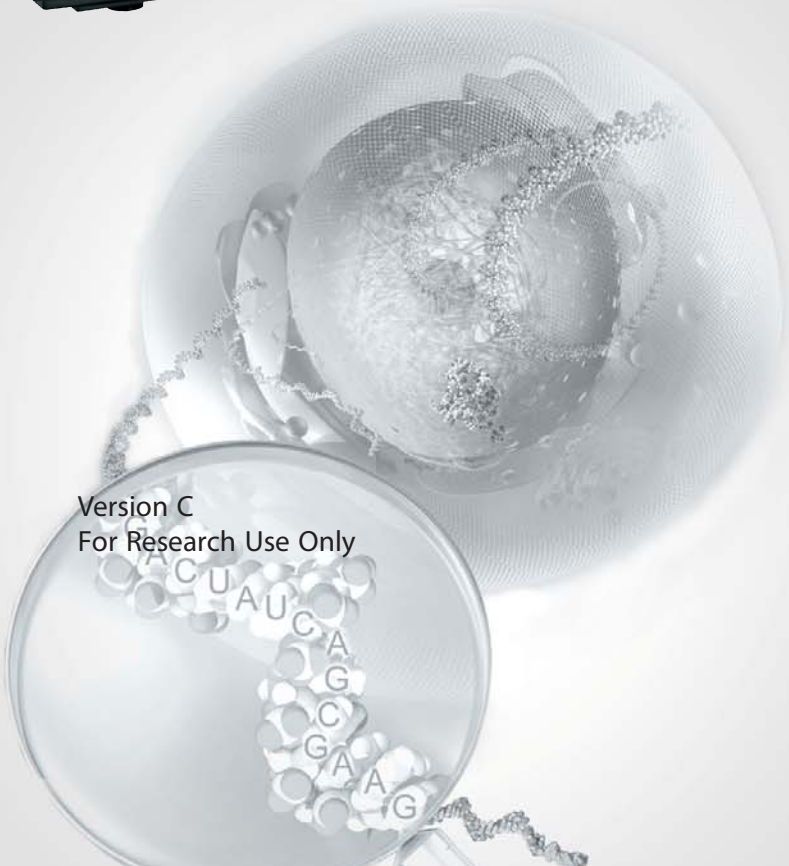
User Guide

VERITAS™ Microdissection Instrument



Version C
For Research Use Only

ARCTURUS

Veritas™ Microdissection Instrument

User Guide

13553-00

Version C

ATTENTION - Please Read!

Improperly moving this instrument, changing settings or installing third party hardware or software may impact the functions of this instrument and result in a billable service call.

Please not move this instrument, change settings, or install any third party hardware without first contacting Arcturus Bioscience.

Phone: (888) 446-7911

Email: support@arctur.com

Legal Notice

Limited Warranty

a) Seller warrants that all products sold hereunder will be free from defects in material and workmanship and perform to Seller's applicable published specifications. All mechanical and electronic parts for the products and assemblies manufactured by Arcturus Bioscience, Inc. are unconditionally warranted to be free from defects in materials and workmanship for a period of one year following delivery of the equipment from the FOB point. Microscope objectives are not covered under warranty. The liability of Seller hereunder shall be limited to replacing, repairing, or refunding the purchase price, at its option, if the product's defect is due to Seller's material and workmanship for any defective units which are returned FOB Seller's facility, Mountain View, California. In no case are goods to be returned without first obtaining permission and a customer Return Material Authorization (RMA) number from Seller. In-warranty repaired or replacement equipment is warranted only for the remaining unexpired portion of the original warranty period applicable to the repaired or replaced equipment.

b) Goods or parts which have been subject to abuse, misuse, accident, alteration, neglect, unauthorized repair or installation are not covered by the warranty. Seller will make the final determination as to the existence and cause of any alleged defect. No liability is assumed for expendable parts such as lamps and fuses, or for reasonable wear and tear. No warranty is made with respect to custom equipment or goods produced to Buyer's specifications except as specifically stated in writing by Seller in the contract for such custom goods.

c) The above warranty statement is valid for units purchased and used in the United States. Products with international destinations may be subject to a warranty surcharge, and/or different warranty conditions.

d) This warranty is the only warranty made by Seller with respect to the goods delivered hereunder and may be modified or amended only by a written instrument that is signed by a duly authorized officer of Seller and accepted by Buyer.

e) This warranty is in lieu of all other warranties, expressed or implied, and does not cover incidental or consequential loss.

f) EXCEPT AS PROVIDED ABOVE, SELLER MAKES NO WARRANTIES, EXPRESSED, IMPLIED, STATUTORY, IN ANY COMMUNICATION WITH THE COMPANY OR OTHERWISE, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

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User Safety

Please review the following precautions carefully to ensure safe and effective use of the Veritas™ Microdissection Instrument.

All models of the Veritas instrument contains a Class 3b infrared laser. The infrared beam from this laser is not visible. **Avoid direct skin and eye exposure to this laser radiation.**

Instruments with a cutting laser (Models 703 and 704) contain a Class 4 ultra-violet laser in addition to the Class 3b laser. The ultra-violet beam from this laser is not visible. **Avoid eye or skin exposure to direct or scattered radiation.**

The instrument itself is classified as a Class 1 laser device.

The Veritas instrument incorporates an interlock system that enables laser operation only when the instrument covers are on and the front door is closed, and the interlock switches are not defeated or bypassed. **Do not modify or override the interlock system.**

Do not remove or modify any of the Veritas optical components or subassemblies, except as described in "User Serviceable Parts" on page 100.

Please note the warning labels on the instrument. They are shown here.

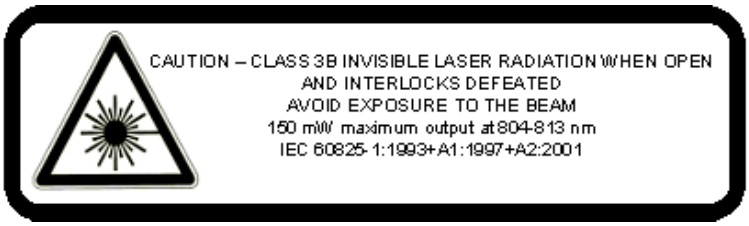


Figure 2-1. Warning for capture laser

Figure 2-2. Warning for cutting laser

Any modifications to the Veritas Microdissection Instrument, will void the system warranty.

The Veritas Microdissection Instrument is for indoor use only.

Symbols

▲**CAUTION**: Consult accompanying documents.

▲**CAUTION**: Hot surface!

▲**CAUTION**: Possible pinching!

▲**CAUTION**: Eye shield required!

Installation

Installing Software Upgrades

Each version of the software is accompanied by detailed instructions for updating an existing installation. Please follow the update instructions distributed with the software.

Instructions for Lifting and Carrying

The Veritas™ Microdissection Instrument is shipped from the factory with internal tie-downs and support hardware for its protection. These should be reinstalled before relocating the instrument. The instrument is heavy, and the use of the hydraulic lift, detachable handles, and support base originally shipped with the instrument is recommended. Place the support base on the lift and slide the instrument onto it applying force to the handles rather than the sides of the instrument.

Detailed Installation Instructions

▲**CAUTION** - Failure to correctly install the instrument may result in damage that is not covered by the warranty.

