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

Rainer Gangnus and Renate Burgemeister
P.A.L.M. Microlaser Technologies AG
Am Neuland 9+13, 82347 Bernried, Germany
(www.palm-microlaser.com)

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, cytospins 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) is a
pulsed UV-A laser coupled into a routine research microscope and focused 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 focused light is used for the transportation of a selected area into a collection device. 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.

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.

Besides that, 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.
The focused 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.

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.