



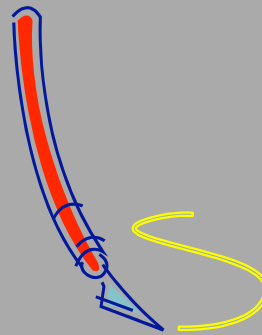
An alternative method in (live) cell sampling

Sample Manipulation with Laser Light

Macrodissection



Microdissection



Laser Microdissection



PALM MicroBeam

Non-contact Laser Capture Microdissection





Non-contact Laser Capture Microdissection (LCM)

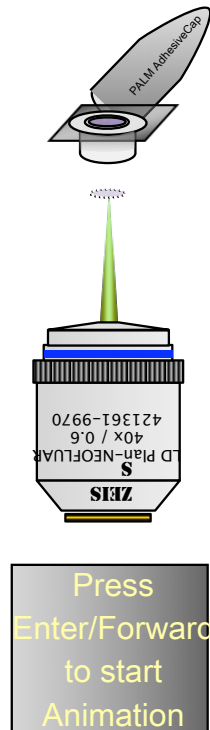
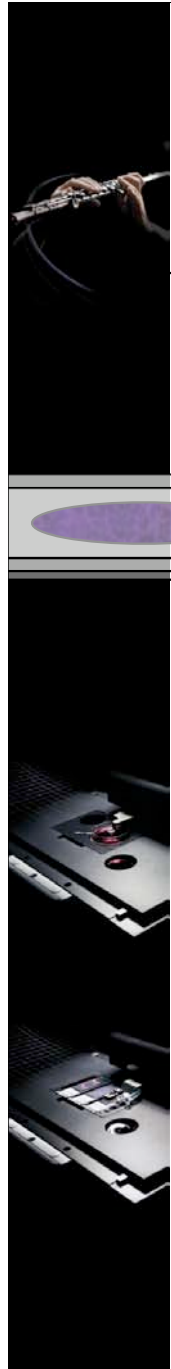
Contamination free
non-contact
Against gravity



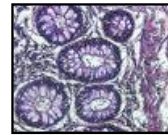
Separate target cells with Laser

PALM MicroBeam

Non-Contact Laser Capture Microdissection



1. Imaging 2. Capture 3. Verification 4. Isolation



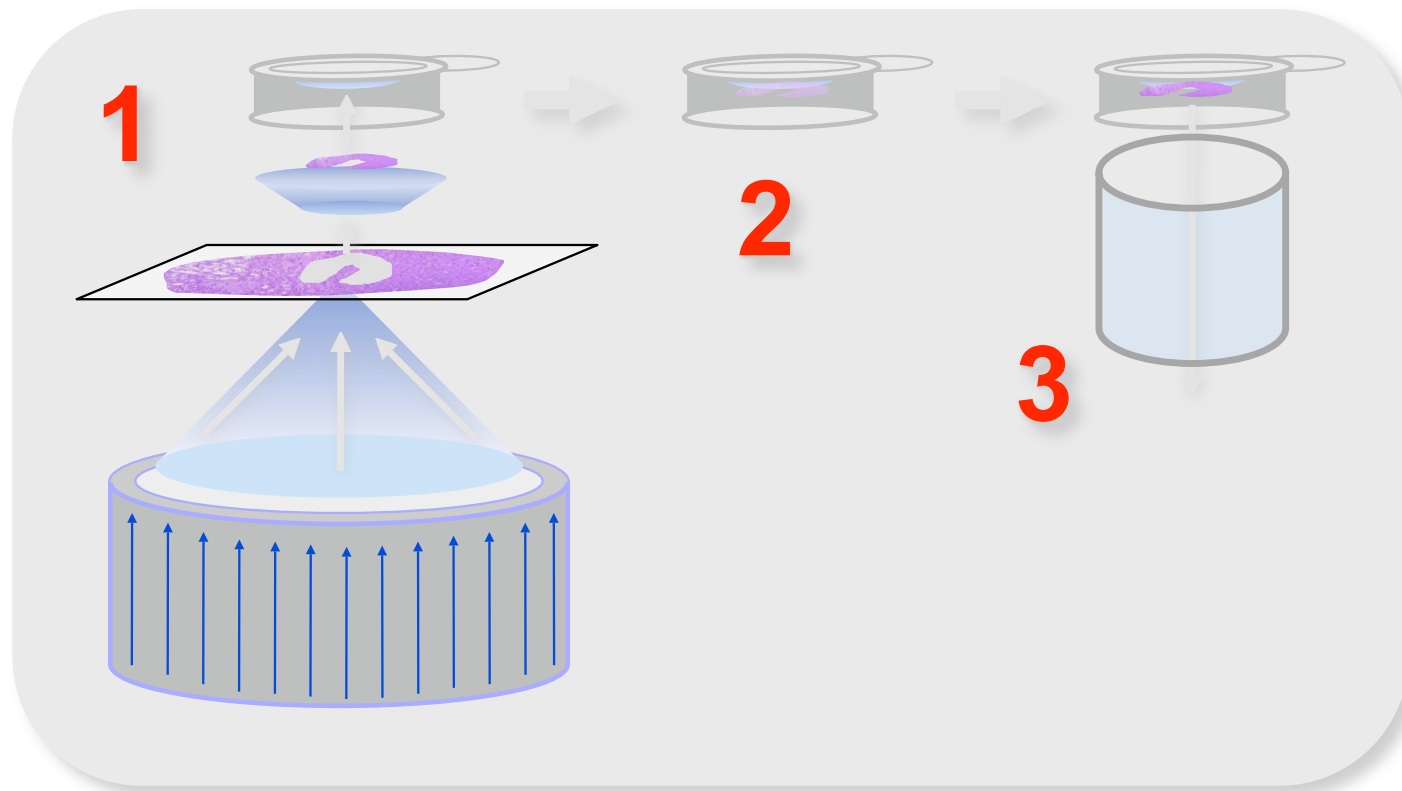
5. Downstream

- DNA analysis
- RNA analysis
- Protein analysis

Isolated sample in your hand



Cut – lift up – spin down



PALM MicroBeam System design



Solid State Laser 355nm

Advantages:

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

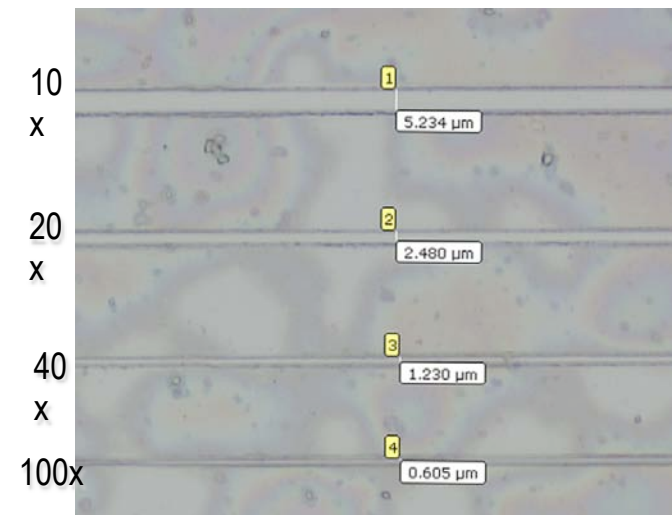
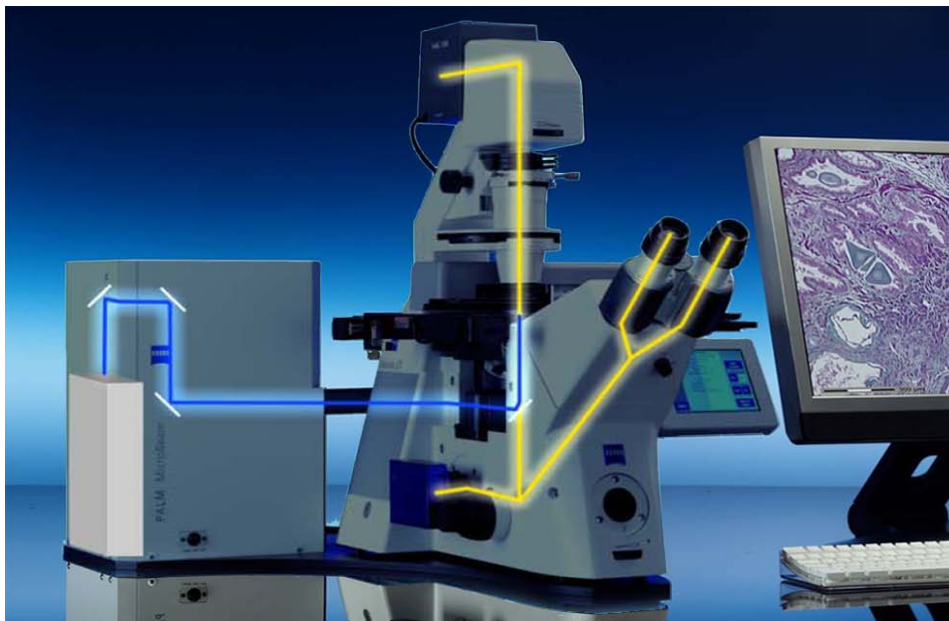
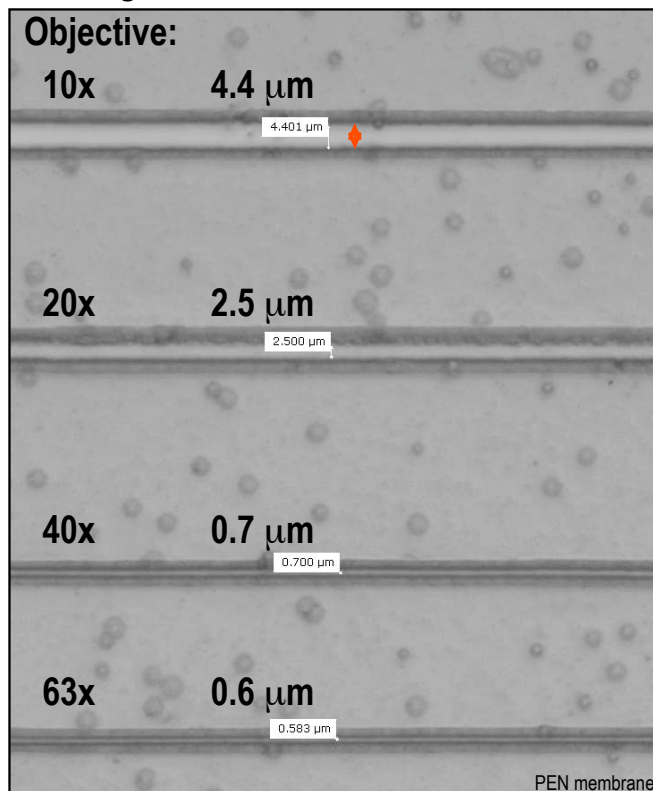


Image taken in 400x magnification

PALM Laser Unit Precision



Cutting Precision of the Laser



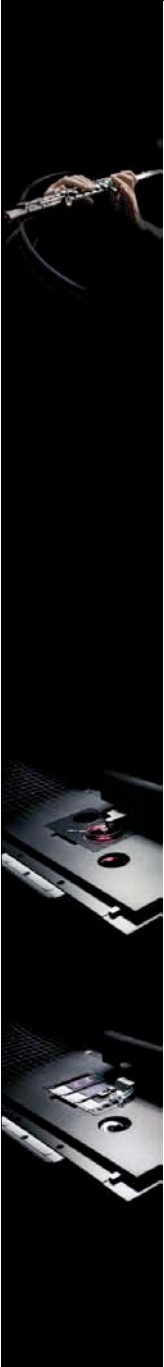
Dimensions:

Mammalian cell >10 μm diameter

Nucleus 5-20 μm diameter

Chromosomes 0.5 x 2-10 μm

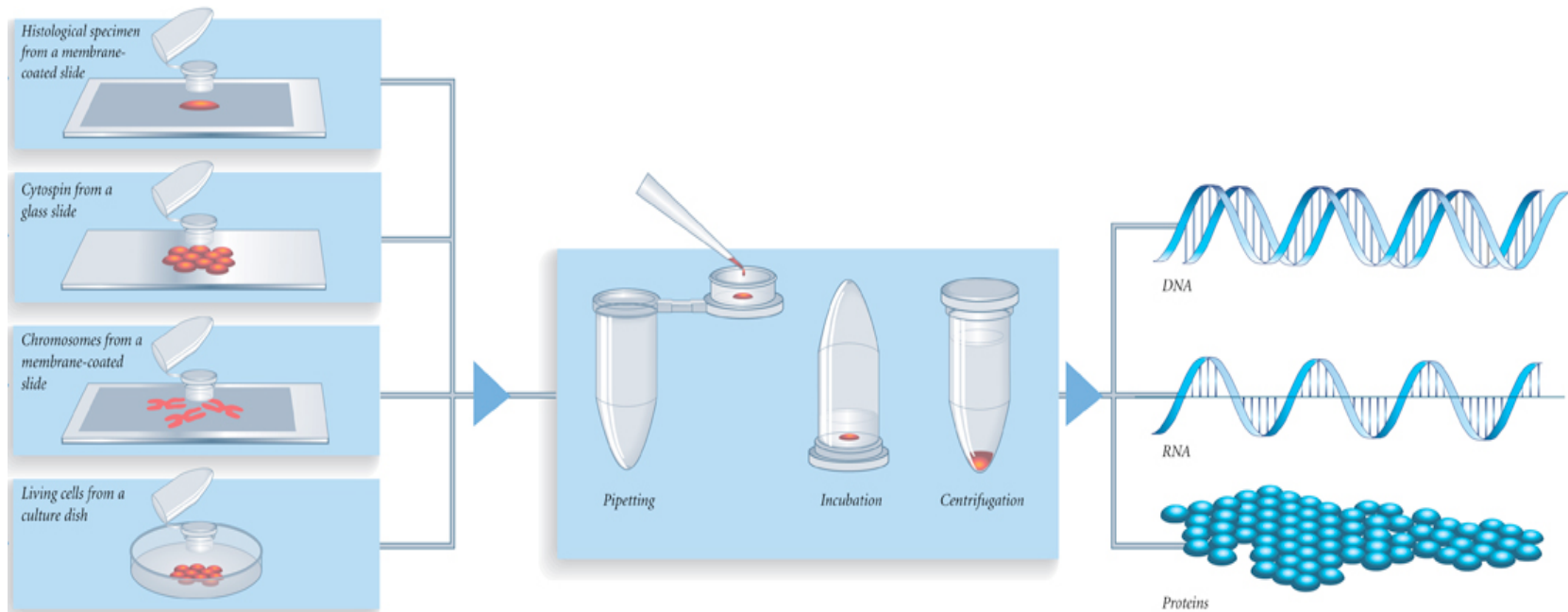
E.coli 1 x 2-4 μm



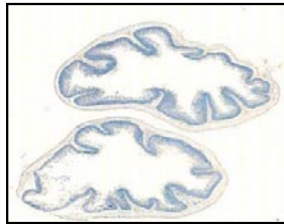
PALM RoboSoftware



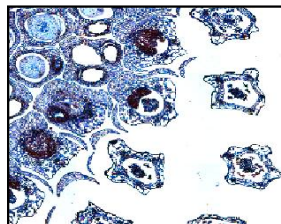
Work-flow methodology



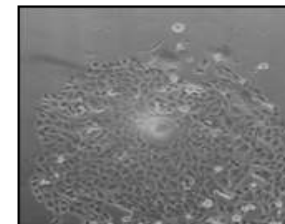
Various Types of Source Material & Applications



