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Laser capture microdissection: Should an ultraviolet or infrared laser be used? ☆

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ABSTRACT

Laser capture microdissection (LCM) is a well-established cell separation technique. It combines microscopy with laser beam technology and allows targeting of specific cells or tissue regions that need to be separated from others. Consequently, this biological material can be used for genome or transcriptome analyses. Appropriate methods of sample preparation, however, are crucial for the success of downstream molecular analysis. The aim of this study was to objectively compare the two main LCM systems, one based on an ultraviolet (UV) laser and the other based on an infrared (IR) laser, on different criteria ranging from user-friendliness to sample quality. The comparison was performed on two types of samples: peripheral blood mononuclear cells and blastocysts. The UV laser LCM system had several advantages over the IR laser LCM system. Not only does the UV system allow faster and more precise sample collection, but also the obtained samples—even single cell samples—can be used for DNA extraction and downstream polymerase chain reaction (PCR) applications. RNA-based applications are more challenging for both LCM systems. Although sufficient RNA can be extracted from as few as 10 cells for reverse transcription quantitative PCR (RT–qPCR) analysis, the low RNA quality should be taken into account when designing the RT–qPCR assays.

Tissue preparations are usually inhomogeneous and consist of a mixture of different cell types [1]. This tissue complexity can affect the outcome and interpretation of molecular studies [2]. In transcriptome analysis, for example, it is very hard to assign expression profiles to specific cell populations if complete tissue extracts are used for messenger RNA (mRNA)[2] extraction [2]. Therefore, isolation of pure cell populations is preferable for molecular analysis.

In the past, manual methods of tissue microdissection were the only way to obtain regions of interest from tissue sections mounted on a glass slide [3,4]. The spectrum of manual methods ranges from crude dissection using conventional tools, such as a scalpel or razor blade [4], to more precise methods using a sterile needle, eventually combined with a micromanipulator [5].

Precision, avoidance of contamination, and efficiency of the procedure are the most important parameters in tissue microdissection [6] that cannot easily be achieved using manual microdissection methods even when performed under a microscope [7].

The problems inherent to manual microdissection were solved with the advent of methods that use the principle of light amplification by stimulated emission of radiation (laser) for tissue microdissection. Meier-Ruge and coworkers introduced the use of a laser in microdissection and described this novel procedure as allowing faster, more precise, more reproducible microdissection than the manual methods [8].

The laser was coupled with a research microscope and focused through the objective lens. This makes it possible to isolate defined target cells or even subcellular components, such as organelles and chromosomes, from heterogeneous cell populations without contamination of unwanted cells [9,10]. Isolated cell populations can then be used for genome or transcriptome analysis.

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[2] *Abbreviations used:* mRNA, messenger RNA; IR, infrared; UV, ultraviolet; LCM, laser capture microdissection; PBMC, peripheral blood mononuclear cell; EDTA, ethylenediaminetetraacetic acid; COC, cumulus–oocyte complex; TE, trophectoderm; ICM, inner cell mass; LPC, laser pressure catapulting; PCR, polymerase chain reaction; RT, reverse transcription; cDNA, complementary DNA; qPCR, quantitative PCR.

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During the late 1990s, two very different novel laser capture microdissection platforms were built almost concurrently. In 1996, Emmert-Buck and coworkers at the National Institutes of Health introduced the infrared (IR) laser capture microdissection system [11]. This system became commercially available by Arcturus Engineering as the PixCell system a year after the first publication describing its use. This platform is based on the placement of a thin transparent thermoplastic film over a tissue section. Consequently, the tissue is visualized microscopically. Cells of interest are selectively adhered to the film with a fixed-position, short-duration, focused pulse from an IR laser [11], as shown in Fig. 1. The adherence of the cells to the film exceeds the adhesion to the glass slide, which allows selective removal of the cells of interest [12]. These cells are detached by lifting of the film, which is then transferred to a microfuge tube containing buffer solutions required for the isolation of DNA or RNA [2].

The second platform, the ultraviolet (UV) laser microbeam microdissection system, was developed by Schütze and Lahr in 1998 [13]. A highly focused laser beam was used to cut out the cells or regions of interest in the tissue. By increasing the power of the laser, the desired cells were subsequently catapulted against gravity into a collection device, as shown in Fig. 1. This system was commercialized by PALM Zeiss Microlaser Technologies.

