SCIENTIFIC PROGRAM

15th Annual Meeting of the German Society for Cytometry

Deutsche Gesellschaft für Zytometrie (DGfZ)

in collaboration with the

**Centre for Environmental Research (UFZ)
Leipzig-Halle GmbH**

**Leipzig, October 19-22, 2005
Germany**

Leipziger Kubus
Centre for Environmental Research Leipzig-Halle GmbH

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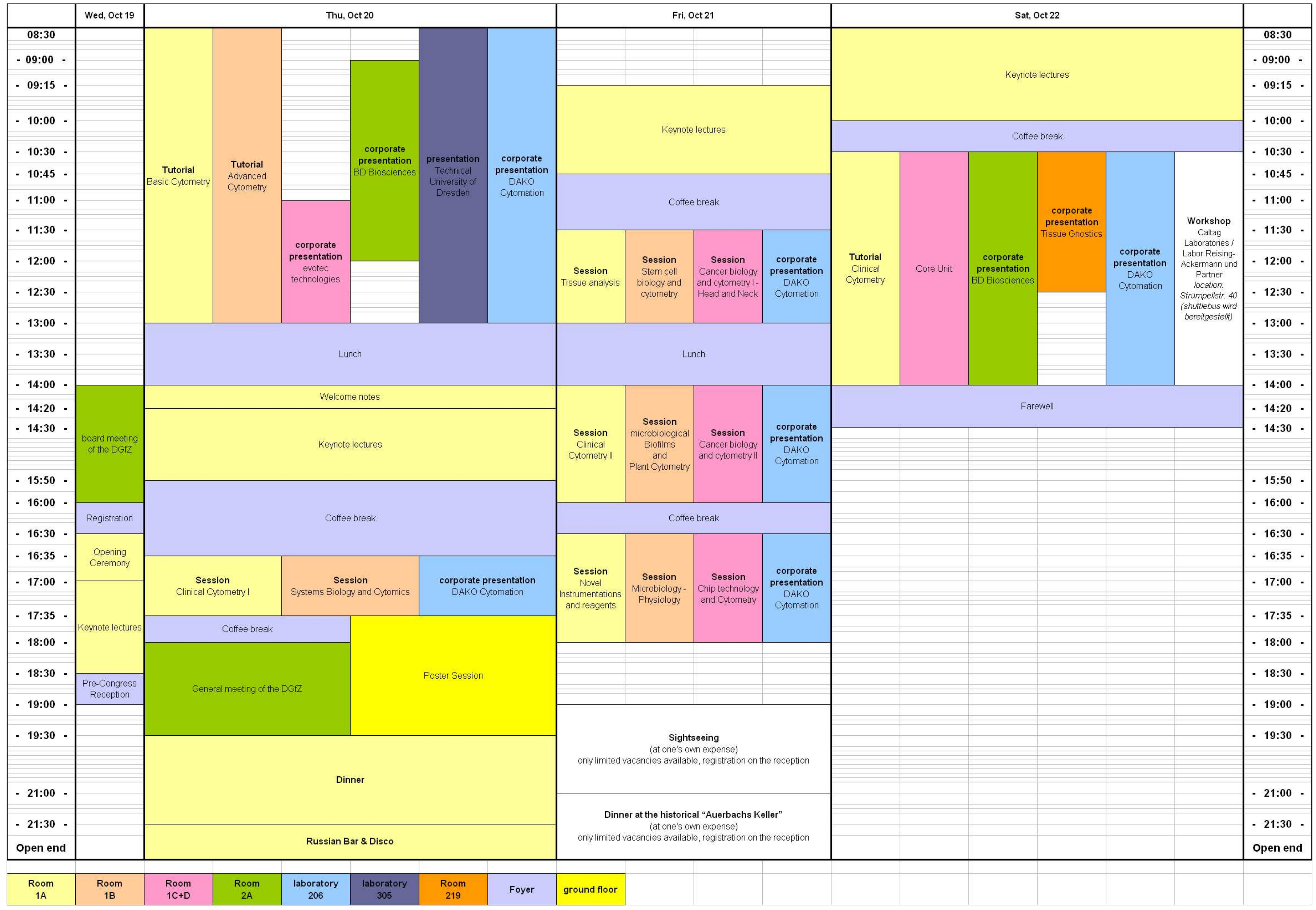
Program overview



Program – Lectures and Tutorials

Wednesday, October 19th

16:00 – 16:30	Registration
16:30 – 17:00	Opening Ceremony Welcome note: ***Schlegel M,*** *Vice-Rector Research of the University of Leipzig* ***Emmrich F,*** *Fraunhofer IZI, Leipzig*
17:00 – 18:30	**Keynote Lectures** Chair: *Emmrich F*
	L63: Stem cells in tissue engineering *Sarraf C* **L51:** Slices of Life: Multispectral Histology *Levenson RM* **L62:** Multi-Colored Fluorescent Protein-Based Imaging in Live Animals: The New Cell Biology *Hoffman RM*
18:30	Pre-Congress Reception

Thursday, October 20th

Time	Track 1	Track 2
08:30 – 13:00	**Tutorial: Basic Cytometry** (Fortbildungsveranstaltung der DGfZ) Chair: *Lenz D*	**Tutorial: Advanced Cytometry** (Fortbildungsveranst. der DGfZ) Chair: *Ulrich H*
	Flow cytometry *Tárnok A; Heart Center Leipzig* Slide based cytometry – possibilities and limitations *Lenz D / Gerstner AOH; University West Lafayette/Bonn* Fluorescent dyes - basics *Glatzel A; Caltag, Hamburg* History of flow cytometry *Valet G; MPI, Martinsried* Analysis and software of cytometric data *Bocsi J; Heart Center Leipzig* Quantitative histology and histopathology *Ecker RC; TissueGnostics, Vienna* FRET and Fluorescence Correlation Spectroscopy *Szöllösi J / Vereb G; University Debrecen* Statistical methods for evaluation of diagnostic tests *Keller T; Acomed, Leipzig*	Cytometric monitoring of biotechnological processes *Bühner J / Weber J; TU Dresden* Diagnosis of agammaglobulinemia – possibilities of flow cytometry *Warnatz K; University Freiburg* Phenotyping of B-cells *Schlesier M; University Freiburg* Analysis of T-cells *Gruber R; University Munich* Circulating blood stem cells *Cross M; University Leipzig* Cell sorting, sorting of stem cells *Cross M; University Leipzig* FACS analysis of specific functions of dentritic cells in the whole blood *Barten MJ; Heart Center Leipzig* Intracellular cytokine staining *Lehmann I; UFZ, Leipzig* Apoptosis *Sarraf C; University Westminster, UK*
13:00 – 14:00	**Lunch**	
14:00 – 14:20	Welcome notes ***Schüürmann G,** Umweltforschungszentrum Leipzig-Halle GmbH*	
14:20 – 15:50	**Keynote Lectures** Chair: *Radbruch A*	
	L73: Cytometry in the post – Genomic Aerea *Radbruch A* **L56:** Flow cytometric quantitation of immunosuppressive drug effects on immune cells *Klupp J* **L52:** Distinguished Photons: Multispectral Fluorescence Imaging In Vivo *Levenson RM*	
15:50 – 16:35	**Coffee break**	
16:35 – 17:35	**Session: Clinical Cytometry I** Chair: *Sack U, Barten MJ*	**Session: Systems Biology and Cytomics** Chair: *Levenson RM, Valet G*
	L06: The role of platelet activity in coronary artery bypass occlusion. Prague-4 trial subanalysis *Osmancik P* **L11:** Quality control of cell analysis in human cerebrospinal fluid (CSF): external pilot trials with CSF controls of the Joint German Society of Clinical Chemistry and Laboratory Medicine (DGKL) *Kleine TO* **L27:** Pharmacodynamics of T-cell functions for monitoring immunosuppression *Barten MJ* **L50:** Implementation of a practicable low-cost assay using a density-based CD4+ T- Cell depletion method for the monitoring of HIV-infected individuals *Bold A*	**L39:** Human Cytome Project, Cytomics and Systems Biology: Towards the Resolution of Biocomplexity *Valet G* **L14:** Focused COMBO-FISH for Selected Nanosized Genomic Regions *Schmitt E* **L43:** Predictive medicine by cytomics and systems biology approaches *Tárnok A*
17:35 – 18:00	**Coffee break**	Poster Session
18:00 – 19:30	General meeting of the DGfZ	
19:30	**Dinner**	
21:30	**Russian Bar & Disco**	

Friday, October 21st

Time	Session		
09:15 – 10:45	**Key note Lectures** Chair: *Knuechel R*		
	L54: Multiparameter flow cytometry of solid tumours *Cornelisse CJ* **L57:** Flow Cytometric Analysis of Oxidative Stress and DNA Repair in E. coli WP2 Tester Strains Deficient in Genes of Antioxidant Defence *O'Connor J-E* **L64:** Development and Dynamics of Pseudomonas aeruginosa Biofilms *Tolker-Nielsen T*		
10:45 – 11:30	**Coffee break**		
11:30 – 13:00	**Session: Tissue analysis** Chair: *Luther E, Ecker RC*	**Session: stem cell biology and cytometry** Chair: *Sarraf C, Scheffold A*	**Session: Cancer biology and cytometry I – Head and Neck** Chair: *Gerstner AOH, Remmerbach TW*
	L08: Microscopy-Based Multicolor Tissue Cytometry (MMTC) *Ecker RC* **L23:** Experiences in Automatic Classification of Colon and Gastric Digital Slides *Ficsór L* **L24:** Combined Serial Section-based 3-D Reconstruction of Cervical Carcinoma with H&E/p16INK4a Alternate Staining *Braumann UD* **L36:** Characterization of the Ectopic Fetal Heart Implant Early Development *Delrée P* **L47:** Analysis of Chromatically Stained Tissues and TMA's by Laser Scanning Cytometry *Luther E*	**L22:** The advances in scanning fluorescent microscopy, (automated slide handling, metal-halide illumination, software features) means significant advantage for routine applications *Varga V* **L34:** Kinin-B1 Receptor Activity during Commitment of P19 Teratocarcinoma Cells to Neuronal Differentiation *Ulrich H* **L44:** Identification of putative target genes of Gfi1 within HSC *Kosan C* **L49:** Stem cell markers associated with the side population of fresh and cryopreserved human umbilical cord blood *Cross M* **L68:** Weibel-Palade bodies as markers for transition of endothelial progenitor cells into matured endothelial cells *Neumüller J*	**L04:** Tumor environment uncouples distinct functions of human plasmacytoid dendritic cells *Brocks C* **L18:** Migration of human myeloid dendritic cells (MDC) is affected by C-reactive protein *Frenzel H* **L19:** Distinct isolation procedures of myeloid dendritic cells (MDC) from peripheral blood *Brocks C* **L20:** Stromal Cell Resistance to Cytostatic Drug Combinations in Head and Neck Carcinoma *Dollner R* **L26:** Effectiveness of liquid based cytology (LBC) in oral brush biopsy: a comparison of conventional cytopreparation techniques *Remmerbach TW* **L33:** Head and Neck Cancer: Multicolor Flow-Cytometric Analysis of CD4+ and CD8+ Tumor Antigen-Specific T-Cells using Peptide-MHC-Class I and II Tetrameric Complexes *Albers A*
13:00 – 14:00	**Lunch**		
14:00 – 16:00	**Session: Clinical Cytometry II** Chair: *Cornelisse CJ, Neumüller J*	**Session: microbiological Biofilms and Plant Cytometry** Chairs: *Tolker-Nielsen T, Neu TR, Meister A, Obermayer R*	**Session: Cancer biology and cytometry II** Chair: *Friedländer E, Brockhoff G*
	L59: Apoptosis of circulating lymphocytes during paediatric cardiac surgery *Bocsi J* **L01:** Changes of active caspase-3 and its inhibitor survivin in leukemic cells in response to initial prednisone therapy in children with acute lymphoblastic leukemia. *Urasinska E* **L16:** Density of expression of CD52 antigen on lymphocytes, CD34+ cells from graft of peripheral blood stem cells and tumor cells from patients with chronic B-cell lymphoproliferative diseases *Klabusay M*	**L65:** Microelectrode technique and Confocal Laser Scanning Microscopy (CLSM) -Investigation of mass transfer and turnover in biopellets of Aspergillus niger *Hille A* **L69:** Investigation of growth characteristics and structure of phototrophic biofilms by confocal laser scanning microscopy (CLSM) *Zippel B* **L71:** Confocal Analysis (ConAn) - a program for digital image analysis of microbial films and aggregates *Staudt C*	**L45:** Double staining of Fluorescence-in-situ-hybridisation-(Urovysion®) and Ki-67 immunohistochemistry for detection of genetic aberrations in precancerous lesions of the bladder. *Koufou S* **L46:** Local stimulation using magnetic microspheres in the assessment of ErbB signalling *Friedländer E* **L09:** An active role of CD97 in the progression of colorectal carcinoma *Galle J*

	L30: Determinants of phagocytic activity of cord blood macrophages *Gille C* **L31:** Effect of Interleukin-10 and Interferon-gamma on cord blood macrophages (CBMF) and MF of adults (PBMF) with respect to their influence on phagocytic activity *Gille C* **L21:** Image spectral cytometry for sperm chromatin testing: new solutions *Tsarev I* **L02:** A new focus algorithm for automated microscopy *Nitschke J*	**L72:** Fluorescence Lifetime IMaging (FLIM) of Syto 13 stained bacteria as a measure for microbial activity *Neu TR* .... **L40:** Impact of environmental and endogenous factors on endopolyploidization of angiosperms *Jovtchev G* **L66:** Genome size in Cirsium (Asteraceae) and its relation to natural hybridization *Bures P* **L67:** Phytoplankton biomass and biodiversity is a key feature of water quality *Wilhelm C* **L70:** Flow-based strategies for the analysis of complex genomes of Triticeae *Bartos J*	**L15:** Herceptin and Omnitarg: two HER2-targeting antibodies with different impact on breast cancer cells *Diermeier S* **L32:** AMIDA – A New Technology for Early Cancer Detection *Lang S* **L42:** A pilot study of combined PCR and flow cytometry aproach: confirmation of leukemic origin of immunophenotypic subsets in bone marrow by PCR *Mejstrikova E* **L17:** Concentration dependent changes in apoptotic and proliferation index of in vitro cultured tumor cells by resveratrol and formaldehyde *Bocsi J*
16:00 – 16:30	**Coffee break**		
16:30 – 18:00	**Session: Novel Instrumentations and reagents** Chair: *Vereb G, Raum K*	**Session: Microbiology - Physiology** Chairs: *O'Connor J-E, Bley T*	**Session: Chip technology and cytometry** Chair: *Gierse A, Ulrich H*
	L05: ElektraTM - Image based Single Cell Selection in Micro Devices *Müller T* **L25:** Assessment of elastic properties of osteoblast cells using quantitative scanning acoustic microscopy *Klemenz F* **L28:** siRNA mediated gene knock-down in primary mouse T cells *Mantei A* **L61:** Automated Analysis Of Nuclear Complexes using SMI Microscopy *Baddeley D*	**L58:** DNA and protein arrays in biotechnology *Stahl F* **L10:** Physiology of living individual Saccharomyces cerevisiae cells - a three colour approach *Achilles J* **L12:** Population profiles of a stable, commensalistic bacterial culture grown with toluene under sulphate-reducing conditions *Vogt C* **L29:** Proliferation and Biocompatible Materials in Bacteria –Population Patterns and Proteome Profiles of Polymer - State based synthesising Subpopulations *Wiacek C* **L55:** Agglutination measurement – a new way for characterization of biotechnological processes working with yeast cells *Esche H-J*	**L07:** Investigation of the effects of Ratjadone on the gene expression level via flow cytometry and DNA Chip technology *Gierse A* **L35:** Development of Anti-kinin B1 Receptor Aptamers as a Tool for Imaging using Laser-scanning Cytometry *Ulrich H* **L41:** A Normalized Meta-Database of Affymetrix Microarrays for transcriptome analysis *Frericks M* **L53:** Development of a new zebrafish (Danio rerio) embryo test system using gene expression profiling to asses the risk of chemical compounds in the aquatic environment. *Vess C*
19:00 – 21:00	**Sightseeing** (at one's own expense, only limited vacancies available, registration on the reception)		
21:00	**Dinner at the historical "Auerbachs Keller"** (at one's own expense, only limited vacancies available, registration on the reception)		

