



Technology Transition Workshop | *Robert Driscoll, M.F.S.*

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# ***Introduction to Laser Microdissection***

# ***Evidence Mixtures***

- **The generation of clean, single source genetic profiles from sexual assault and touch evidence cellular mixtures continually proves to be a difficult challenge in forensics**
- **Evidence of this nature can contain trace amounts of human DNA from mixtures of cell types of various morphologies**
- **Commonly utilized DNA extraction techniques for the purposes of cellular separation are laborious and not always effective**

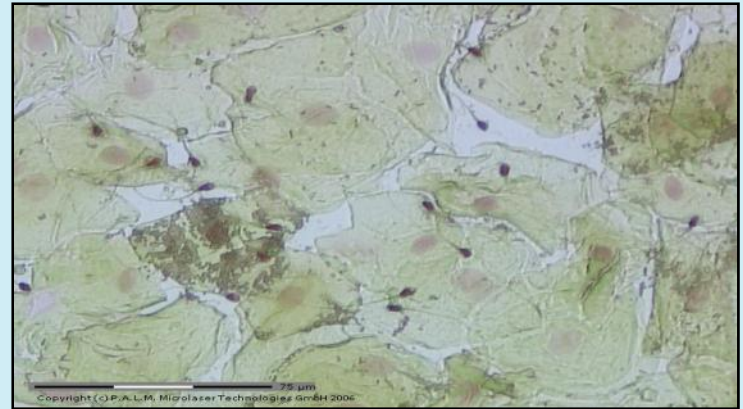


Image courtesy of Rob Driscoll

# *Mixtures of Cells of Different Morphology*

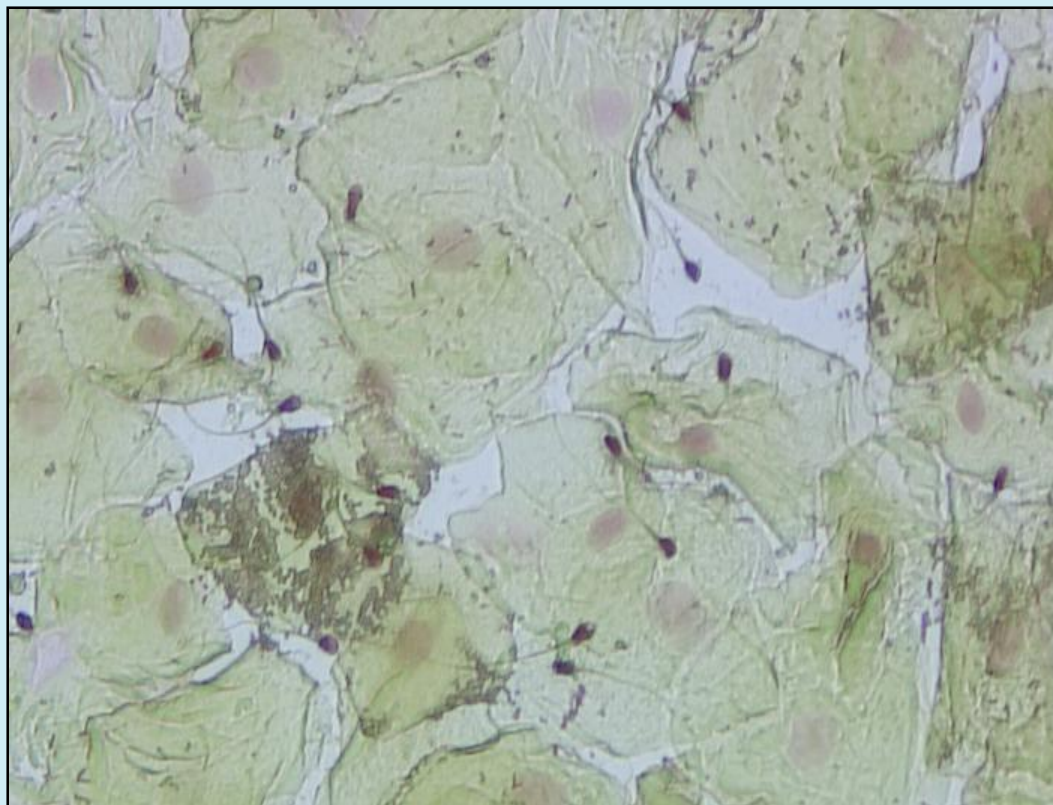


Image courtesy of Rob Driscoll

# *Mixtures of Cells of the Same Morphology*

- Different gender
- Same gender



Image courtesy of Rob Driscoll

# Epithelial Mixture Profile

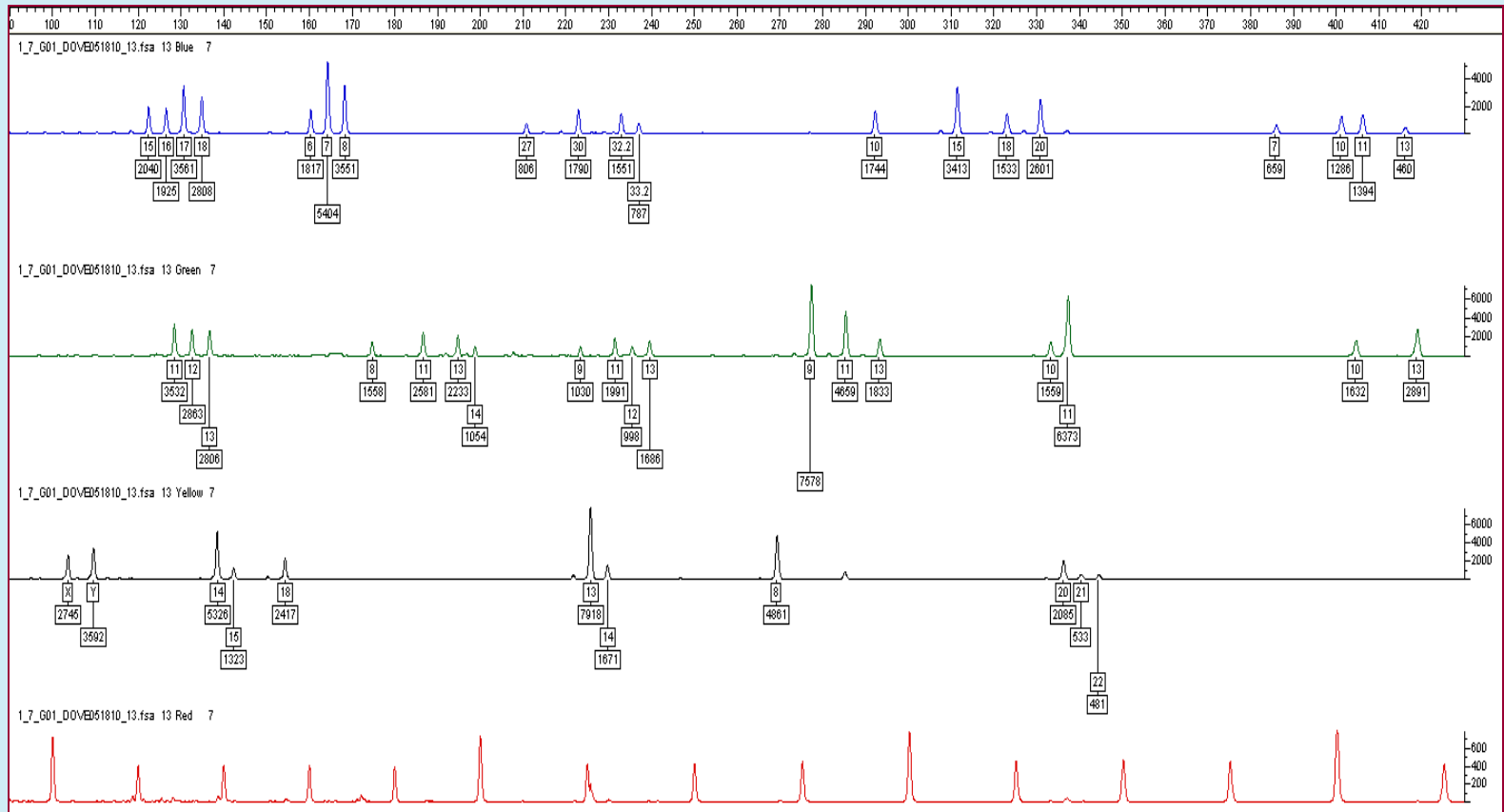
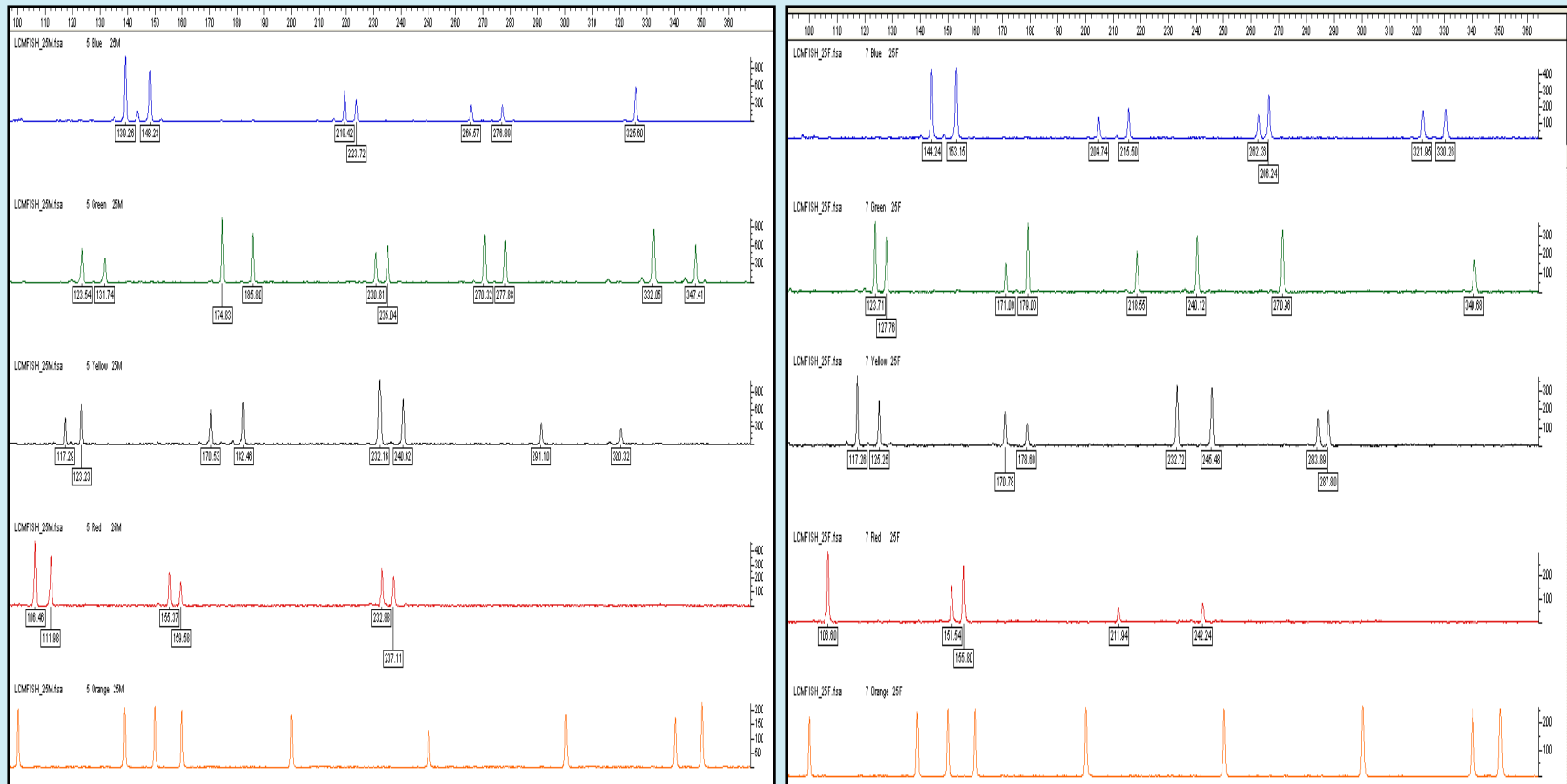


