# How to Use Leica LMD7000

# Step 1

# Turn on the system

# Turn on the power strip

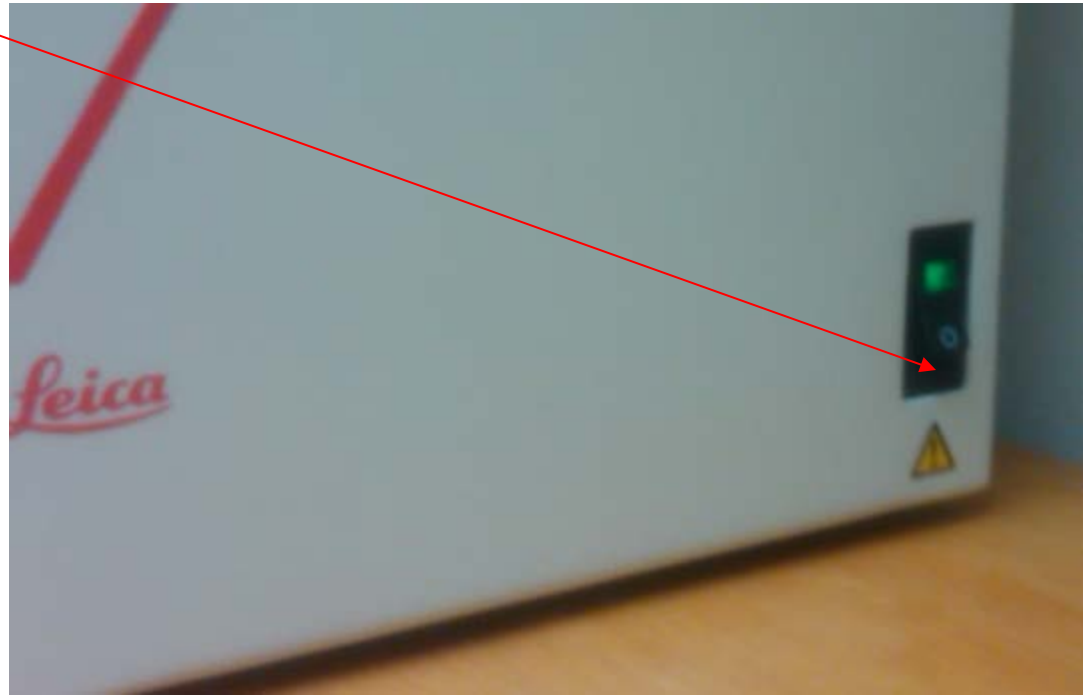
# Turn on the fluorescence light source **ONLY IF** you have fluorescent labels

# Turn on the laser key

- There is a power button on the rear left corner. You may leave it on as we will use the power strip.
- Turn on the laser key
- The ERROR light will be on right after the power is on
- Emission light (blue) will be on after the software started the laser cutting.

# Turn on the CTR6500 controller box

- This is the microscope controller.
- Wait for the CTR6500 started before starting the software.
- Also wait for the PC completely booted before running LMD software.

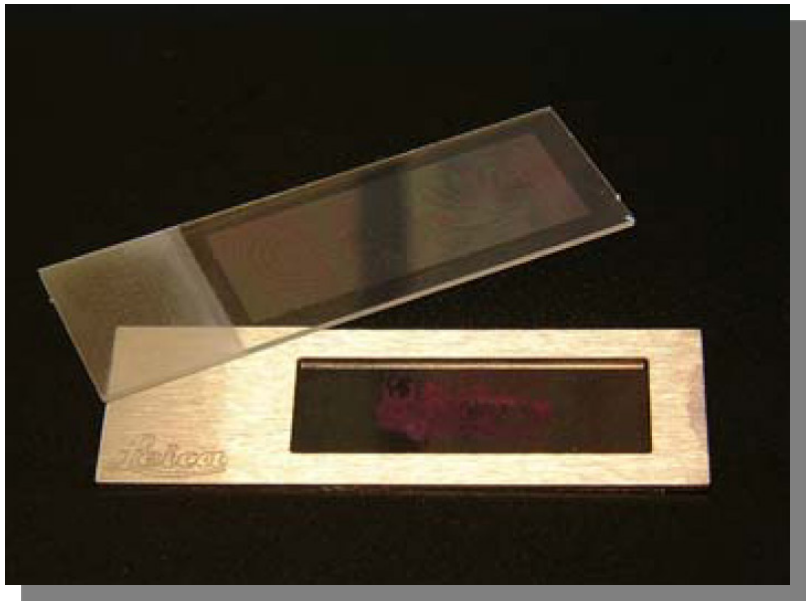


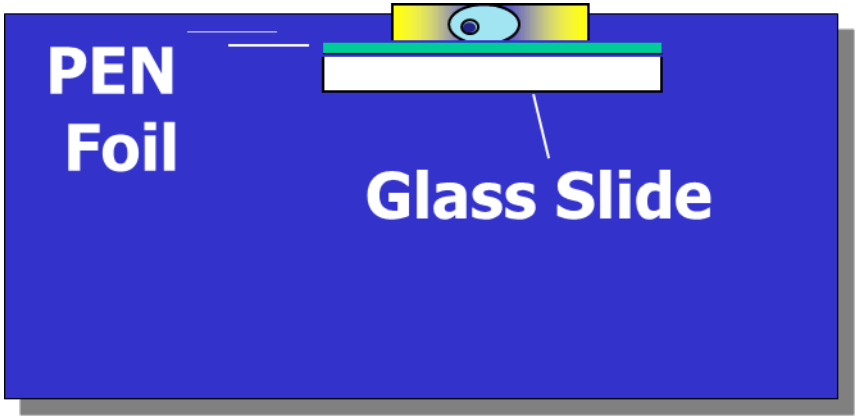
Turn on the computer if it is not on automatically after turning on the power strip

# Step 2

# Prepare the Sample with LMD Membrane Foil Slides

# LMD Slides: Glass vs Frame; PEN vs PET






Tissue Sections placed directly on to the PEN/PET/POL foil anchored to slide.

| | |
|---|---|
| Glass foiled PEN (2.0 u) slides (Poly Ethylene Naphthalate) | 11505158 (50 per box) |
| Frame foiled PET (1.4 u) slides (Poly Ethylene Terephthalate) | 11505151 (50 per box) |
| PEN MembraneSlide (**4µm**), **glass**, 50 pcs | 11600288 |
| PEN FrameSlide, (**4µm**), **steel frames**, 50 pcs | 11600289 |

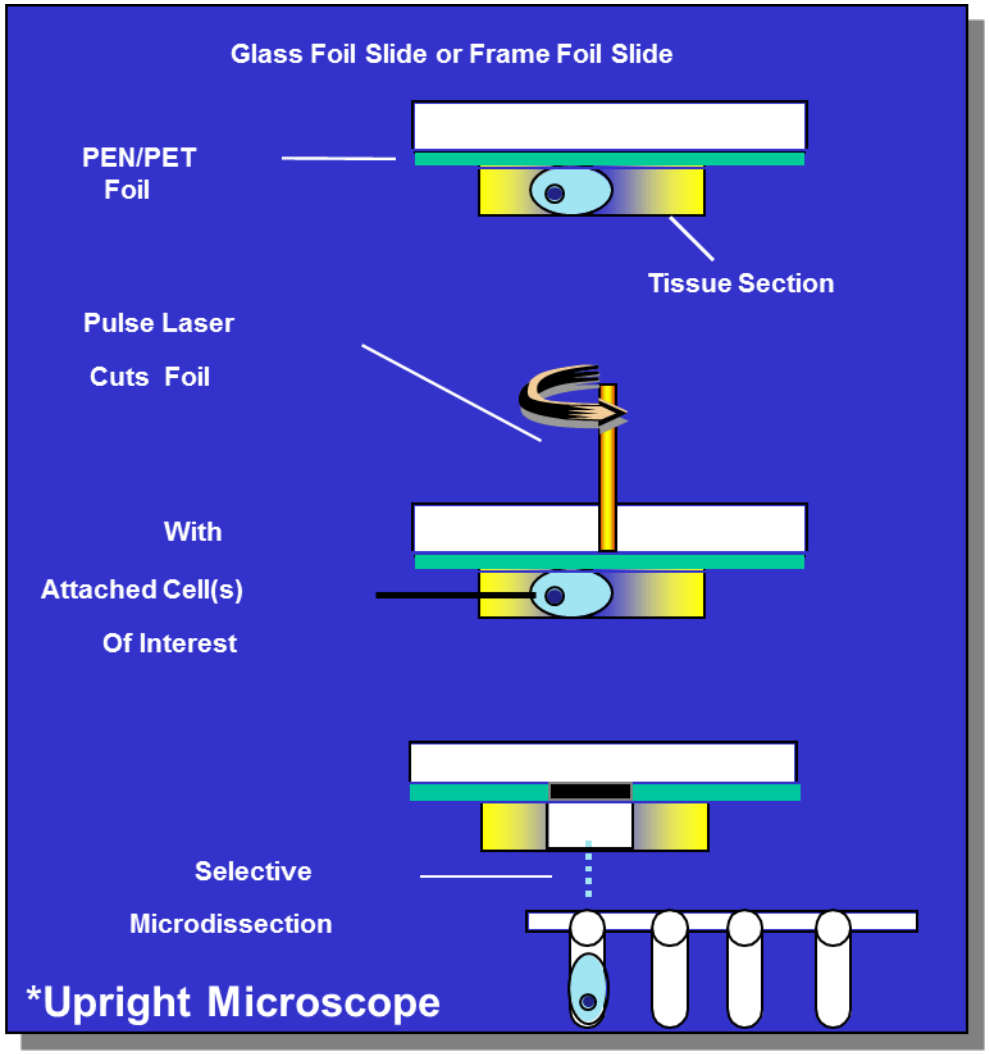
# Typical Protocol for Frozen Sections

- Cut sections 4-20 um
- Mount on special Leica foiled slides
- Fixation with 70% EtOH for 30 sec. minimum
- Wash in DEPC water for 30 sec.
- Stain with Mayer's hematoxlin for 30 sec.
- Wash in DEPC water for 30 sec.
- Counterstain with eosin for 30 sec.
- Rinse with 95% and 100% EtOH for 30 sec.
- Air dry approximately 5 minutes

# Typical Protocol for Paraffin Sections

- Cut sections 4-20 um
- Mount on special Leica foiled slides
- De-wax with xylene for 30-45 sec (1 min maximum)
- Clear with 100%, 95%, 70% EtOH for 30 sec. each
- Rinse with Water for 30 sec.
- Stain with Mayer's hematoxlin for approx. 2 min.
- Wash in water for 60 sec.
- Rinse in 70% EtOH for 30 sec.
- Counterstain with eosin for 10-15 sec.
- Rinse with 95% and 100% EtOH for 30 sec.
- Air dry approximately 5 minutes

# LMD: Non-Contact Method




Section placed directly on to the PEN/PET/POL foil anchored to slide.

Cell(s) of interest are separated from unwanted cells by a cutting Ultra Violet laser.

They are collected directly into the cap of a PCR tube for further downstream analysis.

# Making the Slides RNase Free

- The foil slides can be treated to remove RNases by dipping them into a bath of pure RNase Zap (Ambion Corp) for 15 seconds. Follow this with two rinses in DEPC water to remove all of the RNase Zap. The slides then should be completely dried by placing them in a heater at 37 deg C for as long as is required to dry. Then one may follow with UV treatment as described below.

- Note: Heating in an autoclave may not always ensure that all RNases are destroyed. If this method is performed the heating must ba at 180 deg C for up to 4 hours.

# Preparing the Slide with UV Irradiation

- Prior to placing specimens on the membrane slides, it is advisable to prepare the membrane by placing the blank slides to be used, into a UV Cross-linker device. The slides should be irradiated at 220nm to 260nm at full power for 30 minutes. This will usually destroy RNases, improve the laser cutting of the foil (reducing static), and help make the foil more hydrophilic to improve adherence of the specimen.

