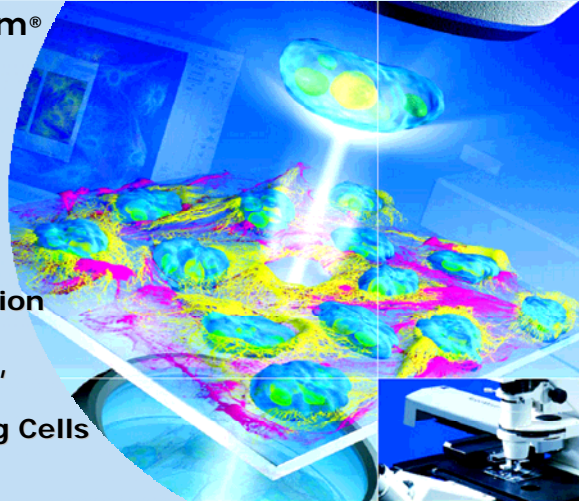


PALM MicroBeam®

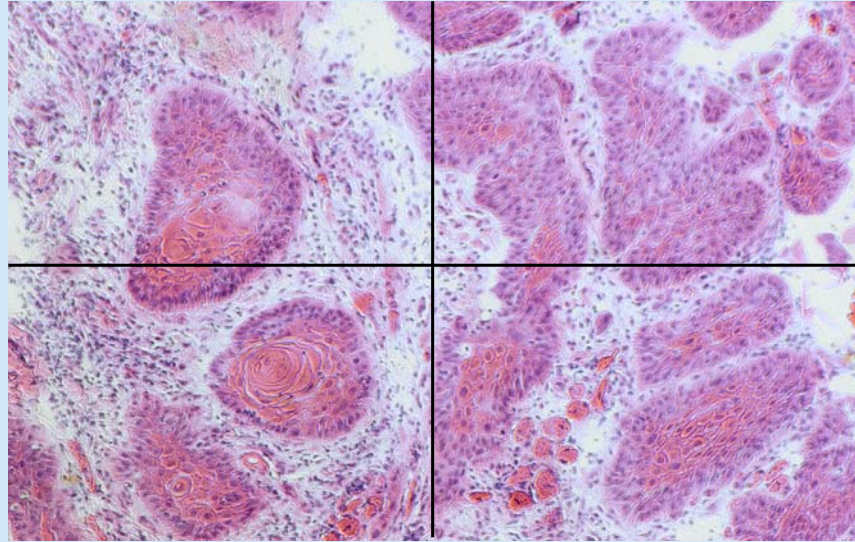
**Non-contact
Laser Microdissection
for pure DNA, RNA,
Proteins and Living Cells**



- 1. What is LMPC?**
- 2. Character of PALM**
- 3. Publication**
- 4. Pathology**
- 5. Living cell manipulation**
- 6. Optical tweezer**
- 7. Upgrade device**
- 8. Summary**



Why Microdissection?



Microdissection & Micromanipulation



Uncovering of cellular mechanisms
by investigating DNA, RNA and Proteins
to resolve the cellular & molecular mechanisms underlying
physiological & pathological changes within Tissue or Living Cells



Samples must be ...

- ... Well Defined
- ... Pure
- ... Contamination Free
- ... No damage



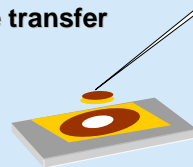
1st Generation Needle transfer



Laser Cutting



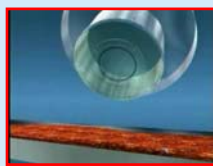
Needle transfer



- Time consuming
- Cross contamination

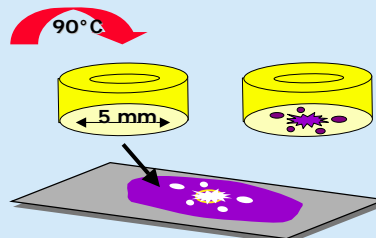


2nd Generation LCM



Principle: A Near Infra-Red (NIR) diode laser point-heats (at $\approx 90^{\circ}\text{C}$) the selected specimen and melts it with an entirely contacting transfer membrane, coated by an heat-activated adhesive film (LCM: "Laser Capture Melting")

NIR-diode laser

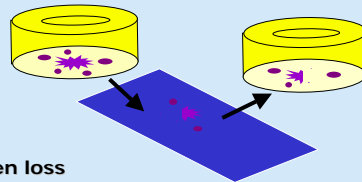


P.A.L.M. Microlaser Technologies AG

2nd Generation LCM



cleaning procedure (!)
with adhesive tape

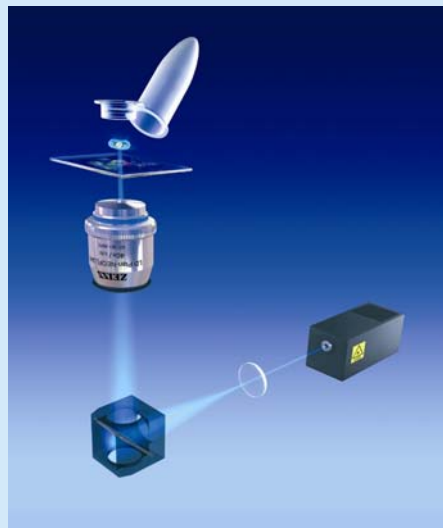


- may cause specimen loss
- does not clear-off all debris!

- Heat damage
- Sample lossing
- Non resued adhesive tape
- Cross contamination



3rd Generation LMPC

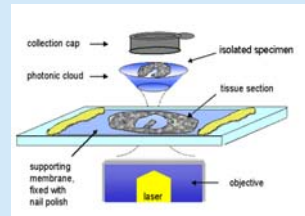


Laser **M**icrodissection
and
Pressure **C**atapulting
(**LMPC**)

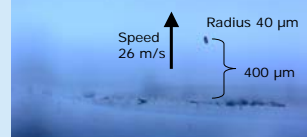
Is really only light-
driven and therefore
contact free



Laser Microdissection & Micromanipulation



Camera delay: 15 ms



Courtesy of A. Vogel, MLL, Lübeck

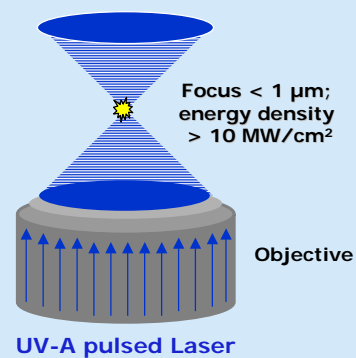
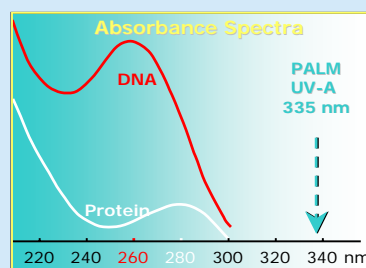
LMPC Laser Microdissection and Pressure Catapulting