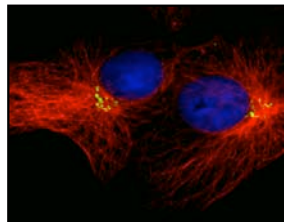
Pathology



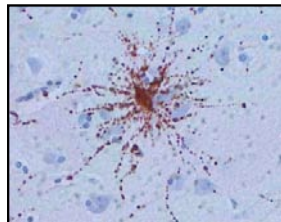
Plant Research



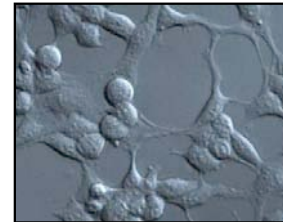
Cell Biology



Fluorescence



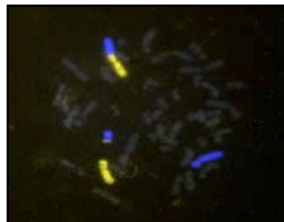
Histochemistry



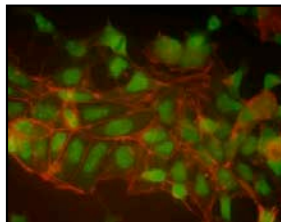
PlasDIC



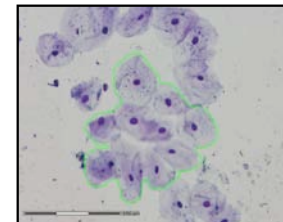
Phase Contrast



Chromosomes



Stem Cells

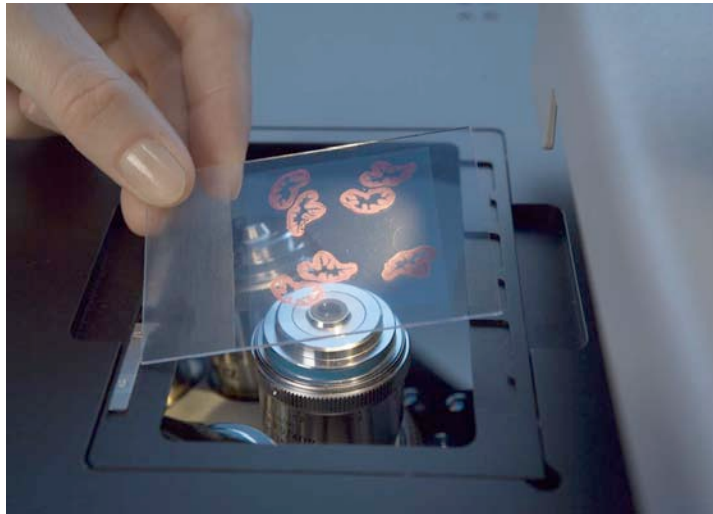


Cells on Forensic Tape



Sperms

Application Examples

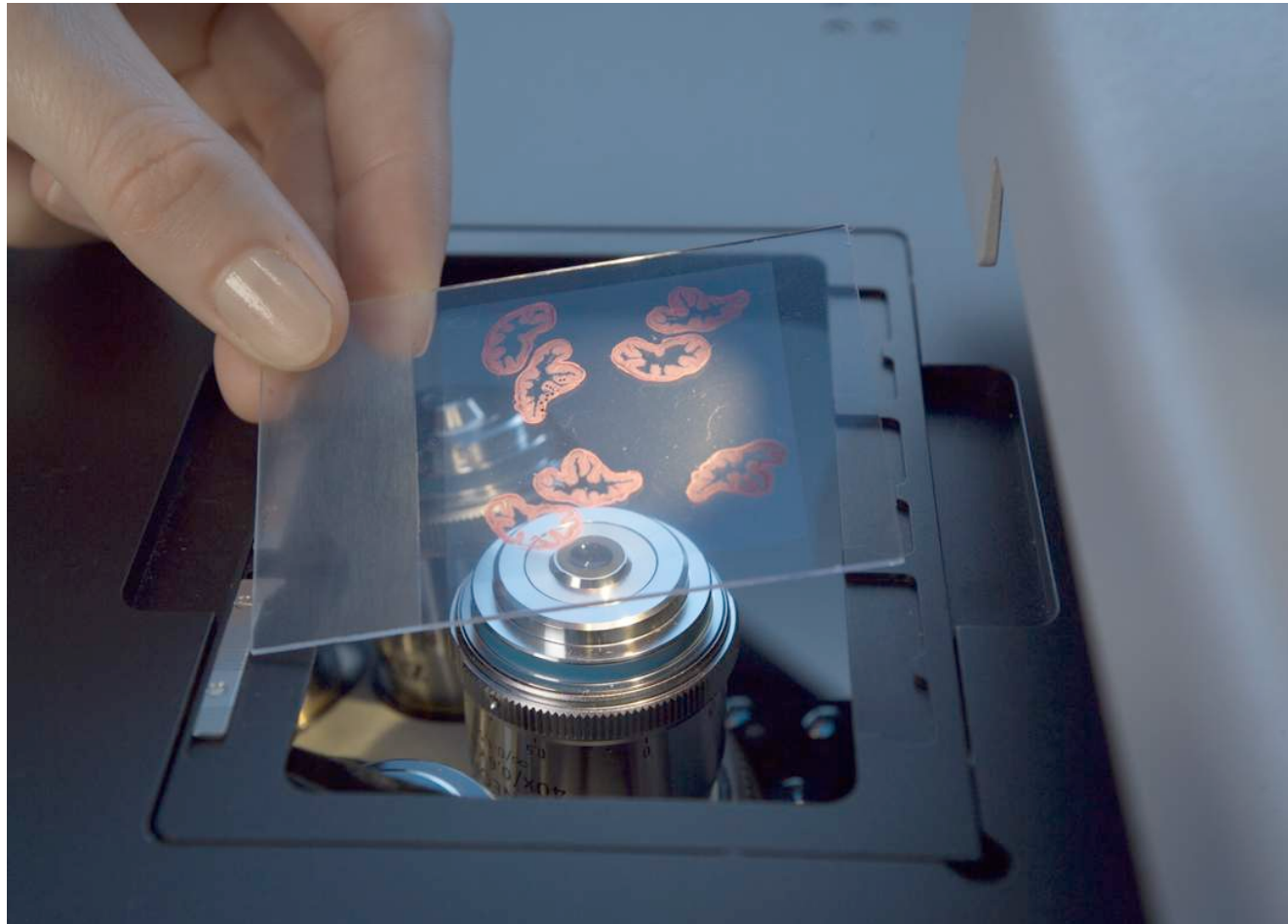


Fixed Materials

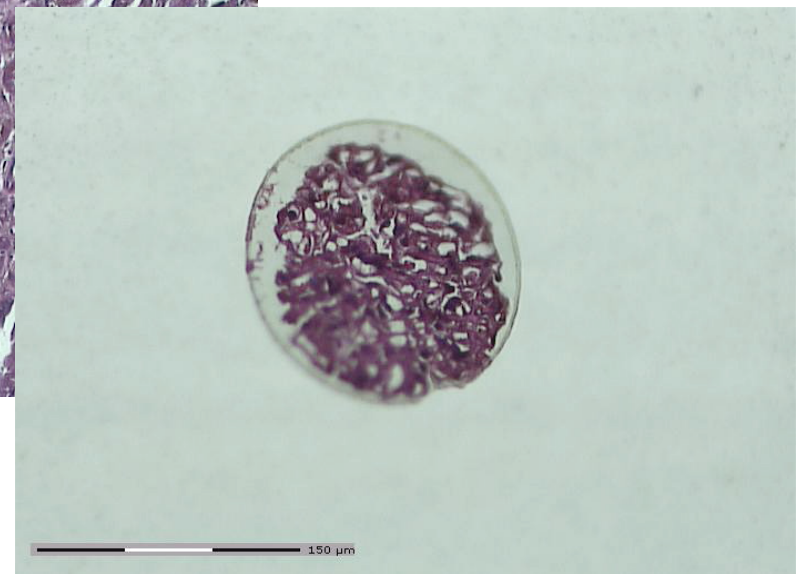
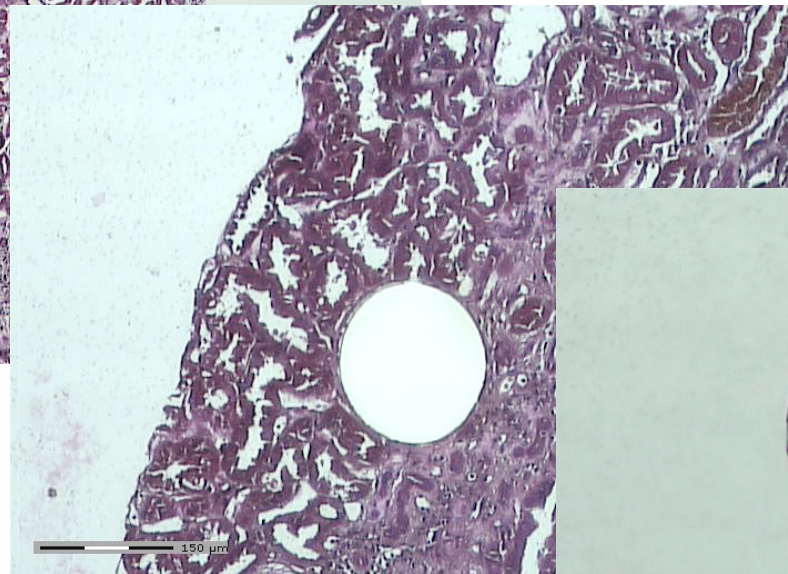
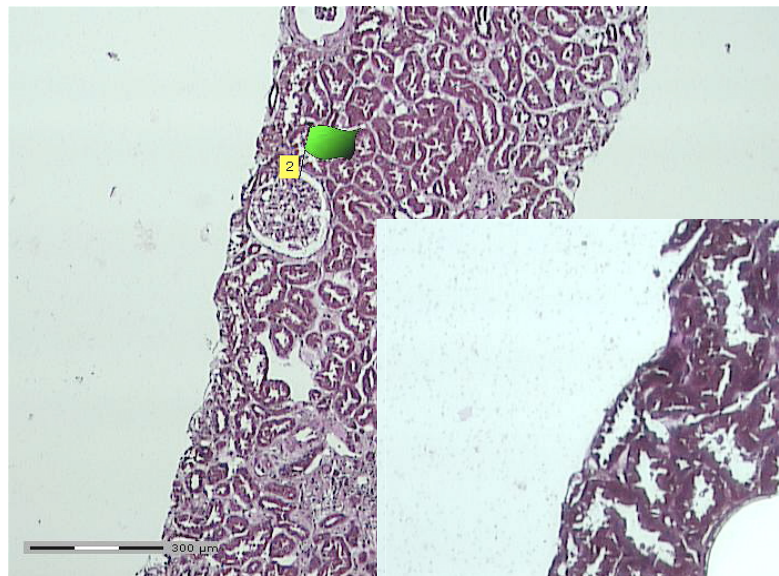


Live Cells

Fixed Materials

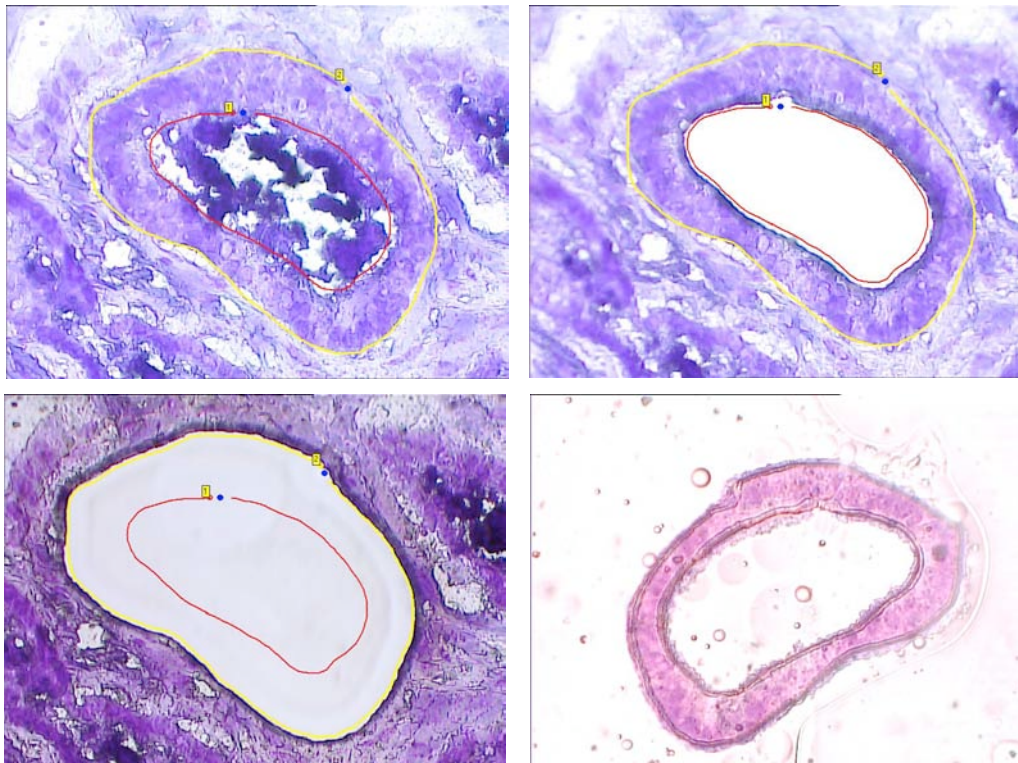


LCM from kidney biopsy (FFPE, H&E)



Selective LCM

frozen section, human prostate duct, CresylViolet stain, 40x



- selective elimination of unwanted material

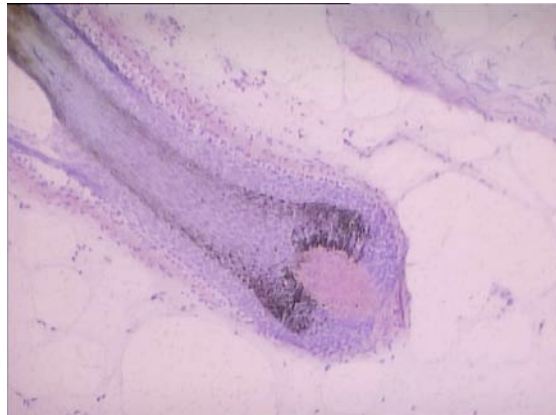
- clear cut gap between selected and unwanted specimen

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

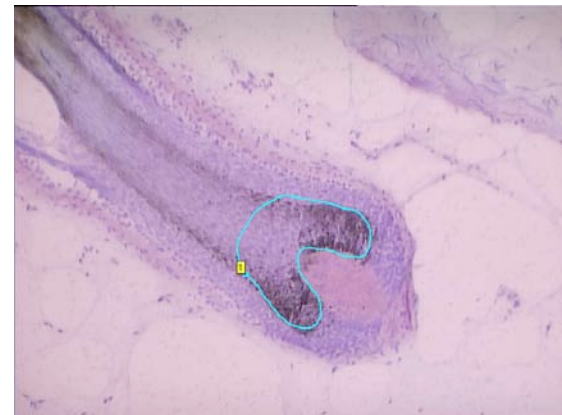
LCM of hair follicle (CresylViolet stain)