Saturday, October 22nd

<table>
<tr><td rowspan="2">08:30 – 10:00</td><td colspan="2">Keynote Lecture
Chair: Hemmer J</td></tr>
<tr><td colspan="2">L48: Ultra Slow Manipulation – A New Way for Stress-reduced and Physiological Handling of Individual Animal and Human Cells
Fuhr GR

L60: Receptor tyrosine kinases driving cell proliferation – their molecular interactions assessed by modern microscopic approaches
Vereb G

L37: The Core Flow Cytometry Facility: current and future challenges
Davies D</td></tr>
<tr><td>10:00 – 10:30</td><td colspan="2">Coffee break</td></tr>
<tr><td rowspan="2">10:30 – 14:00</td><td>Tutorial: Clinical Cytometry (Fortbildungsveranstaltung der DGfZ)
Chair: Valet G</td><td>Core Unit</td></tr>
<tr><td>Selection of fluorescent dyes in multicolor flow cytometry
Schiemann M; University Munich

FACS analysis in primary immune deficiencies
Schulze I; University Berlin

Immunophenotyping by multiparametric flow cytometry – diagnosis and monitoring in acute leukaemia
Ludwig W-D; Charite Berlin

Therapy based prediction of etiopathology of individuals by multiparametric molecular data pattern analysis
Valet G; MPI, Martinsried

Phenotyping of tumor cells
Seliger B; University Halle

Pharmacodynamic monitoring of immune suppressive drugs after heart and lung transplantations
Barten MJ; Heart Center Leipzig

RILIBÄK-adapted realization of flow cytometry
Sack U; University Leipzig</td><td>Aim of the Core Unit meeting is to give support to those who manage one central instrument up to a complete core unit.

Topics: Management, costs, and fees, problems in managing multi user instruments

Endl E; University Bonn

Davies D; FACS Lab, London

Riddel A; EMBL Heidelberg</td></tr>
<tr><td>14:00 – 14:30</td><td colspan="2">Farewell</td></tr>
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Sponsors & Exhibitors

Company	Location
3D Histech	Budapest, Hungary
AHF Analsyentechnik	Tübingen, Germany
American Diagnostics	Hauppauge, NY, USA
BD Biosciences	San Jose, CA USA
Beckman Coulter, Inc.	Fullerton, CA, USA
Caltag Laboratories	Burlingame, CA, USA
Carl Zeiss Vision GmbH	München-Hallbergmoos, Germany
DakoCytomation	Glostrup, Denmark
Evotech Technologies	Hamburg, Germany
Miltenyi	Bergisch Gladbach, Germany
MoBiTec GmbH	Göttingen, Germany
New England Biolabs, Inc.	Ipswich, MA, USA
Olympus Corporation	Tokyo, Japan
Partec	Münster, Germany
Polytec	Waldbronn, Germany
Technical University of Dresden	Dresden, Germany
TissueGnostics	Vienna, Austria

Lectures

L01: Changes of active caspase-3 and its inhibitor survivin in leukemic cells in response to initial prednisone therapy in children with acute lymphoblastic leukemia.

Kamienska K[2], Ociepa T[2], Pawluch L[2], Matysiak M[3], Kurylak A[4], Wysocki M[4], Urasinski T[2], Urasinska E[1]

[1]Dept. of Pathology and [2]I Clinic of Pediatrics, Pomeranian Medical University, Szczecin, Poland, [3]Clinic of Pediatrics, Hematology and Oncology, Medical University, Warszawa, Poland, [4]Clinic of Pediatrics, Hematology and Oncology, Collegium Medicum UMK, Bydgoszcz, Poland

Activation of caspase-3 (CAS) is the crucial stage in prednisone-induced apoptosis. CAS is responsible for proteolysis of ICAD and activation of endonucleases. Survivin (SUR) directly inhibits CAS. This study aimed to assess time resolved changes of CAS and SUR expression and the subpopulations of CAS+/SUR+, CAS+/SUR-, CAS-/SUR+ cells in peripheral blood mononuclear cells in children with acute lymphoblastic leukemia (ALL) in response to prednisone administration. The study comprised 26 children with ALL. Cytospins of mononuclear cells collected prior to and after 6 and 12h of prednisone administration were stained with rabbit anti-CAS antibody followed by swine anti-rabbit IgG/FITC and mouse anti-SUR antibody followed by goat anti-mouse/APC. Cellular DNA was counterstained with PI/RNase. CAS-associated green fluorescence, SUR-associated long red fluorescence and PI-associated red fluorescence were measured by laser scanning cytometer. Values of CAS and SUR-bound fluorescence did not change significantly after 6 and 12h after treatment, however the rate of CAS+/SUR- cells increased after 6h after prednisone administration ($p<0.05$).
Analysis of populations of CAS+/SUR- cells may contribute to better understanding of interaction of proteins promoting and inhibiting apoptosis.

L02: A new focus algorithm for automated microscopy

Hiemann R[1,2], Nitschke J[1], Böhm A[1], Hilger N[2], Weigert M[1], Sack U[2]

[1]Fachhochschule Lausitz, University of Applied Sciences, Großenhainer Straße 58, 01968 Senftenberg, [2]Institute of Clinical Immunology and Transfusion Medicine, Medical Faculty, University of Leipzig

This work describes a novel algorithm for pixel based autofocusing in microscopy applied to CCD camera systems. The algorithm is based on gray-scale co-occurrence matrix. We could show that this procedure measures accurately the focus even under noisy and low contrast imaging conditions with a small computation time even with low information content images. Focus function does not depend on any threshold allowing unattended operation for automated microscopy. We have compared our approach with different standard autofocusing methods and could show that it is a suitable and robust focus measure for use in fluorescence and bright-field microscopy that outperform similar methods. Our results indicate that this function is an optimal focus algorithm for a wide field of use including fully automatized algorithms.

L03: Identification of a Mouse Lymphotoxin beta-Receptor Like Protein

Seegers S[1], Brockhoff G[2], Schwaeble W[3], Hehlgans T[1], Maennel D[1]

[1]Institute of Immunology, [2]Institute of Pathology, University of Regensburg, Germany; [3]Department of Infection, Immunity and Inflammation, University of Leicester, UK

The monoclonal mouse lymphotoxin beta-receptor (mLTbetaR) antibody 1C5 cross-reacts with a protein on thymocytes of wildtype and LTbetaR$^{-/-}$ mice. Since thymocytes do not express the LTbetaR, a LTbetaR-related protein potentially complementing LTbetaR-activity on these cells was expected to be identified.
Applying DNA-profiles of 1C5-treated thymocytes and four-color flow cytometric analyses of splenocytes and thymocytes some unique features of the unknown protein could be revealed. The activation of the unknown protein on thymocytes induces apoptosis *in vitro*, a crucial event during thymocyte development. Furthermore, we found a LTbetaR-independent regulation of the unknown protein with a difference between the CD4 single-positive and the CD8 single-positive pathway. Amazingly only a rare fraction of mature T- and B-cells expresses the unknown protein, whereas recently activated T- and predominantly B-cells upregulate this protein.
These findings together with the observation that a small subset of activated T- and B-cells can re-enter the thymus prompted us to speculate that the unknown protein might be essential for the final thymocyte maturation and furthermore regulate the (re-)access of mature activated T- and B-cells in the thymus. The function of the protein, that we describe here for the first time, remains to be elucidated.

L04: Tumor environment uncouples distinct functions of human plasmacytoid dendritic cells

Pries R, Thiel A, Bohnert N, Wollenberg B

Department of Otorhinolaryngology, University of Schleswig-Holstein Campus Luebeck, 23538 Luebeck, Germany

Dendritic cells (DCs) are most effective amoung antigen-presenting cells and essential for primary immune responses. DCs are marrow-derived leukocytes and can be subdivided in plasmacytoid and myeloid DCs (PDC, MDC) as well as the Langerhans cells of the skin. Human plasmacytoid dendritic cells have been identified in human solid tumor tissue of head and neck squamous cell carcinoma (HNSCC), whereas their cellular functions are strongly affected within this environment and efficient immune responses are impaired. We investigated the influence of HNSCC on distinct functions of human plasmacytoid dendritic cells which were isolated from peripheral blood using magnetic bead separation. Our data reveal contradictory effects of tumor environment on PDC migration and IFNá secretion, which was investigated by chemotaxis assays, ELISA as well as flow cytometric analysis. Whereas IFNá production of PDCs is strongly decreased, we found an increased activity of PDC migration. Taken to gether, our results illustrate that HNSCC uncouples different aspects of PDC function and differentiation which strongly affects an efficient immune response and therefore contributes to 'immune escape' mechanisms in HNSCC.

L05: Elektra™ - Image based Single Cell Selection in Micro Devices

Müller T, Pfennig A, Klein-Vehne A

Evotec Technologies GmbH, Schnackenburgallee 114, 22525 Hamburg, Germany

Conventional methods for clonal analysis are mostly based on limited dilution or FACS sorting. The single cell status has to be controlled microscopically. This process is tedious and time-consuming. Additionally, clonal analysis with co-culture systems complicates the optical verification of deposited single cells. The automated instrument ElektraT improves and expedites the cell cloning process using IACST (Image Activated Cell Selection) providing high-content single cell information. A micro fluidic Sorter Chip containing micro electrodes uses negative dielectrophoretic force to guide, cage and sort cells (about 500 to 1000 cells per run). The system is equipped with a high sensitive CCD camera and a 40x objective lens. Cells are analyzed on-line according to phase contrast, fluorescence and size while passing the micro device. Thresholds can be set for target cell selection. For detected target cells an image series with up to 8 different fluorescence filter sets and individual camera settings can be taken while trapping the cells. ElektraT produces micro titer plates containing single viable clones and broadly documented cells in each well, and within an aseptic environment. 100% pure high quality cell populations are obtained without the need for multiple iterations. This is demonstrated for different cell lines (e.g. CHO, U2-OS, Hybridoma). The performance of clones expressing e.g. endothelin receptors was post-evaluated with a physiological assay and confocal imaging.

L06: The role of platelet activity in coronary artery bypass occlusion. Prague-4 trial subanalysis

Osmancik P[1], Mocikova H[2], Stros P[1], Widimsky P[1]

[1]*Cardiocenter, Department of Cardiology, 3rd Medical Faculty Charles University, University Hospital Kralovske Vinohrady,* [2]*Department of Clinical Hematology, University Hospital Kralovske Vinohrady, Prague, CZ*

Background: Platelets play a key role in acute coronary syndromes as well as in atherosclerosis progression. We tested the hypothesis, whether platelet activity could be associated with bypass graft closure during long follow-up after surgery. Methods: Eighty patients from Prague-4 study were examined retrospectively. Forty examined patients (group A) had at least one occluded graft at one-year coronary angiogram (total graft count: 109, patency rate: 37%). Forty patients (group B) had all grafts patent (total graft count:97, patency rate 100%). Both groups were similar with respect to age, gender, smoking status, diabetes, hypertension, ejection fraction of left ventricle, medication and basal laboratory parameters. Platelet activity was determined by membrane expression of platelet antigen CD41 (part of GpIIb/IIIa integrin), CD42 (von Wilebrand factor receptor) and CD 62P (P-selectin) by flow cytometry as mean fluorescence intensity (CD41, 42b) or % of positive cells (CD62P). Platelet aggregability was measured by ADP-aggregometry. Results: Membrane expression of platelet antigens CD41, CD42b and CD62P were similar in both groups (CD41: 11.8+2.4(A) vs. 12.3+2.3 (B), p=n.s.; CD42b:12.6+1,5(A) vs. 12.7+1.9(B), p=n.s.; CD62P: 1,5+1,7%(A) vs. 1.13+1.4%(B), p=n.s. (Wilcoxon test). Platelet aggregability was also similar between both groups (52.1+15.6%(A) vs. 56.1+16.3%(B), p=n.s., Wilcoxon test). Significant correlation was found between CD41, CD42b expression and glycemia ($r=0.6$, $p<0.001$), CD62P and triglycerides ($r=0.5$, $p<0.05$, both Spearman test). Conclusion: Although higher glycemia and lipid levels are associated with higher platelet activity, higher platelet activity is not associated with lower graft patency in patients after coronary artery bypass surgery. Platelets seem not to play key role in the graft closure.

L07: Investigation of the effects of Ratjadone on the gene expression level via flow cytometry and DNA Chip technology

Gierse A, Stahl F, Burzlaff A, Kasper C, Scheper T

Institut für Technische Chemie der Universität Hannover, Germany

The development of new anti-cancer drugs is of great importance for the pharmaceutical industries. Detailed understanding of the effect of the drugs is often lacking. DNA-Chip Technology was chosen to investigate the effect of a new anti-cancer drug on a gene expression level. Ratjadone is a potential anti-cancer drug and its effect on different tumour cell lines has been investigated by Burzlaff et al. [1]. The results showed that Ratjadone caused a cell cycle arrest in human cancer cells and induces apoptosis. The aim of this work is to find correlations between the responses to Ratjadone and changes in the genetic profile of the cells. In our experiments the human hepatocellular carcinoma Hep-G2 has been used. Self developed, highly integrated arrays of oligonucleotides were used, which hybridise specifically to a large set of drug relevant genes and this grants the possibility to analyse differences in gene expression after treatment with chemical compounds. Hybridisation with fluorescent labelled cDNA of Ratjadone treated Hep-G2 cells, cultured under well defined conditions will result in a specific expression pattern for the determination of the activities of enzymes that play key roles in the Ratjadone metabolism. 1. Burzlaff, A., et al., Multi parameter in vitro testing of ratjadone using flow cytometry. Appl Microbiol Biotechnol, 2003. 62(2-3): p. 174-9.

L08: Microscopy-Based Multicolor Tissue Cytometry (MMTC)

Ecker RC[1], Rogojanu R[2] , Oesterreicher K[3], Steiner GE[1,3]

[1]*TissueGnostics GmbH, R&D,* [2]*Institute for Computer Graphics and Vision, Technical University, Graz,* [3]*Department of Urology, Medical University of Vienna, Vienna, Austria*

The necessity of a standard for automated evaluation of cell stainings in immunohistology has been increasingly emphasized.

