

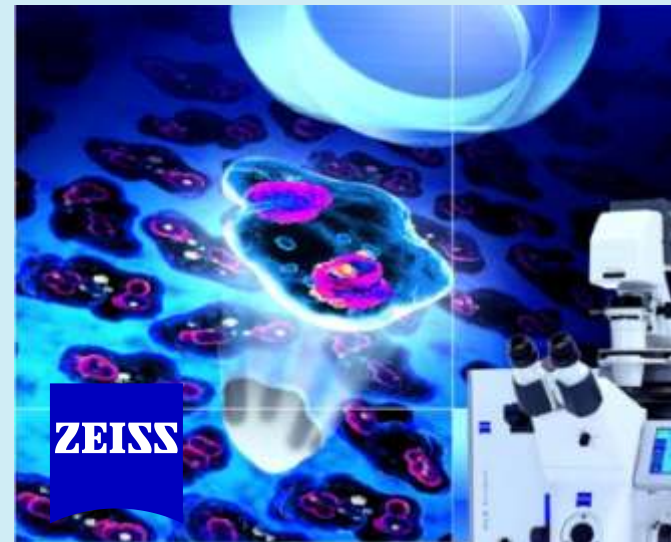


Technology Transition Workshop | *Jack Garner*

Carl Zeiss® PALM®

Laser Microdissection & Pressure Catapulting (LMPC)

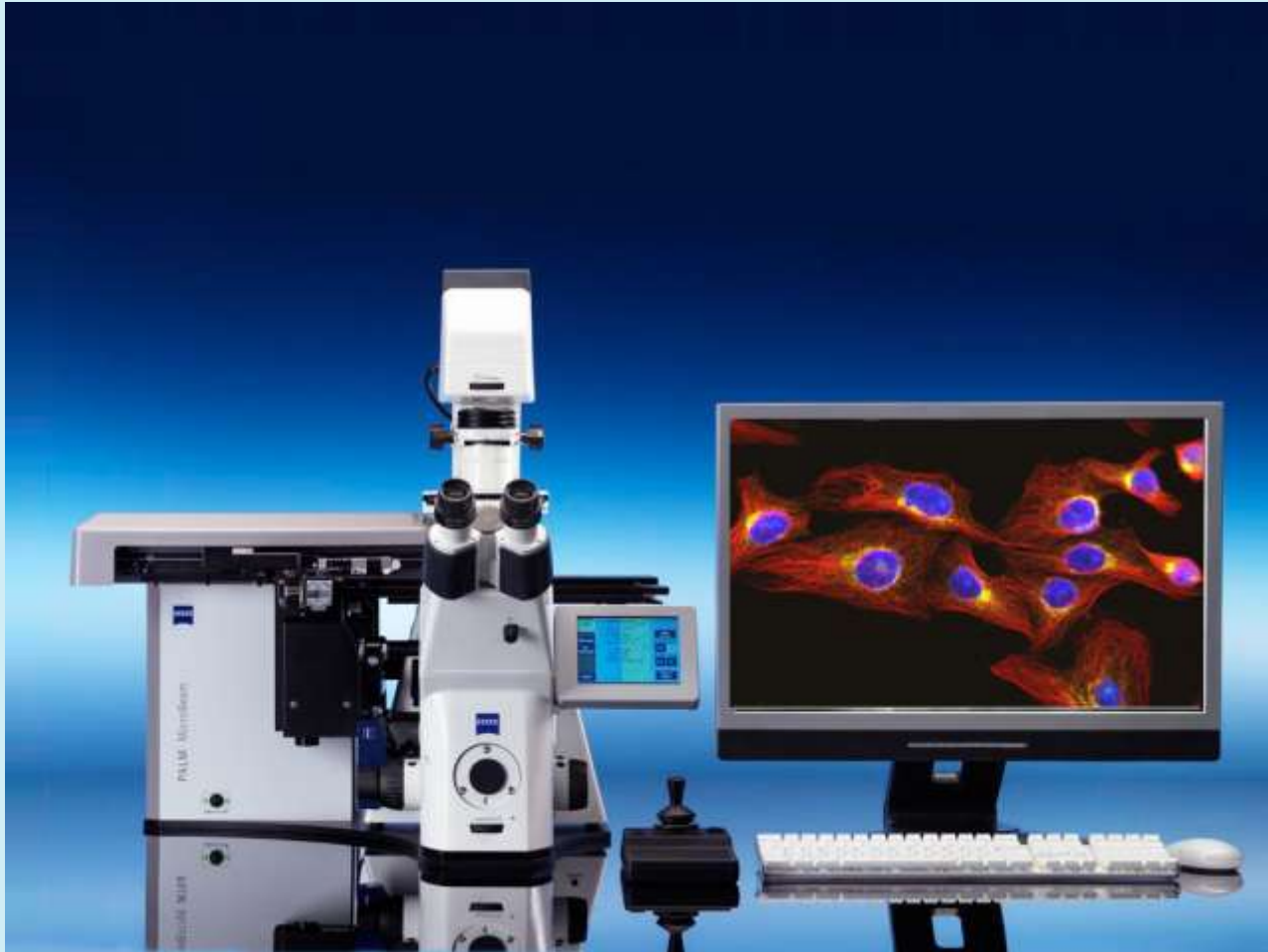
- **New horizons for microgenomics, proteomics and live cell micromanipulation**
 - **Laser capture microscopy**
 - **The technology**
 - **Forensic science application**



We make it visible.

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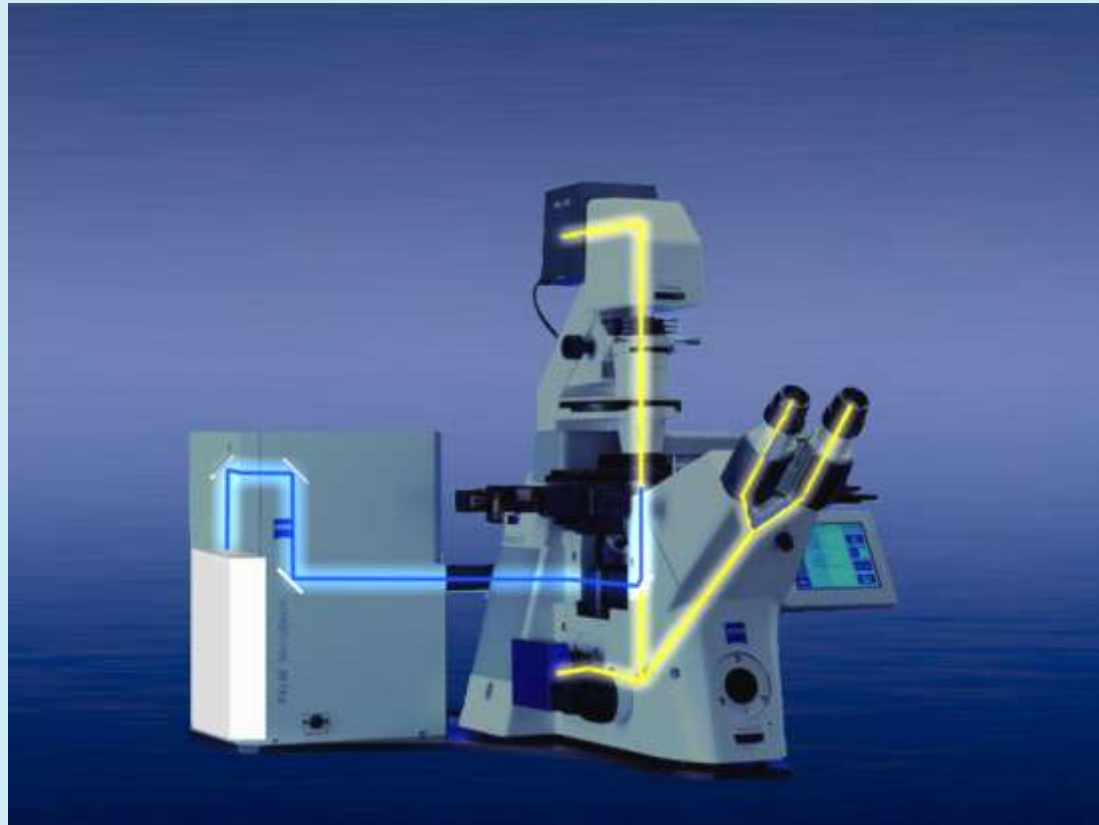
The New Carl Zeiss® PALM® MicroBeam