# DNA, RNA, Protein Downstream Analysis

**DNA**

*1 to 5,000 cells*

- Clonal analysis
- DNA methylation
- Direct sequencing
- CGH
- LOH

**RNA**

*1-1,000 cells*

- RT PCR

*> 500 cells*

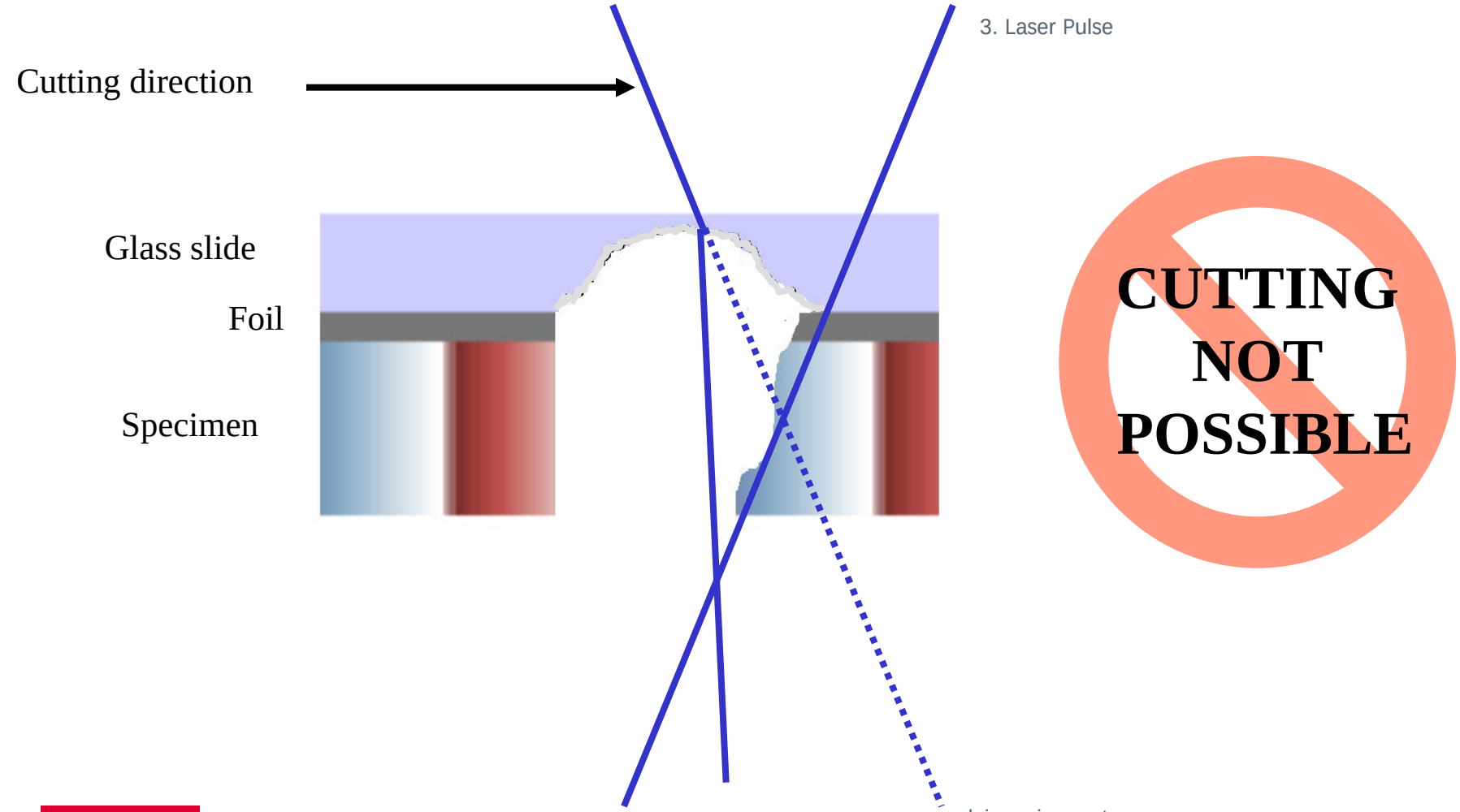
- cDNA library construction
- cDNA microarray probes
- Differential display
- Gene Expression Arrays

**Proteins**

*> 5,000-50,000 cells*

- Resolve
- 2D-PAGE
- Identify
  - Mass Spectrometry
  - Western Immuno-blotting
- MALDI – TOF
- ESI - MS

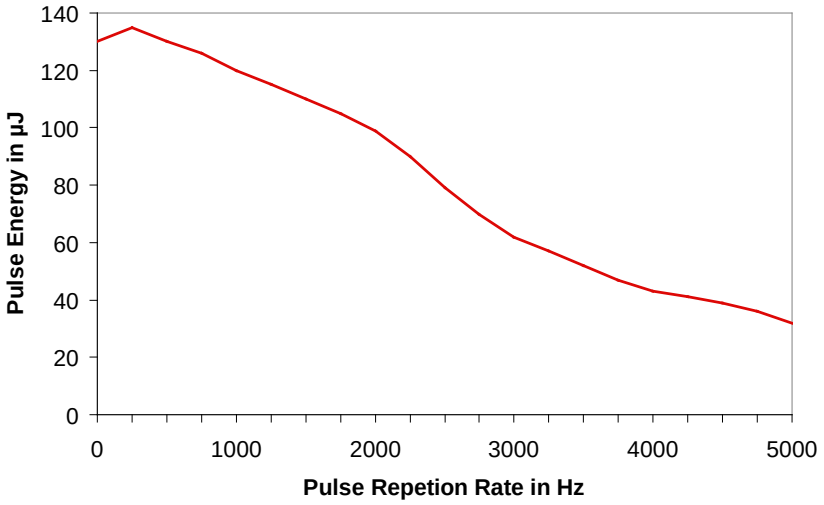
# Laser Induced Glass Etching




**CUTTING NOT POSSIBLE**

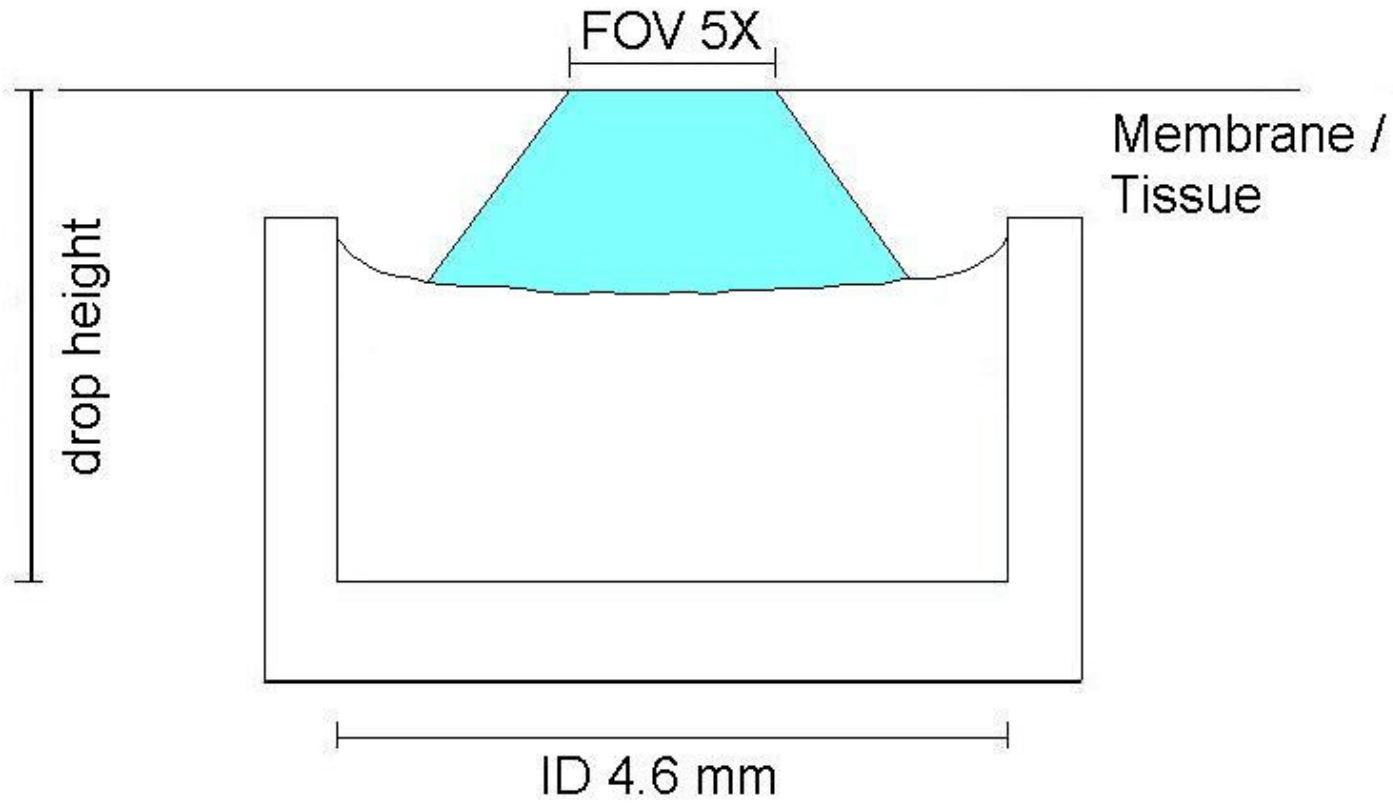
# LMD7000 Laser

- Use lower frequency: 120 Hz
  - Better cutting
  - Less static
- Use slower speed: 3 - 10




| LMD Laser Systems | Leica LMD7000 (SpectraPhysics) |
|---|---|
| Gain medium | Nd:YLF Neodymium, yttrium lithium fluoride |
| Wavelength | 349 nm |
| Max. pulse energy | 120 µJ |
| Q-switch | active |
| Repetition rate | 1 – 5000 Hz |

