LMPC as a high throughput process

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ABSTRACT

Laser microdissection and pressure catapulting (LMPC®; PALM® MicroBeam System) combined with gene expression analyses is useful in a wide range of research and clinical applications. The PALM® RoboMover allows this procedure in a high throughput performance. We analyzed the reliability of automated harvesting and subsequent downstream applications in this process.

INTRODUCTION

Tissue heterogeneity has been a major drawback to the application of genomic and proteomic studies in human disease. A number of strategies have been employed to analyze pure cell populations. Most of these methods (like e.g. enzymatic digestion or short-term cell culture) can substantially alter patterns of gene expression.

P.A.L.M.'s patented laser microdissection and pressure catapulting (LMPC) process renders possible the rapid isolation of pure populations of cells from heterogeneous tissues. This technique allows studying DNA and RNA in even single cells captured from specific areas of tissue under direct microscopic visualization.

The PALM® RoboMover is a special solution appropriate to high throughput processes. It is the ideal complement of the PALM® MicroBeam especially in combination with P.A.L.M.'s new RoboStage II for highly automated harvesting and sorting of different kinds of microdissected specimen into different kinds of collection devices.
The RoboMover is controlled by the PALM® RoboSoftware which allows various ways of collecting and handling. There are different targets available (routine and adhesive 8-CapStrips, microtiter plates and of course a single cap). PALM® RoboSoftware automatically recognizes the type of applied target. For each target well of a microtiter plate or an 8-CapStrip the kind and number of cells to be captured can be preselected and the LMPC process can be operated automatically or manually, respectively.

MATERIALS AND METHODS

Tissue preparation

Snap-frozen murine liver tissue stored at -80°C was cut into 7 µm serial sections on a cryostat at -25°C. The sections were transferred to PALM® MembraneSlides (1 mm glass slides covered with a 1.35 µm thin Polyethylene naphthalate membrane to facilitate the laser pressure catapulting procedure) and air dried for 10 seconds. After a 5 minute dehydration step in 70% ethanol at -20°C the sections were further processed according to standard histochemical procedures.
For DNA analyses sections were stained with Hematoxylin/Eosin (HE) and air-dried after a short increasing ethanol series.
For RNA analyses sections were stained with cresyl violet acetate (1%). After an increasing ethanol series they were air-dried and used immediately.

LMPC

In this study we used 8-CapStrips filled with adhesive material, but it is also possible to use 8-CapStrips filled with 40 µl of lysis buffer.
12 strips were used for the corresponding MultiCap insert for capturing in 96 cavities. In 40x magnification we catapulted three areas per well, each about 8000 μm^2 (288 areas in total). The operation mode "n elements per field" allows determining how many elements will be microdissected and catapulted into one target. As soon as the preset number for the selected target is reached, the RoboMover automatically moves to the next one.
Increasing the number of elements per field from 1 to 3 allows easily catapulting of 288 elements in 96 wells (figure 1 and 2). If more than one slide is needed to collect a sufficient number of cells, it is possible to analyze up to three slides with the RoboStage II. After marking the areas of interest the RoboMover target was loaded and adjusted in the first position (1A corresponding to a conventional microtiter plate).

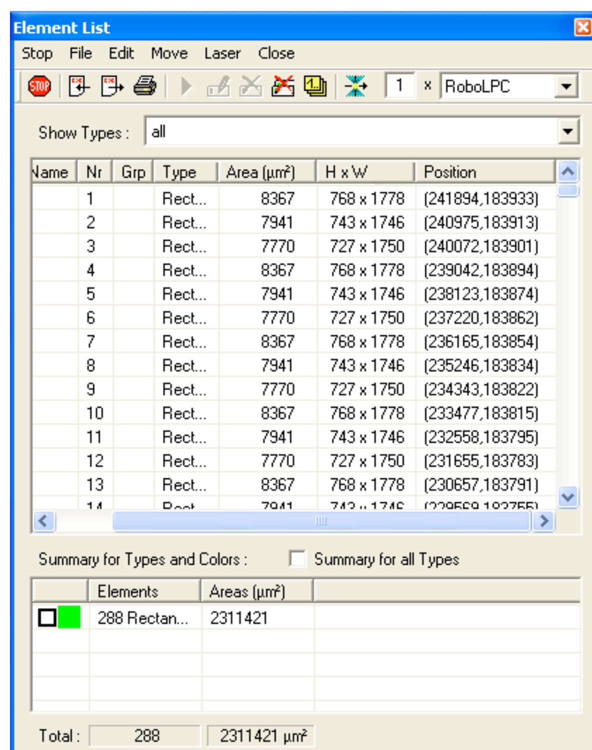

figure 1: Element list

The columns in the table (figure 1) display the following information about each element:

Nr:	number of the element
Type:	type of the element outlined (dot, line, rectangle, Auto-Circle)
Area (μm^2):	Area for elements of type "rectangle" and "circle" in μm^2
Position:	the position (X, Y) relative to the reference position

