Oral Presentations (Abstracts in alphabetical sequence)

Two-stage continuous PHA production: simulation and measurement of polymer distribution within bacterial populations

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Keywords: PHA, two-stage continuous cultivation, flow cytometry, segregated model

PHAs (polyhydroxyalkanoates) are intracellular polyesters produced by a wide variety of bacteria as carbon and energy resource. These thermoplastic polymers became commercially attractive due to their special physico-chemical properties, biodegradability and biocompatibility. Currently different pharmaceutical and medical applications are in the focus of interest. A wide spectrum of properties arose by the possibility to synthesize copolymers and, additionally, by the ability of post-biosynthetic modifications.

The overall process of PHA production comprises three stages, the synthesis of cells, the synthesis and accumulation of the polymer and the isolation of the product from the biomass. Continuous regimes of PHA bioproduction have several advantages compared to the commonly used batch or fed batch processes. Moreover a two-stage continuous process is more favourably than a single chemostat. For a better usability and understanding of the process we developed mathematical models and used flow cytometry for a segregated analysis of the cellular populations.

The two-stage continuous cultivation was performed with two coupled tank reactors. The first one operated as a chemostat for the production of biomass with a low PHA content. The culture liquid together with additional substrate was transferred to the second reactor, in which the product formation took place. The usual analyses of the resulting biomass deliver information about average properties of the cell population. Correspondingly, a non-segregated model was established and simulated with the program *Berkeley Madonna*, which is very helpful for the design and optimization of the cultivation method. However, the population of the second bioreactor consists of cells with very different properties. Therefore, also a segregated consideration was interesting. A structured and segregated model was established on the basis of the program *SigmaPlot*. The model simulates the distribution of age and other cell properties like PHA content creating classes of cells of identical age. Additionally the model provided average date of the population which allowed comparison and showed good conformity as well as to the non-segregated model as to the experimental data obtained with *Ralstonia eutropha* JMP 134.

An experimental approach to obtain individual data of cells and to verify the simulation at least in a qualitative manner was performed by investigations with flow cytometry. Both series of data showed the adjustment of a “quasi” equilibrium, which was faster achieved in reality. This revealed some simplifying assumptions of the model. Nevertheless the model is helpful for a better understanding and use of the process of two-stage continuous cultivation.

Microfluorimetry of the DNA content in individual human chromosomes, using an image analyzer

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We are proposing a method of determination of the DNA content in individual human chromosomes. The method includes three main stages: 1) identification of chromosomes stained differentially with quinacrine mustard, 2) treatment of chromosomes by Feulgen, using the Schiff-type reagent Auramine00-SO2, 3) microfluorimetry of the DNA content in individual chromosomes, using an image analyzer. Metaphase chromosomes were obtained from primary culture of peripheral blood lymphocytes of healthy people by the method of Moorhead et al. (1960). The chromosomes were identified in preparations stained with quinacrine mustard by the method of Caspersson et al. (1970). After identification of chromosomes, the dye was removed using a mixture of methanol-glacial acetic acid-water (1:1:1), and the preparations were stained by Feulgen, using the Schiff-type reagent Auramine00-SO2 (Kudryavtsev, Rozanov, 1974). Identification of Q-stained chromosomes and measurement of their DNA content after the Feulgen staining were performed using an image analyzer (Shtein et al., 1998). Monitoring of the camera, feeding of image from preparation, its correction and storage, and measurements of densitometrical and morphometrical parameters were performed using a software of the VideoTest Company (St. Petersburg). Integral fluorescence of the chromosome 2 in each metaphase plate was taken as unit. Relative to it, the DNA content was calculated in other chromosomes of the karyotype. Comparison of the results of the DNA content determination in individual human chromosomes obtained by means of our method indicates them to correspond to data of other authors. The proposed method has a sufficiently high efficiency and allows detecting differences of the DNA content in chromosomes of the order of 1-2 fg.

RNAi tools to study cancer

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Abstract did not arrive

Automated proliferation/apoptosis determination by tissue cytometry in gastrointestinal fresh frozen sections using triple labeling and scanning fluorescent microscopy

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Background: Proliferation/ apoptosis balance is an important information in gastrointestinal ulcerative and malignant diseases. Until now immunohistochemical staining and visual counting was the routine procedure. Recently we reported a new scanning fluorescent technique for automated motorized microscopes (SFM).
Aims: Development of triple fluorescent labeling method for proliferating / apoptotic / resting cells and application of SFM for the automated analysis and counting on gastric biopsy specimen.
Materials and methods: Routine antral biopsy specimens by gastroscopy (30) were fresh frozen and 5 micron sections were prepared. Proliferation was detected using a PCNA antibody and anti-mouse-rhodamine labeling system. Apoptosis was labeled using the TUNEL reaction with FITC bound nucleotids. DAPI nuclear counter staining was applied.
The labeled sections were scanned and digitized in the three fluorescent channels. SFM was modified to detect epithelial surface, glands in the biopsy specimen. Automated nuclei detection, PCNA and TUNEL detection was performed, ratio was calculated. In parallel standard biopsy specimen were labeled with PCNA and AEC. TUNEL reaction was also performed. Up to 1000 epithelial cells were visually counted.
Results: The mean PCNA labeling in healthy samples were 45,3$\pm$12,4%, positive TUNEL reaction was found in 23,2$\pm$ 7,8%. Significant correlation in apoptosis/proliferation ratio between the SFM and routin methods could be observed ($p<0,05$). The SFM procedure proved to be more time efficient both in the labeling, both in the detection procedures.
Conclusions: Triple fluorescent labeling and automated fluorescent microscopy is an applicable tool for the proliferation, apoptosis determination in fresh frozen samples.

EGFR coexpression modulates susceptibility to Herceptin in HER2/neu overexpressing breast cancer cells

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Background: Growth factors and Herceptin specifically and differentially modulate cell proliferation of tumor cells. However, the mechanism of action on erbB receptor level is incompletely understood. We evaluated Herceptin's capacity to modulate erbB receptor activation and interaction on the cell surface level and thereby potentially impair cell proliferation of HER2/neu (c-erbB2) overexpressing breast cancer cells, both in the presence and absence of relevant growth factors.
Methods: BT474 and SK-BR-3 breast cancer cell lines were treated with EGF, HRG, and Herceptin in different combinations. Kinetics of cell proliferation were evaluated flow cytometrically by BrdU labeling. Fluorescence Resonance Energy Transfer, ELISAs and Western Blotting was performed to investigate erbB receptor interaction and activation.
Results: EGF induced EGFR/EGFR and EGFR/c-erbB2 interactions correlate with stimulation of cell proliferation in BT474 cells. A less pronounced EGFR/c-erbB2 heterodimerization in SK-BR-3 cells is additionally reduced by EGF treatment, causing inhibition of cell proliferation. Growth inhibitory EGF induces Y877 and Y1112 phosphorylation, while growth inhibition by Herceptin is mediated by c-erbB2 phosphorylation at Y877 and Y1248. Herceptin drives BT474 cells more efficiently into quiescence than SK-BR-3. The inhibitory capacity of Herceptin on BT474 and SK-BR-3 cell proliferation depends on the presence and absence of growth factors to a various extent.
Conclusion: The anti-proliferative effect of Herceptin on c-erbB2 overexpressing breast cancer cells is considerably modulated by EGFR coexpression and consequently EGFR/c-erbB2 homo- and heterointeractions, and by availability of growth factors. c-erbB2 overexpression alone is insufficient to predict the impact of growth factors and antibodies on cell proliferation.

