Leica AS LMD – The Latest Advance in Laser Microdissection

by John Ossi, Andy Lee, Janice Zhou, PhD.
Leica Microsystems Inc.
Introduction
Genetic regulations and alterations have been associated with normal development and function as well as diseased states. However, such changes often vary among different cell types in heterogeneous tissues. The ability to isolate specific cell types from heterogeneous tissues allows increased specificity and sensitivity of the molecular signals under analysis. With advances in genomic and proteomic technologies, nucleic acids and proteins can be extracted and quantified from a small sample of pure cell populations. Such advances increasingly require a precise and efficient method to isolate different cell types from heterogeneous tissues.

Laser microdissection emerges as such a technology. It is also frequently referred to as laser capture microdissection (LCM) or laser-assisted microdissection. Manual microdissection has been used for a long time and is still used in certain fields. Manual microdissection allows dissection of large regions and thick sections, but is very labor intensive and not practical for single cell isolation. Laser microdissection, on the other hand, allows faster, easier, and more precise dissecting and transferring of individual cells as well as large regions of interest.

With laser microdissection, heterogeneous tissues are observed under direct microscopic visualization. Cells or tissue regions of interest are identified and marked according to specific morphological and histological properties. Infrared or UV lasers are used to separate specific cells of interest from the surrounding regions, and various methods are employed to collect the dissected cells.

Laser capture microdissection was first developed at the National Cancer Institute for separating cancer cells from normal cells in heterogeneous carcinomas to better analyze and compare molecular differences between diseased and normal cells. With this technique, an IR laser was used to isolate the cells and a contact method was used to transfer them. Since then, many improvements have been made to this technology that makes it easier, faster, more precise, and contamination-free. Laser microdissection technology has also been increasingly used in other research fields, such as neurobiology, developmental biology, plant biology, in addition to cancer biology and other disease-related researches.
Leica AS LMD
Laser Microdissection System

The Leica AS LMD Application Solution Laser MicroDissection system is a new generation instrument, which uses a non-contact method of microdissection. First, tissue samples are mounted on special foil slides. With a user-friendly software interface, targeted cell(s) viewed on the computer monitor are circumscribed with the mouse, and subsequently cut with a UV laser, which allows the sample to fall by gravity directly into a PCR tube cap centered under the tissue. With this method, the regions of interest are not directly exposed to the laser force during cutting or transferring, thereby limiting potential damage to the collected cells. Only dissected cells are transferred by gravity and collected, and this avoids cross-contamination by sticky tape-mediated contact methods.

The Leica AS LMD system utilizes patented laser beam steering technology, by which a laser beam cuts precisely along the line that the user defines, without carryover of unwanted tissue.

Ergonomy
The ergonomic Leica AS LMD is designed to be easy to use. The system is based on a motorized microscope platform with an independently programmable stage for specimen movement and a sub-stage PCR tube holder collection system. Once the sample is loaded onto the stage, this fully motorized system can be controlled via satellite 3-D control and a mouse without touching the microscope, which further limits the possibility of contamination. All laser control functions and collector positions are easily adjusted through the software. Changing magnification or light intensity is as easy as a click of a button on the 3-D controller, which also controls stage movements and focusing. To inspect cell(s) collected in the targeted PCR tube cap, the user clicks on a button, and the system automatically switches to a user-defined low magnification objective, moves away the sample slide, and focuses into the cap. For applications where the molecules under study, such as RNA and protein, are easily degraded and when speed is key, such motorization and automation ensures higher recovery and higher quality for molecular analysis.

In addition, the highly ergonomic design of the system and user-friendly interface make the system easy to learn and operate. In many core facilities, users start collecting samples comfortably and confidently after only several hours’ training and practice.

Flexibility
The Leica AS LMD is a truly flexible system, which allows users to microdissect regions of almost any size or shape. The AS LMD allows dissection of large regions at 4x magnification as well as single cells at 40x and 63x magnifications. Additionally, the AS LMD allows researchers to acquire samples from frozen and paraffin-embedded tissues of various types, including kidney, brain, prostate, ovary, retina, teeth, and much more. When tissue sections thicker than 5–10µm are desired, for example brain tissues, 40–50µm sections are easily microdissected.
Up to four different cell types located on multiple fields or multiple slides can be conveniently collected and pooled into individual PCR tubes in the multi-tube holder system. This is especially useful for comparison studies between several different cell types within the same tissue, or when many individual cells are required from multiple sections for microarray analysis. Relatively large sample areas are quickly acquired from different fields or serial sections, as well as thousands or more cells required for proteomic assays and construction of cDNA libraries. With the introduction of a 150x objective specially designed for laser microdissection, the AS LMD system can also dissect chromosome spreads and small single cells for genetic analysis.

