

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



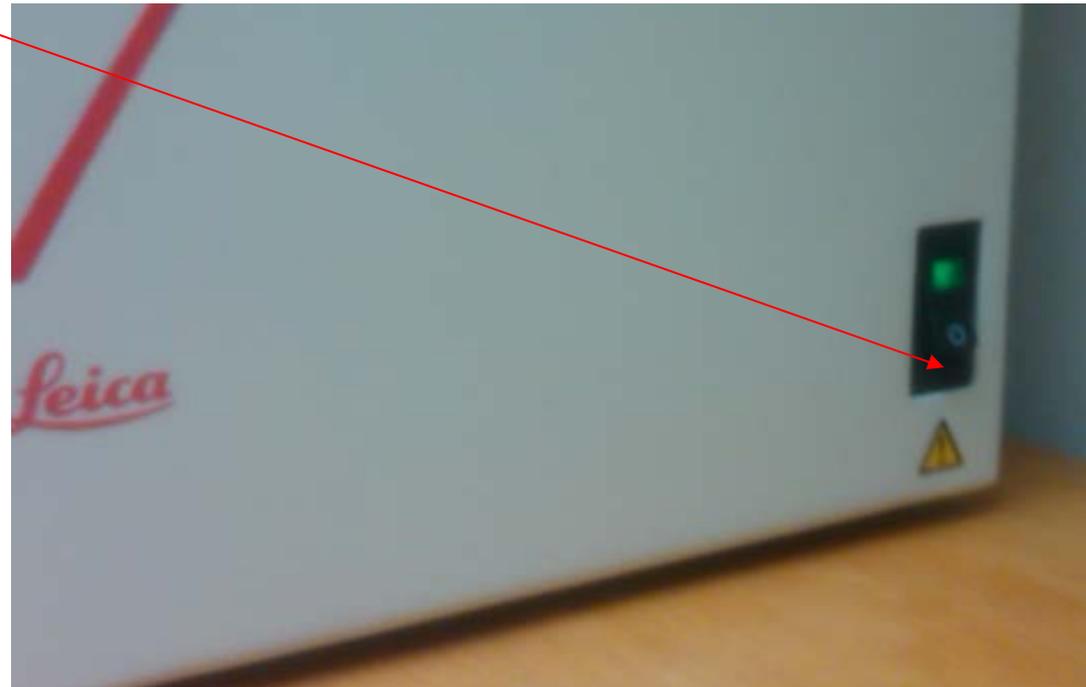
Turn on the laser key

- 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.

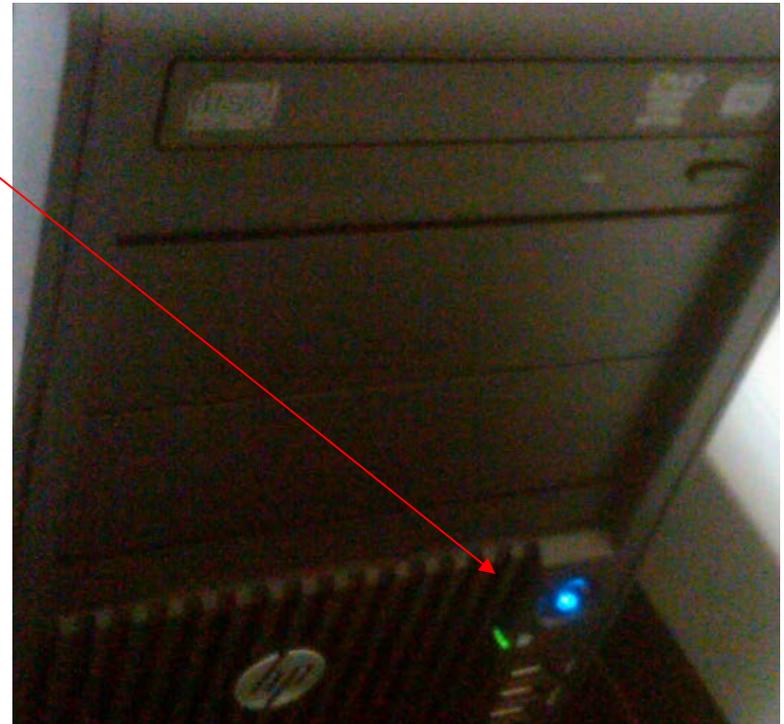


Turn on the CTR6500 controller box

- 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.



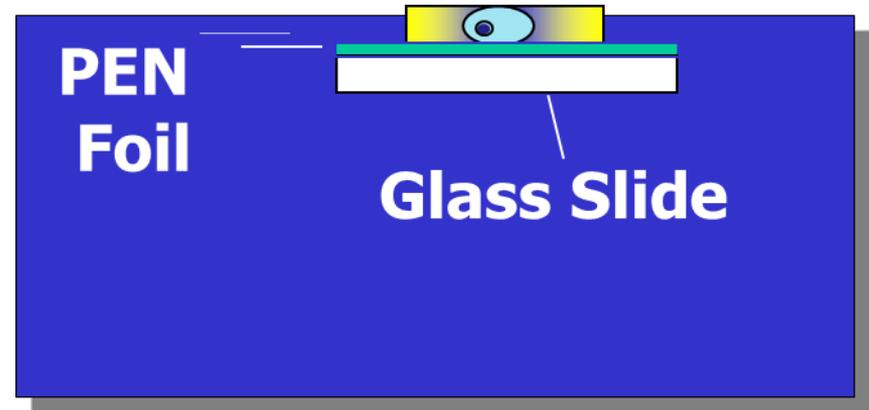
Turn on the computer
if it is **not** on
automatically after
turning on the power
strip



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 (**4 μ m**), **glass**, 50 pcs 11600288

PEN FrameSlide, (**4 μ m**), **steel frames**, 50 pcs 11600289

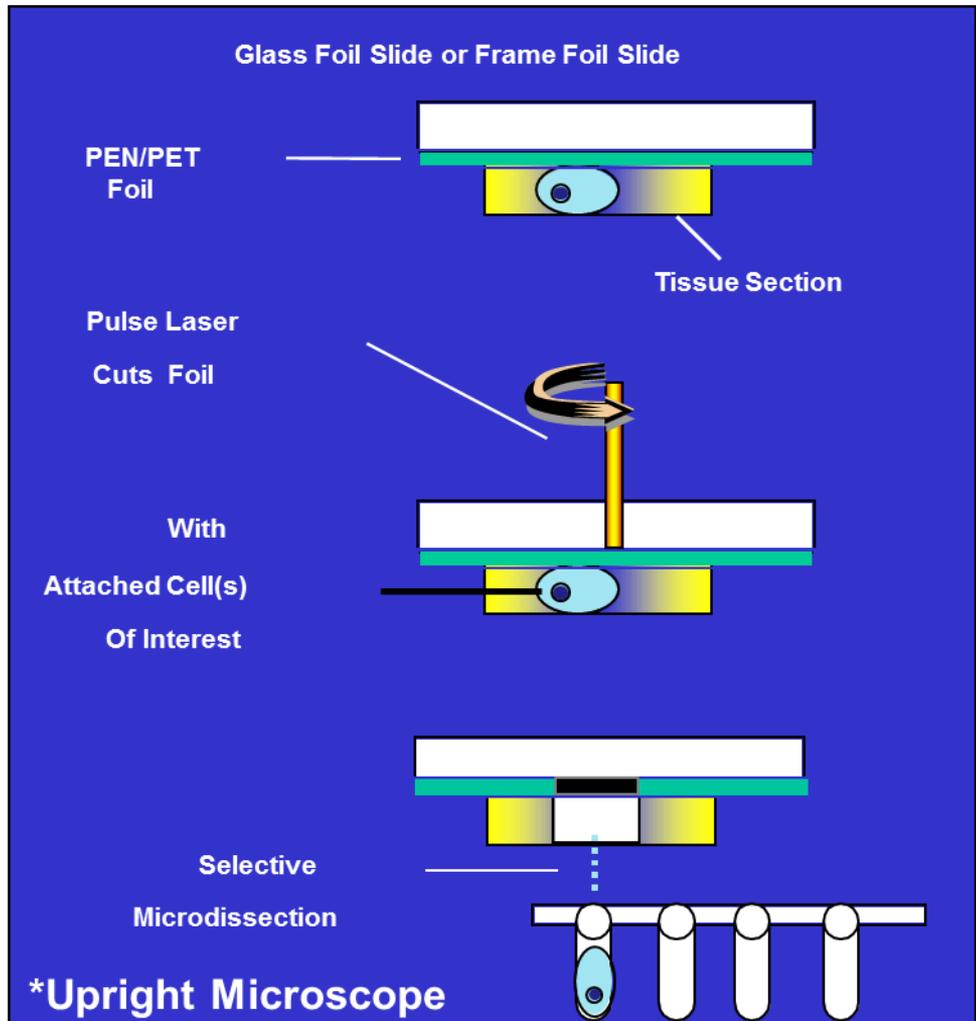
Typical Protocol for Frozen Sections

- 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 be 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

