



Technology Transition Workshop | *Bob Fasulka*

***Leica™ LMD6500 Laser Microdissection
Easy to Use, Fast, Precise, Versatile, Effective***

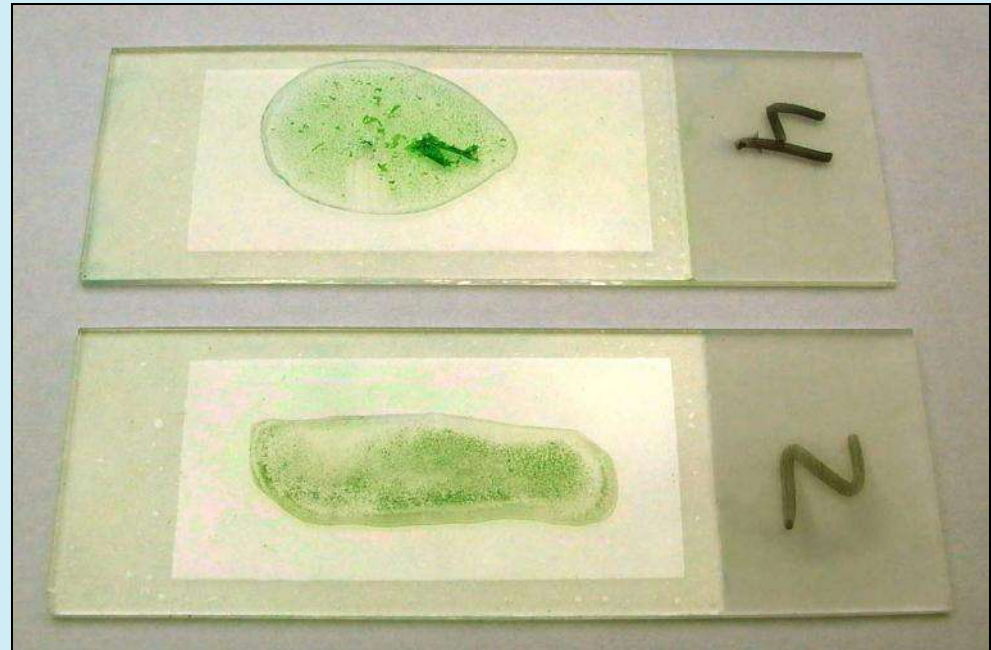
Latest LM Technology

- **The Leica™ LMD6500 represents one of the latest laser microdissection technologies**
 - **Molecular and cell biology researchers want to isolate **specific cell types** from **heterogeneous** tissues to analyze and compare their DNA/RNA/protein contents**
 - **Manual microdissection has been used for a long time in certain fields (such as neurobiology), but is very labor intensive**
 - **Laser microdissection allows faster isolation of cell groups, and makes single cell or group cell isolation possible**

Leica™ LMD6500

Foiled Slide Substrates for Laser Microdissection

- **Glass foiled PEN slides**
 - Polyethylene naphthylate foil on glass slides
 - 2.0 µm PEN foil thickness

