19th Annual Conference of the

German Society for Cytometry

Date

October 14-16, 2009

Location

Leipzig Kubus
Helmholtz-Centre for
Environmental Research Leipzig

Scientific Committee

PD Dr. Susann Müller
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Prof. Dr. Annette Beck-Sickinger
Dr. Wolfgang Beisker
Prof.Dr. Dirk Busch
Dr. Simone Diermeier-Daucher
Dr. Elmar Endl
Andy Riddell
Prof. Dr. Leoni Kunz-Schughart
Prof. Dr. Ulrich Sack
Dipl. Ing. Peter Schwarzmann
Dr. Torsten Viergutz
Prof. Dr. Dieter Weiss
Prof.Dr. Christian Wilhelm
Dr. Johannes Wessels

Organising Committee

Dr. Elmar Endl
PD Dr. Susann Müller
Christine Süring

General Information
www.dgfz.org

Dear Members of the German Society for Cytometry, dear participants of the 19th Annual Conference of the DGfZ, dear guests,

I appreciate the tradition to welcome you to the annual conference of your society in Leipzig.

It is a pleasure for me to do so, since I know that the society has a long standing tradition in a field which is becoming more and more a main focus of the expanding cell biology research in Leipzig and Saxony.

The research landscape in Saxony with a focus on cell research comprises five state universities, institutes of the Scientific Society Gottfried Wilhelm Leibniz, of the Fraunhofer and Max Planck Societies, as well as the Helmholtz Association. Research in Saxonia on the fields of nanotechnology, biotechnology, the neurosciences, medical technologies and anthropogenic impacts on environment is among the world's elite.

Approximately 80 biotechnology and pharmaceutical companies as well as more than 30 university and non-university research institutes focussing on life science found their place within Saxony. According to the Saxon State Minister for Science and Fine Arts Dr. Stange the Free State of Saxony will continue to support research projects within these fields until 2013 with its own state excellence initiative using 160 million € from the European Regional Development Fund and from the Free State of Saxony. Therefore, Saxony and especially Leipzig is an excellent location to realize a conference where the features of a single cell are the favourite universe of its participants.

Leipzig University itself is rich in tradition with a wide spectrum of scientific disciplines. The Faculties of Medicine and of Biosciences, Pharmacy and Psychology are important research institutions and maintain close links to other faculties of the University. With specialized facilities for cell and tissue culture, cytology, histology and immuno-histochemistry, diverse microscopic techniques, flow cytometry and cell sorting, as well as protein biochemistry vital techniques for cell biology research are available.

The Centre for Biotechnology and Biomedicine (BBZ) was established at the University of Leipzig as one of two bio-innovation centres in Saxony in context of the "Biotechnology campaign in Saxony" of the Saxon State Government. The BBZ is a central research institution of the University of Leipzig. With the focus on key issues such as "Molecular Design" and "Medical Biotechnology", the University is aiming at bringing together basic and applied research.

The Translational Centre for Regenerative Medicine (TRM) and the Fraunhofer Institute for Cell Therapy and Immunology (IZI) house national and international experts from research, and clinical application to work on substantive solutions for problems in medical engineering and therapy application.

The researchers of the MPI for Evolutionary Anthropology aim to investigate the history of mankind from an interdisciplinary perspective with the help of comparative analyses of genes, behaviour and languages. Research at the MPI for Human Cognitive and Brain Sciences in Leipzig revolves around cognitive abilities and cerebral processes of people rather than looking at the cellular level. Part of this research, however, will be presented at this conference by providing the Dinner Opener with a talk about ‚Decision making under time pressure' from Mrs. Forstman.

Last but not least, cell related science at the Helmholtz Centre for Environmental Research is the essential ground for understanding environmental processes which drive climate change, which contribute to cleaning up of huge environmental pollutions or which cause allergic reactions to pollutants in air and water.

Since the commencement of the biotechnology campaign in September 2000, Saxony and Leipzig have developed into most dynamic cell based research and biotechnology regions in Germany – and are on the way to approach leading positions in those fields in Germany and even Europe.

Therefore, having you here, presenting your data, discussing results, problems, and providing solutions is exactly the way how Leipzig and the University want to meet the upcoming challenges in cell biology.
I know that your society is characterized by a considerable heterogeneity in research themes, applications in all fields of cell studies as well as in instrumental and technical developments. Although it seems to be complex to house all these themes under one roof it is quite obvious that this might be also the strength of your society. 19 years of meetings show a long standing wish for scientific exchange, learning, presenting, testing instruments and joining in conversations about the many topics applied.
When looking at the history column at your website www.dgfz.org an impressive overview about the science already presented within the frame of the annual conferences can be found.

This year's conference concentrates on three main scientific fields: one is how to get high content information on a single cell level by using new image techniques with an application focus on neuronal cells. Advancements in this research area of cytometry are for the benefit of patients and give an imagination in how information is transmitted, how survival is guaranteed and how life works in general on the microscopic level of a single nerve cell.
The second main topic is the ‚conference within the conference' about stem cells and cancer stem cells which will give deep insight into recent outstanding developments and new opinions about this research. Last but not least there is the application of cytometric techniques beside the traditional fields of basic human cell research. There are many talks and also posters which show usefulness, increased demand and existent problems, but also remarkable new innovations of single cell techniques in microbiology, plant biology and biotechnology.

Moreover, another point is also very important: The daily practice and implicitness of scientific exchange worldwide is also a valuable characteristic of life in the German Society for Cytometry. Speakers and participants of more than ten different countries are participating in the conference. There is now a Session called ‚European Networks' which was introduced to provide a platform for networking and discussion beyond the borders of Germany. There is the advantage of getting people together working in the same region neglecting nearby borders or working on similar very specific themes which may be represented only by few groups or scientists in one country. It will also hopefully increase the possibility to find oneself in scientific consortia being the grain for joint projects within Europe or even together with other countries overseas.

The society is very heterogeneous in its research themes and demands people with an open mind to the various applications used to analyse cells on a single cell level. Usually these people are curious and willing to push the limits of optical resolution and willing to understand structure and function of cells in heterogeneous populations and thereby contributing significantly to urgent problems in life sciences, health care, nutrition or even climate change.

I wish all participants an attractive and fruitful meeting. I hope you will enjoy the meeting at the Helmholtz Centre for Environmental Research and you will benefit from it.

Martin Schlegel

Prorektor für Forschung und wissenschaftlichen Nachwuchs
Universität Leipzig

19th Annual Conference of the German Society for Cytometry

October 14-16, 2009
Helmholtz-Centre for Environmental Research, Leipzig

Wednesday, 14/Oct/2009	
1:30PM - 3:00PM	**ADVANCED IMAGING**
	Chair: Annette G. Beck-Sickinger
3:30PM - 4:30PM	**APPLICATION IN NEUROBIOLOGY**
	Chair: Dieter Weiss
5:00PM - 7:00PM	**NEW DEVELOPMENTS IN INSTRUMENTATION**
	Chair: Wolfgang Beisker
7:00PM - 7:05PM	**MEET THE EDITORIAL BOARD OF CYTOMETRY PART A**
7:05PM - 9:30PM	**CORE FACILITY MANAGER WORKSHOP**
	Chair: Elmar Endl
7:05PM - 9:30PM	
	Get Together and Poster Setup/Session

Thursday, 15/Oct/2009	
9:00AM - 10:30AM	**STEM CELLS AND CANCER**
	Chair: Leoni Kunz-Schughart
11:00AM - 12:30PM	**CANCER STEM CELLS**
	Chair: Leoni Kunz-Schughart
1:30PM - 3:00PM	**PLANTS/ BIOTECHNOLOGY**
	Chair: Christian Wilhelm
3:30PM - 5:00PM	**MICROBIOLOGY**
	Chair: Susann Müller
5:00PM – 7:00PM	**POSTER SESSION**
	Chair: Torsten Viergutz Chair: Elmar Endl
7:00PM - 8:00PM	**OPEN SPECIAL LECTURE**
	Birte Forstmann
8:00PM – 11:30PM	**DINNER**

Friday, 16/Oct/2009	
8:15AM - 8:45AM	**KLAUS-GOERTTLER-PREISTRÄGER**
8:45AM -10:00AM	Mitglieder Versammlung
10:30AM - 12:00AM	**NEW CONCEPTS IN THERAPY CONTROLLING AND PREDICTION**
	Chair: Ulrich Sack
1:00PM - 2:30PM	**IMMUNOLOGY**
	Chair: Dirk H. Busch
3:00PM - 5:00PM	**EUROPEAN NETWORKS**
	Chair: Andy Riddell

19th Annual Conference of the German Society for Cytometry

October 14-16, 2009
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Wednesday, 14/Oct/2009

10:00am – 1:30pm Registration and poster setup

1:30PM - 3:00PM KUBUS, SAAL 1

Advanced Imaging
Session Chair: Annette G. Beck-Sickinger

1:30 - 2:00	Ernst H.K. Stelzer, EMBL-Heidelberg, Germany "Light sheet based fluorescence microscopes (LSFM, SPIM, DSLM) reduce phototoxic effects by several orders of magnitude"
2:00 - 2:15	David Kosel, Leipzig University, Germany "Imaging of Adiponectin Receptor 1 Dimerization Inhibition"
2:15 - 2:30	Thomas Kroneis, Medical University of Graz, Austria "Molecular genetic and cytogenetic analysis following low-volume on-chip whole genome amplification of automatically detected and laser-microdissected single cells"
2:30 - 2:45	Benjamin M. Bader, University of Rostock, Germany Karolinska Institute, Stockholm, Sweden "Quantitative image cytometry: nuclear beta-catenin shuttling during differentiation of human and mouse midbrain progenitor cells"
2:45 - 3:00	Heinz-Georg Jahnke, University of Leipzig, Germany "Impedance spectroscopy based label-free real-time monitoring of 3D-cultures and tissue"

3:00pm - 3:30pm *Coffee break*

3:30PM - 4:30PM KUBUS, SAAL 1

Application in Neurobiology
Session Chair: Dieter Weiss

3:30 – 4:00 Alexander Gottschalk, Universität Frankfurt am Main, Germany
"Optogenetic approaches to control neuronal activity and animal behaviour by light, using microbial-type rhodopsins"

4:00 – 4:15 Dieter G. Weiss, University of Rostock, Germany
"Quantitative determination of electrical activity pattern changes caused by neuro-active compounds in single neurons and whole neuronal networks in vitro by microelectrode arrays"

4:30pm - 5:00pm Coffee break

5:00PM - 7:00PM KUBUS, SAAL 1

New Developments in Instrumentation
Session Chair: Wolfgang Beisker

5:00 - 5:30 Josef Käs, Biophysics at the University of Leipzig, Germany
"Feeling for Cancer with Light"

5:30 - 6:00 James Leary, Purdue University, United States of America
"Ultra high-speed sorting"

6:00 - 6:20 Arkadiusz Pierzchalski, University of Leipzig, Germany
"Label-free single cell analysis with a chip-based impedance flow cytometer"

6:20 - 6:40 Michael W. Olszowy, Life Technologies, United States of America
"The Newest Molecular Probes® Flow Cytometry Reagents from Invitrogen: Qdot® Nanocrystal Conjugates, Reagents for Cell Cycle & Proliferation Analysis, Viability Detection, Detachable Magnetic Beads and a Novel Phagocytosis Assay"

6:40 - 7:00 Marcin Frankowski Physikalisch-Technische Bundesanstalt, Berlin, Germany
"Microfluidic structures for flow cytometric analyses of immunologically stained blood cells"

7:00PM - 7:05PM

Meet the Editorial Board of Cytometry Part A

Attila Tarnok

7:05PM - 9:30PM KUBUS, SAAL 1

Core Facility Manager Workshop
Session Chair: Elmar Endl

Derek Davies, London Research Institute, Cancer Research UK, United Kingdom
"Does size matter? Defining a critical mass for a Core Facility"

7:05PM - 9:30PM KUBUS FOYER

Get Together and Poster Setup/Session

Thursday, 15/Oct/2009

9:00AM - 10:30AM KUBUS, SAAL 1

Stem Cells and Cancer
Session Chair: Leoni Kunz-Schughart

9:00 - 9:10 Leoni Kunz-Schughart, Dresden University of Technology, Germany
"Stem Cells, Cancer Stem Cells, Cancer, Cancer Tissue: Where is the link?"

9:10 - 9:45 Björn Scheffler
"Stem cells as generative, regenerative, and destructive elements in the nervous system"

9:45 - 10:00 Gero Brockhoff, University of Regensburg, Germany
"Presence of HER4 associates with increased sensitivity to Herceptin treatment in patients with metastatic breast cancer"

10:00 - 10:15 Heiko Lemcke, University of Rostock, Germany
"The use of light microscopy techniques and flow cytometric analyses in the search for new synthetic tubulin modulators"

10:15 - 10:30 Anja Kathrin Wege, University Regensburg, Germany
"Flow cytometry- an essential tool in the generation and use of „humanized mice" "

10:30am – 11:00am Coffee break

11:00AM - 12:30PM KUBUS, SAAL 1

Cancer Stem Cells
Session Chair: Leoni Kunz-Schughart

11:00 - 11:35 Dominique Bonnet, London Research Institute, Lincoln's Inn Fields Laboratories, United Kingdom
"Cancer Stem Cells: Lessons from Acute Myeloid Leukemia"

11:35 - 12:10 Mauro Biffoni, Istituto Superiore di Sanità, Italy
"Cancer stem cells from solid tumors: towards clinical applications"

12:10 - 12:30 Claudia Dittfeld, TU Dresden, Germany
"Challenges to identify CD133 expression in cancer cells"

12:30pm – 1:30pm Lunch

1:30PM - 3:00PM KUBUS, SAAL 1

Plants/ Biotechnology
Session Chair: Christian Wilhelm

1:30 - 2:00 David Galbraith, University of Arizona, United States of America
"Novel Cytometric Platforms for Eukaryotic Functional Genomics"

2:00 – 2:15 Kenneth Wayne Berendzen, Center for Plant Molecular Biology - Tübingen, Germany
"How to get the "Flow" of screening protein interaction libraries in living plant cells"

2:15 – 2:30 Sílvia Raquel Castro, Centre of Functional Ecology, Department of Botany, University of Coimbra, Portugal
"Applications of flow cytometry in plant population biology and ecology"

2:30 – 2:45 Pierre Moretti, Institut für Technische Chemie, Leibniz Universität Hannover, Germany
"Mesenchymal stromal cells from human umbilical cord tissue: identification of sub-populations"

2:45 – 3:00 Magda Tomala, Institut für Technische Chemie, Leibniz Universität Hannover, Germany
"Expansion of pluripotent stem cells: Strategies towards large scale cultures"

3:00pm - 3:30pm *Coffee break*

3:30PM - 5:00PM KUBUS, SAAL 1

Microbiology
Session Chair: Susann Müller

3:30 - 4:00 James Leary, Purdue University, United States of America
"High Throughput and Multiplexed Detection of Food-borne Pathogens by a Hybrid Microfluidic SPR and Molecular Imaging Cytometry Device"

4:00 - 4:15 Florian David, Technische Universität Braunschweig, Germany
"Culture heterogeneity in *Bacillus megaterium* cultivations producing antibody fragments regarding the state of production and membrane potential"

4:15 - 4:30 Nico Jehmlich, Helmholtz Centre for Environmental Research, Germany
"Combination of microbial cytomics and proteomics approaches for structural and functional characterisation of bacterial communities"

4:30 - 4:45 Susann Müller, Helmholtz Centre for Environmental Research, Germany
"Community dynamics within a bacterial consortium during growth on toluene under sulfate-reducing conditions"

4:45 - 5:00 Jost Max Weber, Technische Universität Dresden, Germany
"Expression of recombinant hydrophobins in *Schizosaccharomyces pombe* in high cell density fermentation"

5:00PM – 7:00PM KUBUS FOYER

Poster Session
Session Chair: Torsten Viergutz
Session Chair: Elmar Endl

7:00PM - 8:00PM KUBUS, SAAL 1

Open Special Lecture

Birte Forstmann, "Decision Making under Time Pressure"

8:00pm-11:30pm *Conference Dinner*

Friday, 16/Oct/2009

8:15am - 8:45am Klaus-Goerttler-Preisträger KUBUS, SAAL 1

Session Chair: Susann Müller

Frank Alexander Schildberg, Institute of Molecular Medicine, University of Bonn, Germany
"Liver sinusoidal endothelial cells veto CD8 T cell activation"

8:45AM -10:00AM MITGLIEDER VERSAMMLUNG KUBUS, SAAL 1

10:00am – 10:30am *Coffee break*

10:30AM - 12:00AM KUBUS, SAAL 1

New Concepts in Therapy Controlling and Prediction
Session Chair: Ulrich Sack

10:30 - 10:35 Ulrich Sack, Universität Leipzig, Germany
"Introduction into the session"

10:35 - 11:05 Frank WMB Preijers, London Research Institute, UMC St Radboud, The Netherlands
"Multi-colour analyses for immunophenotyping of normal and malignant lymphocyte subsets"

11:05 - 11:35 Sumeet Gujral, Tata Memorial Hospital, Mumbai, India
"Leukemia Phenotype Panels for the Developing Countries"

11:35 - 12:00 Stephan Borte, University of Leipzig, Germany
"Interleukin-21: A new perspective in the treatment of primary antibody deficiency?"

12:00am – 1:00pm *Lunch*

1:00PM - 2:30PM KUBUS, SAAL 1

Immunology
Session Chair: Dirk H. Busch

1:00 – 1:05	Dirk H. Busch, Technische Universität München,Institut für Med. Mikrobiologie, Germany "Introduction into the session"
1:05 – 1:45	Andreas Radbruch, Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), Germany "Tracking Immunologic Memory"
1:45 – 2:00	Marc Beyer, LIMES-Institute,University of Bonn, Germany "FOXP3-mediated inhibition of the global gene regulator SATB1 is required for maintaining regulatory T-cell commitment"
2:00 – 2:15	Elmar Endl, Institute for Molecular Medicine, University Hospital Bonn, Germany "Precise light-induced cell elimination mediated by immune-targeted gold nanoparticles"
2:15 – 2:30	Veit R. Buchholz, Technische Universität München, Germany "Origin of diverse cytokine phenotypes from individual naive precursors"

2:30pm – 3:00pm Coffee break

3:00PM - 5:00PM KUBUS, SAAL 1

European Networks
Session Chair: Andy Riddell

3:00 - 3:30	João Loureiro, Centre of Functional Ecology & Department of Botany, University of Coimbra, Coimbra, Portugal " "Microfluidics, Macrofluidics, Flow and Imaging: Mix and Match" Web based platforms for the flow cytometry community"
3:30 - 4:00	Andy Riddell, EMBL Heidelberg, Germany "The European Cytometry Network"
4:00 - 4:15	Sumeet Gujral, Tata Memorial Hospital, Mumbai, India "Indian Society of Cytometry"

4:15 - 4:30	Attila Tárnok, Cardiac Center GmbH; University of Leipzig, Germany “Education for Publishing in Cytometry Part A”
4:30 - 5:00	Andy Riddell, EMBL Heidelberg, Germany “Discussion about cooperation and communication in Europe”

Sessions

Advanced Imaging

The investigation of biological systems highly depends on the possibilities to visualize and quantify biomolecules and their related activities in real-time and non-invasively. Modern imaging techniques significantly contributed to this topic and advanced cell biology enormously. Next generation techniques will be presented in this session that includes 3D-tissue based imaging to study biochemical reactions under physiological conditions.

