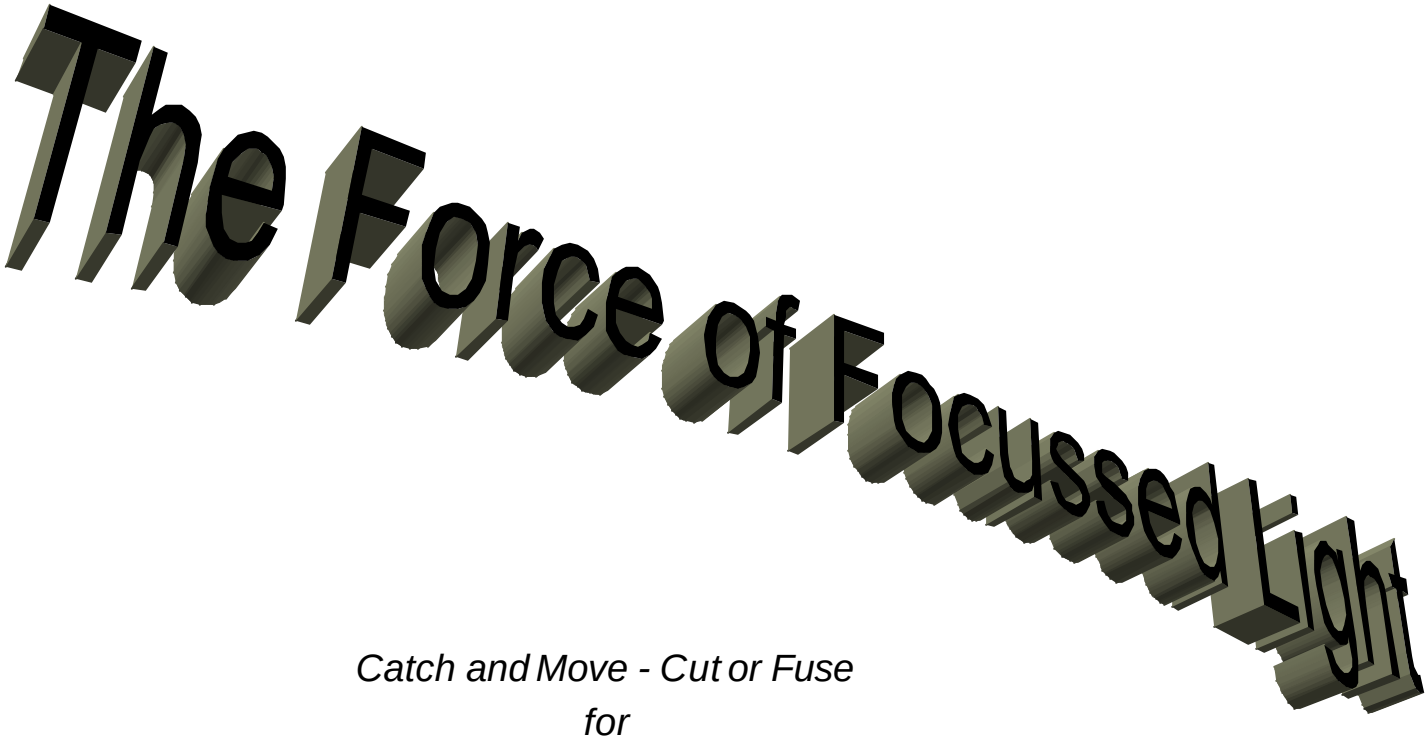
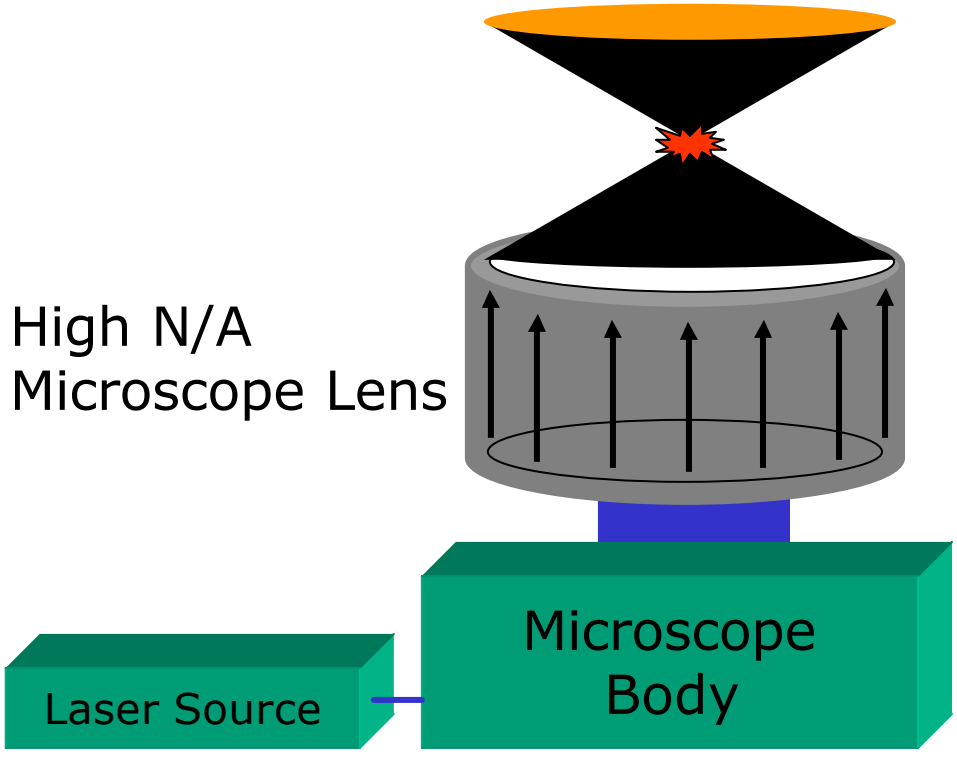


Catch and Move - Cut or Fuse
for
Non-Contact Laser Micromanipulations

Agenda

1. On the Force of Focussed Light
2. Aspects on Laser Micromanipulation and Laser Pressure Catapulting
3. Applications of the Laser Pressure Catapulting

The Principle of Laser Micromanipulation



Laser focus
(< 1 µm in diameter)

Process depends on:

Laser parameters
wavelength
beam quality
focus and energy setting

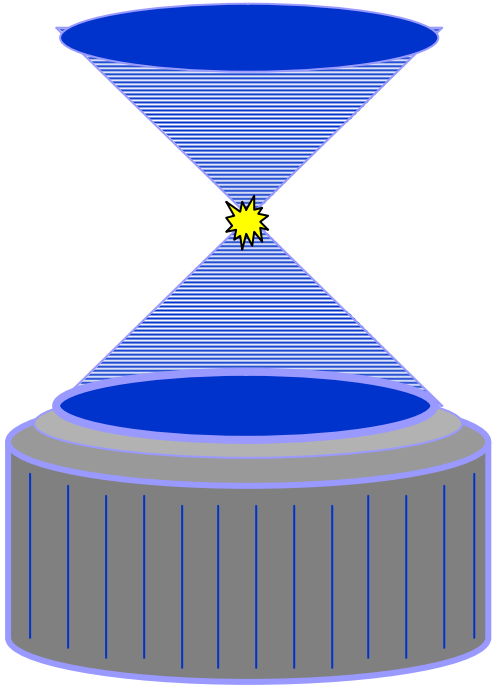
Objective
high numerical aperture

Specimen
absorption behavior

Idea of the Physics Beyond

Photofragmentation
of unwanted material into
ionic molecules, metal ions,
atoms or clusters

A Strongly focus restricted effect
- no lateral damage
- no impairment on living cells
- no impact on DNA, RNA
  or protein recovery



UV-A Nitrogen-Laser
337 nm; 300 µJ;
pulse duration 3 ns; Rep. rate 30 Hz

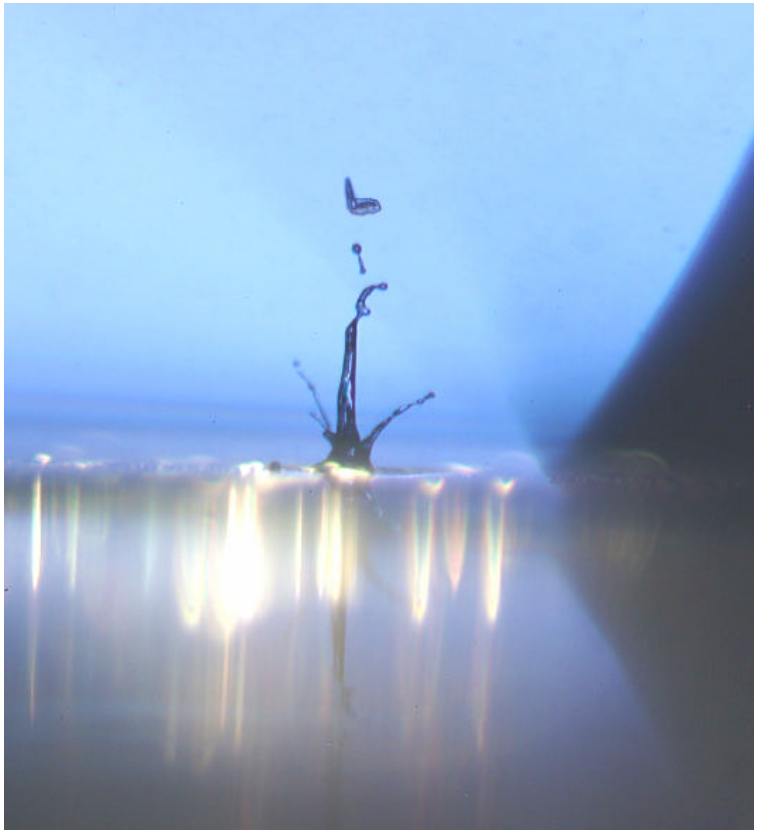
Srinivasan, R.; *Ablation of Polymers and Biological Tissue by Ultraviolet Lasers.* Science 234, 559-565 (1986).

Issues to be Considered Applying UV-A

At safe radiation levels as in the desired use of the system:

1) The viability of the cells is under no impact.
2) There are no single- or double strand-breaks found in the DNA or related.
3) Embryos stay vital.
4) There is no impact on DNA, RNA or protein recovery.
5) The facts are proven in more than 300 publications applying the PALM MicroBeam Laser Micromanipulation System.