Flow cytometry as a research tool in microbial physiology and ecology

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The ability of flow cytometry (FCM) to analyse entire microbial communities on single-cell level provides one of the most exciting new research tools for microbial ecology. Using different treatment techniques, fluorescent antibodies and/or DNA and/or RNA targeting stains, rapid quantification can be made of viruses, parasites, and different bacterial populations in a natural microbial community. A key challenge is measuring, interpreting and understanding bacterial viability. Two different examples are addressed in this presentation. Firstly, it is shown how physiological alterations in *E. coli* JM101 (pSPZ3) with XMO production during fedbatch cultivation are detected with ethidium bromide staining and FCM several hours before conventional methods recognise the problem, which eventually leads to malfunctioning of the reactors. The result is not only quantitative, but also lends an interpretation to the actual causes of the physiological changes in the bacteria. The second example is the detection of microbial inactivation during UV and sunlight disinfection. Comparisons between conventional cultivation and Live/Dead® staining are discussed, while a unique feature of SYTO9 staining for viability detection is shown. In a further application, the ability of FCM to accurately quantify bacteria at relatively low cell concentrations have been used to construct batch growth curves for the growth of bacterial consortia in the ug.L^{-1} carbon range, which was previously not possible with conventional methods.

Cell depletion using laser induced, nanoparticle mediated selective cell eradication

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Absorbing nanoparticles are investigated as a tool for selective manipulation of microscopic biological structures like cells. The inorganic absorber particles are coupled to the target via antibodies and act as well confined heat sources after irradiation with Q-switched and mode–locked lasers. Using cell type specific antibodies coupled to nanoparticles it was possible to selectively damage cells that carry the corresponding antigen, whereas other cells were unaffected. More than 99% percent of the contaminating target cells revealed lethal membrane damages after laser irradiation, whereas 90% of the control cells were still healthy.

Nanoparticle mediated cell eradication can be performed on adherent cells in transparent cell culture systems without the need to detach the cells. This excludes fluid shear stress due to instrumentation or centrifugation known from FACS or MACS Sorting. Identification and analysis on a single cell level is not needed. Using scanning laser irradiation faciliates the elimination of several thousand cells per second.

In conclusion, it is possible to eradicate cells targeted with micro absorbers which are heated with appropriate laser pulses. The damage mechanism is based on physical parameters and can therefore be varied more precisely than chemical methods by choosing appropriate particle size, pulse width and pulse energy. Laser induced, nanoparticle mediated inactivation may therefore represents a new method to effectivly remove contaminating cells even from fragile adherent cell populations without affecting yield and viability of the requested cell type.

Relationship between g-H2AX and Rad51 components of DNA repair in paired radio-resistant and -sensitive tumour cell-lines after irradiation

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Previous studies have shown the anti-apoptotic effect of DNA repair by homologous recombination (HR). However, it is not known whether radiosensitive and radioresistant tumour cells employ this mechanism in the same way. We addressed this question using a pair of lymphoblastoid cell lines derived from the same patient which differ in their p53 status and radioresistance. TK6 is a radiosensitive p53 wild type cell line whilst WI-L2-NS is p53 mutated and radioresistant. Following irradiation with 5 Gy, both cell-lines adapted G1- and were arrested at the G2M-checkpoint. The maximum level of HR, as displayed by the extent of nuclear Rad51 and number of its foci, was observed at the G2M checkpoint in both cell-lines. However, prior to this, when most cells are transit from S-phase to G2, WI-L2-NS cells showed 2-3 times more nuclear Rad51 protein and foci than TK6. Subsequently, by using BrdU pulse labelling we revealed that WI-L2-NS cells were much more active at synthesising DNA than TK6 cells with twice as many completing S-phase. Moreover at 6-24h, a close correspondence in areas occupied by g-H2AX and Rad51 components was observed. However, in TK6 cells the g-H2AX component occupies a much more widespread area than the Rad51 component suggesting only a weak interaction between them. Failed repair, indicated by a lack of interaction between g- H2AX and Rad51 foci, and by the formation of g-H2AX supradomains, was often apparent in TK6 cells. Conclusion: These data indirectly suggest that high efficiency HR in radioresistant p53 mutated cells is facilitated by adaptation of the intra-S-phase checkpoint, allowing stable recruitment of S-phase associated repair proteins by g-H2AX into the repair foci. Conversely, unstable link between the two components favours formation of g-H2AX supradomains, which signal apoptosis.

Trichostatin A induced histone acetylation causes decondensation of interphase chromatin

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Although it is frequently assumed or speculated that histone acetylation affects chromatin structure in vivo to create a more open chromatin conformation direct evidence is missing. Furthermore, it is currently an open question if changes in the histone acetylation state on the nucleosome level will affect the higher order organization of the chromatin fiber. We have studied the effect of trichostatin A (TSA) induced histone acetylation on the interphase chromatin conformation by confocal laser scanning microscopy. The studies were conducted with HeLa cells stably expressing a fusion protein of histone H2A and yellow fluorescent protein (H2A-YFP). H2A-YFP is incorporated into chromatin and provides an in vivo fluorescent label. Upon TSA treatment a reversible decondensation of dense chromatin regions was observed, which led to a more homogeneous chromatin distribution. To quantify this effect, two advanced image analysis methods were used, namely image correlation spectroscopy and spatially resolved scaling analysis. The image analysis revealed that a chromatin reorganization on a length scale from 200 nm to > 1 μm was induced consistent with the opening of condensed chromatin domains containing several Mb of DNA. The kinetics of these conformational changes were also studied and a correlation with the biphasic histone acetylation kinetics was detected.

Changing gears – new ways for stem cell mobilization

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Abstract did not arrive

IMMUNOPHENOTYPING BY SLIDE-BASED CYTOMETRY AND BY FLOW CYTOMETRY ARE COMPARIBLE

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Background: Immunophenotyping of peripheral blood leukocytes (PBLs) is performed by flow cytometry (FCM) as the golden standard. Slide based cytometry systems can give additional information to each individual cell (e.g. repeated staining and scanning, morphology). In order to adequately judge on the clinical usefulness of immunophenotyping by LSC it is obligatory to compare it with the long established FCM assays. We performed this study to systematically compare, FCM and LSC, for immunophenotyping and to test the correlation of the results. Different triggering modalities with the LSC for cell detection were compared.
Methods: Leukocytes from 30 different individuals were stained with directly labeled monoclonal antibodies by the whole blood staining method. One set of samples was additionally stained on the slide following air drying and fixation by aceton with the nuclear DNA marker 7-AAD. Aliquots of the same paraformaldehyde fixed specimens were analyzed with a FACScan (BD-Biosciences) FCM and in parallel with a LSC (CompuCyte). In the LSC different triggering modalities were used to detect cells: 1. 7-AAD staining, 2. forward scatter or 3. Positivity for the pan leukocyte surface antigen CD45 FITC.
Results: The percentage distribution of PBLs obtained by LSC and by FCM shows very good correlation with regression coefficients >0.95 for the major populations (neutrophils, lymphocytes, and monocytes), as well as for the lymphocyte sub-populations (T-helper-, T-cytotoxic-, B-, NK-cells) independent of the trigger signal used for cell detection. The best trigger proved to be the 7-AAD signal followed by CD45 and FSC.
Conclusion: LSC can be recommended for immunophenotyping of PBLs especially in cases where only very limited sample volumes are available or where additional analysis of the cells' morphology is important. There are limitations in the detection of rare leukocytes or weak antigens where appropriate amplification steps for immunofluorescence should be engaged.