# LMD PCR Tube Cap Dimension



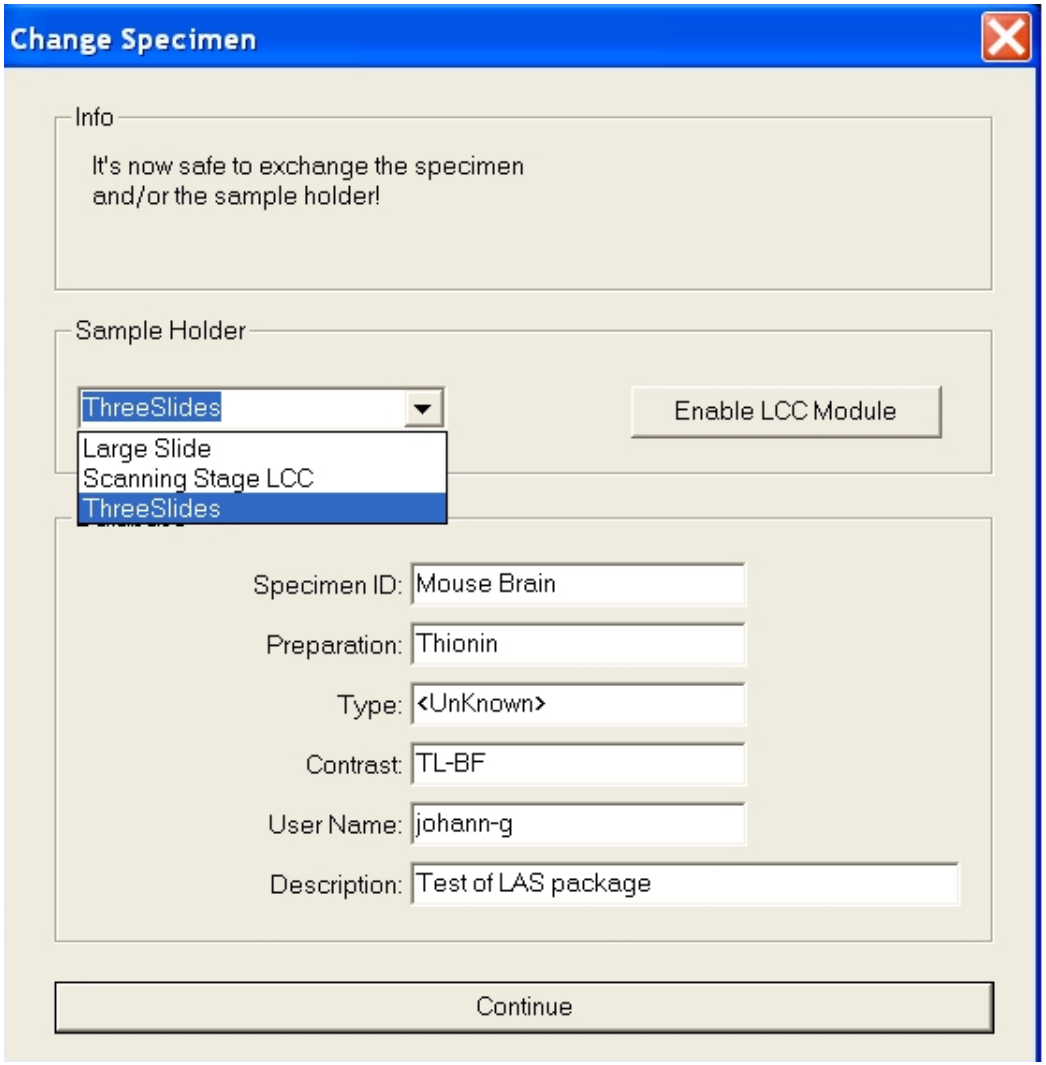

Tissue can only fall down in the lid of the PCR cap

# Step 3

# Start LMD Software

# Load the Specimen

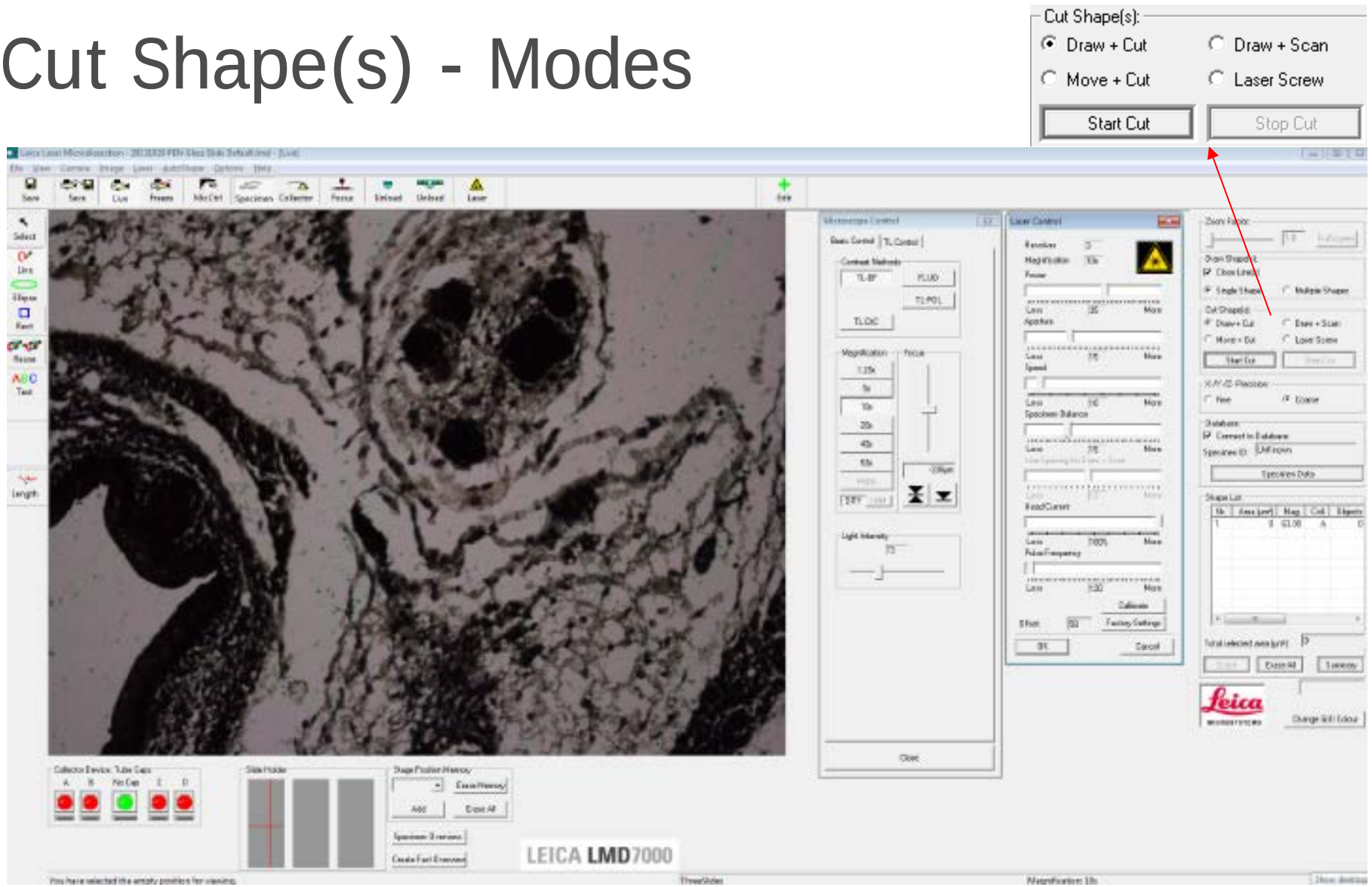
- Click the first “Unload”
- Mount the sample on the slide holder
- Put the slide holder on the stage
- Click “Continue”



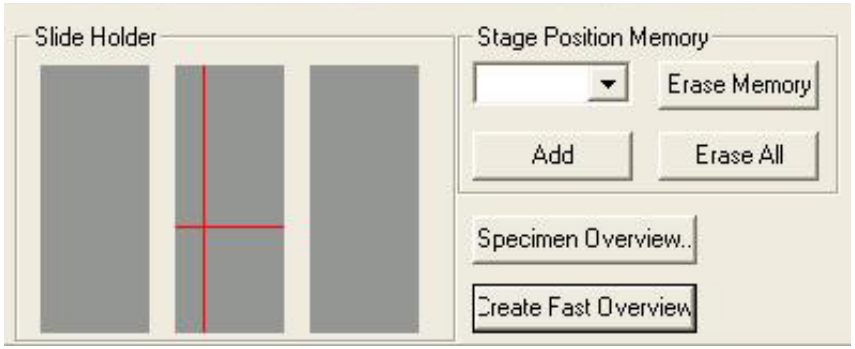
# Load Collector

- Click the 2nd button “Unload”
- Take the collector holder out (0.5ml or 0.2ml – two holders)
- Mount 4 PCR tubes in (0.5ml or 0.2ml flat head PCR tubes).
- Put the holder in. Top left corner has a 45 degree cut.
- Click “Continue”.