C. Elegans, just an Example

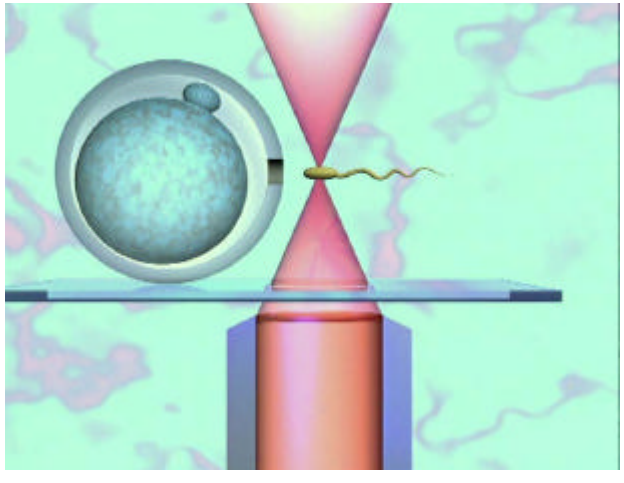


Composite Picture, Courtesy PD A. Vogel, Ph.D., MLL

Two Kinds of Laser Manipulation: Our Products

Optical Tweezers

IR Lasers

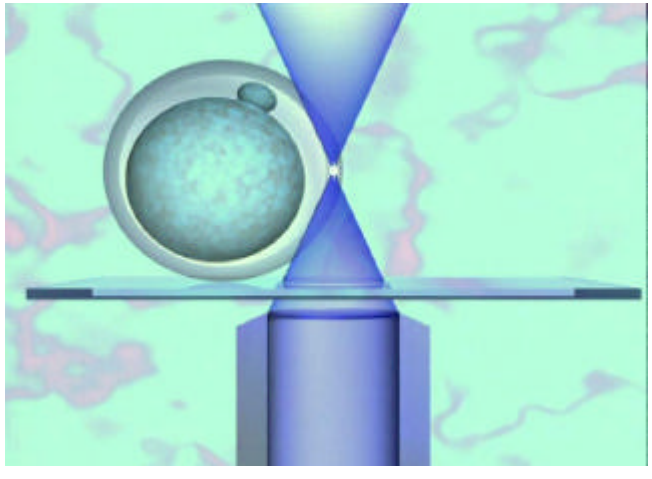


MicroTweezers

Positioning, catching and moving

Laser Ablation

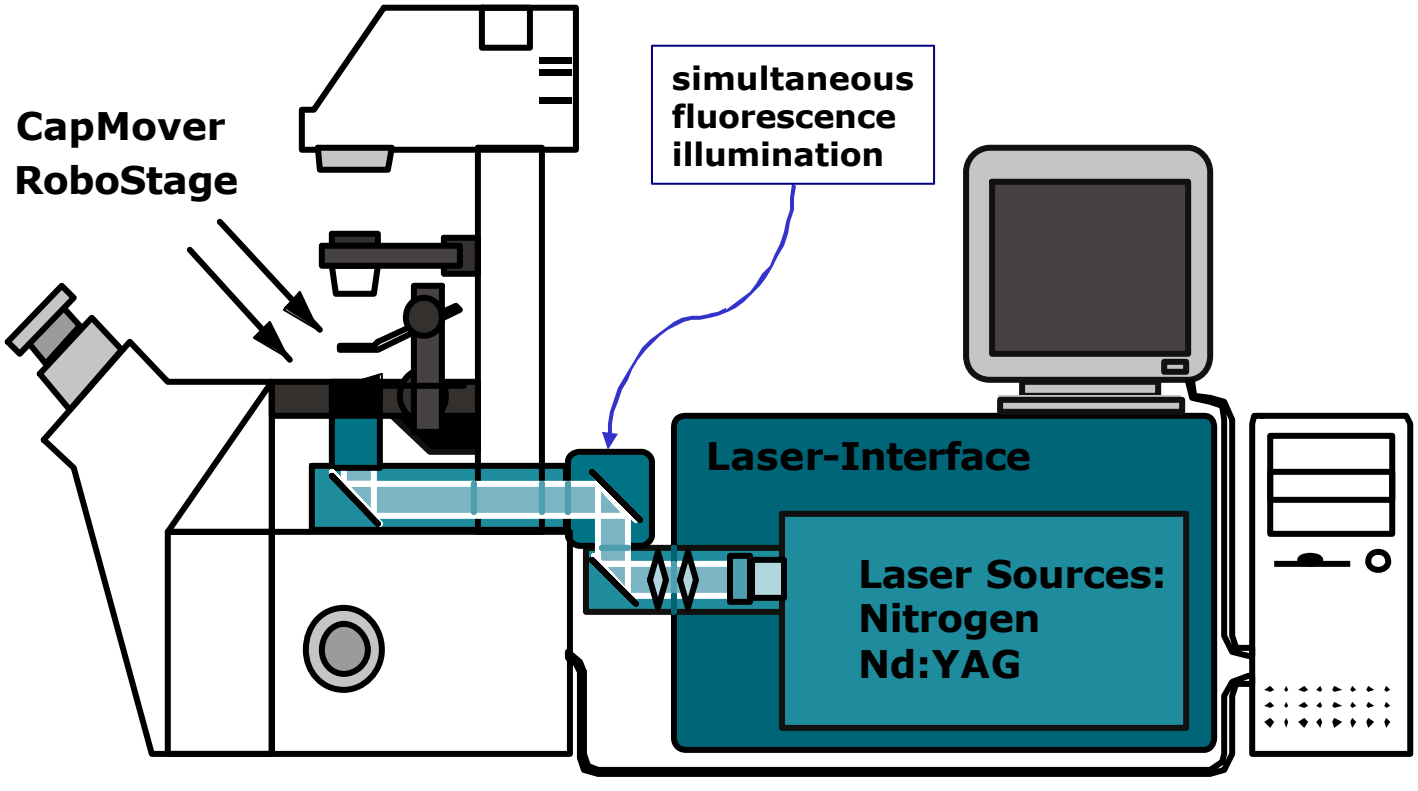
UV-A Lasers



MicroBeam

Ablation, cutting fusing and catapulting

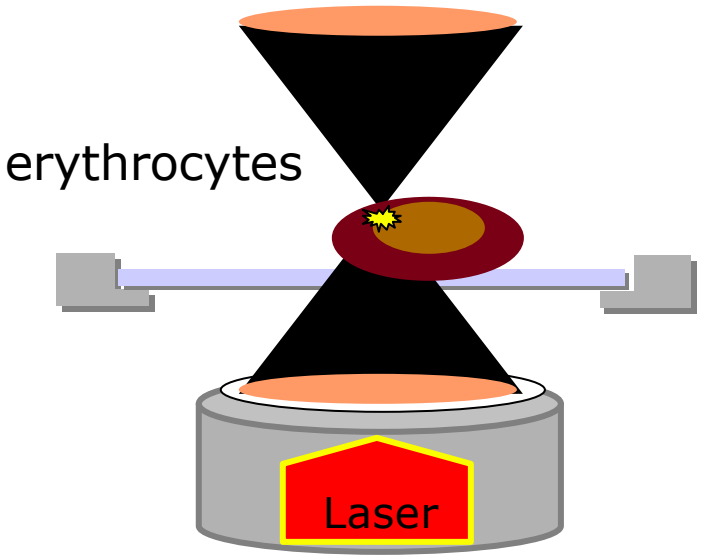
Scheme of the System



Microscopes are inverted, upright; apply LSM or AFM

Interesting Points of the Application Portfolio

Catching, *Sorting* and *Segregation*.



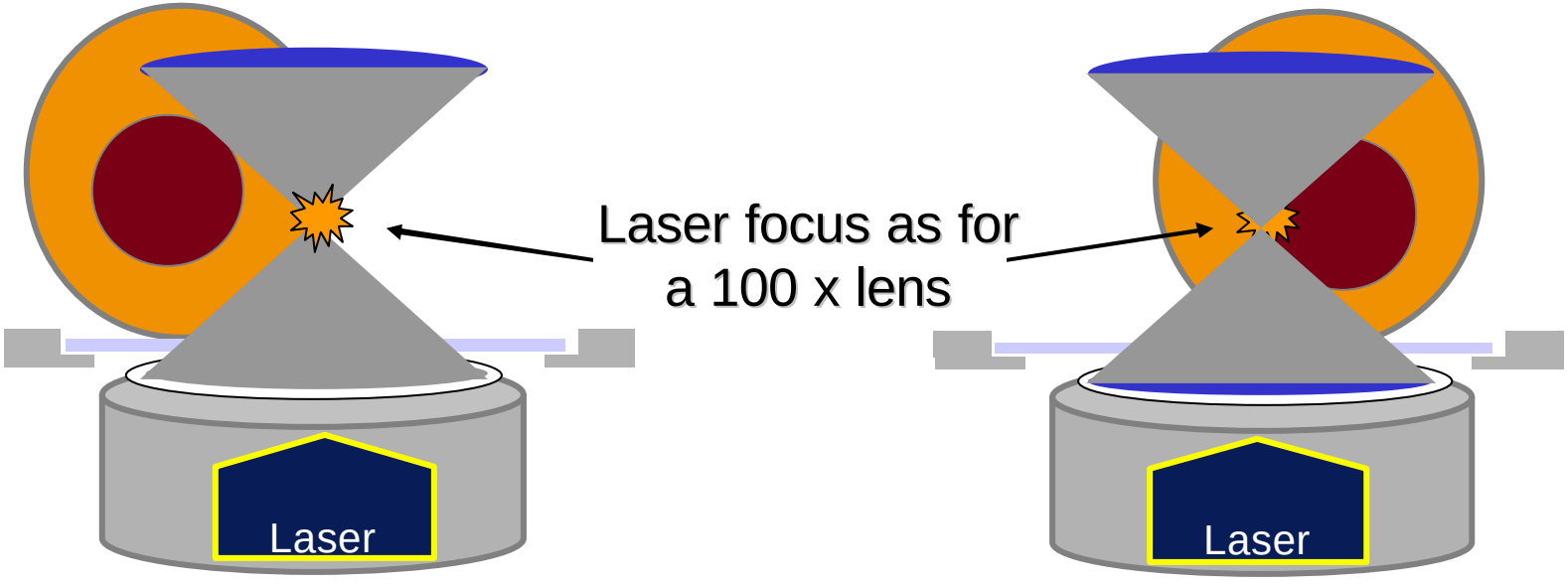
Zur Anzeige wird der QuickTime™ Dekompressor „Video“ benötigt.