Image courtesy of Rob Driscoll

# Single Donor Component Profiles



Images courtesy of Rob Driscoll

# ***Traditional Methods for Mixture Resolution***

- **Sexual assault evidence (male/female mixtures):**
  - **Differential extraction methods**
    - **Separate sperm cells from epithelial using various detergents to lyse membranes**
    - **Process lysates individually**
- **Touch/assault evidence (same gender mixtures):**
  - **None**

# ***Laser Microdissection (LM)***

- **A method of isolating and collecting cells of interest from biological samples and mixtures**
- **Allows for a scientist to perform single source genomic interpretation on samples originating from multi-cellular tissues or mixtures**
- **Utilized primarily in medicine for cancer diagnostics and in forensics for mixture separations**



# ***LM Applications in Forensics***

- **Sexual assault evidence**
  - Separate sperm from epithelial cells
  - Identify and separate male from female epithelial cells based on fluorescent in situ hybridization (FISH) of X and Y chromosome sequences
- **Other evidence**
  - Male/female mixtures of blood/blood or blood/epithelial
  - Bone extraction: collect nucleated cells from bone matrix
  - Hair: collection of nucleated cells from hair
  - Aged slides
  - Botany

# ***LM Forensic Publications***

- Seidl, Stephan, Renate Burgemeister, Roland Hausmann, Peter Betz, and Thomas Lederer. “Contact-free isolation of sperm and epithelial cells by laser microdissection and pressure catapulting.” *Forensic Science, Medicine, and Pathology* 1 (2) (June 2005): 153-157.
  - Complete profiles from 10 epithelial cells
- Sanders, Christine T., Nick Sanchez, Jack Ballantyne, and Daniel A. Peterson. “Laser microdissection separation of pure spermatozoa from epithelial cells for short tandem repeat analysis.” *Journal of Forensic Science* 51 (4) (July 2006): 748-757.
  - Effective recovery of spermatozoa from mixture evidence
- Anoruo , Belinda, Roland van Oorschot, John Mitchell, and David Howells. “Isolating cells from non-sperm cellular mixtures using the PALM<sup>®</sup> microlaser micro dissection system.” *Forensic Science International* 173 (2) (December 2007): 93-96.
  - Effective recovery of spermatozoa from mixture evidence

# ***LM Technique Variation***

- **Laser Microdissection (LM)**
  - Laser cuts target cells and then using laser energy, or adhesion, transfers target cells to a collection tube via:
    - Gravity
    - Pressure catapulting
- **Laser Capture Microdissection (LCM)**
  - A low energy laser melts a thermoplastic film onto the target cell
  - Film and attached cells are then lifted off of the slide
- **Both techniques provide a method of component separation from mixed samples**

# Laser Capture Microdissection

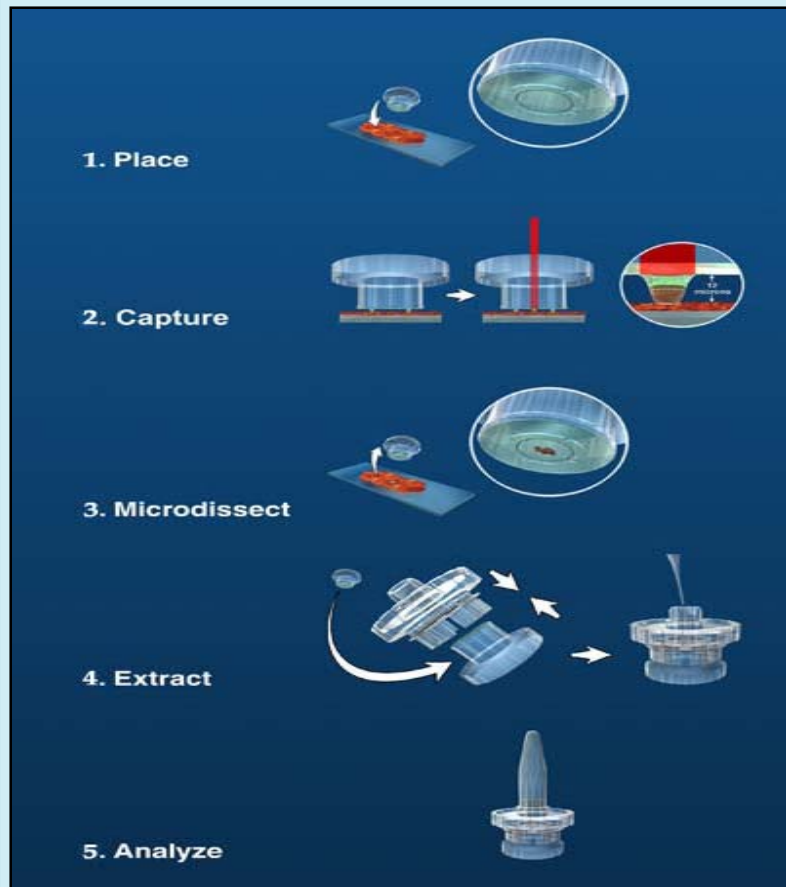


Image from: Arcturus® CapSure® Product Guide

1. Place a polymer cap onto the slide covering the samples of interest.
2. Target and fire the laser through the cap at each cell of interest. The thin film on the cap melts and adheres to the sample.
3. Remove the cap and attached samples from the slide.
4. Place the cap into an extraction collar.
5. Extract the samples.

# *Laser Microdissection*

1. Place a glass slide containing samples of interest on the stage above or below the objective depending on the instrument utilized.
2. Fire the laser equipped with the instrument through the objective onto the sample of interest.
3. Cut the desired areas of the sample.
4. Depending upon the system employed, the sample will either fall or be catapulted into the cap of a collection tube.
5. Extract the samples.

