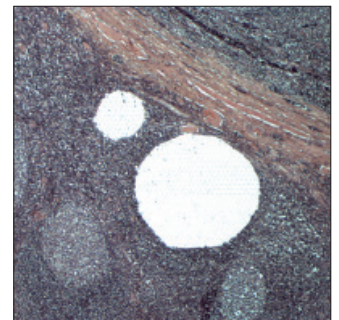
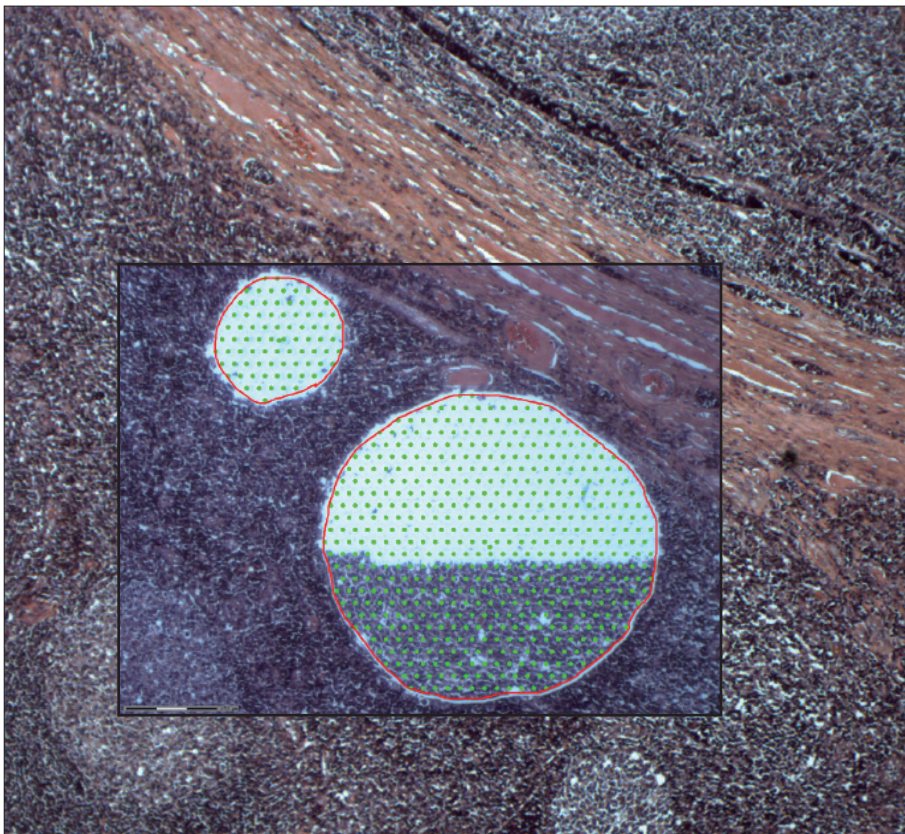


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AutoLPC from glass slides results in good quality RNA



We make it visible.

Front figure: AutoLPC – The power of multiple single pulses

Harvesting glass-mounted tissues is difficult in terms of collecting a complete tissue region. The AutoLPC function „fills“ the outlined desired region with LPC-dots. Each lifted dot transports tissue material into the collection device.



We make it visible.

Non-contact LCM (laser capture microdissection) is the method of choice when differential gene expression shall be analyzed in specific cell types and structures within tissue samples. Due to easier handling and faster work as well as better documentation possibilities a MembraneSlide is normally the recommended LCM-basis for any newly prepared sections especially when later RNA analyses are intended.

For preparations that have to be made on regular glass slides – and this includes even old archived slides (e.g., from Pathology) where the coverslips can be removed by appropriate solvents – PALM MicroBeam offers the unique and proprietary software function of “AutoLPC” (automated laser pressure catapulting).

This feature allows non-contact laser transfer of material directly from the surface of glass slides without the need for any supporting membrane. By automated scanning of the selected area with defined laser pulses few cells to very large tissue areas can be collected. (see Fig. 1)

The small “flakes” of material gained by this method can now be lysed for RNA extraction or used for any other application just like the samples collected from MembraneSlides. Therefore downstream procedures do not require any special adaptation for AutoLPC.