Huber R. et al., Nature, 1995; Beck P. and Huber R., 1997:

syringe

traps control the bacterial mixture

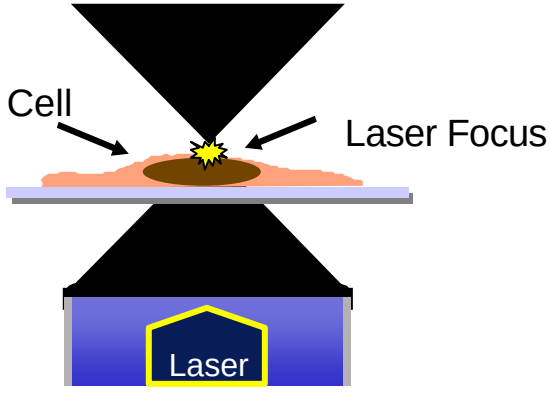
Possible in vital cells without impact on their viability.

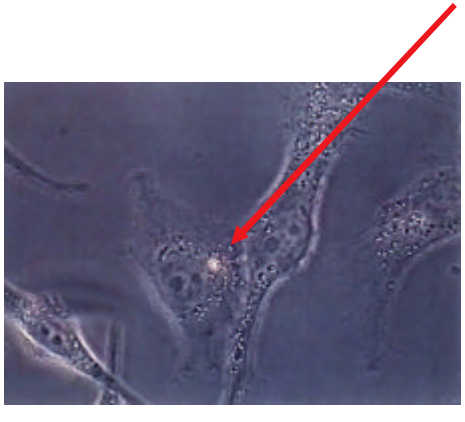


History of Laser Microsurgery in Cell Biology:

Berns M.W. et al.; *Exp. Cell. Res.* 56*: 292-295; 1969.*
Bereiter-Hahn J.; *Umschau* 16*: 601f;1971.*
Monajembashi, S., et al.; *Exp. Cell Res.* 167*: 262 - 265; 1986.*
Greulich et al.; J. Microscopy. 167*: 127 - 51;* 1991.

Non-Contact Laser Microinjection.





Zur Anzeige wird der QuickTime™ Dekompressor „Video“ benötigt.

„Genetic Engineering“

Material Transfer Without Mechanical Contact

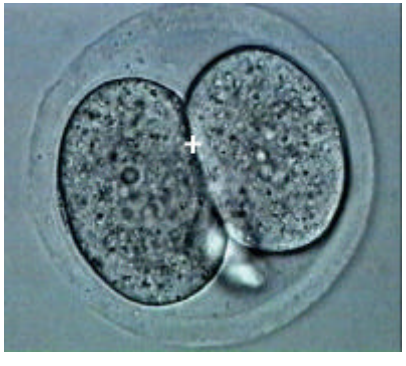
or Virus Transfection

Genuine Laser Microdissection.

Facilitates sperm penetration and assists embryo hatching.

Allows polar body or blastomer extraction for prefertilization and preimplantation diagnosis.

Allows fusion of blastomers for mosaic studies.



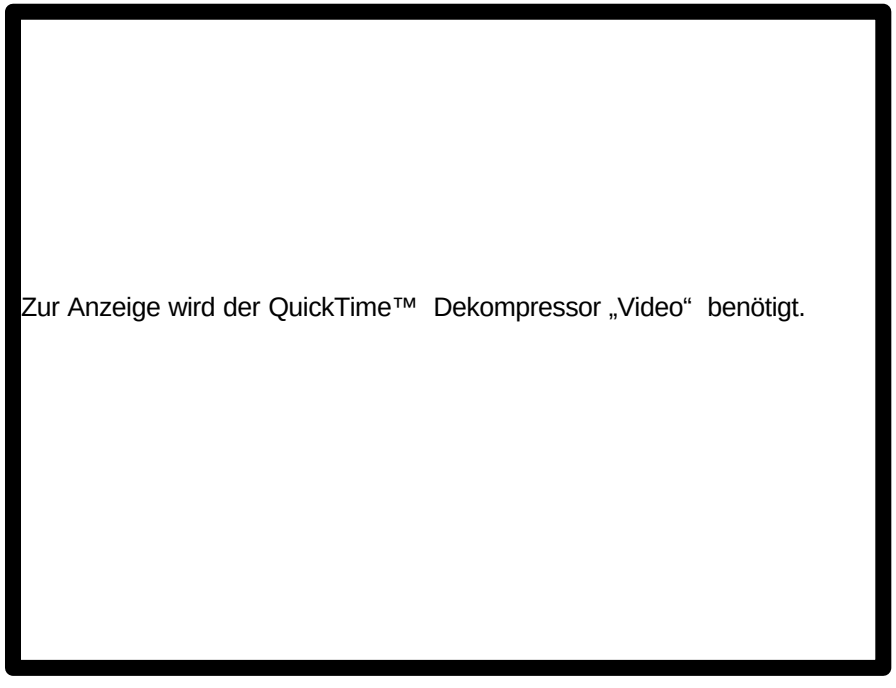
Enables sperm tail micro-dissection for, e.g., IVF.

Laser Pressure Catapulting.

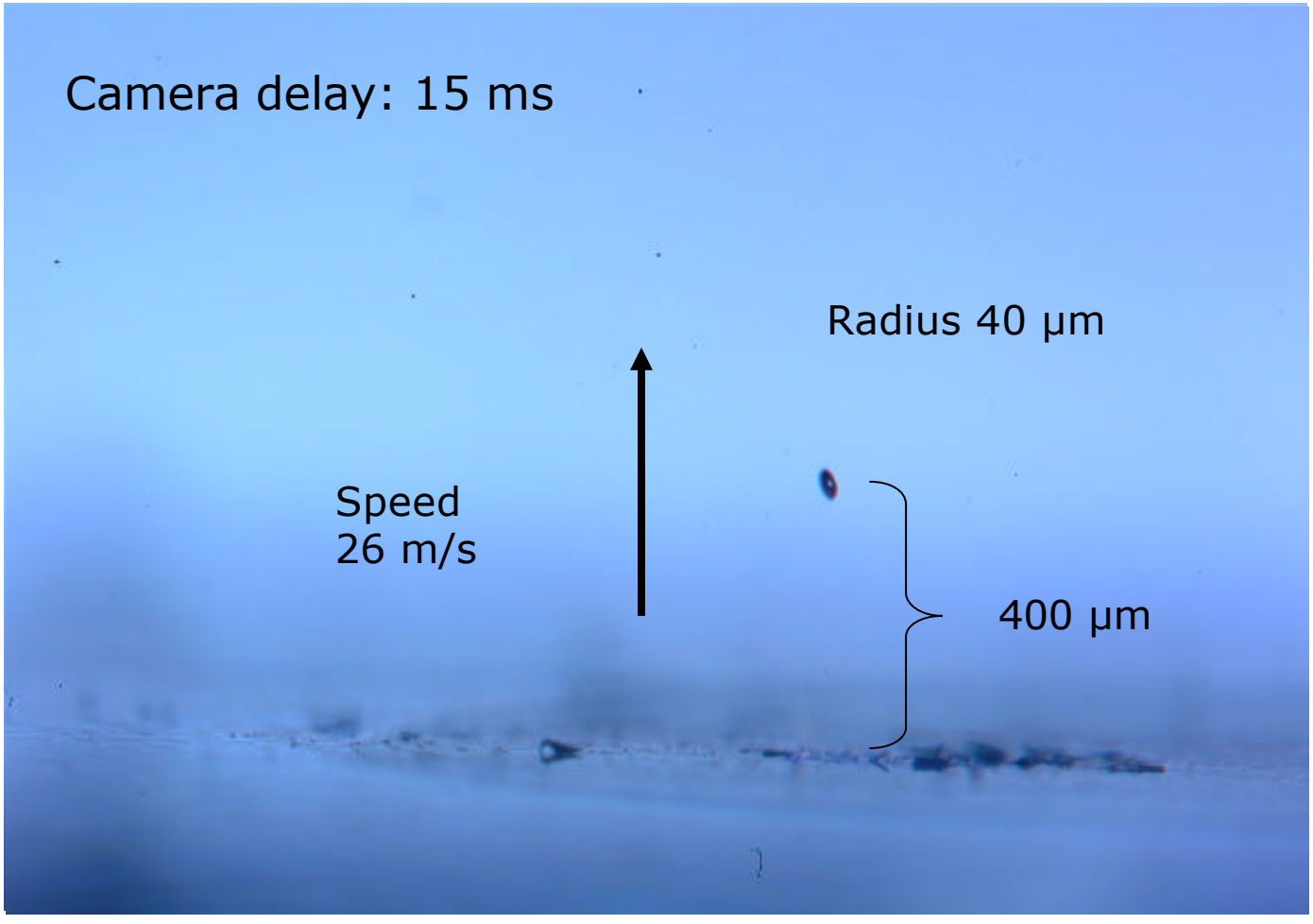
No contact.
LPC against gravity when in an inverted microscope system - no danger of contamination with debris.

No heat.
No impact to subsequent DNA, RNA or protein recovery.

No manual steps.
A high degree of automation is possible with the system.