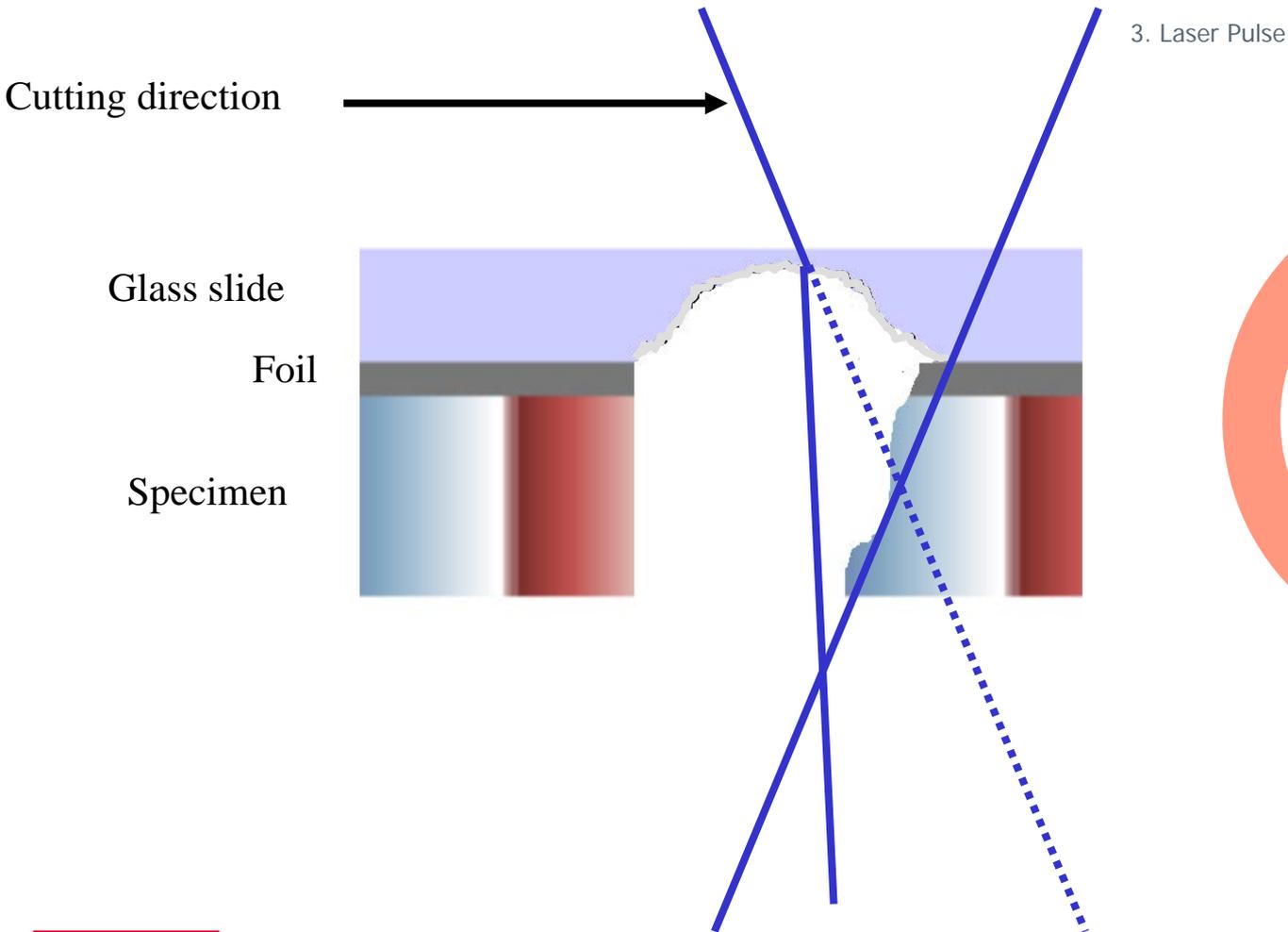
- RT PCR
- > 500 cells*
- cDNA library construction
 - cDNA microarray probes
 - Differential display
 - Gene Expression Arrays

Proteins

> 5,000-50,000 cells

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

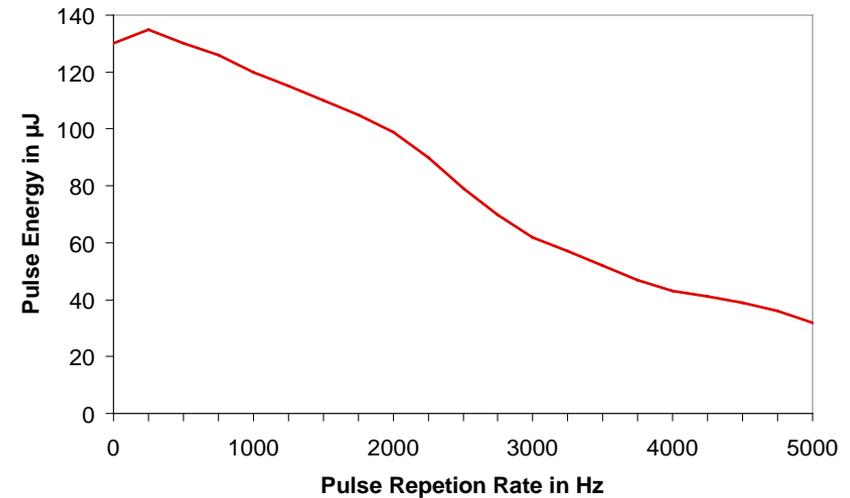
Laser Induced Glass Etching



**CUTTING
NOT
POSSIBLE**

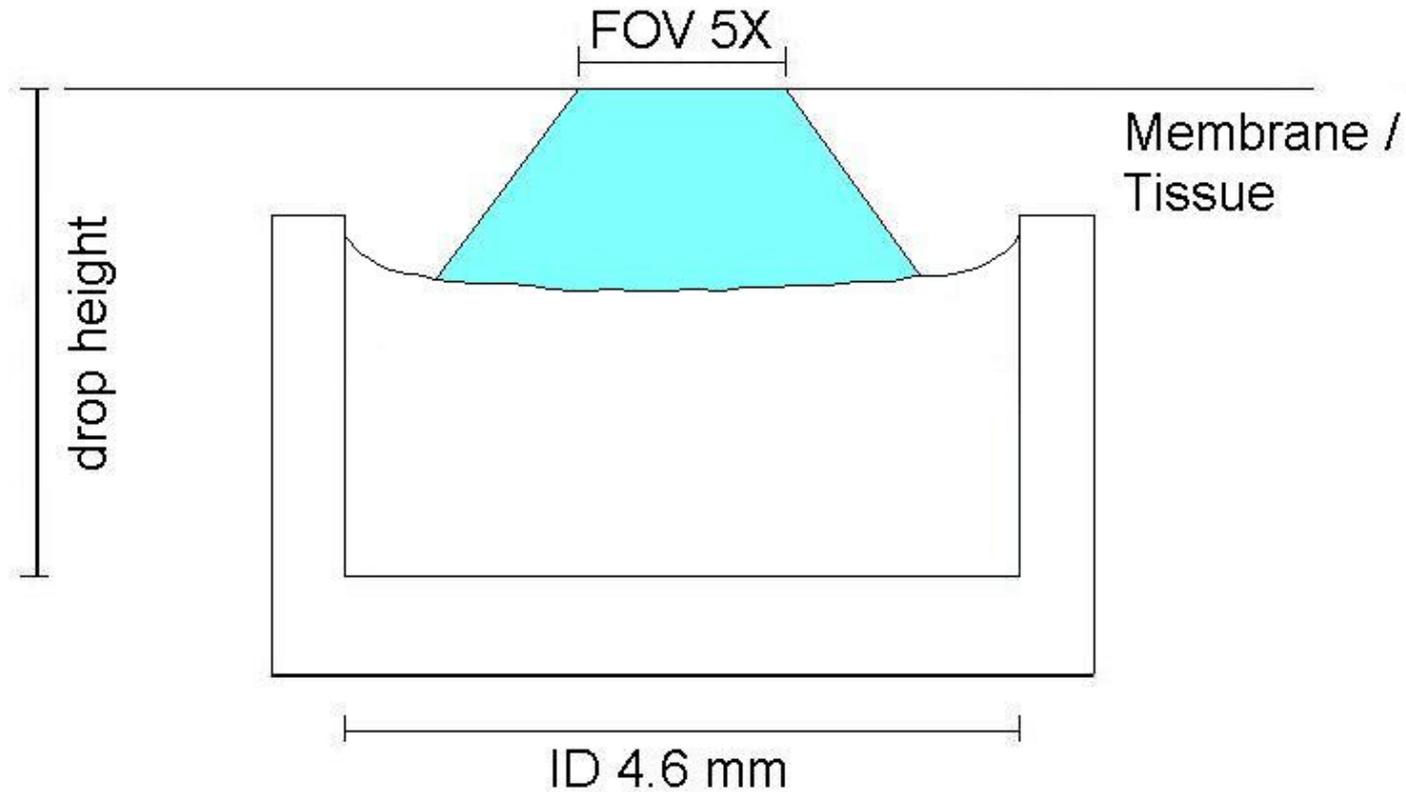
LMD7000 Laser

- 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”

Change Specimen

Info

It's now safe to exchange the specimen and/or the sample holder!

Sample Holder

ThreeSlides

Large Slide

Scanning Stage LCC

ThreeSlides

Enable LCC Module

Specimen ID: Mouse Brain

Preparation: Thionin

Type: <UnKnown>

Contrast: TL-BF

User Name: johann-g

Description: Test of LAS package

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

Cut Shape(s):

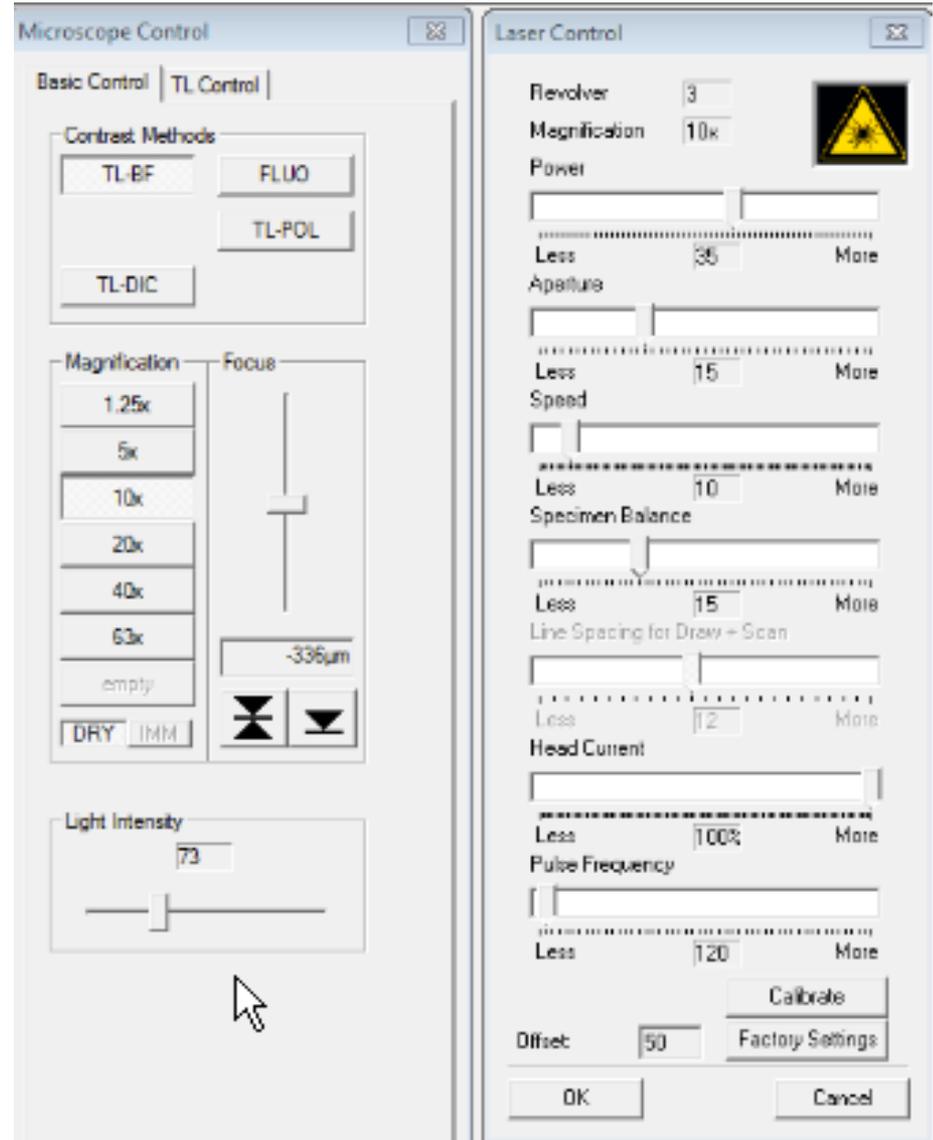
- Draw + Cut
- Draw + Scan
- Move + Cut
- Laser Screw