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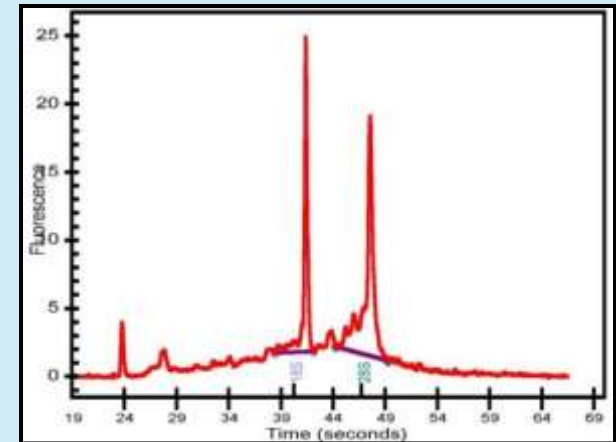
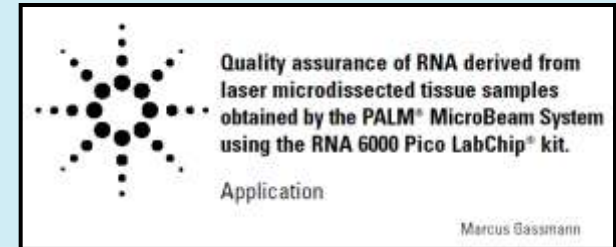
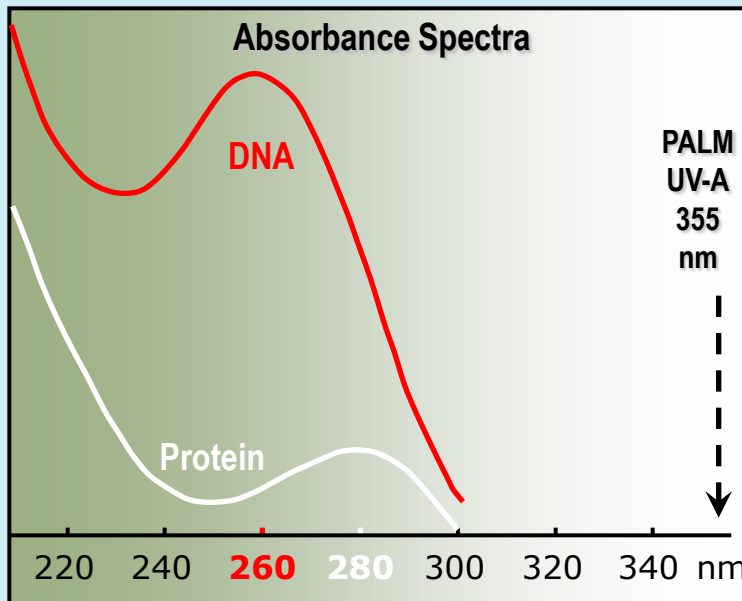
The Next Generation of Laser Microdissection



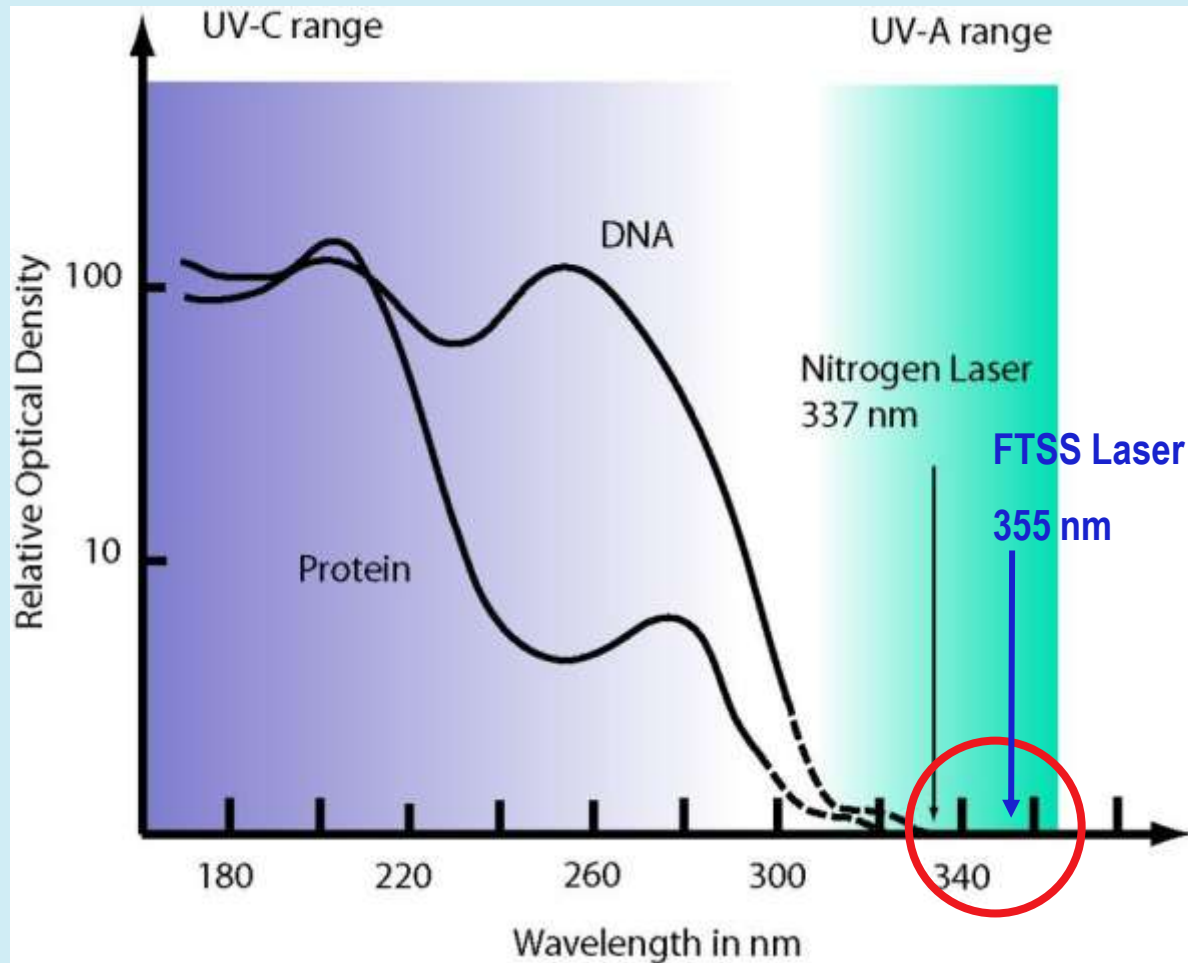
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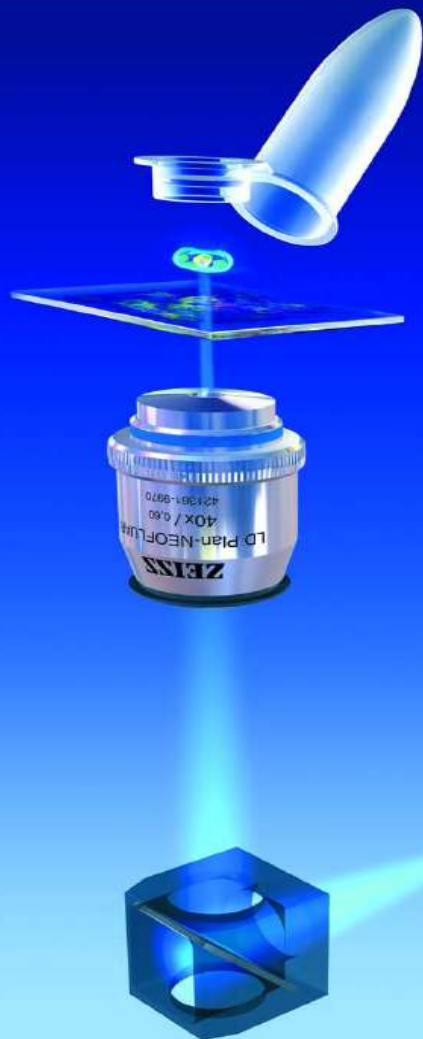
Safety of LMPC for Biological Applications

- Focally restricted adiabatic process
- No UV-A absorbance of DNA, RNA, proteins
- No impact on DNA, RNA, protein recovery



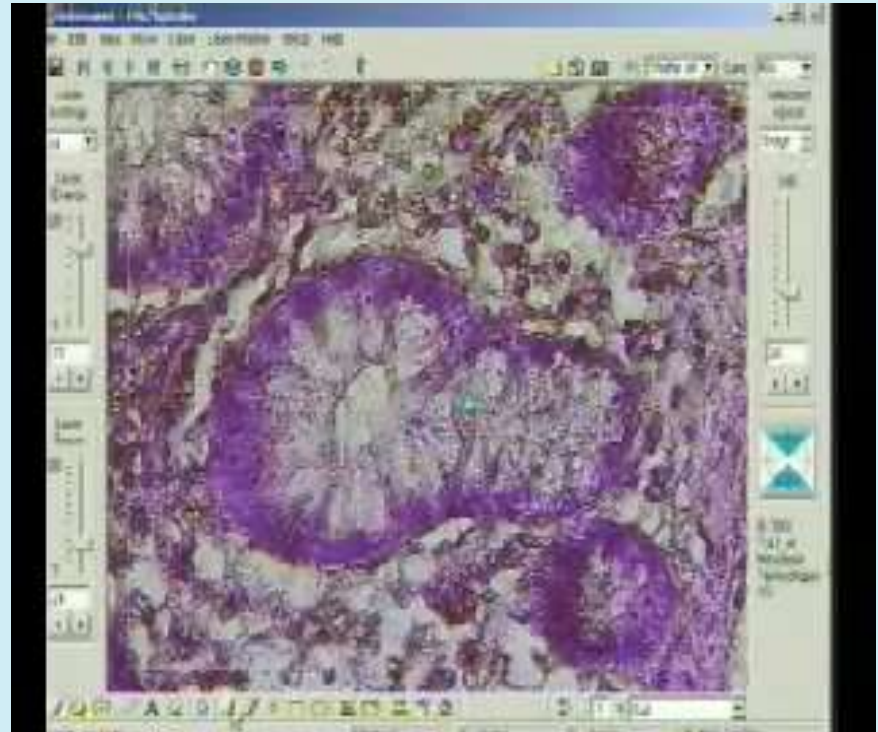
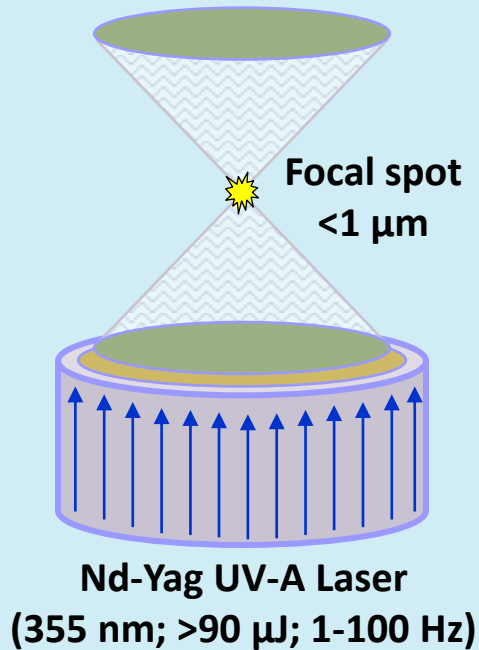
Optical Density of DNA and Protein





- Incise with light, transport with light, work with light
- Non-contact manipulation by the force of focused light!