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COMBO-FISH: A versatile fluorescence labeling technique of genome regions for high resolution microscopy

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The principle of Fluorescence In Situ Hybridization (FISH) with COMBinatorial Oligo (COMBO) probes is presented as an approach that permits specific labelling of any given genomic sites. COMBO-FISH takes advantage of homopurine/homopyrimidine oligo-nucleotides that form double strands as well as triple helices with intact genomic DNA. The latter can be performed without prior thermal denaturation of the target sequence. A set of distinct, uniformly labeled oligo-nucleotide hybridization probes can be configurated from the human genome data base. This set is designed to exclusively co-localize within a 250 kb chromatin domain corresponding to the diffraction limited point volume of a high numerical aperture lens. Some of the oligonucleotides have additional binding sites somewhere else in the genome but clusters with more than 6 probes are excluded. For the proof of principle COMBO FISH was applied to normal lymphocytes, blood preparations of CML patients, formalin-fixed and paraffin-embedded tissue section as well as to bone marrow smears. DNA oligo-probes as well as PNA oligo-probes and hairpin probe configurations were successfully used. Since cell nuclei can be gently treated without denaturation the technique can be used to analyze nanostructures in the genome. As an example the volume and the compaction of the abl-region is measured by high resolution Spatially Modulated Illumination microscopy (SMI-microscopy).

Stem cells and their niche connections determine life fate

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Abstract did not arrive

Large-scale gene silencing using RNAi-based cell arrays

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Abstract did not arrive

Long-term cultivation of Escherichia coli using a substrate-limited, high-cell-density membrane bioreactor system

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To investigate the long-term cultivation of an environmental *Escherichia coli* strain a computer-controlled bioreactor system was set up equipped with an external 0,2 µm pore size crossflow microfiltration membrane.

Membrane bioreactors are employed e.g. in activated sludge processes in municipal wastewater treatments. One main advantage of this technique is low overall sludge production, probably a result of the microorganisms being in a non-growing, though metabolically active state. Under such substrate-limited conditions nutrients are used mainly for maintenance, a mechanism to survive prolonged starvation, which might represent a common physiological state of bacteria in nature.

To study maintenance metabolism and associated metabolic adaptations of bacteria in more detail, *E. coli* cells were grown in a carbon- and energy source-limited membrane bioreactor with 100 % biomass retention in continuous culture. The bacterial population in the fermenter was monitored over a course of several weeks including determination of growth parameters (total cell count, biomass formation, frequency of dividing cells (FDC)) and bacterial activity on a single cell basis (culturability (CFU), membrane integrity (LIVE/DEAD® BacLight™) and respiratory activity (CTC)). The accumulated biomass was investigated by flowcytometric methods to determine the presence of subpopulations.

Population dynamics of a bacterial consortium during growth on diesel fuel in saline environments

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A diesel fuel degrading bacterial consortium enriched from an Argentinean saline soil contaminated with mineral oil was cultivated at different salinities over 84 days to follow changes in the community composition in dependence on salinity and to identify halophilic or halotolerant key degraders. The mixed cultures were grown in 240 ml liquid mineral medium containing 215 mg diesel fuel. The salt concentration of the medium was 0.24%, the different cultures additionally contained 0, 7.5, 15 or 20% NaCl, respectively. The degradation process was continuously monitored by measuring oxygen consumption. After 21, 42 and 63 d as well as at the end of the experiment protein contents, bacterial colony forming units (cfu) on R2A agar as well as total cell counts by epifluorescence microscopy after DAPI staining were recorded. Maximum values for oxygen consumption and protein content were found at 7.5% NaCl, whereas the cfu and total cell counts showed maxima at 15% NaCl. Maximum substrate utilization spectra determined by BIOLOG plates were recorded at 7.5% NaCl. These findings indicate a prevalence of halophilic bacteria.

Shifts in the community composition were monitored by SSCP fingerprinting of the PCR-amplified V4-V5 region of the 16S rRNA gene. Sequencing of extracted dominant SSCP bands revealed different key organisms arising over the time course at the various salinities. In the 0% NaCl culture *Acidovorax* sp. was prevalent after 42 d but disappeared after 84 d whereas *Sphingomonas* sp. and *Streptococcus* sp. arised after 84 d as prominent degraders. At 7.5% NaCl the most prominent band represented *Idiomarina* sp. which was also present at higher salinities. At 15% NaCl a bacterium belonging to the *Alteromonadaceae* was the prevalent degrader, additionally after 42 d a band representing *Alcanivorax* sp. was displayed the intensity of which decreased after 84 d. An unknown alpha-proteobacterium was present at 15% and 20% NaCl displaying most dominant bands after 84 d. *Halomonas* sp. was present over the whole range of salinities and over the whole time course. *Ralstonia* sp. was also present in all samples but showed most prominent bands at 0% NaCl after 42d.

Multiparametric flow cytometry was used to get more detailed information on the consortium based on single cell analysis. Specific population patterns were imaged during growth at the four given salt concentrations. Population dynamics during each cultivation course were followed, which were assumed to vary because of changing substrate composition when diesel fuel is degraded. Such information was available by quantifying the cellular DNA content with DAPI staining and analyzing the cell size of the bacteria employing the forward scatter (FSC) signal. Spatial separation of up to four subpopulations per sample was performed by high speed cell sorting. From DNA extracted from sorted cells the 16S rDNA was PCR-amplified and cloned. The 16S rDNA clone libraries were screened by RFLP analysis. RFLP patterns were grouped by hierarchical cluster analysis and representative clones were partially sequenced to identify the key organisms representing the distinct subpopulations. The results are in accordance with those of the SSCP analysis suggesting that multiparametric flow cytometry is an excellent tool for separating distinct components of complex microbial communities for further phylogenetic identification.

Mechanism of human stem cell homing and repopulation in transplanted NOD/SCID mice

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Abstract did not arrive

Small and cheap: Accurate differential blood count with minimal sample volume by laser scanning cytometry (LSC)

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Aim: In many patients differential blood count is required. For standard analyzers 500 µl blood was taken from patients. This can be a substantial volume, especially after operations with blood loss. Aim of this study was to develop a method to reduce the needed blood volume significantly. This is enabled by a microchamber, which is analyzed cytometrically by LSC.
Methods: 10 µl EDTA blood were mixed with 10 µl of a DRAQ5 solution (100µM, Biostatus) and 10 µl of an antibody mixture (CD45-FITC, CD14-PE, CD16-PECy5). 15 µl of this cell suspension was poured into a Neubauer counting chamber. Due to the defined volume of the chamber it is possible to perform the cell count. The trigger was set on DRAQ5 signal to count leukocytes. Leukocyte subsets were distinguished by fluorescence labeled antibodies. For erythrocyte counting cell suspension was diluted another 150 times. 15 µl of this dilution was analyzed in a microchamber by LSC. Trigger was set on forward scatter signal.
Results: This method allows a decrease of blood sample volume (10 µl instead of 500µl). There was a good correlation between this method and the results of routine laboratory (r^2=0.86,$p<0.0001$;n=20). For all parameters intra-assay variance was less than 9%.
Conclusions: In patients with low blood volume every effort has to be taken to reduce the blood volume needed. With this method only 2% of standard sample volume is needed to generate a differential blood picture. Costs are comparable to routine laboratory.

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Simultaneous HER2/neu FISH and fluorescence immuno staining on paraffin-embedded breast cancer tissue sections

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Background/Aim: HER2/neu is a protooncogene and is overexpressed in 25% to 30% of human breast cancers. The determination of the HER2/neu status has become essential for the selection of patients for Herceptin therapy. Currently, HER2/neu alterations are frequently detected by immunohistochemistry (IHC), showing potential HER2/neu protein overexpression. Alternatively, fluorescence-in-situ-hybridization (FISH) to identify potential HER2/neu gene amplification is used. In order to optimize accuracy and reproducibility of the HER2/neu diagnosis we established a novel diagnostic approach based on the simultaneous detection of HER2/neu gene amplification via FISH and receptor protein expression via fluorescent IHC (FIHC).