If you encounter any difficulties, use the following resources to resolve your problems:

- Refer to "Maintenance and Troubleshooting" on page 95.
- Refer to document #13600-24 on the Veritas installation CD.
- Contact customer support at Arcturus Bioscience 1 (888) 446-7911 (USA toll-free) or at (650) 962-3020. You can also send email to techsupport@arctur.com.

If you prefer, Arcturus will be pleased to arrange for professional installation of your instrument.

Gather Equipment

You will need the following tools for the installation:

- #2 Phillips screwdriver
- 3/8" slotted screwdriver
- 9/16" wrench
- Hex keys of the following dimensions:
  - 7/64"
  - 2.5 mm
  - 3 mm
  - 5 mm
- Cutting pliers or shears (for plastic zip ties)

Prepare the Site

You will need:

- Stable laboratory bench capable of supporting 400 lb. (200 kg)
- Work surface 36 x 72 in (90 x 180 cm) with 32 in (80 cm) vertical clearance.
- Electrical Supply: 100–240 VAC, 50–60 Hz, 600W, voltage fluctuations not to exceed ±10% of nominal supply voltage.
- Temperature 18°–30°C, Relative Humidity < 60%.

Unpack the Instrument

1. Release crate latches and attach front panel as ramp (see photo).

2. Use the #2 Phillips screwdriver to remove small side panels near the bottom of the left and right sides (see photo).
- Using the 9/16" wrench, remove the four screws that secure the Veritas™ Microdissection Instrument platform to the support, near the strap buckles and on the other side.
- Remove the straps. (Crate shown without sides for clarity; see photo.)

3. Verify the instrument platform is secured to the jack with three clamps.

4. Jack the instrument off the shipping supports and roll down the ramp.

5. Remove the bag from the instrument.

Complete Back Side Preparation

1. Remove the top cover.
- Use the slotted screwdriver to loosen the two screws at top rear of instrument securing top panel.
- Slide the top cover back and lift up to remove.
- Place in a secure location.

2. Remove the front cover.
- Loosen the two screws at top back side of the front panel.
- Pull the top of front panel forward until the snap-locks release and tilt the panel forward.

- Disconnect the three cables from the printed circuit board on the front panel in this order: red/black, green/yellow and gray ribbon.
- Tilt the front panel forward and lift to remove.
- Place in a secure location.

3. Remove the center-rear cover by removing screw, tilting the top outward ~5cm (2 in.) and lifting clear.

4. Models 702 and 704 only, at the rear of the instrument:
- Clip and remove orange zip tie from light guide.
- Remove red shipping bracket from light guide.

5. Replace and secure the center-rear metal cover.

Place on Laboratory Bench

▲**CAUTION**: The instrument weighs 120 kg (265 pounds). Use appropriate care.

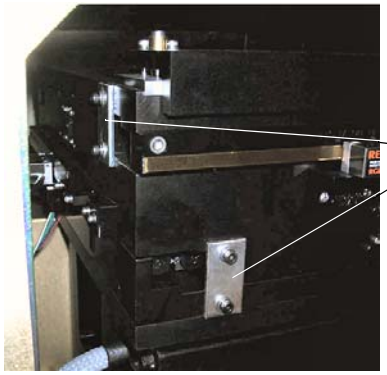
1. Use foot pedal to jack instrument to table height.

2. Use handles to lift instrument over plywood stops and slide onto table.

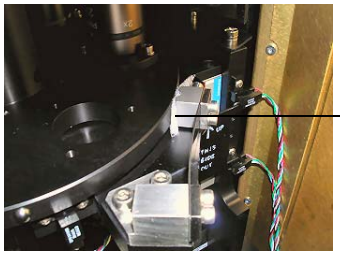
Complete Front Side Preparation

NOTE: Re-install all screws and washers except as noted. Save brackets and foam for future use. Take care not to drop screws or washers.

1. Remove all five red shipping brackets:
- Objective turret support (Use the 7/64" hex key for the top screw, 3 mm hex key for bottom screw.)
- Objective turret rotation (Use the 2.5 mm hex key for two screws.)
- Condenser lens (Use the 2.5 mm hex key for two screws.)
- Two brackets on the X-Y stage (may be silver colored; see photo) (Use the 7/64" hex key for two screws.)

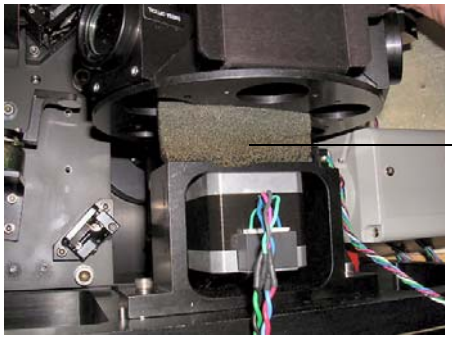


2. Remove protective foam from:
- Between objective turret spring and turret (see photo)



- Models 702 and 704 only: Under filter wheel (see photo)

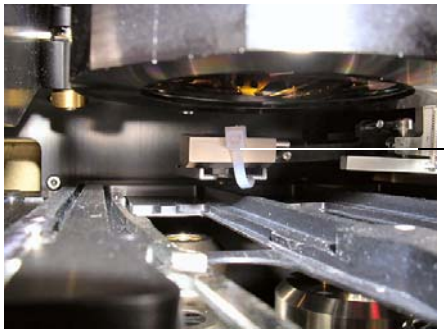
3. Remove green tape from the following locations
- Cap robot front-back motor
- Cap robot up-down motor
- Cap off load tray (2 pieces)



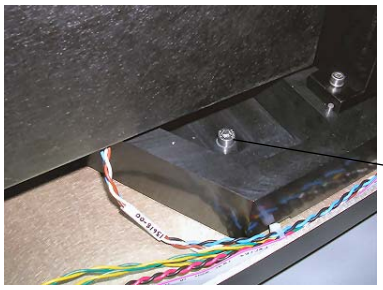
4. Remove zip ties from:
- Cap robot cap fork (white) (see photo)

NOTE: You may need to move fork assembly forward to do this. Verify that the motor is unplugged (see photo on next page) and pull toward you on the right side of the assembly, near the lead screw.

- Front panel above door (orange) (see photo)



5. Remove the two lock-down screws securing the vibration-isolated microscope structure to base. (Use the 5 mm hex key for two screws.)
- M6x20 screw near the front below the stage ribbon cable (see photo)

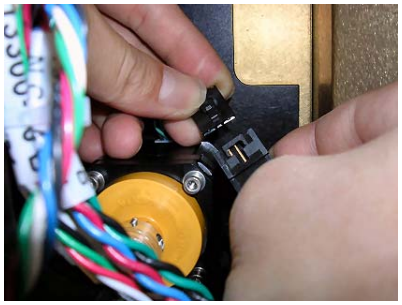


- M6x20 screw behind the Hitachi camera (see photo)

6. Connect the cable at the cap robot front-back motor (see photo).

7. Ensure the stage insert for slides is fully seated by pushing the front edge inward.

8. Verify that the 20x and 40x (or 60x) objective correction rings are at the 1.0 mark. Correct if necessary.



9. Replace front cover.
- Place bottom clips over front edge of base
- Reconnect the three cables to the circuit (gray ribbon, green/yellow, then red/black)
- Align snap clips with mating parts, ensuring cables are out of the way.
- Push the cover foward until it snaps into place.
- Secure with two thumbscrews.

