

Dissection, transport and manipulation: handling cells with the force of light

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The non-contact capture of pure cell populations or tissue samples has become indispensable in modern biomedical research and diagnosis. The combination of laser microdissection and laser catapult fulfils the requirement of many scientists not only in the fields of cell- and developmental biology, molecular pathology and cancer research, but also in botany, in forensic science and even – more recently – in stem cell research.

Lasers implemented in high precision tools commonly play an important role in life sciences and industry. Without contact they penetrate into the depths of a cell and generate images from life processes without harming its viability. Precisely focused laser beams, for example, can “catch, transport and position” microscopic small objects, perform microsurgery within a cell or “drill” minute holes into the cell membrane without touching it. This way,

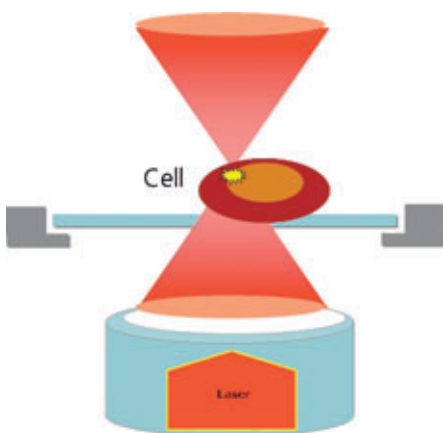


Figure 2: Schematic drawing of Optical Trapping: The micron-sized laser focus catches a much larger cell. Movement of the laser focus causes the cell to follow until suitably positioned. Switching off the laser releases the cell

bio-molecules can be infiltrated without any contact or viral vehicles but also neighbouring cells in contact can be fused.

The non-contact capture of single cells or small cell areas from histological tissue sections or from cell culture is a basic prerequisite in genomic and proteomic research. Cells are precisely microdissected with the laser, ejected from the preparation with one single laser pulse and accurately catapulted into a collection device – all this solely by the force of focussed laser light. These cells are afterwards processed to molecular analyses or – in the case of living cells – are further cultured in corresponding culture vials.

The success of the P.A.L.M. GmbH – founded in 1993 and today a company of Carl Zeiss Microimaging – is due to the technique of laser pressure catapulting (LPC) described here, discovered in 1996 and consecutively advanced to its current precision in subsequent years.

The associated possibilities open a wide spectrum of applications in the entire field of natural sciences, as well as in health care and pharmaceutical research.

The force of focused light

Laser beams coupled into a research microscope and focused through an objective to less than 1 μm in diameter enable the capture or movement of individual cells as well as the high-precision microdissection of tissue, cells or sub-cellular structures or even of inanimate matter (**figure 1**). Depend-

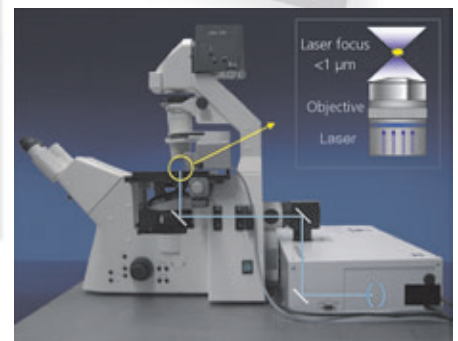


Figure 1: Coupling of the laser through the epifluorescence channel of an inverted research microscope and beam focusing through the objective to less than 1 μm in diameter

ing on the laser system utilised there are two principles of laser micromanipulation available:

• Optical tweezers

For optical tweezers a continuous red or near infrared laser is coupled via the epifluorescence path into the microscope. The extreme focussing of the laser through an objective of high numerical aperture plus its Gaussian beam profile generate the intensity gradient required for optical trapping. Optical tweezers can catch, hold and move microscopic small specimen. For example motile organisms like bacteria or sperm cells can be trapped and kept within the laser focus or individual specimen can be isolated from bulk and pooled for subsequent molecular biological analyses [1]. Additionally, the forces of biomolecular processes can be measured in vitro as well as in vivo without contact [2] (**figure 2**).