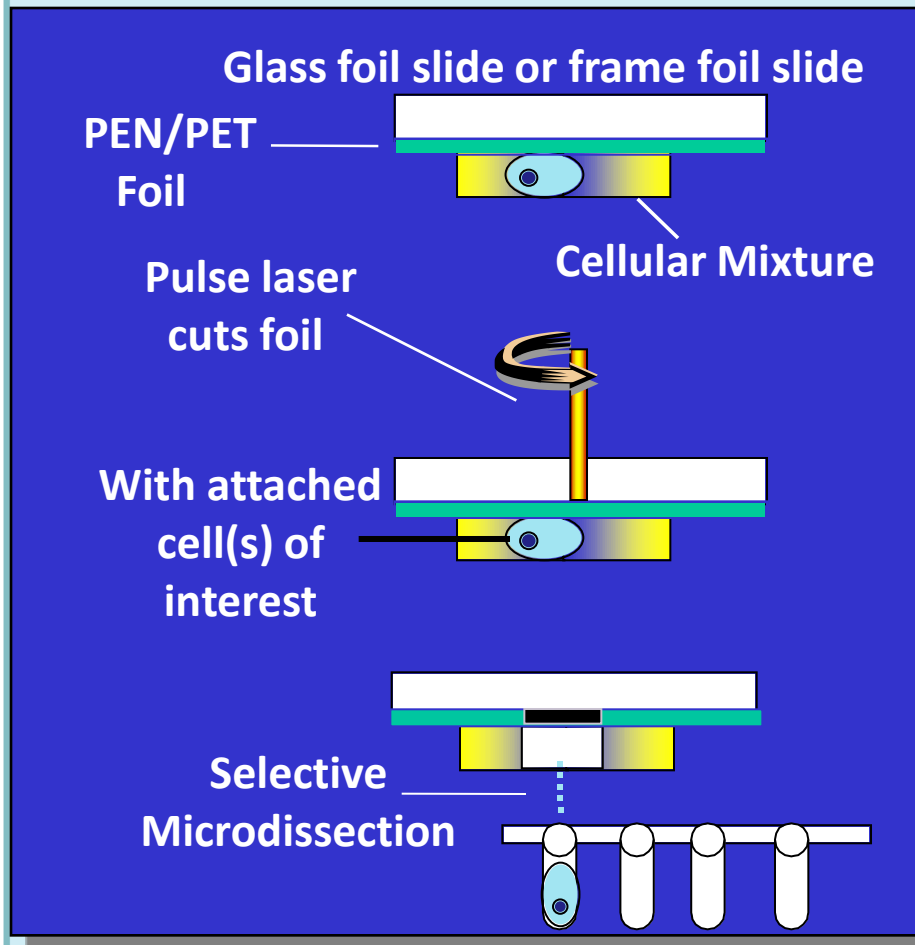


*Spermatozoa and Epithelial Cell Mixture
Stained with “Christmas Tree” Stain*

Samples prepared by Jim Liberty, Bureau of Criminal Apprehension, St. Paul, MN

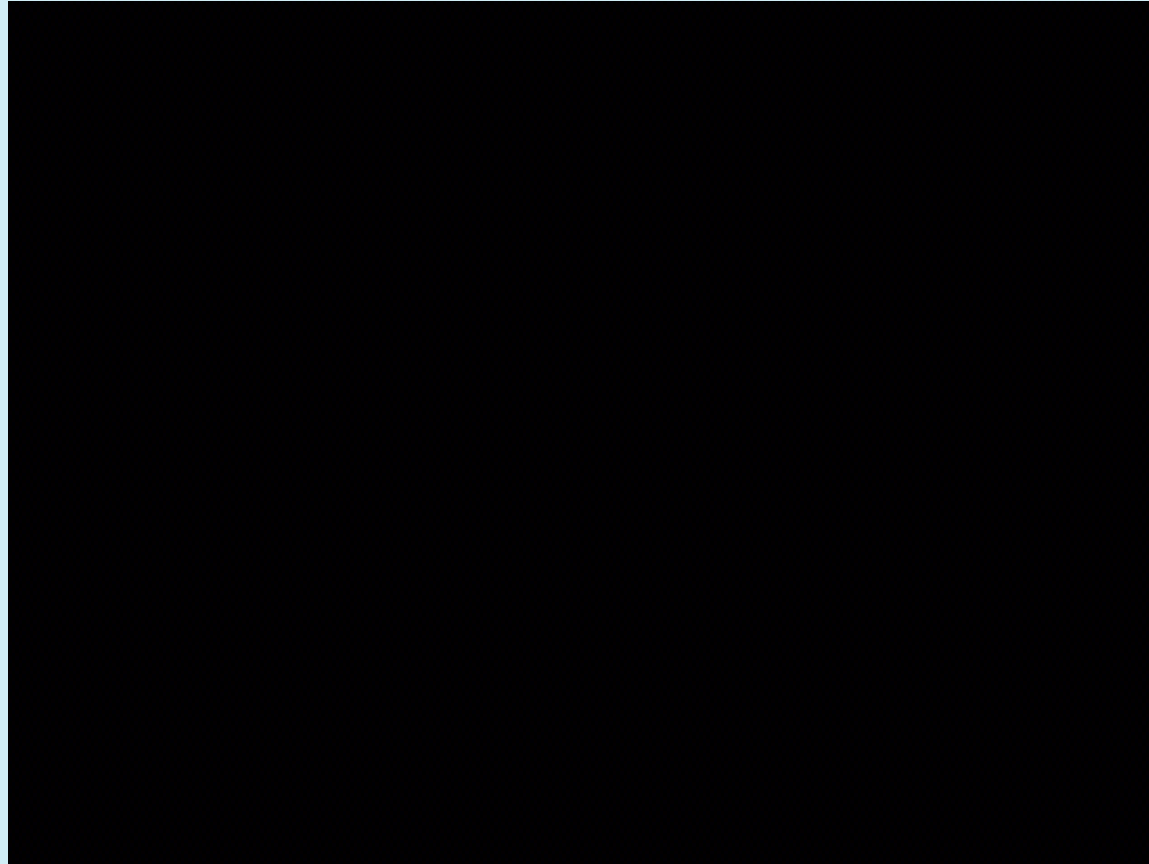
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Non-Contact Method Laser Microdissection (LMD)

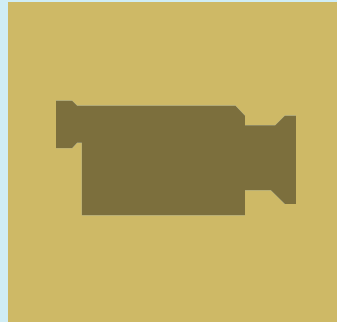


- **Cells or tissue sections are placed directly on to the PEN/PET (polyethylene) foil anchored to slide**
- **Cell(s) of interest are separated from unwanted cells by a cutting ultraviolet laser and collected directly into the cap of a PCR tube for further downstream analysis**

Leica™ LMD6500 Laser Module

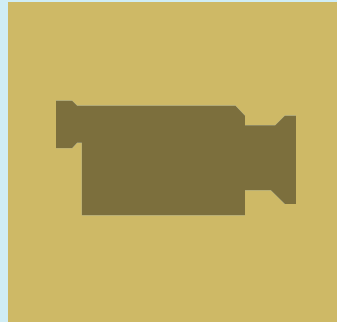


Leica™ LMD6500 Laser Module



600 μ diameter tissue section 10 μ thick free falling after laser microdissection

Leica™ LMD6500 Laser Module



150 μ diameter tissue section 10 μ thick free falling after laser microdissection

Leica™ LMD6500 Laser Module



**300 μ diameter tissue section 10 μ thick free falling into
PCR tube cap after laser microdissection**

Laser Microdissection Morphological Limitations

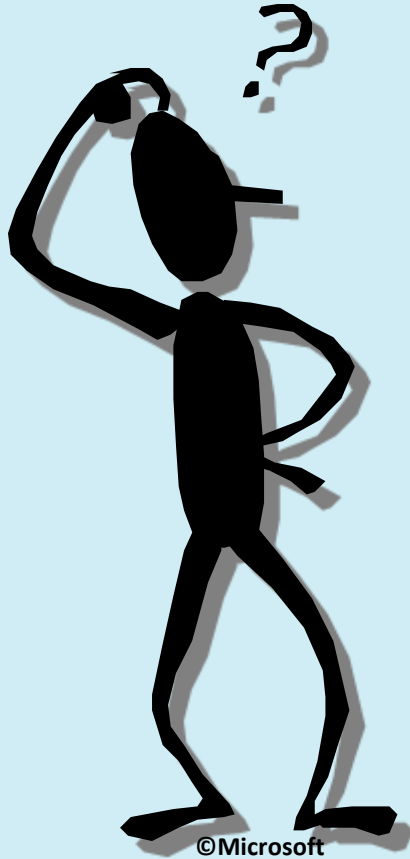
- **No coverslip and no mounting medium**
- **Fixation (if necessary) of tissue needs to be optimal**
- **Multiplicity of fixation techniques and challenges**
- **Issues of morphology versus molecular biology**

Tissue Sample Preparation for LM

- Cellular spreads or cytopspins
- Fresh or frozen, fixed or unfixed
- Fresh or frozen, stained or unstained
- Paraffin embedded, stained or unstained
- Typical tissue sample thickness 4 to 10 μm
- Can cut on sections up to 40 to 50 μm thick (typically brain)
- Xylene dehydration **NOT NECESSARY** or **REQUIRED***

* GFP often inactivated when dehydrated

Laser Microdissection Tissue Protocols



- DNA or RNA or Protein?
- Frozen or Paraffin?