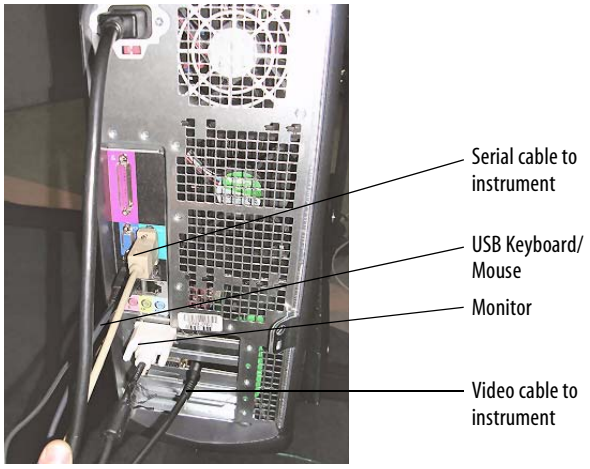
10. Replace top cover.
- Set into place ~10cm (3 in.) from front
- Slide forward.
- Securely tighten two thumbscrews.

11. Remove label covering the power entry module plug.

Set Up and Connect Computer

1. Unpack PC and monitor boxes and set up according to manufacturer's instructions.

2. Connect the computer to the instrument with the serial cable and video cable. See the figure on page 12 for details of the connections.



3. Position the monitor, keyboard and mouse for comfortable operation.

4. When you have completed the installation, please perform the installation qualification below.

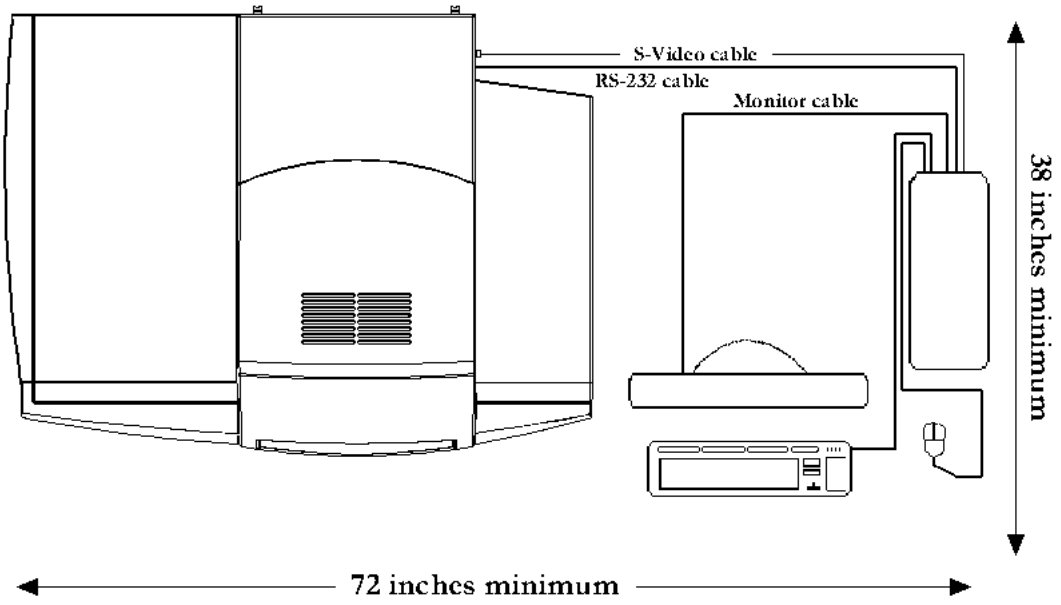


Figure 1. Veritas™ Microdissection Instrument connections (top view)

Installation Qualification

- Verify the instrument is supported on a stable bench or table capable of supporting 200 kg.
- Verify the instrument has at least 8 cm of clearance at the sides, rear, and top for air circulation.
- Verify the monitor, mouse and keyboard are arranged for comfortable operation.
- Verify the laboratory temperature and relative humidity are within the specified limits.
- Verify the electrical supply is compatible with instrument specifications.
- Verify the PC voltage selection matches the electrical supply voltage.

If any of the above requirements is not met, identify and correct the problem before powering the instrument.

- Turn the instrument and computer on and wait 2–3 minutes. Verify the Veritas application launches without error messages.

1. Introduction

The Veritas Microdissection Instrument

The Veritas™ Microdissection Instrument provides an automated approach to microdissecting individual cells or multi-cellular structures from slides containing tissue sections or cytological samples.

The Veritas Microdissection Instrument consists of the Veritas instrument, a computer and the Veritas software application.

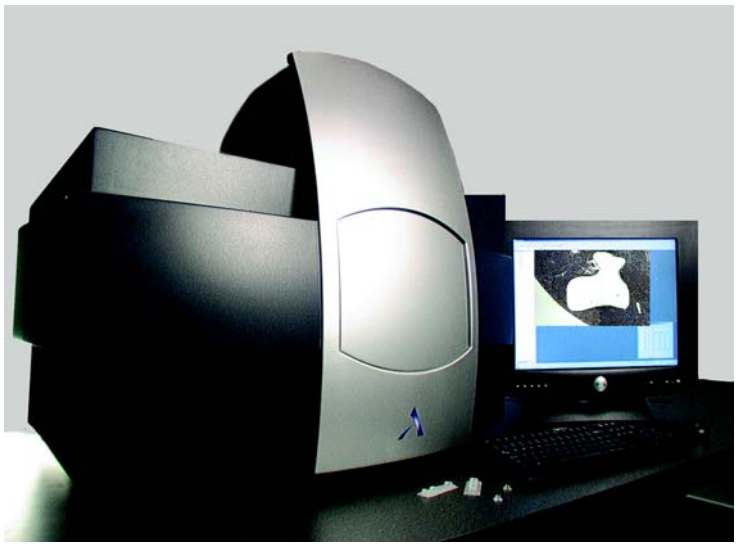


Figure 1-1. Veritas Microdissection Instrument

Veritas Instrument Configurations

There are four models of the Veritas Microdissection Instrument:

- Model 701: IR capture laser only
- Model 702: IR capture laser with epi-fluorescence
- Model 703: IR capture laser and UV cutting laser
- Model 704: IR capture laser and UV cutting laser with epi-fluorescence

With models 703 and 704, the cutting laser allows for quick capture of large regions of tissue. Additionally, a cutting laser allows you to ablate – remove via photo volatilization – tissue that is not of interest, to ensure higher purity samples.

With models 702 and 704, you can perform LCM on fluorescence labeled samples. The fluorescence source is the X-Cite 120 from EXFO Photonics Inc.

The standard system has four objectives for the microscope: 2x, 10x, 20x and 40x. A 60x objective and associated firmware can be installed in place of the 40x by qualified Arcturus service personnel.

For sample visualization the instrument utilizes a PAL-format color video camera.