PALM[®] – Laser Microdissection

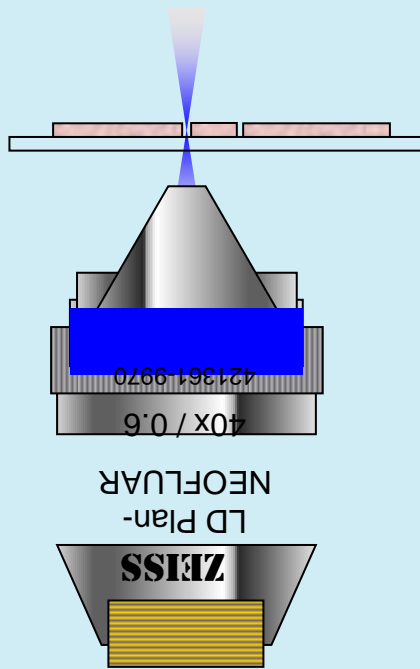


Srinivasan R. "Ablation of Polymers and Biological Tissue by Ultraviolet Lasers."
Science 234 (4776) (October 1986): 559-565.

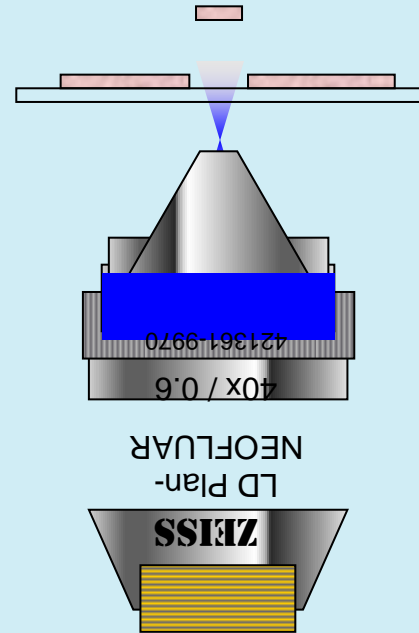
Photodecomposition:

UV laser energy in focal volume causes
breakdown of molecular bonds without lateral heat transfer

How Does LMPC Work?

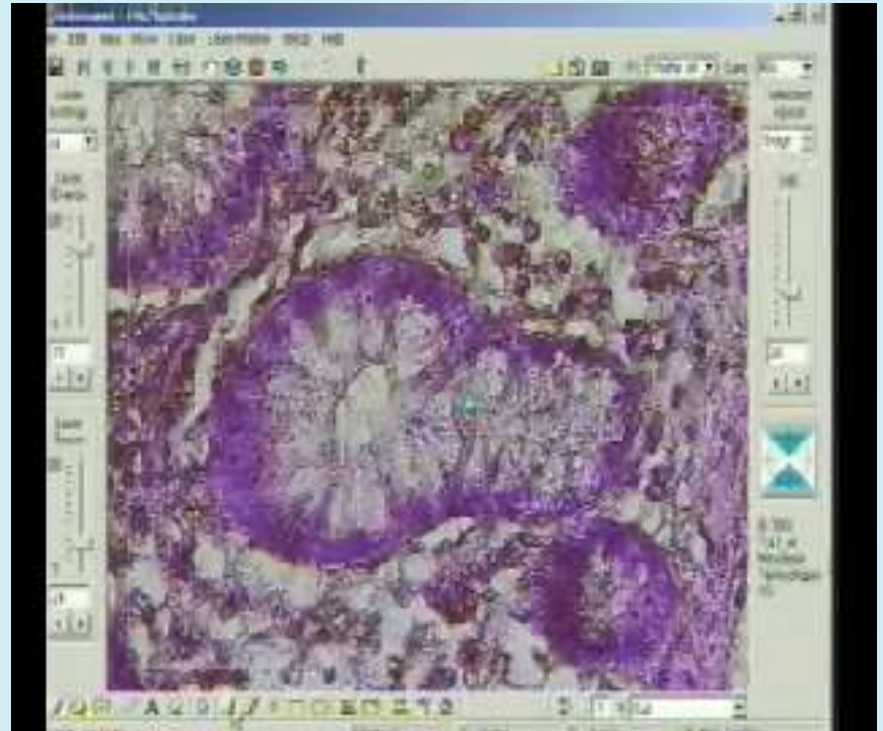


Laser **M**icrodissection



Laser **P**ressure **C**atapulting

PALM[®] – Laser Microdissection



Laser Plasma Formation:

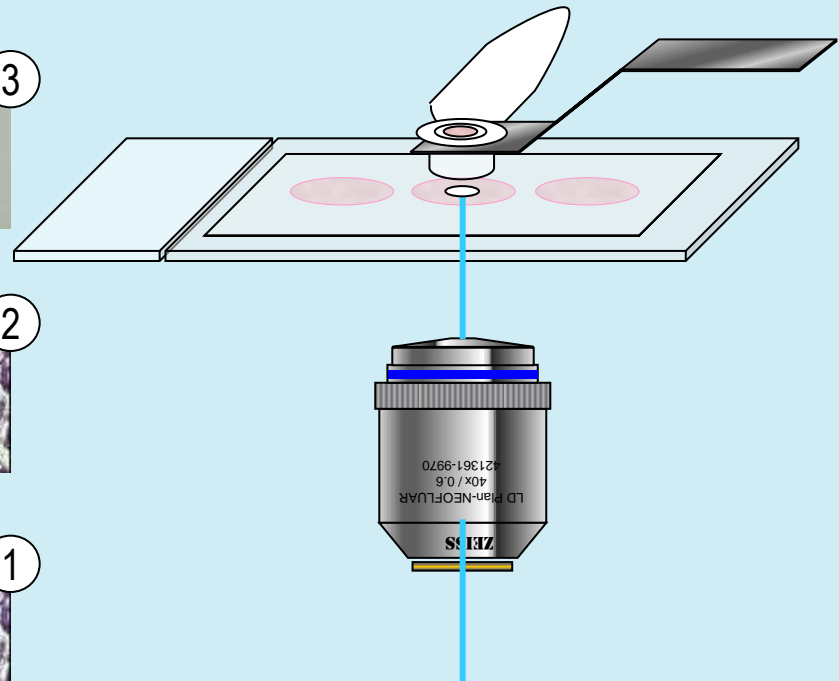
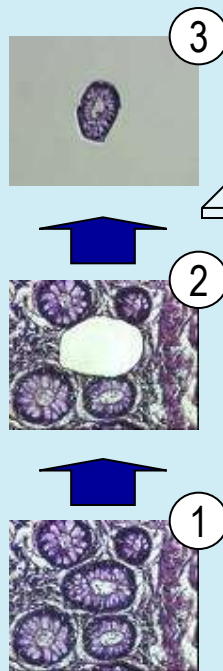
An adiabatic process (no lateral energy transfer during formation & collapse of laser plasma)

To see a video depiction of this principle in action, go to: <http://www.zeiss.com/micro>

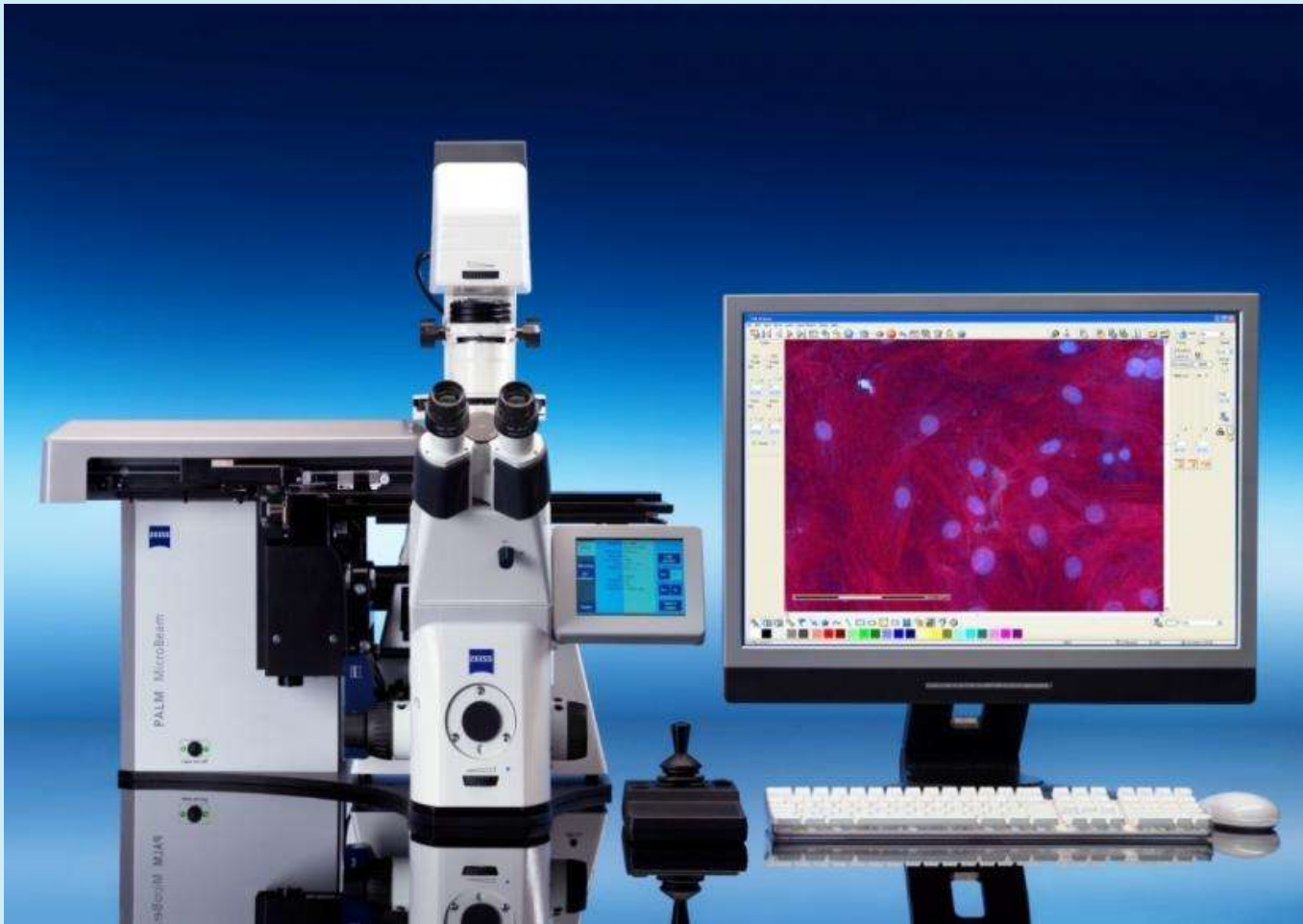
Srinivasan R. "Ablation of Polymers and Biological Tissue by Ultraviolet Lasers."
Science 234 (4776) (October 1986): 559-565.

