Satellite Webinars
September 28 - October 2, 2020

Conference
October 1+2, 2020

DiGifZ 2020
Digitale Konferenz

Deutsche Gesellschaft
für Zytometrie

Abstract booklet

September, 25-27, 2019

DGfZ
Deutsche Gesellschaft
für Zytometrie

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Annual Conference 2020 goes online –
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Deutsche Gesellschaft
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Dear DGfZ members, dear friends of Cytometry,

we are very pleased to invite you to our virtual 30th Anniversary conference of the German Society of Cytometry (DGfZ) from 28.09. – 02.10.2020.

Due to the current pandemic situation, we cannot welcome you personally in Berlin. However, science does not stand still – quite the contrary, cytometry is of great importance now. For that reason, and also because this is the 30th Anniversary of the DGfZ conference, we would like to send a positive signal in the form of a new format, namely an online conference: DGfZ will become DiGiFZ 2020 – a digital forum for cytometry in a completely new, exciting format via ZOOM!

We have adapted the program for this purpose:

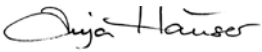
Of course, we will celebrate the society´s 30th Anniversary with a lecture by Günther Valet, one of the founding fathers of the DGfZ. We are especially pleased that we could win exciting speakers as Michael Dustin for the keynote lecture and Andrea Cossarizza for a Cutting Edge Session on the topic of COVID-19 cytometry.

Our popular Product Slam format will be available online this year and an interactive poster session, where even a poster prize will be awarded. Core facility managers will have the chance to exchange their expertise in our Core Facility Session.

Our partners from industry will present their brand new products and developments in form of a virtual exhibition, in the form of webinars, which start even before the conference begins: Between 28.09. and 02.10., there will be nine Satellite Webinars, including a raffle with great prizes!

Today we would like to invite you, to play your part in the anniversary of our society, but also in an experiment to investigate new forms for better online communication of the cytometry community.

Anja Hauser



President of the DGfZ

Pre-Conference Satelite Webinars

Welcome to the DiGifZ 2020, the 30th annual conference of the DGFZ
– this year digital only!

Monday, 28.09.2020

10:00am	Welcome to DiGifZ 2020 by the president of the DGfZ, Anja Hauser
10:05am – 10:40am	Satellite Webinar 1: Becton Dickinson (BD)

Tuesday, 29.09.2020

10:00am	Welcome to DiGifZ 2020 by, Christin Koch
10:05am – 10:35am	Satellite Webinar 2: OMNI Life Science (OLS)
11:00am – 11:35am	Satellite Webinar 3: APE

Wednesday, 30.09.2020

10:00am	Welcome to DiGifZ 2020 by Wolfgang Fritzsche
10:05am – 10:35am	Satellite Webinar 4: Propel Labs
11:00am – 11:35am	Satellite Webinar 5: Luminex

Conference Program

Thursday,01.10.2020

9:00am	Welcome to DiGifZ 2020 by the president of the DGfZ, Anja Hauser
9:05am – 10:00am	Session 1: Jubilee Lecture Speaker: Günter Valet 30 Years DGfZ: A personal view Founding member of the DGfZ Chair: Andreas Radbruch, DRFZ
10:15am – 10:50am	Satellite Webinar 6: Fluidigm
Break	
11:15am – 12:15pm	Session 2: Keynote Lecture Chair: Anja Hauser Lecture by Michael Dustin, Kennedy Institute of Rheumatology, UK
12:30pm – 1:30pm	Session 3: Poster Session
2:30pm – 3:05pm	Satellite Webinar 7: Sony
Break	
3:30pm – 4:30pm	Session 4: Core Facility Session: Spectral Cytometry Speakers: Simone Pöschel, Tübingen Bastian Höchst, München
4:30pm – 6:30pm	Core Facility Networking Event

Friday,02.10.2020

9:00am – 9:35am	Satellite Webinar 8: Beckman Coulter
Break	
10:00am – 10:50am	Session 5: Cutting Edge Sars-CoV-2 – Part 1 Speaker: Andrea Cossarizza, ISAC president, University of Modena, IT
10:50am – 11:50am	Product Slam Selected industrial partners will present their newest innovative technological developments and products
1:00pm – 2:00pm	Session 6: Cutting Edge Sars-CoV-2 – Part 2 Combined approaches for COVID-19 Speaker: Leif Erik Sander, Charité Helena Radbruch, Charité Mir Farzin Mashreghi, DRFZ
2:20pm – 2:55pm	Satellite Webinar 9: Miltenyi Biotec, Pre-existing T cell memory as a risk factor for severe COVID-19 in the elderly, Alexander Scheffold
Break	
3:15pm – 3:45pm	Summary and award ceremony Summary of the online conference Award ceremony: Poster prize and prize for the webinar competition, farewell
Break	
4:00pm – 5:00pm	Annual members assembly of the DGFZ

German Society for Cytometry (DGfZ)

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

Abstracts DiGifZ2020

Session 1:

Thursday, 01/Oct/2020:
9:05am - 10:00am
Session Chair: Andreas Radbruch

Jubilee Lecture: 30 Years DGfZ: A personal view

Günter Valet (Founding member of the DGfZ)

Rudolf Virchow's (1821-1902) introduction of cellular pathology, Torbjörn Caspersson's (1910-1997) quantitative cell DNA and protein measurements and Wallace Coulter's (1913-1998) electrical cell counting and sizing instrument have stimulated the development of the cell sorter (Fulwyler 1965) and of the first flow cytometer in a modern sense (Kamentsky 1965).

The construction of flow cytometers in European scientific institutions resulted in the first commercial fluorescence flow cytometer ICP-11 (W.Göhde 1969, PHYWE), the hydrodynamic focusing cell volume MPV-1 analyzer (R.Thom 1982, AEG-Telefunken), the MPV-Compact flow cytometer (H.Steen 1982, Leitz), the FLUVO-Metricell (V.Kachel 1983, HEKA-Elektronik) and the Kratel cytometer (W.Eisert, W.Beisker, 1984, Kratel Instruments).

The Society for Analytical Cytology (SAC, later ISAC) as scientific background for the new discipline was founded at the 1978 Schloss Elmau conference (K.Goerttler) but developed during the early phase into a scientific marketing organization for US cytometers.