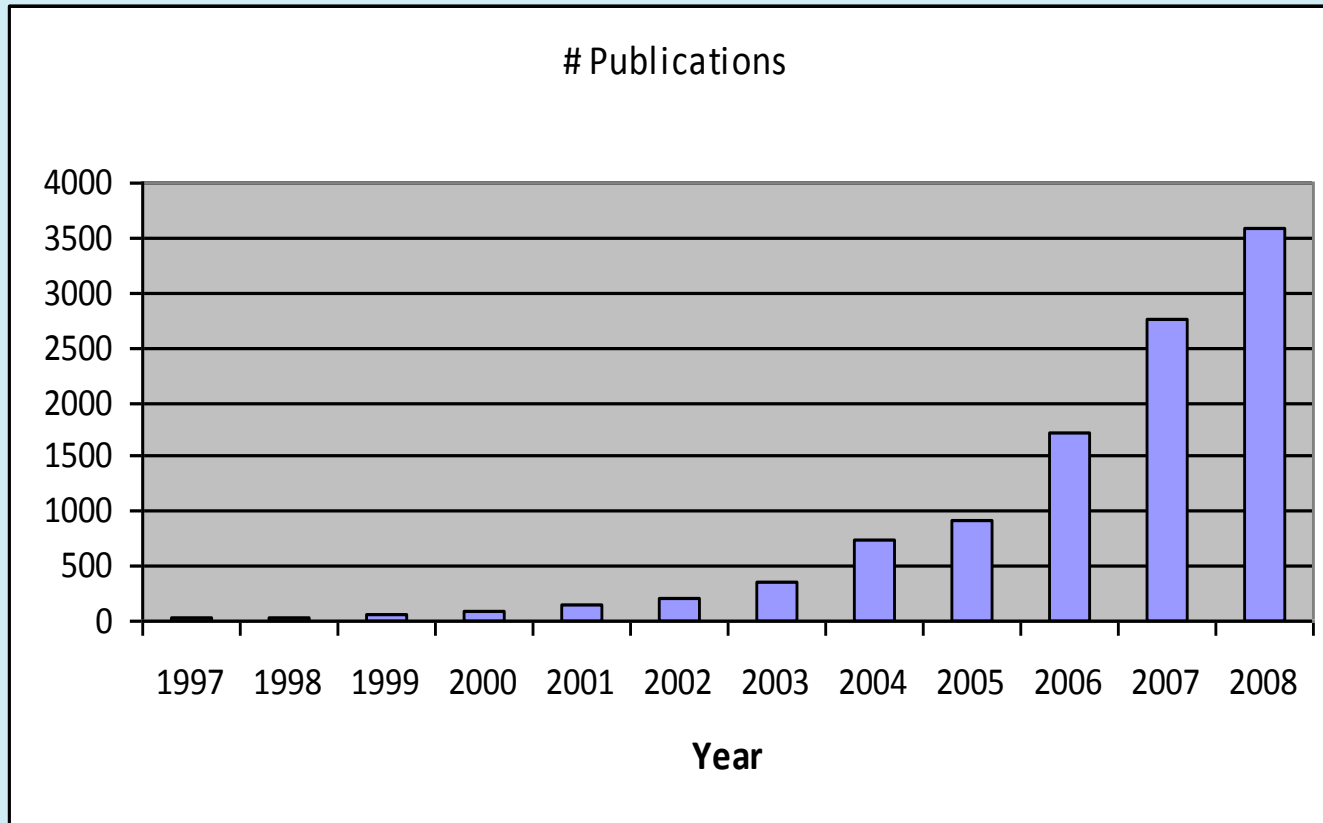
Laser Microdissection Applications

Molecular Analysis

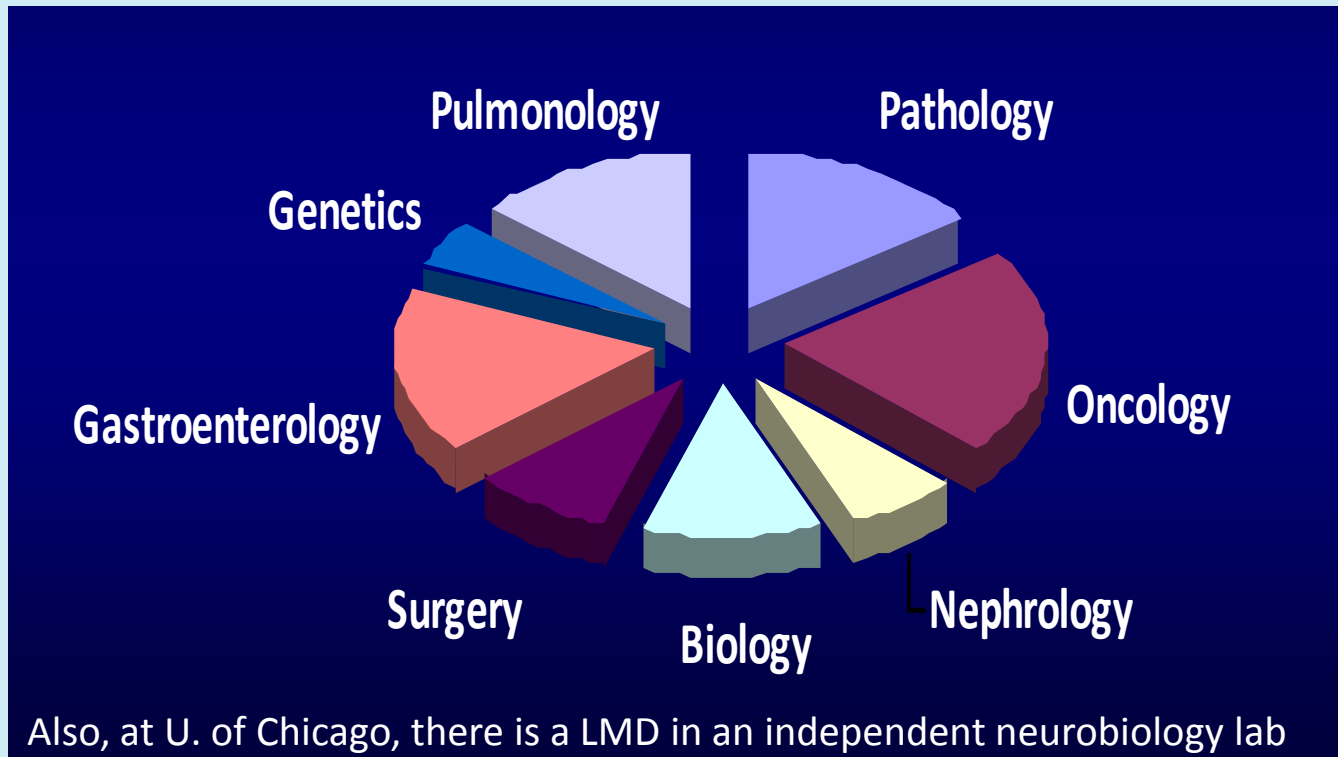
DNA	RNA	Proteins	
1 to 5,000 cells	1 to 1,000 cells	> 5,000 to 50,000 cells	
<ul style="list-style-type: none"> • Clonal analysis • DNA methylation • Direct sequencing • CGH • LOH • PCR 	<ul style="list-style-type: none"> • RT PCR 	<ul style="list-style-type: none"> • Resolve • 2D-PAGE • Identify • Mass spectrometry western immunoblotting 	
	> 500 cells		
	<ul style="list-style-type: none"> • cDNA library construction • cDNA microarray probes • Differential display • Gene expression arrays 		