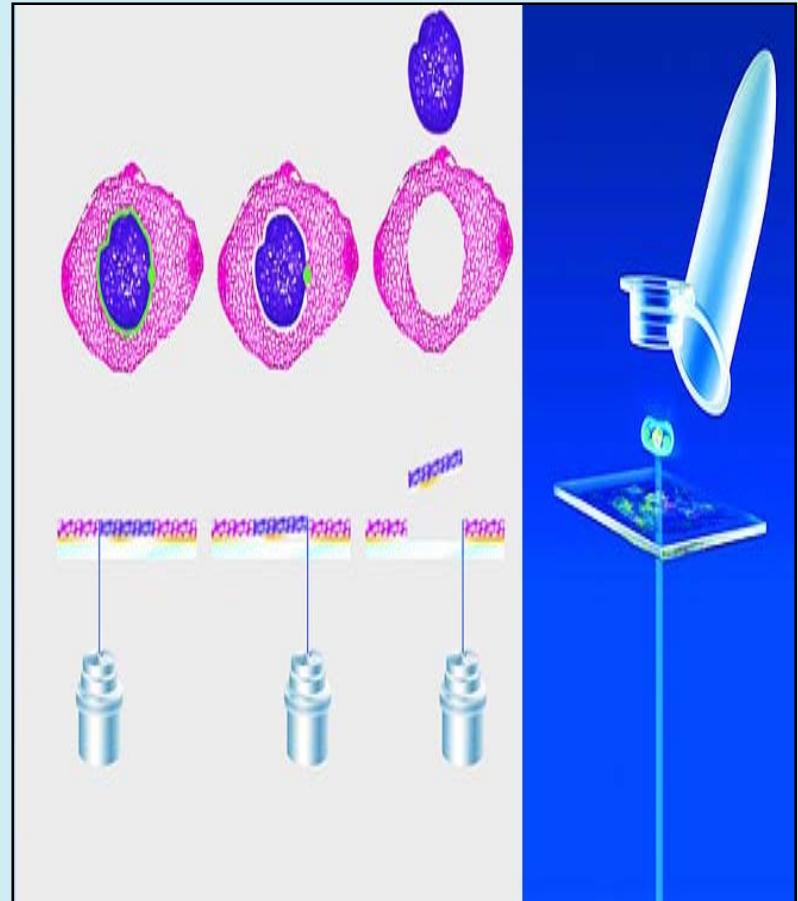


Image from: Carl Zeiss® PALM® Product Guide

# ***LM Instruments***

## ***Carl Zeiss® PALM® MicroBeam System***

- **Flexible applications from archival material to living cells for DNA isolation**
- **Patented laser catapult technology for contact- and contamination-free specimen capture**
- **Automated script search programs for items of interest**
- **Optimal workflow with simple component integration: from individual experiments to automation**

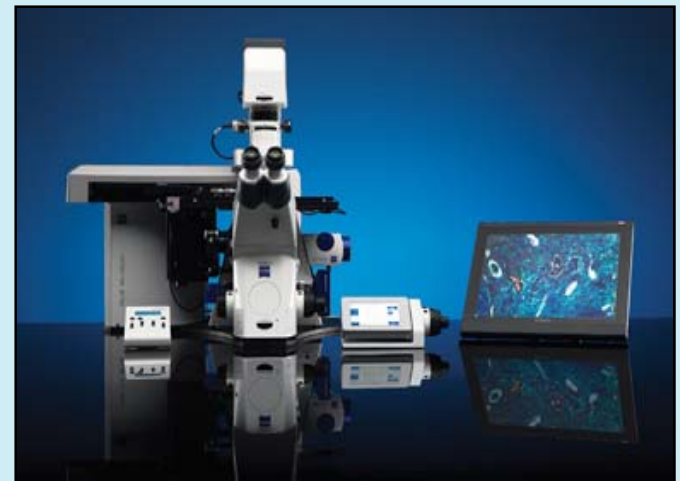


Image courtesy of: <http://www.zeiss.de/microdissection>

# ***LM Instruments***

## ***Arcturus<sup>XT™</sup>***

- **Two methods of collection:**
  - **Laser capture  
Microdissection**
  - **UV Laser Cutting**
- **Offers an open, modular platform with simple, user-friendly operation**
- **Touch screen user interface**
- **Flexibility in sample source and preparation**



Image courtesy of:

<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=607527>

# ***LM Instruments***

## ***mmi CellCut<sup>®</sup>***

- **Combines several proven technologies for precise and quick isolation**
- **Imaging software allows the user to easily select, cut, and collect cells**
- **Utilizes special membrane-covered frame slides which are placed upside down on a glass slide**
- **Samples are cleanly removed using the mmi IsolationCap<sup>®</sup>, which has no direct contact with specimens**

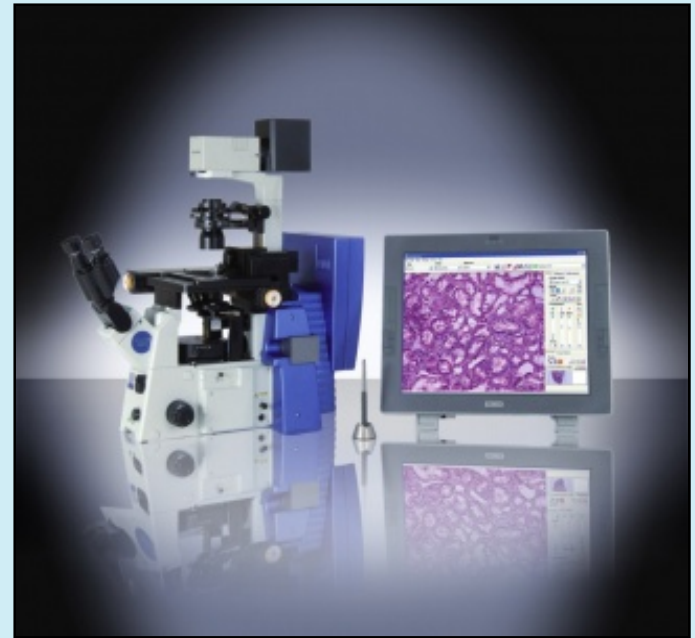


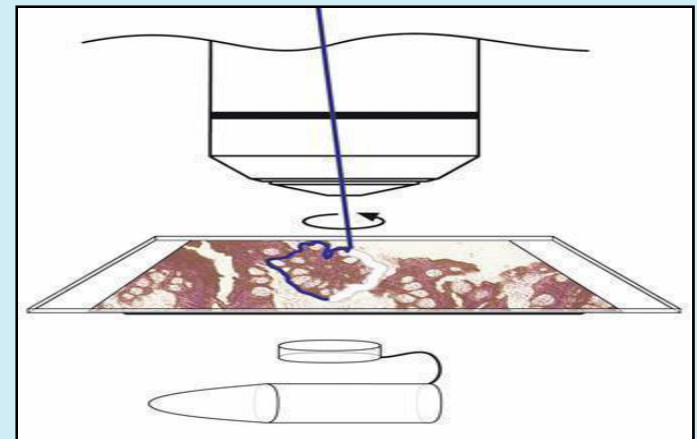
Image courtesy of: <http://www.molecular-machines.com/products/>



