Specimen preparation for Laser Microdissection

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The **PALM® Robot-MicroBeam** allows microdissection of large, homogeneous cell areas, small cell clusters, single cells or chromosomes without any mechanical contact. Thus, there is no danger of contamination with unwanted cellular debris.
The laser works with pulsed UV-c light, yielding „cold ablation“ or photodecomposition of selected specimen without heat formation. This way, cellular material - like unwanted cells within larger areas - can selectively be destroyed. Adjacent cells or tissue remain entirely preserved and there is no enchroachment of DNA, RNA or protein recovery.

With the P.A.L.M. propriety the unique **L**aser **P**ressure **C**atapulting (**LPC**) technique the selected specimen are ejected from common glass mounted tissue, cytocentrifuged samples, or from cell smears and catapulted directly into a common microfuge cap. Single laser shots are used to capture individual cells, whilst larger areas are procured by sweeping over it with a high pulse repetition rate (like gunfire). If required, single cells or larger cell areas can be circumcised by the laser (**L**aser **M**icroBeam **M**icrodissection - **LMM**) prior to catapulting. This way, a micronsized, well defined material-free gap is performed around the selected specimen to avoid any contamination with neighboring cells or tissue warranting entirely clean sample preparations.

LPC of glass mounted samples results in tissue flakes or cell fragments, which however, are perfectly suited for subsequent DNA, RNA or protein analysis. If small cell clusters are required or several cells are pooled, direct catapulting from glass-mounted specimen is suggested.

For larger areas or fragile structures, the morphology is entirely preserved during LPC, if the specimen are mounted on the supporting LPC-membrane. The 1.35 µm thin membrane just holds the selected area or specimen together during the catapulting procedure. This so-called MicroBeam-MOMeNT technique (**M**icrodissection **O**f **Mem**brane mounted **N**ative **T**issue) yields morphologically entirely preserved specimen. For the isolation of rare single cells from tissue or widely spread cells after a cell-smear as well as for fragile structures like chromosomal spreads, we also recommend the MOMeNT-technique, which additionally yields higher efficiency rates (see Bernsen et al. 1998)

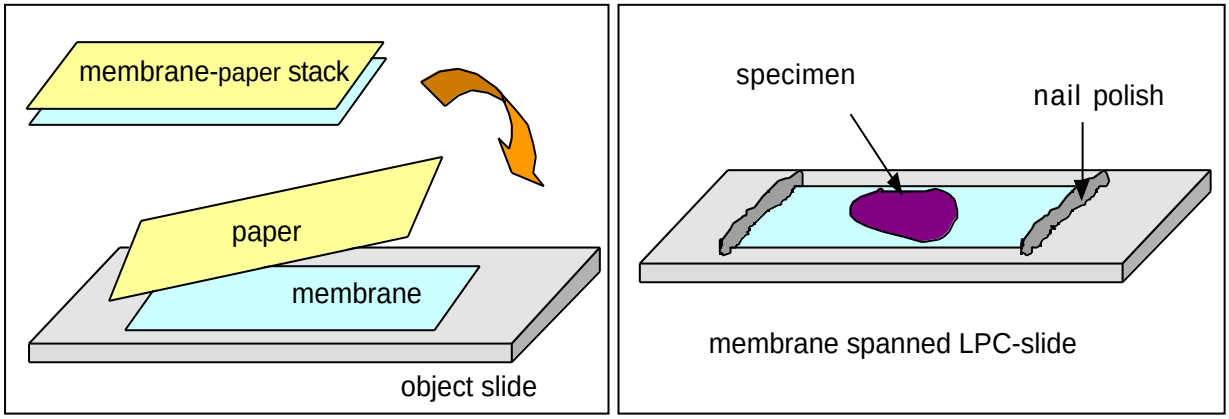
In general:

