture Hall 6 // Session 3: Cutting Edge** |
| **18:15-19:15** | **Lecture Hall 6 // Keynote: Eugen Ermantraut** |
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## THURSDAY, OCTOBER 5ᵀᴴ, 2017

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|---|---|
| **9:15-10:45** | **Lecture Hall 6 // Session 4: Microbiology** |
| 10:45-11:15 | FOYER // COFFEE BREAK |
| **11:15-12:45** | **Lecture Hall 6 // Session 5: European Guest Session** |
| **12:45-13:30** | **Lecture Hall 6 / Foyer // Poster Pitch & Poster Session** |
| 13:30-14:30 | FOYER // LUNCH & POSTER SESSION |
| **14:30-16:00** | **Lecture Hall 6 // Session 6: Klaus-Goerttler Session** |
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## FRIDAY, OCTOBER 6ᵀᴴ, 2017

| | |
|---|---|
| **9:15-10:45** | **Lecture Hall 6 // Session 7: Microscopy** |
| 10:45-11:15 | FOYER // COFFEE BREAK |
| **11:15-12:45** | **Lecture Hall 6 // Session 8: Clinical Applications / ISAC Lecture** |
| 12:45-13:45 | FOYER // FAREWELL & SNACKS |

# Life in Focus

**Dear Cytometrists,**

welcome to the annual DGfZ meeting 2017!

The meeting will cover various aspects of cytometry, in scientific sessions such as on nanotechnology, microscopy, microbiological or clinical applications, on posters, and in the industrial exhibition (Thanks to the sponsors for making this meeting possible!). Technology innovations will be presented in the Cutting Edge session as well as in the company product slam. This years European Guest Session will introduce our French partner organization Association Française Cytométrie (AFC). Jena has a unique tradition of fruitful interactions between technology and science aimed at bringing „Life in Focus", as demonstrated in the work of people like Carl Zeiss and Ernst Abbe, Matthias Schleiden and Hans Knöll. This special spirit reflects the motivation of our society, and shall therefore also guide our meeting.

At this point I would like to thank all funding bodies and industrial partners for their generous support without which this meeting would not have been possible.

I am looking forward to three days of scientific exchange in fascinating presentations and interesting discussions with colleagues, to meet old and to make new friends – let's enjoy the various aspects of the exciting world of cytometry here in Jena!

**Wolfgang Fritzsche** – President DGfZ

# Welcome in Jena – "City of Light"!

Our city is well-known far beyond the borders of our continent as the heart of the optical industry in Germany, maybe even in Europe. The foundation for this development was laid by Carl Zeiss who, in collaboration with Ernst Abbe and Otto Schott, developed microscopes and lenses in Jena that are now known and valued all over the world. The Institute of Photonic Technology is one of the brightest stars in our optical valley.

However, this is not the only reason why Jena is considered a City of Light. Ever since its foundation in 1558, our protestant university has been a place of greatness for plenty of "enlightened" and bright personalities such as philosopher Friedrich Hegel or Friedrich Schiller, whom our alma mater is named after.

You may also find Jena, City of Light reflected in the shining eyes of the children who grow up here amidst beautiful landscapes. From infancy, they find security in an exemplary care system and thrive in the best schools Germany has to offer.

Currently Jena is in the process of becoming a European model city thanks to its thriving, innovative economy, its scientific institutions, its excellent cultural life, its liberal and open-minded atmosphere, and not least its proverbial quality of life.

Have a nice time in Jena – You are most welcome here!

**Albrecht Schröter** – Lord Mayor

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**University of Applied Sciences** // Carl-Zeiss-Promenade 2 // Jena // Germany

CONFERENCE CHAIR

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WIFI-PASSWORD

**Name:** WLAN-PSK
**Key:** r#kuLh3y

# Program

**9:00-12:00**    **Institute of Immunology // Tutorial „Handling of Flow Cytometers" //**
**Thomas Kamradt, Nico Andreas //** Institute of Immunology, Jena

**9:30 -11:30**    **Lecture Hall 6 // Core Facility Networking Event**

**10:30-12:00**    **Seminar Room 04.03.07 // Tutorial „Scientific Publication" //**
**Attila Tarnok //** University of Leipzig, Germany

**11:45-12:45**    **Foyer // Welcome & Registration**

**12:45-14:15**    **Lecture Hall 6 // Session 1: Nanotechnology**
Chair: **Ulrike Taylor, Wolfgang Fritzsche**

**Microplastics – What we know and what we don't know //**
**Gunnar Gerdts //** Alfred Wegener Institute, Helgoland, Germany

**Mass cytometry for detection of silver at the bacterial single cell level //**
**Yuting Guo //** Department of Environmental Microbiology, Helmholtz
Centre for Environmental Research, Leipzig, Germany

**Development of a flow cytometer for nanoparticle detection //**
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**14:15-15:45**    **Lecture Hall 6 // Session 2: Product Slam**
Chair: **Frank Schildberg, Elmar Endl**

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**16:45-18:15**    **Lecture Hall 6 // Session 3: Cutting Edge**
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**differentiation and heterogeneity //**
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**Picturing metabolism of individual cells //**
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**Cell cycle progression: A comparison between vibrational and**
**cytofluorimetric signatures //**
**Lisa Vaccari //** Elettra – Sincrotrone Trieste, Italy

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**18:15-19:15**    **Lecture Hall 6 // Keynote**
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**19:15-21:00**    **Foyer // Welcome Reception at Industry Exhibition**

# THURSDAY, OCTOBER 5TH, 2017

**9:15-10:45**    **Lecture Hall 6 // Session 4: Microbiology**
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Joanna Baginska // University of Luxembourg

**Stronger together: On the benefits of dividing metabolic labor in bacteria //**
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Germany

**10:45-11:15**    FOYER // COFFEE BREAK

**11:15-12:45**    **Lecture Hall 6 // Session 5: European Guest Session –
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Chair: **Wolfgang Fritzsche**

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**The bone marrow microenvironment in MDS: emerging concepts with therapeutic implacations //**
**Carmen Aanei //** CHU St. Etienne

**12:45-13:30**     **Foyer // Poster Pitch & Poster Session**

**13:30-14:30**     FOYER // LUNCH & POSTER SESSION

**14:30-16:00**     **Lecture Hall 6 // Session 6: Klaus-Goerttler-Session**
Chair: **Jonathan Leo Schmid-Burgk, Wolfgang Fritzsche**

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**Julia Reinhardt //** University of Bonn

**Redox proteomics using an MS-cleavable tag //**
**Sebastian Virreira-Winter //** Max Planck Institute Berlin

**Flow cytometric dissection of cellular processes using genome-wide loss of function genetics //**
**Lucas Jae //** Gene Center Munich

**16:00-16:45**     **Foyer // Poster Session**

**16:45-17:30**     **Lecture Hall 6 // Guest Lecture**
Chair: **Wolfgang Fritzsche**

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**17:30-19:00**     **Lecture Hall 6 //** Members Assembly

**19:00-22:00**     VOLKSBAD // SOCIAL EVENT & CONFERENCE DINNER

# Access with conference badge only

# FRIDAY, OCTOBER 6TH, 2017

**9:15-10:45**     **Lecture Hall 6 // Session 7:** Microscopy
Chair: **Anja Hauser, Raluca Niesner**

**Go with the flow – blood flow dynamics and HSPC homing in bone marrow microvessels //**
**Gabriele Bixel** // Max Planck Institute for Molecular Biomedicine, Germany

**The diversity of glial responses in health and disease – combining in vivo 2P imaging and transgenic mice with fluorescent reporter expression //**
**Frank Kirchhoff** // University of Saarland, Germany

**High-throughput 3D-Tomographic-Imaging Flow Cytometry //**
**Thomas Henkel** // Leibniz-Institute of Photonic Technology (Leibniz IPHT)

**Fourier transform infrared micro-spectroscopic imaging of biological samples //**
**Patrick Hoffmann** // Center for Sepsis Control and Care / Jena University Hospital, Germany; Leibniz Institute of Photonic Technology, Germany

**10:45-11:15**     FOYER // COFFEE BREAK

**11:15-12:45**     **Lecture Hall 6 // Session 8:** Clinical Applications – ISAC-Lecture
Chair: **Gergely Toldi, Stephan Schmid**

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**Paul Moss** // University of Birmingham, United Kingdom

**Regulation of pro- and anti-inflammatory human T helper cell functions by the microenvironment //**
**Christina Zielinski** // Technical University of Munich, Germany

**Antibiotics susceptibility testing by machine learning supported image analysis of colour coded microfluidic droplets //**
**Carl-Magnus Svensson** // Hans Knöll Institute, Jena, Germany

**Long-term stabilization of whole blood samples for flow and mass cytometry //**
**Sabine Baumgart** // German Rheumatism Research Centre (DRFZ) Berlin

**12:45-13:45**     FOYER // FAREWELL & SNACKS

# Jena – City of Light

Jena is one of the most popular university and college cities in Central Germany and has an outstanding reputation as high-tech center. It is the second largest city in Thuringia and attractively situated in the picturesque landscape of the Saale Valley. The city surprises visitors with gratifyingly short distances, an almost Mediterranean flair and a contemporary and open-minded atmosphere. Jena's nickname "City of Light" stands for its many scientific institutions, worl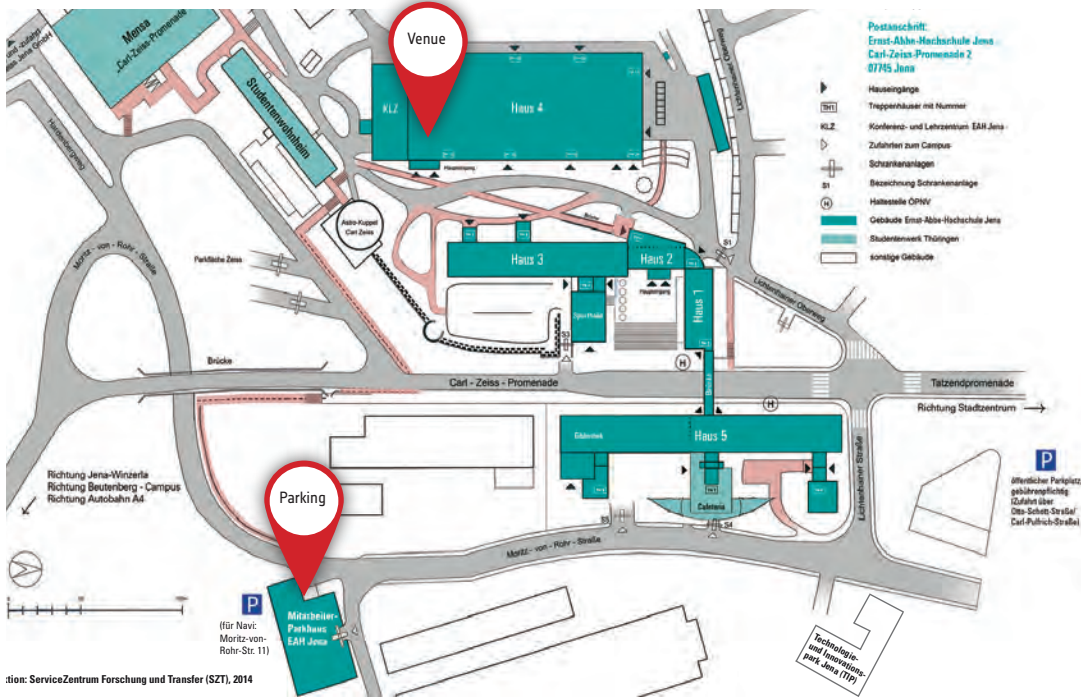d-renowned high-technology companies and young start-up businesses in the field of optics and photonics. Essentially, the name "City of Light" is inspired by Jena's bright minds. Among researchers, Jena is one of the best-known places in Germany. One quarter of the 108.000 inhabitants are students and 4500 scientist work at the universities and research institutes. For many entrepreneurs, the city with one of the highest number of patent applications provides the perfect environment to realize their ideas.
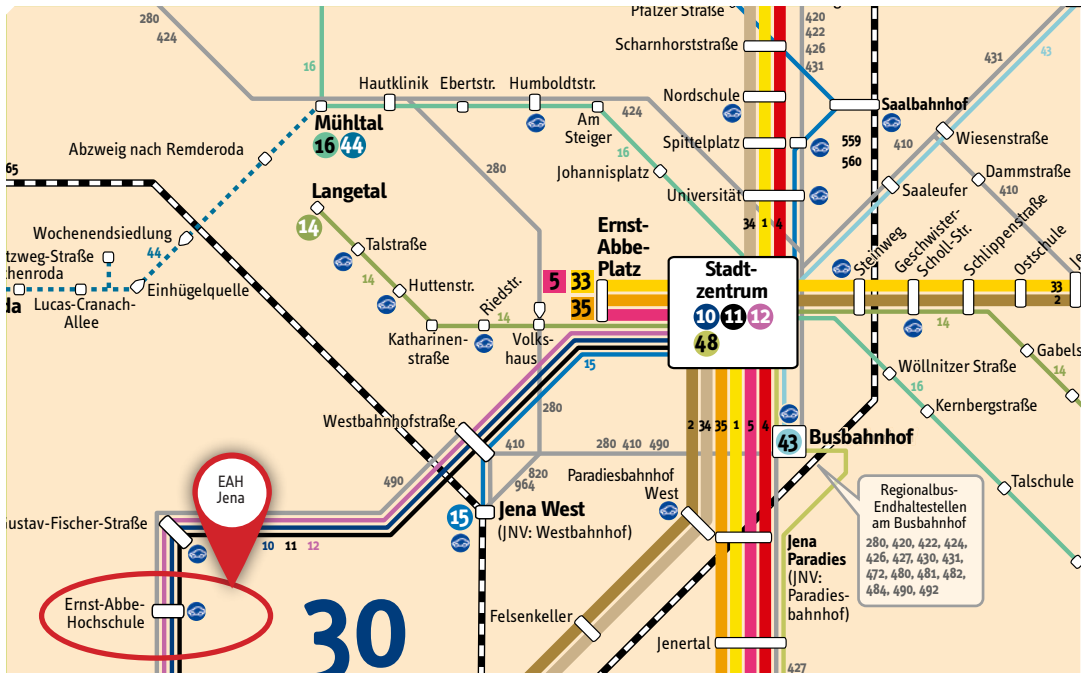
**Map Jena**

Institute of Immu-
nology

Hotel
Schwarzer
Bär

Pulverturm

Johannistor

Jentow

Hotel
IBIS

Goethe Galerie

Volksbad

Friedrich-Schiller-Universität
Jena

Jena Paradies

Märchenbrunnen

EAH
Jena

Rasenmühleninsel

Seidelpark

Jenertal

Jena West

USV Jena e.V

LICHTENHAIN

Mühlenstraße

Obere Aue

WEST

200 m

# Campus Map Jena



Venue

Parking

Mensa
"Carl-Zeiss-Promenade"

Studentenwohnheim

KLZ

Haus 4

Haupteingang

Astro-Kuppel
Carl Zeiss

Parkfläche Zeiss

Haus 3

Haus 2

Haus 1

Haupteingang

Carl - Zeiss - Promenade

Tatzendpromenade

Richtung Stadtzentrum

Richtung Jena-Winzerla
Richtung Beutenberg - Campus
Richtung Autobahn A4

Haus 5

Bibliothek

Cafeteria

P
(für Navi:
Moritz-von-
Rohr-Str. 11)

Mitarbeiter-
Parkhaus
EAH Jena

P
(öffentlicher Parkplatz
gebührenpflichtig
(Zufahrt über
Otto-Schott-Straße/
Carl-Pulfrich-Straße)

Technologie-
und Innovations-
park Jena (TIP)

Moritz - von - Rohr - Straße

tion: ServiceZentrum Forschung und Transfer (SZT), 2014

Postanschrift:
Ernst-Abbe-Hochschule Jena
Carl-Zeiss-Promenade 2
07745 Jena

Hauseingänge
Treppenhäuser mit Nummer
Konferenz- und Lehrzentrum EAH Jena
Zufahrten zum Campus
Schrankenanlagen
Bezeichnung Schrankenanlage
Haltestelle ÖPNV
Gebäude Ernst-Abbe-Hochschule Jena
Studentenwerk Thüringen
sonstige Gebäude

# Bus Map Jena



44

14

34

33
2

10   12

2  34

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Regionalbus-
Endhaltestellen
am Busbahnhof
280, 420, 422, 424,
426, 427, 430, 431,
472, 480, 481, 482,
484, 490, 492

EAH
Jena

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# General Information DGfZ

The Society of Cytometry (Gesellschaft für Zytometrie, GZ) was founded in 1989 in Heidelberg (Germany) by the Foundation Council represented by Cess 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.