Physics of Laser Cutting



Photofragmentation within laser focus
is due to high photon density



- Locally restricted effects
- No heat transfer to adjacent material



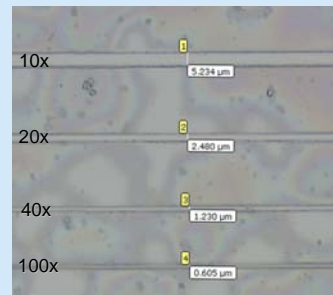
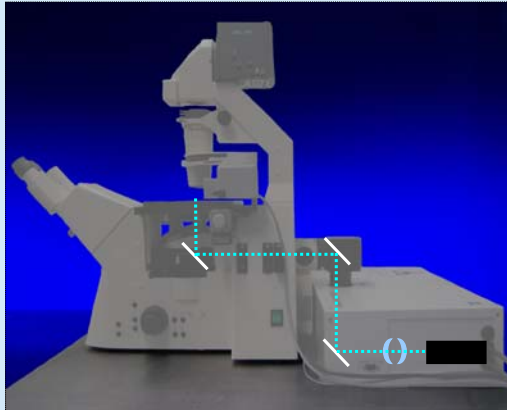
Precision Cutting



Pulsed UV-A Laser 335nm

Advantages:

- No harm to DNA, RNA and Proteins
- Long Lifetime
- High Precision Cutting



Picture taken in 400x magnification

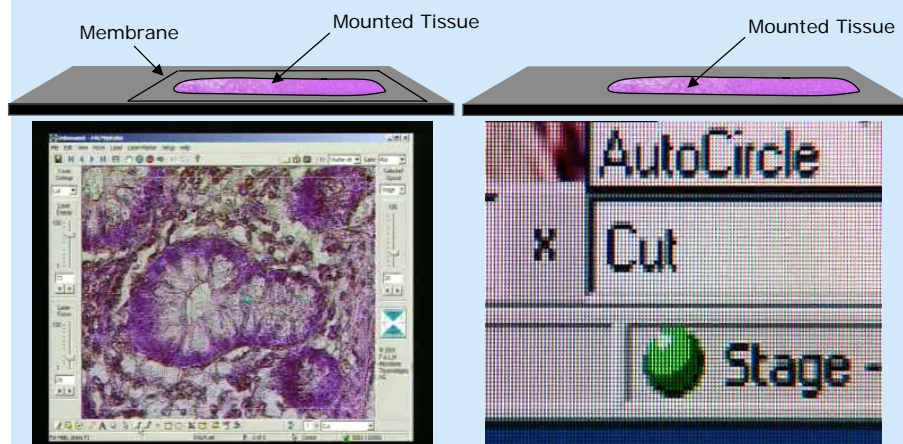


Methods of LMPC



LMPC from Membrane Mounted Tissue

LMPC from Glass Mounted Tissue



Advantage of PALM



High precision cutting

Less than 0.6 μm cutting line

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.

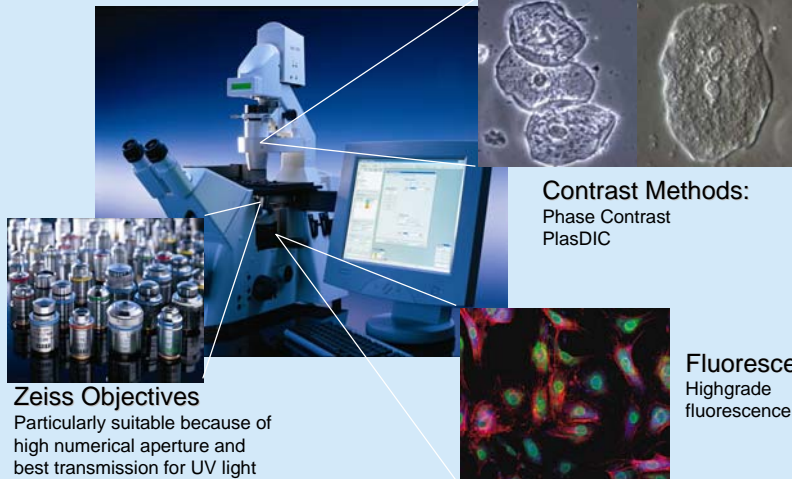
A high degree of automation is possible with the system.



1. What is LMPC?
2. **Character of PALM**
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Zeiss AxioVert 200M



Contrast Methods:
Phase Contrast
PlasDIC

Fluorescence:
Highgrade
fluorescence optics

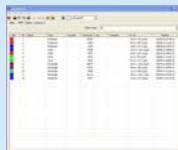
Zeiss Objectives
Particularly suitable because of
high numerical aperture and
best transmission for UV light



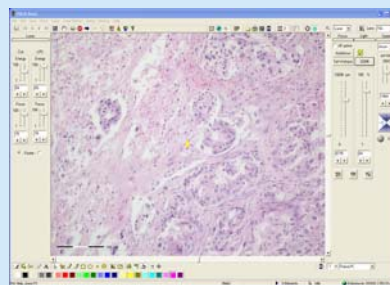
PALM RoboSoftware Features



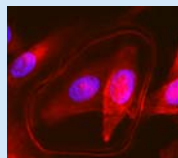
ElementList



PALM RoboSoftware



Multi collecting device



Fluorescence caperture

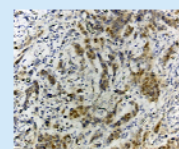
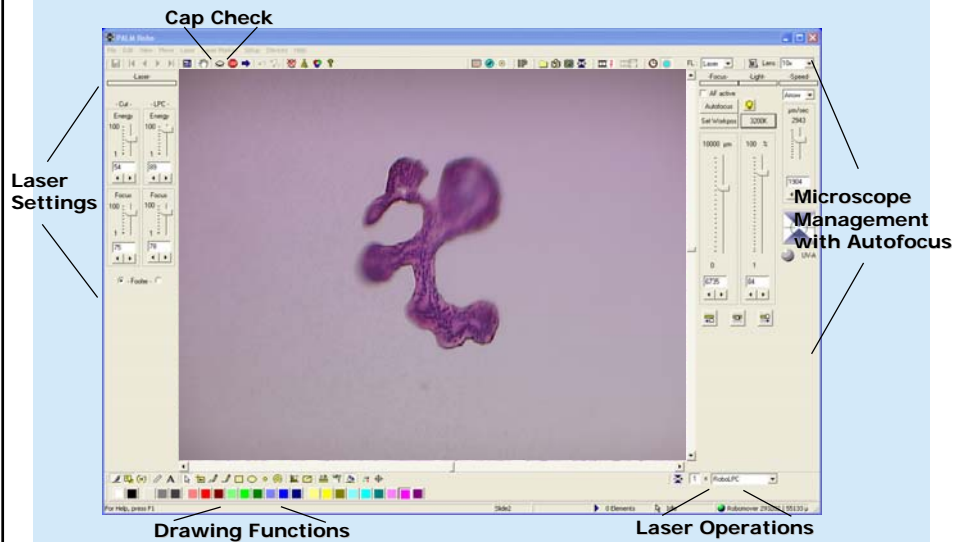