Based on AF-CLSM (Automated Fluorescence Confocal Laser Scanning Microscopy), we developed MMTC as the microscopic equivalent to flow cytometry. The technique consists of a fully automated microscope as well as advanced image processing software (*TissueQuest*). It can be used with immunohistochemical and immunofluorescence staining to quantify tissues on the single cell level. A variety of versatile identification strategies for automated recognition of individual cells, covering fluorescence-based pattern recognition, single cell identification by combination of fluorescent signals, and separate data evaluation for nuclear, cytoplasmic and surface membrane structures is provided. Complex interactions on the cellular as well as subcellular level can be addressed with respect to how many of which cells are where and what is their (functional) status. In histology phenotypic characteristics can be associated with localization and morphologic features in a quantitative manner.

Its versatile functionality makes MMTC appropriate for the search for predictive markers in cancer diagnosis as well as

post transplantation monitoring based on solid tissue sections and biopsy material, respectively. The composition and state of activation of tissue infiltrating leukocytes can be determined in situ. This technique is a versatile tool for histopathology, immunology, single cell cytometry and cytomics.

L09: An active role of CD97 in the progression of colorectal carcinoma

Galle J[1], Sittig D[2], Hanisch I[2], Wobus M[2], Wandel E[2], Kirsten T[1], Aust G[1]

[1]Interdisciplinary Centre for Bioinformatics, University Leipzig; [2]Center of Surgery, Research Laboratories, University Leipzig

The presence of scattered tumor cells at the invasion front of colorectal carcinoma (CC) correlates with bad prognosis. Scattered tumor cells strongly stain for CD97, a TM7 receptor with a long extracellular part containing varying numbers of EGF-domains. Here, we show that CD97 expression in CC cell lines was density dependent, with the strongest presence in isolated cells. Thus, we suggest that CD97 plays an active role in the progression of an environmental regulated type of CC.
We studied the effects of CD97 in CD97-inducible Tet-off HT1080 cells. The smallest CD97 isoform (EGF 1,2,5) but not CD97 (EGF 1-5) up-regulated the transcription and secretion of IL-8, a chemokine known to promote tumur cell migration. Moreover, CD97 (EGF 1,2,5) cells showed the highest proteolytical activity of several matrix-metalloproteinases, increased the migration of HT1080 cells in vitro and promoted growth of HT1080 tumors in scid mice. Tumor cells overexpressing C-terminal truncated CD97 (EGF 1,2,5/TM1), although producing higher IL-8 amounts compared to control, showed impaired in vitro migration and in vivo tumor growth .
Introducing an individual cell based computer model of heterogeneous tumour invasion we derive possible scenarios linking the changes caused by CD97 overexpression on the molecular and cellular level to the results observed in scid mice.

L10: Physiology of living individual Saccharomyces cerevisiae cells - a three colour approach

Achilles J, Müller S

Centre for Environmental Research Leipzig-Halle in the Helmholtz Association, Dept. Environmental Microbiology, Permoserstr. 15, D-04318 Leipzig

The yeast /Saccharomyces cerevisiae/ is one of the most used microorganism in biotechnological processes. Since proliferation, physiological state and, product formation depend on the capacity of the cell to access and metabolise a carbon source, a technique was developed to enable for analysing living /S. cerevisiae/ H155 cells' affinity to extracellular glucose concentrations. The fluorescent glucose analogue 2-NBDglucose was employed to analyse the cells' affinity to glucose. It was found, that the affinity of the cells to 2-NBDglucose was changed depending on the extracellular glucose concentration and, varied at identical extracellular glucose concentration. In order to investigate, whether this behaviour is related to cell cycle events and/ or to distinct modes of metabolism, a method was established for simultaneous determination of the cells affinity to the substrate and of proliferation activity. Hoechst 33342, which is a well known compound for staining DNA of both dead and living cells, was involved for determination of the proliferation activity of the cells. Additionally, Propidium iodide was used to determine the dead cell amount within the population. As a result a three colour flowcytometry approach was developed that enables to get information about proliferation activity, affinity of the cells to the substrate and the amount of dead cells of the population within a few minutes.

L11: Quality control of cell analysis in human cerebrospinal fluid (CSF): external pilot trials with CSF controls of the Joint German Society of Clinical Chemistry and Laboratory Medicine (DGKL)

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Cell counts (leukocytes, erythrocytes) and differential WBC count imply basic indices of CSF routine analysis. Here results of external quality control with standardized CSF samples of DGKL are reported to evaluate CSF cell analysis in routine laboratories. - Two samples (A,B) of native WBC and erythrocytes were prepared from human blood and sent with two samples (C,D)of stabilized blood cells to 200 laboratories to be analysed within 3 days after postage by different procedures: manual counting in Fuchs-Rosenthal chamber of cells native or after vital staining, elelctronic counting and WBC differentiating with optical (laser) or impedance detection, cytocentrifuige techniques Hettich, Shandon,resp. FACScan or immuno-cytochemistry. Target values were established. Method comparision was done acc. to Bablok and Passing. - Cell counting proved to be valid more with simple samples C, D. WBC differentiation could be done only with native samples A, B.

L12: Population profiles of a stable, commensalistic bacterial culture grown with toluene under sulphate-reducing conditions

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The population dynamics of an anoxically grown binary microbial culture composed of the sulphate-reducer Desulfobacula toluolica DSM 7467, and an accompanying bacterium (strain MV1) identified as Cellulosimicrobium sp., was investigated. Flow cytometric analysis and bulk measurements indicate that there was a commensalistic or saprophytic relationship between strain MV1 and D. toluolica. The culture was fed with toluene under sulphate-reducing conditions. The oxidation of toluene only occurred in association with sulphate reduction and growth of D. toluolica. The relationship between the two organisms was investigated at the single cell level by analysing their changing ratio and the proliferation activities of the strains in relation to varying cultivation conditions. A characteristic chromosome pattern, with at least six subpopulations of D. toluolica, appeared during stationary phase, and asymmetric cell division was detected. The accompanying strain MV1 grew repeatedly to a high percentage of the binary culture in lag, early exponential and stationary growth phases of D. toluolica, independently of the feeding substrate toluene. The repeated rapid and frequent changes of the quantities within

population subsets are indicative of very flexible adaptations to changing environmental conditions, reflecting the need for modulated cell states and their probable importance for growth in bacterial communities.

L13: COMBO-FISH of living cells

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Until recently it was impossible to label specific genome targets in cell nuclei of living cells by FISH. The principle shortcoming was the necessity of thermal denaturation in order to hybridize the single stranded DNA probe to the single stranded DNA target sequence. Some years ago it was shown that for certain labelling sites thermal denaturation can be omitted maintaining the chromosomal morphology much better (*Durm et al., Z. Naturforsch. 52c: 82–88, 1997; Winkler et al., J. Microsc. 209: 23–33, 2003*). With the introduction of COMBO-FISH (*Hausmann et al., Biotechniques 35: 564-577, 2003)* it has become possible to specifically label any given genome target without thermal denaturation. COMBO-FISH uses computer selected oligomeres of about 15 – 30 nucleotides that specifically colocalize at a given target site. After microinjection or diffusion of appropriate PNA oligo-probes into *living* HeLa cells or lymphocytes, the cells were further cultivated for another 26 hours during which the probes targeted their complementary genome sequences specifically. For microscopy the cell nuclei were then fixed in order to better visualize the small oligo-labels. The results indicate that specific labelling has taken place during cell culture without any influence on the vitality of the cells. Thus, FISH in living cells should be possible and will offer new perspectives in the investigations of living genomes.

L14: Focused COMBO-FISH for Selected Nanosized Genomic Regions

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In biomedical investigations, it is important to know comparative sequential changes of genomic regions of interest in detail. For investigation of breakpoint regions, local deletions and insertions by labelling specific genomic targets of the size of about 20 to 250 kb length, we use a set of oligomeres of about 15–30 nucleotides that specifically colocalize at the given site (COMBO-FISH, *Hausmann et al., Biotechniques 35: 564-577, 2003).* We restrict to homooligopurines or homooligopyrimidines for double as well as triple helical hybridizations with known binding dynamics. Depending on the target length and the density of such stretches, 10 to 50 colocalizing oligomeres are selected, excluding further visible clusters. In experimental design, it is desirable to have larger oligonucleotide sets at hand which can be reduced dynamically to a small set focusing on the region of interest, maintaining a maximal signal to noise ratio. For the efficient design of such focusing sets, enhanced algorithms on compactly coded genome data banks are implemented. Results on their statistical distribution within introns, exons, and non-coding regions in different genomes (of mice and men) are inferred. Labelled sites, include breakpoint regions of translocating cancer genes, can be clearly distinguished from the background using different hybridization molecules (DNAs, PNAs) and different types of microscopy (confocal, SMI, etc.).

L15: Herceptin and Omnitarg: two HER2-targeting antibodies with different impact on breast cancer cells

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Background: The therapeutic antibodies Herceptin and Omnitarg, both targeting the human epidermal growth factor type 2 (HER2), bind to different epitopes on the extracellular domain (ECD) resulting in different cellular mechanisms that are not yet completely understood. We evaluated their capacity to modulate HER-family receptor (EGFR/HER1 and c-erbB2/HER2) activation, dimerization, internalization, and shedding of HER2-ECD in two different breast cancer cell lines.
Methods: BT474 and SK-BR-3 breast cancer cell lines, both overexpressing HER2, were treated with EGF, HRG, Herceptin, and Omnitarg in various combinations. HER1 and HER2 internalization was evaluated flow cytometrically. FRET, Protein Array Technology, and Western Blotting were applied to investigate HER2 activation, dimerization and shedding of HER2-ECD.
Results: In both SK-BR-3 and BT474 cells HER2 homodimerization was inhibited by Omnitarg, but HER2 homodimers seemed to be stabilized by Herceptin treatment. Both antibodies increased HER2 phosphorylation at Y877 and Y1248, irrespective of growth factor treatment. Herceptin inhibited HER2-shedding in both cell lines, whereas Omnitarg reduced it solely in SK-BR-3. Both antibodies do not induce HER2 or EGFR internalization.
Conclusion: Significant differences on HER-family receptor dimerization caused by Herceptin and Omnitarg result in different cellular behavior, e.g. cell proliferation. A detailed understanding of their impact on receptor function and interaction will improve tumor treatment with monoclonal antibodies in the future.

L16: Density of expression of CD52 antigen on lymphocytes, CD34+ cells from graft of peripheral blood stem cells and tumor cells from patients with chronic B-cell lymphoproliferative diseases

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CD52 is expressed with relative high density on the surface of most mononuclear cells. Monoclonal antibody anti-CD52 (alemtuzumab) is used for treatment of B-CLL, PLL and T-cell lymphomas and also for T-cell depletion in vitro in PBSC grafts. Samples from patients with new B-cell lymphoproliferative diseases were analyzed. Samples of CD34+ cells were analyzed in allografts of PBSC. Control samples were obtained from healthy donors. CD52 expression was evaluated by flow cytometry on the target cell population. The values of mean intensity of fluorescence were transferred to MESF units. The median intensity of CD52 expression on CD19+ B-lymphocytes in the control samples was 350x10e3 MESF. In patients with SCLL the intensity of CD52 expression on tumor population (344x10e3 MESF) did not differ significantly from that of control

samples. Lower intensity of expression was observed in B-CLL samples (293x10e3 MESF). CD52 antigen was present on 70% of CD34+ cells in PBSC graft with low intensity of expression: 156x10e3 MESF. The level of CD52 expression is relatively high on B-CLL cells and comparable to the expression of normal B-lymphocytes. Most of CD34+ cells from PBSC graft express CD52 antigen at lower density. The density of CD52 expression can correlate with the dose-response curve and therapeutic ratio of alemtuzumab.

L17: Concentration dependent changes in apoptotic and proliferation index of in vitro cultured tumor cells by resveratrol and formaldehyde

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Resveratrol (3,5,4'-trihydroxystilbene), a natural compound occurring in grape and wine, is considered having a protective action against arteriosclerosis and affects the proliferative and apoptotic process in tumor cells. One of the concepts of mechanism of its actions based on reaction with formaldehyde generating through the biochemical pathway of cells. In this study we evaluated the effect of resveratrol and formaldehyde on apoptotic or proliferation index of tumor cells if they are given directly into the medium. Human tumor cells were cultured for 24h in various concentrations (in range of 0 - 0.1 mM) of resveratrol and formaldehyde and combinations of them. Cell cycle distribution and frequency of apoptotic cells was investigated by flow cytometry. Resveratrol at higher (0.1mM) concentration increased S-phase to 47% (18.7% control) which was nearly doubled (78%) in combination with 0.01 mM formaldehyde. The apoptotic rate was increased in all concentrations and combinations compared to the control but 0.001mM formaldehyde or combination with 0.001mM resveratrol. Resveratrol collect cells in S-phase and induce apoptotic activity of tumor cells in vitro in concentration dependent manner. Extracellular formaldehyde could potentiate the resveratrol effect on cell cycle block in S-phase.

L18: Migration of human myeloid dendritic cells (MDC) is affected by C-reactive protein

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Myeloid dendritic cells (MDCs) are rare bone marrow derived cells and the major function is sensitizing naive T cells to protein antigens. C-reactive protein (CRP) is known to be an acute phase protein produced by the liver in response to Il-6 secretion. It is involved in complement activation, binds to numerous cellular antigens and affects apoptosis. Influence of CRP on immune cells as an immune modulator is strongly suggested but detailed mechanisms still remain unclear. Recently, extrahepatic expression and secretion of CRP in malignant or inflamed tissue has been shown but the benefit of a local CRP-production is yet unknown.
We investigated the influence of CRP on distinct functions of MDCs, isolated from human peripheral blood using magnetic bead separation. Expression patterns of costimulatory molecules before and after incubation with CRP were analysed by flow cytometry. Migration activity analysis was performed using a chemotaxis assay.
Our data reveal a strongly decreased migration activity of MDCs in response to CRP exposure dependent on the CRP concentration. Expression patterns of costimulatory molecules are not affected.
Our data suggest the existence of a specific and regulated CRP-receptor pathway on MDCs influencing the cellular migration activity. Therefore further investigations concerning the physiological scale and function of CRP in vivo have to be carried out.

L19: Distinct isolation procedures of myeloid dendritic cells (MDC) from peripheral blood

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Dendritic Cells (DC) are the most potent antigen-presenting cells.
Since myeloid DC (MDC) contribute only about 0.5% of total peripheral blood mononuclear cells (PBMC), molecular investigations require highly efficient as well as gentle procedures of cell enrichment. We use magnetic bead separation which is based on magnetically labelled antibodies. Isolated cells were analyzed by flow cytometry.
To improve isolation efficiencies, we elucidated advantages and disadvantages of two different methods.
MDC were isolated from PBMC using magnetic labeled anti-BDCA-1 antibodies. With the first method, PBMC were obtained from buffy coats by Ficoll-Hypaque density gradient centrifugation by magnetic column-isolation.
Secondly, MDCs were isolated from leukaphareses of human peripheral blood with AutoMACS™ system.
The first technique is more time-expense and reveals to a total average cell amount of 5x10^8 MDC which can be used for migrationassays and cytokine detection. The second technique is slightly faster but more expensive. A 10 fold increased cell amount gives the opportunity for protein analysis.
Flow cytometry showed no difference in cell surface protein expression.
We show here two different but equally efficient methods for MDC isolation which can individually be performed for specific use in isolating distinct cell amounts of MDC.