# Council

## EXECUTIVE BOARD

**President //** **(APL) Prof. Wolfgang Fritzsche //** Institute of Photonic Technology (IPHT), Nanobiophotonics Department

**Vice President //** **Prof. Anja Hauser //** Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin, Immundynamik

**Secretary //** **Dr. Thomas Kroneis //** Medical University Graz, Center for Molecular Medicine Institute of Cell Biology, Histology & Embryology

**Treasurer //** **Dr. Torsten Viergutz //** Leibniz-Institut für Nutztierbiologie (FBN)

## ADVISORY BOARD

**Dr. Wolfgang Beisker //** GSF – Institut für Toxikologie, Labor Durchflußzytometrie

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**Dr. Henrik Mei //** Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin

**Dr. Raghav Palankar //** Institute of Immunology and Transfusion Medicine, University Medicine Greifswald

**Dr. Frank A. Schildberg //** Klinik und Poliklinik für Orthopädie und Unfallchirurgie der Uniklinik Bonn

**Dr. Stephan Schmid //** Uniklinikum Regensburg, Klinik für Innere Medizin 1

**Dr. Frank Schmidt //** University of Greifswald Functional Genomics

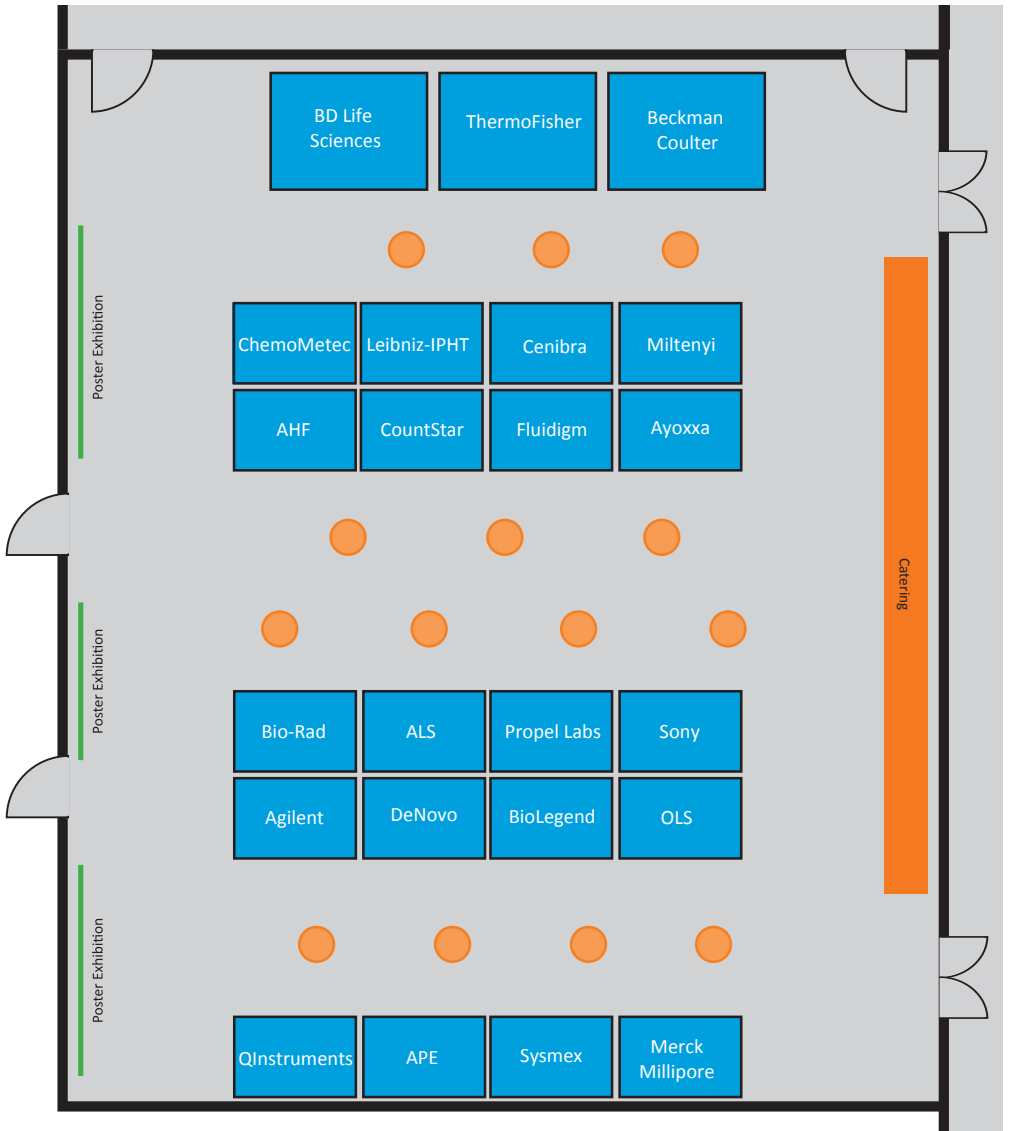**Dr. Gergely Toldy //** Birmingham Women's Hospital, Birmingham, UK

# Excellent Plus Sponsors



# Excellent Sponsors

# Floorplan Exhibition Hall



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Poster Exhibition

ChemoMetec | Leibniz-IPHT | Cenibra | Miltenyi

AHF | CountStar | Fluidigm | Ayoxxa

Poster Exhibition

Bio-Rad | ALS | Propel Labs | Sony

Agilent | DeNovo | BioLegend | OLS

Poster Exhibition

QInstruments | APE | Sysmex | Merck Millipore

Catering

# Core Facility Networking Event

This event should be an opportunity to meet and share your experiences and challenges in working in a core facility. We will report on the latest developments regarding the platform „cytometry.de", including:

- follow up on the activities
- cytometry.de as a resource for education material
- job shadowing program - from the view of a user and a host ...

We will also give you an overview about what the international society (ISAC) has in place for core facility staff and summarize the ‚Cytometry Part A' publication on best practices in a flow cytometry shared resource lab (SRL).

We hope to spend a motivating morning session with you for a good start into the DGfZ-Meeting 2017.

# Tutorials

It is a good tradition of the Annual Conference of the German Society for Cytometry to offer pre-conference tutorials. The tutorials are a great opportunity to gain deeper insights and practical skills with a special focus on cytometry related content. Therefore the tutorials are recommended especially, but not solely for students. The tutorials are free of charge for registered participants of the conference.

## Tutorial 1

**9:00-Noon**  **Presentation and demonstration of flow cytometers //**
**Nico Andreas / Thomas Kamradt //** University Hospital Jena, Institute of Immunology

## Tutorial 2

**10:30-Noon**  **Scientific publication with special aspects for Cytometry Part A //**
**Attila Tárnok //** Seminar Room 04.03.07, University of Applied Sciences

# October 4th // 12:45-14:15

# Session 1: Nanotechnologies

Chair: **Ulrike Taylor**
Chair: **Wolfgang Fritzsche**

The increasing usage of nanomaterials for a plethora of purposes causes a steep rise in exposure, intended as well as uninteded, of such materials to a wide range of organisms. The inadvertend creation of micro- and nanoparticles, for instance by decay of plastic rubbish in the oceans, adds to the scenario. As a major point in all nanomaterial related studies is the reliable particle detection and characterisation, this years session emphasizes on this vital component for studying the consequences of interaction between nanomaterials and biological matter.

**Gunnar Gerdts //**
Alfred Wegener Institute (AWI), Helgoland, Germany

## Microplastics – What we know and what we don't know

Since the middle of last century, the rapidly increasing global production of plastics has been accompanied by an accumulation of plastic litter in the marine environment. Dispersal by currents and winds does not diminish the persistence of plastic items which degrade and become fragmented over time. Together with micro-sized primary plastic litter from consumer products these degraded secondary micro-fragments lead to an increasing amount of small plastic particles (smaller than 5 mm), so called "microplastics". The ubiquitous presence and massive accumulation of microplastics in marine habitats and the uptake of microplastics by various marine biota is now well recognized by scientists and authorities worldwide. Although awareness of the potential risks is emerging, the impact of plastic particles on aquatic ecosystems is far from being understood. A fundamental issue precluding assessment of the environmental risks arising from microplastics is the lack of standard operation protocols for sampling, detection and analysis microplastics. Consequently, there is a lack of reliable data on concentrations of microplastics and the composition of polymers within the marine environment. Comparability of data on microplastics concentrations is currently hampered by a huge variety of different methods, each generating data of extremely different quality and resolution. Although microplastics are recognized as an emerging contaminant in the environment, currently neither sampling, extraction, purification nor identification approaches are standardized, making the increasing number of microplastics studies hardly -if at all- comparable.

**Abstract – Session 1: Nanotechnologies**

<u>Yuting Guo[1]</u>, Sabine Baumgart[2], Hans-Joachim Stärk[3], Hauke Harms[1], Susann Müller[1]* //
1_Department of Environmental Microbiology, Helmholtz Centre for Environmental Research, Leipzig, Germany, 2_Department of Immune Monitoring, German Rheumatism Research Centre, Berlin, An-Institute of the Leibniz Association, Germany, 3_Department of Analytical Chemistry, Helmholtz Centre for Environmental Research, Leipzig, Germany
**\*Correspondence to: Susann Müller, E-mail: susann.mueller@ufz.de**

# Mass cytometry for detection of silver at the bacterial single cell level

**Background:** Mass cytometry (CyTOF) allows single-cell characterization on the basis of specific metal-based cell markers. In addition, other metals in the mass range such as silver can be detected per cell. Bacteria are known to be sensible to silver and a protocol was developed to measure both the number of affected cells per population and the quantities of silver per cell.

**Methods:** For mass cytometry ruthenium red was used as a marker for all cells of a population while parallel application of cisplatin discriminated live from dead cells. Silver quantities per cell and frequencies of silver containing cells in a population were measured by mass cytometry. In addition, live/dead subpopulations were analyzed by flow cytometry and distinguished by cell sorting based on ruthenium red and propidium iodide double staining. Verification of the cells' silver load was performed on the bulk level by using ICP-MS in combination with cell sorting. The protocol was developed by conveying both, fast and non-growing Pseudomonas putida cells as test organisms.

**Results:** A workflow for labeling bacteria in order to be analyzed by mass cytometry was developed. Three different parameters were tested: ruthenium red provided counts for all bacterial cells in a population while consecutively applied cisplatin marked the frequency of dead cells. Apparent population heterogeneity was detected by different frequencies of silver containing cells. Silver quantities per cell were also well measurable. Generally, AgNP-10 treatment caused higher frequencies of dead cells, higher frequencies of silver containing cells and higher per-cell silver quantities. Due to an assumed chemical equilibrium of free and bound silver ions live and dead cells were associated with silver in equal quantities and this preferably during exponential growth. With ICP-MS up to 1.5 fg silver per bacterial cell were detected.

**Conclusion:** An effective mass cytometry protocol was developed for the detection and quantification of silver in single bacterial cells of different physiological states. The silver quantities were generally heterogeneously distributed among cells in a population, the degree of which was dependent on micro-environmental conditions and on silver applied either in ion or nanoparticle-aggregated form.

Martin Hussels[1], Elia Wollik[1], Dietmar Lerche[2] //

1_Physikalisch-Technische Bundesanstalt, Abbestr. 2-12, 10587 Berlin, Germany, 2_LUM GmbH, Justus-von-Liebig-Str. 3,12489 Berlin, Germany

## Development of a flow cytometer for nanoparticle detection

Detection of nanoparticles is of major and increasing interest in research and industry e. g. pharmaceutic industry. In cytometry, especially detection of extracellular vesicles (EVs) is a rapidly developing field. However, detection of nanoparticles and in particular EVs is challenging and quite different detection methods are typically used, such as dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), flow cytometry, and even sophisticated methods as cryo electron microscopy and electrospray-differential mobility analysis [1,2]. All these methods have particular advantages and disadvantages. For example, flow cytometry for nanoparticle detection has the advantage of high throughput single particle detection, enabling analysis of broad size distributions and polydispers systems. But, conventional flow cytometers lack sensitivity to detect the majority of EVs and a lot of other nanoparticles.

We are developing a flow cytometer with significantly enhanced sensitivity in light scattering detection, which enables detection of nanoparticles smaller than 100 nm using only light scattering. In combination with the accurate sample volume measurement developed at PTB [3] this enables determination of nanoparticle concentration with low measurement uncertainty. Additionally, we are increasing the dynamic detection range to detect micro and nanoparticles within one measurement. This may be helpful for example to detect EVs and cells at once making EV detection with simplified sample preparation possible.

The current setup at PTB has a lower detection limit of approximately 65 nm for polystyrene particles in side scatter and approximately 100 nm for forward scatter. Additionally, the setup shows a very good resolution in signal intensity enabling accurate particle sizing. However, the lower detection limit for detectable particle size by light scattering strongly depends on the index of refraction of the material which the particles consist of.

**References:**
1. U. Erdbrügger, and J. Lannigan, Cytometry Part A, 2016, 89A, 123-134.
2. N. Cloulet-Foraison, F. Gaie-Leverel, L. Coquelin, G. Ebrard, P. Gillery, V. Delatour, Analytical Chemistry, 2017, 89, 2242-2249
3. S. Reitz, A. Kummrow, M. Kammel, J. Neukammer, Measurement Science and Technology, 2010, 21, 074006

# October 4th // 14:15-15:45

## Session 2: Product Slam

Chair: **Frank Schildberg**
Chair: **Elmar Endl**

Industrial partners of the conference will get the chance to present their newest innovations and products within three minutes in this latest event. This type of appetizer is a fantastic marketing opportunity supplying talking points for later conversations. As bonus, there will be a prize for the best performance – chosen by vote of the audience.

| Exhibitor | Presenter |
| --- | --- |
| **Agilent Technologies** | Joseph Liotta |
| **ALS Automated Lab Solutions** | Katharina Uhlig |
| **AquArray** | Dr. Simon Widmaier |
| **Ayoxxa GmbH** | David Sharp |
| **Beckman Coulter** | Nadine Kärner |
| **Becton Dickinson** | Wieland Keilholz |
| **BioLegend** | Stefanie Ries |
| **Cenibra** | Geziel Aguilar |
| **Countstar** | Bodo Kohring |
| **De Novo Software** | Andrea Valle |
| **Fluidigm** | Roberto Spada |
| **Merck Millipore SAS** | Peter Rhein |
| **OLS OMNI Life Science** | Peter Engel |
| **Propel Labs Europe** | Mark Cheetham |
| **Sony Europe** | Sebastian Weissmann |
| **Sysmex** | Christoph Rothfuchs |

# October 4th // 16:45-18:15

# Session 3: Cutting Edge

Chair: **Elmar Endl**
Chair: **Wolfgang Fritzsche**

Cutting-edge research not only evolves from newly invented and optimized technologies and strategies, but may arise from combining well-known technologies in an new and optimized way. The combinations of flow cytometry and Next Generation Sequencing or microscopy and mass spectrometry are just recent examples. By now it is not only the challenge to get sufficient and reproducible data, but to handle the massive amount of data in a way so that meaningful results can be derived in a reasonable time. The keynotes of this session will give you an impression on that. The session will be supplemented by talks on spectral tools to provide a new look on cells and add some fresh points for discussion.

Marc Beyer //
German Center for Neurodegenerative Diseases (DZNE), Bonn

# Single cell transcriptomics to better understand immune cell differentiation and heterogeneity

Immunology has greatly benefited from advances in cellular phenotyping using mass and flow cytometry. With this new subpopulations could be unraveled and their function for the immune system resolved.

Since a few years, immunology is witnessing a similar revolution as recent technological innovations, such as single-cell transcriptomics have become available which possess the power to measure thousands of features for thousands to millions of cells in parallel, thereby allowing the deep characterization of complex cellular networks in homeostasis as well as under disease conditions. These multidimensional approaches dramatically accelerate the discovery of novel cell populations but also shed light on the heterogeneity of immune cells.

These approaches now allow for the characterization of small amounts of material on the transcriptome level to allow for an unbiased, high-dimensional, and bioinformatically supported systems biology approach which enables a better understanding of immune cell function in a rapid fashion.

Taking myeloid cells as an example the analysis of differentiation processes as well as cellular ontogeny has allowed us to answer questions in this field, which so far could not be sufficiently answered.

**Dr. Theodore Alexandrov //**
EMBL Heidelberg, Germany

## Picturing metabolism of individual cells

Every cell is unique. Metabolites define the composition of each cell and play key roles in essential intracellular processes of energy production and uptake, signaling, regulation, and cell death. Obtaining metabolite signatures of individual cells and linking them to cellular phenotypes is of paramount importance for a holistic understanding of these processes. We recently developed a novel approach to obtain metabolite profiles from tens of thousands of individual cells in adherent culture and link them to the cells phenotype, in particular by correlating with fluorescent microscopy. We have applied this approach to study human hepatocytes cultured with inflammatory cytokines and were able to molecularly characterize the lipid droplet formation due to the immune response. This approach opens completely novel avenues in studying metabolism inside single cells.