Application in Neurobiology

Single cell techniques are of special importance in neuroscience because - more than for other organs - for the understanding of the nervous system function one has to take into account the individuality and the special contribution of the single neuron to the network activity. Cell morphology, gene expression, and electrical activities can be studied and even manipulated by single cell imaging techniques, but also flow cytometry deserves the attention of neurobiologists, e.g. in studies on neural progenitor cell differentiation.

New Developments in Instrumentation

New technologies and instrumentation in microscopy and cytometry have always driven scientific progress and open new horizons. Three-dimensional imaging, low level fluorescence detection of molecules to high speed cell sorting and high efficient data analysis on the more technical side is complemented by methods in biology and biochemistry to gain new insights in some of the most important questions in life sciences. This session will present concepts, ideas, applications and scientific results of high priority for the scientific community.

Stem Cells and Cancer

This first session of a two session series in the field of stem cells and oncology will try to interdigitate our knowledge in stem cell and progenitor cell phenotype with cancer research and treatment. Today, stem and progenitor cells are an important source to replace diseased tissues. An overview of how stem cell research has affected and may further impact cancer therapy will be given. One of the plenary talks will particularly focus on brain development and tumors. However, all abstracts dealing with new insights into bone marrow or organ-specific stem and progenitor cell phenotype and function including those on the identification and use of causal or non-causal surrogate markers for stem/progenitor cells are welcome and will be considered for oral presentation. The lectures are expected to lead to some discussion to introduce the term "cancer stem cell" which will be further emphasized in the second session.

Cancer Stem Cells

Over the past decade, research in the field of oncology has led to the hypothesis of a so-called *cancer stem cell* (CSC) population. According to the consensus definition of the AACR "Cancer stem cell" Workshop in 2006, CSC are a small subset of cancer cells within the tumor mass that (i) constitute a reservoir of self-sustaining cells with the exclusive ability for self-renewal and tumor maintenance and (ii) have the capacity to both divide and expand the CSC pool and to differentiate into the heterogeneous (non-tumorigenic) cancer cell lineages. Experimental evidence supports the hypothesis of CSC in various solid tumor entities such as brain tumors, melanomas and carcinomas of the breast, prostate, pancreas or intestinal tract. However, it is still unclear whether such CSC originate from organ stem cells that retain selfrenewal properties but acquire epigenetic and genetic changes required for tumorigenicity or from proliferative progenitors that acquire self-renewal capacity. Either mechanism would be different from the widely held notion that most cells in a solid tumor are competent for tumor formation. However, because of lack of clarity, many researchers prefer an alternative nomenclature for the cell population(s) of interest, e.g. tumor-initiating or tumorigenic cells, to avoid over-interpretation of their data. Many theoretical and practical approaches to identify and study potential CSC are driven by lessons that were learned from hematologic malignancies. Accordingly, the role and origin of CSC in leucemia will be discussed in the session. In addition, the challenge to identify and characterize the behavior of CSC in solid tumors shall be highlighted with respect to anti-tumor therapy and recurrence of disease.

Plants/ Biotechnology

Interest has been strongly growing in the application of flow cytometry in plant sciences. The session will be open to single cell analysis with regard to investigations of ecosystems stability, use of plants in white biotechnology and as renewable resources for energy and food production. A number of important aspects might be considered like plant genomes analysis, e.g. size, ploidy, endopolyploidy, and aneuploidy changes. The session presents also investigations to functions of distinct compartments within plant cells. Since fluorescent reporter proteins are informative tools in higher plant protoplast studies which are used to monitor signal transduction, co-transfection, transformation, protein trafficking and localization, and protein-protein interactions the session invites talks within these fields and will start with a key scientist in this area: David Galbraith.

Poster Session

During the poster session you have the chance to describe your work in a short presentation and to explain the significance of your results during a discussion with the chairs. The time limit is 5 min. The best poster presenter will get a poster price (certificate and 200 Euro).

Open Special Lecture
Birte Forstmann, "Decision Making under Time Pressure"

Microbiology
In microbiological and biotechnical research areas cells are often considered to be uniform populations which can be adequately described by average values. Consequently, measurements of physiology and biochemistry of these cells often rely upon analysing either the culture supernatant or a lysate of all the cells in the population. New methods for single cell analysis have improved possibilities for the in depth analysis of what is happening in cells and why. Although flow cytometry has been widely shown to successfully investigate and describe the dynamics of eukaryotic populations, the technique is now increasingly applied to prokaryotic organisms. Additional techniques now extend structural and functional information (by involving phylogenetic, genomic, transcriptomic and proteomic approaches) on microorganisms. The development of biotechnical applications where microbial cells interact within an artificially engineered environment (e.g. fermentation, bio-remediation, bio-transformation etc) is important for both human health and industry.

New Concepts in Therapy Controlling and Prediction
Flow cytometry allows high throughput single-cell based multiparametrical analysis based on multiple morphological, immunological, and functional parameters of cells in suspension. Novel technical developments in multicolor flow cytometry require adequate design of staining protocols, quality control, and data analysis that allow generation of reproducible results. Interpretation of acquired data depends on reliable diagnostic and prognostic values. Further information can be found by combining flow cytometric data with additional genetic, immunological, and metabolic parameters as well as imaging procedures. In this session, novel developments and concepts for improved and individualized diagnostic procedures are presented. This includes aspects of flow cytometry but also study design and standardization.

Immunology
Improved flow cytometry and cell purification strategies are main driving forces to modern immunological research. In this session, the speakers will provide examples for advanced flow cytometry applications. These comprise in-depths analyses of immunological memory and developmental programs of T cell lineage commitment (e.g. regulatory T cells), as well visualization of 'extremely rare' events within complex cell mixtures. In addition, flow cytometry-based sorting and novel cell depletion strategies will be presented, which are currently evaluated in preclinical mouse models.

Sessions for Communication

European Networks

In Europe there is a disconnect of information ranging from workshops to meetings involving cytometry, and the users of cytometry techniques. Although at a country base level we are well supported, however, coordination and information exchange is often poor between countries and different nationalities. In response to this situation we created a network of cytometrists whose remit encompasses all of Europe. A meeting of established cytometrists was held on the 28-29th of February at EMBL Heidelberg Germany to discuss the creation of such a Network. It was decided to host the network through web 2.0 tools, in particular social networking infrastructure. The meeting ascertained the purpose of the network and the issues that made communication difficult. The meeting also established the goals of the network and armed with this information we created the European Cytometry Network (ECN). From the launch in September 2008, the ECN has undergone rapid growth in membership with nearly 600 members and 30 interest groups (at the time of writing). In this session I will summarise the creation and current activities of the network to date.

Core Facility Manager Workshop

The aim of the workshop is to share knowledge and experience among people that run or work within a flow or image core facility. Talks and discussion should be of interest for anyone managing daily life in a core facility or especially for people thinking about setting up a core facility. Refreshments will be there during the workshop.

ABSTRACTS
(ORAL PRESENTATIONS)

Session Chair: Annette G. Beck-Sickinger

LIGHT SHEET BASED FLUORESCENCE MICROSCOPES (LSFM, SPIM, DSLM) REDUCE PHOTOTOXIC EFFECTS BY SEVERAL ORDERS OF MAGNITUDE

Ernst H.K. Stelzer

EMBL-Heidelberg,Germany

Most optical technologies (microscopy, optical tweezers, laser nanoscalpel are applied to two-dimensional cellular systems, i.e. they are used in a cellular context that is defined by hard and flat surfaces. However, physiological meaningful information relies e.g. on the morphology, the mechanical properties and the biochemistry of a cell's context. A physiological context is certainly not found in single cells cultivated on cover slips. It requires the complex three-dimensional relationship of cells cultivated e.g. in an ECM-based gel or in naturally developing small embryos of flies or embryos and, of course, in tissue sections. However, the observation as well as the optical manipulation of extended biological specimens suffers from at least two severe problems. 1) The specimens are optically dense, i.e. they scatter and absorb light. Thus, the delivery of the probing light and the collection of the signal light tend to become inefficient. 2) Many biochemical compounds apart from fluorophores also absorb light and suffer degradation of some sort (photo-toxicity), which induces malfunction or death of a specimen. The situation is particularly dramatic in conventional and confocal fluorescence microscopy. Even though only a single plane is observed, the entire specimen is illuminated. Recording stacks of images along the optical z-axis thus illuminates the entire specimen once for each plane. Hence cells are illuminated 10-20 and fish embryos even 100-300 times more often than they are observed. Surprisingly, this can be avoided by a simple change of the optical arrangement. The basic idea is to use light sheets, which are fed into the specimen from the side and overlap with the focal plane of a wide-field fluo-rescence microscope. Thus, in contrast to an epi-fluorescence arrangement, which uses the same lens, an azimuthal fluorescence arrangement uses two independently operated lenses for illu-mination and detection. Optical sectioning and no photo-toxic damage or photo-bleaching outside a small volume close to the focal plane are its intrinsic properties. Light sheet based fluorescence mi-croscopes (LSFM) take advantage of modern camera technologies to generate images with a signal to noise ratio that is at least thirty times better than that of a confocal fluorescence microscope. LSFM can be combined with essentially every contrast and specimen manipulation tool to operate in a truly three-dimensional fashion. In a recent application, they were used to record early zebra-fish (Danio rerio) development in vivo and in toto from the early 32-cell stage until late neurulation with sub-cellular resolution and very short sampling periods (60-90 sec/stack). The recording speed was more than five four Megapixel large frames/sec with a dynamic range of 12-14 bit.

We followed the cell movements during gastrulation and revealed its development during cell migration processes. We can show that an LSFM exposes an embryo to 200 times less energy than a conven-tional and 5,000-6,000 times less than a confocal fluorescence microscope. Based on this outstand-ing performance, we claim that our novel, truly three-dimensional approach will have a dramatic impact on developmental, and cell biology as well as on biophysics. It is particularly useful in the observation of tissue sections.

IMAGING OF ADIPONECTIN RECEPTOR 1 DIMERIZATION INHIBITION

David Kosel, John T. Heiker, Cornelia M. Wottawah,
Matthias Blüher, Karin Mörl, Annette G. Beck-Sickinger

Leipzig University, Germany

Adiponectin receptors (AdipoR) 1 and 2 are newly discovered members of the huge family of seven-transmembrane receptors, but both receptors are structurally and functionally different from G-protein-coupled receptors. Little is known about the oligomerization behaviour of the AdipoRs. Here, we show the presence of endogenous AdipoR1 dimers in various cell lines and human femoral muscle tissue. To directly follow the dimerization we applied bimolecular fluorescence complementation (BiFC) in combination with fluorescence microscopy and flow cytometry. Indeed, we could visualize and quantify AdipoR1 homodimers in HEK293 cells. Moreover, we identified a GXXXG dimerization motif in the fifth transmembrane domain of the AdipoR1. By mutating both glycines to phenylalanine or glutamic acid we were able to modulate the dimerization of the AdipoR1, implicating the contribution of the GXXXG motif in AdipoR1 dimerization. We also addressed the question, whether adiponectin as natural ligand for AdipoR1 has any influence on receptor dimerization. Interestingly, flow cytometry and Western blot analysis revealed that adiponectin decreases in a concentration dependent manner for both, the wild-type and mutant receptor. Accordingly, this is the first direct read-out signal of adiponectin at the AdipoR1 receptor and the first report which revealed the involvement of specific amino acids modulating the quaternary structure of the AdipoR1.

MOLECULAR GENETIC AND CYTOGENETIC ANALYSIS FOLLOWING LOW-VOLUME ON-CHIP WHOLE GENOME AMPLIFICATION OF AUTOMATICALLY DETECTED AND LASER-MICRODISSECTED SINGLE CELLS

Thomas Kroneis[1], Jochen Bernd Geigl[2], Martina Auer[2], Peter Ulz[2], Thomas Schwarzbraun[2], Michael Speicher[2], Peter Sedlmayr[1]

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Accurate analysis of rare cells (such as microchimeric fetal cells in the peripheral blood of pregnant women or of circulating tumor cells in the blood of cancer patients) requires unambiguous markers for distinguishing them against the background of maternal or non-tumor cells as well as sufficient DNA for analysis. Based on previous protocols we developed a low-volume on-chip isothermal whole genome amplification (WGA) method allowing us to identify and analyse single cells at the same time. We spiked HT-29 (human adenocarcinoma cell line) cells into peripheral blood mononuclear cells, cytocentrifuged them onto membrane slides, and labelled the cells using FITC-conjugated A45B/B3 antibodies. FITC-positive candidate cells were automatically detected (RCDetect, MetaSystems) and laser microdissected (PALM, Carl Zeiss MicroImaging) onto reaction sites of a surface modified chip (AmpliGrid, Olympus Life Science Research Europa). On this chip, we performed low-volume cell lysis and isothermal WGA in less than 2 µl. WGA samples were recovered and water was added up to 10 µl. For evaluation WGA aliquots were forwarded to (1) DNA fingerprint analysis, (2) sequencing, and (3) chromosomal-comparative genome hybridization (CGH). In a first run we performed WGA of pools of ten and five unfixed HT-29 cells, respectively, followed by DNA fingerprint analysis (PowerPlex 16 System, Promega). High mean PCR efficiency of 95.5% (range 92.3% – 100%, n = 6) proved the WGA protocol to be compatible with subsequent DNA fingerprint analysis. WGA aliquots used in sequencing analysis showed heterozygous pattern in 7 of 18 analyzed HT-29 specific SNP yielding a total sequencing efficiency of 69.4%. Following that, we performed WGA of three A45B/B3-positive single HT-29 cells. WGA aliquots were forwarded to DNA fingerprint analysis resulting in a mean PCR efficiency of 97.4% (range 92.3% - 100%). Sequencing of WGA aliquots showed heterozygous patterns in three of nine analyzed SNP giving a total sequencing efficiency of 66.7%. Additionally, WGA samples forwarded to chromosomal-CGH were in high accordance with CGH profiles from genomic samples of HT-29 cells. We showed that our single cell WGA method is compatible with both molecular genetic (DNA fingerprint analysis, sequencing) and cytogenetic (chromosomal-CGH) analysis. In addition, our method works on the single cell level and even allows for using fixed and labelled cells. As this method meets the above mentioned requirements it is especially suitable for the analysis of microchimeric cells.

QUANTITATIVE IMAGE CYTOMETRY: NUCLEAR BETA-CATENIN SHUTTLING DURING DIFFERENTIATION OF HUMAN AND MOUSE MIDBRAIN PROGENITOR CELLS

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Quantitative image analysis is a powerful tool to gain deeper insights into signalling cascades and networks. Spatio-temporal changes of protein distribution and the transformation of the cellular architecture are regulated by such signalling networks like the beta-catenin dependent (canonical) Wnt-pathway where, after being activated, beta-catenin accumulates and translocates into the cell nucleus, acting as transcriptional activator. We quantified nuclear fluorescence signals by semi-automatic quantitative 3D-analysis of confocal images. We measured the active and total beta-catenin signals in vitro during the differentiation of human embryonic neural progenitor cells (ReNcell VM, derived from midbrain) into neurons and glia cells. To investigate the influence of Wnt-signalling activity on the localization of beta-catenin *in vitro*, the cells were treated with the glycogen synthase kinase-3ß inhibitor SB216763 and the upstream effectors WIF-I, Wnt3a, and Dickkopf-1. The results show that the nuclear beta-catenin concentration increases after induction of cell differentiation. Treatment with SB216763 and Wnt-3a enhanced this effect, whereas WIF-I and Dkk1 treatment suppressed it. The neuronal cell number correlates with the altered nuclear beta-catenin concentration. To investigate whether these results are comparable with the *in vivo* situation, we measured beta-catenin fluorescence signals in the cell nuclei of embryonic mouse ventral midbrain cryo-sections. The results show that this image cytometry approach can also be used in cryo-sections to quantify sub-cellular protein distribution and hence allow the comparison of protein translocation processes during neuronal differentiation *in vitro* and *in vivo*.

IMPEDANCE SPECTROSCOPY BASED LABEL-FREE REAL-TIME MONITORING OF 3D-CULTURES AND TISSUE

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University of Leipzig, Germany

With the help of novel 3D microcavity arrays various 3D tissues can be used for a non-invasive, label-free and continuous screening of cellular alterations in real-time. Thereby, an automated high-content and/or high throughput screening of biologic active compounds or drugs on three-dimensional tissues derived from already established cell lines, primary cell cultures or even biopsies of e.g. tumours can be achieved. Thus, the presented technique can be of great benefit for diagnostic in anti tumour therapy as well as the pharmaceutical industry, especially in respect of functional lead identification and safe drug development process. For non-invasive real-time measurement techniques impedance spectroscopy - was used. By applying an alternating voltage the cell itself acts as a resistor and capacitor affecting the recorded impedance. Depending on the frequency and the dielectric properties of sub-cellular structures, it is possible to analyse different cellular processes occurring under native conditions or after application of drugs, toxins or other active compounds. Up to now, a comparable screening system of spherical in vivo equivalents is not known. For demonstrating proof of principle, we used cell line based models to screen common cytostatica that are used in chemotherapy. The results give evidences that not all cytostatica lead to degradation of tumour aggregates, more strikingly some of them lead to an increase of cell proliferation in our 3D model. Encouraged by our first studies we now take use of our novel screening system for analysing biopsy material from melanoma metastases with the aim of finding optimum cytostatica for individual therapy of patients.