Methods: Paraffin-embedded breast cancer cell lines with known HER2/neu status on DNA and protein level were used to establish a standardized simultaneous expression and gene amplification scoring. The diagnostic applicability was verified on 215 primary breast cancer tissue sections using the tissue microarray technology (TMA) and an epifluorescence microscope.

Results: The multiparametric, simultaneous HER2/neu protein and gene detection is reliable and in concordance with the FDA approved scoring, ranging from 0 to 3+, conventionally applied in the HercepTest (DAKO). Using this novel, fast one step approach breast carcinomas with HER2/neu protein overexpression and simultaneous gene amplification were detected with 100% sensitivity. 2.3% (n = 5/215) cases were disclosed, showing HER2/neu gene amplification without protein overexpression.

Conclusion: Information on polysomy, aneuploidy, gene amplification and protein content can be obtained in the same cell by the novel single step analysis and might upgrade the value of HER2/neu diagnosis with respect to a more precise patient stratification.

Kinin B2 receptor function during neuronal differentiation of embryonal P19 cells

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The kinin B2 receptor (B2R) is widely expressed in several organs including the brain and participates in inflammation responses, blood pressure control, neuromodulation and development. We have studied B2R expression and function during in vitro neuronal differentiation of embryonal P19 cells by cytometry methods such as calcium imaging and confocal microscopy. Steady state levels of B2R mRNA and protein expression are modulated along the differentiation of embryonal P19 cells to neurons. Following induction to differentiation by retinoic acid the cells start expressing neuronal markers, as high molecular weight neurofilaments (NF-200) reaching the highest expression level after nine days. P19 neurons develop functional synapses and express neurotransmitter receptors, such as acetylcholine receptors. Immunochemical staining followed by confocal microscopy revealed that B2R protein is co-expressed with the neuronal marker NF-200 in differentiated P19 cells. Mea!

surement of changes in [Ca2+]i by confocal microscopy in the presence of bradykinin (BK) revealed that embryonal P19 cells are irresponsive to bradykinin application, but neuronal differentiated cells respond with a transient rise in [Ca2+]i. Specific inhibition of B2R activity by the antagonist HOE-140 at day 5 results in a reduction of the Ca2+-response to the acetylcholine receptor agonist carbamoylcholine at day 8, implicating that B2R action is necessary for development of synaptic plasticity.

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Appropriateness of different heat measurement principles and flow cytometry used to control the continuous synthesis of polyhydroxyalcanoates

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Optimum continuous growth-associated synthesis means a maximum substrate-carbon channeling into the desired product and the carryover is even sufficient to regenerate and maintain the microbial catalyst. This point is characterized by a certain Gibbs energy dissipation and cellular state. Therefore, different calorimetric measurement principles and flowcytometry were applied to the example of the continuous growth-associated synthesis of polyhydroxyalcanoates (PHA) from phenol and fructose by *Variovorax paradoxus* DSM4065. By controlling the supply of the respective carbon substrate to the reactor, the growth rate, formation rate of the target product were heighten gradually until the capacity of carbon-substrate assimilation was exhausted. After this point the carbon-substrate accumulated. This accumulation was indicated by changed slope of heat production rate in complete heat balanced bioreactors. The amount of the change depended tightly on the toxicity of the substrate. In case of employing a flow calorimeter as "measurement loop" in connection with the bioreactor the carbon-substrate accumulation was indicated by a sudden change in the heat production rate independent on substrate toxicity. The latter fact was exploited for the long term control of phenol conversion into PHA.

Flowcytometry revealed that at the optimum two subpopulations are present with complete different PHA-content. Although, intoxication by phenol accumulation was indicated by increasing the ratio of cells with quadruple set of DNA to such with a double set, this correlation was not tight enough to control the process. Surprisingly, phenol grown cells contained a subpopulation with a single set of DNA, which was not found in case of fructose grown cells.

Keywords: Chemostat, calorimetry, flow cytometry, polyhydroxyalcanoates (PHA), phenol, *Variovorax paradoxus* DSM 4065.

Two- and threedimensional distribution of nuclei and neurons in brain tissue using Laser Scanning Cytometry (LSC)

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Background:
In tissue cytometry a solid trigger is necessary in order to unequivocally differenciate between cellular and non-cellular events. This can be best acchieved by nuclear staining. Aim of this study was to analyze a brain tissue section by laser scanning cytometry (LSC) in order to depict the threedimensional distribution of nuclei in the tissue. To this end the section was measured in several foci and different nuclei detected in several depths were accessed to the respective layer.

Materials and methods:
Frozen sections of formalin-fixed rat brain tissue (120µm thickness) were incubated with propidium iodide (PI) (50µg/ml) and covered on slides. For analysis by the LSC PI was used as trigger. After a first analysis focussed on the top of the tissue the focus was moved 30µm down into the tissue. Per analysis data of at least 50,000 cells were acquired. After finishing all measurements all data files were merged, i.e. data were combined.

Results:
With the special features of the LSC it was possible to develop a method to depict the threedimensional distribution of the nuclei in solid tissue sections.

Discussion:
LSC can be an useful tool for this field of solid tissue cytometry. After evaluating methods like this, so far not available data can be analysed for diagnostic value. We hope to demonstrate the power of the LSC for the routine pathological use. This should add up to the versatility of applications for the LSC as a cytometric instrument suitable for high throughput and high content analysis.

Immunological and ultrastructural characterization of endothelial cell cultures differentiated from human cord blood derived endothelial progenitor cells

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Bioenergeening of endothelium from endothelial progenitor cells (EPCs) for therapeutic use in order to improve the vascular status of ischemic organs is now in focus of vascular research. The aim of our studies was to find an appropriate method to differentiate human cord blood EPCs to mature or premature endothelial progenitor derived cells (EPDCs). We investigated EPCs and EPDCs by immunological characterization using flow cytometry, phase contrast and fluorescence microscopy as well as confocal laser scanning microscopy. In addition, the two cell types were investigated ultrastructurally using transmission and scanning electron microscopy. While EPCs can be easily propagated from clusters of MNCs, the approach to obtain EPDCs was only successful if isolated CD34+ progenitor cells were used as starting population. Both cell types took up Dil-Ac-LDL and could be positively stained for CD31, CD105, VEGFR-2, UEA-1 at the cell surface. EPC showed surface expressi on of CD54 and CD106. However, only a small portion of EPDCs were positive for CD54 but negative for CD106. Intracellular staining for vWF provided a homogenous stain in EPCs while in EPDCs a typical punctuated staining pattern related to Weibel-Palade bodies was visible. In phase contrast and scanning electron microscopy the arrangement of EPCs in cord-like structures could be demonstrated. In these formations, cells are parallel aligned but not connected by cell contacts. In contrast, EPDCs develop adherence junctions, interdigitating junctions and syndesmos. EPDCs display spindle-like and cobblestone-like cell types whereas the former contain Weibel-Palade bodies abundantly. These organelles display bizarre shapes and are concentrated in close neighborhood to mitochondria-rich areas. Weibel-Palade bodies could never be found in EPCs. In EPDCs, signs of a high metabolic activity are a well developed RER and multiple Golgi complexes. These morphological features correlate well with a high growing capacity. In this respect, we suggest that these highly active cells might be appropriate for therapeutic use in order to induce vasculogenesis and angiogenesis in several vascular disorders.

