

CUSTOMER MAGAZINE FOR LIFE SCIENCE RESEARCH

reSOLUTION

TIRF Microscopy Turned Upside Down

Fluorescence Microscopy
of the Apical Membrane of Polarized Epithelial Cells

Good Vibrations

Perspectives of CARS Microscopy in Life Science Research

Where are Memories Stored?

Mapping Billions of Synapses with Microscopy and Mathematics

Ten Years of Laser Microdissection by Leica Microsystems

Dissection Perfection!

Kerstin Pingel, Leica Microsystems

Laser microdissection is a relatively new technique, having first been developed in the 1990s. It makes it possible to obtain homogenous, ultrapure samples from heterogenous starting material. A researcher can selectively and routinely analyze regions of interest down to single cells to obtain results that are reproducible, and specific. Laser microdissection uses a microscope to visualize individual cells or cell clusters. Regions of interest are selected by software, excised from the surrounding tissue by a laser, and collected by gravity into specialized devices for analysis. There has been enormous progress in the development of laser microdissection instrumentation in recent years and Leica Microsystems is one of the major players.

Leica Microsystems was the first company to use an upright microscope for laser microdissection. The advantage is that material cut out for analysis, regardless of its size or shape, falls due to gravity and lands in a collection container – without additional steps and without contamination.

One of the technological advances that only Leica LMD systems feature is the adjustable laser that cuts even nonstandard specimens like wood, bones, teeth or tissue cultures. Dedicated objectives for the Leica LMD systems guarantee the highest possible laser power. As the laser beam movement is controlled by high precision optics, fast and precise cuts can be made. The microscope stage and the sample both remain fixed. Only Leica LMD systems enable specimens to be cut during fluorescence, because there are separate beam paths for the laser and fluorescence.

Fig. 1: The Leica LMD7000 laser microdissection system combines high laser power and high repetition rates.

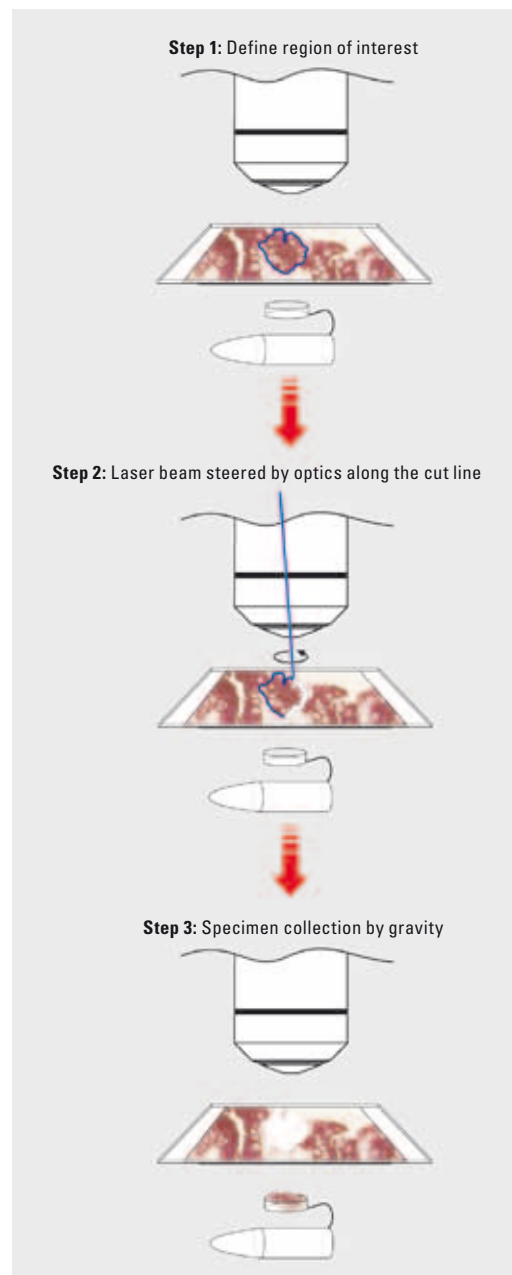


Fig. 2: The process of microdissection

In the year 2000 the first laser microdissection system from Leica Microsystems, the Leica AS LMD, was launched. With the Leica LMD7000, the third generation system and the most successful of Leica

