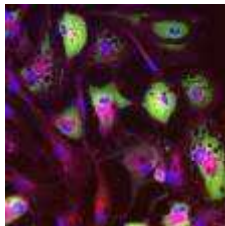


**Arcturus<sup>XT</sup> Laser Capture Microdissection System:  
Optimized Protocol for Laser dissection of living  
cells and Microgenomics**



**Dr. Amy Lam  
Field Application Scientist**





# Agenda

- Introduction to Laser Capture Microdissection (LCM)
  - Why Use LCM?
  - LCM Technology
  - Arcturus® LCM Systems
- Arcturus® LCM System Applications
- The Microgenomics Process
- LCM Consumables



# Why Use Laser Capture Microdissection (LCM)?

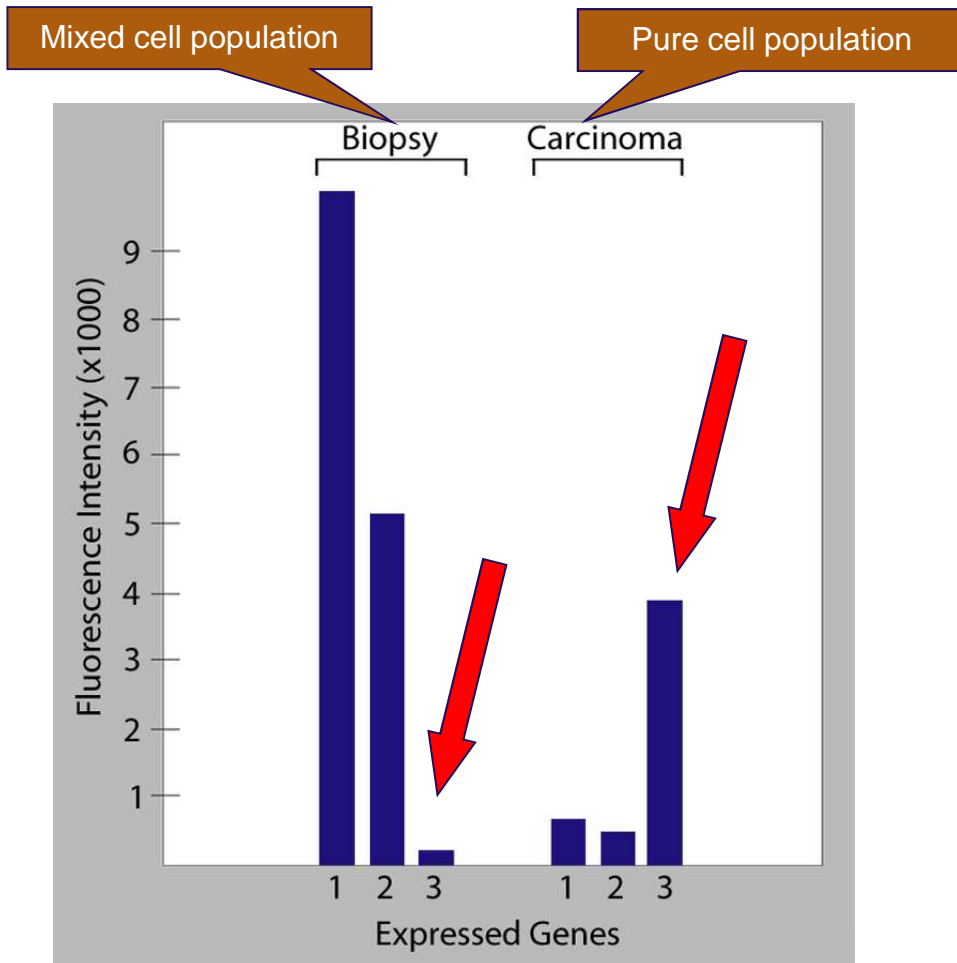
“Pure populations”

versus

“mixed populations”

To help reveal accurate, cell-specific data otherwise obscured in mixed cell samples

# See What You've Been Missing



Whole tissue biopsy or tissue section



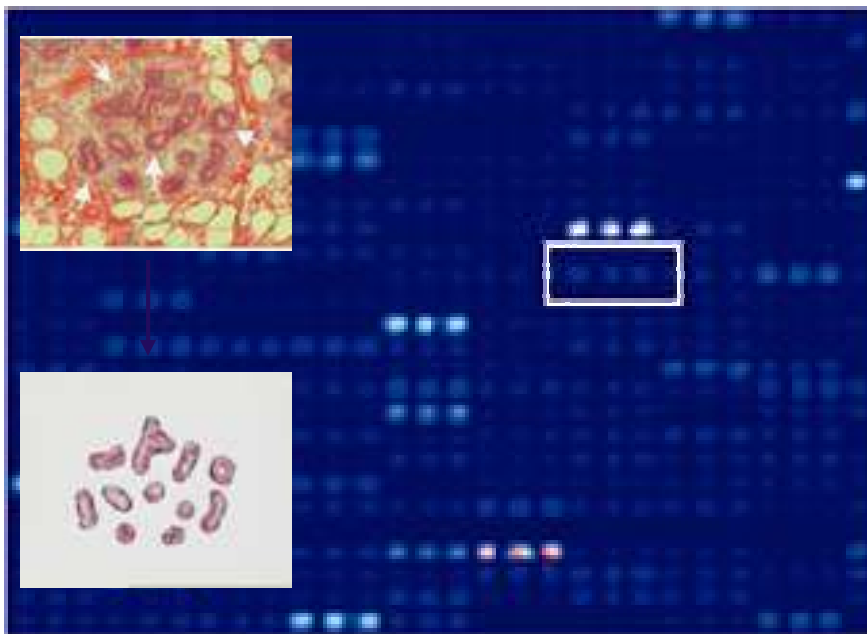
Microdissected cells



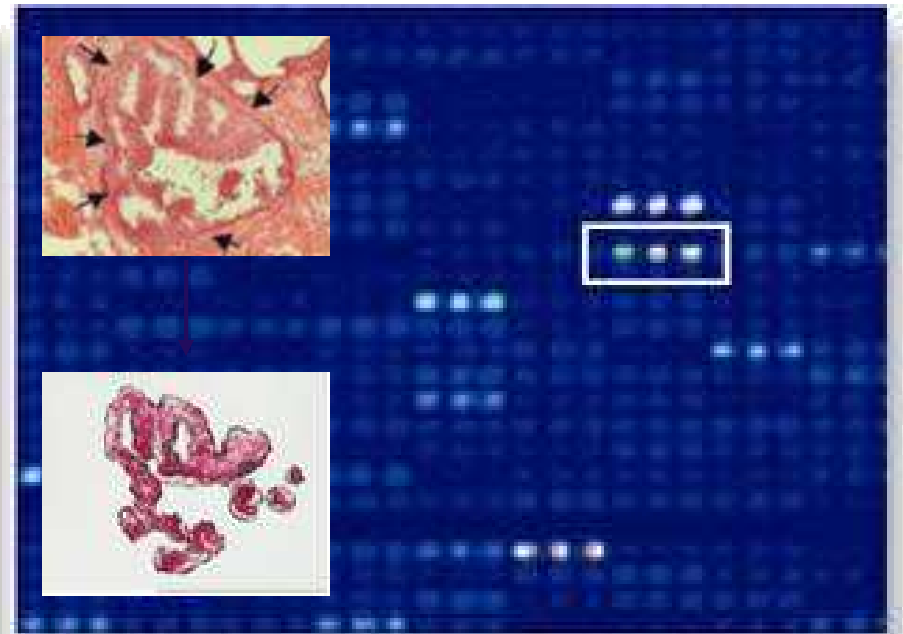
LCM uncovers molecular signatures hidden by whole biopsies and tissue scrapes

# Differential Gene Expression

Normal Breast Tissue

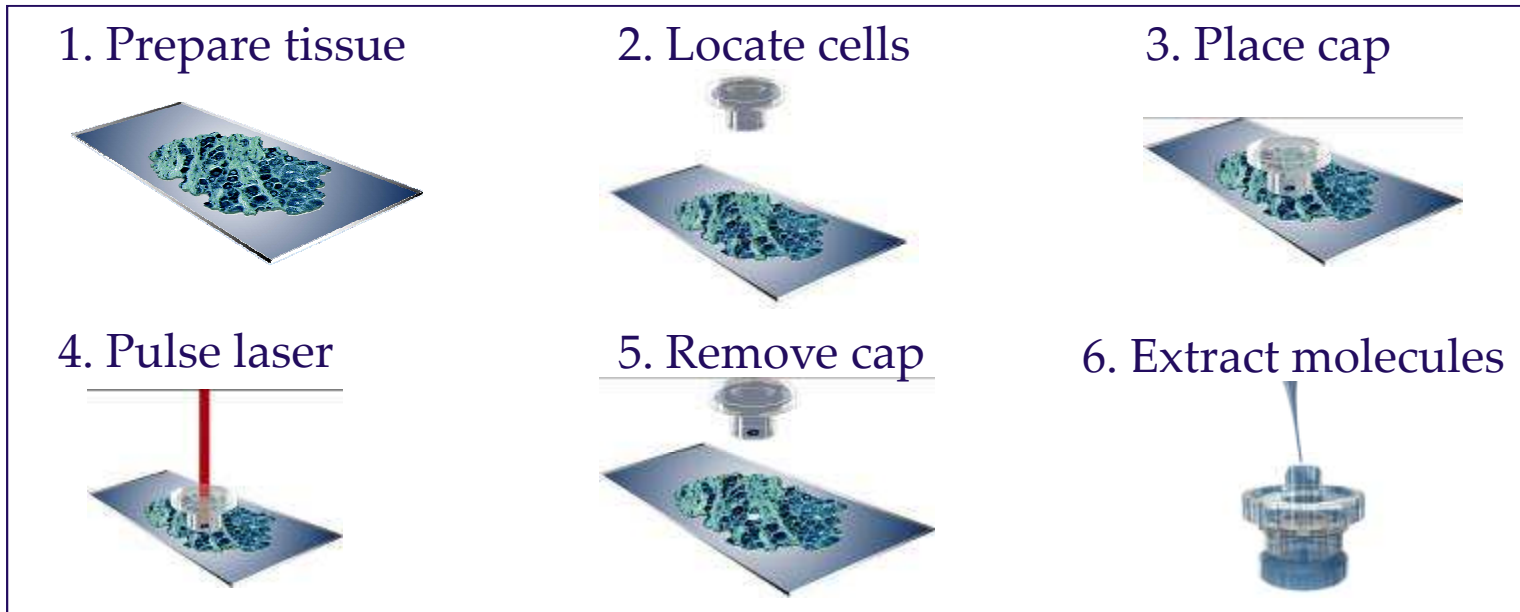


Breast Carcinoma

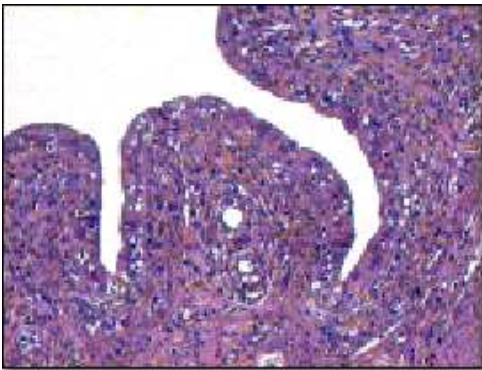


LCM reveals breast cancer-specific molecular signatures

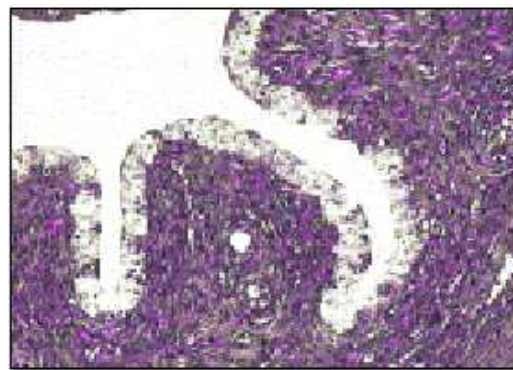
# The LCM Process



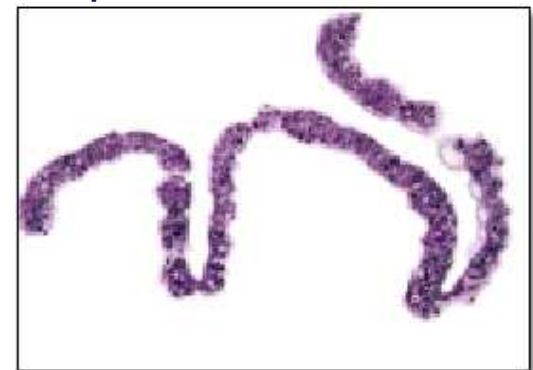
Before LCM



After LCM



Captured LCM Cells





# How LCM Works

- Near-Infrared (IR) laser activates thermoplastic polymer transfer film
  - Thermoplastic film:
    - > Absorbs IR laser energy
      - Prevents laser energy from reaching sample
      - Laser energy never directly absorbed by sample
    - > Becomes adhesive
      - Polymer film activates and melts near 70° C
      - Sticks to cells of interest
    - > Increasing IR energy increases activated film area
    - > Distends predictably, evenly, reproducibly to enable selective targeting
    - > Adhesion overcomes opposing forces to enable selective capture
- Cell(s) removed with polymer

# Arcturus<sup>XT</sup>™ LCM Instrument



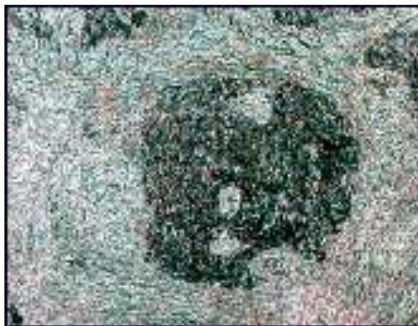
**Flexible and Modular  
Dual IR and UV LCM System**



# Advantages of Arcturus LCM

- Low energy near-IR laser does not damage proteins or nucleic acids
- Single cells, small # of cells and intricately shaped areas are easily captured
- Maintains the morphology of captured cells
- Adjacent tissue is neither altered nor destroyed
- Contact capture method: cells are precisely located, inspected and imaged after microdissection

Never lose custody of your sample!



