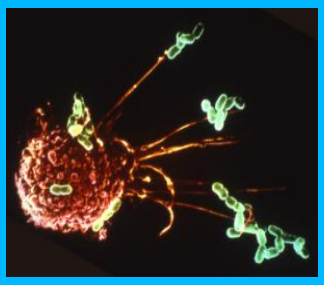


2nd Münster Conference on

Single Cell Analysis

Progress in Research and Technology

Münster, December 1-2, 2005

Program

Thursday, Dec. 1

Opening and Introduction **Georg Peters, Münster**

17:00 **James H. Eberwine, University of Pennsylvania**
Molecular biology of the single cell: Unique insight into cellular functioning

18:00 **Get-together**

Friday, Dec. 2

Introduction The organizers

8:30 **Edgar A. Arriaga, University of Minnesota**
Heterogeneity in the mitochondria of single cells revealed by individual organelle capillary electrophoretic analysis

Session I: Genomics Chair: Heinrich F. Arlinghaus, Münster

09:00 **Ralf Küppers, University Clinic Essen**
Gene expression profiling of microdissected Hodgkin and Reed/Sternberg cells of Hodgkin´s lymphoma

09:30 **Christoph Klein, Ludwig Maximilian University Munich**
Combined genome and transcriptome analysis of single disseminated tumor cells

10:00 **Jörg D. Hoheisel, DKFZ Heidelberg**
Toward single-molecule detection on DNA-, RNA- and protein-level

10:30 *Coffee Break*

Session II: Proteomics Chair: Klaus Dreisewerd. Münster

10:45 **Jørn Koch, Aarhus University**
Single cell and single molecule analysis in cell and tissue preparations

11:15 **Ka Wan Li, University of Amsterdam**

Analysis of a simple neural circuit by single cell profiling

11:45 **Dave A. Barrett, University of Nottingham**
Combined optical trapping and chemical analysis of single cells within a capillar

12:15 **Jonas Bergquist, University of Uppsala**
Analytical tools for the single cell

12:45 Lunch Break

Session III: Technologies

Chair: Cornelia Denz, Münster

14:00 **Ulrich Kubitschek, University of Bonn**
Intracellular dynamics of single molecules

14:30 **Claus Seidel, University of Düsseldorf**
Multiparameter fluorescence spectroscopy and imaging with single-molecule sensitivity

15:00 **Gert von Bally, University of Münster**
Digital holographic microscopy – a new technology for living single cell analysis

15:30 *Coffee Break*

Instrumentation

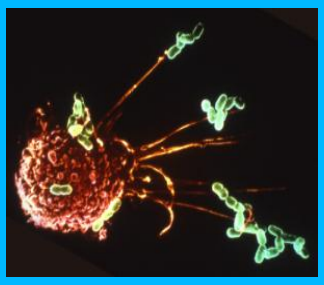
Chair: Simone König, Münster

15:45 **Andreas Bosio, Miltenyi Biotec**
Gene expression profiling of single and few cells sorted by MACS, FACS and microdissection

16:00 **Detlev Suckau, Bruker Daltonics**
High-speed MALDI-TOF imaging: The future of biomarkerdiscovery and proteomics?

16:15 **Marc Kipping, Waters Corporation**
New horizons by the use of parallel qualitative and quantitative proteomics without isotopic labeling

16:30 **Yilman Niyaz, PALM Microlaser Technlogies**
Laser based isolation of single living cells – New approaches in cell and molecular biology



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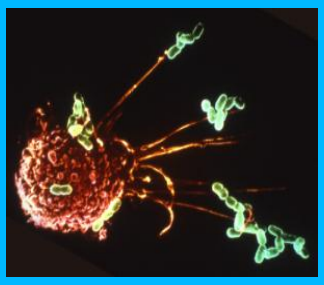
"Quantitative Single Cell Molecular Biology"