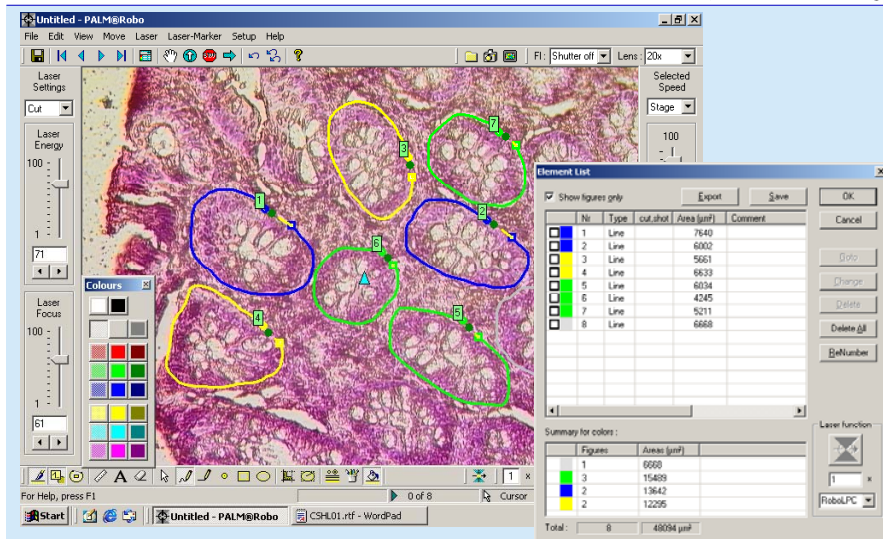
Image caperture



PALM Software



Selective Caperture

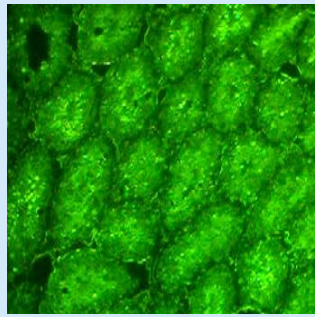
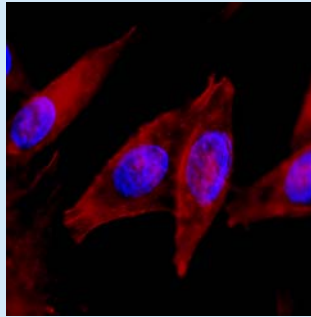


Fluorescence mode



Fluorescence attachment

- consisting of: light source for fluorescence illumination
- Filter wheel for 6 excitation filters



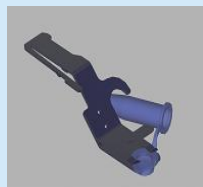
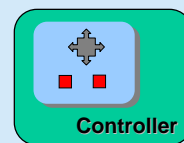
HEP-G2 cells fixed on supporting LMPC-membrane
- F-actin labelled with TexasRed-Phalloidin
- interphase nuclei counterstained with DAPI



CapMover



CapMover



Single tube



8 caps strip

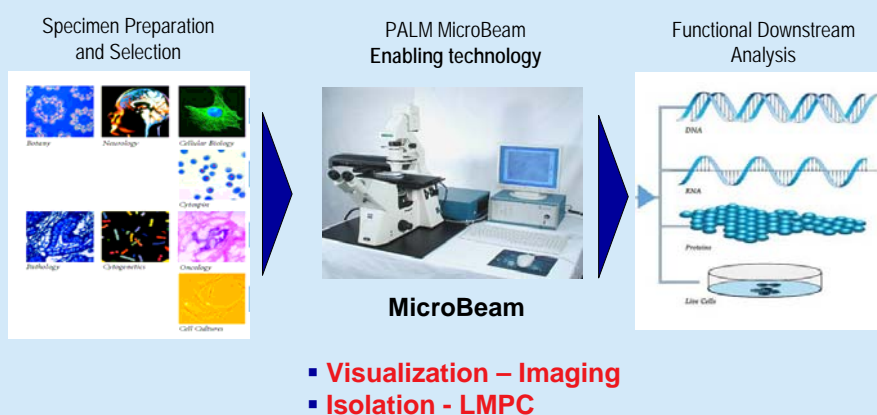


Living cell



1. What is LMPC?
2. Character of PALM
3. **Publication**
4. Pathology
5. Living cell manipulation
6. Optical tweezer
7. Upgrade device
8. Summary

Application of PALM



Publication of PALM



> 700 publication

Bibliography of PALM MicroLaser Systems

1 PALM MicroBeam

1.1 DNA and RNA Research

Aarts WM, Bende RJ, Bossenbroek JG, Pals ST and van Noesel CJM

Variable heavy-chain gene analysis of follicular lymphomas: subclone selection rather than clonal evolution over time.
Blood, 2001, 98,1: 238-240

Aarts WM, Bende RJ, Vaandrager J-W, Kluin PM, Langerak AW, Pals ST and van Noesel CJM

In situ analysis of the variable heavy chain gene of an IgM/IgG-expressing follicular lymphoma.
Am J Pathol, 2002, 160,3: 883-891

Aida M, Irié T, Aida T and Tachikawa T

Expression of protein kinases C β I, β II and VEGF during the differentiation of enamel epithelium in tooth development.
J Dent Res, 2005, 84,3: 234-239

Ardigó M, Borroni G, Muscardin L, Kerl H and Carroni L

Hypopigmented mycosis fungoides in Caucasian patients: A clinicopathologic study of 7 cases.
J Am Acad Dermatol, 2003, 49,2: 264-270



Publication of PALM



- 神經醫學 : Biol Reprod 63, 643 (2000)
- 分子醫學 : Nature Medicine 4, 1329 (1998)
- 癌症醫學 : Int. J. Cancer 85, 82 (2000)
- 細胞生物學 : Nature 409, 1, 630 (2001)
- 生殖醫學 : Photomed. in Gynecol. and Reprod., 340 (2000)
- 發育學 : Nature 376, 6, 57 (1995)
- 遺傳學 : PNAS, 102, 25, 8905 (2005)
- 新生兒診斷 : CMLS Cell Mol Life Sci, 57: 96 (2000)
- 病理學 : Am J Pathol, 156, 1, 57 (2000)



Protocol of PALM



Application note

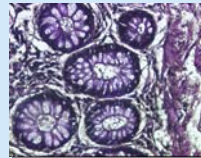
1. DNA & RNA
2. Tissue section and staining
3. Living cell
4. Chromosome



1. What is LMPC?
2. Character of PALM
3. Publication
4. Pathology
5. Living cell manipulation
6. Optical tweezer
7. Upgrade device
8. Summary



Tissue Section and Cell Culture



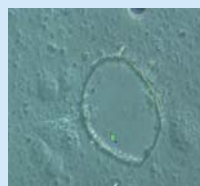
Before cut



After cut



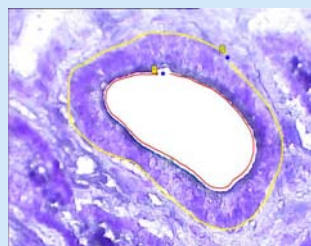
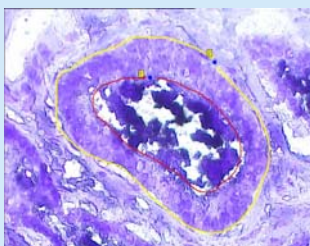
Tube cap



Selective LMPC



Cryo section, human prostate duct, cresylviolet stain, 40x



- selective elimination of unwanted material

- clear cut gap between selected and unwanted specimen

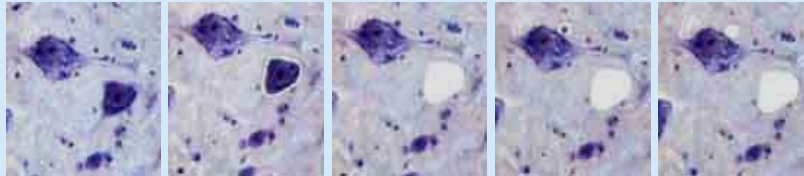
Images by courtesy of Dr. T. Schlomm, UKE Hamburg



Application in NeuroScience



Rat spinal cord - cells grown on LPC membrane



native tissue

cut
motoneuron

catapult
motoneuron

cut glial cell

catapult
glial cell

Benefits:

Selectively prepared single cells, cell clusters or other clustering material without any contamination with unwanted surrounding material.