**L. Vaccari[1], S. Kenig[1,2], R. Mendoza-Maldonado[3], P. Zucchiatti[1,4], D. Bedolla[1], G. Birarda[1], E. Mitri[1] //**
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## Cell cycle progression: A comparison of spectroscopic and cytofluorimetric signatures

Cellular proliferation, differentiation and death are tiny regulated events at the basis of organism life and the interplay between them is crucial for hindering disease occurrence and progression and can eventually enable a successful therapy. The knowledge of cell cycle phase distribution is of paramount importance for understanding cellular behavior under normal and stressed conditions, and this information is normally obtained by Flow Cytometry (FC) or immunohistochemistry, which usually requires fixation and labelling procedures. This talk is intended to present the most relevant results in the field of cell-cycle assessment by means of Fourier Transform Infrared Microscopy (FTIRM) obtained by the research team working at the infrared beamline of Elettra Sincrotrone Trieste, SISSI (Synchrotron Infrared Source for Spectroscopy and Imaging) [1].

In particular, asynchronous, S- and G0-synchronized B16 mouse melanoma cells were studied by running parallel experiments of FTIRM on living unlabeled cells and FC using Propidium Iodide (PI) staining. Hierarchical Cluster Analysis (HCA) of cellular spectroscopic data in the $1300\text{-}1000\,cm^{-1}$ region pointed out a distribution of cells among G0/G1, S and G2/M phases which was in good agreement with FC results (See Fig 1, left panel). The differentiation was mostly driven by the intensity of the IR bands associated to Nucleic Acid (NAs), both DNA and RNA [2]. We envisaged the possibility to exploit this dual dependence for discriminating between G1-progressive and G0-resting phase on the base of their different total amount of nucleic acids, and corroborated this hypothesis by correlating FTIRM results with FC-PI data coupled with immunohistochemistry results [3].

On the same topic, we will also present results of FC assay based on Acridin Orange (AO) fluorescent dye, which allows the simultaneous evaluation of both DNA and RNA content. FTIRM experiments were done in parallel, on both B16 and T98G single hydrated cells, and the spectral contributions of both NAs were separately evaluated by deconvolving the symmetric and asymmetric stretching bands of phosphate moieties [4]. The good agreement among the techniques (See Fig. 1, right panel) further demonstrates the capability of FTIRM to assess all the stages of the cell cycle at the level of a single live cell, without the use of any label and providing more information than conventional FC-PI.

It is possible to summarize that, at the current stage of knowledge and IR-transparent fluidic devices development [5], FTIRM can be considered a valuable technique for in situ cell sorting: upon localization of individual cells within the device and assessment of their cell-cycle stage, the effects of stressor agents can be followed by FTIRM itself (See Fig 1, central panel). Further steps in technique maturation needed for its broader exploitation will be envisaged in this presentation.
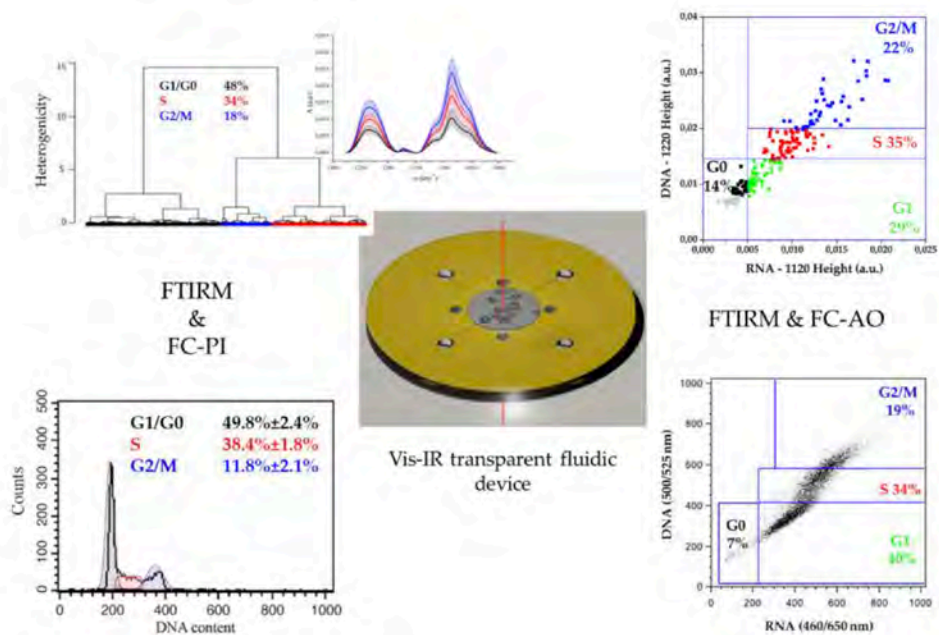


**Figure 1:** Central panel: drawing of Vis-IR transparent fluidic devices, suitable for in situ cell sorting and probing; Left Panel: comparison of the result achieved of FC-PI and FTIRM; Right panel: comparison of the results achieved by FC-AO and FTIRM.

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5. G. Birarda, A. Ravasio, M. Suryana, S. Maniam, H.-Y. N. Holman, G. Grenci, Lab Chip, 2016, 16, 1644-1651

## Abstract – Session 3: Cutting Edge

**Toralf Kaiser[2], Konrad v. Volkmann[1], Claudia Giesecke[2], Jenny Kirsch[2], Kristen Feher[2]**
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## Adding multispectral capabilities to a standard flow cytometer – a report on benefits and promising first results

In recent years, multispectral flow cytometry systems have come to attention. They differ from conventional flow cytometers in two key ways: a multispectral flow cytometer collects the full spectral information at the single cell level by using a dispersing element and the detector configuration is fixed and not explicitly tuned to a particular staining panel. We present an approach of transforming a standard commercial flow cytometer into a multispectral cytometer which is able to measure the entire emission spectrum of fluorophores excited by a 488nm laser. In our instrument, the emitted fluorescence light of the cells is spectrally dispersed by optical bandpass filters and processed by extremely low noise electronics. Moreover, compensation is not required due to the robust instrumentation (fixed optical configuration, one laser) and unbiased data analysis. This offer new possibilities for unsupervised flow cytometry data acquisition and analysis such as high throughput analysis.

We show that the increased light collection of our multispectral flow cytometry lead to increased sensitivity, an improved detection limit, and a higher resolution.

An eight-color immunophenotyping T-cell staining was used to validate the multispectral setup. Data were analyzed using a novel algorithm based upon Common Principal Analysis and FMO controls.

# October 4th // 18:15-19:15

# Keynote

Chair: **Wolfgang Fritzsche**

Eugen Ermantraut started his first company with QUANTIFOIL Micro Tools in the field of Electron Microscopy while working on his Master thesis in 1995. He developed an interest in Microarrays and founded CLONDIAG together with colleagues where he pioneered the integration of microarrays into diagnostic analysis systems and led the development of a nucleic acid Point-of-Care product that later was commercialized by ALERE under its Alere Q brand. After the acquisition of CLONDIAG by ALERE he led the site in Jena during its transformation from a R&D facility into a IVD development and manufacturing site. With the PIMA CD4 system his team launched the world's first commercial image cytometer for Point-of-Care use. Over a short time the product became the standard of care for HIV staging in many countries all over the world. After leaving ALERE in 2015 together with his team Eugen started BLINK to develop a novel diagnostic product platform combining different analytes in one format and providing access to third party developers.

**Eugen Ermantraut //**
BLINK AG, Winzerlaer Strasse 2, Jena, Germany
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## Cytometry at the Point of Care – why and how this makes sense.

The analysis of cells and cell associated parameters is an integral component of comprehensive laboratory diagnostics. Driven by the need to establish diagnostic capacity outside of a working laboratory infrastructure predominantly in resource poor settings dedicated technologies and products have been developed. Our group was involved in the development and deployment of the first truly mobile cytometer system. With the Pima CD4 product disease staging became available right after HIV diagnosis. Millions of HIV infected individuals in the most remote settings have been linked to care. In spite of its specific success the use and application of this product like for any other point of care product remained limited to a narrow application niche. Notwithstanding available technologies the bulk of diagnostic testing still remains with the centralized laboratory. The fundamental reasons for this will be discussed and possible solutions will be presented that may be leading to a paradigm change in the delivery of diagnostic services. By integrating cytometric analysis into holistic diagnostic workflows including nucleic acid tests, immunoassays and clinical chemistry analysis decentral diagnostics may step out of its application niche and become the standard of care.

# October 5th // 9:15-10:45
## Session 4: Microbiology

## Microbiology and beyond

Chair: **Christin Koch**
Chair: **Alexander Grünberger**

"The whole is greater than the sum of its parts" – with this concept in mind we will look at different aspects of microbial behavior and interaction and challenge the concept of autonomous individual cells.

The human body is only vital as meta-organism consisting of eukaryotic and microbial cells. Representative in vitro models to investigate their interactions were recently developed and allow new insights into this unique interface. Likewise, complex microbial interactions challenge our understanding of the individual physiological potential and metabolic autonomy. This session will focus on new technologies and methods to study microbial cells and their interactions on single cell level. This lays the foundation to develop a detailed understanding of microbial behaviour and to provide personalized model systems for microbiome and immunology research.

Joanna Baginska, Paul Wilmes //
Luxembourg Centre for Systems Biomedicine, Université du Luxembourg

## Microfluidics-based co-culture device, HuMiX, allows proximal co-culture of human epithelial cells and microbes

General awareness of the microbiome and its effect on human health and disease has grown in recent years and today attracts major interest from the global pharmaceutical, nutritional and biotechnological industries. Driven by technological innovation, the growing depth of microbiome knowledge has dissected associations between specific microbial communities and different pathological conditions and diseases. In this context, we recently developed a novel microfluidics-based human-microbial co-culture system – HuMiX. The HuMiX platform consists of three parallel microfluidic channels acting as microbial, epithelial, and immune microchambers, respectively. Each microchamber has dedicated inlets and outlets, which allow for the inoculation of the relevant cell lines and the precise control of the physiochemical conditions through the perfusion of dedicated cell growth media. HuMiX is the first representative model of the human gastrointestinal interface which allows for the systematic investigation of molecular interactions between immune, epithelial and bacterial cells in vitro. A better understanding of the interaction between the microbiome and the gut will lead to the development of new guidelines for personalized treatment, help to improve global human health, and establish ways to prevent and treat various diseases via personalized pharmaceutical and/or dietary interventions. Therefore, we use HuMiX for testing the efficacy of drugs as well as dietary compounds to address a clear industrial demand and meet the needs of a developing market on the edge of microbiome science, immunology, oncology and nutrition sectors.

**Christian Kost** //
Department of Ecology, School of Biology / Chemistry, University of
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## Stronger together:
## On the benefits of dividing metabolic labor in bacteria

Cross-feeding interactions, in which bacteria exchange essential metabolites, are very common in the microbial world. However, due to difficulties to study these often tightly interwoven interactions, the factors that facilitate the evolution of these obligate interactions as well as the consequences that result for the interacting partners, remain poorly understood. We solve this problem by analysing synthetically engineered as well as naturally evolved cooperative cross-feeding interactions between two or more bacterial genotypes. Our experiments reveal that strong adaptive fitness benefits drive the loss of seemingly essential biosynthetic genes. The energy bacteria save when dividing their metabolic labour in this way facilitates the entering into obligate cross-feeding interactions. Moreover, we discovered that bacteria use nanotubes to directly transfer cytoplasmic nutrients between bacterial cells and that this exchange can couple the metabolism of two interacting partners in a source-sink-like relationship. Taken together, our results suggest that strong selective advantages drive the loss of metabolic autonomy in bacteria and the evolution of metabolic cross-feeding interactions. The possibility that by connecting via nanotubes bacterial cells can significantly extend their biochemical repertoire without the need for genetic change, suggests bacteria may function as multicellular, interconnected entities rather than as individual, physiologically autonomous units.

## Abstract – Session 4: Microbiology

**Raphael Freiherr von Boeselager**, Tino Polen, Julia Frunzke //
Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, Germany

# Cytometry meets Next Generation Sequencing – Towards systems-level analyses of phenotypic heterogeneity

Even clonal microbial populations may develop significant phenotypic differences at the single-cell level thereby fostering the optimal adaptation of the particular species. Therefore, novel tools need to be developed to address various questions of microbial population dynamics [1]. Here we developed a novel workflow to analyse the transcriptome of FACS-sorted bacterial subpopulations. For proof-of-concept, we studied the activation of the cryptic prophage CGP3, which shows a heterogeneous response in clonal populations of Corynebacterium glutamicum [2]. Overall, the transcriptome profile of 105 to 106 sorted cells matched the unsorted control but also revealed effects previously obscured by the analysis of bulk data. Further attempts focus on the analysis of cell sorting effects on the transcriptome and the optimization of storage conditions. In summary, we present a novel workflow combining FACS and RNA-Seq enabling to unravel differences in transcriptome profiles of microbial subpopulations.

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**Zishu Liu**, **Nicolas Cichocki, Florian Schattenberg, Florian Centler, Fabian Bonk, Susann Müller //**
Helmholtz-Zentrum für Umweltforschung GmbH, Leipzig, Germany

## Natural microbial community cytometric analysis

Microbial communities play a key role in both natural water body self-purification and waste water treatment engineering. Research on their composition and function are important and open possibilities for community support and biotechnological process control.

Microbes are extremely small in size, have short generation times and are vulnerable to surrounding environments. In nature, the majority of microorganisms cannot easily be distinguished by physiological characteristics and, in addition, most of them cannot be cultivated in laboratory. Thus, monitoring and analysing environmental microbes need specific requirements. One of those tools is the application of cultivation - independent molecular techniques, e. g. NGS, which allows a comprehensive observation of microorganisms in nature, and a gradual understanding of their behaviours. However, the NGS technology is still too expensive and time consuming with regard to conventional pipelines of data evaluation to be applied with a frequency which is similar to the generation time of the species that are part of microbial communities. Recently, single cell based high-throughout flow cytometry has been used and applied to the analysis of natural microbial communities and shown to describe structural dynamics of microbial communities in high resolution [1]. Different to the NGS technology the costs are low, and the time for measurements and evaluation of the data is fast and can be performed nearly automatically.

To explain the behaviour of microbial communities, advances on applying ecological theories from macroecology to cytometric data have been made recently where the contribution of species sorting and mass transfer paradigms to community assembly were investigated [2]. In addition to describing community assembly, calculation of both dynamics and evolution of microbial communities is a further step in understanding community behaviour. Also diversity metrics can be identified now. In this presentation, a workflow for calculation changes in natural microbial community structures is displayed in several steps. Metrics to quantify variations in community structure and how to connect those variations with theoretical ecological terms will be shown.

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# October 5<sup>th</sup> // 11:15-12:45

## Session 5: European Guest Session

Chair: **Wolfgang Fritzsche**

For more than 20 years, the French Association of Cytometry has brought together people, laboratories and companies all with a common interest in flow and / or image cytometry. Its mission is:

· to promote and organize reflection and action on the themes of cytometry and histometry in all their aspects and in particular flow cytometry, image cytometry and quantitative microscopy
· to bring together the people concerned by the scientific, methodological and technical problems of the field, which include, among others, the fundamental, experimental and applied aspects
· to take initiatives to establish links with relevant national and international bodies
· to carry out actions to ensure the promotion of „young cytometrists"

Lydia Campos-Catafal, Tiphanie Picot, Amandine Fayard, Carmen Aanei,  Emmanuelle Tavernier, Pascale-Flandrin-Gresta, Sylvie Tondeur, Denis Guyotat //
Association Française Cytomētrie (AFC), CHU St. Etienne, France

## High expression of the pluripotency-associated transcription factor OCT¾ associated with differentiation anomalies in acute myeloid leukemia and myelodysplastic syndromes

**Introduction:** Acute Myeloid Leukemia (AML) and high-grade myelodysplastic syndromes (MDS) are characterized by the expansion and resistance to apoptosis of poorly differentiated myeloid cells. Long term dissemination of the disease is mainly due to a subset of cells termed leukemic stem cells (LSC), which reacquire self renewing properties and give rise to the leukemic clone. Several studies have suggested that LSC mostly belong to the CD34+CD38- compartment and represent the malignant counterpart of normal hematopoietic stem or progenitor cells (HSC). Self-renewal and lack of differentiation are also features of embryonic stem cells, characterized by the expression of specific genes including those known as "Yamanaka's factors", including the pluripotency-associated transcription factor OCT¾. A few reports show an abnormal expression of OCT¾ in solid tumors in which it induces pluripotency and suppresses differentiation, but no study is available in AML. We studied the potential role OCT¾ in leukemogenesis by evaluating its expression in stem cells from AML and MDS patients compared to normal HSC subsets (CD34+CD38- and CD34+CD38+).

**Methods:** We studied 150 AML and 42 MDS newly diagnosed cases by Multicolor Flow Cytometry (MFC) and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). We also evaluated in HL60 leukemic line its potential involvement in myeloid differentiation by inhibiting its expression by short hairpin RNA (shRNA).