L20: Stromal Cell Resistance to Cytostatic Drug Combinations in Head and Neck Carcinoma

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Recent studies focusing on response prediction to chemotherapy gave hints on a possible role of stromal cells in the chemoresponse of solid tumors. So far the chemosensitivity of tumor explants has only been studied by exposure to single cytostatic drugs, in contrast to the clinical application of drug combinations. The present study aims to determine and compare the quantitative chemoreactivity of stromal and epithelial cells of Head and Neck Squamous Cell Carcinoma (HNSCC) to cytostatic drug combinations. Specimens from twelve histologically confirmed HNSCC were investigated. Using an ex vivo colony formation assay, the individual cellular chemoreactivity was determined quantitatively for combinations of four cytostatic drugs: cis-platinum (cis-DDP), carboplatin (CBDCA), 5-fluorouracil (5-FU), and docetaxel (DTX). The tests were performed using

drug combinations according to recent clinical therapy regimens in the treatment of solid tumors: 1) cis-DDP + 5FU, 2) CBDCA + 5FU, 3) cis-DDP + DTX, and 4) CBDCA + DTX. The approach provides individual drug response patterns of epithelial and of stromal cells. Individual, selective sensitivities were found for each drug combination tested. Stromal and epithelial chemoreactivity profiles differed in most of the specimens. Moreover, stromal cell chemoresistance dominated selective epithelial chemosensitivities in the majority of cases. The surprising finding of stromal cell chemoresistance needs further investigations, in particular, concerning its significance for the clinical chemoresponse of HNSCC.

L21: Image spectral cytometry for sperm chromatin testing: new solutions

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Recently, we have proposed the Toluidine Blue (TB) test (at pH 3.5) as an alternative to existing methods for sperm DNA integrity evaluation. In this study, we have developed two new approaches for evaluation of sperm chromatin structure and stability using modification of this TB test.
The first approach, based on pH shift, has been tried on the group of 82 patients. Staining results at pH 4.5 with that at pH 3.5 were compared. In this way a group of sperm cells with non-impaired DNA integrity and stable DNA-protein structure can be discriminated. Proportion of such cells significantly higher in donor group compared to infertile patient group. No correlation between proportion of these cells and standard semen parameters were found.
Second approach is based on the on data published futher by Erenpreisa et al., 1992; 1997 on somatic cells stained by TB, which showed that impairment of DNA integrity in early apoptosis causes increase of TB absorption in the blue-green part of spectrum, while disorder of the chromatin in late apoptosis induces additional increase of absorption in its red part. Therefore we used the ratio of red/green optical density of TB stained sperm cells, measured by camera, in order to discriminate of the cells with disordered chromatin structure by mean of special mathematical analysis.
Clinical signifficance of both approaches for males' fecundity is under current investigation.

L22: The advances in scanning fluorescent microscopy, (automated slide handling, metal-halide illumination, software features) means significant advantage for routine applications

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Background: we reported recently about the application of single slide fluorescent scanning technology on a routine motorized microscopy. Several technical limitations became known as scanning speed, scanning area determination.
Aims: Development and evaluation of a fully automated new fluorescent slide scanner.
Materials and methods: Hi-Scope (3DHISTECH Ltd, Budapest Hungary) features automated slide box movement (up to 6 boxes, 300 slides), slide loading and barcode identification. Automated scanning software features multi-channel slide digitization. Coverslips are fixed using Pro-Long anti fade media (Molecular Probes, USA). Illumination was enhanced using a fluorescent light source with liquid fiber scrambler. Cytometric calibration and standardization was done using Coulter beads.
Results: Focus determination worked without error. CV of the density measurements on the calibration beads were 3.9 %. The determination of area of interest using pre-labeled coverslips was automatically performed without failures. Resolution of the system is 0.37 µm / pixel. Digitization of a field of view took upto 0.1 sec in FITC, 0.1 sec in DAPI and upto 0.2 sec in the Rhodamine channel. This way a cytospin area could be scanned in three channels in less then 10 minutes.
Conclusions: Hi-Scope can contribute to high volume fluorescent slide scanning. The produced image quality is acceptable for routine use.

L23: Experiences in Automatic Classification of Colon and Gastric Digital Slides

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INTRODUCTION Thanks to the fast evolution of digital microscopy new ways have been opened for image analysis on whole histological tissue slides. AIMS The aims of present study were to adopt new development of image processing algorithm for whole biopsy slides to detect higher structures as gland, epithelium surface and finally to automatically classify gastric and colon samples using digital slide format. METHODS Altogether 69 colon and 79 gastric biopsy specimens were selected. To digitalize the selected H/E stained slides Zeiss Mirax slide scanner system was used. Automatic histological evaluation modules were developed in C++. Altogether 45 parameters described the area, cell density and cellular characteristics of the basic tissue components as the surface epithelium, glands, connective tissue and the inflammatory cell compartment. Area and contained cell number ratios of different tissue compartments were calculated (tissue cytometric features). RESULTS We could find that that newly developed tissue cytometric features efficiently can be used to classify both gastric and colon digital slides by their disease state. The most important parameter was the ratio of total cell number and cell number in interstitial region (Colon: healthy 1.57 ± 0.17; aspecific colitis 1.34 ± 0.16; colitis ulcerosa 1.18 ± 0.09; Crohn 1.28 ± 0.11, $p<0.01$ | Gastritis: healthy 1.43 ± 0.12; gastritis 1.23 ± 0.13; carcinoma 1.1 ± 0.05 $p<0.01$). CONCLUSION This preliminary study proved that the development and evaluation of quantitative tissuemetric features can be used in the automated classification of gastrointestinal specimens.

L24: Combined Serial Section-based 3-D Reconstruction of Cervical Carcinoma with H&E/p16INK4a Alternate Staining

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The 3-D reconstruction of cervical cancer invasion fronts at spatial resolutions of 10µm was successfully accomplished in the past. H&E-stained serial sections of remarkable extent were processed applying a reference-free automated coarse-to-fine image registration strategy. This reconstruction process is expected to improve using p16INK4a, a novel specific immunohistochemical marker for cervical cytology/histology.
350 serial sections of a cervical carcinoma were alternately stained with p16INK4a and H&E to determine the influence

of the staining principle on the 3-D tumor reconstruction. The tumor segmentation step applies different modes for H&E and p16INK4a and utilizes the fuzzy c-means approach.
The segmented tumor volume, its surface, its discrete compactness, and a visual inspection using 3-D visualization was compared between the two staining techniques. For p16INK4a, the segmented tumor volume was twice as large ($23mm^3$ vs. $46mm^3$), while its surface is 12% smaller compared to H&E ($1589mm^2$ vs. $1401mm^2$). This corresponds with the compactness numbers (0.71 vs. 0.9). The p16INK4a-derived tumor invasion front exhibits rather smooth visible margins, while the H&E-derived invasion front is more craggy. The uneven invasion front derived from H&E-staining originates from under-segmentation related to inflammatory changes adjacent to the tumor.
In this study, we show the first combined serial section-based 3-D reconstruction with two different histological stainings. Alternate staining is successfully processed by our algorithm and might be relevant for various tissue analysis questions.

L25: Assessment of elastic properties of osteoblast cells using quantitative scanning acoustic microscopy

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The contrast of a scanning acoustic microscope (SAM) arises from local variations of acoustic and elastic cell properties. Therefore it is possible to image living cells with negligible energy deposition and without the necessity for staining. This study aimed at i) validating SAM for the assessment of cellular properties of cultured human osteoblasts and ii) relating changes of acoustic properties to variations of the intracellular deposition of calcium induced by treatment with vitamin D or ascorbic acid.
The V(z) signature was measured at 900 MHz in two dimensions. From this 3D data set cell thickness, acoustic attenuation and the change of the surface acoustic wave (dSAW) velocity of the substrate were locally derived. N-way ANOVA was used to determine the effects of the anatomical region, treatment and duration of treatment on the parameter estimations. While attenuation was not sensitive to treatment but to the anatomical location, dSAW was predominantly affected both by type and duration of treatment.
Localized intracellular calcium deposition effects physical properties (mass density, elasticity) that are closely related to acoustic attenuation and the speed of sound. These parameters can be assessed either directly or via the measurement of the change of the SAW velocity. Therefore SAM proves to be a powerful research tool for the study of dynamic changes of cell properties *in vivo.*

L26: Effectivnes of liquid based cytology (LBC) in oral brush biopsy: a comparison of conventional cytopreparation techniques

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BACKGROUND: The objective of the current split sample study was to evaluate the applicability of LBC of oral brush biopsies in a screening program for oral cancer. Cytological diagnoses were compared with follow-ups of the patients METHODS: Two different preparation methods were investigated: the conventional transfer procedure to glass slides and the liquid-based SurePath-System (TriPath Imaging, Burlington, NC, USA) for the detection of squamous cell carcinomas of the oral cavity. The obtainments of epithelial cells were performed five times with a cell collector (ORCA-Brush, DGOD, Leipzig, Germany) 550 conventional slides and 113 thin layers from a total of 113 oral lesions were reviewed with both techniques. RESULTS: Sensitivity of our cytological diagnosis of conventional prepared slides was 96,30%, specificity 90,63%, positive predictive value 96,30% and negative predictive value 90,63%. Sensitivity of our cytological diagnosis of thin layers was 97,53%, specificity 68,75%, positive predictive value 96,30% and negative predictive value 91,67% under consideration that only remained cell amount was investigated. CONCLUSION: Our findings indicate that in oral cytology, thin layers can safely replace other types of wet-fixed preparations, resulting in enhanced specimen quality and diminished false negative rates in a direct to vial procedure.

L27: Pharmacodynamics of T-cell functions for monitoring immunosuppression

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Background: Therapeutic drug monitoring relying on measuring blood concentrations (pharmacokinetics) is still problematic in the clinic due to drug interactions, toxicities and individual responses to drug effects. Therefore, in this study we monitored the pharmacodynamics of immunosuppressants using our established T-cell function assays and investigated both pharmacokinetic and pharmacodynamic approaches after heart transplantation (HTx).
Methods: HTx recipients of two groups were studied: group-I: recipients taking cyclosporine (CsA); group-II: recipients taking tacrolimus (TRL) after conversion from CsA because of side effects. PD effects were analyzed by FACS of expression of T-cell functions (cytokines:IL-2, IFN-g; proliferation: PCNA; activation: CD25).
Results: In group-I before dosing and two hours after dosing (C2) increased pharmakokinetics of CsA produced a significant decrease of expression of T-cell functions ($p<0,05$). Correlations (r^2) at C2 between inhibition of T-cell functions with drug concentrations and with drug doses were: CsA-concentration:0.71-0.91; CsA-dose:0.73-0.87. In group-II pharmacokinetics within the respective target values produced pharmacodynamic effects of TRL which were equally high on expression of T-cell proliferation and activation, but significantly higher on cytokine expression compared to pharmacodynamics of CsA-therapy before conversion ($p<0,05$).
Conclusions: Our results showed that monitoring pharmacodynamics of T-cell functions in combination with pharmacokinetics provide the use for monitoring immunosuppression to increase the efficacy and safety of individual immunosuppressive therapy.

L28: siRNA mediated gene knock-down in primary mouse T cells

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siRNA mediated knock-down of target genes in somatic cells is a promising technology for functional gene analysis and potential therapeutic applications. So far the technique is mainly used in vitro in immortalized cell lines which can easily be transfected with siRNAor siRNA expression plasmids. In contrast, the application of siRNA in primary cells such as lymphocytes was so far limited because these cells are difficult to transfect with conventional techniques. Using the Nucleofectorâ technology we show here that primary mouse T cells can be transfected with siRNA with an efficieny of almost 100%. Using CD4 as a target gene we show that a specific knock-down of up to 70-80% on the mRNA level and 50-75% on the protein level can be achieved in resting as well as activated T cells. Maximal knock-down can be observed already 24 hours after transfection and is stable for up to 5 days depending on the concentration of siRNA. Transfected cells are viable and can be activated for functional analysis. Our results show that siRNA mediated knock-down can now also be used for functional gene analysis in primary mouse T cells in vitro and in vivo following adoptive transfer of transfected cells.

L29: Proliferation and Biocompatible Materials in Bacteria –Population Patterns and Proteome Profiles of Polymer - State based synthesising Subpopulations

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The activity, physiological state and, biomedical product formations (PHA) of microbial individuals very often depend on the state of the cell in the cell cycle. Since these syntheses are subjected to individual states, flow cytometry was used to deliver insights in cell cycle related product formation mechanisms. Though the genetic equipment is identical in all individuals of the cultivated bacterium *Cupriavidus necator* and the applied microenvironmental conditions were stably designed, the metabolic fluxes were found to be directed into different product formation rates at the population level. To get knowledge about the cellular strategies, proteome analysis was involved for detailed information about individual expression of chosen metabolic pathways. Therefore subpopulations were flow cytometrically separated by proliferation and product formation status, two-dimensional gelelectrophoresis were performed and the resulting subproteome patterns were compared.
The protein patterns released information about more than 130 proteins per subpopulation when sorting up to 1.5 x 10^9 cells per status. Depending on proliferation phase more than 12 differences of protein expression profiles were detected; some of them were newly induced whereas others were down- or up-regulated.
Multiparametric flow cytometric and subpopulation proteome analysis represent a new break through for discovering metabolic phenotypes and thus to overcome cell systems heterogeneity by developing cytomic concepts for biomedical polymer production.

L30: Determinants of phagocytic activity of cord blood macrophages

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Background: Labour plays a role in modulating host defences in newborns. Cord blood macrophages (CBMΦ) of vaginally delivered term neonates (VDN) produce higher levels of proinflammatory cytokines compared to those with elective cesarean section (ECSN). Hypothesis: Phagocytic activity and intracellular degradation of *E. coli* by CBMΦ are not influenced by mode of delivery or multiplet pregnangy, gender or birth weight. Methods: CBMΦ of VDN (n = 8), ECSN (n = 22), twins (n = 6) were isolated. *E. coli* DH5á, expressing green fluorescent protein (*E. coli*-gfp) were added (bacteria: cells = 50: 1). Phagocytosis index (CD14+gfp+: CD14+), -capacity (gfp MFI of CD14+) and CD14 expression were analyzed. Results: CBMÖ from VDN vs. ECSN were comparable in phagocytosis index (39 ± 14% vs. 39% ± 22; p = 0.6) and phagocytic capacity (95 MFI ± 55 vs. 124 MFI ± 52; p = 0.2). Phagocytosis index of first vs. second born multiplets (51% ± 17 vs. 53% ± 17; p = 0.6) and phagocytic capacity (139 MFI ± 57 vs. 121 MFI ± 40; p = 0.6) were comparable. There was no correlation between gender or birth weight and both parameters. Conclusion: Our data suggest that none of the investigated parameters influence phagocytic activity of CBMÖ. These data based on a single cell assay are in contrast to results from other groups, obtained from plating experiments.