European identity in this environment was maintained by the foundation of the European Society for Analytical Cellular Pathology (ESACP, 1986), the journal Analytical Cellular Pathology (ACP, today Cellular Oncology, IF 4.191 in 2019) and amongst others the Deutsche Gesellschaft für Zytometrie (DGfZ, 1989). In addition, the first scientific society servers (ESACP, DGfZ 1994) were set up on the Internet and practical cytometry knowledge was communicated during the first European flow cytometry courses at the Max-Planck-Institut in Martinsried to more than 200 scientists (1985-93), using initially only European instrumentation.

Cytometry, in contrast to genomics or proteomics analysis is not affected by mixed cell populations during sample preparation, constituting an important advantage for

clinical use. Therapy is presently conceived for patient cohorts. The establishment of individualized predictions for therapy dependent disease progression or outcome is highly desirable and constitutes an important scientific challenge for clinicians and clinical cytometrists. Data pattern analysis for individual patients seems promising for clinical research projects at the European level (https://www.classimed.de/classif1.html).

Session 2: Keynote Lecture

Thursday, 01/Oct/2020:
11:15am - 12:15pm
Session Chair: Anja Hauser

Use of multiparameter flow cytometry to quantify the output of the immunological synapse

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The Immunological Synapse is a pivotal hub for the regulation of adaptive immunity. The identity of receptor and ligands interacting and clustering at the cell-cell interface is crucial in defining the global features of the triggered immune response. Significant efforts have been invested in developing multiparametric analyses deciphering T cell transcriptional phenotypes driving disease, however scalable methods allowing their effector characterization are still to be developed. Here, we harness Bead Supported Lipid Bilayers (BSLB) and multiparameter flow cytometry as a general method for the "enhanced functional interrogation" of the effector phase of T-cell immune synapses allowing the identification of protein and RNA species packed into synaptic vesicles (SV). SV can come from at least two sources- exosomes released from multivesicular bodies (intracellular stores) by directed exocytosis or ectosomes that bud directly from the pre-synaptic plasma membrane. Antigen, co-stimulation, co-repression, adhesion molecules and viral proteins are loaded on the surface of BSLB to generate tailored, synthetic antigen presenting cells. By providing these signals, BSLB instigate synapse formation and the release of patches of SV containing a variety of effectors, including CD40L, Perforin, ectonucleotidases, tetraspanins and small RNA species. Using CD40L as a model helper T cell effector we then explored the dynamics influencing the release of CD40L+ SV, and demonstrate the role of T cell phenotypes, antigen and CD40 density, antigen potency, co-stimulation, co-repression, enzymatic processing by ADAM10 and the facilitating role of structural proteins including bone marrow

stromal cell Bone Marrow Stromal Cell Antigen 2 (BST2, CD317) and CD81. Altogether, we demonstrate the broad applicability of BSLB for the dissection of T cell effectors delivered in the form of synaptic vesicles born either from the plasma membrane or intracellular stores.

Session 3: Poster Session

Thursday, 01/Oct/2020:
12:30pm - 1:30pm
Session Chairs: Attila Tarnok, Torsten Viergutz, Raluca Niesner

Abstracts:

A comprehensive analysis of intestinal microbiota by multi-parameter flow cytometry

Lisa Budzinski

Toni Sempert, René Maier, Alexander Beller, Gitta A Heinz, Ute Hoffmann, Pawel Durek, Mir-Farzin Mashreghi, Hyun-Dong Chang

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The intestinal bacterial flora, or microbiota, is associated to a variety of diseases including cancer, neurological diseases, and autoimmune diseases. Analyses of stool samples of patients showed that in such diseases the gut microbiota composition is altered, a condition termed dysbiosis. So far, dysbiosis is mainly detected via 16 S rDNA sequencing and manifests as altered abundancy of bacterial phyla, class, family or even certain genera. Albeit the determination of altered bacterial composition is highly relevant, any information on the condition of the microbial community is missing. Cellular properties of the bacteria and immunoglobulin-mediated immune responses shaping the microbiota are relevant parameters to reconstruct the host-microbe interaction. To achieve a more comprehensive understanding of the human intestinal microbiota, we apply multi-parameter flow cytometry revealing the complexity of the bacterial community through quantitative DNA label and light scatter determination, analysis of endogenous coating of bacteria with IgA1, IgA2, IgM and IgG and different lectin-binding sugar moieties exposed on the bacterial cell surface. As such we have characterized healthy human stool samples as proof-of-concept for the feasibility and strength of microbiota flow cytometry to depict the complexity and individuality of the intestinal microbiota.

Characterization of chemical-specific CD4+ T cells in human blood

Caterina Curato1,2,

Marina Aparicio-Soto1,2, Franziska Riedel1,2, Ingrun Wehl1,2, Alev Basaran1,2, Melanie Leddermann1,2, Uwe Hillen3, Hermann-Josef Thierse2, Andreas Luch1,2, Katherina Siewert1,2

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Allergic contact dermatitis (ACD) is a widespread skin condition mediated by T cells (delayed type IV hypersensitivity reaction). It occurs in response to exogenous agents and external stimuli (Militello et al., 2020) and clinically manifests with a dermatitis or "eczema". Although it is estimated that about 30% of chemicals may induce an ACD reaction prediction of the sensitizing potential remains difficult. At present, no validated *in vitro* T cell-based tests are available.

Recently, we described a CD154 (CD40L) upregulation assay to detect nickel-specific CD4+ T cells in human blood (Aparicio-Soto et al., 2020). CD154 is an activation marker transiently expressed by specific naïve and memory CD4+ T cells shortly after antigen stimulation. We here adapted the CD154-upregulation assay to detect human non-metal chemical specific CD4+ T cells. As model allergen, we use 2,4,6-trinitrobenzenesulfonic acid (TNBS), which possesses extreme sensitizing capacity (Gerberick et al., 1992).