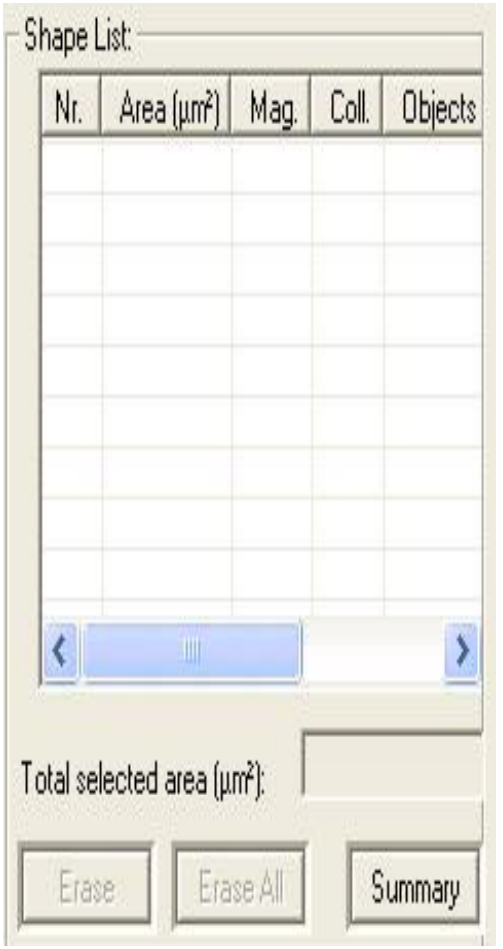
# Cut Shape(s) - Modes



# Easy to Use Software

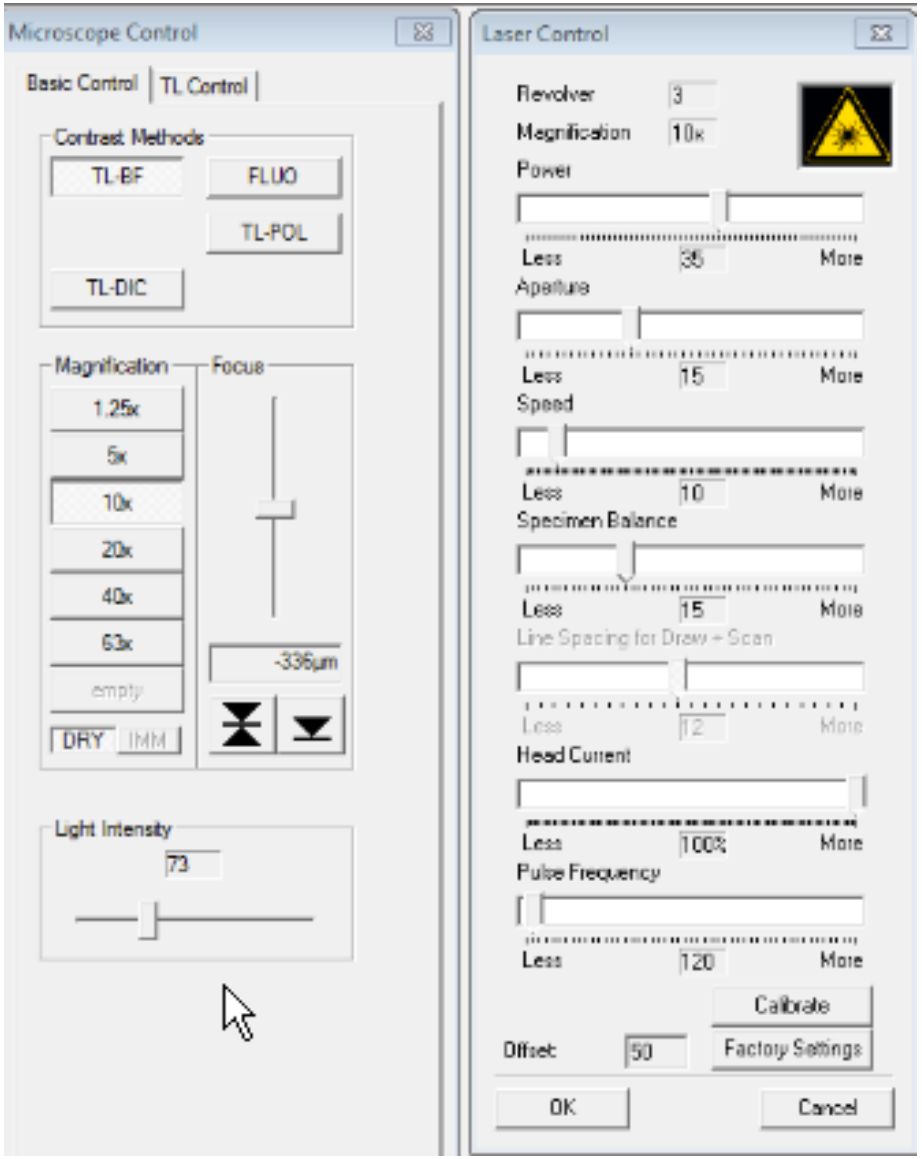





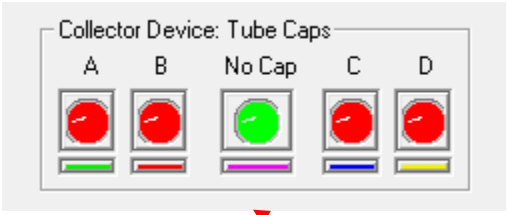


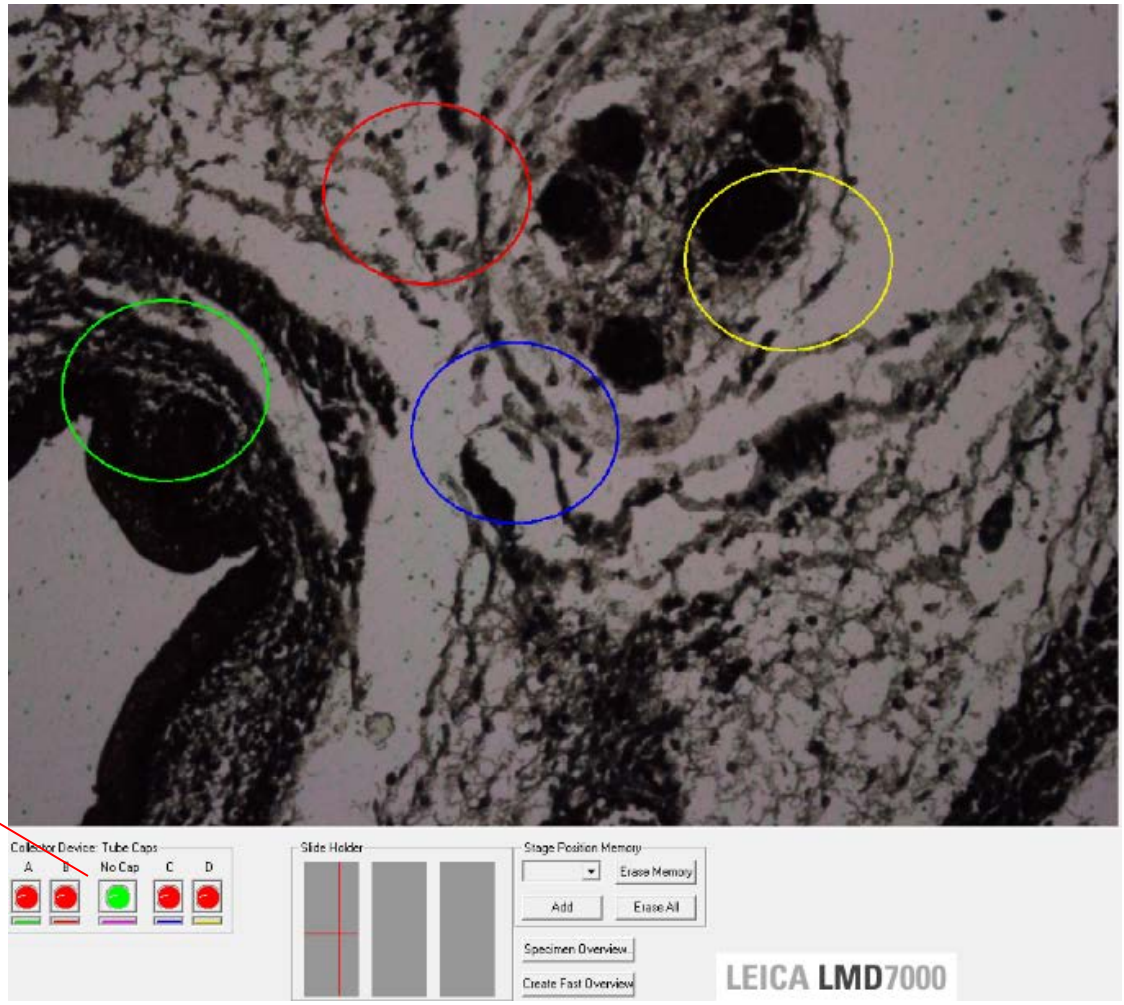
# Automated Control

- Microscope Control
- Laser Control



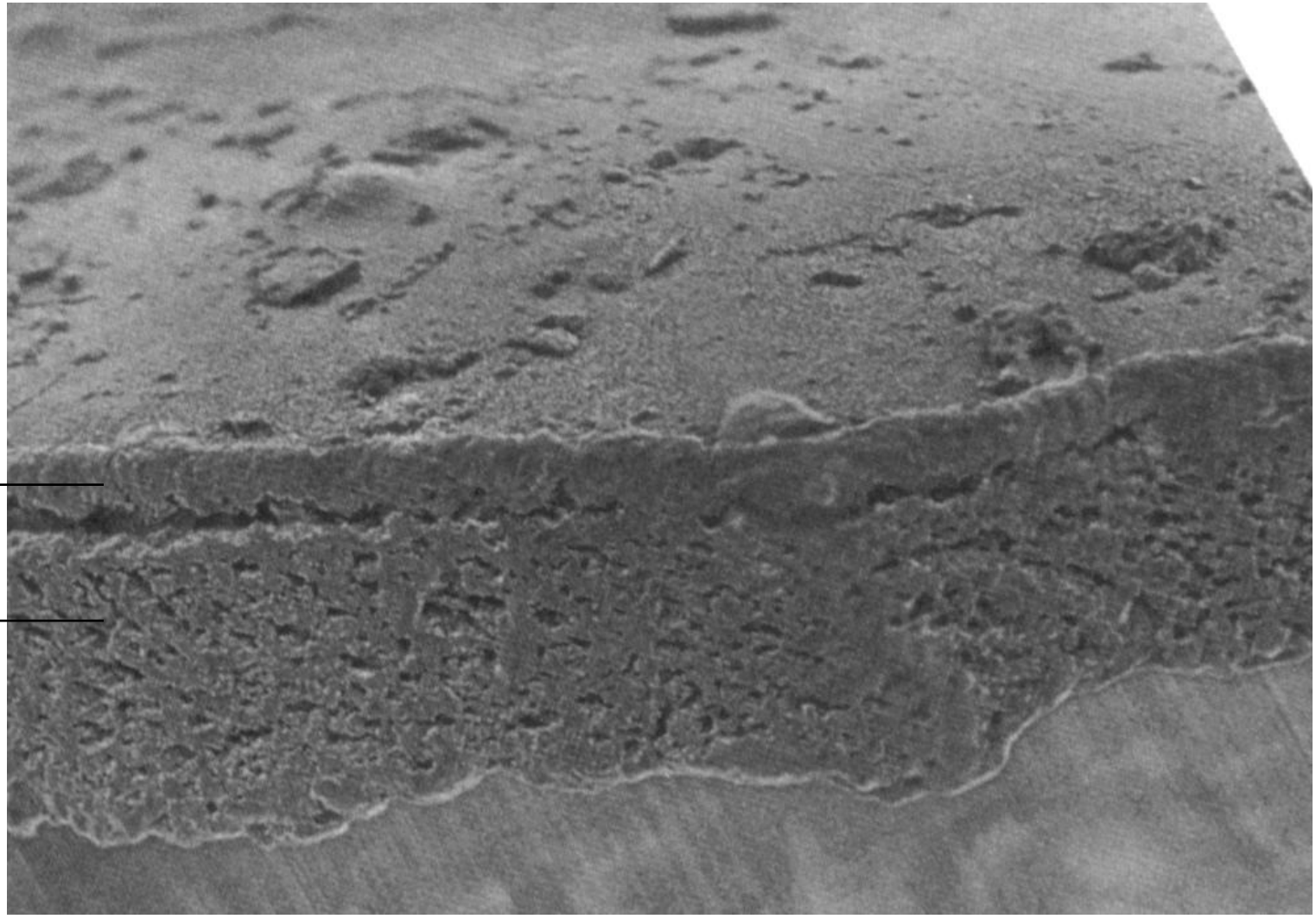
# Multiple ROI Cutting



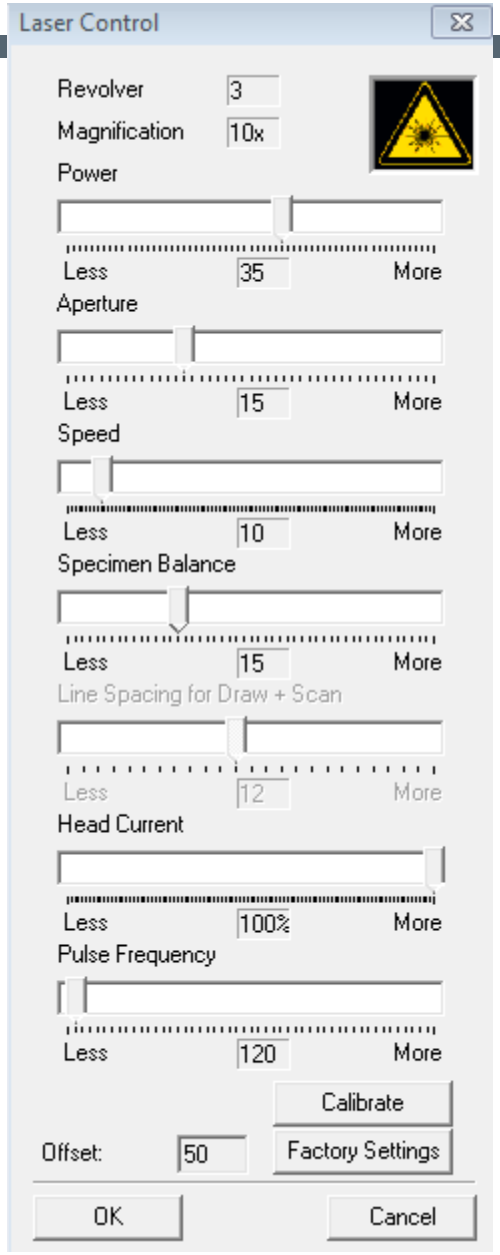
# Step 4
# Laser Microdissection Parameters

# Laser Cold Ablation

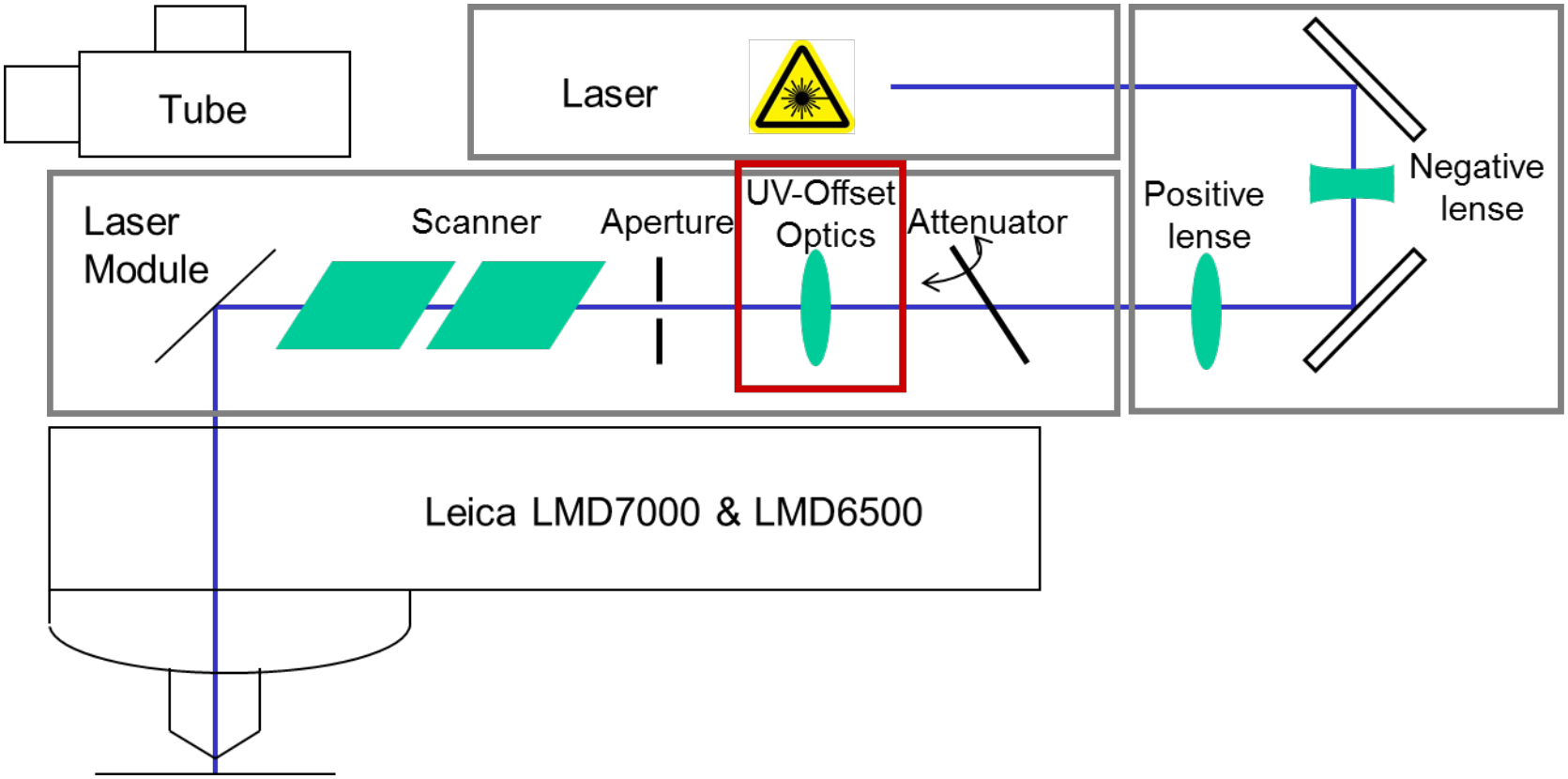


# Laser Parameters

- For LMD7000 laser:
  - Frequency (choose 120 Hz, up to 800Hz)
  - Laser current
    Always use 100%
- Power and Aperture
  - For glass slide too much power could cause glass etching.