Session Chair: Dieter Weiss

OPTOGENETIC APPROACHES TO CONTROL NEURONAL ACTIVITY AND ANIMAL BEHAVIOUR BY LIGHT, USING MICROBIAL-TYPE RHODOPSINS

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It was always a dream of Neuroscientists (but also other cell biologists) to exactly control cellular activity in a fast, reversible and non-invasive manner. In the nervous system, this would allow to connect activity of individual neurons or neuronal circuits with a behavioural output. To achieve non-invasive control, applying light would be an ideal approach, provided that the cells of interest can be rendered light-sensitive in a specific fashion and that the light can be delivered to the relevant tissue. In the past few years, the field of optogenetics rapidly expanded. Several approaches were taken to achieve light-control of neurons: These make use of chemically modified photo-switchable ion channels, photo-sensitive enzymes, artificial photosensitive proteins, or rhodopsins from microbial origin, to depolarize or hyperpolarize membrane potential, or to manipulate intracellular 2nd messengers. Of particular interest, due to the simplicity of their use, are the microbial-type rhodopsins: These are small proteins that can be easily introduced by transgenic techniques, and that require only retinal as a co-factor, which is either present in the tissue of interest, or can be easily supplied: Channelrhodopsin-2 (ChR2) is a blue-light activated cation channel, and Halorhodopsin (NpHR) is a yellow-light driven chloride pump. Using these proteins, we could achieve fast, bidirectional and reversible control of membrane potential in neurons, which allowed to trigger or inhibit neurotransmitter release and consequently, behaviour, in the nematode Caenorhabditis elegans. For example, we could stimulate sensory neurons or motor neurons, initiating particular behaviours like escape responses, directional changes or alterations in body posture, but also body contraction or elongation. Coupled with electrophysiology and Ca2+-imaging, this allows to functionally dissect neuronal circuits, but also chemical synaptic transmission, in unprecedented detail. An overview of our current work will be given, as well as about some approaches to improve the utility of ChR2 and NpHR, e.g. to achieve highly cell-specific expression, alteration of the absorption spectrum and sub-cellular localization.

QUANTITATIVE DETERMINATION OF ELECTRICAL ACTIVITY PATTERN CHANGES CAUSED BY NEURO-ACTIVE COMPOUNDS IN SINGLE NEURONS AND WHOLE NEURONAL NETWORKS IN VITRO BY MICROELECTRODE ARRAYS

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Neuronal networks of primary dissociated cell cultures from mouse brain grown on microelectrode arrays (MEA) are an interesting alternative for substance screening in drug development and safety pharmacology. Action potentials of up to four individual neurons can be recorded at each of 64 micro-electrodes in an 8x8 array. Alternatively, data for two networks can be recorded simultaneously through 32 electrodes for each network. A large number of parameters allow a quantitative description of the temporal distribution of both action potentials and their bursts for more than 100 individual neurons and also for the whole neuronal ensemble. The activity pattern changes of the networks which are observed following chemical stimulation are both reproducible and substance-specific, so that they are suitable as a new read-out system for cell-based drug screening. Here we describe data analysis and classification methods of activity patterns of neuronal network cultures on MEA neurochips. The spike train parameters that describe the activity patterns of spontaneously active neuronal cell cultures are different from those needed for stimulation experiments of brain-slice cultures. The parameters which are most suitable to distinguish between native activity and the effects of certain compounds are being evaluated by performing classification experiments based on our activity pattern data base of more than 80 well-characterized neuro-active substances. Since the substance effects depend on the concentration, it is also being investigated whether a classification approach with different concentrations is possible. In this study electrical pattern changes caused by different established anticonvulsive drugs were compared. Spike train parameters were grouped into categories describing the overall electrical activity, the oscillatory properties, the synchronicity aspects and the structure of the busts. Anticonvulsive drugs showed similar pattern changes such as reduction of overall activity and of synchronicity. On the base of these investigations we discovered a very singular activity pattern caused only by baclofen and gabapentin. They reveal a unique pattern for their bursting behaviour. These results were compared to results from computational neuroscience which also show similar effects on general activity and burst structure and they are discussed in the context of the suitability of this approach for receptor type determination.

Session Chair: Wolfgang Beisker

FEELING FOR CANCER WITH LIGHT

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The cytoskeleton a compound of highly dynamic filamentous proteins and nano-sized molecular motors inside mammalian cells is responsible for a cells stability and organization. It mechanically senses a cells environment and generates cellular forces sufficiently strong to push rigid AFM-cantilevers out of the way. These forces are generated in the lamellipodium by molecular motor-based nano-muscles, and by polymerization through mechanisms similar to Feynmans hypothetical thermal ratchet. The active polymer networks as basic element of the lamellipodium are described by a new type of polymer physics since nano-sized molecular motors and active polymerization overcome the inherently slow, often glass-like brownian polymer dynamics. Light has been used to observe cells since Leeuwenhoeks times; however, we use the forces caused by light described by Maxwells surface tensor to mechano-optically feel the cytoskeleton without loss of viability and use of molecular markers. For that purpose a laser trap, the optical stretcher, has been developed that deforms cells with optical surface forces unlike the gradient and scattering forces used in optical tweezers. The optical stretcher exploits the nonlinear, thus amplified response of a cells mechanical strength to small changes between different cytoskeletal proteomic compositions as a high precision cell marker that uniquely characterizes different cell types. Malignantly transformed cells become softer with increasing aggressiveness. Thereby an increase in cell motility and cell proliferation serves as indicators for the aggressiveness. Preclinical trials discover two typical cell populations in breast and lung tumors not found in normal tissue. These cells are either significantly softer than cells from normal tissue or highly mechano-active (i.e. respond with active contractions to minimal mechanical stress). The distribution of these cells varies between different tumors. Further molecular biological characterization will determine the importance of these two cell types for tumor progression. Furthermore, proof-of-premise demonstrates that the throughput of the current microfluidic optical stretcher can be increased from 200 cells/h to 5000 cells/sec.

ULTRA HIGH-SPEED SORTING

James Leary

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Cell sorting has a history dating back approximately 40 years. The main limitation has been that, although flow cytometry is a science, cell sorting has been an art during most of this time. Recent advances in assisting technologies have helped to decrease the amount of expertise necessary to perform sorting. Droplet-based sorting is based on a controlled disturbance of a jet stream dependent on surface tension. Sorting yield and purity are highly dependent on stable jet break-off position. System pressures and orifice diameters dictate the number of droplets per second, which is the sort rate limiting step because modern electronics can more than handle the higher cell signal processing rates. Cell sorting still requires considerable expertise. Complex multicolor sorting also requires new and more sophisticated sort decisions, especially when cell subpopulations are rare and need to be extracted from background. High-speed sorting continues to pose major problems in terms of biosafety due to the aerosols generated.
Cell sorting has become more stable and predictable and requires less expertise to operate. However, the problems of aerosol containment continue to make droplet-based cell sorting problematical. Fluid physics and cell viability restraints pose practical limits for high-speed sorting that have almost been reached. Over the next 5 years there may be advances in fluidic switching sorting in lab-on-a-chip microfluidic systems that could not only solve the aerosol and viability problems but also make ultra high-speed sorting possible and practical through massively parallel and exponential staging microfluidic architectures.

LABEL-FREE SINGLE CELL ANALYSIS WITH A CHIP-BASED IMPEDANCE FLOW CYTOMETER

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Label-free identification of phenotype and physiology of a large number of individual cells is a requirement in clinical research and cell therapy. Impedance flow cytometry (IFC/ Leister/Axetris) is a promising alternative to fluorescence-based flow cytometry (FCM). IFC measures impedance of hundreds of single cells/sec simultaneously at various frequencies. The frequencies used for signal acquisition range from 0.1 to 20 MHz. The impedance signal provides information about cell volume (<1 MHz), membrane capacitance (~1-4 MHz) and cytoplasmic conductivity (4-10 MHz), parameters directly related to the physiological conditions of single cells. Hybridoma cells were treated with cytotoxic agents to induce cell death. Impedance analysis showed discrimination between viable and dead cells. The changes were clearly visible at 4 MHz suggesting that differentiation was possible based on cell membrane capacitance. IFC results were confirmed by FCM measurement of AnnexinV/PI labeled aliquots. Also changes in cell membrane potential and [Ca2+]i are detectable by IFC. Peripheral blood mononuclear cells were loaded with membrane potential sensitive dye (DiBAC4) or with calcium sensitive dye (Fluo-3). The cells were then treated with the ionophores: valinomycin or A23187, respectively. Changes in membrane potential and [Ca2+]i were detectable at the level of cytoplasm conductivity (>4 MHz) and membrane capacitance (1-4 MHz), respectively. Our data indicate that IFC could become a valuable alternative to conventional FCM. As measurement by IFC is label-free it could be applied in future for quality control of precious samples such as isolated stem cells or for continuous monitoring in bioreactors. In combination with cell sorting IFC may gain additional relevance in cell isolation for therapeutic use. The work will be extended to address further applications in biotechnology and biomedical cell analysis.

THE NEWEST MOLECULAR PROBES® FLOW CYTOMETRY REAGENTS FROM INVITROGEN: QDOT® NANOCRYSTAL CONJUGATES, REAGENTS FOR CELL CYCLE & PROLIFERATION ANALYSIS, VIABILITY DETECTION, DETACHABLE MAGNETIC BEADS AND A NOVEL PHAGOCYTOSIS ASSAY

Michael W. Olszowy

Life Technologies, United States of America

The presentation will cover the latest releases of new Molecular Probes® reagents from Invitrogen - reagents and assays for flow cytometry that take significant steps forward in the analysis of cellular function, far beyond immunophenotyping. Much anticipated Qdot® nanocrystal-conjugated primary antibodies will be reviewed in detail including the newest releases with application notes in regard to optimal filer use and instrument configuration. Newer cell cycle analysis reagents can be used on violet, blue, and red lasers, are less toxic than other reagents, and can be used to look at stem cells using side population analysis. Our new Click-iT™ EdU assay replaces the tedious BrdU assay with a 2 hour assay that can be used with simple fixation and permeabilization – no harsh denaturation required. One of our new dead cell stains allow researchers to look at GFP expression, replace PI, stain with two antibodies and still look at dead cells – in the APC channel - SYTOX® Red. The newest of our dead cell indicators, SYTOX® AADvanced, is a much improved analogue of 7-AAD that penetrates cells as quickly as PI with brighter staining of dead cells and lower CVs in cell cycle analysis. We've also added new fixable dead cell dyes and beads to facilitate compensation of the same. With recent advances in attachment chemistries we now offer a fully detachable magnetic separation bead. Our novel phagocytosis assay allows for the simple, rapid and unambiguous measurement of fully functional phagocytic activity. This presentation will cover a number of these assays, including cutting edge novel approaches, with practical examples and detail and any other exciting releases that are introduced in time for the meeting.

MICROFLUIDIC STRUCTURES FOR FLOW CYTOMETRIC ANALYSES OF IMMUNOLOGICALLY STAINED BLOOD CELLS

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Disposable microfluidic platforms are of particular relevance as a part of simple and robust analytical systems for point-of-care in vitro diagnostics. Integration of optical elements and electrical connectors can be adapted to particular medical applications. In this paper we present measurement platforms applying microfabricated flow cytometers for differentiation and counting of human blood cells based on the detection of scattered light, fluorescence and impedance changes. To assure sufficient statistics in flow cytometric analysis blood cells must be counted at relatively high throughput. Implementation of stable and efficient hydrodynamic focusing in flow cytometric microchip systems is thus recommended. Microfluidic structures are manufactured here using a hot embossing technique where mould inserts are prepared by ultra-precision milling [1]. Such an approach avoids restrictions of commonly used photolithographic fabrication techniques. The complex three dimensional polycarbonate structures featuring stable two-stage cascade hydrodynamic focusing or one-stage spin hydrodynamic focusing enable high throughput measurements with count rates up to 5 kHz. The core flow is confined to typically 5-10 µm diameter at velocities of up to 3 m/s. The width of the sample flow was determined by imaging the fluorescence of dye solutions. Hydrodynamic focusing perpendicular to the assembling plane was inspected by an on-chip integrated mirror. The capabilities of micro flow cytometers are demonstrated by counting red blood cells and platelets as well as through differentiation of white blood cells (leucocytes) which were immunologically stained simultaneously with up to three different types of fluorescently labelled monoclonal antibodies. Measurements of subsets of leucocytes including CD14+ monocytes and CD3+/CD4+ lymphocytes, clearly demonstrate the potential of using such microsystems. Optical excitation and detection of fluorescence and light scatter was performed either by optical fibres or using lenses and mirrors. Exploration of these alternative optical pathways utilizing fibre- and free-space optics results in pulse height distributions comparable to those of conventional instruments. Measurements of fluorescence signals from calibration particles yield coefficients of variation (CV) of less than 2% for optimised settings.

[1] A. Kummrow, J. Theisen, M. Frankowski, A. Tuchscheerer, H. Yildirim, K. Brattke, M. Schmidt and J. Neukammer, Lab Chip 9 (2009) 972-981

Session Chair: Elmar Endl

DOES SIZE MATTER? DEFINING A CRITICAL MASS FOR A CORE FACILITY

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Core facilities are increasingly common in many areas of biomedical research. Such facilities are an effective means of centralising equipment and expertise where resources such as capital equipment budgets and staff are limiting. The advantages are clear where equipment that demands specialised expertise and maintenance is considered – multiple applications may be performed on a single instrument, there is a cost-effective use of time and money and users benefit from a successful service.
However establishing a successful core facility is not as simple as identifying space, equipment and personnel. A successful facility must be self-promoting, economically viable and scientifically sound as well as providing support and expertise for users. Although many high profile core facilities will have a range of equipment available, most will not start that way. This presentation will address what is needed to make the transition from a small base of Laboratory equipment used by a few specific researchers to a central resource available to all.

Session Chair: Leoni Kunz-Schughart

STEM CELLS AS GENERATIVE, REGENERATIVE, AND DESTRUCTIVE ELEMENTS IN THE CENTRAL NERVOUS SYSTEM

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Stem cells can be found at various stages of neural development. They build the brain and the spinal cord, and they can remain active in circumscribed niches of the CNS from which they attempt to regenerate lost nervous structures throughout adulthood. During the last years it has furthermore become evident that malignant brain tumors, e.g. glioblastoma multiforme can contain sub-populations of Œcancerous[1] stem cells that may represent the source for disease maintenance, expansion, and the stunning resistance to treatment. There is general consensus that the various classes of stem cells share functional characteristics, i.e. they can self-renew and they can give rise to variety of mature phenotypes. It is less clear, however, whether and how these cells relate to each other. This lecture will present an overview of the field, examples from own research experience will be demonstrated, and current developments in neural stem cell research will be discussed.

PRESENCE OF HER4 ASSOCIATES WITH INCREASED SENSITIVITY TO HERCEPTIN TREATMENT IN PATIENTS WITH METASTATIC BREAST CANCER

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Introduction: HER2 overexpression or rather HER2 gene amplification is indicative for Herceptin therapy in both metastatic and pre-metastatic breast cancer patients. Individual patient's sensitivity to Herceptin treatment however varies enormously and spans from effectual responsiveness over acquired insensitivity to complete resistance from the outset. Thus no predictive information can be deduced from HER2 determination so that molecular biomarkers indicative for Herceptin sensitivity / resistance to Herceptin are needed to be identified. HER2 related ErbB receptor tyrosine kinases, known to mutually interact and to crossregulate each other, are prime candidates to be involved in cellular susceptibility to Herceptin. Methods: Using immunohistochemistry and fluorescence in situ hybridization we retrospectively investigated primary breast cancer tissues from 48 patients who were under Herceptin treatment. We quantified the gene copy numbers of all HER receptors and evaluated their coexpression profile. Moreover the HER2 phosphorylation state, the ratio of native to truncated HER2, p27(kip1) and PTEN expression were objects of this study. Results: Kaplan-Meier and Cox regression analysis revealed a significant positive impact of HER4 (co-)expression on overall survival from the outset of antibody therapy. Both HER4 expression and HER4 gene amplification emerged as independent prognostic markers in Herceptin treated breast cancer patients. Responsiveness to Herceptin turned out to be more efficient if tumor cells coexpress HER4. Conclusions: HER4 has been demonstrated to potentially exert tumor suppressing activity and in turn to have a favourable impact on the course of breast cancer disease. Moreover we show here that HER4 expression even prolongs overall survival of Herceptin treated patients which indicates a functional integration of HER4 into anti-HER2 targeting. Complementing functional studies will elucidate the special role of HER4 in the context of Herceptin treatment and will advance the design of highly efficient and individualized receptor targeting.

THE USE OF LIGHT MICROSCOPY TECHNIQUES AND FLOW CYTOMETRIC ANALYSES IN THE SEARCH FOR NEW SYNTHETIC TUBULIN MODULATORS

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Light microscopy techniques and flow cytometry are powerful tools for the investigation of new microtubule-interfering drugs. We analysed the ability of newly synthesised tubulin modulators to affect microtubule dynamics and distribution as well as their antimitotic activity on human A549 lung carcinoma cells. We found that a series of novel 1,3,4 oxadiazole derivatives, synthesised by V. Semenov (1), demonstrated strong microtubule-disrupting activity, which was in some cases even stronger than that of nocodazole and colchicine. Moreover, we analysed new hybrid molecules, synthesised by O. Zefirova (2), containing colchicine and adamantane-based moieties. Immunofluorescence staining of drug treated cells revealed that these compounds possess both microtubule-destabilising activity and tubulin polymer-stabilising activity as taxol or vinblastine would do. Additional flow cytometric analyses showed that tested oxadiazoles and hybrid molecules in nM concentrations induce mitotic block at the G2/M stage. Our data indicate that the ability of analysed drugs to induce mitotic arrest by binding to tubulin makes them new promising microtubule-interfering anticancer drugs for chemotherapy.

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2. Zefirova ON, Nurieva EV, Lemcke H, Ivanov AA, Shishov DV, Weiss DG, Kuznetsov SA, Zefirov NS. Design, synthesis, and bioactivity of putative tubulin ligands with adamantane core. 2008, Bioorg. Med. Chem. Lett.,18:5091-5094.

FLOW CYTOMETRY- AN ESSENTIAL TOOL IN THE GENERATION AND USE OF „HUMANIZED MICE“

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Since the nude mouse was discovered by Isaason & Cattanach in 1962, many immunodeficient mice strains have been generated. All of these mice have one thing in common: they can be reconstituted with human hematopoietic stem cells more or less without rejection. In the beginning, these mice insufficiently produced human T cells to maintain the human level of hematopoietic cells which and in turn showed short lifespan, a continuing murine NK cell activity, and likely developed thymomas. Recently, Ito and his group were able to eliminate these problems by back-crossing the common-γ chain null allele into the NOD/Scid background. These mice develop all human subsets of the immune system like T-, B-, and NK-cells as well as myeloid cells and develop a humoral and cellular immune response to antigens. These mice can be taken as a powerful new tool in the research field of infectious diseases (e. g. HIV, EBV, and HTLV), in medication and vaccine studies, and investigations of human hematopoiesis. We are using these NOD/scid IL2Rγ null mice strain for xenotransplantation with human hematopoietic stem cells separated from cord blood (intrahepatic transplantation into neonates). Flow cytometry is not only important in the purity control of the separated human stem cells after magnetic bead separation (>90% CD34+ are necessary), but is even more crucial in the quantification of engraftment levels during the lifespan of these humanized mice. It is also the method of choice to quantify and qualify the engraftment levels not only in the peripheral blood, but also in many other organs as spleen, bone marrow, lung, liver, and lymph nodes. We are currently utilizing the humanized mice as an infection model by analyzing the infection course of microbial diseases. A main goals we are heading for is the characterization of the ongoing immune response and the influence of medication. Besides immunohistochemistry, quantitative PCR, and other molecular biological methods, flow cytometry is the major technique for characterizing the immune response looking for generation of memory and effector T cells, maturation of B cells, and the activation of dendritic cells. In conclusion, generating “humanized mice“ without utilization of multiparametric flow cytometry would be doomed to failure.

