Laser Microdissection and Manipulation

Laser microdissection and pressure catapulting (LMPC) technology enables the non-contact microdissection and manipulation of specific tissue samples and even living cells, and is becoming a standard tool for structural and functional genomics, and proteomics research.

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Modern technologies in molecular biology and life sciences require homogeneous starting materials for reliable analyses and results. The extraction of specific samples from a morphologically well-defined origin has become one of the most challenging tasks in genomic and proteomic research and application.

At P.A.L.M. Microlaser Technologies, we have countered this challenge by the development of a range of high-quality laser devices. These not only allow microdissection – that is, cutting and ablation of microscopic specimens – but also micromanipulation of objects in a liquid environment. This modular concept enables tailor-made solutions to be implemented in research institutes and industrial pharmaceutical laboratories throughout the world.

NEW INSIGHTS INTO THE MOLECULAR WORLD

Laser microdissection has evolved from a technique for the isolation of tissue samples to a modern technology finding use in a variety of applications. New insights into molecular mechanisms in biotechnology, medicine, cancer research and drug therapy can be found by using laser microdissection. Wherever control of sample composition is needed, laser microdissection is the gold standard for research at the molecular level. It is a prerequisite for genomic analyses such as DNA/RNA microarrays or qRT-PCR, proteomic analyses such as MALDI/SELDI mass spectrometry and other highly sensitive downstream applications.

Any biological sample is suitable for non-contact Laser Microdissection and Pressure Catapulting (LMPC). So far, no restrictions regarding the origin of a selected specimen, or applied preparation and staining procedures, have been reported. Even archived material can be catapulted directly from glass slides, but, for the sake of convenience, most samples are mounted on membrane slides. This preserves the morphology, enabling automated routine processes in combination with image analysis.

With LMPC, it is possible to capture thousands of cells within a short time, or to isolate single cells or subcellular particles; the automated finding of rare cells is also possible. A variety of tailor-made tools is available for working with living cells in liquids for contamination-free isolation and re-cultivation without affecting cell viability.
The powerful laser-based technology enables the circumcision of a desired sample, creating a clear-cut gap between the sample and its surrounding tissue. The circumcised sample is then lifted up into a collecting vessel – only with the help of light. The complete process of sample generation is entirely 'non-contact' and thus absolutely free from contamination (Figure 1). Thus, molecular information about the sample is totally preserved and can be analysed in subsequent genomic and proteomic studies.

Lasers of high beam quality (a pulsed UV-A cutting laser and a continuously emitting infrared laser for optical trapping) are interfaced into research microscopes and focused to a minimum possible spot size. At this size, the user is able to manipulate at the level of single cells or even sub-cellular components. The pulsed UV-A laser beam used for LMPC reaches a high energy density (≈10 MW/cm²) within the focal spot. The energy transfer is sufficient for fragmentation of the matter without any physical contact with the specimen. And as the cutting is a fast process, devoid of any heat transfer, adjacent biologic matter or biomolecules – DNA, RNA or proteins – outside of the focus are not affected.
After the cutting procedure, the selected area is ejected from the object plane – usually with a single laser impulse. This process is named Laser Pressure Catapulting (LPC) and marks a breakthrough in modern laser capture methods enabling the entire non-contact preparation of pure and homogeneous samples. The sample can be transported several millimetres against gravity directly into a capture device. Single particles, such as chromosomes, up to larger tissue areas (1mm²) can be successfully transported applying LPC without disturbing the biological information or the viability of the specimen.

P.A.L.M. has developed special lens and mirror holders which ensure that the laser beam path is in parallel with the optical axis of the light microscope and that the laser focus is stable in its preset position without any drift. This is a prerequisite for precise laser micromanipulation with the highest possible level accuracy for all-day use.

**HIGH AUTOMATION FOR SPECIAL APPLICATIONS**

At the heart of the MicroLaser System lies a very sophisticated piece of software – RoboSoftware. From the very beginning, the software was conceived to meet the special needs of microdissection and, within 10 years of being used in the field, it has matured to a multifunctional tool offering all possibilities in a user-friendly, self-explanatory interface. Different cutting routines for glass- and membrane-mounted sections can be chosen to optimise operating conditions from the first laser pulse. With a large collection of drawing tools, cells or sample areas can be pre-selected easily and then assigned to special collection vessels. With only one mouse-click, the system is able to automatically perform the cutting and catapulting actions (Figure 2).