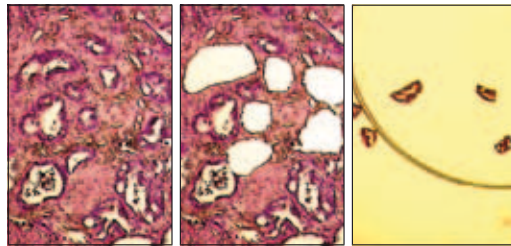
Microsystems' widefield imaging products is now on the market, complementing the product portfolio in addition to Leica LMD6500. Laser microdissection plays a major role in a wide variety of applications. Here, Leica customers report on the research results they have attained by using laser microdissection.

Improving pharmacogenetic analyses of tumor cells

Dr. Niccola Funel from the Department of Surgery, Unit of Experimental Surgical Pathology, University of Pisa, studies the Pancreatic Ductal Adenocarcinoma (PDAC). A key focus of his research is identifying new techniques to tailor adequate treatments.



Fig. 3: Frozen section of Pancreatic ductal adenocarcinoma (PDAC) stained with hematoxylin and Eosin, objective 10x (before cutting, after cutting and inspection)



"PDAC is a very aggressive pancreatic tumor, and overall survival of PDAC patients is very poor. The PDAC tissue exhibits an intense desmoplastic reaction which can mask the true tumor expression. However, the target of drugs is referred to the epithelial cells and not the stromal cells (desmoplastic reaction). We have demonstrated that laser microdissection improves pharmacogenetic analyses on PDAC, as it helps the pathologist to harvest tumor cells only and thus establish their real genetic expression. We compared LMD tissues versus non LMD tissues and found a significant difference in terms of mRNA and miRNA expression. Finally, working with laser microdissection, we found a progressive upgrade of methodological approach, increasing the efficiency of molecular tests."

The Morbus Parkinson puzzle

Dr. Falk Schlaudraff researched Morbus Parkinson at the molecular level as a doctoral candidate under the supervision of Prof. Dr. Birgit Liss in the Molecular Neurophysiology team at the Institute of Applied Physiology, University of Ulm.



"There are several theories on how the disease originates. M. Parkinson can be compared to a puzzle.

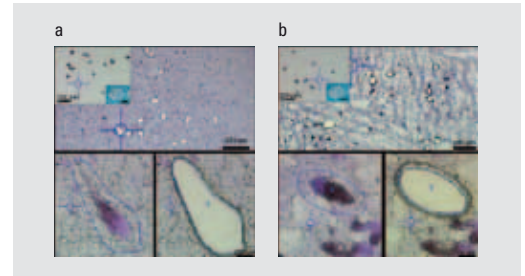


Fig. 4: Laser microdissection and mRNA-expression analysis of individual substantia nigra (SN) neurons from human PD and control postmortem brains. Pools of neuromelanin-positive [NM (+)] neurons were isolated via LMD of cresylviolet-stained horizontal midbrain cryosections from PD (a) and control brains (b) (published in: Gründemann et al., Nucleic Acids Research, 2008, 1–16 doi:10.1093/nar/gkn084, © Oxford University Press).

We have already found many of the pieces and can put some of them together, but we don't know what the whole picture looks like. We wanted to selectively view the midbrain dopaminergic neurons that are involved in the pathogenic process and used laser microdissection for validated comparison at the single-cell level. This technique makes it possible to accurately cut individual dopaminergic neurons out of complex tissue, without contact or contamination, and analyze the gene expression in individual cells.

The most prevalent type of tissue in the brain is supporting tissue: without laser microdissection, it would be almost impossible to clearly characterize the relatively rare nerve cells on a molecular level; they would not be distinguishable from background noise. The analysis of single cells frequently leads to different results from those obtained from a complete tissue examination. Studies have shown that the expression of certain microRNAs is changed in the tissue of M. Parkinson patients. We were able to confirm the results for the whole tissue. However, we also examined microdissected cells in parallel. Here we found that the investigated microRNA expression is not changed on a single cell level. This tissue artifact was detected with the aid of laser microdissection."

