



**Technology Transition Workshop | *Robert Bever, Ph.D.***  
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## ***Closing Discussion***

# ***Laser Microdissection – Summary***

- **LM instruments are well established and utilized throughout the medical research community**
- **FISH techniques are routinely utilized in clinical cytogenetic laboratories**
- **LM and FISH have only just recently (< 6 years) been utilized within the forensic community**

# ***Laser Microdissection – Summary***

## ***Advantages***

- **LM techniques provide a method of component separation from mixed samples**
- **Clean, single donor male profiles have been consistently seen from:**
  - **Varying cellular ratio slides**
  - **Aged samples**
  - **Mock evidence items**

# ***Laser Microdissection – Summary***

## ***Advantages***

- **LM instruments are ideal for labs attempting to process difficult evidence containing:**
  - **Low copy number (LCN) samples**
  - **Sexual assault items**
  - **Touch evidence cellular mixtures**
- **The PALM<sup>®</sup> MicroBeam and Arcturus<sup>®</sup> PixCell<sup>®</sup> II have proven to be effective tools for the separation of samples at Bode Technology**

# ***Laser Microdissection – Summary***

## ***Limitations***

- **These are tools that should be considered a functional option when difficult and imperative evidence arrives in the laboratory**
- **LM should be employed when there is a known mixture sample located on items of evidence**

# ***Laser Microdissection – Summary***

## ***Limitations***

- **Incorporation of procedures of this type would provide alternative methods of sample processing for those labs utilizing LM technologies**
- **LM processing is not intended to be a high-throughput technique**

# ***Laser Microdissection Evidence Types***

- **LM techniques can provide a method of component separation from the following samples:**
  - **Sexual assault evidence mixtures:**
    - **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**

# ***Laser Microdissection Evidence Types (Continued)***

- **Touch evidence mixture types:**
  - **Male/female mixtures of blood/blood or blood/epithelial**
- **Other evidence types:**
  - **Bone extraction: collect nucleated cells from bone matrix**
  - **Hair: collection of nucleated cells from hair**
  - **Aged slides**
  - **Botany**



# ***Tested LM Processing Techniques***

- **When utilized, the following processing techniques reliably produce full and/or high partial profiles when used with LM:**
  - **Extraction**
    - **QIAGEN® QIAamp® DNA Micro Kits**
    - **QIAGEN® EZ1® Kits**
    - **ZyGEM™ forensicGEM™ Kits**
  - **Concentration**
    - **Microcon® YM-100 Centrifugal Columns**
  - **Amplification**
    - **Promega® PowerPlex® 16/PowerPlex® 16 HS Amplification Kits**
    - **ABI Identifler®/Identifler® Plus Amplification Kits**

# ***Tested LM Processing Techniques***

- **Recommended Workflow**
  - **LM Cells → ZyGEM™ Extraction → Microcon® Concentration → PowerPlex® 16 HS Amplification**
    - **With this workflow, as few as 25 cells may be successfully amplified without any alterations to manufacturer's protocols**
    - **Laboratories considering employing LM techniques should evaluate what extraction methods and amplification systems work best for them**

# *LM/FISH Summary*



- The combination of LM and FISH methods has created a tool to effectively process previously unusable items of evidence
- FISH processing utilizing X/Y probes is a novel method for differentiating male and female cells




Image courtesy of Rob Driscoll

# ***Future FISH Studies***

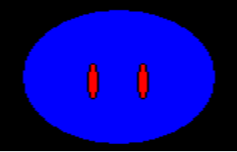
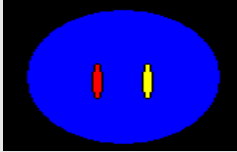
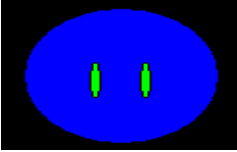
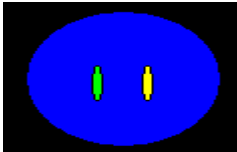
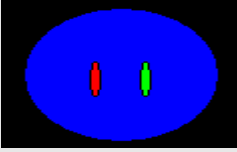
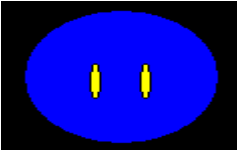
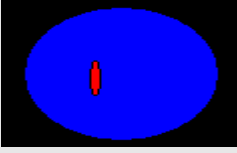
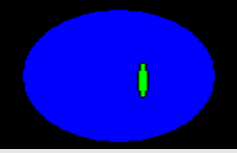
- **New systems are being explored that may allow for differentiation of cell mixtures of same morphology and gender**
  - **FISH probes designed to visually identify the genetic differences in the ABO blood group**
  - **FISH probes designed to visually identify single nucleotide polymorphisms (SNPs) differences in individuals**

# ***Future FISH Studies***

## ***Proposed ABO Probe Screening System***


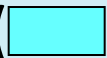

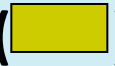


- The ABO screening system will consist of three fluorescent marking dyes (TRITC, FITC, and CY5)
  - Allele A subtype-specific probes will be tagged with red dyes  (TRITC)
  - Allele B subtype-specific probes will be marked with green dyes  (FITC)
  - Allele O subtype-specific probes will be labeled with yellow dyes  (CY5)
- Homozygous samples will exhibit monochromatic fluorescence while heterozygotes will be identified by the presence of two colors

# ABO Blood Group FISH Assay Scenarios

ABO Genotype	Visual #1	Visual #2
AA or AO		
BB or BO		
AB		None
OO		None
Failed (examples)		

# ***Future FISH Studies***

## ***Proposed SNP Probe Screening System***

- **The proposed SNP probe screening system will consist of six fluorescent marking dyes:**
  - Red ()
  - Aqua ()
  - Green ()
  - Gold ()
  - Orange ()
  - Far red ()

# ***Future FISH Studies***

## ***Proposed SNP Probe Screening System***

- **The screening panels will target 10 SNP loci that have high heterozygosity, low genetic variance, and are unlinked**
- **A testing set of two panels (slides) will be constructed for an evidence sample with each panel targeting five specific sites**



# *LM/FISH Implementation*

- **Bode Technology anticipates the validation and implementation of LM/FISH techniques in the near future**



Images courtesy of Rob Driscoll and Abby Bathrick

# Commerically Available LM Instruments

- **Arcturus<sup>XT™</sup>**
  - Life Technologies:
  - [www.appliedbiosystems.com](http://www.appliedbiosystems.com)
- **PALM<sup>®</sup> MicroBeam**
  - Zeiss:
  - [www.palm-microlaser.com](http://www.palm-microlaser.com)
- **mmi CellCut<sup>®</sup>**
  - Molecular Machines and Industries:
  - [www.molecular-machines.com](http://www.molecular-machines.com)
- **Leica<sup>™</sup> LMD7000**
  - Molecular Devices:
  - [www.leica-microsystems.com](http://www.leica-microsystems.com)

# ***Discussion for Labs with LM Capabilities***

- **What instrument does your lab possess?**
- **What challenges have you faced with implementing the new technology?**
- **Have you experienced any successes?**
- **Any advice for those labs considering a purchase or encountering difficulties?**

# ***Discussion for Labs without LM Capabilities***

- **Do you see LM instrumentation as a useful tool for your laboratory?**
- **Do you see FISH as a useful tool for your laboratory?**
- **Do you have cases these techniques may be useful for?**
- **What do you see as the advantages and disadvantages of LM/FISH technology?**

***Final Questions?***  
***Final Comments?***

# Contact Information

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