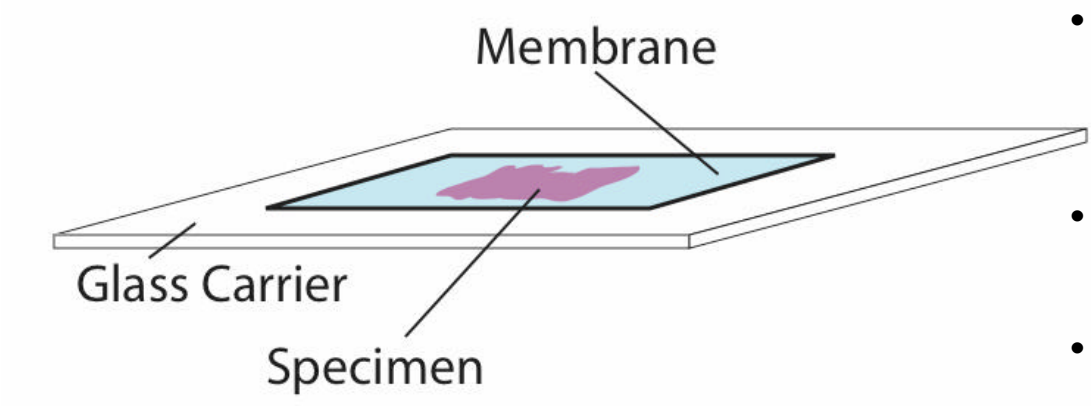


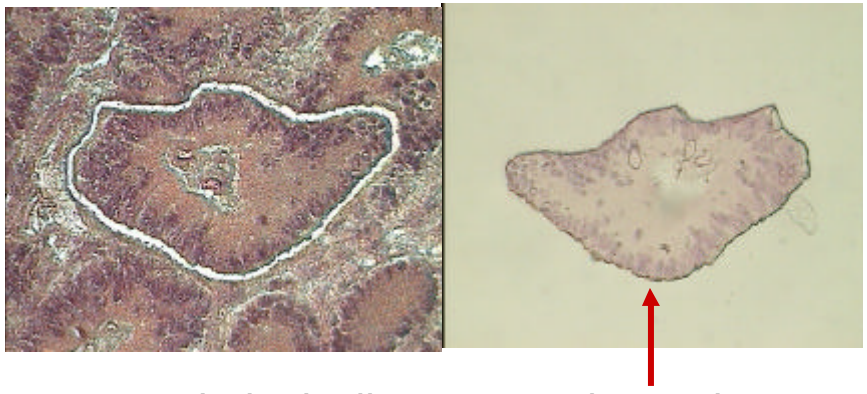
Result of a catapult impulse



Courtesy of A. Vogel, MLL, Lübeck

LPC of Membrane Mount Specimen





morphologically preserved samples
after LPC

- The supporting membrane serves as a backbone.
- It holds specimen together.
- It preserves the morphology during catapulting.
- It enables cut and catapult of geometries of any size and shape.
- It allows usual fixation and staining procedures.

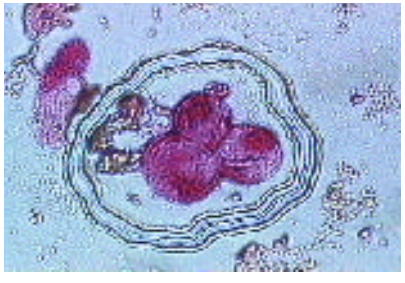
Results of Laser Pressure Catapulting: Glass

On a glass mount tissue the result are tissue flakes:

➲ No impairment of DNA, RNA or protein recovery.

Results of Laser Pressure Catapulting: Membrane

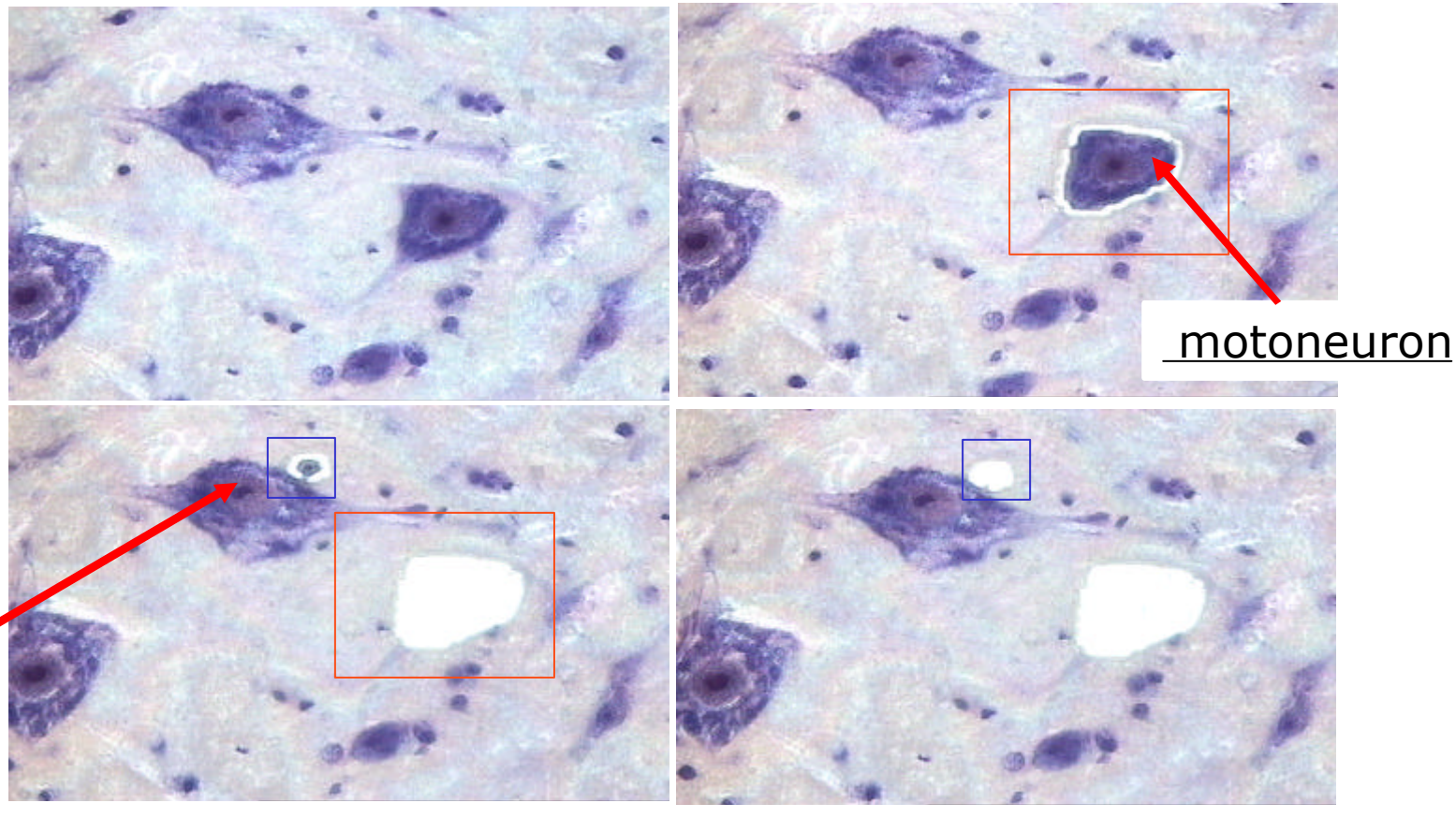
Catapult of a membrane mount tissue results in morphologically preserved samples:



➲ No impairment of DNA, RNA or protein recovery.

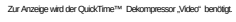
Motoneurons and Glial Cells (5 µm) from Rat Spinal Cord

Cells grown on LPC membrane (PEN)



B. Meurers / The R.W. Johnson Pharmaceutical Research Inst. San Diego, USA

Life Cell Capture: The Process

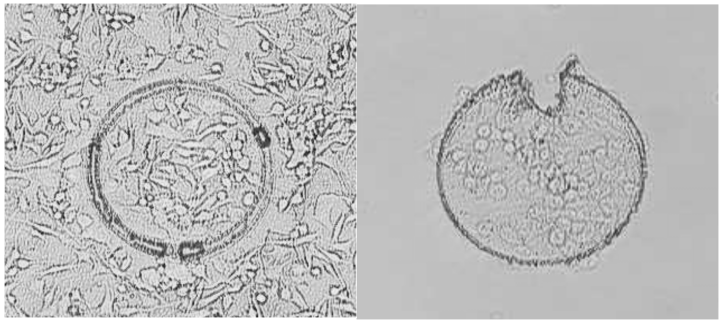


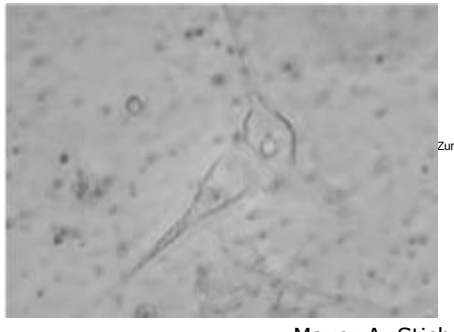
Pictures from PD Dr. Georgia Lahr, Munich.

- The vital cells are nearly not visible due to their index match to the surrounding water and liquid fertilizer.
- The bubbles arise from accumulation of cavity bubbles according to the optical breakthrough in the medium *water* upon the membrane.

LMPC of living cells

Capture of living cells for subsequent cultivation or cloning





Zur Anzeige wird der QuickTime™ Dekompressor „YUV420 codec“ benötigt.

Mayer A, Stich M, Brocksch D, Schütze K and Lahr G (2002) Going *in vivo* with laser-microdissection. *Methods Enzymology*, Series: Laser Capture Microscopy *356:* 25-33

The cells survive the cutting and catapulting procedure and can be recultivated even after a **second** or **third** LMPC-cycle.