James Eberwine, Kevin Miyashiro, Lindy Barrett, Theresa Kannanayakal, Jeanine Jochems, Tiina Peritz, Kalle Kilk, Emelia Eriksdottir, Ulo Langel, Jai-Yoon Sul, Hajime Takano, Phillip Hayden, Elizabeth Van Bockstaele and Jason Glanzer, University of Pennsylvania, University of Stockholm, Thomas Jefferson University

There are an estimated 1012 neurons and glia in the mammalian brain, the interactions of which give rise to the functional central nervous system. To examine specific neuronal systems we have been developing methodologies that permit single cell resolution and quantitation of mRNA and protein abundances. These approaches including single cell aRNA amplification, single cell PCR and IDAT (immunodetection amplified by RNA polymerase) have recently been complimented with approaches that permit analysis of interactions between proteins and RNA. Using a combination of these methodologies, single dendrite transfection, APRA (antibody positioned RNA amplification) and PAIR (PNA-assisted identification of RNA binding proteins), we have been able to prove that protein synthesis is differentially regulated in distinct subcellular regions and have been able to show that RNA binding proteins differentially bind to the same RNA dependent upon particular neuronal stimulation paradigms. Application of these methodologies to the characterization of live cell physiologies as well as fixed cell biochemical states will be presented. Finally the use of single cells for functional genomics analysis, using novel methodologies, will also be discussed.



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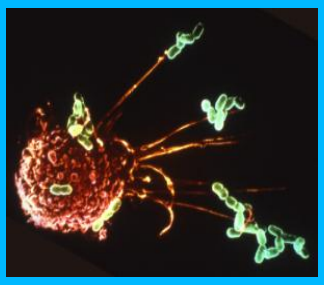
"Heterogeneity in the Mitochondria of Single Cells Revealed by Individual Organelle Capillary Electrophoretic Analysis"

Capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) has been previously utilized to investigate properties of whole single cells. In these studies, the information at the subcellular level is lost because the entire cell contents are solubilized and mixed. In this presentation, we describe a novel approach to analyze by CE-LIF individual mitochondria after their release from a single mammalian cell. The results of this analysis are data clusters that reveal the heterogeneity in the organelle population of the analyzed cell. Data clusters that contain the number of detected organelles per cell, their individual electrophoretic mobilities, and their individual organelle properties measured via fluorescent reporters will be illustrated with studies using the human osteosarcoma 143B and leukemia CCRF-CEM cell lines. The implications of detecting the anti-cancer agent doxorubicin in individual mitochondria from single CCRF-CEM cell lines will also be discussed. The single cell data clusters of individual organelle CE-LIF measurements are a new approach to more comprehensively describe single cell properties.



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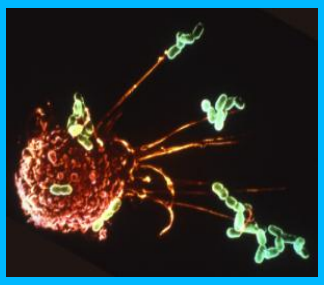
„Gene expression profiling of microdissected Hodgkin and Reed/Sternberg (hrs) cells“

Institute for Cell Biology (Tumor Research), University of Duisburg-Essen Medical School, Essen, Germany

In classical Hogkin lymphoma (cHL) the neoplastic HRS cells represent <1% of the lymph node cellularity. Attempts to reveal the largely unknown pathogenesis of cHL through gene expression profiling have so far been restricted to HL cell lines. However, these lines most likely do not retain all important features of primary HRS cells. To generate gene expression profiles from primary HRS cells, ~1000 HRS cells were laser-microdissected from H&E-stained frozen sections of cHL biopsies. After two rounds of *in vitro* transcription, RNA was hybridized to Affymetrix HG-U133 Plus 2.0 chips. Expression profiles were also generated from similar cell numbers of HL cell lines, microdissected tumour cells from various other B cell lymphomas and lymphocyte-predominant HL (LPHL), and normal mature B-cell subsets that were MACS/FACS-sorted from tonsil or peripheral blood of healthy donors. Unsupervised hierarchical clustering of the first 55 samples grouped the 22 normal B-cell samples separately from the 33 tumour samples. The further branching of the dendrogram showed that each of the four B-cell subsets tended to form discrete clusters, and that, among tumour samples, cell lines grouped apart from primary cases. The latter were further split in two sub-branches: one with diffuse large B cell lmphomas and follicular lymphomas, and the