Number of Publications

- PubMed search for “Laser Microdissection”



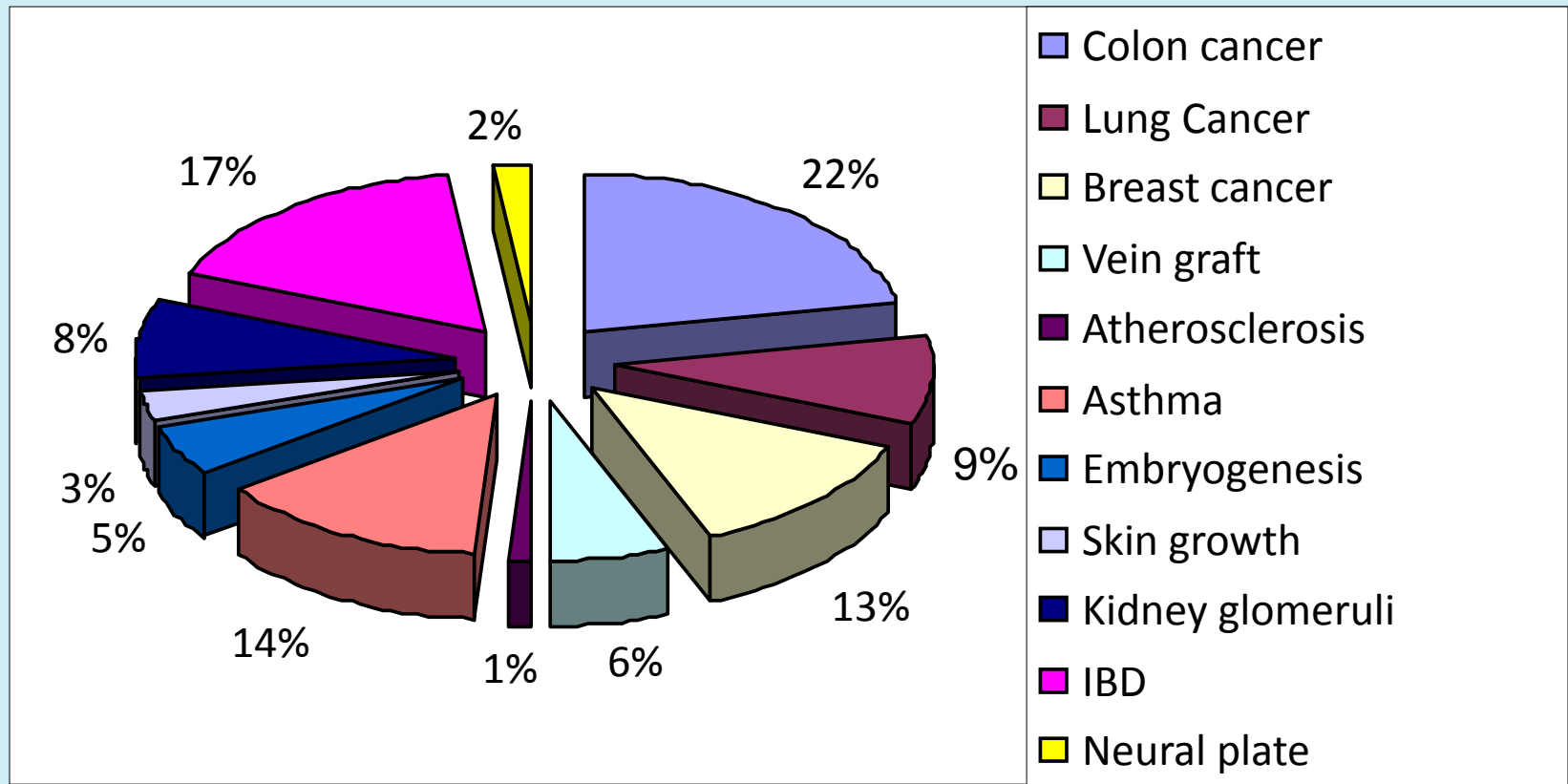
Who Uses Laser Microdissection?



Data based on Core Facility at Department of Pathology, University of Chicago
Courtesy of Dr. Maria Tretiakova

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Projects with Laser Microdissection



Data based on Core Facility at Department of Pathology, University of Chicago
Courtesy of Dr. Maria Tretiakova

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The Scientific Community and Leica™ Microsystems – LMD

Papers:

Hippocampal Dendritic

Yugo Fukui, Kaoru Inoue, J. Neurosci., 2002

Distinct Gene Expression

Mueller, A. PNAS 2002

Detection of the Renin-Angiotensin System

Inoue K., Virchowsch Arch Anat Hist Embol Microscop

HLA-G gene analysis

Bulmer J. Prenat. D. 2002

Laser Capture Microdissection

D.L. Ellis, BioTechniques 2002

GABAergic mRNAs

Costa E., Neuropharmacology 2002

Large Scale Salivary

Henry F.J. Su, Jun C. American J. Pathol. 2002

Restriction on a Hepatic

German J. Pathol. 2002

Sauvage, Pincon, J Biol Chem 2002

Whole Cell

Colleen, (book ed)