Session Chair: Leoni Kunz-Schughart

"CANCER STEM CELLS: LESSONS FROM ACUTE MYELOID LEUKEMIA"

Dominique Bonnet

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Acute myeloid leukaemia (AML) is a clonal disorder defined by the accumulation of abnormally differentiated myeloid blasts. Because leukaemic blasts have very limited proliferative capacity, it is believed that leukaemic clone is maintained by a rare population of leukaemic stem cells (LSC) that have extensive proliferation and self-renewal capacities. Elucidating the nature of the target cell that undergoes leukaemic transformation and characterising the LSC is essential for both the understanding of the leukaemogenic process and for the design of effective therapies. The development of an in vivo model that replicates many aspects of human AML had provided a mean to identify leukaemic stem cells (termed the SCID-Leukaemia Initiating Cells, SL-IC). SL-IC is defined by the ability of that cell to initiate AML in NOD/SCID mice. This in vivo assay provides the foundation of an assay to define the biological and molecular properties of such leukaemic stem cells (LSC) (1). The SL-IC have been originally purified as CD34+CD38– cells, regardless of the phenotype of the bulk blast population, and represented the only AML cells capable of self-renewal (1).
Since this early study, further heterogeneities have been identified. Using cell-tracking analysis, the Dick's group identified different sub-clone of SL-ICs (2). Recently, we also show phenotypic heterogeneity of the SL-ICs between patients and also within the same patients (3). This heterogeneity not only indicates a potential differential origin or progression of the disease but also have important implications in the development of new therapies to eradicate these cells.
1- Bonnet, D., and J. E. Dick. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature Med., 3, 730-7.
2- Hope, K. J., Jin, L. & Dick, J. E. (2004) Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol, 5, 738-43. 3- Taussig D, Miraki-Moud F, Anjos-Afonso F, Pearce DJ, Allen K et al. (2008) Anti-CD38 antibody mediated clearance of human repopulating cells masks the heterogeneity of leukemia initiating cells. Blood; 112: 568-75.

CANCER STEM CELLS FROM SOLID TUMORS: TOWARDS CLINICAL APPLICATIONS

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Cancer stem cells (CSCs) are the rare population of undifferentiated tumorigenic cells that are thought to be responsible for tumor initiation, maintenance and spreading. Their existence might also explain why tumours are resistant to many conventional therapies, which typically target the rapidly proliferating tumor cells but spare the slow dividing tumor stem cell population. The concept of CSCs has profound implications for our understanding of tumor biology and for the development of more effective cancer therapeutics. The selective targeting of these cells offers a potential revolutionary advance in the treatment of cancer, by attacking the roots of the disease. Such cell population should therefore represent the target of new therapies aimed at eradicating the tumor. We developed a technology that allowed us to isolate and expand in vitro CSCs from several solid tumors, including glioblastoma, melanoma, breast, lung, colon, thyroid and ovary cancers. We are currently characterizing these tumorigenic cell populations at different levels, including genome-wide expression of mRNA, microRNA and proteome profiling. Such extensive characterization may allow the identification of more specific CSC markers, while providing key information on the relevant pathways to be targeted for successful therapies. Moreover, the use of CSC-based xenografts which closely reproduce the parental tumor, as assessed by morphological and molecular analysis, offers a unique opportunity to test new anticancer treatments and potentially optimize individualized therapies. Thus, although the identification of CSCs is relatively recent, this research area appears extremely promising as it may significantly contribute to the rational design of novel targeted therapies for cancer.

CHALLENGES TO IDENTIFY CD133 EXPRESSION IN CANCER CELLS

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Background and Aim: The identification and characterization of small cancer subpopulations that exclusively exhibit tumor-initiating cell (TIC) character has become an ambitious focus in cancer research. Various surface markers have been used to define, enrich and isolate such subpopulations from primary tumor material and cancer cell line models. One of these putative markers is CD133 (human Prominin-1), a 92-110 kDa transmembrane glycoprotein. Its function is still not entirely understood. The antibody clone most frequently used for the isolation of CD133-positive cancer cells is AC133 which binds to a glycosylated extracellular domain of CD133. We applied this antibody to monitor CD133 expression in numerous colorectal cancer (CRC) cell lines and faced a variety of technical and scientific challenges that shall be discussed on the basis of experimental data using other antibodies and various detection methods. Material and Methods: Ten CRC cell lines were analyzed for CD133 cell surface expression using flow cytometry. To clearly distinguish the two subpopulations, fluorescence signal enhancement was required and realized by the FASER technology. Western blotting was performed on whole cell protein extracts. Different antibodies that recognize either the glycosylated CD133 or total CD133 protein were used. Some CRC cell line populations were sorted according to their surface expression and reanalyzed by Western blotting. Results and Conclusions: CRC lines were classified according to their CD133 cell surface presentation as monitored by flow cytometry: (I) cell lines that do not express CD133, (II) lines with roughly all cells expressing the protein and (III) those that contain two distinct CD133+ and CD133- subpopulations (including HCT-116, DLD1, HCC2998, KM20L2 and HT29). The mean proportion of the CD133+ subpopulation in these cell lines ranged from about 20% in KM20L2 to 90% in HT29 cells. In most cells lines, flow cytometric data could be confirmed by Western blot analyses using an antibody that detects total CD133. Unexpectedly, DLD1 and HCC2998 were entirely negative in Western blot analyses leading to the literature-driven hypothesis of a truncated membrane-located form of CD133. This, however, is unlikely since an alternative antibody for Western blotting towards the glycosylated epitope also did not reveal the positive signal in flow cytometry. This discrepancy should be elucidated. The potential for false-negatives in flow cytometry, e.g. the exclusive presence of non-glycosylated CD133 and/or intracellular CD133 will also be highlighted. Lack of AC133 staining signal in flow cytometry demands for an independent confirmation.

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Session Chair: Christian Wilhelm

NOVEL CYTOMETRIC PLATFORMS FOR EUKARYOTIC FUNCTIONAL GENOMICS

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Eukaryotic organisms comprise complex mixtures of cells which coordinate gene expression between themselves in a way that efficiently regulates development and response of the organism to its environment. Elucidating these patterns of regulation, and the functions of the underlying and responding genes, loosely termed functional genomics, has been greatly facilitated by the recent development of high-throughput platforms for characterization of gene expression. Drawing from examples in my laboratory, in this talk, I describe three of these platforms, the way that they have been implemented and the results obtained. The first explores global analysis of cell type-specific gene expression, and employs expression of the Green Fluorescent Protein within cells and within nuclei as a marker for selective purification, respectively, of cells and nuclei through fluorescence-activated sorting (Birnbaum et al. Science 302:1956 (2003), Nature Methods 2:1 (2005); Zhang et al. Plant Methods 1:7 (2005), Plant Physiology 147:30 (2008)). These are then used as sources of polyadenylated RNA for microarray-based global gene expression profiling. The second allows characterization of the influence of genotype on global gene expression within plant populations, focusing on the important crop, rice. It is based on the identification in silico of Insertion-Deletion elements, which are then employed for design of microarray elements (Edwards et al. Plant Methods 4:13 (2008)). These microarrays provide a highly cost-effective means for determining the genotypes of Recombinant Inbred Lines as well as their parents. Combining genotyping with expression profiling then permits elucidation of regulatory mechanisms as well as assignment of candidate genes to QTLs. The third implements a low-cost, high-throughput microarray platform for use in chemical genomics. Based on a quantitative nuclease protection assay, this platform permits accurate querying of the expression levels of the transcripts of up to 2500 genes, and of miRNAs, within a microplate format. This allows screening of large chemical libraries for small molecule effector leads that specifically modulate transcript levels for individual genes or that alter specific gene signatures (Kris et al. Plant Physiology 144:1256 (2007)). The future prospects of these platforms and others, either in development or envisaged, will be discussed.

HOW TO GET THE "FLOW" OF SCREENING PROTEIN INTERACTION LIBRARIES IN LIVING PLANT CELLS

Kenneth Wayne Berendzen

Center for Plant Molecular Biology - Tübingen, Germany

Screening for protein-protein interactions of plant proteins has been limited primarily to heterologous systems, which excludes many aspects of native regulation that can influence interaction. Bimolecular fluorescence complementation (BiFC) is the restoration of fluorescence from a split YFP molecule, when they are brought near each other by interacting proteins fused to the complementary YFP-halves. The simplicity and effectiveness of BiFC makes it favourable to search for unknown interactions in order to identify novel interaction partners. However, in order to screen a library of sufficient complexity in vivo, one needs a method for identifying and extracting positive interactions; flow cytometry (FC) provides this solution.

APPLICATIONS OF FLOW CYTOMETRY IN PLANT POPULATION BIOLOGY AND ECOLOGY

Sílvia Raquel Castro[1,2], Zuzana Münzbergová[2,3], Luis Navarro[4], Pavel Trávníček[3,2], Jan Suda[2,3], João Loureiro[1]

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Flow cytometry (FCM) is a high-throughput technique that simultaneously measures and analyses multiple parameters of individual particles. Over the last decade, the applications and use of FCM in plant ecology have increased dramatically. The unsurpassed speed and reliability on estimating differences in nuclear DNA content by FCM allowed large-scale surveys at the landscape, population, individual and tissue levels, with the majority of the studies being focused in spatial distribution and evolutionary significance of genome duplication (polyploidy). Also, representative sampling opened the possibility to gain novel insights into the extent of intra- and inter-population ploidy variation, niche differentiation, and ecological preferences of particular cytotypes. In combination with molecular and phenotypic approaches, FCM promises qualitative advances in our understanding of genome multiplication and the population biology of vascular plants. In the present talk we will demonstrate the importance of FCM in plant ecology by presenting several case studies (e.g., cytotype distribution in *Aster amellus* agg. and *Gymnadenia conopsea* agg., invasive species *Oxalis pes-caprae*) that we have been exploring in the past years.

MESENCHYMAL STROMAL CELLS FROM HUMAN UMBILICAL CORD TISSUE: IDENTIFICATION OF SUB-POPULATIONS.

Pierre Moretti[1], Tim Hatlapatka[1], Ingrida Majore[1], Stefanie Boehm[1], Ralf Hass[2], Thomas Scheper[1], Cornelia Kasper[1]

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The human umbilical cord (UC) is a non-controversial source of autologous cells harbouring a high frequency of mesenchymal stromal cells (MSC). We used an explant culture approach to isolate MSCs from whole umbilical cord tissue. Using our protocol, high number of fibroblastic cells exhibiting a MSC immunophenotype (CD31-, CD34-, CD44+, CD45-, CD73+, CD90+; CD105+) and mesodermal differentiation potential were isolated with high success rates in a reproducible manner. The isolated cells demonstrated a high proliferation potential with population doubling times measured by 27.5 ± 1.6 hours. Under our culture conditions the cells could be expanded without loss of proliferative activity and viability at least for 20 population doublings. Furthermore UC-derived cells could be efficiently cryopreserved and revitalized with cell survival rate reaching 75 ± 12.8%. Thus, compared to other MSC-sources, UC-derived MSCs can be rapidly expanded to a large number of cells and efficiently stored, which is highly interesting in term of cell engineering and clinical applications. Despite their homogeneous MSC immunophenotype, a growing number of evidences suggest that UC-derived MSCs are rather heterogeneous. We observed a broad cell-size distribution and marked morphological differences in isolated UC-MSCs cultures. After fractionation of the population via Counterflow Centrifugal Elutriation (CCE) according to the cell size, we obtained two sub-populations with significant differences in cell size, growth properties and biochemical markers expression. Where small-sized subpopulations exhibited the highest proliferative capacity and the most pronounced expression of MSC markers, large-sized cells were identified as senescent via β-Galactosidase staining. We conclude that the CCE approach may be a valuable and fast method in order to deliver high quality cells for clinical applications.

EXPANSION OF PLURIPOTENT STEM CELLS: STRATEGIES TOWARDS LARGE SCALE CULTURES

Magda Tomala[1], Antonina Lavrentieva[1], Pierre Moretti[1], Ursula Rinas[1], Cornelia Kasper[1], Frank Stahl[1], Axel Schambach[2], Ulrich Martin[3], Thomas Scheper[1]

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[3]LEBAO, Hannover Medical School

For current research within the field of Tissue Engineering and drug discovery different kinds of stem cells are used. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have a great potential for this purpose due to their pluripotentiality. However, large numbers of cells with defined characteristics are needed. At this point, suitable cytokines and proper cultivation systems are key factors for an efficient expansion of the cells. We developed a production and purification process for recombinant Leukemia Inhibitory Factor (LIF). This cytokine is essential for murine ES and iPS cell cultures since it maintains the pluripotent state of these cells. After expression and purification from recombinant *E. coli*, the biological activity of LIF was successfully demonstrated with regard to the proliferation, morphology and specific marker expression of pluripotent cells. Furthermore, we tested different suspension cultivation systems for cultures of ES cells. Adherently growing murine ES cells were adapted to suspension conditions and then static, stirred and orbitally shaken cultivation systems were compared with respect to the ability to generate undifferentiated ES cells. Besides the evaluation of biomass production, pluripotency markers were analysed via flow cytometry and RT-PCR. Finally, the differentiation potential of the ES cells was investigated. This study demonstrates that the expansion of suspended ES cells highly depends on the design of the culture vessel as well as the stirrer design and stirring speed whereas a similar differentiation potential was observed for all tested cultivation systems.

Session Chair: Susann Müller

HIGH THROUGHPUT AND MULTIPLEXED DETECTION OF FOOD-BORNE PATHOGENS BY A HYBRID MICROFLUIDIC SPR AND MOLECULAR IMAGING CYTOMETRY DEVICE

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Purdue University, United States of America

The goal of this project is to construct a new, rapid, and highly sensitive peptide array, microfluidic, flow-through image cytometry system based on a combination of surface plasmon resonance (SPR) imaging and molecular imaging. The SPR imaging allows label-free detection of any object attached to the surface coated with a thin layer of gold on coded arrays that permit multiplexed assays and actual capture of the pathogenic bacteria. Immobilization of specific peptide sequences to the surface results in specific adhesion of only the target bacteria which can be detected by SPR imaging. The SPR imaging is also done from the opposite side of the slide so that the system is not in direct contact with the food particulates and pathogens. This allows much easier design of a small portable device, with swappable array slides to take out to the field on location. Simultaneous molecular imaging permits multi-color fluorescence assays of pathogen status (e.g. live/dead and other variables) in a fluorescence image-based cytometry system on the other side of the slide from the SPR detection system (Patent pending). The objective of this project is to develop a prototype SPR imaging device based on an inexpensive, portable biosensor sensor that can be taken out into the field to perform flow-through image cytometry at the source. The proposed biosensor device upon successful development should be capable of very sensitively detecting pathogenic bacteria at threshold concentrations (as few as a few bacteria per milliliter of sample) in 5-10 min. Part of this rare cell sampling in relatively large volumes of water requires the prior use of magnetic nanoparticle labeling to highly concentrate rare pathogenic bacteria for subsequent analysis of micro-volumes in the microfluidic device. Current detection methods for pathogenic bacteria takes 8 hours to days before the results are known, and clearly this is too slow for prevention of other outbreaks of disease due to food-borne pathogens. There is an urgent need to develop a device that can be low cost and portable, so that the device can be used where samples are collected, including where foods are processed or used.

CULTURE HETEROGENEITY IN *BACILLUS MEGATERIUM* CULTIVATIONS PRODUCING ANTIBODY FRAGMENTS REGARDING THE STATE OF PRODUCTION AND MEMBRANE POTENTIAL

Florian David, Claudia Korneli, Mandy Schön, Antje Berger, Ezequiel Franco-Lara

Technische Universität Braunschweig, Germany

Recombinant antibodies and antibody fragments are indispensable tools for research, diagnostics and therapy. In mammalian systems the titres are usually very high, but at the cost of very long process times. In most of microbial systems the production itself is also very efficient, but it is associated with very high downstream processing costs, since the intracellular product must be gained through cell disruption and purification. For these reasons efficient, less cost intensive microbial production systems are desirable. A promising alternative is the use of the gram positive *Bacillus megaterium*, which is an expression host with high secretion capacities. Due to the lack of the outer membrane, produced antibody fragments can directly be harvested from the medium supernatant. In this work the production and secretion of specific antibody fragments anti-lysozyme (D1.3 scFv) and the anti-C-reactive protein (LA13-IIE3 scFv) in *B. megaterium* were investigated. The antibody fragment D1.3 scFv, as a single chain fragment, was chosen as model system for antibody production in *B. megaterium*. The methodology was extended to the anti-CRP scFv production, an antibody fragment binding CRP, a serum protein which can be used as an unspecific marker protein resulting from inflammation or infection processes. Several methods and stains like DiOC2, DiBAC and DiOC6 were evaluated and established to determine cell heterogeneity with at-line measurements during cultivations of *B. megaterium*. Culture heterogeneity was detected in cell morphology, membrane properties and productivity by means of flow cytometry. To characterize the production capabilities of *B. megaterium*, two different approaches were followed. The first approach focussed on the characterization of the membrane properties, since the membrane influences not only the product secretion, but also plays a very important role in cell physiology. Therefore by measuring the membrane potential metabolic active cells can be distinguished from non-active cells giving a hint on the effectiveness of a bioprocess regarding its productivity. In a second approach, the state of antibody fragment production itself was determined via immunofluorescence. First an antibody directed against the His-tag linked to the produced antibody fragment and a second antibody fluorescently labelled were used for detection of producing cells. Fluorescence data from stained cells was normalized to their single cell volume allowing a very easy assay to differentiate between producing and non-producing cells. By these approaches batch and fed-batch cultivations could be characterized enabling the implementation of taylor-made cultivation strategies to optimize the whole process of antibody production and secretion.