other mainly comprising HLs. A preliminary supervised comparison of primary HRS with HL cell lines showed a highly differential expression (≥ 4fold change) of ~1200 genes, including many involved in intercellular signaling, chemotaxis, and immune/inflammatory response. These preliminary results suggest that expression profiles can be reliably generated from small numbers of microdissected cells, and that primary HRS cells and HL cell lines differ in a number of biological features.



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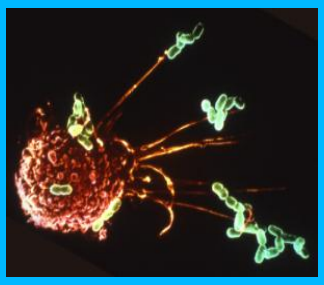
„Combined genome and transcriptome analysis of single disseminated cancer cells“

Single disseminated cancer cells reside in various organs after so-called “curative” surgery and may eventually grow out to lethal metastases. Adjuvant therapies are currently tested to prevent metastatic outgrowth, however detailed knowledge about the target cells is lacking. Unfortunately, the analysis of the progenitor cells of metastases is hampered by their extreme rareness, because they are detected in bone marrow or lymph nodes at a frequency of 10^{-5} to 10^{-6}. Therefore, we started to develop techniques for the study of single cells and to investigate the early stages of systemic tumor progression. Thus far we succeeded in establishing protocols for global analysis of the genome and transcriptome of a single cell. While genome analysis was initially established for comparative genomic hybridization on metaphase chromosomes, recent development now enables the high-resolution analysis using BAC arrays. Global gene expression analysis of single cells using high-densitiy oligonucleotide arrays has been achieved by several modifications of our previously published protocol. The first results obtained from samples of cancer patients already have an impact on current models about systemic cancer progression. We show that dissemination is a very early event in the genomic and morphologic development of a tumor and provide novel strategies to search for efficient target structures of adjuvant therapies.



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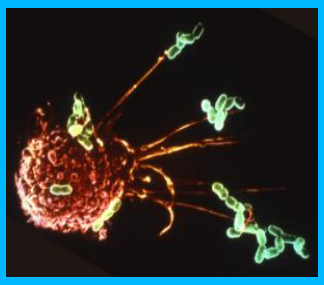
„Toward single-molecule detection on DNA-, RNA- and protein-level“

In the DKFZ Division of Functional Genome Analysis, we are developing technologies for the identification, description and evaluation of cellular functions and their regulation by producing and processing biological information on a genomic scale. One emphasis in our efforts is work on DNA-, protein- and peptide-microarrays. Many chemical and biophysical issues are being addressed as part of this work in an attempt to understand the underlying procedural aspects, thereby eventually establishing superior analysis procedures. Concerning the analysis of human material, systems are being developed toward early diagnosis, prognosis and evaluation of the success of disease treatment with accentuation on cancer.

An important issue is the sensitivity of the analyses not only for the purpose of detecting small numbers of molecules but also for achieving a good measurement dynamics. Based on self-assembly processes, we designed a system that allows a very sensitive detection of chip-based analyses, avoiding both labelling and amplification prior to microarray hybridisation. In another approach, secondary ion mass spectrometry is being applied for detecting binding events directly, again avoiding any amplification or labelling. Last, also in protein-protein binding assays, we established conditions, which led to a significant improvement of detection limits.



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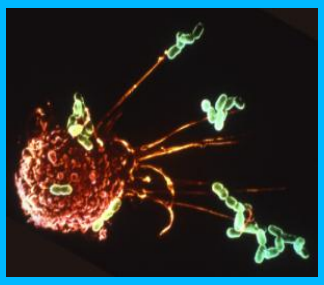
„Single cell and single molecule analysis in cell and tissue preparations."