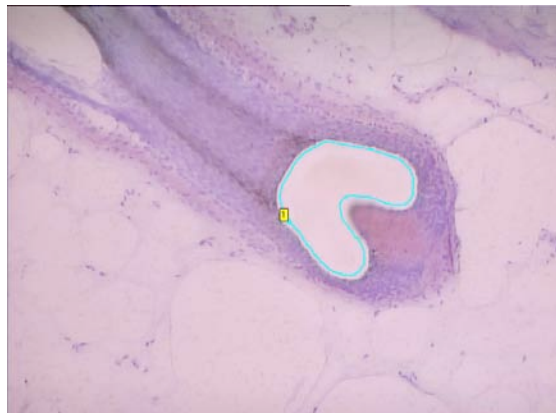
1



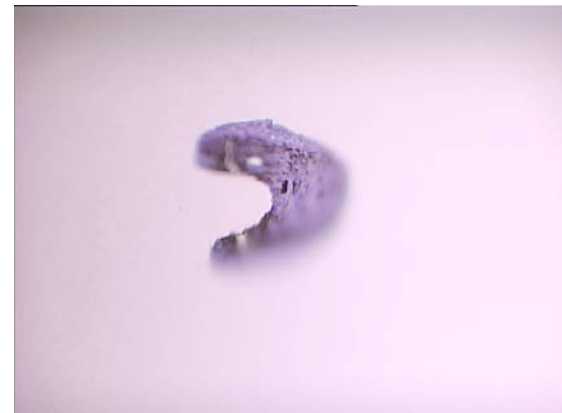
2



3



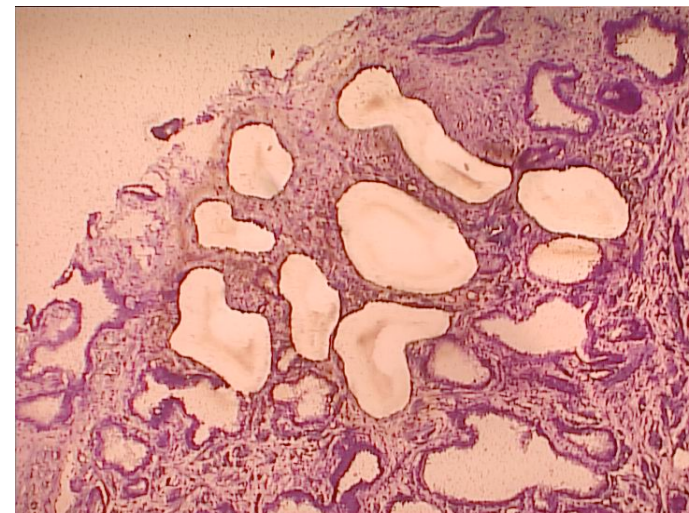
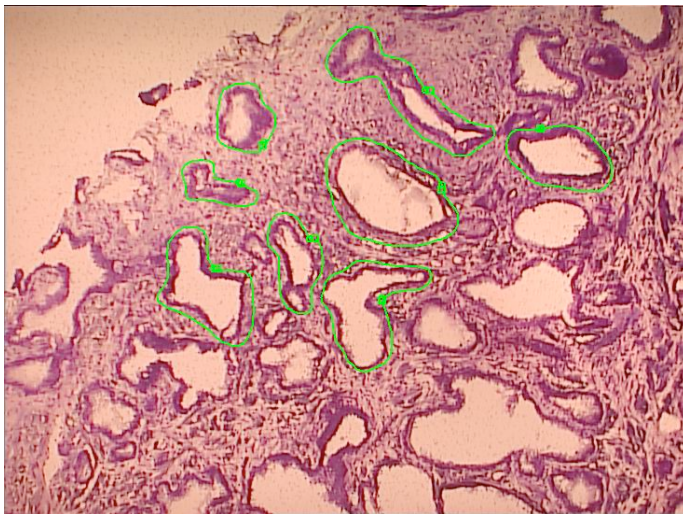
4



LCM of gut nerve cells
(CV stain, frozen section)



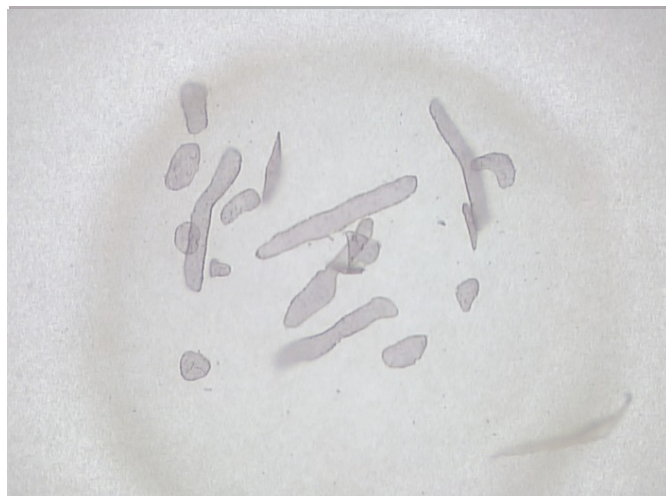
Homogenous sample collection with LCM



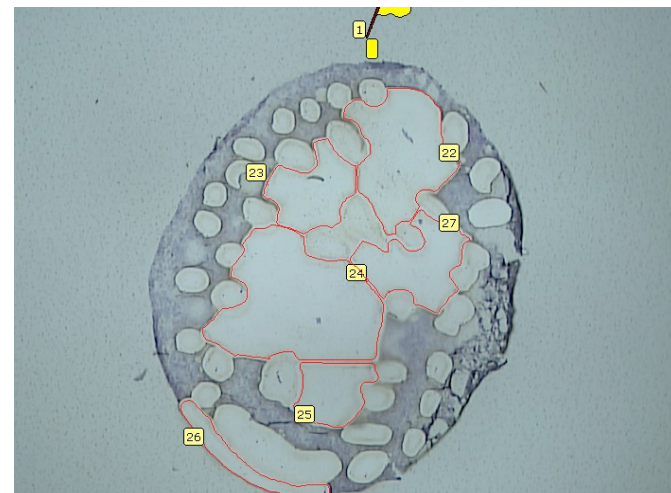
Images by courtesy of Dr. T. Schlomm, UKE Hamburg

RNA in Toxicology (cDNA-arrays)

Identification of region-specific gene expression changes and signalling pathways affected by dibutyl phthalate in fetal rat testes.



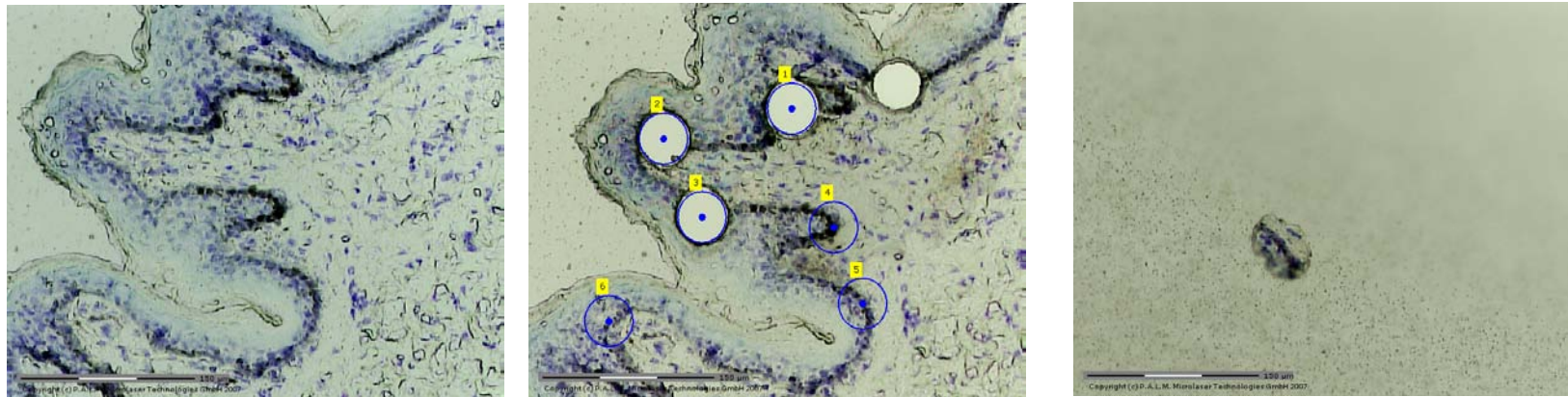
TUB (Sertoli cell region)



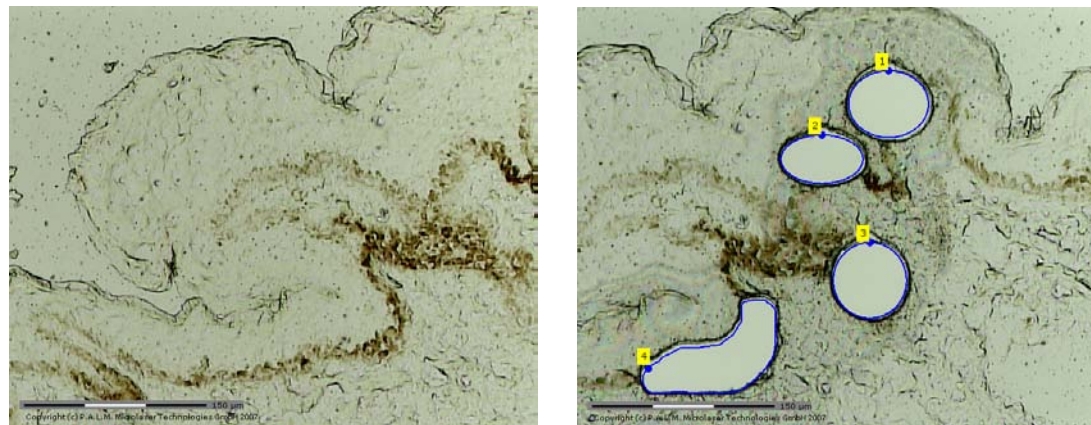
INT (Leydig cell region)

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

LCM of Skin Samples



Skin samples mounted on PALM FrameSlides, cryo sections (7 µm), Cresyl-Violett staining, 20x obj.



Skin sample on PALM FrameSlide, unstained, 20x obj.

Protein applications

Example: Identifying candidate



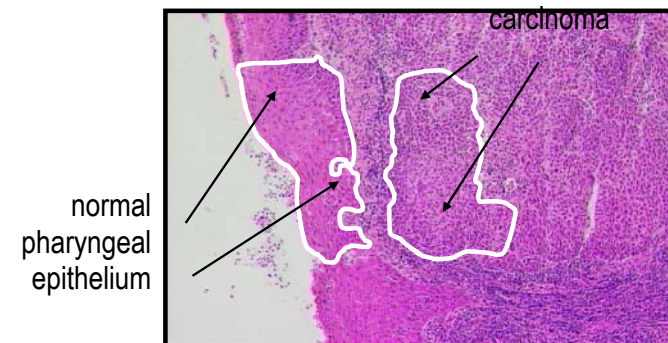
Preselection of putative candidate proteins with pure samples

Laser microdissection:

Selective collection of

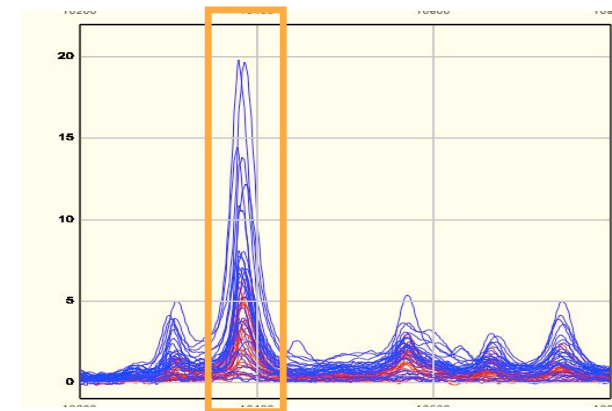
- a) Tumor tissue
- b) Non-tumor tissue

1000 cells were collected



SELDI Analysis with microdissected samples:

By comparing tumor versus non-tumor cells it was possible to find a **protein** which is **higher abundant** in carcinoma tissue than in unaffected tissue



Candidate protein with 36 kDa

By courtesy of Dr. F. v. Eggeling, Core Unit Chip Application, Jena

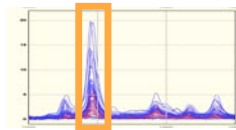
Protein applications

Using information from LCM approach

Analysis of bulk material in a 2D-PAGE Gel

-> is the search for the needle in a haystack ...

Analysis of bulk material combined with information of preliminary approach ...



Result of preliminary approach:
Candidate protein with 36 kDa



Mr (kDa) 119
94

51.1

35.4

28.5

20.1

7

Mr (kDa) 119
94

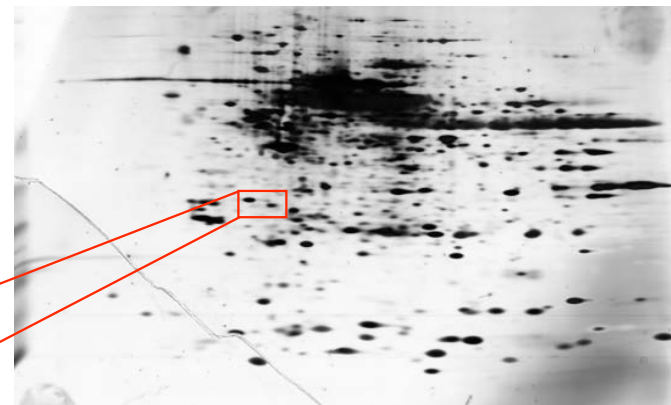
51.1

35.4

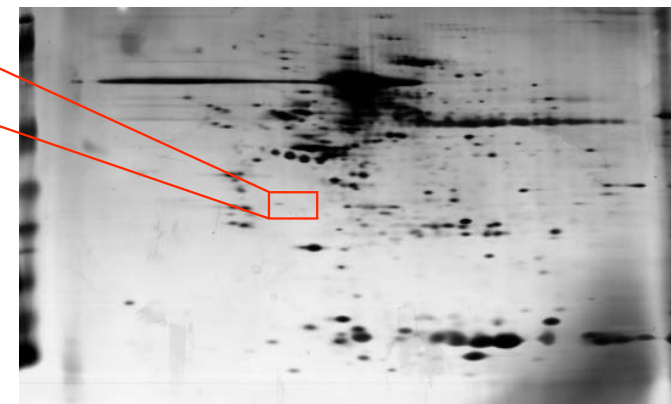
28.5

20.1

7



2D Gel from tumor



2D Gel from non-tumor

... can facilitate search for candidate for further analysis steps

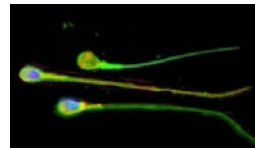
By courtesy of Dr. F. v. Eggeling, Core Unit Chip Application, Jena

Forensic Medicine

From sparse sample materials to genetic profiles



sperms



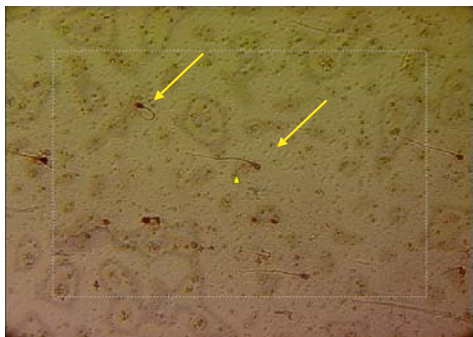
epithelial cells

flakes of dandruff



Forensic Medicine

Sperms on MembraneSlide



Vaginal smear from a rape case

Collection of identified rapist`s sperms from mucus smear of rape victim. Subsequent DNA fingerprint may allow identification of culprit.



