

An Introduction to Laser-Based Tissue Microdissection Techniques

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Summary

The development and application of laser-based tissue microdissection techniques has provided a major impetus to the sensitive and specific molecular analysis of solid tissues and tumors. This chapter provides an overview of the different laser-based microdissection systems and an introduction to the principles involved in the function and applications of these individual systems.

Key Words: Laser capture microdissection; laser microbeam microdissection; molecular analysis.

1. Introduction

Tissues, especially diseased tissues, are complex three-dimensional structures composed of heterogeneous mixtures of morphologically and phenotypically distinct cell types. The meaningful molecular analysis of morphologically and/or phenotypically distinct cell types from such tissues requires rapid, efficient, and accurate methods for obtaining specific population of cells.

The molecular investigation of solid tissues, especially tumors, has been revolutionized over the past decade by the development of accurate, rapid, and effective laser-based methods of tissue microdissection (*1,2*). This has provided an extremely valuable and sophisticated tool to fully utilize the power and sensitivity of modern molecular analytical technologies in the detailed investigation of many different diseases and provided significant new insights into the pathogenesis of these diseases. Many of the investigations using laser microdissected cells have focused on specific types of cancers, where the morphological and phenotypic heterogeneity and complexity of tissues is often the greatest (*1-3*).

From: *Methods in Molecular Biology*, vol. 293: *Laser Capture Microdissection: Methods and Protocols*
Edited by: G. I. Murray and S. Curran © Humana Press Inc., Totowa, NJ

Individual studies have usually based cell selection on specific morphological criteria of stained histological sections, but phenotypic characteristics as defined by immunohistochemistry of antigen expression (4) or genotypic features as demonstrated by *in situ* hybridization have also been used as selection criteria (5) and demonstrate the power of laser-based microdissection techniques. One of the major advantages of using laser microdissection methods to obtain specific cells for molecular analysis, especially from the viewpoint of the pathologist, is that the procedure is carried out under direct-light microscopic visualization of the cells. Whereas other technologies used to isolate specific cell populations for molecular analysis—e.g., fluorescent-activated cell sorting or magnetic bead-based cell separation—are indirect techniques with no microscopic visualization of the cells and require the availability of suitable antibodies to aid cell selection. Moreover, the methods used to prepare single cell suspensions (e.g., proteolytic enzyme digestion) that are necessary for antibody-based cell separation techniques from solid tissues may result in alteration or modification of cellular constituents. There is no doubt that the availability of laser-based microdissection technologies has provided a major impetus to molecular pathology research and this technology is now found in many laboratories worldwide (as represented in the diverse geographic locations of contributors to this volume). The wide availability of this easy-to-use technology has allowed many questions in a range of research disciplines to be answered that previously could not be asked or answered using manual methods of tissue microdissection because of the imprecise nature of manual methods of microdissection or the time required to obtain tissue.

There are two major systems that have been developed for performing laser-assisted tissue microdissection—namely, laser capture microdissection and laser microbeam microdissection. Both types of systems have now been commercially available for several years. An overview of the principles, advantages, and potential disadvantages of each of the systems will be provided in this introductory chapter, detailed descriptions and applications of the individual systems are given in the relevant chapters in this volume.

2. Overview of Laser Microdissection Systems

2.1. Laser Capture Microdissection

The laser capture microdissection system was developed in the mid-1990s at the National Institutes of Health by Emmert-Buck and colleagues (6,7), who recognized the need to develop a microscope-based microdissection system for accurately and efficiently microdissecting cells from histological tissue sections to fully exploit emerging molecular analytical technologies. They developed this system primarily to facilitate the molecular analysis of solid tumors.

This system rapidly moved into commercial production by Arcturus Engineering (8), which has further developed the system since then. The laser capture microdissection system is now probably the most widely used laser-based microdissection system worldwide and our own experience of more than 5 yr is with this system, in particular the Arcturus PixCell II laser capture microdissection system.