Analysis of

Mori M, Matsuura, Surgery 2002

Microdissection

Elhoum, Adv Anat Hist Embol Microscop 2002

Monoclonal

Pruneri, Hum Pathol 2002

Abstracts

Comparative

M. Taur, (I. Drug Society) 2002

Microarray

K.A. Ba, Jackson Society, 2002

Optimizing

M.J. Ros, H.Hiem, German Society, 2002

Multiple and Simultaneous Measurements of Inflammatory Mediators After Excitotoxic Injury in Hippocampal Organ Cultures

R.P.Kraig, P.E. Kunkler, J. P. Fedynshyn (Dept Neurology, Univ of Chicago, IL.)
Society for Neuroscience 2002 abstract 391.1

Age-dependent Changes of Gene Expression Profiling in Laser Capture Microdissected Mitral Cells in Mouse Olfactory Bulb

Y. Cao^{1*}, L. Hsieh¹, W. Wu¹, R. Gillespie², V. Meyers², S. Thierianos², P. Coleman² (1. Affymetrix and 2. Univ. of Rochester)
Society for Neuroscience 2002 abstract 903.5

Molecular analyses of B-cell clusters from rabbit splenic tissue sections by Leica-LMD pulsed UV laser

Guibin Yang, Harold Obiakor, Robert Bonner, Rose Mage (NIAID and NICHD, NIH)
NIH LCM02 abstract

Impact of laser microdissection and linear amplification on microarray profiles

M. Treiakova, H. Dyanov, J. Zhou, B. Qigg, J. Hart (U of Chicago, Dept. Pathology and Dept Medicine)
NIH LCM02 abstract

Detection of tissue localization of NOD2 Gene using laser microdissection

M. Turkyilmaz, M. Treiakova, R. Anders, J. Chu, and J. Hari (U of Chicago, Dept Pathology)
NIH LCM02 abstract

Identification of Bacteria in Human Root Caries Using Laser Microdissection and DNA-DNA Hybridization

Lily Hwang (Harvard School of Dental Medicine)
NIH LCM02 abstract

Analysis of WNT pathway components in human colorectal cancer: LMD and QRT-PCR in tandem

Konrad Koelble (Charite Berlin, Germany)
NIH LCM01 abstract

Laser-assisted microdissection of neurons

Michael McKinney (Mayo Clinic, Dept. of Pharmacology)
NIH LCM01 abstract

- References and publications
- Proven world-wide success with peer reviewed research journals and publications

The Leica™ AS LMD

- **Application Solution – Laser Microdissection System**
 - Introduced in USA, October 2000
 - First public showing at Neuroscience Exhibition New Orleans, November 2000
- **The Leica™ LMD6000**
 - Introduced in USA, November 2005
 - First public showing at Neuroscience Exhibition Washington, DC, November 2005
- **The Leica™ LMD6500 and LMD7000**
 - Introduced November 2008

Leica™ LMD6500

- **Based on the Leica™ DM6000B**
- **DM6000B upright automated research microscope**
 - **Intelligent automation of all microscope functions**
 - **Constant Color Intensity Correction (CCIC)**
 - **Fluorescence Intensity Manager (FIM)**
 - **8 position fluorescence filter turret**
 - **Precision z-focus**
 - **Capable of all contrast techniques, BF, PH, DIC, POL, FLZ**
 - **Change technique at the touch of a button**
 - **Ergonomic satellite control**



Leica™ LMD6500 LM System

- **Patented laser beam steering technology for precise cutting**
- **Region of Interest (ROI) for laser microdissection, any size and any shape**
- **Optimized to cut with up to seven objectives with automatic UV offset compensation for laser focus**
- **No need to focus the laser**



New LMD Module

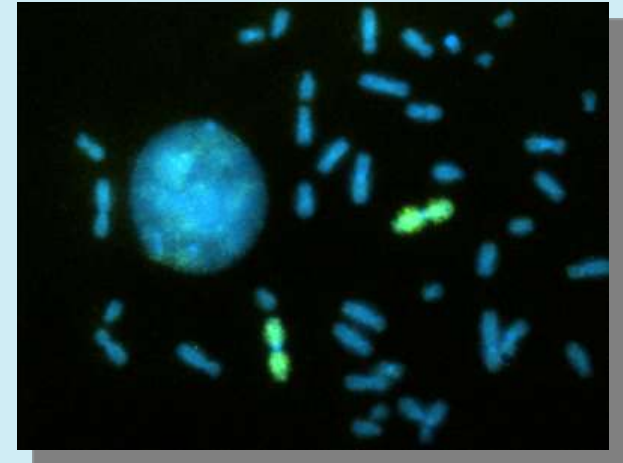
Leica™ LMD6500 LM System

- Tissue on slide faces downward with a protective shield to minimize ambient contamination
- Leica™ LMD Systems have **Class 1 safety laser rating** with safety interlock protective shield