Start Cut Stop Cut

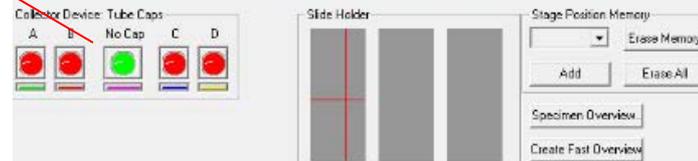
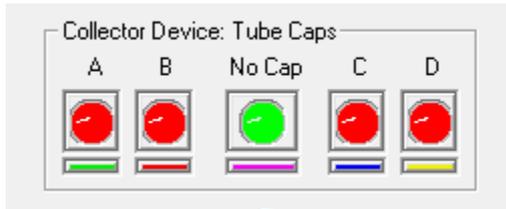
The screenshot displays the Leica LMD7000 software interface. The main window shows a grayscale micrograph of a biological specimen. On the right side, the 'Cut Shape(s)' dialog box is open, showing the 'Draw + Cut' mode selected. Below the dialog, the 'Microscope Control' and 'Laser Control' panels are visible, containing various sliders and buttons for adjusting the system. At the bottom, there are status indicators for 'Collection Device', 'Tube Caps', and 'Stage Position History'. The Leica logo and 'LEICA LMD7000' are visible at the bottom center.