The basic principle of the laser capture microdissection system is the capture of groups of cells or even individual cells onto a thermoplastic membrane from stained tissue histological sections (frozen sections or fixed wax-embedded sections) or cytological preparations. This plastic membrane that overlies the tissue section is attached to a specially designed “cap” and ensures that the plastic membrane is held in direct contact with the tissue section. The plastic is transiently melted by a low-power narrow-beam infrared laser directed at the cells of interest under microscope control. As the plastic cools and solidifies again, the cells are embedded into the plastic membrane and are removed from the tissue section by lifting the cap along with the plastic membrane off the tissue sections. Multiple groups of cells can be readily captured onto the same membrane. The laser beam in the current model of the laser capture microdissection system, the Arcturus PixCell II system, has three settings of the diameter of the laser beam, and using the laser beam at its narrowest diameter (7.5 μm) setting permits even single cells to be microdissected (9). Subsequently cellular components including DNA, RNA, and protein can all be readily extracted with appropriate procedures and used for an extensive range of molecular analysis including PCR, gene expression studies, and proteomics (6,7,10). To date, laser capture microdissected cells have been found to be compatible with all the molecular analytical techniques that have been used. Histological staining of the tissue, which is generally necessary for laser capture microdissection to allow morphological visualization, does not appear to significantly alter most cellular constituents. Moreover, the process of acquiring the cells onto the thermoplastic membrane does not appear to alter or damage the integrity of DNA, RNA, or protein, nor does capturing the cells onto a thermoplastic membrane appear to prevent a subsequent high rate of recovery of such material.

The procedure of laser capture microdissection is generally rapid, although morphological visualization of the tissue sections and cells is only moderate in comparison with stained and mounted histological slides as the tissue sections are not mounted with a coverslip. The inferior-quality morphology that is observed with this system in our experience only requires a little readjustment by an operator used to observing and interpreting high-quality morphology and we have found that operating this technology is easily learnt. In our laboratory we have also found that even relatively inexperienced operators, often with no

background in histology/morphology, can easily be taught how to use the system, including recognition of cell types of interest, and thus rapidly and successfully acquire appropriate cells for downstream molecular analysis.

2.2. Laser Microbeam Microdissection

This method of laser microdissection operates on an entirely different principle from that used in laser capture microdissection (11,12). The basic principle is that a pulsed very narrow-beam ultraviolet light laser is used to “draw” around the cell or cells of interest and unwanted tissue is photoablated (12). Three different manufacturers have produced laser microbeam microdissection systems, all of which vary in the precise details of operation, especially with regard to the method of collection and transfer of the microdissected tissue for subsequent molecular analysis. In the PALM system the laser can be used to “catapult” the microdissected cells into a collecting tube, whereas in the Leica AS LMD system the section is inverted so that after microdissection the microdissected cells fall into the collecting tube under the influence of gravity. The advantages of this method is that there is no physical contact between the cells and plastic, unlike in laser capture microdissection, and this method clearly avoids the potential risk of modification of the molecules of interest by especially the heating and cooling of the thermoplastic membrane. In the microcut MMI system transfer of the microdissected cells is by a very fine needle or glass micropipet, this can run the risk of loss of microdissected cells during the transfer process or contamination of the transfer needle.

All laser microbeam microdissection systems use a much finer diameter laser beam (smallest laser diameter is 0.5 μm) compared with the laser capture microdissection system (smallest laser diameter size is 7.5 μm), making these systems ideally suited for the precise microdissection of single cells. However, this makes operating these systems potentially more time-consuming compared with laser capture microdissection, especially when a large number of cells require to be microdissected. For nucleic acid-based molecular analysis this is unlikely to be a significant issue, as analysis can often be performed on very few cells, but the time taken to acquire the large number of cells required for many types of proteomic studies may then become a significant factor in experimental planning and design.

3. Conclusions

Both types of laser microdissecting systems greatly facilitate the acquisition of specific cells for a wide range of downstream molecular analysis. For both laser capture microdissection and laser microbeam microdissection, cell selection can be based on morphological features or phenotypic criteria; both systems have their advantages and possible limitations

The further development of these laser-based microdissection systems—especially the integration of sophisticated image analysis software to permit the automatic dissection of pre-defined cells of interest—will greatly facilitate the more widespread application of these technologies. Indeed, Arcturus (**8**) has recently introduced an automated laser capture microdissection system (AutoPix™ Automated Laser Capture Microdissection Instrument). This instrument allows the user to predefine the areas of each tissue section to be microdissected and then automatically microdissects the areas of interest. PALM Microlaser Technologies has also introduced an automated system to its PALM microbeam system allowing highly automated specimen handling (isolation, transportation, and capturing) without mechanical contact for pure contamination-free target cells (**13**). The development of automated laser microdissection systems will greatly enhance the speed and accuracy at which cells can be microdissected and acquired for molecular analysis and will support higher-throughput and larger-scale molecular analysis of specific cell types.

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