The PALM[®] MicroBeam LMPC Method

- Laser microdissection pressure catapulting
 - Contamination-free
 - Non-contact
 - Against gravity



The Next Generation: PALM[®] MicroBeam IV

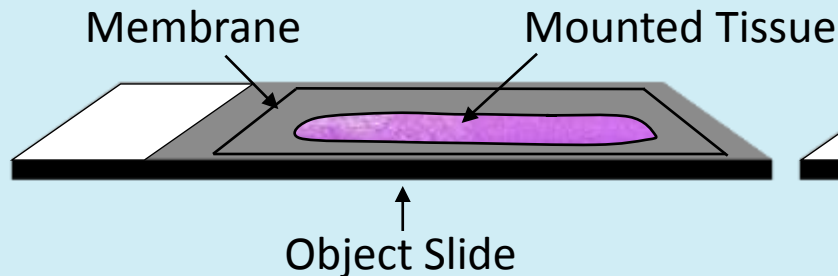


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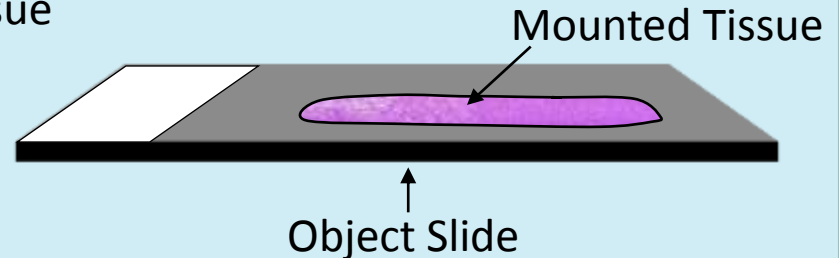


Tissue Microdissection from Membrane and Glass Slides

Laser Microdissection from Membrane-mounted Tissue



Laser Microdissection from Glass-mounted Tissue



PEN (Polyethylene-Naphtalene) Membrane

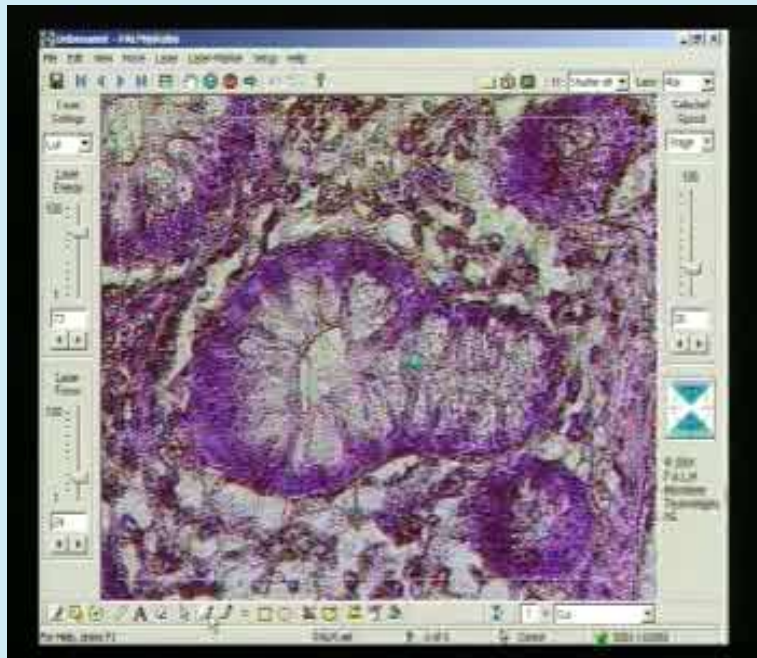
- **Serves as a backbone**
 - Preserves tissue morphology
- **Holds selected specimen together**
 - Facilitates ablation and tissue separation
- **Enables dissection of any shape and size**
 - **Allows fixation and staining**

Glass-mounted Tissue

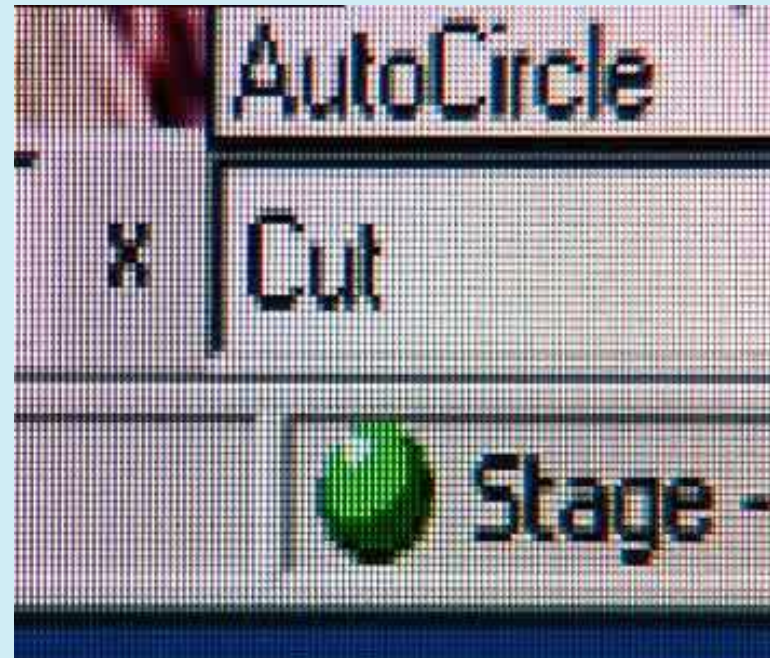
- **Can be used on archival material**
- **Unique PALM® feature**

Tissue Microdissection from Membrane and Glass Slides

Laser Microdissection from Membrane-mounted Tissue



Laser Microdissection from Glass-mounted Tissue



The Zeiss® Advantage

- **Collection accuracy and efficiency**
 - **Stage movement to sub-micron accuracy**
 - **Laser cutting remains perpendicular to focal plane**
 - **Laser pressure catapulting provides increased efficiency under normal laboratory conditions, and better collection efficiency under challenging laboratory conditions**



Laser Microdissection: Understanding the Complete Forensic Workflow

Specimen Preparation and Selection



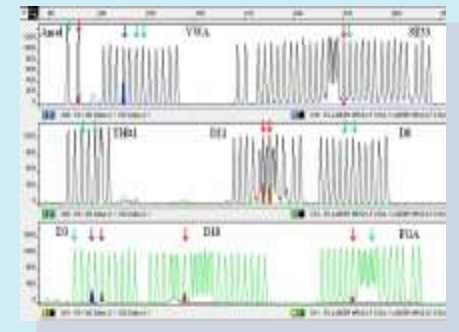
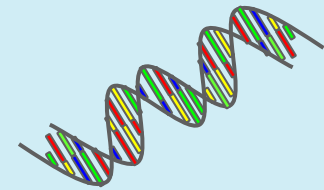
Forensic Specimen