- **The specimen can be mounted on regular glass slides.** Charged (+)slides are well suited. Thin object slides are only required in combination with short working distance objectives.
- **All common tissue preparations are suitable for LPC.** There are no restrictions regarding the dehydration steps or staining procedures. Just apply any regular histological staining (like hematoxilin-eosin, methyl-green etc) as well as immuno-histological staining and so on. (For subsequent RNA or DNA recovery see also: Burton, MP., Schneider, BG., Brown, R., Escamilla-Ponce, N., Gulley, ML.1998. Comparison of histologic stains for use in PCR analysis of microdissected, paraffin-embedded tissuses. *Biotechniques.* **24, 1**: 86-92.
- Allow freshly prepared specimen to dry some minutes. Do not use specimen directly out of the fridge or freezer, since humidity will form due to condensed water.
- **The specimen should tightly stick to the glass surface or onto the MOMeNT-membrane.** For paraffin embedded specimen coating with some tissue-adhesive like Poly-L-Lysine might be advisable prior to specimen application. Frozen sections could be used without coating. **(Note:** Do not use Eucit, Gelantine etc., as they always remain moist!)
- **In case of high humidity**, dry the specimen in a heater at 30-40°C. Storage together with some silica beads etc. might help to keep the specimen dry.

1. MicroBeam-MOMeNT: Membrane application:

The membrane comes on a sheet of paper having a pattern, which allows to cut the membrane in aliquots of the right size for object slide mounting (about 2x4 cm). Just take good scissors and cut along the lines. Dip the object slide into 100 % ethanol and bring the paper-membrane stack close to the object slide. The

membrane will immediately stick to the glass upon approach. Position the membrane with gloved fingers and fix it with some Fixogum (*Marabu*) nail polish, Rubber Cement, Eucit or Cristal mount) as demonstrated in the figure below to prevent the membrane from floating away while the specimen is processed. (Note: please use normal object slides without any adhesive coating!) (Note: if nail polish is used, „long-lasting“ or “high resistance” brands are preferable. Additional taping with selected tapes might be useful to keep the membrane tightly attached on the slide.)



Time for membrane mounting: approximately 1 minute per slide. The membrane-mounted slides can be stored at room temperature until needed. (“Ready-to-use“ LPC-slides are available through P.A.L.M. GmbH: order number 8150). Please note: to facilitate membrane mounting without wrinkles, the membrane should be smaller than the object slide. Pieces of about 2x3,5 cm are well suited. For sterilization you may use UV-light.

2. Sample preparation:
It is extremely important to tightly adhere the tissue onto the membrane!
0,1% poly-L-lysine is advised to be used (apply some droplets onto the membrane and wipe with Kleenex or a brush). Allow at least 10 minutes to dry. (Do not use Eukit, gelantine or any comparable sticky material, as this will prevent catapulting)

3. Tissue mounting of fixed and paraffinized sections

Priska Banko (Department of Pathology, Academic Hopital München-Harlaching, Munich, Germany)

a. Treat membrane with Poly-Lysine on the object-slide
b. Put paraffin-section on the membrane
c. Dry section overnight at 40°C in the incubator

d. How to get rid of the paraffin:
   - Xylol 0,5 to 2 min.
   - Et-OH abs. 2 min.
   - Et-OH 96% 2 min.
   - Et-OH 70% 2 min.
   - rinse with Aqua dest

f. Optional: Turn membrane-tissue stack up-side down if desired (see Bernsen et.al)