Molecules do not function in cells by simply existing, but rather by interacting with and affecting other molecules. It is therefore important to know where a molecule is, when it is there, and what other molecules are in the neighbourhood. A rather simple example of this is an mRNA molecule with a mutation disabling its export into the cytoplasm. While such a molecule would exist, possibly in great abundance since the cell might increase its production in response to a lack of effect, it would get no further than the cellular nucleus, and therefore would be unable to mediate the synthesis of the appropriate protein. It is similarly important to know the context in which a cell exists. To control and modulate the functions of the individual cells, any multicellular organism relies on communication between cells. Therefore, no cell exists in splendid isolation, but is constantly influenced by its neighbours. To understand what goes on inside a cell, it may therefore be necessary to know what other cells are present and what they do. A prime example of this is found in embryology, where one single starting cell eventually develops into a great variety of vastly different cells depending upon what organ or tissue they end up in. Many pathological processes can be understood in this context as well. This is not least the case for cancer, in which cancer cells, among other things, rely on ignoring appropriate signalling and on producing their own signalling to fool the organism into not eradicating them.

For the analysis of this, we are developing new detection reactions with probes capable of resolving minor molecular details in bio-molecules and amplification reactions capable of visualising single probe molecule *in situ*. The technology uses oligonucleotide probes to identify targets, if necessary at single nucleotide resolution, and localised DNA synthesis at sites binding the probe as the amplification medium. More particularly, various formats of circular hybridisation probes are employed for target identification, whereupon the target molecule primes an “endless” DNA synthesis in a rolling circle format copying the probe over and over again to produce a long tandem array of DNA extending from the hybridisation target and covalently linked to it. The circular probes can resolve single nucleotide differences in the target molecules, and the rolling circle DNA synthesis produces enough copies of the probe to enable single probe molecules to show up under the microscope.



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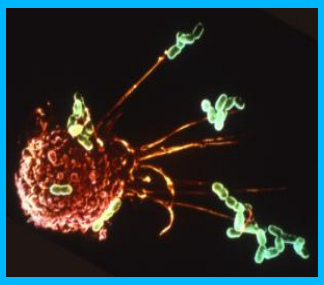
"Analysis of a simple neural circuit by single cell profiling"

Neuropeptides form a large group of signaling molecules with multiple effects on the target cells. Given that even closely apposed neurons may have distinct peptide contents and serve different functions, it becomes clear that the analysis of peptides at the single cell level is the first step toward the understanding of the functioning of the neuronal circuit of interest. In the present study, we utilized the fact that functionally connected neurons share a common nerve innervating the same or overlapping targets, and therefore, they can be retrograde-labeled from the nerve. Next, these labeled cells can be isolated for subsequent analysis. To demonstrate proof of principle, we analyzed single stained neurons from the parietal ganglion of several individual freshwater snail. Direct single cell MALDI-TOF-MS peptide profiling reveals the presence of several putative peptides. From a single labeled neuron, the peptides were structurally characterized by MALDI time-of-flight/time-of-flight MS/MS. Single cell peptide profiling is (semi)quantitative; the relative signal intensities of the distinct peptides displayed on the mass spectrum correspond closely to the expected ratio of these peptides expressed in the cell. Currently, hundreds of single neurons have been analyzed, and reveals that closely apposed neurons often contain overlapping sets of peptides suggesting that these cells may have similar functions. LC-MS/MS analysis of peptides contained in the nerve confirms the transport of these peptide messengers to the target organ.



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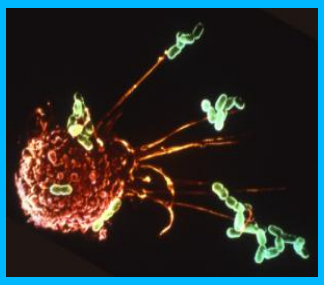
„Combined Optical Trapping and Chemical Analysis of Single Cells Within a Capillary“

An approach is described which has potential for combined trapping and chemical analysis of a single cell. Optical tweezers have been used to facilitate the rapid trapping of cells within a narrow glass capillary. A single cell can be isolated and held using a infrared Nd: YAG laser beam focussed through the capillary at a point just above the inner surface. Preliminary studies with polystyrene microspheres (radius 6.8 μm) established the basic parameters required for successful optical trapping. Individual yeast cells (*Saccharomyces Cerevisae*) could be isolated from an injected 100 nL suspension of many thousands of cells, the process taking 2-3 minutes. The relationship between capillary flow rate and the power of the optical trap was measured to optimise the conditions for isolation of single cells. A single cell could be readily held in the optical trap for periods of tens of minutes or more and it was established that cell viability was not compromised. by monitoring yeast budding rates.