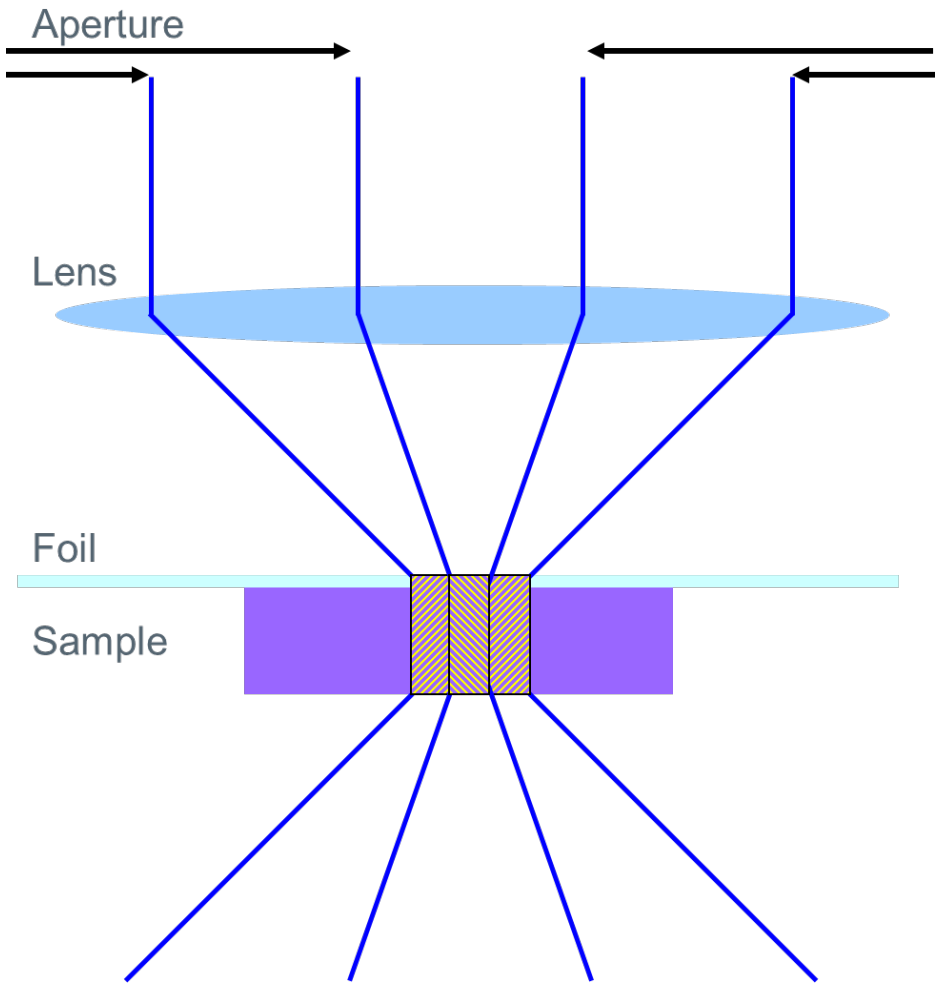


# LMD Laser Module Light Path

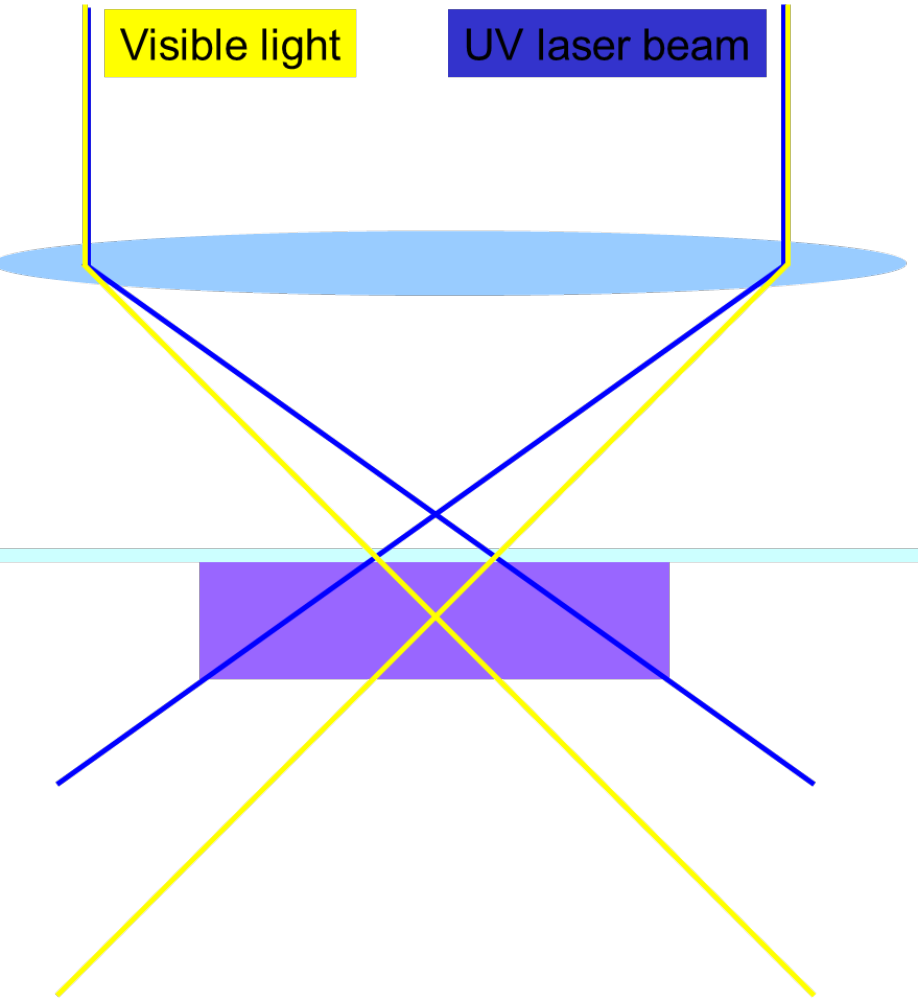


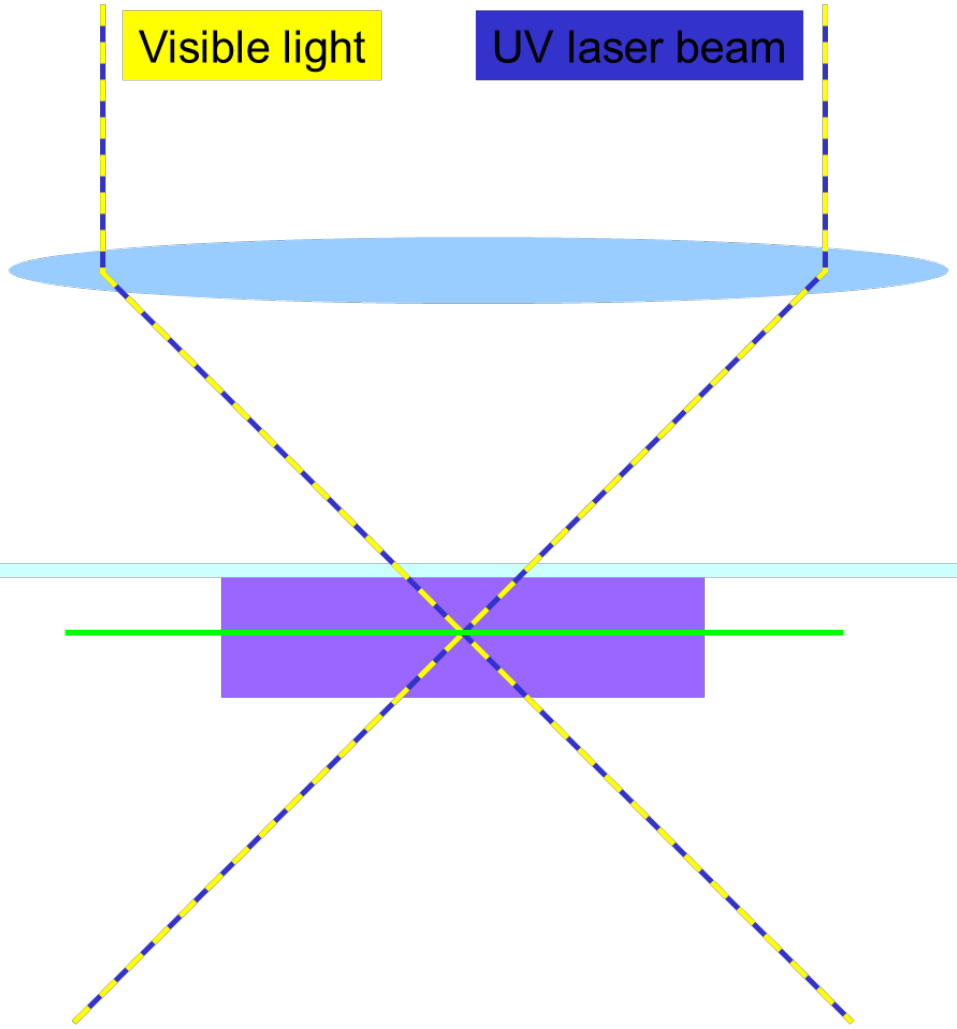
# Laser Aperture Control

- The aperture allows to control the width of the cutting line
- Aperture ⇑ Pulse Energy ⇑
  Aperture ⇓ Pulse Energy ⇓
- Always optimize aperture settings for every objective