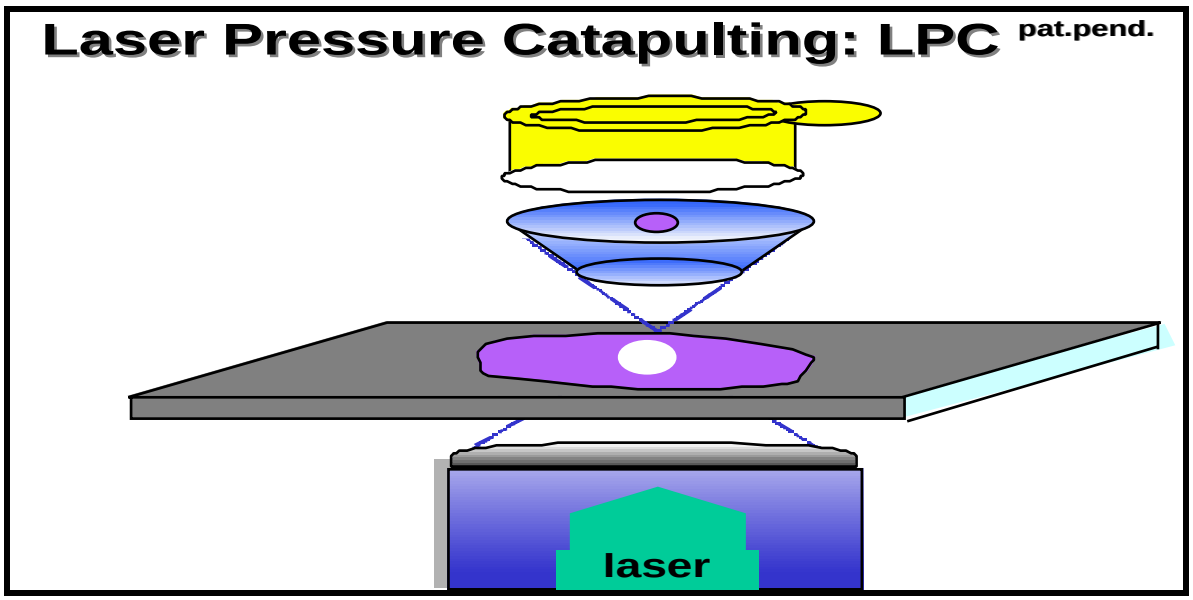
e. Staining:
   - Haemalaun described by Mayer et.al. 5 min.
   - water5 min.
   - Eosin 2% 1 min.
   - rinse with water
   - Et-OH 96% 1 min.
   - Et-OH 96% 1 min.
   - Et-OH abs. 1 min.
   - Et-OH abs. 1 min.

4. Tissue mounting of frozen sections
You can also use frozen sections for laser microdissection and catapulting. Membrane mounting of frozen sections provides higher efficiency rates, if single cells are collected for subsequent genetic analysis (see also Bernsen et al. 1998). Just apply the section onto the membrane. In this case, usually no poly-L-lysine is required .

5. Membrane cover:
You can either use the preparation right away for laser microdissection. For protection against contamination, the membrane-tissue stack may be turned upside down and mounted on a separate object slide.

- onto a **normal** object slide: laser microdissection is possible with long distance objectives only; magnification of 40x and less!
- or onto a **0.17 mm thin** (P.A.L.M. GmbH) object slide: working with high magnifying objectives for single cell or chromosome micropreparation!

Use some ethanol to adhere and flattened the probe. You may fix with nail polish as described above.
This way, a flat tissue slice is achieved, covered by the supporting membrane, which additionally serves as protection against contamination.