All commercially available laser microdissection systems are essentially based on one of these two platforms. The main variations concern system configuration and intended applications. A variety of instruments exist, but laser capture microdissection (LCM) is the standard terminology used regardless of laser method type [14].

In this study, the two main LCM types (IR and UV laser systems) have been compared in terms of user-friendliness, speed, precision, sample preparation necessities, and effect on DNA and RNA quality. Because LCM is widely applied to several kinds of cell and tissue types, the comparison was performed on two types of samples: bovine peripheral blood mononuclear cells (PBMCs) cytocentrifuged on a glass slide and sections of bovine blastocysts.

Materials and methods

Blood sample collection

This study was approved by the ethics committee of the Faculty of Veterinary Medicine at Ghent University (EC 2012/140). One Belgian Blue bull and one Holstein Friesian cow from the herd of the Faculty of Veterinary Medicine were used as blood donors. Peripheral blood (5 ml) was collected from the tail vein by

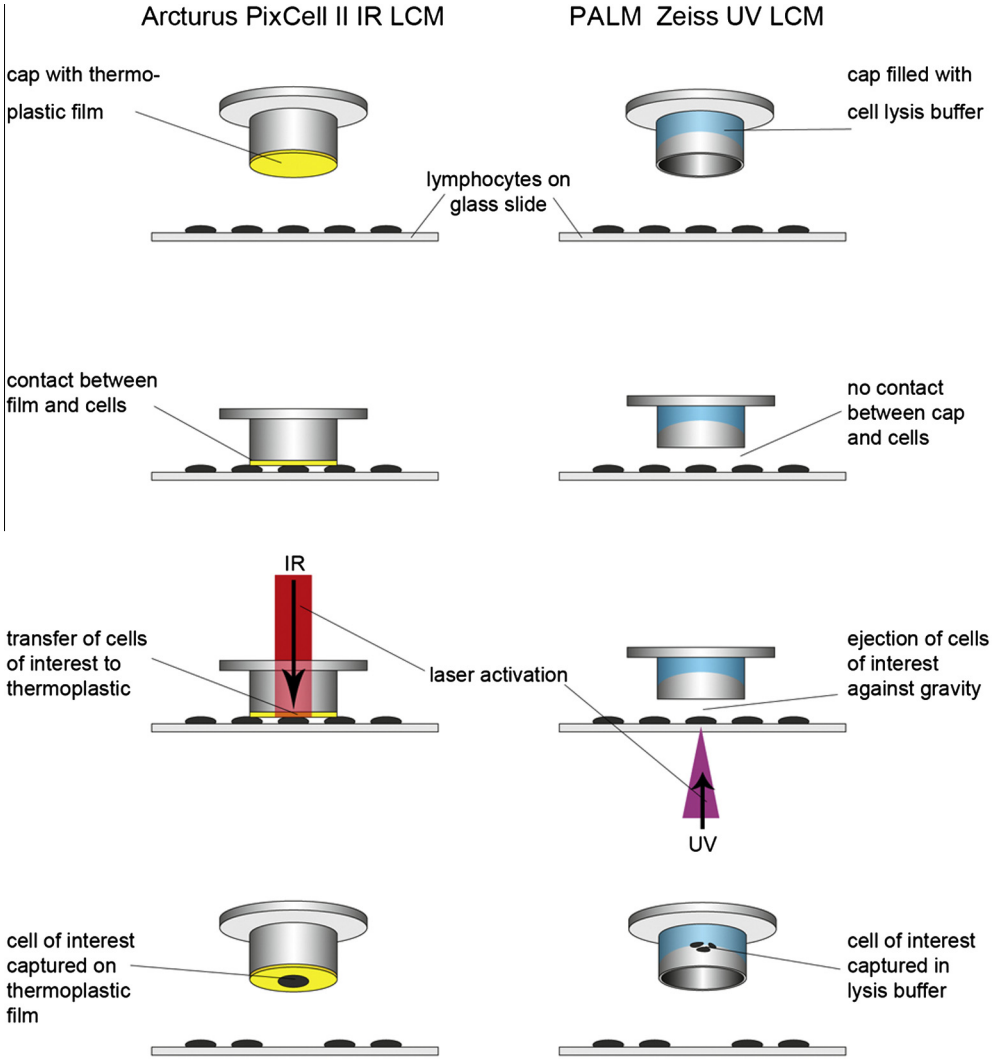

Fig. 1. Schematic representation of the two main LCM systems: the Arcturus PixCell II IR laser system and the PALM Zeiss UV laser system.