• The MicroBeam

The second laser micromanipulation system utilises a pulsed UVA-laser that is coupled via the epifluorescence path into the microscope and focused through the objective down to a few micrometers in diameter. Within the focal point extreme

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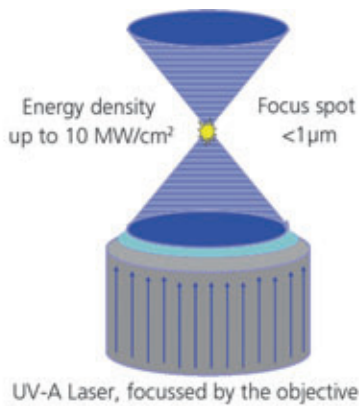
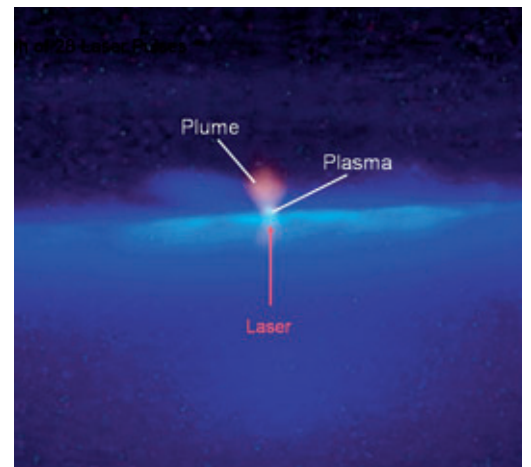


Figure 3: Schematic drawing of optical ablation: Within the narrow laser focus extremely high energy densities are created, whilst beyond the focal point the energy density is not sufficient to cause ablation

energy densities are created that can cut biological and inanimate matter (such as glass) and disrupt it into separate molecules and atoms (**figure 3**).

Laser cutting is based on the physical phenomenon known as “ablative photodecomposition”, a photochemical process initiated by the extreme photon density within the narrow laser focal point. This process results in an ultra-short, adiabatic expanding micro-plasma confined to the sub-micron sized laser focus. Due to extremely high temperature and pressure, all chemical bonds are disrupted within this narrow region generating a gaseous efflux that consists of molecular debris and atoms [3]. The micro-plasma carries a high energy (bluish colour in **figure 4**), whilst the gaseous efflux, also called “plume” (reddish colour in figure 4) comprises less energy [4]. Within the micro-plasma no intact molecules are detectable. Immediately outside the laser focus, however, all the biomolecules and cellular compounds remain undisturbed. Here, the photon density is not sufficient to generate ablation or disruption. In addition, the micro-plasma collapses within a fraction of a nanosecond – a time too short for the transfer of heat into the surroundings. The lack of heat transfer is an important criterion and is the reason why the procedure is called “cold ablation”. The laser activity is confined to the minute focal area, thus enabling micromanipulation and microdissection of fixed or living cells without loss of bio-information or cell viability. Furthermore, the laser wavelength of 355 nm is sufficiently

Figure 4: Formation of a micro-plasma: Within the laser focus a locally restricted micro-plasma (arrow) is created, that collapses within a fraction of nanoseconds – too short for heat transfer. The gaseous efflux (“plume”, red) consists of atoms and molecular debris (high-speed photography courtesy of, A.Vogel, BMO Lübeck)



far away from the absorption maxima of DNA, RNA and proteins, so that neither the genetic nor the proteomic characteristics are altered or influenced. Most biological specimens are transparent at this wavelength, thus permitting manipulation within the interior of living cells [5]. Therapeutically, the phenomenon of “cold ablation” is utilised in the so-called “LASIK” method (Laser In Situ Keratomileusis) to ablate and thus reshape the cornea in order to correct vision errors [6].