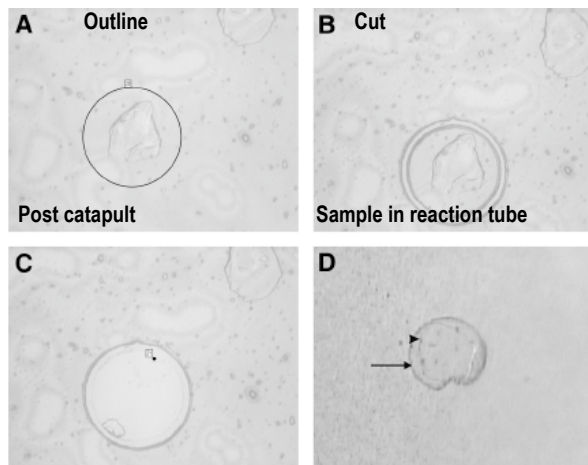
Optionally the sperms can be collected altogether („pooled“) into one collection device or into different caps.

Tests performed by Dr. Seidl and Dr. Lederer,
Institute of Forensic Medicine, University Erlangen, Germany.

Forensic Medicine

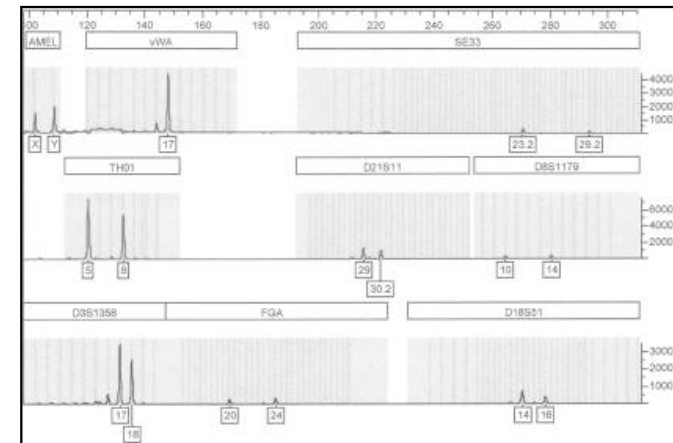


Laser microdissection of epithelial cell



DNA extraction & PCR

Genetic profile of 10 epithelial cells



Genetic profile of 4 sperm cells



Seidl *et al.*, Forensic Science, Medicine, and Pathology 2005

Complete profile in one single sperm



Available online at www.sciencedirect.com



Forensic Science International: Genetics Supplement Series 1 (2008) 437–438



www.elsevier.com/locate/FSIGSS

Research article

An efficient novel method for analyzing STR loci from a single sperm captured by laser microdissection

T. Miyazaki^{a,*}, M. Hara^b, A. Ichiki^c, Y. Yamamoto^d, A. Takada^b,
A. Kido^c, M. Nodera^a, H. Yanagisawa^a, H. Suzuki^a, Saito K^b

^aDepartment of Health Science & Preventive Medicine, Community Health Science Center, Japan

^bDepartment of Forensic Medicine, Faculty of Medicine, Saitama Medical University, Japan

^cDepartment of Anesthesiology, Mita Hospital, International University of Welfare & Health, Japan

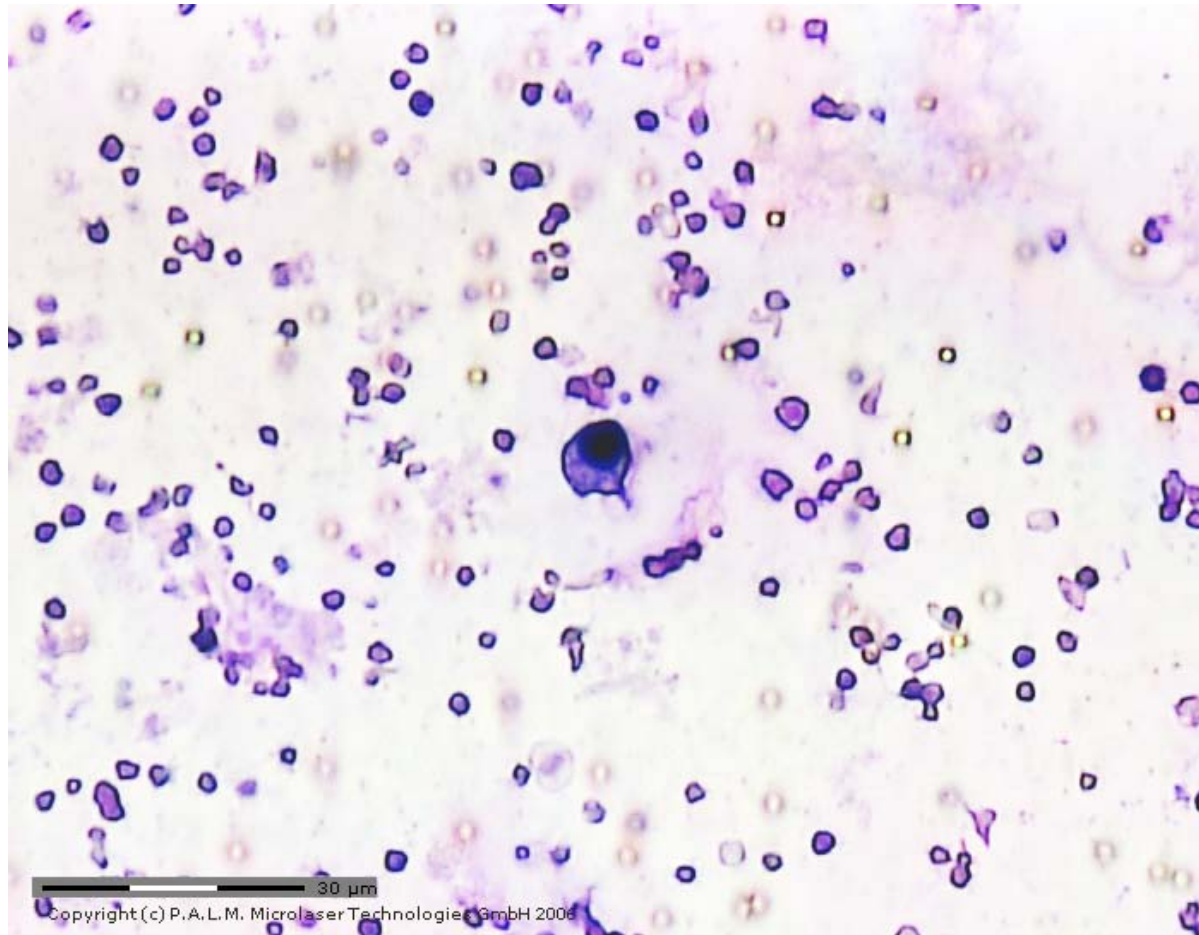
^dCriminal Investigation Laboratory, Saitama Prefectural Police Headquarters, Japan

^eDepartment of Legal Medicine, Faculty of Medicine, University of Yamanashi, Japan

Prenatal Medicine
Single Cell Handling: NRBC in maternal blood
(nucleated red blood cells)



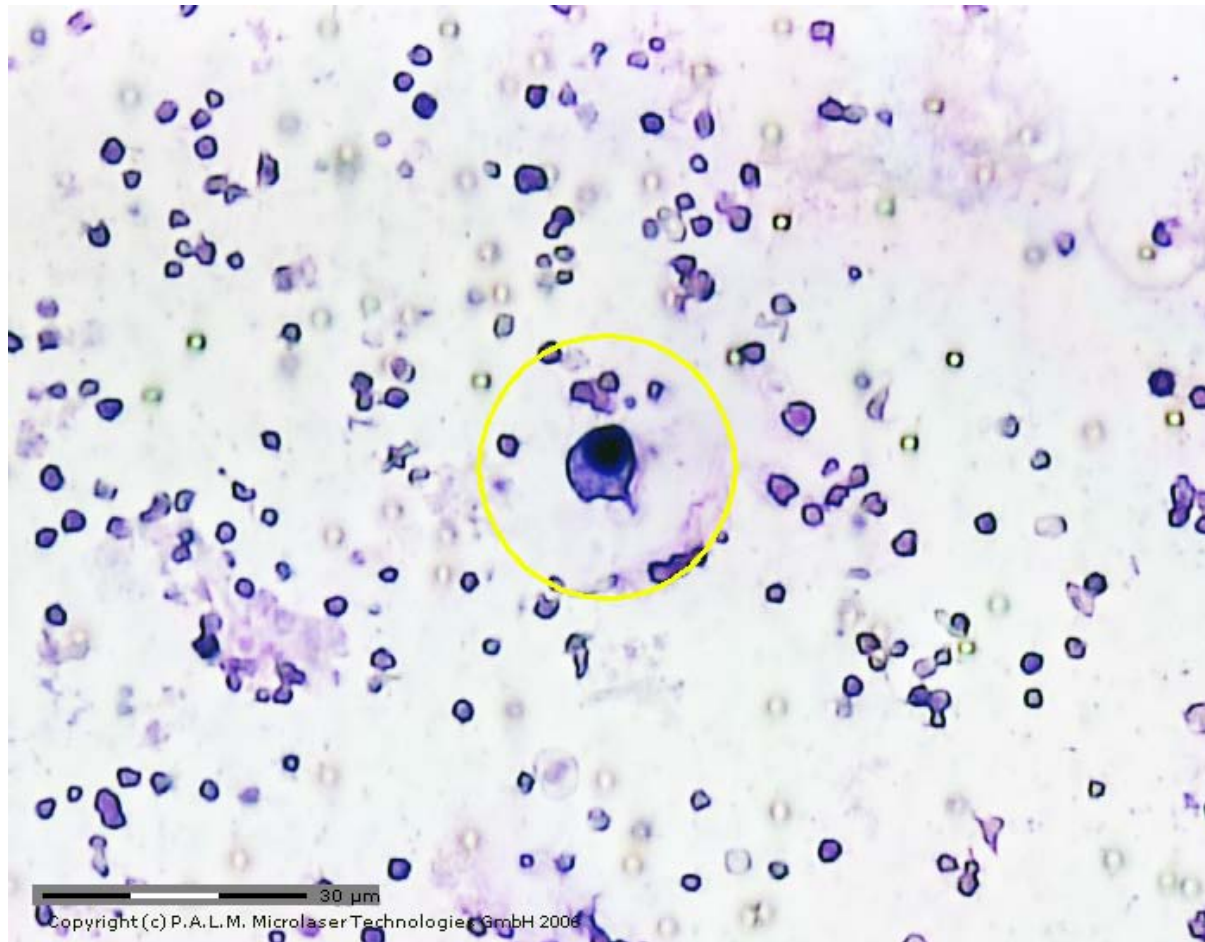
63x objective



Prenatal Medicine
Single Cell Handling: NRBC in maternal blood
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63x objective



Prenatal Medicine
Single Cell Handling: NRBC in maternal blood
(nucleated red blood cells)