Automated Control

- Microscope Control
- Laser Control



Multiple ROI Cutting



LEICA LMD7000

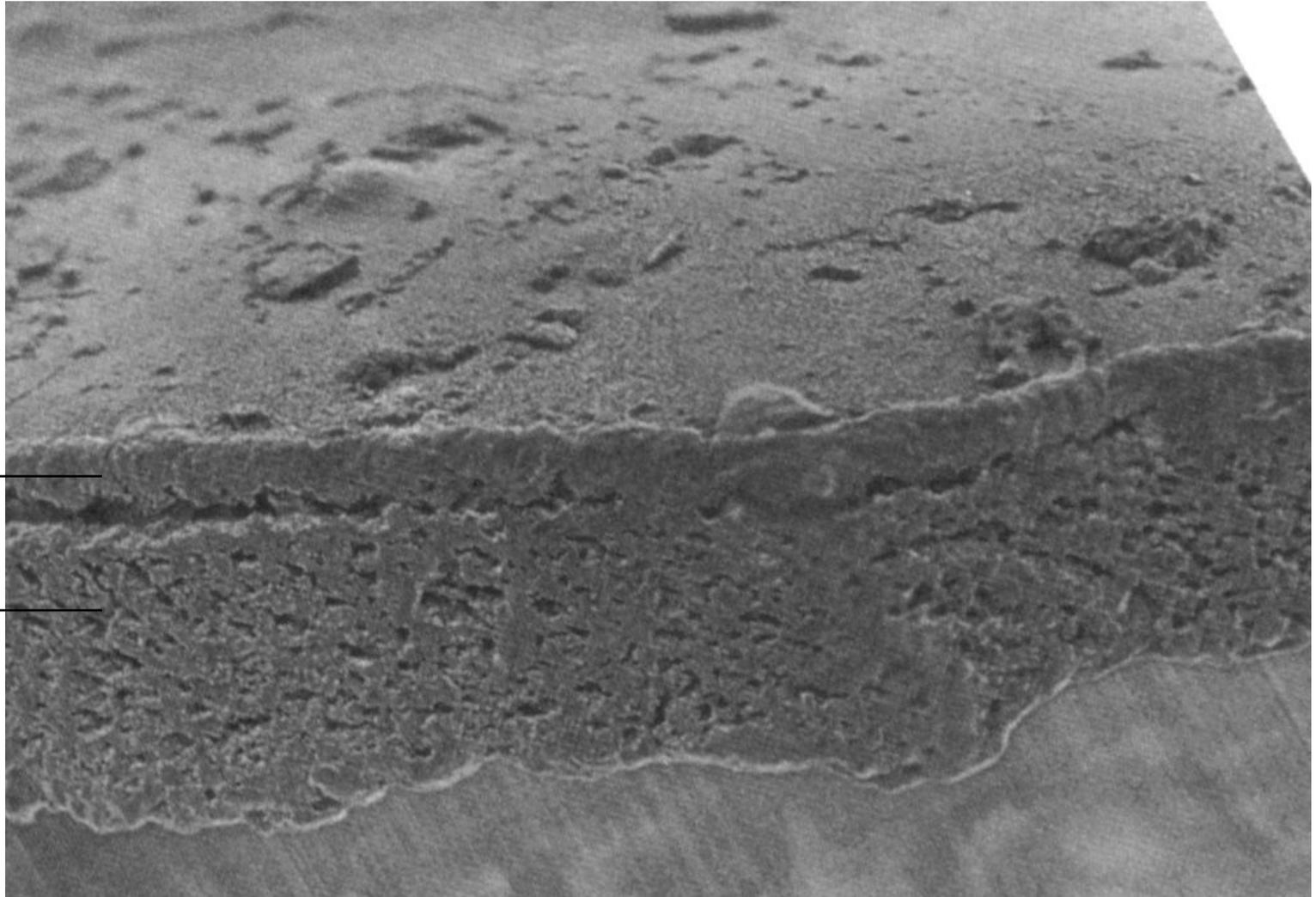
Step 4

Laser Microdissection Parameters

Laser Cold Ablation

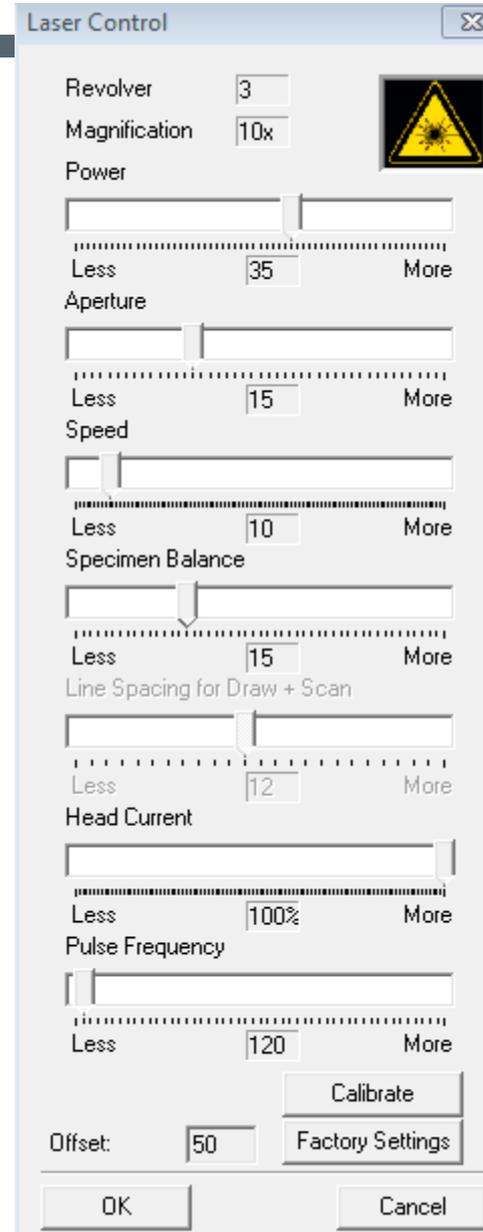
Membrane

Tissue

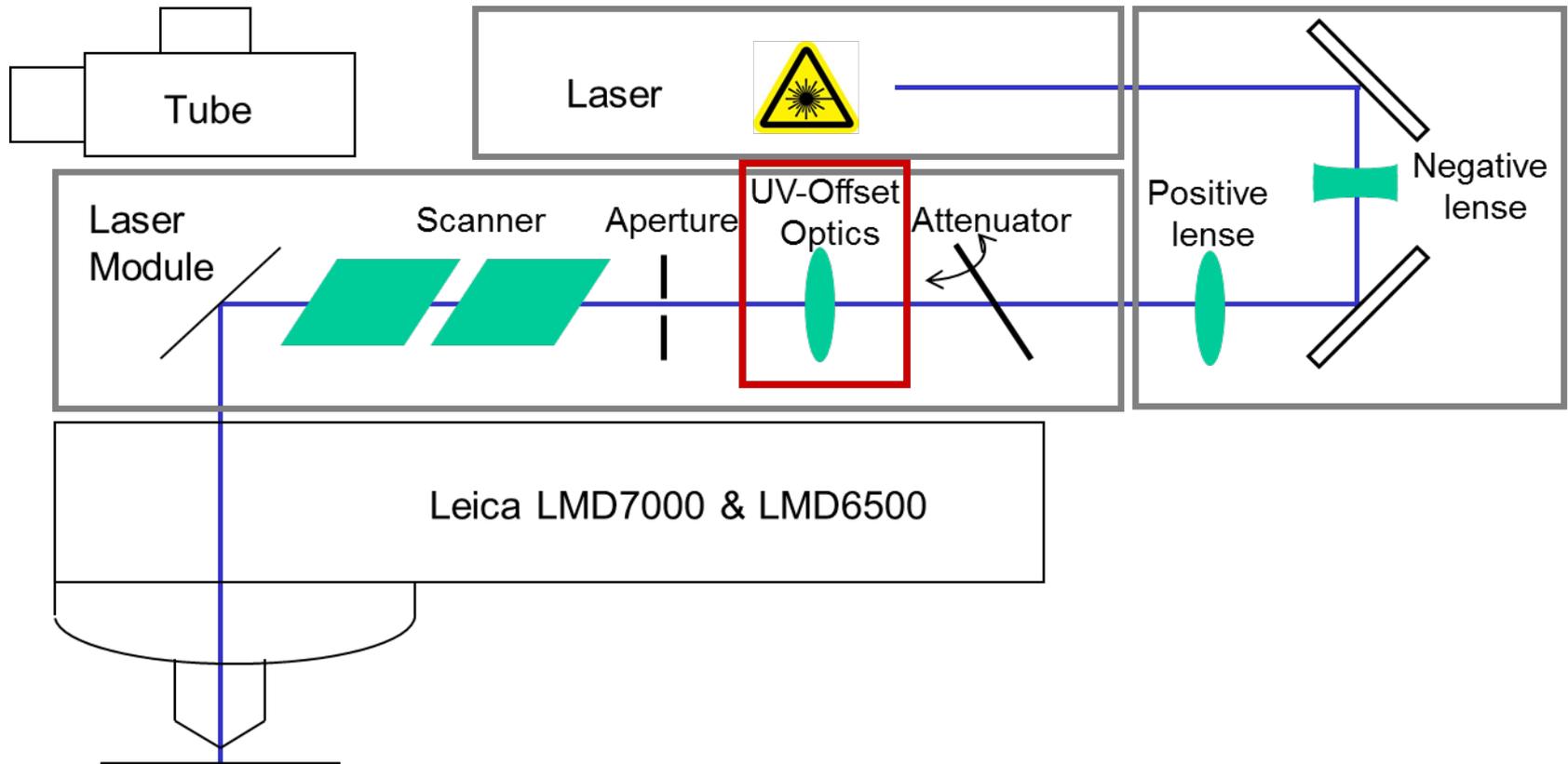


Laser Parameters

- 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.

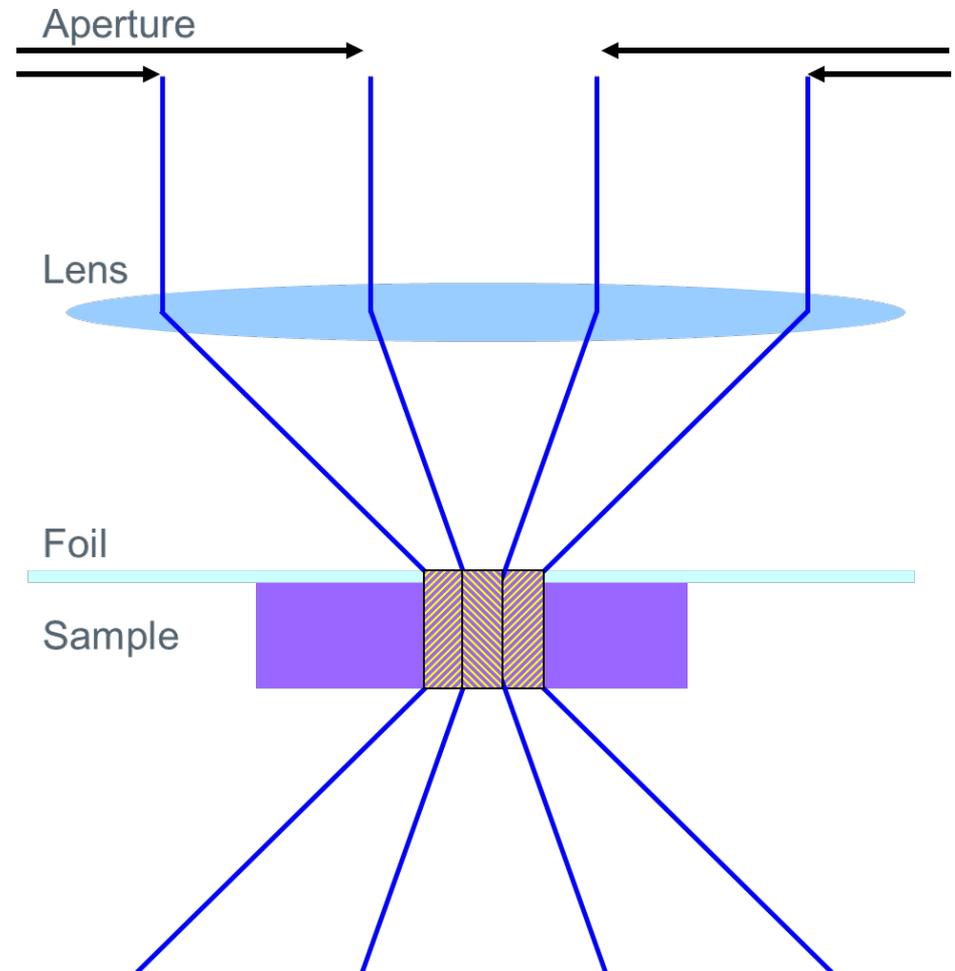


LMD Laser Module Light Path

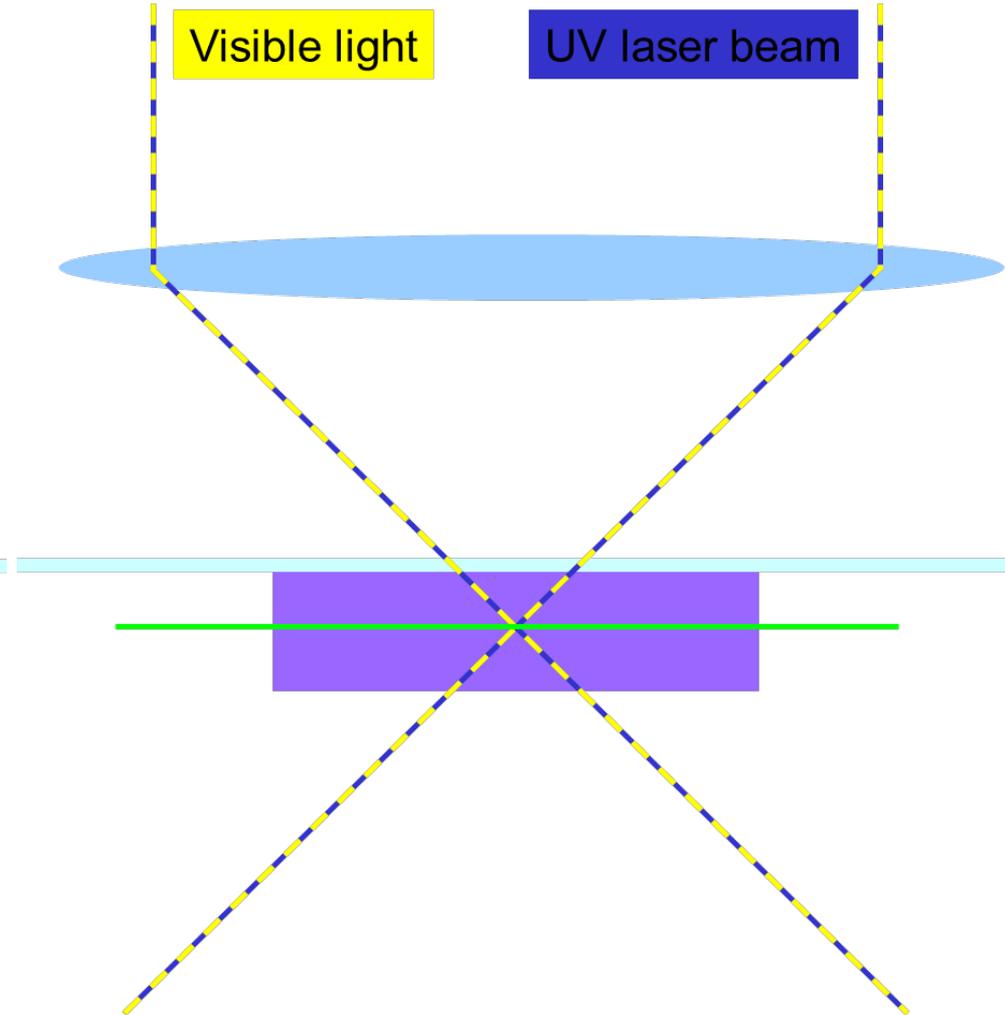
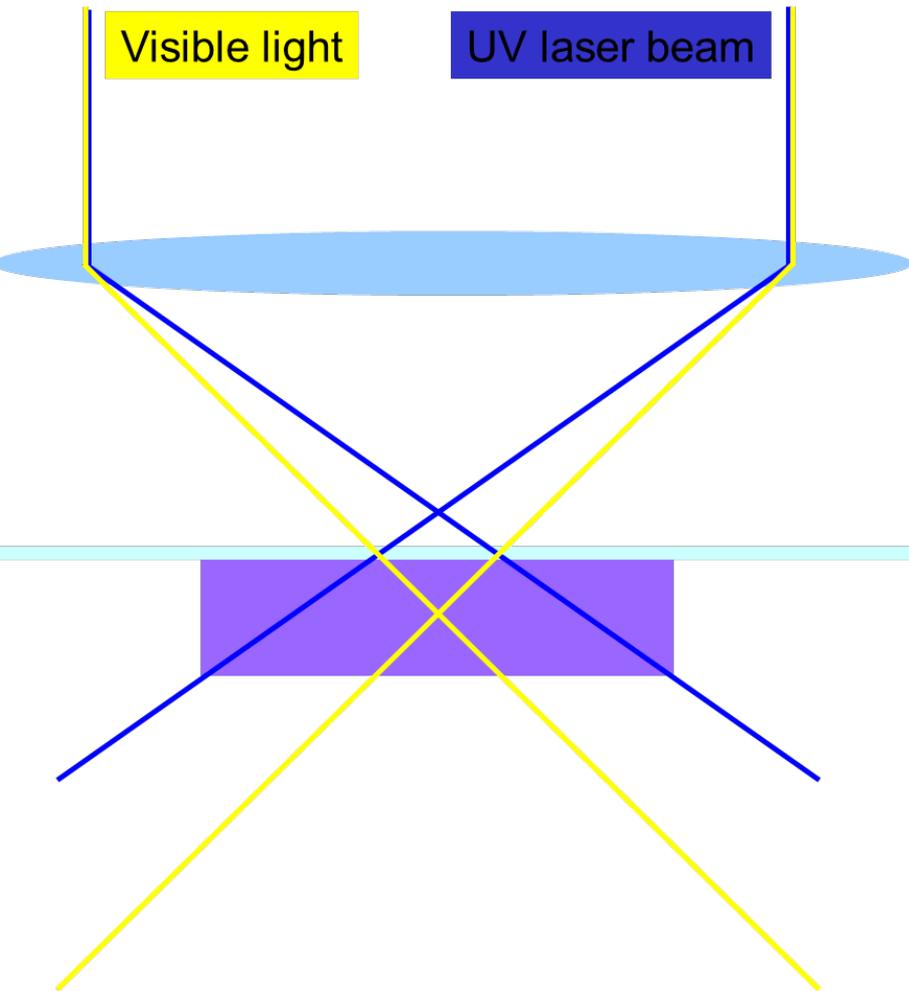