PALM® MicroBeam Enabling Technology



MicroBeam

Functional Downstream Analysis



DNA Fingerprint Analysis

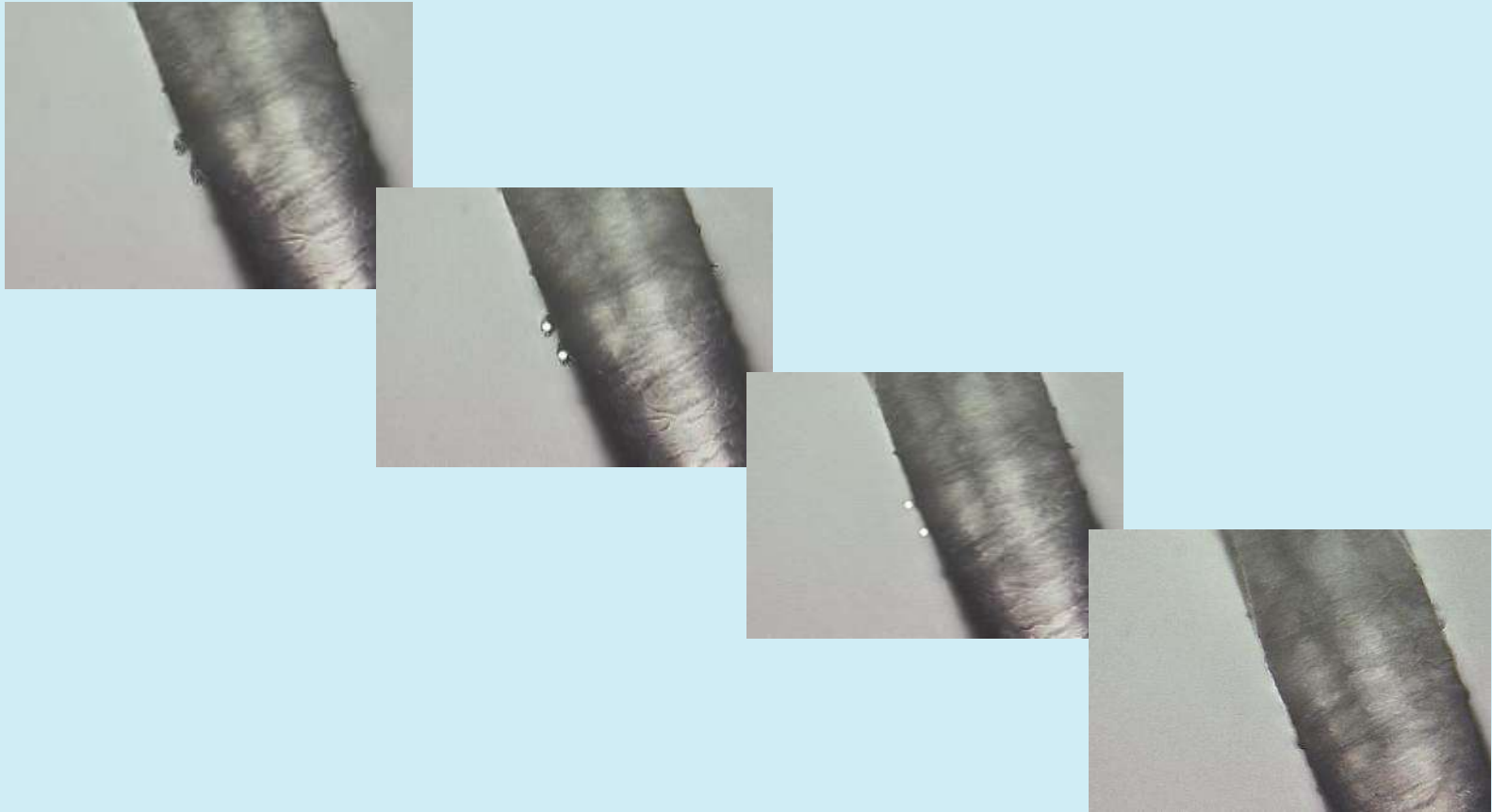
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Contact-free Collection of Forensic Evidence with PALM[®] MicroBeam

- Adhesive tape
- Sperm cells
- Epithelial cells
- Hair
- DNA fingerprinting

Forensic Evidence – Hair



Sperm Identification Methods

- **Manual**
 - Visual identification and selection by laboratory personnel
- **Automatic**
 - Identification with pattern recognition feature of AxioVision® software
 - This is a significant time saver where larger numbers of samples are required

Fluorescence



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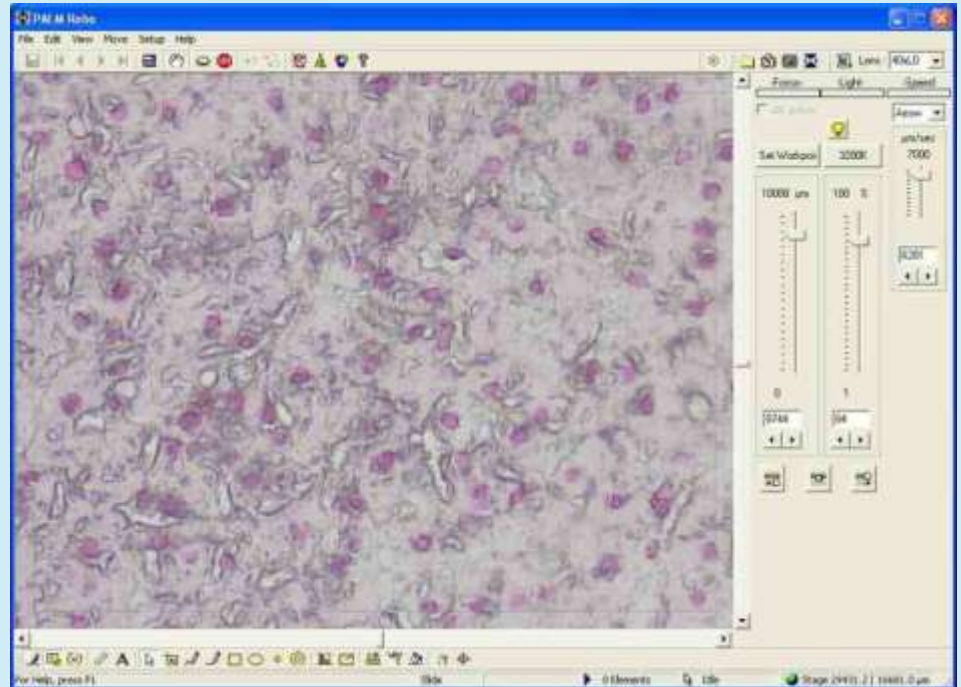
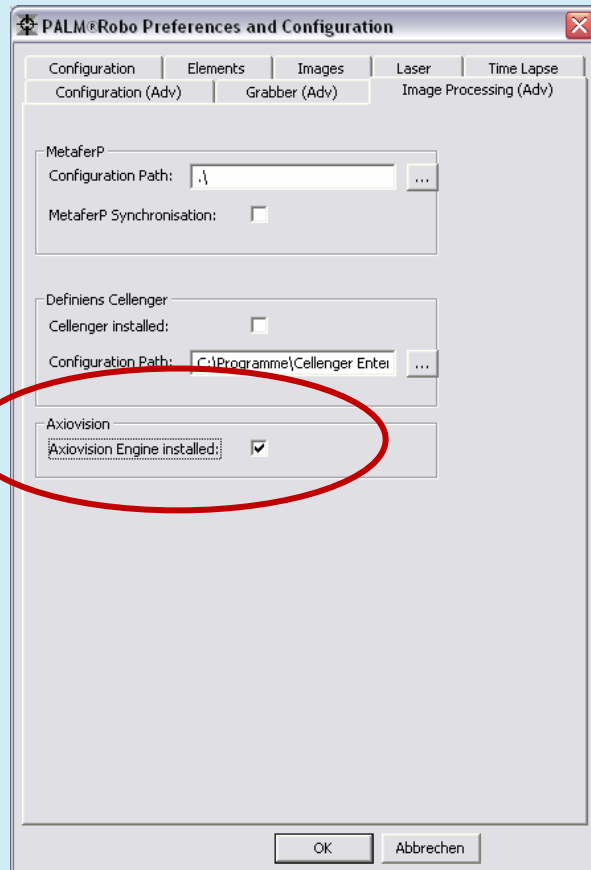
PALM[®] Sample Selection and Identification

AxioVision[®] Example Script

High Throughput: Image Recognition Software Interfaces

Carl Zeiss® AxioVision®

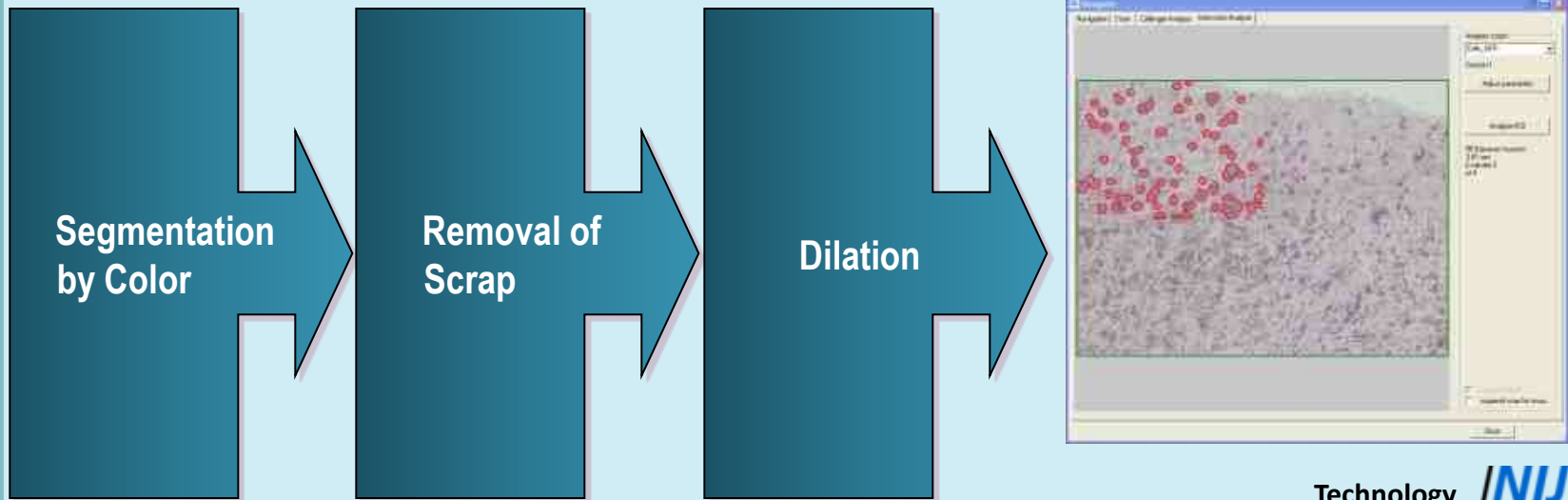
Goal Example: Selection of Nuclei in Liver Tissue