Laser micro-surgery is an interesting and important research tool giving new insight into many cellular and developmental questions, whereby the laser may be used to cut chromosomes, both in vitro as well as in vivo, to precisely and selectively micro-inject distinct cells, to open oocytes or to fuse cells or blastomers [7,8]. Both laser principles are very exciting per se and provide highly interesting applica-

tion possibilities within several cell biological areas.

Combined and implemented in a compact, easy to handle laser-microscope system, it opens up entirely new dimensions in the processing of individual cells or sub-cellular particles [9]. For example, it has been shown that optical tweezers could introduce sperm cells into oocytes through the zona pellucida via a drilled hole previously prepared with the microbeam – i.e. artificial insemination solely by the force of light [10].

Sample capture

The PALM-MicroBeam is a unique tool for the quick and safe generation of pure and homogeneous cell samples, contributing to rapid and meaningful analysis. Without difficulty the laser precisely cuts out and separates single cells or small cell areas.

A precisely cut gap between the selected and surrounding material prevents any contamination. Problematic, however, was the proper detachment of the isolated cells and their transfer into a collection device. During experimentation it was repeatedly observed that the isolated cells were flung out of the object plane during the cutting procedure, demonstrating that the same laser could not only be used for microdissection but also to transport the specimen. This effect was patented and has become known as “LMPC – Laser Microdissection and Pressure Catapulting”, subsequently being developed further into an entirely non-contact, reliable and fast laser capture method.

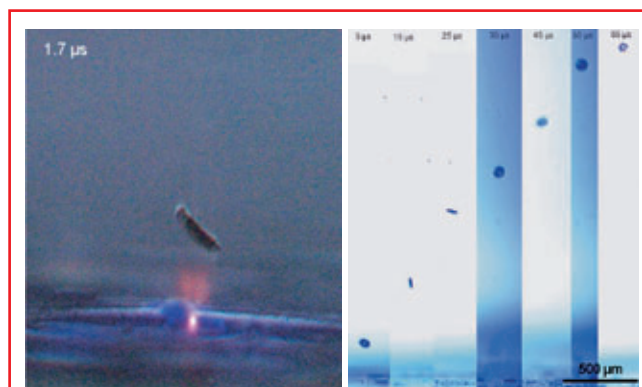


Figure 5: Trajectory: A microdissected cell area (40 µm in diameter) is propelled atop a micro-plasma plume. The image illustrates the glowing micro-plasma and the distance travelled by the sample in 1.7 µs. The linear trajectory is illustrated with high-speed photography of the trajectories of similar samples, showing time intervals from 5 to 95 µs (Images courtesy of A. Vogel, BMO Lübeck)

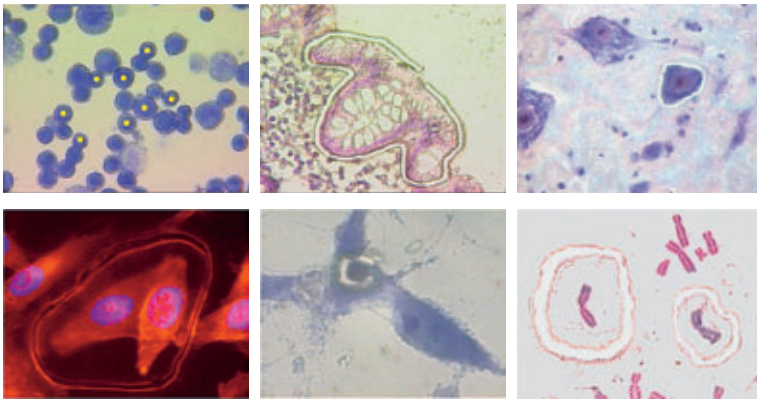


Figure 6: Application examples for laser microdissection (from left), top: cells marked with a yellow dot are catapulted directly from the object slide; entire cell areas are microdissected from a histological tissue section and catapulted with a single laser pulse; single neuron cells are precisely separated from the closely attached glial cells. Below: isolation of individual cells under fluorescence observation; separation of a nucleus from the rest of the cytoplasm; microdissection and catapulting of chromosomes