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



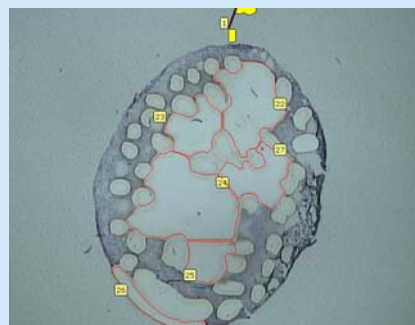
RNA in Toxicology (cDNA-arrays)



Identification of region-specific gene expression changes and signalling pathways affected by dibutyl phthalate in foetal rat testes. (GD19)



TUB (Sertoli cell region)

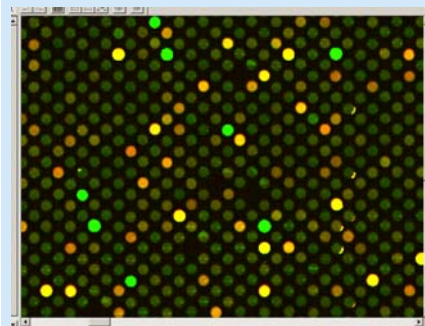


INT (Leydig cell region)

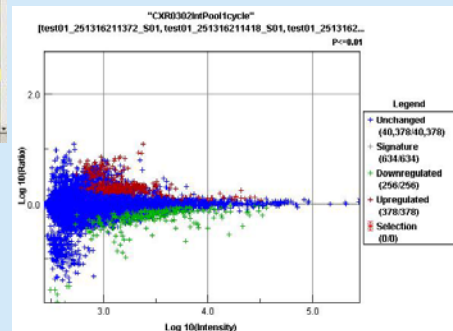
By courtesy of S. Plummer, CXR Biosciences, UK; Society of Toxicology Annual meeting 2006



Microarray analysis



60mer oligo array hybridised with RNA from foetal testes (Cy3 or 5)



By courtesy of S. Plummer, CXR Biosciences, UK; Society of Toxicology Annual meeting 2006



RNA/Protein analysis Prostate Cancer



40 different patients:
Each with tumor and non-tumor material

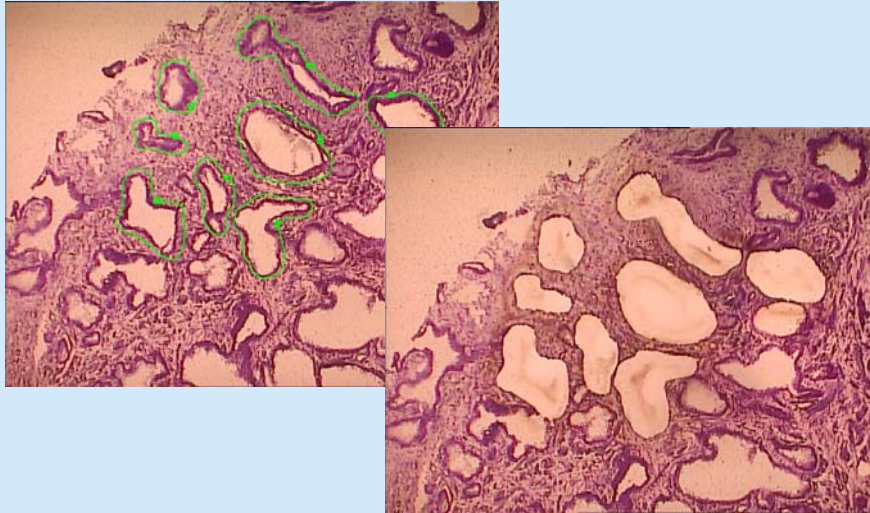


Sample collection for
RNA / Protein analysis with P.A.L.M. MicroBeam

Schlomm T et al. *Int J Oncol*, 27, 3: 713-720 (2005)



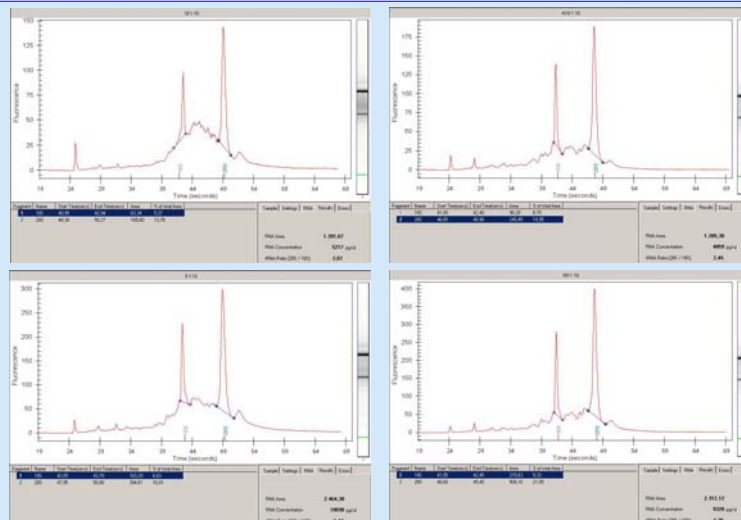
P.A.L.M. MicroBeam sample collection



Schlomm T et al. *Int J Oncol*, 27, 3: 713-720 (2005)



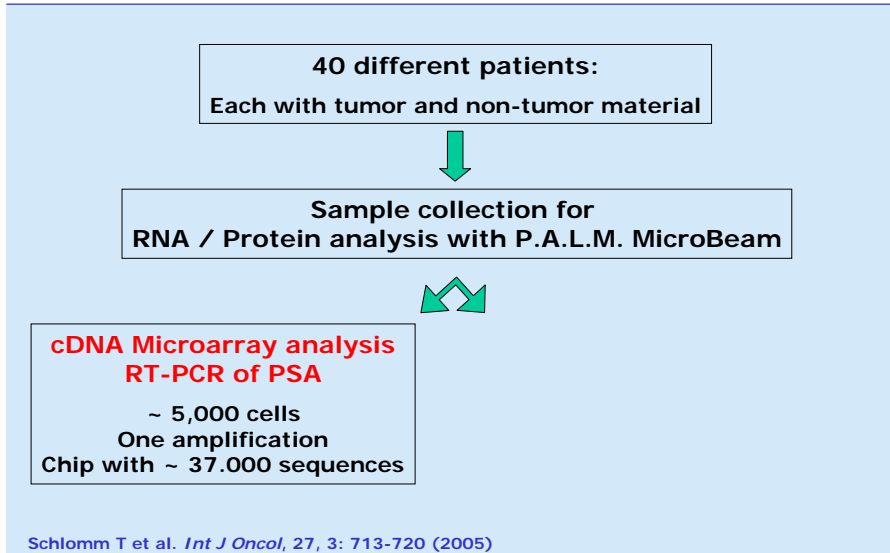
RNA analysis Agilent Bioanalyzer results



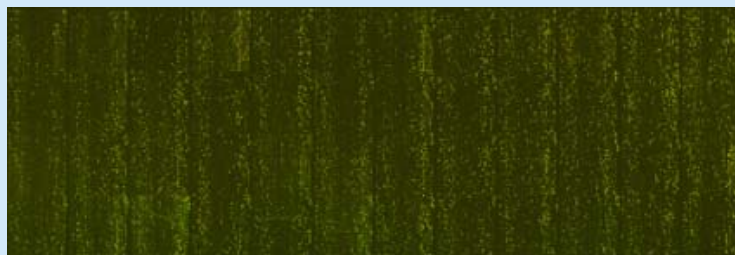
Schlomm T et al. *Int J Oncol*, 27, 3: 713-720 (2005)



RNA / Protein analysis Prostate Cancer



RNA analysis Microarray results



Array result of 5 patients (tumor against non-tumor)

- 200 ng total RNA amplified with Ambion MessageAmp Kit
- label: Cy3 and Cy5 with „dye-swap“
- Chip with 37,531 cDNA Clones

Result: in tumor **191 genes upregulated**, **25 downregulated**
among these some **prostate cancer relevant genes**, like
AMACR (upreg.), CAV1, CLU, THBS1 (downreg.)