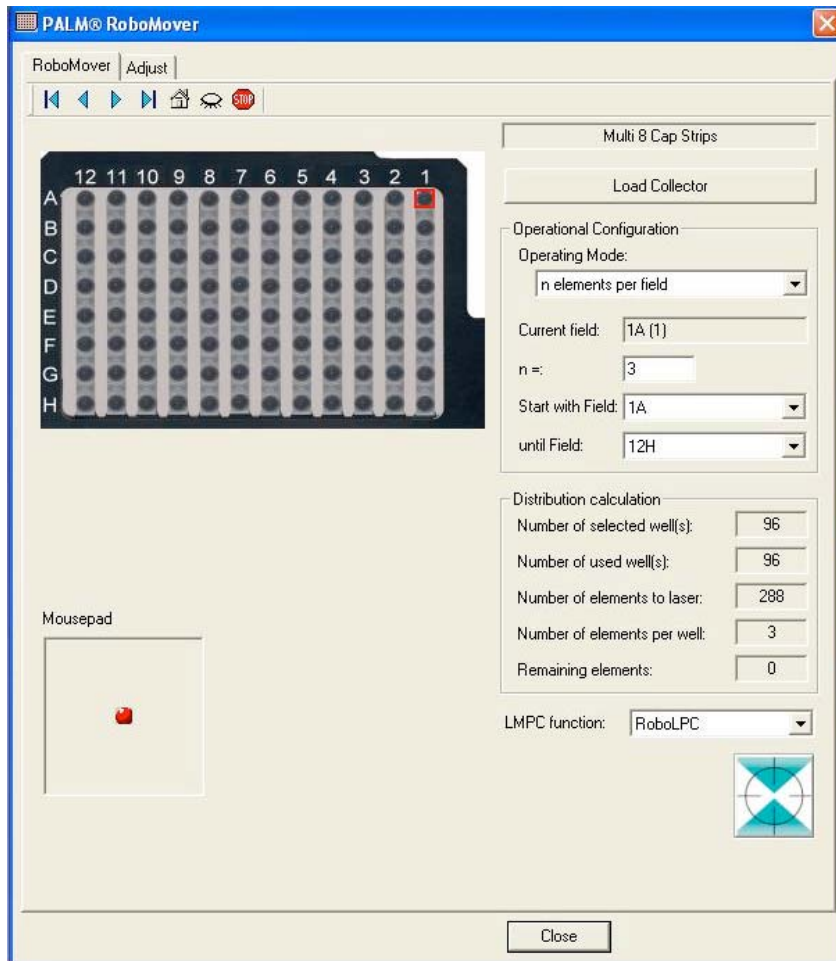

figure 2: Software window PALM® RoboMover

In the operational configuration the number of elements and the field of the first cap and the last one into which elements have to be catapulted are determined. The distribution calculation allows an overview of the experiment actually. The laser function (LPC, AutoLPC, CloseCut + AutoLPC, RoboLPC or AutoCircle) can be selected in the field "LMPC function". The "Mousepad" allows the control of the catapulted specimen inside the cap and a positioning.
Further details can be found in the software manual.

Activation of the Autofocus function allows a real automation as each element is catapulted from its optimal focus point. After the selection of the desired laser function "RoboLPC" the automated laser function was started.
To check catapulting efficiency we controlled arrival of the catapulted cells inside the collection device. For this purpose the cap was moved to the checkpoint where the microscope is focused into the cap automatically. With a small mouse pad at the left bottom of the RoboMover window (see figure 2), the cap was fine-positioned and the microdissected area or single cell can be documented by taking an image (figure 3). For subsequent PCR analyses DNA or RNA was extracted.

figure 3: Captured areas; view inside the collection cap

DNA isolation

The captured samples were purified using the *CST*® Genomic DNA Purification Kit (DNA Research Innovations Ltd.). Tubes corresponding to the 8-CapStrips were filled with 125 µl lysis mix containing Proteinase K. The insert containing the 8-CapStrips was used to stamp the caps to the tubes. Samples were incubated overnight at 55°C upside down and then centrifuged for 5 minutes at 5000 rpm. After incubation with RNase A the purification buffer and *CST*® coated magnetic beads were added. Magnetic beads were separated, washed and purified DNA was eluted from the beads by addition of 20 µl elution buffer. DNA was directly used for PCR based amplification.
For PCR analysis the Eppendorf Mastercycler was used to amplify a 130 bp fragment of the murine porphobilinogen deaminase (PBGD) gene in a nested PCR.

RNA isolation

Captured samples were purified using the Qiagen RNeasy® Micro Kit. Tubes were filled with 75 µl buffer RLT and vortexed for 30 sec upside down after stamping the caps to the tubes. Samples then were centrifuged for 5 minutes at 5000 rpm. After homogenization with 70 % ethanol samples were applied to an RNeasy MinElute Spin Column, DNase treated, washed and eluted in 14 µl RNase-free water. Total RNA quality was analyzed using Agilent's Bioanalyzer. RNA 6000 Pico LabChip®kit chips were prepared according to the provider's instruction.