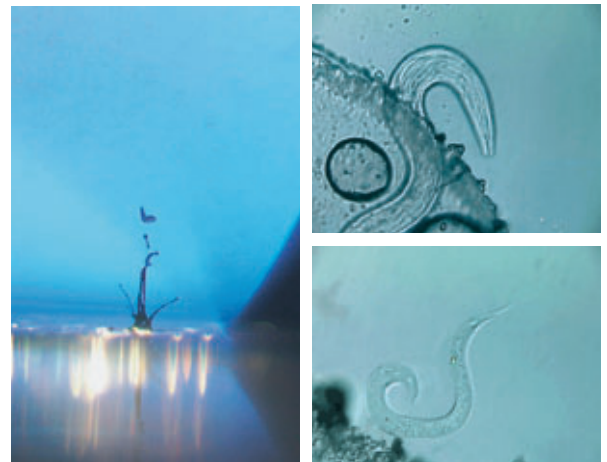


Figure 7: A juvenile nematode (*Caenorhabditis elegans*) catapulted from the culture medium (high-speed photography A. Vogel, BMO Lübeck). After having been catapulted a distance of about 2 mm, the nematode was paralysed for a short time before it happily wiggled away as if nothing had happened

First experiments using laser microdissection and laser catapulting clearly proved that the laser process does not impact even sensitive molecules such as RNA [11]. Thus, LMPC enables the capture of single cells or entire cell areas without violation of their biomolecules. In combination with specially adapted working protocols for small cell amounts the PALM-MicroBeam is meanwhile well established in molecular biological research and biomedical analysis [12].

The physics behind laser catapulting is presumably the same as for the cold ablation process, i.e. the formation of a micro-plasma below the selected area at the border between the specimen carrier (i.e. object slide or supporting membrane) and the isolated specimen sample. The resulting pressure wave and the atomic efflux ("plume") propel the isolated cells out of the object plane and catapult them with high speed into a collection device positioned above.

One single laser pulse is sufficient to detach and propel single cells or even entire cell areas with a diameter up to 1 mm out of the object plane (figure 5)

and provides targeted transport even over some centimetres.

Application examples

This non-contact and contamination-free sample preparation has strongly influenced the scientific world. The allocation of pure cell populations for subsequent molecular biological evaluations or clonal expansion is of immense importance for biomedical research. Only on the basis of pure starting material can meaningful molecular biological analyses be generated and functional correlations established without doubt. These pure samples thus enable faster and more definite diagnosis of malfunctions.

Increasing automation and user-orientated software control facilitate the capture of homogeneous cell samples. Peer referees of scientific journals now require that studies dealing with the evaluation of genetic defects or diseased cells have to be undertaken with selected cell material. This explains the rapid increase on citations of laser "microdissection" within common data banks as well as the comprehensive lists of scientific publica-

tions citing the use of PALM systems (www.palm-microlaser.com).

The fields of application of laser microdissection are manifold, ranging from the isolation of individual chromosomes or various cell compartments, the capture of selected cells from fixed histological tissue sections, up to patient material like sputum, cell smears or biopsies (figure 6).

Notably, even cells from living cell cultures can be captured alive for subsequent clonal expansion and even entire organisms survive the laser based "high speed" transportation with up to 25 m/s, as has been remarkably demonstrated with the successful catapulting of a living nematode (*Caenorhabditis elegans*) (figure 7).

The PALM-MicroBeam has not only become established in molecular pathology and cancer research to study gene expression, but is now rapidly penetrating the botany fields through studies of cellular correlations.