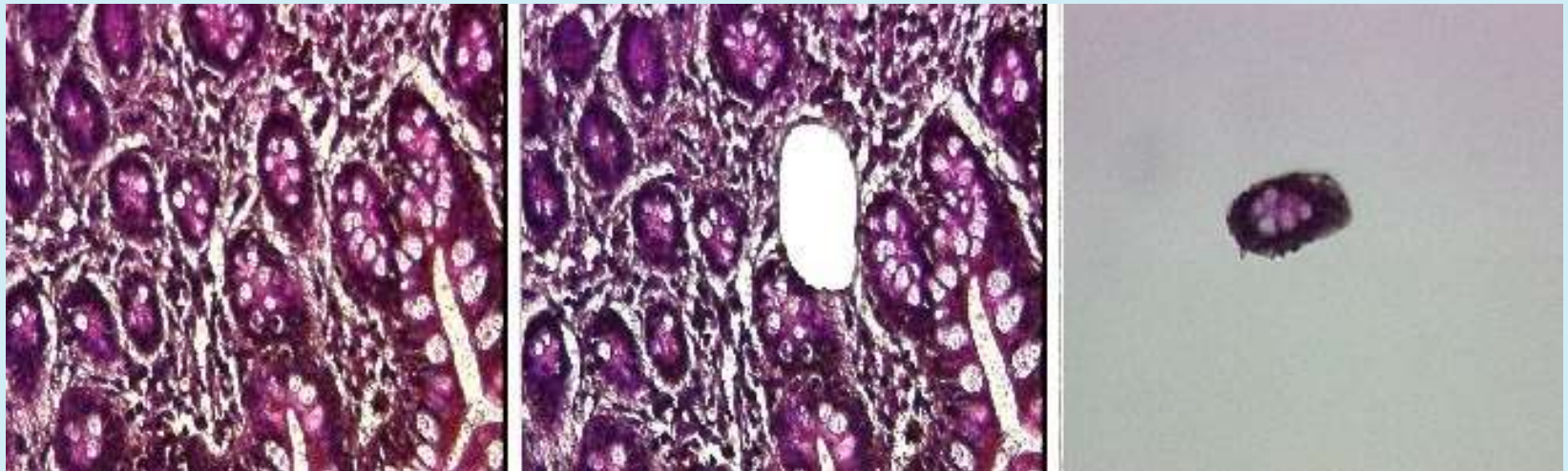


Microscopy Techniques for LM

- **Brightfield**
 - 4x - 150x Dry, 100x Oil
- **Fluorescence**
 - Simple to retrofit, economical & modular
- **Phase Contrast 10x - 63x PH1/PH2**
 - For culture cells/unstained tissues
- **DIC-Nomarski 10x - 63x**
 - For cellular or cell organelle ablation



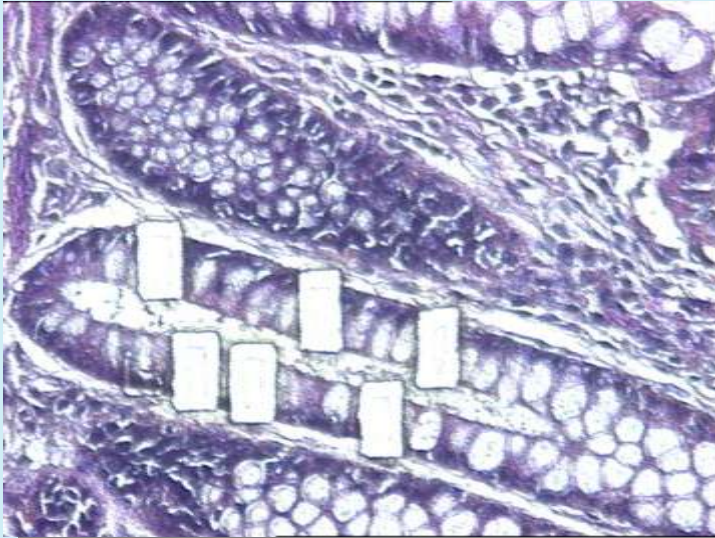
Innovative Control and Imaging Software for the Entire LM Process



- **Direct visual inspection of PCR cap**
- **Automatic image capture (optional)**