Change in cell surface expression of adhesion molecules during differentiation of human cord blood derived endothelial progenitor cells

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Endothelial progenitor cells derived from human cord blood have a high potential in respect to the reconstitution of altered microvasculature in ischemic areas of the heart and limbs. We were interested to demonstrate the differences in cell surface receptor expression between endothelial progenitor cells (EPC), isolated from the MNC population or isolated CD34+ stem cells, differentiated endothelial progenitor derived cells (EPDC) and human umbilical vein endothelial cells (HUVEC) qualitatively by laser confocal microscopy and quantitatively by flow cytometry measuring the antigen binding capacity using the Sigma-Aldrich Quantum Simply Cellular Kit. In addition, the tumor necrosis factor alpha (TNF-alpha)- and lipopolysaccharide (LPS)-induced activation and upregulation of the adhesion molecules CD62E, CD54 and CD106 were compared in EPDC and HUVEC. The results show that EPC are strongly positive for CD31 but only weakly positive for CD34, CD105 and the vascu lar endothelial growth factor receptor 2 (KDR). In MNC derived EPC, CD31 positive cells are also able to bind ulex europaeus lectin 1 (UEA-1) and are positive for the von Willebrand factor (vWF) after permeabilization of the cell membrane. During differentiation, KDR and CD34 are downregulated (CD34 can be demonstrated intracellularly in about 20% of EPDC but also in HUVEC), CD105 is upregulated while CD31 remains relatively unchanged at the cell surface. Comparing EPDC with HUVEC, there is a stronger expression of CD31 but a weaker expression of CD105 in EPDC. The activation process due to LPS and TNF-alpha, reflected by upregulation of CD54, CD106 and CD62E, is significantly higher in EPDC than in HUVEC. Morphologically, not all cells of the EPDC population display the cobblestone-like morphology of HUVEC because of the presence of fibroblast-like cells. Nevertheless, these fibroblast-like EPDC contain a high number of Weibel-Palade bodies, staining strongly positive for vWF. Our investigation supports the view of a high phenotypic plasticity of maturing cells derived from hematopoetic stem cells.

Simultaneous measurement of p53 and MDM2 in acute lymphoblastic leukemia cells in response to prednisone

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p53 plays a crucial role in triggering apoptosis of acute lymphoblastic leukemia (ALL) cells in response to prednisone treatment. MDM2 is a cellular protein that inhibits p53 expression on a negative feedback loop. Several studies indicate that MDM2 overexpression is associated with poor prognosis in children with ALL.

The study comprised 30 children with de novo ALL. Peripheral blood samples were taken at diagnosis and after 6 and 12 hours from prednisone administration. Cytospin preparations of mononuclear cells isolated on density gradient were stained with mouse anti-MDM2 protein antibody followed by goat anti-mouse IgG conjugated with APC and then mouse anti-p53 protein antibody directly conjugated with FITC. Cellular DNA was counterstained with PI in the presence of RNase A. FITC – associated green fluorescence excited by Ar laser (488 nm) and MDM2 – associated long red fluorescence excited by HeNe laser (633nm) were measured separately over the nucleus and the rim of cytoplasm by laser scanning cytometer (CompuCyte).

Changes of p53 and MDM2 expression were inversely correlated. The ratio p53/MDM2 expression measured over the cytoplasm was higher after 6 hours followed by the rise of the ratio over the nucleus after 12 hours. This delay may reflect the time needed for cytoplasmic synthesis of proteins and their subsequent translocation from cytoplasm to nucleus.

Our data seem to indicate that prednisone not only activates p53 but may also inhibit MDM2 in peripheral blood mononuclear cells of children with ALL.

Evaluation of in vitro-effects of 50 toxic reference chemicals using an electrical current exclusion assay versus MTT- and NRU- assay

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In health hazard determination of chemical compounds *in vitro* screening methods using cell cultures became increasingly important. Good correlation between *in vitro* cytotoxicity (IC_{50}) and *in vivo* animal lethality (LD_{50}) were verified in various studies.

Cytotoxic properties of chemical compounds are generally determined and quantified by inhibition of cell proliferation. Decrease in cell viability, e.g. drug-induced alteration in metabolic pathways based on colorimetric assays and/or cytoplasmic membrane integrity based on dye exclusion or enzyme release are measured. These methods require manipulation of the cells, coloration and/or fixation. Reproducibility decreases with increasing manipulation required for the assay.

Two validated *in vitro* assays (neutral red uptake NRU and MTT/WST-8 assay) based on quantification of metabolic processes necessary for cell proliferation and two membrane integrity assays (digital cell analyser CASY® Model TTC with electrical current exclusion assay, and trypan blue dye exclusion assay) were performed.

L929 mouse fibroblast cells were cultured as monolayer for NRU and WST-8 assays and in single cell suspension manner for trypan blue and electrical current exclusion assays. IC_{50} values were evaluated using 50 chemicals covering six logs from low to high toxicity.

High correlation between IC_{50} values obtained in this study and published data was found. Sensitivity of the assays was highest with the electronic cell assay and decreased from MTT/WST-8 assay to NRU to trypan blue assay.

Advantage of the electronic assay is, that the result is achieved without manipulating the cells. The method is fully 21CFR11 compliant and interfacable with existing data analysis software.

Use of RNA interference for gene function analysis in mammalian cells

THOMAS RUDEL

MPI für Infektionsbiologie, Berlin

Abstract did not arrive

Towards in vivo application of siRNA technology

SASCHA RUTZ

Deutsches Rheuma-Forschungszentrum Berlin

Abstract did not arrive

Statistical Geometrical Cytometry

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Recent developments in apparative cytometry have enabled researchers to attack scientific questions by investigating statistically significant amounts of cells and drawing conclusions from a multitude of measurements rather than from the examination of a single cell. Geometric relations between objects like telomeres, centromeres, and gene loci in cell nuclei as encountered in different cell cycle phases can change in time or due to physiological or environmental conditions. To characterize dynamical processes within the cell, radial or angular distributions of these loci or the distribution of their distances are measured in 3D or, more conveniently, via 2D projections to a single image plane.

For statistically relevant discrimination of such distributions, e.g. as consequence of varying experimental treatments, as well as for the reconstruction of 3D distributions from 2D measurements, very simplistic approaches like MC simulations as well as advanced analytical methods like the solution of singular integral equations are derived and compared with respect to their practical performance.

Moreover, 3D distributions lead to structure-function correlations to which, actually very often, statistical explanations are taken to draw unequivocal conclusions, e.g. in epistasis. This has to be considered very carefully in order to avoid “given in – coming out” results. Of course, the presentation can only give a short introduction to scientifically general problems of the kind within the framework of the cytogeometrostatisticome.

Combined application of flow cytometry & retroviral vector technology for the functional genomic analysis of human myelopoiesis and dendritic cell development

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Simultaneous flow cytometric analysis of cell surface and intracellular differentiation antigens recently led to a refined understanding of hematopoietic differentiation processes. Additionally, differentiation models of CD34+ umbilical cord blood progenitor/stem cells have been established and these models led to advancement of our understanding of hematopoietic lineage commitment and differentiation processes at the molecular/fuctional level. CD34+ progenitor cells can be induced to extensively proliferate and to differentiate along defined leukocyte lineages under controlled conditions, depending on specific cytokine conditions. Here we uitilized this knowledge in conjunction with retroviral vector gene transfer technology for pursuing molecular studies. Retroviral vectors stably integrate candidate genes into target cells, and depending on gene transfer efficacy, gene transduced cells may carry one or only a few gene integrations per target cell. Additiona!

lly, random stable integration allows for gene expression in progeny cell populations. Based on this methodology, we have established FACS-based assays to: (1) clone dominant effector molecules from complex cDNA libraries based on their capacity to inhibit monocyte/macrophage differentiation; (2) assess enforced or inhibited candidate gene expression on myeloid/dendritic cell (DC) subset development; (3) study molecular mechanisms involved in the regulation of human DC maturation using tetracycline-inducible retroviral vectors applied to CD34+ primary cells.