This process does not require special micro-manipulation skills; the cells can simply be injected directly into the capillary. This allows rapid cell isolation even for inexperienced users. One a cell is trapped within the capillary, it offers the possibility of directly analysing release of biomolecules from the cell or the entire cell contents after *in situ* lysis using integrated capillary electrophoresis with high sensitivity detection using laser-induced fluorescence or mass spectrometry.



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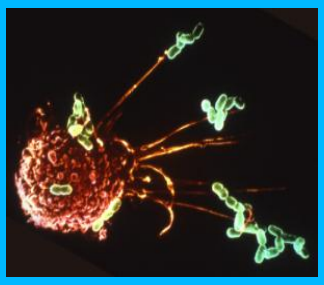
"Analytical tools for the single cell"

Mammalian tissues are composed of very heterogeneous assortments of different cell types and their biologically consequential analysis necessitates the preparation of pure samples of the cells of interest. A variety of microdissection techniques have been used in attempts to overcome the heterogeneity of tissues. Initially, these involved scraping off selected parts the tissue of interest from a histological section, very slow, tedious, and highly operator dependent approaches. In recent years new methods for single cell analysis have improved our possibilities for in depth analysis of what is happening in cells, and why. Clearly cytomics (and its sub-disciplines proteomics and peptidomics), if defined as the global analysis of cell function (or protein and peptide content respectively) at the cellular level, becomes an important addition to the available tools in cell biology. To fully appreciate e.g. single-cell proteomics, consider that a typical mammalian cell is a mere 10 µm in diameter, has a volume of about 500 fL, and contains about 50 pg (2 femtomoles) of total protein. In this presentation mass spectrometric approaches to obtain "fingerprints" of a single-cell proteome or peptidome will be presented. We have developed a methodology able to study on-target grown single cells using MALDI-TOF MS technology that enables studies of variation in protein expression among individual cells, cell-cell interactions, and to follow on-target manipulations of cells including e.g. stimulation with growth factors. Yet another methodological approach utilises a highly sensitive Fourier transform ion cyclotron resonance

mass spectrometry (FTICR MS) system for proteinanalyis in minimal amounts of spinal cord from patients with neurodegenerative disease. This approach also includes the technique of laser microdissection in combination with pressure catapulting (LMPC) for the dissection of samples and specific neurons. This proteomic approach promises to allow the identification of qualitative or quantitative differences in proteins between normal and diseased cells. As well as providing insights into pathogenesis, such information might yield new screening or therapeutic targets.



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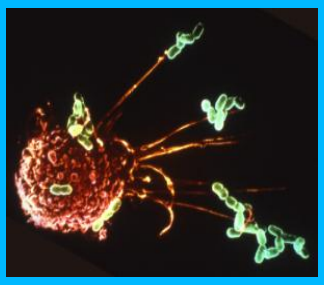
“Intracellular dynamics of single molecules”

The nuclei of mammalian cells are highly organized structures, in which complex tasks like DNA transcription and replication occur in dedicated nuclear domains. We examine the structure and dynamics of such molecular assemblies at the single-molecule level with a time resolution of ≥ 2 ms and spatial precision of ≥ 20 nm using state-of-the-art fluorescence microscopy. New results on the dynamics of the large pore complex in the nuclear envelope, on functional splicing factors, and on non-functional tracer molecules within the cell nucleus will be presented. These examples show that the light-microscopic imaging, tracking and nanolocalization of single protein molecules allows fascinating insights into the intracellular pathways of single protein factors, the structure and dynamics of supramolecular arrangements and the elucidation of biomolecular interactions at the single cell level.



