Laser microdissection for adherent live cells: what you see is what you get

Cells in culture can be distinguished by microscopy on the basis of different morphology features or fluorescence labeling. By applying laser microdissection from Carl Zeiss, it is possible to access these cells. The desired cells are visualized, selected and transported into a collection device with PALM MicroBeam. The single-step procedure does not require treatment of cells with enzymes or chemicals before or after collection. The process is gentle and preserves cell morphology and label.

Single-step isolation: no trypsinization, no dilution series
So far, the common way to remove single cells from an adherent cell culture is a multistep process. First, the cells are detached, usually using a mixture of trypsin and EDTA. Then a dilution series is done to ensure that individual cells are examined. This relies on chance because one never can be sure that the experiment starts with one cell, no cells, or two or three cells. Another drawback of this method is that enzyme treatment may lead to changes in the cellular state such as the differentiation status of stem cells. In addition, after such treatment various cell types lose their characteristic morphology that makes them easily distinguishable in the adherent state.

Laser microdissection with PALM MicroBeam is the only certain method to isolate selected individual cells without intermediate steps or enzyme treatment. The advantage of the method is that one can remove a ‘true’ single cell from a bulk culture. This single cell can be applied for specific molecular analyses, or homogeneous cell colonies can be obtained from isolated cells by recultivation and clonal expansion.

Contact-free laser capture via LMPC
By means of a precisely controlled laser pulse the selected cell is cut out and vertically ejected out of the object plane into a collection device. In contrast to other laser capture microdissection machines, the PALM system from Carl Zeiss cuts and transports material using only laser light, avoiding any contamination with surrounding material because the transport direction is against the force of gravity. This so-called laser microdissection and pressure catapulting (LMPC) process is unique to Carl Zeiss and has proven to be an invaluable tool for the collection of adherent cells. The current generation of these instruments features a strong modular concept and digital image capabilities, and is expandable with several hardware and software options including the laser Tweezers module.

Figure 1 | Obtaining homogeneous cell populations by positive- and negative-selection methods.

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If the collection device is filled with appropriate medium, the cells can be easily recultivated and clonally proliferated under routine cell culture conditions.

For direct molecular analysis the cells usually are collected in devices filled with dry and sticky material. Cells are lysed in these devices and then analyzed as other samples.

**Individual cell analysis**

The structural and functional characteristics of cells are dependent on their specific gene expression profile. The ability to study and compare gene expression at the single-cell level provides valuable insights into the physiology of a cell. LMPC combined with real-time PCR for expression profiling allows several investigations of individual cells. High-quality RNA can be extracted even from a single cell quickly and conveniently. Efficient cell collection, quick RNA isolation and optimized real-time PCR protocols allow reliable analysis.

With the PALM system, no useful cells of a cell culture have to be wasted. When one or a few cells are removed for analysis, the remaining cells can be kept in culture and used for experiments at a later date. This may be especially helpful for work with slowly growing and valuable cells.

**From mixed to homogeneous**

Even pure cell populations can be far from homogeneous. Noncontact isolation of cells allows easy separation of different cell types from a cell layer for analysis or recultivation. Candidate cells can be identified and expanded using a positive- or negative-selection method.

Using positive selection the desired cells are isolated from the parent culture via LMPC and recultivated in a new vessel. In the negative-selection approach unwanted cells are eliminated (ablated), leaving only the desired cells in the parent culture vessel. Both methods are simple and effective ways to obtain homogeneous populations of a specific cell type (Fig. 1). Cultures showing low transfection rates can be switched to a nearly 100% transfection-positive culture.

Many relevant cells in research are commonly surrounded by different cell types or are difficult to distinguish in a mixed culture. Such cells are often identified by immunological staining with specific surface markers such as fluorescently labeled antibodies or by GFP transfection. Unlike fluorescence-activated cell sorting and magnetic cell separation methods, the PALM system from Carl Zeiss allows individual cells to be sorted directly from the culture, even under fluorescent illumination, and affords the enrichment through clonal expansion after LMPC. Under microscopic view of the specific labeled cells, the selected cell type becomes accessible for characterization in a single purification step without any enzyme treatment or selective stimulation. Also, time-consuming repetitive enrichments can be avoided.

**Stem cells**

Stem cells express a variety of specific pluripotency markers whose expression levels are important for maintaining these cells in an undifferentiated state. Manipulation of embryonic or adult stem cells may trigger differentiation accompanied by changes in morphology and pluripotency marker expression.

Using LMPC stem cells can be isolated and propagated without changing their marker expression. Furthermore, stem cell cultures with diversely differentiated cells can be separated into homogeneous subpopulations, and even embryoid bodies can be sorted (Fig. 2).

**Summary**

The laser-driven isolation of living cells out of a cell culture is a useful innovation. The new release of PALM systems offers a gentle and convenient method to select single cells or small groups of cells that can be used for direct molecular analysis or recultivation.

Additional information is available on our website (http://www.zeiss.de/microdissection).


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