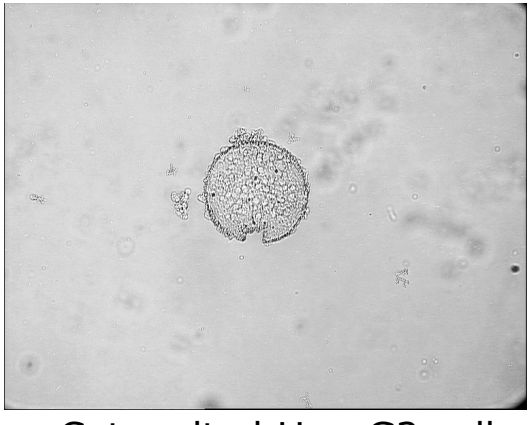
Recultivation of living cells after LMPC

Fill cap with 40 µl culture medium

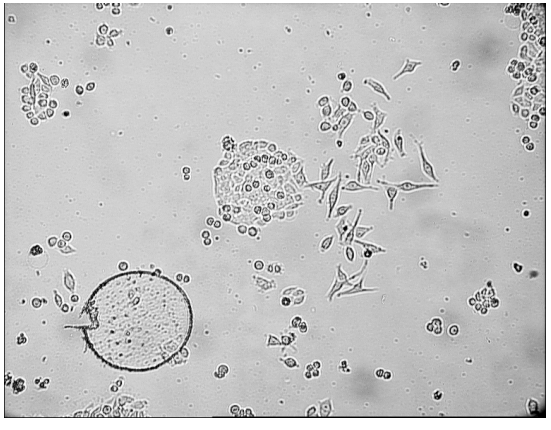
↘ Select, dissect and catapult the cell area

↘ Centrifge into tube and resuspend pellet in additional 50 µl

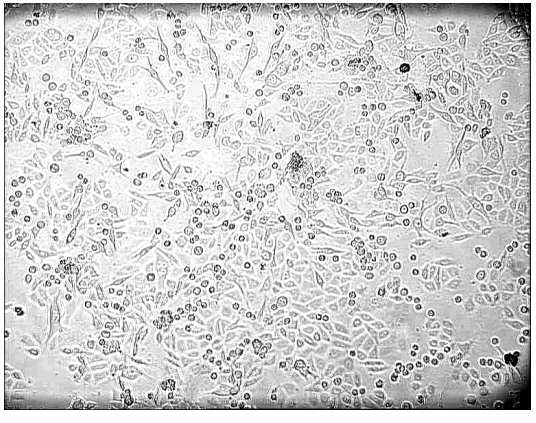
↘ Transfer the droplet in a multiwell plate, 1 ml culture mediu



Catapulted Hep-G2 cell cluster in multiwell plate

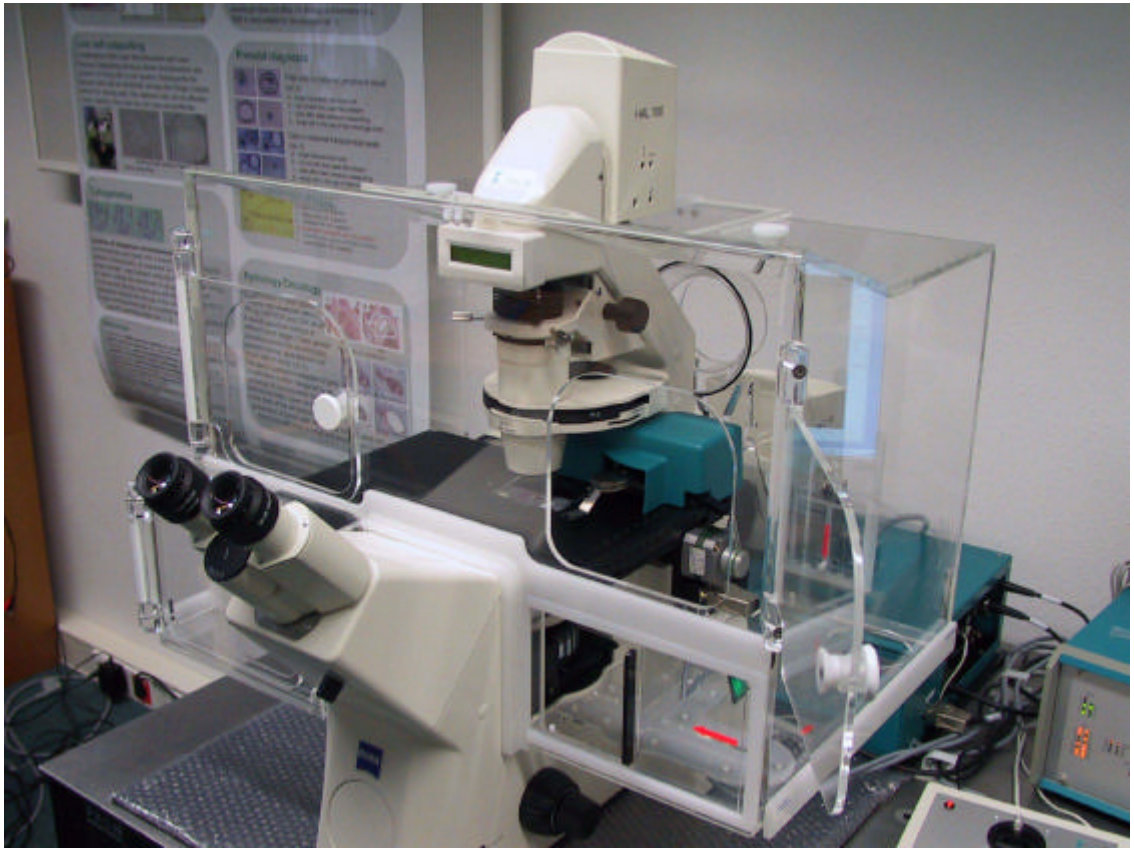


2 days after LMPC



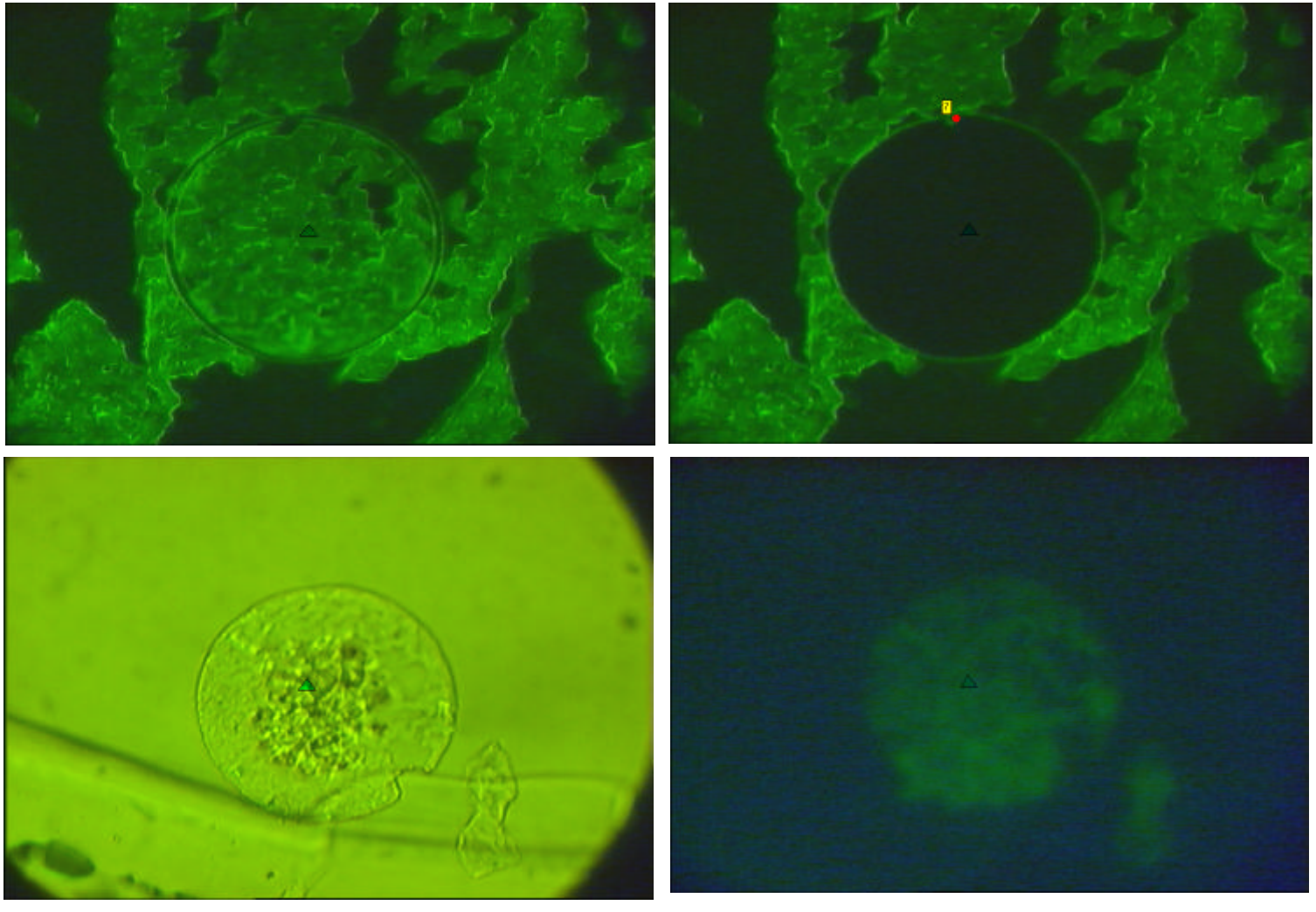
3 weeks after LMPC (10x)

Workflow for live-cell preparation



The incubator provides a clean environment and enables working under a precise environmental control.

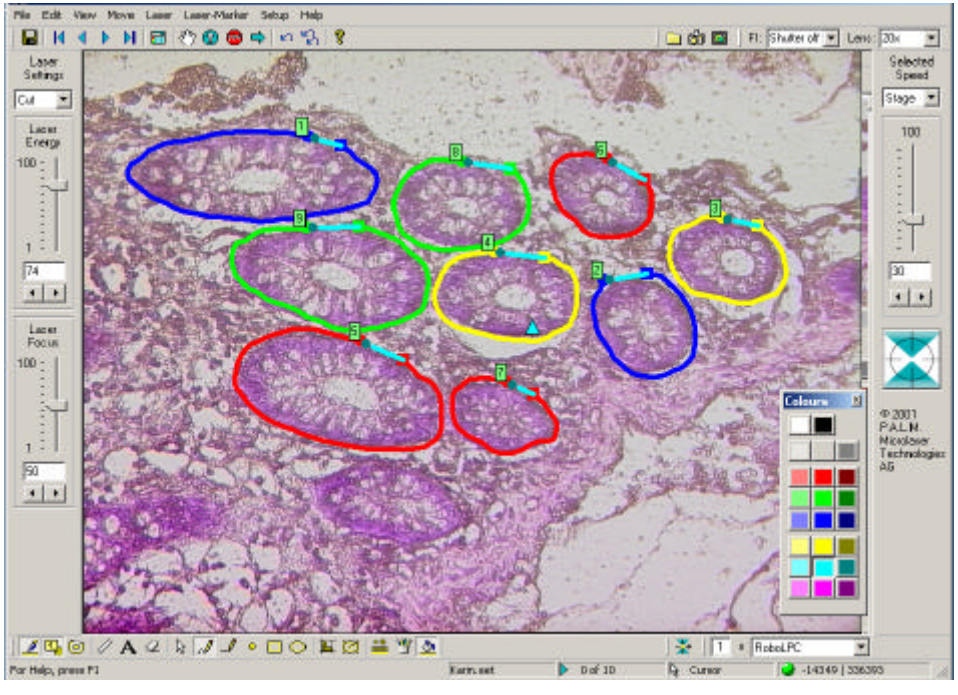
Microdissection under Fluorescence Observation