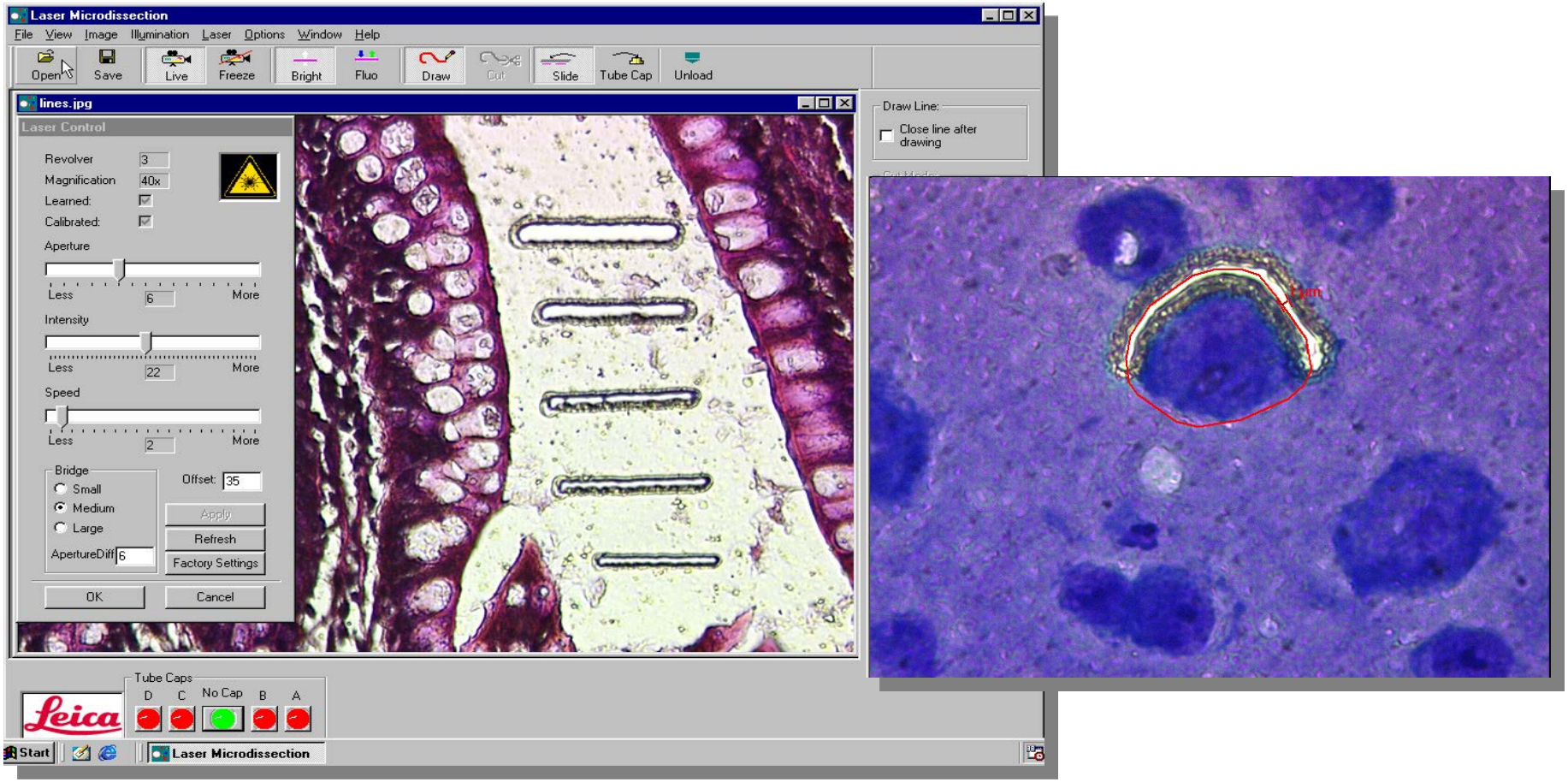


# UV Offset Control

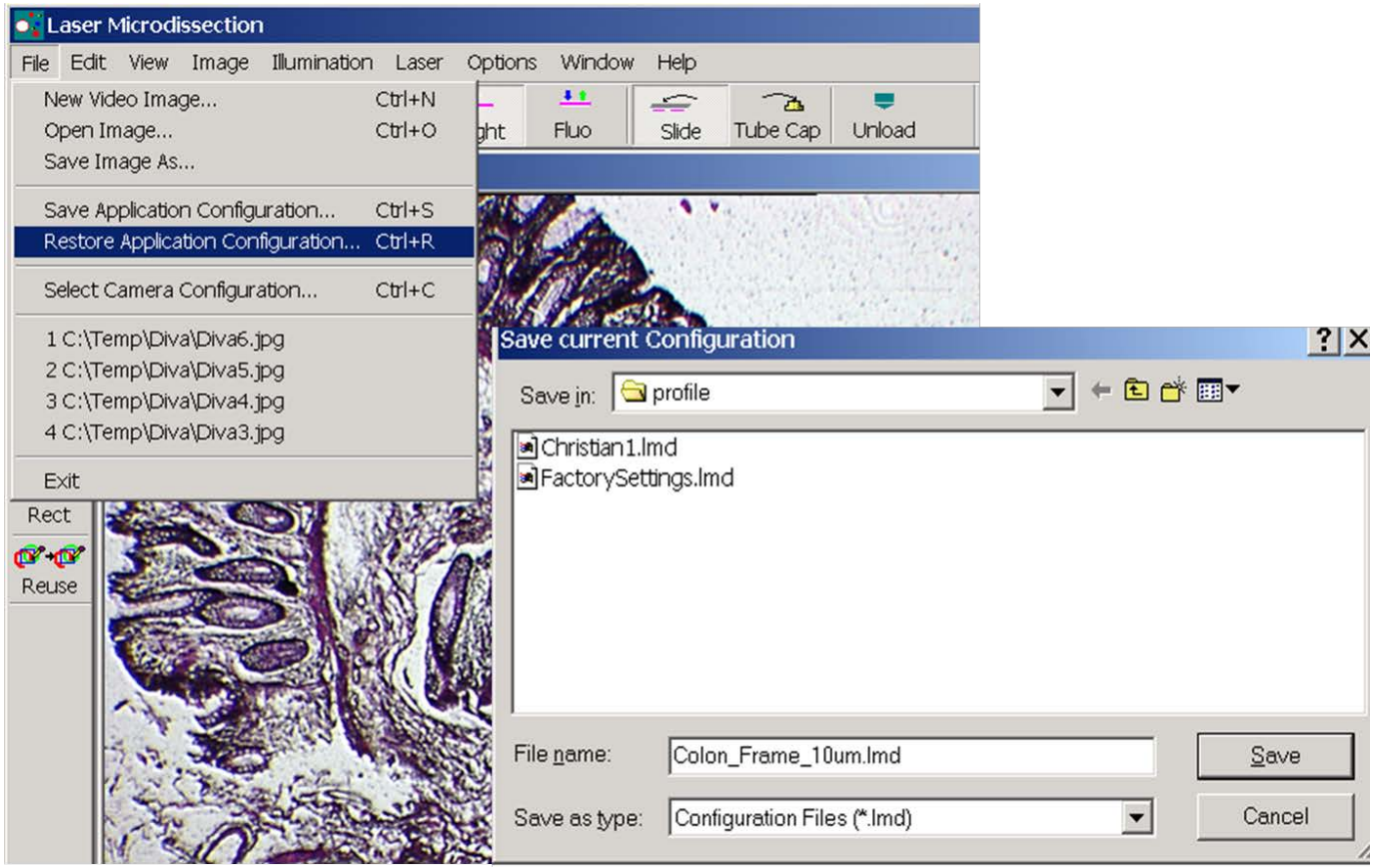


# Laser Cut Line

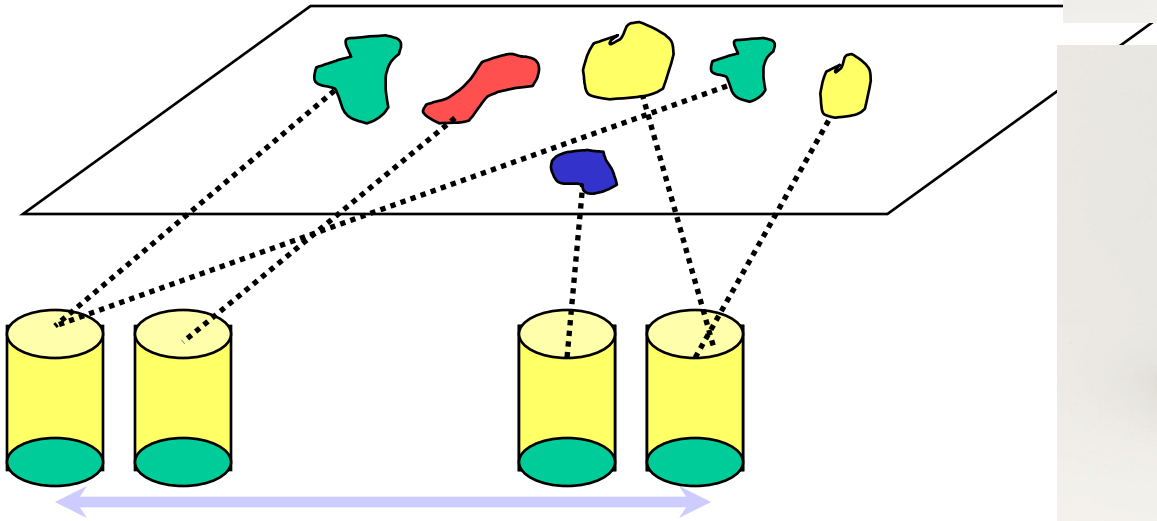


# Multi-User Settings

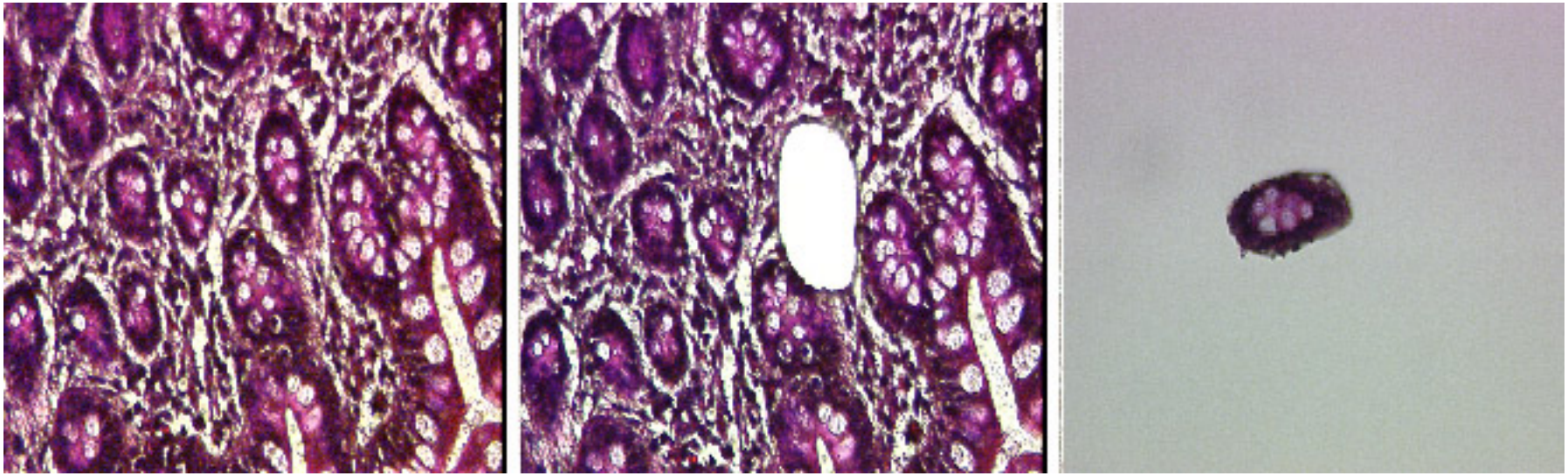


# Step 5
# Collector Inspection

# Collector (PCR Tube CAP)



# Direct Visual Inspection of PCR Cap

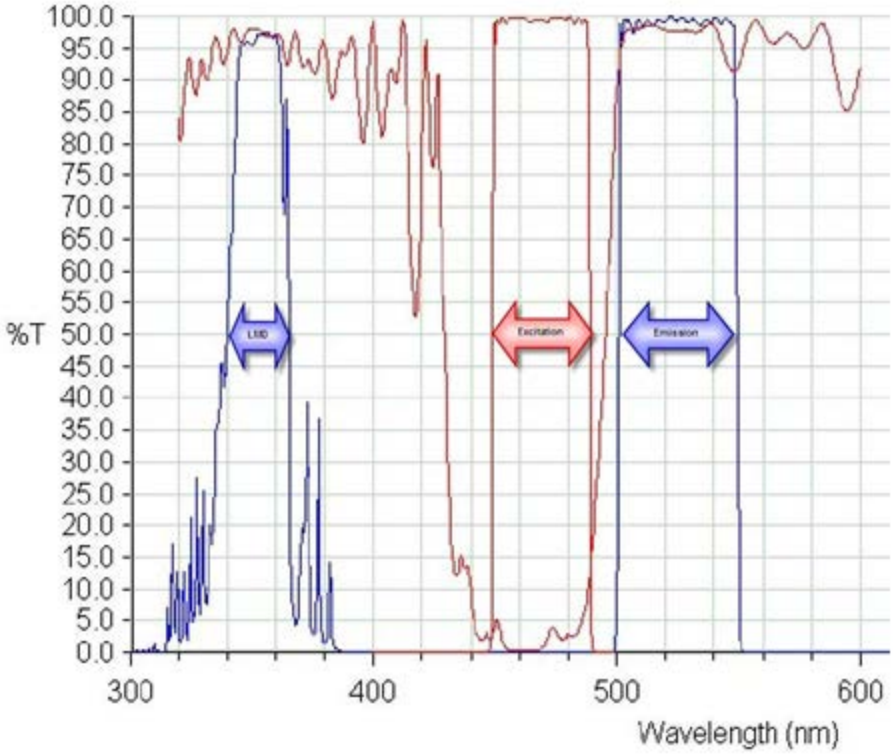


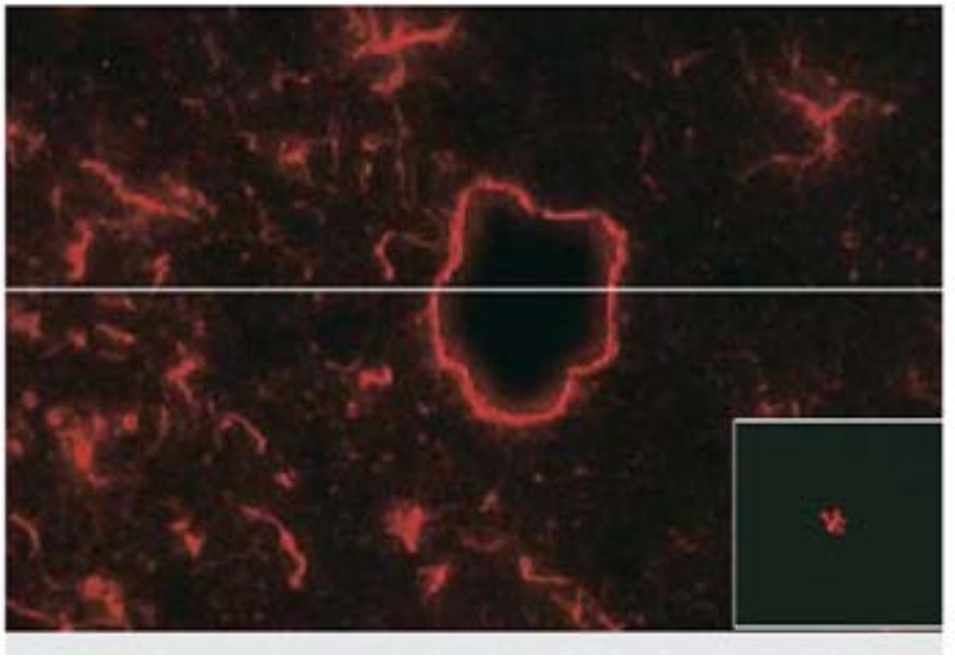
# Reference Point Calibration

- Unload collector first
- Switch to 5x
- Remove the sample holder
- Click “Go to Reference Point”
- Focus on the white light hole (reference point)
- Use the mouse, click the four arrow button to move the reference point to the center of the image view window
- Click OK.

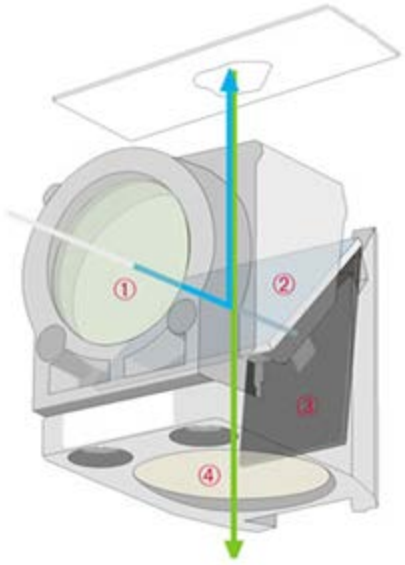
# Step 6
# Fluorescence Mode; Specimen Overview; Database Module

# Fluorescence Mode



# Fluorescence Filter BGR




Light trap for the efficient suppression of noise.
① Excitation filters
② Dichroic mirrors
③ Leica light trap
④ Emission filters

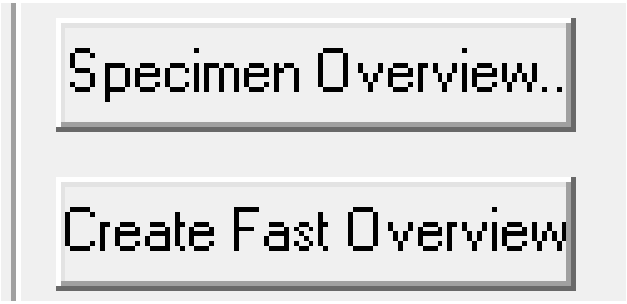
BGR three excitation ranges:
405 to 435 (420/30)
487 to 502 (495/15)
560 to 580 (570/20)

BGR three emission ranges:
465/20
530/30
640/40

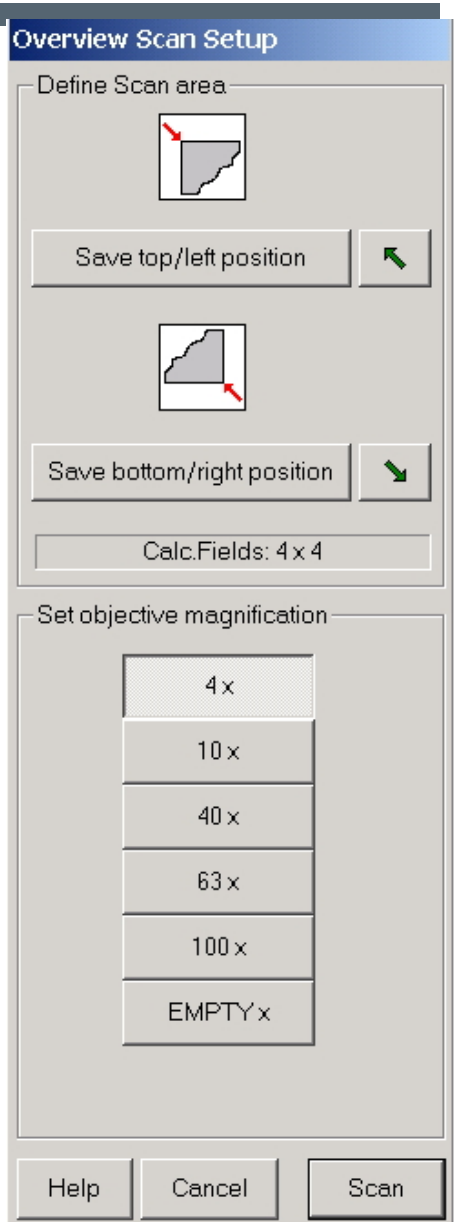
Internal Fast Filterwheel (IFW):
Short pass 460 filter
Band pass 460 to 515
Long pass 520 and higher

