



# Laser Micro Dissection Systems: An Alternative Method of Defined Cell Isolation for Molecular Profiling

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## Abstract

Laser micro-dissection permits isolation groups of cells from heterogeneous tissue sections under direct microscopic visualization. This technology not only allows isolation of homogenous subpopulation of cells but also allows immunophenotypically similar cells for RNA/DNA or protein extraction for downstream applications like western blot, mass spectrophotometry, real time quantitative polymerase chain reaction, microarray and RNA sequencing. Isolation of dead cells unsuitable for functional assays, high cost of equipment and technical expertise required for operation are few limitations to laser microdissection systems.

**Keywords:** Laser microdissection; mammary tissue; carcinoma; immunolabelled cell; transcriptomics; proteomics

**Abbreviations:** LMD: Laser microdissection; LCM: Laser capture microdissection; UV: Ultraviolet; IR: Infrared rays; PEN: Polyethylene naphthalate; PPS: polyphenylenesulphide; RNAseq: RNA sequencing; BrdU: bromodeoxyuridine; qPCR: quantitative polymerase chain reaction; xMD: expression microdissection; 2D: Two dimensional; iTRAQ: Isobaric tag for relative and absolute quantitation; LREC: Label retaining epithelial cells

## Introduction

### Principles of laser microdissection systems

There are two types of laser based micro dissection systems, ultra-violet (UV) and Infrared (IR) laser based systems. IR laser micro dissection system transfers cells into thermoplastic polymer (called "capture") from which molecules are extracted for downstream applications. Therefore, this system is called laser capture micro dissection (LCM) system. The principle of LCM is depicted in which tissue sections are placed on the stage of microscope. Thermoplastic sheet (a transparent ethylene vinyl acetate layer, usually 6 mm in diameter) is placed onto the tissue section. When IR laser beam is focussed, thermoplastic sheet just above the tissue section melts and surrounds (i.e. captures) the tissue/cells of interest [1,2]. Later on thermoplastic sheet, embedded tissue/cells are harvested in a tube's cap for nucleic acids or protein isolation for downstream applications. Initially, this technique was applicable on the frozen tissue sections [3]. Later on, this technique is used on formalin fixed, paraffin embedded tissue sections, smears, cytopspins, live cells for RNA, DNA or protein isolations [4,5].

Laser micro-dissection (LMD) is UV laser based system where tissue sections are placed on glass membrane slides. Polyethylene naphthalate (PEN) or polyphenylene sulphide (PPS) membranes are cemented onto glass slides, commonly called glass membrane slides. Tissue sections placed on the top of the membrane and slides are kept inverted, facing tissue sections downwards towards collector. When UV laser passed from upward passes through the glass slides, it cuts the membrane. The areas of interest are selected on computer screen and membranes associated with area of interest are deposited into collector through gravity. Unfortunately, UV laser damages the molecules that comes in the cut perimeter especially when area of interests are very small like single cell.

The combination of IR laser capture and UV laser cutting (Arcturus XT™ micro dissection system, from Life Technology, as an example) is really a promising one to perform both types of work under the same platform. Three different types of laser micro-dissection systems include, IR laser system, UV laser system and combined laser systems (Table 1). In this review,

usefulness LMD on isolation of different type of cells, in particular mammary tissue, including isolation of pathogens from the tissue section, has been discussed. Author believes that there is not much reviews focussed on application of LMD for isolation of various types of cells from complex tissue in veterinary and

animal sciences are present. Furthermore, molecular profiling such as RNA seq or proteomics study of micro dissected cells would certainly provide insights of biology of harvested cells from their tissue.