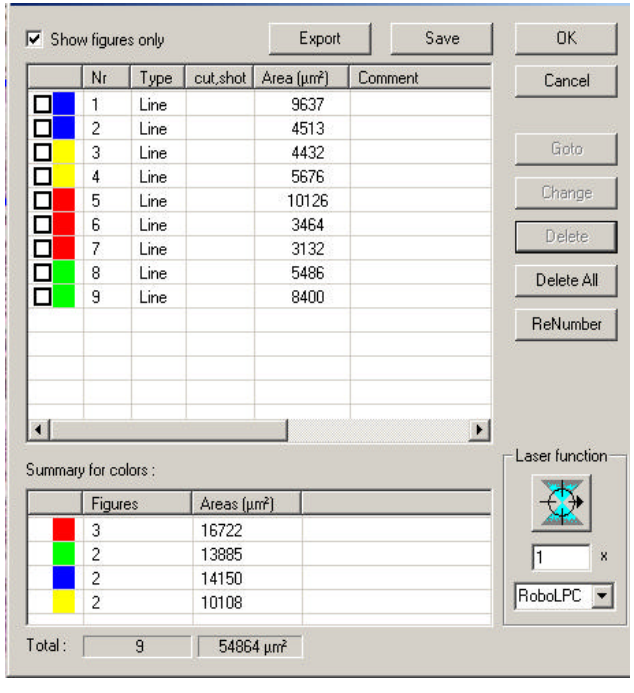


Phalloidin-FITC stained Actin filaments of chicken heart muscle

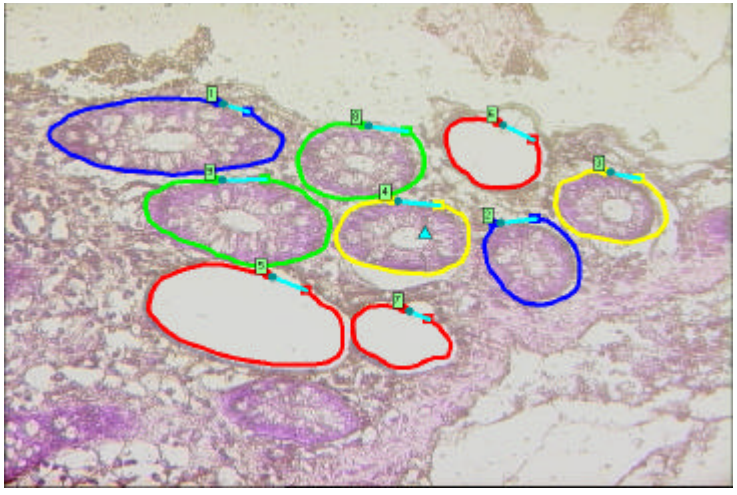
Aspects of The Software

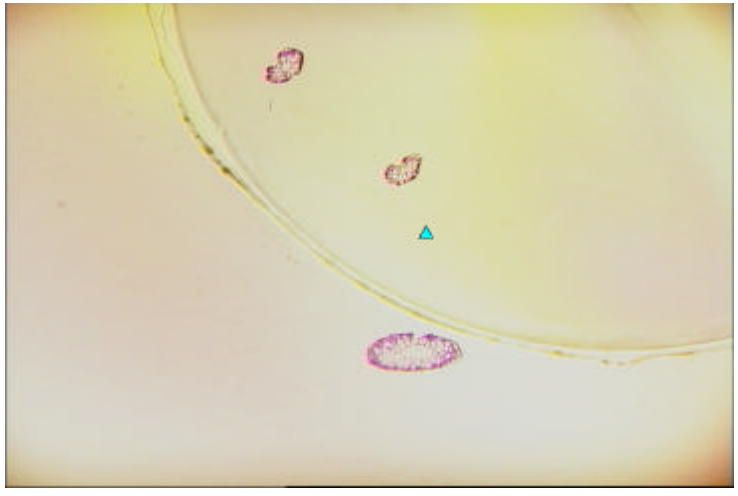
Layout of the PALM RoboSoftware

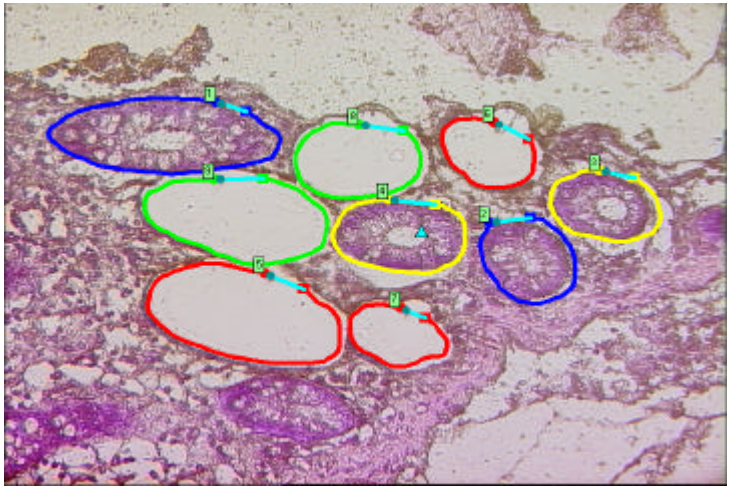


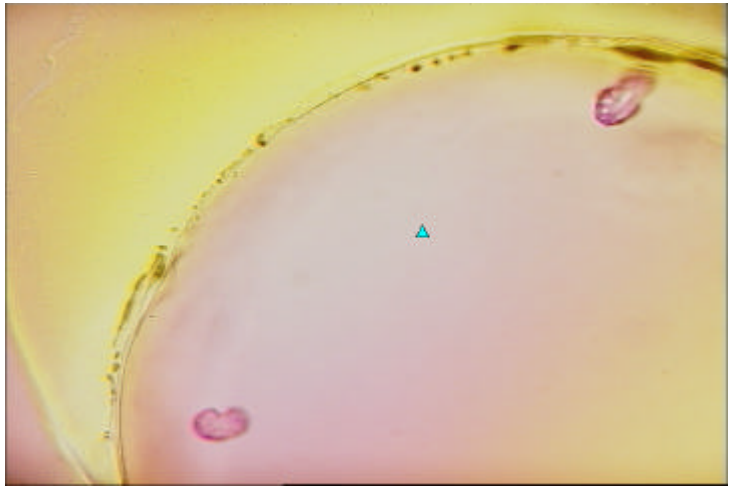


Distinction of the Cell Types





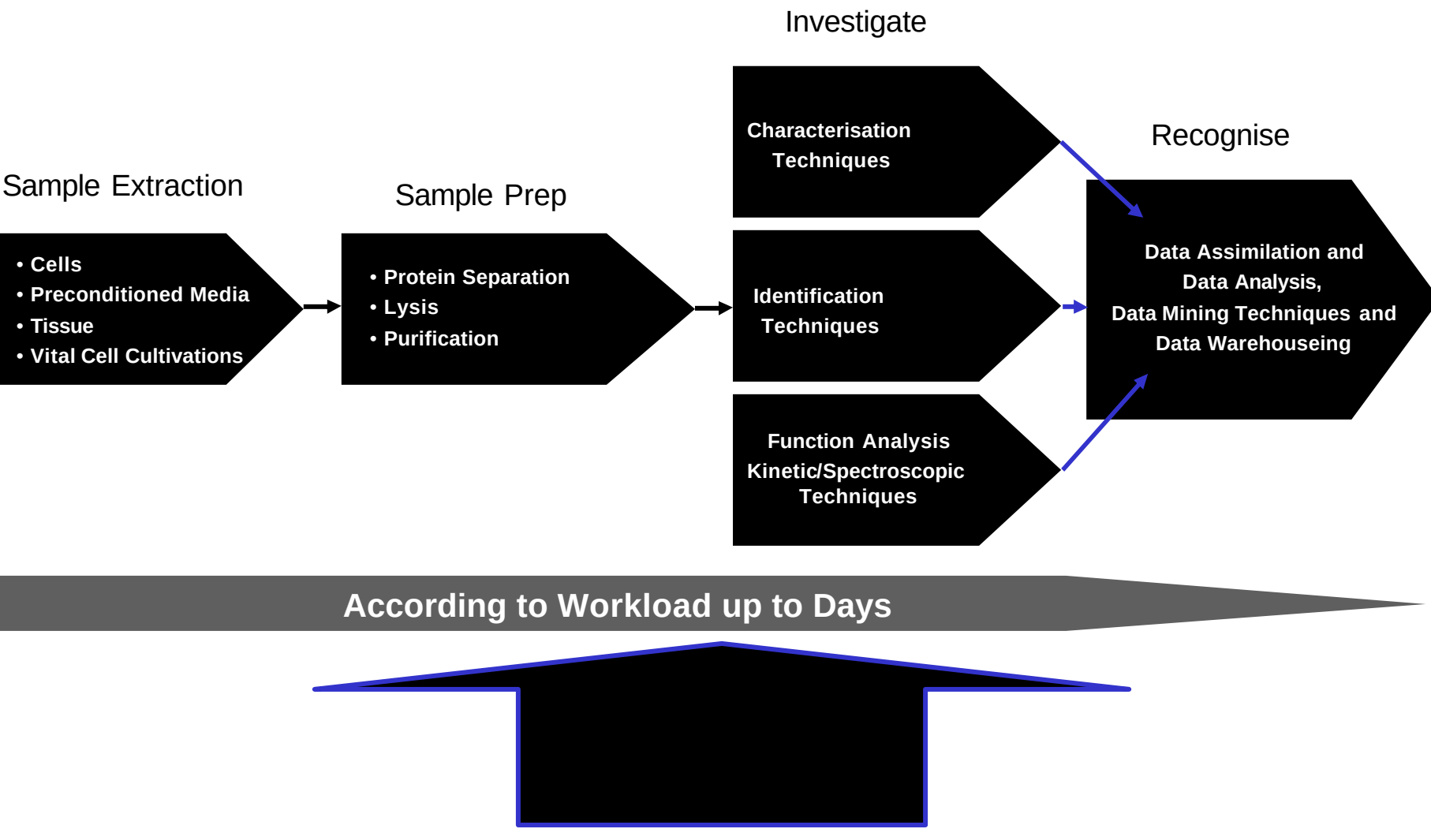




Proteomics, Genomics and Related

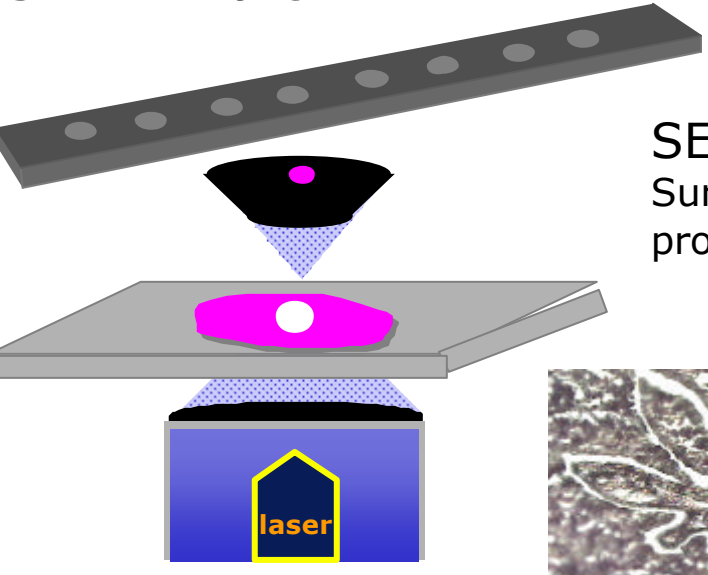
- Purity: Pure samples from morphological defined origin (homogeneity), to be accumulated.
- Safety: No impact to DNA, RNA or protein recovery.
- Precision: Specimen sampling of any shape and any size.
- Automation: Quick access to thousands of cells for DNA-array or proteomic studies.
