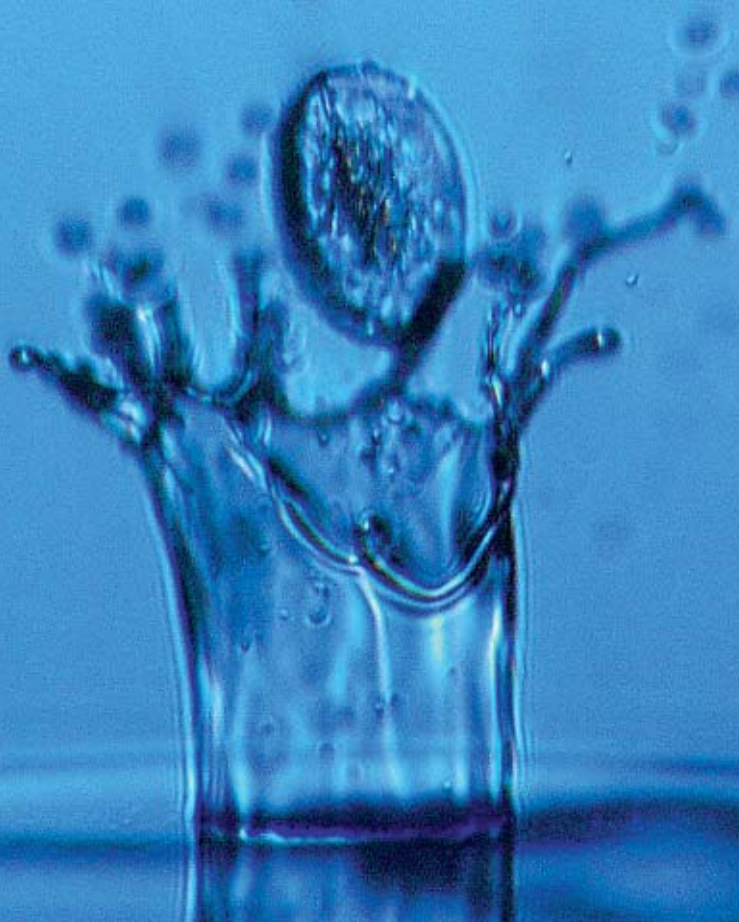
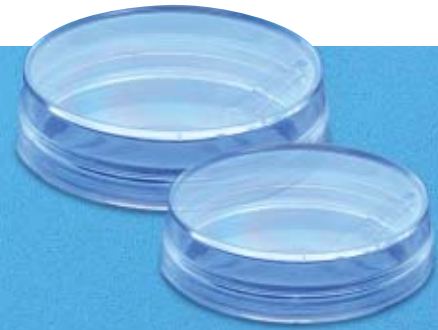


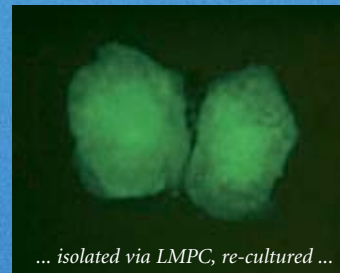
Cell Biology

Cell Culture - Clonal Expansion - Stem Cells

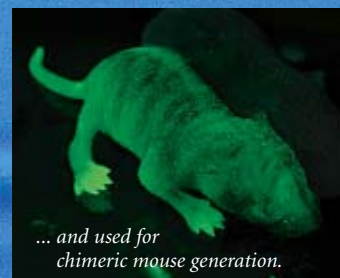
Trypsination steps are usually required to separate and re-culture living cells *in vitro*. Combined Laser Microdissection and Pressure Catapulting (LMPC) is the unique technique that enables single-step cell isolation without the need for Trypsin or a dilution series. Using the LMPC process it is possible to generate pure cultures that are cloned from a single cell. Simply identify the desired cell under the microscope, isolate it with LMPC, and then re-culture. Even embryoid bodies can be recovered with their three dimensional structures intact.



Murine embryonic stem cell
grown on PALM DuplexDish ...



... isolated via LMPC, re-cultured ...



... and used for
chimeric mouse generation.

PALM MicroBeam:
Contact-free Isolation of Living Cells
without Trypsination or Dilution Series



We make it visible.

Visualize Cells with PlasDIC

Image contrast using transmitted light illumination is an essential part of the live cell microscope.

PlasDIC is a completely new contrast technique specially developed by Carl Zeiss for living cell applications. As well as increased depth of field, PlasDIC also enables brilliant and sharp relief-like contrast through plastic cell culture ware.