# Specimen Overview

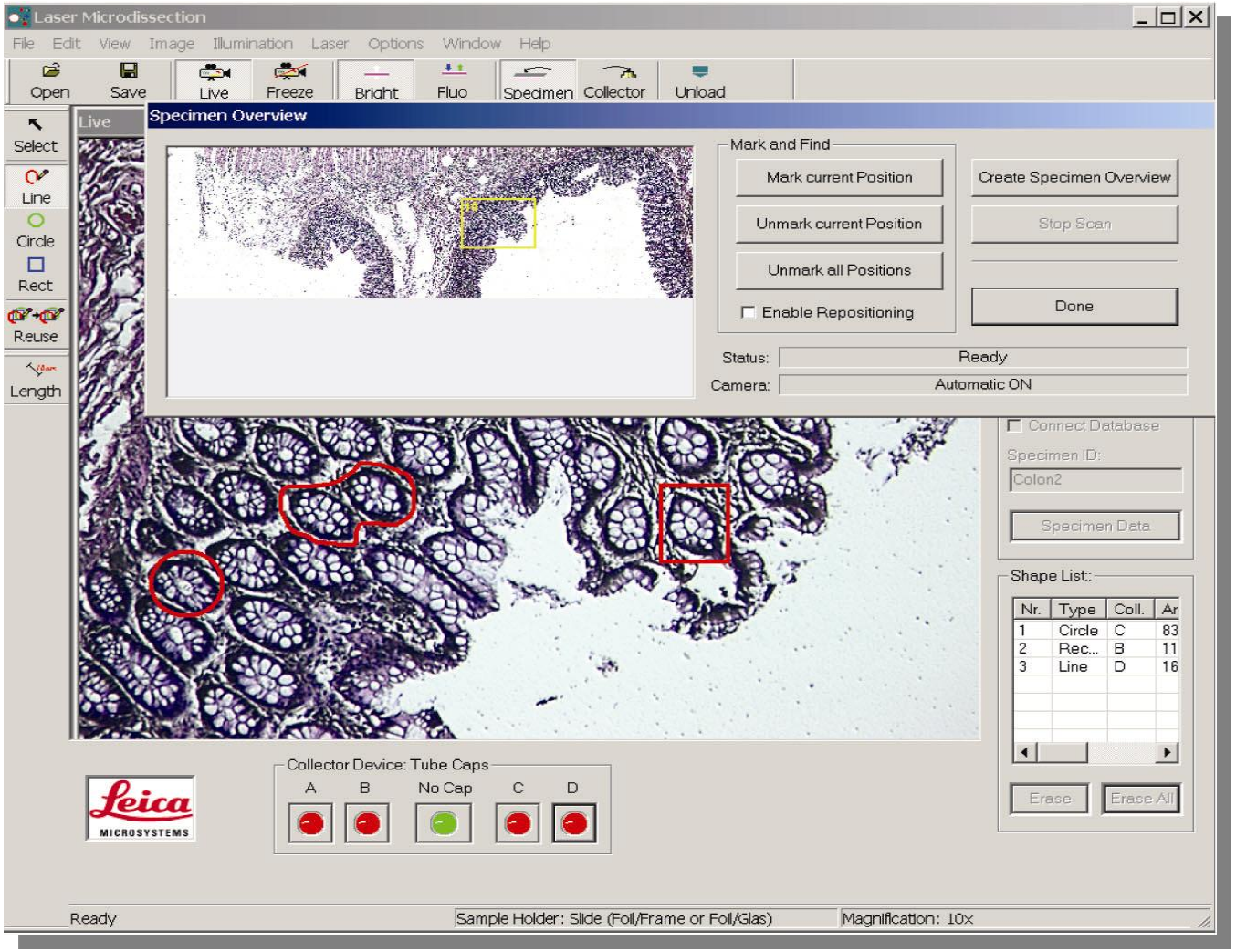
- Switch to any lens, camera auto exposure
- Then define top/left position; bottom/right position; then Scan.




- If using “Fast Specimen Overview”, the maximum slide area (defined in Options → Settings) will be used. It will only use 1.25x.

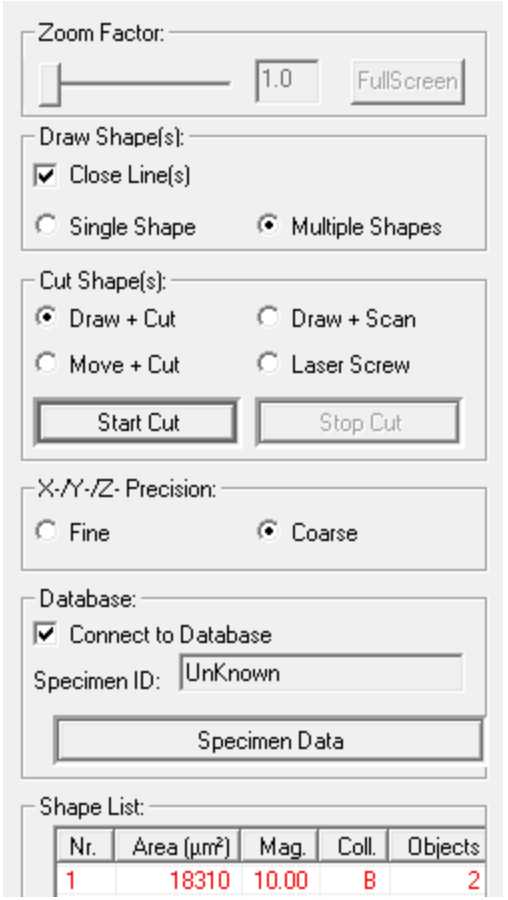


# Specimen Overview



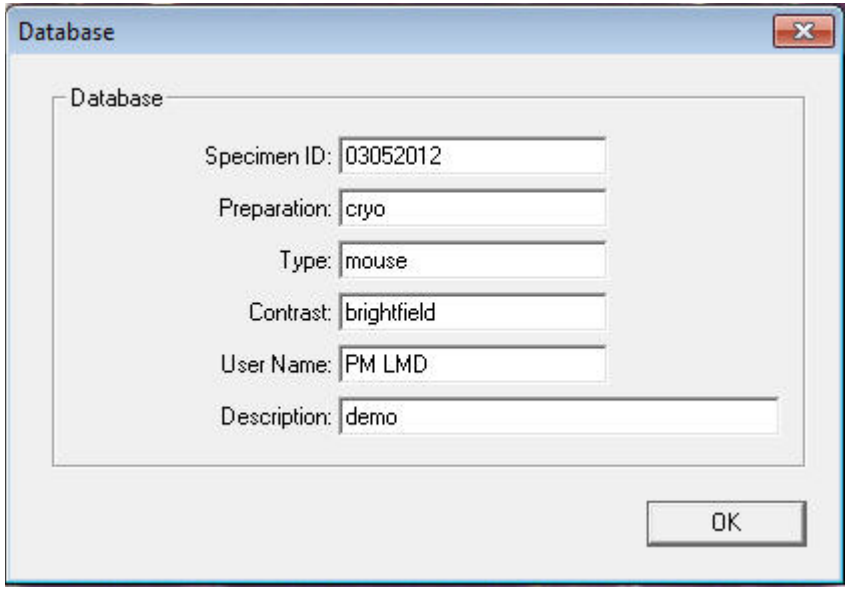
# Enable Database Module

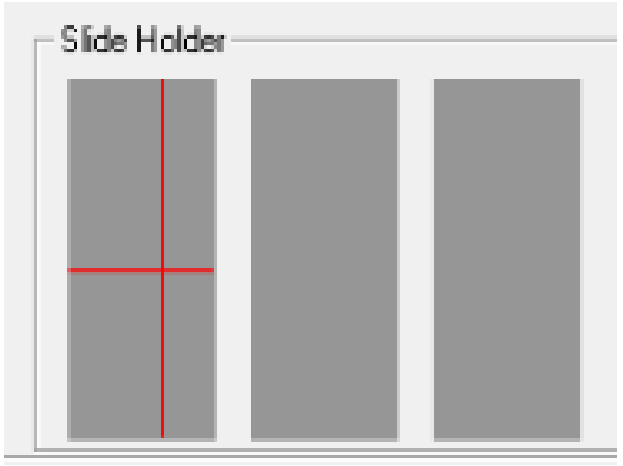
- Check the box: Connect to Database (Dongle protected – make sure the dongle is plugged in the PC)



# Type in Specimen Data

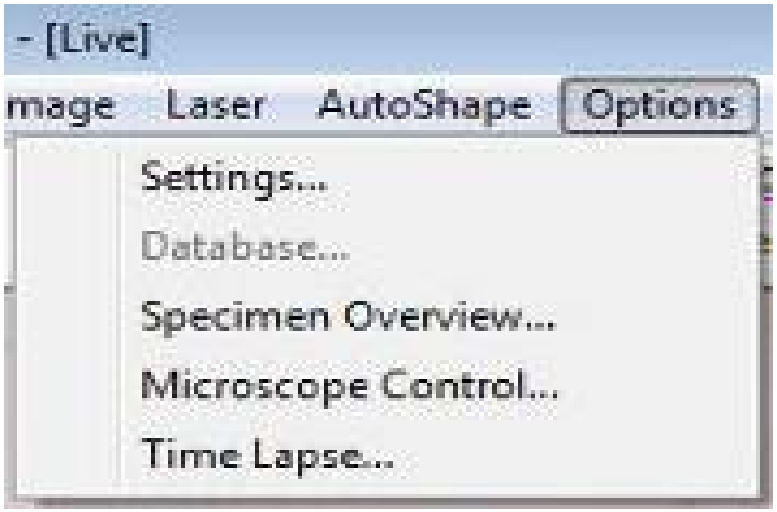
- The “Specimen Data” is unique for each slide
- Double click on one of the three slides, then click the “Specimen Data” to enter information such as “Specimen ID”, “Preparation”, “Type” etc.

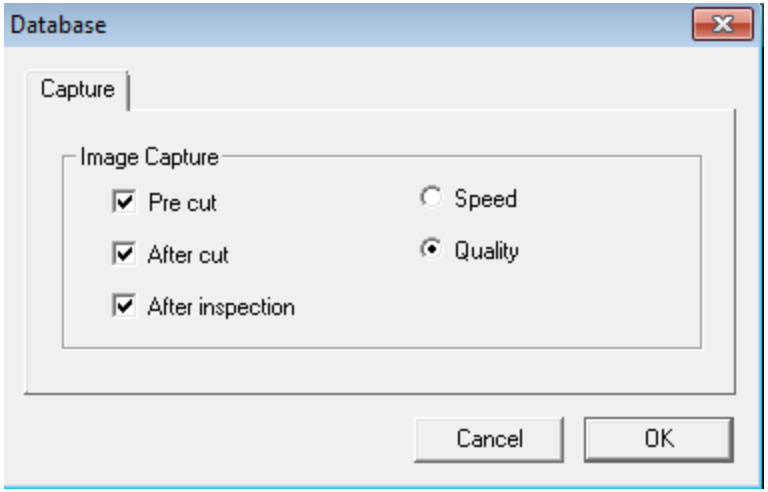


# Capture Images

- Options → Database menu settings:



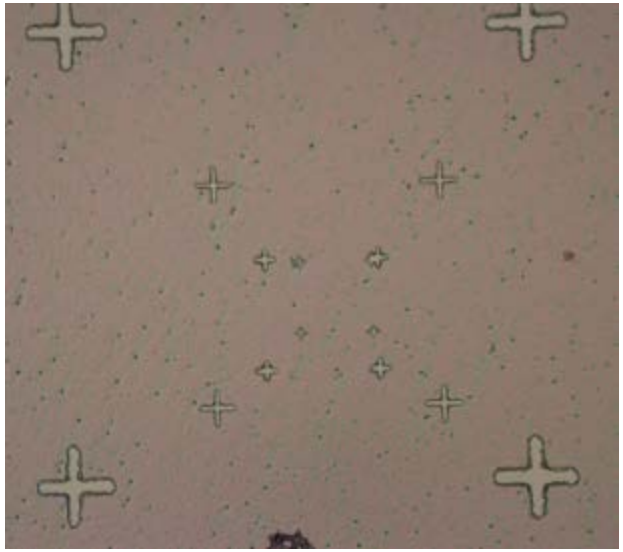
# LIF File Viewer

- “LAS-AF-Lite_3.1.0_8587_Setup.exe” - for PC only

- “Specimen ID.lif” can be copied to another location, and opened by LAS AF Lite free image viewer.

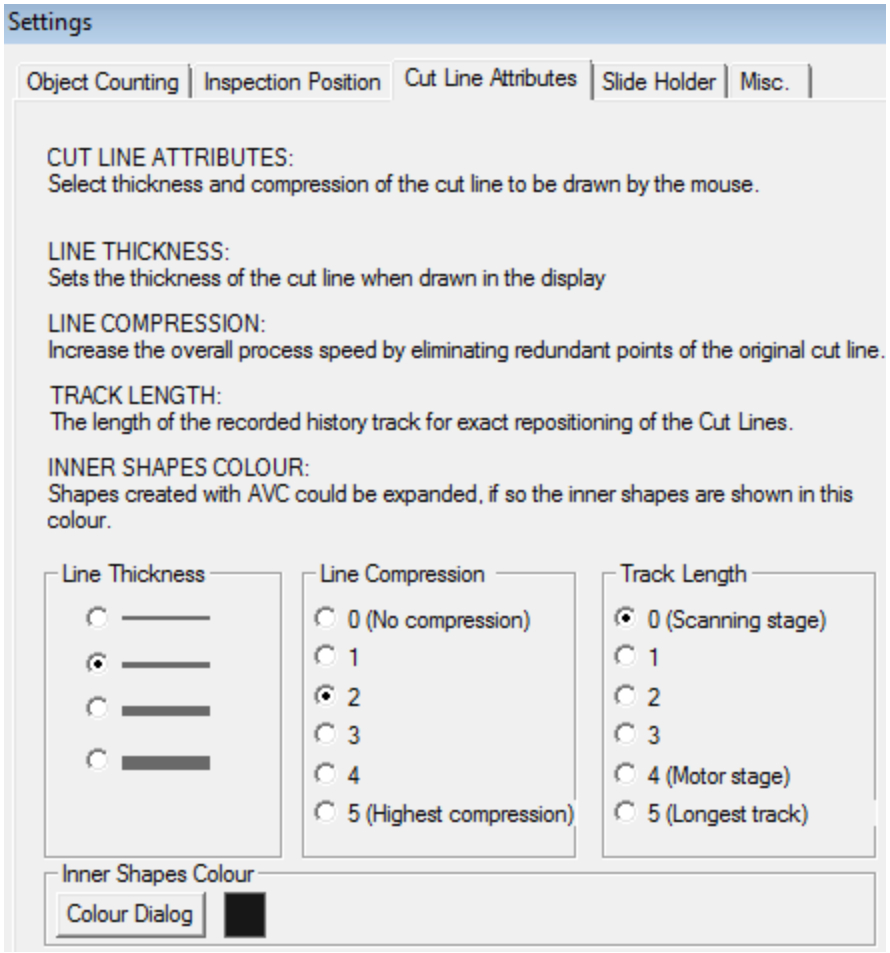
# Step 7
# Fine Tuning

- Laser calibration
  -- Focus on the sample
  -- Adjust the laser power
  -- Go to “Laser” menu, choose “Calibration”
  -- Follow the instruction to click on the center of each cross (total four crosses will be cut)