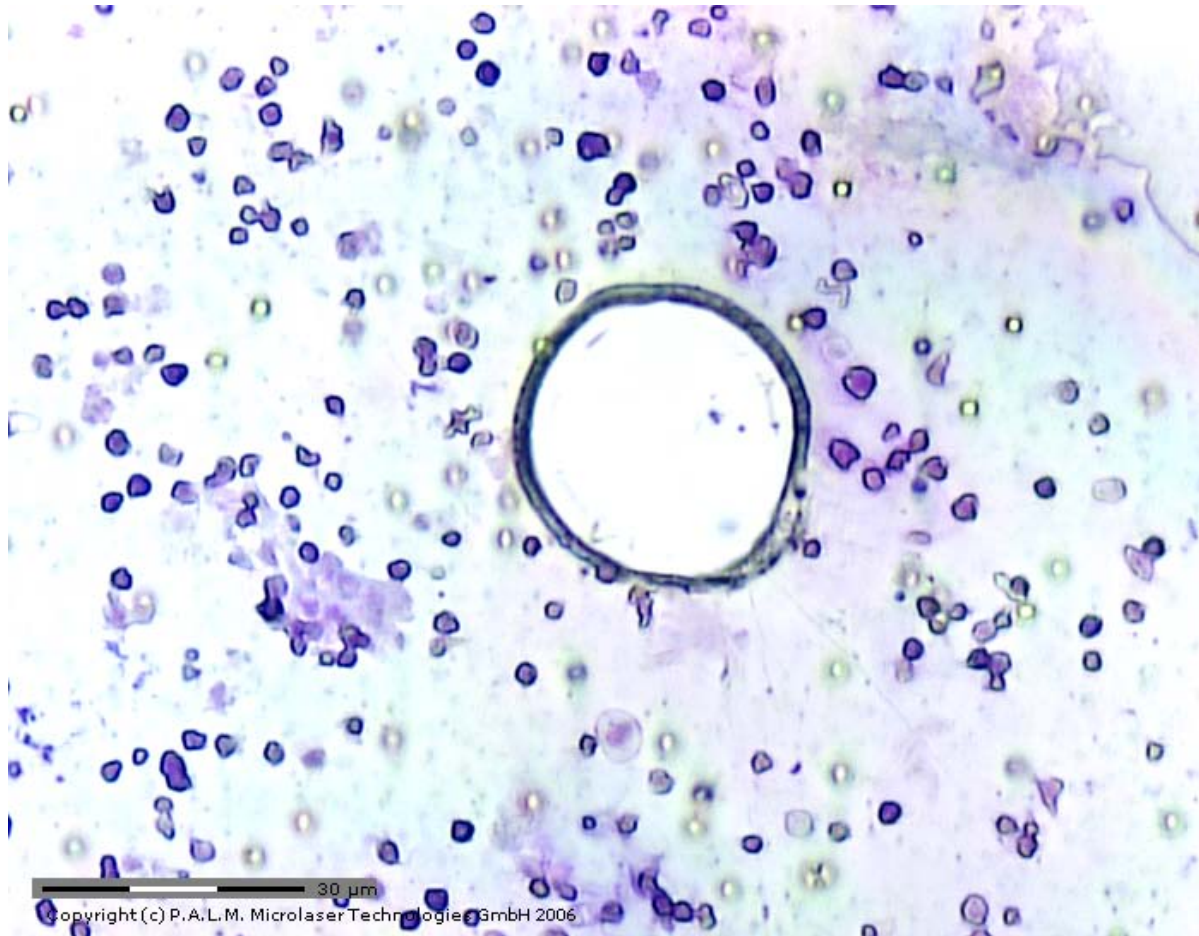
63x objective



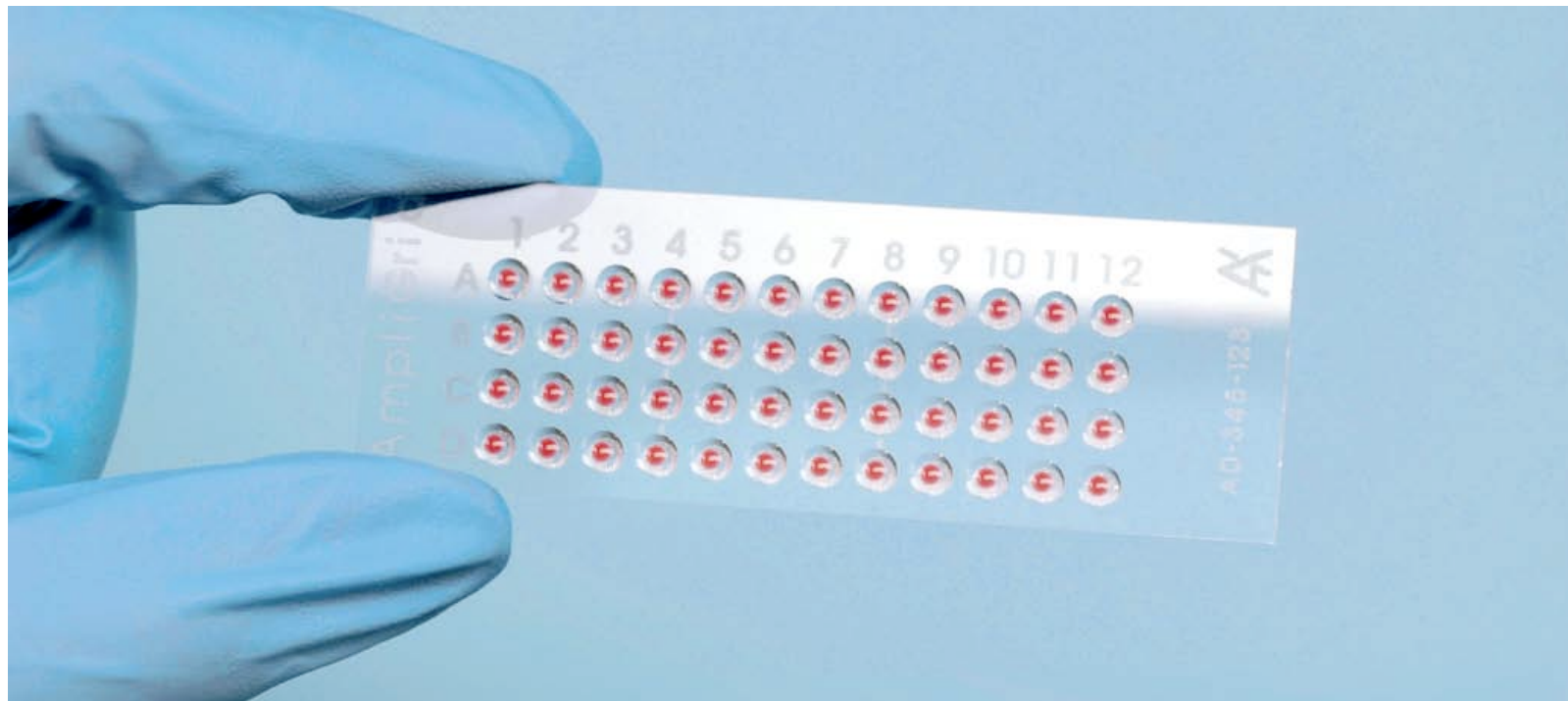
Prenatal Medicine
Single Cell Handling: NRBC in maternal blood
(nucleated red blood cells)



63x objective



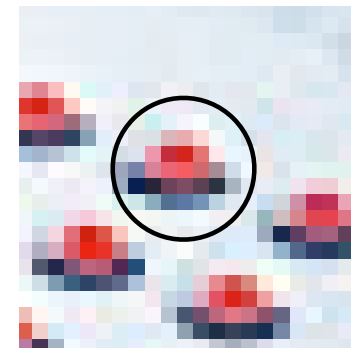
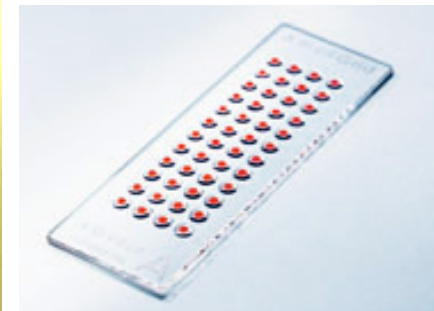
Slide48: Don't pool cells, pool results



NRBC in maternal blood
(nucleated red blood cells)



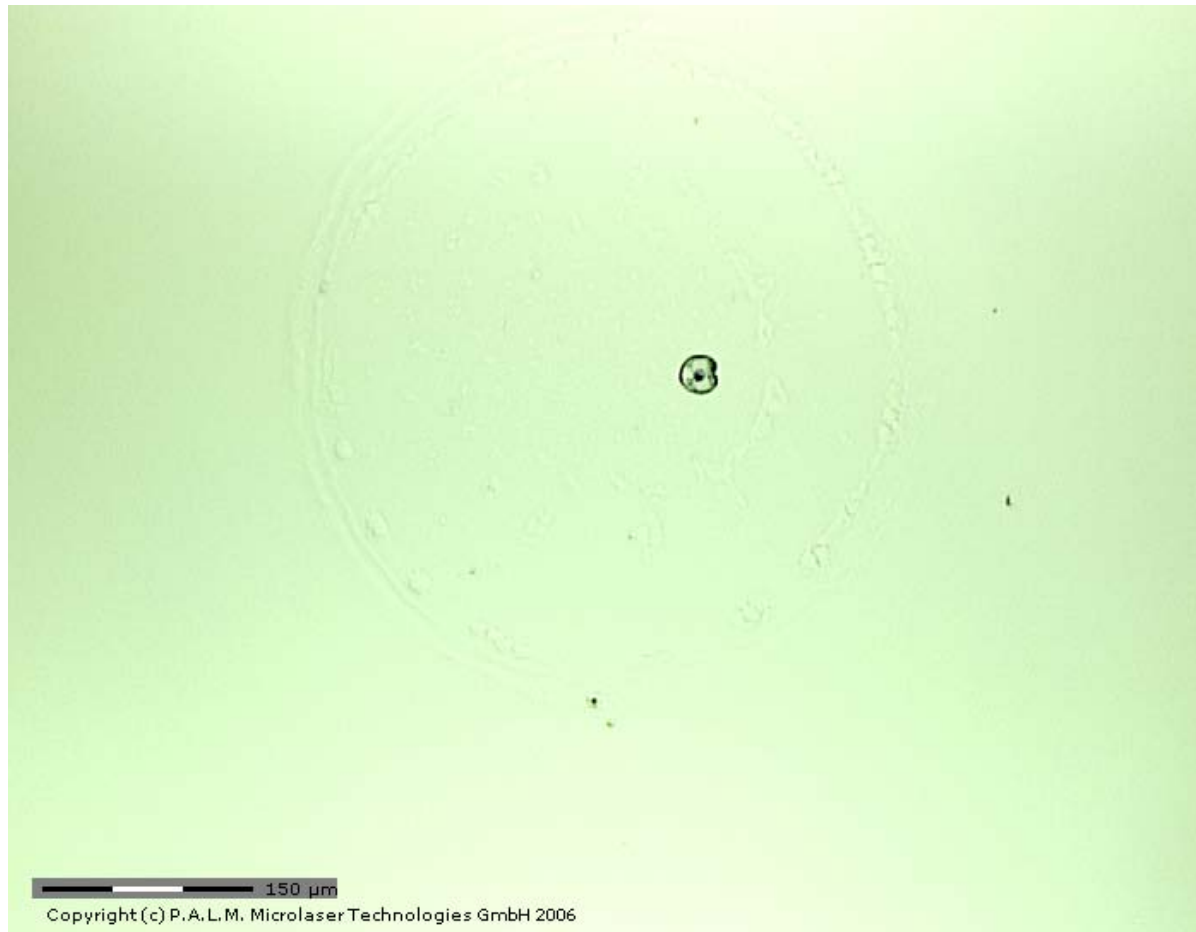
5x objective



NRBC in maternal blood
(nucleated red blood cells)



10x objective



NRBC in maternal blood
(nucleated red blood cells)



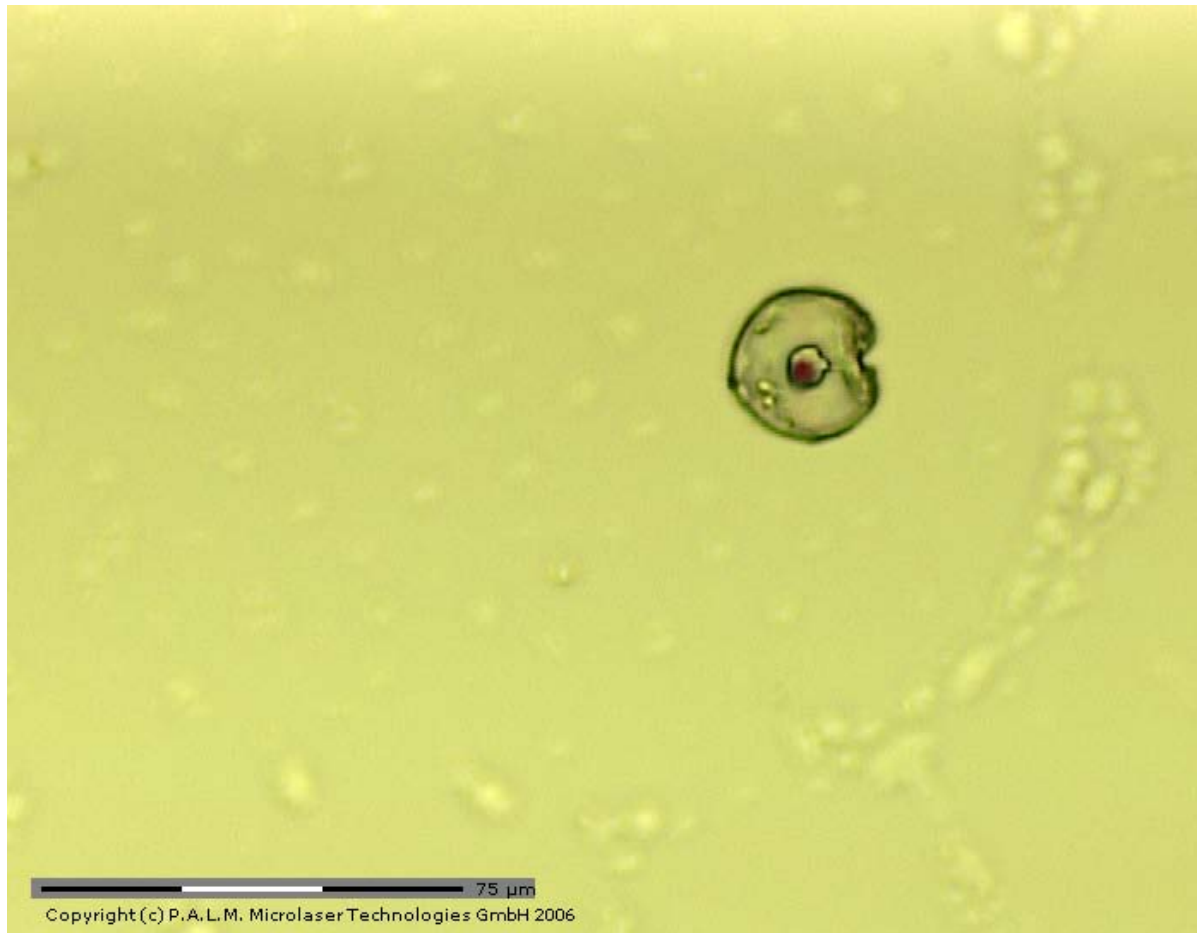
20x objective



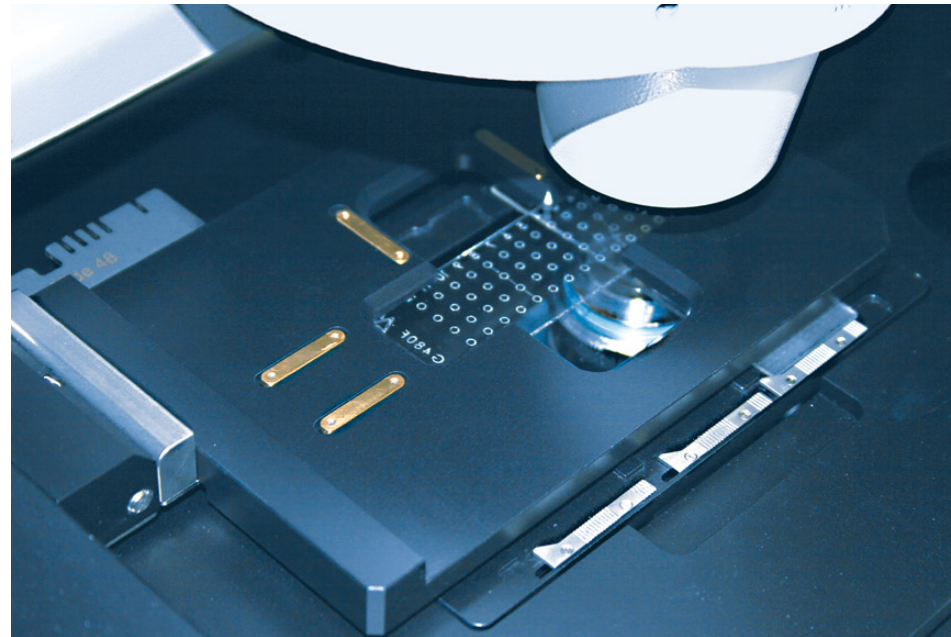
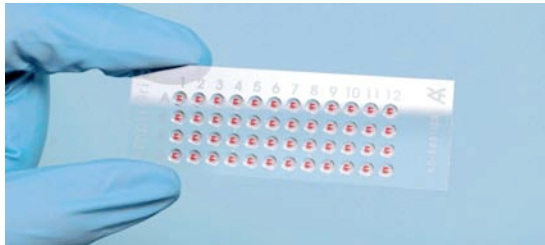
NRBC in maternal blood
(nucleated red blood cells)

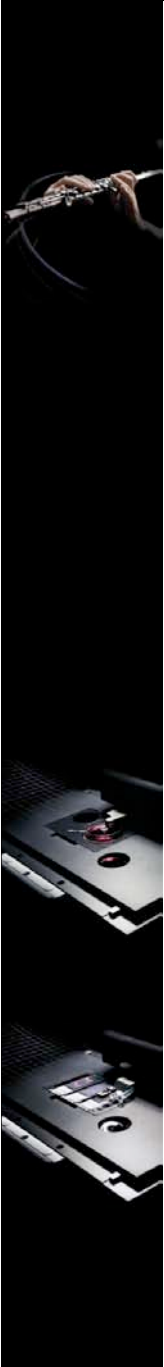


40x objective



AmpliGrid Slide (chip) combined with PALM MicroBeam





Sampling in an automatic way



Image Processing AxioVision

Image Analysis
NRBCs from peripheral blood - Kolialexi et al.