PlasDIC is the perfect contrast solution for live-cell laser microdissection.



*Visualization
in brightfield...*



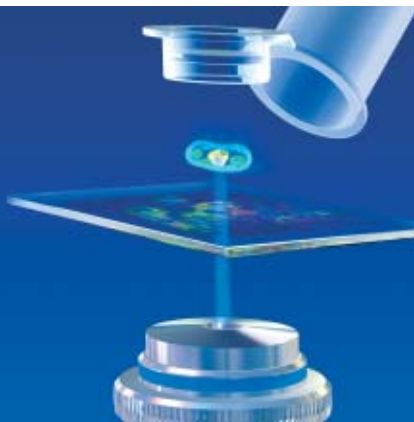
...and in PlasDIC



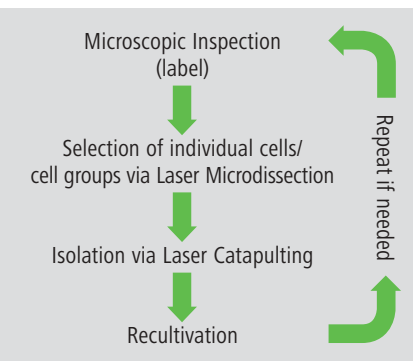
*PALM MicroBeam
from Carl Zeiss:
analyzing purest samples*

Contact-free Contamination-free How LMPC works

PALM MicroBeam from Carl Zeiss enables contact-free sampling from biological specimens including living cells in culture. By means of a precisely controlled laser pulse the selected specimen is vertically ejected out of the object plane and into a collection device - against the force of gravity. This so-called LMPC (Laser Microdissection and Pressure Catapulting) process is unique to the PALM MicroBeam system and has proven to be an invaluable innovation for scientific research. Its non-invasive and contact free mode of action makes LMPC beneficial in the field of cell biology, particularly stem cell research.



Workflow



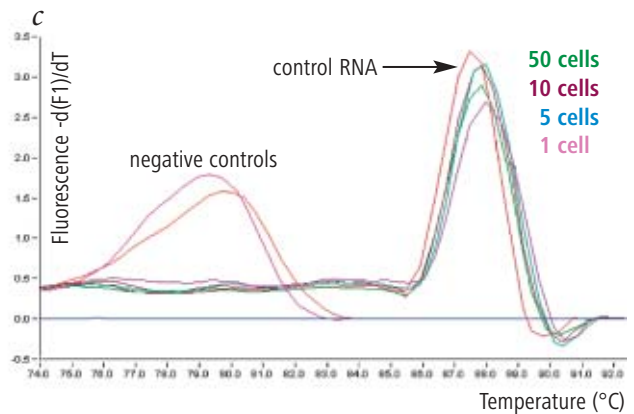
Individual Cell Analysis

Single cell analysis is dependent on purity gained through precision.

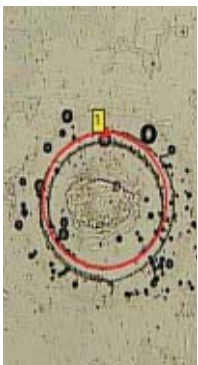
The structural and functional characteristics of cells are dependent on their specific gene expression profile. The ability to study and compare gene expression at the cellular level will provide valuable insights into the physiology of the cell and is useful in a wide range of research and clinical applications.

Even pure cell populations can be far from homogeneous. New methods for single cell analysis have improved the possibilities for detailed analysis of processes inside cells. LMPC combined with different analysis methods allows versatile investigations of individual cells.

- a) EJ28 bladder carcinoma cells are grown on PALM DuplexDish.
- b) One single cell catapulted into a collection device for further mRNA isolation and RT-PCR.
- c) Specific melting curves prove the specificity of the PCR products.



One single murine embryonic stem cell transfected with EGFP for generation of a transgenic mouse (see frontpage). Check for correct cloning by gene-specific PCR.