L31: Effect of Interleukin-10 and Interferon-gamma on cord blood macrophages (CBMF) and MF of adults (PBMF) with respect to their influence on phagocytic activity

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Background: We showed that IFN-γ and IL-10 diametrically induce distinct MΦ subpopulations. The sensitivity of CBMΦ towards both, IFN-γ and IL-10, were found to be reduced compared PBMΦ. Hypothesis: In contrast to their different sensitivity on specific MΦ functions, phagocytic activity of *E. coli* are influenced equally by the cytokines. Methods: To MΦ of neonates (n = 8) or adults (n = 6) IFN-ã (5 - 500 I.E./ ml) or IL-10 (IL-10; 0.5 – 50 ìg/ml) were added for 24 h. *E. coli* DH5á, expressing green fluorescent protein (*E. coli*-gfp) were added. Phagocytosis index (CD14+gfp+: CD14+), phagocytic capacity (gfp MFI of CD14+) and CD14, CD80, CD16, and HLA-DR were analyzed. Results: IFN-γ dose-dependently enhanced CD80 and HLA-DR, with PBMÖ showing a pronounced effect (73% vs. 40% on CBMΦ; $p < 0.05$). IL-10 downmodulated HLA-DR on PBMÖ vs. CBMÖ by 700% vs. 30% and upregulated CD16 by 200% vs. 17% ($p < 0.05$). In contrast to phenotypic changes, IFN-γ equally reduced phagocytosis index of PBMÖ and CBMÖ by 20% vs. 23% (p = 0.6) and phagocytic capacity by 37% vs. 29% (p = 0.7). IL-10 equally elevated both indices (17% vs. 24% and 9% vs. 13%, p = 0.2). Conclusion: CBMΦ do not exhibit a general hyporesponsiveness towards IL-10 and IFN-γ.

L32: AMIDA – A New Technology for Early Cancer Detection

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In many patients suffering from carcinomas of the upper aerodigestive tract advanced stage disease and therefore late therapeutic intervention much contributes to the bad clinical prognosis. Early detection of malignomas is a promising strategy for a better long-term survival. We have established a new technology termed AMIDA (autoantibody-mediated identification of antigens), which allows the identification of potential tumor-associated antigens (TAAs) based on the immunoprecipitation of these antigens by autologous serum antibodies followed by two-dimensional electrophoretic separation and mass spectrometry. As today, we identified approximately 50 potential TAAs, one of which being Cytokeratin 8 (CK8). We demonstrated by means of an adapted Bio-Plex system that levels of CK8-specific antibodies were significantly elevated in sera of cancer patients as compared to healthy donors. The overall sensitivity and specificity of the test were 83.3% and 89.5% respectively. Interestingly, we observed the highest concentrations in patients with early stage cancer (pT1/pT2), making CK8-specific antibodies a promising circulating tumor marker for head and neck cancer. In conclusion, AMIDA is a proprietary technology for the fast and efficient identification of disease-associated autoantibodies, i.e. biomarkers, developing early in the course of cancer.

L33: Head and Neck Cancer: Multicolor Flow-Cytometric Analysis of CD4+ and CD8+ Tumor Antigen-Specific T-Cells using Peptide-MHC-Class I and II Tetrameric Complexes

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The enumeration and characterization of tumor antigen-specific T-cells in cancer patients is important for the planning-, performing- and follow-up-phase of immunotherapy. Recently, peptide-MHC-class-I and -II tetrameric complexes (tetramers) have been introduced allowing for the detailed evaluation of epitope-specific CD8+ T-cells as well as CD4+ T-helper cells. The application of tetramers is technically easy if the number of epitope-specific T-cells is high. However, this is not the case if T-cells are investigated which display specificity for tumor antigens– a rare event analysis is necessary.

Here we describe a multicolor-flow-cytometry-assay allowing for detection of rare tumor-specific T-cells. Background staining was decreased by a modified gating strategy and specificity was increased by determining competition between tetramer and anti-CD3 antibodies. This was the basis for the enumeration and characterization of tumor-specific T-cells which are of relevance in head and neck cancer (HNC). A panel of p53 derived epitopes was investigated and number as well as characteristics of specific T-cells was correlated with the antigen status in the patient´s tumor. This correlation gave insight in the in vivo interaction of T-cells with HNC and might have implications for future immunization strategies.

L34: Kinin-B1 Receptor Activity during Commitment of P19 Teratocarcinoma Cells to Neuronal Differentiation

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Kinins are vasoactive oligopeptides generated upon proteolytic cleavage of low and high molecular weight kininogens by kallikreins. The kinin-B2 receptor, stimulated by its specific agonist bradykinin has a well established role in inflammation and homeostasis, whereas the kinin-B1 receptor, stimulated by des-arg9-bradykinin, is expressed in conditions of inflammation, but has also been related to inhibition of mitogenesis, blocking transition from G1 to S phase. Kinin-B2 receptor activity has been rela bradykinin together with kinin-B1 receptor gene expression was detected on the embryonal body stage at day 2 following stimulation to neuronal differentiation. Therefore, we suggest that B1 receptor activity may be involved in early proliferation prior to onset of neuronal differentiation. Cell cycle analysis of P19 cells induced to neuronal differentiation by retinoic acid in the presence of the B1 receptor antagonist lys-des-arg9-leu8-bradykinin showed an increase in S and G2 phase, compared to cells.

L35: Development of Anti-kinin B1 Receptor Aptamers as a Tool for Imaging using Laser-scanning Cytometry

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The objective of this present work is the identification of non-peptidergic fluorescent antagonists of the kinin-B1 receptor as a tool for imaging in cytometry applications and possible therapeutics. As this receptor is mainly expressed during pathological conditions such as inflammation and trauma and is possibly related to regulation of proliferation, stable high-affinity inhibitors of the receptor function would help to halt inflammation processes where B1 receptor activity is involved, and could be used as diagnostic tools for B1 receptor detection. A combinatorial library denominated SELEX technique (Systematic Evolution of Ligands by EXponential enrichment) has been employed to select for nuclease-resistant RNA aptamers that bind to recombinant rat B1 expressed in CHO cell membranes and are displaced by B1 antagonist lys-des-arg^{9}-leu^{8}-bradykinin. Following nine cycles of in vitro selection, we have identified a group of RNA aptamers with structural similarities, specifically binding to kinin-B1 receptors. Following adding a biotin-moiety to the aptamers and coupling to streptavidin-fluorescein, fluorescent-labeled aptamers were used as tools for quantification of B1-kinin receptor expression in vascular smooth muscle cells (VSMC) by laser-scanning cytometry. Fluorescence-labeled aptamers bound to VSMC, and could be displaced by an excess of the kinin-B1 receptor antagonist lys-des-arg^{9}-leu^{8}-bradykinin. This study confirms the

feasibility of developing high-affinity ligands for cytometry applications by using the SELEX technique.

L36: Characterization of the Ectopic Fetal Heart Implant Early Development

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Background: Authors have noted that fetal organ implant ectopic growth begins after a first destructive stage. Aims: to part degenerative and proliferation processes during this early period after fetal heart implantation, to precise the role of stem and precursor committed cells in the implant reconstruction, their origin (host, implant). Material , methods: Recipients were 25 adult Fischer rats. Donors were Fischer fetuses aged 15-19 days. Fetal hearts were implanted into an ear subcutaneous pouch. Biopsies were provided till 90 days for microscopy. Staining: hematoxylin eosin, immunohistochemistry: actin, myosin, desmin, nestin, vimentin and Islet-1. Results: Day 0: the heart implant is well formed, positive for actin, desmin and focally nestin, vimentin). Day 2: infarct is observed (necrosis, apoptosis, actin disappearance). Day 4: vimentin appears in many isolated cells, nestin positive reaction is noted especially in the implant capillaries but also in surrounding host hair follicles. Day 8: the implant becomes an organ (endothelium lined heart-like cavities, actin, desmin positive reaction, vimentin disappearance). Nestin is found later in the ear nerves, skin. During the whole observation period no Islet-1 positive cells (main cardiac stem cells) were observed. Conclusion: During the first week after implantation, fetal heart looses its differentiation phenotype. Immediately, the graft acquires a "regenerative" phenotype (vimentin, nestin positive cells). The cellular origin of the implant re-growth seems to be graft undifferentiated committed cells, though a host participation cannot be excluded. The absence of Islet-1 stem cells may decrease tumor development danger.

L37: The Core Flow Cytometry Facility: current and future challenges

Davies D

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Flow cytometry is a widely used resource in many clinical and research Laboratories. Many Institutes now recognise the value of a core facility for such equipment but these are not always adequately supported. The basic function of a Core Facility Manager is to ensure that users understand the principles of cytometry and are capable of operating flow analysers to generate meaningful data. In addition, depending on the size of the facilty, the manager may also operate or supervise staff using cell sorters. There are many facets that a Core Manager must consider which are often overlooked including budget management, strategic planning, staffing and career development, R&D work, promotion of the facility internally and externally and possibly revenue generation. As cytometer manufacturers strive to make their machines more user-friendly, it is more vital than ever that a designated expert – the Core Manger – be present to oversee the output. A multi-user environment demands that a designated manager be seen as a local point of contact for troubleshooting of machine problems, experimental problems and data analysis, interpretation and presentation. The benefits of a Core facility and competent manager and the recognition of cytometry as a key technology will be illustrated here with reference to the Flow Laboratory at the London Research Institute.

L38: A short history of fluorescent proteins and an overview of their applications

Ludin B

Life Imaging Services GmbH, Reinach, Switzerland

Only a decade ago, D. Prasher, W. Ward, M. Chalfie, and co-workers cloned and sequenced the Green Fluorescent Protein (GFP) from the jellyfish Aequorea victoria and showed that it could be expressed functionally in other organisms as a marker for gene expression. When it was shown soon after that GFP could also be used as a genetically encoded tag to visualize proteins in living cells, many people recognised that GFP had a true potential to cause a small revolution in biological research. But only few, if any, would have foreseen the multitude of tools that GFP and related proteins, isolated more recently from other species, would be - and keep being - turned into. This presentation attempts to tell the story in a nutshell and to give an overview of the wide range applications that fluorescent proteins serve today.

L39: Human Cytome Project, Cytomics and Systems Biology: Towards the Resolution of Biocomplexity

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BACKGROUND: Biocomplexity is frequently explored bottom-up from gene over biomolecule, cell, organism and disease mechanism levels to model molecular pathways by systems biology and to predict individualized reactivity of entire organisms towards external influences. Given the high biocomplexity of mammalian organisms resulting from their multiple heterogeneities at the various levels including the many variable exposure influences, this approach is tedious. CONCEPT: Alternatively, a top-down single cell, single patient oriented approach significantly simplifies the exploratory effort and is of immediate medical use. With cells as elementary function units of organisms and diseases emerging from molecular alterations in cells and cell systems (cytomes), the hypothesis-driven multiparametric determination of single cell characteristics by cytometry in combination with the hypothesis-free knowledge extraction from the entire single cell heterogeneity (cytomics) provides differential molecular cell phenotypes, representing molecular disease correlates resulting from genotype and exposure influences in individual patients. POTENTIAL: The differential molecular cell phenotypes are expressed as differential data patterns and suitable for therapy dependant disease course prediction in patients at diagnosis (predictive medicine by cytomics). Molecular reverse engineering of differential data patterns by systems biology provides information about disease inducing molecular pathways, favoring the detection of new target molecules for drug discovery. Disease induced differential changes of molecular cell phenotype provide furthermore the potential to systematically uncover organismal biocomplexity at the level of its basic function unit, the cell, thus enabling in an human cytome project the establishment of a standardized periodic system of cells, tissue components and disease states at the biomolecule level. http://www.biochem.mpg.de/valet/cellbio.html

L40: Impact of environmental and endogenous factors on endopolyploidization of angiosperms

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Endopolyploidy (EP) is due to consecutive replication cycles not alternating with mitotic nuclear divisions. EP shows a strong relation to the phylogenetic position of a taxon, a weaker to the life strategy and slightly negative correlation to the genome size. We have tested i) the EP level in natural polyploid versus diploid individuals of some taxa and ii) the impact of different growth conditions on EP of taxa with different EP level. Different nutritive supply does not significantly alter the EP-level, while temperature may influence EP in opposite directions. Sinapis arvensis and Brassica napus showed no altered EP levels when grown at different temperatures, while Lapsana communis switched from non-EP to EP, when grown at suboptimally low temperature. Natural polyploids showed a lower EP level than the corresponding diploids, whereas artificially generated polyploids revealed no immediate reduction of EP level compared to their diploid ancestors. From 7 species of 5 families, growing in untypical habitats or showing untypical life strategies, only the annual Galinsoga parviflora is endopolyploid i.e. differs from non-EP typical for Asteraceae. We conclude that immediate alterations of the EP level are the exception rather than the rule when environmental conditions or basic ploidy levels are modified, while in evolutionary terms selective effects may well occur.

L41: A Normalized Meta-Database of Affymetrix Microarrays for transcriptome analysis

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Gene expression profiles derived from microarrays describe cellular dynamics and can be used to generate hypotheses for many biological questions. In addition, microarrays provide huge amounts of data beyond the scope of the original investigation. GEO and ArrayExpress, are open access repository databases for thousands of expression profiles published in the literature. Direct comparison of these data is currently not possible (i) within one chip type ("platform") due to inter-chip fluorescence variation, and (ii) between different array platforms. We performed a linear normalization procedure for 1971 profiles from the murine Affymetrix U74v2a GeneChip platform deposited in GEO. We calculated a correction factor using the median signal intensity across profiles, allowing us direct comparison between expression profiles from this platform. This meta-database was used to analyse the expression of genes relevant in dioxin signalling. We could confirm the differential tissue expression levels of the arylhydrocarbon receptor AHR, and its partner ARNT. The AHR-associated protein XAP2 was highly expressed in all arrays, with a very small CV, suggesting a more general physiological role as a "housekeeping" gene. HPRT, often used for this purpose was not expressed in all tissues. In a first analysis we also identified congruent tissue expression of Rag1, Rag2 and TdT. In conclusion, our database will be useful for comparable gene expression in the analysis of new functions and interactions between genes currently not under investigation.

L42: A pilot study of combined PCR and flow cytometry aproach: confirmation of leukemic origin of immunophenotypic subsets in bone marrow by PCR

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Acute lymphoblastic leukemia(ALL) is the most common malignancy in childhood. Although almost all patients achieve complete remission, 20% of patients suffer from relapse. Patients with risk of relapse can be discerned by presence of minimal residual disease(MRD). Flow cytometry is often used for MRD detection but still has limited impact on clinical decisions. B-cell regeneration presents major challenge for specificity of cytometric MRD evaluation. Multicolor cytometry-FACS sorting, detection of rearranged immunoglobulin and/or TCR genes using RQ PCR and fusion genes' transcripts were combined for MRD detection. 14 patients entered the pilot study (8 with newly diagnosed ALL, 2 with extramedullary relapse, 4 during allogenic transplantation). Cell subsets with leukemic and nonleukemic B-cell precursor immunophenotype were FACS sorted and analyzed by quantitative PCR. Combination of these two methods allows: 1. PCR quantification of leukemic cells with greater sensitivity 2. Testing the specificity of chosen immunophenotypic characteristic of residual leukemic cells Using these combined techniques we evaluate 4 to 8 color panels for use in MRD monitoring. Goal is to find panels that will be sensitive, standardized, useful in all patients and reserve the more laborious and more expensive RQ-PCR methods only for preselected cases. Supported by GAUK43/2005, GAUK65/2004, GAUK62/2004, IGA MZdNR8269-3/2005 and VZMSMTMSM0021620813

L43: Predictive medicine by cytomics and systems biology approaches

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Surgical procedures, combined with medication, stimulate the immune system. Patients with an activated immune system can develop a more pronounced immune response that in turn can contribute to postoperative complications. Personalized preoperative prediction of risk patient could provide the rationale for individual prophylactic treatment prior to or during surgery. Cytomics methods are capable for multiple characterization of the actual immune status. We demonstrate in three different clinical studies that by multiparametric cytometry with computational algorithms it is possible to early detect patients at risk for adverse outcome after cardiosurgery. Predictive medicine by cytomics and systems biology approaches may be useful for early therapy guidance in regenerative therapies.