Schlomm T et al. *Int J Oncol*, 27, 3: 713-720 (2005)



RNA/Protein analysis Prostate Cancer



40 different patients:
Each with tumor and non-tumor material



Sample collection for
RNA / Protein analysis with P.A.L.M. MicroBeam



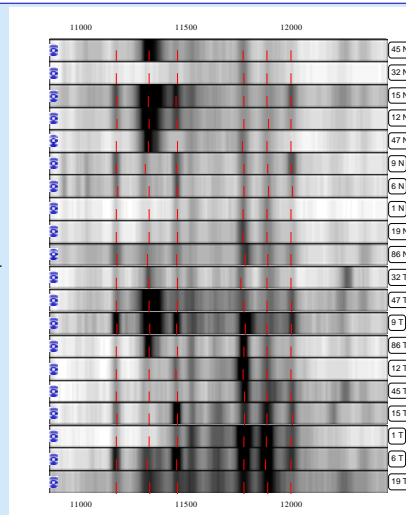
cDNA Microarray analysis
RT-PCR of PSA
~ 5,000 cells
One amplification
Chip with ~ 37.000 sequences

SELDI Protein analysis
~ 1,500 cells

Schlomm T et al. *Int J Oncol*, 27, 3: 713-720 (2005)



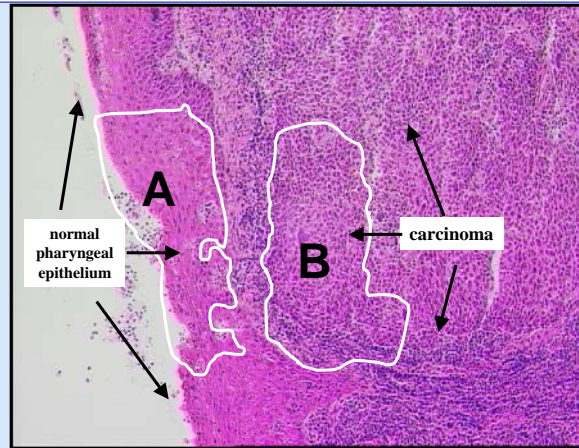
Protein analysis SELDI results



Schlomm T et al. *Int J Oncol*, 27, 3: 713-720 (2005)



Laser Microdissection for Proteomics

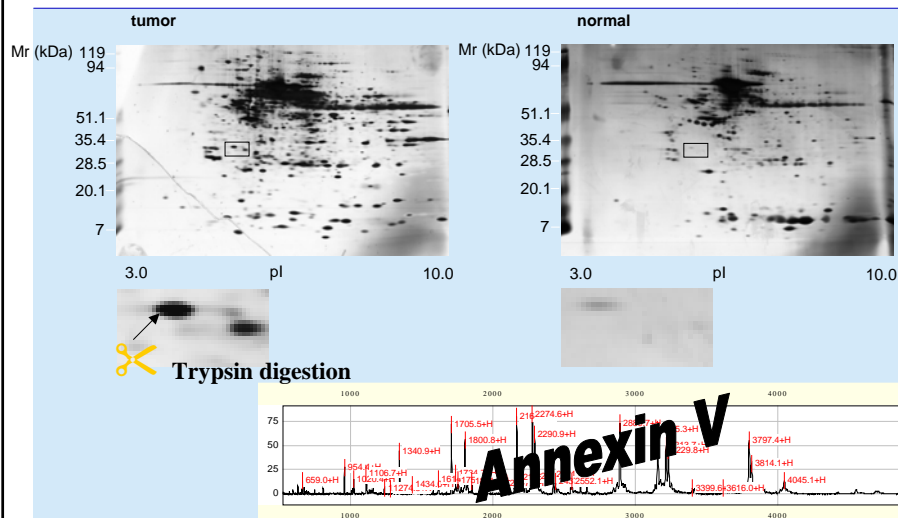


Histological cancer section (HNC); the white line shows the area to be excised by LMPC

Christian M et al. *Molecular & Cellular Proteomics* 27, 443-452 (2005)



Signal identification: 2-DE; in-gel-digestion



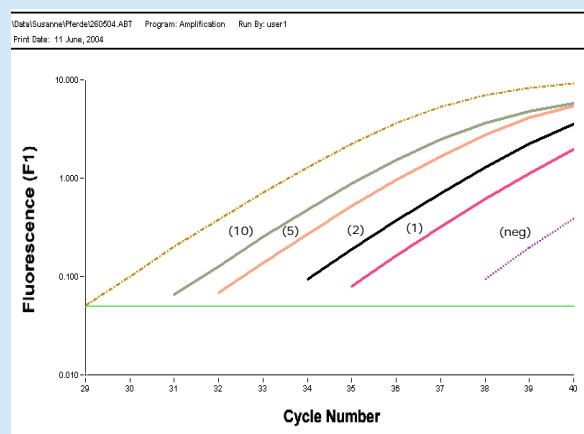
Christian M et al. *Molecular & Cellular Proteomics* 27, 443-452 (2005)



Single Cell Analysis



LightCycler PCR: single cells from glass



DNA isolation with:

CST® Forensic DNA Purification Kit (ChargeSwitch™, UK) + 10 mM DTT in the Lysis Buffer

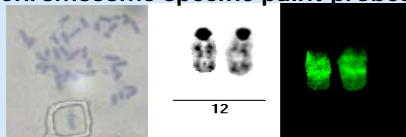


Single Chromosome Preparation

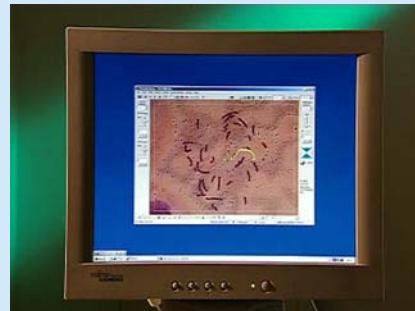


Metaphase spread on LMPC-membrane
Laser ablation of unwanted chromatin

Chromosome specific paint probes



Laser-isolated mouse chromosomes;
FISH after MSE-adaptor PCR



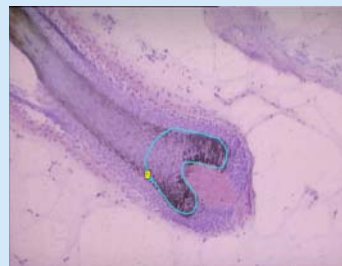
LMPC of hair follicle (CresylViolet stain)