COMBINATION OF MICROBIAL CYTOMICS AND PROTEOMICS APPROACHES FOR STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF BACTERIAL COMMUNITIES

Nico Jehmlich[1], Thomas Hübschmann[2], Jana Seifert[1], Manuela Gesell Salazar[3], Uwe Völker[3], Dirk Benndorf[4], Susann Müller[2], Martin von Bergen[1],Frank Schmidt[3]

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Knowledge of the structure and function of microbial communities is needed to improve our understanding of physiology and ecology. The dynamic in microbial communities in terms of the relative abundances of strains, migration, and the heterogeneity of microbial cell states presents a key challenge in cytomics and proteomics studies. Thus, the precise quantification of bacterial cellular constituents like the DNA contents performed by flow cytometrics with subsequent cell sorting of subpopulations in combination with proteomics, provide accurate data for comprehensive functional proteomic analysis. Since large amount of microbial cells were needed, due to their small size and low protein amount, in this study, we applied a newly developed filter system workflow with a high recovery rate of proteins without the need of centrifugation steps after cell sorting. We therefore applied an artificial community consisting of *E. coli* K-12 and *P. putida* KT2440 and identified in total 903 for *E. coli* K-12 and 867 for *P. putida* KT2440 mostly cytoplasmic proteins after cell sorting and LTQ-FT-ICR mass spectrometry from 5x106 cells of each. The proteome coverages of identified proteins were approx. 30-40% of the predicted number accessible by mass spectrometry. The high proteome coverage after elaborated wash, transfer and digest procedures shows the usefulness of the developed protocol. Furthermore, the bias of the Mr, the *pI* and the hydropathicity was further investigated. As a result, the data demonstrated that the influence of cell sorting by flow cytometry is marginal and in combination with consecutive proteomic investigations and this workflow was useful for comprehensive analysis of all aspects of physiology.

COMMUNITY DYNAMICS WITHIN A BACTERIAL CONSORTIUM DURING GROWTH ON TOLUENE UNDER SULFATE-REDUCING CONDITIONS

Susann Müller, Sabine Kleinsteuber, Carsten Vogt, Hauke Harms

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A sulfate-reducing bacterial consortium was enriched from an anoxic aquifer contaminated with BTEX compounds, using toluene as growth substrate. About 13 morphotypes were visible by microscopic observation. A clone library of PCR-amplified 16S rRNA genes revealed 12 major phylotypes belonging to several Deltaproteobacteria as well as members of the Epsilonproteobacteria, the Bacteroidetes, the Spirochaetaceae and an unclassified bacterial clade. The most prominent phylotype comprising 34% of all clones was affiliated to the Desulfobulbaceae and was closely related to environmental clones retrieved from hydrocarbon-contaminated aquifers. Growth of the consortium on toluene at 14°C and 25°C was followed based on sulfide production, protein content and total cell counts. The community dynamics at both temperatures was studied by combining flow-cytometric cell sorting and T-RFLP profiling of the 16S rRNA genes. Flow cytometry based on DNA contents and side scatter behavior of individual cells was used to visualize dominant and newly emerging clusters of cells. Up to seven subcommunities, two of them dominant, were detected and physically separated by high resolution cell sorting. Subsequent T-RFLP profiling revealed that the Desulfobulbaceae phylotype accounted for up to 87% in proliferating subcommunities, indicating that it represents the key organism of toluene degradation within this complex anaerobic consortium.

EXPRESSION OF RECOMBINANT HYDROPHOBINS IN *SCHIZOSACCHAROMYCES POMBE* IN HIGH CELL DENSITY FERMENTATION

Jost Max Weber, Theresa Pöschel, Katja Geipel, Susann Kurz, Thomas Bley, Gerhard Rödel, Kai Ostermann

Institut für Lebensmittel- und Bioverfahrenstechnik, Technische Universität Dresden, Germany

The fission yeast *Schizosaccharomyces pombe* is an established model organism in molecular biology. The great evolutionary distance to other yeasts that are commonly used in biotechnology, makes it an attractive alternate expression system for technical and pharmaceutical proteins. However, efficient use of *S. pombe* as an expression system requires optimization of the fermentation conditions. To follow the expression of recombinant hydrophobin in *S. pombe* an expression plasmid coding for a hydrophobin tagged with EGFP was constructed. Fluorescence of yeast transformants was monitored by Flow Cytometry to determine the fraction of EGFP expressing cells and the EGFP expression level. Due to the strong crabtree effect, batch cultivations with high initial concentrations of glucose are not an efficient approach for this yeast. Chemostat experiments were performed to estimate the onset and the degree of the fermentative metabolism of the *S. pombe* strain. Applying carbon limited fed batch fermentations we were able to increase the cell dry weight by more than a factor of 30 compared to the batch fermentations. Both the fraction of EGFP expressing cells and their level of EGFP expression remain at an elevated level, even during the prolonged fed batch experiments.

Birte Forstmann

DECISION-MAKING UNDER TIME PRESSURE

Birte U. Forstmann[1], Gilles Dutilh[1], Scott Brown[2], Jane Neumann[3], D. Yves von Cramon[3], Richard Ridderinkhof[1], Eric-Jan Wagenmakers[1]

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Human decision-making almost always takes place under time pressure. When people are engaged in activities such as shopping, driving, or playing chess, they have to continually balance the demands for fast decisions against the demands for accurate decisions. In the cognitive sciences, this balance is thought to be modulated by a response threshold, the neural substrate which is currently subject to speculation. In a speeded decision-making experiment, we presented participants with cues that indicated different requirements for response speed. Participants had to indicate with a left or right response button press whether a cloud of dots moved to the left or the right on the screen, respectively. While performing the task participants´ brain activity was scanned using functional magnetic resonance imaging. The results revealed that cueing for speed activates the striatum and the pre-supplementary motor area (pre-SMA), brain structures that are part of a closed-loop motor circuit involved in the preparation of voluntary action plans. Moreover, it is known that the striatum disinhibits the cortex thereby facilitating speeded but also more error prone decision-making. Finally, the data show that individual variation in the activation of striatum and pre-SMA is selectively associated with individual variation in the amplitude of the adjustments in the response threshold estimated by the mathematical model. These results demonstrate that when people have to make decisions under time pressure their striatum and pre-SMA show increased levels of activation.

Friday, 16/Oct/2009: 8:15am - 8:45am

Klaus-Goerttler-Preisträger

Session Chair: Susann Müller

LIVER SINUSOIDAL ENDOTHELIAL CELLS VETO CD8 T CELL ACTIVATION

Frank Alexander Schildberg

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The liver is known to induce tolerance rather than immunity through tolerogenic antigen presentation or elimination of effector T cells. In particular, hepatic dendritic cells (DC) are known to be little immunogenic for CD8 T cells. We investigated whether this peculiar phenotype resulted from interaction with resident hepatic cell populations. Contact of DC with liver sinusoidal endothelial cells (LSEC) but not hepatocytes or B cells vetoed antigen-presenting DC to fully activate naive CD8 T cells. This MHC-independent regulatory effect of LSEC on DC function was not connected to soluble mediators but required physical contact. Because interaction with third-party LSEC still allowed antigen-presenting DC to stimulate expression of initial activation markers on naive CD8 T cells and to stimulate activated CD8 T cells, we hypothesized that LSEC controlled the DC co-stimulatory function. Indeed, contact with LSEC led to reduced DC expression levels of CD80/86 or IL-12, but supplementation of these signals failed to rescue the ability to prime naive CD8 T cells, indicating involvement of further molecules. Like LSEC, also fibroblasts are able to modulate T cell activation, which is mediated by DC. This suggests that mesenchymal cells are generally competent for this regulation. Besides an indirect inhibition of T cell activation by a de-licensing of DC, also a direct inhibition could be found due to a direct regulation of T cells by LSEC. One possible mechanism could be based on enhanced intracellular cAMP concentrations. Taken together, these results reveal a novel principle operative in hepatic tolerance induction, in which LSEC not only tolerize T cells themselves but also suppress neighboring APC normally capable of inducing T cell immunity.

Session Chair: Ulrich Sack

MULTI-COLOUR ANALYSES FOR IMMUNOPHENOTYPING OF NORMAL AND MALIGNANT LYMPHOCYTE SUBSETS

Frank WMB Preijers

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Flow cytometry is increasingly used in the clinical laboratory for the diagnosis and monitoring of various hematological diseases. The rapid and sensitive multiparameter detection, even at a single cell level, renders flow cytometry to a powerful tool to distinguish malignant cells from normal cells. Due to technical aspects and the limitation of the currently used flow cytometers most immunophenotypical analyses are still performed by 3-to 4-colour analyses. This approach is usually adequate for diagnosis posing in case of contamination of the blood or bone marrow with high numbers of malignant cells. The restricted number of markers that can be simultaneously determined on these cells, however, hampers an optimal immunophenotypical characterization of small leukemia and lymphoma populations and non-malignant lymphocyte subpopulations. In particular, minimal residual disease determination or characterization of very small cell populations needs high-level multi-colour analyses. Recent developments in flow cytometry enable analyses up to 10-colour phenotyping by using a flow cytometer with high sensitivity, resolution and dynamic range with high-speed data collection combined with fast and easy-to-use software. Such new generation flow cytometers that are recently launched are the Gallios and the Navios from BeckmanCoulter, by which 10 colors can be simultaneously and rapidly determined. Although this high-level multi-colour flow cytometry offers many advantages over the current 4-colour analyses, the technical problems and pit falls are increasing with the number of applied parameters. Besides the availability of sufficient labeled conjugates, the balancing of the combination of MoAbs and fluorochromes is crucial. By example, MoAbs directed against dimly expressed antigens should be conjugated to fluorochromes with high quantum yields. Furthermore the different MoAbs-fluorochrome conjugates can influence each other resulting in quenching or FRET effects. We defined prerequisites in 8- and 10-colour analyses using the Navios and analyzed normal and malignant lymphocyte subpopulation.

LEUKEMIA PHENOTYPE PANELS FOR THE DEVELOPING COUNTRIES

Sumeet Gujral, PG Subramanian, Ashok Kumar, Y Badrinath
Tata Memorial Hospital, Mumbai, India

Introduction: Levels of development may vary widely within so-called developing countries, with some developing countries having high average standards of living (vice versa). Evidence-based guidelines are recommendations for management. They should be clear, concise, easy to read and follow with easy applicability in routine practice. Immunophenotyping is a must for accurately diagnosing and prognosticating various subtypes. Antibody panel selection plays a vital role in obtaining an accurate diagnosis. Numerous guidelines have addressed antibody panels. Majority of the guidelines are from North America and Europe. Lot of diversity in panel selection and it is mirrored in the various published guidelines. Goals: We critially evaluate Indian guidelines with various western guidelines. 2006 Bethesda International Consensus Guideline:: Unlike all the aforementioned guidelines which use a panel of antibodies to diagnose a specific disease condition for e.g., AL or CLPD, it uses a panel of antibodies which is sensitive to pick up cells of a particular lineage. A combination of markers is used for a particular medical indication or symptom (for example lymphadenopathy or blasts in the blood). It recommends an initial evaluation panel comprising of 31 antibodies including B, T/NK cells, myelomonocytic cells and plasma cells markers. This is followed by a more extensive lineage specific panel of antibodies depending upon the results of the primary panel. Similarities and differences in US and Indian approach

- US – indication based, Indian - morphology (and clinical) based
- Both rely on a screening panel - 33 versus 10 antibodies
- US - comprehensive panels, more T-cell reagents in screening
- Secondary reagents differ
- Indian - donot address maturation pattern, CD45 gating optional
- Indian panel includes CD23, FMC7 in primary screen

Indications based panels and India
Conditions like anemia, primary lymphadenopathy, splenomegaly are common in India. Staging bone marrow biopsy might not require immunophenotyping in all cases
Conclusion:: Medical insurance is available to a miniscule Indian population. At TMH- Approximately 70% pediatric and 50% adult patients of hematolymphoid neoplasms are treated with a curative intent (protocol based) and these patients are worked up with all available prognostic and predictive markers. Diagnostic tests constitute 2 - 6% of total cost of management (BMT excluded) thereby underlying the importance of more comprehensive testing for the patients who are on a protocol based treatment. Rapid advances in instrumentation, newer antibodies and fluorochromes. Importance of standardization and need of guidelines. It was a learning exercise, hopefully it bridges the divide between low cost reporting and an accurate diagnosis (it is not a cost cutting exercise) Indian Guidelines may need a re-look / revision. Global Guidelines may be formed to help improve management, especially in countries with limited resources (Different Levels). Program may be specific to each country's unique situation. Diagnostic guidelines for hematolymphoid malignancies should include immunophenotyping and molecular genetics.

INTERLEUKIN-21: A NEW PERSPECTIVE IN THE TREATMENT OF PRIMARY ANTIBODY DEFICIENCY?

Stephan Borte[1], Qiang Pan-Hammarström[2], Ulrich Sack[1], Michael Borte[3], Ulf Wagner[1], Dagmar Graf[4], Chonghai Liu[2], Lennart Hammarström[2]

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Selective IgA deficiency (IgAD) and common variable immunodeficiency (CVID) represent the most prevalent primary immunodeficiency diseases in Caucasians, characterized by low or absent levels of serum IgA, or of all switched immunoglobulin isotypes, respectively. While many individuals with IgAD are asymptomatic, some suffer from an increased susceptibility to infections of the respiratory and gastrointestinal tract. CVID shows a highly variable clinical presentation and outcome that is dominated by upper and lower respiratory tract infections, leading to chronic lung disease, bronchiectasis, and eventually death. Today, the main treatment of primary antibody deficiencies is replacement immunoglobulin therapy. This restoration of physiological levels of IgG in the patient's blood has been a profound therapeutic advance, preventing, or alleviating, the severity of infections. Whilst a distinct etiology remains unclear in the vast majority of patient cases, B cell maturation defects and defective class switch recombination, blocks in plasma cell differentiation, and abnormal apoptosis of B cells provide a basis for CVID and IgAD. Here, we report for the first time that that a combination of IL-21, IL-4 and anti-CD40 stimulation induces class switch recombination to IgG and IgA and differentiation of Ig-secreting cells, consisting of both sIgG+ and sIgA+ B cells and CD138+ plasma cells, in patients with CVID or IgAD, implying that B cells from these patients are not lacking the molecular machinery necessary for such processes. Moreover, interruption of CSR by silencing of AID expression only partly abrogated IL-21 induced Ig-production, suggesting the presence of already-switched, isotype-committed B cells in patients with CVID. Furthermore, spontaneous apoptosis of CD19+ B cells from patients with CVID or IgAD was prevented by a combination of IL-21, IL-4 and anti-CD40 stimulation, indicating that an IL-21 based therapeutic approach also prevents abnormal apoptotic pathways in CVID and IgAD. Given that IL-21 is a crucial factor for the differentiation of normal mature human B cells into Ig-secreting cells, we hypothesized that alterations in the IL-21 gene could be responsible for the lack of switched Ig production in CVID. However, analysis of IL-21 and IL-21R mRNA expression upon anti-CD3 stimulation of T cells showed no evidence for defective IL-21 expression in CVID patients and sequencing of the coding regions of the IL-21 gene did not reveal any mutations, suggesting a regulatory defect. Thus, our work provides an initial basis for a therapeutic role of IL-21 to reconstitute the immunoglobulin production in patients with CVID or IgAD.

Session Chair: Dirk H. Busch

TRACKING IMMUNOLOGIC MEMORY

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Immunological memory is still one of the enigmas of modern immunology. We poorly understand the generation of memory cells from their precursors, the lifestyle of memory cells or their maintenance, reactivation and termination. There are clear indications that in autoimmune and allergic diseases the immune system has developed a "pathogenic memory" for autoantigens or allergens. Therefore, it is of major importance to track and compare the development of a dysregulated and a protective immunologic memory in appropriate animal models. This strategy will allow identifying memory-associated target molecules useful for a resetting of dysregulated immune systems. In this lecture, the latest findings in this field will be summarized with a special emphasis to the experimental strategy applied by combining global molecular and single cell-based cytometric approaches to characterize and immunoscope short- and long-lived plasma cells, and memory T lymphocytes in mice and human. In this context the significance of stroma cells which are mainly responsible for the formation of survival niches in which long lived memory lymphocytes reside in will be highlighted. Finally, the successful clinical implementation of these technologies has shown the reset of autoreactive immune systems in therapy refractory systemic lupus erythematodes patients by immunoablation and subsequent autologous stem cell transplantation.

FOXP3-MEDIATED INHIBITION OF THE GLOBAL GENE REGULATOR SATB1 IS REQUIRED FOR MAINTAINING REGULATORY T-CELL COMMITMENT

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FOXP3-expressing regulatory T (Treg) cells are critical for the establishment and maintenance of self tolerance and immune homeostasis thereby preventing autoimmunity while at the same time suppressing immune responses to pathogens and tumors. Expression of FOXP3 is essential for the development and suppressive function of Treg cells. Mutations in FOXP3 cause massive multi-organ autoimmunity in mice and humans, while induction of FOXP3 in conventional CD4+ T cells confers a Treg-cell phenotype. FOXP3 regulates the transcription of several known target genes still the exact molecular mechanisms how lineage definition in the thymus and maintenance of the phenotype in the periphery is engraved by FOXP3 in Treg cells are elusive. Using a subtraction approach based on 171 genome-wide transcriptional profiling experiments interrogating human Treg cells and conventional CD4+ T cells in different states of activation and differentiation combined with interrogation of 735 human miRNA, inverse miRNA-mRNA correlations, and miRNA binding site prediction, we demonstrate the chromatin remodelling enzyme SATB1 (special AT-rich sequence-binding protein-1) to be an important target of FOXP3 that is actively suppressed in Treg cells to sustain Treg-cell properties. SATB1 is required for normal thymic T-cell development, peripheral T-cell homeostasis, TH1/TH2 polarization, and reprogramming of gene expression. By gain- and loss-of-function experiments we establish SATB1 regulation in Treg cells both on transcriptional and translational level. FOXP3 directly controls transcription at the SATB1 gene locus in vivo and in vitro. Moreover, the FOXP3-induced miRNA miR-155 interferes with translation by binding to the 3' UTR of the mature SATB1 mRNA thereby further decreasing SATB1 protein expression, while epigenetic modifications are not involved in the regulation of SATB1 expression. Overall, our data strongly support an active and dominant suppression of TH1/TH2 differentiation by FOXP3 in Treg cells mediated via SATB1 downregulation. Loss of SATB1 expression and function prevents global chromatin remodeling usually required for T helper cell differentiation. These data place modulation of global chromatin remodelling central during the decision process between effector and regulatory T-cell function.