Before LCM

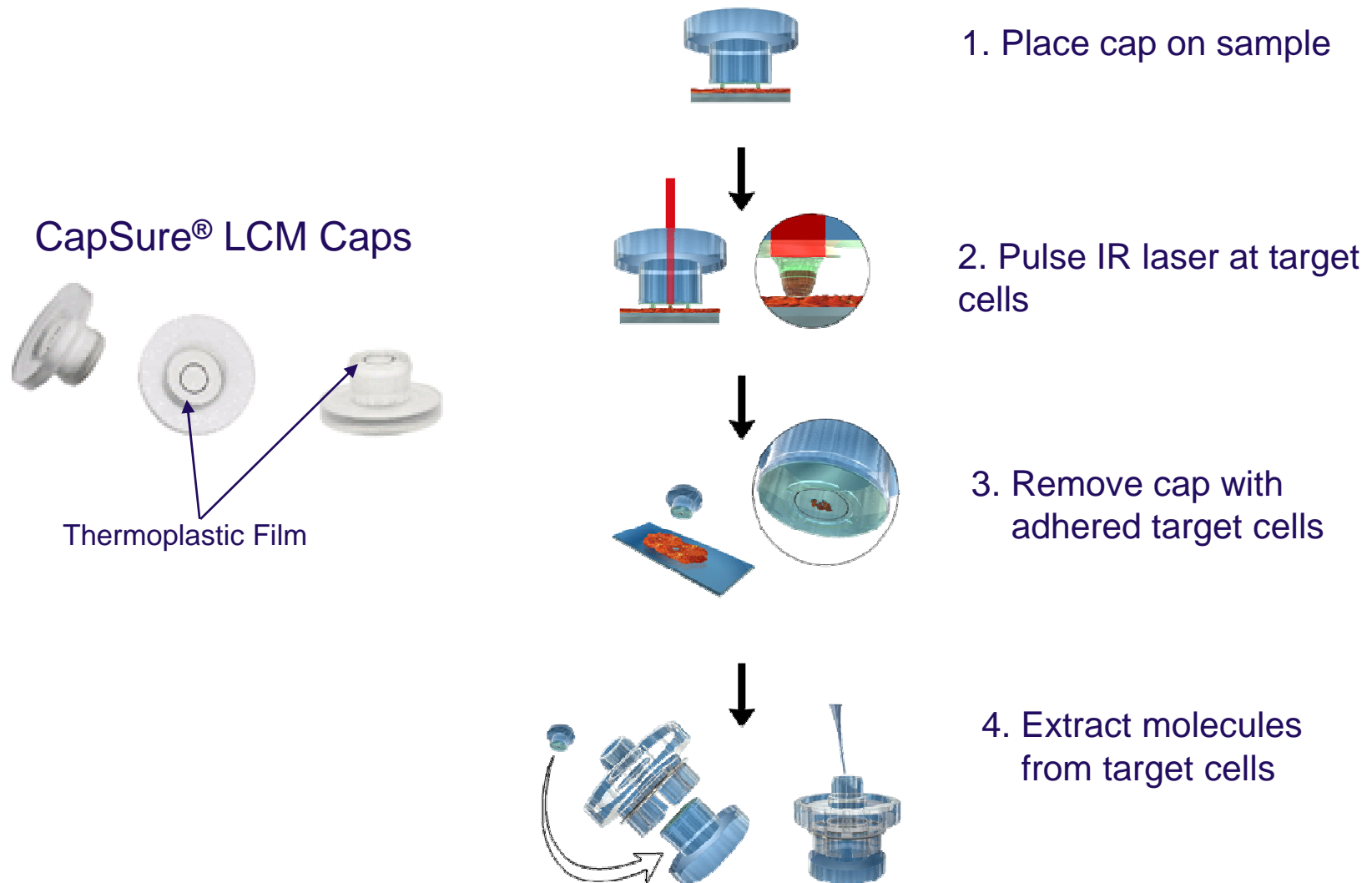


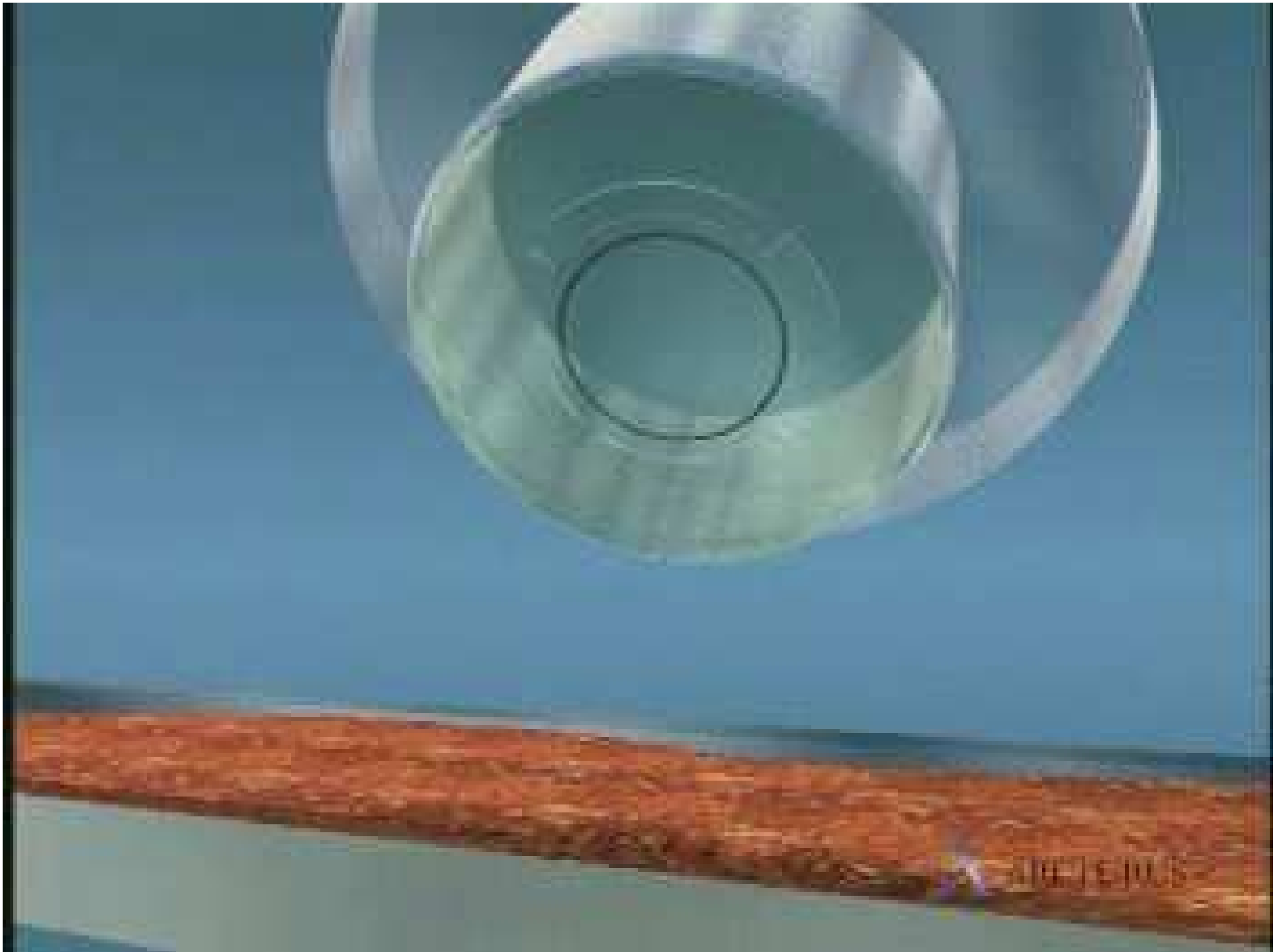
After LCM



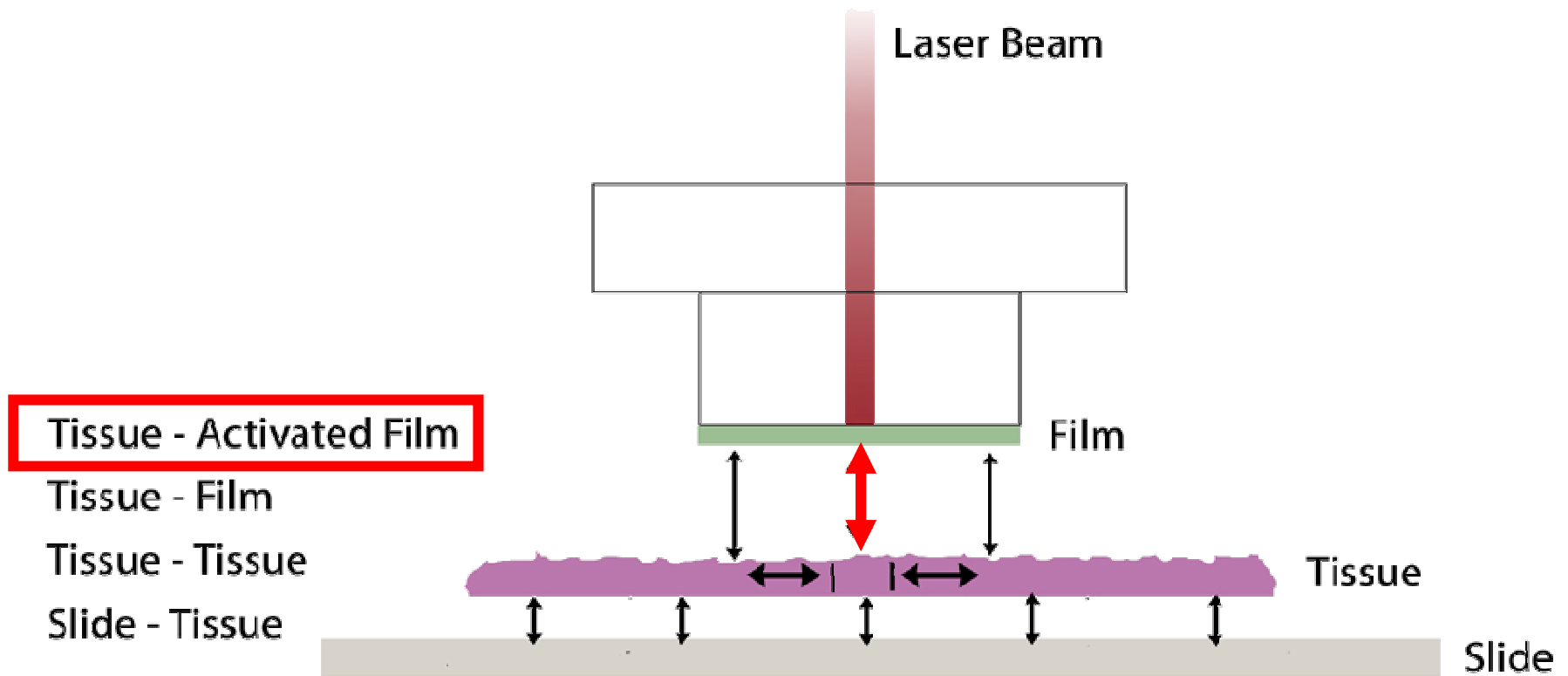
CapSure® Cap

# Arcturus<sup>®</sup> LCM System Process



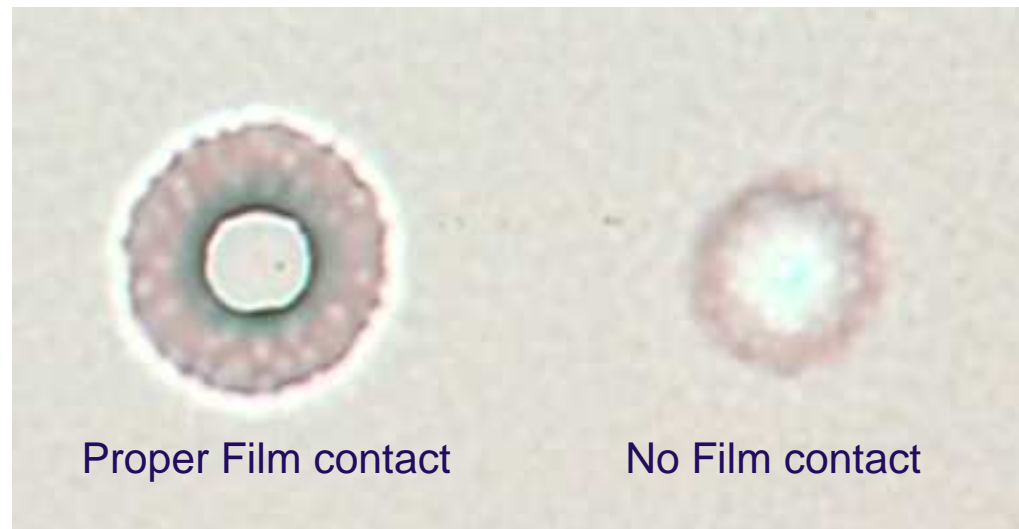


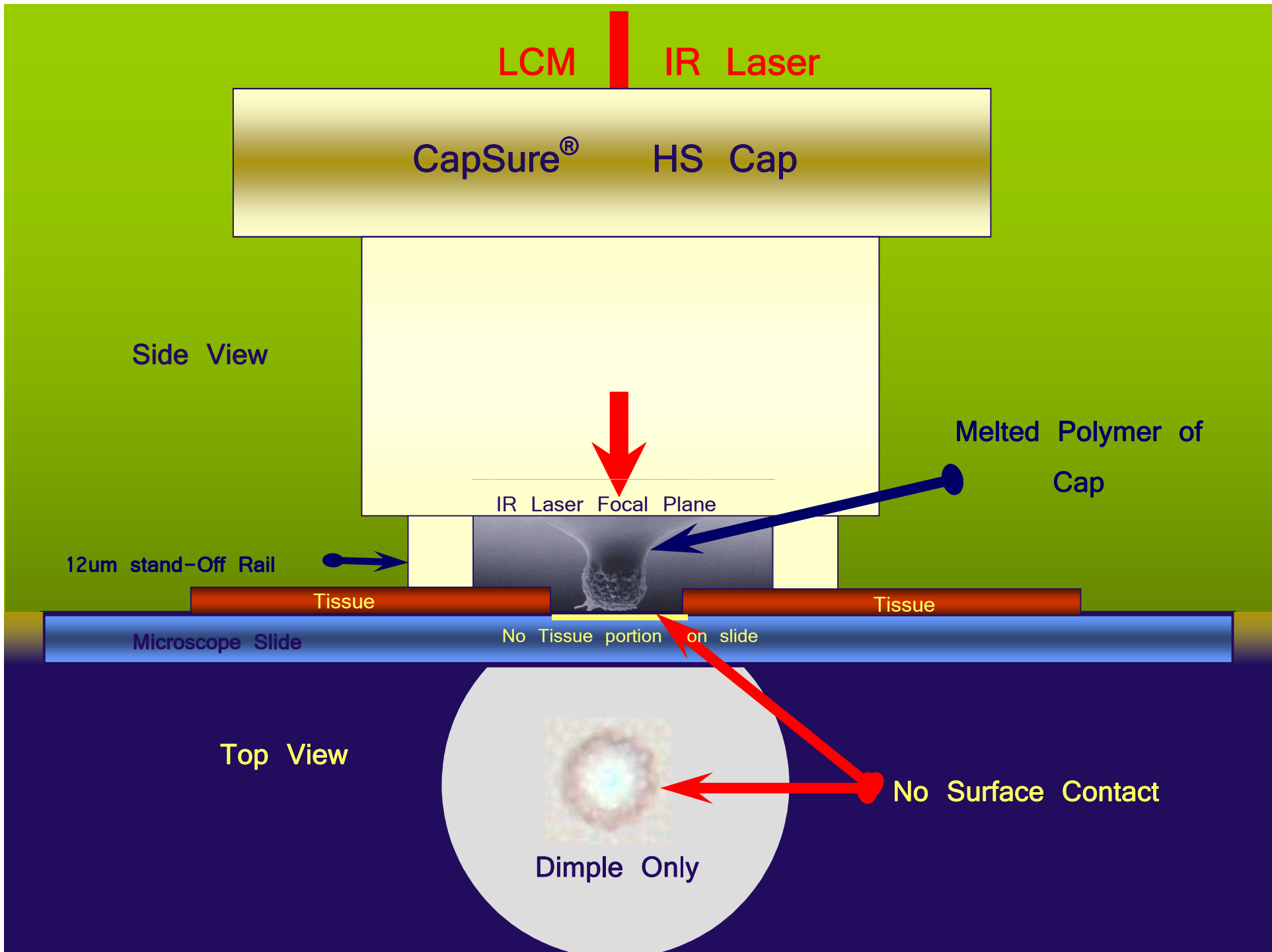
# Mechanical Forces of LCM

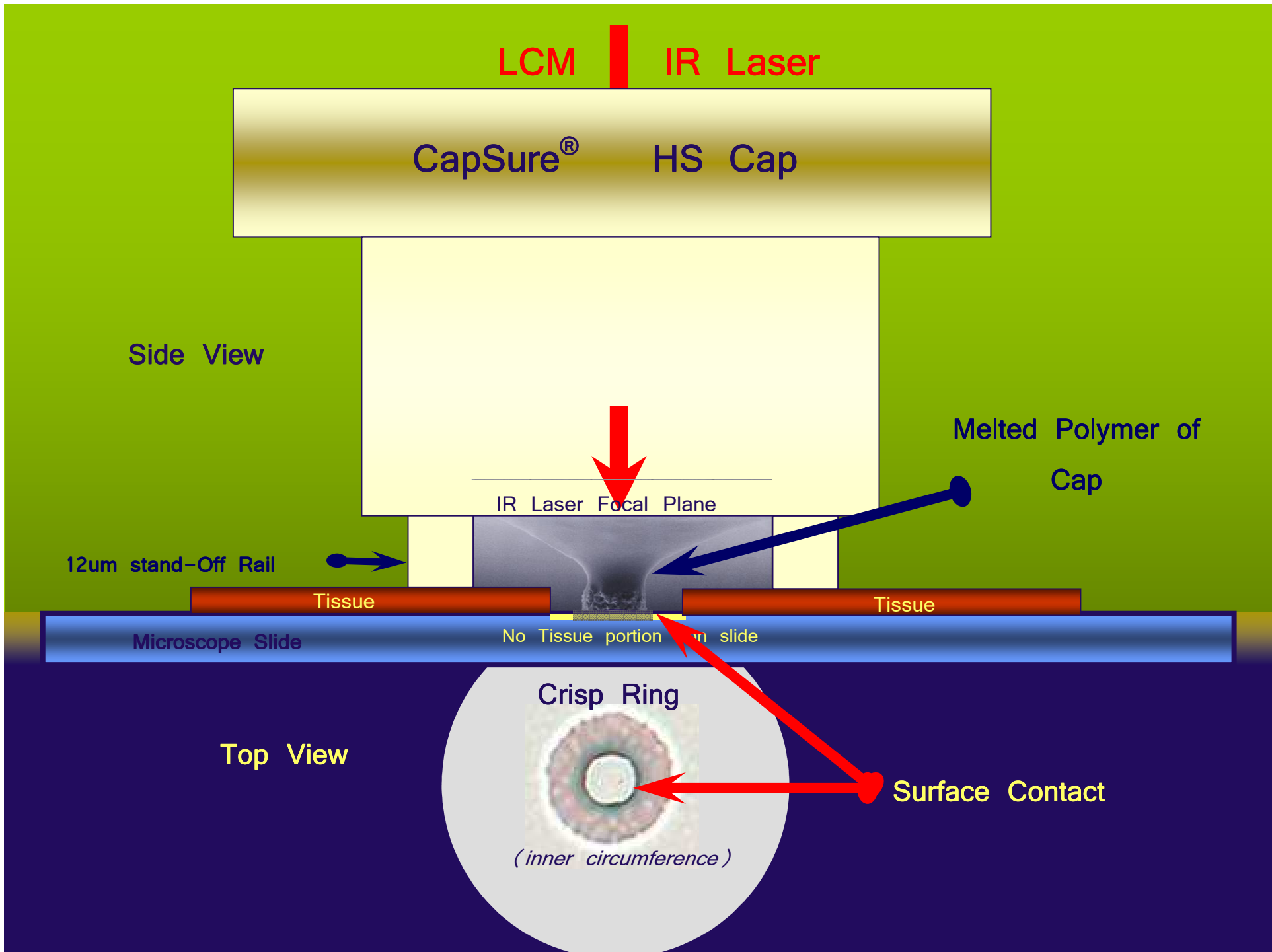


# The LCM “Spot”

- Activated film must contact the specimen
  - Adjust IR laser power and duration settings to ensure polymer film touches the glass
  - If film touches glass, IR laser and LCM cap are ready for capture



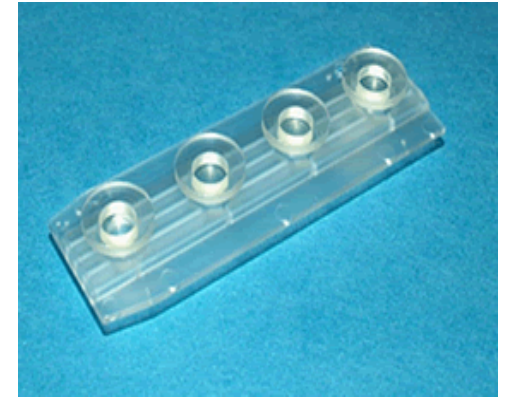




# CapSure<sup>®</sup> LCM Caps



- CapSure<sup>®</sup> Macro LCM Caps
  - Macro Cap ideal for large area capture, many cells
  - Contacts specimen surface for optimal adhesion
  - 50 microliters of extraction volume needed
  - Couples directly with 0.5 microfuge tubes



- CapSure<sup>®</sup> HS LCM Caps
  - “HS” = High Specificity
  - 12  $\mu\text{m}$  stand-off rails
  - Optimal for smaller #'s of cells, especially rare single cells
  - Only 10 microliters of extraction volume needed
  - Captures cells with speed and high specificity
  - Use of ExtracSure Device, Alignment Tray, Incubation Block

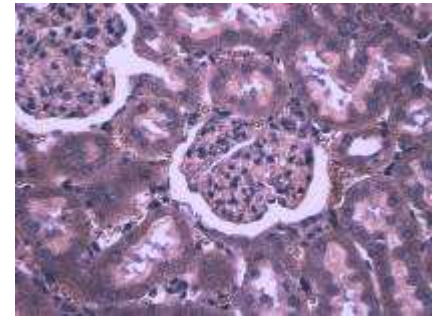
→ CapSure LCM caps maintain custody of the sample at all times



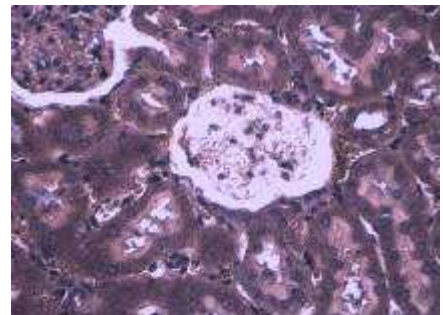
# Arcturus LCM Instruments

## The Best of Both Worlds

- Arcturus LCM systems are the only commercially available platforms to offer **both** IR and UV lasers
  - **IR Laser Capture (LCM)**
    - > Exclusive to Arcturus LCM
    - > Delivers a gentle technique, ensuring bio-molecule integrity
    - > Best choice for single cell or small number of cells
    - > Allows reliable use of plain glass slide preparations
      - ***Never lose custody of your sample!***
  - **UV Laser Cutting**
    - > Provides additional speed and flexibility
    - > Ideal for non-soft tissues and large number of cells
    - > Allows use of membrane slide preparations
      - Contact or non-contact microdissection