**Table 1:** Various types of laser microdissection systems and their characteristic of tissue/cell isolation.

Types of Laser microdissection system	Name of Laser microdissection system	Vendors	Properties
Infra-red (IR) laser capture system	PixCell II	Arcturus	A laser capture microdissection (LCM) system, utilizes low-power infrared laser to melt a thermoplastic film over the cells of interest. Caps are collected with adherent target cells.
Ultra-violet (UV) laser microdissection system	Laser microdissection	Leica	A laser microdissection system utilizes ultra-violet light to melt thin film over the tissue or interest. Contact and contamination free method, as isolated cells are falls into collector by gravity.
	PALM Microbeam	Zeiss	A non-contact sampling method that combines laser microdissection and pressure catapulting technology, a laser assisted transfer of sample.
	MMI-CellCut Plus	Molecular Machines and Industries (MMI)	Tissue section is sandwiched between membrane slide and glass slide. UV laser is passed through glass slide that cuts the tissue/cell of interest along with the membrane. Lifting adhesive caps of collector collects isolated target area.
Combined IR and UV laser systems	<ul style="list-style-type: none"> <li>• Veritas</li> <li>• Arcturus XT</li> </ul>	Arcturus	This system applies powers of two lasers, gentle infrared laser for cutting and UV laser for capturing harvested cells.

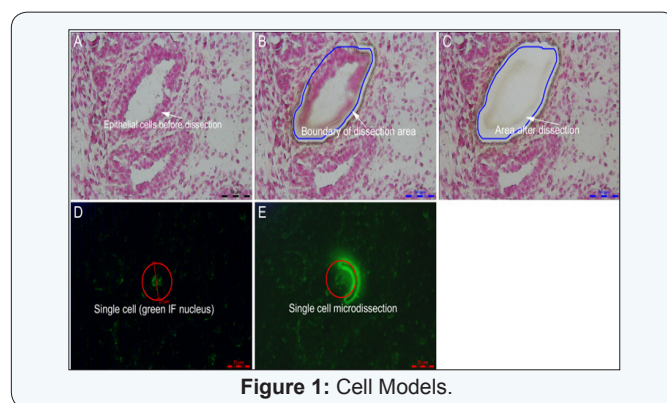
In this review, usefulness LMD on isolation of different type of cells, in particular mammary tissue, including isolation of pathogens from the tissue section, has been discussed. Author believes that there is not much reviews focussed on application of LMD for isolation of various types of cells from complex tissue in veterinary and animal sciences are present. Furthermore, molecular profiling such as RNAseq or proteomics study of microdissected cells would certainly provide insights of biology of harvested cells from their tissue.

**Isolation and molecular profiling of defined cells from tissue**

**Mammary epithelial cells and stroma**

Information on use of laser micro dissection system to characterize mammary tissue is limited. Mammary tissue is composed of mammary epithelium and stroma embedded in the adipose tissue. Isolation of epithelial cells and stroma individually could provide unique and common characteristics of mammary epithelial cells and stroma, respectively that could provide reliable cell-specific gene expression and functions. Considering that RNA quality decides transcriptomic analysis of tissue harvested after micro dissection, it is important to process frozen section quickly. Staining of frozen sections using

haematoxylin or cresyl violet or fast red or toluidine blue stains in nuclease free conditions provided isolation of good quality RNA from mammary epithelium and stroma [6-10] (Figure 1A-C).



**Figure 1:** Cell Models.

Laser micro dissection also permitted investigators to study the immune-modulatory role of mammary epithelial cells during mastitis. In goats, mammary gland (one quarter) infected with pathogens while the other half gland (other quarter) served as control. Mammary epithelial cells were dissected out leaving contaminating inflammatory cells. Investigators examined epithelial cells specific gene expression profiles [13]. In addition

to the transcriptomic analysis of micro dissected mammary tissues using microarray and real time qPCR, RNA Sequencing (RNA seq) also examined gene profile [14]. RNA seq is the advent of next generation sequencing that offer more advantages over microarray because of its ability to identify all genes including novel gene, splice variants, allele-specific expression, independent of genome annotation and absolute quantification of copy numbers transcripts [15,16].