Modification of peripheral blood mononuclear cells (PBMC) with TNBS induced the generation of T cell epitopes. After CFSE labeling, 'modified' cells were co-cultured for 5 hours with unmodified 'responder' cells. We monitored the toxicity of different TNBS concentrations in both 'modified' and 'responder' cells, as well as the upregulation and co-expression of different activation markers (e.g. CD154, CD69, CD137) by the 'responder' cells. The highest frequencies of CD154+ CD4+ memory T cells were observed with 3 mM TNBS (mean ~0.05%). On average 80% of these cells co-expressed CD69. This finding and specific restimulation of TNBS-specific single cell clones confirmed TCR-mediated activation.

Summarizing, CD4+ T cell activation by organic contact allergen-induced epitopes may be detected with the CD154 upregulation assay. In the future, this approach may be optimized to *in vitro* test, diagnose and eventually predict the sensitization ability of other contact allergens.

Deeper insights in neutrophil metabolism by systematic enzyme mapping based on phasor-analyzed label-free NAD(P)H-FLIM

Ruth Leben1

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The ubiquitous co-enzymes NAD+ (oxidized)/ NADH (reduced) and their phosphorized variants NADP+/NADPH form the basis of the cellular energy metabolism including glycolysis and OxPhos as well as of pathogen-defense provided by NADPH oxidases (NOX). Since the oxidized forms are autofluorescent, they can serve as markers to monitor these life-sustaining mechanisms label-free *in vivo*. In contrast to their emission wavelength, their fluorescence lifetime changes with the enzyme to which they bind to.

We performed NAD(P)H fluorescence lifetime imaging microscopy (FLIM) in isolated human neutrophils in a two-photon microscope (in time domain) and analyzed the data using the phasor approach [Digman, 2008]. Both the evaluation and interpretation of such measurements in a real biological environment are challenging, especially since the two-photon action cross-sections of NAD(P)H are comparatively low and the exact values of the enzyme-bound NAD(P)H lifetimes are controversially discussed in this field [Ranjit, 2019]. Here we measured the fluorescence lifetime of the eleven most abundant NAD(P)H-dependent enzymes in solution. In order to support a systematic interpretation of NAD(P)H-FLIM data, we developed an algorithm, which allocates each pixel of a FLIM-image to one of these enzymes by vector-analysis. In this way, discrete enzyme-maps as well as maps of their general metabolic activity are created. [Leben, 2019].

Applied to FLIM-images of phagocyting neutrophils, this method reveals deeper insights in their metabolism, which go beyond the NOX activation leading to the oxidative burst. In some cells we found a different group of enzymes activated than in most others. This could be a sign of activation, since most activated immune cells switch their metabolism form the efficient, but slow OxPhos to an upregulated faster glycolysis, to face an infection [Gaber, 2017].

In this way, we demonstrated the power of our systematic evaluation framework for

NAD(P)H-FLIM in retrieving the complexity of metabolism in cells and tissues.

High-throughput cell and spheroid mechanics in virtual fluidic channels

Muzaffar H.

Panhwar1, Fabian Czerwinski1, Venkata A. S. Dabbiru1, Yesaswini Komaragiri1, Bob Fregin1, Doreen Biedenweg2, Peter Nestler1, Ricardo H. Pires1, Oliver Otto1

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Microfluidic techniques have proven to be of key importance for achieving high-throughput cell mechanical measurements. However, their design modifications require sophisticated cleanroom equipment. Here, we introduce virtual fluidic channels as a flexible and robust alternative to Poly-dimethylsiloxane (PDMS) chips. Virtual channels are liquid-bound flows that can be created in almost arbitrary fluidic systems, e.g. standard flow cytometer cuvettes, and tailored in three dimensions within seconds for rheological studies on a wide size range of biological samples. We show that cell deformation in narrow virtual channels inside micrometer-sized systems is mainly driven by shear stress .By contrast, cells inside virtual channels of a large cuvette or capillary are deformed by an interfacial normal stress originating from the liquid-liquid interface. We demonstrate that this liquid-liquid interface acts as a high-frequency liquid cantilever for probing cell rheology on a millisecond timescale. As a proof-ofprinciple experiment, cells are treated with the actin depolymerizing drug cytochalasin D. A significant reduction in elastic modulus is found compared to untreated cells. Our results highlight that Young's modulus of single cells exceeds the one of tissue by one order of magnitude. In summary, we show that virtual channels might offer the ability for high-throughput mechanical cell and tissue characterization in almost arbitrary geometries.

Integrated Cytomics platform for improved environmental monitoring of pollination, air and water quality, combining high throughput (imaging) cytometry and deep learning

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Pollination, good air and water quality as ecosystem services are crucial aspects of our daily life and directly related to our well-being. An impairment of these ecosystem services has dramatic consequences for health and quality of life, e.g. in form of loss of agricultural products, respiratory diseases or restricted drinking water supply. Regular monitoring of pollination, air and water quality is therefore extremely important and traditionally done via microscopy. Microscopic investigations are of high quality but come with several restrictions like taxonomic expert requirement, difficulties in providing standardized taxonomic knowledge and taking long time, limiting the samples which can be processed in total. A new integrative Cytomics platform has been established to address different aspects of environmental research, including pollen and phytoplankton research. The platform includes an ImageStream X Mk II, a FACS Aria II, an Accuri C6 and a Multisizer 4, allowing for rapid flow cytometric assarys, but also for detailed image-based analytics or sorting. The innovative combination of high throughput imaging flow cytometry and deep learning is suggested as a promising tool to enable faster analyses, by keeping the microscopic evaluation of samples.The methods which will be developed should directly inform scientist or local stake holders about aspects of pollination, as well as air and water quality. This will help to deliver early warning information for toxic organisms in drinking or bathing water, inform about relevance of certain pollinators or supports physicians with allergic pollen forecast.

Light scattering pulse shape analysis with multiple forward scatter detectors in flow cytometry

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In a flow cytometer, objects of interest (e.g. cells, bacteria, extracellular vesicles) passing the laser spot create a time-dependent scattering intensity signal, resulting in a signal pulse. In standard instruments, this pulse shape is normally reduced to three read-out values: height, area, and width. Consequently, valuable information can be lost since these three values hardly represent a sample's complex light scattering behavior. Moreover, the scattered light is commonly detected within a large solid angle which inhibits angular resolution.