Laser Aperture Control

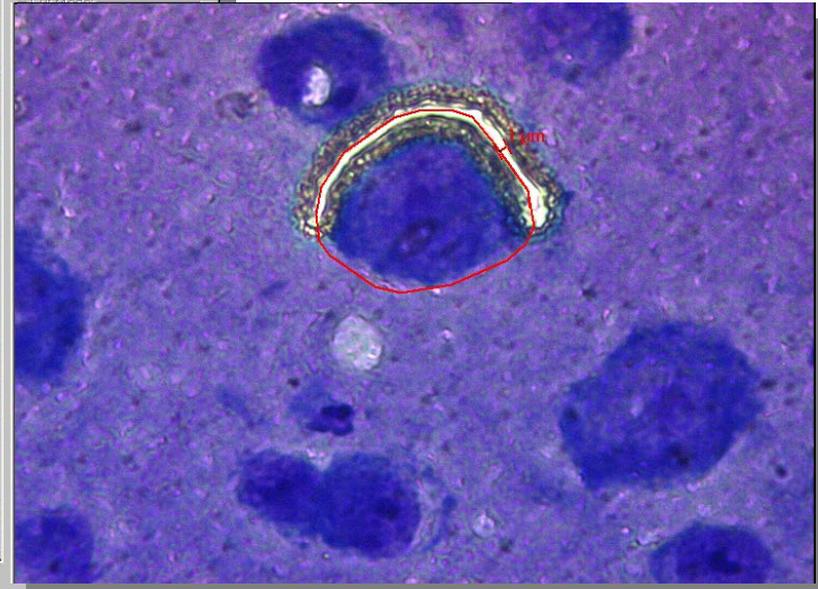
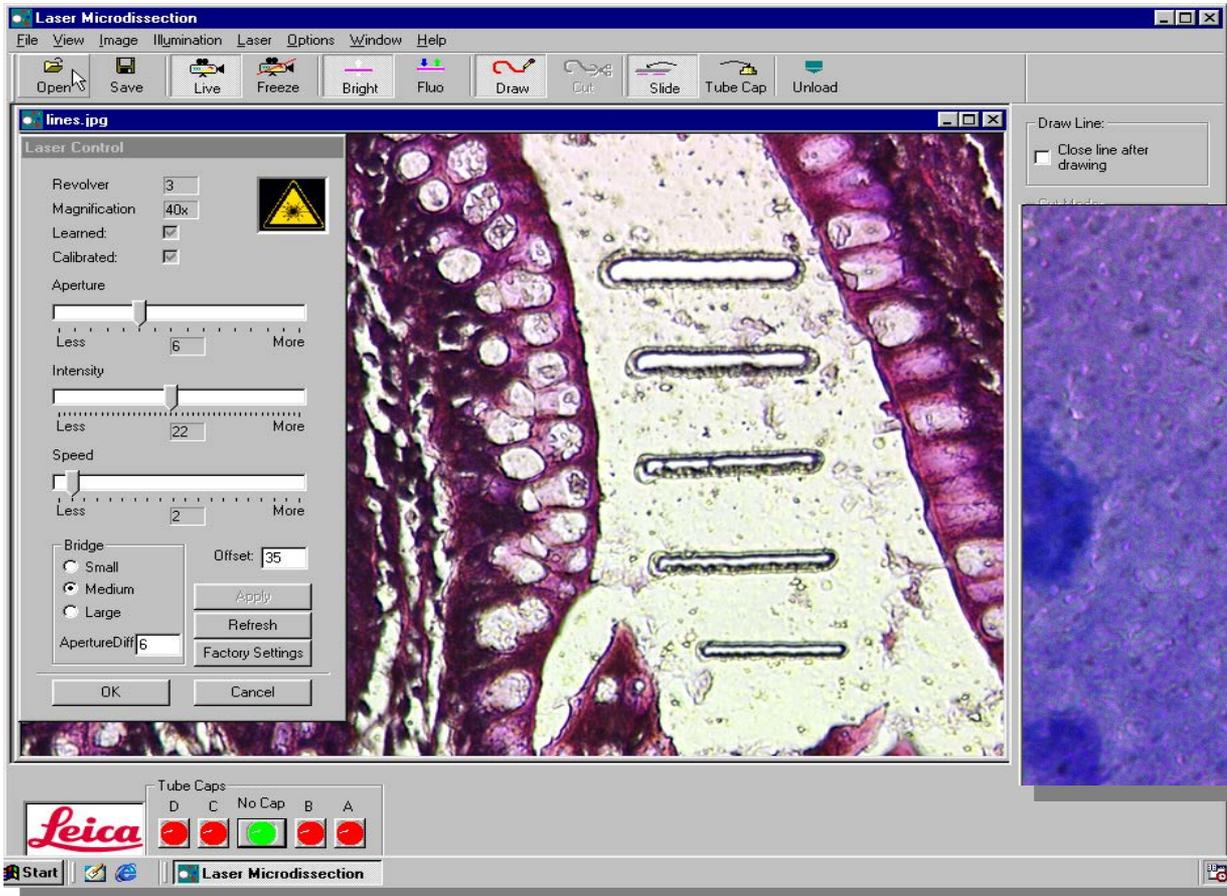
- The aperture allows to control the width of the cutting line
- Aperture \uparrow Pulse Energy \uparrow
Aperture \downarrow Pulse Energy \downarrow
- Always optimize aperture settings for every objective