Of particular interest are the recent applications in forensic science, where for example laser isolated and catapulted sperm cells or epithelial cells from fingerprints, sputum or hair are examined and compared with

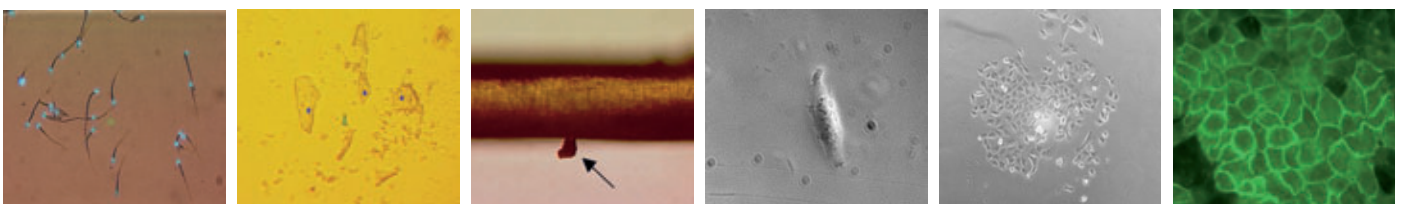


Figure 8: Laser microdissection (from left): sperm from fabrics, marked epithelial cells and cells attached to hairs are catapulted without any problem. Mouse stem cells isolated and catapulted from cell culture – grown into a clone within 10 days – confirmation of stem cell nature through fluorescence tags (courtesy of Dr. A. Buchstaller)

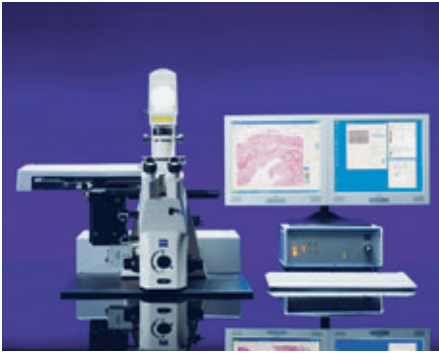


Figure 9: PALM-MicroBeam: consisting of a motorised Axiovert200 microscope, the laser unit in the background and the Robo-Mover as collection device (left) with image analysis software AxioVision (right monitor)

“genetic fingerprinting” to convict a suspect (**figure 8**).

The isolation and “purification” of living cells is especially interesting for stem cell research. Even delicate stem cells can be catapulted without losing their stem cell character, enabling selective isolation of differentiated from non-differentiated stem cells and their capture for ongoing cultivation (**figure 8**). It has been demonstrated that laser capture of embryonic stem cell derived cardiomyocytes, as required for cardiotoxicity tests, saves months in pharmaceutical drug development [13].

The potential of laser micromanipulation has still not been fully tapped. The combination of laser micromanipulation such as microinjection or cell fusion together with LMPC technology in order to capture successfully manipulated cells is a new

challenge that, combined with adequate automation, offers innovative approaches for stem cell research and pharmaceutical drug development with the promise of tailored patient treatments.

The systems

In 1993 an original CombiSystem consisting of optical tweezers and a MicroBeam was developed and provided the basis for the first commercially available system. The combination of “Positioning and Ablation with Laser Microbeams” gave the PALM system its name. Laser energy, laser focus and positioning of the samples within the laser beam had to be controlled manually. Initial product development was undertaken in the family home, with all the rooms doubling as office space, research and development laboratory, manufacturing and quality control assessment areas. An especially important philosophy from the very start was the goal of achieving a synergy between technology and its application so that both can thrive and prosper together.

The new system generation (**figure 9**) is computer controlled and allows even better sample capture with higher throughput. The microscope stage can hold multiple object slides or entire plates in microtiter-format and a cleverly devised robotic system sorts the catapulted specimen in up to 96 individual wells of a specially designed CapturePlate.

Adapted to the composition of the specimen there are different laser functions available that automatically excise, colour-

sort and catapult the outlined cells or cell areas into individual wells. Image enhancing software assists the user in locating the relevant cells (**figure 10**).

Acknowledgement

We would like to thank Prof. Berthold Leibinger for the idea and formation and his daughter Dr. Nicola Leibinger-Kammüller for her committed continuation of the Berthold Leibinger Foundation.