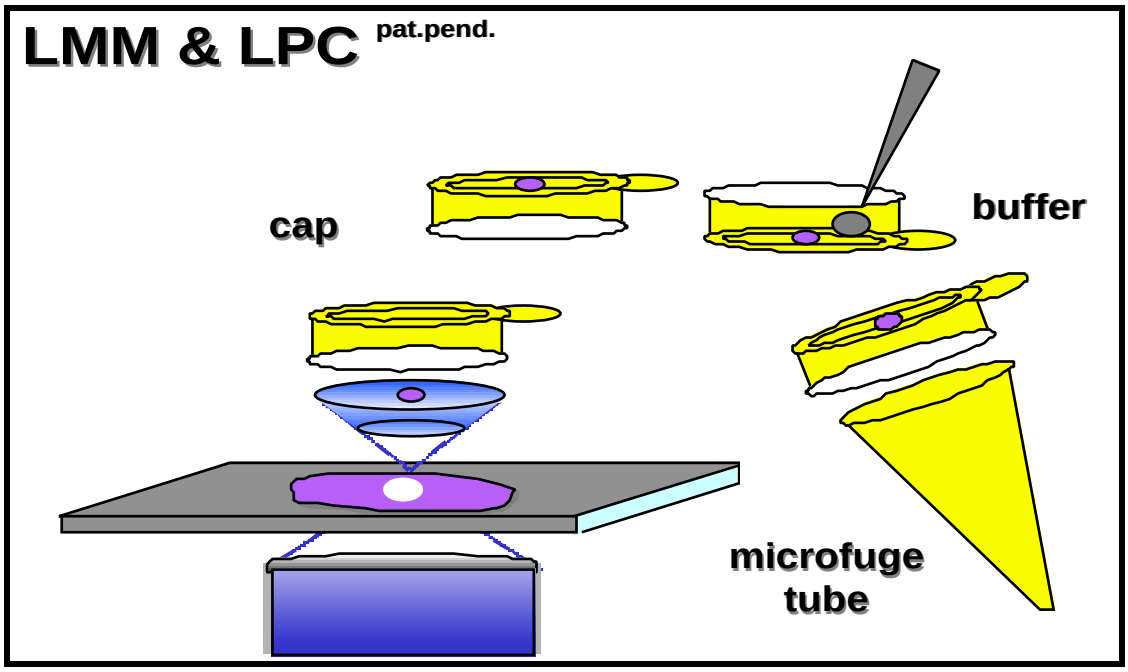
LMM&LPC

The laser precisely cuts around the selected specimen leaving a micron sized gap, which separates selected from non-selected material. Unwanted cells within a large area can be selectively eliminated with a few laser shots prior to microdissection, yielding entirely homogeneous tissue probes. The laser isolated specimen are subsequently ejected from the slide with one single, precisely aimed laser shot in an entirely ”non-contact” manner. The ejected specimens are either caught on a small piece of cover glass, or directly catapulted into the cap of a common microfuge tube - both mounted on the PALM® **LPC**-collector.

Isolation and transfer of a single cell into a microfuge tube cap takes less than 30 seconds. The isolation and transfer of cell clusters was depending on their size and the objective used. For example, a cell cluster with a diameter of 100 µm took less than 60 seconds, whereas a 1000 µm cluster required 1 to 2 minutes.

Preparation of the microfuge cap:

- **paraffine oil:** a thin film of paraffine oil is spread onto the cap surface. The paraffine oil is inert and not interferring with subsequent PCR analysis. This way, the specimen will tightly stick onto the cap surface until needed.The topped cap can even be sent all over the world. Furthermore, the specimen will beautifully show up in the microscope, since the oil acts as a sort of embedding material.
- **PCR-buffer**: It is also possible to apply a small droplet of PCR-buffer, which has to be placed into the inner ring of the mcirofuge cap (Dr. Dries, personal communication). The droplet will be held by the plastic ring and stay for some time before it evaporates. This means, the specimen has to be processed right away after catapulting.
- **take as it is:** the catapulted specimen may stick to the inner surface by electrostatic forces. However, there might be a decharging and the specimen may uncontrollable fall into the tube, where it will stick anywhere onto the tube surface - thus, it is lost for subsequent analysis.



Specimen recovery

The specimen within the cap are either topped with the remaining tube and stored until needed. Otherwise, the specimen are simply covered by proteinase-K containing (PK)-buffer or with the buffer for total RNA isolation (Schütze and Lahr 1998) or any routinely used buffer. To dissolve the specimen the droplet should be soaked-up and down several times using the pipette. Thereafter, the cap is topped with the remaining tube, the specimen is spinned down into the tube and processed as usual. Dissolving of the specimen can also be achieved by simply letting the specimen with the buffer react overnight with the tube standing upside down. Centrifugation thereafter will spin down the sample into the tip of the tube.

LMM&LPC can also be used with cytocentrifuged cells mounted directly onto the glass slide or onto the supporting membrane. The same technique can be used with all cell and blood smears or chromosomal spreads. You may also culture cells directly on the 1-1.5 µm thick membrane. Just mount the membrane onto a thin object slide as described above and submerge it in the culture medium. Let the cells grow, fix them and mount the foil, if desired, upside down onto the object slide. Fix with nail polish.

The LMM&LPC-technique allows to selectively cut out and procure any kind of native or fixed tissue specimen, independent of its size or shape. Single cells or entire tissue areas can be captured in a quick and convenient manner and above all without any mechanical contact (Schütze & Lahr, 1999).

Good luck!

For further questions please directly contact the P.A.L.M. GmbH in Bernried:
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Please also visit ***our website: www.PALM-Mikrolaser.com***

Some current references:

Schütze K. et al., Nature Biotechnology, 16,8: 737-742 (1998)
Schütze K. et al., J. Amer.Laboratory (March 1999)
Bernsen M. et al.; Lab. Invest. 78: 1267-1273 (1998)
Fink L. et al., Nature Medicine, 4,11: 1329-1333 (1998)