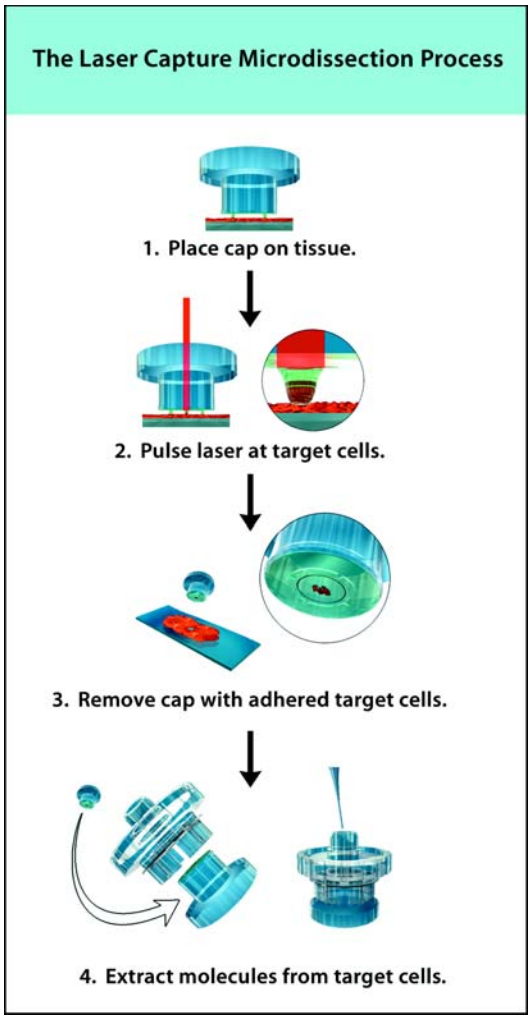
For sample visualization and dissection for fluorescence applications, the system uses an illuminator and three filter cubes – red, green and blue excitation – as well as a fourth position for a user-installed special purpose cube or factory-installed optional UV excitation filter cube.

See "Specifications" on page 105 for excitation and emission wavelengths and "Interchanging Fluorescence Filter Cubes" on page 101 for instructions for installing filter cubes.

What is LCM?

Laser capture microdissection (LCM) is a method to quickly and easily procure specific cell populations from standard histological slides, using a low-power infrared laser (referred to as the "capture laser" in the rest of the document) to activate a special thermoplastic film over the cells or tissue of interest. The activated transfer film adheres to the cells that are located within the laser beam diameter. The laser does not affect the tissue sample; the quality of nucleic acids and proteins within the sample and the cell morphology are not compromised.

In the Veritas™ Microdissection Instrument, specially designed CapSure™ HS or CapSure™ Macro Caps that are coated with this film are placed on the tissue section of interest. The instrument directs the laser through the cap to activate the film onto the selected cells. The cells adhere to the cap surface when it is lifted from the tissue section while the surrounding tissue remains intact on the slide. The cap can be placed directly into a microcentrifuge tube for extracting DNA, RNA or protein.



What is "Cut and Capture"?

Photoablation, the volatilization of tissue by light emitted from an ultraviolet laser, can be used in conjunction with the IR capture laser. In one application of photoablation, a relatively wide "moat" is ablated around the region of interest and then the remaining cells are captured by the capture laser. This minimizes contamination of the cells due to collateral pick up during the capture process. This "cut and capture" method can be used for tissue mounted on regular glass slides.

An alternate "cut and capture" method can be used for tissue mounted on membrane (such as 2 µm thick polyethylene napthalate (PEN), either on glass or in a metal frame). Here, the cutting laser is used to cut a narrower outline around the region of interest after which the entire region within the outline is captured.

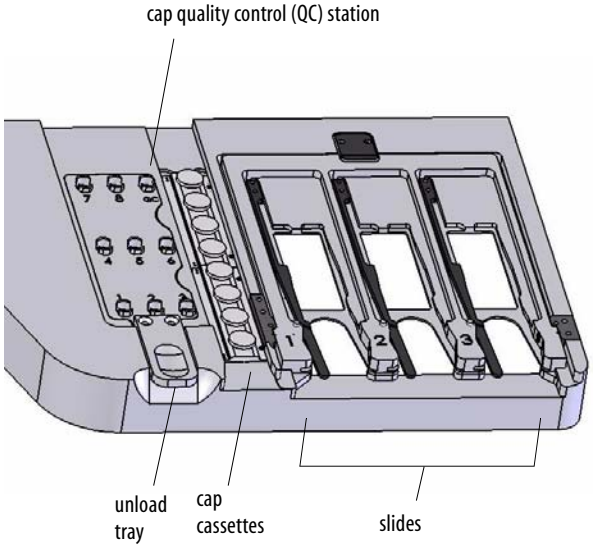
Other Uses of the Veritas Microdissection Instrument

The cutting laser can also be used for negative selection. In this application, undesired material is ablated, leaving behind the

material of interest. You may also remove the region of interest manually (i.e. the capture laser is not used).

Work Surface

The Veritas instrument's work surface is where the slides and caps are located, as well as the stage for the microscope. Use the following guidelines when loading materials on the work surface:



Unload Tray
Place the unload tray in the unload slot with the handle facing towards the front.

NOTE: To avoid instrument damage, always be sure the unload tray is correctly positioned.

Caps
Push cap cassettes all the way back within the slot. If you are using a single cassette, be sure to push it all the way to the back.

Slides
Push the tension lever to the left and place the slide in the opening of the slot. Push the slide all the way back in the slot. The tension lever will keep it securely in place.

Computer

The Veritas Microdissection Instrument includes a computer and an LCD monitor. The computer includes the Windows operating system and basic Windows applications, as well as the Veritas application to control instrument operation.

Additional Materials

CapSure™ Macro Caps
CapSure Macro Caps provide a large surface area to capture several thousand cells on a single cap. With CapSure Macro Caps, the polymer surface is completely flat, so the entire surface can be used to capture cells. Following LCM, the caps can be placed directly onto a 0.5-mL microcentrifuge tube containing extraction buffer.

CapSure™ HS Caps
CapSure HS Caps provide low-volume extraction and analysis of highly specific LCM-captured material. The surface of the CapSure HS Cap has a 12-µm discontinuous circular ridge that sits on the surface of the sample during LCM. This ridge keeps the full surface of the cap elevated above the sample during LCM. Thus, the polymer on the cap surface comes in contact with the sample only where the polymer is activated by the laser.

CapSure™ LCM Sample Preparation System
Includes:

- CapSure HS Alignment Tray

- Incubation Block
- PrepStrips™ Slide Preparation Strips
- ExtracSure™ Sample Extraction Devices

Cap Insertion Tool
The Cap Insertion tool is used to facilitate removal of CapSure Caps from the unload tray.

Membrane Slides
With instruments equipped with a cutting laser and PEN (polyethylene-napthalate) membrane slides, you can swiftly and precisely capture large regions using the cut and capture method described above.

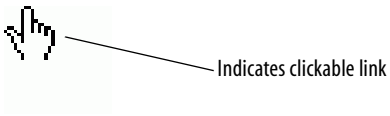
Figure 1-2. Membrane slides

These slides are available from Arcturus.

Slide	Catalog Number
PEN membrane frame slides	LCM0521
PEN membrane glass slides	LCM0522

About the PDF Version of the Manual

This manual is also distributed as a PDF (portable document format) file, installed with the Veritas application. You will need Adobe® Reader®, version 5.0 or greater, to open the file.



In the PDF version, when the document references a different section or chapter, the cursor in Acrobat Reader will change to a finger. You can click on the text with the section name or the page number to go immediately to that section. For example, if the sentence says: See "View Menu" on page 63 click on "View Menu" or the page number to go to page 63.

You may also click on any page number in the table of contents or the index to go immediately to the corresponding item.

2. Easy Access to Microdissection

This chapter explains how to use the Veritas™ application to perform laser capture microdissection (LCM). For a more detailed introduction to the application, please see Chapter 3, "System Overview" on page 31.

LCM consists of the following steps. Each step is described in more detail on the corresponding page.

