

Laser mediated live cell handling: Detection, selection and collection of single live cells by Laser Microdissection and Pressure Catapulting (LMPC)

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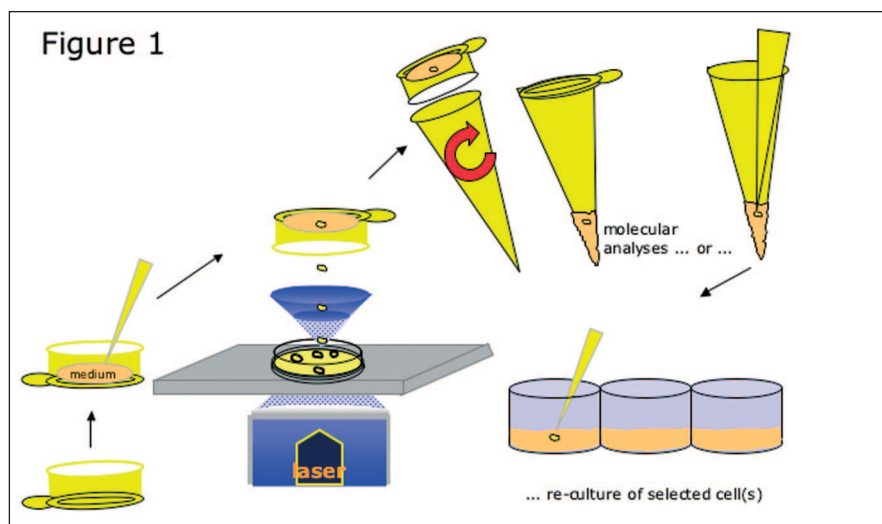


Fig. 1: Principle of LMPC of live cells
The UV-A laser is focussed through the objective. The microdissected cell is catapulted into a medium filled cap of a reaction tube. After spinning down the cell can be analyzed or re-cultured.

Modern molecular research relies on the capability of getting access to pure samples. Laser Microdissection and Pressure Catapulting (LMPC) is a well-known method to isolate and collect specific cells from complex tissues for subsequent molecular analyses. Tissue preparation and extraction protocols allow the utilization of microsamples for quantitative molecular analyses like, e.g., PCR and RT-PCR amplification, microarray analysis, and MALDI/SELDI spectrometry. Up to now, LMPC mostly has been applied on paraffin and cryosections, cell smears, cytopspins and chromosome preparations. An important innovation is the laser driven isolation of live cells out of a cell culture. Individual or small groups of cultured cells can be used for direct molecular analysis or re-cultivation. This helps scientists to isolate cell clones and separate different cell types by morphology or fluorescent label. The work with selected

live cells is extremely facilitated with this new approach and opens a wide field of new applications and research possibilities in molecular biology and medicine as well as cell biology.

The Principle of LMPC (Laser Microdissection and Pressure Catapulting)

A pulsed UV-A laser is coupled into a routine research microscope and focussed via the objective lenses to a micron-sized spot diameter. Within the narrow laser focal spot forces are generated that allow ablation of material (that is cutting: Laser Microdissection); whilst the surrounding tissue remains fully intact. Using the same laser the separated cell(s) or selected tissue area can be lifted up (Laser Pressure Catapulting) and captured in a collection device. This is a totally non-contact process, as only focussed light is used for the transportation of a selected area into a collection device. The

sample is travelling for several millimeters against gravity (Schütze et al, 2002). Targets, from parts of chromosomes up to an entire living organism, as the nematode *C. elegans* are successfully transported without impairing the biological information or the viability of the specimen.

The same principle is applicable for the collection of live cells from a cell culture. The catapulted material subsequently will be spun down and analyzed, or used for further experiments (Figure 1).

New kind of Live cell handling: Tissue Culture, LMPC and re-cultivation

Our proof-of-principle experiments were performed using adherently growing liver carcinoma cells (Hep-G2) and bladder carcinoma cells (EJ-28) (Stich et al., 2003). The cells were cultured in a special culture dish (PALM® DuplexDish), a modified sterile packed double membrane Petriperm dish (Figure 2).

To perform isolation and collection of desired cells, the culture medium was removed; only a humidity layer remains on top of the cells. Microdissection was carried out by laser cutting around the selected cells. The isolated cell-membrane stack was then catapulted into a medium filled cap of a reaction tube.

The cap containing the collected cells was put onto a corresponding reaction tube and centrifuged at 250g for 3 minutes. The cells, now in the tip of the tube, were gently resuspended in additional 40 µl of medium and transferred into a 24-multiwell dish filled with 1ml of medium. Culture was performed in 37°C and 5% CO₂ as usual.