### Nerve cells

Molecular analysis of class specific neuronal development requires identification of pure population of class specific neurons. Identification and isolation of pure population of peripheral neurons that were performed using magnetic bead sorting [17] and florescent activated cell sorting did not procure accurate isolation of specific cells. However, laser capture micro dissection provided accurate and precise isolation of dendritic neurons, motor nerves, dopaminergic, catecholamine neurons and astrocytes for understanding molecular mechanism of class-specific dendrite morphogenesis and progression of disease like Parkinson's [18-26]. Identification of similar cells that differs immune phenol typically coupled with laser micro dissection enabled RNA isolation [3,1][27-30] for gene expression analysis like qPCR. Alternatively, in thin serial sections, parvalbumin neurons were identified immune histo chemically in one section and adjacent sections simply stained with neutral red.

In neutral red sections, Neuronal profiles of parvalbumin immune stained section identified cell bodies, subjected to laser micro-dissection. This method located parvalbumin neurons without immune histo chemical processing and provided intact RNA [29]. Since neuronal cells are big in size, it was possible to compare immune positive cells profile of one section to the serial section. Utility of this alternative method to identify cells of interest among the other tissue needed to evaluate. Molecular signature of glioma has been investigated where invading glioma tissue were harvested from rim of tumour cells mass [31-33]. These studies investigated gene signature of glioblastoma of brains. However, these studies do not emphasize on the role of tissue microenvironment on development of glioblastoma. Microenvironment plays an important role in orchestrating invasion of cancerous cells into normal tissue. Laser micro-dissection allows isolation of different types of tissue- glioma cancer cells, invading glioma cells and cells of normal cortex. This comprehensive type of tissue sampling permitted study of tumour microenvironment and gene regulatory networks and pathways responsive for glioma invasion [34].

Laser micro dissection can also be used to identify gene knockout cells at genomic level. Micro dissection of motor neurons from spinal cord for DNA isolation led identification of neuron specific ablation of *Mtmr2* gene inknock out mice using PCR [35]. Identification of dopaminergic neurons from substantia nigra pars compacta using laser captured micro dissection determined transcriptional changes in aged-matched

and young control individuals and revealed mitochondrial DNA deletions in substantia nigra neurons in ageing [36]. One of the causes for Parkinson's disease was the substantial degradation of somatic mitochondrial DNA in aged-matched individual. In addition to finding a reason to prevent loss of somatic mitochondrial DNA loss in the disease, pathway analysis of gene regulatory networks responsible for neuronal loss could result in therapeutic interventions of Parkinson's disease [36,24].

Underlying mechanisms of cell survival rheostat switch which controls dying or surviving cells after traumatic brain injury, was the result of single neuronal cell (injured vs healthy cells from adjacent area) isolation using laser micro dissection systems [37]. Degeneration of motor neurons in amyotrophic lateral sclerosis has identified involvement of RNA binding proteins, TDP43 and FUS. The genes involved with these proteins are normally transcribed, evidenced by RNA sequencing of motor neurons of mutant vs. wild-type mice [38] that were purely isolated using laser micro dissection. Molecular basis of diseases of nervous systems like Parkinson's disease or schizophrenia, where particular types of neurons are vulnerable for degeneration, can be studied in detail upon their isolation individually in pure form [39].

