Technology Transition Workshop|

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Welcome and Introductions

Introductions

- **Bode Technology**
  - **Robert Bever, Ph.D.**
  - **Robert Driscoll, M.F.S.**
  - **Heather Cunningham, M.S.**
  - **Abigail Bathrick, M.F.S.**
- **NFSTC**
  - **Joan Ring, M.S.**
  - **Karolyn Tontarski, M.S.**

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Bode Technology Overview

- **Provides forensic DNA analysis, DNA collection products, and research services to law enforcement agencies, federal and state governments, crime laboratories, and disaster management organizations**
- **Identified criminals in nearly every U.S. state, as well as victims of war, terrorism, crime, and natural disasters throughout the world**

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Bode Technology Overview

- **Identified remains of U.S. soldiers and victims from the World Trade Center attack, the war in Bosnia, Hurricane Katrina, and the Thailand tsunami**
- **Over 12 years of experience in the development of techniques associated with the analysis of challenging DNA forensic samples**
- **Processed over 1 million convicted offender samples, thousands of touch evidence samples from burglary and property crimes, and hundreds of chemically processed fingerprints samples**

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Bode Technology Facilities

- **ASCLD/LAB, FQS-I ISO/IEC 17025 accredited**
- **Laboratories have seamless floors, pass-through windows, HEPA-filters, and controlled access**
- **Large pre-amp laboratory with smaller connected laboratories for screening, extraction and research and development work**

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Bode Technology Facilities

- **Post-amp laboratory equipped with:**
  - **Nine 3100 and four 3130*xl* Applied Biosystems™ Genetic Analyzers**
  - **Two Applied Biosystems™ 7000 Real-Time PCR Systems and three 7500 Real-Time PCR Systems**
  - **Twenty-four 9700 and three 9600 Applied Biosystems™ Thermal Cyclers**
- **Arcturus® PixCell® II and Carl Zeiss® PALM® MicroBeam laser microdissection (LM) systems**

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Safety

- **Personal Protective Equipment (PPE)**
  - **Always wear proper clothing and footwear**
    - **Legs and feet must be completely covered at all times**
  - **Always wear a lab coat, goggles, and gloves in the labs**
  - **Wear a respirator when handling hazardous chemicals**
- **Eyewash/shower stations**
  - **Stations are located in each of the labs**
    - **Running water stations (Main Lab and Research)**
    - **Saline eye rinse stations (Serology only)**
  - **Flush eyes or stand under shower for at least 15 minutes**

Safety

- **MSDS - Read every MSDS prior to chemical usage**
  - **Hazardous**
    - **Hybridization Buffer - contains formamide**
    - **Formalin - formaldehyde solution; toxic by inhalation, absorption, and consumption; corrosive and carcinogenic**
    - **Magnesium chloride hexahydrate - requires extreme caution; has delayed effects on CNS, kidneys, and GI tract through inhalation, absorption, and consumption**
    - **Hydrochloric acid - corrosive**
  - **Most others are irritants**
  - **Wear respirator when handling hazardous chemicals**
  - **Wash your hands upon exiting the lab**

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Safety

- **Evacuation procedures**
  - **In the event of an emergency, listen to and follow the instructor to the designated meeting point outside of the building**
- **Chemical spill or medical emergency**
  - **If a chemical is spilled or someone needs medical attention, immediately ask the lab instructor to contact the Assistant Safety Officer, Hannah Gillis**
  - **If for some reason the lab instructor is unavailable, use the intercom to page Hannah Gillis (x601)**
- **If you have any questions, please ask**

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Experience with LM Techniques

- **FBI Contract J-FBI-05-178**
  - **Investigate and determine which LM instrument is best for separating the individual components of sexual assault mixtures**
  - **Develop automated sperm search programs for the chosen LM instrument**
  - **Evaluate effectiveness of instrument when used with samples of various age, ratio, and originating substance**

Experience with LM Techniques

- **NIJ Contract # 2006-DN-BX-K032**
  - **Physical separation and collection of male and female cells using the Arcturus® PixCell® II and Carl Zeiss ® PALM® Microbeam laser microdissection systems**
  - **Identification of male and female cells using fluorescent in situ hybridization (FISH) techniques**
  - **Optimization of downstream processing for LM collected samples**