Before LCM



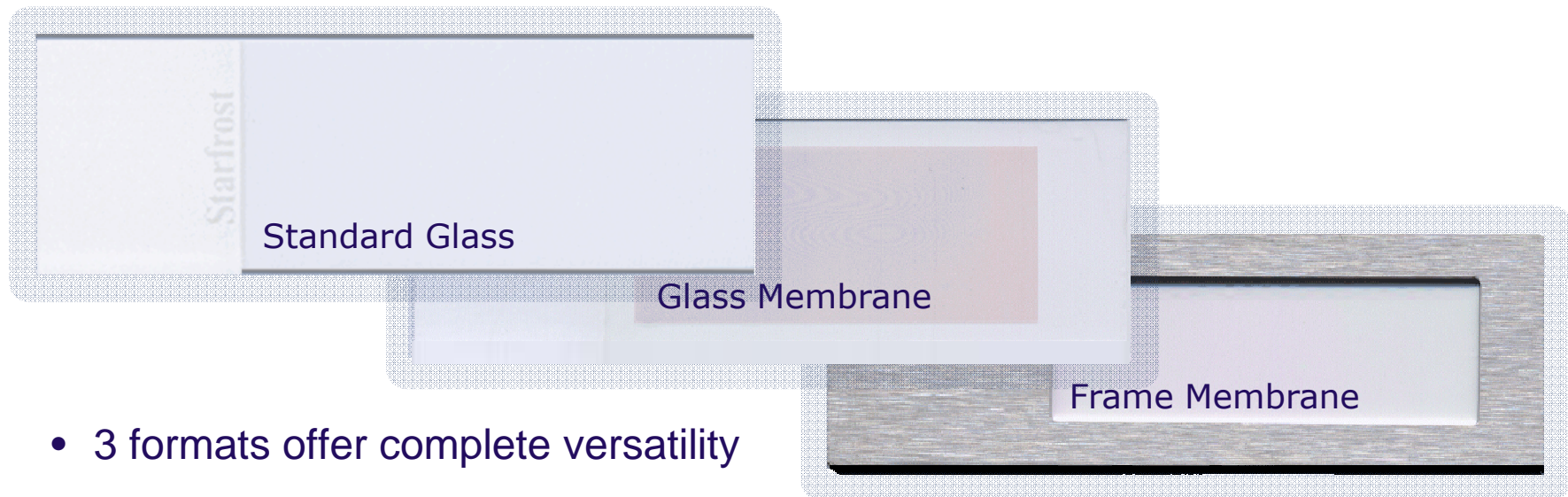
After LCM



CapSure Cap

# Arcturus® LCM Instruments

Enables sample preparation flexibility

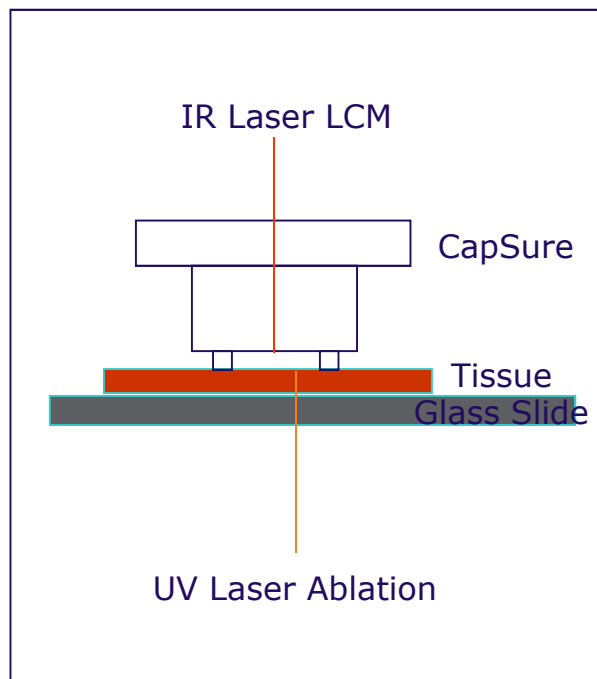


- 3 formats offer complete versatility
- Allow for unique applications
  - Hydrated or dehydrated
  - Contact or non-contact microdissection
- Help to standardize difficult tissues
- **Arcturus<sup>XT</sup>™ LCM System is only instrument that enables efficient use of all slide formats**

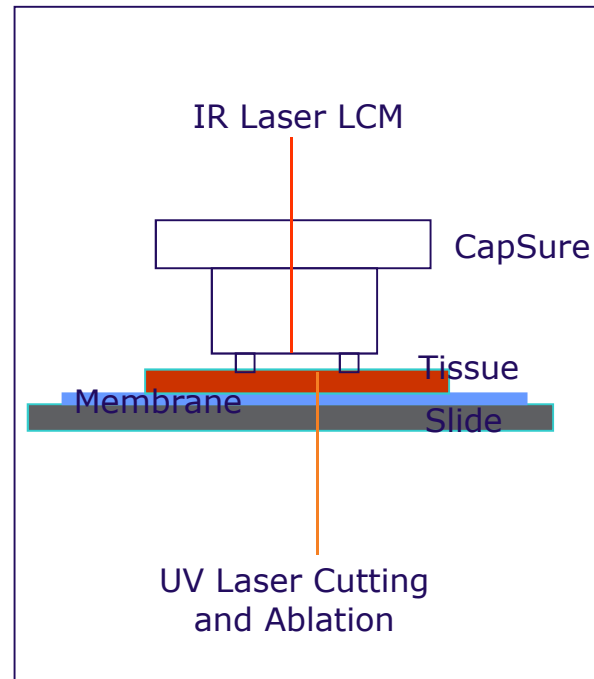
# Arcturus® LCM Instruments

- Multiple Slide Formats Allow LCM and LC/LCM operation

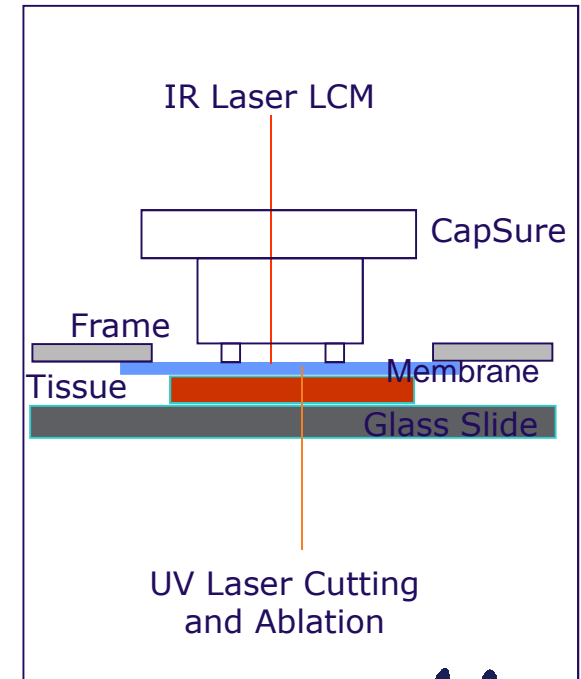
## Standard Glass Slides



## Glass Membrane Slides



## Frame Membrane Slides





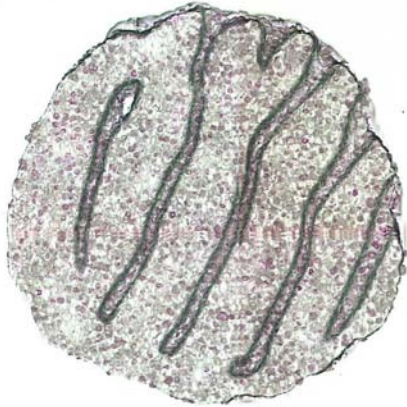
# When to Use IR Capture (LCM) vs. UV Cutting ?

*Depends on.....*

- Sample type
- Sample area
- Number of cells
- Downstream application

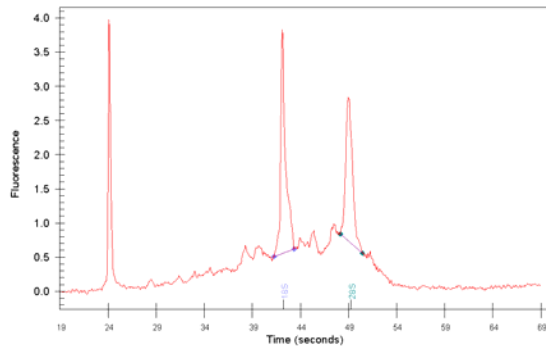
# Rapid Large Area Capture

## IR - Laser Capture



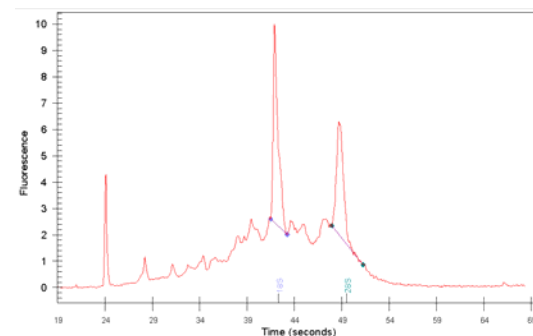
Same size  
dissections

650  $\mu\text{m}$   
diameter



Comparable  
RNA quality

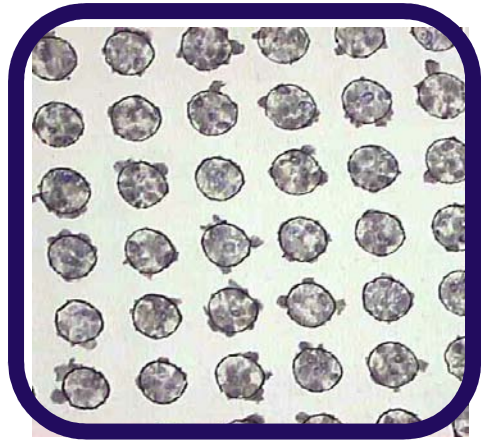
## UV - Laser Cutting



UV laser cutting: quick dissection of **large areas** and **hard** and **thick tissues**

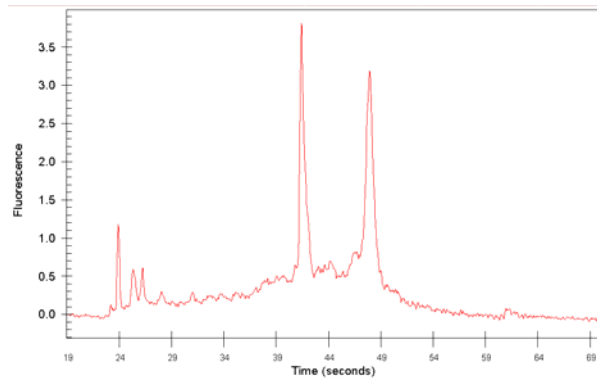
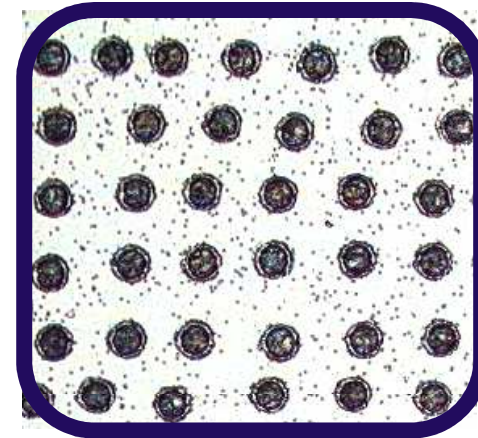
# Precise Small Area Capture

IR - Laser Capture

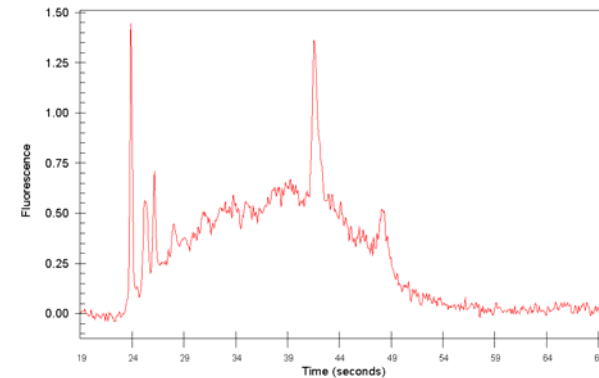


Same size  
dissections  
30  $\mu$ m diameter

UV - Laser Cutting



Large  
difference  
in RNA  
quality



IR Laser Capture preserves RNA quality for small areas  
and single cell dissections

# Arcturus<sup>XT</sup> Instrument– Key Features

- Arcturus exclusive IR laser-enabled LCM and UV laser cutting for ultimate microdissection flexibility
- High-performance Nikon® Eclipse Ti-E inverted research microscope base
- Intuitive user interface
- Phase contrast and Differential Interference Contrast (DIC)
- Superior optics: 2X – 100X (air and oil)
- Binoculars for standard microscope functions
- Optional high resolution imaging camera, including MetaVue® Imaging System
- Optional AutoScanXT™ Image Analysis Software simplifies and automates identification of areas for LCM
- Validated Systems for Microgenomics® with full applications support



LED Brightfield Illumination

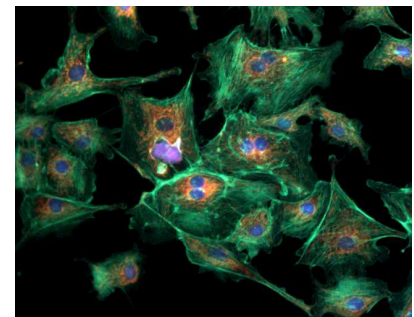
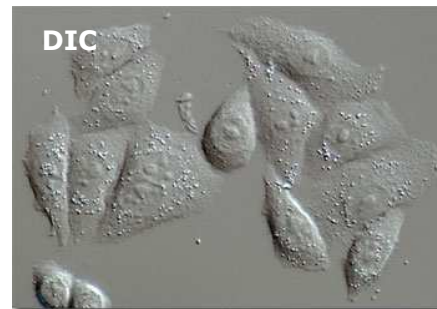
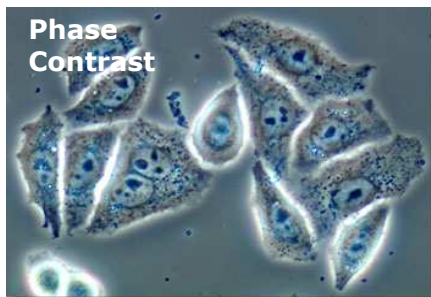
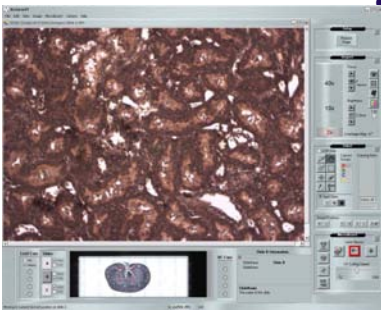


Nikon Phase Contrast Illumination

# Arcturus<sup>XT</sup>™ Instrument– Key Features



- Open, modular design – each system tailored to user needs
- Can be used as a stand-alone research microscope
  - Contrast illumination (Phase and DIC)
  - Epi-fluorescence
  - Manual or motorized
- Fully extendable to grow as the research grows, and to incorporate future offerings





# Arcturus<sup>XT</sup> Instrument– Contrast Imaging

- Phase contrast and Differential Interference Contrast (DIC)
  - Manual or motorized condenser
  - Optional DIC Analyzer cube
- Ideal for live cell applications and unstained tissue
  - Living cells can be examined in natural state without being killed, fixed, and stained
  - Retain nucleic acid integrity in tissue samples by skipping staining steps
- Compatible objectives
  - Phase contrast: 4X, 10X, 20X, 40X, 60X
  - DIC: 10X, 20X, 40X, 60X

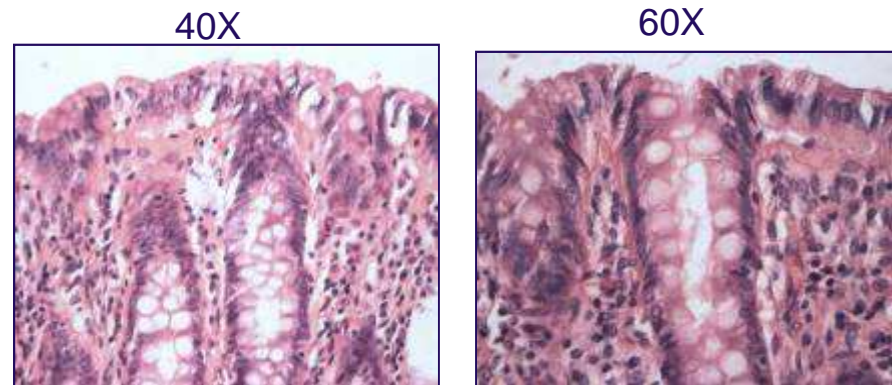
Learn more at: <http://www.microscopyu.com/>



Images courtesy of Nikon

# Arcturus<sup>XT</sup> Instrument– High Performance Optics

- Motorized six-position objective turret
- Standard objectives: 2x, 10x, 40x
  - Optional objectives
    - > 4X, 20X, 60X, 100X Air, 100X Oil
- Nikon® CFI60 objectives
  - Optimized for universal applicability
    - > Brightfield, Fluorescence, Phase Contrast, DIC, UV transmission
- Super Plan Fluor objectives: 20x, 40x, 60x
  - Increased working distance
  - Improved transmission
  - Lower chromatic aberration



Colon tissue, H&E stain, visualized at 40X and 60X

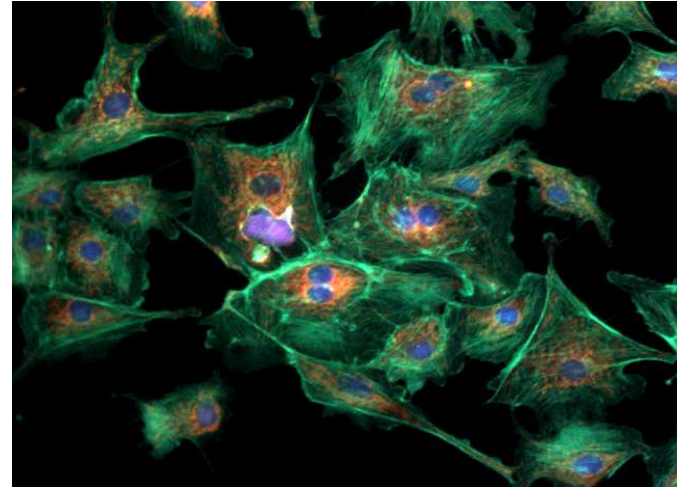


100X

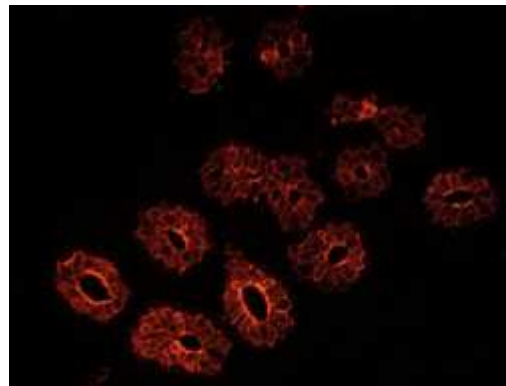
Human chromosome metaphase spread, giemsa stained and visualized at 100X.

# Arcturus<sup>XT</sup> Instrument– Fluorescence

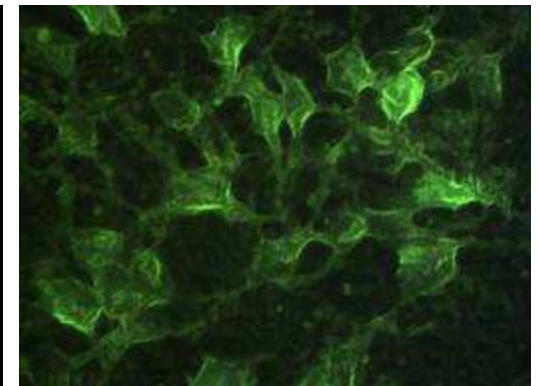
- Higher signal-to-noise ratio by eliminating stray light
  - Nikon's exclusive "Noise Terminator" technology
  - Eliminates possibility of stray light escaping from the fluorescence filter cube, reducing contrast and introducing photon noise into the image path
- 6-position filter turret
  - Manual or motorized turret
  - Red, Blue and Green included
  - UV filter cube optional
  - Triple dichroic filter cube optional
- Filter cubes allow easy changing and modification



Triple labeled bovine pulmonary artery endothelial (BPAE) cells. Mitochondria = red, F-actin=green and Nuclei= blue. Visualized simultaneously using an Omega triple band dichroic filter.