Especially new users of laser microdissection often feel some uncertainty about the possibility of impairment of RNA induced by the laser without the “protecting” membrane.

To show the reliability of this technique we compared equal samples collected either from MembraneSlides or with AutoLPC directly from regular glass slides.

Method:

We chose frozen mouse liver tissue as a model system because its homogenous structure allows preparation of multiple samples from the same section without the risk of strong local variations in expression. Furthermore, fresh material stained with our quick CresylViolet (CV) protocol allows precise direct RNA quality analysis on a Bioanalyzer (Agilent Technologies) without distraction by fixation artefacts.

Serial sections of 10 µm thickness were placed in parallel on MembraneSlides or glass slides, CV-stained and samples of 0.75 and 0.50 mm² were collected in duplicate from both preparations.

Total RNA was purified from the captured samples with the Qiagen RNeasy Micro kit and 1 µl of each eluate analyzed on a Bioanalyzer Pico-chip.

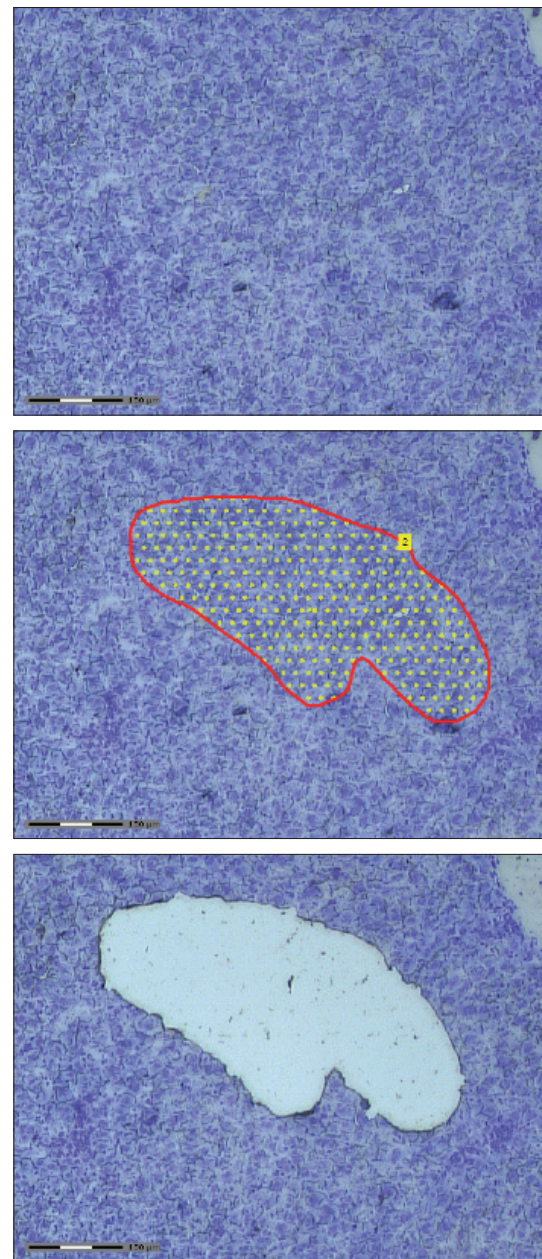


Figure 1: AutoLPC
(Mouse liver, frozen section, CresylViolet staining)

Results – RNA integrity:

The assessed RNA qualities, mainly demonstrated by the RIN (RNA integrity number) values as well as the isolated total amounts did not vary significantly between the samples collected from membrane by RoboLPC and those collected from glass by AutoLPC.