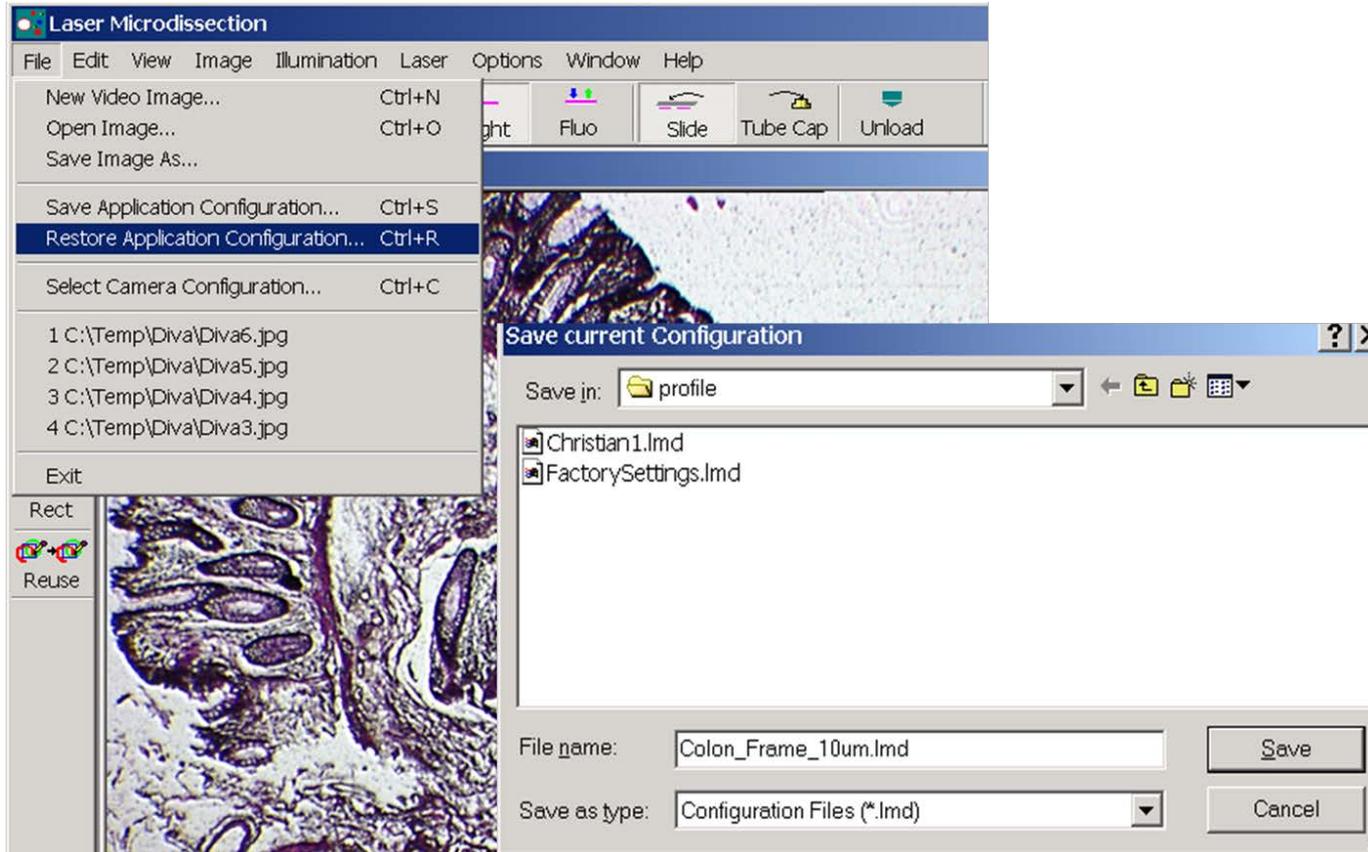
UV Offset Control



Laser Cut Line



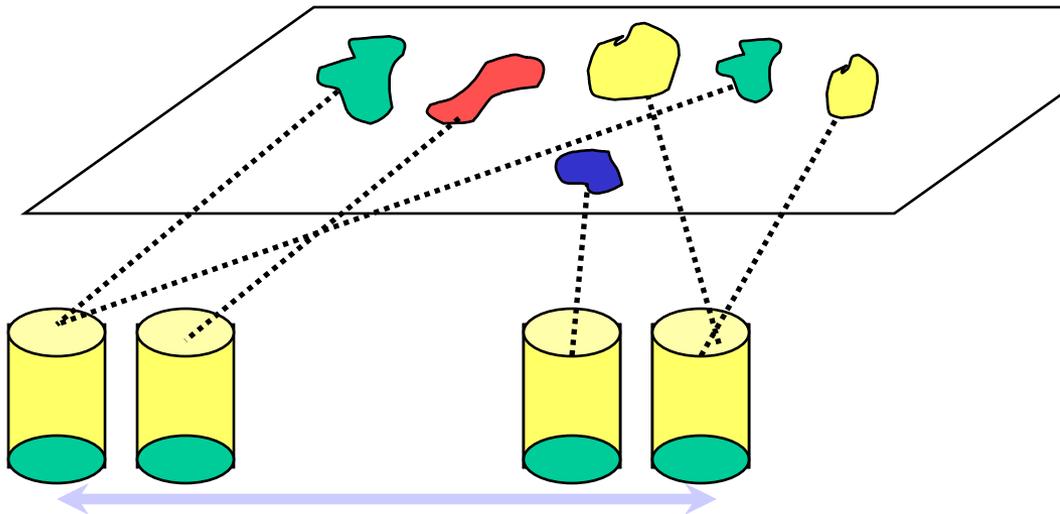
Multi-User Settings



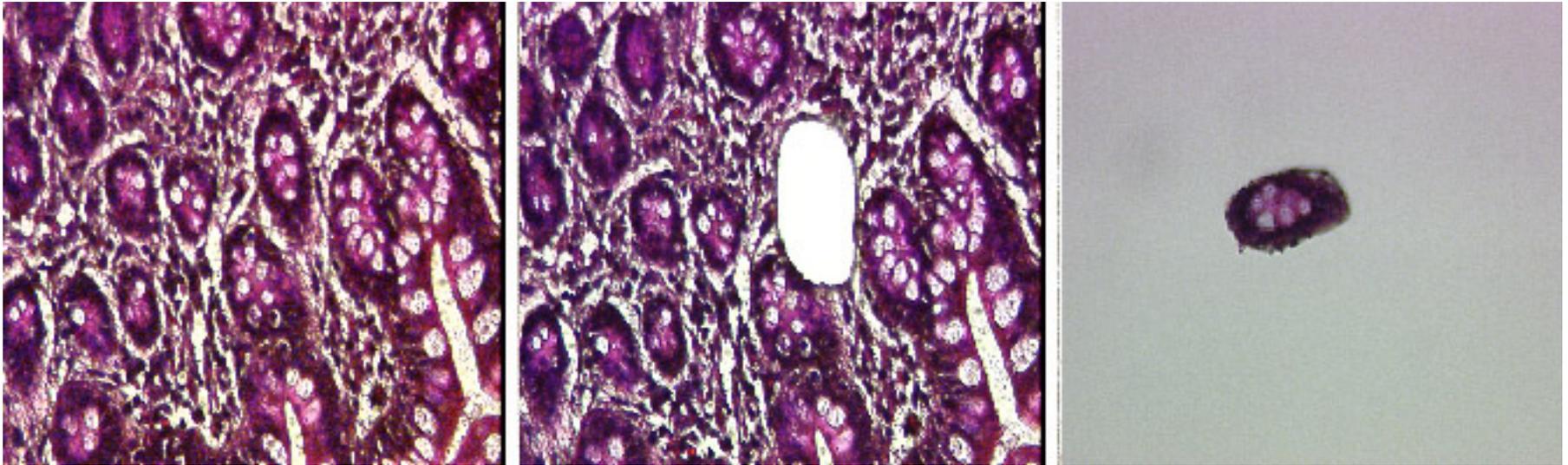
Step 5

Collector Inspection

Collector (PCR Tube CAP)



Direct Visual Inspection of PCR Cap



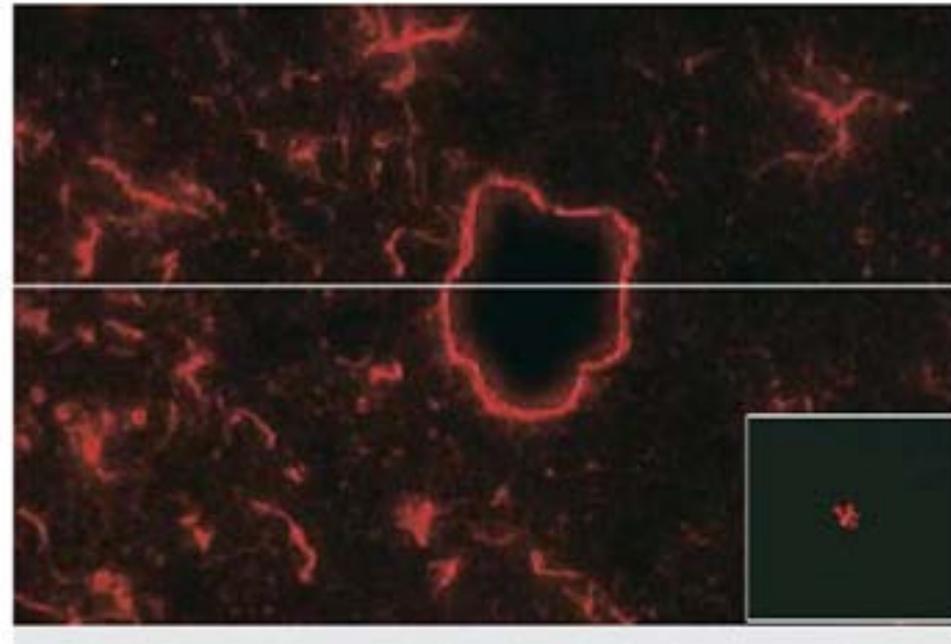
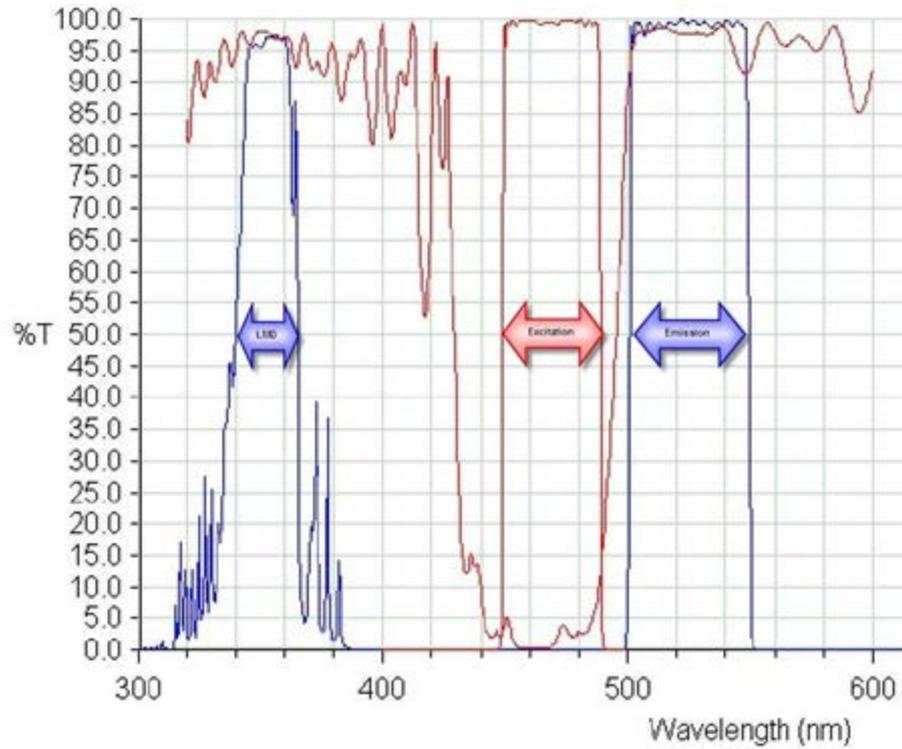
Reference Point Calibration

- 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.