Aptamers as Modulators of Protein Functions and Tools for Cytomics Research

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The SELEX technique (systematic evolution of ligands by exponential enrichment) is a combinatorial library approach in which DNA or RNA molecules (aptamers) are selected by their ability to bind and modulate the activity of their protein targets with high affinity and specificity, comparable to that of monoclonal antibodies. In contrast to antibodies conventionally selected in animals, aptamers are generated by an in vitro selection process, and can be directed against almost every target. The potential utility of aptamers as therapeutic agents is considerably enhanced by chemical modifications that lend resistance to nuclease attack. Aptamers are also ideal candidates for cytomics, as they can be attached to fluorescent reporters or nanoparticles in order to study biological function by fluorescence microscopy, flow cytometry, or quantifying the concentration of its target using ELISA, RIA and Western blot assays. The potential therapeutic importance of RNA an!
d DNA aptamers will be demonstrated by the following examples: The selection of nuclease-resistant RNA aptamers that block cell-adhesion events and inhibit cell infection by Trypanosoma cruzi, and the selection of aptamers that bind to acetylcholine receptors and counteract receptor inhibition by cocaine. Currently, we are developing fluorescent-labeled anti-kinin B1 receptor aptamers as tools for imaging of receptor expression and inflammation processes.

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siRNA-mediated silencing of EGFR has no inhibitory effect on human glioma cells in vitro.

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The *epidermal growth factor receptor* (EGFR, ErbB1) is frequently dysregulated in a variety of solid human tumors, including malignant glioma. Expression has been associated with disease progression, resistance to standard therapies and poor survival.
The application of *small interfering* RNAs (siRNAs) has become an effective and highly specific tool to modulate gene expression, and until today, a wide range of oncogenes have been silenced successfully. Here we show the siRNA-mediated *knockdown* of EGFR in two established glioma cell lines (U373MG, LN18). The expression of EGFR mRNA and protein was downregulated by 80-90%. We could demonstrate the higher efficiency of the siRNA-mediated silencing compared to an EGFR-specific antisense-oligonucleotide. However, siRNA treatment had no inhibitory effect on cell proliferation, migration and activation status of EGFR-coupled signaling cascades (Erk1/2, PKB). In accordance with these results, gene expression analysis with microarrays revealed only small changes in expression patterns. In contrast, the use of the synthetic EGFR inhibitor AG1478 resulted in the inhibition of the aforementioned cellular properties. Several possibilities for this inconsistency can be considered. First, the residual amount of EGFR protein might still be sufficient to transduce EGFR-dependent signals. Second, other members of the ErbB-family or different receptor tyrosine kinases might compensate for the decreased EGFR. Finally, the studied cell lines might be largely independent of EGFR. The effects seen with AG1478 might then be unspecific, a possibility that is not unlikely considering the high concentrations of inhibitor (up to 20 μM) needed.

Genome organization in human cancer: basic principles and clinical implications

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Recent advances in the specific fluorescent labelling of chromatin in fixed cells in combination with three-dimensional (3D) fluorescence microscopy and image analysis along with the vast amount of sequence data of the human genome have opened the way for detailed studies of genetic architecture of cancer cells. Implicit in this approach is the ability to compare histomorphological and molecular alterations associated with cancer development as normal cells progress toward the malignant phenotype. An initial screening with one-dimensional (1D) methods for genomic imbalances by array-CGH and oligonucleotide microarrays is a critical first step in dissecting oncological pathways as described here for the malignant transformation of esophageal, breast and pancreatic cancer. Mapping these alterations back by Fluorescence-in-situ hybridization within an architectural context simultaneously for both, the histomorphological and genetic level in the cancer genome opens an unique insight into complex moleculargenetic events in situ (2D). We illustrate this powerful approach by applying 2D- and 3D-Fluorescence microscopy to invasive esophageal, breast and pancreatic cancer and their associated premalignant lesions. Instead of generalized presence or absence of gene amplification, we could demonstrate different levels of chromosomal polysomy in combination with changing patterns of distinct locus specific copy number changes within three-dimensional territories of individual tumour cell nuclei being dependent upon each other. The study of such complex genetic variation can elucidate critical determinants in cancer, which could have future implications for diagnostic, preventive and early intervention strategies.

The Hansemann–Boveri Hypothesis on the Origin of Tumours

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Cancer is now known to be a genetic disease. In tumour development, cell nuclei undergo mutations, which can result in cytologically visible chromosome aberrations. The aneuploid errors may involve amplification or deletion of whole chromosomes or segments thereof. David Hansemann [1858 – 1920, anatomist in Berlin] and Theodor Boveri [1862 – 1915, zoologist in Würzburg] were major contributors to early debates on the relationship between chromosomal defects, tumorigenesis and malignancies. In 1890, Hansemann observed asymmetric nuclear divisions in human epithelial cancers. In these abnormal, but bipolar, divisions, a fraction of the chromosomes failed to segregate properly. However, he remained a lifelong sceptic with regard to whether such events could be considered the underlying cause of tumours. Almost a quarter of a century after Hansemann's initial observations, Boveri considered the origin of tumours based on his earlier recognition of the functional specificity of each chromosome. He also explicitly drew on Hansemann's observations in proposing a model for tumorigenesis. Its central tenet was that a tumour typically originates from a single cell that has inherited a defined, but incorrectly combined, set of chromosomes. The rare occurrence of a pluripolar spindle represented Boveri's paradigm for a type of abnormal mitosis that can produce a host of random chromosomal combinations. He suggested that some of these combinations will induce tumorous transformation, and will inevitably arise occasionally. Since pluripolar and unbalanced bipolar divisions fail to distribute the hereditary chromatic material correctly, Boveri suggested that both of these mechanisms could give rise to tumour progenitors. Nowadays, aneuploidy of interphase nuclei in tumours is quantified by microphotometry.

Flow cytometric characterization of a new monoclonal antibody specific to fetal erythroid cells

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Fetal nucleated red blood cells (FNRBCs) are in the focus of interest for non-invasive prenatal diagnostics. The lack of markers which specifically identify fetal cells circulating in maternal peripheral blood is the crucial obstacle for the development of a reliable diagnostic method. The aim of this approach was to generate a monoclonal antibody (mab) that exclusively labels fetal erythroid cells.

Therefore, B-cell-hybridoma clones were screened on pooled mononuclear cord blood cells. The screening procedure, a six parameter flow cytometric analysis, included the simultaneous identification of erythroid precursor cells, leukocytes, enucleated erythrocytes and antibodies reacting specifically with FNRBCs. Besides immunohistochemical analyses the binding of the new mab was checked by flow cytometric examinations of mononuclear cells of adult blood, adult bone marrow and erythroid cells lines K-562 and KMOE.

A clone with specificity for a surface antigen exclusively expressed on fetal erythroid cells has been identified. Detailed examinations showed no surface reactivity with adult erythrocytes, erythroblasts or lymphatic and myeloid cells. Additionally, well-known markers for erythroid cells and major blood group antigens could be excluded as potential antigens for the new antibody. Moreover, the flow experiments helped to set up an expression profile of the new mab in comparison to well-known surface markers like the transferrin receptor (CD71) and glycophorin A.