**Live Cell Cutting**

The introduction of the new optional Live Cell Cutting (LCC) module further extends the Leica AS LMD’s capabilities to microdissect live cells in culture. The LCC module isolates individual or small groups of cultured cells for direct molecular analysis or re-culturing. This aids researchers to isolate cell colonies or separate different cell types by morphology or fluorescence. Cells growing on special foil-bottom dishes are microdissected with a laser beam and dropped directly into one well of an 8-well strip, which can be fitted onto a 96-well plate rack and returned to the incubator for re-culturing. The non-contact method used by the AS LMD ensures the least impact on the live cells during dissection or transferring, and increases the survival of the isolated cells.

**Laser Illumination Module**

To match the focal planes of observation (visible range) and dissection (UV range), the Leica AS LMD laser illumination module employs automatic or user-defined laser offset correction for up to six imaging objectives with magnifications from 4–150x. For greater optimized cutting, Leica recently developed several objective lenses especially for laser microdissection with high UV transmission at 337nm. Superb optical performance of the upright microscope with Koehler illumination facilitates identification of cell types at low and high magnifications. Cells can be easily identified in brightfield, phase contrast, DIC-Nomarski, or fluorescence modes.

**Software Enhancements**

New Leica AS LMD software developments provide researchers with more information and control while maintaining the easy-to-use interface:

The “user profile” function allows laser control settings to be saved in user-defined files and recalled for future use. It is especially useful for multi-user facilities or for users working with multiple sample types that require different laser settings.

The “specimen overview” function generates an overview image of the whole slide (or part of the slide(s) as the user defines) in a separate window, and allows a quick survey and switching to the field of interest. The shape and size of each marked area are displayed in a table for easy selection and calculation. An extended shape selection and drawing tool allows the user to repeat the last user-defined shape for multiple cuttings.
An optional “image acquisition” function allows digital images of the tissue regions to be automatically captured prior to and after laser microdissection and after tube cap inspection for future morphological analysis and documentation. The image acquisition software is part of a powerful relational database archival system where all vital system information, such as objective magnification, laser settings, stage position, and more, is automatically associated with each acquired image.

Sample Preparation and Molecular Analysis

The success of molecular analysis depends on high-quality samples. For example, successful RNA isolation requires samples with minimal RNA degradation. Isolation of specific cell groups from heterogeneous tissues increases the specificity, selectivity, and reproducibility of the signals in RNA amplification and microarray studies. The quality of the final results requires optimization at each step of sample preparation, laser microdissection, and downstream molecular analysis.

Samples prepared for laser microdissection need to strike a balance between the morphology and integrity of the molecules being studied. In general, fresh frozen samples are preferred for isolation of RNA and protein, because they are quicker to prepare and the integrity of the molecules are better preserved with limited enzyme activity at low temperatures. On the other hand, paraffin-embedded samples are frequently used in pathology and histology. Paraffin-embedding provides samples of better morphology, which may be critical for identifying different cell groups, and the samples can be easily stored over a longer period of time. Nevertheless, for better molecular recovery, prolonged storage is not recommended for samples intended for laser microdissection. Instead, archived slides offer a great source of historical data and can often preserve the morphology information. However, in most cases, the RNA and protein molecules recovered from these slides are largely degraded, inconsistent, and extremely difficult to recover.

Slide Staining

To enhance the morphological differences or to differentiate various cell groups, certain staining methods are frequently used. Commonly used histological staining methods, such as H&E staining, toluidine blue, or methyl green, are quick and easy, and enhance the structural integrity of tissues. Also, with expanding knowledge of cell-specific markers, immunostaining is increasingly used for laser microdissection samples.

Based on antigen-antibody recognition, immunostaining offers high specificity to discern morphologically similar cells. However, it is a long and complicated procedure, and may require prolonged incubation of samples in aqueous solution, which is not favorable for RNA recovery. This is again a balance between morphology and molecular integrity. Use of highly specific antibodies and customized procedures with shortened incubation times will help to improve molecular recovery while minimizing the impact on morphology.
For some tissues, no staining is necessary. Some cells can be distinguished by their morphology. In such cases, special contrast methods should be used. An easy switch between brightfield and phase contrast or DIC will facilitate the observation of such tissues. Fluorescence is another increasingly popular method to help identify specific cell types. In some transgenic animals, where certain group of cells express GFP-labeled proteins, tissue sections can be visualized under fluorescence without staining. With immunostaining, the availability of multiple fluorochromes offers the opportunity of multi-labeling and identifying different cell types in the same tissue section. Because xylene will reduce fluorescence, xylene treatment should be avoid or limited for fluorescence labeled samples.

**Morphological Challenges**

Special morphologic challenges for laser microdissection samples are due to the fact that coverslips cannot be used on the slides. Without a flattened surface, image quality may be compromised at higher objective magnifications. Recently, van Dijk et al. reported that a special mounting method greatly improves the morphological quality of tissue sections while allowing laser microdissection of the tissues.

Additionally, without a coverslip and mounting media, the samples are exposed to air. For some fluorochromes prone to oxidation and photo-bleaching, fluorescence will quickly be lost before or during laser microdissection. Therefore, when choosing a fluorochrome to label the tissue sample, probes of higher resilience to photo-bleaching should be considered. A motorized shutter for fluorescence and image software that allows integration of fluorescence signals will help to decrease the exposure time of the sample and thus reduce photo-bleaching.