# ***LM Instruments***

## ***Leica™ LMD7000***

- **Combines both high power laser power and high pulse repetition rates**
- **High pulse repetition rates are ideal for the fast excision of single cells or clusters**
- **Utilizes gravity to gently collect samples in a contact-free manner**
- **Laser beam is controlled by high precision optics while the sample remains in fixed position**



Images courtesy of: <http://www.leica-microsystems.com/products/>

# ***Laser Microdissection: Processing Costs***

- **Normal LM processing:**
  - **Instrument purchase:**
    - ~\$175,000 - \$275,000
  - **Slides:**
    - Glass slides - \$0.25
    - PEN slides - \$4.00
    - PET slides - \$3.50
  - **Collection vessels:**
    - 0.5 ml tubes - \$0.15
    - Adhesive caps - \$5.00
- **FISH LM Processing:**
  - **Probes:**
    - ~\$30.00 - \$60.00 per slide
  - **Buffers and reagents:**
    - ~\$5.00 - \$10.00 per slide

# ***Laser Microdissection: Processing Costs (Continued)***

- **Totals:**
  - **LM additional costs per slide:**
    - ~\$0.40 - \$9.00 per slide
  - **FISH additional costs per slide:**
    - ~\$35.00 - \$70.00 per slide

# ***LM Forensic Research***

## ***Overall Objectives***

- **Develop and implement LM as a tool to resolve cellular evidence mixtures**
- **Develop protocols incorporating LM techniques to optimize low copy sample processing**
- **Identify and separate male from female cells of similar morphology based on FISH probe signals**



Image courtesy of Rob Driscoll

# *LM Forensic Research Systems Utilized*



Image courtesy of Rob Driscoll

## **Arcturus® PixCell® II**

- **Laser capture**

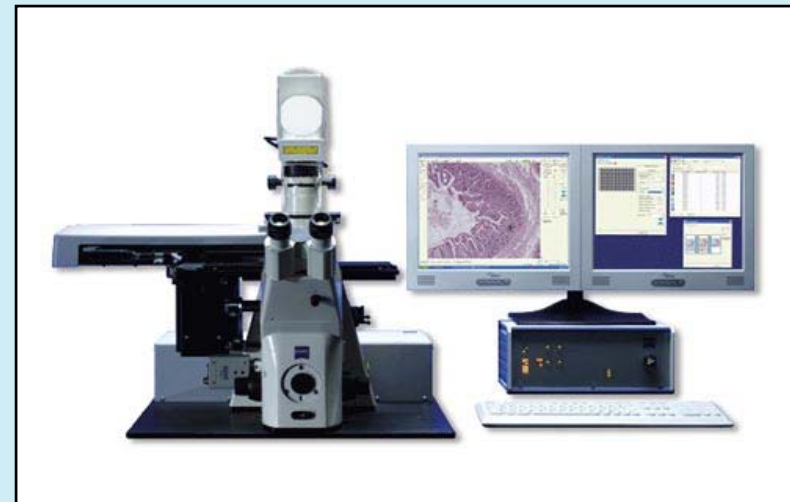


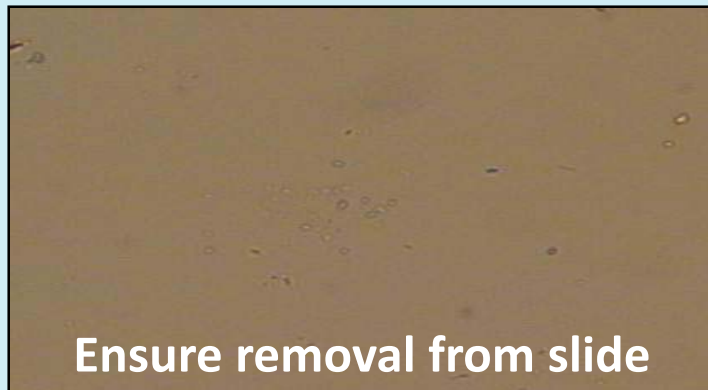
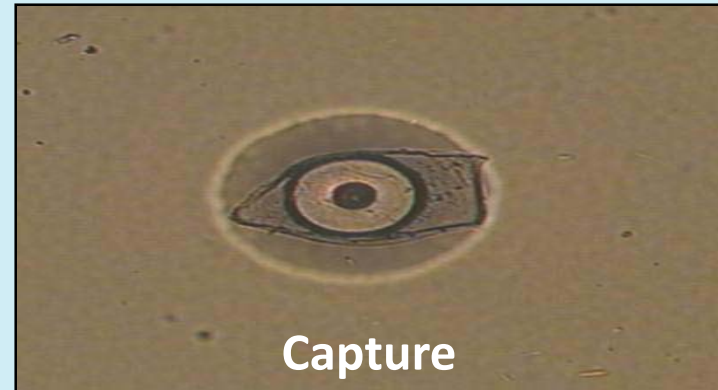
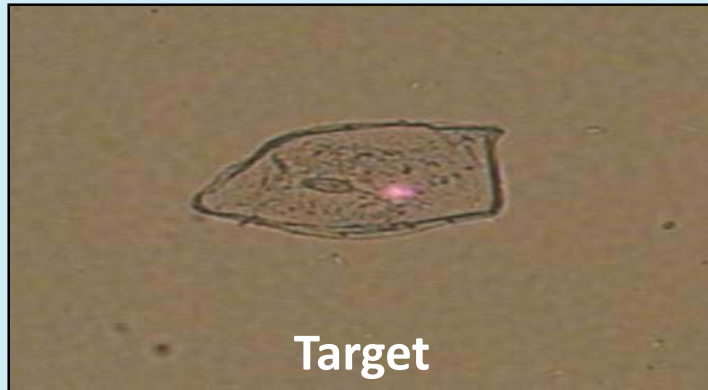
Image courtesy of: <http://www.zeiss.de/microdissection>