The investigations showed that the new mab specifically binds differentiated fetal erythroid cells. Because of the expression of the related antigen on FNRBCs a non-invasive prenatal diagnostic may be feasible. This antibody may be useful for different enrichment techniques and for the identification and characterization of fetal erythroid cells.

Poster Exhibition (Abstracts in alphabetical sequence)

Affinity of individual Saccharomyces cerevisiae cells to glucose during transient state cultivations

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Saccharomyces cerevisiae is a widely employed microorganism in biotechnological processes. Since proliferation 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 the S. cerevisiae H155 cells' affinity to extracellular glucose concentrations.

The fluorescent glucose analogue 2-NBD-glucose was employed as a functional parameter to analyse the cells' affinity to glucose. Structural parameters (proliferation, neutral lipid content, granularity, and cell size) were additionally investigated. Cells were grown in chemostat regimes.

The 2-NBDglucose uptake in individual cells proceeds in a time- and concentration-dependent manner and is affected by respiratory and respiro-fermentative modes of growth. The affinity of the individual cells to 2-NBD-glucose was found to be high at low extracellular glucose concentrations, and weak at high concentrations. An additional, underlying pattern in the cells' affinity to glucose was detected, illustrated by the recurrent appearance of two sub-populations showing distinctly differing quantities of this substrate.

A multiparameter flow cytometry approach is presented that enables, for the first time, for analysis of the affinity of individual S. cerevisiae cells to glucose. Besides the adjustment of the yeast cell metabolism to extracellular glucose concentrations by altering their affinity to glucose, at least one further mechanism is clearly involved. Two sub-populations of cells were resolved, with different affinities not correlated with other cellular parameters measured.

A double staining method with Hoechst 33342, known as a vital DNA dye, and 2-NBD-glucose shows that the individual quantities of 2-NBD-glucose can not obviously related to a stage in cell cycle.

Microfluorimetry of the DNA content in individual human chromosomes, using an image analyzer

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We are proposing a method of determination of the DNA content in individual human chromosomes. The method includes three main stages: 1) identification of chromosomes stained differentially with quinacrine mustard, 2) treatment of chromosomes by Feulgen, using the Schiff-type reagent Auramine00-SO2, 3) microfluorimetry of the DNA content in individual chromosomes, using an image analyzer. Metaphase chromosomes were obtained from primary culture of peripheral blood lymphocytes of healthy people by the method of Moorhead et al. (1960). The chromosomes were identified in preparations stained with quinacrine mustard by the method of Caspersson et al. (1970). After identification of chromosomes, the dye was removed using a mixture of methanol-glacial acetic acid-water (1:1:1), and the preparations were stained by Feulgen, using the Schiff-type reagent Auramine00-SO2 (Kudryavtsev, Rozanov, 1974). Identification of Q-stained chromosomes and measurement of their DNA c!
ontent after the Feulgen staining were performed using an image analyzer (Shtein et al., 1998). Monitoring of the camera, feeding of image from preparation, its correction and storage, and measurements of densitometrical and morphometrical parameters were performed using a software of the VideoTest Company (St. Petersburg). Integral fluorescence of the chromosome 2 in each metaphase plate was taken as unit. Relative to it, the DNA content was calculated in other chromosomes of the karyotype. Comparison of the results of the DNA content determination in individual human chromosomes obtained by means of our method indicates them to correspond to data of other authors. The proposed method has a sufficiently high efficiency and allows detecting differences of the DNA content in chromosomes of the order of 1-2 fg.

Automated Three Color CD4/CD8 Analysis of Leukocytes by Scanning Fluorescent Microscopy using quantumdots

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Background: Flow cytometer and Laser scanning cytometer are usually used for the three color phenotyping of leukocytes. The Scanning Fluorescent Microscope (SFM), the new technique for automated motorized microscopes could also be able to measure triple fluorescent labeled slides (Bocsi et al. Cytometry 2004). CD4+-CD8+ lymphocyte cell count and ratio are important features of the immune system for immune diagnistics. With the spread of HIV there is emerging demand for automatic methods to determine the T-Helper/ T-suppressor cell ratio.
Aims: Development of triple fluorescent labeling method for leukocytes and application of SFM for their automated analysis and counting on peripheral blood specimen. Study the bleaching of some currently developed dyes.
Materials and methods: EDTA anticoagulated blood samples were stained by the whole blood method by CD4 PE-Alexa-610 (Caltag) and CD8 FITC (BD-Biosciences) antibodies, parallel staining was made by CD4 FITC (BD-Biosciences) and CD8 biotin/Streptavidin Qdot-605 or Qdot-655. An aliquot was measured by flow cytometer. The remaining suspension was transferred to glass slides and antifade mounting medium was used for saving the fluorescence. The specimens were scanned and digitized in the three fluorescent channels. Automated cell detection, CD4 and CD8 detection was performed, ratio was calculated.
Results: Fluorescence signals were well separable. Bleaching of dyes were affected by mounting. Significant correlation between the SFM and FCM CD4/CD8 ratio results could be observed ($p<0,05$).
Conclusions: Triple fluorescent labeling and automated SFM is an applicable tool for the CD4/CD8 ratio determination in peripheral blood samples.

MedisEL laser-scan-cytometer (LSC) optimizing chemo-therapy

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CellScan from MedisEL Co. is a novell Laser Scan Cytomer(LSC).
It contains an high resolution Laser Scanner combined with a fine CCD camera and a PC controlled system.
The reflected laser beam from tissue is digitized and stored in a unique computer file. According to reflection and polarization, the cell status is being defined and classified.
New about this device is that Chemo-medications can be applied to the tissue, scanned and evaluated.
According to the scan results, the best medication could be located among many possible.
This makes therapy more transparent, saves time and money and decreases therapy duration, mostly raising Chemo-therapy results. Systems are already installed in Europe and USA.

Online analysis of yeast cell cultivations

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Online measurements of cultivations are an increasing issue in biotechnology. They help to improve the systems e.g. via regulation or by determining the optimal time to harvest the product.

In our approach we used an oscillating Saccharomyces cervisae as model strain for cultivation. Besides the off gas analytic as online method an in-situ microscope and 2D-fluorescence spectroscopy has been used.

The 2D-fluorescence spectrometer is used for the continuous monitoring of all fluorophores present in the cell culture medium covering a wide range of excitation and emission wavelengths. The spectrometer is connected to a standard 25 mm port to the reactor and as a multichannel fluorescence detector it excites at 270 – 550 nm at 20 nm intervals and collects the fluorescence signals at 310 – 590 nm at 20 nm intervals. Therefore a whole spectrum consists of 150 data points and is collected within 1 minute.

The in-situ microscope is based on a direct light technique and consists of a measuring chamber and a digital camera and has been used to determine the cell density and cell size. The data obtained are processed by an automatic data image analysis system. It is also connected via a 25 mm port to the reactor.

As a reference flow cytometry has been used offline to determine the DNA, protein and lipid content of the yeast cells. The data obtained by the 2D-Fluorescence spectroscopy and the flow cytometry have been used to generate a chemo metric model which describes online the distribution off the yeast cells in the cell cycle.

Currently a method is being developed to distinguish live yeast cells by their cell cycle state and sort them for further analysis by chip technology. Further investigations should result in developing a chemo metric model which is able to describe online the DNA expression in the yeast cell culture.