Isolation of single cell

Green fluorescent single cell inside collection device

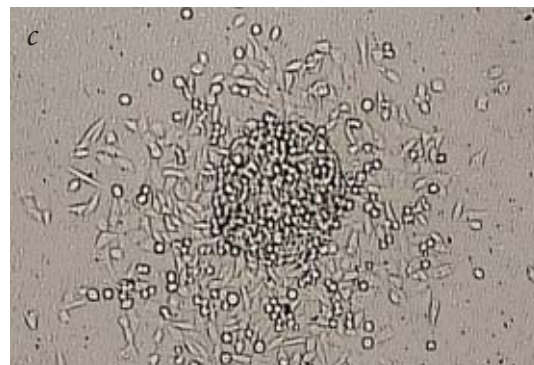
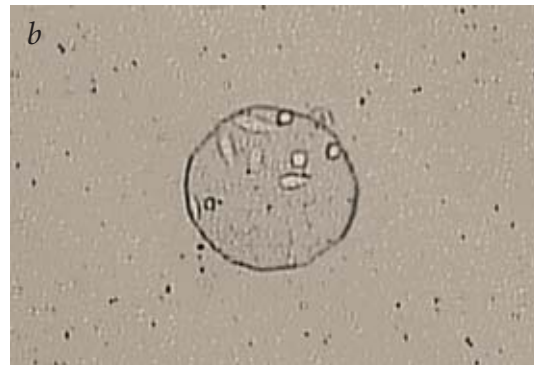
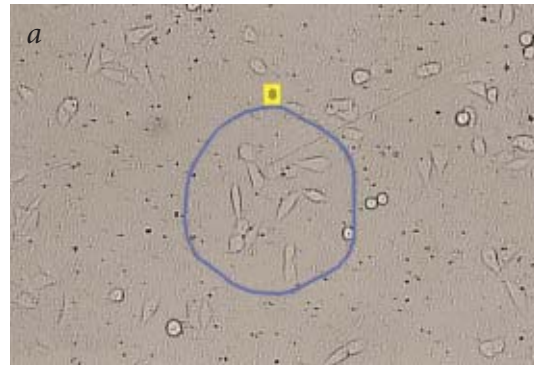
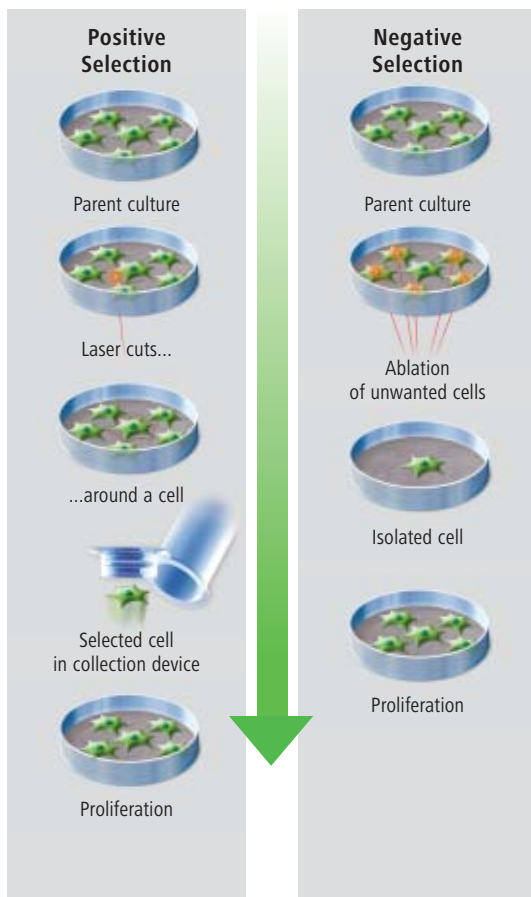
PCR genotyping of inserted EGFP gene

Clonal Expansion of Living Cells

Non-contact isolation of living cells enables easy separation of different cell types from a cell layer for analysis or re-cultivation.

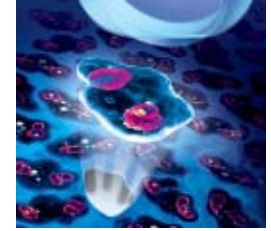
The ability to identify candidate cells for LMPC and clonal expansion is an essential function of the PALM MicroBeam. PlasDIC, Fluorescence, or a combination of both, can be used to discriminate target cells from the background of unwanted material. Access to pure cell colonies enables many new applications in molecular and cell biology as well as in medicine.

Using the 'positive selection' method, desired cells are isolated from the parent culture via LMPC and re-cultivated in a new vessel. The flexibility of PALM MicroBeam enables a second method known as 'negative selection' where unwanted cells can be ablated leaving only the desired cells in the parent culture vessel. Both methods are simple and effective ways to obtain homogeneous populations of a specific cell type.



a) Selected group of EJ28 bladder carcinoma cells.
b) View into the medium filled collection device to check harvested area.
c) Clonal expansion of the dissected area after transfer to fresh culture vessel.