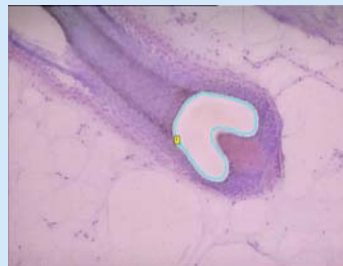
1



2



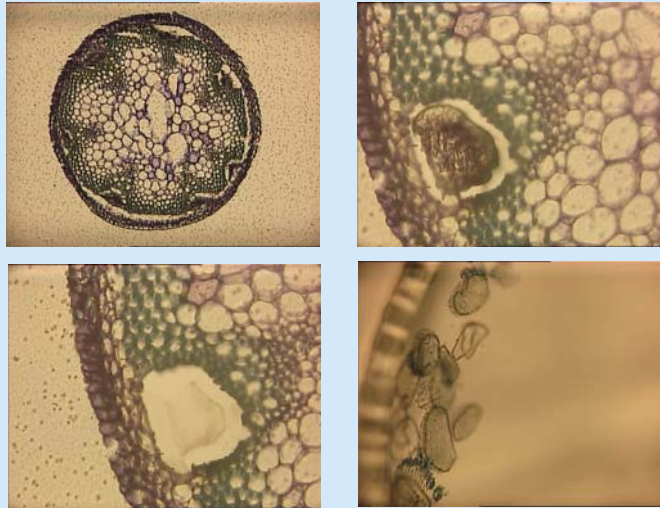
3



4



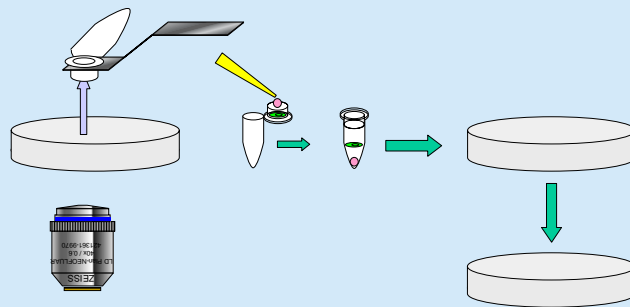
Arabidopsis stalk, phloem, LMPC



1. What is LMPC?
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6. Optical tweezer
7. Upgrade device
8. Summary



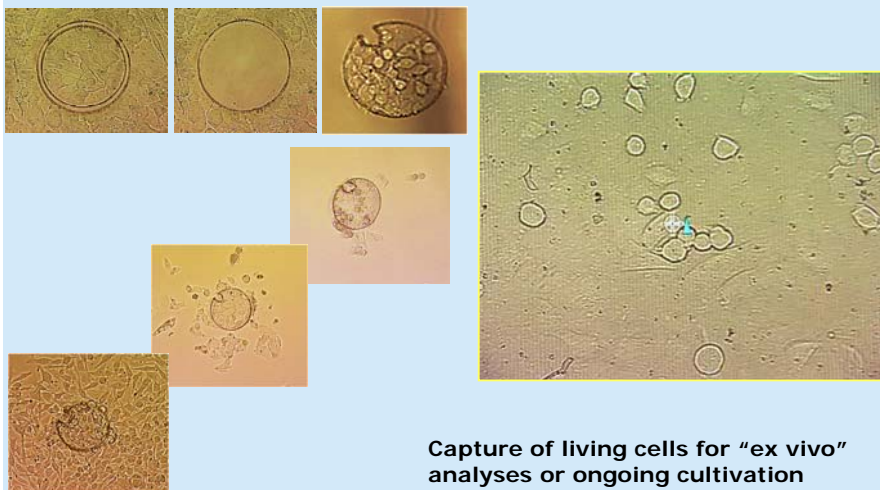
Working with Living Cells



Cloning from "picked" cells



LMPC of Living Cells



Capture of living cells for "ex vivo" analyses or ongoing cultivation

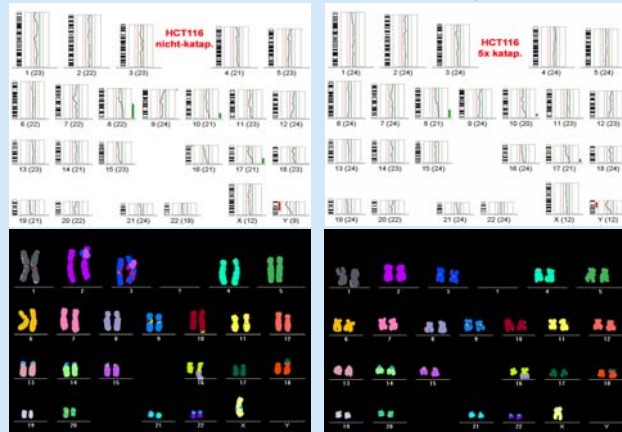


PALM® MicroBeam Genetic Proof



Not catapulted

5x catapulted



CGH

M-FISH

Both have the same karyotype :

45,X,der(10)dup(10)t(10;16),der(16)t(8;16),der(18)t(17,18)

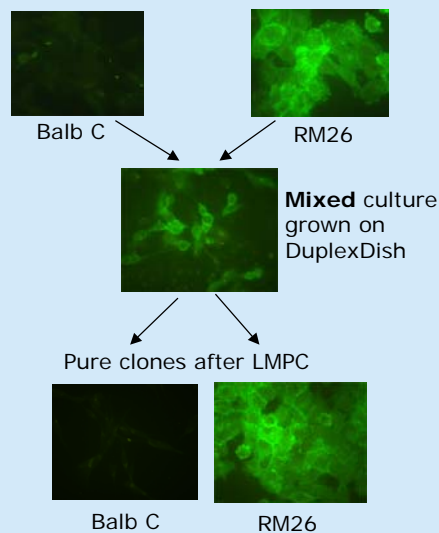


Anger S. et al. *Cancer Genetics and Cytogenetics* **161**: 174-177(2005)

Generation of Pure Cell Populations



Cell cultures of
two different murine cell lines,
immunostained against CD34

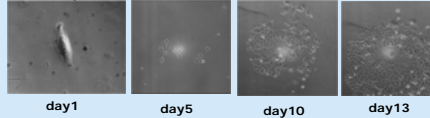


Images by courtesy of Dr. A. Buchstaller, LMU Munich

Embryonic Stem Cells

Do they survive the LMPC - procedure ? ✓

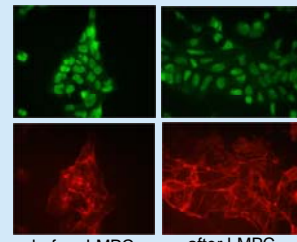
LMPC of murine stem cell line RM26 and subsequent recultivation



The expression of the pluripotency-marker „Oct-4“

Do they keep their stem cell character ? ✓

i.e. can they maintain themselves indefinitely in a stem cell state by "self-renewal", while also producing (through division) more specialized cells



Oct-4

Actin Cytoskeleton

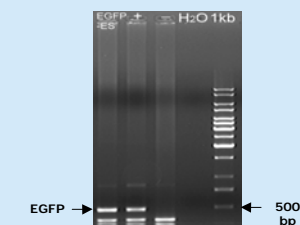
By courtesy of Dr. A. Buchstaller, LMU Munich

Mouse Embryonic Stem Cell LMPC

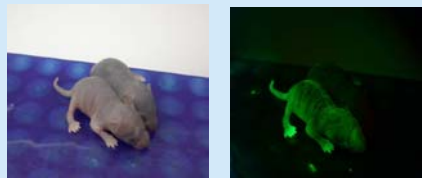


Murine stem cell (ES) clones with transgenic EGFP (enhanced green fluorescent protein) expression

After re-cultivation of this single cell on a feeder layer the cell divided in two daughter cells



PCR genotyping of transgenic EGFP in ES clone



EGFP mouse chimera production

Courtesy of
Level Biotechnology INC.
Research & Development
Division Associate Scientist
Wen-Jen Yu Ph. D.
mailto: jacksonyu@mail.level.com.tw
Marketing & Sales Dept.