This innovation award is a stimulus for anyone not to waive from the hurdles of the innovation process and to courageously face its challenges.

Hearty thanks also goes to all colleagues of the P.A.L.M. company who have supported the realisation of PALM innovations with energetic engagement and thus have helped to propel the company to international success.

Many thanks to our two daughters Kim and Mayke Schütze who have shared their parents and initially also family rooms with the company, often foregoing family activities for the sake of the company.

We would like to thank Carl Zeiss AG for its financial engagement – the combination of applicative competence with optoelectronic know-how and worldwide presence has greatly benefited the worldwide distribution of PALM technology and its transfer into new application fields.

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Figure 10: Colour-coded areas are catapulted accordingly; Robo-Mover and RoboStage enable improved sample capture with enhanced throughput (image lower right courtesy of Berthold Leibinger Foundation)

Literature:

- [1] A. Ashkin, J.M. Dziedzic, T. Yamane, *Optical trapping and manipulation of single cells using infrared laser beams*, Nature 1987, 330 (6150): 769-771
- [2] A. Ashkin, K. Schütze, J.M. Dziedzic, U. Euteneuer, M. Schliwa, *Force generation of organelle transport measured in vivo by an infrared laser trap*, Nature 1990, 348 (6299): 346-348
- [3] R. Srinivasan, *Ablation of polymers and biological tissue by ultraviolet lasers*, Science 1986, 234: 559-565
- [4] A. Vogel, V. Venugopalan, *Mechanisms of Pulsed Laser Ablation of Biological Tissues*, Chem. Rev. 2003, 103, 577-644
- [5] S. K. Mohanty, A. Rapp, S. Monajembashi, P.K. Gupta, K.O. Greulich, *Comet Assay Measurements of DNA Damage in Cells by Laser Microbeams and Trapping Beams with Wavelengths Spanning a Range of 308 nm to 1064 nm*, Radiat. Res. 2002, 157, 378-385
- [6] W.J. Dupps Jr., S.E. Wilson, *Biomechanics and wound healing in the cornea*, Exp Eye Res., 2006 Oct, 83(4): 709-20
- [7] E.L. Botvinick, M.W. Berns, *Internet-based robotic laser scissors and tweezers microscopy*. Microsc Res Tech. 2005 Oct;68(2):65-74
- [8] A. Clement-Sengewald, K. Schütze, S. Sandow, C. Nevinny, H. Pösl, *PALM Robot-MicroBeam for laser-assisted fertilization, embryo hatching and single-cell prenatal diagnosis*, Photomedicine in Gynecology and Reproduction, 2000, (eds. P. Wyss, Y. Tadir, B.J. Tromberg, U. Haller), Karger, Basel, 340-351
- [9] A. Holzinger, S. Monajembashi, K.O. Greulich, U. Lutz-Meindl, *Impairment of cytoskeleton-dependent vesicle and organelle translocation in green algae: combined use of a microfocused infrared laser as microbeam and optical tweezers*, J Microscopy, 2002 Nov; 208 (Pt2): 77-83
- [10] K. Schütze, A. Clement-Sengewald, *Catch and move-cut or fuse*, Nature, 1994, a, 368: 667-670
- [11] K. Schütze, G. Lahr, *Identification of expressed genes by laser-mediated manipulation of single cells*, Nature Biotechnol, 1998, 16,8: 737-742
- [12] K. Schütze, B. Becker et al., *Part 1: Manual dissection and laser capture microdissection: 307-313, Part 2: Laser pressure catapulting: 331-356*, In DNA Microarrays – A molecular cloning manual (ed. D. Bowtell, J. Sambrook), Chap. 5, Tissue Microdissection, CSHL Press New York, 2002
- [13] K.W. Chaudhary, et al., *Embryonic Stem Cells in Predictive Cardiotoxicity: Laser Capture Microscopy Enables Assay Development*, Toxicological Sciences 2006, 90(1), 149-158

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