The cells on the membrane stack start to

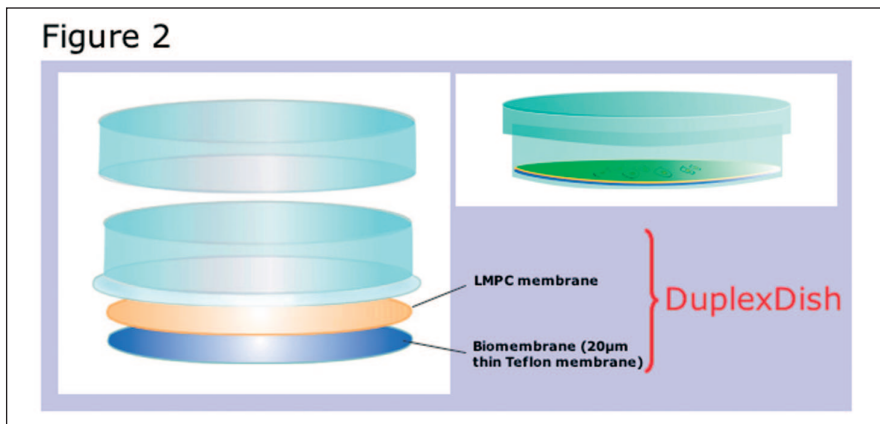


Fig. 2: PALM® DuplexDish
A modified sterile packed double membrane Petriperm dish. The upper LMPC membrane serves as a support and is dissected together with the selected cells.

grow out of the membrane area and proliferate. The proliferation rate of the cells depends on the status of growth at the time of LMPC. If only three cells or less are catapulted the time until proliferation starts may be prolonged to a few days (Figure 3).

After 8 to 10 days of re-culture most of the cell colonies grew to a confluent cell layer in the multiwell plate and have to be passaged again. The trypsinized cells were then seeded out again in PALM® DuplexDishes and the complete procedure of LMPC was repeated. Also these cells remain viable and start to proliferate. Cells in the original DuplexDish already used for LMPC can be long term cultivated and ensure reproducible experiments. Recent results were created by catapulting and re-culturing of murine embryonic stem cells (Wen-Jen Yu, 2003). He was able to show that single stem cells proliferate after LMPC and that even the transgene was expressed.

Live cell handling: Laser Microinjection, Microsurgery, Cell Fusion

For a lot of years lasers are used for micromanipulation of live cells. The laser can cut the cell membrane of mammalian cells, or drill holes into the solid wall of plant cells. Even *within* live cells entire organelles, chromosomes or other cellular parts have selectively been opened, cut or eliminated without impairing cell viability. Within an entire organism, *C. elegans*, single cells have been selectively eliminated or fused by distinct laser shots (Hutter & Schnabel, 1994; Rajcan-Separovic et al., 1995). Also in botanical applications the technology has its use (Connolly 2001).

The focussed laser allows to poke minute holes into cells and nuclear cell walls, which were closed by the cell itself within a few seconds or minutes. This enables injection of, e.g., drugs or genetic material without using viral vectors or chemical treatment of the cells.

Outlook

There is high interest in new methods to handle single live cells. For example stem cell isolation, selective ablation of unwanted cells in a cell culture, creation and maintenance of mixed cell cultures, and maintaining specific cell type ratios in mixed cultures in general is very hard work in cell biology. With the development of a protocol to select and collect (even single) live cells in a non-contact way that kind of work will be dramatically simplified and accelerated.

Using the easy to handle protocol of catapulting live cells allows getting access to clearly selected single or few cells for, e.g., all kind of cloning experiments. This positive selection allows catapulting of desired cells and their re-culture. Negative selection, this means

elimination of unwanted cells in a cell culture, is done by ablating undesired cells from a mixture and ongoing culture of only the remaining cells. This way it is easy to obtain homogeneous cell populations.

The PALM® MicroBeam is the state-of-the-art laser system for non-contact microdissection, pressure catapulting and microsurgery. The method of laser mediated live cell handling promises to take a big step forward in all fields of science related to the study of live cells.

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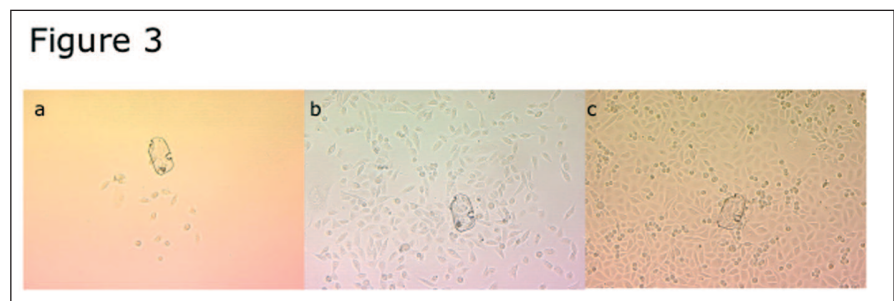


Fig. 3: LMPC of E3-28 cells
Three catapulted cells grown on the LMPC membrane were cultured in a multiwell plate. Images show cells at different days after start of re-culture. a) 4 days b) 7 days c) 10 days after re-culture.