Both pulse shape analysis with time resolution and the detection of scattered light with improved angular resolution have already been discussed. However, neither have they been considered to be combined nor did they become standard methods.

We are combining both angular and temporal resolution of scattered light signals for flow cytometry. To that end, a flow cytometer setup was equipped with modified optics for angle-resolved detection of forward scattered light. In addition, custom-made signal acquisition electronics and software were employed to measure light scattering pulse shapes and acquire cell-specific information beyond height, area, and width signals.

With this setup, we analyzed cells from two cell lines (HEK and Jurkat). Based on the pulse shape analysis by means of wavelet decomposition and k-means clustering, we were able to distinguish between cells in different phases of the cell cycle (G1, S, G2/M) using the angle-resolved forward scatter signals. The identification of the cell cycle populations was confirmed by fluorescent staining (PI and BrdU-FITC) and analysis of samples that were sorted based on the fluorescent staining.

In-depth pulse shape analysis for flow cytometry data enables label-free analysis of cells and could help in increasing the number of accessible parameters. Future work aims at discovering further applications and the implementation of label-free cell

sorting based on pulse shape analysis, i.e. pulse-shape activated cell sorting (PACS).

Limbostomy: Longitudinal Intravital Microendoscopy in Murine Osteotomies

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1. BACKGROUND

Dedicated interplay of immune cells, mesenchymal cells, and vasculature over the time course of regeneration is required for successful bone healing. In order to quantify the spatiotemporal aspects of the underlying cell processes, new imaging methods are needed.

2. RESULTS

We developed a method termed Limbostomy[1] by combining LIMB[2] - our intravital two-photon fluorescence microendoscopy approach - with osteotomy, to quantify parameters of successful endochondral bone regeneration.

The internal fixator plate stabilizes the bone and hosts a modular endoscope based on gradient refractive index (GRIN) lenses. To correct for intrinsic optical aberrations of the GRIN lenses, we designed an image post-processing algorithm: Image plane deformations as well as background- and noise effects on the image quality were minimized. We are now able to observe sub-cellular processes and quantitatively analyze dynamic angiogenesis, cell motility and - interactions during bone

regeneration.

Utilizing a transgenic reporter mouse strain with nuclear-GFP and membrane-tdTomato under the Cadherin-5 promoter, we identified two distinct phases of vascularization after injury. In an initial rapid vessel-sprouting phase the field of view is pervaded within 3-4 days post-osteotomy. This is followed by a remodeling phase of the vessel network until the end of our observation time, 14 days post-surgery.

3. CONCLUSION

Hematopoiesis, cellular niches, immunological memory, vascularization and the underlying environmental and cellular interactions are only fully analyzable with approaches that allow fine-meshed continuous intravital time-lapse imaging with high-resolution in the bone marrow. Therefore, Limbostomy creates a unique set of opportunities to gain insights on spatiotemporal aspects of bone marrow biology during health and disease.

[1] J. Stefanowski, A.F. Fiedler, "Limbostomy: Longitudinal Intravital Microendoscopy in Murine Osteotomies." Cytometry A (2020)

[2] D. Reismann, J. Stefanowski, "Longitudinal intravital imaging of the femoral bone marrow reveals plasticity within marrow vasculature." Nature Communications (2017)

Longitudinal Intravital Multiphoton Microendoscopy Reveals Vascularization during Bone Regeneration To Occur in Two Distinct Phases and To Be Preceded by CX3CR1+ Myeloid Cells

Jonathan Stefanowski1,2,

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Successful vascularization and osteoimmunological interactions are essential for a good healing outcome after fractures. Absence of macrophages has been associated with delayed healing in long bone osteotomies. However, the dynamics of how the injury site is vascularized and the role of macrophage subsets remain elusive. Using longitudinal intravital multiphoton microendoscopy in murine osteotomies (Limbostomy) we observed two phases of vascularization shown in Cadherin-5 reporter mice: 1. An early vascularization at 3-4 days post-osteotomy, which was completed within 24 hours and 2. a continuous remodeling of the vascular network until 14 days post-surgery. Immunofluorescence histology of the bone marrow revealed the vasculature in both phases to resemble the immunophenotype of CD31+Endomucin+ endothelial cells (type H vessels) an endothelial cell type that is linked to bone formation. During early vascularization of the fracture hematoma is preceded by motile CX3CR1+ cells shown in CX3CR1GFP reporter mice. Furthermore, immunofluorescence histology showed CX3CR1+ cells to express the pan-macrophage marker F4/80 in the second phase of vascularization. Of all cells in the osteotomy gap, CX3CR1+F4/80+Gr-1- mononuclear phagocytes, which resemble the immunophenotype of non-classical monocytes, constitute the major population. These results suggest that non-classical monocytes actively participate in the vascularization process of type H vessels to support bone formation.

ROS induced cell mechanical alterations in suspension and adherent cells

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Increase in oxidative stress has been linked to many haematological and neurological disorders. Reactive oxygen species (ROS) are one of the primary sources of oxidative stress which are associated with essential alterations in cell physiology1. Mechanical properties have long been established as a label-free biomarker, but their interplay with alternating levels of ROS has not been thoroughly investigated. This study focusses on understanding the impact of oxidative stress on the mechanical properties of the human leukaemia cell line (HL-60) and immortalized rat brain C6 glioma cells. In an in-vitro assay, for ROS was generated by exposing cells to varying concentrations of

hydrogen peroxide. Using real-time fluorescence deformability cytometry2, we link for the first time the molecular phenotype of ROS using MitoSOX-red a fluorescent marker to changes in the mechanical phenotype which is a label-free biomarker. We show for micromolar concentrations of H2O2 induces different alterations in cell mechanical properties between both the cell types. For adherent cells, we find no changes in Young's modulus, but for suspended cells we observe a different cell response to oxidative stress of increased elastic modulus. Alterations in Young's modulus are not accompanied by significant changes in levels of microtubule and F-actin levels as detected by flow cytometry analysis but can be attributed to significant changes in cytoplasmic pH.

1. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018. doi:10.2147/CIA.S158513

2. Rosendahl P, Plak K, Jacobi A, et al. Real-time fluorescence and deformability cytometry. Nat Methods. 2018. doi:10.1038/nmeth.4639

SARS-Cov-2 induces enhanced intestinal immune responses in severe COVID-19 patients

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The Coronavirus disease 2019 (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2). Initial infection occurs through binding of the Receptor binding domain (RBD) of the viral Spike protein (S) to the ACE2 receptor, which is expressed in the nasopharynx, but also vastly on enterocytes of the gastrointestinal tract. It was shown that a SARS-Cov-2 infection could lead to gastrointestinal manifestations, raising the question how the mucosal immune response towards the virus is regulated. We analyzed fecal samples of intensive care unit (ICU) COVID-19 patients and could observe a highly disbiotic microbiota profile by flow cytometrie of bacterial populations. Furthermore, the commensal bacterial coating with IgA2 and IgG was enhanced in patients compared to healthy

individuals, which correlated with the levels of unbound immunoglobulins in the fecal supernatants. Subsequently, we could determine that IgA2 and IgA1 fecal antibodies from COVID-19 patients are specific to the SARS-Cov-2 Spike protein. Thus, our data show that COVID-19 is associated with intestinal, mucosal immune response, which is characterized by an aberrant production of IgA2 and IgG antibodies.

Spatially resolved, in-depth cell profiling and quantification in human tissues by Multiplexed Histology and its biological applications

Anna Pascual Reguant1,2,

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The phenotype and function of cells are essentially linked to their tissue localization and to the interactions with the microenvironment. Based on that premise, we established a highly multiplexed immunofluorescence microcopy technique and combined it with a customized analysis pipeline that allows investigation of the overall tissue composition, retaining both spatial and single-cell resolution. Thereby, we have identified major immune cell populations in several organs, but also rare cell types, such as innate lymphoid cells (ILCs) and plasma cells, two cell types that we found to localize closely together in particular areas of the tonsils. We have identified new markers for ILC characterization, which were subsequently validated by RNA-seq, and we identified conserved stromal landmarks for ILC localization within and across tissues. We are currently studying local immune responses in several tissue pathologies, such as kidney tumors and transplants, colon samples from Crohn´s disease patients, as well as lungs and SLOs from COVID-19-deceased patients, in order to gain insights on the interrelation between immune cell types and structural tissue components, possibly mediating tissue inflammation and, ultimately, disease.

Stress-strain relation in virtual fluidic channels analyzed by particle image velocimetry

Peter Nestler

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Real-Time Deformability Cytometry (RT-DC) is a label-free technique for biomechanical phenotyping with high-throughput. However, in order to apply well-defined hydrodynamic stress on co-flowing cells dedicated channels with micrometer-precision are required. Recently, we demonstrated that virtual fluidic channels provide a versatile alternative to lithography based channels. Virtual fluidic channels are tunable liquid-liquid interfaces capable to constrain and deform cells that are created within a glass cannula. The cells mechanical properties can be deduced from a simple Kelvin-Voigt model, i.e. the proportionality between interfacial stress and cell strain. However, so far the virtual channel interfacial stress is poorly understood since it is highly connected to flow velocity distribution within the channel. To shed some light on the relation between flow profile and interfacial stress acting on cells we utilize micrometer resolution particle image velocimetry. Small tracer particles are introduced to the liquid and follow the flow profile faithfully. Observing the motion of the entrained tracer particles enables to calculate the flow velocity and direction both inside and outside the virtual fluidic channel. The flow profile of a virtual fluidic channel is characterized by a steep velocity gradient in close vicinity to the liquid-liquid interface. Linking cell deformation with flow velocity gradients promises a deeper understanding between the mechanical stress distribution on the cell surface and the consequential cell strain.

Superposition of viscosity maps and metabolism in parasitically infested intestinal tissue using BODIPY and NAD(P)H fluorecence lifetimes

Wjatscheslaw Liublin1,

Ruth Leben1, Robert Günther2, Jonathan Stefanowski3, Juliane Liebeskind3, Ingeborg Beckers4, Ingo Röhle4, Alina Liebheit3, Carolin Ulbricht3, Alexander F. Fiedler1, Anja E. Hauser3, Sebastian Rausch5, Susanne Hartmann5, Raluca Niesner1

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Background

Previous findings suggest a strong correlation of interactions between viscosity changes of the mucus of parasitically infested intestine, its metabolism and interaction processes with the parasites (especially as far as the behavior of the co-enzymes NAD(P)H is concerned). Thereby, defense mechanisms of the host, e.g. based on the enzymes NOX2 and NOX4, as well as dynamic parasite migration within the intestine as a counter-reaction are expected to be highly relevant in this context. Combining these physical parameters with spatiotemporal resolution in tissue opens new perspectives on the metabolic behaviour of host and parasites.

Results

The fluorescence lifetime of the lipofilic dye BODIPY varies with the viscosity of the medium in which it is dissolved. On the other hand, the fluorescence lifetime of NAD(P)H depends both on general metabolic activity and specific enzymatic function. Due to different emission spectra of BODIPY and NAD(P)H, we are able to synchroniously monitor by fluorescence lifetime imaging (FLIM) NAD(P)H-dependent metabolism and, based on BODIPY fluorescence, mucus viscosity, in one and the same ROI within murine intestine samples. Based on a calibration curve of BODIPY fluorescence lifetime as a function of viscosity, we were able to determine absolute, local changes in mucus viscosity. Together with NAD(P)H-FLIM, we created a metabolic and viscosity 3D map of healthy intestine tissue.We extended our investigations to duodenum of mice infected with Heligmosomoides polygyrus. We observed changes in viscosity in close proximity to the worms and were able to visualise fluid dynamic flow processes in the stained mucus while simultaneously recording the worm movements over time.

Conclusion

By using BODIPY and NAD(P)H-FLIM, we could show that we are able to gain reliable results in measuring mucus-viscosity and NAD(P)H-based metabolic processes.