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Experience with LM Techniques

- **NIJ Contract # 2008-IJ-X-K016**
  - **Separate cellular mixtures of similar morphology and same gender by using sequence-specific FISH probes based upon the genetic polymorphisms associated with the Duffy and ABO blood groups**
- **NIJ Contract # 2009-DN-BX-K250**
  - **Separate cellular mixtures of similar morphology and same gender by using sequence-specific FISH probes based upon human SNP genetic variations**
  - **Use of laser microdissection to optimize front-end LCN sample processing techniques**

Workshop Purpose

- **Provide workshop attendees with an introduction to the:**
  - **Forensic uses of LM instruments**
  - **Various platforms available**
  - **Techniques available for sample processing**

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Agenda Review

Workshop attendees will split into two groups of 6:

<u>Lecture Session</u>:

- **Introduction to LM**
- **Introduction to FISH**
- **LM methods**
- **Role of LM and FISH in the forensic laboratory**
- **Overview of commercially available LM instruments**
- **Closing remarks**

<u>Laboratory session</u>:

- **Slide preparation**
- **FISH methods**
- **Separation of mixture samples via LM**
- **Sample extraction, amplification, and data interpretation**

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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**

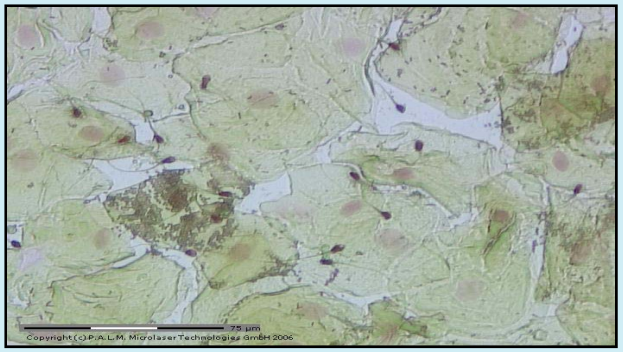

Image courtesy of Rob Driscoll

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Laser Microdissection

- **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 History

- **A biomedical technique used for isolating cells of interest from tissues or mixtures**
- **Laser is focused upon the surface of a specimen slide to "cut" or "separate" sections of interest**
- **Technology first emerged in the late 1960s and early 1970s using argon lasers**
  - **Instrumentation was not suitable for routine use**
  - **Specificity of dissection points was not optimal**

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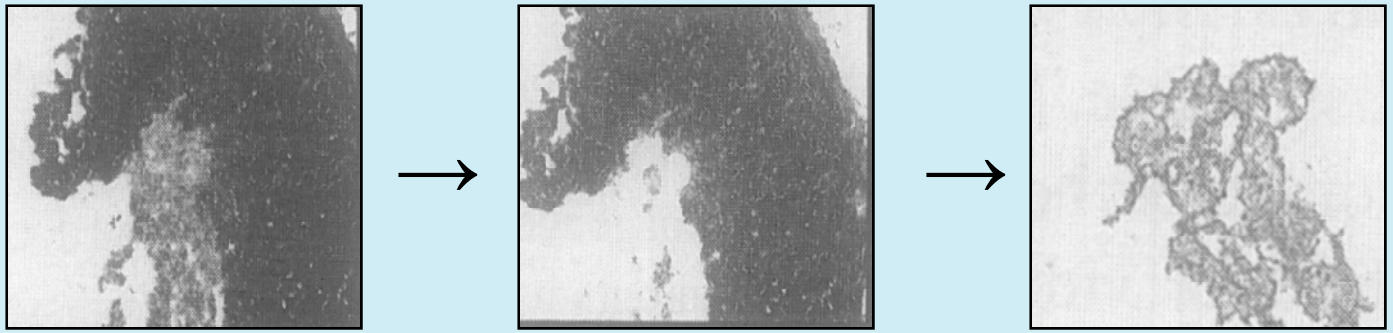
LM History

- **Revival of technique occurred during the mid-1990s at the National Institutes of Health (NIH)**
  - **UV lasers and laser capture microdissection**
- **Now routinely used in medical research for DNA, RNA, and protein analysis**



Images from reference # 10 (See Handout, Relevant Scientific Literature)