**Results:** In MCF, we observed an up-regulation of OCT¾ in AML cells as compared to normal cells (Mean of fluorescence 4.5 vs 3.2, $p < 0.05$, cycle threshold 26 vs 37, $p < 0.05$). This expression was higher in CD34+CD38- than in CD34+CD38+ subsets in normal as well as in leukemic marrows. There was no evidence of preferential association with cytogenetic or molecular characteristics of AML. Furthermore, an over-expression of OCT¾ was observed in MDS, more specifically in forms with excess of blasts. We also evaluated the prognostic value of OCT¾ expression in the 103 AML patients who received an intensive treatment. The rate of complete remission was influenced by the OCT¾ level. There was a significant difference ($p=0.005$) for better overall and leukemia-free survival for patients with high OCT¾ expression. Finally, HL60 differentiation with retinoic acid induced a decrease of OCT¾ expression. Inhibition experiments by shRNA showed a strong downregulation of OCT¾ and an arrest of proliferation associated with myeloid differentiation.

**Conclusion:** These results suggest that aberrant expression of OCT¾ in AML and MDS is associated with differentiation blockade and that it could be a therapeutic target.

**Carmen Aanei //**
Department of Hematology, University Hospital of Saint-Etienne,
France

## The bone marrow microenvironment in MDS: emerging concepts with therapeutic implications

Myelodysplastic syndromes (MDS) are heterogeneous, clonal hematopoietic stem cells disorders, with different prognostic and increased risk of transformation to acute myeloid leukemia (AML)1. MDS predominates in the elderly and the number of newly diagnosed patients will be expected to increase in the future due to the ageing of the population and increasing number of long-term cancer survivors2. In MDS, malignant hematopoietic stem progenitor cells (HSPCs) acquire multiple genetic and epigenetic abnormalities, which progressively drive the disease evolution1.  The therapies directed against the MDS hematopoietic clone has limited success, which reflects our inadequate insight into disease pathogenesis.

Nowadays, emerging evidence indicates that apart from intrinsic HSPC abnormalities, the bone marrow microenvironment may also contribute to the ineffective, malignant hematopoiesis3-5. Mesenchymal stromal cells (MSC) represent one of the main cellular components of the bone marrow microenvironment, which express a number of adhesion molecules and secreted factors that regulate blood regeneration by contributing to hematopoietic stem and progenitor cell maintenance, self-renewal and differentiation.

Concept of niche-induced dysplasia in the hematopoietic system has been demonstrated by several seminal studies. Inactivation of the micro-RNA-processing enzyme dicer in immature osterix-expressing progenitors causes MDS6. Loss of function mutations in SBDS gene (SHWACHMANN-BODIAN-DIAMOND Syndrome) in Cebpa-expressing HSPCs cells induces a multisystemic disorder in mice only in presence of the SBDS gene mutation in MSCs 7. Nuclear localization of $\beta$-catenin associated with activation of Notch signaling in osteoblasts induces MDS/AML in mice8. In addition, Meydouf et al. showed that the engraftment of CD34+ MDS derived cells in NSG mice fails in the absence of co-transplantation with patient-derived MSCs that allows efficient and long-term MDS reinstallment in NSG mice5.

Previous work from our team showed that in hi-risk MDS the hyper-phosphorylation of the Focal adhesion kinase (FAK) at Y937 site induce its colocalization with HSP90$\alpha$/$\beta$ and paxillin, which than promote nuclear sequestration of paxillin with increased MSC proliferation and, indirectly, with impairment of hematopoiesis support9,10.  Moreover, we observed the gradual augmentation of FAK expression and activation during disease progression11.  The increased expression and activation of FAK could therefore be considered as a surrogate marker for disease progression and as potential therapeutic target to improve hematopoiesis support (in low-risk MDS) and decrease chemo-resistance (in high-risk MDS and AML).

We also noticed that in low risk-MDS (LR-MDS), the MSCs showed impaired growth and

clonogenic capacity, which were independent of cellular senescence and apoptosis, but related with the decreased adhesion markers expression, especially with diminution of CD44 and CD49e markers9,11. In addition, the MSCs selected from MDS patients showed a higher propensity toward the pro-adipogenic differentiation and an attenuated osteogenic capacity along with reduced SDF-1 expression, characteristics that might be involved in creating an unfavorable microenvironment for hematopoiesis11. This abnormal differentiation ability correlates with the significant FAK downregulation and hypophosphorylation11.

However, we still have to determine whether FAK defect is directly responsible for these abnormalities in LR-MDS MSCs and how it influences MSC-HSCP interaction leading to ineffective hematopoiesis.

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**Abstracts**

# October 5th // 12:45-13:30
# Poster Pitch & Poster Session

# P01

Rico Bongaarts – Union Biometrica //

## Comparison of Total Fluorescence in Cell Clusters versus Fluorescence of the Individual Cells in the Cluster

In the analysis of cell clusters composed of a mixture of fluorescent and non-fluorescent cells it has been questioned how the amount of fluorescent cells in the clusters can be discriminated. The level of fluorescence in a cell cluster is a result of the number of positive cells in the cluster and the intensity of light emitted from the fluorescent marker on/in those cells. In this QTN we compare the analysis of the fluorescence level of an entire cell cluster with the fluorescence measurement of the individual cells of that particular cell cluster. The BioSorter® was used to measure and dispense an individual cell cluster into the well of a multiwall plate. Then, cells of the cluster were separated from each other and analysed by conventional single cell flow cytometry. We compare the fluorescence mean intensity of the cluster as measured on the BioSorter with the total number and mean channel of fluorescent cells within the cluster measured on a conventional single cell flow cytometer.

# P02

Mohammad Dabaghi, Ingrid Hilger //
Department of Experimental Radiology, Institute for Diagnostic and Interventional Radiology, University Hospital Jena, Germany

## Influence of coating formulation, cell culture medium and cancer cell type on the uptake of magnetic nanoparticles

This research aimed to study the characterization and behavioral changes of magnetic nanoparticles (MNP) with different coating formulations suspended in biological solutions (cell culture mediums) and their influence on cellular uptake of MNPs in in vitro cancer therapy studies. For this purpose, four iron oxide magnetic nanoparticles (MNP) coated with different formulations called chitosan (CS), polyvinyl alcohol (PVA), carboxymethyldextran (CMX) and polydimethylamine (PEA) were functionalized with 5-Fluorouracil (5FU) and characterized in distilled water. 5FU functionalization was done in order to investigate the drug delivery potential of MNPs. Also, six different cell lines were used in this research in order to study the cytotoxic effect of MNPs and the uptake rate differences. The cytotoxic effect of MNPs/5FU-MNPs was evaluated using AlamarBlue test and Prussian blue staining method was used in order to stain MNPs which were taken up by cells. Then, the MNPs were suspended in four different cell culture mediums and were characterized again. Characterizations (hydrodynamic

diameter, surface charge, polydispersity index and zeta-potential) were performed via dynamic light scattering (DLS) method. The sedimentation (Vs) and diffusion (Vd) velocities of MNPs were calculated using formulas reported in a recent study (1), for all mediums used in this research. The uptake of PVA and CMX MNPs by cells were quantified through atomic absorption spectroscopy (AAS) method.

Our results showed that all 5FU functionalized MNPs were cytotoxic to cells but not the native MNPs. We also found that all MNPs have almost the same hydrodynamic diameter in distilled water whiles, the size of CMX, CS and PEA increased notably after suspension in cell culture mediums probably due to adsorption of different proteins (2). However, PVA MNP hydrodynamic diameter increased slightly. The calculations indicated that CMX, CS and PEA MNPs have higher Vs and less Vd than PVA MNP. We showed that CMX MNP, which sediments faster than PVA MNP, interacted more with cell and was taken up more by all cell lines used in this research. Cell viability results showed that 5FU can bind electrostatically to the surface of MNPs in absence of any cross-linker. Also, the results, in confirmation of the previous findings, indicated that the changes of MNPs characterizations such as the agglomeration behavior, surface charge, Vs and Vd are governed by surface coating formulations and vary among different cell culture mediums (3). This research suggests that MNPs with higher sedimentation rate interact more with cells and consequently are taken up by cells in a higher extent.

This research suggests that the sedimentation rate plays the most important role in the extent of interaction between MNPs and cells in in vitro studies. However, the coating formulation of MNPs and the cell culture medium and its protein content dictate the characterizations and agglomeration behavior of MNPs and this in turn determines the sedimentation rate.

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# P03

**Roy Edward //**
BioStatus Ltd., Shepshed, Leicestershire, UK

## Channel- & compensation-free dead cell exclusion: N+1 panel design

**Introduction:** Exclusion of dead cells is a common requirement for flow cytometry using a viability dye, historically with DAPI, PI and 7-AAD. A new far-red fluorescing alternative DRAQ7 has been robustly tested (e.g. Akagi, et al., 2013; Smith, et al., 2013) and shown to have improved separation between negative and positive events (Moshaver et al., EBMT Conference 2013; Beckman Coulter, FLOW-1710APP05.16-A, 2016). Conventional flow cytometry is limited by spectral "space" and preference for bright chromophores to label antibodies to aid sensitivity increases need for compensation. At its simplest, far-red DRAQ7 benefits panel design e.g. no overlap with FITC/R-PE pairs makes it highly compatible with single platform CD34+ cell counting. Of specific interest, DRAQ7 is optimally orange-red excited (599nm, 644nm) yet is usefully excited with blue, green, yellow laser lines, permitting deployment on basic cytometers. In principle, DRAQ7 occupies multiple fluorescence channels but this enables a unique display of dead cell events in a "virtual channel"; plotting pairs of red/far-red channels from different excitations against each other one can choose a suitable pairing that gives the preferred separation of a double-positive population for a chromophore panel. These double-positive events define dead-cells to be excluded by a live cell gate and from other channels without compensation.

**Methods:** We employed a selection of common flow cytometry platforms to fully exemplify the concept. Multi-colour phenotyping panels containing various red/far-red chromophores were compensated for optimal performance and when applied to cell preparations had DRAQ7 added. In all cases, data was displayed to show bivariate plots for available combinations of relevant fluorescence channels. Initially, each was examined for suitability to describe a live cell gate excluding double-positive DRAQ7 events for that panel.

**Results:** In all cases it was possible to determine a bivariate plot that reliably and simply described the dual-excited DRAQ7 dead cell events to the exclusion of the single-excited chromophores on either axis of the bivariate plots.
Evidently, care should be taken, where possible, to avoid the labeling of co-expressed antigens with red/far-red chromophore tagged antibodies. However, it should remain possible to display sufficient bivariate plots that this combination can be overcome and a suitable alternative chosen.

**Conclusions:** This method permits "N+1" cytometry to design a phenotyping panel without prior consideration for inclusion of a viability probe, that can be added subsequently into samples alongside antibody cocktails without requirement for compensation, and where that viability dye had been accommodated originally.

# P04

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## Influence of the FACS Diva software threshold function on cell sorting

The BD FACS Diva software, which operates the BD FACS Aria cell sorter, offers a function to exclude particles from the analysis that is called threshold. It can be applied to any analyzed parameter whereby particles that carry signal intensity below the given threshold are not processed by the software. In this study the influence of the threshold on the sort purity and yield as well as on the RNA quality and gene expression of sorted murine hematopoietic stem cells are investigated. First, experiments were performed with standardized FITC and PE Calibrite beads, which were mixed in the same proportion. The FITC beads are sorted with a purity of nearly 100 % in the reanalysis. Adding a threshold to the FITC parameter so that the PE beads are excluded from the processing caused a reduction in the purity of the sorted FITC beads. In the reanalysis the PE beads were detected whereby the extent of contamination increased with the rate of excluded PE beads per second to a maximum of 27 % when 3800 PE beads per second were excluded. On the other hand the absence of the threshold caused a reduction of the sort yield in the same extent like the impurity observed in the sorts performed with threshold for the FITC parameter. In additional experiments it was investigated whether these observations depend also on the fraction of the sorted FITC bead population, on the used nozzle (70 μm, 85 μm, 100 μm) and on the sort precision mode (purity, 4-way purity, yield). It is shown that the percentage of the FITC bead population did not influence the sort purity and yield when the threshold was applied as long as the excluded number of PE beads per second was kept stable. In contrast to that, the nozzle influenced highly the outcome whereby purity and yield decreased with increasing nozzle size. The comparison of the purity and 4-way purity mode revealed that there is no difference in the sort yield if no threshold is applied, while the threshold induced decrease of the purity is around two times higher in the purity compared to the 4-way purity mode. When sorting in yield mode applying of the threshold did neither influence the sort purity nor the yield. The contamination with PE beads in the sorts performed with the yield mode was in the same range as the contamination induced by the threshold in the sorts using the purity mode. Taken together, these data provide the proof that exclusion of particles by the threshold leads to a significant contamination of the sorted sample with these particles. These contaminations might influence assays performed with sorted cells. To answer this question experiments in which murine hematopoietic stem cells are sorted with different threshold settings were performed. By increasing the threshold on the forward scatter during the sort the yield of extracted RNA increases. On the other hand, the RNA integrity in these samples is impaired. Additionally, the gene expression profiles were determined. The analysis of the data

is still ongoing. The data will help to optimize the sort settings in order to obtain samples with the maximal quality necessary to perform successful downstream assays like gene expression profiling or DNA sequencing.

# P05

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## Fully automated enumeration of CD34$^+$ hematopoietic stem cells using the MACSQuant Analyzer 10

Introduction: Flow cytometric enumeration of viable CD34$^+$ hematopoietic stem cells (HSC) from different sources provides critical information in various applications, e.g. the evaluation of stem cell grafts or the determination of optimal timing for donor apheresis. Due to the low frequency of HSCs, this rare-event analysis can prove to be a demanding and complicated procedure, creating the need for a standardized and reproducible workflow. The guidelines established by the International Society of Haematotherapy and Graft Engineering (ISHAGE) were a major step towards standardization, but operator variance can still be an issue. Here we demonstrate a fully automated process of HSC enumeration, including staining, lysis, acquisition and analysis using the MACSQuant Analyzer 10 (Miltenyi Biotec).

Methods: Automation of the complete workflow was realized by an Express Mode, including automated pipetting, sample transfer, acquisition and analysis of samples according to the ISHAGE guidelines. Finally, a report summarizing the results of each measurement, including the volumetrically determined absolute HSC count, can be generated. For the evaluation of the process, cord blood and the CD-Chex CD34 control reagent (Streck) were used. The results of the Express Mode samples were then compared to manually handled samples.

Results: With the automated workflow described here, it was possible to process six samples in parallel within less than 45 minutes for determination of viable CD34$^+$ HSC counts in cord blood or CD-Chex CD34 control reagent. These automatically obtained cell counts of HSCs were within the expected range for the different levels of the control reagent and similar to the manual controls. In addition, a spike-in experiment with magnetically isolated HSCs confirmed the linearity of the HSC count determination.

Conclusions: The results shown here demonstrate that the MACSQuant Analyzer 10 can be utilized to establish a standardized and automated workflow for HSC enumeration in order to reduce the intra- and interassay variability.

# P06

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## More from Less – Multiplex Protein Analysis for Translational Research

LUNARIS™ is AYOXXA's innovative and patented bead-on-a-chip technology platform to measure picogram quantities of proteins in precious biological samples. It represents a fully-integrated multiplex protein analysis system consisting of a dedicated reader, integrated analysis software and an expanding menu of kits enabling protein detection in translational biomedicine – all the way from basic research to clinically relevant studies. Due to extremely low sample volumes of just 3µl, LUNARIS™ high-quality immunoassays greatly expand types and numbers of precious samples. Ready-to-use kits are validated for the analysis of samples from serum and cell culture supernatant while numerous further matrices have been tested (e. g. CSF, saliva and ocular fluids).

AYOXXA is now launching dedicated LUNARIS™ kits for the analysis of cytokines relevant for the T helper 17 (Th17) related pathway in human and murine samples. Th17 cells are a subset of pro-inflammatory T helper cells defined by their production of interleukin 17 (IL-17). Cytokines secreted by Th17 cells play an important role in maintaining mucosal barriers and eliminating harmful microbes but they have also been implicated in autoimmune and inflammatory disorders, including rheumatoid arthritis, multiple sclerosis, and inflammatory bowel diseases.

Furthermore new Ophthalmology panels are available for the analysis of biomarkers relevant for diseases such as age-related macular degeneration (AMD) and diabetic retinopathy (DR).

# P07

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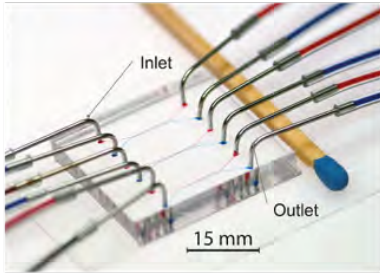
## Microfluidic Single-cell (co-) cultivation: From proof of concept to first application

Microbial communities represent one of the most fascinating complex systems in nature offering an unlimited diversity of biological processes that are of utmost importance at a global scale, but by far not understood. Microbial consortia play an important role in wastewater treatment, biogas production, bioremediation and have a high potential for future bioprocesses [1, 2]. Deeper insights into the communication and interaction between multiple strains and species is mandatory to understand naturally occurring and synthetic communities. Therefore, novel analytical methods need to be developed to understand these processes in detail.