## **Carl Zeiss® PALM® MicroBeam**

- **Pulsed laser catapulting**

# ***LM Forensic Research***

## ***PixCell® II System Processing***



Images courtesy of Dane Plaza

# ***LM Forensic Research***

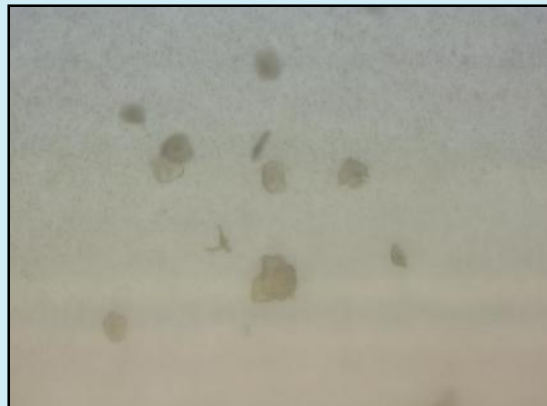
## ***PALM<sup>®</sup> MicroBeam System Processing***



**Locate cells**



**Empty cap**



**Cells in cap after catapulting**

Images courtesy of Rob Driscoll

# ***LM Forensic Research***

## ***Sperm Search Script Development***

- **The PALM<sup>®</sup> system includes automated scanning programs which allow one to differentiate and select targets based on size, shape and color**
- **Scripts were developed for the automated identification of sperm cells stained with:**
  - **Christmas tree stain**
  - **Haematoxylin**



Image courtesy of Rob Driscoll



# ***LM Forensic Research Sperm Search Script Development***

- **Overall, the scripts serve as an effective sperm search method for automated purposes but will not eliminate the need of an analyst to review the findings**



Image courtesy of Rob Driscoll

# ***LM Forensic Research***

## ***Epithelial Cell/Sperm Ratio Studies***

- **PALM<sup>®</sup> System was used to separate sperm cells from various samples containing sperm/vaginal cell mixtures:**

| <b><u>Ratio Slides</u></b> | <b><u>Aged Slides</u></b> | <b><u>Mock Evidence</u></b> |
|----------------------------|---------------------------|-----------------------------|
| <b>1:1</b>                 | <b>1 day</b>              | <b>100% cotton</b>          |
| <b>1:10</b>                | <b>7 days</b>             | <b>Blue denim</b>           |
| <b>1:100</b>               | <b>30 days</b>            | <b>Latex condoms</b>        |
| <b>1:1000</b>              | <b>6 months</b>           |                             |
|                            | <b>1 year</b>             |                             |

# ***LM Forensic Research***

## ***Epithelial Cell/Sperm Ratio Studies***

- **Approximately 200 sperm cells were separated and collected from the mixture located on each item of interest**
- **Approximately 150 individual collections were performed**
- **Clean, single donor male profiles were consistently seen from all ratio, aged, and mock evidence samples**
- **PALM<sup>®</sup> MicroBeam proved to be an effective tool for the separation of individual components of mixture samples**

# ***LM Forensic Research***

## ***LM as a Tool to Resolve Difficult Mixtures***

- **The work performed for the National Institute of Justice has focused on the further investigation of the various aspects and benefits of this burgeoning technology:**
  - **Physical separation and collection of male and female cells using the Arcturus® PixCell® II LCM and Carl Zeiss® PALM® MicroBeam systems**
  - **LM compatibility assessments of multiple extraction methods**
  - **The amplification of LM samples using various Applied Biosystems® and Promega™ STR kits**
  - **Identification of male and female cells using FISH techniques with Vysis CEP X® Alpha Satellite and CEP Y® Satellite III probes**

# ***LM Forensic Research***

## ***Investigated LM Extraction Techniques***

- **QIAGEN® QIAamp® DNA Micro and EZ1® Investigator Kits:**
  - Post LM, samples incubated in Proteinase K/ Buffer mixtures at 56°C for 1 to 2 hours
  - DTT added to sperm extractions
- **ZyGEM™ forensicGEM™ Kits:**
  - Post LM, samples incubated with thermophilic enzyme and optimized buffer mixture at 75°C for 15 minutes and at 95°C for 5 minutes
- **Sample concentration: Microcon® YM-100 columns**