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„Multiparameter Fluorescence Spectroscopy and Imaging with single-molecule Sensitivity“

Using a confocal fluorescence microscope the newly developed multiparameter fluorescence detection (MFD) enables us to simultaneously collect all fluorescence information [1-4]. MFD is applied to perform single-molecule fluorescence-resonance-energy-transfer (FRET) studies on biological systems labeled with a fluorescent donor and acceptor dye. Thus, it is possible to circumvent the classical pitfalls of the FRET method in ensemble measurements (incomplete labelling, uncertainty about assumptions concerning dye anisotropy and quantum yield). Direct access to the time trajectories of the FRET efficiency allows one to reveal the dynamics of the system in real time. Work on HIV-1 Reverse Transcriptase:DNA/DNA complexes and the SNARE-protein Syntaxin and on various DNAs will be presented showing that MFD has developed to a powerful tool in single-molecule biophysics [5-8]. Moreover it will be shown that the MFD principles can also be applied to imaging.

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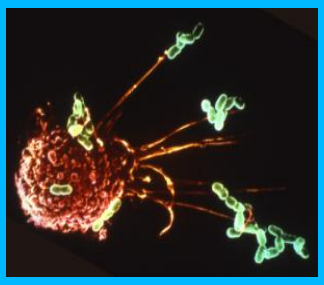

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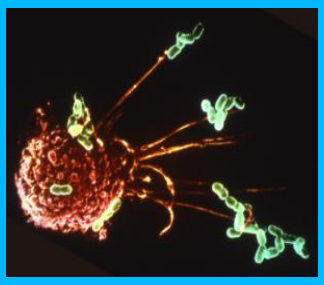
„Digital holographic microscopy - a new technology for living single cell analysis“

Marker free, in vivo screening methods actually gain increasing importance, as well as stand alone analysis technique as in combination with fluorescence labelled methods as reference. Especially nanoholographic methods like digital holographic microscopy provide simultaneously a label free, non destructive, non contactive, full field quantitative online 3D-imaging of living cells with an axial resolution of several nanometers. Therefore, nanointerferometric holographic techniques open new possibilities for the analysis of morphology, form and volume, specifically the analysis of new parameters in dynamic processes like volume changes, micromovements and (localisation of) elasticity differences at supracellular, cellular and subcellular level. In this way, important new application domains in dynamic living cell analysis in the fields of biophotonics, life sciences and pharmacy are opened. At the Laboratory of Biophysics, University of Muenster, modular holographic, nanointerferometric microscopy systems in combination with conventional fluorescence microscopes, laser scanning microscopes and laser micromanipulation systems were developed in cooperation with partners from industry, biology and medicine. Investigations of migration and reaction to drug traffiting of living tumor cells demonstrate a new way of functional imaging by holographic analysis of dynamic processes down to the nanometer scale.



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"Gene expression profiling of single and few cells sorted by MACS, FACS and microdissection"

The molecular analysis of single or few cells is a fascinating topic which is gaining increasing awareness in the scientific community. The demands and possible applications are increasing as both, technologies for cell sorting via MACS, FACS and microdissection as well as tools for molecular analysis like microarrays have reached a high level.

Stem cells, grafted cells or certain neurons have individual characteristics and must therefore sometimes be analysed as a single cell. Furthermore, multiparametric sorting of an ever increasing number of different cell subsets via MACS or FACS results in decreasing numbers of cells for downstream analysis.

We have a established a single cell amplification procedure (SuperAmp) combining bead based mRNA extraction and conversion, global PCR amplification and Klenow fragment labelling. Our amplification protocol circumvents two main experimental obstacles of published protocols: i) all cDNA fragments have a comparable length avoiding a PCR bias due to different transcript length ii) PCR amplification is performed with one single primer avoiding PCR bias due to different primer annealing conditions. To gain sensitivity, all reactions are performed in a volume of 10 - 25 µl.