Testing the B cell surfaceome by a bar-coded cytometry assay

Michael Reth,

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We are studying the nanoscale organization of receptors on the B cell surface and found that most molecules are highly organized at nano-distances. In our study we provided evidence of the functional relevance of this nanoscale membrane organization. Thus, different receptor systems on the lymphocyte surface are not residing in perfect isolation from, but are functionally connected to each other. To learn more about the functional association of receptors on the B lymphocyte we are using the human Burkitt lymphoma cell line Ramos that we render receptor deficient with the CRISPR/Cas9 technique. For our study of the alteration of proteins on the Ramos B cell surface before and after a CRISPR/Cas9-mediated gene deletion we developed a barcoded flow cytometry assay, which allows us to compare the surface protein expression pattern of up to 16 differently color-coded Ramos B cells. For this we expose different genetically modified Ramos cell lines to 4 increasing concentrations of CytoTell Blue and/or CytoTell Green. The cells are then mixed together and distributed over separate tubes for further staining with fluorescent antibodies each specific for a given B cell surface marker. This barcoding technique is then used to directly compare wild type Ramos B cells to either normal human B cells or to Ramos cell mutants. These studies demonstrate the of receptors on the B cell surface. We also use Ramos cells and the barcoding technique for the detection of antibodies directed against the SARS-CoV-2 spike protein in the blood of infected or vaccinated persons.

This project was supported by the Deutsche Forschungsgemeinschaft through the TRR130-P02 and R01 grant 1R01AI145656-01.

The role of surface contacts in 2D and 3D microenvironments for cell mechanical properties

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Cells form with their microenvironment a network of biological and physicochemical signals that stem from cell-cell and cell-matrix contacts. Several pathologies including oncological disorders are associated with changes in such contacts but a comparative investigation by different approaches substantiating their relevance towards cell mechanics has, to our knowledge, never been conducted.

Here, we examine the role played by the substrate for the mechanical properties of HEK293T cells grown in 2D monolayers and spheroids as a 3D cell culture model. Experiments are performed using real-time deformability cytometry (RT-DC) and atomic force microscopy (AFM) in comparative assays.

Our AFM results show that cells cultured in 2D have a Young's modulus that is significantly higher than that of 3D cultured cells. Interestingly, when cells are detached from the 2D substrate or the 3D matrix and captured in suspension, they become considerably stiffer. Comparing our AFM data to RT-DC results, which probe cells in suspension, we observe the same increase in elastic modulus independent of cell culture geometry. Our findings suggest, that the mechanical phenotype of adherent cells is to a large extent dominated by the presence of a substrate and less by the dimensionality of the cell environment. Furthermore, we look into the molecular basis of cell mechanics by deducing the levels of cytoskeletal proteins such as β actin, α/β tubulin, vinculin and talin by fluorescent flowcytometry and western blotting.

Session 4: Core Facility Session: Spectral Cytometry

Thursday, 01/Oct/2020:
3:30pm - 4:30pm
Session Chair: Frank Schildberg, Christian Kukat

Implementing spectral cytometry in a core facility: one lab's experience with the Cytek Aurora

Simone Pöschel

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You want to take flow cytometry to the next level of performance?

The Cytec AURORA allows separation of fluorochromes with very close emission profiles by capturing the entire emission spectrum signature of the fluorescent dyes rather than just a section of it. This enables the use of a wide range of new fluorochrome combinations and opens the door to high complexity applications.

Having recently implemented the AURORA in our Core Facility, we would like to introduce you to this interesting technique and share our experiences of the establishing procedure. We will talk about advantages, challenges, pitfalls of technique and software and present first data and results.

Did we arouse your interest? We do look forward to exchanging our experiences with this powerful technique.

The metabolic momentum of MDSCs

Bastian Höchst

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Myeloid derived suppressor cells (MDSCs) develop in the response to chronic inflammation and are key players in blunting immune responses against cancer. Despite the high importance of these cells in local regulation of effector T cells in cancer and intensive research efforts in this filed, no molecular marker has been identified that defines MDSCs.

We could show that MDSCs are characterized by a strongly reduced metabolism and that they transfer this hibernation-like state to CD8 T cells and thereby paralyze them.

This effect is caused by the accumulation of the dicarbonyl radical methylglyoxal, which is produced by the semicarbazide sensitive amine oxidase (SSAO). Methylglyoxal is transferred to the effector cells where it leads to a depletion of l-arginine.

In a preclinical model, we demonstrated that neutralization of methylglyoxal overcomes MDSC-mediated suppression and, in combination with checkpoint inhibition, leads to significantly improved cancer immunotherapy.

Our results identify methylglyoxal as a functional marker metabolite for MDSCs that mediates T-cell paralysis and may serve as a target for improving cancer immunotherapy.

Session 5: Cutting Edge (Sars-CoV-2) Part 1

Friday, 02/Oct/2020:
10:00am - 10:50am
Session Chair: Raluca Niesner, Anja Hauser

Immune changes during COVID-19 pneumonia

Andrea Cossarizza

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During the last months, the outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed a great challenge to the human health, worldwide, with million of infections. We were immediately involved in studying the role of immune system in fighting SARS-CoV2, and have focused our attention on T lymphocytes, B cells and monocytes to investigate the mechanisms underlying the progressive leukopenia observed in patients.

Multiparameter flow cytometry, coupled with unsupervised data analysis, allowed us to find that the T cell compartment in COVID-19 patients with severe pneumonia has several alterations that involve naïve, central memory, effector memory and terminally differentiated cells, as well as regulatory T cells and PD1+CD57+ exhausted T cells. Several lineage-specifying transcription factors and chemokine receptors are also altered. Terminally differentiated T cells from patients proliferate less than those from healthy controls, whereas their mitochondria functionality is similar in CD4+ T cells from both groups. Dramatic simultaneous increases of proinflammatory or anti-inflammatory cytokines, including T helper type-1 and type-2 cytokines, chemokines and galectins were observed in plasma. We then interrogated B cells in these patients, who displayed normal plasma level of the main immunoglobulin classes, of antibodies against common antigens or against antigens present in common vaccines. A decreased number of total and naïve B cells was found, along with decreased percentages and numbers of memory switched and unswitched B cells. On the contrary, IgM+ and IgM- plasmablasts were significantly increased. In vitro cell activation revealed that B lymphocytes showed a normal proliferation index and number of dividing cells per cycle.