Examples



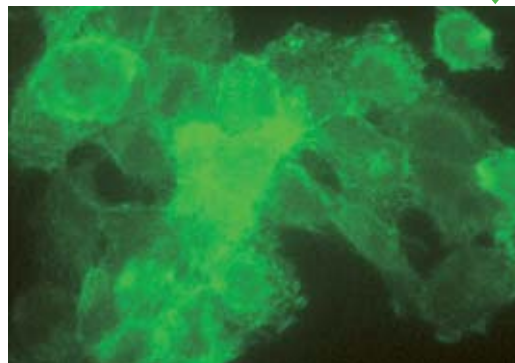
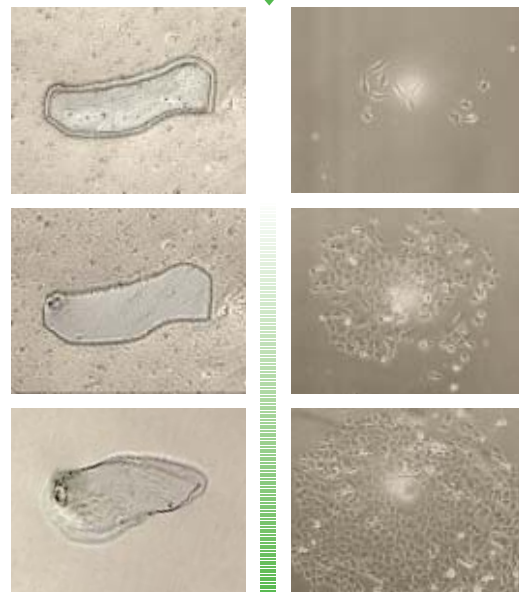
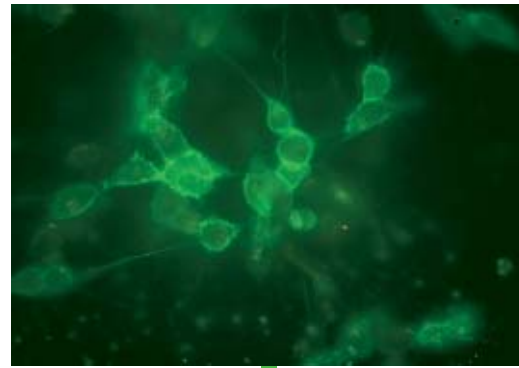
Immunostaining of Living Cells

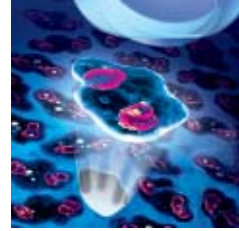
Find the right cells, select and collect.

Many relevant cells in research are commonly complexed with different types of surrounding cells or are rather difficult to distinguish in a mixed culture. The discrimination between those cells often is performed by immunostaining with specific surface markers like fluorescent-labeled antibodies or GFP transfection. The unparalleled fluorescence capabilities of the Axio Observer with AxioCam digital cameras allow for perfect visualization of selected live cells. In addition, the ApoTome from Carl Zeiss can be added to the system for optical sectioning or 3D imaging to gain new insights into your specimen.

Unlike FACS or MACS methods, the PALM MicroBeam allows individual adherent cells to be sorted from a mixture under fluorescent illumination and affords the enrichment through clonal expansion after LMPC. Under microscopic control of the specific marker, the selected cell type becomes accessible for characterization within one single purification step without enzyme treatment i.e. of trypsin or harmful selective reagents. Also time consuming repetitive enrichments can be avoided.

The mixed culture (top panel) was used to separate both cell types back to purity again. One of each cell (middle panel) was isolated using the LMPC technology and recultivated in fresh culture vessels. It could be proven that pure colonies of each cell type were reconstituted again (bottom panel).