L44: Identification of putative target genes of Gfi1 within HSC

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The generation of all blood cells depends on haematopoietic stem cells (HSC), representing only 0.05-0.1% of total bone marrow cells. They are characterised by extensive proliferation and self-renewal. The zinc finger protein Gfi1 is known as a transcriptional repressor and plays an essential

role in self-renewal and proliferation of HSCs. Mice which are deficient for Gfi1 (Gfi1-/-) show a highly reduced HSC population, but the total number of bone marrow cells is not altered (Zeng et al., 2004). To ana plification Kit Nano Version (Artus, Germany), a special method to enhance the amount of total RNA. After in vitro transcription labeled cDNA was used to probe Affymetrix DNA arrays. This analysis revealed around 415 differently regulated genes. Within these genes 127 were upregulated whereas 288 genes were downregulated in Gfi1 deficient mice. For validation of putative candidate genes additional quantitative RT-PCR analyses were performed on two separate sorted cell populations.

L45: Double staining of Fluorescence-in-situ-hybridisation-(Urovysion®) and Ki-67 immunohistochemistry for detection of genetic aberrations in precancerous lesions of the bladder.

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Aims: Bladder cancer is known as a frequently multifocal and recurrent tumour entity. In order to better understand the evolution and spread of bladder tumours a method was established to relate genetic aberrations to proliferating cells.
Material and Methods: Double staining of fluorescence-in-situ-hybridisation (FISH) and Ki-67 immunohistochemistry was established on frozen section of bladder tumours and consequently carried out on frozen tissue sections from 15 patients with precancerous lesions of th ghbourhood in precancerous lesions of the bladder cells also show genetic aberrations. Proliferating basal cells have rather than proliferating intermediate cells a normal diploid FISH signal and no loss of the chromosomal 9p21 (p16) locus.
Conclusion: The method established is apt to show that genetic aberrations detected in early bladder lesions or normal urothelium are biologically relevant since found in proliferating cells. This work has been supported by the German Science Foundation (DFG, grant).

L46: Local stimulation using magnetic microspheres in the assessment of ErbB signalling

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ErbB2 can drive cell proliferation by forming complexes with members of the ErbB family and undergoing trans-activation of its potent kinase activity. ErbB2 overexpressed in breast carcinomas is targeted by the humanized monoclonal antibody, trastuzumab, which is effective in only 20-30 % of tumors. We have used EGF- and trastuzumab-covered paramagnetic microspheres, and quantitative confocal laser scanning microscopy and digital image processing to investigate the (trans)activation of, and local signal propagation from, erbB1 and erbB2 on trastuzumab sensitive and resistant carcinoma cell lines. On A4-erbB2-mYFP cells expressing high levels of endogenous erbB1 and transfected erbB2-mYFP EGF-microspheres activated erbB1 and also trans-activated erbB2-mYFP. In two other cell lines with comparable erbB2 expression but lower levels of erbB1, EGF-microspheres trans-activated erbB2 less efficiently. Trastuzumab in solution activated erbB2 on A4-erbB2-mYFP and the trastuzumab-sensitive SKBR-3 cells, but only negligibly on the resistant JIMT-1 cells that showed a ten times higher Kd for the antibody. Nevertheless, pronounced erbB2 activation and tyrosine phosphorylation could be detected upon stimulation with trastuzumab-coupled microspheres in all cell lines, although trans-activation of erbB1 was negligible. Receptor phosphorylation was restricted to the immediate proximity of the microspheres. Coupling trastuzumab to microspheres can override the inertia of erbB2 to activation by trastuzumab in solution.

L47: Analysis of Chromatically Stained Tissues and TMA's by Laser Scanning Cytometry

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Introduction: Morphologic assessment of chromatically stained sections forms the basis of most pathologic diagnosis. The ability to obtain quantitative data from chromatic dyes complements fluorescence, making it easier to validate the findings by visual confirmation. Using Her2 as a model we have constructed and evaluated a paradigm for laser scanning cytometric assessment of immunoperoxidase (IPOX)-stained sections.
Methods: Tissue microarrays from a CAP survey stained for HER2/neu expression were evaluated on an iCyte® Automated Imaging Cytometer. Additionally, serial sections of breast tumor tissue microarrays (TMAs) were stained for HER2/neu and the therapeutic agent Herceptin. Breast tissue sections were stained with antibodies to progesterone and estrogen. Labeling was with DAB and hematoxylin. Laser scanning techniques for fluorescent tissue analysis were adapted using multicolor laser light absorbance combined with autofluorescence detection.
Results: There was good agreement between the automated results, the pathologist's evaluation and FISH probe spot counts in the CAP survey analysis. In the TMA study, most, but not all, of the HER2/neu core elements showed overexpression of Her2 and bound Herceptin. The discordance suggests that assessing binding capacity of the actual therapeutic antibody agent may be a better method for predicting responsiveness to Herceptin. Nuclear based segmentation provided quantitative assessment of the number of estrogen- and progesterone-positive cells in the tissue sections.

L48: Ultra Slow Manipulation – A New Way for Stress-reduced and Physiological Handling of Individual Animal and Human Cells

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Gentle cell handling in vitro is a key problem of biotechnology and regenerative medicine. Due to the complex surface sensitivity of cells there are two general cases:
1. Manipulation of cells in solution avoiding any surface contact.
2. Manipulation of cells adherently growing on biocompatible surfaces.
Key problems in cell manipulation related to point 2 are the molecular adhesion and slow migration velocity of higher

animal cells. These cytoskeletally controlled processes occur in minutes or hours. These times clash with the current technical trend of making all cell separation and manipulation procedures as fast as possible. With selected examples, the uses and advantages of extremely slow instrument motion to manipulate cells in an automated way are presented and discussed. Mechanical cell cutting without cell destruction and mechanical induced cell fusion are demonstrated in time laps video sequences. Perspectives and further applications of this new technique in relation to stem cell in vitro culture are discussed.

L49: Stem cell markers associated with the side population of fresh and cryopreserved human umbilical cord blood

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The side population phenotype resulting from efflux of Hoechst 33342 has been associated both with multidrug resistance, and with populations of high quality stem cells from both haemopietic and non-haemopoietic organs. However, the relationship between the SP phenotype and the expression of surface markers associated with haemopoietic stem cell activity is incompletely characterised, and appears to depend on the cell source. Here, we combine four-colour FACS analysis with two-colour detection of the SP phenotype to compare the characteristics of SP cells in fresh and cryopreserved human umbilical cord blood. Furthermore, we present evidence that expression of the transporter protein ABCG2, which is commonly associated with the SP phenotype of murine bone marrow cells, is not a feature of the SP population from human umbilical cord blood.

L50: Implementation of a practicable low-cost assay using a density-based CD4+ T- Cell depletion method for the monitoring of HIV-infected individuals

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Background: The CD4+ T-cell count is considered to be the best surrogate marker for monitoring the clinical course of infection with HIV. Flow cytometry, as the standard method for the enumeration of CD4+ T-cells, requires expensive equipment and well-trained technicians. Therefore, in most developing countries, flow cytometry is not affordable for most patients, since the average monthly income there is only 10$ per person.
Objective: In order to make the monitoring of this important surrogate marker widely available in these countries, the present study introduces a new practicable low-cost assay as an alternative to flow cytometry.
Method: Whole venous blood taken from 10 HIV-patients as well as 10 healthy blood donors was incubated with a cocktail of bi-specific tetrameric antibody complexes which cross-links unwanted nucleated cells (NC) to red blood cells (RBC) by forming RBC rosettes around targeted NC. After the following erythrocyte lysis and centrifugation over a density medium, the enriched CD4+ T-cells were harvested and then counted on a hematocytometer using a light microscope.
Results: The CD4 counts obtained by the introduced technique correlated significantly with those determined by flow cytometry ($r = 0{,}945$ [$p<0{,}0005$]). The cost of the examination of one blood sample is below 0,25€
Conclusion: These results suggest that this technique is an appropriate diagnostic method for widespread use, especially in resource-limited situations.

L51: Slices of Life: Multispectral Histology

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The ability to detect multiple molecular species at once is becoming increasingly important. Multispectral imaging systems can be used to capture multiplexed molecular signals, and can be applied to the analysis of chromogenically stained slides in brightfield mode and of samples stained with a variety of light-emitting dyes (from the visible to the NIR range) in fluorescence mode. Quantum dots make a particularly good match with this imaging technology, which is also extremely helpful for the identification and elimination of interfering autofluorescence. The ability to accurately determine the spectral qualities of dyes in-situ is also valuable. Multispectral imaging has proven to be useful for multicolor FISH, for resolving multiple species of GFP with overlapping emission spectra and for resolving red/brown double-labeled histopathology stains.
The uses of spectral imaging in clinical pathology are still being explored and need to be matched to appropriate software tools. Appropriately constrained linear unmixing algorithms and novel automated tools have recently been developed to provide simple, accurate analysis procedures. Conventional hematoxylin-and-eosin- or Papanicolaou-stained pathology sections can have sufficient spectral content to allow the classification of cells of different lineage or to separate normal from neoplastic cells. Analysis of such specimens may succeed using spectral “signatures” and simple segmentation algorithms. The rich data sets also reward the use of more advanced analysis techniques. These can include a number of approaches pioneered for remote sensing purposes, such as spectral similarity mapping, automated clustering algorithms in n dimensions, principal component analysis, as well as other more sophisticated techniques.

L52: Distinguished Photons: Multispectral Fluorescence Imaging In Vivo

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Non-invasive in-vivo imaging is a rapidly growing field with applications in basic biology, drug discovery and clinical medicine, with new technologies and techniques being constantly developed. Because of the high cost of MR- and CT-based systems, a great deal of effort has gone into developing optical imaging methods, which offer, in some modalities, the promise of high spatial resolution and the ability to detect multiple markers simultaneously The ability to image and quantitate fluorescently labeled tumors and other fluorescently labeled markers in vivo has generally been limited by the autofluorescence of the tissue. The presence of any autofluorescence reduces the sensitivity of detection and accuracy of quantitation of the labeled tumor. One solution to this problem is to use a multispectral imaging methodology to spectrally characterize and computationally eliminate autofluorescence, enhancing signal-to-background dramatically, and revealing otherwise invisible labeled targets. Effective use of spectral tools to remove autofluorescence signal requires accurate spectra of the individual components. Frequently, however, the measured emission spectra in vivo may be different from those

measured in vitro due to combinations of effects of the local environment, absorbance and scattering. Additional analysis tools are needed to determine appropriate component spectra using what is available from the sample itself. Such tools have recently been developed and their use greatly simplifies the application of spectral imaging in vivo.

L53: Development of a new zebrafish (Danio rerio) embryo test system using gene expression profiling to asses the risk of chemical compounds in the aquatic environment.

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Background: The zebrafish Danio rerio has emerged as one of the most important model organisms for developmental genetics. Furthermore, it gained importance for the identification and risk assessment of toxic compounds. One example of this is the establishment of the Danio rerio embryo test (DarT) as an alternative acute toxicity tests.

Gene expression profiling using microarrays can be applied to assess hazardous effects of chemical compounds. Marker genes identified by toxicogenomic approaches could potentially be used to (i) identify sub-acute effects, (ii) predict chronic effects, (iii) classify chemicals according to their gene expression signature and (vi) understand the mode of action of compounds.

In order to extend the DarT to the molecular level and to identify genes indicative of chemical stress, we performed microarray experiments with zebrafish embryos exposed for 48 h to the model substance 3,4-dichloroaniline.

Data analysis of the microarray experiments revealed 21 significant differentially expressed genes. Among these genes, four could be confirmed independently using quantitative RT-PCR. These genes encode for cyp1a1, ahr2, fzr1 and hsp70. Moreover, their expression levels remained altered in early larvae (5days post fertilization) exposed to various concentrations of 3,4-dichloroaniline.

Taken together this approach may help to unravel mechanisms of chemical toxicity and to identify unknown substances by their mode of action. It is also a first step toward predicting chronic effects based on changes to gene expression by means of in vitro models, which may in the future lead to a further reduction of animal experiments in toxicology.

L54: Multiparameter flow cytometry of solid tumours

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Technical as well as logistic problems long have hampered the development of multiparameter flow cytometry of solid tumours. For this reason the potentialities of this technique for quantitative and simultaneous analysis of variation in protein expression in subpopulations of normal and neoplastic cells in solid tumours have been under exploited. In the past 10 years we have taken a systematic approach to the development of sample preparation and staining protocols for multiparameter flow cytometry of solid tumours. This resulted in robust protocols for fresh tumour samples that enabled studies on genotype-phenotype relationships of loss of HLA class I expression in cervical and colorectal carcinomas. The possibility to rapidly enrich discrete tumour cell (sub) populations on the basis of protein expression and DNA ploidy for subsequent genotyping is one of the main advantages of this technique, particularly for LOH analysis where DNA from contaminating normal cells usually is a disturbing factor. However, the inherently prospective nature of studies on fresh material limits their clinical relevance. Not surprisingly therefore, archival paraffin-embedded specimens are now increasingly recognized as precious sources of molecular information that directly can be linked to clinico-pathological follow-up data. We recently developed a protocol for multiparameter flow cytometry of deparaffinized samples from solid tumours enabling flow sorting of cells on the basis of (high-resolution) DNA content measurements and expression of cell lineage markers. Examples from application on cervical, colorectal and BRCA1 and BRCA2-associated breast cancers will be presented.

L55: Development and Dynamics of Pseudomonas aeruginosa Biofilms

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No abstract submitted

L56: Agglutination measurement – a new way for characterization of biotechnological processes working with yeast cells

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Various biotechnological processes work with yeast cells, e.g. the brewing process. Such processes can be characterized by various parameters; such are temperature, pH, pressure, color and others.

The agglutination measuring method gives direct information about the present process situation. This method uses the effect, that the carbohydrate composition on the yeast cell surfaces reflects the individual process history.

For measurement will take out only few milliliter of process suspension. After washing the yeast cells will be suspended in a photometer cell and some µl lectin will be added. Influenced by the carbohydrate composition on the cell surfaces will be built bridges between yeast cells; they agglutinate. The delay-time after START, the velocity of agglutination and the mean-number of yeast cells per flake characterizes very well the present process situation.

This agglutination kinetics will be observed with a photometer arrangement. The results will be stored, computed and assessed by a classifier unit. By the way of correlation of typical process situations with agglutination kinetics and their classifying results is given a powerful characterization tool for biotechnological processes working with yeast cells.

The paper presents the working principle of such agglutimeter and typical agglutination kinetics of yeast cell samples taken out during the brewing process on different moments. Also some sampling influences will be discussed.