Molecular network in Arabidopsis

The research of Prof. Lucia Colombo and Raffaella Battaglia, Ph.D. from the Department of Biology at the University of Milan aims at understanding the molecular mechanisms that control plant reproduction. The lab uses *Arabidopsis thaliana* as model species. Molecular information obtained from this small model species has often been fundamental to highlighting molecular networks in agronomically important species like rice.



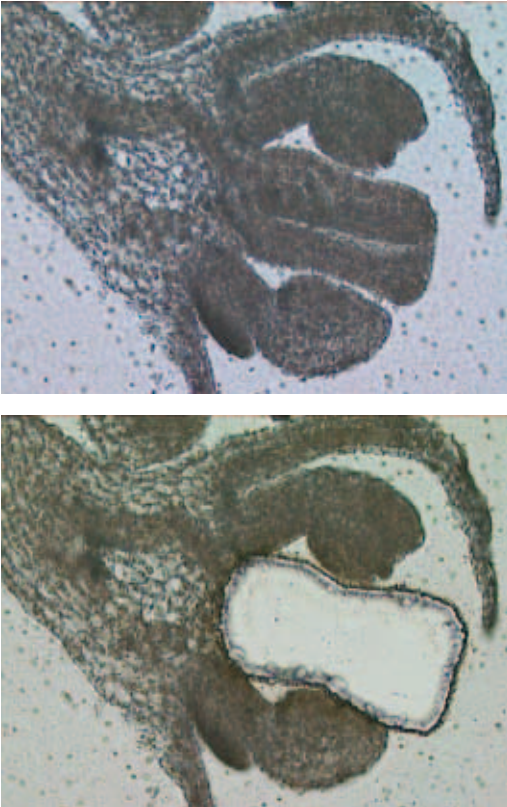


Fig. 5: Laser microdissection helps to identify and functionally characterize genes which play a role during female organ formation and fertilization in *Arabidopsis thaliana*.

“We want to identify and functionally characterize those genes which play a role during female organ formation and fertilization in *Arabidopsis thaliana*. Our lab has strongly contributed to the comprehension of the molecular mechanisms at the base of ovule differentiation. We participated in the identification and functional characterization of those genes, mainly encoding for transcription factors, which give important signals to start the ovule differentiation process.

MADS-box transcription factors represent an important group of key regulators which act as molecular switches to activate molecular cascades leading to mature organ formation. Despite the fact that the importance of MADS-box proteins for such functions as organ identity determination and flowering time control is well established, few direct target genes of MADS-box transcription factors have been identified in plants until now. Using laser microdissection, we have recently identified the first gene regulated by MADS-box transcription factors involved in ovule differentiation.

We know which genes are switched off as a consequence of the lack of activity of MADS-box factors. We have identified and functionally described the *VERDANDI (VDD)* gene as a direct target of ovule

identity MADS-box factors but also playing a fundamental role in female gametophyte differentiation. The possibility to combine single cell analysis with genome-wide approaches is giving a powerful impulse to developmental biology.”

The mitochondrial hypothesis of aging

Why do we grow old? Scientists have been looking for an answer to this question for many years – particularly against the background of the increase in neurodegenerative diseases among older people such as Morbus Parkinson. PD Dr. med. Andreas Bender from the Neurological Hospital of the University of Munich-Großhadern is researching the mitochondrial hypothesis of aging, which regards a vicious molecular circle of oxidative damage and mtDNA mutations as being a cause of neuronal functional disorders.



“Our aim is to investigate at single-cell level why some cell types are more susceptible to neurodegenerative diseases like Morbus Parkinson than others. After extracting these cells from human post mortem brain tissue by laser microdissection we subject them to molecular-biological examination. In particular, we are looking for damage to mitochondrial DNA (mtDNA).

Mitochondria have not been thoroughly researched with single cell analysis so far, although evidence is growing that they could play a major role in neurodegenerative diseases and in the aging process.

What surprised us was that there were a lot of mtDNA mutations in 60- to 70-year-old control patients as well – although distinctly fewer than in the Parkinson group. In a second step we therefore examined control samples of people of all ages, from a few months to a hundred years old. We found that the mtDNA deletions increase with age. We are born with no or extremely few deletions and at some stage in our life the critical threshold of 50 to 60 % of mutations is reached.