All samples showed similar high qualities between RIN 8 and 9. (see Fig. 2; A,B = duplicates)

Even by hundreds of AutoLPC pulses no apparent fragmentation occurs which again proves the safety of UV-laser microdissection. (see Fig. 3a, 3b)

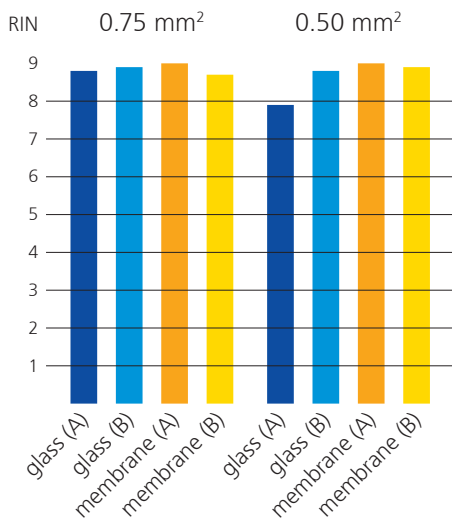


Figure 2

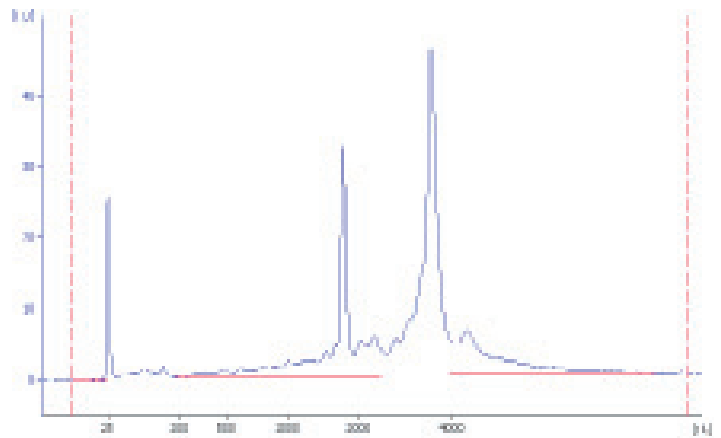


Figure 3a RNA Quality – AutoLPC **glass** 0.75 mm²

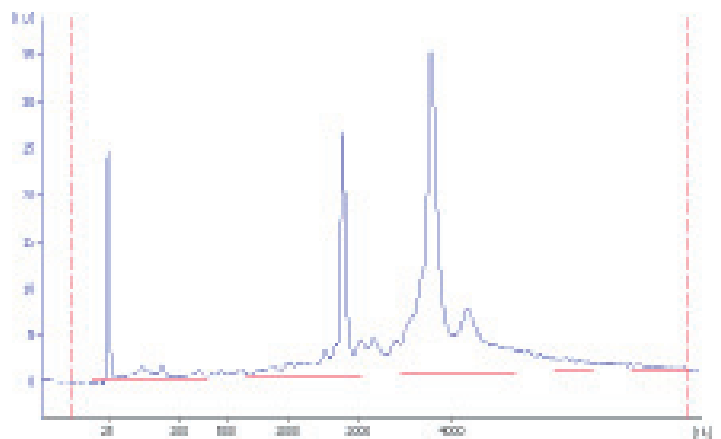


Figure 3b RNA Quality – RoboLPC **membrane** 0.75 mm²

Results – RNA yields:

When few cells or smaller areas from tissue sections are isolated - especially when using higher magnifying objectives like 20x or 40x for higher precision - the possible amount of extracted RNA is mostly far too low to be analyzed directly.

Often the lower concentration limit for the presently highest possible sensitivity of analysis just cannot be reached (Bioanalyzer Pico-chip: about 50-200 pg/ μ l total RNA). So comparison of RNA yields from such small samples is only possible after reverse transcription and quantitative PCR analysis.