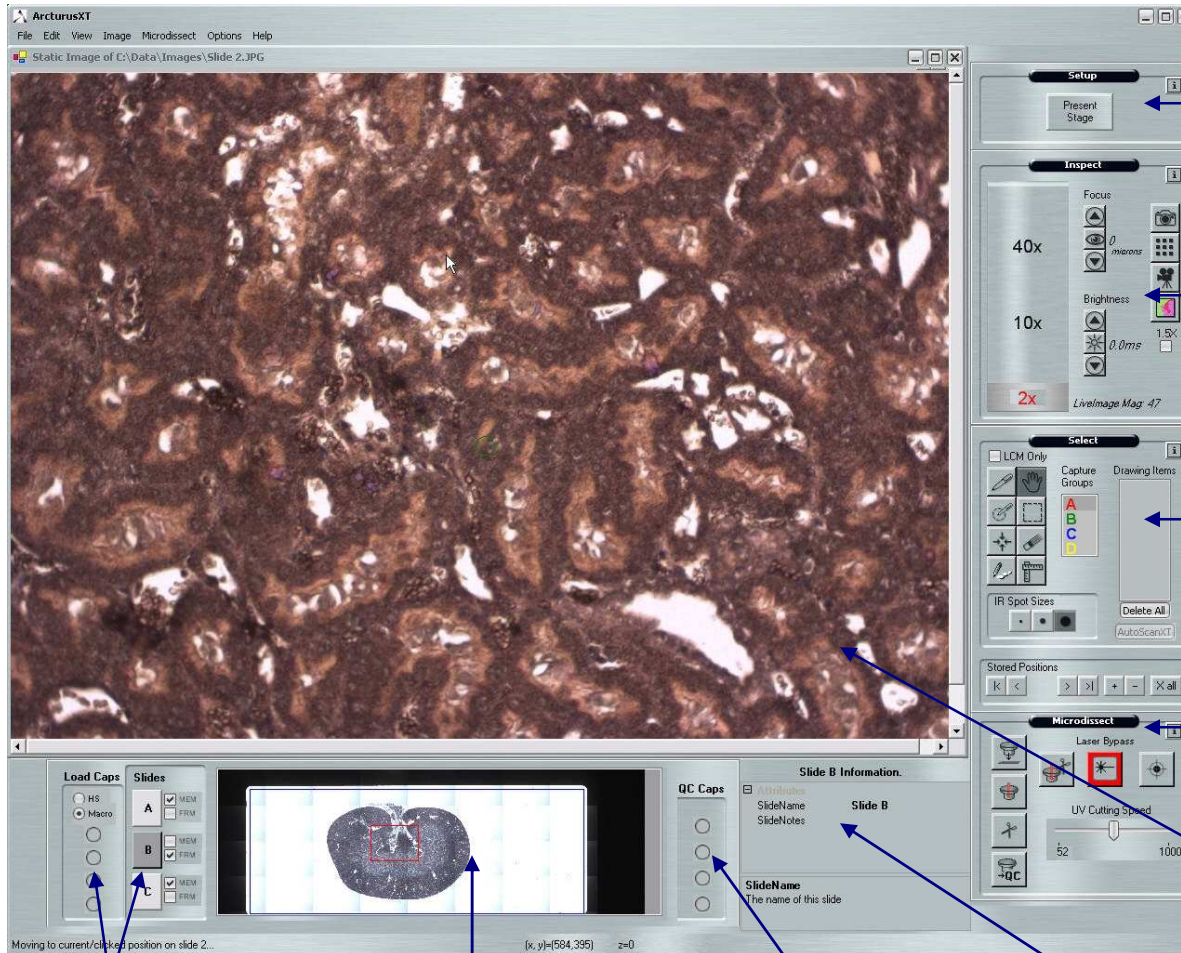


Human Breast Carcinoma, anti-cytokeratin/Cy3.



Cultured HELA cells exposed to BCECF, a cytoplasmic pH indicator.

# Arcturus<sup>XT</sup> Software Graphical User Interface



Microdissection  
Tools Panel

Live Video Image

Cap and Slide Load  
Details

Slide Overview  
Image

Cap QC /Offload  
Station

Materials Information

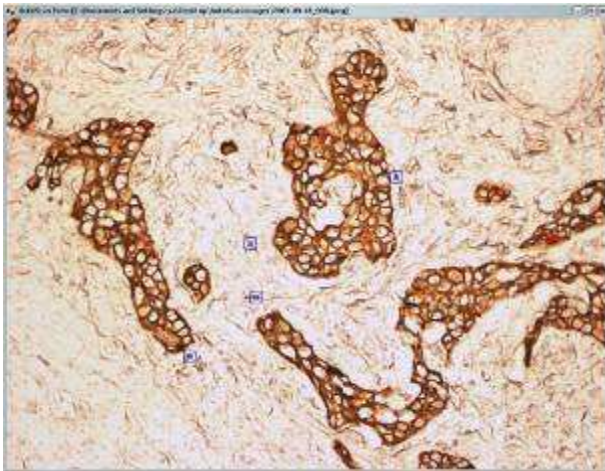
# Arcturus<sup>XT</sup> Software Graphical User Interface

The screenshot displays the ArcturusXT software interface. The main window shows a large micrograph of a tissue sample. On the right side, there is a vertical toolbar with several sections: 'Setup' (containing 'Present Stage'), 'Inspect' (containing 'Focus', '40x', '10x', '2x', 'Brightness', and 'LiveImage Mag: 47'), 'Select' (containing 'Capture Groups', 'Drawing Items', and 'IR Spot Sizes'), and 'Microdissect' (containing 'Laser Bypass' and 'UV Cutting Speed'). At the bottom left, there are 'Load Caps' and 'Slides' sections. At the bottom right, there is a 'Slide B Information' panel. Five numbered callouts point to specific features: 1. 'Load Materials' points to the 'Present Stage' button; 2. 'Inspect Samples' points to the 'Brightness' control; 3. 'Select Cells' points to the 'Capture Groups' section; 4. 'Microdissect' points to the 'Laser Bypass' button; 5. 'QC' points to the 'UV Cutting Speed' control.

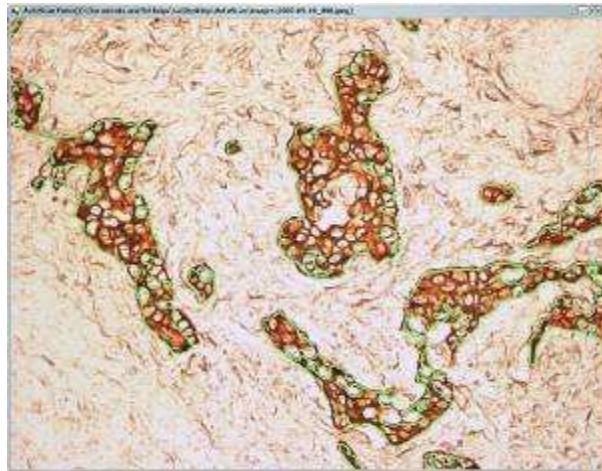
1. Load Materials
2. Inspect Samples
3. Select Cells
4. Microdissect
5. QC

# AutoScanXT Image Analysis Module

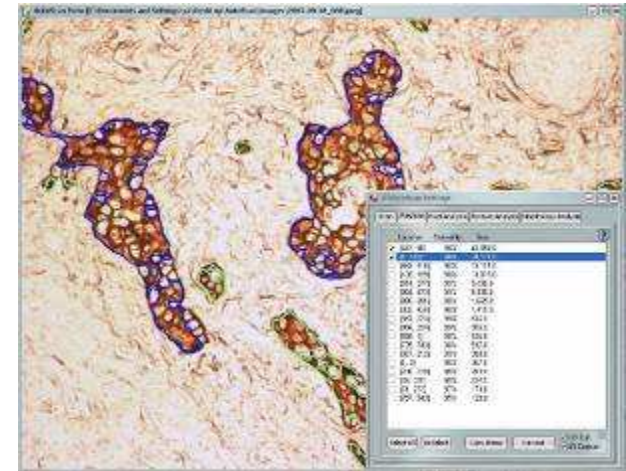
## Human Breast Carcinoma: IHC with DAB, anti-Cytokeratin



Selection of Regions of Interest (ROI) and Background



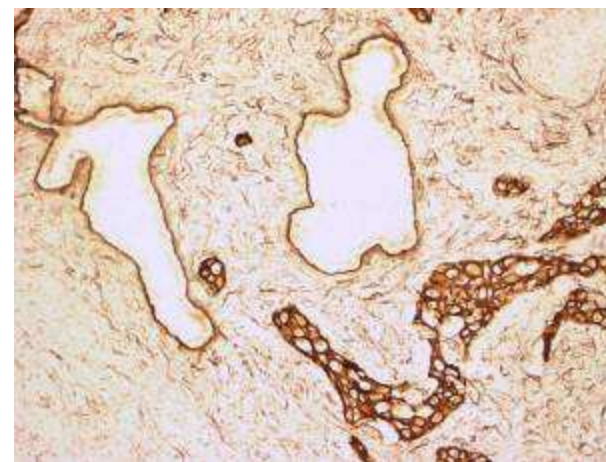
Post-AutoScan Analysis  
Items identified green outline



2 Items (blue outlined) selected for Microdissection



Post-Microdissection: Cap



Post-Microdissection: Tissue

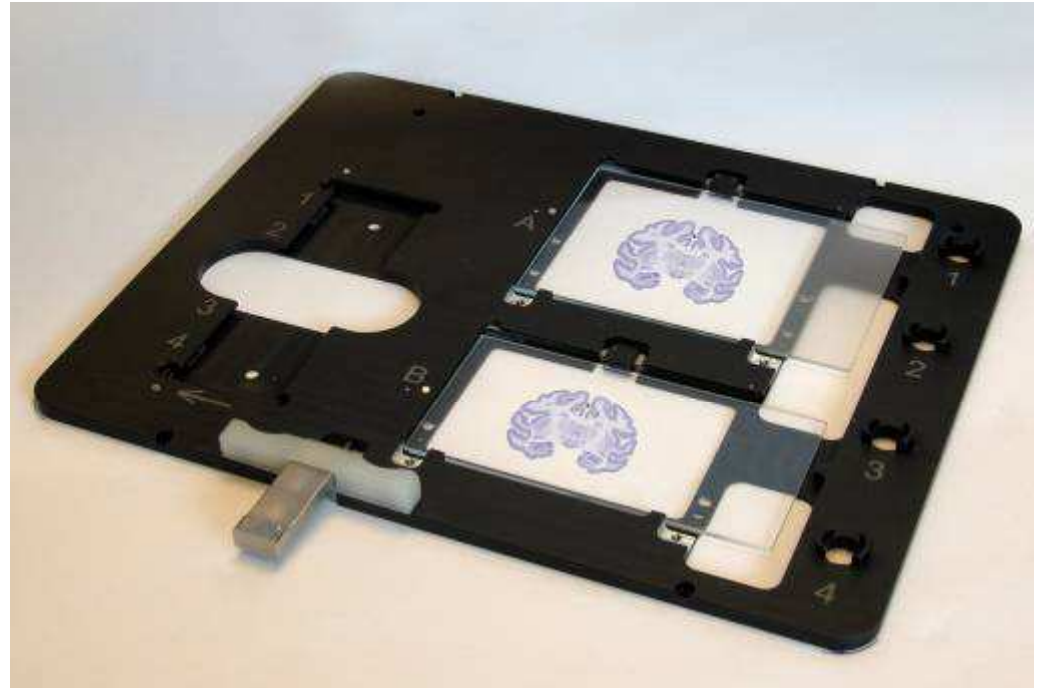
# Arcturus<sup>XT</sup> Instrument Materials Handling

- Easily accessible materials
  - Load up to three slides
  - Load up to four caps
- Modular stage insert for alternate sample formats
  - Petri dish
  - Wide slide format



# Arcturus<sup>XT</sup> Instrument Neurological Slide Stage Insert

- Optimized for Neurobiology
  - Large format slide stage insert
  - Common neurological slide preparations
  - User-adjustable positions
    - > 25 mm, 38 mm and 50 mm





# Arcturus<sup>XT</sup> Instrument Petri Dish Stage Insert

- Optimized for:
  - Live cell imaging
  - Live cell microdissection
- Stage insert accommodates 50mm x 7mm petri dishes
- Easy swap out for live cell or tissue based applications





# Arcturus LCM Instruments and Microgenomics Reagents

*Fueling Discovery in Diverse Research Areas*

## Key Research Areas

- Oncology
- Developmental Biology
- Neuroscience
- Proteomics
- Inflammation
- Cardiovascular
- Diabetes
- Hard tissues (bone, skin, cartilage)
- Sub-cellular structures (chromosomes, organelles, etc)

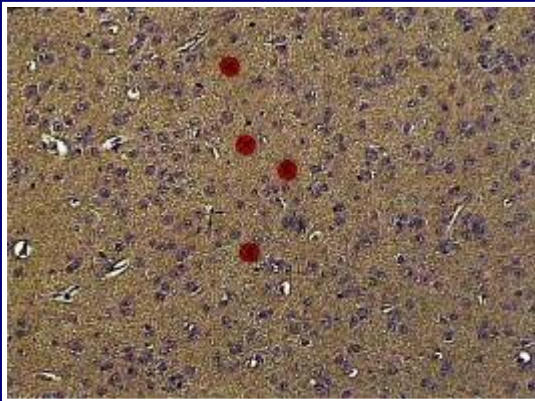
## Emerging Application Areas

- Live cells / stem cells
- Forensics
- Plant biology

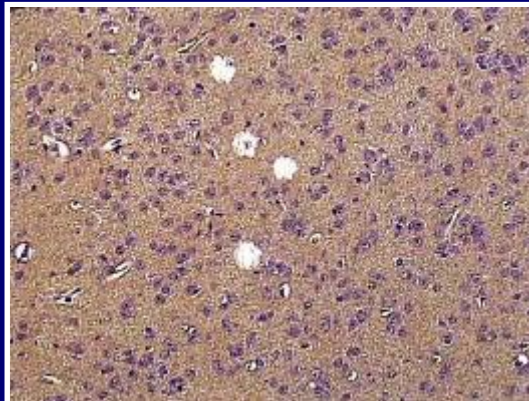
# LCM of Single Cells

Frozen Rat Brain

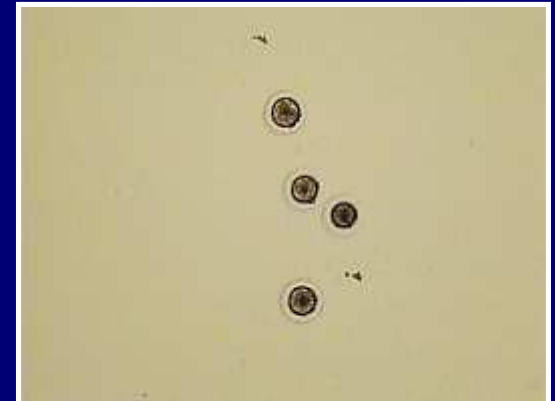
HistoGene® Kit stained Single Neurons



Before LCM



After LCM



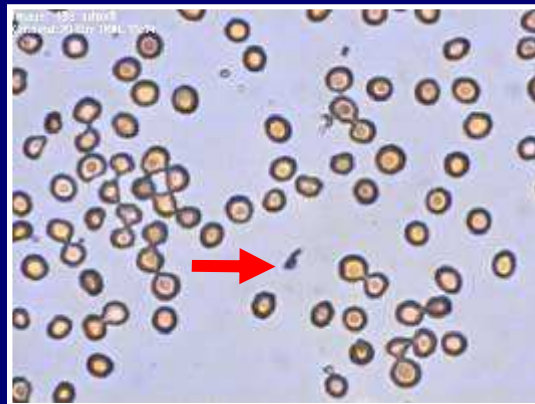
CapSure® Cap

# LCM of Single Cells

## Human Blood Cells



Before LCM

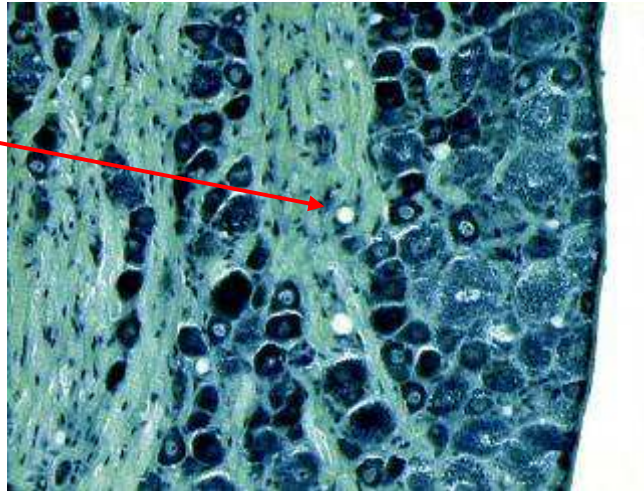


After LCM

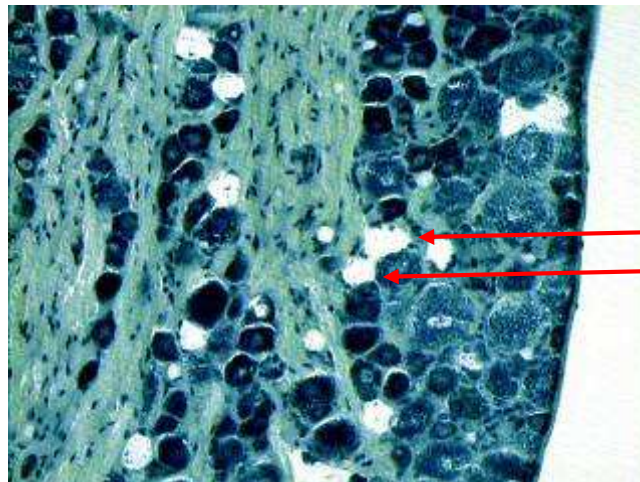


CapSure<sup>®</sup> Cap

# LCM of Adjacent Cells



Small Dorsal Root Ganglion  
Rat Neurons, Nissl stained

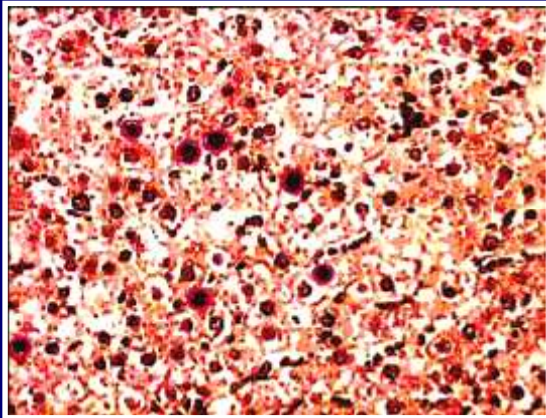


Large Dorsal Root Ganglion  
Rat Neurons, Nissl stained

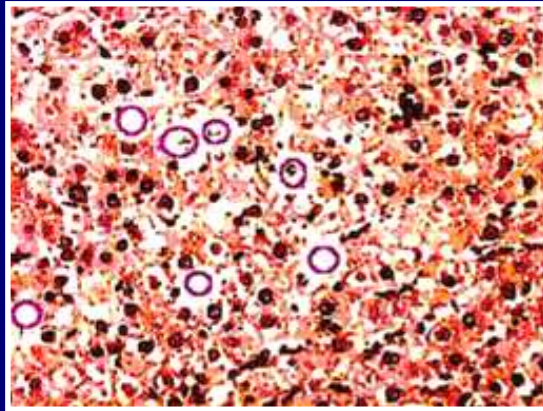
*Courtesy of Dr. Lin Luo and Dr. Mark Erlander, R. W. Johnson Pharmaceuticals*

# LCM of Single Cells

Proliferating Rat Hepatocytes Immunostained (IHC)



Before LCM



After LCM

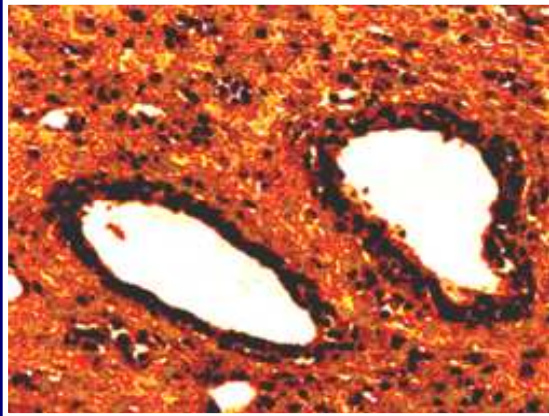


CapSure® Cap

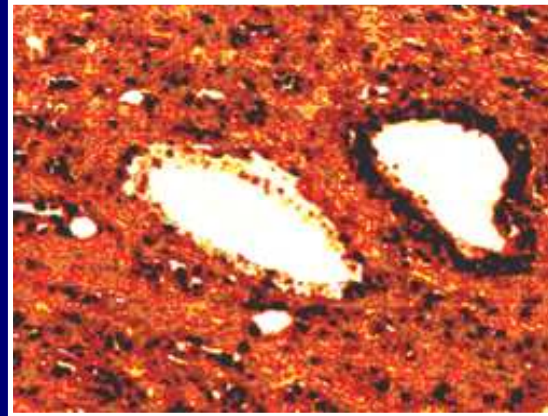
Source: Dr. Nicola Wallis, Zeneca Pharmaceuticals, Inc.