In this contribution, we demonstrate the realisation of a picoliter bioreactor for two strain co-cultures (Figure 1) and give first insights into the behavior of a synthetic microbial model consortia with full spatio-temporal resolution of single cells. Similar to previously developed microfluidic cultivation systems [3,4], which have been solely applied for the investigation of isogenic colonies of only a single strain, the present device incorporates interconnected chambers of picoliter volumes (Figure 1B). Two different strains can be grown in defined regions which are separated by sub-micrometer sized sieve structures, thereby facilitating diffusion based interaction, cellular communication, cross-feeding, etc. The chamber height of approx. 1 μm restricts bacterial growth to cell monolayers, which ideally suited for image based live-cell microscopy. The system can be used to determine growth rates, interactions and cell-to-cell heterogeneity of two interacting microbial strains. As a proof of principle the behavior of Corynebacterium glutamicum Δ LysA, a lysine auxotroph strain, and its interaction to different C. glutamicum lysine production strains was investigated.

Our results show that the concept of microfluidic single-cell co-cultivation has the potential to investigate bacterial interactions of multiple strains and species in more detail. This lays the foundation for an improved understanding of natural and synthetic co-cultures and could lead to novel strategies of designing and establishing novel microbial bioprocesses.
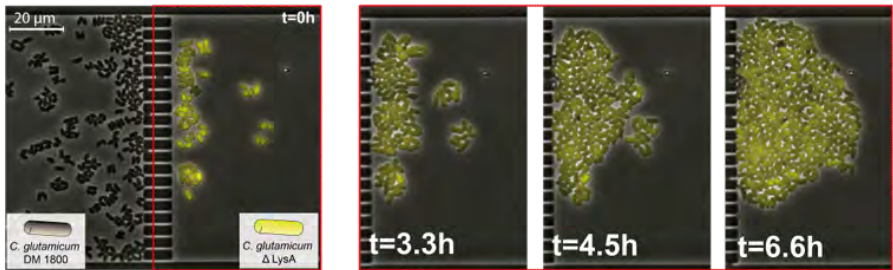
**Figure 1:** Microfluidic single-cell co-cultivation setup (A) Image of the microfluidic chip system, showing inlet and outlet for continues nutrient supply (B) Working principle of the picoliter cultivation chambers, which allows to cultivate and investigate the interactions of two interacting microcolonies on single-cell level. (C) Proof of principle cultivation of C. glutamicum Δ LysA, a lysine auxotroph strain (yfp labelled), which is fed with lysine produced by C. glutamicum DM 800 lysine production strain (not labelled). Successfully growth over several hours is shown on the right side.

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# P08

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## One sensor and multiple colors: fluorescence detection in droplet microfluidics and flow cytometry using frequency-division multiplexing

The vast majority of analytical measurements currently performed in flow cytometry and droplet microfluidics are fluorescence dependent. With the vision and necessity of multiparametric analysis, most biological applications demand the simultaneous usage of multiple color channels. To this end, excitation light sources have to be carefully paired with arrays of filters and multiple detectors. Such a complicated set-up not only increases cost and complexity, but also strongly restricts the selection of fluorescent markers. In order to break free from the restrictions of optical set-up complexity we introduce a system for laser frequency modulation and frequency-division multiplexing of the emitted light, detected in a single sensor. As a very common technique in telecommunications, frequency-division multiplexing (FDM) is used to separate signals in different non-overlapping frequencies. Here, we control the frequency at which the sample is exited using non-overlapping MHz frequencies for each of four lasers. All the emitted light is simultaneously collected by a single optical fiber and detected in a single pmt. The signal is processed with a lock-in amplifier to isolate the values of the emitted light originating from a specific channel in real-time. This method not only reduces complexity and provides set-up flexibility, but also results in improved signal intensities and higher signal to noise ratios. This translates into higher sensitivity and significantly improved precision. Furthermore, the combination of frequency demodulation and using an optical fiber for fluorescent light collection enables the possibility of parallel transmitted light microscopy, essential for process monitoring and quality control, and also for the implementation of image-based measurements. We showcase the presented strategy by performing multiparametric detection and classification of microfluidic droplets with mCherry producing *E. coli* cells cultured in five different conditions labelled with different fluorescent dyes.

# P09

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## Bringing Innovative Technologies Together: An Integrated Workflow for Single Cell Analysis Uncovering Hidden Events in a Solid Tumor

BD Genomics offers a suite of products that enable broad biological research through a fully integrated workflow. Tissue Dissociation combined with FACS analysis based on complex surface marker panels is capable of detecting cellular heterogeneity within a tissue sample. Furthermore, in conjunction with FACS sorting technology and RNA-Seq on the sorted cells, the picture is sharpened by linking proteomic and transcriptomic data.

# P10

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## Advanced imaging flow cytometry

The combination of imaging flow cytometry (IFC) with sorting options opens new opportunities in clinical and biological fields. Beside the powerful conventional flow cytometry, IFC provides additional high resolution morphological information's and can be seen as an extending technology in the field of flow through particle analyzes. The objective of our work is to develop a microfluidic system for conventional and tomographic IFC. The system shell be able to guarantee a viable processing of different bio-particles unattached by their population. In addition with software tools for particle tracking and feature extraction, our imaging flow cytometer provides the user with numerous classification possibilities for identifying bio-particles. Both, conventional and tomographic IFC is realized by advanced 3D hydrodynamic focusing which automatically aligns all particles as a sheet at a controllable z-position. For post processing of the detected bio-particles a future combination of the IFC chips with our image-based piezo-sorting system is under development.
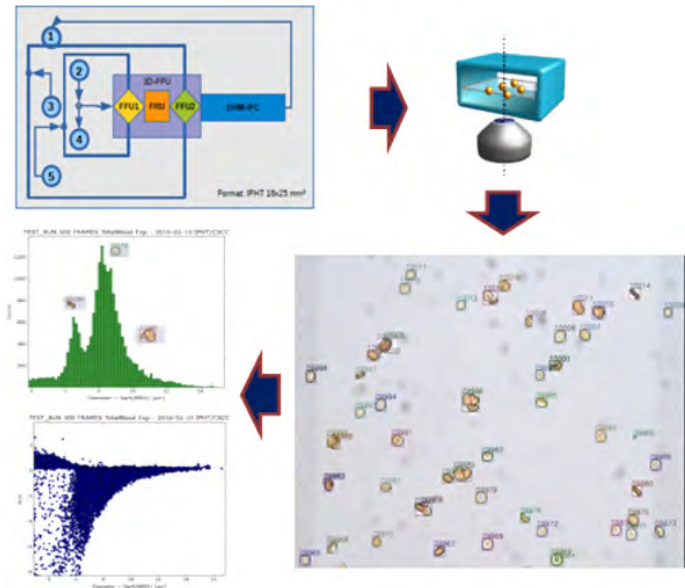
**Figure 1:** For a sharp imaging off all particles they must be aligned within the focal plane of the microscope. Three dimensional hydrodynamic focusing is utilized to align all objects within the focal plane of the microscope objective. Particles finally pass the flow cell as a planar sheet.

# P11

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## Flow cytometric quantification, sorting and sequencing of methanogenic archaea based on F420 autofluorescence

The industrial scale anaerobic digestion of biomass to $CH_4$ is widely established and may play a vital role in the future global energy supply. It relies on methanogenic archaea as key organisms which represent the bottleneck in the process. The quantitative analysis of these organisms can help to maximize process performance and uncover disturbances before failure. It may ultimately lead to community based process control schemes. Existing qPCR and fluorescence microscopy based methods are very attractive but can be cost intensive and laborious.

We present an autofluorescence based, flow cytometric method for the fast, low-cost quantification of methanogenic archaea in complex microbial communities and crude substrates (Lambrecht et al.). The method was employed to a methanogenic enrichment culture (MEC) and digester samples (DS). The methanogenic archaea were identified by the distinct fluorescence of their cofactor F420 in a range from $3.7 \times 10^8$ ($\pm 3.3 \times 10^6$) cells mL-1 and $1.8 \times 10^9$ ($\pm 1.1 \times 10^8$) cells mL-1. We evaluated different fixation methods and tested the sample stability. Steady abundance and fluorescence intensity were recorded up to 26 d during aerobic storage in PBS at 6 °C. The discrimination of the whole microbial community from the ubiquitous particle noise was facilitated by SYBR Green I staining and enabled calculation of relative methanogenic archaea abundances of up to 9.64% ± 0.23% in the MEC and up to 4.43 % ± 0.74 % in the DS. Illumina sequencing of the mcrA gene amplicons in sorted F420 fluorescent and non-fluorescent subcommunities verified the flow cytometric findings and confirmed the dominance of methanogenic archaea in autofluorescent subcommunities.

The presented method allows for fast and reliable quantification of methanogenic archaea in microbial communities under authentic digester conditions and can thus be useful for process monitoring and control in biogas digesters.

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# P12

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## Alternative live cell barcoding strategies in mass cytometry

Sample barcoding and pooling facilitates assay and data accuracy in single-cell cytometry by establishing identical sample preparation and acquisition conditions for multiple samples. We have previously introduced cell surface barcoding using CD45 antibodies (Ab) for multiplexed mass cytometry of PBMC, allowing for joint cell surface staining and sample processing prior to cell fixation and permeabilization. However, this approach spares cells characterized by variable, low, or no expression of CD45, limiting the applicability of CD45-based cell surface barcoding in some scenarios.

We here present an antibody-based cell surface barcoding solutiuon for leukocytes from human blood and bone marrow, using alternative cell surface antigens.

We evaluated the expression of CD45, HLA-ABC, 2 microglobulin (B2M), and CD298 on major subsets of human leukocytes by mass cytometry and confirmed that CD45 expression was low or variable on granulocytes and plasma cells, respectively. By contrast, HLA-ABC and B2M were expressed by all leukocyte subsets tested, including granulocytes and plasma cells, with signal intensities suitable for barcoding. Leukocytes stained with CD298 Ab showed high median signal intensity (MSI: 52-271) except for CD56br NK cells (MSI: 8). Isothiocyanobenzyl-EDTA-based and alternative MAXPAR-based Ab conjugation performed equally well for palladium labelling of B2M Ab.

Next, combinatorial barcoding of peripheral blood leukocyte samples was performed with a series of B2M antibodies, labeled with Pd104, Pd106, Pd108, Pd110, and Pt198. Here, CD45hi lymphocytes, monocytes, and CD45low granulocytes were recovered with comparable efficiencies from the sample convolute, demonstrating the versatility of B2M-based barcoding. Moreover, B2M-based barcoding was successfully applied to human bone marrow samples, comprising CD45low/neg plasma cells, for which CD45-based cell surface barcoding was inapplicable.

By introducing B2M-based cell surface barcoding, we extend live cell barcoding and its benefits to additional cell types such as granulocytes and plasma cells. Now, leukocyte preparations of blood and bone marrow and potentially also non-leukocyte preparations can be stained and processed as pools of B2M-barcoded samples without prior fixation and permeabilization, permitting uncompromised joint surface staining of cells.

# P13

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## Preservation of antibody cocktails for mass cytometry

Mass cytometry facilitates high-dimensional phenotyping of single cells and is ideally suited for monitoring leukocytes in various clinical situations, such as chronic inflammation. Data accuracy and consistency can be maximized by sample barcoding, data normalization, and a careful instrument setup. Involuntary variation in the preparation of antibody (Ab) cocktails, however, represents an additional and significant source of unwanted data variability. While stably storable Ab cocktails are an apparent solution to this problem, previous attempts to preserve liquid metal-labeled Ab cocktails at routine Ab storage conditions at 4 °C were unsuccessful.

We here evaluate different means of stabilizing cocktails of metal-labeled Ab.

Multiple Ab cocktails spanning different Ab clones and containing lanthanide, palladium and platinum conjugates were subjected to freezing at 20 °C, 80 °C or in liquid nitrogen, to in-house lyophilization, and to the commercial dry-down approach (DuraClone). Staining patterns of the differently treated Ab cocktails were evaluated with a single batch of cryopreserved PBMC at different time points, for up to 8 months.

Cocktails kept at 4 °C and 20 °C produced unexpected staining patterns after 4 weeks of storage. In-house lyophilization resulted in altered binding patterns of some Ab conjugates and was excluded from further evaluation. In contrast, cocktails stored at 80 °C or in liquid

nitrogen for 6 to 8 months, showed expected staining patterns on PBMC, with little variation. Of the commercially dried Ab cocktail, two out eleven antibody conjugates failed, while the remaining conjugates produced consistent staining patterns of PBMC for at least 6 months, even if stored at 37 °C.

In summary, both, commercial drying and in-house freezing solutions maintain the integrity of metal-labeled Ab cocktails, and can be used to preserve them for mass cytometry assays over a long period of time. Both approaches were compatible with all tested metal labeling procedures.

The Ab dry-down approach requires further optimization to generalize the concept, but appears as the more convenient option, since cocktails can be stored and shipped at room temperature. By contrast, freezing Ab cocktails is the more economic, flexible and independent approach.

Stabilized Ab cocktails promise to minimize unwanted data variability resulting from the repeated preparation of individual cocktails, which is of fundamental importance for data accuracy in longitudinal and multi-center mass cytometry studies.

# P14

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## Rapid single cell analysis using Raman spectroscopy

In the last two decades there has been extensive research on biomedical applications of Raman spectroscopy. It is based on an inelastic scattering between a photon and a molecule, and provides a complete molecular fingerprint of a sample. Due to its label-free nature of the signal generation, especially cell biology experiments have raised significant popularity, because it can provide information about molecular changes label-free and without any preparation steps. Applications range from drug-cell interaction, differentiate between apoptotic and proliferate cells, toxicology, and many more. [1,2] While many results are promising, large-scale biomedical and clinical studies are missing. This is specifically due to its lower acquisition speeds, complex data acquisition, and tedious data processing procedures for single cell analysis. [3] Additionally the whole manual process limits the investigation of cells to a minute number, and is often not inadequate for reliable statistical viability.

Here we present a novel data acquisition approach for spectroscopic Raman measurements of single cells, by removing human dependency. The proposed approach allows the acquisition of a large number of cells in short time, and allows to perform an entire experimental series of single cells Raman spectroscopy efficiently without prior knowledge of the technology. New applications using the new approach will be presented.

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# P15

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## Deformable Three-Dimensional Micro-architectures for Quantifying Platelet Contractile Forces

Platelets are small (3-5µm) discoidal cellular fragments produced by megakaryocytes in the bone marrow. The balance between hemostasis and thrombosis relies on a finely tuned adhesive and mechanosensitive response of blood platelets. At the site of sub-endothelial lesions, platelet-extracellular matrix (ECM) interactions play key roles in initiation of thrombus formation. This process is associated with generation of contractile forces through the coordination of physicochemical interactions between platelet cytoskeletal components (e.g. actin, myosin and tubulin) and surface receptors (e.g. glycoprotein Ib-V-IX complex, integrin's $\alpha 2 \beta 1$ and $\alpha IIb \beta 3$) that recognize the exposed ECM. Thus, platelet contractile forces (PCF) play key role in stability of blood clots and the eventual clot retraction is mainly dependent on the release of coagulation factors from platelets trapped in the fibrin network of the clot. Under in vivo physiological conditions and in blood flow, platelets encounter a complex and dynamically changing three-dimensional (3D) biophysical and biomechanical microenvironment. So far, little is known about PCF generated during platelet adhesion, spreading and activation on 3D microarchitectures immobilized with platelet interacting ligands. Within this context, we are currently developing deformable 3D micro-architectures through direct writing using two-photon lithography (TPL). We use finite element mechanics (FEM) simulations and TPL to

optimize the design, dimensions, choice of photopolymer materials and their elastic properties and bio-functionalization approaches for development of deformable 3D micro-architectures. Our long-term goal is to assess platelet generated contractile forces due to 'loss-of-function' mutations in key platelet cytoskeletal proteins from individuals diagnosed with platelet cytoskeleton defects with bleeding phenotypes.

# P16

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## Towards label-free imaging flow cytometry for full blood assay

The current state-of-the-art in flow cytometry is fluorescence labeling and the application of light scattering on blood cells for their discrimination. While information about granularity and size can be provided using single point measurements, imaging of the cell of interest does provide significantly more information. So far, only few imaging flow cytometers are commercially available all of which rely on fluorescence detection. In this context, label-free techniques are applied to imaging flow cytometry providing chemical contrast.

Non-linear optical imaging (NLOI) techniques, particularly coherent anti-Stokes Raman scattering (CARS), two-photon excited fluorescence (TPEF) and two-photon fluorescence lifetime imaging microscopy (2P-FLIM), are ideally suited to derive chemomorphic information of biological samples, non-destructive and without the use of labeling. NLOI techniques only generate signals in the laser focus providing 3D sectioning capability and low background levels. CARS enables to demonstrate the distribution of abundant marker molecules like lipids, proteins and DNA by characteristic vibrations, while TPEF visualizes the distribution of autofluorescing species, in particular cofactors like NAD(P)H and flavins, which are closely connected to the cellular metabolism and the concentration changes, e.g., with cell activation. In this contribution NLOI techniques are used for label-free imaging based cell sorting of blood cells.