Finally, the analysis of monocytes revealed a consistent redistribution of their subsets,

with a significant expansion of intermediate/pro-inflammatory cells, a concomitant compression of classical monocytes, and an increased expression of inhibitory checkpoints, including PD-1/PD-L1. Altered bioenergetics and mitochondrial dysfunction was found, that included a reduced basal and maximal respiration, reduced spare respiratory capacity and decreased proton leak.

Session 7: Cutting Edge (Sars-CoV-2) Part 2 - Combined approaches for COVID-19 Cytometry in Berlin

Friday, 02/Oct/2020:
1:00pm - 2:00pm
Session Chair: Attila Tarnok, Session Chair: Asylkhan Rakhymzhan

Dissecting innate immune responses to SARS-CoV-2

Leif Erik Sander

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Coronavirus disease 2019 (COVID-19) is a mild to moderate respiratory tract infection, however, a subset of patients progresses to severe disease and respiratory failure. The mechanisms of protective immunity in mild forms and the pathogenesis of severe COVID-19 associated with increased neutrophil counts and dysregulated immune responses remain unclear. In a dual-center study we combined single-cell RNA-sequencing and single-cell proteomics of whole-blood and peripheral-blood mononuclear cells to determine changes in immune cell composition and activation in mild versus severe COVID-19 (242 samples from 109 individuals) over time. Highly activated HLA-DRhiCD11chi monocytes with a strong interferon-stimulated gene (ISG) signature were elevated in mild COVID-19. In contrast, severe COVID-19 was marked by the occurrence of neutrophil precursors, as evidence of emergency myelopoiesis, and dysfunctional mature neutrophils. Moreover, monocytes in patients with severe COVID-19 showed signs of alternative activation and expressed low levels of HLA-DR. Single cell RNA-Seq analysis of bronchoalveolar lavage and lung tissue from different disease stages, combined with Multi-epitope-ligand cartography (MELC), revealed an enrichment of alternatively activated monocyte-derived macrophages in patients with COVID-19 ARDS. Our study provides detailed insights into the innate immune response to SARS-CoV-2 infection and reveals profound alterations in the myeloid cell compartment associated with severe COVID-19 and ARDS.

Olfactory transmucosal SARS-CoV-2 invasion as port of Central Nervous System entry in COVID-19 patients

Helena Radbruch

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The newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19, a pandemic respiratory disease presenting with fever, cough, and often pneumonia. Moreover, thromboembolic events throughout the body including the central nervous system (CNS) have been described. Given first indication for viral RNA presence in the brain and cerebrospinal fluid and in light of neurological symptoms in a large majority of COVID-19 patients, SARS-CoV-2-penetrance of the CNS is likely. By investigating and anatomically mapping oro- and pharyngeal regions and brains of 33 patients dying from COVID-19, we not only describe CNS infarction due to cerebral thromboembolism, but also demonstrate SARS-CoV-2 neurotropism. SARS-CoV-2 enters the nervous system via trespassing the neuro-mucosal interface in the olfactory mucosa by exploiting the close vicinity of olfactory mucosal and nervous tissue including delicate olfactory and sensitive nerve endings. Subsequently, SARS-CoV-2 follows defined neuroanatomical structures, penetrating defined neuroanatomical areas, including the primary respiratory and cardiovascular control center in the medulla oblongata.

In severe COVID-19, SARS-CoV-2 induces a chronic, TGF-β-dominated adaptive immune response

Mir-Farzin Mashreghi

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The human immune response to SARS-CoV-2 infection is highly variable, with less than 10% of infections resulting in severe COVID-19 requiring intensive care unit (ICU) treatment. Here we have analyzed the dynamics of the adaptive immune response in COVID-19 ICU patients at the level of single cell transcriptomes and B cell and T cell receptor (BCR, TCR) repertoires. Early after ICU admission, before seroconversion in response to SARS-CoV-2 spike protein, patients generate activated peripheral B cells with a type 1 interferon-induced gene expression signature. After seroconversion, patients display circulating activated B cells expressing an IL-21-induced gene expression signature and mainly IgG1 and IgA1, two isotypes induced by IL-21 and TGF-β, respectively. In sustained COVID-19, the persistent immune reaction is shifted to IgA2-expressing activated peripheral B cells, displaying somatic hypermutation, and expressing TGF-β-induced signature genes, like IgA germline transcripts. The switch from an IgG1 to an IgA2-dominated B cell response correlates with the appearance of SARS-CoV-2 reactive follicular T helper cells expressing IL-21 and/or TGF-β in the blood. Despite the continued presence of IgA2-expressing B cells and IgA antibodies in the blood of progressed COVID-19 patients, IgA2 secreting cells were scarce in the lungs of deceased COVID-19 patients. In summary, in severely affected COVID-19 patients SARS-CoV-2 triggers chronic immune reactions which are controlled by TGF-β, with most of the activated B cells being no longer specific for the SARS-CoV-2 spike protein and its receptor binding domain, nor for nucleoprotein. TGF-β may candidate as a target to ameliorate detrimental immunopathology in those patients.

Industry Webinars

Becton Dickinson – BD

Chair: Anja Hauser, DGfZ

Combination of single-cell transcriptomics and multi-parameter flow cytometry to better understand cellular heterogeneity and function in humans

Jonas Schulte-Schrepping, Lorenzo Bonaguro, Marc Beyer

German Center for Neurodegenerative Diseases (DZNE), Bonn

Recent technical advances in the fields of flow cytometry and single-cell Omics have greatly increased our understanding of cellular heterogeneity and paved the way for initiatives charting the cells of the immune system and the human body.

We have set out to harness these new technologies to characterize the cellular heterogeneity in the CNS as well as the human immune system in chronic and acute infections and its influence during the development and progression of neurodegenerative diseases.

By combining multi-parameter flow cytometry with single-cell transcriptomics we could recently fine-map the human blood-borne immune response to COVID-19 infections and describe the occurrence of neutrophil precursors, as evidence of emergency myelopoiesis, as well as dysfunctional mature neutrophils as a hallmark of severe COVID-19 infections.

Taken together, these data clearly demonstrate the usefulness of this approach to address questions focusing on cellular differentiation processes as well as ontogeny and function, which so far could not be sufficiently answered.

Omni Life Science, OLS

Chair: Christin Koch, DGfZ

We invite you to celebrate the virtual 30th Anniversary conference of the German Society of Cytometry (DGfZ) with OLS.