LMPC of Whole Living Organisms



A. Vogel MLL-Lübeck



Caenorhabditis elegans
Courtesy of Ralf Baumeister

And it stays alive !



Microinjection and cell fusion

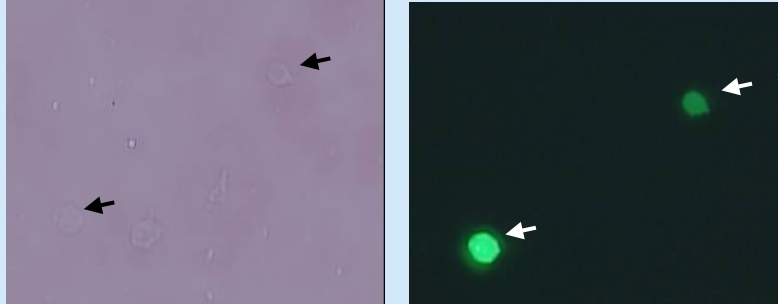


Images provided by PALM

Microinjection



Microinjection (2 $\mu\text{g/ml}$ EGFP plasmid in medium)



Precise Single Cell Transfection !!



WJ. Yu, Dept.R&D, Level Biotechnology, 2003



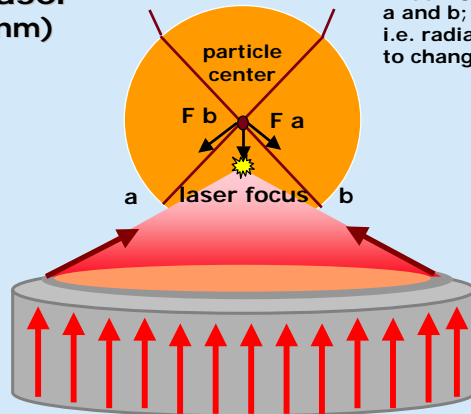
1. What is LMPC?
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7. Upgrade device
8. Summary



Optical tweezer



**NIR laser
(1064nm)**



MIE REGIME:
trapping due to refraction of rays
a and b;
i.e. radiation pressure force due
to change in momentum

Objective

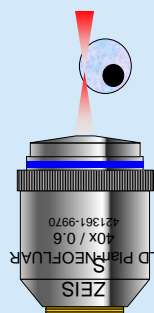
Ashkin, A. et al. PNAS 94: 4853 (1997)



The Dual-system concept



Laser Microdissection



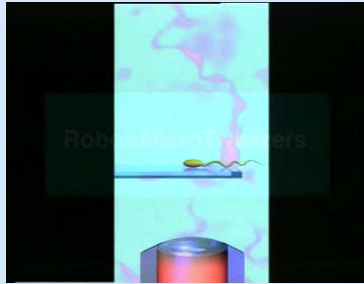
Laser Tweezers



Optical Tweezer

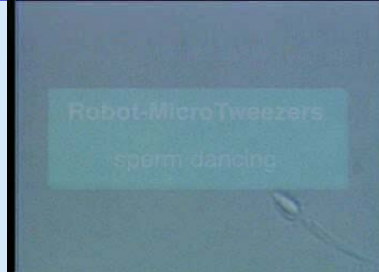


Optical Tweezers NIR laser



Robo-MicroTweezers
Positioning, catching
- catch and move -

Sperm
Catching
(I)



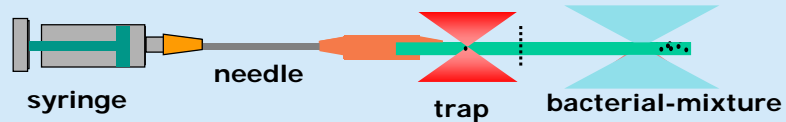
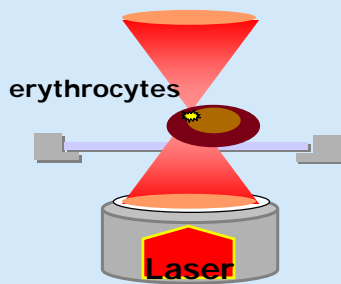
Sperm
Catching
(II)
(micro-surgery)



Optical Tweezer



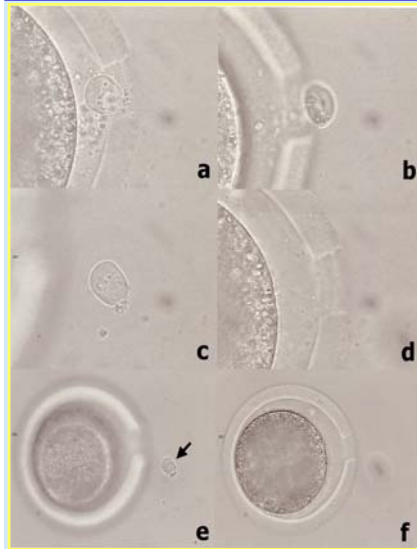
Specimen Segregation



Huber R. et al. Nature, 1995, 376: 57-58
P.Beck and R.Huber FEMS Microbiol.Lett. 147:11-14 (1997)



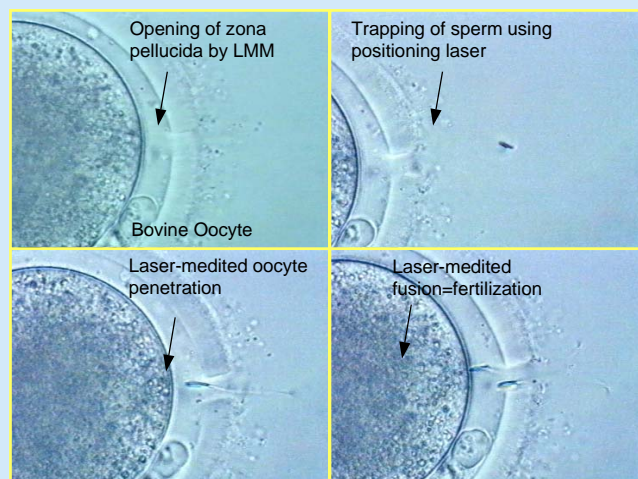
Prefertilization Diagnostics



- a. Opening of the zona pellucida with laser zona drilling
- b. Polar body trapping and dragging with optical tweezers
- c. Isolated polar body
- d. Empty oocyte
- e. Isolated polar body (40x)
- f. Empty oocyte (40x)



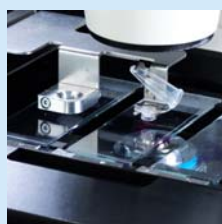
In vitro fertilization



Thalhammer S, AG NanoScience, Munich

1. What is LMPC?
2. Character of PALM
3. Publication
4. Pathology
5. Living cell manipulation
6. Optical tweezer
7. Upgrade device
8. Summary