# LM Forensic Research Results

## PowerPlex® 16 Profile Generated from 25 Epithelial Cells

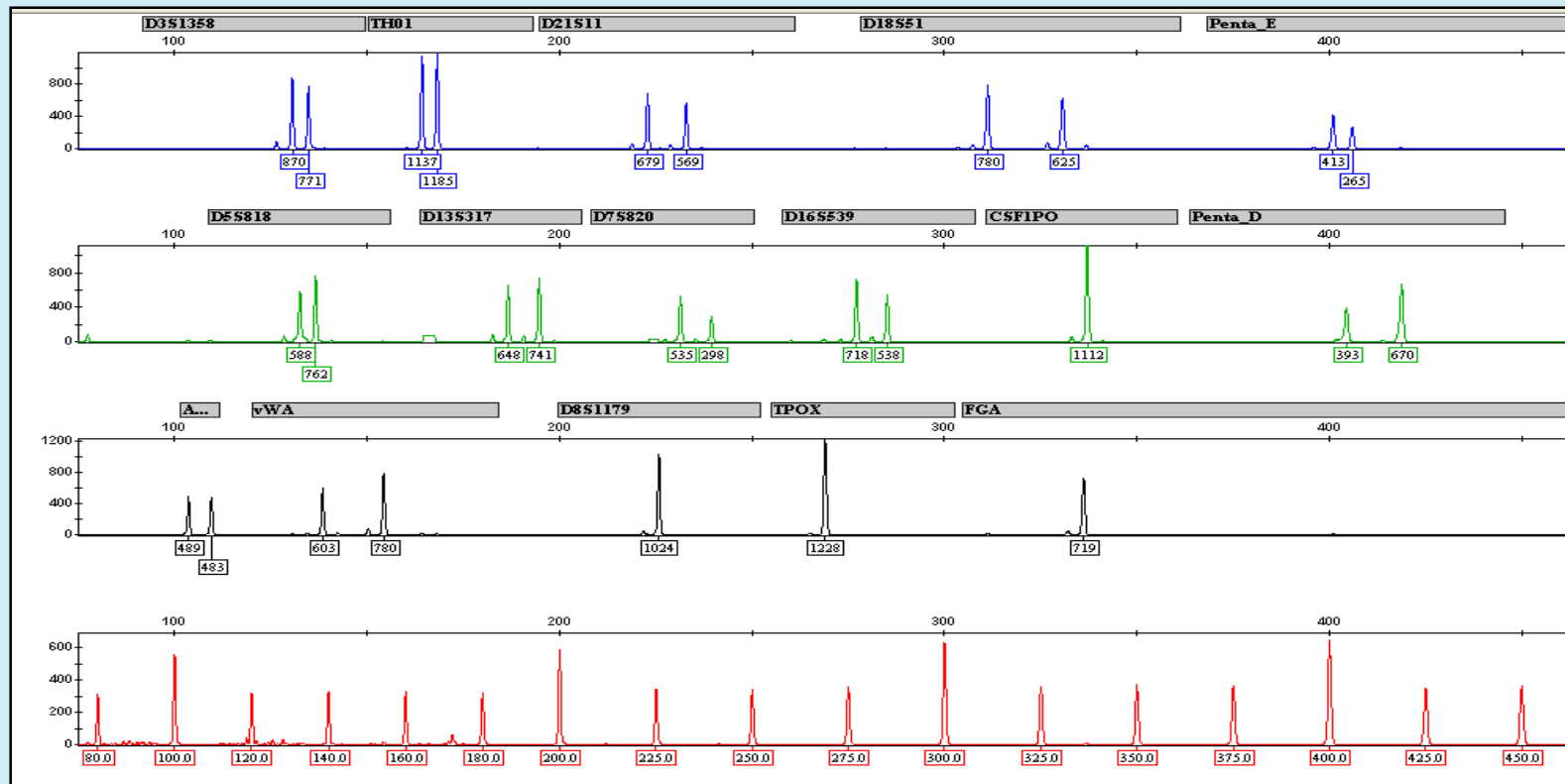


Image courtesy of Abby Bathrick

# LM Forensic Research Results

## Identifiler® Profile Generated from 10 Captured Epithelial Cells



Image courtesy of Dane Plaza

# LM Forensic Research Results

## Identifiler® Profile Generated from 5 Vaginal Cells Collected From a Sperm/Vaginal Epithelial Mixture

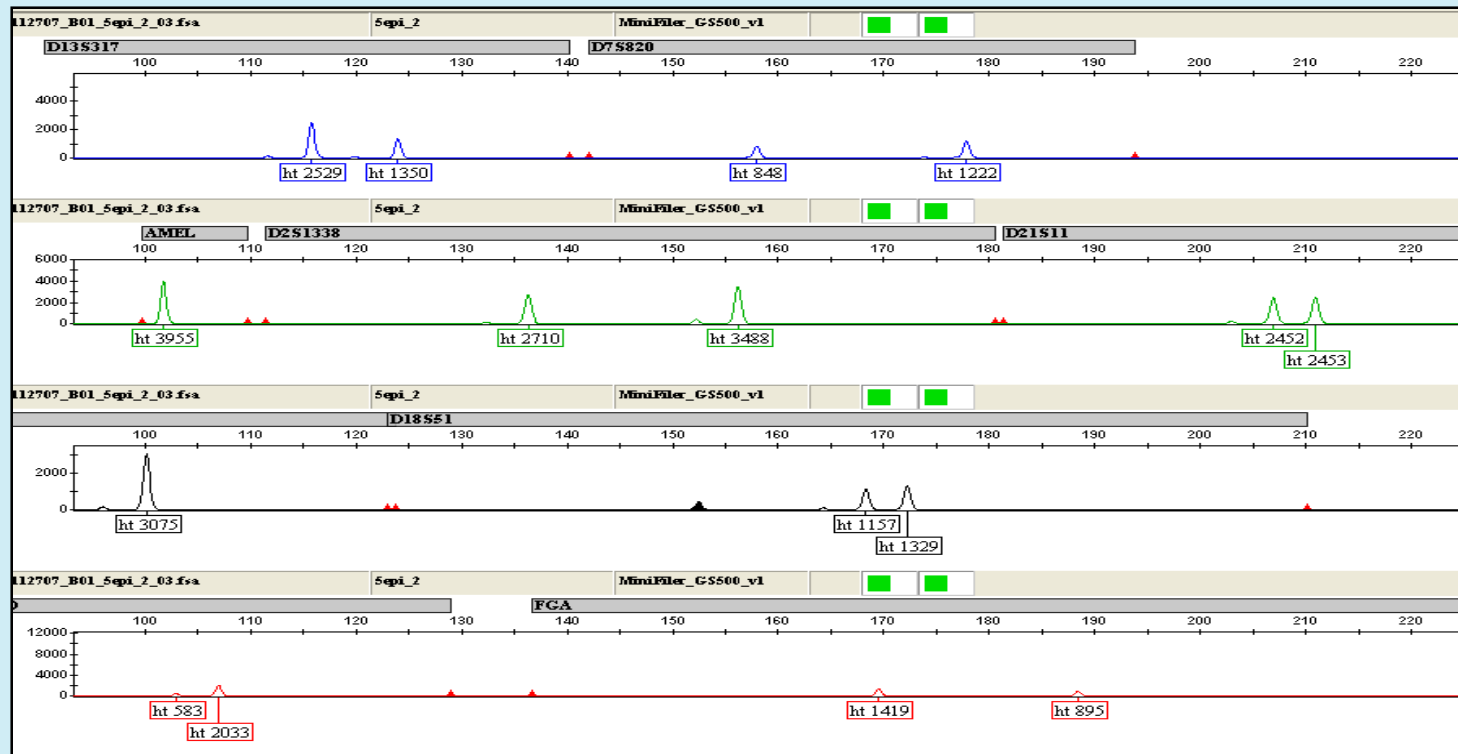


Image courtesy of Dane Plaza

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# LM Forensic Research Results

