# LASER CAPTURE MICRODISSECTION

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***Abstract:*** *Such comprehensive task has been accomplished by laser capture microdissection (LCM), a powerful tool used in molecular biology and epigenetics of eukaryotes generally and plant particularly. LCM combines microscopy with laser beam technology and allows targeting of specific cells or tissue regions that need to be separated from others. There are many LCM systems with their own advantages and disadvantages regarding to distinct subjects and purposes. This essay will focus on different LCM systems and its applications on plant studies*

***Keywords:*** *Laser capture microdissection (LCM), ribonucleic acid (RNA), cells, tissue.*

Received 10.1.2021; accepted for publication 25.1.2021

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## 1. INTRODUCTION

Eukaryotic tissues are composed of distinct cells with specific morphologies, functions and genetic, protein, and metabolite profiles (Teixeira, RT; Pereira, H, 2010). This heterogeneous nature of tissues, however, can affect the outcome and interpretation of profiling studies where homogeneous cells are needed for high-resolution data (Vandewoestyne, et al., 2013). Previously, different dissection approaches, such as manual techniques using razor blades, and sterile needles of fine glass pipettes (micropipettes), microcapillary technique, tissue digestion, and cell sorting, were developed to isolate specific cell types from the complex tissues. However, these methods have performed many drawbacks which are time-consuming and lack of precision (manual techniques), failure of the amplification of extracted RNA and failure of obtaining internal cells (microcapillary technique), extensive manipulation (tissue digestion), and changes of gene expression pattern during protoplast preparation (cell sorting) (Teixeira, RT; Pereira, H, 2010). Therefore, it is necessary to develope a cell separation method with high precision, efficiency, and avoidance of contamination.

## 2. CONTENT

### 2.1. LCM systems

LCM is a technique for isolation of specific cell populations from heterogeneous tissue sections, which is described as a faster, more precise, more reproducible and free from contamination method. The first LCM platform, which employed infrared (IR) laser to capture the cells, were introduced by Emmert-Buck and colleagues at the National Institutes of Health in 1996; almost concurrently, another LCM platform employed ultraviolet (UV) laser was developed by Schütze and Lahr in 1998 (Vandewoestyne, et al., 2013). In general, all LCM systems include a microscope for visualisation of the cells coupled with laser generators for capturing (IR systems) or cutting (UV systems) the cells of interest from the surrounding sections (Espina, et al., 2006). The cell-collecting principals of the two platforms, however, are different. In IR LCM, the targeted cells are obtained by using a low-power infrared (IR) laser to activate a special thermoplastic film that is placed over a membrane slide containing tissue sections. The activated transfer film adheres to the cells that are located within the laser beam diameter; these cells, subsequently, are detached by lifting of the film, and then transferred to a microfuge tube containing DNA or RNA extraction buffers (AppliedBiosystems, 2010).

The later platforms, UV LCM, uses a highly focused laser beam to cut out the targeted cells or regions from the tissues, and then the cells of interest were catapulted against gravity into a collection device (Vandewoestyne, et al., 2013). There is another system that combines IR and UV laser for a "cut and capture" method which uses UV cutting laser to cut a narrow outline around the region of interest, and then uses the IR laser to capture entire region within the outline onto the CapSure cap (AppliedBiosystems, 2010). To date, various LCM systems have been developed and commercially available such as Arcturus PixCell, and Arcturus AutoPix (IR LCM systems), Arcturus Veritas (combined IR/UV system), and Zeiss P.A.L.M. Microbeam, Leica LMD6000 and Molecular Machines & Industries mmi CellCut (UV-only systems) (Espina, et al., 2006).

The advantages and disadvantages of LCM systems are evaluated depending on their starting material and following downstream applications (Vandewoestyne, et al., 2013). A comparison between UV- and IR-based systems has been conducted by Vandewoestyne, et al. in 2013, with the peripheral blood mononuclear cells and blastocysts as starting materials to extract DNA and RNA for RT-qPCR. The two LCM systems (PALM Zeiss UV LCM system and Arcturus PixCell II IR LCM system) are compared in terms of user-friendliness, speed, precision, sample preparation necessities, and effect on DNA and RNA quality. The results showed that both systems are equipped with user-friendly, application-dedicated, and easy-to-learn software. However, working with IR system is approximately two times more

time-consuming than working with the UV LCM, this makes UV systems are more efficient when working with RNA.