- No Restrictions: Specimen of variable source, any routine preparation and staining procedure.
- Expenses: Low running costs.

Analysis Workflow



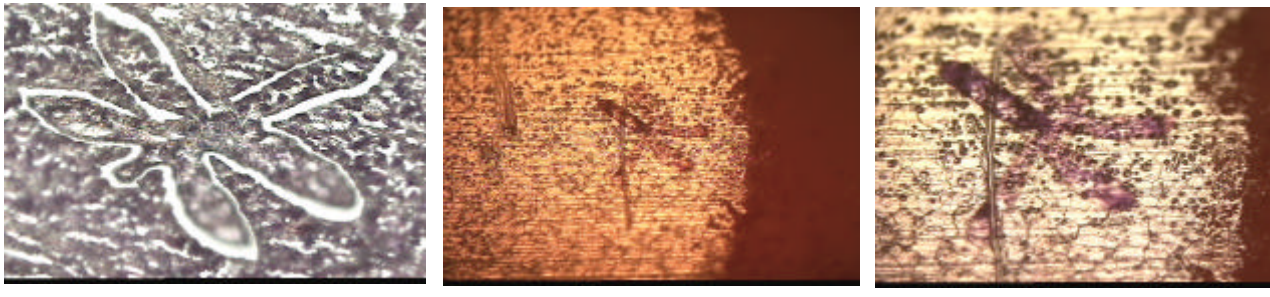
Proteomics Applying SELDI and MALDI

SELDI wafer



MALDI
Matrix Assisted Laser Desorption Ionization:
protein characterization for fractionated protein samples;
i.e. in combination with 2D-gel or liquid chromatography

SELDI
Surface Enhanced Laser Desorption Ionization:
protein profile analysis of complex biological samples

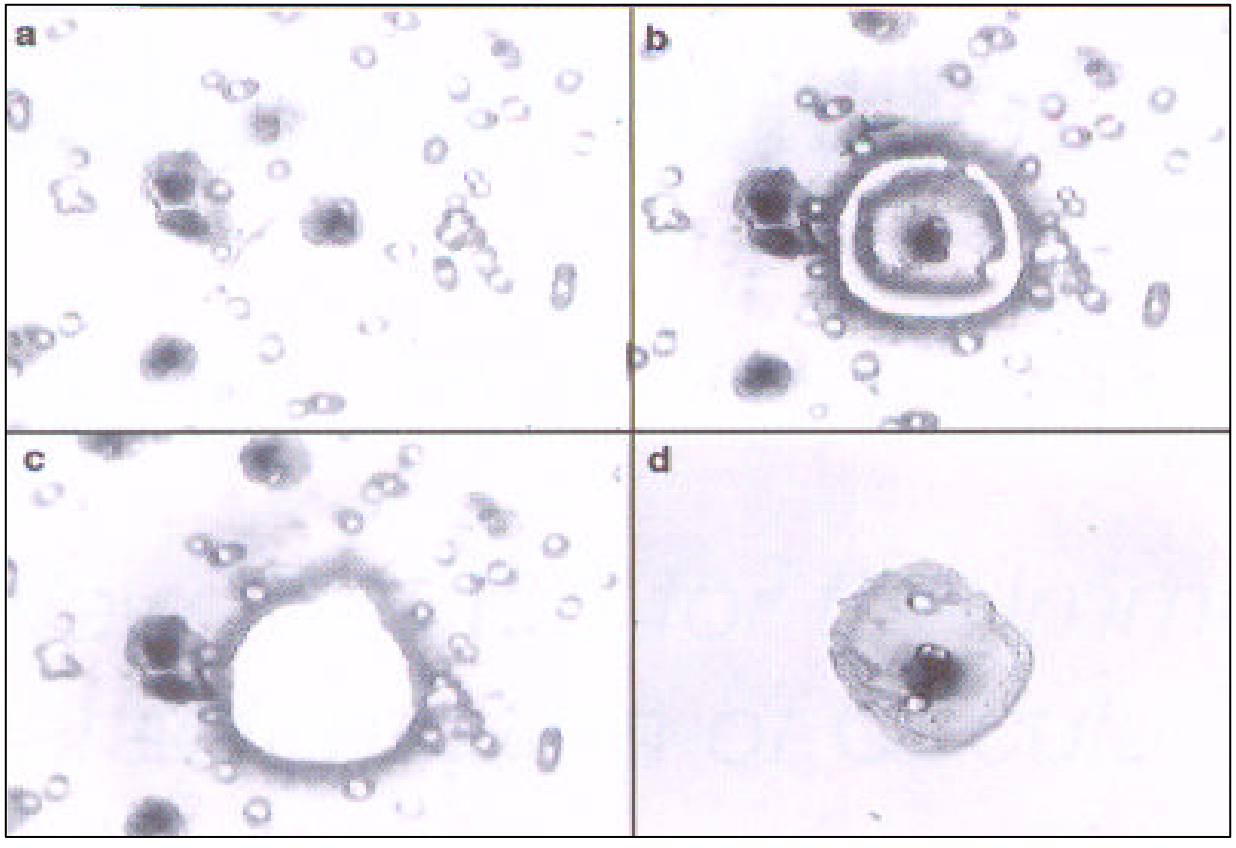


Catapulting of membrane-mounted specimen directly onto a Ciphergen SELDI-chip

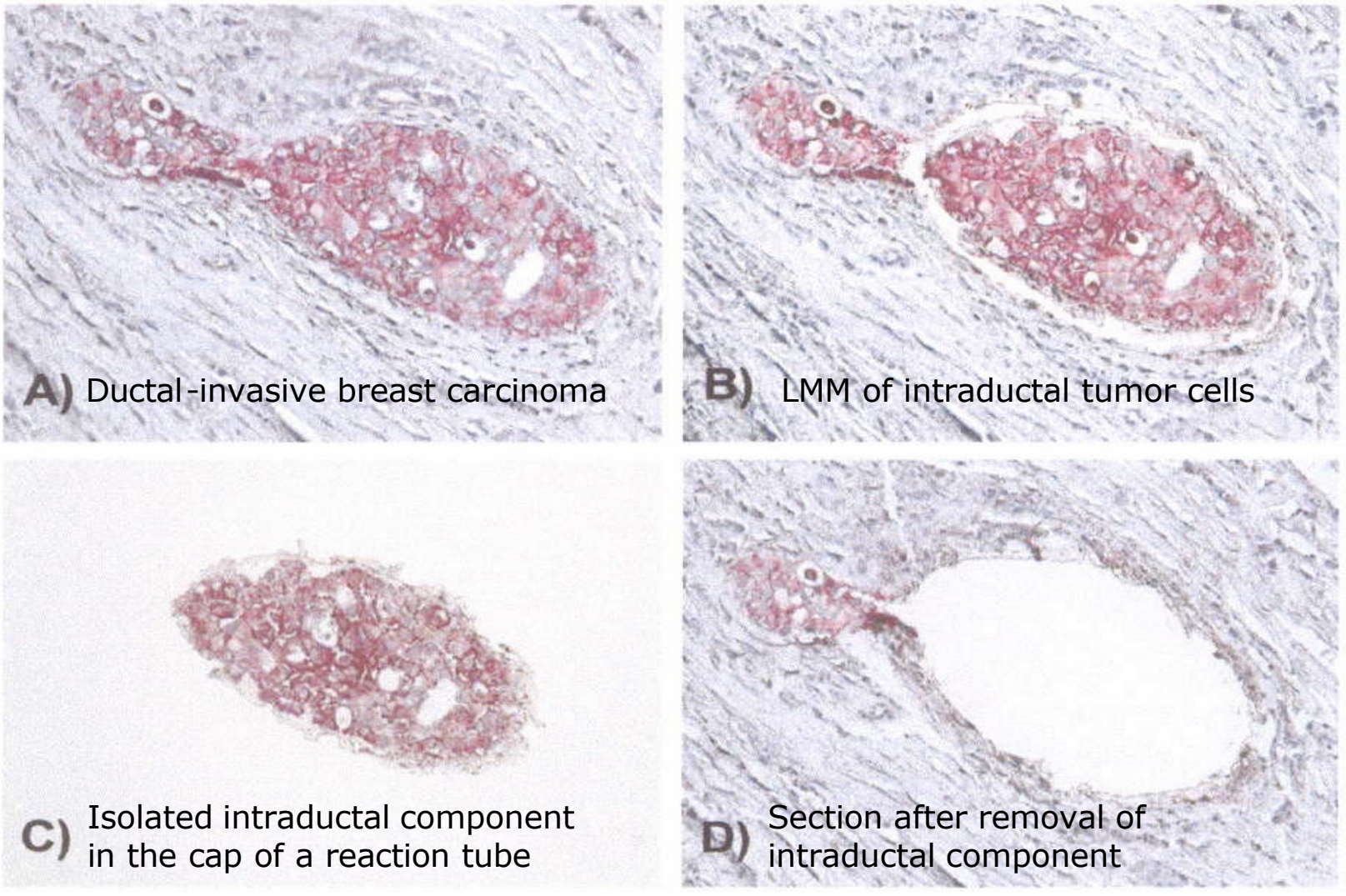
Applications of the Laser Microdissection and Catapult