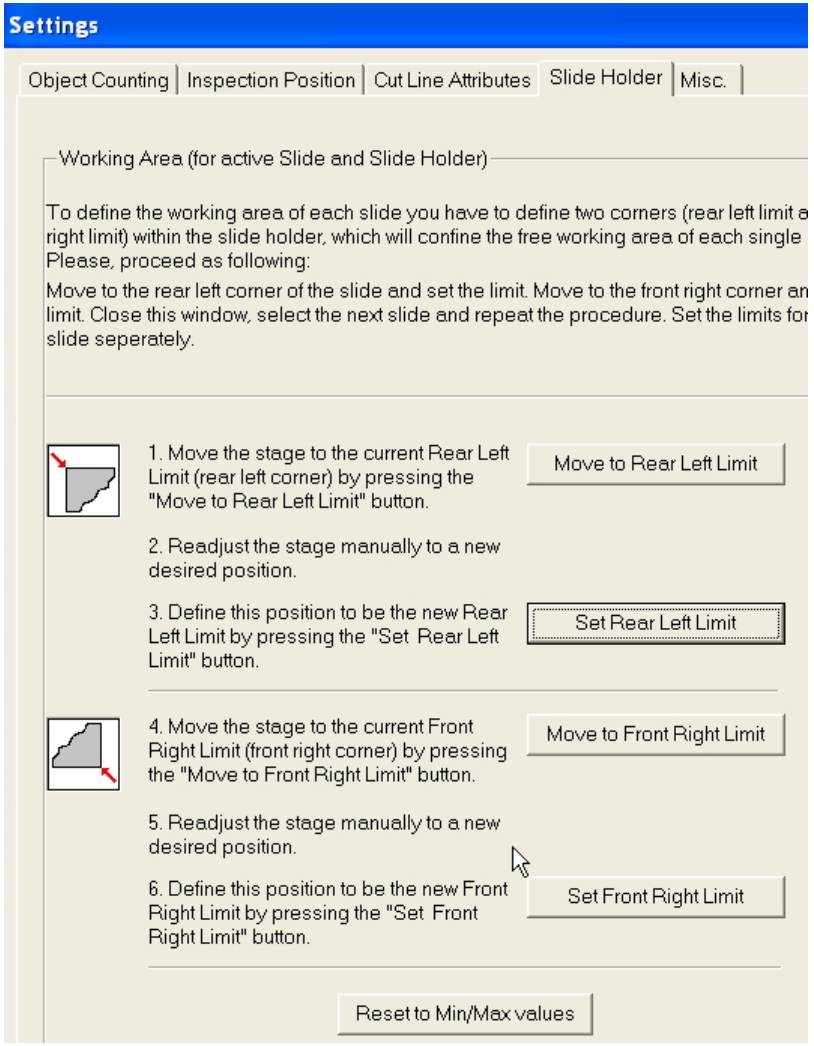
# Option – Settings - Cut Line Attributes

- Line Thickness: top one
- Line Compression: 2
- Track Length: 0 (scanning stage)



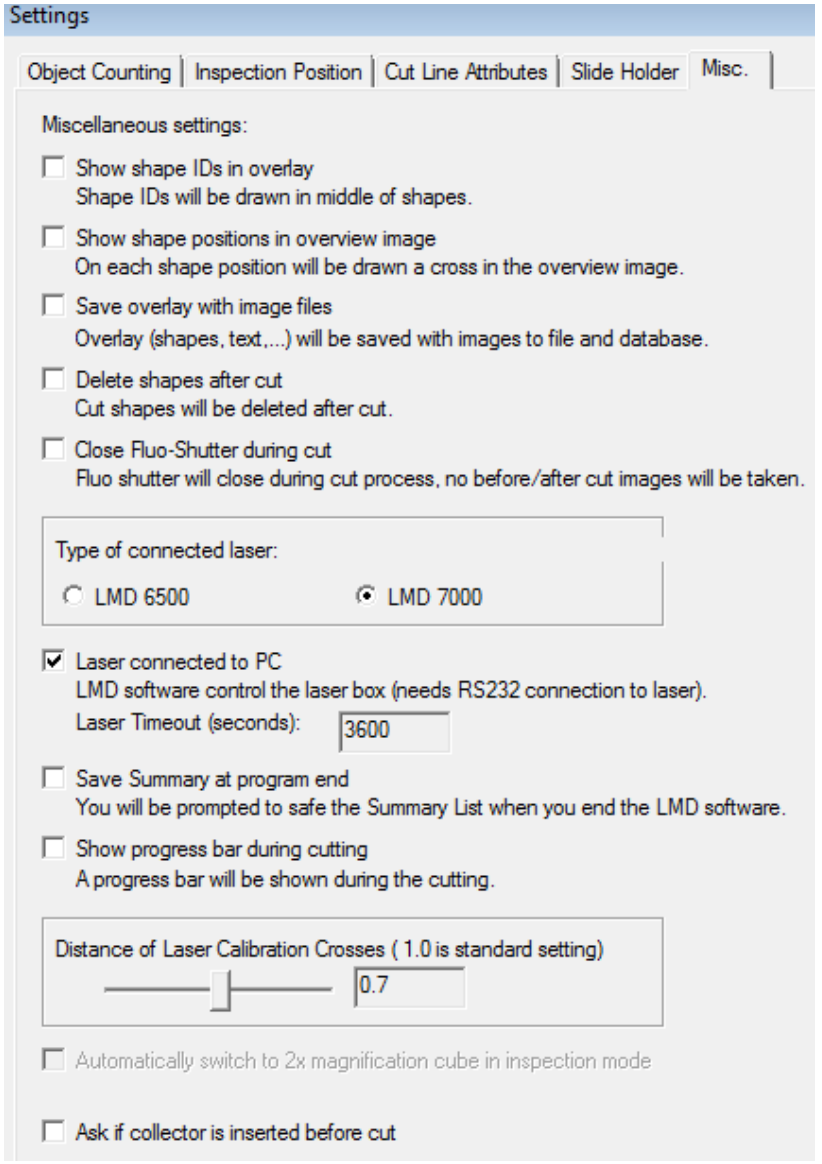
# Slider Holder Scan Area

- This area is an arbitrary boundary for the slide
- It is used for the Fast Specimen Overview



# Misc. Settings

- Save overlay with image
- Laser connected to PC
  - 3600 seconds
- Distance of laser calibration
  - 0.7
- LMD7000



# Step 8

# Troubleshooting

# Not cutting; Static; Camera; Microscope, etc.

# Not Cutting

- Laser power/aperture is enough?
- Laser speed and frequency?
- Laser offset is off?
- Tissue too thick?
- Tissue too wet?

# Static

- Blank slide could be UV treated before mounting tissue
- Tissue dehydrated too much
- Tissue air dried too long – use a enclosed chamber with wet paper towel
- Put liquid buffer in the PCR tube cap
- Humidifier in the room
- Lower laser frequency
- Glass PEN slide better than metal frame slide
- Metal frame PEN slide is better than metal PET slide

# Camera, Microscope

- Camera no image:
  - Lever switched from eyepiece to camera?
  - Auto exposure? Manually set to high exposure to see if there is image
  - Light intensity (INT), FD(field diaphragm), AP (aperture) enough?
  - Collector is blocking the light? Diffusor is eating the light?

- Microscope:
  - Another user is logged in and using the microscope?
  - Log off or restart the computer
  - Wait for microscope completely started.

# Step 9

# Microscope Standalone Operation

# DM6000B Touch Screen

# DM6000B Buttons (Preset)


Illumination Manager

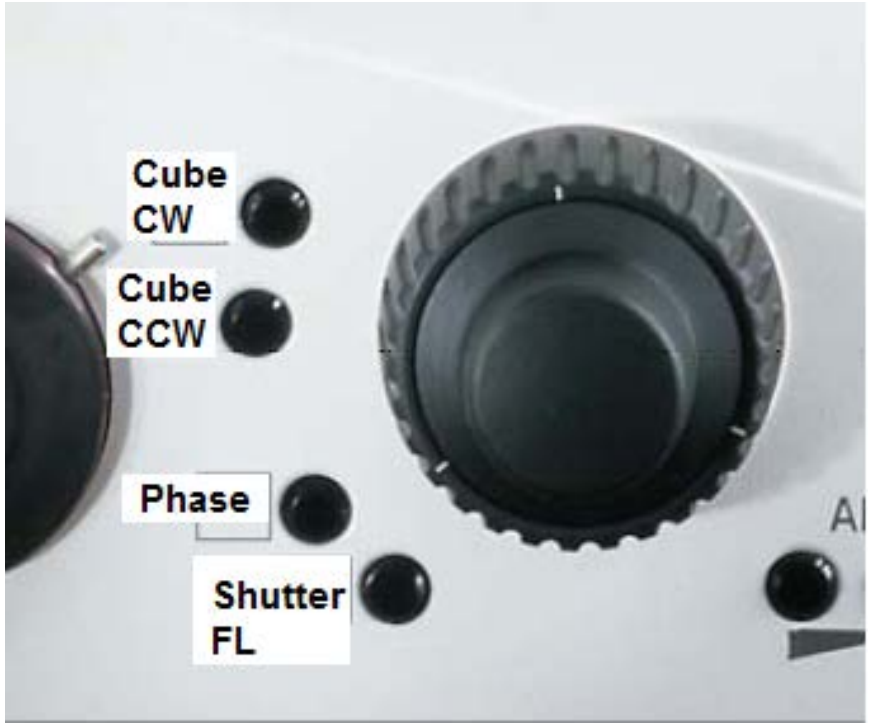
*TL & FL Intensity Controls

*TL / IL Toggle

*Field Diaphragm Controls

- 100% T
- 55% T
- 30% T
- 17% T
- 10% T
- 0% Shutter

# DM6000 Buttons (User Defined)




User Defined Function Buttons

4 Left Side / 3 Right Side

# Objective Correction Collar


Constant Focus Correction Collar

For select N Plan L & Fluotar L objectives.

Simplifies viewing the best images possible.

# Step 10

# Support / Troubleshooting

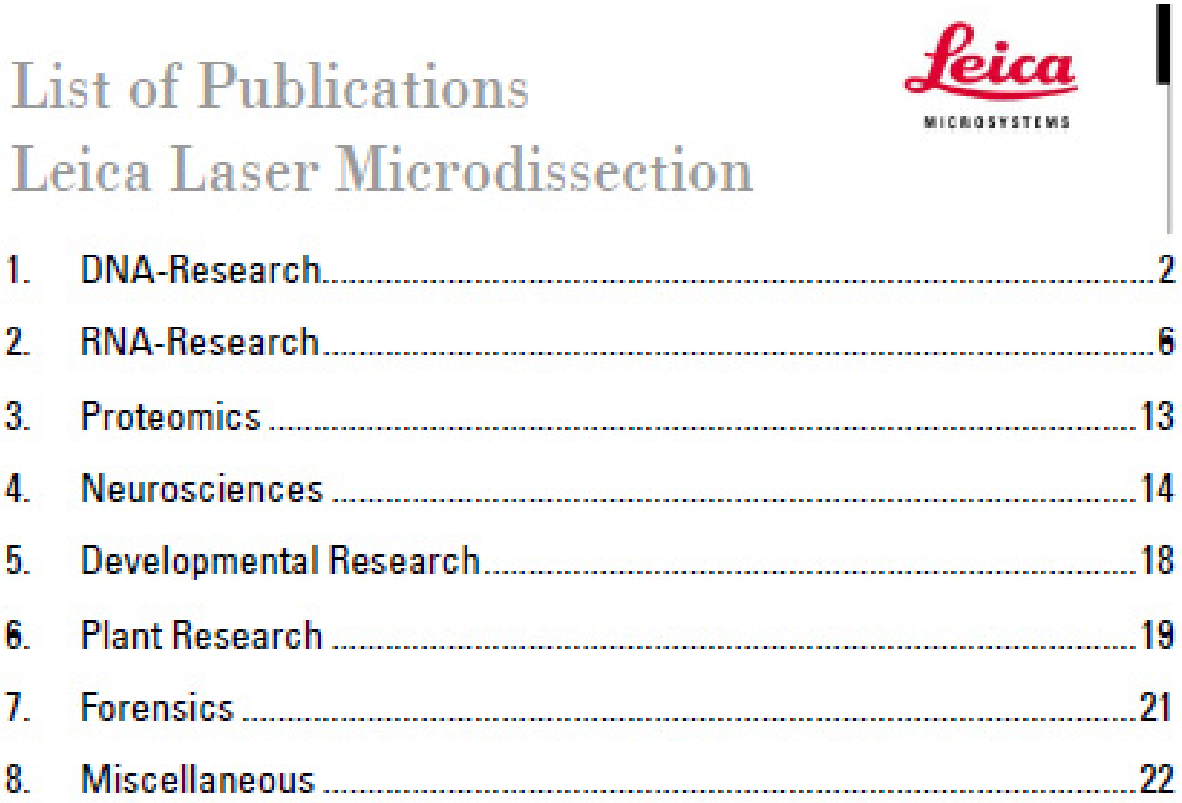
# LMD Protocol Guide



Sample Preparation
For Leica Laser Microdissection

Protocol Guide for Leica Microsystems Laser Microdissection Systems

# LMD Citations



List of Publications
Leica Laser Microdissection



- *http://www.leica-microsystems.com/lmd*

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(Internet: Refer to the Word document to avoid real time virus scan of LMD software folders: “LMD_Networking_3rdParty_Software_Policy_V1.0_Oct13_FS.doc”)