Collaboration

Task:
Find NRBCs

Staining:
May Grünwald, Giemsa

Objective: 40 x

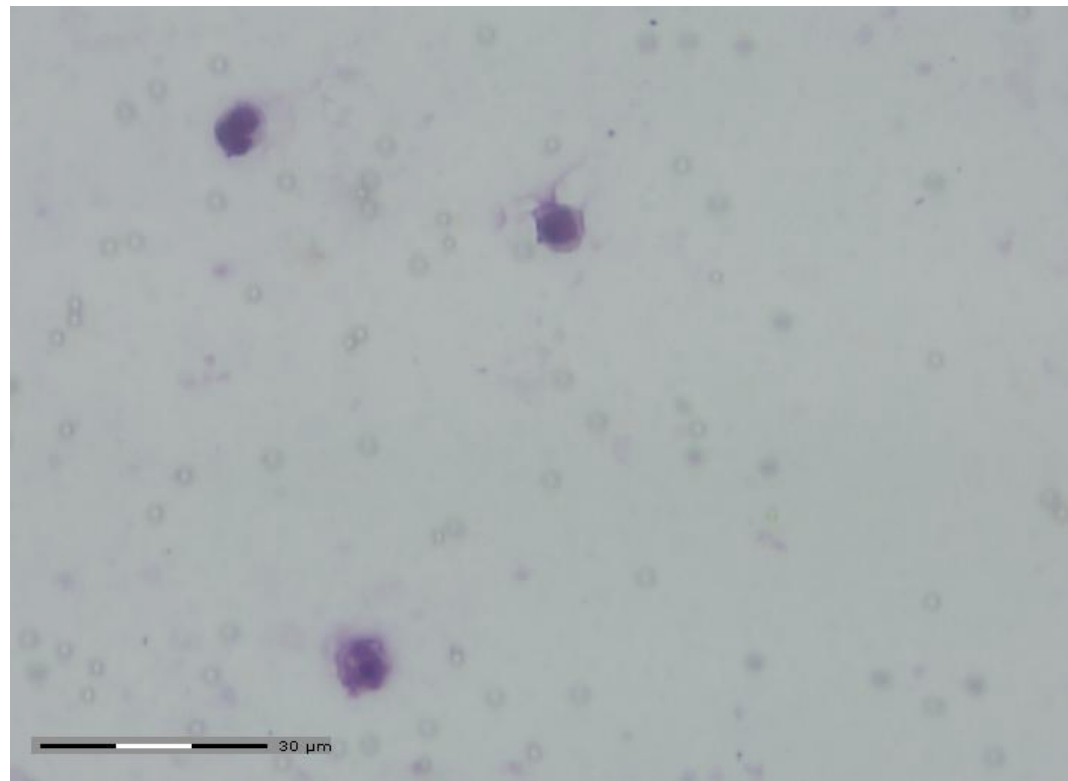


Image Analysis
NRBCs from peripheral blood - Kolialexi et al.



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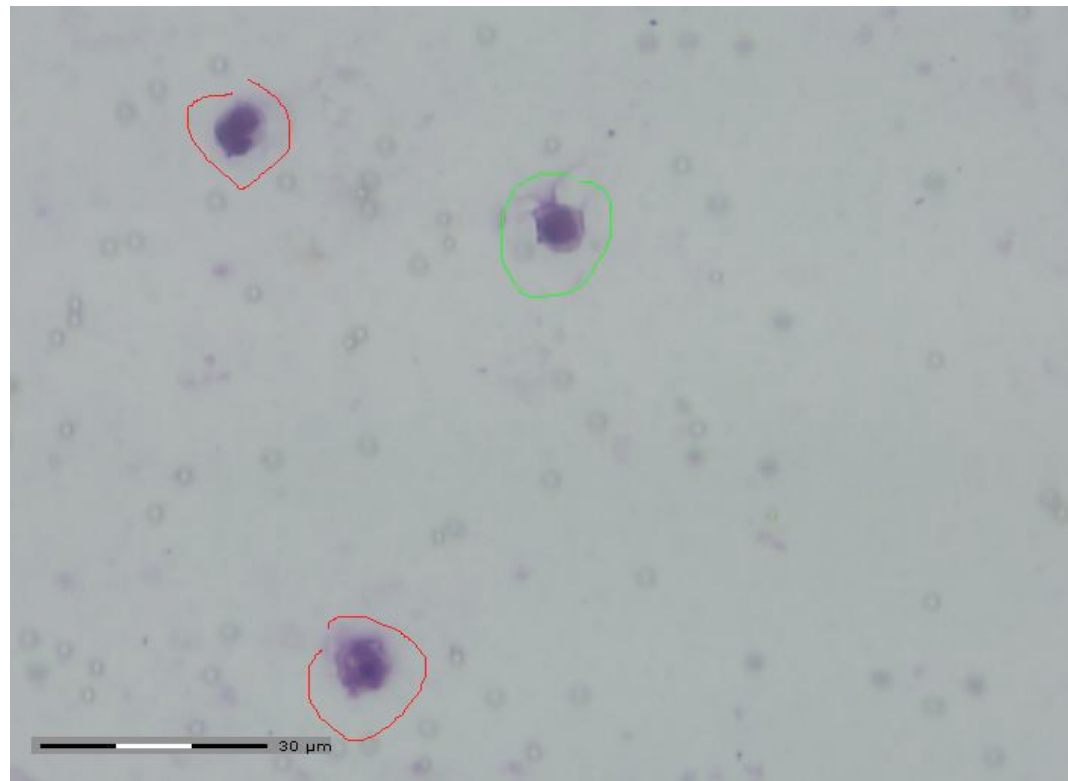
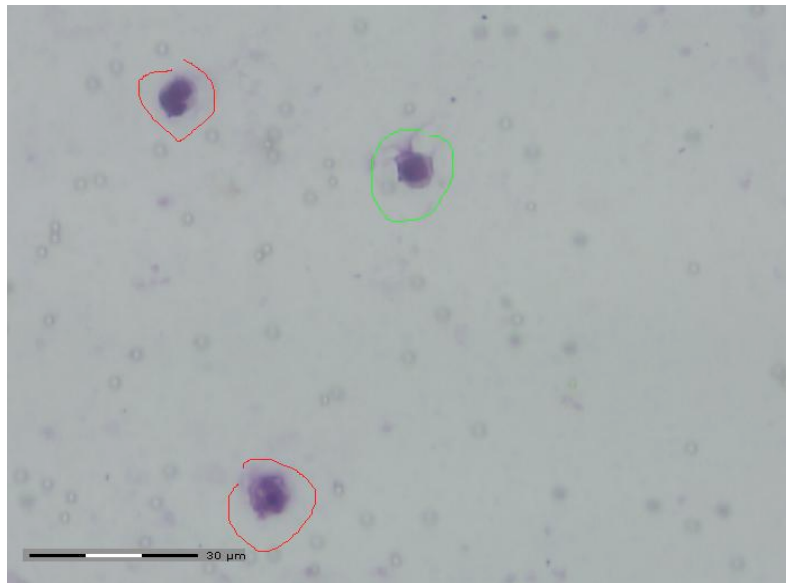


Image Analysis

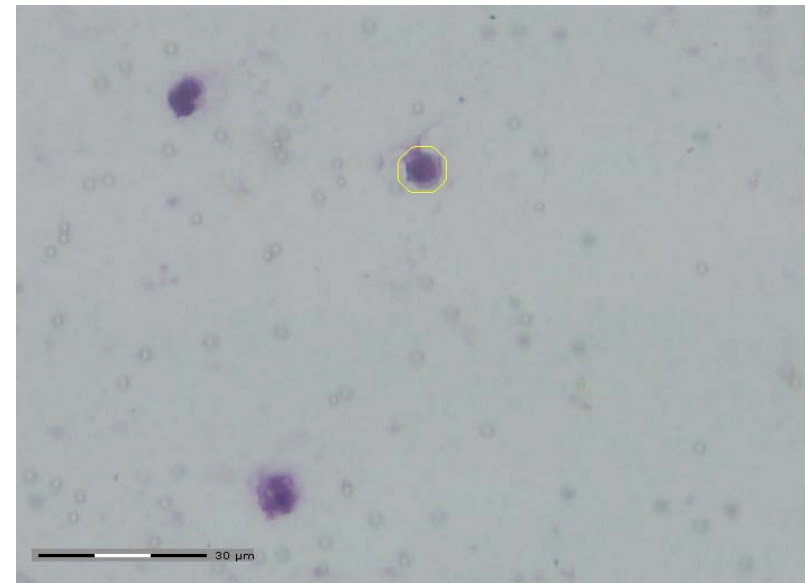
NRBCs from peripheral blood - Kolialexi et al.



Should be found



Result with AxioVision



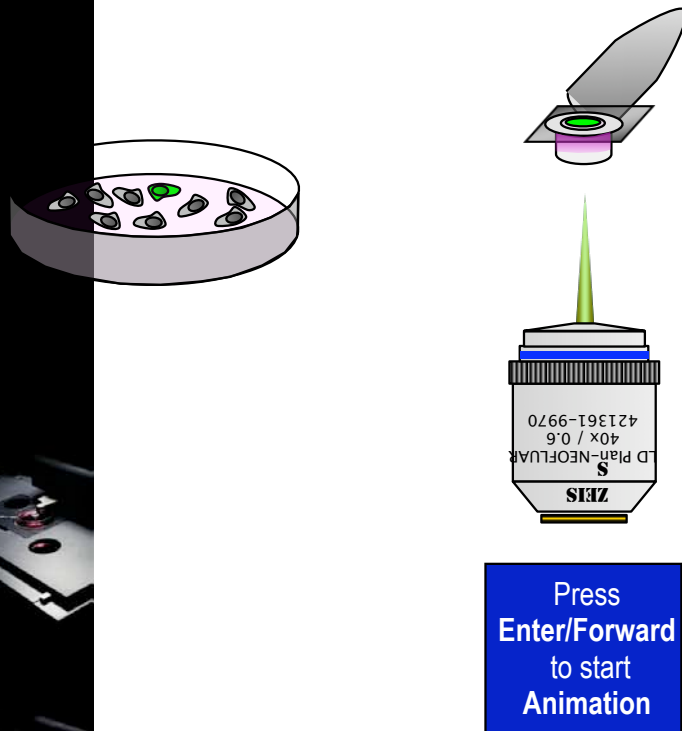
Application Examples

Live Cells

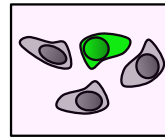


Non-Contact Laser Microdissection

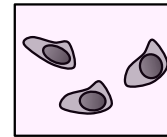
Live Cells - Performance



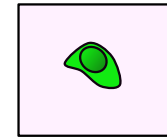
1. Imaging



2. Capture



3. Verification

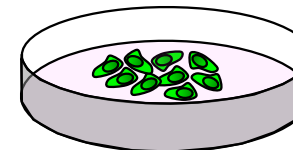


4. Isolation

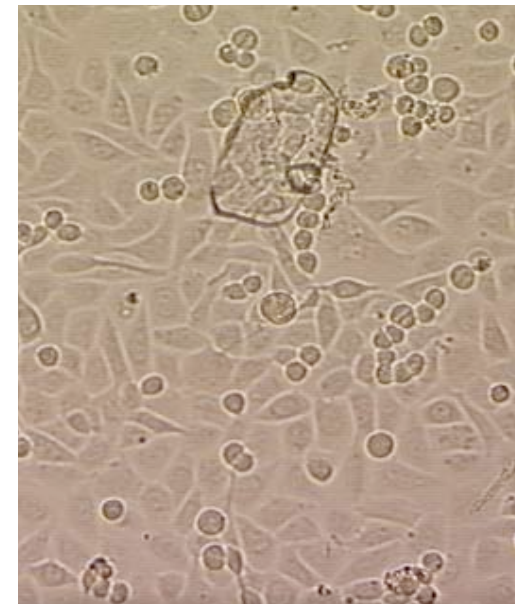


5. Downstream

- Live cell proliferation/
- clone expansion
- DNA analysis
- RNA analysis
- Protein analysis



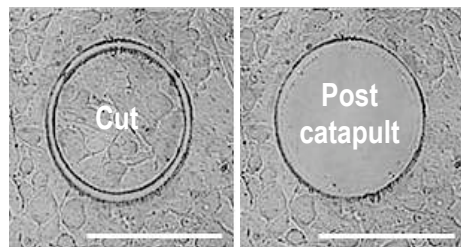
Cell growth after catapulting



Laser Microdissection of Live Cells

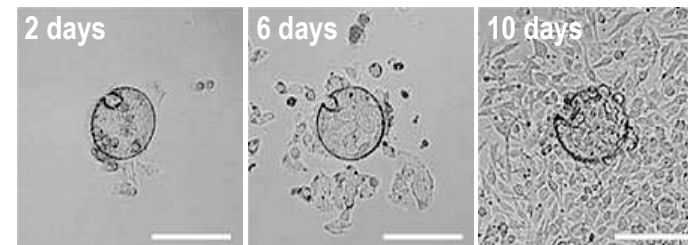
Live Cell PALM Laser Microdissection

- Performance in wet conditions/cell culture medium ✓
- Re-cultivation of isolated cells ✓
- No change in cell growth-rate or phenotype ✓



Laser Microdissection

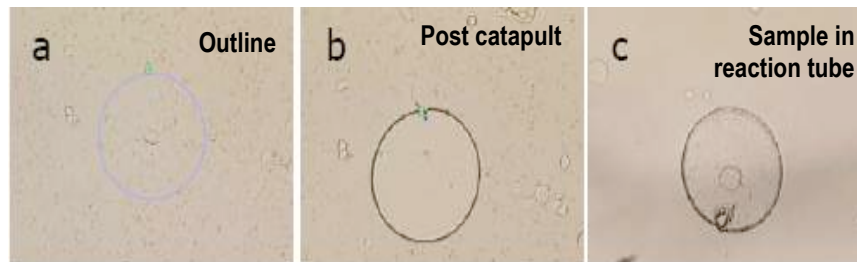
Re-cultivation



Proliferation of isolated cell sample over time

Single Cell Laser Microdissection and RT-PCR

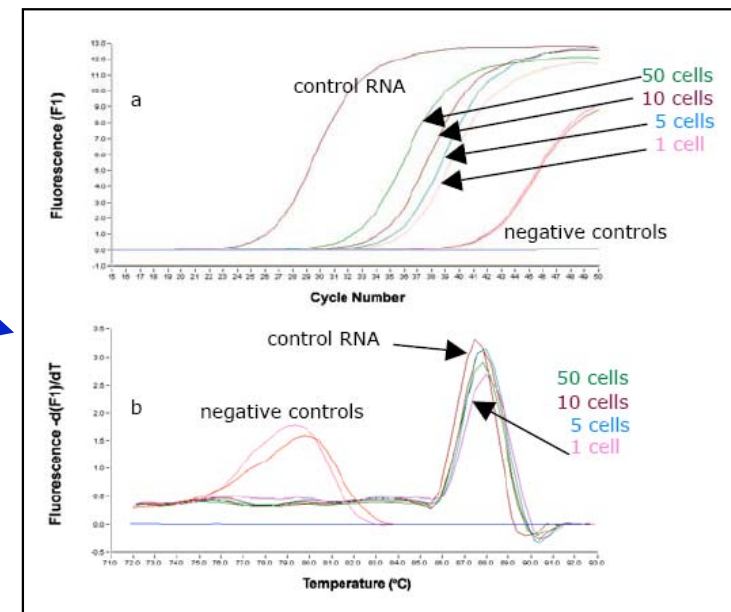
EJ 28 bladder carcinoma cell



Downstream Protocol:

- RNA Extraction with QuickPick mRNA Kit (Bio-Nobile)
- Gene-specific RT-PCR analyses (Negative control: Membrane without cell)

(Bio-



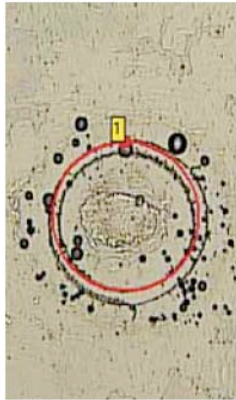
Roche LightCycler CT values (a) and melting curves (b)

PALM Lab Application Note 2004

Creation of Chimeric GFP Mouse Live Cell Isolation for Transgenic Studies



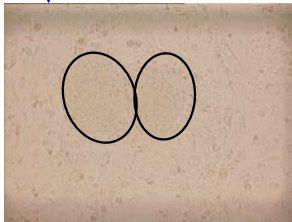
PALM Laser Microdissection



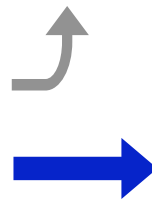
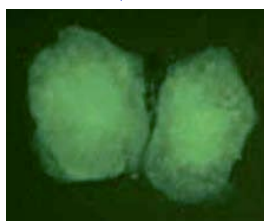
Isolation of single
GFP expressing
murine ES cell