PRECISE LIGHT-INDUCED CELL ELIMINATION MEDIATED BY IMMUNE-TARGETED GOLD NANOPARTICLES

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Advancements in cell purification strategies have a significant impact on basic and applied immunological research and are also translated in therapeutic applications. In this field of research, immune-targeting particles (Magnetic-Activated Cell Sorting, MACS) and fluorescence activation by laser irradiation (Fluorescence Activated Cell Sorting, FACS) plays an important role. The combination of define immune-targeting by nanoparticles using monoclonal antibodies together with the activation by complementary short pulsed laser irradiation leads to selective cell elimination by photothermolysis. This physical effect is based on superior optical properties of spherical gold nanoparticles that act as a local heat source after laser energy absorption. The here applied Light-Induced Nanoparticle-Activated Cell-Selection (LINACS) allows an highly efficient, selective and contact free in vitro or ex vivo cell elimination over five orders of magnitude without causing collateral damage to non-target cells resulting in a high yield of intact purified cells. In a preclinical animal model we could demonstrate for the first time, that LINACS can be used to efficiently eliminate tumor cells from bone marrow. The bone marrow of healthy mice was spiked with leukemia cells and subsequent treated by LINACS, using gold nanoparticles specific for the contaminating tumor cells. Transplantation of purified bone marrow in myeloablative treated mice resulted in tumor free survival of these mice for at least twelve weeks, in contrast to mice transplanted with unpurged bone marrow. In addition, no collateral damage to the purified bone marrow stem cells by the treatment could be observed. The successful elimination of tumor cells by LINACS did not interfere with stem cell functions, because the bone marrow reconstitution and subsequent blood cell reconstitution was unaltered. Furthermore, the development of a functional immune system was observed after transplantation of purified bone marrow, tested with an immune challenge by adenovirus infection at the end of this study. Since LINACS is first not restricted to cells in suspension, as well adherent cells can be purified and is second not restricted to antibody targeting, as well peptides or aptamers can be used for immune-targeting, this method can be capable as a platform technology for a broad field of application in immunology.

ORIGIN OF DIVERSE CYTOKINE PHENOTYPES FROM INDIVIDUAL NAIVE PRECURSORS

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Concerning the secretion of Interferon gamma, Interleukin 2 and Tumor Necrosis Factor alpha, antigen-specific CD4 T helper 1 (Th1) populations show a complex subdivision into mono-, bi- and tri-functional cytokine producing cells. We show in vivo that these diverse cytokine phenotypes are simultaneously present and similarly distributed in Th1-polarized progeny, derived from 10000 down to as few as 100 naïve TCR-transgenic CD4 T cells. However, we find that a progeny's phenotypic diversity is markedly reduced when decreasing starting population size to a single cell. This hints towards an early determination of Th1 cytokine patterns before significant clonal expansion has taken place.

Session Chair: Andy Riddell

WEB BASED PLATFORMS FOR THE FLOW CYTOMETRY COMMUNITY

João Loureiro[1], Sílvia Castro[1,2], Jaroslav Dolezel[3], Eleazar Rodriguez[4], Conceição Santos[4], Jan Suda[2]

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In the last 5 years, especially after the emergence of the Web 2.0, an increasing number of tools aimed to facilitate communication and knowledge dissemination, information sharing and collaboration between researchers have been developed. The world of flow cytometry (FCM) has also been profoundly influenced and more and more web-based platforms directed to the flow cytometry community became available. In the present communication, some of these tools will be presented, from the initial but still prevailing mailing lists to the blogs, forums, databases and the latest social networks. A special focus will be given to the efforts that are being made within the plant FCM community. An ever-increasing number of users and applications in plant FCM have called for the better awareness of potential methodological flaws and limitations, and for the development of tools for assessing quality standards. In response to these needs we built three interrelated Internet resources, the Plant DNA Flow Cytometry Database (FLOWER database; http://flower.web.ua.pt/), the Plant Flow Cytometry Forum http://flowerdatabase.20.forumer.com/index.php) and the Plant Flow Cytometry Blog (http://flowerdatabase.blogspot.com/. In addition, we have been involved in the European Cytometry Network (ECN), having established a research group directed to the applications of FCM in plant sciences. During the relatively short period of their inception, all these web tools have demonstrated their value and usefulness. The FLOWER database is a comprehensive and easily accessible source of information (a search tool) and a platform for performing quantitative analyses of relevant parameters related to FCM practice (a survey tool). The Plant Flow Cytometry Forum, the Blog and the Group in the ECN have all contributed to better knowledge dissemination, presentation of scientific events, and most importantly, to the improvement of communication between researchers engaged in plant FCM. We are convinced that these new web-based platforms filled the existing gaps and will stimulate further development of plant DNA flow cytometry.

THE EUROPEAN CYTOMETRY NETWORK

Andy Riddell

EMBL Heidelberg, Germany

A meeting of established European cytometrists was help on the 28th-29th of February 2008 at EMBL Heidelberg Germany to discuss the creation of a network for cytometrists. The meeting highlighted the need for such a network and established the aims and issues that such a network would need to address. After the meeting work began to identify an appropriate platform to structure the network on. A Web 2.0 based solution was selected called NING, a social networking tool, and it was converted for use in the intervening months prior to the launch of the network in September 2008. The European Cytometry Network (ECN) was created whose reach encompasses all of Europe. Through the WEB 2.0 tools, the ECN has a modern infrastructure that builds rapid links between professionals in cytometry. By supporting novel connections for multidisciplinary scientific exchange, the ECN promotes new collaborations and initiatives with the aim to lead to independently funded projects. From the launch in September 2008, the ECN has undergone rapid growth in membership with nearly 600 members and 30 interest groups (at the time of writing). In this talk I will summarise the creation and current activities of the ECN to date.

EDUCATION FOR PUBLISHING IN CYTOMETRY PART A

Attila Tárnok

Cardiac Center GmbH; University of Leipzig, Germany

Education for Publishing in Cytometry Part A

INDIAN SOCIETY OF CYTOMETRY

Sumeet Gujral

Tata Memorial Hospital, Mumbai, India

Prof. Sumeet Gujral will talk about foundation, work and members of the cytometry society in India, its issues and challenges faced by the society under the current conditions. He will specially emphasise on the work within the clinical wing of the society.

ABSTRACTS
(POSTER PRESENTATIONS)

A1

A STUDY OF GLYCOGEN STRUCTURE IN HEPATOCYTES BY FRET METHOD

Natalia Nikolaevna Bezborodkina, Grigory Israilovich Stein, Ekaterina Vasilievna Mushinskaya, Ekaterina Vladimirovna Sivova, Boris Nikolaevich Kudryavtsev
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The phenomenon of FRET (Förster (fluorescence) resonance energy transfer) is used in modern light microscopy to determine of the small distances between molecules (up to 6 nm), the nature of their microenvironment and interaction.The method is very sensitive and far surpasses the resolution of widefield light microscopy. Using this method, molecules are marked two fluorochromes, conventionally called the donor and acceptor. In our work we have analyzed the structure of glycogen in individual hepatocytes of normal liver after glucose administration to fasting rats by FRET method. To mark labile (LF) and stable (SF) fractions of glycogen were employed fluorescent Schiff-type reagents auramine-SO2 (donor) and ethidiumbromide-SO2 (acceptor), respectively. Max wavelength of the donor emission and acceptor absorption were 526 and 546 nm, respectively. Registration FRET between the donor and the acceptor was carried out on a LSCM Leica TCS SP5 with the application of FRET AB (acceptor photobleaching) procedure. For the donor excitation was employed laser 405 nm. Acceptor bleaching performed argon laser at a wavelength of 514 nm. Objective HCX PL APO 20×/0.70 imm was applied. As an immersion and enclosing medium of the isolated hepatocytes smears used glycerine. The distance between the glucose residues in the glycogen molecule was determined by efficiency of FRET calculating as: E = (Dpost – Dpre) / Dpost, where Dpre and Dpost are donor fluorescence intensity before and after acceptor's photobleaching respectively. It was shown that the E in individual hepatocytes at different stages of refeeding ranges from 0 to 25 % and depends both on the value of LF/SF ratio, and the total glycogen content in hepatocytes. It is known that the full molecule of glycogen, also called macroglycogen, has 12 concentric tiers and the distance between tiers makes about 1.7 nm. Only the four most external ones are usually involved in the regular turnover of the synthesis– degradation cycle. We assume that 9, 10, 11 and 12 tiers are LF, and 8 internal tiers of a molecule represent SF of glycogen. Because this method works for limited distances (up to 6 nm), the transfer of energy from donor to acceptor can occur only between 10-8, 10-7, 9-8, 9-7 and 9-6 tiers of molecules. Thus, the FRET technique allows to estimate the distance between the glucose residues in glycogen molecule and to obtain valuable information about its structure.

The work was supported by Russian Found of Basic Research (RFBR № 08-04-00971).

PLASMA MEMBRANE RECEPTORS DEFINE ANNEXIN-DEPENDENT INTERACTION OF MATURE PHAGOSOMES WITH F-ACTIN

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Phagocytosis is an essential component of innate immune response. Phagocytic cells, like macrophages and dendritic cells, internalize pathogens and form phagosomes, which subsequently mature to phagolysosomes and kill pathogens. Highly complex and very well orchestrated rearrangements of actin cytoskeleton play a key role in both internalization and phagosome maturation processes. Furthermore, during phagocytosis different pathogens are recognized by specific receptors. These different receptors, when activated, have distinct effects on the F-actin rearrangement. However, the precise molecular mechanism of the interaction between phagosome and actin cytoskeleton is not clearly understood. We initially observed that purified mature Latex Bead Phagosomes (LBP), formed after uptake of latex beads coated with Fc-fragment or mannan, have different affinities to F-actin networks in vitro. Both mass spectrometry and immuno-fluorescence microscopy revealed that actin binding proteins annexin A1 and annexin A2 were present on Fc-LBP, but only annexin A2 was found on mannan-LBP. Interestingly, bacterial expressed annexin A2 also specifically stimulated the binding of both Fc-LBP and mannan-LBP to F-actin in a Ca2+ dependent manner. However, recombinant annexin A1 stimulated both, Fc-LBP and mannan-LBP interaction with F-actin. In order to confirm that endogenous macrophage annexins A1 and A2 are also involved in LBP-F-actin interaction, we used gel-filtration and ion-exchange chromatography to isolate an active cytosolic fraction, containing these proteins. The phagosome-F-actin binding activity of this fraction was strongly inhibited by antibodies against annexin A1 and A2. These results indicate that annexin A1 and A2 are involved in LBP binding to F-actin. Furthermore, already the initial step of phagocytosis, namely the ligand-receptor interaction, specifies annexin association with the LBP membrane and thereby the affinity of LBP to actin cytoskeleton. Acknowledgement: This project is supported by DFG (KU 152813/1) and Marie Curie Research Training Network (MRTN-CT-2006-035946)

A3

FLOWCYTOMETRICAL ANALYSIS OF RADIATION-INDUCED GAMMA-H2AX FOCI

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OBJECTIVES: Phosphorylation of histone H2AX (gamma-H2AX) occurs at sites flanking DNA double-strand breaks (DSBs) and can provide a measure of the number of DSBs within a cell (gamma-H2AX-assay). We investigated whether the mean intensity and the mean number of radiation-induced gamma-H2AX foci vary as a function of radiation quality and dose. MATERIALS AND METHODS: Jurkat cells were irradiated with different doses of either low linear energy transfer (LET) Cs-137 Gamma-rays (662 keV) or high LET Am-241 α-particles (5.48 MeV). The gamma-H2AX foci were detected using immunocytochemistry (anti-phospho-Histone H2AX(Ser139), Millipore) and quantified by counting the number of gamma-H2AX foci employing fluorescence microscopy and by measuring the mean signal intensity in single cell nuclei by flow cytometry. RESULTS: The mean number of gamma-H2AX foci is increased in a dose dependent manner for both radiation qualities and are similar at identical absorbed radiation dose for both investigated radiation qualities. The mean gamma-H2AX signal intensity of single nuclei is increased after exposure to alpha-particles when compared to Gamma-irradiation at iso radiation dose. CONCLUSIONS: α-particle induced gamma-H2AX foci show higher signal intensities compared to Gamma-ray-induced gamma-H2AX foci. The mean intensity of radiation-induced gamma-H2AX foci in Jurkat cells depends on the LET.
Supported by BMU and BMBF; KVSF - Kompetenzverbund Strahlenforschung (03NUK005A)

A4

ACTIVATION OF APOPTOSIS PATHWAYS DURING THE ON-SET OF DIFFERENTIATION OF HUMAN NEURAL PROGENITOR CELLS IN VITRO

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Human brain development is strictly regulated and driven by basic physiological processes at the cellular level: cell proliferation, cell differentiation and programmed cell death. We investigated in vitro the cross-talk between apoptosis signaling pathways, cell proliferation and differentiation in ReNcell VM197 cells, a human neural progenitor cell line, which differentiates within few days after removal of growth factors into astrocytes, oligodendrocytes and neurons. Our comparative studies of proliferating versus differentiating VM197 cells emphasize the importance of apoptotic processes. Cell cycle analysis of proliferating and differentiating VM197 cells at the very early stage of differentiation shows a cell cycle distribution of about 50 % G1/G0-, 40 % S- and 10 % G2/M- phase cells. At later stages of differentiation (8 h to 72 h post initiation of differentiation) we observe a significant increase of G1/G0 cells of about 40 % and the number of cells in S-Phase drops significantly to about 7 %. The accumulation of cells in G1/G0 goes along with characteristic changes in cell morphology and an increased apoptotic loss of cells. Expression analysis of apoptosis-related genes and proteins show a rapid activation of apoptosis signaling pathways shortly after removal of growth factors. Both, pro- and anti-apoptotic pathway components are involved in the formation of a fully differentiated neural network. We observe e.g. an activation of Bax and caspase-3, PARP cleavage as well as an increased expression of Bcl-2. We conclude from our results that the very early stage of differentiation is of particular importance for the life or death decision of human neural progenitor cells.

Supported by DFG, Graduiertenkolleg 1387 - dIEM oSiRiS

A5

SPECIFIC INHIBITION OF BCL-2 LEADS TO DIMINISHED NEURONAL DIFFERENTIATION IN VM197 CELLS.

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Apoptosis is of crucial importance for neural differentiation and can be observed during human neurogenesis in vivo. The anti-apoptotic protein Bcl-2 is one of the key players of apoptosis and modulates apoptotic cell death during embryogenesis by competitively binding and antagonizing apoptogenic proteins. The human neural progenitor cell line ReNcell VM197 differentiates within few days into oligodendrocytes, astrocytes and neurons after removal of growth factors EGF, bFGF. This process is accompanied by apoptotic cell loss and a significantly changed Bcl-2 gene expression. The presented data show that functional inhibition of Bcl-2 by its competitive ligand HA14-1 results in a temporally altered and delayed pattern of differentiation of VM197 cells as detected by light microscopy. This observation was accompanied by a diminished Bcl-2 expression on protein level during differentiation and an increased activation of effector caspase-3 at an early stage of differentiation. Flow cytometry analysis of VM197 cell cultures with functionally disabled Bcl-2 revealed a diminished differentiation into neurons when compared to untreated control cells. We conclude therefore, that Bcl-2 has a neuroprotective function during differentiation of the neural progenitor cell line VM197.
Supported by DFG, Graduiertenkolleg 1387 - dIEM oSiRiS

A6

CYTOSENSE – FLOW CYTOMETRY DETECTION OF PLANKTON

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The CytoSense is an instrument to perform flow cytometric measurements for the study of microorganisms in complex and dynamic (eco)systems. The recirculating sheath fluid system of CytoSense allows to perform measurements autonomously also for long time periods. The ultrawide (1x1 mm) flow cuvette with 2-stage hydrodynamic sheath fluid injection system makes detection of even large planktonic particles (size maximum: diameter 700 um, length up to several mm) possible. CytoSense accommodates maximally 2 lasers, 10 detectors and (optional) imaging function. The applied CytoSense is equipped with a continuous-wave diode-pumped solid state laser operating at 491 nm, measuring FWS, SWS and fluorescence signals (FL Red, FL Orange). The system includes the picoplankton option - an extra detection unit which allows detection of picoplankton (particle size < 2 um) and contains a higher laser output power (75 mW instead of 20 mW). The bench-top flow cytometer CytoSense can be placed inside a small spherical buoy (CytoBuoy) to enable in situ operation in open water environments. The telemetry between CytoBuoy and shore base is held by a long distance Wi-Fi connection. Energy is generated by solar radiation through 8 solar panels on the buoy cover charging two lead-acid batteries inside. Depending on available light, up to 5 samplings per day are possible.

REDUCING RELATIVITY IN FLOW CYTOMETRIC ANALYSIS

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Flow cytometric analysis relies on the interaction of a number of systems, each having inherent variability. These include the functional systems of the instrument itself (fluidics, optics, electronics and software) as well as factors such as sample preparation, reagent quality and stability, and the skill and experience of the human operator. This variability at every step in the process often frustrates efforts to standardize analysis and to compare data collected on different types of instruments at various locations. The Accuri C6 Flow Cytometer is manufactured to optimize overall instrument performance before leaving the factory. The optical alignment of lasers, flow cell, light filters and detectors is "locked down" during the manufacturing process, and the voltage and gain on photomultipliers and diode detectors is factory-set, using industry standard beads (Spherotech 8 Peak Rainbow). The result is a cytometer with highly predictable and reproducible performance. The advantages of this for the user are multiple: 1) Routine instrument characterization and quality assurance procedures are greatly simplified 2) Spectral overlap is highly predictable 3) Quantitative fluorescence measurements are easy to obtain and 4) Instruments within any given manufacturing generation show low instrument-to-instrument variation in performance and fluorescence detection characteristics. This allows users of different C6 instruments to create, and share, analysis templates including pre-set gates and compensation values specific to particular applications and sets of fluorochromes.

SOLID STATE LASER UPGRADE FOR BD™ FACSDIVA™ CELLSORTER

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Successful research depends on the availability of and access to shared resources, like complex technologies, databases, instrumentation facilities, and the expertise to run them. The application of flow cytometry to problems in the area of life sciences and medicine has shown that this technology has significant impact on research value. The mission of the Flow Cytometry Core Facility, at the Institute of Molecular Medicine, at the University of Bonn, is to enhance the scope and quality of scientific research and facilitate communication amongst scientists on methods of common interest in flow cytometry. We provide all investigators access to high quality, state of the art flow cytometry instrumentation as well as the scientific expertise necessary to effectively integrate this technology into their research projects. We therefore upgraded our FACSDiva cellsorter with Solid State Lasers, to enhance the capabilities of the cell sorter and ensure proper operation, for the next years. The generally installed gas Lasers of the instrument had a lifetime of approximately five years (3500h). After break down of our gas lasers we decided to switch to Solid State Lasers, which have the following advantages: Longer lifespan (up to 10.000 hours). Reduced power consumptions and power efficiencies, hence no need of water-cooling and lower energy costs. And last but not least Solid State Lasers are very small. We also took the opportunity to replace the original prisms and mirrors built in for laser alignment with standardized optical components, giving a better performance. A further advantage is, that the possibilities of excitation (405nm, 488nm and 647nm) are now identical to current analysers and protocols can now easily be transferred. In the following we describe the procedure of upgrading of the instrument and the material needed. We further illustrate the upgrading with a gallery of corresponding images. We think this is of general interest for all users, still working on FACSDiva systems, which are considering upgrading or replacing their current instrument configuration.