Conclusion

The PALM® RoboMover is a reliable and convenient solution for highly automated harvesting and sorting of different kinds of microdissected specimen in a high throughput process. In this study 288 areas of interest (see figure 4) were captured and analyzed for DNA and RNA (see figure 5).

12A +++	11A +++	10A +++	9A +++	8A +++	7A +++	6A +++	5A +++	4A +++	3A +++	2A +++	1A +++
12B +++	11B +++	10B +++	9B +++	8B +++	7B +++	6B +++	5B +++	4B +++	3B +++	2B +++	1B +++
12C +++	11C +++	10C +++	9C +++	8C +++	7C +++	6C +++	5C +++	4C +++	3C +++	2C +++	1C +++
12D +++	11D +++	10D +++	9D +++	8D +++	7D +++	6D +++	5D +++	4D +++	3D +++	2D +++	1D +++
12E +++	11E ++-	10E +++	9E +++	8E +++	7E +++	6E +++	5E +++	4E +++	3E +++	2E +++	1E +++
12F +++	11F +++	10F +++	9F +++	8F +++	7F +++	6F +++	5F +++	4F +++	3F +++	2F +++	1F +++
12G +++	11G +++	10G +++	9G +++	8G +++	7G +++	6G +++	5G +++	4G +++	3G +++	2G ++-	1G +++
12H +++	11H +++	10H +++	9H +++	8H +++	7H +++	6H +++	5H +++	4H +++	3H +++	2H +++	1H +++

figure 4: automatic LMPC procedure; catapulting of 288 elements
+ LMPC successful: 286 of 288 elements corresponding to 99.3 %
- not automatically catapulted (cutting process not successful): 2 of 288 elements

Analyzed samples show RNA of excellent quality. (figure 5)

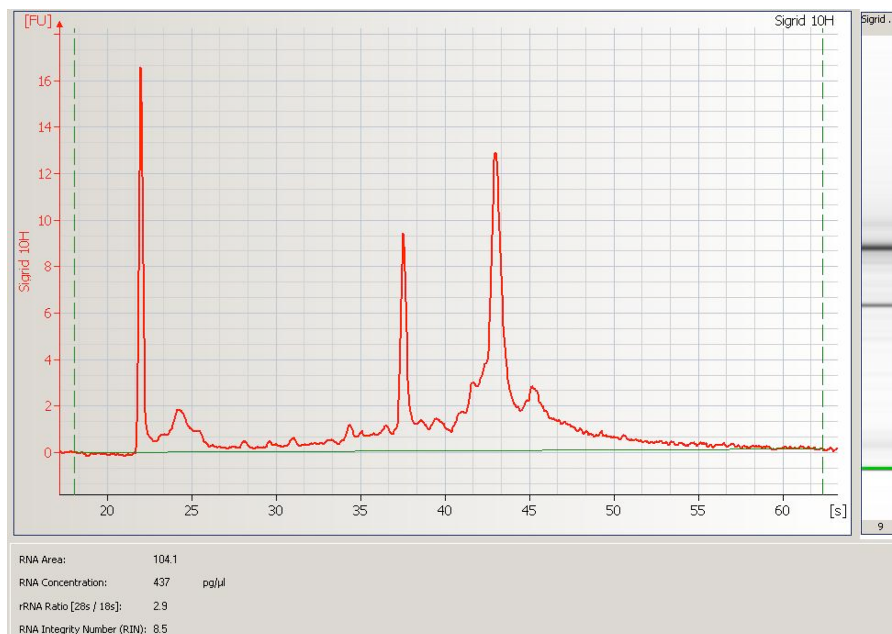

figure 5: RNA quality after LMPC and RNA isolation using the RNA 6000 Pico LabChip® kit

Interestingly even extended processing times for large specimen quantities did not influence RNA integrity. RNA extracted from samples collected in the last well showed no difference in quality compared with RNA extracted from samples collected in the first well.

The use of caps filled with adhesive material (PALM® AdhesiveCaps) allows collecting samples in a completely dry way, which protects RNA from degradation.

Possible further applications for the PALM® RoboMover

Cell-specific mutation detection to evaluate genotype in malignant, surrounding premalignant and nonmalignant cells, resp., in a variety of tumors is extremely useful. Gene expression analysis of complex tissues, for example in normal, metastatic and invasive breast cancer cells, resp., as well as proteome analysis of microdissected tissues is a nifty and outstanding way to detect differences between specific cells. These challenging applications need to separate between cells and have to avoid using bulk material. The development of automated microdissection technology in combination with automated cell identification (e.g. MetaferP or Cellenger) will become an extremely useful tool to apply genetic and proteomic applications in research and routine.

Wherever specific material from a complex tissue should be sampled into different collection devices, the automatic high throughput collection option of the PALM® MicroBeam, the RoboMover, is a reliable, convenient and time saving alternative to manual collection.