# LCM Only

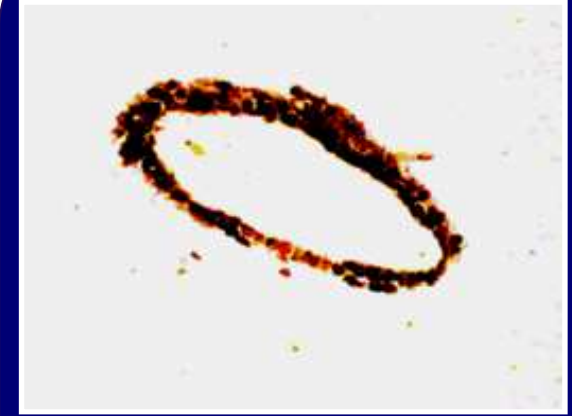
Prostate Epithelium Immunostained for bcl-2



Before LCM



After LCM



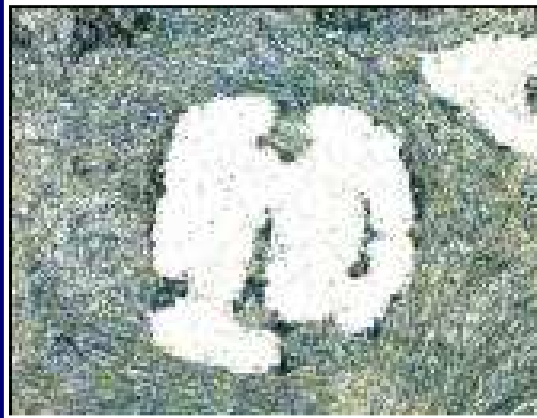
CapSure® Cap

# LCM Only

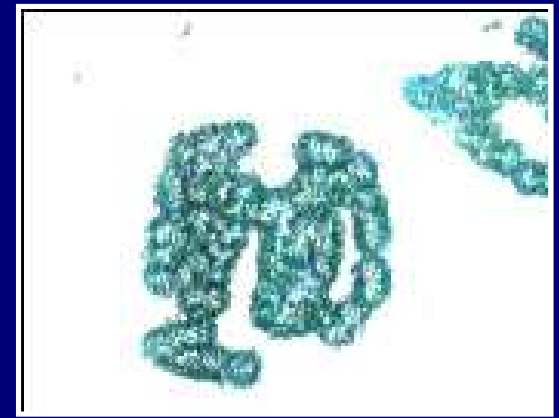
Nasopharyngeal Carcinoma Immunostained for keratin



Before LCM



After LCM



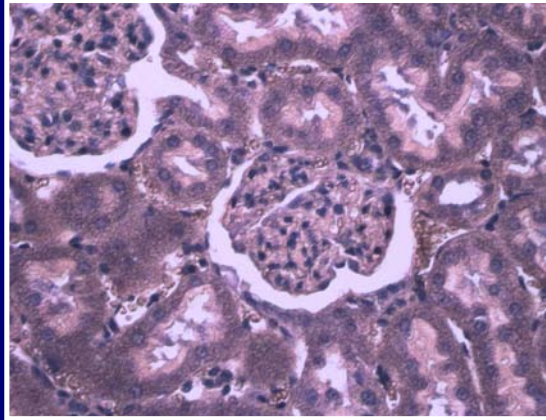
CapSure® Cap

*Source: Dr. Margaret Gulley, University of Texas Health Science Center at San Antonio, Dr. Mark Burton, Wilford Hall Medical Center, and Dr. Barbara Schneider, Louisiana State University Medical Center*

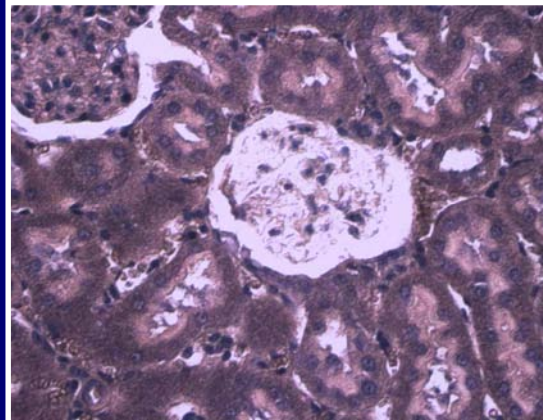


# LCM Only

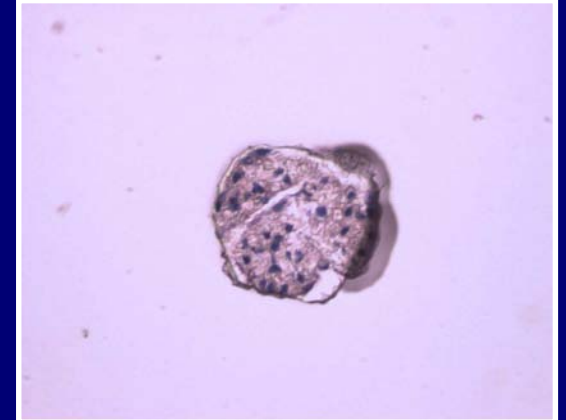
Rat FFPE kidney section Paradise<sup>®</sup> PLUS Reagent Kit stain



Before LCM



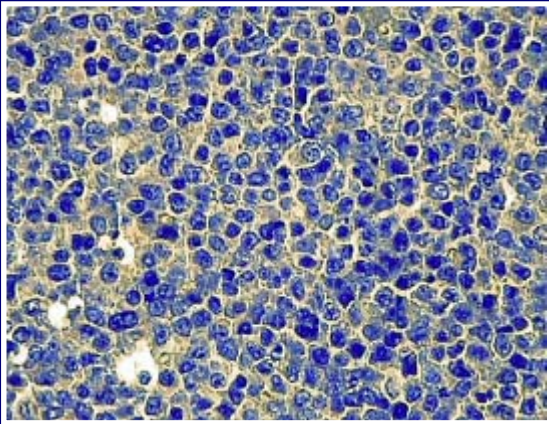
After LCM



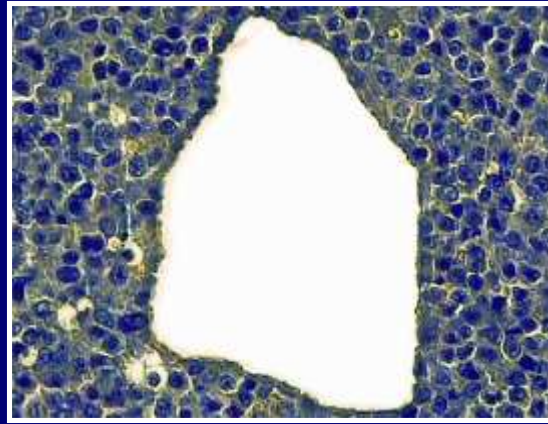
CapSure<sup>®</sup> Cap

# LCM and UV Laser Cutting

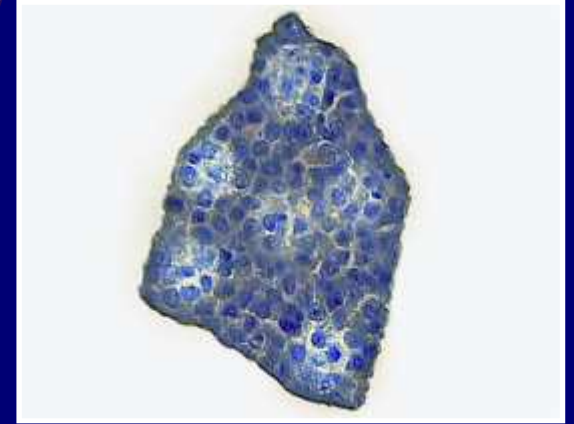
Hard Tissue Microdissection - Human Bone Marrow  
FFPE Cresyl Violet - Stained



Before LCM



After LCM

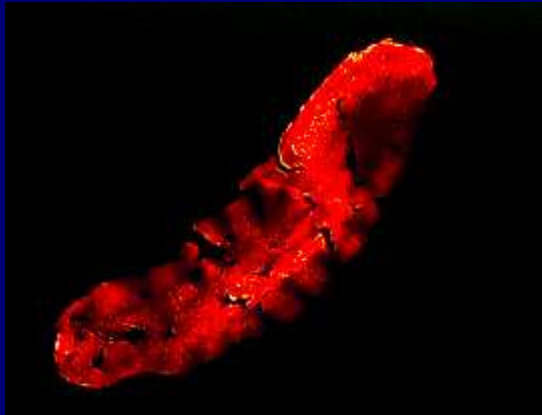


CapSure<sup>®</sup> Cap

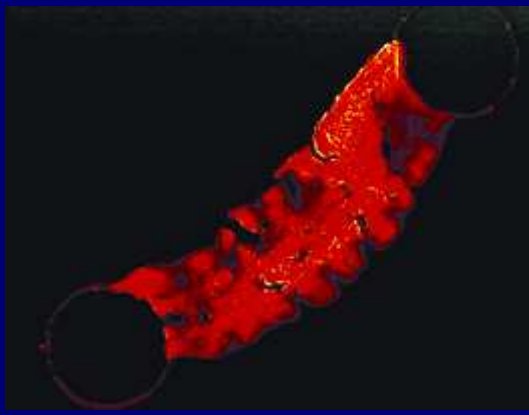
# LCM and UV Laser Cutting

## Developmental Biology

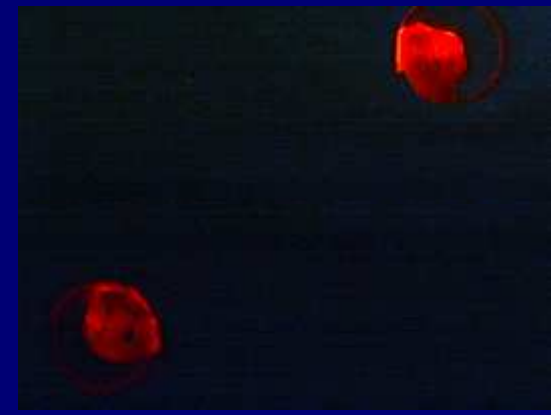
Fluorescence Microdissection - Drosophila Embryo  
X-gal-Stained



Before LCM



After LCM

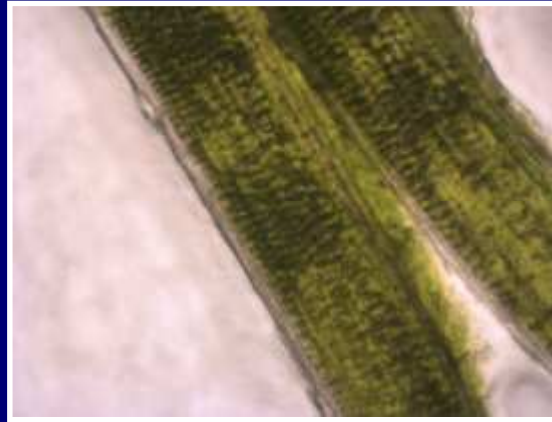


CapSure<sup>®</sup> Cap

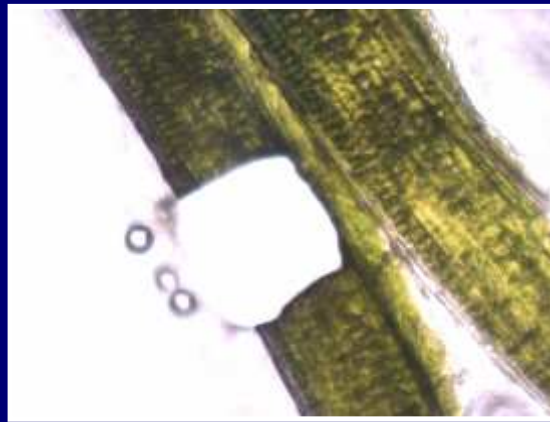
# LCM and UV Laser Cutting

## Plant Biology

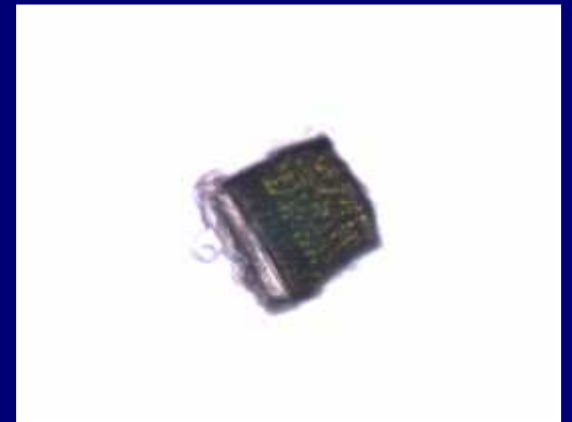
Live Plant Microdissection - Blade of Grass  
Whole mount preparation on frame membrane slide



Before LCM



After LCM



CapSure® Cap

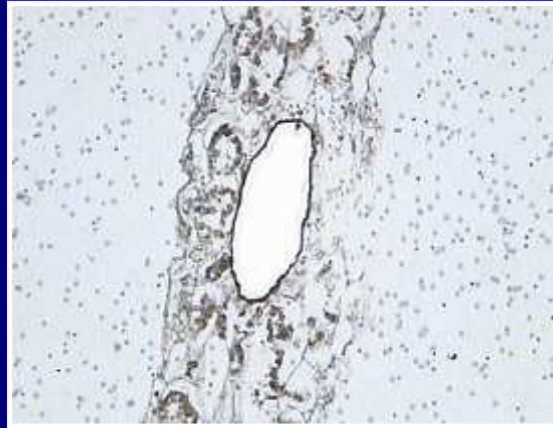
# LCM and UV Laser Cutting

## Plant Biology

Plant Tissue Microdissection – Arabidopsis Seedling  
FFPE X-GlcA Substrate-Stained



Before LCM



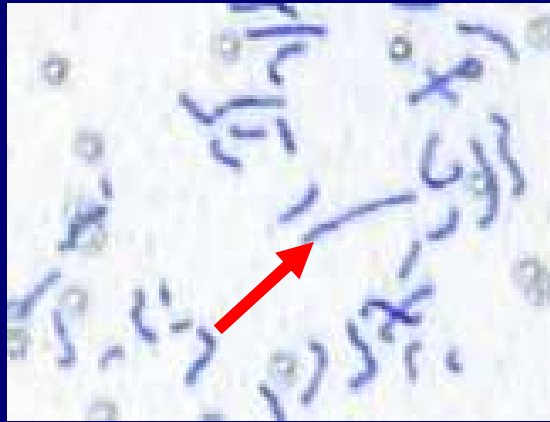
After LCM



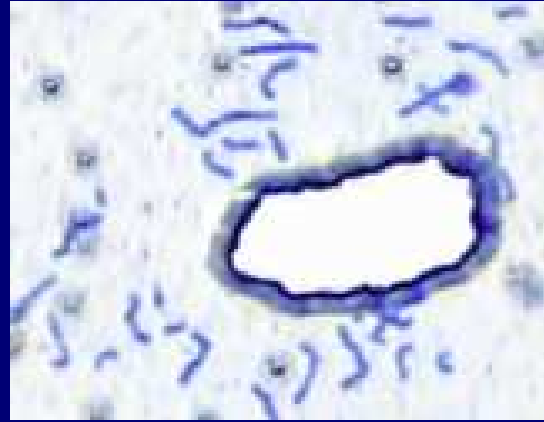
CapSure® Cap

# LCM and UV Laser Cutting Cytogenetics

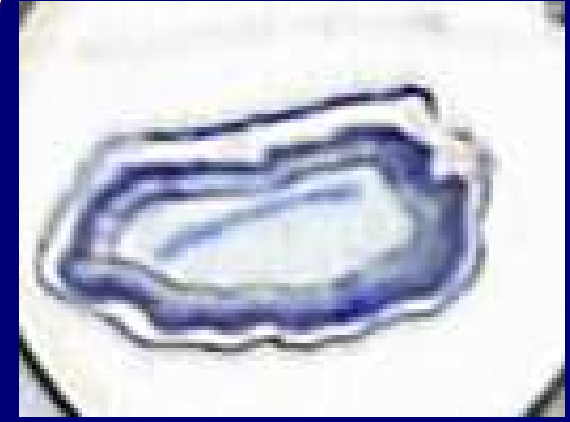
Sub-Cellular Microdissection – Human Chromosome  
Peripheral Lymphocyte, Giemsa stained



Before LCM



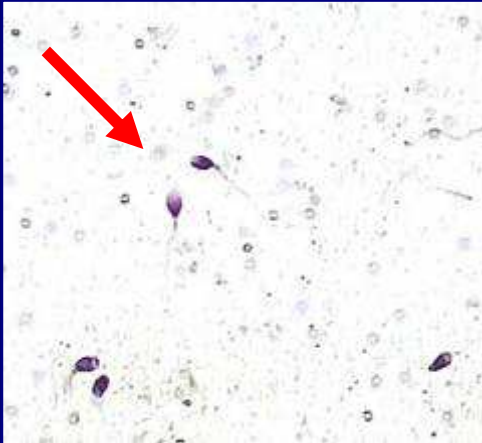
After LCM



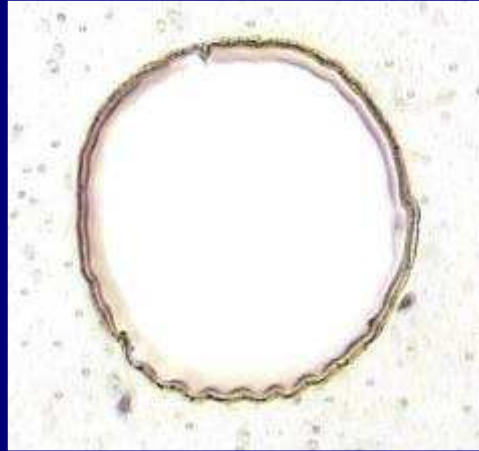
CapSure® Cap

# LCM and UV Laser Cutting Forensics

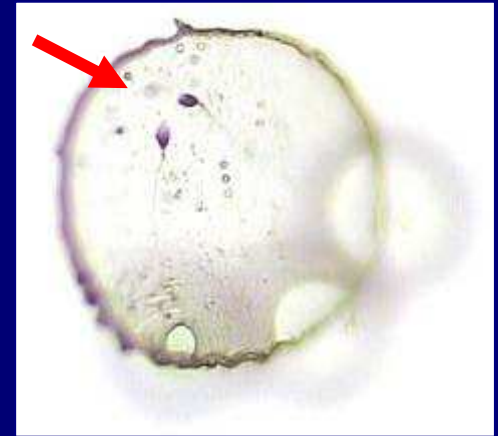
Sperm from mixed forensic smear  
Christmas tree stain