L57: Flow cytometric quantitation of immunosuppressive drug effects on immune cells

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In contrast to the measurement of immunosuppressant blood levels (pharmacokinetics), pharmacodynamics directly measures the biological effects of immunosuppressive drugs on immune cells. Novel assays quantitate diverse cell functions and measure immunosuppressive drug effects that correlate highly with immunosuppression of organ graft rejection. After extensive assay development work in rodents, this technology has been optimized for use in non-human primates and humans. The methods described only require microliter volumes of whole blood, which is briefly stimulated ex vivo to activate different T cell, B cell and monocyte activation pathways. Pharmacodynamic effects were assessed by multicolor flow cytometry. This technique enables immune cell proliferation, cytokine synthesis and expression of cell surface activation antigens to be measured in specific immune cell lineages in different species. In the near term, these potential surrogate markers of immunosuppressive drug efficacies are being exploited for more rational and efficient drug development preclinically in rats and non-human primates and during clinical trials of immunosuppressants. Pharmacodynamics may be a valuable addition to the traditional reliance on pharmacokinetics for these phases of drug development. In the medium term, pharmacodynamic techniques similar to ones we describe may maximize the efficacies and minimize mechanism-based toxicities of immunosuppressants by enabling drug doses to be tailored to produce immune suppression that is optimized for each patient.

L58: Flow Cytometric Analysis of Oxidative Stress and DNA Repair in E. coli WP2 Tester Strains Deficient in Genes of Antioxidant Defence

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Background: The functional analysis by flow cytometry (FCM) in bacteria is difficult because of outer membrane impermeability. We have previously shown that E. coli WP2 tester strains are suitable to FCM functional assays and have obtained several strains deficient in one or several genes involved in antioxidant defence and DNA repair
Aim: To investigate oxidative stress pathways and their effects in E. coli, while providing a simple model for in vitro evaluation of pro-oxidant compounds and antioxidants treatments.
Methods: Cultures of wild-type and gene-deficient E. coli WP2 are exposed to exogenous prooxidants with or without pretreatment with antioxidants. Intracellular reactive oxygen species (ROS) and nitric oxide (NO) are quantified by end-point or kinetic FCM using fluorogenic substrates. Oxidative damage to DNA is determined by FCM assay of 8-oxoguanine and propidium iodide.
Results: Our data show that E. coli WP2 strains deficient in key genes of antioxidant defence are far more sensitive to oxidative stress than wild-type and provide a suitable model for assessing ROS- and NO-induced cytotoxic and genotoxic effects in live bacteria.
Conclusions: Our system can be applied to investigate bacterial physiology as well as to assess the toxicity of prooxidants and the protective potency of antioxidants.

L59: DNA and protein arrays in biotechnology

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Alongside metabolome analysis using membrane sensors and immunosensors, analysis of various components of the proteome, the transcriptome or the genome will become increasingly valuable. New biosensors known as biochips will be required. DNA chip technology has already opened up new ways of studying disease in more depth and identifying far more possible targets. DNA chip technology therefore enables large numbers of genes to be screened simultaneously, giving a comprehensive, detailed picture of changes in gene expression, shedding light on complex regulatory interactions. By applying highly advanced DNA chip technology to the fields of protein analysis it will be possible to analyse cellular processes. But fabrication of protein arrays is particulary challenging and protein arrays lagged behind in development because of the more complex coupling chemistry, the instability of the immobilized protein, and far weaker detection signals.
Thus the major significance of the present work is to develop new membrane surfaces (activated nylon, nitrocellulose and others) that remains the most ideal surface for protein arrays and to transfer the established coupling chemistry developed for DNA arrays by the use of aptamers. Futhermore, we want to validate and perhaps improve known biosensors through a functional proteomics study in which the expression of several hundred proteins is detected simultaneously.

L60: Apoptosis of circulating lymphocytes during paediatric cardiac surgery

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Background: Surgical trauma is associated with elevated apoptosis of circulating leukocytes. Increased apoptosis leads to partial removal of immune competent cells and could therefore in part be responsible for reduced immune defence. Cardiovascular surgery with but not without cardiopulmonary bypass (CPB) induces transient immunosuppression. Its effect on T-lymphocyte apoptosis has not been shown yet.
Methods: Flow-cytometric data of blood samples from 90 children (age 3-16 yr.) who underwent cardiac surgery with (65) or without (25) CPB were analysed. Apoptotic T-lymphocytes were detected based on light scatter and surface antigen (CD45/CD3) expression (Clin Exp Immunol 2000;120:454). Additionally, in vitro leukocytes from healthy volunteers were incubated (1h,37°C) with serum samples from the same patients obtained perioperatively. Apoptosis was determined cytometrically by AnnexinV binding and DNA condensation.
Results: Patients with but not without CPB surgery had elevated lymphocyte apoptosis. T-cell apoptosis increased from 0.45% (baseline) to 1.34% (4h postoperative, ANOVA $p=0.0034$). These results were in accordance with in vitro findings demonstrating elevated apoptotic ratio for lymphocytes and neutrophils after incubation with serum from patients with CPB up to 3d after surgery ($p<0.01$). No activity was found without CPB.
Conclusion: Increased apoptosis of circulating lymphocytes and neutrophils further contributes to the immune suppressive response to surgery with CPB. (Support Pipek: Deutsche Herzstiftung, Frankfurt, Germany)

L61: Receptor tyrosine kinases driving cell proliferation – their molecular interactions assessed by modern microscopic approaches

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Receptor tyrosine kinases, such as members of the ErbB and PDGFR families are frequently involved in cell proliferation and survival. Their di- or higher order oligomerization either with each other or similar family members is thought to be central to their activation by trans-phosphorylation. Furthermore, their interactions with other transmembrane molecules and their lipid environment could be important in modulating their activity. Since these receptors are increasingly targeted in tumor therapy, the proper understanding of their interactions is a key to choosing the appropriate drug or drug combinations. Interactions and molecular organization of proteins can be detected at two hierarchical levels. At the submicron level, confocal microscopy and scanning nearfield optical microscopy can be used to determine spatial auto- and crosscorrelation functions that characterize molecular clusters and colocalizations, respectively. At the nanometer level, measuring fluorescence resonance energy transfer (FRET) in the microscope allows for rendering molecular interactions to various subcellular compartments. The data gained in diverse modalities of microscopic FRET are averaged over an ensemble of molecules in the voxel of interest and represent a momentary interaction. Fluorescence correlation spectroscopy on the other hand is capable of detecting the diffusion of single molecules in femtolitre volumes, and its cross-correlation version can provide information about the co-diffusion of fluorescently labeled molecular entities which hints at their stable association.

L62: Stem cells in tissue engineering

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No abstract submitted

Poster

P01: Surface modification of polyurethane for optimizing growth of human endothelial cells

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In biomedicine metals, ceramics, polymers are used, whereby polymers show increasing importance. Optimal implant characteristics depend on surface properties of the part: The surface has to provide optimal conditions for the selective attachment and growth of the particular cell type. For improving biocompatibility, different technologies were developed. In our paper, we will focus on cold plasma technology, which we have applied to improve the properties of polymer surfaces.
Our goal was to make a polymer surface modification allowing an optimized biocompatibility and biofunctionality for human endothelial cells of vascular implants. As polymer substrate, we have used a transparent and flexible polyurethane (PUR). The cold plasma treatment was carried out by an microwave generator, by varying power, exposure time, gas and gas flowing rate. The resulting surface modification was analysed by X-ray photoelectron spectroscopy and contact angle measurements. The influence of different surface modifications on the HUVEC and their gene expression was assessed by microscopic visualisation and realtime-PCR.
Our data suggest that surface characteristics of modified PUR have profound influence on endothelial gene expression patterns. On unmodified PUR surfaces a high expression of adhesion molecules and low expression of connexin could be observed. These observations can be augmented thrombogenicity and diminished biocompatibility. In contrast, on modified PUR surface we have found an improved biocompatibility.

P02: Bioreactors

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In vivo, blood vessels are subjected to mechanical forces in a form of radial distention, encompassing cyclic mechanical strain due to the pulsatile nature of blood flow, and the hydrodynamic wall shear stress. In many cases the functionality of growing cells reduces without the mechanical stress. E. g., vascular smooth muscle tissues engineered in vitro with a conventional tissue engineering technique may not be functional. Pulsatile strain and shear stress stimulate not only the functionality of the cells but increase the velocity of cell growth in vitro. For optimal effect the hydrodynamic stress must be combined with a soft subsurface. We present different bioreactors with and without soft subsurfaces. Our bioreactors can be equiped with exchangeable subsurfaces with modified surfaces. So the optimal properties of the surface can be adapted to the used cell types.
Additionally we demonstrate a system of pump, valve, thermostat and suitable adapters to realize the pulsatile hydrodynamic stress. With this system flow-time curves of the human blood can be realized with an excellent accuracy.

P03: The analysis of cytoplasmic fluorescence using Laser Scanning Cytometry

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The Laser Scanning Cytometer (LSC) is a microscope-based instrument which fills the gap between high throughput multiparametric cytometry and morphological analysis. For each fluorescent event several fluorochromes can be detected and quantified simultaneously. Thereby the intensity of the brightest pixel (max pixel) and the average brightness (integral) is recorded, supplemented by the size (area) of the cell and the exact x-y position on the object slide. The spectrum of biological material in LSC applications ranges from cell cultures and blood cells to tissue sections and polyploidy, apoptosis and the cell cycle is investigated. The LSC also offers the possibility to quantify the cytoplasmic fluorescence of single cells. In our study we used a kidney cell line (cos-7) to establish a protocol for the quantification of cytoplasmic fluorescence signals. The cells were transfected with a plasmid coding for the enhanced green fluorescent protein (EGFP). DNA staining was used to define cells and immunolabelled ß-actin was chosen as trigger for

the LSC measurement. The integral of the EGFP-fluorescence signal was analysed and the amount of protein was calculated. This technique is a suitable completion to microscopy and biochemical methods and allows the quantification of proteins on the level of single cells.

P04: Difference in fluorescence pattern of cytoplasmic and nuclear antigens in cultivated human cells dependend on the applied fixation procedure

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Localisation of proteins using immunodetection is common in research labs and diagnostic institutes with clinical impact, e.g. identification of autoantibodies in patients' sera via their specific fluorescence pattern on cultivated cells. Preparation and staining methods are mostly not known for commercially available products or there is a lack of validating different preparation steps and materials prior to analysing fluorescence patterns. To analyse the influence of fixation procedures on the antigenicity of special proteins two human cell lines (MEL-HO and HEp-2) were grown on slides, fixed according to different protocols and treated with antibodies directed against typical cytoplasmic (vimentin, cytokeratins) and nuclear antigens (lamins, nucleolar helicase), followed by Cy3-labeled secondary antibody. Fluorescence microscopy revealed quite different patterns for cytoskeleton proteins depending on the used fixatives. These patterns range from a specific fibrous staining (4% buffered cold formalin) up to a crude granular detection (acidic ethanol at room temperature) of cytokeratins. Vimentin patterns demonstrate in part the expected fibers (formalin) or untypical striations (acidic ethanol or cold methanol-acetone). Lamin patterns showed only few variations and nucleolar helicase patterns were nearly consistent after six different fixation procedures. These results demonstrate the necessity to validate fixation procedures prior to analysis of fluorescence patterns, especially in clinically relevant applications.

P05: Phenotypic and functional characteristics of monocytes from coronary heart disease (CHD) patients

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Monocytes are the principal inflammatory cells recruited into the atheroscleritic lesion where they phagocytose lipids and differentiate into foam cells . Circulating monocytes contribute to plaque instability by increased cell adhesiveness, procoagulant activity and by influencing function of endothelial cells. Peripheral blood monocytes can be divided into two subpopulations: CD14+CD16+ and CD14+CD16- cells. This study focuses on phenotyp and activity of circulating monocytes in CHD patients undergoing coronary artery bypass surgery. Using flow cytometry we examined the percentage of CD14+CD16+ monocytes among all circulating monocytes in 79 patients and 25 healthy controls. We evaluated also the spontaneous and LPS-challanged intracellular production of IL-6 by monocytes as well as their antigen presenting capacity in alloMLR assay. In CHD cases we observed higher percentage and number of circulating monocytes and their proinflammatory subpopulation CD14+CD16+ . Percentage of monocytes producing IL-6 was higher in patients than in controls, whereas after stimulation with LPS percentage of IL-6+ monocytes was higher in controls. In both studied groups proinflammatory monocytes CD14+CD16+ produced significantly more IL-6 than CD14+CD16- monocytes. Additionally, in patients slightly decreased antigen presenting capacity of monocytes was observed. Our results indicate functional impairment of monocytes from CHD cases, which is propably compensated by increased number of CD14+CD16+ cells.

P06: NK cell compartment in patients with coronary heart disease

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There are two main NK cells subsets: CD3-CD56dim - cytotoxic subset and CD3-CD56bright - regulatory subset. Viral infection has been considered as a risk factor of Coronary Heart Disease (CHD). Furthermore, inflammatory state connected with CHD may affect the activity of immune system. Thus, the main aim of our study was to determine status of NK cell compartment in patients with CHD qualified for coronary artery by-pass surgery. Thirty six patients with CHD were included into the study, control group consisted of 26 people without diagnosed CHD.
NK cells activity was measured by Cytotoxicity Detection Kit. Percentage and number of the CD3-CD56+, CD3-CD56dim and CD3-CD56bright, was evaluated by flow cytometry. IL-2 serum level was estimated by bioassay.
The CHD patients had lower NK cytotoxic activity in comparison to control group. The CHD group had also decreased number and percentage of total NK cells, as well as the CD3-CD56dim cells. The number of the CD3-CD56bright cells was similar in both populations studied. Serum level of IL-2 was higher in the control group.
This data indicate that CHD is connected with suppression of NK cells function. Decreased NK activity may be explained by lower number of the CD3-CD56dim cells and by decrease of IL-2 levels in serum.

P07: Identification of myeloid and plasmacytoid dendritic cells and surface marker analysis in different tissues of the human body by 6-color flow cytometry

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The appearance of plasmacytoid and myeloid dendritic cells (DC) in different tissues like blood, tonsils, lymph nodes, adenoid vegetations, nasal mucosa and head and neck squamous cell cancer has been published earlier. For the understanding of interaction between T-cells and DCs and interference by tumor environment it is essential to discriminate and analyse vital DCs. We compared several methods for the dissociation of tissue to obtain a single cell suspension containing vital DCs. The tested methods variated from mechanical milling to enzymatic digestion and continuous or discontinuous density gradient centrifugation. Single cell suspensions were either subsequently stained for three to six colour flow cytometric analysis or resuspended in DC friendly medium for further incubation with stimulating additives like oligonucleotides. Identification of the major DC subtypes the myeloid and plasmacytoid DC was possible

by staining the cell surface marker CD11c, CD123, lineage-cocktail-1 (CD3, CD14, CD16, CD19, CD20, CD56) and anti-HLA-DR. Additional staining for CCR7 chemokine receptor and expression of costimulatory molecules CD40/80/86 was performed. Flow cytometric analysis was performed on a FacsCanto from Becton Dickinson capable for simultaneous analysis of eight parameters. The compensation was automatically calculated by the software after FACS analysis of specific stained micro beads. Our result show a standardized reliable application for repetitive processing, staining and FACS analysis of different tissues.