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Speaker: Dr. Thorsten Rieling

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Chair: Asylkhan Rakhymzhan, DGfZ

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Speaker: Konrad von Volkmann

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Propel Labs

Chair: Wolfgang Fritzsche, DGfZ

Discover the Bigfoot Spectral Cell Sorter

Speaker: Mark Cheetham

The BIGFOOT Cell Sorter with Sasquatch Software (SQS) provides the power, safety, performance, and flexibility for your lab today and into the future.

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Propel Labs Europe
Cambridge, UK.

Luminex

Chair: Frank Schildberg, DGfZ

Amnis® AI Image Analysis Software

Speaker: Sebastian Thalmann

Amnis® AI incorporates the latest advancements in computer vision to create an intuitive and powerful image analysis tool for the imaging flow cytometers FlowSight® and ImageStream®X. Amnis® AI leverages a number of recent advancements in machine learning, including computer-aided hand-tagging, clustering with object map plots, generation of a novel experimental model using deep learning convolutional neural networks (CNN), and confusion matrix tables with accuracy analytics. Combined with Amnis® high-throughput imaging systems, Amnis® AI is an easy-to-use analysis tool, allowing researchers to harness the power of artificial intelligence to further their scientific discoveries. The presentation will introduce the workflow using Amnis® AI software by means of exemplary applications.

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Fluidigm

Deep & spatial immune profiling by mass cytometry

Mayur Bakshi

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Immune profiling & monitoring are commonly applied in translational and clinical research settings to provide phenotypic understanding of immune states prior to and following treatment (e.g. COVID-19). Mass cytometry, which utilizes CyTOF(r) technology, is a single-cell analysis platform that uses metal-tagged antibodies and can resolve over 50 markers in a single sample tube. We have developed a sample-to-report solution for human immune profiling using mass cytometry, the Maxpar(r) Direct(tm) Immune Profiling System. It includes an optimized 30-marker immune profiling panel provided in a dry, single-tube format, protocols for human whole blood and PBMC staining, a Helios™ data acquisition template, and Maxpar Pathsetter™ software for automated data analysis. The Pathsetter software analyzes FCS 3.0 files generated with the kit and automatically reports cell counts and percentages for 37 immune cell types with analytical validation data on repeatability, reproducibility, software precision, and software accuracy. The Hyperion Imaging System brings proven CyTOF® technology together with imaging capability to empower simultaneous interrogation up to 40 protein markers using Imaging Mass Cytometry™ (IMC™). Using this system, you can deeply interrogate tissues and tumors at subcellular resolution while preserving the information in tissue architecture and cellular morphology to uncover new biomarker correlations and cell interactions.

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Sony

Chair: Asylkhan Rakhymzhan, DGfZ

Sony Biotechnology Innovations

Speaker: Sebastian Weissmann

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To improve stability, Sony systems allow direct monitoring of particle flow status using technology originally developed for the Blu-ray disk that delivers error free performance while operating at very high speed. In conventional flow cytometers, this accuracy largely depends on skillful operators.

To deliver very low noise electronics, Sony Cytometers incorporate technologies that have brought pure, true sound to music. This Sony know-how reduces signal noise from the electric circuit currents to achieve very low noise electronics so that rogue signal noise does not get in the way of delivering accurate results to scientists.

In addition, Sony Biotechnology has developed an innovative approach to capturing and decoding the information gained through flow cytometry. Spectral analysis technology unique to Sony flow cytometers enables scientists to better visualize flow cytometric data by collecting and saving a wider spectrum of visual information and allowing scientists to examine that information with greater granularity—down to individual spectral fingerprints. As a result, spectral technology reduces complexities associated with flow cytometric analysis, improves detection of rare populations and accuracy of fluorescent data including independent signal detection of autofluorescence –all of these are critical to biomedical discovery.

Sony Europe B.V.

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Beckmann Coulter

Chair: Anja Hauser, DGfZ

A New Addition to the CytoFLEX Platform, Introducing the CytoFLEX SRT Cell Sorter

Speaker: Andreas Wicovsky

Field Marketing Manager, Beckman Coulter Life Sciences

Built on patented CytoFLEX platform technology, which simplified performing common to complex flow cytometric experiments, the CytoFLEX SRT empowers researchers with scalable sorting options in a benchtop format. Available in multiple configurations for maximum budget flexibility, allowing the same sorter to easily grow to fit your current and future demands. CytoFLEX SRT includes automation technology not previously seen in a benchtop platform. Setup and QC is smart and simplified with a single bead and sort setup has been fully automated allowing novice users to easily create stable droplets, find drop delay, and establish stable sort streams. Stability of sorting is maintained through advanced automation including droplet and side stream monitoring paired with auto-recovery of the sort. While benchtop sized and automated for ease of use, Beckman Coulter has kept important features such as missed mode sorting, abort recovery, and index sorting available on all configurations.

Topics:

- See available configurations for CytoFLEX SRT
- See a preview of anticipated specifications
- Watch an overview of creating a sort in CytExpert

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Miltenyi

Chair: Elmar Endl, DGfZ and Thomas Bauer

Pre-existing T cell memory as a risk factor for severe COVID-19 in the elderly

Alexander Scheffold and Petra Bacher

Institute of Immunology, Christian-Albrechts-Universität zu Kiel

Coronavirus disease 2019 (COVID-19) displays high clinical variability but the parameters that determine disease severity of COVID-19 are unclear. Pre-existing T-cell memory generated by frequent infections with related "common cold" Coronavirus (CCCoV) has been hypothesized as protective mechanism, but conclusive evidence is lacking. We used antigen-reactive T cell enrichment (ARTE) a sensitive technology to identify antigen-reactive T cells, for deep profiling of SARS-CoV-2 and CCCoV-specific T cells from healthy donors and COVID-19 patients, employing multiparameter cytometry, single cell gene expression profiling and TCR avidity and cross-reactivity measurements. The data I will present suggest that pre-existing SARS-CoV-2 specific memory is not primarily induced by CCCoV and is not protective but mainly increased in the elderly. Pre-existing memory may instead contribute to severe COVID19 disease observed in this age group.

Participants

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