Before LCM



After LCM

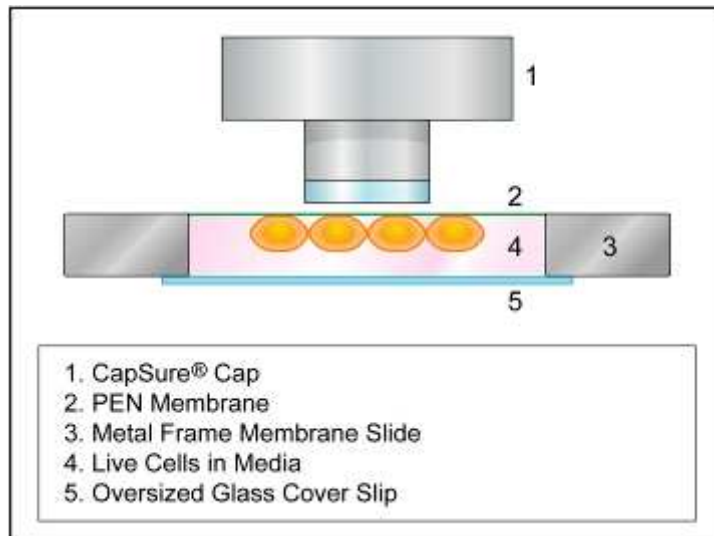
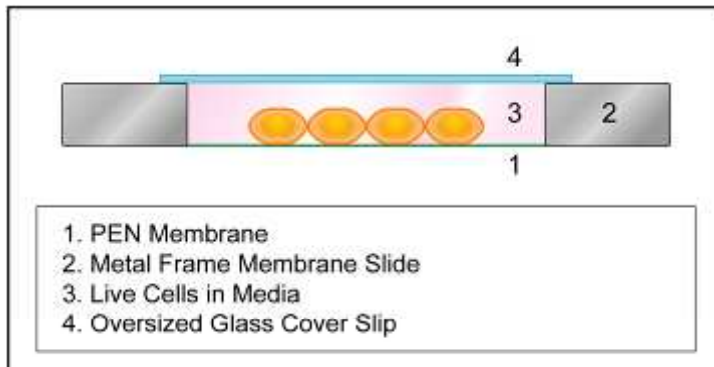


CapSure® Cap

# Arcturus<sup>XT</sup> Instrument Live Cell Microdissection

## Application Note #11

Isolate living cells for re-culture or molecular analysis



3T3 cells  
visualized  
with DIC  
optics

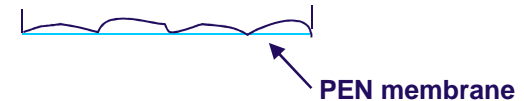
*life*  
technologies™



# ArcturusXT Instrument Live Cell Microdissection Protocol

1. Cells cultured in a PEN membrane “growth chamber”
  1. Standard sterile culture conditions
    - Growth chamber inside closed Petri dish
  2. Growth chamber containing live cells placed inside covered Arcturus<sup>XT</sup> Instrument Petri dish
    - Silicone-coated dish
    - Done in culture hood
  3. Covered Arcturus<sup>XT</sup> Instrument Petri dish / growth chamber brought to Arcturus<sup>XT</sup> Instrument

1.



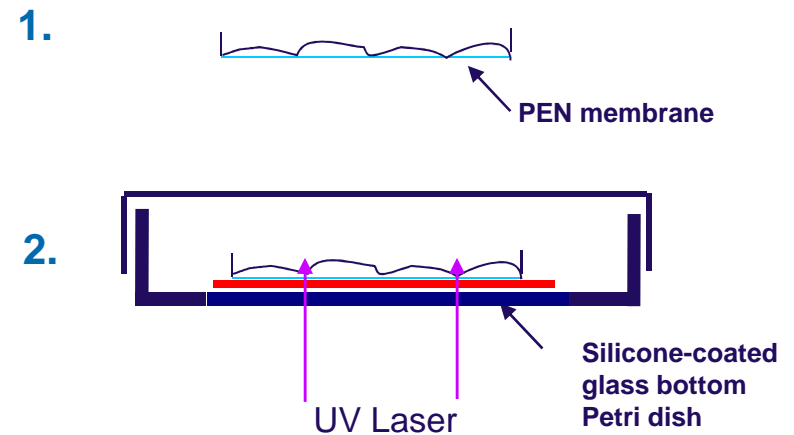
# Arcturus<sup>XT</sup> Instrument Live Cell Microdissection Protocol

## 1. Cells cultured in a PEN membrane “growth chamber”

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  - Growth chamber inside closed Petri dish
2. Growth chamber containing live cells placed inside covered Arcturus<sup>XT</sup> Instrument petri dish
  - Silicone-coated dish
  - Done in culture hood
3. Covered Arcturus<sup>XT</sup> Instrument Petri dish / growth chamber brought to Arcturus<sup>XT</sup> Instrument

## 2. UV laser used to cut through the membrane and around the cell(s) of interest

1. Covered Arcturus<sup>XT</sup> Instrument Petri dish



# ArcturusXT Live Cell Microdissection Protocol

## 1. Cells cultured in a PEN membrane “growth chamber”

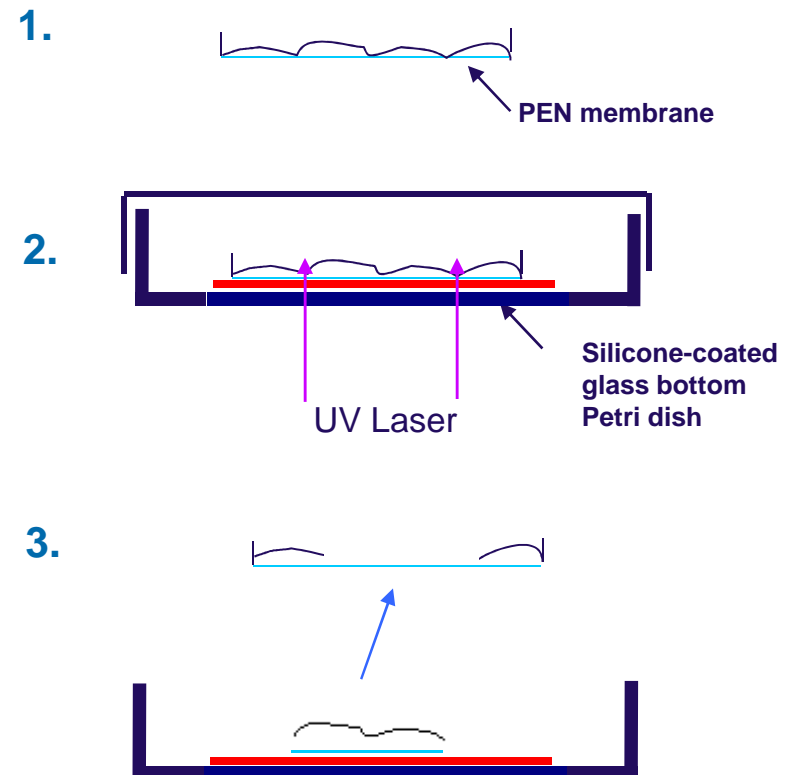
1. Standard sterile culture conditions
  - Growth chamber inside closed Petri dish
2. Growth chamber containing live cells placed inside covered Arcturus<sup>XT</sup> Instrument Petri dish
  - Silicone-coated dish
  - Done in culture hood
3. Covered Arcturus<sup>XT</sup> Instrument Petri dish / growth chamber brought to Arcturus<sup>XT</sup> Instrument

## 2. UV laser used to cut through the membrane and around the cell(s) of interest

1. Covered Arcturus<sup>XT</sup> Instrument Petri dish

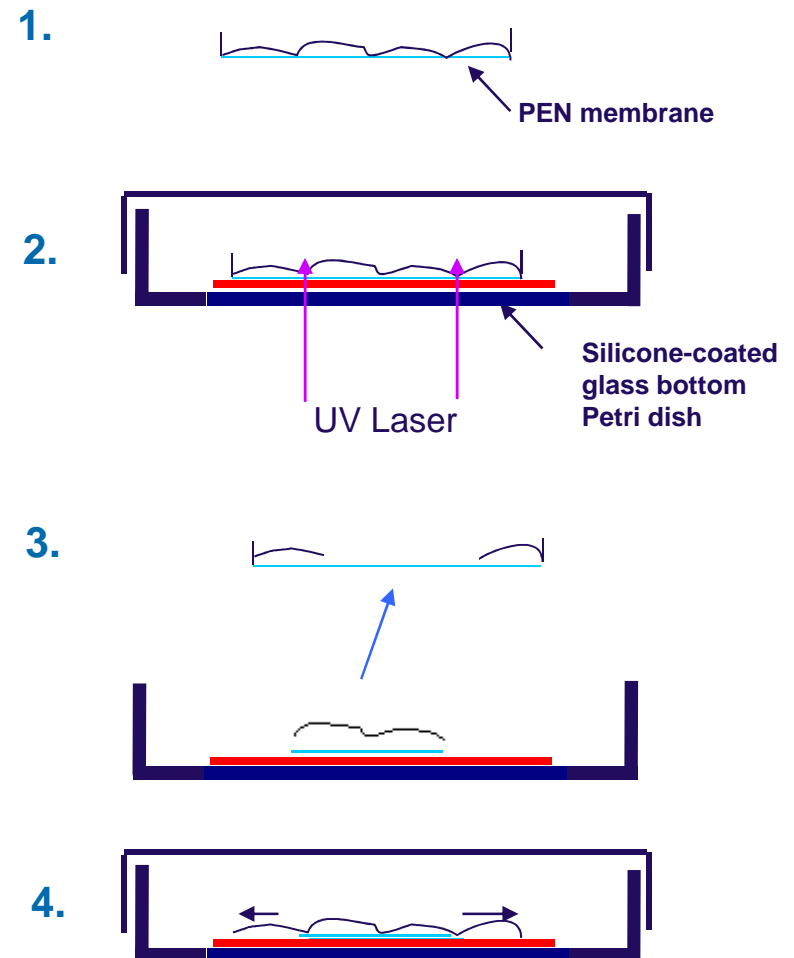
## 3. When finished with microdissection:

1. Arcturus<sup>XT</sup> Instrument Petri dish / growth chamber brought back to culture hood
2. Growth chamber insert removed
3. Cut areas are left behind, attached to the Arcturus<sup>XT</sup> Instrument Petri dish surface



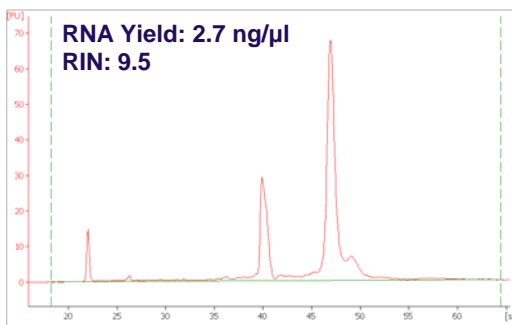
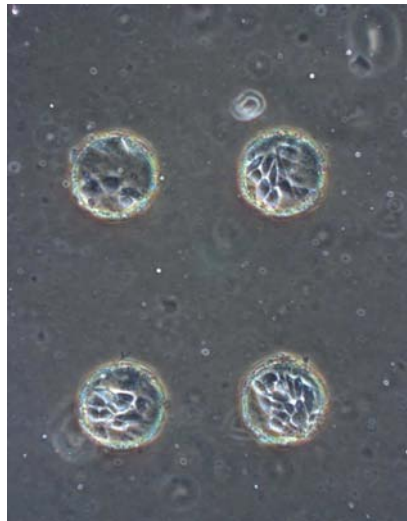
# ArcturusXT Live Cell Microdissection Protocol

- 1. Cells cultured in a PEN membrane “growth chamber”**
  1. Standard sterile culture conditions
    - Growth chamber inside closed Petri dish
  2. Growth chamber containing live cells placed inside covered Arcturus<sup>XT</sup> Instrument Petri dish
    - Silicone-coated dish
    - Done in culture hood
  3. Covered AXT Petri dish / growth chamber brought to Arcturus<sup>XT</sup> Instrument
- 2. UV laser used to cut through the membrane and around the cell(s) of interest**
  1. Covered Arcturus<sup>XT</sup> Instrument Petri dish
- 3. When finished with microdissection:**
  1. Arcturus<sup>XT</sup> Instrument Petri dish / growth chamber brought back to culture hood
  2. Growth chamber insert removed
  3. Cut areas are left behind, attached to the AXT Petri dish surface
- 4. After microdissection:**
  1. Cells left to outgrow in Arcturus<sup>XT</sup> Instrument Petri dish
  2. Growth chamber can be discarded, **or**
    - Placed into another Arcturus<sup>XT</sup> Instrument Petri dish for additional microdissection, **or**
    - Kept for continued culturing of the cells

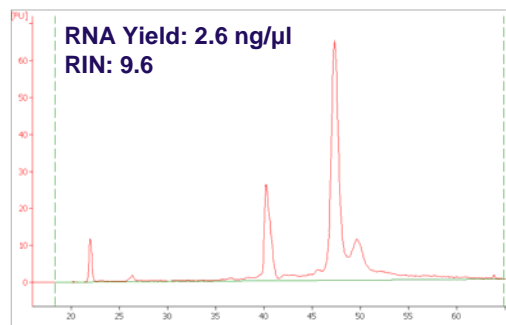
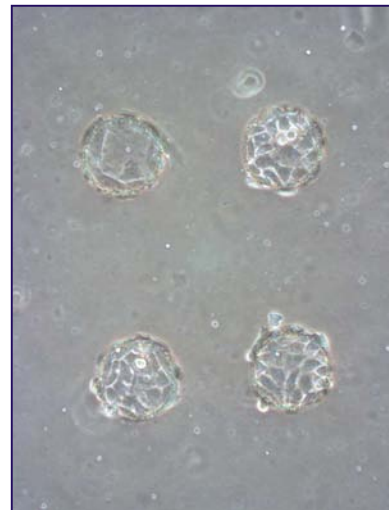


# Arcturus<sup>XT</sup> Instrument Live Cell Microdissection (Petri Dish)

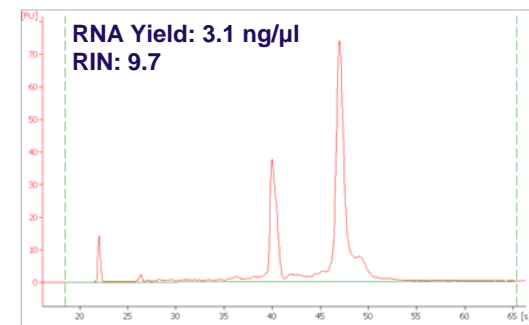
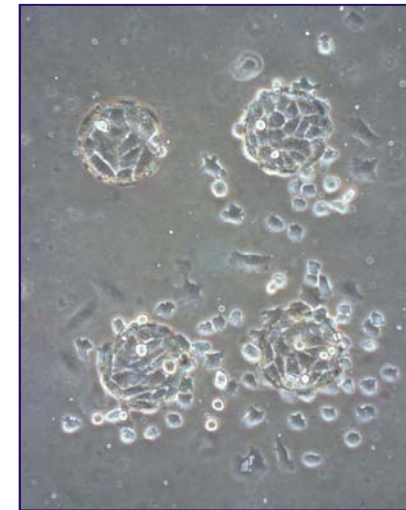
0 Hours  
Post-Microdissection



24 Hours  
Post-Microdissection



48 Hours  
Post-Microdissection

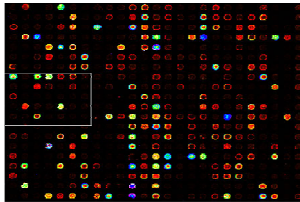


Cultured CHO and TE671 cells

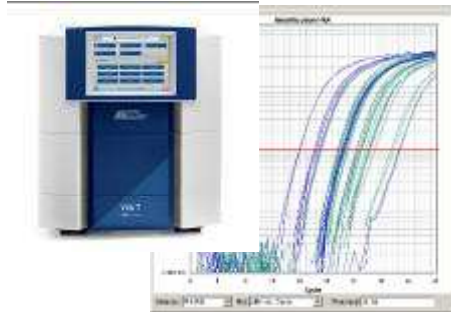
→ Microdissection does not affect cell or nucleic acid viability

# Arcturus<sup>®</sup> System LCM Applications

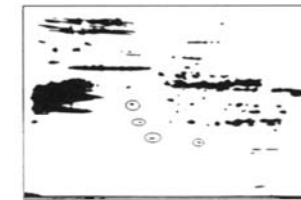
Gene Expression –  
Microarray



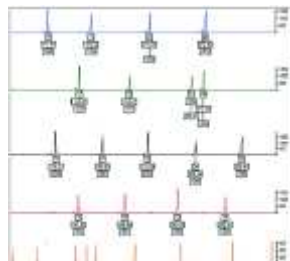
Mutation Analysis



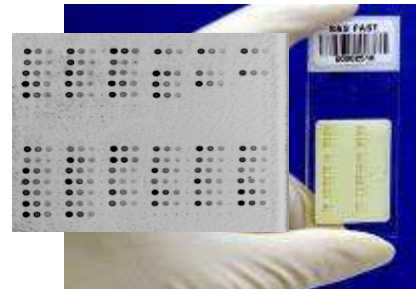
Gene Expression –  
Real-Time PCR



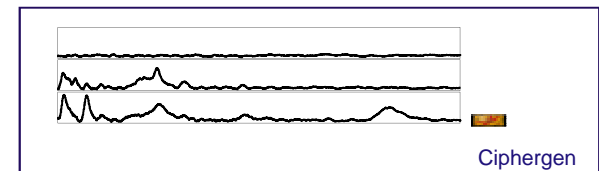
Proteomics – 2D Gels



Forensics – STR  
analysis



Proteomics – Reverse  
Phase Protein Arrays



Proteomics – Protein  
Chip/Mass Spec

# Cell Quantities and Downstream Applications

**TABLE 1** | Recommended cellular yields from microdissected tissue for downstream analyses.

Molecule	Methodology/assay	Cellular yield/area of microdissection	References
DNA	Loss of heterozygosity	100–1,000 cells	50,53
DNA	Imprinting/DNA methylation	200 cells	48
gDNA	Genetic mosaic analysis	2,000 cells	77
RNA	cDNA library construction	25,000 cells (93 ng total RNA)	54,55
		5,000 cells (14.7–18.6 ng total RNA)	
RNA	Gene-expression arrays	100 cells from FFPE	57
RNA	Real-time RT-PCR	1,400 cells	15,56
		0.8–1.0 × 10 <sup>6</sup> μm <sup>2</sup>	80
		200 and 1,000 cells	58
		22,000 cells/37.5 ng RNA	81
		10,000 cells/40 ng RNA from maize	30
		single cell	82–84
RNA	QRT-PCR	100 cells/1 reaction or 2,000 cells/200 μl	59
		4,000–5,000 cells	15
Protein	Western blot	500 cells (optimized blotting procedure)	64
		2,500 cells	63
		8,000–10,000 cells	62
Protein	2D gel electrophoresis	50,000–100,000 cells	5
		3.7 mm <sup>2</sup> area	85
		10,000 cells (100–200 μg in 350 μl)	86
		20,000–25,000 cells	87
		50,000 cells	63
Protein	2D-DIGE	30,000 cells/40 μl	88
Protein	Molecular profiling: reverse-phase protein microarray	5,000–30,000 cells	12,19,20,22,59
Protein	Mass spectrometry: MALDI or LC/MS-MS	50,000–100,000 cells (ICAT and LC/MS)	89
		10,000–15,000 cells	90
		25,000 μm <sup>2</sup>	90
		300 microvessels	91
Protein	Mass spectrometry: SELDI	1,500 cells	66
		3,000–5,000 cells	67