P08: Flow cytometry investigation of in vitro intracellular production of proinflammatory cytokines by peripheral blood CD3+ cells in women with endometriosis after phorbol 12-myristate-13-acetate and ionomycin stimulation

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In this study we investigated the ability of activated, primary peripheral blood CD3+cells derived from women with endometriosis to produce intracellular proinflammatory cytokines in vitro compared with those from adenomyosis patients, women with uterine myomas, and healthy women. Isolated peripheral mononuclear cells were stimulated for 6h with 50ng/ml of PMA and 1ìg/ml of ionomycin in the presence of 2ìg/ml of brefeldin A. Intracellular cytokine production of TNF-alpha, IFN-gamma, and IL-8 was evaluated with three-color flow cytometry in the CD3+, CD3+CD8- and CD3+CD8+populations. We noted that intracellular production of IFN-gamma (CD3+ and CD3+CD8-cell populations) in endometriosis patients was significantly lower than in both women with adenomyosis and those with uterine myomas. In turn, production of IL-8 was significantly higher in women with endometriosis than in adenomyosis and uterine myoma patients in all the analyzed lymphocyte populations and also higher than in healthy women (CD3+and CD3+CD8+cell populations). Moreover, IFN-gamma production was significantly decreased in advanced endometriosis compared with mild endometriosis, adenomyosis, uterine myomas (CD3+and CD3+CD8-populations), and healthy women (CD3+CD8-population). On the other hand, IL-8 production was significantly higher in advanced endometriosis than in uterine myomas, healthy women (all analyzed populations), mild endometriosis (CD3+CD8-population), and adenomyosis (CD3+, CD3+CD8-populations). Significantly IL-8 production was also observed in mild endometriosis compared with healthy women, uterine myomas, and adenomyosis (CD3+, CD3+CD8+populations). The findings of our study may indicate that the progression of endometriosis, connected with decreased lymphocyte cytotoxicity and enhanced angiogenesis, may be related to decreased IFN-gamma and increased IL-8 production of peripheral blood lymphocytes.

P09: Local and systemic intracellular in vitro production of cytokines by peripheral and peritoneal CD3+ and CD14+ cells in women with advanced endometriosis after stimulation in vitro

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The aim of this study was to investigate the intracellular in vitro cytokine production in T cells (CD3+) and monocytes/macrophages (CD14+) of PB and PF after appropriate phorbol 12-myristate-13-acetate (PMA) and lipopolysaccharide (LPS) stimulation. Isolated peripheral mononuclear cells from both PB and PF of women with advanced endometriosis (n=16) were stimulated for 6h with 50ng/ml of PMA and 1ìg/ml of ionomycin or 1ìg/ml of LPS in the presence of 2ìg/ml of brefeldin A to produce the following intracellular cytokines TNF-alpha, IFN-gamma, and IL-8 (CD3+cells) or TNF-alpha, IL-6, IL-10, MCP-1, and IL-8 (CD14+cells) Intracellular cytokine production was evaluated with, three or two-color flow cytometry in following cells populations: CD3+, CD3+CD8-, and CD3+CD8+ and CD14+ cells. We observed that intracellular in vitro production of IL-6, MCP-1, and IL-8 was significantly increased in monocytes compared with macrophages. We also observed a significantly increased production of TNF-alpha (CD3+ and CD3+CD8+populations) and IFN-gamma (all analyzed T-cell populations) in PF compared with PB. In turn, the production of TNF-alpha by CD3+CD8- and IL-8 by all analyzed T-cell populations was significantly increased in PB compared with PF. In our view differential production of cytokines, in PF and PB could be responsible for the inflammatory state and modulation of the proliferation process characteristic of advanced endometriosis.

P10: Immunophenotyping in the diagnosis of haematologic malignancies: Standardization and quality control rounds

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For the diagnosis of haematologic malignancies, clinicians frequently have to rely on the results of immunophenotyping (IPT). Standardization of this complex laboratory procedure constitutes the basis for making the correct diagnosis and treatment stratification. Various international recommendations on the standardization of IPT have been published. For their national implantation, we established quality control rounds (QCR) in the context of our project "central immunophenotyping" within the network "akute und chronische Leukämien". We aimed at evaluating all three phases of IPT, namely the pre-analytical (i.e. panel selection), analytical (i.e. data acquisition) and the post-analytical phase (i.e. data interpretation) of the laboratory process. Therefore we performed a QCR with the dispatch of viable cells in 2004. Participants (n=80) were assessed according to their gating strategy and the correctness of the diagnosis. The results displayed a high congruence in making the right diagnosis. However, there is a wide range in the amount of cells which were designated as "malignant", "blasts" and

physiological haematopoetic cells. Also there are great differences in the number of used antibodies (8 to over 20) and the quality of the used panels (from single to five colour analysis). Thus, the major aim of further QCR remains to unify analysis strategies by clear guidelines.

P11: Protein expression and quantitative mRNA levels of Ikaros transcription factors in human leukemia

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Transcription factors from the Ikaros family, Ikaros, Aiolos and Helios are of major importance during lymphocyte differentiation and a misbalance in their expression results in tumor development in mice. The aim of our study is to tackle the question whether a change in Ikaros expression is of relevance also for human leukemogenesis.
We performed an extensive study of lymphocyte Ikaros mRNA expression in patients with B and T-cell acute and chronic lymphocytic leukemia. By means of RT-PCR analysis in most patients we found a variation in mRNA expression of all Ikaros family members, from undetectable to very high. We also were able to confirm the splicing variants expression in 5 out of 35 patients tested. These results confirmed that similarly to the KO mice experimental model, also in human malignancies there are detectable differences in Ikaros expression. Therefore we further analyzed the total amount of mRNA by real time PCR to look for a quantitative difference in Ikaros expression in leukemia patients. By means of flow cytometry with antibodies for both cell surface and intracellular proteins we determined the developmental stage of malignant cells and correlated it with Ikaros protein expression.
In conclusion we show here that Ikaros family members are relevant for diagnostics and prognostics of leukemia patients.

P12: On-chip non-invasive and label-free cell discrimination by Impedance Spectroscopy

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A novel cell discrimination method based on the measurement of electrical cell properties in a microfluidic chip is presented. The method can be used as a general cell analysis technique that simplifies, miniaturizes and shortens extensive and costly cell diagnoses. The impedance measurement provides information on volume, membrane capacity and cytoplasm conductivity in order to discriminate size, structural aspects, metabolism or composition. By applying dielectrophoresis within the microfluidic chip as gentle cell handling technology also issues of non-physiological conditions stress can be overcome.
Applications: Model: Discrimination of treated erythrocytes (gramicidin). *Cell biology*: Discrimination of cell line types; differentiated mouse fibroblasts/adipocytes; differentiated monocytes; dead and viable cells. *Microbiology*: Discrimination of species from yeast, bacteria, fungi and phases of live cycle. *Haematology*: Separation of lymphoblasts, granulocytes and monocytes.
Conclusions: Impedance spectroscopy and dielectrophoresis have been used for long time for the analysis of cell suspensions, bulk tissues and particle size. With our device, using impedance for the measurement of single cells, we could show promising results with the discrimination of different cells types. The measurements provide further potential applications in the field of oncology, apoptosis, stem cell research, parasitology and infectiology. The combination of these technologies in a microfluidic chip to measure in gentle way single cells underlines the potential of this new device as a valuable complement to the known cytometers and other cell detection systems.

P13: Terminal differentiation of preovulatory follicles involves thecal platelet-activating factor expression

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The ovarian follicle is an important functional unit of the female reproductive system, consisting of an oocyte surrounded by somatic (granulosa and theca) cells. Oocyte growth and maturation are coordinated with somatic cell proliferation and differentiation through intrafollicular signals secreted by the oocyte and somatic cells. Terminal follicle growth is triggered by a surge of luteinizing hormone (LH). LH initiates the preovulatory stage capable of releasing oocytes for fertilization and luteinization of the residual follicle with a strong increase in the synthesis of progesterone (P4). P4 protects from apoptosis by cessation of granulosal proliferation. P4-mediated withdrawal from cell cycle does not take place in thecal cell layers and the theca develops an inflammatory reaction leading to follicle rupture. The platelet-activating factor (PAF), present in the follicle fluid, is a potent inflammatory mediator acting through its receptor (PAF-R), but thecal expression of PAF-R is unknown, thereby role of PAF remains speculative. We determined thecal PAF-R expression in preovulatory follicles by quantitative RT-PCR and immunohistochemical staining. PAF-R was detected in the thecal layer but not in granulosa cells. The results suggest that thecal compartment is sensitised to develop inflammatory reaction by LH-induced PAF-R expression. This response may also contribute to thecal maintenance of proliferative potential, vascularization, vascular permeabilization and sensitivity to apoptotic signals required for follicle rupture.

P14: Immunosuppressive drugs and lymphocyte-apoptosis: a new human whole blood assay

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We designed this study to develop a novel whole blood assay to assess the effects of different immunosuppressive drugs on apoptosis in T-,B-and NK-cells, determining the pharmacodynamic effects on immunosuppressive therapy in human heart transplanted (HTx) recipients. Healthy peripheral blood for six experiments for each drug was drawn and whole blood was treated either with cyclosporin-A (CsA,1µM), mycophenolate acid (MPA,10µM), tacrolimus (TRL,100nM) or rapamycin (RAPA,100nM). Whole blood was stimulated with eight different concentrations of actinomycin-D (0-2µg/ml), an apoptosis inductor via caspase pathways. Apoptotis was measured by TUNEL and Annexin-V expression using FACS. Drug effects were calculated by taking the effects of actinomycin-D as baseline values. Drug treatment with CsA, MPA, TRL and RAPA significantly ($p<0,05$) decreased the apoptotic effect of actinomycin-D in CD3 positive cells in a non-competitive manner. Furthermore, the number of apoptotic cells after RAPA treatment was significantly lower compared to CsA and MPA

treatment ($p<0,05$). Actinomycin-D-induced apoptosis in CD16 and CD19 positive cells was not influenced by drug treatment. All drug effects reached maximum expression of apoptotic cells after stimulation with 1µg/ml actinomycin-D, which could be completely blocked by caspase inhibitor zVAD.
Our results indicate that CsA, MPA, TRL and RAPA are non-competitive inhibitors of apoptosis in T-cells, RAPA as the most potent. This assay may be helpful to discriminate between the mechanisms of action of different immunosuppressants in different lymphocyte subtypes in a therapy undergoing HTx.

P15: The Preliminary Studies of Novel Phthalocyanines and Their Potential for Photodynamic Therapy.

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Photodynamic therapy (PDT) is based on the use of photosensitizer and light to produce reactive oxygen species, which cause death of target cancer cells. Finding a suitable photosensitizer is crucial in improving the efficiency of PDT. Especially, due to their excellent photochemical properties and potential photosensitizers for the photodynamic therapy, we focused on Phthalocyanines (Pcs) and their cellular responses. For this purpose, human cancer cell lines were treated with our newly synthesized Pcs and the cell proliferation was determined by using MTS assay. The cells which treated for 24h in various concentrations of two novel Pcs didn't show any toxicities. Interestingly, treatment in high concentrations of Pcs cause more proliferation effects, that confirmed no toxicities of our Pcs without illumination. Additionally, the cellular fluorescence distribution was observed with a laser scanning microscope and all fluorescence of Pcs were observed in the cytoplasm and not in the cell nucleus. Following our preliminary results we will attempt to determine the mechanism of cell death type (apoptosis or necrosis) and related molecules by using Pcs and light combination.

P16: A Simple Method for Estimation of Duration of Cell Cycle Phases and Growth Fraction Using Bromodeoxyuridine-Flow Cytometry Data from a Single Sample

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Presently available flow cytometric methods of bromodeoxyuridine (BrdUrd) labelling do not provide information on the cell cycle time (Tc) and the growth fraction (GF).
We describe a novel and simple method for estimation of cell cycle phases and GF from flow cytometric analysis of a single tumour sample afer BrdUrd labelling.
The total numbers of labelled divided G1 cells, labelled divided S cells, labelled undivided S cells, and labelled undivided G2 cells were obtained for DNA histograms of BrdUrd-positive cells in a collected sample. These cell numbers were used to write equations to determine the durations of cell cycle phases, Tc and GF. To illustrate the practical application and suitability of the proposed formulae, cell cycle kinetics parameters were analyzed in solid SL2 tumors growing in DBA/2 mice and in human T-leukaemia Jurkat cells in culture. In addition, Ts and G2 values were estimated also using currently usable relative movement (RM) methods.
The suitability of the proposed method was demonstrated. Development of suitable software enabling more objective interpretation of the DNA profile in this method would be desirable.

P17: Hyperchromatic Cytometry using Laser Scanning Cytometry

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The increasing knowledge of the complexity of the immune system leads to the necessity of poly- and hyperchromatic cytometry. In most cases the simultaneous analysis of many fluorochromes is limited due to the number of available lasers and PMTs. Unlike Flow Cytometry, Slide based Cytometry is a non consumptive method, i.e. the analyzed sample is not lost during analysis. In contrast, it can be used for further analyses, e.g. remeasuring after restaining, changing of filters or bleaching or activation of selected fluorochromes thereby increasing the information density. In theory one can perform n re-measurements in order to obtain more information on a single cell level. This is only limited by the number of available antibodies and by sterical hindrance. Hyperchromatic cytometry is the way towards single cell proteomics and genomics.

P18: Photodynamic therapy combined with a cystein protease inhibitor decreases VEGF production and promotes tumor necrosis in a rat mammary carcinoma

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Photodynamic therapy (PDT) and inhibition of cathepsin B proteases by cystatin (CPI) are potential new tumour treatment modalities. We have investigated the efficacy of PDT and CPI alone and in combination on an ErbB2 negative solid mammary carcinoma transplanted in Wistar rats. Intraperitoneally injected single does of chlorin e6 or HpD as photosensitizers were excited at 630 nm (90 J/cm2). CPI (500 mg/animal) was injected around the tumor daily during the 8-day treatment. Inoculation of tumor was either on day 1 of the protocol, or 8 days before the protocol. Tumor size, tumor necrosis and vascularization based on HE stained sections and serum VEGF levels using an ELISA kit on day 8 were determined. No differences (2-way ANOVA) were found for treatments started at vaious lags. At doses where CPI or PDT alone had no or negligible effect on the parameters examined, their combination caused a marked ($p<0.001$) decrease of serum VEGF, paralleled by a significant decrease of tumor size, capillary vessels, and increase of necrosis up to 80% of the tumor tissue. Thus, the combined use of PDT and CPI could be a useful approach in tumor therapy based on their synergistic effect.

P19: Slide Based Cytometry - possibilities and limitations

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Slide Based Cytometry (SBC) is well evaluated and accepted in parallel to Flow Cytometry (FCM) as the gold standard. Above all, several studies have shown that with special features of the SBC, it is possible to excite and distinguish more fluorochromes at the same time, than with an equally equipped Flow Cytometer. This results mainly from SBC's non-consumptive character, and the known and stored x-y-position of the fixed cells on the slide. The non-consumptive character allows several measurements of the same sample. If changes (e.g. restaining with new fluorochrome-tagged antibodies) appear between measurements, they can be merged into a single virtual data file thus increasing the information density. In contrast to FCM, SBC allows analysis of solid tissue that has already been the subject of several studies. Significant differences in cellular colonization of lymph nodes were shown between HIV-infected and healthy patients. In another study, neurons of patients with Alzheimer's disease displayed a significantly higher cell cycle activity than neurons in healthy patients. Another study depicted the two- and three-dimensional distribution of neurons in brain tissue using SBC. However, quantitative tissue cytometry is very difficult to perform since its quality is limited due to sectioning artifacts and auto fluorescence.