LASER MICRODISSECTION

What is laser microdissection?

- LMD or LCM (Laser Capture Microdissection),
- contact- and contamination-free method for isolating specific single cells or entire areas of tissue from a wide variety of tissue samples.
- The thickness, texture and preparation technique of the original tissue are relatively unimportant.
- The dissectate is then available for further molecular biological methods such as PCR.

Laser microdissection is used in a large number of research fields:

- neurology,
- cancer research,
- plant analysis,
- forensics.

PRINCIPLE

- LCM technology can harvest the cells of interest directly or can isolate specific cells by cutting away unwanted cells to give histologically pure enriched cells.

- A variety of downstream applications exist: DNA genotyping and loss-of-heterozygosity (LOH) analysis,
- RNA transcript profiling, cDNA library generation, proteomics discovery and signal-pathway profiling.

- The total time required to carry out this protocol is typically 1–1.5 h.

"catapulting" technology.

- The sample can be catapulted from a slide or special culture dish by a defocused U.V laser pulse which generates a photonic force to propel the material off the slide/dish, a technique also called Laser Micro-dissection Pressure Catapulting (LMPC).
- The dissected material is sent upward to a collector which contains either a buffer.
- This process avoids some of the static problems while using membrane-coated slides.

gravity-assisted microdissection

- turns on gravity to collect samples in tube cap under the slide used(used by ION LMD system, Jungwoo F&B).
- it moves the motorized stage to cut the cells of interests, keeping the laser beam fixed. And the system uses a 355 nm Solid-state laser(UV-A) which is the safest way to cut the tissues without RNA or DNA damage.

LCM process (used by Leica)

- cuts the sample from above and the sample drops via gravity (gravity-assisted microdissection) into a capture device below the sample.

- the laser beam move to cut tissue by moving dichroic mirror.

UV based technology (used by Molecular Machines and Industries AG)

- essentially creating a sandwich of sorts with slide>sample>and membrane overlying the sample by the use of a frame slide whose membrane surface is cut by the laser and ultimately picked up from above by a special adhesive cap.

UV based technology uses standard glass slides coated with an inert energy transfer coating and a UV based laser microdissection system

- Tissue sections are mounted on top of the energy transfer coating. The energy from a UV laser is converted to kinetic energy upon striking the coating, vaporizing it, instantly propelling selected tissue features into the collection tube.
- The energy transfer coated slides, commercialized under the trade name DIRECTOR slides by Expression Pathology Inc., offer several advantages for proteomic work.
- They also do not autofluoresce, so they can be used for applications using fluorescent stains, DIC (Differential interference contrast microscopy) or polarized light.

- In addition to tissue sections, LCM can be performed on living cells/organisms, cell smears, chromosome preparations, and plant tissue.

- The process does not alter or damage the morphology and chemistry of the sample collected, nor the surrounding cells.
- useful method of collecting selected cells for DNA, RNA and/or protein analyses.
- It is possible to isolate acellular structures, such as amyloid plaques.
- LCM can be performed on a variety of tissue samples including blood smears, cytologic preparations, cell cultures and aliquots of solid tissue. Frozen and paraffin embedded archival tissue may also be used.