The present study focuses on the differentiation of leukocytes for diagnosing and monitoring the status of infectious diseases using NLOI techniques.

By combining microfluidics with an inverted laser scanning microscope (LSM), preliminary NLOI experiments were performed with full blood as well as with isolated leukocytes. In addition, fixed leukocytes have been imaged by nonlinear microscopy combining CARS, TPEF

and 2P-FLIM. By colocalization analysis of hyperspectral CARS measurements within the C-H-stretching region, false-colour images of the lipid ($2850\,cm^{-1}$, $CH_2$), protein ($2930\,cm^{-1}$, CH) and DNA ($2967\,cm^{-1}$, CH) distribution within blood cells are generated. [1], [2] TPEF and FLIM are applied to visualize the distribution of endogenous fluorophores like NAD(P)H and FAD. Furthermore FLIM is used to assess the molecular environment of these autofluorophores. [3]

In Figure 1 results from NLOI modalities of a fixed neutrophil granulocyte are shown.
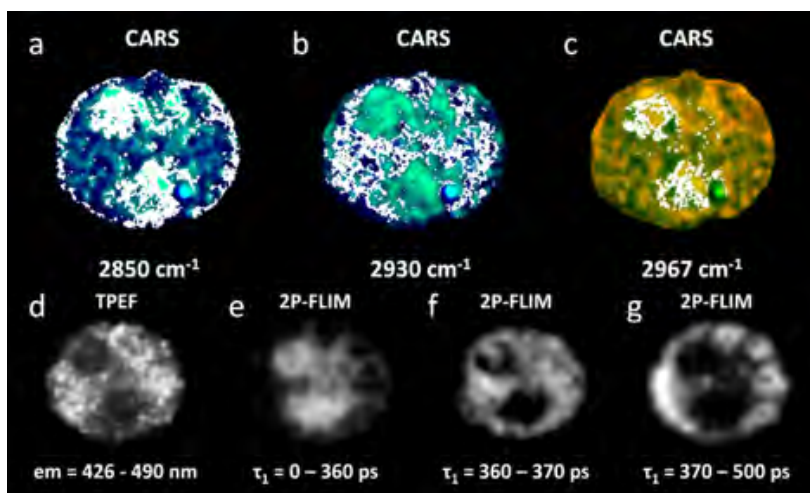


**Figure 1:** (a – c) Colocalized CARS false-colour images of an isolated, fixed neutrophil. $CH_2$ = $2850\,cm^{-1}$ (blue), $CH_3$ = $2930\,cm^{-1}$ (green), CH = $2967\,cm^{-1}$ (red). (d) TPEF image and (e-g) image processed 2P-FLIM measurements scaled to the first fluorescence lifetime constant, which is related to free NAD(P)H ($\tau 1$ = 300 ps). Stack size: 14.3 μm.

Differentiation of blood cells is based on the detection of biopolymer related CARS resonances as well as autofluorescence and fluorescence lifetime. By automatic quantification of cell morphology and chemical composition based on non-linear images as well as by expanding the sensitivity and flow stability of the microfluidic-LSM-setup label-free, non-linear optical imaging cytometry of blood cells can be realized.

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# P17

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## Assessing autophagic flux by measuring LC3, p62 and LAMP1 co-localization using imaging flow cytometry

Autophagy is a catabolic pathway in which normal or dysfunctional cellular components that accumulate during growth and differentiation are degraded via the lysosome; for instance, during starvation it is a survival mechanism that reallocates nutrients from unnecessary processes to more vital processes in the cell. However, autophagy is an important cellular homeostatic process and can both promote and inhibit cell death under different cellular contexts. And recent studies have elucidated several mechanistic links between autophagy and apoptosis.

In autophagy, cytoplasmic LC3 protein is processed and recruited to the autophagosomal membranes. The autophagosome then fuses with the lysosome to cause the breakdown of the autophagosome vesicle and its contents. The ubiquitin-associated protein p62 which binds to LC3 is also used to monitor autophagic flux.  Immunofluorescence microscopy has been used to visually identify LC3 punctca, p62 and/or lysosomes on a per-cell basis; however, an objective and statistically rigorous assessment can be difficult to obtain.  To overcome these problems, the ImageStreamX Mark II imaging cytometry platform was used to collect large numbers of cell images. Using a new analytical feature (Bright Detail Colocalization 3) the localization of 3 autophagy markers; LC3, p62 and lysosomal LAMP1 could be assessed in an objective, quantitative, and statistically robust manner.  The ability to co-localize 3 markers of autophagy in a single assay could lead to novel insights to the induction and regulation of autophagy.

# P18

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## Topology of Multiple Antigen Expression in Tonsillar Lymphoid Follicles and Stroma Analyzed by Imaging Mass Cytometry

Investigation of co-distribution of 35 biomarkers simultaneously (major cell type and immuno-oncology, stromal, and tissue architecture markers, as well as cell proliferation and nuclear markers) in normal lymphoid sections using imaging mass cytometry (IMC) (Chang

et al., Cytometry: Part A, 2017) is presented. Tonsil tissue revealed primary lymph follicles as dense clusters of lymphocytes expressing B cell markers, surrounded by CD3, CD4, and CD8 T cells. Secondary lymph follicles could be distinguished by proliferating B cells with high Ki-67 expression. Strong expression of Bcl-6 was detected in germinal center B cells. Tonsillar FoxP3 CD8 T cells exhibit a Treg phenotype with high CTLA-4 and CD45RO. CD68+ mac/mono were found in germinal centers and rarely in stroma. Squamous epithelium of crypts was beta-catenin-positive and infiltrated by PD-L1+ T cells, while PD-1+ cells were also found within the follicle centers. Images acquired on the Hyperion Imaging System (Fluidigm) were compared to immunofluorescence (IF) of sequential sections stained with the same antibodies (Ab) conjugated to fluorophores (FL). For direct comparison of IF and IMC, dual-tagged Abs were created by attaching a metal tag first (Maxpar®, Fluidigm), then conjugating to FL using Click Chemistry. CD3 and CD19 were labeled with metals and FL and tested in combination with the full panel on 8 µm frozen tonsil sections. CD3+ and CD19+ lymphocytes were identified by IF first, followed by IMC analysis. Results show that IMC is comparable to IF images and allows identification, characterization, and localization of cell populations and tissue architecture elements in the absence of autofluorescence, photobleaching, and with low background for acquisition of up to 50 targets.

# P19

**Jonathan Stefanowski[1,2], David Reismann[1], Robert Günther[1], Asylkhan Rakhymnzhan[1], Reto Nützi[3], Romano Matthys[3], Sandra Zehentmeier[1,2,5], Katharina Schmidt-Bleek[4], Georg Petkau[1], Fritz Melchers[1], Georg Duda[4], Anja Hauser[1,2], Raluca Niesner[1] //**
1_Deutsches Rheuma-Forschungszentrum, Berlin, Germany, 2_Immune Dynamics, Charité – Universitätsmedizin, Berlin, Germany, 3_RISystem AG, Davos, Switzerland, 4_Julius Wolff Institute, Charité – Universitätsmedizin, Berlin, Germany, 5_Dept. of Immunobiology, Yale University School of Medicine, New Haven, USA

## Longitudinal Intravital Multiphoton Endomicroscopy In Murine Bone Marrow (LIMB)

Intravital two-photon microscopy (2PM) is the method of choice to study interactions, migration, or activation of cells of the immune and skeletal system in bone marrow (BM). Both cell types show high plasticity in normal tissue and especially during bone regeneration. To date, 2PM in the BM is limited to a few hours in duration and depths < 150 µm. We have developed a reproducible microendoscopy technique based on GRIN lenses, which allows for longitudinal in vivo imaging of the marrow cavity.

The design of the implant for murine femurs facilitates the precise guidance of a 350 µm thin GRIN endoscope into the center of the marrow cavity. The implant is fixed with two bone screws to the right femur in a lateral approach. Thus, it allows repeated access to the BM

without further surgical procedures for more than 100 days.

Ex vivo µCT, immunofluorescence-based histology and histochemical methods were used to monitor tissue recovery post-surgery and to proof that tissue homeostasis is reached within two to three weeks.

In a first approach, we repeatedly imaged B cells in CD19:RFP the vasculature by i.v. injection of Qdots. Surprisingly, high plasticity of vessels was observed in the bone marrow of long bones.

Therefore, we demonstrated that this technique permits longitudinal intravital multiphoton microendoscopy in murine bone marrow (LIMB). We further plan to use this approach to study immune reactions in bone injury models and to elucidate the role of immune and bone cells in bone healing.

# P20

**K.-B. Suttner[1, 2], T. Meyer[1, 2], T. Bocklitz[1, 2], M. Schmitt[b], J. Popp[a,b]**
1_Leibniz Institute of Photonic Technology, Albert-Einstein Straße 9, Jena, Germany, 2_Institut für physikalische Chemie and Abbe Center of Photonics, Albert-Einstein Straße 6, Jena, Germany

## Label-free nonlinear imaging cytometry for high throughput bacterial culturing experiments

Excessive use of antibiotics in the past resulted in the occurrence of multi resistant bacteria and subsequently an increase of infectious diseases also in developed countries. Hence, there is an urgent need for novel antibiotics, which is a major focus in current drug research. One promising strategy is large scale screening for novel antibiotic substances released by microorganisms, which requires extensive culturing of microbes. Currently the state of the art is culturing on agar plates, which is a time and space consuming and error prone process. Many limitations of this process can be avoided by culturing microorganisms in a microfluidic environment, allowing highly controlled variations of growth media conditions. Particularly the number of culturing experiments can be significantly increased by reducing the volume to a nl-sized droplet, a small number of microorganisms per experiment and a high throughput up to kHz rates. However, currently there is no established method to accurately read-out these type of culturing experiment, especially at the high detection speeds needed. While established methods rely on fluorescent dyes, there is still an urgent need for fast label-free imaging with subcellular spatial resolution and molecule specific contrast for real-time monitoring. Vibrational spectroscopy provides label-free chemical contrast. In particular coherent Raman scattering (CRS) techniques allow for video rate image acquisition at diffraction limited spatial resolution when imaging a single vibrational band [1, 2], but the chemical information gained in these experiments is limited. New methods

for multispectral, hyperspectral or broadband coherent Raman spectroscopy bridge this gap by providing spectral information with high speed for application in life sciences [3-8]. This comes at the cost of significantly increasing the experimental complexity and the need for sophisticated data analysis in order to extract the spontaneous Raman [9] from the acquired BCARS spectra.

In this contribution multimodal nonlinear microscopy combining two photon excited autofluorescence (TPEF) of endogenous fluorophores and CARS is applied to fast 3D-imaging of droplets filled with polystyrene beads and bacteria in order to determine bacteria number, size and shape noninvasively. For further insight into the molecular processes during culturing a broadband coherent anti-Stokes Raman scattering (BCARS) spectroscopy approach has been developed to assess the vibrational spectrum of culturing media and bacteria. The setup for combining BCARS spectroscopy and microfluidic experiment is schematically depicted in Fig. 1a. Key parts are a photonic crystal fiber for generating a broad band emission which is subsequently compressed by a chirped mirror compressor. The broadband pulse is synchronized to the CARS pump pulse by a delay line. A microscope objective focuses the lasers into sample, where the CARS spectrum is generated and subsequently detected using a spectrometer.

First results from BCARS spectroscopy of solutions in combination with data analysis routines for retrieving the spontaneous Raman spectrum are presented, paving the way towards label-free and non-invasive monitoring of bacterial growth and microbial interactions in a microfluidic based culturing experiment in the future.
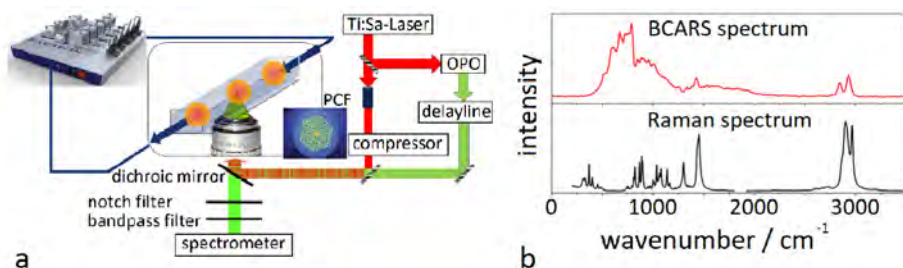


**Figure 1:** a) The setup for BCARS spectroscopy applied to microfluidics contains a PCF for generating the broadband laser source which is compressed before being recombined with and synchronized to the pump pulse from an OPO. b) Comparison of a BCARS spectrum with a spontaneous Raman spectrum of hexane. Phase retrieval methods are required for extraction of the Raman spectrum.

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# P21

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## *In vivo*-analysis of B cell receptor activation in germinal centers

High-affinity memory B cells and antibody-secreting plasma cells are formed in B cell-follicular structures within secondary lymphoid organs, the germinal centers (GCs). Selection of the best suited B cell clone to undergo terminal differentiation into antibody secreting plasma cells is based upon affinity of the B cell receptor (BCR) to the antigen. B cells with higher affinity BCRs outcompete those with lower affinity BCRs. We here want to study this process called affinity maturation in vivo.

Intravital twophoton microscopy helped to elucidate many features of germinal center initiation and B cell selection. With twophoton microscopy it was possible to observe how B cells acquire native antigen on follicular dendritic cells (FDCs) to become activated. Furthermore, it helped to redefine the role of T follicular helper cells that select B cells based upon the amount of antigen presented to them. However, a functional spatiotemporal analysis of the events which happen during and directly after BCR-activation in GCs has not yet been performed.

We want to elucidate if different signal strengths received via BCR within the germinal center influence the motility behaviour of the cells, in terms of velocity or direction. We

therefore chose Calcium (Ca2+) as a read out for BCR signaling. With the development of the YellowCaB mouse that shows expression of the genetically encoded Ca2+ sensor Tn-XXL, we here present a powerful tool to assess B cell activation upon engagement of the BCR via acquisition of Förster Resonance Energy Transfer (FRET) between the two fluorophores of the sensor. We were able to perform intravital 5D-imaging of B cells in the popliteal lymph node and could show increased Ca2+ levels that were sustained over several minutes, when cells had prolonged contacts with FDCs or resting B cells. Individual tracking of single cells revealed that signaling led to simultaneous motility arrest. We therefore asked if the relation between motility and BCR signaling is changing in different contexts: by comparing instantaneous velocity and FRET ratio, we could indeed detect distinct populations among B cells that were shifting with ongoing differentiation of GC B cells into plasma blasts. Importantly, we could show that higher affinities to antigen led to the presence of a population with higher FRET and smaller velocities. Thus, we developed a powerful tool to further study open questions of GC B cell selection, for example, if there is a certain threshold level of Ca2+ flux into the cell that initally has to be reached for the cell to be selected accordingly and that decides over cell fate.

# P22

**Marco Vahldieck, Susanne Krauthaeuser, Anne Richter and Christian Dose //**
Research & Development, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

## Vio®Dyes meet REAfinity™ – High Resolution Antibody-Fluorochrome Conjugates for Multi-Parameter Flow Cytometry Analysis

State-of-the-art sensitivity and target specificity of fluorochrome-conjugated antibodies limit applications in flow cytometry. Identification of cell subsets can be challenging due to low expression levels of surface and intracellular markers. The combination of Vio®Dyes and REAfinity™ antibodies provides researchers with the most powerful and reliable tools for flow cytometric cells analysis.

REAfinity™ are recombinant antibodies of human IgG1 isotype with a mutated region within the Fc part, which prevents Fcγ receptor interactions. They are characterized by high antigen specificity and consistent quality compared to conventional hybridoma-derived monoclonal antibodies. Vio®Dyes, e. g. Vio®515, VioBright®515, Vio®667, VioBright®667, and PE-Vio®615, allow due to their excellent fluorescent properties the detection of low expressed markers and provide new choices for multi-parameter analysis. To demonstrate the potential of the combination of REAfinity™ antibodies and Vio®Dyes, we developed detection panels

and protocols for the characterization of FOXP3+, HELIOS+, and CD45RA+ in CD4+ CD25+ CD127low/- human regulatory T cells (Tregs). Tregs were detected and enumerated in whole blood, PBMC, and in samples of magnetically enriched Tregs utilizing the newly developed reagents. In addition, we set-up new and fast staining protocols using 96-well plates or magnetic columns, which allow improved identification of Treg subsets.

In summary, the portfolio of Vio®Dyes conjugated REAfinity and special protocols open up new ways for designing and implementing multicolor panels towards highly sensitive, reproducible, and rapid cell profiling approaches.