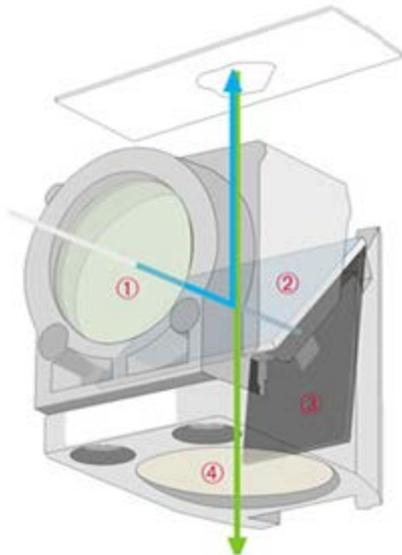
Step 6

Fluorescence Mode; Specimen Overview; Database Module

Fluorescence Mode

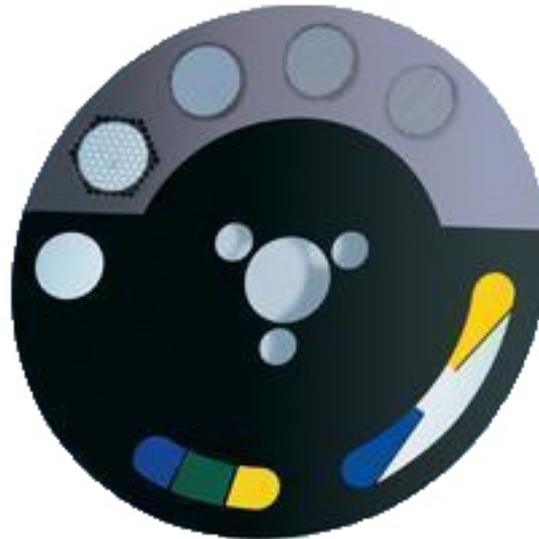


Fluorescence Filter BGR



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):

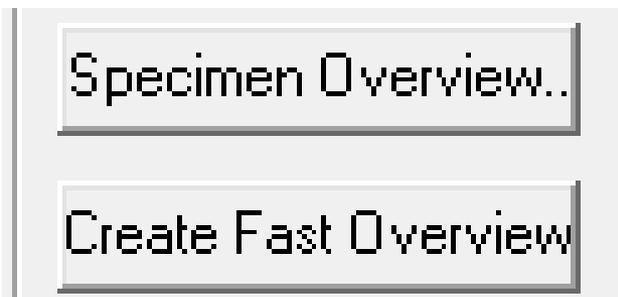
Short pass 460 filter

Band pass 460 to 515

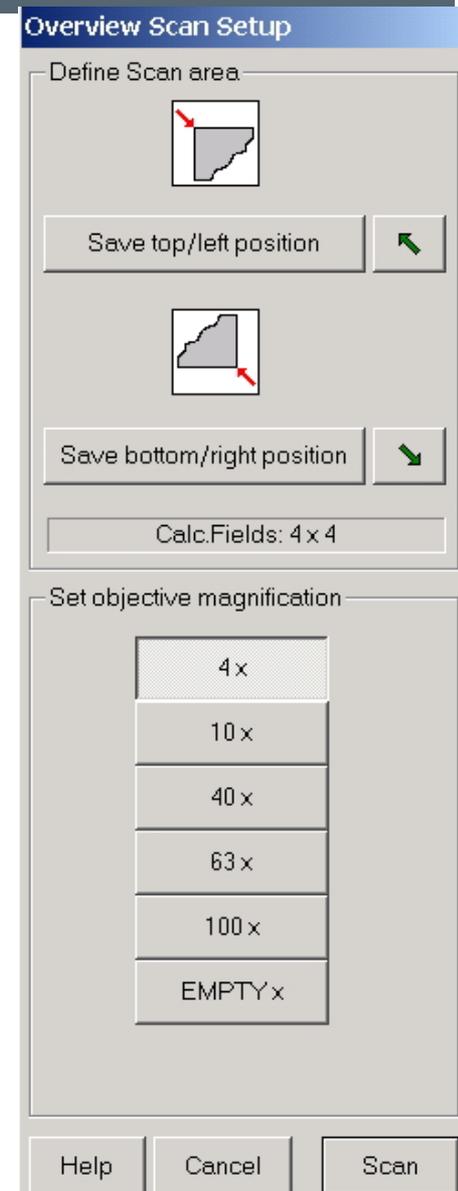
Long pass 520 and higher

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

The screenshot displays the 'Laser Microdissection' software interface. The main window is titled 'Specimen Overview' and features a central histological image of a tissue section. A yellow rectangular box highlights a specific region of interest within the tissue. To the left of the image is a vertical toolbar with icons for 'Select', 'Line', 'Circle', 'Rect', 'Reuse', and 'Length'. Below the image is a 'Collector Device: Tube Caps' control panel with five buttons labeled A, B, No Cap, C, and D, each with a corresponding colored indicator light (A, B, C, and D are red; No Cap is green). To the right of the image is a 'Mark and Find' control panel with buttons for 'Mark current Position', 'Unmark current Position', and 'Unmark all Positions', along with an 'Enable Repositioning' checkbox. Further right are buttons for 'Create Specimen Overview', 'Stop Scan', and 'Done'. Below these are status indicators for 'Status: Ready' and 'Camera: Automatic ON'. At the bottom right, there is a 'Specimen ID' field containing 'Colon2', a 'Specimen Data' button, and a 'Shape List' table.

Nr.	Type	Coll.	Ar
1	Circle	C	83
2	Rec...	B	11
3	Line	D	16

At the bottom of the interface, the status bar shows 'Ready', 'Sample Holder: Slide (Foil/Frame or Foil/Glas)', and 'Magnification: 10x'.

Enable Database Module

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

Zoom Factor:

Draw Shape(s):
 Close Line(s)
 Single Shape Multiple Shapes

Cut Shape(s):
 Draw + Cut Draw + Scan
 Move + Cut Laser Screw

X-/Y-/Z- Precision:
 Fine Coarse

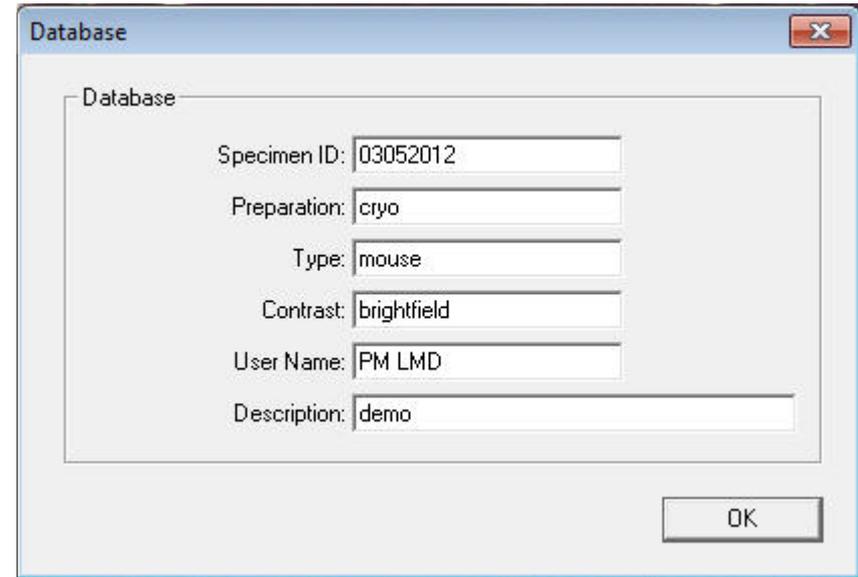
Database:
 Connect to Database
 Specimen ID:

Shape List:

Nr.	Area (μm ²)	Mag.	Coll.	Objects
1	18310	10.00	B	2

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.



Database

Database

Specimen ID: 03052012

Preparation: cryo

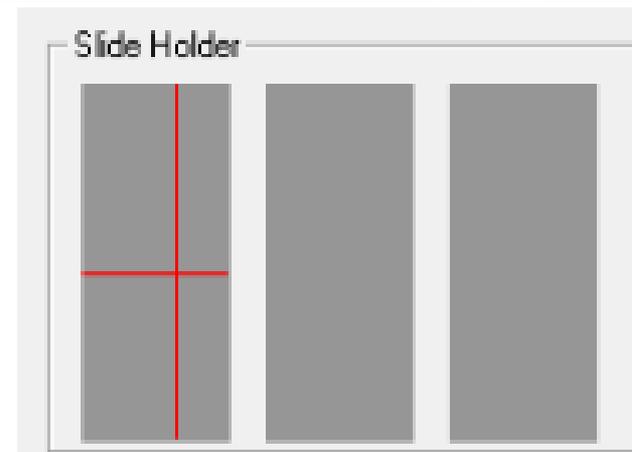
Type: mouse

Contrast: brightfield

User Name: PM LMD

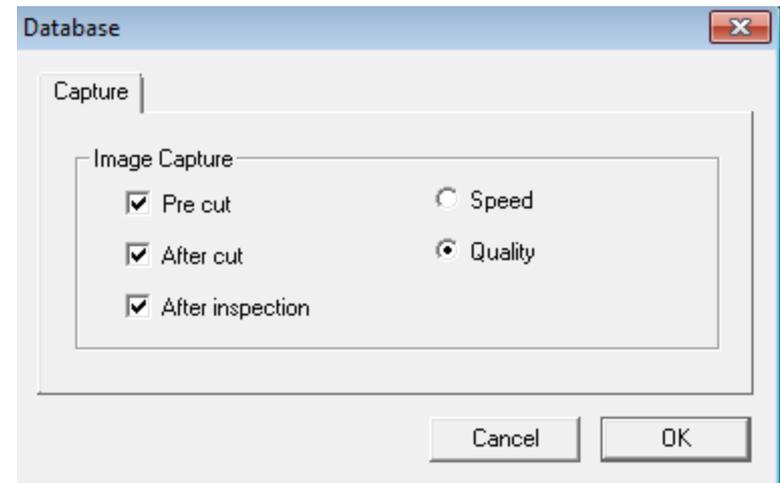
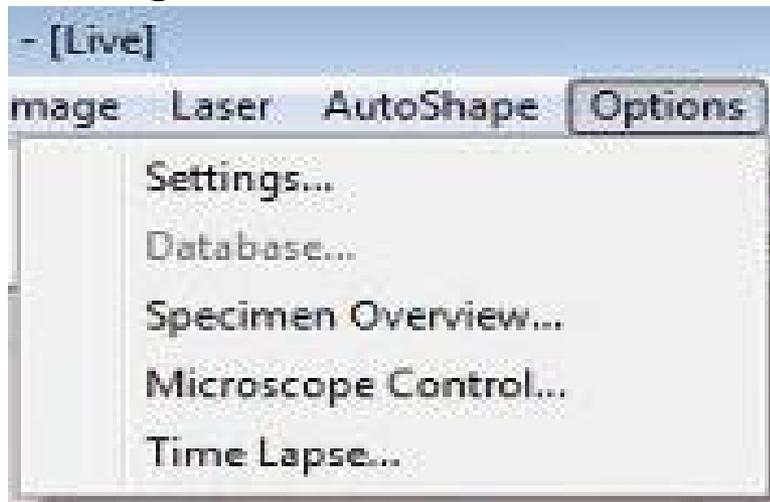
Description: demo

OK



Capture Images

- Options → Database menu settings:



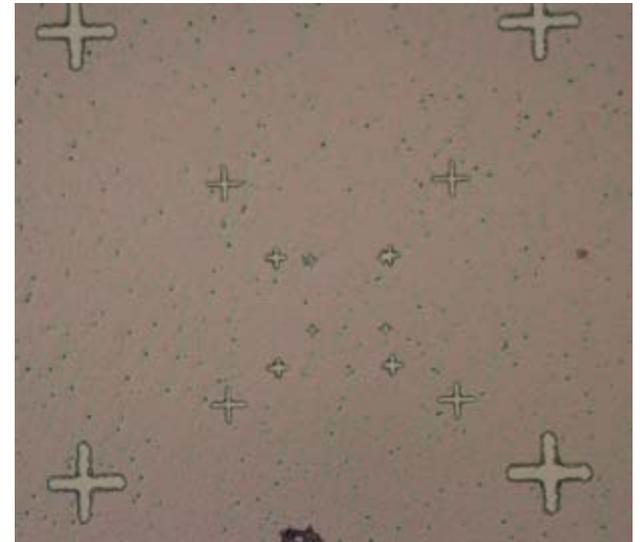
LIF File Viewer

- “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

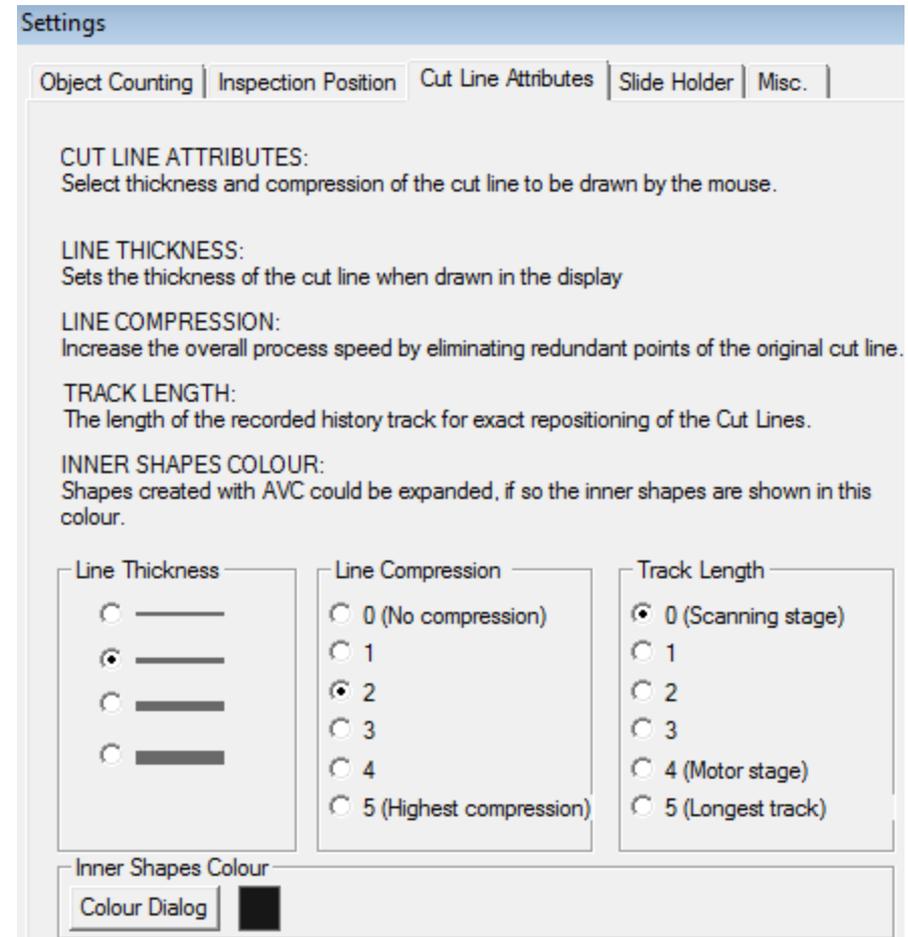
Fine Tuning

- 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

Settings

Object Counting | Inspection Position | Cut Line Attributes | Slide Holder | Misc.

Working Area (for active Slide and Slide Holder)

To define the working area of each slide you have to define two corners (rear left limit a right limit) within the slide holder, which will confine the free working area of each single. Please, proceed as following:
Move to the rear left corner of the slide and set the limit. Move to the front right corner an limit. Close this window, select the next slide and repeat the procedure. Set the limits for slide seperately.



1. Move the stage to the current Rear Left Limit (rear left corner) by pressing the "Move to Rear Left Limit" button.

2. Readjust the stage manually to a new desired position.
3. Define this position to be the new Rear Left Limit by pressing the "Set Rear Left Limit" button.



4. Move the stage to the current Front Right Limit (front right corner) by pressing the "Move to Front Right Limit" button.

5. Readjust the stage manually to a new desired position.
6. Define this position to be the new Front Right Limit by pressing the "Set Front Right Limit" button.

Misc. Settings

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

Settings

Object Counting | Inspection Position | Cut Line Attributes | Slide Holder | Misc.

Miscellaneous settings:

- Show shape IDs in overlay
Shape IDs will be drawn in middle of shapes.
- Show shape positions in overview image
On each shape position will be drawn a cross in the overview image.
- Save overlay with image files
Overlay (shapes, text,...) will be saved with images to file and database.
- Delete shapes after cut
Cut shapes will be deleted after cut.
- Close Fluo-Shutter during cut
Fluo shutter will close during cut process, no before/after cut images will be taken.

Type of connected laser:

LMD 6500 LMD 7000

- Laser connected to PC
LMD software control the laser box (needs RS232 connection to laser).
Laser Timeout (seconds):
- Save Summary at program end
You will be prompted to save the Summary List when you end the LMD software.
- Show progress bar during cutting
A progress bar will be shown during the cutting.

Distance of Laser Calibration Crosses (1.0 is standard setting)

- Automatically switch to 2x magnification cube in inspection mode
- Ask if collector is inserted before cut

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.

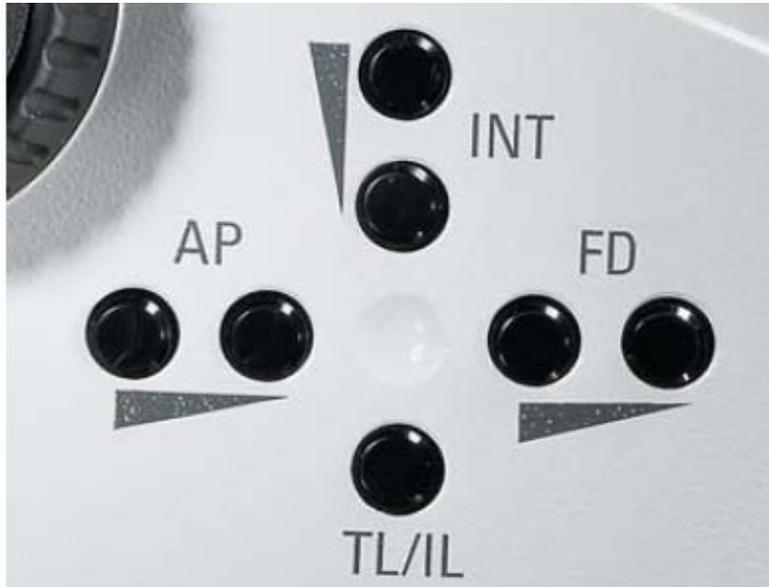
Step 9

Microscope Standalone Operation

DM6000B Touch Screen



DM6000B Buttons (Preset)



Illumination Manager

*TL & FL Intensity Controls

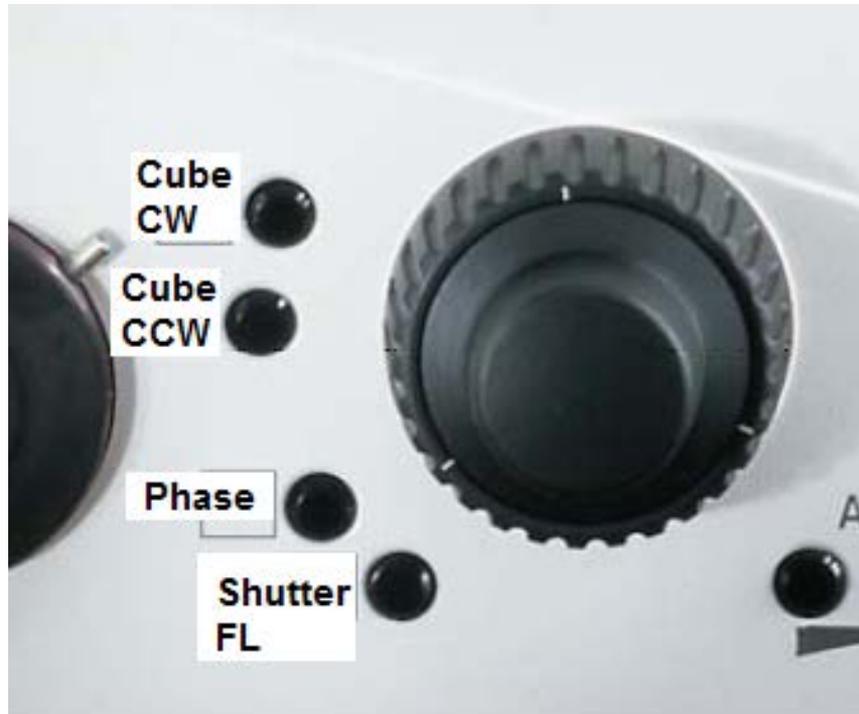
*TL / IL Toggle

*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

Section 2. How to Prepare Tissue Sections

Preparing Frozen Sections.....

Precautions for working with RNA

Workflow for Preparation of Frozen Sections ...

Flash-freezing Methods

Sectioning.....

Preparing Paraffin Sections

Tissue Fixation

Paraffin embedding and Sectioning

Dissectate collection from plain glass slides via LMD

Preparation of archived slides for LMD



Sample Preparation For Leica Laser Microdissection

Protocol Guide for Leica Microsystems Laser Microdissection Systems

LMD Citations

List of Publications Leica Laser Microdissection



1. DNA-Research.....	2
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4. Neurosciences	14
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6. Plant Research	19
7. Forensics	21
8. Miscellaneous	22

- <http://www.leica-microsystems.com/lmd>

Leica Contacts

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617-817-0481, qing.tang@leica-microsystems.com

Leica Application and Technology Support Center:
866-830-0735 Option 3 (Usually Louise Bertrand)
(Remote webex support: <http://support.e-leica.com>)

(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")