High Throughput: Image Recognition Software Interfaces

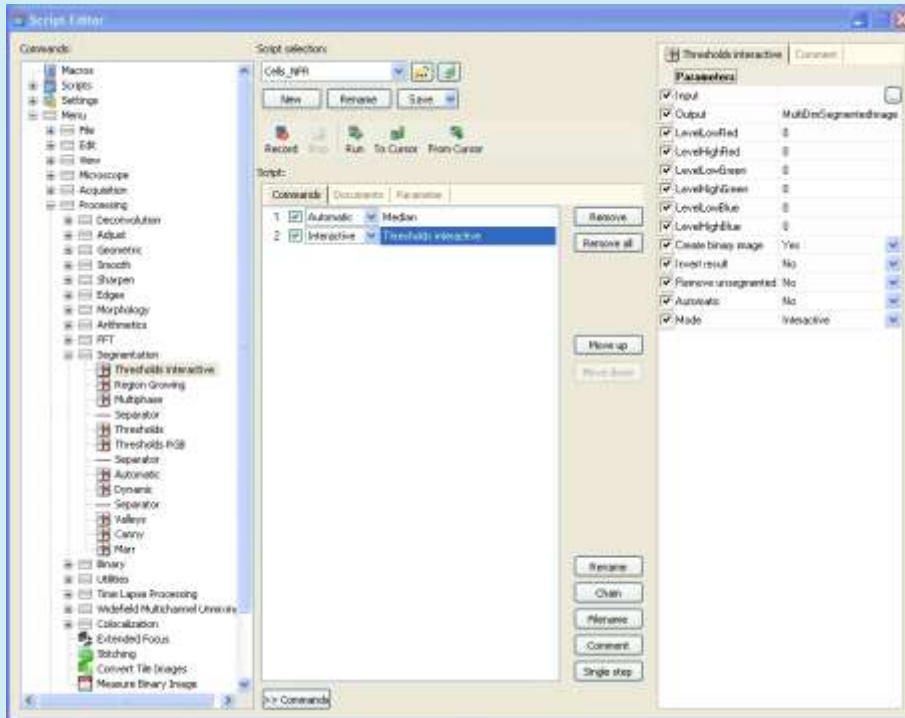
Carl Zeiss® AxioVision®

- Find **sperm**/forensic evidence according to staining/morphology
- Reject particles of wrong morphologies
- Prepare correct objects for LMPC
- Export to RoboSoftware®



High Throughput: Image Recognition Software Interfaces

Carl Zeiss® AxioVision®



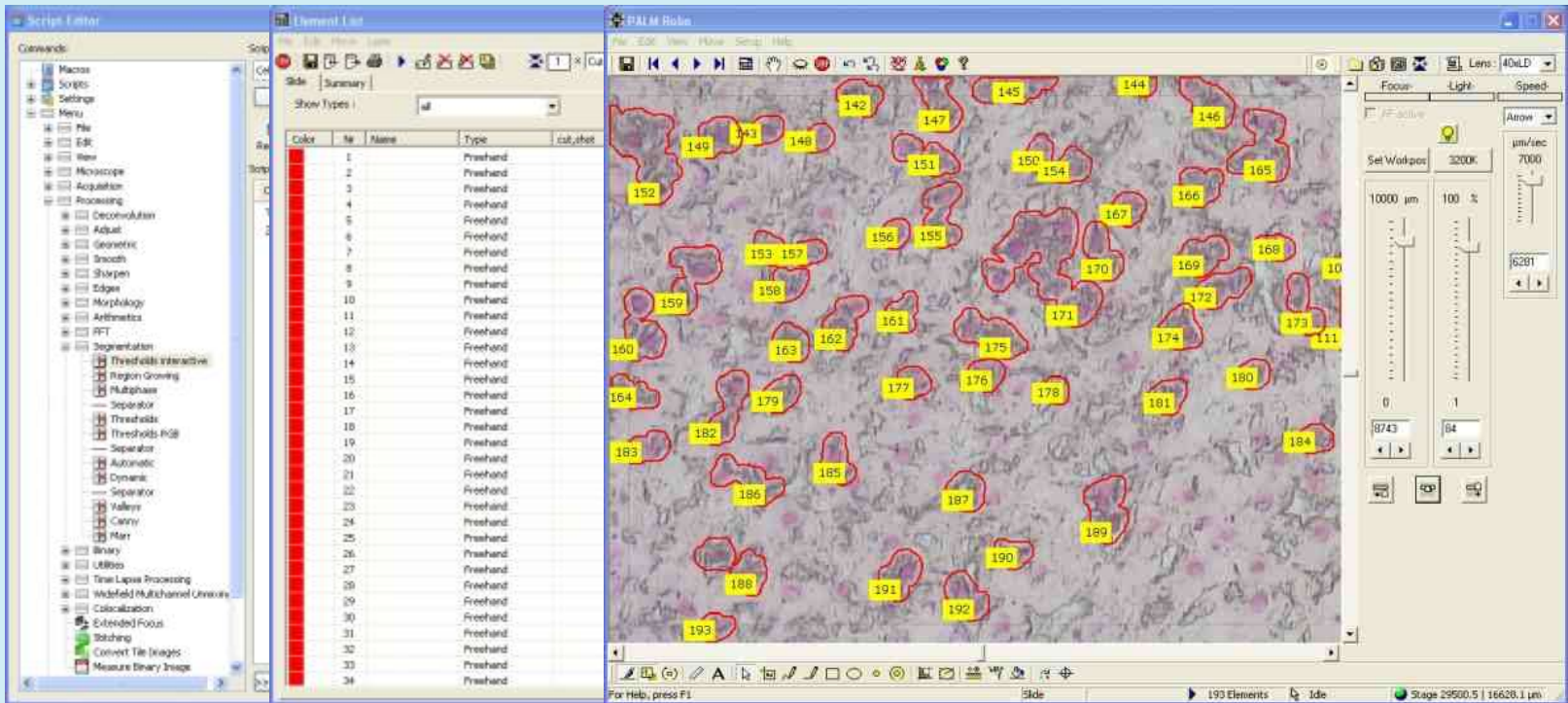
High Throughput: Image Recognition Software Interfaces

Carl Zeiss® AxioVision®

Color	Nr	Name	Type	Cat_shot	Area (µm ²)	Obj	Comment	H x W	Position
	1		Freehand		40			8.6 x 5.8 (µm)	(29461.8, 16967.0)
	2		Freehand		67			9.8 x 8.2 (µm)	(29474.8, 16963.7)
	3		Freehand		60			8.5 x 8.5 (µm)	(29531.3, 16956.1)
	4		Freehand		105			12.4 x 12.4 (µm)	(29545.2, 16946.8)
	5		Freehand		69			9.2 x 8.8 (µm)	(29574.4, 16942.4)
	6		Freehand		51			7.9 x 7.9 (µm)	(29462.7, 16946.5)
	7		Freehand		97			8.9 x 8.2 (µm)	(29399.6, 16947.6)
	8		Freehand		161			15.2 x 14.2 (µm)	(29434.5, 16944.6)
	9		Freehand		67			10.5 x 10.4 (µm)	(29474.5, 16935.1)
	10		Freehand		163			17.8 x 13.1 (µm)	(29215.4, 16934.5)
	11		Freehand		72			10.5 x 8.2 (µm)	(29563.3, 16932.1)
	12		Freehand		136			14.9 x 16.4 (µm)	(29372.5, 16917.2)
	13		Freehand		97			10.5 x 10.9 (µm)	(29539.5, 16913.0)
	14		Freehand		107			12.4 x 10.9 (µm)	(29561.7, 16911.8)
	15		Freehand		68			9.5 x 8.3 (µm)	(29446.1, 16924.5)
	16		Freehand		228			22.9 x 17.1 (µm)	(29421.2, 16919.3)
	17		Freehand		52			7.3 x 8.5 (µm)	(29480.8, 16915.7)
	18		Freehand		103			13.3 x 9.4 (µm)	(29498.5, 16907.5)
	19		Freehand		59			8.9 x 8.5 (µm)	(29449.8, 16906.3)
	20		Freehand		68			9.6 x 8.5 (µm)	(29429.4, 16903.6)
	21		Freehand		67			8.4 x 9.6 (µm)	(29539.5, 16796.8)
	22		Freehand		71			10.1 x 8.8 (µm)	(29467.0, 16796.8)
	23		Freehand		91			16.5 x 9.8 (µm)	(29535.1, 16785.0)
	24		Freehand		58			7.9 x 10.0 (µm)	(29439.9, 16784.4)
	25		Freehand		66			10.4 x 8.5 (µm)	(29562.7, 16775.0)
	26		Freehand		47			5.7 x 9.5 (µm)	(29375.5, 16794.4)
	27		Freehand		169			18.7 x 16.4 (µm)	(29410.7, 16779.0)
	28		Freehand		76			9.0 x 9.1 (µm)	(29484.3, 16773.8)
	29		Freehand		66			7.6 x 11.2 (µm)	(29526.5, 16770.8)
	30		Freehand		67			9.6 x 9.4 (µm)	(29475.2, 16764.1)
	31		Freehand		51			7.7 x 7.6 (µm)	(29465.0, 16760.1)
	32		Freehand		78			10.8 x 8.8 (µm)	(29514.8, 16758.8)
	33		Freehand		73			12.1 x 7.3 (µm)	(29556.7, 16749.5)
	34		Freehand		358			27.6 x 22.2 (µm)	(29588.7, 16739.2)