# P23

**Konrad v. Volkmann[1], Kristen Feher[2], Claudia Giesecke[2], Jenny Kirsch[2], Toralf Kaiser[2] //**
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## Photonic calibration, determination of background, signal-to-noise and dynamic range of a flow cytometer - a novel practical method for instrument characterization and standardization

A well-defined scale calibration in flow cytometry can improve many aspects of data acquisition such as cytometer setup, instrument comparison and sample comparison. The theory for scale calibration was proposed by Steen over two decades ago [1], but it has never been put into regular use due to the lack of a widely available precision light source. The introduction of such a light source, the quantiFlash®, gave this possibility. Here, we want to describe how this light source can be used to characterize a cytometer's PMT performance and the instrument's response over the entire PMT voltage range.

As a consequence, we propose a practical method to characterize a cytometer's signal-to-noise ratio (SNR) and dynamic range (DNR). This allows the selection of a voltage / gain corresponding to a PMT's maximum efficiency and hence the lowest electronic noise, which can help with experiment design [2]. We further introduced a Decibel (dB) scale for the presentation of SNR and DNR values. SNR and DNR are stand-alone values that allow the direct comparison of different instruments.

Finally we link these two new parameters to the outcome of the so far widely accepted method to characterize flow cytometer in terms of detection efficiency (Q) and the signal background (B) [3], [4]. We will show that the combination of SNR and DNR together with the detection efficiency Q indeed yields a powerful set of parameters to characterize and compare flow cytometers.

## Abstract – Poster Pitch & Poster Session

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**Abstracts**

# October 5th // 14:30-16:00
# Session 6 – Klaus-Goerttler-Session

Chair: **Jonathan Leo Schmid-Burgk**
Chair: **Wolfgang Fritzsche**

## 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, Bachelo, Ph.D. or an equivalent work) and was selected out of several submissions by the council board of the society.

Reinhardt J.[1], Landsberg J.[2], Schmid-Burgk J.[3,4], Bald T.[2,5], Lopez-Ramos D.[2], Glodde N.[1,2], Nettersheim D.[6], Schorle H.[6], Quast T.[7], Kolanus W.[7], Tumeh P.[8], Ribas A.[8], Tüting T.[2,9], Hölzel M.[1] //
1_Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany, 2_Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, University of Bonn, Bonn, Germany, 3_Institute of Molecular Medicine, University of Bonn, Bonn, Germany, 4_Broad Institute of MIT/Harvard, Cambridge, Massachusetts, USA, 5_Immunology in Cancer and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston, 4006 QLD, Australia, 6_Department of Developmental Pathology, Institute of Pathology, University of Bonn, Bonn, Germany, 7_Molecular Immunology and Cell Biology, Life and Medical Sciences Institute, University of Bonn, Bonn, Germany, 8_University of California Los Angeles (UCLA), Los Angeles, California 90095, USA, 9_Institute of Dermatology and Venereology, University Hospital Magdeburg, Magdeburg, Germany

## MAPK signaling and inflammation link melanoma phenotype switching to induction of CD73 during immunotherapy

Cancer cell plasticity is a key driver of tumor immune escape and tumor progression. However still little is known about the mechanisms that shape cancer cell states. CD73 is a cell surface 5' ectonucleotidase expressed by melanoma and immune cells that converts extracellular AMP to immunosuppressive adenosine and a recently described component of tumor immune escape mechanisms.

Here we identify CD73 as a marker of an intermediate cell state recording an early inflammatory memory. Methylation of CpG islands in the 5' regulatory sequence of CD73 is only found in MITFhigh EMT-negative cell lines. We show that inflammatory mitogenic stimulation induces CD73 in a MEK-ERK signaling dependent manner. Consistently, many melanoma cell lines with activating mutations in BRAF or NRAS exhibit high basal CD73 expression, which is robustly suppressed by the treatment with BRAF or MEK inhibitors.

Previously, we could show that melanoma plasticity is regulated by a MITF/c-JUN antagonism. In line we find that c-JUN strongly induces CD73 expression in melanoma cells. Using ChIP-qPCR analysis and an arrayed functional CRISPR-Cas9-based genome editing and FACS sorting screening approach we identify a first intron enhancer in the CD73 gene with a canonical AP-1 binding site to be the key genomic region for c-JUN dependent regulation of CD73 expression.

In line, also in our mouse model inflammation-induced tumor cell plasticity leading to immune escape is accompanied by upregulation of CD73. Finally, we detect CD73 upregulation in melanoma patients progressing under adoptive T cell transfer or checkpoint immunotherapy arguing for an adaptive resistance mechanism.

In summary, our findings link immunosuppressive CD73 expression in melanoma cells to oncogenic MEK-ERK signaling and shed light on the induction of CD73 in a regenerative microenvironment, substantiating CD73 as target to combine with current immunotherapies.

**Sebastian Virreira Winter //**
Max Planck Institute of Biochemistry, Martinsried, Germany

## Redox proteomics using an MS-cleavable tag

Emerging data suggest that reactive oxygen species (ROS) can act as second messengers that modulate intracellular signaling via redox modifications of proteins. However, the molecular targets of ROS that are reversibly oxidized during redox signaling remain largely unknown. We used CRISPR/Cas9 technology to generate cells with deficiencies in specific enzymes of the antioxidant defense system. The cells were transfected simultaneously with three gRNAs per gene to increase the frequency of biallelic gene disruption. In addition, cell sorting by flow cytometry enabled the selection of cells expressing large amounts of Cas9, thereby further increasing the gene editing efficiency. We used these cells to identify the targets of key antioxidant enzymes by mass spectrometry analysis of reversibly oxidized cysteines. Our findings contribute to a better understanding of redundancy and specificity of the cellular antioxidant defense system.

**Lucas Jae //**
LMU Munich, Germany

# Redox proteomics using an MS-cleavable tag

Loss-of-function forward genetics have proven to be a tremendously successful tool for linking genes to phenotypes in model organisms. This is particularly true for yeast, for which scientists have generated highly detailed genetic wiring maps that illuminate the genetic cross-talk underlying different phenotypes. However, the application of loss-of-function genetics to human cells has long been hindered by technical barriers. With advances in programmable bacterial nucleases and ultra-deep genome mutagenesis of haploid human cells, we find ourselves at an exciting point in time, where a systematic dissection of human phenotypes on the genetic level seems to be within reach.

Using a combination of CRISPR-Cas guided genome engineering, ultradeep haploid mutagenesis and flow cytometry, we seek to better understand the genetic basis of cellular phenotypes relevant to human disease. Despite decades of biomedical research, the genetic contribution to many disease-associated phenotypes, such as mitochondrial dysfunction, secretory pathway defects, altered metabolism etc., remains uncharted or incompletely understood. Additionally, in cases where individual genes have been linked to the phenotype of interest, it is usually unclear how these contributions are affected by interactions among genes. This constitutes a bottleneck for therapeutic intervention, as a detailed understanding of the genetic crosstalk underlying a disease phenotype could potentially offer novel treatment avenues (such as exploitation of synthetic lethality in cancer or extragenic suppression in malfunctioning pathways).

Using flow cytometry, we seek to quantify disease-relevant cellular phenotypes on the level of individual cells that have been subjected to ultradeep genome mutagenesis. Coupling of the quantitative trait and next-generation sequencing of tens of millions of mutations throughout the genome allows us to generate detailed maps of genes affecting the process in question. Subsequently, we can mechanistically dissect the role of identified regulators by mapping their genetic interactions in modifier screens using isogenic mutants generated by CRISPR-Cas. Importantly, an efficient mutagenesis and mutation mapping approach circumvents the need to amplify living cells after flow cytometric isolation, which allows us to study a rich repertoire of intracellular phenotypes that can be quantified in dead cells using fluorescent probes. We are applying this FACS-based genetic screening strategy to interrogate diverse disease-related cellular processes ranging from organelle dynamics over metabolism to immune checkpoints and signal transduction.

# October 5th // 16:45-17:30

# Guest Lecture

Chair: **Wolfgang Fritzsche**

Markus Knaden started his work on insect behavior already during his diploma thesis in Bonn, when he investigated decision making in subsocial beetles. From then on, and especially when he startet to work on the desert ant Cataglyphis, Markus was fascinated how insects with their small brains often become much better navigators than humans. Since 2006 Markus is a group leader at the Max Planck Institute for Chemical Ecology in Jena focussing on odor-guided behavior in flies, moths, and ants. Flies, i. e. Drosophila, offer great molecular tools to e.g. shut down whatever pathway to investigate its involvement in a specific task. Regarding flies and moths Markus' group is trying to understand the valence of olfactory cues. Why are some odors innately attractive or repellent, what is their ecological relevance, and which neuronal circuits are involved from detection to decision making. While the desert ant Cataglyphis fortis does not offer elaborate molecular tools yet, it still  has become a famous model just because of its phantastic navigational performance. Current research in Markus' group is focussing on how olfaction helps individual ants to localize food and find their way back to the nest after up to 1500m-long foraging journeys.

**Markus Knaden** //
Max Planck Institute for Chemical Ecology, Jena

## Navigating in an extreme environment: how desert ants forage efficiently

Desert ants feeding on dead arthropods forage for dead arthropods that are distributed unpredictably in space and time in the food-scarce terrain of the Saharan salt pans. Scavengers of the genus *Cataglyphis* forage individually and do not lay pheromone trails. They rely primarily on path integration for navigation and, in addition, use visual and olfactory cues. While foraging the ants are exposed to extreme heat stress and predators. Here I show, how the ants cope with this situation by using a navigation system that always brings them back to the nest as fast as possible.

## October 5th // 17:30-19:00

## Members Assembly

**Lecture Hall 6** // Members Assembly
Chair: **Wolfgang Fritzsche**

# Conference Dinner (Access with conference badge only)

**19:00-22:00**     **Volksbad // Social Event & Conference Dinner**

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The Volksbad in Jena was a public swimming bath, built between 1907 and 1909 by the Jena Volksbad society. During that time public swimming baths were developed in many european cities. The architect Wilhelm Werdelmann, director of Barmen Art College, was selected to construct the Volksbad in Jena. He had already had experiences with the construction of baths and understood how to combine functionality and contemporary architectural appreciation.

The construction costs amounted to 450,000 D-mark. The building was financially supported by the Carl-Zeiss-Foundation, the savings bank Jena and the city authorities. The Volksbad Jena has an angular design with non-centered tower and oriels. The facade is made of domestic limestone. Originally it was equipped with swimming pools, baths, shower baths, medical baths, a laundry as well as a restaurant.

The building was remodeled between 1983 and 1987. The original equipment was only partially maintained. Afterwards, following a ten-year interruption, the bathing activities could be resumed.



Quelle: JenaKultur, Foto: Andreas Hub

# October 6th // 9:15-10:45
# Session 7 – Microscopy

Chair: **Anja Hauser**
Chair: **Raluca Niesner**

In the thirties of the last century, fluorescence was introduced to microscopy and caused a break-through in the analysis of biological specimens, both in health and disease. In the last two decades of this century, we experienced several similar revolutionary events. Microscopy in various organs of living mammals became available and provided unique insight into the „motion of life" and into cellular and tissue function, super-resolution nanoscopy taught us how single molecules are orchestrated in cells, and innovative techniques such as optogenetics let us even manipulate the cellular function. Without the rapid development of fluorescent reporter animals, this revolutionary progress in the field of microscopy would have never taken place. The present session gives insight into newest developments, especially related to the field of in vivo microscopy, expanding our horizon in the field of tissue cytometry.

**Dr. Gabriele Bixel //**
Max Planck Institute for Molecular Biomedicine, Münster

## Go with the flow – blood flow dynamics and HSPC homing in bone marrow microvessels

Bone marrow (BM) is the principle site of postnatal hematopoiesis and requires a specialized microenvironment, the hematopoietic niche, which controls maintenance and self-renewal of hematopoietic stem cells. Deeper insight into the functional structures of the BM strongly depends on a better understanding of the blood vessel microarchitecture and blood flow dynamics in the various types of BM microvessels.

The BM microvasculature forms a complex and irregular network of interconnecting sinusoidal microvessels, which are located within the BM cavities of long and flat bones. The functional organization and blood flow dynamics within the different types of microvessels have remained poorly understood due to technical challenges associated with in vivo high-resolution imaging in the intact bone. We developed an intravital multiphoton imaging approach to visualize blood flow dynamics and to measure velocities in multiple vessel segments by capturing the motion of red blood cells (RBCs). We used high-resolution spatio-temporal measurements through a cranial window in the mouse to determine short-time dynamics of flowing RBCs and repetitive centerline scans to obtain a detailed flow map with hemodynamic parameters.

Arterioles which branch from larger arteries were found to control the blood flow into the BM compartment. Blood flow slowed down considerably in downstream sinusoidal capillaries. Most interestingly, we observed highly variable RBC flow densities with few sinusoidal capillaries showing barely any flow. To analyze the impact of blood flow on hematopoietic stem and progenitor cell (HSPC) homing behavior in BM microvessels we monitored the initial phase of this multistep process by single cell resolution imaging. We observed considerable variability in HSPC rolling and adhesion in irregularly shaped sinusoidal capillaries, while no homing events were detected in arterial vessels. HSPCs prefer sinusoids with slow blood flow and low shear stress as sites for homing to the BM compartment.

**Frank Kirchhoff //**
Molecular Physiology, Center for Integrative Physiology and Molecular
Medicine (CIPMM), University of Saarland, Homburg, Germany

## The diversity of glial responses in health and disease – combining in vivo 2P imaging and transgenic mice with fluorescent reporter expression

Acute brain injuries activate signaling cascades essential for scar formation. Here, we report that acute lesions associated with a disruption of the blood-brain barrier (BBB) trigger re-programming of the oligodendrocyte lineage. Differentiated oligodendrocytes and their precursor cells can generate another neuroglial cell type: astrocytes. By in vivo 2P-LSM we followed oligodendrocytes after injury and demonstrate their plastic differentiation potential. Epileptic seizures are not only characterized by electric discharges of neurons, but also by a variety of $Ca^{2+}$ signals in adjacent glia such as astrocytes or oligodendrocytes. For that purpose, glial cells express various receptor to sense neuronal transmitter release. Encoding the spatial and temporal patterns of glial $Ca^{2+}$ signals will provide a novel understanding of brain function.

**Andreas Kleiber[1], Anuradha Ramoji[2], Günther Mayer[1], Thomas Henkel[1]** //
1_Leibniz-Insitute of Photonic Technology, Albert Einstein Straße 9, Jena, Germany,
2_University Hospital Jena, Centre of Sepsis control and Care, Am Klinkum 1, Jena, Germany

# High-throughput 3D-Tomographic-Imaging Flow Cytometry

**Abstract**

Tomographic imaging Flow Cytometry extends conventional imaging flow cytometry for the image based measurement of 3D-geometrical features of cells. The required multi-directional views are generated by rotating all cells while passing the imaging window of the developed microfluidic chip. Rotation is implemented by guiding the particles at a shear flow position of the parabolic velocity profile of the capillary slit detection chamber. All cells pass the detection chamber within a two dimensional sheet under controlled rotation. Images are recorded in parallel for the bright field and the fluorescence channel with an angular resolution better than 15 degree. The high potential of the approach is demonstrated by the analysis of White Blood Cells with fluorescent stained nucleus. The 3D-features were directly derived from the tomographic image sequences. Its application in the classification-model significantly improves the capabilities for the computer based cell-identification.

**Introduction**

Particles are ubiquitous entities of life and matter and the requirements for particle shape and structure analysis are important in material research and heterogeneous catalysis, nutrition, health, life sciences, plant breeding and many other fields. In flow cytometry each particle is measured while passing a flow cell. During the last twenty years conventional flow cytometry became the gold standard in high-throughput analysis of particle collections with the focus on bio-particles. Here particles pass a flow cell one by one as a single file arrangement for measuring their optical properties for multiple detection channels in parallel. For each detection channel a single value measure is provided. However, spatial distribution of texture and compartmentalization are not resolved by this technique. This limitation has been overcome by the imaging flow cytometry which is providing a microscopy image for each particle and imaging channel. Different bright field contrast methods can be combined with multichannel fluorescence imaging for more than 12 channels and an imaging rate of up to 10000 cells per second. Therefore, this method significantly extends the conventional flow cytometry for detailed informations on the compartmentalization and spatial distribution of fluorescent labeled biomarkers in bioparticles. However, each image represents only a 2D-projection of the three dimensional bioparticle which makes the obtained data dependent on the particular orientation of the particle at the time of imaging.

Three dimensional imaging has the opportunity, to resolve this issue, but it is still unavailable as a high throughput approach. Therefore, many groups are active in developing new concepts for 3D-imaging flow cytometry with enhanced throughput and spatial resolution. For gaining three dimensional data each cell has to be imaged either in multiple slices or from multiple directions.

In axial tomography an object is imaged from multiple directions and 3D-features with the opportunity for 3D reconstruction are calculated from these images. Hence, for implementing tomographic imaging flow cytometry, a particle has to rotate while passing the flow cell. Particle rotation is automatically induced by the shear flow of a fluid when the shear rate grad(u) deviates from zero. The fluid mechanical analysis and derivation of mathematical models has been initially performed by A. Einstein[Einstein1906] and extended to non-spherical particles by Trevelyan [Trevelyan1951]. Therefore it seems natural to combine this simple and self-acting principle for particle rotation with flow-through microscopy in order to implement a powerful but inexpensive system for high throughput tomographic imaging flow cytometry.