### Muscle cells

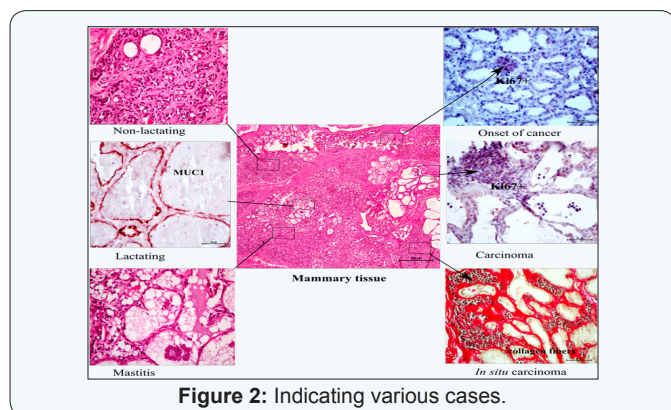
Skeletal muscle fibre is composed of various types of cells including microsatellite stem cells, neurons and vascular endothelium enabling molecular profiling of specific cell types at myofibril level difficult. Laser micro dissection systems offer expression profile of individual cell types like myofibrils, satellite stem cells, vascular elements and neuronal elements from muscle fibres [40-43]. Identification of type I, type IIA, type IIB and type IIC of myofibrils and then isolation of these different types of myofibrils using laser micro dissection provided insight in understanding the biology of muscular atrophy [44].

Molecular signature of laser micro dissected smooth muscle cells resulted in identification of gene involved in diseases like asthma, atopic and hypoxia-induced hypertension [45-47]. Asthma and atopy are the respiratory tract diseases having similar symptoms except asthma obstructs airways whereas atopy does not. Out of one hundred differentially expressed genes between asthma and non-asthma (including atopy) patients, a set of eight genes distinguished asthmatic from non-asthmatic patients [47].

### Cancer cells

Cancer tissues are well known for tissue heterogeneity having different types of tissues e.g., healthy cells, cancerous cells, invasive cancerous cells, infiltrating immune cells, proliferating cells of blood vessels and surrounding stroma (Figure 2). Breast carcinoma, for example, is a mixture of different cells types as revealed by molecular analysis of the tissue [48-50]. Because of such heterogeneity of tissues, the RNA or protein isolated from cancer tissues actually represents transcripts or proteins

of various cell types present in the cancerous tissue under examination. Thus, pool of transcripts or protein derived from various cell types of tumour, prohibits prognostic discovery of cell-specific markers and drug discovery. Isolation of primary breast tissue and corresponding metastatic lymph nodes using laser micro dissection provided insights of metastatic progression of breast cancer [51].



**Figure 2:** Indicating various cases.

Protocol of performing staining, micro dissection of cancer specific cells from heterogeneous mixture of breast tumours, protein isolation from micro dissected cells and protein biomarker discovery using mass spectrophotometric are provided from frozen tissue (Braakman et al. 2011)[52]. Microenvironment often drives cells for normal or abnormal phenotypes. In breast cancer, understanding stromal component of tumour thus offers promise to understand tumour-associated changes. Isolation of tumour cells and stromal cells surrounding cancer cells using laser micro dissection offered study of tumour associated microenvironments [53]. Proteomic analysis of different stages of colorectal cancers, it was found that tenascin-C was a potential stromal biomarker for colorectal cancer metastasis [54]. It was a combined methodology of LCM, iTRAQ labelling and liquid chromatography-tandem mass spectrometry (2D LC-MS/MS) of stromal proteins from various stages of colorectal cancer.

Potential markers (AnnexinA2, HSP27, CK19 and 14-3-3 $\sigma$ ) of lymph node metastasis of lung squamous cell carcinoma identified using 2D-gel electrophoresis of laser captured micro dissected cell in human lungs. Such differentially expressed proteins may serve as protein biomarker of metastatic lung squamous cell carcinoma and provides molecular basis of metastasis that might provide a way for predictive diagnosis and outcome of patient's treatment [55]. A proteomic profiling of various types of lung adenocarcinomas using LMD identified 840 proteins in which early lepidic-types of lung adenocarcinomas was associated with numerous tumour progression pathways [56].