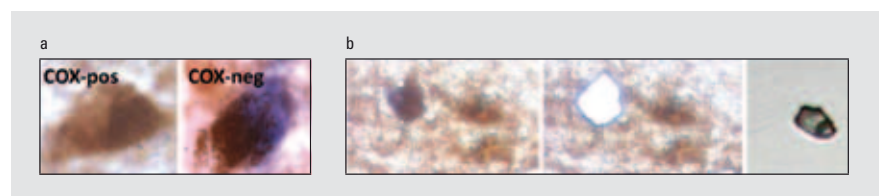


Fig. 6a–b: Cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) staining of dopaminergic substantia nigra neurons (a). Normal neurons are COX-positive (brown). Neurons with high levels of mtDNA deletions are COX-negative (blue). b: A COX-negative neuron is cut out with laser capture microdissection (middle) and identified in the lid of a PCR tube (right).

Laser microdissection plays an extremely important role, as the precise dissection of individual cells ensures that we really examine the cells that interest us. Before the age of laser microdissection, most examinations were done on homogenates. These contained different types of cells, so that the molecular connections we were researching sometimes disappeared in a great background noise. If a disease only affects one particular cell type, as in the case of Parkinson's, we only obtain meaningful results when we are able to analyze homogeneous cell material."

Understanding asymmetric stem cell division

Prof. Monica Gotta from the Department of Genetic Medicine and Development at the University of Geneva uses the LMD for the study of asymmetric cell division, which allows the generation of cell diversity during development and stem cell renewal.



"Establishment of cell polarity and positioning of the mitotic spindle are essential prerequisites for asymmetric cell division. While many players in these processes have been identified, the big challenge is now to understand how these regulate each other and how they regulate polarity and spindle positioning. My lab studies these processes using the *C. elegans* embryo as a model system. This is a great system for studying asymmetric cell division as the one cell embryo is polarized and the first division is asymmetric.

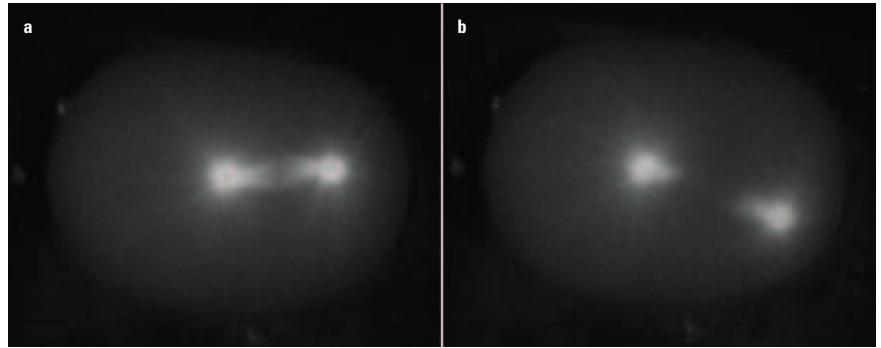


Fig. 7a–b: Mitotic spindle ablation in a wild type one-cell embryo. A *C. elegans* one-cell embryo expressing α -tubulin fused to GFP. The mitotic spindle just before (a) and after laser ablation (b). The two spindle poles separate fast after the ablation. (Grill, S. et al, 2001, Nature)

Furthermore we can combine genetics, live cell imaging, biochemistry and genomics/proteomics.

One limitation of the embryo is the egg shell, which does not allow the use of inhibitors. To overcome this problem we use the LMD. It allows us to permeabilize the tough and impermeable eggshell by producing small holes that allow penetration of drugs dissolved in the embryo mounting medium and therefore inhibition of a pathway or protein of interest with high spatial and temporal resolution.

We also use the LMD for the mechanical destruction of other structures such as centrosomes and microtubules, which allows measurements of movement and velocities in wild type and mutant backgrounds. The system has also been used to ablate specific cells during development to study cell function and/or lineage."

Register for Upcoming reSOLUTION Issues

Would you like to read future issues of reSOLUTION, too? The following link will take you straight to the registration form:

www.leica-microsystems.com/registration.01

Choose between different reSOLUTION editions and we would also be glad to hear your opinion of our magazine and any suggestions for topics.

You can find this issue and all past issues of reSOLUTION on our website at:

www.leica-microsystems.com/magazines.01