- Tissue Preparation – page 17
- Startup – page 19
- Loading Materials – page 19
- Marking the Cells for Capture – page 21
- Creating Additional Capture Groups – page 24
- Capturing Cells – page 24
- Inspecting Captured Material – page 25
- Capturing Additional Capture Groups – page 27
- Unloading and Removing Caps – page 26
- Extracting Captured Tissue from the Caps – page 27

See "Overview of Additional Microdissection Methods" on page 53 for information about other microdissection modes available with the Veritas Microdissection Instrument.

Tissue Preparation

Tissue sections prepared from both frozen and formalin-fixed paraffin-embedded tissue can be used for LCM. Freezing tissue helps ensure the integrity of the biological molecules within the cells. Thus, cells captured from frozen tissue sections provide material that is suitable for many downstream molecular biology applications. This is especially true for applications requiring intact RNA. While the integrity of the RNA from formalin-fixed tissue may not be as optimal as that from frozen tissue, using the recommended reagents will allow you to use these samples for molecular biology applications as well.

Arcturus provides an application note describing the recommended protocol for working with frozen samples: Application Note #1, *Optimized Protocol for Preparing and Staining LCM Samples from Frozen Tissue and Extraction of High Quality RNA*. This note can be found on the Arcturus web site at www.arctur.com.

NOTE: For optimal sample preparations of **frozen tissue samples** and downstream processing, Arcturus recommends the following reagent kits:

Reagent Kit	Catalog Number
HistoGene™ LCM Frozen Section Staining Kit	KIT0401
HistoGene LCM Immunofluorescence Staining Kit	KIT0420
PicoPure™ RNA Isolation Kit	KIT0204
PicoPure DNA Isolation Kit	KIT0103
RiboAmp® HS RNA Amplification Kit	KIT0205

Formalin-fixed, paraffin-embedded tissue may also be used for LCM for DNA and protein work. Suggested protocols based on the experience of Arcturus customers are available on the Arcturus web site. For gene expression profiling studies using formalin-fixed paraffin-embedded tissue, Arcturus recommends using the Paradise™ Reagent System. This system provides all the reagents for sample preparation, RNA extraction, isolation and linear amplification of the RNA, for use in microarray or quantitative real-time PCR applications.

NOTE: For optimal sample preparations of **formalin-fixed, paraffin-embedded tissue samples** and downstream processing, Arcturus recommends the following reagent systems:

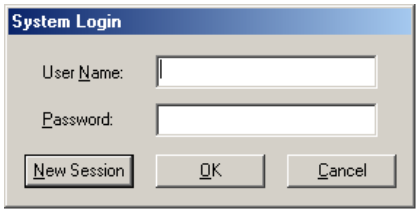
Reagent System	Catalog Number
Paradise™ Reagent System, 1.5 rounds of amplification for use with Affymetrix GeneChip® Arrays	KIT0301 (48 reactions) KIT0311 (12 reactions)
Paradise Reagent System, 2 rounds of amplification for use with cDNA arrays	KIT0302 (48 reactions) KIT0312 (12 reactions)
Paradise Reagent System for Quantitative Real Time PCR	KIT0300L (48 reactions)

Startup

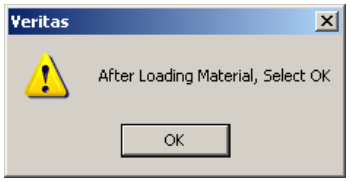
1. Turn on the computer.
2. Turn on the Veritas™ Microdissection Instrument.
   The power button is located on the left side of the instrument, towards the back.
3. Start the application by double-clicking the **Veritas** icon on the desktop.
   – or –
   Click **Start**, point to **Programs** and click **Veritas**.

Loading Materials

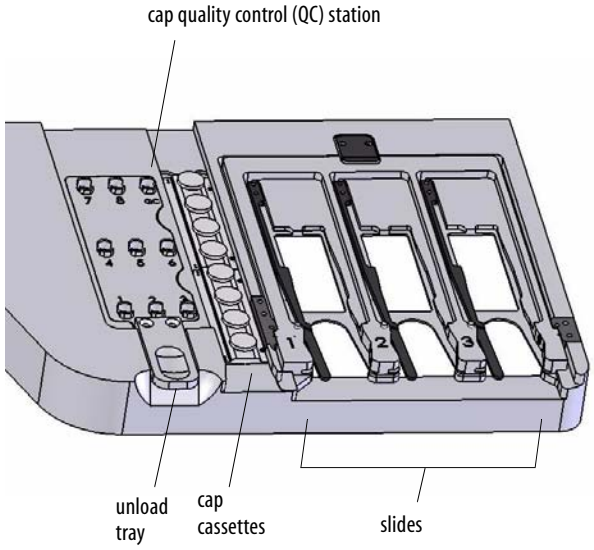
1. Log in.
   The System Login dialog box appears automatically when you start the Veritas application.
   - Enter your user name and password.
   - Click **New Session**.



   The instrument door opens, the work surface slides out and the application displays following message:



2. Load your slides and caps.
   NOTE: If needed, remove any caps that may have been left in the QC station, the unload tray or on any slides.
   - Place your slides in the appropriate locations on the work surface. Push the tension lever to the left and place the slide in the opening of the slot. Push the slide all the way back in the slot. Make sure the slides are seated properly.
   - Slide the CapSure™ cassettes into the appropriate slot on the work surface. If you are using a single cassette, push it all the way to the back of the slot.
   - Place the unload tray in the slot with the handle facing towards the front. Make sure the unload tray is securely in place.



3. Click **OK** in the message.
   The work surface slides in, the instrument door closes and the system automatically performs material detection, identifying the number and types of slides and caps you have loaded.
   If you do not have the **Use Material Detection** option set (see "Setting User Preferences" on page 36), the Materials

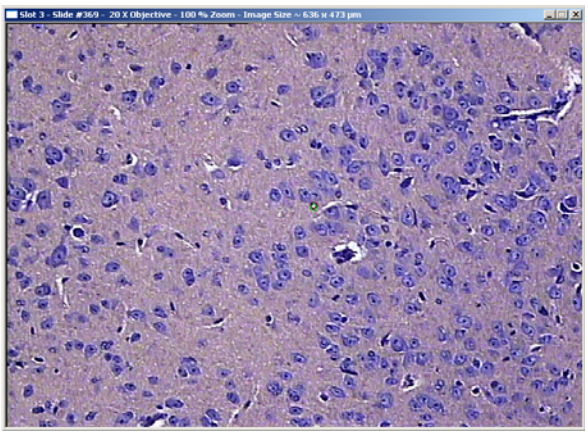
Loading window appears and you must manually enter the details about your slides and caps, as described in "Entering Information in the Materials Loading Window" on page 67.

If you are using the AutoPix 100e, the Materials Loading window will *always* appear and you must enter the details for your slides and caps.

4. Once materials detection is complete, the system automatically performs the following actions for each slide, starting with the slide in the highest numbered slot:

- Displays the live video window.
- Once the live video window is open, the system automatically adjusts the brightness and focuses the live video image.

  NOTE: If you do not have the **Auto Focus** and/or **Auto Brightness Adjust** options set (see "Setting User Preferences" on page 36) you will need to manually set the brightness and/or focus the live video; see "Manually Focusing the Live Video" on page 51.



- Acquires and displays a roadmap for the slide.

  NOTE: If you do not have the **Auto-acquire Roadmap Images** option set (see "Setting User Preferences" on page 36), you will need to manually acquire a roadmap. To do this, activate a slide in the Materials Tool by clicking it, then right-click and choose **Acquire Roadmap Image**.