## Yfiler® Profile Generated from 10 Sperm Cells

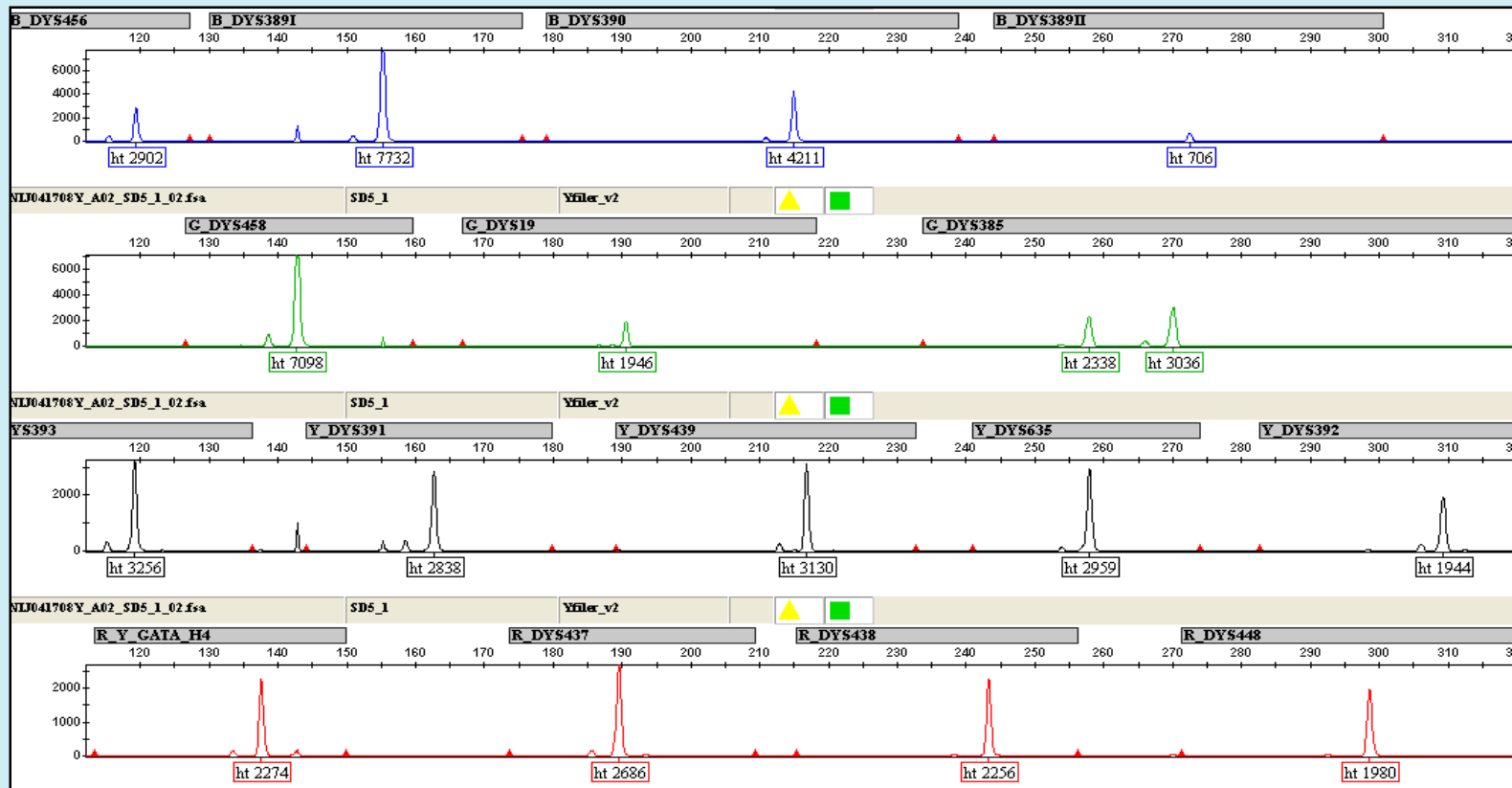
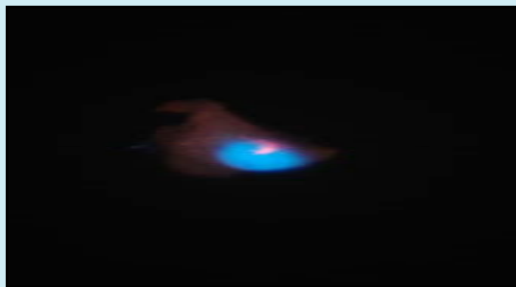


Image courtesy of Dane Plaza

# ***LM Forensic Research Results***

## ***Methodology of LM-FISH Processing***

- **FISH is a cytogenetic technique used to detect the presence or absence of specific genetic sequences**



- **Isolate cells of evidence mixtures, identify contributor by gender, separate using LM methods**
- **Profiles were generated from the separation of approximately 30 FISH treated male and female epithelial cells from a 1:1 mixture**

# ***LM Forensic Research Summary and Conclusions***

- **LM techniques provide a method of component separation from mixed samples in order to obtain a single source DNA profile**
- **The PALM<sup>®</sup> MicroBeam and Arcturus<sup>®</sup> PixCell<sup>®</sup> II have proven to be effective tools for the separation of mixture samples and the processing of low copy sample evidence types**
- **Clean, single donor male profiles were consistently seen from all ratio, aged, and mock evidence samples**

# ***LM Forensic Research Summary and Conclusions***

- **The combination of software scanning and human manual review can ensure the efficient processing of slides containing sperm and epithelial cell mixtures**
- **The QIAGEN® QIAamp® DNA Micro and EZ1® kits and ZyGEM™ forensicGEM™ extraction techniques reliably produce full/high partial profiles when used with LM**

# ***LM Forensic Research Summary and Conclusions***

- **The combination of LM and FISH methods has created a tool to effectively process previously unusable items of evidence**
- **Utilization of LM instrumentation and associated optimized techniques has allowed for the successful resolution of low copy number, sexual assault, and touch evidence cellular mixtures**
- **LM instruments are ideal for labs attempting to process difficult evidence containing low copy number (LCN) cellular mixtures**

***Questions?***

# ***Contact Information***

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