Source: Espina V et al, “Laser-Capture Microdissection”, Nature Protocols (1:2), 2006

<http://www.nature.com/nprot/journal/v1/n2/pdf/nprot.2006.85.pdf>

# Challenges of Small Sample Research

- Collect a pure cell population from heterogeneous tissue
- Obtain enough material for analysis
- Minimize sample loss during processing
- Maintain biomolecule integrity
- Reproducible analysis
- Quality control

LCM

Microgenomics





# What is Microgenomics?

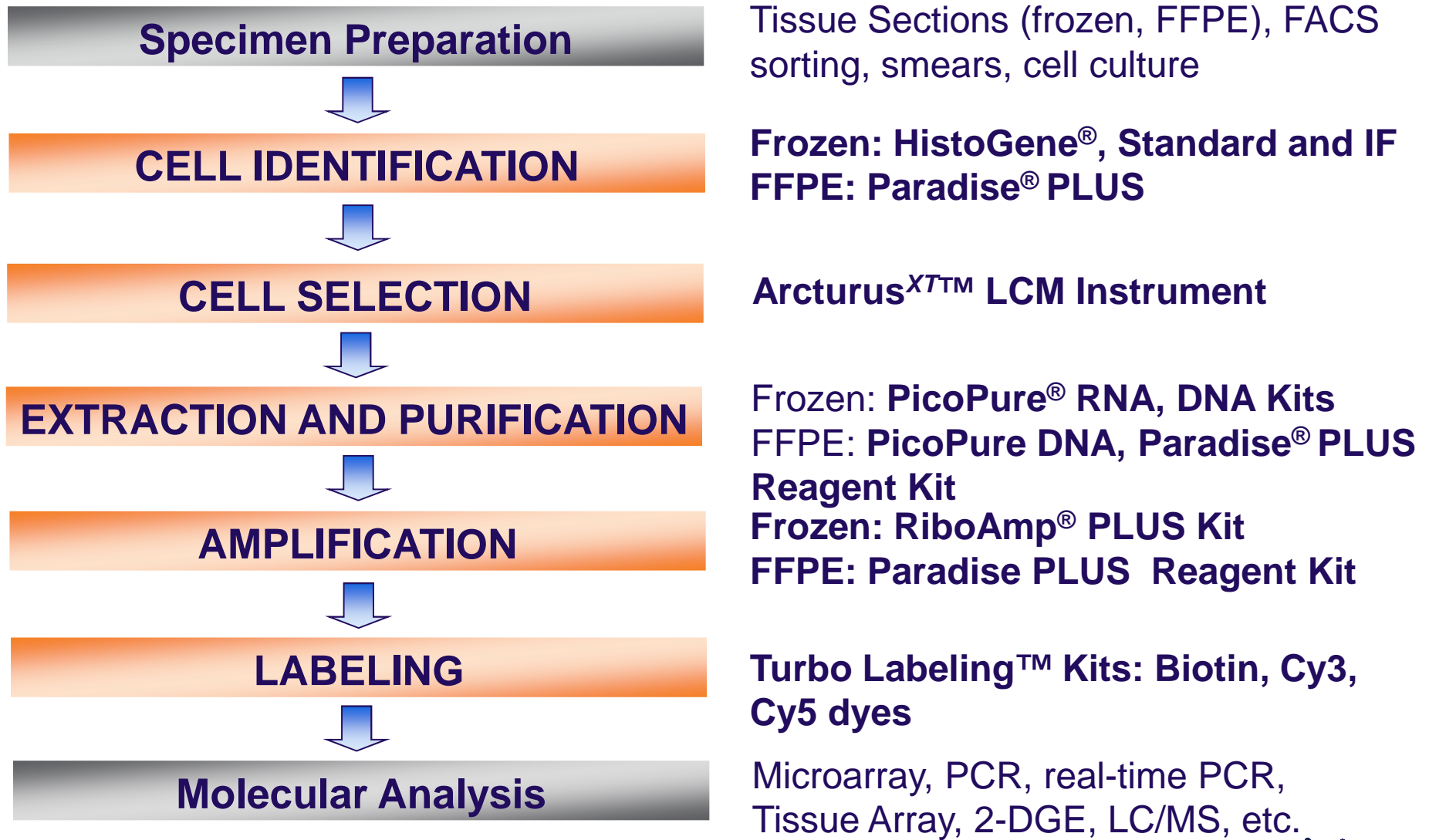
## Microgenomics

Quantitative genomic (and proteomic) molecular analysis of single cells or small groups of cells

## Systems for Microgenomics<sup>®</sup>

Integrated and complete sets of instruments, reagents and protocols for the study of microgenomics

# The Microgenomics Process



# The Microgenomics Process

**Specimen Preparation**

Tissue Sections (frozen, FFPE), FACS sorting, smears, cell culture

**CELL IDENTIFICATION**

Frozen: HistoGene<sup>®</sup>, Standard and IF  
FFPE: Paradise<sup>®</sup> PLUS

**CELL SELECTION**

Arcturus<sup>XT™</sup> LCM Instrument

**EXTRACTION AND PURIFICATION**

Frozen: PicoPure<sup>®</sup> RNA, DNA Kits  
FFPE: PicoPure DNA, Paradise<sup>®</sup> PLUS Reagent Kit

**AMPLIFICATION**

Frozen: RiboAmp<sup>®</sup> PLUS Kit  
FFPE: Paradise PLUS Reagent Kit

**LABELING**

Turbo Labeling<sup>™</sup> Kits: Biotin, Cy3, Cy5 dyes

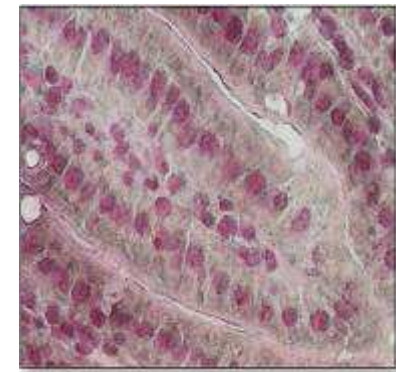
**Molecular Analysis**

Microarray, PCR, real-time PCR, Tissue Array, 2-DGE, LC/MS, etc.

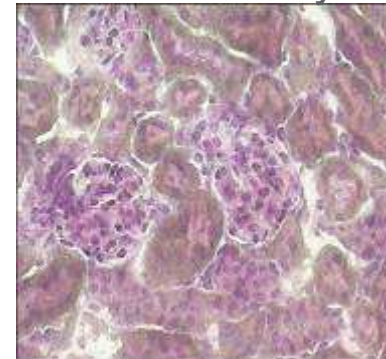
# HistoGene<sup>®</sup> LCM Frozen Section Staining Kit

HIGH QUALITY mRNA  
AND PROTEIN  
FROM FROZEN  
SECTIONS

Mouse Intestine

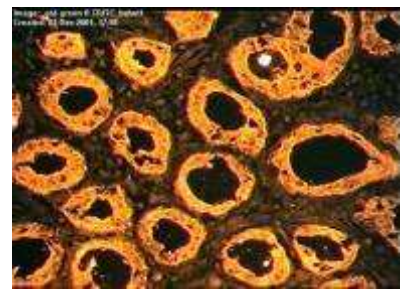


Mouse Kidney



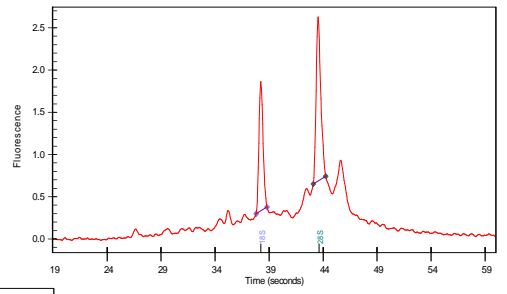
# HistoGene<sup>®</sup> LCM Immunofluorescence Kit

## RNA PRESERVATION & EXCELLENT VISUALIZATION

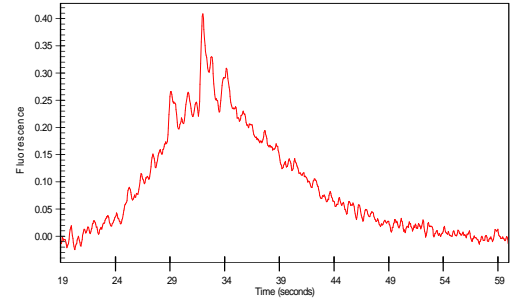


Human jejunum  
+ anti-cytokeratin

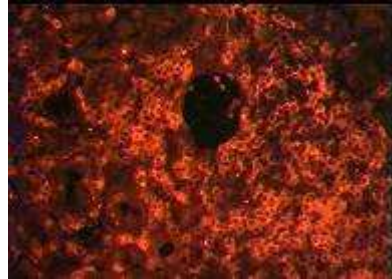
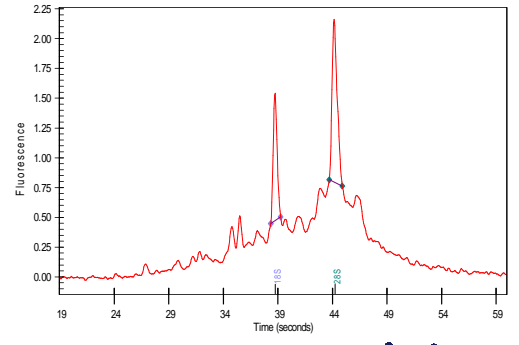
Dehydration only



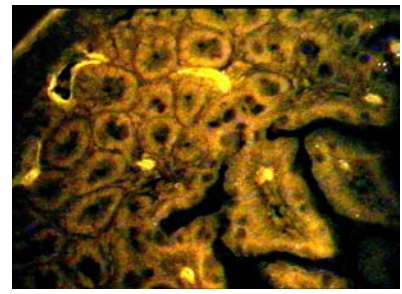
Conventional  
Immuno-staining



HistoGene  
Immunofluorescence Kit



Inflamed mouse  
lacrimal gland  
+ anti-CD4



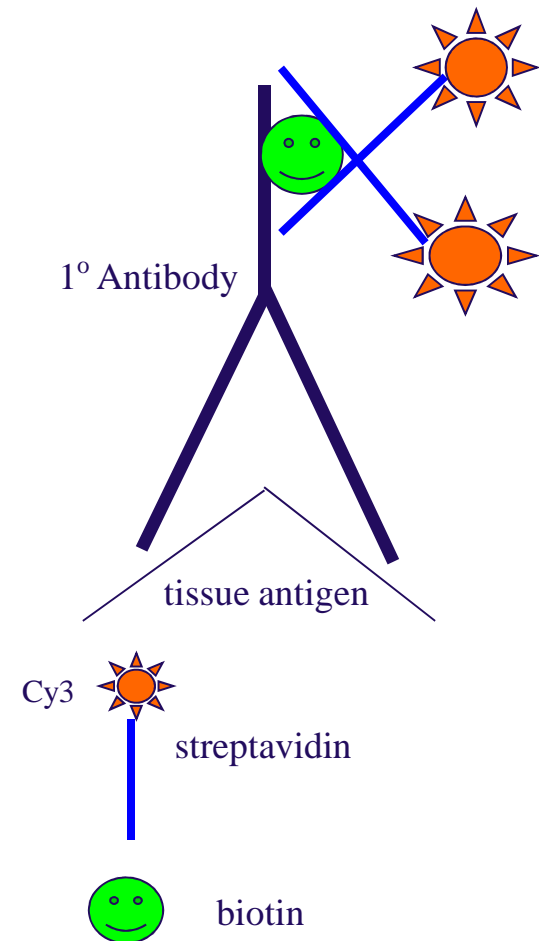
Mouse small  
intestine  
+ anti-MAAdCAM-1

# HistoGene<sup>®</sup> LCM Immunofluorescence Kit

## HistoGene LCM Immunofluorescence Staining Process

- Prepare antibody solutions
- Thaw tissue section slides
- Fix in Acetone (2')
- Rehydrate in staining buffer
- Incubate with biotinylated 1°AB (3')
- Rinse with staining buffer
- Incubate with Cy3-Streptavidin (1')
- Rinse with staining buffer
- Dehydrate
- Perform LCM

**Total staining time = 17 mins**



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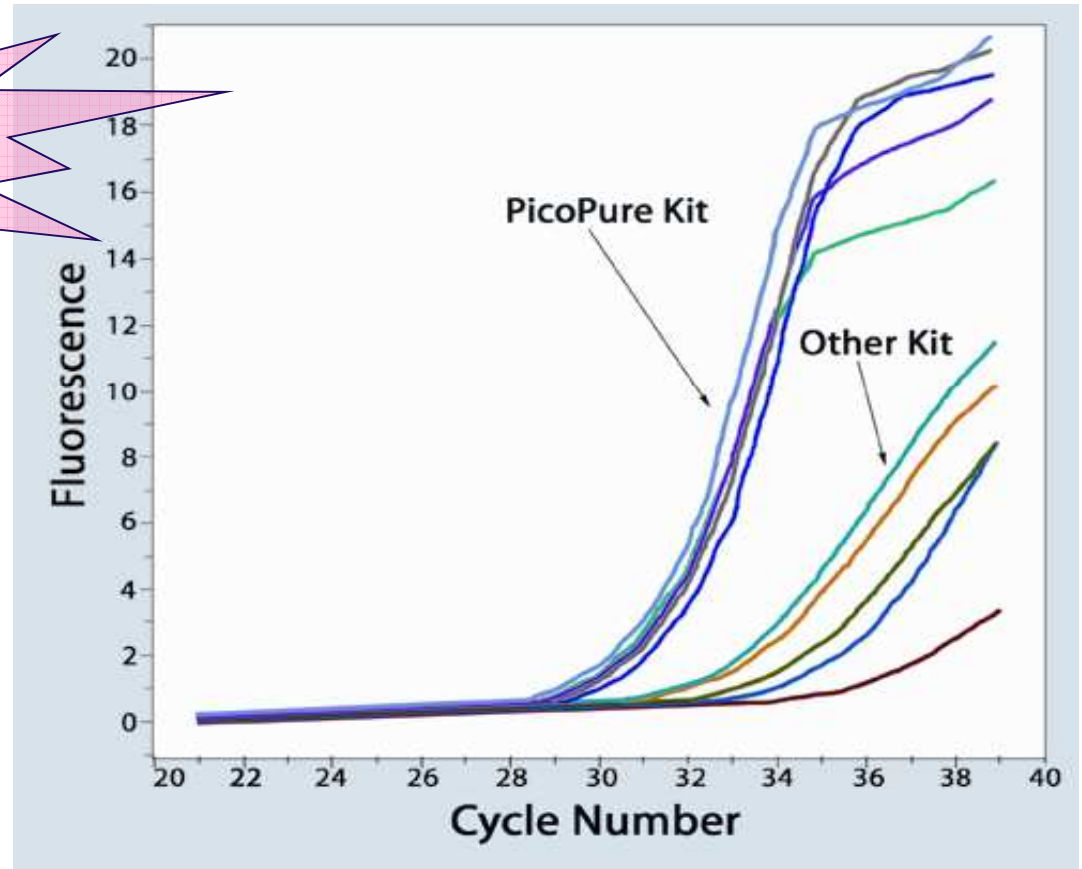
Microarray, PCR, real-time PCR, Tissue Array, 2-DGE, LC/MS, etc.



# PicoPure<sup>®</sup> DNA Extraction Kit

OPTIMIZED FOR SMALL SAMPLE RECOVERY

No purification needed  
prior to PCR

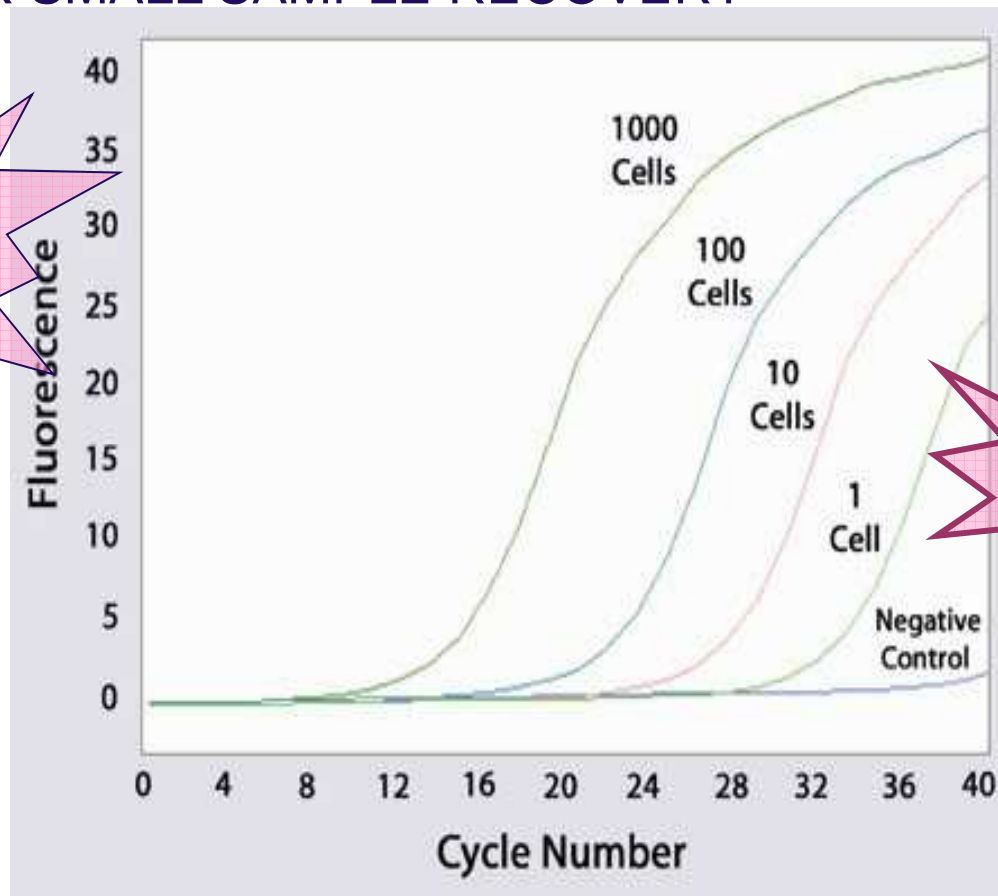


Cycle thresholds for the PicoPure Kit samples were  $28.9 \pm 0.28$  and  $32.4 \pm 1.35$  for the column-purified samples, respectively.  
Samples amplify earlier (more DNA present)  
Samples amplify more consistently (less sample variation)

# PicoPure RNA Isolation Kit

OPTIMIZED FOR SMALL SAMPLE RECOVERY

*>100 $\mu$ g  
capacity*



**Total RNA  
from 1 cell**

Replicate samples of 1, 10, 100, and 1000 cells were microdissected using the PixCell Ite LCM Instrument. RNA was isolated using the PicoPure RNA Isolation Kit, reverse-transcribed and subjected to a QRT-PCR assay for GAPDH using a LightCycler (Roche). Quantifiable message can be detected from a single cell, and the fluorescence signal is proportional to the cell number for all samples studied.