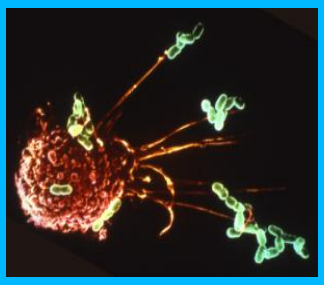
In more than 200 set up experiments we have varied and optimised all possible parameters. The resulting protocol shows a robust amplification down to a single cell. Regression coeffients of samples independently amplified and subsequently hybridised to the same array as well as pearson correlation coeffients of repeated hybridisations of different cells

hybridised against each other are in the range of 0.2 for a single cell, > 0.6 for 10 cells and > 0.9 for 100 cells.

The amplification procedure has been used to perform repeated analysis of 12 different T cell subsets sorted by FACS from the same donor allowing to dissect and compare differentiantion pathways of T cells.

Furthermore, analysis of four different Natural Killer cell subsets sorted by novel magnetic cell sorting (MACS) methods from the same donor have illuminated underlying molecular mechanisms of NK cell identity and reactivity.

The analysis of laser microdissected samples from snap frozen and paraffin embedded tissue has also shown very promising results which are currently repeated and evaluated.



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„High-speed MALDI-TOF imaging: The future of biomarker discovery and proteomics?“

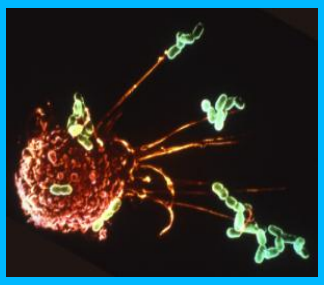
Martin Schürenberg, Sören-Oliver Deininger, Arne Fütterer and Detlev Suckau

Biomarkers detected by clinical proteomics approaches are expected to increase the sensitivity and specificity of diagnostic assays for various diseases. However, the direct analysis of serum/plasma did not provide reliable biomarkers so far that were of diagnostic or prognostic value, as the actual analytical sensitivity is not sufficient to deal with typical biomarker concentrations. The concentration of such biomarkers in diseased tissue, such as cancer, is expected to be a lot higher and more amenable to mass spectrometric detection. Therefore, tissue as a direct platform for marker discovery is becoming very interesting and its direct analysis by MALDI imaging.

MALDI imaging allows the characterization of the protein composition of a number of cells covering approx. 500-5000 µm² - at best 20 µm² - on the MALDI sample carrier. Typically, thin tissue slices from a microtome are deposited on an electrically conductive microscope slide, photographed and then covered by MALDI matrix. The prepared tissue on the microscope slide is then scanned by MALDI with a 200 Hz Smartbeam or a 50 Hz N_2 laser and the spectra are displayed as a heatmap for each selected molecular weight. These maps allow to determine the special distribution of proteins and other biomolecules in the mass range of 500-20,000 Da.

However, the identification of detected biomarkers still requires the isolation of regions of interest from the tissue (e.g., by laser capture micro dissection) and the subsequent isolation/digestion of the markers for subsequent MS analysis.

First examples from recent work will be discussed, covering technical aspects as well as applications in cancer diagnostics and fundamental research.



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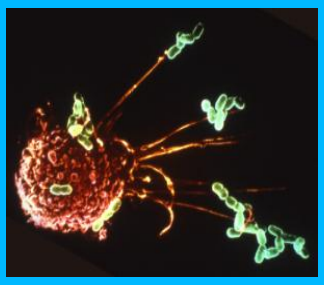
„New horizons by the use of parallel qualitative and quantitative proteomics without isotopic labelling“

Proteins are the key players in living processes of the cell. Mass spectrometry proved to be an ideal technique to identify and characterize cellular proteins with high sensitivity, therefore proteomics was mainly based on qualitative mass spectrometric techniques in the last years. In order to understand the complex interactions from thousands of proteins within a cell it becomes more and more important to get information about quantitative changes of protein expression levels in different cell states or under distinct cellular conditions. So far approaches have been used which utilise stable isotope labelling of samples with separation and subsequent analysis by mass spectrometry. Despite the ability of these techniques to quantitatively compare protein levels between samples and across conditions, complexity of samples, sample amount, speed of analysis and costs are limiting factors. To improve these shortcomings Waters has introduced a label-free technique. The Protein Expression System is an exact mass LC-MS strategy, where quantification is achieved via normalisation of the LC-MS datasets and comparison of the observed tryptic peptide intensities across samples. This completely new approach allows to open up new possibilities in proteomic research.