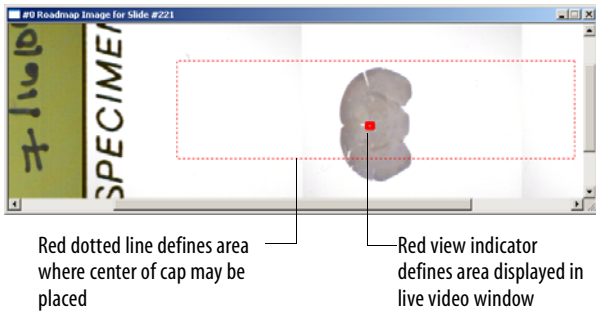
5. Move the stage to display an area of interest in the live video image. To use a slide other than what is displayed, double-click on the slide in the Materials Tool or click the **Slot** button corresponding to the slide of interest. The roadmap for the selected slide will be displayed.

When the live video window is the active window, the cursor turns into a hand. Click and drag the mouse to move the stage to display a regions of interest.

In addition to moving the stage with the hand, you may move the stage in any of the following ways:

- Press the UP, DOWN, LEFT and RIGHT arrow keys to move the stage a small amount.
- Hold down the CONTROL key while pressing an arrow key to move one half of the field of view at a time.

You can save the locations of areas of interest so you may easily return to them. See "Saving Positions of Interest" on page 84 for more information.



6. If you are performing fluorescence LCM, turn on the fluorescence lamp by clicking the **Fluorescence** button in the Microscope Tools. The fluorescence lamp needs several

minutes to warm up to reach its maximum light intensity. When it is ready, the message "EPI Ready" appears in the lower-right corner of the screen.
(See "Microdissection of Fluorescent Samples" on page 52 for more information.)

7. To view the cells and tissue you wish to capture, change the objective as needed, by clicking in the Microscope Tools. Each time you change the objective, the instrument should automatically adjust the image brightness and focus the microscope.

Marking the Cells for Capture

Use the individual tools in the Microdissection Tools to mark the cells for capture and/or ablation. These marks are referred to collectively as "dissection marks".

NOTE: If your instrument is equipped with a cutting laser (Models 703 and 704), you should mark your cells at *one* objective power and then initiate cut and capture. This ensures the cutting laser will most accurately follow the dissection marks.

The Microdissection Tools are arranged on three tabs:

- Cut and Capture (Models 703 and 704 only); use these tools to mark larger regions for capture using a combination of laser cutting and capture.
- LCM; use these tools to mark single cells or regions for capture.
- Ablate (Models 703 and 704 only); use these tools to mark areas to be ablated.

See "The Microdissection Tools" on page 80 for more detailed information about these tools.

Common Microdissection Tools

In addition to the tools used to mark cells, there are four tools common to each tab:

- **Hand**

  Use this tool to move the stage. By default, this tool is always active in the live video.

- **Ruler**

  Use this tool to measure distances in the live video window. See page 80 for information on using the ruler.

- **Go Capture**

  Once you have marked cells, this tool initiates the cut and capture process. See "Capturing Cells" on page 24 for more information.

- **Autoscan**

Click this to begin training the system to automatically identify cells for capture. See "Introduction to Auto Scan on a Static Image" on page 55 for detailed instructions on this feature.

Activating a Microdissection Tool

To activate a tool, click on it. The tool remains active until you click the tool again, which will turn it off. When you turn off a tool, the Hand tool becomes active so you can move the stage. You can use the arrow keys to move the stage while a tool is active.

Changing Properties of the Dissection Marks

You may change the properties of any dissection marks, such as the number of capture laser spots or width of the cut. See "Adjusting Dissection Mark Properties" on page 83 for more information.

Cut and Capture Tools

If you have a cutting laser in your Veritas™ instrument (Models 703 and 704), the Cut and Capture tools are available and allow you to mark larger regions for capture.

If you wish to capture single cells or smaller regions, use the LCM Dissection Tools, described below.

If you wish to ablate an area, use the Ablation Tools, described on page 23.

The tools on the Cut and Capture tab are:

- Free-Form Cut and Capture Region
- Circular Cut and Capture Region

These tools are described briefly below.

Free-Form Cut and Capture Region – Use this tool to draw a free form region to be captured.

Circular Cut and Capture Region – Use this tool to draw a circular region to be captured.

After you have marked cells with either of these tools, the application will display the outline of the region and the capture laser spots on the live video image.

LCM Dissection Tools

These tools allow you to mark cells and smaller regions for capture.

To capture larger regions more quickly, use the Cut and Capture Tools, described above.

If you wish to ablate an area around a region to be captured, use the Ablation Tools, described below.

The following tools are available on the LCM tab:

- Single Point Dissection
- Dissection Line
- Dissection Region
- Dissection Exclusion Region

These tools are described briefly below.

Single Dissection Point – Use this tool to target individual cells for capture.

Dissection Line – Use this tool to draw a line on the image to capture a layer or line of cells, for example, to capture an epithelial cell layer.

Dissection Region – Use this tool to draw a region on the image to identify an area to be captured.

Dissection Exclusion Region – Use this tool to deselect a specific area that you don't want to capture within a previously marked region. This tool is only enabled when you have a dissection region selected.

After you have marked cells with these tools, the application will show the marked regions as spots on the live video image, using the spot size of the capture laser.

Ablation Tools

If you have a cutting laser in your Veritas™ instrument (Models 703 and 704), the following tools are available and allow you to mark regions of tissue to be ablated.

To capture larger regions quickly, use the Cut and Capture Tools, described on page 22.

To capture single cells or smaller regions, use the LCM Dissection Tools, described on page 22.

The tools on this Ablate tab are:

- Free-Form Ablation Region
- Ablation Exclusion Region

These tools are described briefly below.

Free-Form Ablation Region – Use this tool to draw a region to be ablated.

Ablation Exclusion Region – Use this tool to draw a region that is excluded from a region to be ablated. This tool is only enabled when a free-form ablation region is selected.

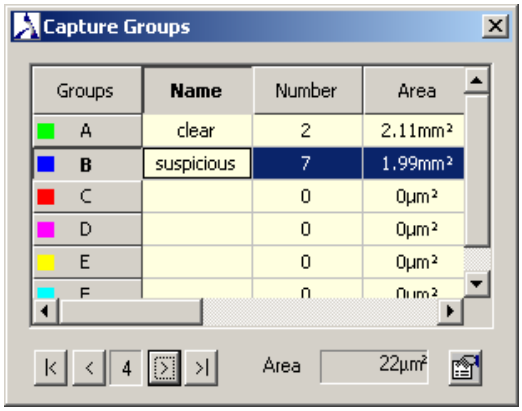
After you have marked cells with these tools, the application will display the regions to be ablated as a solid area in the color for the current capture group.

Creating Additional Capture Groups

You may wish to designate groups of tissue to be captured together. For instance, you may have two different types of cells on one slide, both of which you are interested in assaying. You can create different groups, one for each type of cell. When you capture, you can capture the first group on one cap and the second group on another cap.

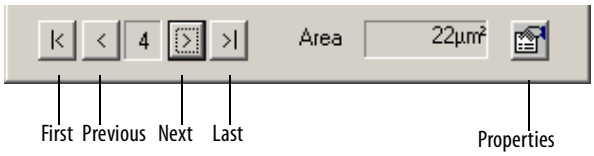
By default, all marked cells belong to the first capture group.