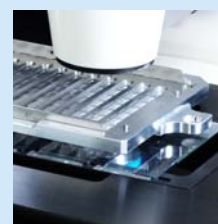
PALM RoboMover



Single Tube



8 Tubes



8-Strip Collector

AxioCam



AxioCam MR family

- Highly sensitive 2/3" CCD image sensor with **1.4 megapixels**

For brightfield

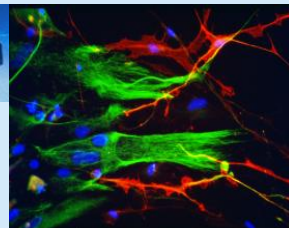
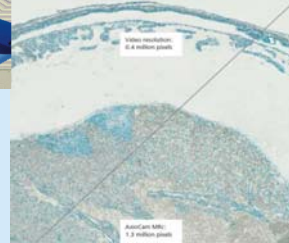
AxioCam MRc

- Excellent for high-quality bright field color images

For fluorescence

AxioCam MRm

- Excellent for high-quality fluorescence imaging with variable exposure time
- Obtain **low-noise images** in extraordinary quality

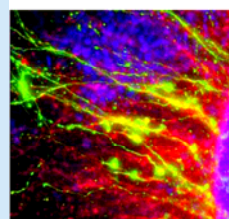


Apotome

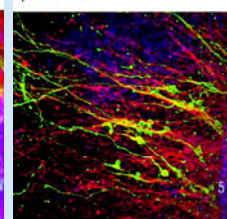


Axons of a dorsal root ganglia

Conventional



Apotome mode



Drosophila melanogaster embryo

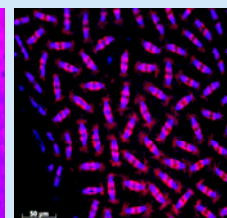
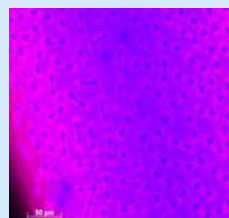
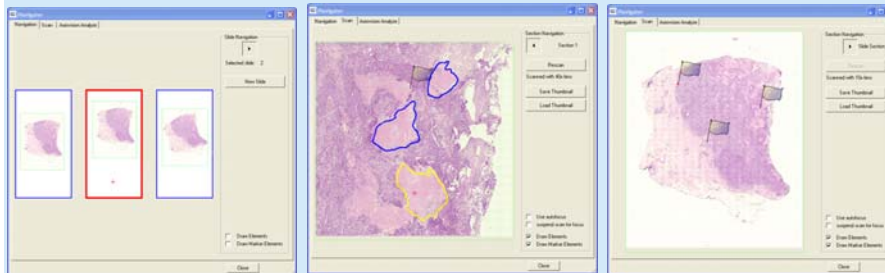


Image Scan and Navigator



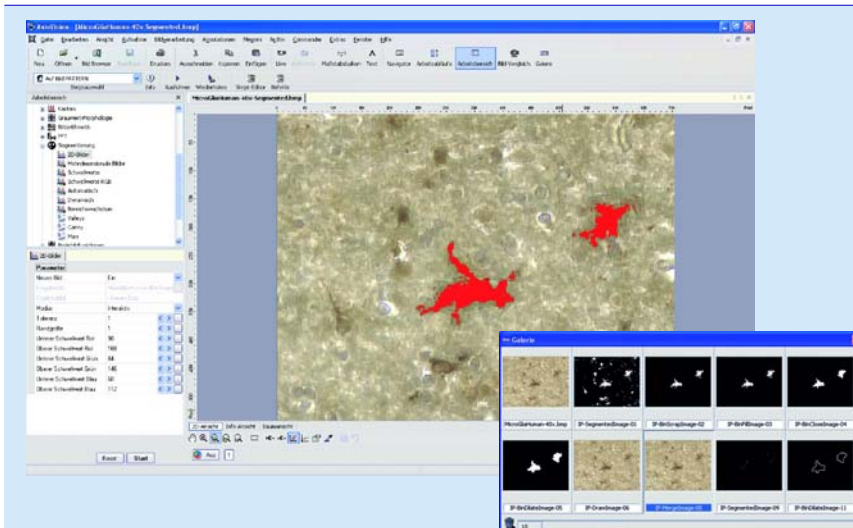
Scan 3 slides
to get overview

ReScan in higher
magnification to get a
closer look

Set markers at
regions of interest



Axiovision



1. What is LMPC?
2. Character of PALM
3. Publication
4. Pathology
5. Proteomic
6. Living cell manipulation
7. Optical tweezer
8. **Summary**

One System – Many Advantages

- Flexible applications from archival material to living cells – DNA, RNA, Protein
- Patented LMPC system for non-contact and, thus, contamination-free capture
- Upgradeable technology with additional solutions from Carl Zeiss
- PALM Application Laboratory: years of experience and specialized know-how

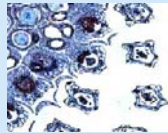


PALM MicroBeam

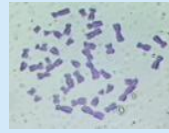
One System – Many Application



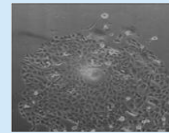
Cancer Research



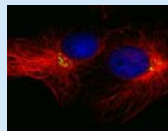
Plant Research



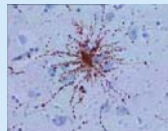
Cytogenetics



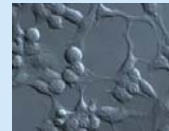
Cell Biology



Fluorescence



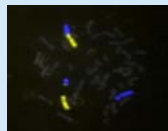
Immunohistochemistry



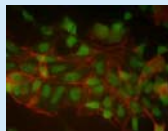
Phase Contrast



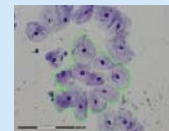
Phase Contrast



Chromosomes



Stem Cells



Cells on Forensic Tape



Sperms



Comparison of LCM



	LMPC	LCM
General features	Inverted microscope, Cold ablation, Broadest application	Inverted microscope, Heat direct contact, restricted application
Purity	High, LPC	Low, Direct contact
Precision	Ranged from 0.6 μ m to 1mm in any shape	Microdissection with 40X objective only
Automation	Quick access, automated multi-group sample collection, automated multi-caps positioning	Automated multi-disk positioning
Living cells	Yes	No
Routine Slide	Yes	No
SALDI Compatible	Yes	N.A.
Optical Tweezer	Combisystem	N.A.
Local R&D support	Yes	N.A.
Consumable cost	Low	High



Summary



Specimen Preparation and Selection

Sources (a selection)

- Histological specimens
- Living cells and cell cultures
- Plant material
- Chromosome spreads
- Forensic preparations

Preparation

- Cryofixation or FFPE material
- Living or fixed
- Stained or unstained
- For fluorescence and transmitted light

LMPC

Laser Microdissection & Pressure Catapulting

Precision

- Laser focus diameter < 1 μm
- Reproducible precision of stages < 1 μm
- Precise control of microscope and laser
- Perfect component compatibility

Automation

- Reliable and reproducible selection of target areas
- Choice of automated or manual microdissection
- Efficient specimen collection

Subsequent Analysis

DNA

PCR, mutation analysis, SNPs, genetic fingerprinting, LOH, FISH

RNA

RT-PCR, expression analysis, microarrays

Proteins

2-D PAGE, SELDI-TOF, MALDI-TOF, immunoblot, nLC/MS/MS

Living Cells

Regenerative medicine, stem cell research, cloning, tissue cultures, primary cultures



Laser Microdissection & non-contact Pressure Catapulting

BEAM ME UP...

Thank You

with PALM® MicroLaser Systems