Applications for Laser Microdissection

- Small area or single cell laser microdissection



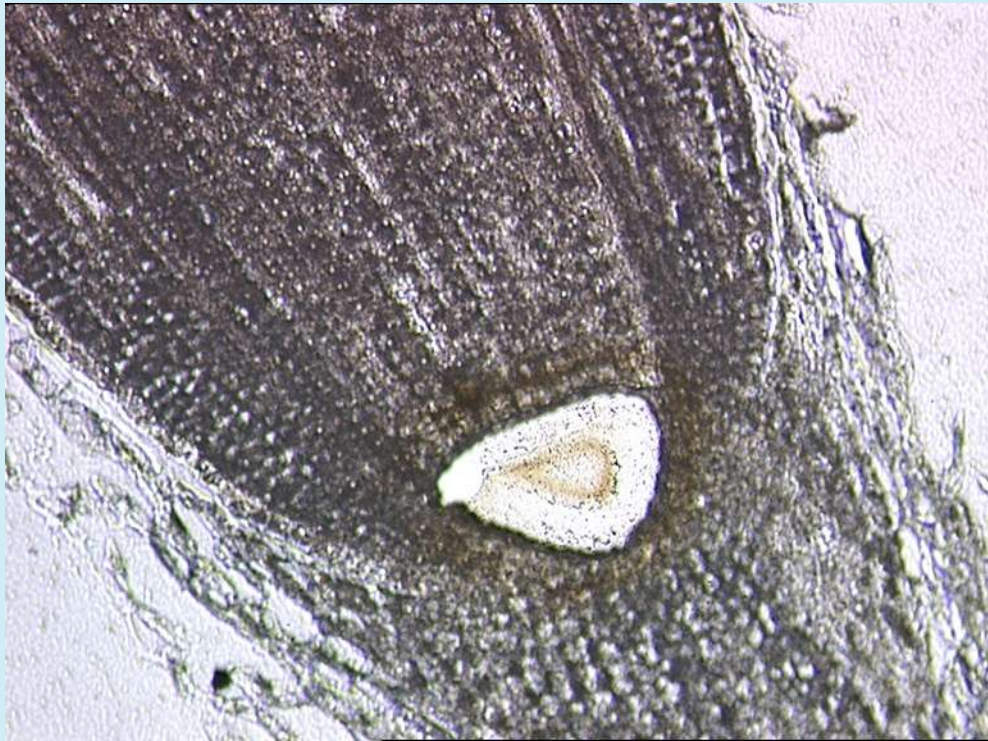
Colon H&E, 40x Objective



PCR Tube Cap Inspection 4x and 10x Objectives

Applications for Laser Microdissection

- **Plant cell laser microdissection**

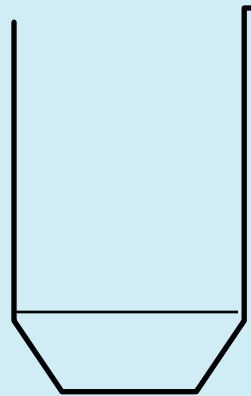


Root Meristem Cells, Maize, 20x Objective

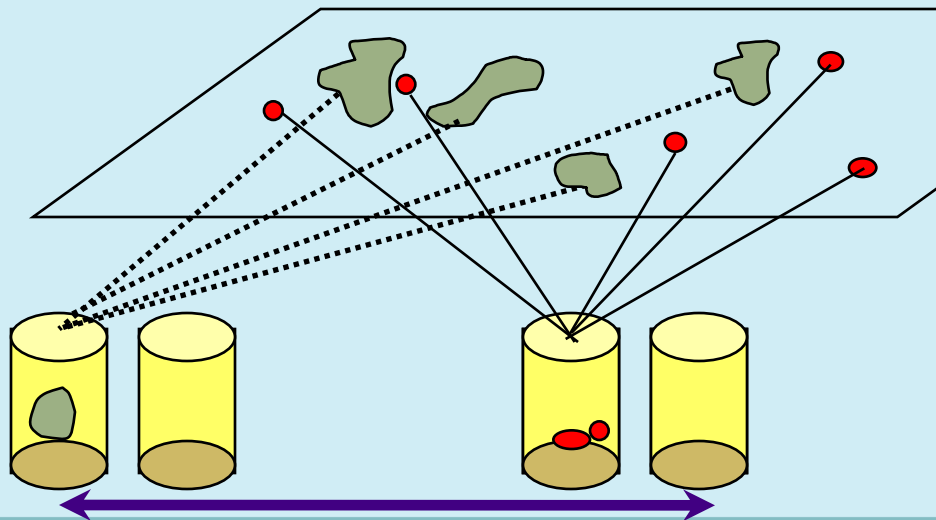


**View in PCR Cap
20x Objective**

Collection Tray for Multiple PCR Tube Support



- Collection and cutting from anywhere on a slide (or another slide)
- Automated support for 4 or more PCR tubes (0.2 or 0.5 ml)
- Motorized PCR tube holder tray



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Rapid Separation of Spermatozoa and Epithelial Cell Mixture Using Laser Microdissection

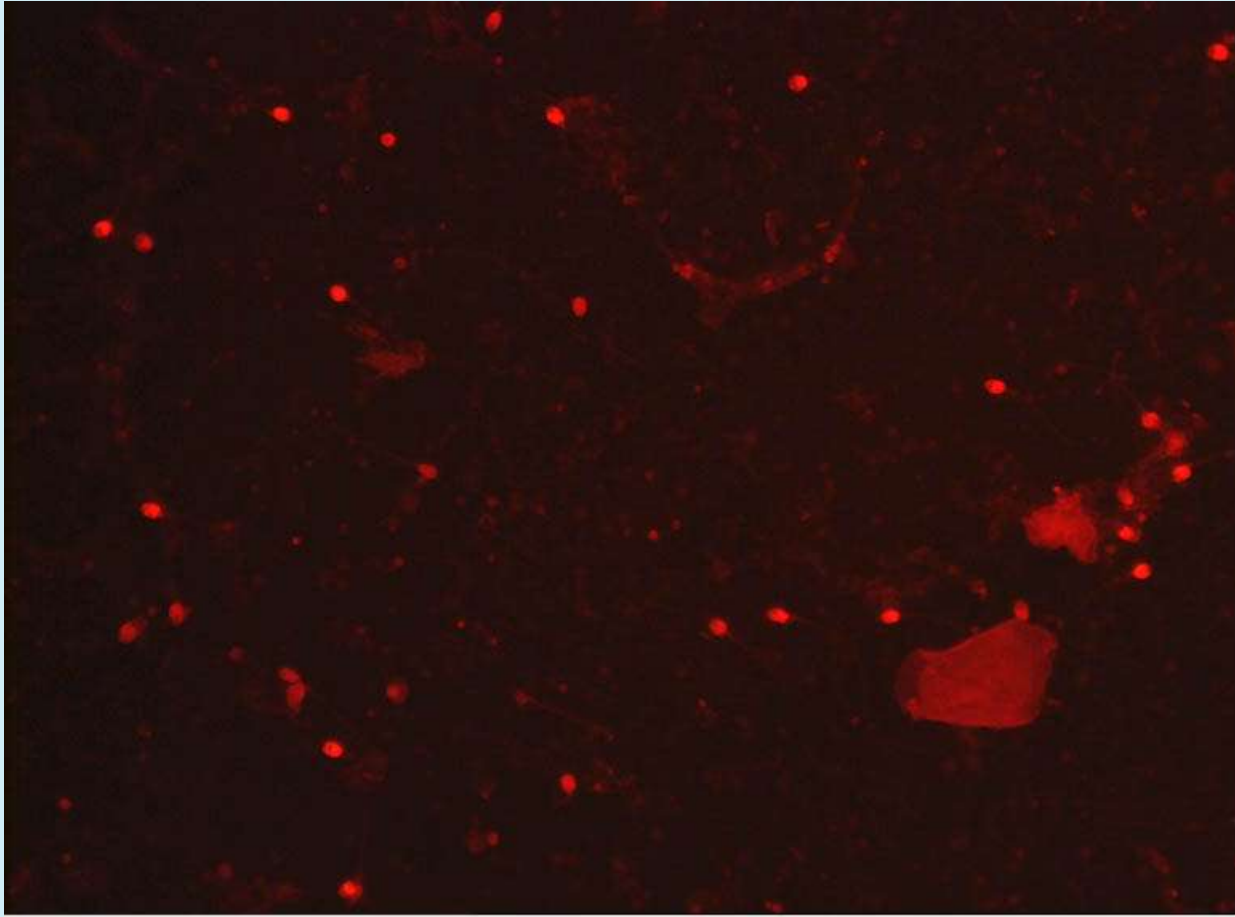


- 40x objective
- Christmas tree stain

Sample prepared by Jim Liberty, Bureau of Criminal Apprehension, St. Paul, MN

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Fluorescence Detection



Sample prepared by Jim Liberty, Bureau of Criminal Apprehension, St. Paul, MN

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AVC Autodetection

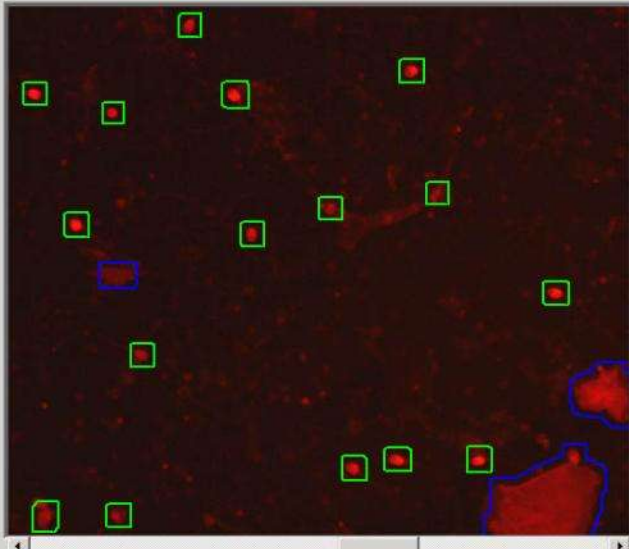
AutoShape Adjustments

Fluorescence Mode
 Remove Boundary Touching Objects
 Remove Small Objects
 Size:

Recognition Method
 Gray Scale - Auto Threshold
 Gray Scale - Manual Threshold
 Color (RGB)

Gray Scale Recognition
 Threshold:

Color (RGB) Recognition
 Red Channel Threshold:
 Green Channel Threshold:
 Blue Channel Threshold:



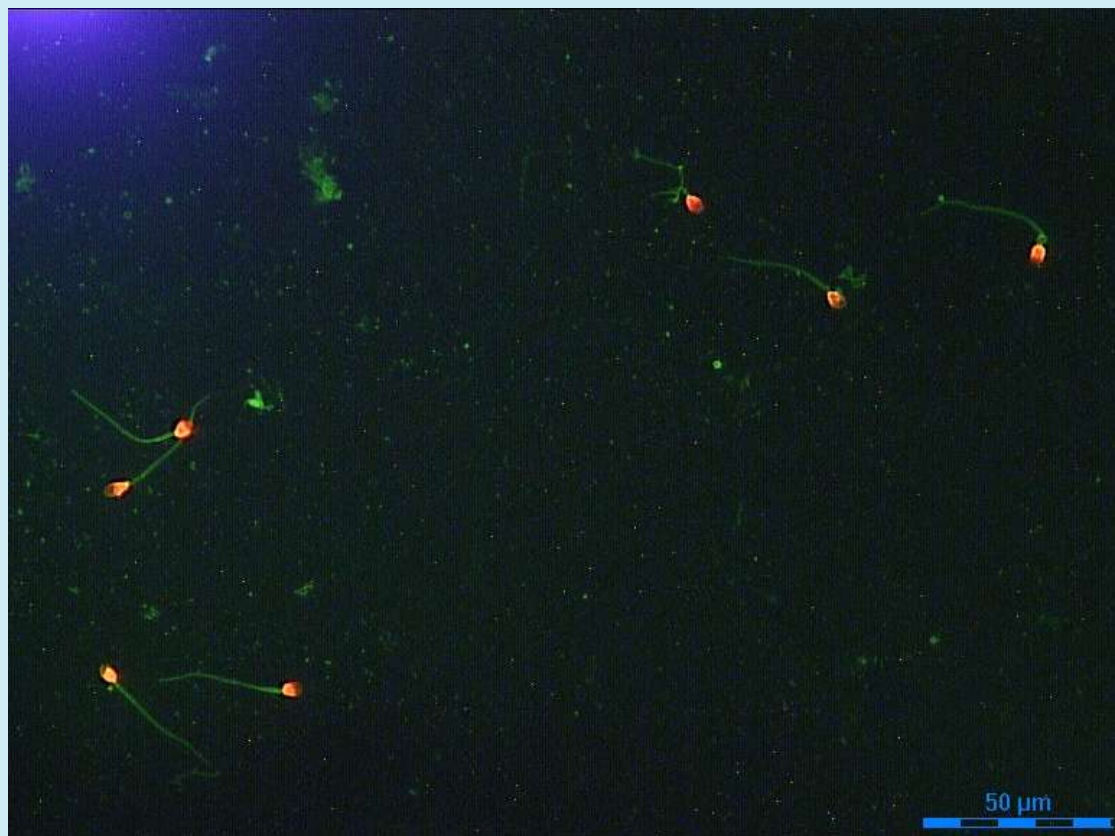
Feature Parameter Limits
 Perimeter: Lower On
 Upper:
 Area: Lower On
 Upper:
 Horizontal Projection: Lower On
 Upper:
 Vertical Projection: Lower On
 Upper:
 Roundness: Lower On
 Upper:

Shape Expansion:

Objects: Accepted Objects: Not Accepted Objects:

Index	Object	Perime...	Area	Horz.P...	Vert.Pr...	Round...	FCPX	FCPY	Object...	XMAX	YMAX
0	OK	88.485...	565	24	24	0.906810	229	5	300	230	28
1	OK	78.828...	438	21	21	0.885764	364	73	301	364	93
2	OK	84.485...	519	24	22	0.913724	218	94	302	219	115
3	not acc...	354.02...	6956	106	90	0.697434	638	112	303	664	201
4	OK	91.313...	599	23	27	0.902744	191	126	304	195	152
5	OK	80.828...	459	22	21	0.882865	257	130	305	257	150
6	OK	85.071...	518	23	23	0.899448	87	166	306	91	188
7	OK	81.656...	477	22	22	0.898965	459	170	307	459	191
8	OK	83.656...	499	22	23	0.895999	564	177	308	566	199
9	OK	83.656...	499	22	23	0.895999	497	177	308	497	199

New Fluorescence Stains for Auto-detection



Leica™ LMD6000

Other Possible Forensic Science Applications

- **Laser microdissection of specific cells from fetal tissue sections or maternal blood to determine paternal parentage through DNA profiling**
 - **DNA profile of chorionic villi and of the actual fetus for a “match” to the “father”**
 - **DNA profile of maternal cells**

Acknowledgements:

- **Jim Liberty**
 - Bureau of Criminal Apprehension
 - St. Paul, MN
- **Janice Zhou, Ph.D.**
 - Leica™ Microsystems, Inc.
 - Bannockburn, IL
- **Andy Lee**
 - Leica™ Microsystems, Inc.
 - Bannockburn, IL

References

- Sanders, C., Peterson, D. and Sanchez, N. “Separation of spermatozoa and epithelial cell mixtures by laser microdissection for forensic DNA analysis.” *Proceedings of the American Academy of Forensic Sciences 56th Annual Meeting 10* (2004): 36.
- Valentine, J.A., Wojtkiewicz, P., and Williams, B.J. “Separation of diploid cells using FISH and LMD applications in the analysis of sexual assault evidence.” *17th International Symposium on Human Identification Poster Abstract 17* (2006, Nashville).

Questions?

Contact Information

Bob Fasulka

**Leica™ Microsystems, Inc.
Applied Optical Microscopy
25 Berkeley Ave.
Westward, NJ 07675
201-723-8963**

bob.fasulka@leica-microsystems.com

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