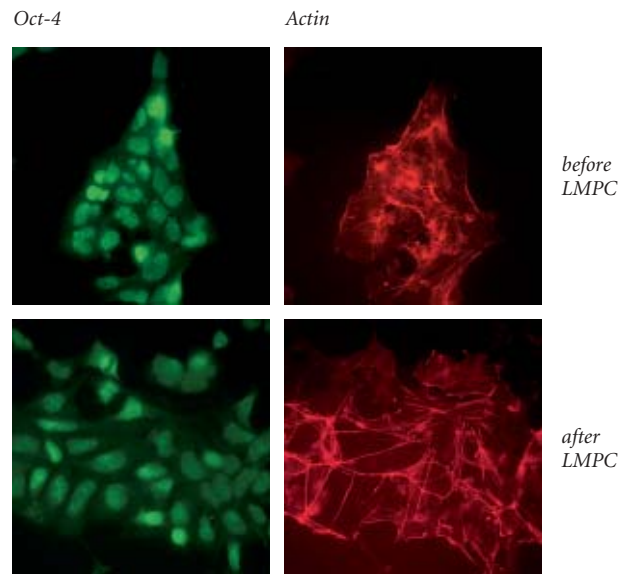


Stem Cell Applications

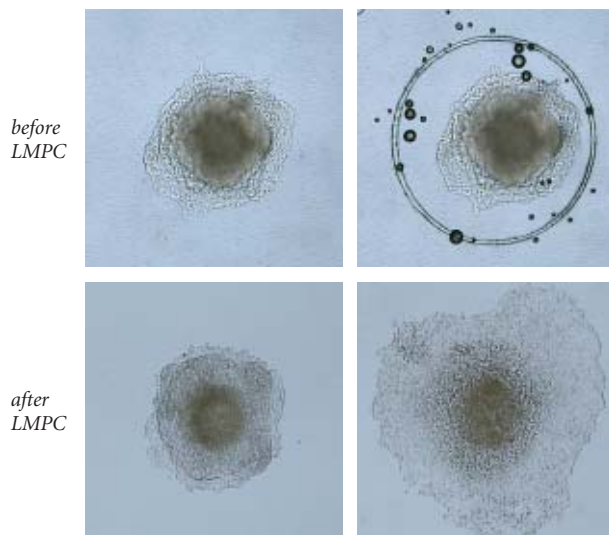
Stem cells are already used extensively; with careful handling and the right conditions, they can yield precious insights into all areas of biomedical research.

Stem cells express a variety of specific pluripotency markers, whose expression levels are significant for an undifferentiated state. Manipulation of embryonic or adult stem cells may trigger differentiation accompanied by changes in morphology and pluripotency marker expression.

By employing the PALM MicroBeam with its unique LMPC technology, stem cells can be isolated and propagated without changing their marker expression. Furthermore, stem cell cultures with diversely differentiated cells can be separated into homogeneous subpopulations, and even embryoid bodies can be sorted.



The murine embryonic stem cell line P19 was immunostained for Oct-4 and Actin before and after re-cultivation following LMPC isolation. The expression level of the pluripotency marker Oct-4 remains unaltered (left panel). Also the cytoskeletal Actin, which served as a control, is unchanged (right panel).



The murine embryonic stem cell line P19 is able to form embryoid bodies (upper left), from which differentiation in, e.g., beating heart muscle cells can be triggered. One of these embryoid bodies was isolated using LMPC (upper right) and recultivated in a fresh culture vessel (lower left). After 3 days, the embryoid body starts proliferation from the outer borders (lower right).



The new generation of PALM MicroBeam



*Hellen C. Ishikawa,
Max Planck Institute of Biochemistry,
Germany*

“I have been using the PALM MicroBeam very successfully. The application was isolation of rare cells expressing GFP-tagged proteins in eukaryotic cells that were hard to transfect. Under these circumstances the transfection efficiency was very low and labelled cells were rare. In the past it has never been possible to purify and recultivate these cells by any method. However, with the LMPC technique of the PALM MicroBeam I was able to generate highly purified transfected cultures.”



*Dr. Gabriela Cezar,
University of Wisconsin-Madison,
USA*

“The heterogeneity observed in differentiating embryonic stem cells stands in the way of obtaining valid in-vitro models for drug discovery. Elimination of undesired cell types by genetic modification is complicated and extremely time-consuming. The innovative PALM MicroBeam technology enabled us to isolate relevant stem cell cultures quickly, precisely and effectively. As a result, pharmacological evaluations could be realized within a very short time frame.”

Provided images in this brochure

Cover picture: The transport pulse is lifting a laser microdissected membrane out of a liquid.

Prof. Dr. A. Vogel, BMO, Lübeck, Germany

Cover sequence and page 4: Generation of a transgenic mouse.

Dr. Wen-Jen Yu, Level Biotechnology Inc., Taiwan

Page 7: Expression experiments.

Dr. A. Buchstaller, LMU München, Germany

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The products described herein are protected

by one or more of the following patents: US 5,689,109

US 5,998,129

US 6,930,764