Single GFP-positive
cell in collection
device



Re-cultivation



Transgenic GFP mouse



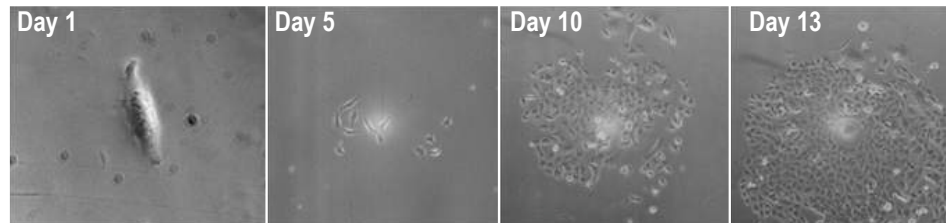
Courtesy of Dr. W.-J. Yu, Level Biotechnology Inc., Taiwan

Stem Cell Cloning & Characterization

P19 embryonic stem cells



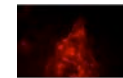
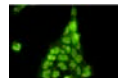
Stem Cell Cloning



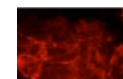
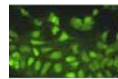
Phenotyping

P19 clone kept
stem cell characteristics
as shown by
pluripotency marker **Oct-4**
before and after
laser microdissection

Before

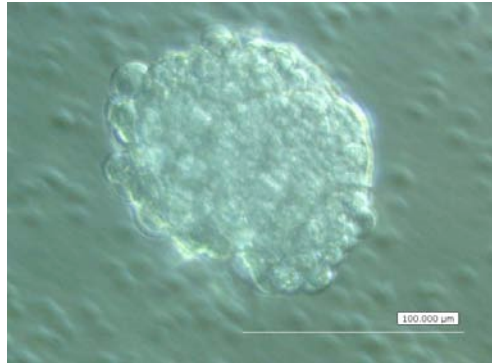


After

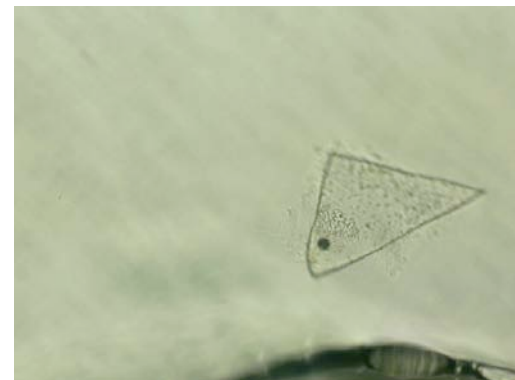
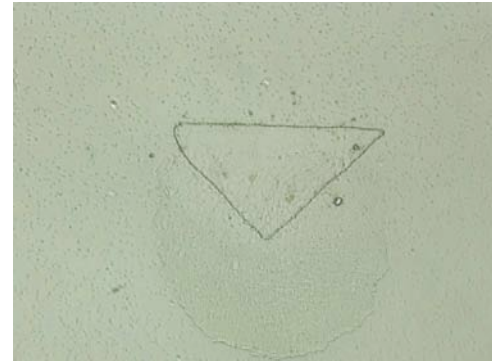


Courtesy of Dr. A. Buchstaller, LMU Munich

LCM of P19 stem cell aggregates

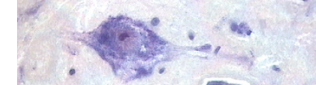
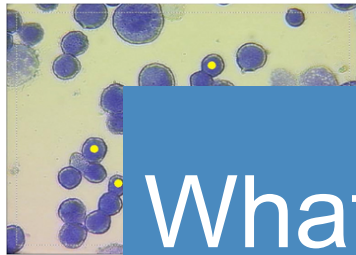


Plas-DIC, 40x

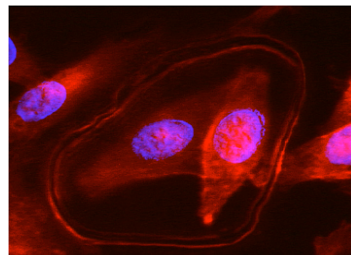




Various LCM Applications



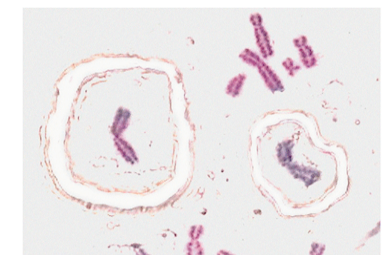
What you see is what you get



Fluorescence



Reduction
Refinement
Replacement



Chromosomes



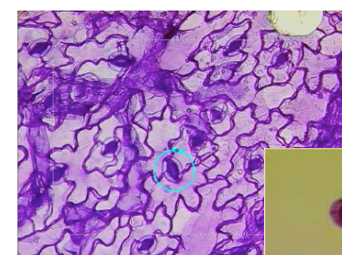
Cells on Filter



Sperm



Hair

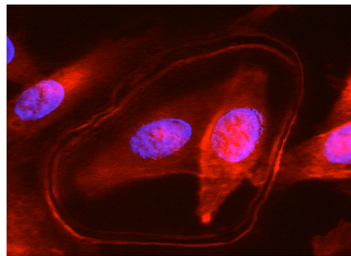


**Stomata cells of
Arabidopsis**



Various LCM Applications

What you see is what you get



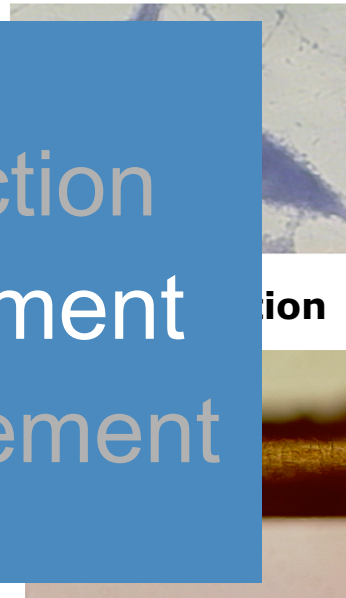
Fluorescence



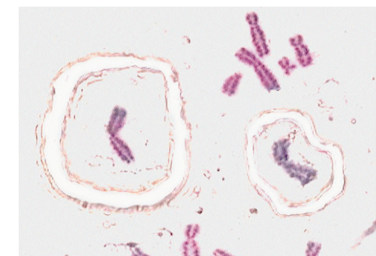
Cells on Filter



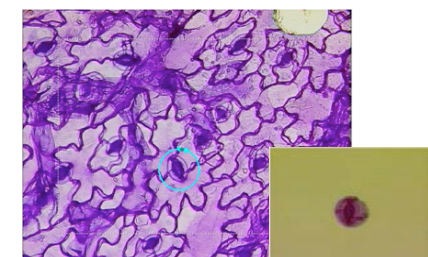
Sperm



Hair



Chromosomes



Stomata cells of Arabidopsis

SUPPORT



PALM Laboratories
Munich

PALM Laboratories



“You don’t have to reinvent the wheel again ...”

palm-labs@zeiss.de

PALM Laboratories at Carl Zeiss

in Munich, Kistlerhofstr. 75

palm-labs@zeiss.de



After our move to a central site in Munich, our molecular biological lab is up and running again in brand new rooms. We are there for you, to support you in any aspect of laser microdissection and your daily research.

- Expert consultation service
- RentalLab
- Application training
- Validation service
- R&D
- Protocol development
- Proof-of-principles

■ 7 scientists, 2 assistants

S 1



Thank you for your attention!



www.zeiss.de/microdissection

burgemeister@zeiss.de



We make it visible.

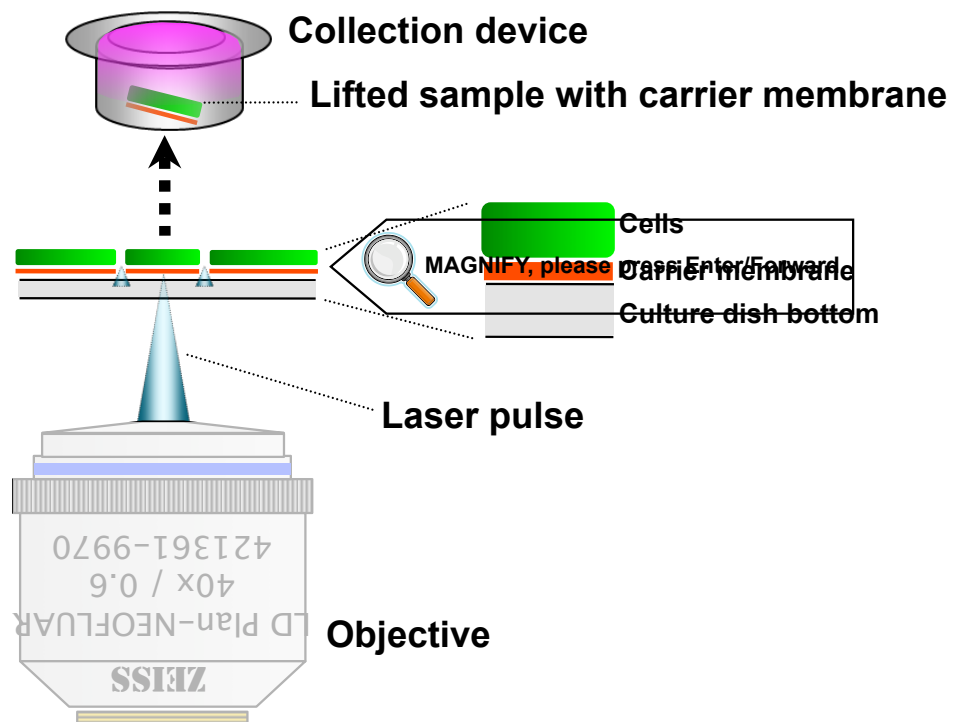
Laser

- FTSS laser (Frequency tripled solid state laser):

- wavelength 355 nm
- pulse energy 100 μ J
- pulse duration 1 nsec
- pulse frequency 100 pulses per second
- beam divergence 3 mrad
- frequency 50/60 Hz
- voltage 115/230 V AC

PALM Laser Microdissection of Live Cells

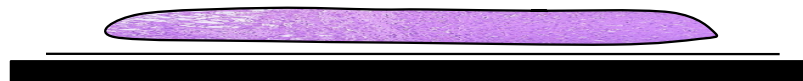
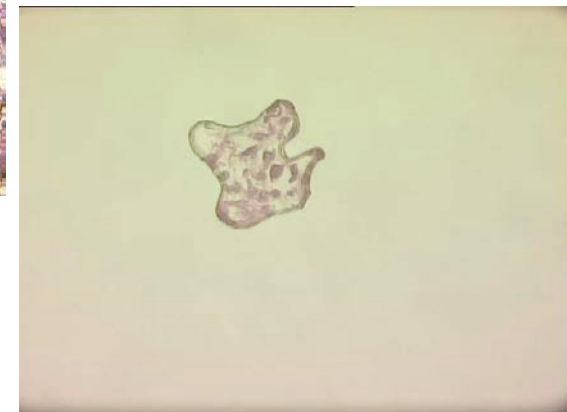
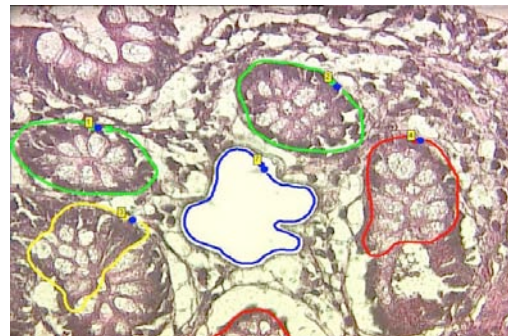
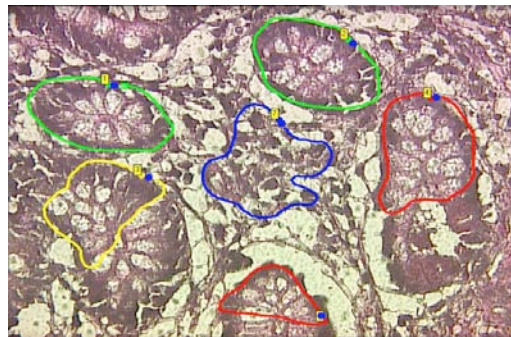
Live cells are shielded and supported by an underlying carrier membrane:



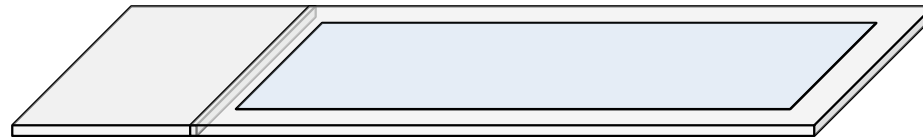
non-contact LCM
membrane mounted tissue



- results in morphologically preserved samples



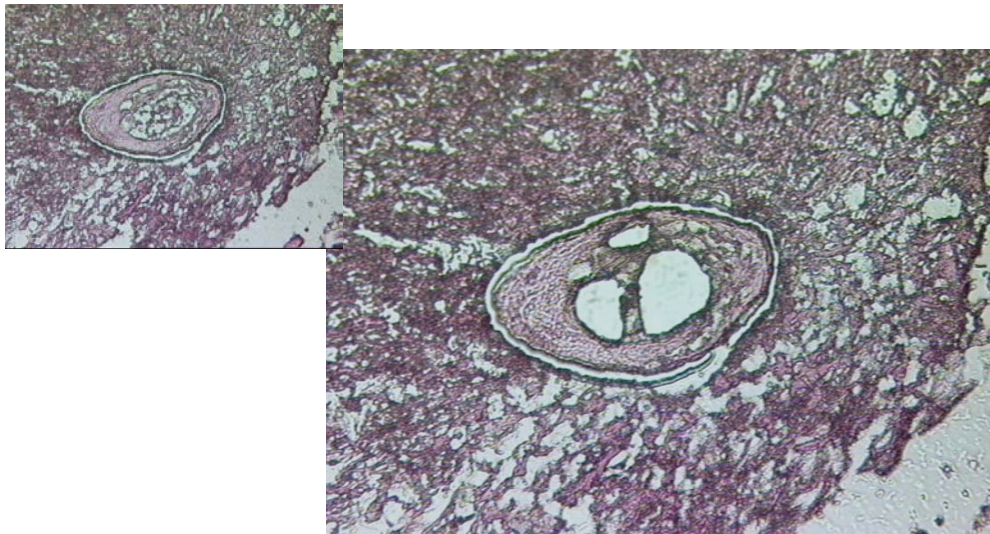
PALM MembraneSlide



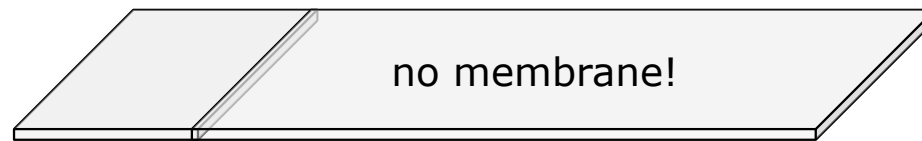
- Serves as a backbone
- Holds selected specimen together
- Facilitates ablation and tissue separation
- Enables dissection of any shape and any size
- Preserves tissue morphology during catapulting
- Allows usual fixation and staining procedures