Laser microdissection produces a small quantity of sample material, and this poses a special challenge for downstream molecular analysis. Many molecular companies have developed kits and protocols especially for laser microdissected samples. For example, QIAGEN introduced the RNeasy Micro Kit and QIAamp DNA Micro Kit, which produce efficient and reliable nucleic acid isolation of high-quality and yield. Other resin-column based methods include the Absolutely RNA Microprep and Nanoprep Kits (Stratagene), and the RNAsqueous Micro Kit (Ambion). Phenol extraction, TRizol extraction and other customized protocols are also frequently used. The Leica AS LMD allows adding lysis buffer into the PCR tube cap to preserve the molecular integrity of the sample immediately after its dissection. Additionally, automatic pooling of multiple dissects into the same PCR tube cap reduces downstream sample handling and facilitates molecular analysis.

**Applications of Laser Microdissection Technology**

Laser microdissection was first developed for cancer research, and molecular pathology. Now, this advanced technology is rapidly gaining use in many other research fields, such as neurobiology, molecular genetics, gynecology, cardiology, dental research, developmental biology, plant biology, and many more.
Gene Expression
The most common use of laser microdissection technology so far is the study of gene expression in heterogeneous tissues. Laser microdissection separates different cell types and allows comparison of expression levels of different genes in various cell types. In combination with microarray studies, hundreds to tens of thousands of genes can be compared simultaneously from the same tissue sample. Real time RT-PCR offers a qualitative method to study the expression level of specific genes in more detail.

- With laser microdissection and large-scale microarray analysis, genes whose expression in adenoid cystic carcinomas (ACC) was altered relative to those in normal salivary glands, and those uniquely expressed in ACC compared to 175 other carcinomas, were identified, which could be exploited for therapeutic targets for this cancer.
- Similarly, gene expression profiles among normal cells, primary carcinoma cells, and metastatic carcinoma cells in gastric carcinomas were studied with cDNA microarray, and validated by means of semi-quantitative RT-PCR.
- The combination of these two technologies (above) was also used to identify and characterize molecular markers of different histopathological stages of the gastric B cell mucosa-associated lymphoid tissue (MALT) lymphoma, and to study the gene expression profile in heart transplants.
- GABAA receptor expression was quantified with nested RT-PCR from regions of layer I dendrites and individual cells of layer V pyramidal neuron somata in the brain; as well as the vascular endothelial growth factor (VEGF) and its receptor flk-1 in glomeruli of kidneys by real time PCR.
- In addition to human patient tissue samples, laser microdissection has also been successfully used to dissect mouse tissues.

Cytogenetics
Loss of Heterozygosity (LOH) has been widely used to identify tumor suppressor genes, however, contamination by stromal tissue may decrease the sensitivity and accuracy of LOH.

- Laser microdissection in combination with whole genomic amplification techniques produces a sufficient amount of DNA for high throughput genome-wide allelotyping.
- Comparative genomic hybridization (CGH) was used in combination with laser microdissection technology to study molecular cytogenetic abnormalities in Hodgkin’s lymphoma. Such studies provide a basis for identification of tumor-related genes and help to classify tumors.
- PCR of laser microdissection samples from a cutaneous melanoma patient helped to find monoclonal rearrangement of the lymphoid cells in the follicle.

Proteomics
More and more researchers start their study of gene regulation at the protein level, to complement their findings at the mRNA level and to study post-translational modifications:
• Laser microdissection was combined with western blot to study the level of ADF/cofilin phosphorylation in the dentate gyrus after late LTP (long-term potentiation).

However, since there hasn’t been a method for protein amplification, such as PCR for nucleic acid amplification, the amount of protein samples required for conventional protein detection and analysis methods is so large that it makes laser microdissection a tedious and sometimes impossible task. The AS LMD helps streamline the task through automation and the ability to cut and collect relatively large and thick regions. The fully motorized microscope and automated sample collection make isolation of multiple samples easy and fast; the powerful laser and superb optics provide enough power to cut through certain tissues, such as brain, as thick as 40–50µm.

Additionally, advances in technologies for highly sensitive protein detection and analysis allow proteomic studies at the cellular level:

• Using sensitive fluorescent dyes before 2-D PAGE separation, less than 6.6µg of protein was enough to generate a 2-D profile with approximately 1500 protein spots.
• Using a bead-based analysis technology, multiple inflammatory mediators can be simultaneously measured within the pg/ml range, demonstrating a differential distribution pattern in the brain.

Conclusion

The past several years have seen great advances in laser microdissection technology, both in the development of the instruments and the applications in different research areas. Based on feedback from users and the emergence of new applications, Leica Microsystems is continually developing the AS LMD system to make it even faster, more precise, more flexible, and easier to use. More and more researchers have found it to be a valuable instrument to tackle complex scientific questions that were not possible to address in the past.

References


Internet: www.leica-microsystems.com/down_ossi_et_al_en