High Throughput: Image Recognition Software Interfaces

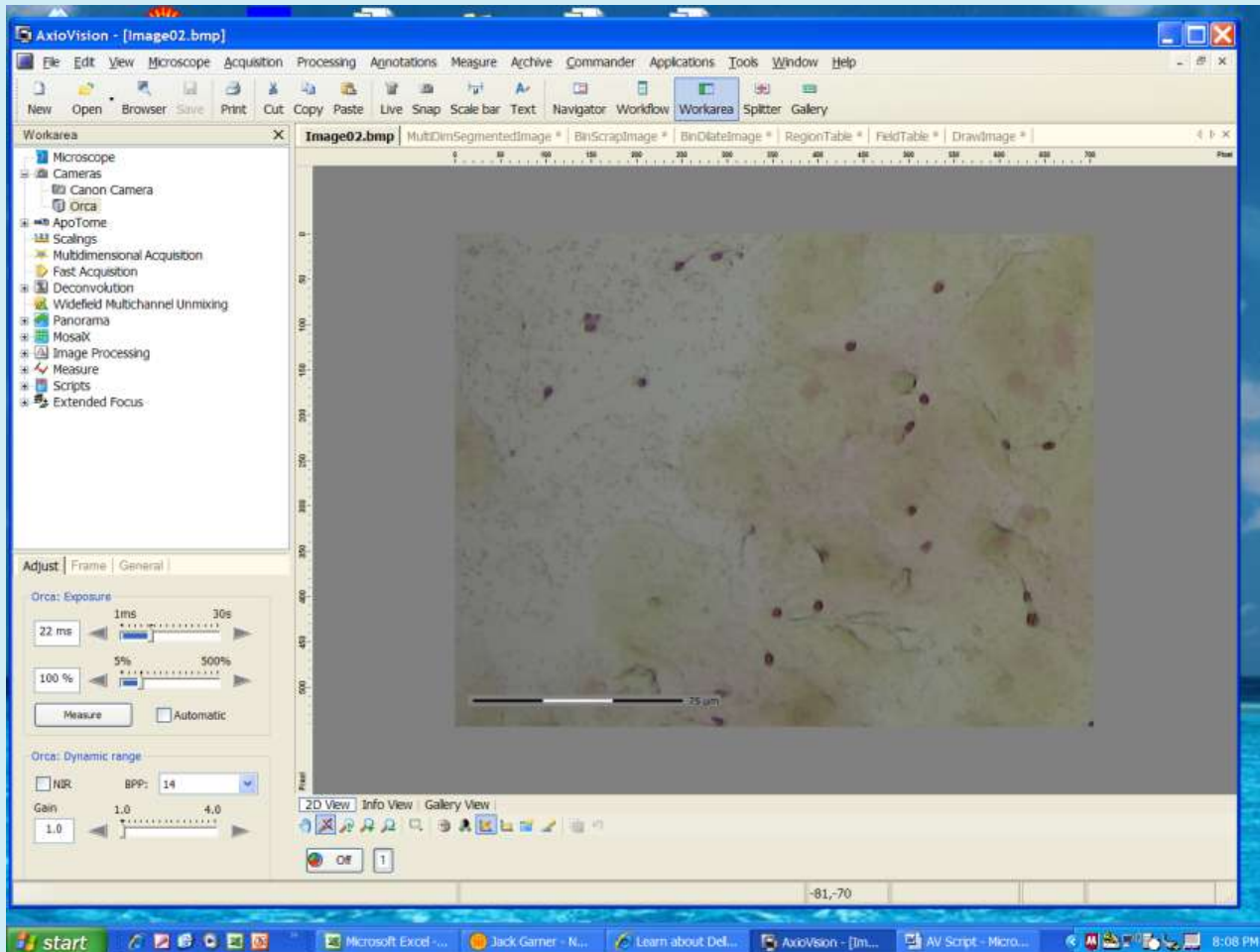
Carl Zeiss® AxioVision®



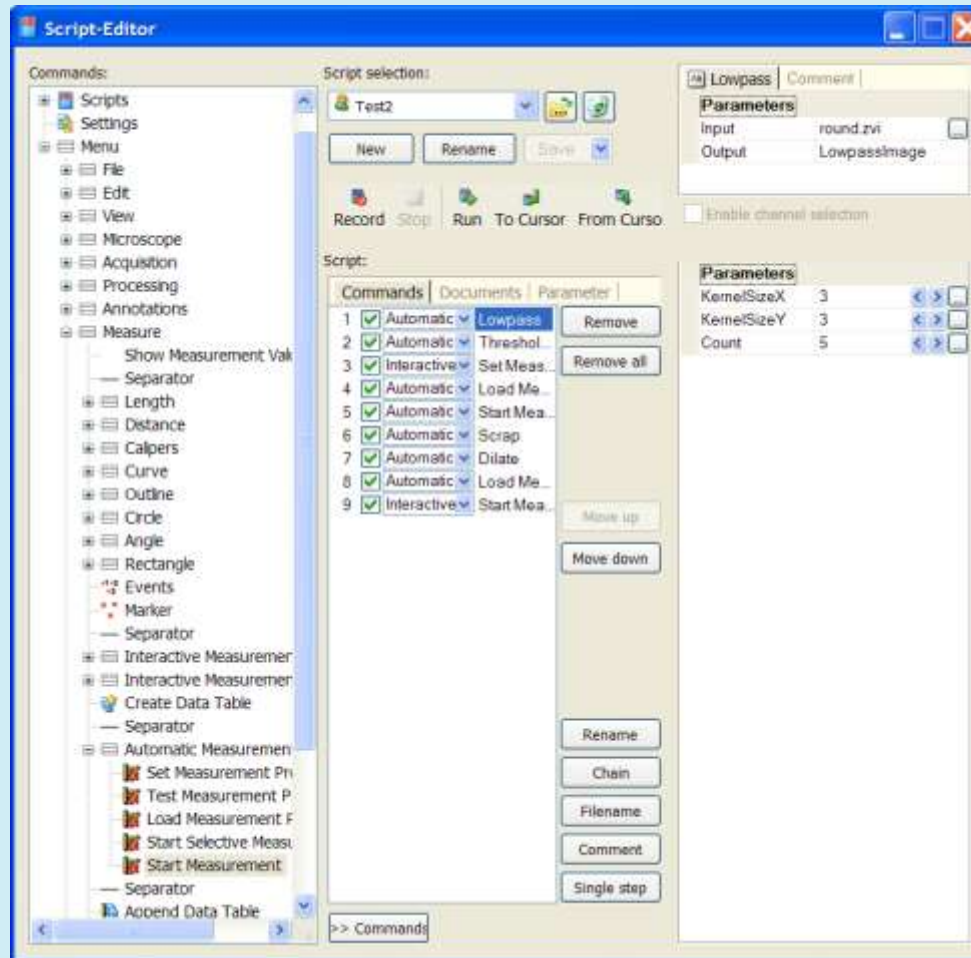
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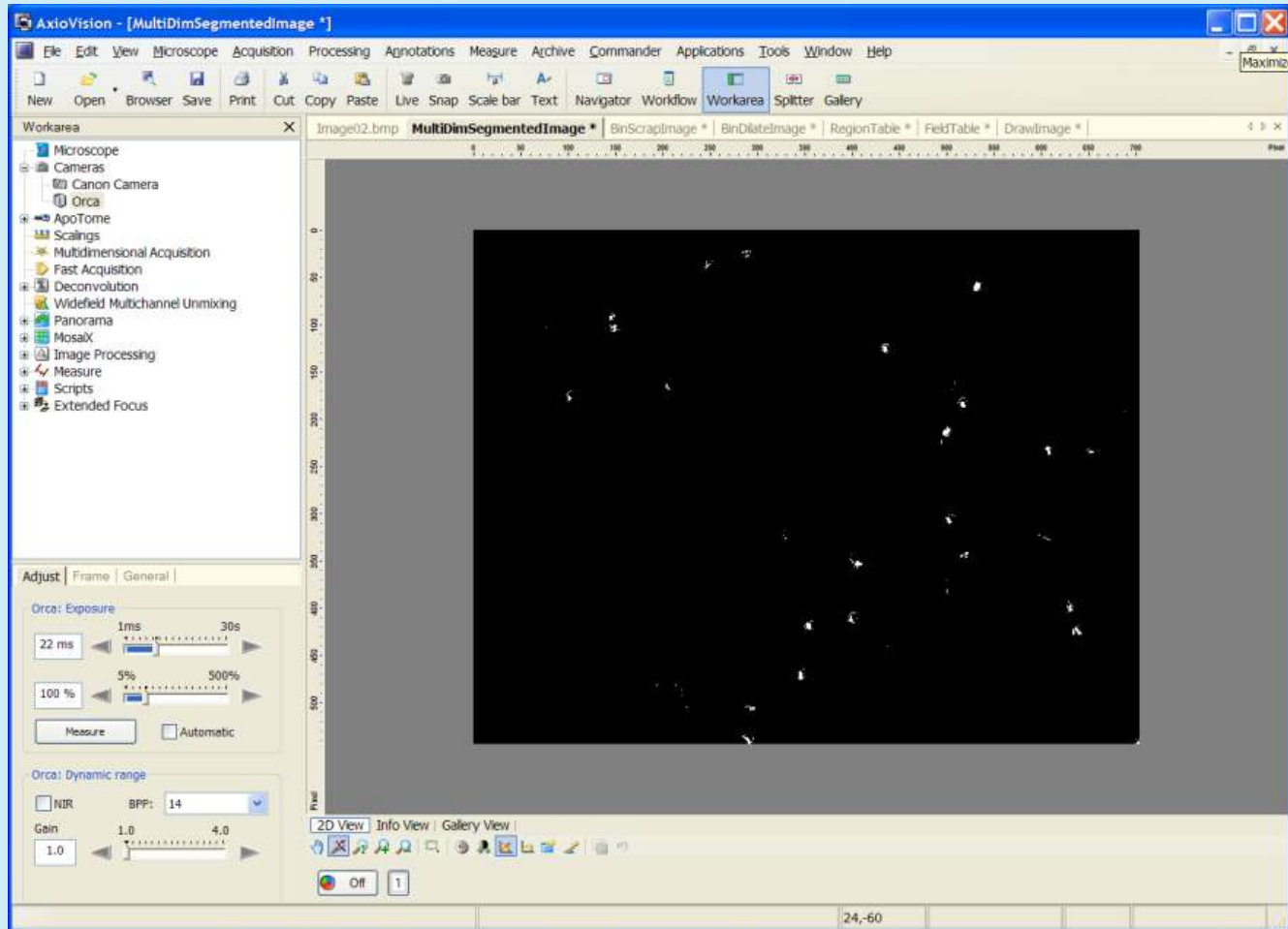
Imaged Sample in AxioVision®