Combination of LCM and mass spectrophotometry revealed 14-3-3-mediated signalling pathways as the most relevant canonical pathway of colorectal cancer [56]. Likewise, proteomic study of LCM islets, LCM acinar tissue and EIC islets proteomes enabled accurate and unbiased profiling of islet proteome. Such

studies could enable study of progression of type1 diabetes of pancreas and progression of other diseases where heterogeneous types of cells are involved [57]. Thus, laser micro dissection offers a reliable method to enrich specific cell population for proteome analysis. Proteome profiling of 38 breast cancer tissues in which, comparison of whole cell lysates and LCM enriched tumour epithelial cells were compared, identified more number of proteins in captured epithelial cells [58]. LCM and LMD also overcome limitation of working with small sample size such as the early lesion of cancer of frozen or formalin fixed paraffin embedded tissue sections [59]. Thus, laser micro dissection disregards influence of tissue heterogeneity, low interference from surrounding tissue and offers a better tool for biomarker discovery in meagre of sample.

### Microorganisms

Less known application of laser micro dissection is the isolation and characterization of selective microorganisms from biological samples. Bart De Spiegeleer and colleagues [60] described selective isolation of microorganisms from milk using laser capture micro dissection. Investigators have identified *Methylobacterium spp.* using DNA sequencing of 16SrRNA gene. Phylogenetic identification of unidentified and uncultured microorganisms has been done reliably on the basis of ribosomal RNA sequence [61,62]. Likewise, a combined approach of fluorescent in situ hybridization and laser capture micro dissection has identified *Brachyspira spp* from human gut. Bacteria were targeted with oligo nucleotide probe against 16S RNA in paraffin embedded formalin fixed tissue sections, fluoresced green and regions of interest were micro dissected for DNA sequencing [63].

Use of LCM to harvest *Lawsonia intracellularis* infected pigs enterocytes has enabled investigator to characterize host-bacterial interactions. With the help of RNA sequencing of bacterial infected enterocytes, it was shown that bacteria prevent differentiation of enterocytes by repressing membrane transporters related nutrient uptake and prevented themselves from oxidative stress by utilizing host's Zn/cu superoxide dismutase machinery [64]. In a recent study, LCM successfully used to harvest intracellular malarial parasites from avian erythrocytes for whole genome sequencing [65]. LCM offer investigator to isolate parasitic DNA without contamination of host DNA. Thus, LCM might offer isolation of individual parasites from co-infected hosts and also help in isolation of rear species of parasites from museum/achieved samples [65].

### Molecular profiling of immune-selected cells

In majority of the situations, precise identification of phenotypically similar but immune phenotypically dissimilar cell types are not useful by standard histo chemical staining procedures. Only immune staining with immune specific markers offer unequivocal identification of precise cell types. Mammary stem/progenitor cells of bovine mammary gland have been identified using bromodeoxy uridine (BrdU) label retention

study in which BrdU label retaining epithelial cells (LREC) were micro dissected and expression study were performed using microarray analysis [13]. In this study, LREC (mammary stem/progenitor cells) and non-LREC (differentiated cells from adjacent locations as control cells) were harvested using laser micro dissection and total RNA isolated. Microarray analysis revealed up-regulation of stem cells related genes in LREC in comparisons to non-LREC, provided the evidence of LREC as putative stem/progenitor cells. Immuno staining protocol using glial fibrillary acidic protein marker permitted precise and accurate identification of astrocytes and enabled expression analysis of laser micro dissected astrocytes using quantitative PCR [66].

To understand biology of different cells of pancreas namely,  $\beta$ -cells, alpha-cells and islets, laser micro dissections employed in normal and pathological conditions to harvest cells of interest. Isolating  $\beta$ -cells of pancreas by identifying fluorescence of anti-insulin antibody has enriched  $\beta$ -cells isolation by LCM [67,68]. This protocol prevented contamination of isolated cells from exocrine cells of pancreas. Protocols for obtaining pancreatic  $\beta$ -cells [69] and pancreatic duct glands [70] for transcriptome analysis have been proposed. Immuno-selected specific cells could also use for comprehensive protein profiling. In human pancreatic adenocarcinoma cells, CD24+ cells were micro dissected and protein profiles were compared with adjacent normal tissue that were CD24- cells [71]. In this study, investigators have discovered 27 candidate proteins expressed significantly different in CD24+ cells than normal tissue and suggested these proteins as potential biomarkers for pancreatic cancers as potential therapeutic targets.