We therefore performed similar laser capture comparisons of AutoLPC and membrane samples as described above with smaller areas and various objectives (5x - 40x). After laser capture and RNA extraction a quarter of each RNA sample was reverse transcribed to cDNA and equal aliquots were then used as templates for real time PCR in a Light Cycler Instrument (Roche Diagnostics - mPBGD; Quantifast Sybr Green PCR kit). Pairwise comparisons of corresponding AutoLPC or membrane samples were based on the Ct values within the same PCR run. (see Fig. 4 for example)

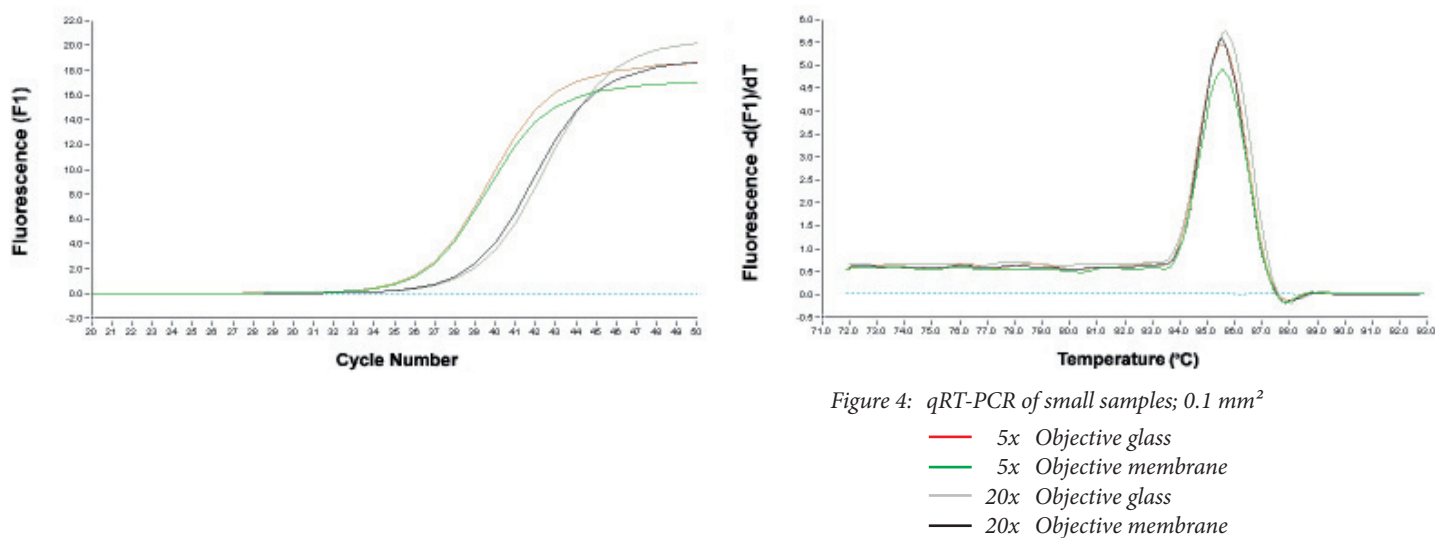


Figure 4: qRT-PCR of small samples; 0.1 mm²

- 5x Objective glass
- 5x Objective membrane
- 20x Objective glass
- 20x Objective membrane

Conclusion:

Down to areas of about 20000 μ m² (corresponding to about 50 cell equivalents) reproducible results could be achieved without significant difference between membrane and glass slides. Larger areas can be collected easier and quicker with lower magnifications due to the creation of larger “flakes” with each single laser pulse of AutoLPC. Nevertheless, the RNA yields as estimated from the crossing points (Ct-values) of the real time RT-PCR seemed not to be influenced strongly by the used objectives (only up to 1 cycle difference when overall sample to sample variation is already between 0.5 to 1 cycle). Anyway, in real experiments the objective has to be selected according to the wanted precision and parallel samples for expression analyses should always be collected under the same conditions within one experiment.

Concerning the RNA yields from small samples we could detect quite some variations from experiment to experiment, especially when different users performed the laser capturing. To a large extent this probably reflects the more demanding optimization of settings when working with AutoLPC. Therefore some investment of time may be necessary to find the ideal combination of laser energy, laser focus and distance of AutoLPC shots for each specific tissue or sample type. Once the above mentioned factors are perfectly tuned, the RNA yield and quality can be the same as those from membrane samples.

Summary:

Our experiments show that non-contact laser capture microdissection by AutoLPC from glass slides does not influence the RNA quality. Furthermore, the performance of optimized AutoLPC is comparable to membrane based microdissection.

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