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LM History

- **LM can be performed on a variety of biological specimens:**
  - **Solid tissue**
  - **Cytologic preparations**
  - **Blood smears**
  - **Paraffin embedded tissue**
  - **Archival slides**
- **LM has only just recently (< 6 years) been utilized within the forensic community**

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Interphase FISH Techniques

- **FISH is a cytogenetic technique used to detect the presence or absence of specific chromosomes and/or sequences**
- **Interphase FISH techniques incorporate probes which pass through cellular membranes and into the nucleus eliminating the need to lyse cells during processing**
- **Fluorescence compatible microscopes are typically employed to visualize the multicolor probes used in these hybridizations**

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FISH History

- **In situ hybridizations (ISH) utilizing probes labeled with radioisotopes were first performed in the late 1960s**
- **Fluorescent in situ hybridization (FISH) was developed as an alternative method in the 1980s**
  - **Use of fluorescent probes led to increased resolution, speed, and safety**
  - **Allowed for detection of multiple targets, quantitative analysis, and live cell imaging**

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FISH History

- **Use in cytogenetics**
  - **Whole chromosome painting**
    - **Deletions and duplications of chromosomal regions are detected by different fluorescent signals**
  - **Detection of diseases caused by chromosomal abnormalities**
    - **Down Syndrome**
  - **Cancer prognosis**
    - **Breast cancer – detection of multiple copies of gene HER2**

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FISH History

- **Chromosome painting: FISH identification of human chromosomes**
  - **Fluorescent probes specific to regions of particular chromosomes are hybridized to a chromosome spread**
  - **Small variations in fluorescence are detected and enhanced**
  - **The resulting computer generated image is called a "false color" image**

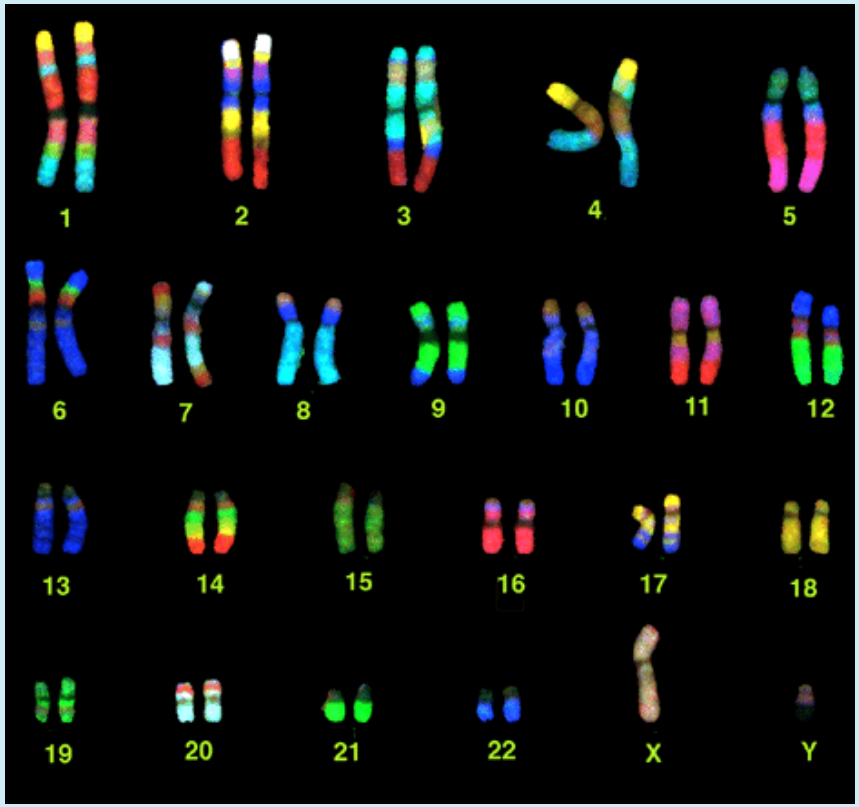

Image from reference # 5 (See Handout, Relevant Scientific Literature)

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LM and FISH in Forensics

- **LM and FISH processing provide a tool for processing previously unusable items of evidence**
  - **Mixture is detected**
  - **Sample is processed with LM/FISH**
  - **Clean, interpretable profiles are generated**

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Questions?

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