In our work we report on a microfluidic system and method for tomographic imaging flow cytometry. The potential of the approach is demonstrated by its application for the discrimination and classification of White Blood Cell-subtypes by combining two and tree dimensional features derived from the tomographic imaging data in the statistical classification model.

**Results and Discussion**
A tomographic imaging flow cytometry setup has been implemented which allows the parallel acquisition of two fluorescence and two bright field imaging channels.
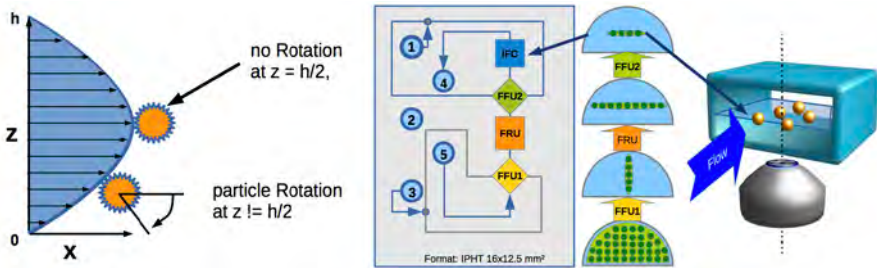


**Fig. 1:** Particle Rotation in shear flow and implemented chip concept
In contrast to conventional approaches, where the particles pass the imaging window in single file arrangement we are using a 2D arrangement of the particles as a planar sheet (see Fig 1, right part). Due to the integrated 3D-hydrodynamic focusing step all particles pass the imaging window within the depth of focus. Out of focus effects are eliminated and all particles move with the same velocity. The angular velocity of particle rotation can be controlled by the control of the z-position of the sheet in the parabolic velocity profile of the capillary slit detection chamber (Fig. 1, left part).



**Fig. 2:** Fluorescence stained nucleus of a white blood cell. Object boundaries (Contours) for feature measurement by Digital Image Analysis are drawn as circumference lines in the thumbnail images. A 180 degree rotation is recorded within a single transition of the cell trough the detection chamber of the chip.

While passing the detection chamber a series of images is acquired for each particle with an angular resolution of 10-15°. A full 360 degree rotation can be realized within the imaged region. Up to 100 particles can be measured per second in 3D. The systems usability has been confirmed for the analysis of White Blood Cells with fluorescent stained nucleus. Availability of the 3D-information on the shape of the nucleus enhances the precision in the classification and identification of different  subtypes of White Blood Cells. Work is in progress on the application of the system as an research tool in cell biology and clinical diagnostics within collaborative research tasks.

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# Fourier transform infrared micro-spectroscopic imaging of biological samples

Fourier transform infrared (FT-IR) micro-spectroscopy is a non-invasive imaging technique for biological material, that does not require any staining or labeling.[1] Biochemical information is obtained based on the intrinsic vibrational properties of the sample molecules. A FT-IR spectrometer coupled with a microscope and a multi-element detector allows spatially resolved hyperspectral images. The method is well suited for studying the cellular uptake and release of compounds with characteristic absorptions in an IR region usually silent for biological samples (e. g., 1800 to 2200 cm$^{-1}$, Fig. 1). Therefore, this approach was applied for the non-destructive investigation of mammalian cells treated with novel UV-Vis light sensitive CO-releasing molecules (photoCORMs).

Spectroscopic measurements were performed in transmission mode on an Agilent Cary 670 FT-IR spectrometer connected with an Agilent Cary 620 FTIR microscope equipped with a 25× objective (0.81 NA) and a 64 × 64 focal plane array (FPA) detector resulting in a field of view of 210 μm × 210 μm with a spatial resolution of 3.3 μm per pixel. The recorded hyperspectral images were evaluated using R, a free programming environment for statistical computing and graphics applying the N-FINDR algorithm.[2] With the help of spectral unmixing the cellular distribution of CORM was visualized (Fig. 1).

Capability of infrared micro-spectroscopic imaging as a rapid, non-invasive and non-destructive method for detection of the cellular uptake of photoCORMs was successfully and reproducibly demonstrated. Based on confirmation of the cellular CORM uptake, these results the pave the way for future investigation of the effect of CO against human cell lines by application of innovative water-soluble and non-toxic CORMs.
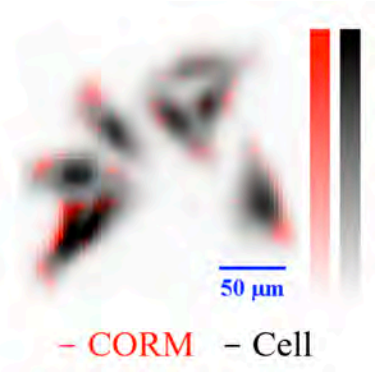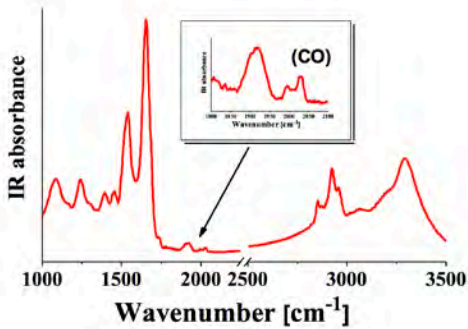
**Figure 1:** IR mean spectrum of mammalian cells after treatment with aqueous CORM solution showing specific CO marker bands (left) and the corresponding IR false color abundance plot of cell and CORM fractions (right).

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1. M. J. Baker et al., Nat Protoc., 2007, 9, 1771–1791.
2. C. Beleites et al., "A New N-FINDR Algorithm and the unmixR Package for Spectral Unmixing" in: Proc. FT-IR Spectroscopy in Microbiological and Medical Diagnostics (RKI Berlin, Germany), 2015, 10, 43

**Abstracts**

# October 6th // 11:15-12:45
# Session 8 – Clinical Applications

Chair: **Gergely Toldi**
Chair: **Stephan Schmid**

In this year's Clinical Applications session, we are exploring new exciting avenues of research on how the immune system interacts with its environment in health and disease. Cytometry plays a key role in deciphering these relationships and establishing how these mechanisms can be turned into therapeutic benefit for patients. Selected abstracts offer an insight into the latest methodological advances in the field of laboratory diagnostics.

**Paul Moss //**
University of Birmingham, UK

## Use of flow cytometry to determine the phenotype and function of tumour infiltrating lymphocytes in human cancer

Malignant cells are immunogenic and induce an immune response that plays an important role in controlling disease. Indeed the success of recent immunotherapy agents, such as antibodies that block checkpoint proteins, has uncovered the importance of established immune responses in limiting disease progression.

Tumours often establish an inflammatory environment and tumour infiltrating lymphocytes (TIL) are a feature of most tumour subtypes. TIL are likely to represent a combination of immune cells that can directly recognise tumour tissue, and may therefore play an important role in disease control, as well as cells that are recruited indirectly into the site of inflammation. As such, the analysis of TIL subsets now represents one of the most fascinating areas of cancer research and one in which flow cytometry plays a central role.
Using examples from human skin lymphoma, testicular cancer and pancreatic cancer, the presentation will describe how flow cytometry is uncovering a range of novel features about the biology of TIL. These will include phenotypic features such as the pattern of checkpoint protein expression on TIL and tumour cells; functional assessment through analysis of properties including cytokine expression, and the use of cytometric sorting to allow downstream applications such as transcriptomic and epigenetic analysis. The potential contribution of novel technologies such as CyTOF will also be addressed.

The current renaissance within tumour immunology is such that the analysis of tumour infiltrating lymphocytes through flow cytometry is one of the most important areas of biology. This technology holds the potential to uncover novel therapeutic pathways and may serve to guide appropriate clinical management of disease.

Paul Moss is Professor of Hematology and Director of Research in the College of Medicine at the University of Birmingham. He is also Chair of the Infections and Immunity Board and member of Strategy Board at the UK Medical Research Council. Professor Moss's research programme is within the immunology of human disease, where he undertakes a range of translational studies within cancer and transplant immunology. Professor Moss played a leading role in the development of HLA-peptide tetramers to detect antigen-specific T cells by flow cytometry and went on to use these as a novel form of cell therapy for patients with viral infection in the post-transplant setting.

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## Regulation of pro- and anti-inflammatory human T helper cell functions by the microenvironment

The adaptive T helper cell compartment is highly specialized in the elimination of microbes by a broad range of effector functions. A division of labour is implemented on the cellular level by distinct T helper cell subsets. Th17 cells have previously been identified as major players in autoimmunity. We could identify an anti-inflammatory Th17 cell counterpart, which was induced by specific commensal microbes such as *S. aureus*. Pro-inflammatory Th17 cells, instead, were induced by other microbes and distinct cytokine requirements. IL-1$\beta$ represented the switch factor that was differentially induced by different microbes and that determined T cell pathogenicity. Therapeutic blockade of IL-1$\beta$ shifted the balance from pro- to anti-inflammatory Th17 cell functionalities and correlated with complete clinical remission in autoinflammatory syndromes. This revealed two distinct types of Th17 cells that differed in functionalities, priming requirements and microbial TCR specificities with implications for human health and disease.

Other factors beyond microbe induced cytokine environments also contribute to shaping T helper cell functionalities. Tonicity signals have been largely neglected so far despite great variations in different tissues and disease states. Sodium chloride (NaCl) has recently been demonstrated to strongly enhance the generation of Th17 cells. This finding has helped consolidate the epidemiological correlation of enhanced dietary salt intake with the increase in the incidence of autoimmune diseases. Our data support the Th17 cell promoting effect of NaCl on human Th17 cell priming. However, Th17 cells acquired anti-inflammatory properties upon stimulation in NaCl enriched conditions. We therefore propose an anti-inflammatory role for increased salt osmolarities, which can be found in lymph nodes and skin in physiological conditions and in the tumor microenvironment in pathological conditions.

Together, we will demonstrate how the balance of pro- and anti-inflammatory human T helper cell subsets is regulated by microbes as well as tonicity signals from the microenvironment.

**Carl-Magnus Svensson**[1]**, Oksana Shvydkiv**[1]**, Lisa Mahler**[1, 2]**, Stefanie Dietrich**[1, 2]**, Thomas Weber**[1, 2]**, Mahipal Choudhary**[1]**, Miguel Tovar**[1, 2]**, Martin Roth**[1]**, Marc Thilo Figge**[1, 2] **//**
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## Antibiotics susceptibility testing by machine learning supported image analysis of colour coded microfluidic droplets

To battle the growing threat of antibiotic resistance, new methods to test the effectiveness of antibiotics against different bacteria are needed. Here we present an image-based analysis method for the determination of minimum inhibitory concentration (MIC) of antibiotics in setting that uses microfluidic droplets. The use of droplet microfluidics offers enormous decrease in sample volumes as each individual sample is encapsulated into a 200 pL aqueous droplet dispersed in an oil phase. The entire droplet population, normally in the range of several hundred-thousand droplets, are divided into a number of experimental conditions that are encoded by a unique combination of coloured beads. We use polystyrene beads in eight different colours and use single colours as well as pairs of colours to encode the different conditions. The practical number of conditions we are able to encode and decode with the current experimental setup is at least twenty and each condition is determined by the biological and chemical composition of the droplets. Each droplet is imaged after microbe/ antibiotics co-incubation and the condition is determined by an image analysis algorithm that finds the beads, assign each bead a colour using a random forest classifier and then decode the droplet using a probabilistic classifier. The probabilistic classifier assumes that the random forest classification is not perfect and assigns the droplets to the condition that is most likely according to Bayes theorem. The bead classification and decoding procedures described are adaptive, so that the classifiers are automatically adjusted for each experiment, and the decoding of twenty codes have accuracy over 99%. Besides the decoding, the microbial growth in each droplet is automatically determined by image analysis and the percentage of the droplet area that contains microbial growth is calculated. For the microbe Escherichia coli, eight hours of incubation time are needed to discriminate between microbial growth and lack thereof. The percentage of droplets that display growth is recorded and used as a measure for the effectiveness of the antibiotics. This microfluidic platform can be used for antibiotic susceptibility testing, screening for new antibiotics and determination of the MIC. Besides the relatively short incubation time, all the experimental conditions are incubated together under identical external circumstances. In other applications of droplet-based screening, fluorescent dyes have been used as labels for different conditions and in other cases fluo-

rescence emitted by the microbe is used as an indicator of microbial growth. In our study, both the decoding of experimental conditions and the measurement of biological activity are image-based. We thereby avoid both the addition of dyes into the droplets and laser illumination of the droplets. Both these factors can affect the biological activity in the droplets in a way that may be hard to predict and correct for. It is known that fluorescent dyes are affected by the interdroplet transport and dyes can affect the metabolic activity of living cells. As a proof of concept we determine the MIC of the antibiotic Tetracycline hydrochloride which is co-incubated with E. coli. Our study confirms the previously determined MIC range of 1-2 mg/L for Tetracycline hydrochloride, as we see a sharp drop in microbial growth in this range and no significant growth at higher concentrations of the antibiotic. In conclusion we present a fast and efficient method to monitor antibiotic and microbe interaction under multiple conditions in parallel. We exemplify this by confirming the MIC of Tetracycline hydrochloride.

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# Long-term stabilization of whole blood samples for flow and mass cytometry

**Background:** Long-term stabilization of whole blood samples for subsequent cytometric analyses is desired in longitudinal and multicenter patient leukocyte immune phenotyping studies to minimize technical error in sample preparation and data acquisition. While cryopreservation of PBMCs is commonly employed, this method excludes granulocytes and poses concerns regarding the integrity of sensitive cell types such as monocytes and plasma cells. Commercial whole blood preservatives (e.g. TransFix and Cyto-Chex) promise to stabilize blood cells for several days, yet are not suited for longer-term storage. Here, we explored a new fixative-based whole blood preservative ("stabilizer", Smart Tube Inc.) for comprehensive immunophenotyping by flow and mass cytometry.

**Methods:** The stabilizer was tested on heparinized human whole blood. Blood was mixed at a 1:1,4 v/v ratio with the stabilizer, incubated for 12 minutes at ambient temperature and frozen at -80 ℃ for storage up to 21 months. After sample thawing and hypotonic lysis of erythrocytes, leukocytes were prepared for immune cell phenotyping by flow and mass cytometry according to routine protocols, with optional addition of heparin to buffers to prevent artificial staining of eosinophils (doi: 10.1002/cyto.a.22826).

**Results:** Consistent with their fixation, leukocytes derived from stabilizer-treated blood samples showed changes in their light scatter characteristics when compared to fresh leukocytes. Some fluorochrome-tagged antibodies, e.g. Cy7 tandem conjugates, FITC and PerCp showed a tendency for increased non-specific binding to e.g. neutrophils compared to fresh, untreated whole blood samples. However, careful adjustment of antibody concentrations permitted the design of an 8-parameter immune phenotyping panel suitable to distinguish major leukocyte fractions, i.e. neutrophils, T cells, and B cells. The addition of heparin to buffers reduced background binding of fluorescent antibodies. Major leukocyte populations were detectable at consistent frequencies after storage of stabilizer-treated blood samples up to 21 months. Thirty antibodies targeting common leukocyte lineage markers such as CD3, CD4, CD8, CD20, CD16 etc. (Baumgart et al., doi: 10.1002/cyto.a.22894) were tested on leukocytes prepared from stabilizer-treated blood samples. Most antibodies exhibited expected staining patterns, while, antibodies targeting chemokine receptors, except CCR7, did not yield any staining.

Finally, previously established antibody-based and intracellular sample barcoding approaches were successfully validated for leukocytes after cryostorage in stabilizer.

**Conclusion:** Whole blood cryopreservation using a new fixative-based stabilizer is compatible with immune phenotyping and sample barcoding in both, flow and mass cytometry. Sample stability can be assumed for at least 2 years of storage. However, cytometric assays need to be optimized for the processing of leukocytes derived from stabilizer-treated whole blood. Application of the proposed procedure facilitates blood sample collection for immune phenotypical analyses in clinical longitudinal or multicenter studies for bundled, post-hoc analyses in central labs.

# Farewell & Snacks

**12:45-13:45**          **Auditorium / Foyer**

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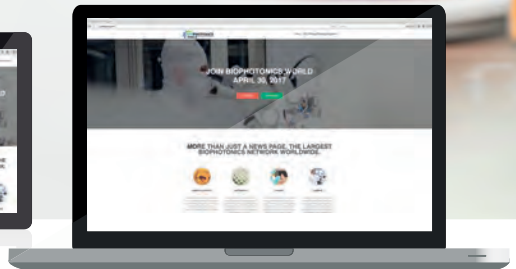**Würch, Andreas //** wuerch@immunbio.mpg.de // MPI for IE Freiburg

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# Cytometry 2018

## Life in Focus.

**28th Annual Conference of the German Society for Cytometry**

## See you next year!

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