You can designate a total of eight capture groups, as follows:



1. In the Capture Groups Tool, click on the row for the new group.
2. If desired, type the name of the group in the **Name** cell in the selected row.
3. Mark the cells for capture with any of the Microdissection Tools, as described above.
4. You may set different properties for each capture group, such as the color that the capture group will be displayed on screen or the cut width of the cutting laser. See "Adjusting Capture Group Properties" on page 74 for details.

Capturing Cells



1. If desired, review the items in each capture group. Click the row for the capture group of interest, then click buttons at the bottom of the Capture Groups Tool to move through the items in that capture group. The stage will move and the live video image will update as needed to display each item in the capture group. The area of the currently selected item is shown in the **Area** field.

2. Click the **Go Capture** button. The instrument does the following:
   - Performs all cuts and/or any ablation.

   If the 2x objective is selected when you click **Go Capture**, the instrument switches to the 10x objective and auto-focuses before initiating any cuts. Once cell capture is complete, the instrument switches back to the 2x objective.

   NOTE: The cut may be too narrow to be visible at 2x. To inspect the cut, switch to a higher objective.

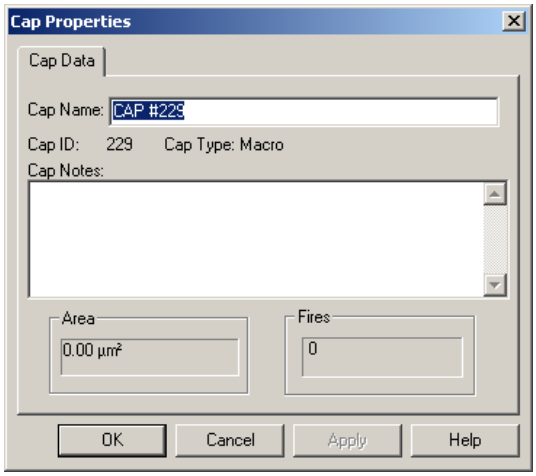
   Depending upon the type of slide you have loaded, the details of the cutting vary.

   - *For glass slides* – The instrument cuts a moat around the region of interest. The width of the moat is set in the **Cut Width** field in the Cut Properties tab of the Capture

Group Properties dialog (see "Adjusting Capture Group Properties" on page 74).

- *For membrane slides (including membrane frame slides)* – The instrument cuts around the region of interest, leaving tabs. Tabs are short stretches where the cutting laser will not cut. Tabs keep the tissue from curling up from the surface of the slide before the capture laser can fuse it to the cap. The number of tabs, their size and spacing are set in the Cut Properties tab of the Capture Group Properties dialog (see "Adjusting Capture Group Properties" on page 74).

3. When the cutting laser has completed cutting, the instrument places a cap over the slide and the Cap Properties window appears.



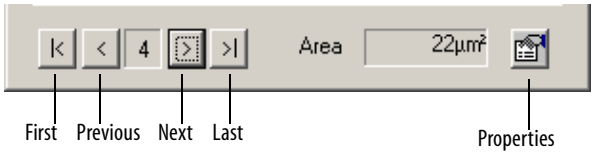
4. In the Cap Properties window, enter a name for the cap (or use the default name) and any notes you wish to save with the cap data. Click **OK**.
An outline of the cap appears on the roadmap indicating its location.

5. Once the cap has been placed, the instrument continues with the capture process, and does the following:

- Locates the capture laser and focuses it.
  See "Manually Locating the Capture Laser" on page 50 and "Manually Focusing the Capture Laser Beam" on page 49, if necessary.
- Fires the capture laser at the position of each capture laser spot, to fuse the cells to the cap.

Inspecting Captured Material

Following cell capture, you should inspect the slide and the cap to verify that the collection was successful. To view the cap, you should move it to the QC station.



1. Review each item in the capture group on the slide to verify it was completely captured, using the buttons at the bottom of the Capture Groups Tool.

2. If cutting or capture was incomplete, you may cut and/or capture again. See page 64 for instructions on how to repeat cutting and capture.

3. Right-click the cap on the active slide and choose **Move To**, then select **QC**.

– or –

Click and drag the cap from the active slide to the QC station.

NOTE: To automatically move the cap from the slide to the QC station at the completion of a capture or after Auto Scan,

select the **Move Cap To** setting in User Preferences (see "Setting User Preferences" on page 36).

When the cap has been moved to the QC station, the application will display the cap in the live video window, where you may inspect the captured tissue.

4. If you have a cutting laser, you may ablate any unwanted tissue from the material on the cap. There are two modes for ablating:

- Click ALT+X to turn on the cutting laser, and use the mouse to move the region you wish to ablate under the laser spot. When you are finished, click ALT+X to turn off the cutting laser.
- Hold down the CONTROL and SHIFT keys at the same time. Move the mouse to move the area of tissue under the laser spot. The cutting laser fires only while the keys are depressed. When you release the keys, the laser turns off.

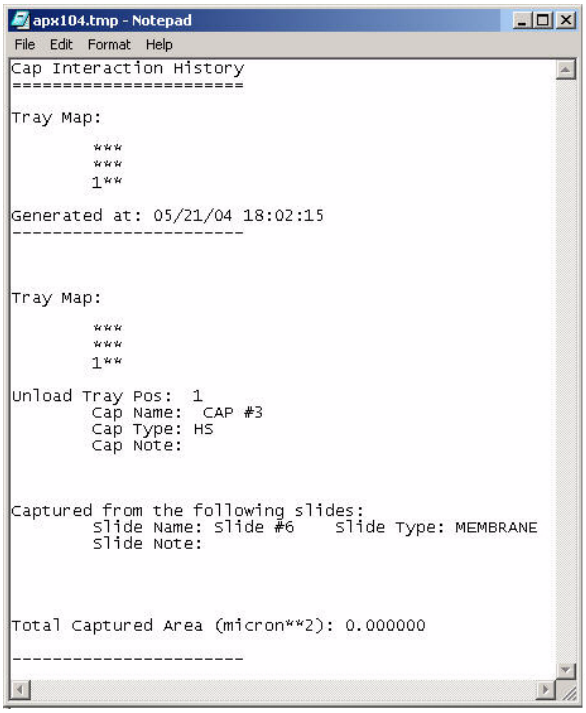
Unloading and Removing Caps

1. Move the cap to the unload tray in one of the following ways: Click and drag the cap from the QC station (or the slide, if you did not move it to the QC station) to any position in the unload tray.

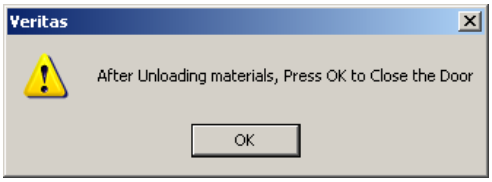
– or –

Right-click the cap in the QC station and choose **Move To**, then select **Unload**. The cap is placed in the next available location on the unload tray.

2. Click **Open Door** in the Materials Tool. The instrument door opens and the work surface slides out.
The Cap Interaction History appears on the screen, in Notepad. (If you have not placed a cap, the Cap Interaction History is not shown.)



3. Close Notepad, saving the history if desired. The unload materials message appears.



4. Slide the cap insertion tool onto the unload tray. Make sure the open end of the insertion tool faces the suspended cap and slides over the empty cap receptacles.