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„Laser based isolation of single living cells – New approaches in cell and molecular biology“

Y. Niyaz*, M. Stich, A. Buchstaller, R. Gangnus, R. Burgemeister

The understanding of the molecular mechanisms of cellular interactions depends on identification, isolation and finally characterization of a single cell or a population of cells and thereafter of their specific subsets of biomolecules. Thus, the generation of homogeneous samples is a very crucial step for high-resolution downstream applications.

Laser Microdissection and Pressure Catapulting (LMPC) is a well-known method to isolate and collect selected cells from complex human/animal or plant tissues, resulting in an eminent increase in the specificity of downstream molecular analyses like PCR and RT-PCR amplification of DNA or RNA, respectively, and protein analysis via, e.g., MALDI/SELDI spectrometry.

With laser microdissection, focused laser light is utilized to excise selected tissue areas or single cells of a specimen down to a resolution of subcellular components like organelles or chromosomes, respectively. After microdissection, the separated sample is directly transferred against gravity into an appropriate collection device by using the same laser (Laser Pressure Catapulting, LPC). As this process is working entirely without any mechanical contact, LMPC enables pure and homogeneous sample retrieval from e.g., paraffin and cryosections, cell smears and virtually any other preparation on microscope slides.

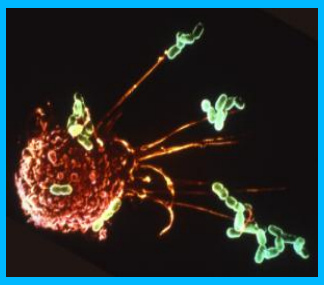
An important innovation is PALMs laser driven isolation of cultured cells. The work with selected living cells is extremely facilitated with this new approach and opens a wide field of new applications and research possibilities. Small groups or individual cultured cells can be separated by morphology or label for direct molecular analysis, clonal expansion or re-cultivation (e.g., stem cells).

Here we present the proof-of-principle for generation of pure stem cell cultures from a heterogeneous, mixed starting culture. Two different murine stem cell lines, either expressing a specific marker (CD-34) or not, were co-cultured. CD-34 expressing cells were then separated by LMPC from those not expressing this marker. These isolated cell types were individually re-cultivated. It could be shown, that generation of pure stem cell lines is feasible with LMPC assisted isolation.

The applied UV-A laser does not affect the viability of the specimens: There is no impairment of viability or biological information, even after several cycles of catapulting and recultivation, as is proven by, e.g., Comparative Genomic Hybridization (CGH) or Multiplex Fluorescence In-Situ Hybridization (M-FISH) analysis.

The efficacy of this method was also recently shown by LMPC-mediated isolation of transgenic murine embryonic stem cells, which were successfully used to generate chimeric mice.

Keywords: Laser Microdissection and Pressure Catapulting (LMPC), high-resolution sample preparation and cell analysis, stem cells



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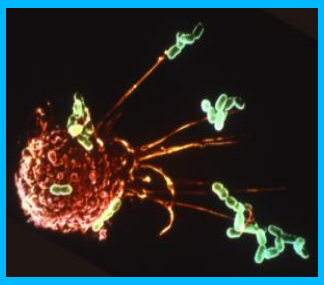

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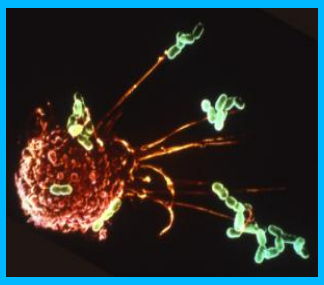

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