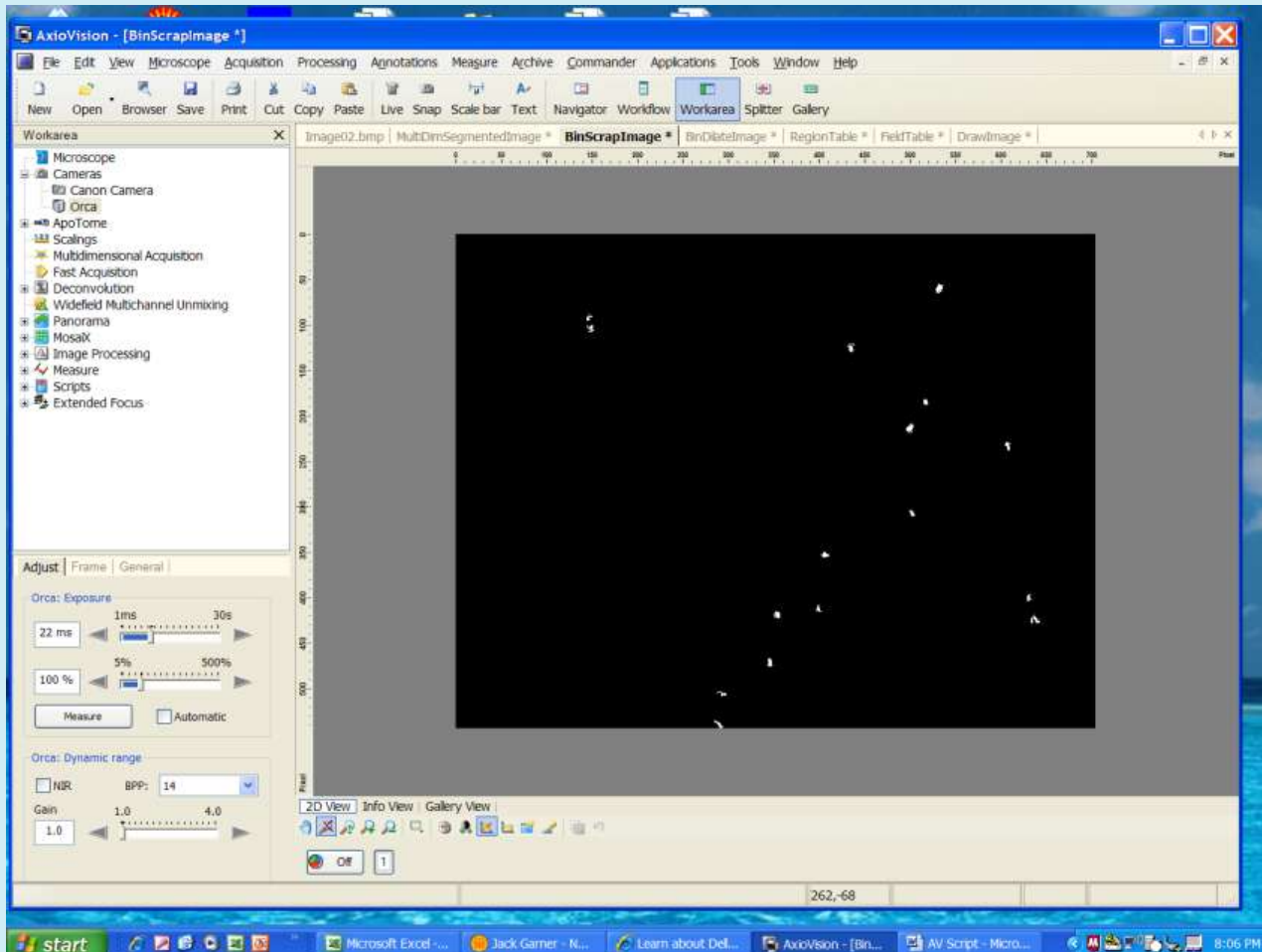
Example Script



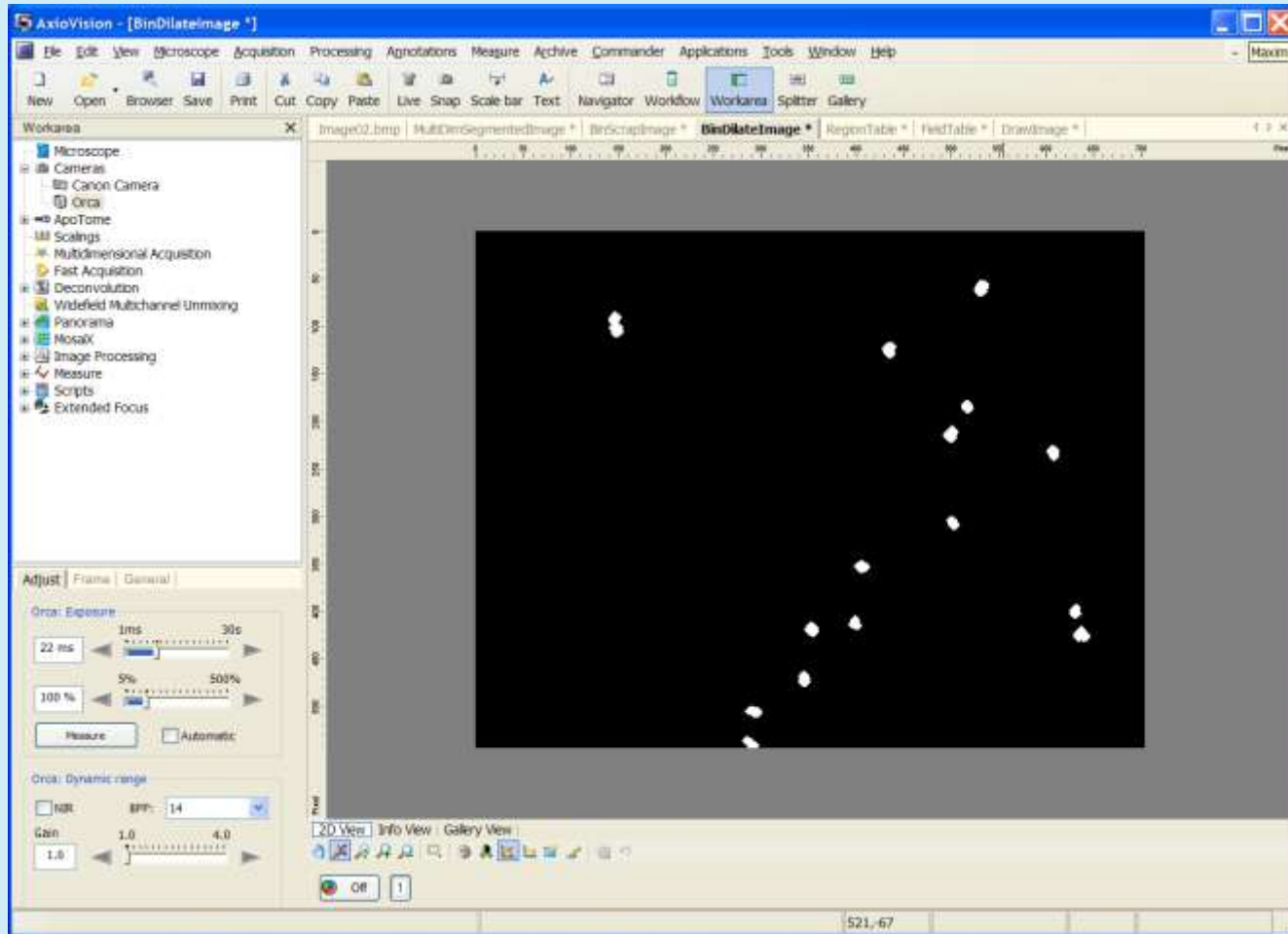
Binary Results of Auto ID



Discriminatory Selection



Selected Samples Filled



Samples Selected for Collection

The screenshot displays the AxioVision software interface. The main window shows a microscope image with 14 red spots, labeled '14' in the top left corner. The 'Element List' window is open, showing a table of 9 selected elements. The table columns are Color, Nr, Name, Type, cut_shot, Area (µm²), Grp, Comment, H x W, and Position.

Color	Nr	Name	Type	cut_shot	Area (µm²)	Grp	Comment	H x W	Position
Green	1		Freehand		23898			305.2 x 165.2 (µm)	(101172.2,46252.0)
Green	2		Freehand		27044			252.9 x 188.3 (µm)	(101304.9,46413.4)
Red	3		Freehand		10057			156.0 x 126.8 (µm)	(100787.6,46422.0)
Cyan	4		Freehand		4627			90.1 x 65.3 (µm)	(101105.3,46561.3)
Cyan	5		Freehand		11186			243.2 x 166.2 (µm)	(100877.7,46299.1)
Red	6		Freehand		7773			77.5 x 190.2 (µm)	(100721.8,46599.6)
Green	7		Freehand		26681			227.6 x 214.1 (µm)	(100992.1,46466.1)
Green	9		Freehand		9984			134.6 x 120.1 (µm)	(100828.4,46550.6)

Questions?

Contact Information

Jack Garner
PALM[®] Microlaser Technologies
Carl Zeiss[®] MicroImaging Group

All images courtesy of Carl Zeiss[®] unless otherwise noted.

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