10-COLOR FLOW CYTOMETRY PROTOCOLS FOR THE CLINICAL LABORATORY

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The latest development of commercial flow cytometers (FCM) is that they are equipped with three (blue, red, violet) or more lasers and many PMT detectors. Nowadays routine clinical instruments are capable of detecting 10 or more fluorescence colors simultaneously. Thereby presenting opportunities for getting detailed information on single cell level for cytomics and systems biology to improve diagnostics and monitor of patients. University Leipzig (Germany) recently started a cluster of excellence to study the molecular background of life style and environment associated diseases, enrolling 25000 individuals. To this end a most comprehensive FCM protocol has to be developed for this study. We aimed to optimize fluorochrome and antibody combinations to the characteristics of the instrument for successful 10-color FCM in this clinical setting. Systematic review of issues related to sampling, preparation, instrument settings, spillover and compensation matrix, reagent performance, and general principles of panel construction was performed. 10-color FCM enables for increased accuracy in cell subpopulation identification, the ability to obtain detailed information from blood specimens, improved laboratory efficiency, and the means to consistently detect major and rare cell populations. Careful attention to details of instrument and reagent performance allows for the development of panels suitable for screening of samples from healthy and ill donors. The characteristics of this technique are particularly well suited to the analysis in broad human population cohorts and have the potential to reach the everyday practice on standardized way in the clinical laboratory.

A10

CELL VIABILITY ANALYSIS BY IMPEDANCE MICROFLOW CYTOMETRY

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Impedance-based flow cytometry is not a totally new inspiration for characterising particles or cells. The electronic cell volume or Coulter volume in the 1950-ies was in fact one of the first cell parameters measured using a flow cytometric approach. Compared to the achievements of fluorescence-based flow cytometry, traditional single cell impedance analyzers provide only limited information content with their enumerating and sizing capability. However, the advent of micro-fabrication technologies in the last decade promises a boost in sensitivity related to that of macro-scale impedance devices, enabling thereby true single cell characterisation. One of these devices, based on a microfluidic impedance chip, is the Axetris microflow cytometer . It is able to characterise cells without the need of fluorochromes or other dyes, just by measuring at various electrical frequencies simultaneously. The measured impedance signals provide information about cell volume, membrane capacitance and cytoplasmic conductivity , parameters that are directly related to the physiological conditions of single cells. In the last two years applications in the fields of cell viability and differentiation, parasitemia, hematology and microbiology were addressed with the Axetris microflow cytometer prototype. Here we present some data on viability of yeast cells and leukocyte cell lines . Yeast cells killed by heat or by different chemicals can be clearly distinguished from viable yeast cells by impedance microflow cytometry. Also leukocyte cells treated with cytotoxic agents can be differentiated from viable cells. These capabilities permit near-inline monitoring of cell density and viability in cell culture and biotechnology laboratories.
In general, the presented technology is most suitable for routine and quality control analyses of any kind of cells.

A11

EXPRESSION AND FUNCTION OF THE LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR (LOX-1) IN BOVINE OVARIAN FOLLICLE AND CORPUS LUTEUM

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The concentration of oxidized lipids, including oxidatively modified low-density lipoprotein (ox-LDL), is known to increase with milk performance. The plasma ox-LDL can access to both periovulatory follicle (due to the luteotropin surge-induced thecal vascularization and periovulatory permeabilization of the microvessels) and the strongly vascularized corpus luteum. The entry of ox-LDL into the vessel wall and vascular cells is known to be mediated by LOX-1 whereas information about receptor-mediated uptake by follicle cells and luteal cells is lacking. Therefore, we determined the expression of LOX-1 at the level of mRNA and protein in follicular and luteal tissue cells, respectively. The function of LOX-1 was assessed by changes in the cell cycle progress after targeting the receptor. The generation of nitric oxide (NO) was measured in the medium of cultured cells by a commercial kit. Results: 1) Moderate expression of LOX-1 in mural granulosa cells from growing (n = 3) was observed in contrast to periovulatory (n = 3) follicles. The expression of LOX-1 in the early (n = 3) and midphase (n = 4) corpus luteum resembled that of the periovulatory thecal expression. Corpora lutea (n = 2) in regression expressed LOX-1 at the detection limit of the immunohistochemical technique used. 2) Inhibition of LOX-1 by LOX-1 antibodies decreased generation of NO in mural granulosa cells from periovulatory follicle (n = 6) and luteal cells from early (n = 3) and midphase (n = 3) corpus luteum. Targeting of LOX-1 by ox-LDL increased the generation of NO in these cell types. 3) Mural granulosa cells from periovulatory follicle responded to LOX-1 blockage with an increase in mitotic cells on the contrary to luteal cells from any phase of corpus luteum. Conclusion: The data indicate that LOX-1 is not only markedly expressed in periovulatory follicle and luteal cells from healthy corpora lutea but also functions as a regulator of the generation of NO, a potent vasorelaxant. Furthermore, LOX-1 plays a specific role in the ovulatory regulation, since granulosa cell number is known as a critical factor in ovulation. Thus, ox-LDL can interfere with ovulation using LOX-1 as mediator.

QUANTITATIVE CELL ANALYSES – IN-VIVO, EX-VIVO AND IN-VITRO

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Cytometric analyses can be used for a wide range of applications. The Translational Center for Regenerative Medicine (TRM) in cooperation with the research laboratory of the Dept. of Pediatric Cardiology at the Heart Center Leipzig offers the possibility for quantitative cell analyses of different kinds of samples. Whereas flow cytometric techniques are available in many laboratories, also cells in culture and tissue sections can here be analyzed quantitatively. With two laser scanning cytometers (LSC® with an upright microscope and iCys® with an inverted microscopic unit) this lab is one of the leading working groups in Slide-based Cytometry (SBC) analyses and multicolor experiments. Maestro fluorescence in-vivo imaging system allows for small animal imaging. With that system e.g. tumor development via injected labeled cells can be observed or tumor growth can be monitored after e.g. pharmacological treatment. Due to the detection of the whole emission spectrum of the used fluorescent dyes or proteins the system overcomes the limitations of analyses with bandpass filters and allows multicolor measurements in-vivo. Next to flow cytometry (with the possibility to analyze 10 fluorescence and 2 scatter parameters simultaneously) a new technique, impedance flow cytometry, is available, too. Impedance analysis allows for cell analysis without the need for specific labeling. Frequencies used for cell analysis range from 0.1 to 20 MHz and provide information about cell volume, membrane capacity, and cytoplasmic conductivity of analyzed cells.

A13

WHAT CAN WE LEARN FROM FLUORESCENCE LIFETIME MEASUREMENTS ABOUT FREE RADICALS IN LIVING CELLS ?

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Free radicals are an important part of reactive oxygen species (ROS). They are involved in numerous cellular processes, they can act as signal transducers, and their presence is essential for correct cell function. But ROS can also cause oxidative stress, and they can induce cell death. Several diseases are caused by aberrant ROS production. However, direct quantification of ROS remains difficult. Since their detection on cellular level is of great interest for biomedical researches new methods are continually developed, such as spin trapping techniques or new fluorescent probes. We developed a new method based on the fluorescence lifetime measurement of well-known oxygen probes. We demonstrated that in solution, both time-resolved and steady-state fluorescence measurements show that free radicals can quench the fluorescence of oxygen probes in millimolar concentrations [4]. In living cells, ROS are detected with 1-pyrenebutyric acid (PBA) [1]. We record fluorescence lifetime decays using time-resolved microfluorimetry in PBA-loaded cells. The emission is recorded through an adapted band-pass filter (404 nm) after excitation of individual cells with a pulsed nitrogen laser (337 nm). Both PBA and free and bound NAD(P)H fluorescence lifetimes are simultaneously measured. The complex decay is resolved into three exponential curves. The variations of ROS concentrations are calculated from the Stern-Volmer equation. We demonstrated that ROS concentrations change upon treatment with inductor and inhibitor of intracellular ROS levels [2]. Recently, our studies were focused on ROS involvement in the improvement of adriamycin anti-tumoural effect resulting from the use of hyperoxygenation as adjuvant therapy [3].

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A14

THE CORE UNIT FLUORESCENCE-TECHNOLOGIES IN THE IZKF LEIPZIG

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The overall purpose of the Core Unit Fluorescence-Technologies in the Interdisciplinary Centre for Clinical Research (IZKF) Leipzig is to offer access to expert assistance to various techniques in flow cytometry/cell sorting, slide-based cytometry and laser scanning confocal and multiphoton microscopy. The specific objectives of this shared resources are to provide the users with a powerful array of cytometric and microscopic techniques, with expert consultation also in experimental design to optimise data generation, as well as in data presentation and publication. Currently the Core Unit houses a BD LSR II digital benchtop analyser, a Laser Scanning Cytometer , a FACSVantage SE high-speed cell sorter, two confocal Laser Scanning Microscopes and one multiphoton Laser Scanning Microscope. The cytometers are equipped with up to four lasers (UV, 405 nm, 488 nm and 633 nm) and up to 12 parameters can be measured at the same time. With the microscopes six laser lines (364 nm, 405 nm, 458 nm, 488 nm, 543 nm and 633 nm) are available. The Core Unit is open to all scientists from the Faculty of Medicine and other faculties of the University of Leipzig as well as to external researchers from other institutions. It is designed to provide services, like training users to operate the analytical cytometers and the microscopes, performing high-speed cell sorting by the staff only, ensuring that all instruments are properly calibrated on a daily basis, advising users concerning the proper settings for their experiments, advising investigators on experimental design and data analysis and performing further training of graduate students, postdoctoral fellows and other colleagues.

A15

DEVELOPMENT OF A WILD TYPE F1 MOUSE MODEL FOR INDUCTION OF ACUTE AND CHRONIC GVHD AND CHARACTERISATION BY FLOW CYTOMETRY AND IMMUNOFLUORESCENCE MICROSCOPY

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Introduction: Graft versus host disease (GvHD) is one of the major complications after hematopoietic stem cell transplantation (HSCT) and occurs approximately in 40-70% of all cases. This life-threatening disease is initiated by donor T lymphocytes, which recognize the MHC antigens of the recipients. Diagnostic and therapeutic opportunities for GvHD are still limited. Therefore, GvHD models for testing of new diagnostic and therapeutic strategies are urgently required. The aim of this study was the establishment of a haploidentical murine parent (C57Bl/6 [H-2Kb]) into (C57Bl/6 x BALB/c) F1 (CB6F1 [H-2Kdxb]) bone marrow transplantation (BMT) model for induction of acute and chronic GvHD. Material and Methods: Bone marrow and splenocytes from male donor mice were freshly prepared and transplanted into female CB6F1 mice after lethal irradiation (8.5 Gy). All experimental groups were monitored daily for weight-loss and signs of GvHD (ruffled fur, hunched posture). Blood counts and flow cytometric analysis of host animals are performed weekly to assess reconstitution of hematopoiesis. Molecular biological, histological and immunohistological examinations of different organs were added. Invasion of intraepithelial lymphocytes (IELs), which are probably responsible for the induction of gut GvHD, was observed by means of fully-automated, quantitative fluorescence microscopy and flow cytometry of isolated IELs from recipient mice. Results: Following irradiation, WBC decreases from 8.61 ±2.44x10^3/mm^3 (day -2) to 1.33 ±0.88x10^3/mm^3 (day 5). After transplantation of 2.5x106 BM and 2.5x106 splenocytes, 33% of recipient mice survive until day 33 after BMT. In comparison, transplantation of 5x106 BM and 5x106 splenocytes leads to survival of all recipients (>day 50). Reconstitution of hematopoiesis (WBC >1x10^3/mm^3; day 12 vs. day 19) and full donor-type chimerism (H-2Kd-; day 26 vs. day 33) was faster in this group. Isolation of IEL from wild-type and recipient mice yields a population predominantly consisting of CD8+/CD103+ T cells shown by flow cytometry. IELs isolated from transplanted mice on day 54 after BMT show donor-type (H-2Kd-) MHC expression. Immunofluorescence analysis shows localization of CD8+ T cells and allows quantification (CD8+/nuclei [DAPI]). Conclusion: We establish a haploidentical murine parent into F1 BMT model with reliable and reproducible determination between donor and host cells by flow cytometry. An innovative analysis of IELs by fully-automated, quantitative fluorescence microscopy and flow cytometry is possible. The role of CD103+ IEL in inducing gut GvHD is currently under investigation.

FIFTY HERTZ EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELD CAUSES CHANGES OF THE REDOX STATUS IN HUMAN MONO MAC 6 CELLS

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The current study was designed to establish whether 50 Hz extremely low frequency magnetic fields (ELF-MF) can induce a cell-activating capacity through a redox-sensitive mechanism. The release of reactive oxygen species (ROS) and the influence on the antioxidant level in human Mono Mac 6 cells were investigated at low flux densities (10-200 µT) and during co-exposure conditions (PMA-phorbol 12-myristate 13-acetate, LPS-Lipopolysaccharide). Cells were sham, PMA (1 µM), LPS (1 µg/ml) and/or co-exposed to 50 Hz MF for 45 min at flux densities of 10, 25, and 200 µT. Helmholtz coil systems were used for ELF-MF and sham exposure. The background flux density in both sham and exposure incubators was 1–6 µT. The release of total ROS was detected by using the dye dihydrorhodamine 123 (DHR), which is oxidized by hydrogen peroxide, hypochlorous acid and peroxynitrite anion to the fluorescence dye rhodamine. Rhodamine fluorescence intensity (X-mean) was measured by flow cytometry (Epics Altra, EXPO32 vs. 1.2 analysis software; Beckman Coulter). Furthermore, catalase activity was measured at 240 nm by spectrophotometer (UV-2401 PC, Shimadzu, Germany) every 15s for 5 minutes, by following the rate of reduction of hydrogen peroxide (decrease in absorbance), according to Aebi (Methods. Enzymol., 1984). Cells were characterised as total ungated cells, gated responding cells (60-65 % of total cell number) or non-responding cells (27-35 %) to ELF-MF exposure. Selective gating and further analysis of responding and non-responding cells showed an additional increase in ROS release compared to results obtained after non-selective analysis after 10, 25 and 200 µT ELF-MF exposure. The positive controls (PMA, LPS) induced a statistically significant higher level of free radical release via their different mechanisms. ELF-MF and LPS (CD14 receptor-dependent activator) treatment of Mono Mac 6 cells showed a similar double peak distribution (responding and non-responding cells; rhodamine fluorescence) indicating the possible involvement of similar signal transductions pathways in these cells. No significant differences in the catalase activity was detected after 45 min ELF-MF exposure of human Mono Mac 6 cells for 45 min. Taken together the present study showed that 10, 25 and 200 µT exposure induced a significant increase of ROS levels when compared to control, which was found to be dose independent.
This study was supported by Bundesamt für Strahlenschutz (StSch 4556).

LONG-TERM REGROWTH OF TUMOR SPHEROIDS TO MONITOR THERAPY RESPONSE

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Background and Aim: Multicellular tumor spheroids are a well-established in vitro culture model for sophisticated experimental therapy testing in a three-dimensional cellular context since they more closely reflect the in vivo pathophysiological milieu conditions than monolayer or suspension cultures. Clonogenic survival is one of the endpoints for evaluating radioresponse, and classical colony formation assays after dissociation of treated spheroids have thus been considered and frequently used. The preparation of single cell suspensions from spheroids, however, is critical and may be affected by the treatment. We therefore intend to evaluate alternative endpoints for monitoring therapeutic efficacy in spheroid cultures. The present study was designed to long-term monitor irradiated colorectal cancer spheroids using a spheroid-based screen platform originally developed to investigate drug efficacy. Material and Methods: HT29 cell spheroids were cultured in liquid overlay using a standardized semi-automated set-up and single dose (0-30 Gy) irradiation at defined diameters of 380-400 µm. Spheroids were imaged by phase contrast microscopy for a period of up to 56 days after irradiation. The following parameters were studied: (I) spheroid integrity, growth/regrowth and growth delay, (II) cell viability using acid phosphatase (APH) activity post irradiation, (III) membrane integrity via propidium iodide and calcein AM (CAM) co-staining monitored by fluorescence microscopy and (IV) cellular adherence and proliferative capacity on adherent surfaces after long-term 3-D culturing. Results and Conclusion: All spheroids irradiated with single doses up to 10 Gy regrew despite potential spheroid destruction within 6-8 days after irradiation. Here, a growth delay could be calculated by determining the times to reach 5x the initial spheroid volume relative to untreated controls. At 15 Gy, only a subfraction of the spheroids regrew while doses of 20 Gy or higher diminished spheroid regrowth. Lack of spheroid regrowth, however, was not reflected by complete loss of membrane integrity but some of the remaining, non-spheroidal cells and cell clusters were membrane intact even 56 days after irradiation and showed some, yet poor CAM enzymatic activity. Some of these cell clusters were able to adhere and proliferate after transfer onto adherent surfaces. In parallel, morphological alterations, e.g. abnormal giant-cells, were frequently seen which did not multiply. The adaptation of the APH assay to monitor spheroid cell survival 5-7 post irradiation was not successful so far, however, the calculation of spheroid control doses according to a tumor control dose in vivo (TCD50) is envisioned as a new aspect in advanced therapy testing.