non-contact LCM
selective elimination of unwanted material



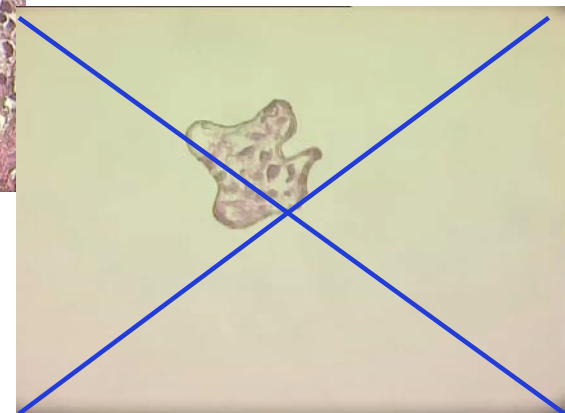
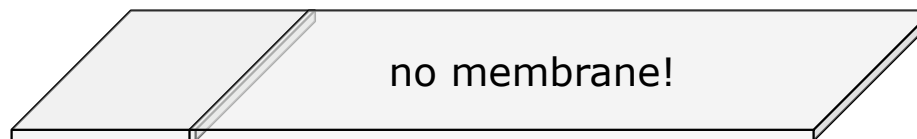
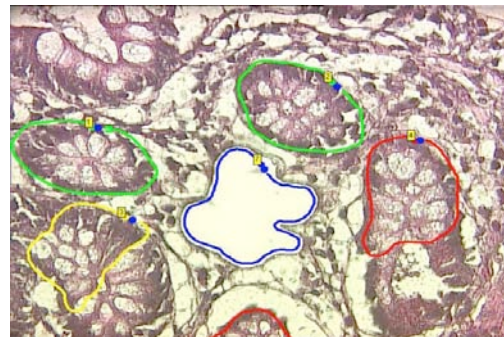
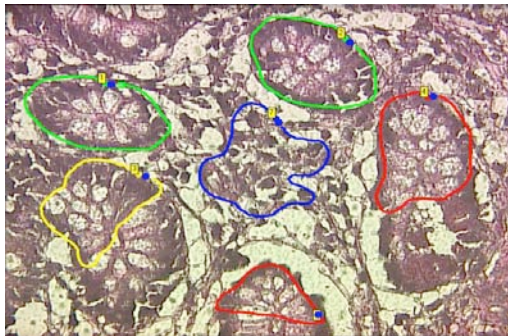
Archived Glass Slides

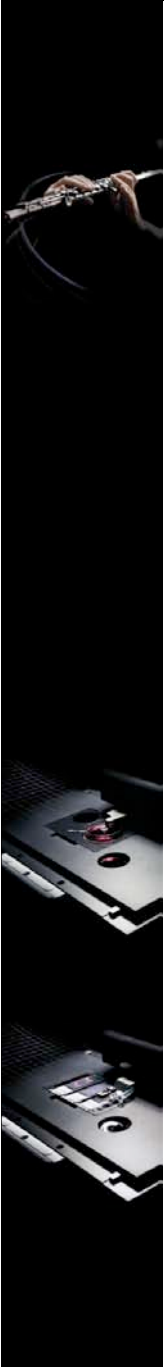


non-contact LCM
membrane mounted tissue



- results in morphologically preserved samples





AutoLPC

Tissue on glass slide

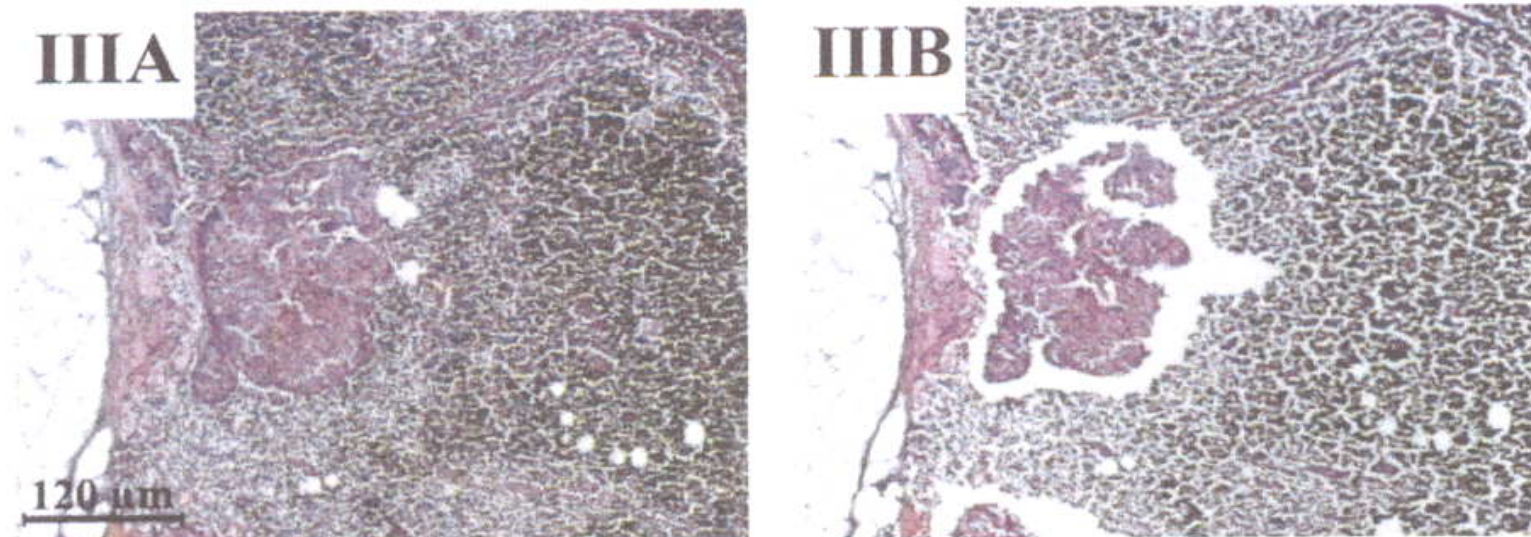


Lymph node metastases

selective elimination of unwanted material



Aubele et al., Int. J. Cancer, 2000



before

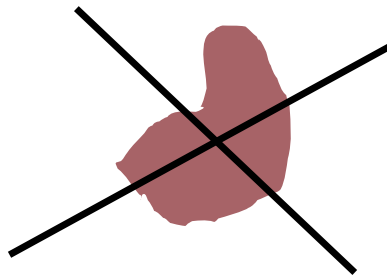
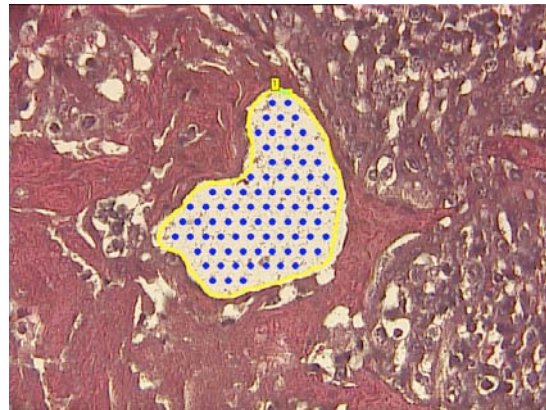
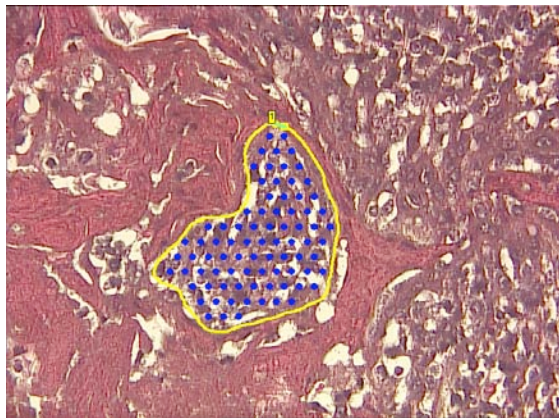
laser ablation of unwanted cells

after

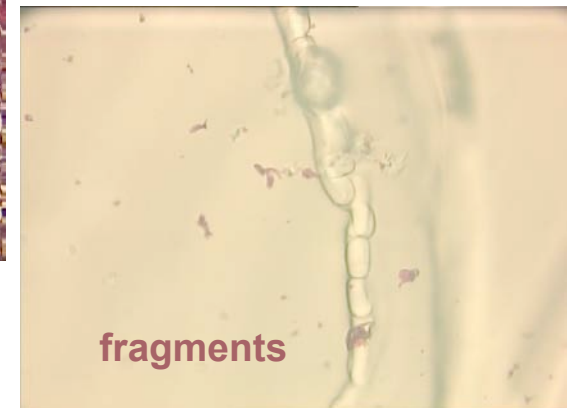
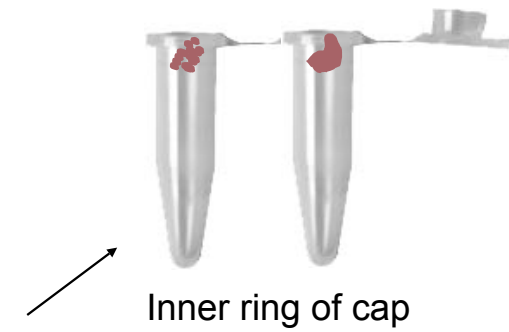
non-contact LCM
Glass mounted tissue



- results in tissue fragments



Renate Burgemeister

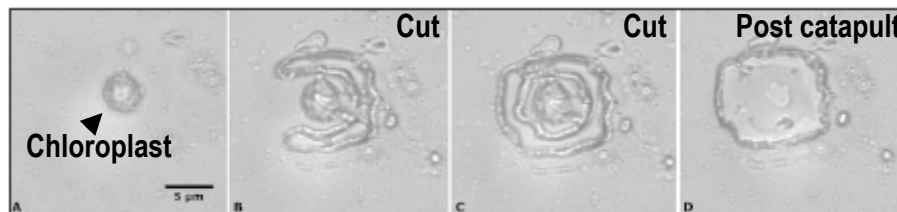


Plant Research

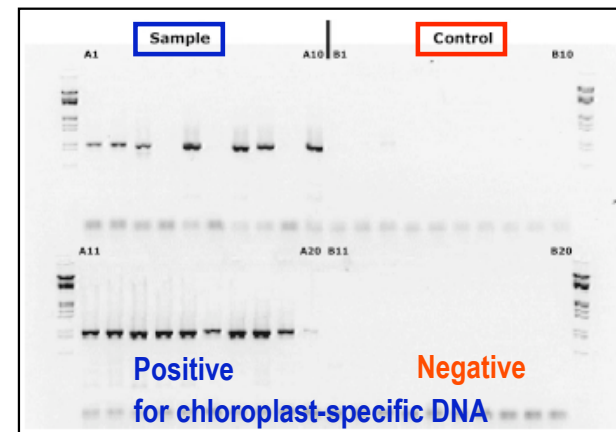
Single-organelle PCR Analysis



Isolation of single chloroplasts by PALM Laser Microdissection



PCR amplification



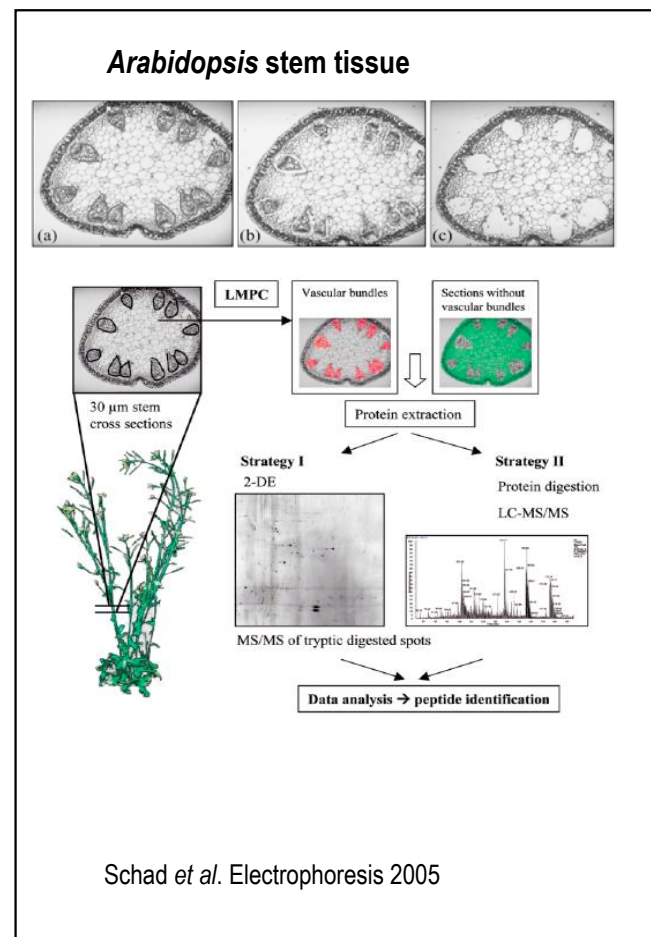
Agarose gel electrophoresis

Meimberg *et al.* BioTechniques 2003

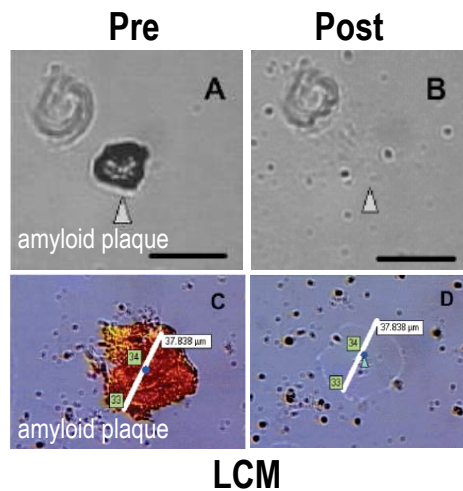
Plant Research Protein Analysis



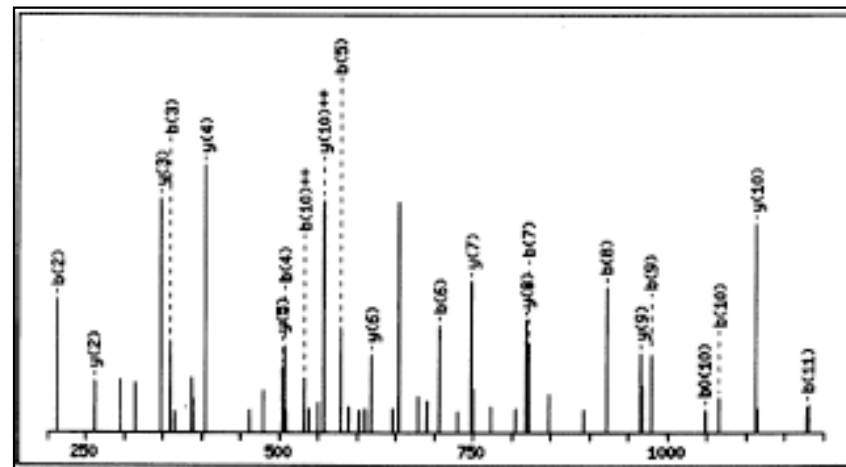
Protocol: Tissue isolation from native sections → Protein extraction → 2D electrophoresis/ mass spec analysis



Neuropathology Protein Aggregates



Identification of plaque protein composition



Mass spec analysis from single aggregate

Söderberg *et al.* Biochemistry 2006

Summary