### Expression micro dissection (xMD)

It is an immune-based cell or sub cellular isolation tool when combined with laser micro dissection system provides rapid procurement of target of interest from cytological/histological tissue samples. This is very useful in where immune positive cells are harvested from the tissue section with increased speed of dissection in multiple magnitude [72,73]. In one recent study, tumour cells with estrogen receptor-positive nuclei harvested for large region of tissue in contiguous fashion thus offered precise sub cellular dissection of targets [74]. In cancer research, this tool is going to provide information of neoplastic transformation of cells from normal to cancer type. It has been observed that within the same tissue section, various types of tissues are found (Figure 2). Especially in carcinoma, where cells are invasive carcinoma, hyperplasia, partially transformed cells to normal healthy cells is present. xMD is going to offer enrichment of scanty cells from admixture of cell population including pathogens for next generation sequencing, proteomics and genomics study.

### Limitations of laser micro dissection system

Major consideration during immune staining and laser micro dissection of tissue or cells is the integrity of RNA for gene

expression analysis and quality of protein for protein analysis. Reduction in immune staining protocol by short incubation time, maintaining RNase free environment by addition of RNase inhibitor and nuclease free water and buffers are imperative for successful isolation of intact RNA. In addition to that, testing of RNA quality using standard procedures like Nano Drop reading and RNA denaturing gel electrophoresis to visualize 28S and 18S band might not be applicable as the quantity of RNA is so meagre. Only capillary-based electrophoresis system like Agilent bioanalyzer or Bio Rad experion systems could determine quantity of RNA in nano- and pico-grams and quality in terms of RNA integrity number.

Working with limited amount of RNA introduces problems related to signal to noise ratio in gene expression analysis. Quantity of RNA obtained from laser micro dissected cells range between nano- to pico-grams and the amount of RNA needed for microarray or RNA sequencing is in microgram. Therefore, it is required to amplify total RNA from pico- to micro-gram quantity. Various techniques exists to amplify picogram of RNA into microgram quantity that are required for expression analysis [75] indicating that it is feasible and reliable to accurately perform expression profiling of sub-optimal quantity of RNA.

Another limitation is the ineffective isolation of homogenous cell types due to lack of distinct cell types of haematoxylin stained sections. Adoption of immune histo chemical staining prior to LCM will assist researchers for immune phenotypic identification of cells within tissue. In such cases, identified cells are dead in nature and hence could not be propagated to test their characteristics and also studying of expression dynamics of the immune stained protein is limited due to binding of antibody with the protein of interest. Another limitation of laser micro dissection is that a skilful person and therefore, it usually identify various cell types of tissue could cyto architecture of tissue of interest should be known to the investigators [76].

### Conclusion

Laser micro dissection system is a powerful and useful tool in many areas of biological and medical research. The technique is now able to produce meaningful, accurate and reproducible biology at cellular and molecular level. In cell biology, it is used in neuroscience research to study various types of nerve cells. In cancer biology, laser micro dissection is used for study the microenvironment of cancer cells, biology of various cell types and cancer metastasis. Profiling of various cell types within epithelial and stromal compartment of mammary tissue is helpful in delineating their molecular phenotypes. Isolation of microorganisms from tissue is a novel research towards understanding host-pathogen interactions and hence sheds light into progression of the disease.

Laser micro dissection could be the most beneficial and advantageous tool in the context that the area of transcriptomics and proteomics of selective cells with almost nil contamination of other cell types. It would be interesting to see advancement in

this technique to isolate live cells so that isolated cells would be suitable for studying cell kinetics and propagation. Additionally, isolation of cell organelles, low cost system and high throughput micro dissection like expression micro dissection system (xMD) would like help in studying progress of neoplastic transformation of cells and their microenvironments.

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