Even critical work requiring observation of fluorescence can easily be realised due to a sophisticated fluorescence shutter mode (‘freeze’ mode) which allows sample pre-selection in the frozen image to avoid bleaching effects. All available standard fluorescence filters can be used after sample pre-selection with the freeze mode.

L MPC is also possible under direct fluorescence illumination requiring special filter sets. It can be complemented with an intelligent image-processing software for detection and identification of single rare cells and complex specific tissue areas.

**MICROLASER SYSTEMS**

The PALM® range of MicroLaser Systems is a collection of up-to-date tools for precise, non-contact laser-based micromanipulation as is needed in all areas of biotechnical and molecular research. The range of products starts with the MicroBeam as its centerpiece; this is a state-of-the-art device for modern laser microdissection and subsequent pressure catapulting. It is intended for the collection of precisely defined cells (living or fixed) or subcellular particles from all kinds of samples as they are routinely used in pathology, oncology, and neurology, or are needed for proteomics and cytogenetics, as well as genetic engineering and other fields of biological, medical or pharmaceutical research.

The MicroBeam is available in several modular configurations. The image-processing model has already been developed with the needs of life science research in mind. This device combines the proven technology of non-contact laser manipulation and pressure catapulting with advanced image-scanning software features.

MicroTweezers are the main feature for catching and moving particles in liquids. The CombiSystem combines the cutting properties of the MicroBeam with the trapping and manipulation capabilities of the MicroTweezers, and adds up to an all-purpose working station for the manipulation treatment of all kinds of microscopic particles. Only the forces of light are used to cut, catapult, trap, and sort or position with extremely high precision.

The latest model in the MicroBeam range is the high-throughput MicroBeam HT (Figure 3). The combination of non-contact microdissection with automated target identification for rare cells, and specific tissue and target capture is specially configured to meet the needs of high-throughput applications.

To achieve the high level of precision required, P.A.L.M. developed a motorised, computer-controlled microscope...
stage (RoboStage); this enables object positioning with sub-micron precision, as well as partly automated micromanipulation procedures. The latest generation of the microscope stage offers the possibility to use customised sample holders or – in combination with special software functions – even serial sections from one sample by linking up to three slides.

A tailor-made device, the RoboMover, enables high-speed collection of the microdissected sample pieces and areas into a variety of customised collecting repositories. This is a prerequisite for establishing microwebs as a standard procedure in high-throughput processes. The capturing of thousands of cells within a short time, isolation of single cells or sub-cellular particles, and automated finding of rare cells are all made possible (Figure 4).

The high-speed automated collection device allows time- and cost-saving procedures to be introduced in routine as well as research laboratory work. The company as a whole solution provider runs a Service & Application Laboratory, which was the first of its kind worldwide, and the P.A.L.M. specialists have 10 years’ experience in micromanipulation and following downstream procedures. The P.A.L.M. specialists are ready to give individual and detailed advice whenever it is required. The qualified services range from RentalLab, where the customer can prepare his own samples with the aid of the experienced P.A.L.M. staff, to ScientificSupport in special questions with practical advice directly to the bench. P.A.L.M. not only offers technology hardware with an excellent, worldwide background, but is also the platform for microdissection and contamination-free sample generation for modern molecular research. Furthermore, for routine and automated procedures, plenty of custom-made consumables have been developed and much routinely available laboratory equipment has been adapted to the needs of routine microdissection.

THE FUTURE

The purity of sample preparation is of paramount importance for research at the molecular level; for the future, microlaser dissection systems will help researchers to achieve unambiguous results right from the beginning of their work. The available technologies and handling protocols, backed up by high-quality maintenance and technical support, enable users to focus on breakthrough biopharmaceutical research – rather than spending a lot time and money on routine laboratory work.

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