In terms of precision and avoidance of contamination, the cells of interest are collected by being adhered to the transfer film in IR systems, while the UV systems use the force of UV light to catapult the cells directly into extraction buffer without physical contact with the specimen; thus, uses of UV LCM can avoid contamination from unwanted cells and experimental tools. The xylene dehydration step is compulsory in IR system, which causes problems in release of targeted cells from the slides due to wetness of cells, whereas this step is optional for UV system by alternating fixation method. The DNA extracted using 2 systems were qualified by PCR assays, DNA samples obtained from UV system performed higher quality in comparison to that from IR system. The RNA products of the two systems, however, performed very low integrity (RIN) when being tested with the Bioanalyzer system (Vandewoestyne, et al., 2013). Although IR system seem to performed more limitations in the study of Vandewoestyne, et al., it possesses many advantages such as minimizing tissue damage, suitable for small and scattered targets, and working with plain glass slides (Nelson, Tausta, Gandotra, & Liu, 2006). The UV laser systems, meanwhile, also have a potential limitation which is damage of cells lying directly under the UV laser cutting path, these cells may contribute significantly to the final molecular signal if the number of cells in the perimeter of the cut area is high (410%) compared to the overall microdissected area (Espina, et al., 2006). The IR/UV systems combine the uses of both IR and UV, the former is ideal for capture of single cell and small number of cells, and the later offers unprecedented speed and precision optimal for dense tissue structures and capturing large amount of cells (Thermo Fisher Scientific, 2016).

In short, every system has its own benefits and limitations. Thus, it has been suggested that users should consider key issues before starting their LCM work: (1) type of tissue, animal or plant, (2) fixation method, (3) compatibility with downstream technique, (4) the number of cells needed, (5) extraction method.

**2.2. LCM applications on plants: achievements and challenges**

LCM allows separation of homogeneous cells only which enables profiling studies of transcriptome, proteome and metabolome of specific cell types through microarray analysis or high-throughput sequencing (Nakazono, Qiu, Borsuk, & Schnable, 2003). However, LCM had not been applied in plants until 2002 due to plant cell walls making it difficult to isolate the desirable cells and the formation of ice crystals in the air spaces between cells when preparing frozen sections (Nakazono, Qiu, Borsuk, & Schnable, 2003). In 2002, the UV system were first employed in the plants by Asano et al. to study the gene expression of rice phloem through construction of a specialized cDNA library (Asano, et al., 2002). Almost

concurrently, in 2003, Nakazono et al. published an article on identification of genes expressed differentially in epidermal cells or vascular tissues of maize by using LCM coupled to microarrays (Nakazono, Qiu, Borsuk, & Schnable, 2003). Recently, UV laser system were employed to study the gene expression in the aleurone layer and starchy endosperm of developing rice caryopses in the early storage phase (Ishimaru, et al., 2015). Although LCM has been getting more and more popular on profiling study (transcriptome, proteosome, metabolosome and methylome) of plants, it is mainly used for transcript expression profiles studies in plants due to the possibility of RNA amplification (Teixeira, RT; Pereira, H, 2010).

Despite the fact that LCM has been widely used in both animals and plants, many challenges are remaining. Firstly, LCM is used to isolate single cells or small groups of cells, the quantity of desirable samples is very low and inadequate for downstream processing. As mentioned above, one of the reasons making LCM unpopular in proteomics and metabolomics is lack of amplification techniques for these macromolecules (Teixeira, RT; Pereira, H, 2010). Secondly, there are many manual sample preparation steps involved in LCM methods including fixation, embedding, sectioning, visualization, extraction and amplification causing degradation of macromolecules, especially RNA; whereas most downstream processes require biological molecules with high integrity. Efforts have been made to remedy these challenges by developing alternative methods for each steps and optimizing the protocol for a specific cell type. For instance, microwave processor is employed to reduce dehydration and paraffin-embedding time from one week to 5 hours, and various extraction methods are developed for more specific cell types and smaller amount of materials (Teixeira, RT; Pereira, H, 2010). Many labs have successfully optimized LCM protocol for a targeting tissue; however, protocols work differently from lab to lab. Besides, commercially available extraction and amplification kits are very limited to different types of biological molecules and at picogram level.

## 3. CONCLUSIONS

In conclusion, LCM technique has proved its essential role in profiling study in both plant and animal, especially transcript expression. A pure and homogenous cell population can be precisely isolated from surrounding cells promising better understanding in genomic functions of individual tissue or even individual cell. However, numerous problems for LCM method related to quantity and quality of biological molecules, availability of extraction and amplification kits, and current compatible downstream techniques have remained and need to be addressed by further studies.

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## CHỤP ẢNH VI MÔ BẰNG TIA LASER


***Tóm tắt:*** *Chụp ảnh vi mô bằng tia laser. Nhiệm vụ toàn diện này đã được hoàn thành nhờ vi phân cắt tia laser (LCM), một công cụ mạnh mẽ được sử dụng trong sinh học phân tử và di truyền biểu sinh của sinh vật nhân chuẩn nói chung và thực vật nói riêng. LCM kết hợp kính hiển vi với công nghệ chùm tia laze và cho phép nhắm mục tiêu các tế bào hoặc vùng mô cụ thể cần tách biệt với những tế bào khác. Có nhiều hệ thống LCM với những ưu điểm và nhược điểm riêng liên quan đến các đối tượng và mục đích riêng biệt. Bài tiểu luận này sẽ tập trung vào các hệ thống LCM khác nhau và các ứng dụng của nó trong nghiên cứu thực vật.*



***Từ Khóa:*** *Chụp ảnh vi mô bằng tia laser (LCM), axit ribonucleic (RNA), tế bào, mô.*