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THE AMBIGUOUS BEHAVIOR OF CD133+/- SUBPOPULATIONS FROM DIFFERENT COLORECTAL CANCER CELL LINES

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Background: The existence of subpopulations of cancer cells with stem-like characteristics that might be responsible for recurrence of disease and therapy resistance is under controversial discussion. In colorectal carcinoma (CRC), the cell surface expression of CD133 has been used as a surrogate to enrich CRC cell populations with particular tumorigenic potential. Such enrichment was also shown in a CRC study based on the established cell line HT29. In contrast we found no difference in tumorigenic potential of CD133+ vs. CD133- HCT-116 subpopulations. These cell lines, however, reflect two pathways of colorectal carcinogenesis associated with an aneuploid, chromosome instable (CIN) or a pseudodiploid, microsatellite instable (MSI) phenotype. Yet, the relevance of this coincidence is unclear. It is hypothesized that environmental conditions affect CD133 expression profile. The aim of our study was therefore to compare the in vitro behavior of CD133+/- subpopulations isolated from HCT-116 and HT29 cells grown under identical culture conditions. The objective was to provide clear evidence for an extended project comparing the tumorigenic potential of CD133+/- subpopulations from various CIN vs. MSI cell lines. Material and Methods: HCT-116 and HT29 monolayer cultures show two clearly distinguishable CD133+/- subpopulations (CD133+: 74.3 ± 6.2% and 90.9 ± 6.4% respectively). These were sorted via FACS and analyzed regarding colony formation, spheroid formation and growth in liquid overlay. The distribution of CD133 expressing populations was monitored by flow cytometry. A subcutaneous xenograft model in NMRI(nu/nu) mice was used to assess tumorigenic potential. Results and Conclusions: Colony formation capacity, spheroid volume at day 4 after inoculation and spheroid growth of CD133+ and CD133- HCT-116 subpopulations did not differ. This is in contrast to HT29 subpopulations. Here, plating efficiency and spheroid volume after initiation were significantly higher in the CD133+ subfraction compared to the CD133- counterpart. However, this discrepancy was not preserved throughout spheroid growth and the CD133+ fraction increased to reach the distribution of unsorted HT29 cultures after 20 days. Such redistribution of CD133+/CD133- fractions after sorting and reculturing in 3-D was not found in the HCT-116 model. The hypothesis of CD133 re-expression on the surface of CD133- HT29 cells grown in 3-D is under further investigation as it seems contradictory to previous observations. First in vivo transplantation assays imply that the tumorigenic potential of CD133+/- HCT-116 populations does not differ. The respective experimental series for HT29 is underway. Supported by the BMBF and the DFG (KU 971/7-1 / GR 3376/2-1 and KFO179).

A19

ISOLATING HIGH PRODUCING CELLS: A NOVEL ALTERNATIVE AFFINITY CAPTURE SYSTEM FOR HYBRIDOMA CELLS

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The most important recombinant proteins for pharmaceutical use are produced in cultures of mammalian cell lines. These cells have high requirements towards the cultivations conditions resulting in a cost-intensive business. These cell lines often show a comparative low productivity which is often a limiting factor for a productive process. To increase the productivity of these cell lines the affinity matrix capture system is a customary method. The present work describes a newly developed variant of this method. This technique is used to identify and separate high producing cells from a suspension cell culture. By capturing the product on the cell surface, the productivity of each single cell can be detected. The bound products are stained with fluorescence labeled antibodies. Since the amount of surface product binding correlates with the productivity, the cells can easily separate by FACS. The sorted cells represent a subpopulation of high producers. Usually this method works with biotinylated antibodies to capture the produced antibodies. We use biotinylated Protein A to perform this. Protein A binds with high affinity onto the Fc region of the produced antibody. To investigate the functionality of the different bindings a protein microarray was performed. We show that the new capture system is feasible and can be adopted on the cell surface. A human hybridoma cell line is used as a model system for further works.

GROWTH PATTERNS OF *CHLAMYDOMONAS REINHARDTII* AND *MICROCYSTIS AERUGINOSA* ANALYSED WITH THE PAS III AND THE CYTOSENSE

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Chlamydomonas reinhardtii (eukaryotic green alga) and *Microcystis aeruginosa* (Cyanobacteria) were analysed on a PAS III (PARTEC, Germany) and a CytoSense (CytoBuoy b.v., Netherlands) flow cytometer in parallel to train for upcoming CytoBuoy in situ operations and to ensure reliable data interpretation. The microorganisms were autotrophically and aerobically cultivated on Kuhl (*C. reinhardtii*) and BG 11 (*M. aeruginosa*) medium, respectively, over a time period of 21 days (*C. reinhardtii*) and 14 days (*M. aeruginosa*). Scatter behaviour (SSC, FSC) and autofluorescence intensities (FL Red) were analysed. The two instruments gave comparable information about the growth status of the strains. In addition to the usual cluster distribution analysis the CytoSense resolves every event with the respective silico-imaging function (one-dimensional scanned particle shape and fluorescence intensity). In consequence of this increased information on morphological and fluorescence variations of cells is offered. Variations in FWS, SWS and FL Red intensities were mirrored by significant changes in the silico-imaging signals which also provided additional information on events of the main cell cluster and deviant cells outside this cluster area.

CYTOBUOY – FLOW CYTOMETRY ANALYSIS OF PLANKTON DYNAMICS IN A FRESH WATER POND

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The natural environment of a pond was investigated with CytoSense flow cytometer over one summer period to observe population dynamics of planktonic microorganisms. The modifiable parameters of CytoSense trigger-level and flow rate of the sample-pump were varied with each sample to figure out optimal parameters for cluster-gating. The abundance of cells in the clusters were dependent on the choosen combination of trigger-level and sample-pump flow rate. Small particles (bacteria, picoplankton, etc.) can be better detected with low device settings while huge particles (makroplankton, zooplankton etc.) can be observed with high device settings. This fact demands gating of populations in the adequate dotPlots adjusted with the different CytoSense settings of the same sample. With CytoBuoy direct daily *in situ* measurements will be possible. The aim of long duration is to acquire a planktonic library of fresh water environments.

FLOW CYTOMETRIC DNA MEASUREMENTS OF RED BEET INTACT PLANTS AND IN VITRO CULTURES

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Plants are a literally source of valuable secondary metabolites used for centuries as pharmaceuticals, food additives, fragrances, dyes and agrochemicals. Production of plant derived metabolites through classical technologies lead to several difficulties, resulting mainly from seasonal geographical and soil characteristics. Plant cell, tissues and organ cultures offer an alternative opportunity for the production of biological active substances. For efficient processes constant and high yields are necessary and a good analytical understanding of the physiology of the cells or tissue facilitate this. Therefore the ploidy profiles of different tissues (leaves, petiols and roots) at different ages of intact plants from *Beta vulgaris* cv. Detroit Dark Red, as well as different *in vitro* systems (transformed hairy roots, calli derived from leaves and rhizogenic calli) obtained from it were investigated by flow cytometry. In most cases older tissues of intact plants undergo in most cases more cycles of endoreduplication than young ones. Transformed Hairy root tissue show similar ploidy pattern like *in vivo* roots, whereas rhizogenic calli exhibit a distinct ploidy profile. The obtained rhizogenic callus was morphologically heterogeneous and two lines with red and yellow phenotypes were derived by mechanical separation and examined as well. The DNA profiles of these two phenotypes differed, possibly reflecting differences in their metabolism.

DIPLOID AND POLYPLOID CYTOTYPES DISTRIBUTION IN THE WHITE-RAYED COMPLEX OF MELAMPODIUM (HELIANTHEAE, ASTERACEAE)

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The genus *Melampodium* (Heliantheae, Asteraceae) comprises 39 recognized species distributed throughout Mexico, Central America and the southwestern U.S.A, All species have yellow rays except for three shrubby, xerophytic taxa, *M. leucanthum* , *M. cinereum* and *M. argophyllum* , which form the so-called white-rayed complex and mark the northern limits of the distribution range. Based on morphological characters, *M. cinereum* has been further divided into three varieties: *var. cinereum* , *var. hirtellum* , and *var. ramosissimum* . The three *Melampodium* species are clearly separated by their distributions and ecology. They also are well supported in molecular analyses. Previous studies revealed the presence of three different ploidy levels within the white-rayed complex: diploids and tetraploids (plus occasional triploids) in *M. cinereum* and *M. leucanthum* , and exclusively hexaploids in *M. argophyllum* . In both *M. cinereum* and *M. leucanthum* diploid cytotypes prevail in the western part of their distribution area, while tetraploids are dominating in the east. It is hypothesized that *M. cinereum* evolved out of peripheral populations of *M. leucanthum* in low mountains of northeastern Mexico. Further dispersal northeastward led to divergence now recognized as taxonomic varieties. Tetraploidy developed within the easternmost populations of *M. cinereum var. cinereum* . *M. argophyllum* was hypothesised to be of allopolyploid origin, involving *M. leucanthum* and *M. cinereum* as parents. The current study concentrates on ploidy distribution in *M. leucanthum* , *M. argophyllum* and *M. cinereum* . It presents the data on the ploidy levels estimations for 148 populations (2094 individuals) of *M. leucanthum* , for 2 populations (36 individuals) of *M. argophyllum* , and for 30 populations (450 individuals) of *M. cinereum* covering the varieties: *M. cinereum var. cinereum* (21 populations, 274 individuals), *M. cinereum var. hirtellum* (6 populations, 139 individuals) and *M. cinereum var. ramosissimum* (3 populations, 37 individuals). The data obtained in this study agree well with the reports on ploidy level distribution (on the smaller scale) in the white rayed complex performed 40 years ago, suggesting that the polyploid establishment and maintenance is connected to ecological and perhaps historical biogeographical factors. The polyploid individuals within *M. cinereum var. cinereum* form a distinct genetic entity clearly separated from the diploid populations. The correlation of ploidy levels in *M. cinereum* with the molecular and cytogenetic data suggests single (or few time) origin of polyploids and their subsequent establishment in a new ecological niche. For ploidy determination, flow cytometry of DAPI stained silica gel-dried material has been applied, using Glycine max 'Merlin' as the internal standard.

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BIOMASS MEASUREMENT IN A SOLID STATE FERMENTATION PROCESS WITH WHITE ROT FUNGI BY FLOW CYTOMETRY

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Solid state fermentation (SSF) – fermenting microorganisms on a mostly humid solid substrate without or nearly without any free liquid – is a fermentation technique that has a very long tradition. A group of products that can be produced by SSF are different enzymes like cellulases or oxioreductases from white rot fungi. A handicap for the implementation of solid state fermentations into industrial processes is the difficulty to control the process due to the impossibility to measure the amount of biomass directly during and after the process. We developed an alternative and rapid method for the measurement of biomass in a SSF process by measuring the number of fungal nuclei by flow cytometry and compared the calculated results of fungal biomass with the amount of biomass calculated by measurement of ergosterol in the fungal cell wall. We found that both methods revealed a comparable amount of biomass in the SSF process, with the advantage that the measurement of nuclei by flow cytometry is quicker and less elaborate.

FLOW CYTOMETRIC ANALYSIS OF A MEDICALLY RELEVANT MICROBIAL COMMUNITY USING SPECIES-SPECIFIC IMMUNOFLUORESCENCE PROBES AND DNA STAINING

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Most microorganisms live in natural environments in microbial communities forming complex networks. The spectrum of interactions reaches from cooperation to competition. Their characterisation within heterogeneous microbial systems is of increasing interest in medical, environmental and biotechnology research and corresponding applications. In particular, it is essential to gain a more detailed insight into the dynamics of such a complex system, which cannot be obtained from pure culture laboratory studies. For our studies a defined mixed culture of Burkholderia cepacia , Pseudomonas aeruginosa and Staphylococcus aureus , which cause infections in the lung of cystic fibrosis patients, was established as a model community. Experimental data as well as mathematical models describing growth and metabolism in pure and mixed cultures revealed strong interactions [1, 2]. Currently the community is investigated by flow cytometry to analyse its heterogeneity on a single cell level. Here, a defined binary mixed culture of B. cepacia and P. aeruginosa was investigated. A specific antibody for B. cepacia was successfully tested for immunofluorescence detection. The antibody binds specifically to the outer membrane of B. cepacia and is commercially available. After incubation with this primary antibody, cells were stained indirectly by a R-phycoerythrin labelled secondary antibody The staining procedure was optimised with respect to antibody concentration. Unspecific binding of the primary antibody against P. aeruginosa as well as unspecific binding of the secondary antibody against both strains were tested by flow cytometry and fluorescence microscopy. No crossreactivity was detected. In order to discriminate bacteria from electronic background noise the samples were additionally stained against DNA with DAPI. Experimental results showed that B. cepacia and P. aeruginosa could be detected and distinguished from each other in mixed culture samples using the above described double staining approach.

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A26

EVALUATION OF DIAGNOSTIC PANEL FOR RHEUMATOID ARTHRITIS BY LSC ANALYSIS

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Diagnoses often are based on visual inspection of patients' tissue by pathologists. Common immunohistochemical staining (e.g. H&E) gives evidence for cell infiltration, inflammation, etc. However, in most cases it is not clear which cellular subtypes are present or are involved in physiological reactions. Rheumatoid arthritis (RA), for example, is sometimes hard to diagnose or to differentiate from other types of arthritis. Therefore, aim of this study was to evaluate a diagnostic panel for RA by quantitative fluorescence analysis of human synovial tissue. Surface markers were used for labeling various cell types known to be involved in RA (fibroblasts, lymphocytes, macrophages, dendritic cells). Single, double, and triple staining was done on tissue samples of patients with RA. Fluorescence of the sections was quantitatively analyzed by Laser Scanning Cytometry (LSC). Whereas a single cell based analysis (i.e. by DNA triggering) provides information of marker expression for individual cells in the tissue, the aim of this study was a quantitative analysis of the whole tissue section. Therefore, phantom contouring (non-cell based analysis algorithm) was used to obtain an overview of an overall marker expression. The applicability of this method for RA diagnosis was tested by combining markers proven to be indicators for RA. The excised synovium was stained for three surface markers simultaneously with the same fluorochrome (FITC, PE, or APC). In single cell analysis higher fluorescence intensity would only be obtained if the same cell type is stained with two or more markers (comparable to activation markers). Since different cell types were labeled phantom contouring turned out to be the optimal analytical method of choice. In comparison to single or double labeling fluorescence intensity of triple stained tissue (mean intensity of the phantoms) increased. The additive effect of marker combination, stained with the same fluorochrome, leads therefore to quantitative fluorescence information of cells involved in RA; i.e. this method provides data about the overall amount of labeled antigens in the tissue but not on a single cell level. This additive effect could be observed for different markers and fluorochromes. Therefore, combination of carefully chosen markers, as analysis panel, might be a tool for RA diagnosis.

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A FLOW CYTOMETRY-BASED METHOD FOR COUNTING AND ANALYSING THE FIRST LARVAL STAGE OF A PARASITIC WORM

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Lymphatic filariasis caused by infection with filarial worms affects over 120 million people; 40 million of them are seriously incapacitated and disfigured by the disease. The main goal of the WHO Global Program for the elimination of lymphatic filariasis is to interrupt the transmission of infective larval stages by blood-feeding mosquitos.
In the murine model of filariasis research, infective third larval stage (L3) are transmitted by a mite vector into the murine host. These L3 migrate to the thoracic cavity, develop into adults, mate and release the first larval stage, the microfilaria (L1). Those enter the bloodstream and henceforward can be taken up by the arthropode vector. After two moltings from L1 to L3 the infective stage is ready to be transmitted into another host. A vaccine that would target the circulating L1 in the peripheral blood stream of the host would be a major step towards elimination of lymphatic filariasis. Of course the survey of these circulating L1 is a key parameter to monitor success of vaccination in the animal model. Here we show a fast cytometry-based method to count the 80-100µm tall L1 directly from murine peripheral blood. With the newest portable generation of flow cytometers it may also allow high-throughput monitoring of patients in the field. Furthermore it offers new possibilities in filariasis research: using commercial available panels of fluorochrome-conjugated antibodies, it facilitates the search for molecules on the L1 surface that may interact with the host immune system, e.g. as ligands for inhibiting receptors.

EVALUATION OF MARKERS FOR DIFFERENTIATION OF CHRONIC AND ACUTE INFLAMMATION OF SYNOVIUM IN JOINT DISEASES BY SLIDE BASED CYTOMETRY

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune-disease of unknown origin that primarily affects joints and leads to their destruction. However, in contrast to our incomplete understanding of the etiology of RA, a large number of studies have identified a variety of different cells such as lymphocytes, synovial fibroblasts (SF), macrophages or dendritic cells (DC) involved in the pathogenesis of RA. Especially synovial fibroblasts and dendritic cells constitute unique cell types that might be specific for RA and could be suitable to distinguish RA from other inflammatory conditions in the joint. Methods: Here we investigated the occurrence and distribution of lymphocytes, SFs, DCs and macrophages in synovial tissues of RA patients in comparison to patients with traumatic joint injury (i.e. patients with a rupture of the cruciate ligament). Visual inspection of H&E stained synovial tissue by pathologist provided evidence about cellular allocation. The expression of markers such as CD90, CD29, CD11b and HLA-DR was indentified by immunofluorescence on cryosections of synovial tissue. To obtain information about the overall marker expression, quantitative fluorescence analysis of complete cryosections was performed on a Laser Scanning Cytometer (LSC). Results & Conclusion: A significantly higher expression of CD90, CD29, CD11b and HLA-DR could be detected in synovial tissue of RA patients in comparison to patients with traumatic joint injury. This indicates an increased number of cells and/ or a higher state of activation (e.g. CD11b) of these cells. For this reason markers as described above can be used for differentiation between chronic and acute inflammation in joint diseases. Hence, the quantitative fluorescence analysis by LSC might be a pivotal tool for differential diagnosis in arthritic disease and to analyze further cellular subtypes and their involvement in RA.

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THE EUROPEAN CYTOMETRY NETWORK

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A number of Flow Cytometry societies and associations are spread across the European continent. Each group delivers a number of high quality meetings and courses, but some overlap in subject matter making duplication of effort and resources a problem. In bringing European cytometrists together in one defined structure, we could coordinate resources for courses and meetings more effectively. We would be able to promote Flow Cytometry in the new member states and the current member states of Europe in a more cohesive way. Including Cytometry manufacturers and supporting companies in this network will result in better feedback on technical issues and forge new scientific technological challenges for the future. We could develop coordinated Pan-European teaching courses, workshops and tutorials. Initiatives, such as standards, health and safety, best practice, core management and assay development, can be coordinated and pursued at a European level. Umbrella, national and local level organisations would be strengthened through access to resources and coordination. We created a network of cytometrists whose remit encompasses all of Europe. The goals of such a Network were discussed at a meeting of established cytometrists representing 16 European Countries on 28-29th of February 2008 at EMBL Heidelberg Germany. It was decided to host the network through web 2.0 tools, in particular utilising a social networking infrastructure. From the launch of the European Cytometry Network (ECN) platform in September 2008, we have undergone a rapid growth in membership with 618 members and 32 interest groups. Members can promote their interest to the community through access to a personal webpage with professional information and several web 2.0 publishing tools, cytometry groups, discussion forums, shared educational resources, detailed information on European events and access to Cytometry experts across Europe. Over 98% of our membership comes from 23 European countries. Most importantly, 8 National Cytometry societies are represented inside the ECN and membership has expanded to include Eastern European cytometrists and societies. More than a year after its conception, the European Cytometry has succeeded in gathering a membership of mixed background and expertise in all areas of Cytometry supporting the promotion of new ideas into scientific research. Clearly the ECN has lots of room for growth in the way collaborations are structured. It is still ‘early days’ in this regard as the potential of the ECN for interactions are still being discovered.

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