Application in Oncology / Pathology

ISET (isolation by size of epithelial tumor cells)

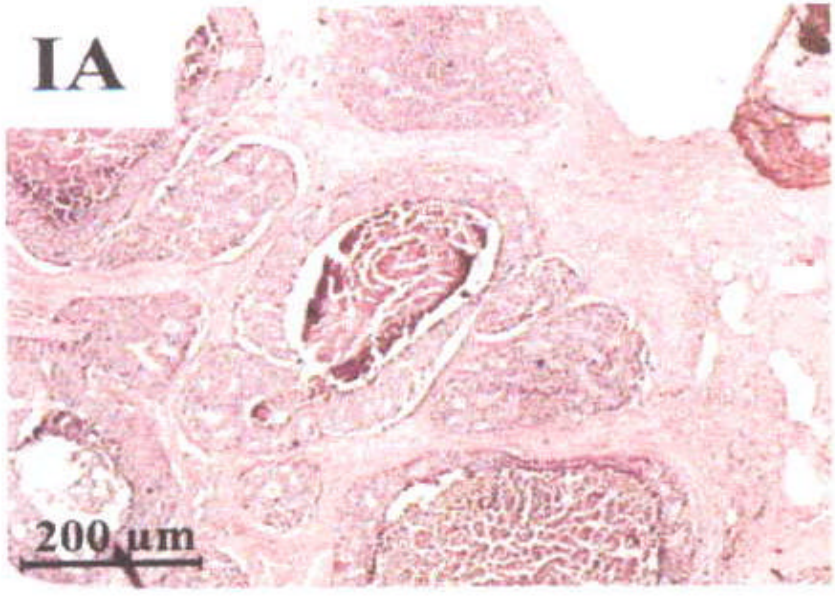


Application in Brest Cancer

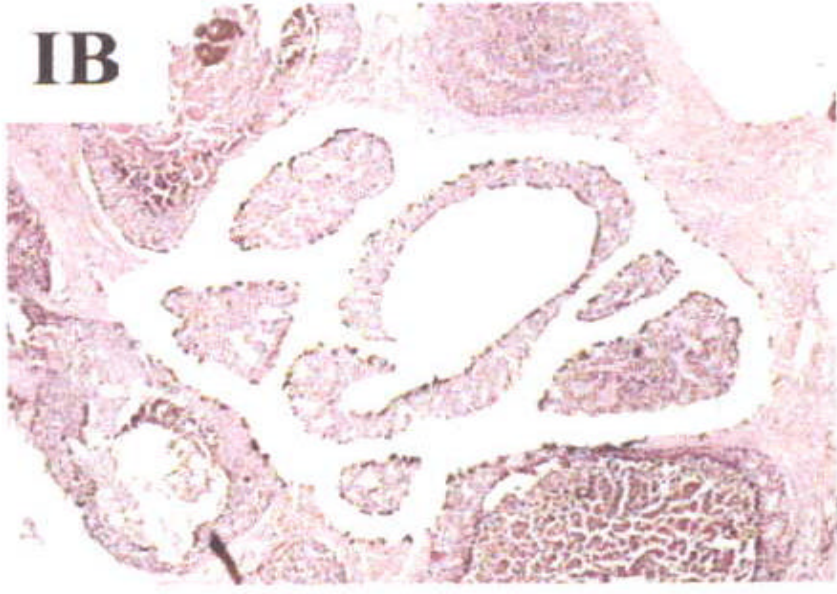


Lehmann et al., 2000

Laser Ablation of Unwanted Cells



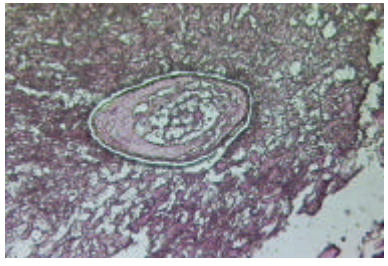
Prior to

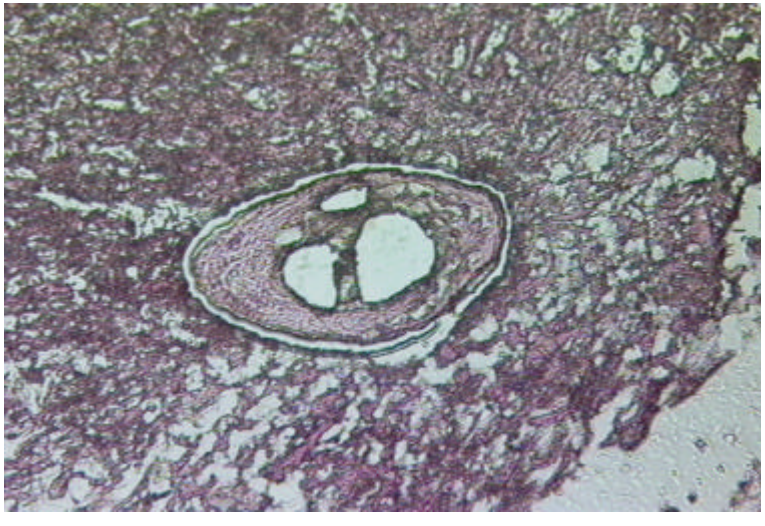


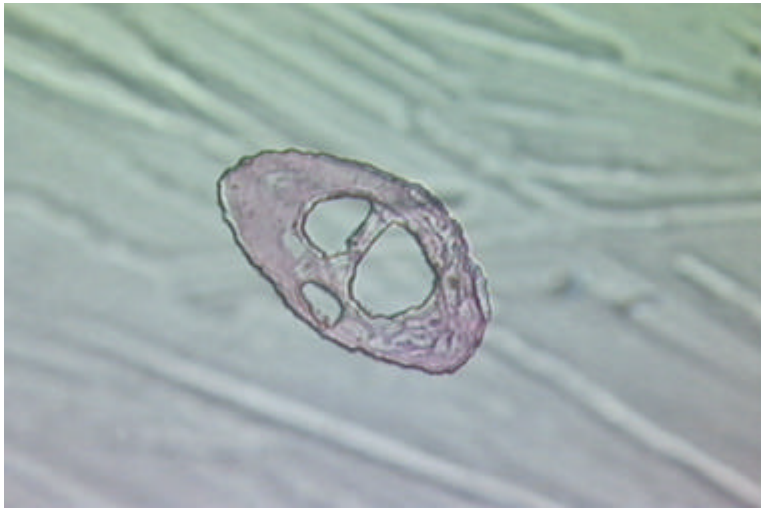
and after removal of unwanted cells

Summing Up The Applications of The PALM MicroBeam
Laser Microdissection and Laser Pressure Catapult

Advantages







No danger of contamination with unwanted material.

Unique Features of Laser Microdissection and Pressure Catapult

- LMPC are very precise and tough forces - leaves unwanted material back.
- LMPC directly into the buffer/onto a wafer or into analysis hardware inlet.
- LMPC from any routine biological preparation.
- LMPC from morphologically defined areas.
- LMPC from geographical different areas and/or from multiple slides.

Laser Pressure Catapulting is...

... a reliable and easy tool to handle and

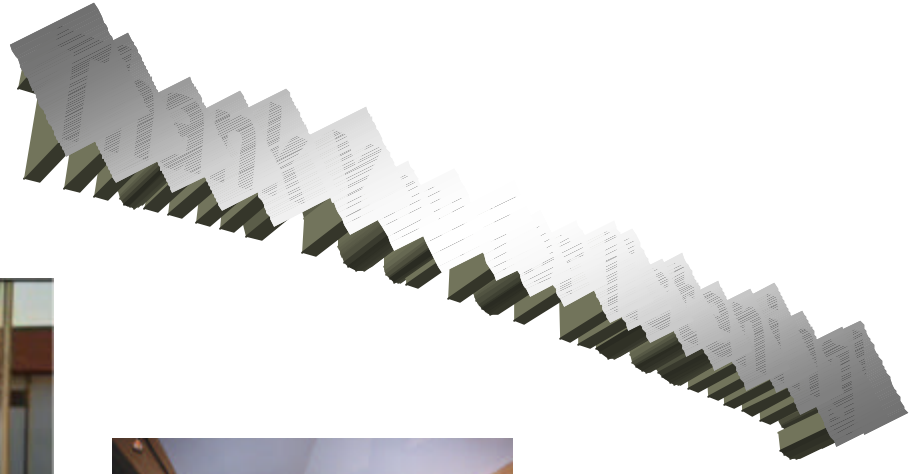
to prepare

- homogeneous cell populations
- single cells
- chromosomes
- tissues without unwanted neighbourhood

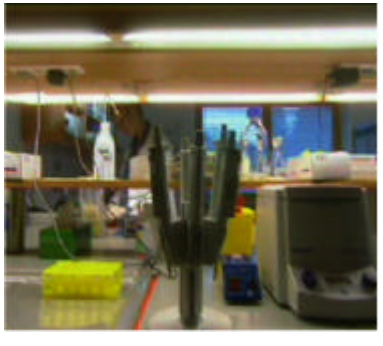
for subsequent molecular or proteomic analysis.

Sales and Administration

Research, Development, and Production all together about 2000m²



The PALM Team (59 employees)



Service and Application Laboratory