Radioresistant tumours have increased capacity of recombination DNA repair by polyploid cells and employ this mechanism for the DNA sorting

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We have recently shown the potential of endopolyploidy to repair DNA by homologous recombination (HR) and to support cell survival. In the present work the relationship between DNA repair by HR and mechanism of the DNA sorting from endopolyploid cells were studied in human lymphoblastoid cell lines. Cells were irradiated with 5 and 10 Gy and stained for Rad 51, Rad 52, g-H2AX and DNA (DAPI). Ploidy was determined by the nuclear area, functional repair foci - as bright round sharply delineated Rad51 spots colocalized with g-H2AX. The mean nuclear area of Rad51 component is increasing at 24-48 h in radioresistant WI-L2-NS cells (with increasing of ploidy) while it is decreasing in its radiosensitive counterpart, TK6. WI-L2-NS cells displayed a higher capacity than TK6 cells to form complex functional foci of DNA repair which were heterogenous by size. The largest were seen releasing as micronuclei with tight association between Rad51 and g-H2AX. Another mechanis!

m of DNA sorting was elaborated by resistant cells after higher DNA damage (10 Gy). Large domains of the chromatin were sequestered in autophagosomes of alive giant cells accompanied by disarranging suprastructures of Rad52 and Rad51 recombinases. Thus endoreduplication is employed in DNA repair by HR in resistant tumour and is not in the radiosensitive one. Besides tumour cells are capable to form megafoci of repair which can be released as micronuclei.

Influence of live strategy, habitats and nutritive supply on endopolyploidy in higher plants

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The phenomena endopolyploidy is widespread over the plant kingdom and is present in more than 90% of angiosperms. It can be defined as an increase in the number of chromosome sets caused by replication without cell division. It is not clear whether endopolyploidy is more genetically or more environmentally dependent. Some authors suggested that endoreduplication is influenced by plant age, phytochromes and environmental conditions, such as water, temperature and nutrition.

In this study we report about the effect of untypical life strategy, habitats and nutritions on the degree of endopolyploidy of some chosen species of plant families, known as predominantly endopolyploid or non-endopolyploid.

Plants grown on sand substrate were clearly smaller than plants growing on standard potting soil. Arabidopsis thaliana and Barbarea stricta (fam. Brassicaceae) and Bidens tripartita (fam. Asteraceae) shows organ specify in the degree of endopolyploidy and weak differences in the cultivation variants without changes of the common endopolyploidy state of their family.

Species with untypical growing characteristics were compared with other species from the same family.

Only one of all examined species shows opposite state of endopolyploidization. Galinsoga parviflora is an annual herb and likes nitrogen-rich disturbed habitats and shows to be endopolyploid in cotyledons, under leafs and flowers.

Data shows that the family affinity is stronger important for the state of endopolyploidy than nutritive supply, life strategy and habitat.

Development of anti-kinin B1 receptor aptamers as a tool for imaging and therapeutics

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The objective of this present work is the identification of non-peptidergic 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 upon presentation to bacterial lipopolysaccharides, stable high-affinity inhibitors of the receptor function would help to halt inflammation process where B1 receptor activity is involved. 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-Arg9-bradykinin. The post SELEX 6-RNA pool labeled in Western blot analysis targeted a protein of 38 kDa (in accordance with the molecular weight of the B1 receptor), that was not present in non-transfected cell!

s. For use in cytometry research, nuclease-resistant RNA aptamers are conjugated with FITC-biotin-streptavidin and tested for their specificity with vascular smooth muscle cells expressing the B1 receptor and CHO cells which do not express the receptor.

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Monitoring ploidy patterns in the arctic-alpine polyploid *Draba lactea* Adams (Brassicaceae) and its low-ploid relatives

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Ploidal level information is of particular importance in intricate complexes such as in arctic-alpine *Draba*. Relative DNA content was measured for the tetra- and hexaploid *D. lactea* and seven of its low-ploid relatives, in altogether 200 plants from 93 populations, using rapid flow cytometry screening. Absolute DNA content (C-value) was determined by internally standardised Feulgen densitometry (image analysis) for 13 plants from seven species. In addition, reference chromosome numbers were determined in 12 plants representing six species. The plants grouped into diploids ($2n = 16$), tetraploids ($2n = 32$), hexaploids ($2n = 48$), and two odd triploids. The diploid level was confirmed in *D. nivalis*, *D. subcapitata*, *D. fladnizensis*, and *D. lonchocarpa*. *Draba palanderiana,* previously reported as di-, tetra- and octoploid, was diploid in all investigated accessions. Hexa- and tetraploids were observed in *D. lactea*, in approximately the same ratio (8:1) as previously reported. The ploidal levels of the Central Asian *D. altaica* and *D. turczaninovii* were reported for the first time, as diploid and tetraploid, respectively. Whereas the polyploid *D. lactea* occurs both as hexa- and tetraploid, ploidal levels seem constant for the diploid taxa. The variation both within and between species in relative DNA content as well as the deviations from absolute DNA content could be caused by the base preferency of the fluorochrome DAPI used in the flow cytometry screening. This method is sufficient for ploidy analyses but not for obtaining C-value data. In the present study, the DNA amounts of the polyploids increased in direct proportion to ploidal level. In combination with molecular data, these results are pointing to a relatively recent polyploid origin. For a more detailed investigation of occurrence and extent of intra- and interspecific C-value variation in *Draba*, a separate study focusing on this topic is necessary.

3D-image analysis of the topology of chromatin in cell nuclei of formalin-fixed, paraffin-embedded tissue sections

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The higher order chromatin architecture is not completely understood especially in correlation to tumour induction and progression. By means of appropriate FISH labelling and high resolution microscopy of cell nuclei details of the nuclear architecture can be further elucidated. Therefore, novel tools are required to analyse 3D-digital images and to determine appropriate parameters for the quantification of the genome architecture and organisation and their changes. A versatile software package with a user friendly shell script was developed for the analysis of FISH labelled cell nuclei after 3D-fluorescence microscopy using e.g. a confocal laser scanning microscope or an Apotome epi-fluorescence microscope. After image acquisition of optical sections through the tissue specimen, the cell nuclei were segmented interactively by visual inspection, whereas the segmentation of FISH signals was based on a local 26 relationship. The criterion for a voxel to be inside a local 26 neighbourhood of another voxel was, that the voxels must be connected at their sides, edges or corners. To ensure that measurements of image parameters are threshold independent, the whole intensity range was analysed. It was feasible to get information about the topology and the interior organisation of chromatin, e.g. the radial position in the cell nucleus, relative distances to other markers, or the shape of the labelled site etc. The software was successfully tested and applied on several chromosome territories and gene loci in human cells. Territories of chromosome #8 in nuclei of pancreatic adenocarcinoma cells in formalin-fixed, paraffin-embedded tissue sections were studied in more detail. The results indicate the possibility to characterise differences in the architecture and organisation of the genome in normal and aberrant cell nuclei.

High Throughput Viability and Cell Cycle Analysis by RNAi using FACS

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After the completion of whole genome sequences, a key challenge remains to address the functional properties of each predicted gene. Model organisms and cell culture systems have contributed significantly to our current models of cellular pathways and one of the important next steps will be to use systematic approaches to dissect gene function on a genome-wide scale.

Previously, we have used RNA interference in Drosophila cells as a model system to rapidly identify genes that are required for cell growth and viability. We have developed a library of RNAi reagents that target every predicted gene in the Drosophila genome. Cell growth and viability was measured using homogenous assay formats (1). As a next step, we are developing high-content screening approaches using flow cytometry to asses the proportions of cells in different parts of the cell cycle, as well as cells having abnormal DNA amounts.

RNAi against every gene in the library is performed in a high-throughput format and depletion of corresponding transcript and specific phenotype is detected by BD 96-well FACSArray ™ Bioanalyzer. We will present methods and results for automated procedures for RNAi, staining steps and phenotype analysis by flow cytometry.

(1) Boutros et al. (2004). Genome-wide RNAi Analysis of Growth and Viability in Drosophila Cells. Science 306:832-835