# PicoPure RNA Isolation Kit

## Quantitative Recovery of Total Cellular RNA

- Isolate total RNA from one or more cells
- Maintain high quality of total cellular RNA
- Retains low abundance messages
- Columns designed to bind up to 140 micrograms
- 10  $\mu$ l elution volume allows optimal integration into down-stream processes such as reverse transcription and linear amplification

# The Microgenomics Process

**Specimen Preparation**

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Microarray, PCR, real-time PCR, Tissue Array, 2-DGE, LC/MS, etc.

# RiboAmp<sup>Plus</sup> RNA Amplification Kits

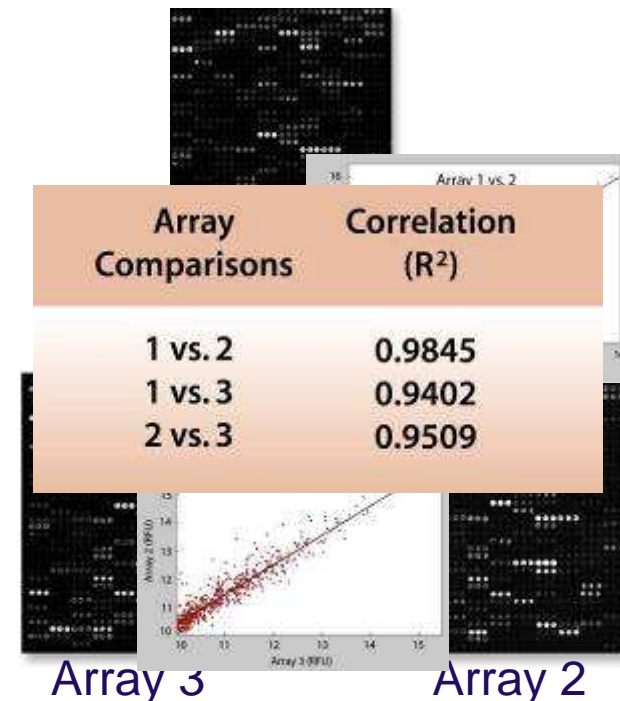
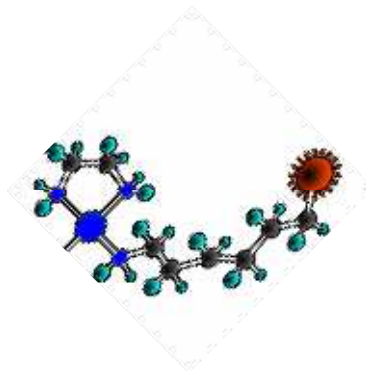
Amplify enough RNA for microarray analysis consistently and robustly from a few nanograms of sample

RiboAmp Plus → 5 ng - 40 ng  
RiboAmp HS Plus → 100 pg - 5 ng

aRNA yields enough material for a microarray (up to 50 µg) with 2 rounds of amplification

RiboAmp PLUS is available in a wide variety of configurations customized for your research:

- Biotin labeling
- Cy3 Labeling
- Cy5 Labeling
- Amino-allyl incorporation
- Natural nucleotides
- qrtPCR



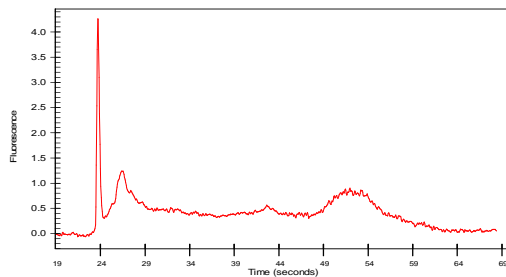
# Which RiboAmp PLUS Kit Should I Choose?

	RiboAmp HS PLUS 100pg to 5ng of Total RNA (10 to 500 Cells)		RiboAmp PLUS greater than 5ng Total RNA (> 500 cells)	
Microarray Platform	Turbo	Alternative Labeling	Turbo	Alternative Labeling
Biotin based platforms <i>(Illumina, Affymetrix, CodeLink)</i>	KIT0515B	KIT0528	KIT0511B	KIT0526
Cy3, Cy5 based platforms <i>(Agilent, BlueGnome, NimbleGen, Oligonucleotide arrays)</i>	KIT0515C	KIT0525aa	KIT0511C	KIT0525aa
	KIT0515D	KIT0528	KIT0511D	KIT0526
QRT-PCR <i>(ABI, Fluidigm, BioTrove)</i>	100pg to 5ng of Total RNA		greater than 5ng Total RNA	
few genes	KIT0528		KIT0526	
many genes	KIT0525		KIT0521	

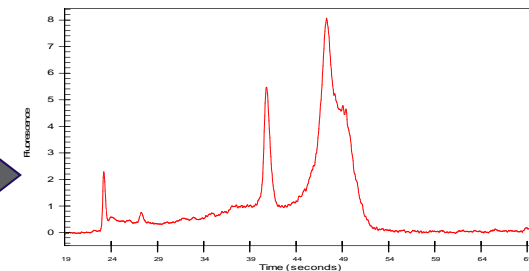
\*aa = for amino allyl

# Gene Expression Analysis of Formalin-Fixed Tissue

- The Problem:
  - Cross-links nucleic acids and proteins
  - Interferes with RNA Amplification and RT processes



← Got This  
But  
Want This? →



- The Solution: Arcturus' Paradise<sup>®</sup> Plus FFPE Reagent System
  - Gene expression studies of FFPE samples are possible
  - Retrospective and prospective studies
- The Paradise Plus system is analogous to:
  - HistoGene Staining,
  - PicoPure RNA isolation and
  - RiboAmp PLUS Amplification

... In one kit optimized for FFPE material

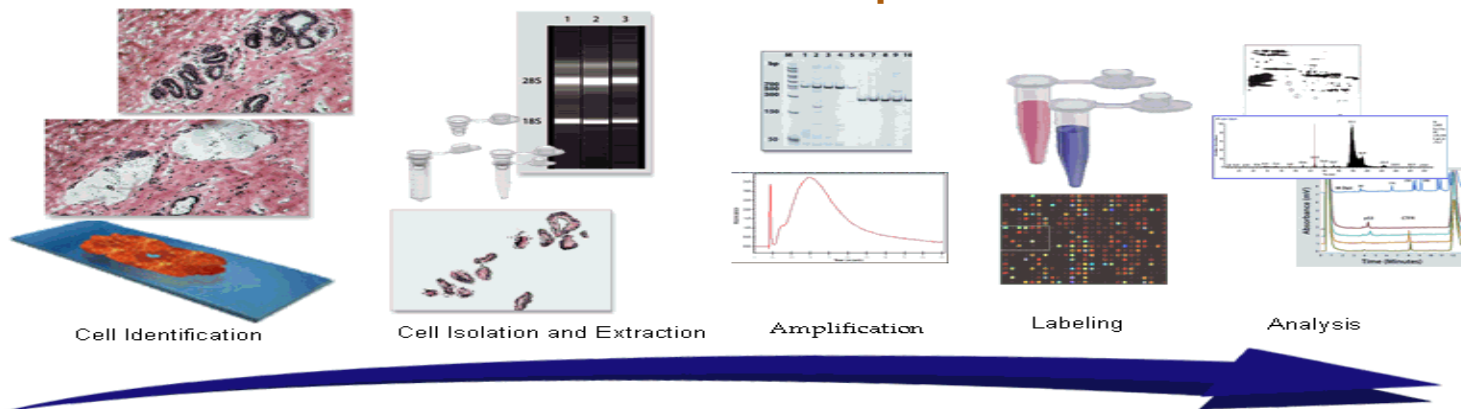
# Paradise<sup>®</sup> Plus Reagent System



**Paradise Plus**  
**Reagent System**  
**Unlock your tissue archives**

Paradise available with options customized for your research:

- Biotin labeling
- Cy3 Labeling
- Cy5 Labeling
- Amino-allyl incorporation
- Natural nucleotides
- qRT-PCR

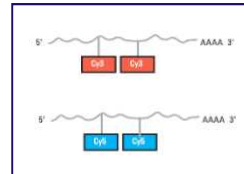
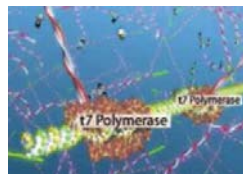
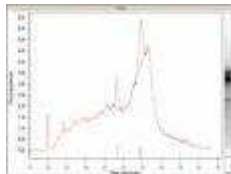
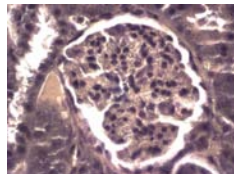


FFPE Tissue staining, extraction, isolation, amplification, and microarray labeling – all in one convenient kit



# Paradise<sup>®</sup> Plus Product Family

- Paradise<sup>®</sup> Plus Reagent System -
  - Gene Expression Analysis for FFPE Specimens and LCM Samples
- Paradise<sup>®</sup> Plus QC Kit
  - Qualify your FFPE samples
- Paradise<sup>®</sup> Plus Quantitative Real Time PCR (qRT-PCR) Kit
  - Quantify Your Genes of Interest
- Paradise<sup>®</sup> Plus Whole Transcript Reverse Transcription (WT-RT) Reagent System
  - Formulated for Real-Time PCR (RT-PCR)



# Which kit to chose?

## ParadisePLUS Amplification Kits

Paradise	minimum 5ng Total RNA	
Microarray Platform	Turbo	Alternative Labeling
Affymetrix (biotin based platforms)	KIT0312B	KIT0311
Agilent oligoarrays (Cy3, Cy5 based platforms)	KIT0312C KIT0312D	KIT0314 KIT0311
qRT-PCR	minimum 5ng Total RNA	
1round amplification	KIT0310	
FFPE sections	KIT0315	

- KIT0314 – amino allyl
- KIT0315 (WT-RT) is RNA extraction and cDNA synthesis only (no amplification)

# The Microgenomics Process

**Specimen Preparation**

Tissue Sections (frozen, FFPE), FACS sorting, smears, cell culture

**CELL IDENTIFICATION**

Frozen: HistoGene<sup>®</sup>, Standard and IF  
FFPE: Paradise<sup>®</sup> PLUS

**CELL SELECTION**

Arcturus<sup>XT™</sup> LCM Instrument

**EXTRACTION AND PURIFICATION**

Frozen: PicoPure<sup>®</sup> RNA, DNA Kits  
FFPE: PicoPure DNA, Paradise<sup>®</sup> PLUS Reagent Kit

**AMPLIFICATION**

Frozen: RiboAmp<sup>®</sup> PLUS Kit  
FFPE: Paradise PLUS Reagent Kit

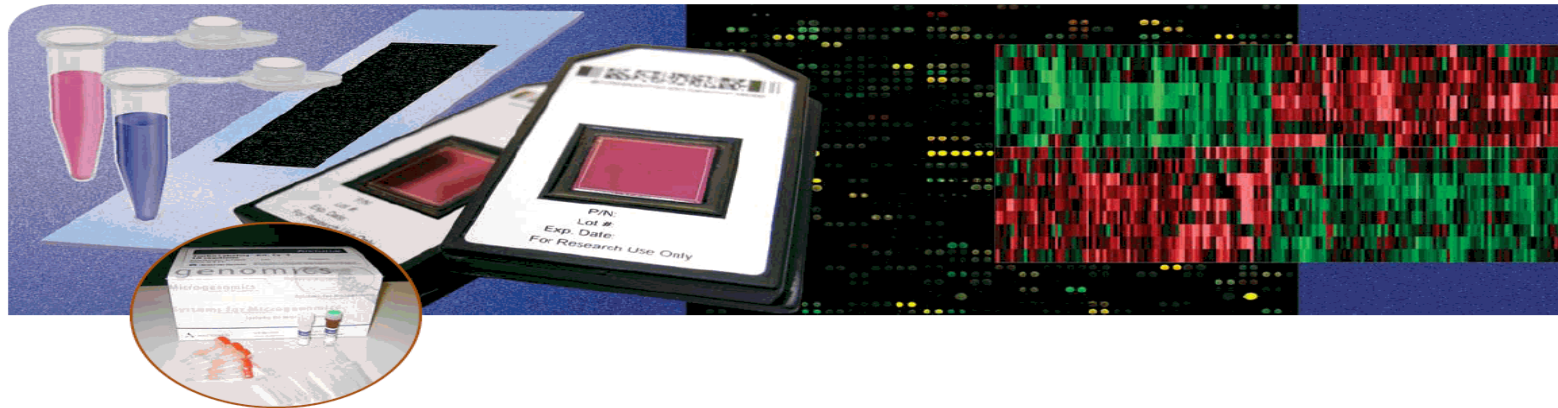
**LABELING**

Turbo Labeling<sup>™</sup> Kits: Biotin, Cy3, Cy5 dyes

**Molecular Analysis**

Microarray, PCR, real-time PCR, Tissue Array, 2-DGE, LC/MS, etc.

# Turbo Labeling™ Kits



- Non-enzymatic technology for labeling of DNA or RNA for any microarray application including Oligo, cDNA and CGH arrays
  - Easy protocol takes less than an hour
  - Allows splitting samples for comparative studies and differential labeling
- Especially useful when sample needs to be amplified
  - Enables using natural nucleotides in the amplification process
  - Results in unmodified aRNA with higher yields and longer aRNA fragments
  - Better representation of the mRNA transcript for downstream analysis
  - Higher %P calls



# Turbo Labeling™ – Features and Benefits

- Non-enzymatic labeling done after amplification
- Labels aRNA generated from both frozen and FFPE samples
- Labels any nucleic acid:
  - Total RNA
  - Amplified RNA (aRNA or cRNA)
  - Genomic DNA
  - cDNA
- By labeling post amplification, customer is not committed to a particular array platform
- Samples can be split for comparative studies:
  - Using different array platforms (Affymetrix vs. Agilent)
  - Array versus qRT-PCR
- Labeling can be done using a simple protocol (< 30 minutes)

# Arcturus<sup>®</sup> System LCM Consumables

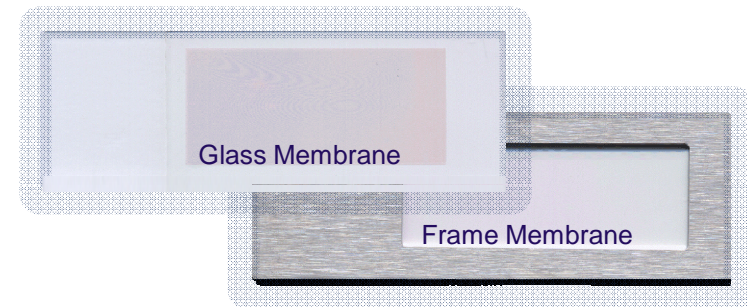
- CapSure<sup>®</sup> LCM Caps

- Macro LCM Caps
- HS LCM Caps



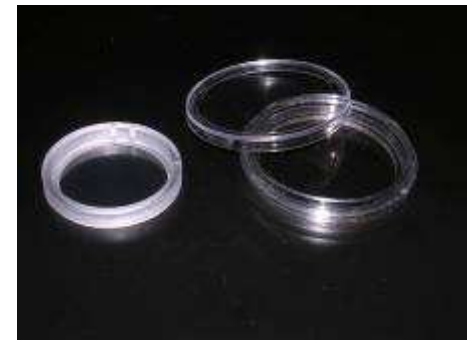
- Tissue Microdissection Slides

- Membrane Glass Slides
- Membrane Frame Slides

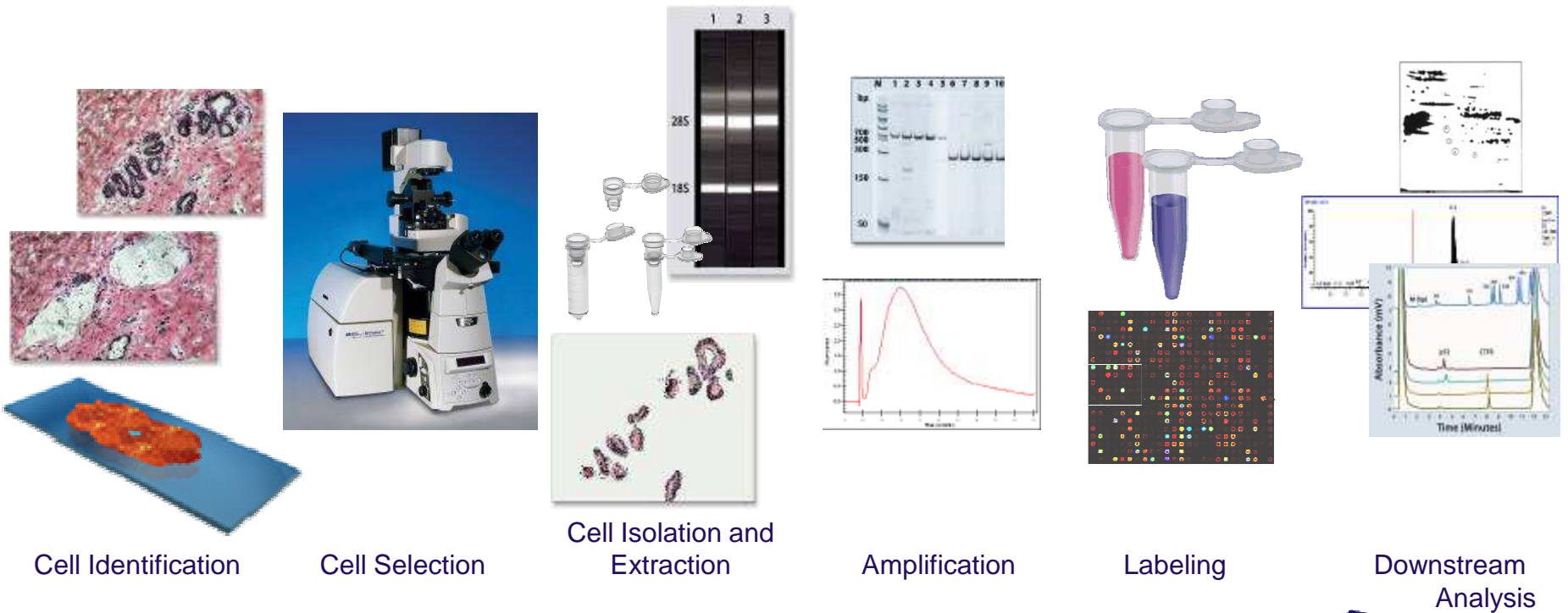


- Live Cell Microdissection

- Membrane Frame Slides (Untreated)
- Arcturus<sup>XT</sup> Instrument Live Cell Growth Chamber, Sterile
- Arcturus<sup>XT</sup> Instrument Microdissection Petri Dish, Sterile



# Integrated Systems for Microgenomics®



HistoGene®  
Kits

Arcturus<sup>XT</sup>™  
LCM  
System

PicoPure® Kit  
(frozen)  
Paradise®  
PLUS Kits  
(FFPE)

RiboAmp® PLUS  
Kit (frozen)  
Paradise® PLUS  
Kit (FFPE)

Turbo  
Labeling™  
Kit



# Q&A

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