5. Slide the insertion tool towards the cap until the cap is engaged.

6. Remove the insertion tool from the unload tray. The cap should be attached to it.

   NOTE: Be sure you do not touch the polymer surface that holds the captured cells as you remove the caps from the unload tray.

7. Repeat steps 4., 5., and 6. for each cap in the unload tray.

8. Click **OK** to close the instrument door.

9. If you only have one capture group, log out and exit the application as described on page 31, under "Shutdown".

Capturing Additional Capture Groups

If you assigned dissection marks to more than one capture group in the Capture Groups Tool, you can capture the next group.

1. In the Capture Groups tool, click on the row corresponding to the group you wish to capture next.

2. Click **Go Capture**.
   The instrument will place a cap and display the Cap Properties window for the cap.

3. Enter any information in the window and click **OK.**

4. The instrument will proceed to cut and capture the items in this capture group.

5. Inspect the captured material, as described previously on page 25.

6. Repeat for each capture group.

Extracting Captured Tissue from the Caps

Extraction Kits Available from Arcturus

Arcturus offers the PicoPure™ RNA Isolation Kit (cat.# KIT0202) and the DNA Extraction Kit (cat.# KIT0103) specifically designed to work with the CapSure™ LCM Sample Preparation System. These kits provide detailed step-by-step protocols for extracting DNA and RNA from frozen cells. The Paradise™ Reagent System, also from Arcturus, is designed specifically for extracting DNA or RNA from formalin-fixed paraffin-embedded tissue.

Extracting from CapSure HS Caps

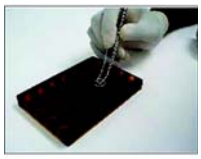
Following LCM, place the ExtracSure Extraction Devices onto the CapSure HS Caps containing the microdissected cells. The ExtracSure Device seals around the perimeter of the cap surface and covers the circular ridge that was in contact with the sample during LCM. With the ExtracSure Device you can incubate the captured cells in a small volume of extraction buffer.

NOTE: Due to the way the ExtracSure device fits onto the CapSure HS Cap, LCM captures should be made in the center of the CapSure HS Cap, within the black capture ring.

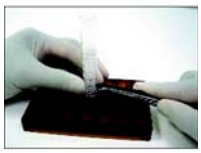
Detailed steps are listed below.

1. Use clean tweezers to remove the cap from the cap insertion tool and place the cap with the sample facing up into the Alignment Tray. Make sure the cap is properly seated in the Alignment Tray following the directions in the CapSure HS manual.

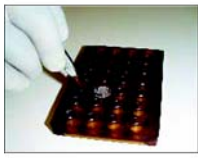
2. Use clean tweezers to remove and position the ExtracSure Device over the cap. The fill port on the ExtracSure Device should be facing up.



3. Use tweezers to firmly push down the ExtracSure Device onto the cap. The ExtracSure Device should fit securely into place.
   At this point, the ExtracSure Device should be firmly sealed to the CapSure HS Cap.



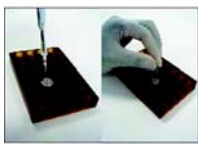
4. You may add extraction buffer to the device. Do not remove the assembled ExtracSure/CapSure HS Device from the alignment try until incubation is completed.



5. After adding the buffer, place a 0.5 mL microcentrifuge tube over the fill port and allow the samples to incubate as described in the appropriate extraction procedure.

Extracting from CapSure Macro Caps

Following LCM, you can place CapSure Macro Caps directly onto a 0.5 mL microcentrifuge tube containing extraction buffer. For best results, Arcturus recommends the use of MicroAmp® Autoclaved Thin-Walled Reaction tubes, available from Applied Biosystems (part number N8010611).



1. Place at least 40 µL of extraction buffer into a 0.5 mL microcentrifuge tube, or if using a PicoPure™ RNA Isolation Kit or the DNA Extraction Kit, follow the instructions in the user's guide.

   NOTE: Less than 40 µL of buffer may not provide enough volume to cover the surface of the CapSure Macro Cap.

2. Use the CapSure Insertion Tool to insert the CapSure Macro Cap into the microcentrifuge tube.

3. Press down firmly and rotate the insertion tool to ensure the Macro Cap is tightly and evenly sealed with the microcentrifuge tube.

4. Invert the tube so that all the extraction buffer comes in contact with the captured cells on the cap surface.

5. Incubate the sample as described in the appropriate extraction procedure, then place the tube into a microcentrifuge and briefly spin to bring the buffer to the bottom of the tube.

3. System Overview

This section gives you a brief description of the Veritas™ application. Also included are user and study information, as well as descriptions of the key components of the system — caps and slides, and the images created for each.

Shutdown

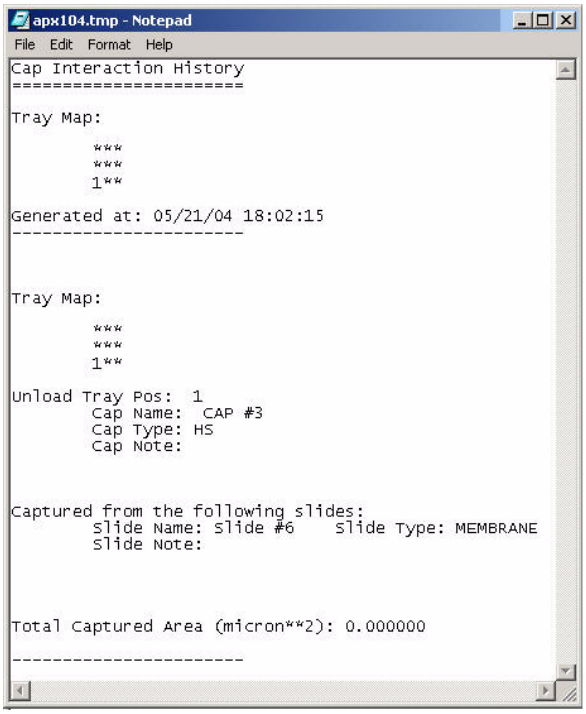
1. When you have finished the microdissection session, you can:

- Choose **Log Out** from the User menu. This will allow a new user to log on and start a new session.
- Choose **Exit** from the File menu. This will close the application.

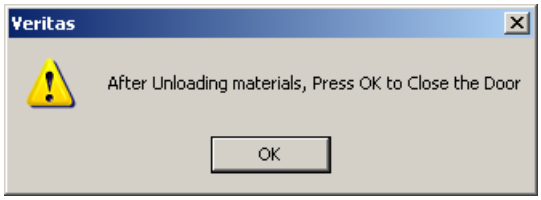
Whether you choose **Log Out** or **Exit**, the instrument automatically moves the active cap to the unload tray, opens the instrument door and slides the work surface out.

The Cap Interaction History appears, opened in Notepad, where you may save it or print it. This file shows the location of each cap in the unload tray. For each cap, the cap name, total area captured and its location are also shown.

NOTE: If you did not place a cap, the Cap Interaction History is not shown.



2. Close Notepad, saving the history if desired. The unload materials message appears.



3. Remove all materials from the work surface and then click **OK** in the message.
The work surface will slide back in and the instrument door will close.

The application automatically saves all images generated this session.

If you selected **Log Out**, the application will open the System Login dialog so another user may log in.

Application Window

When you log in to start a new session and enter the appropriate information in the Material Loading window, the application window appears. The Veritas™ application allows you to control the entire microdissection procedure using the menus, tools and toolbars in the application window.

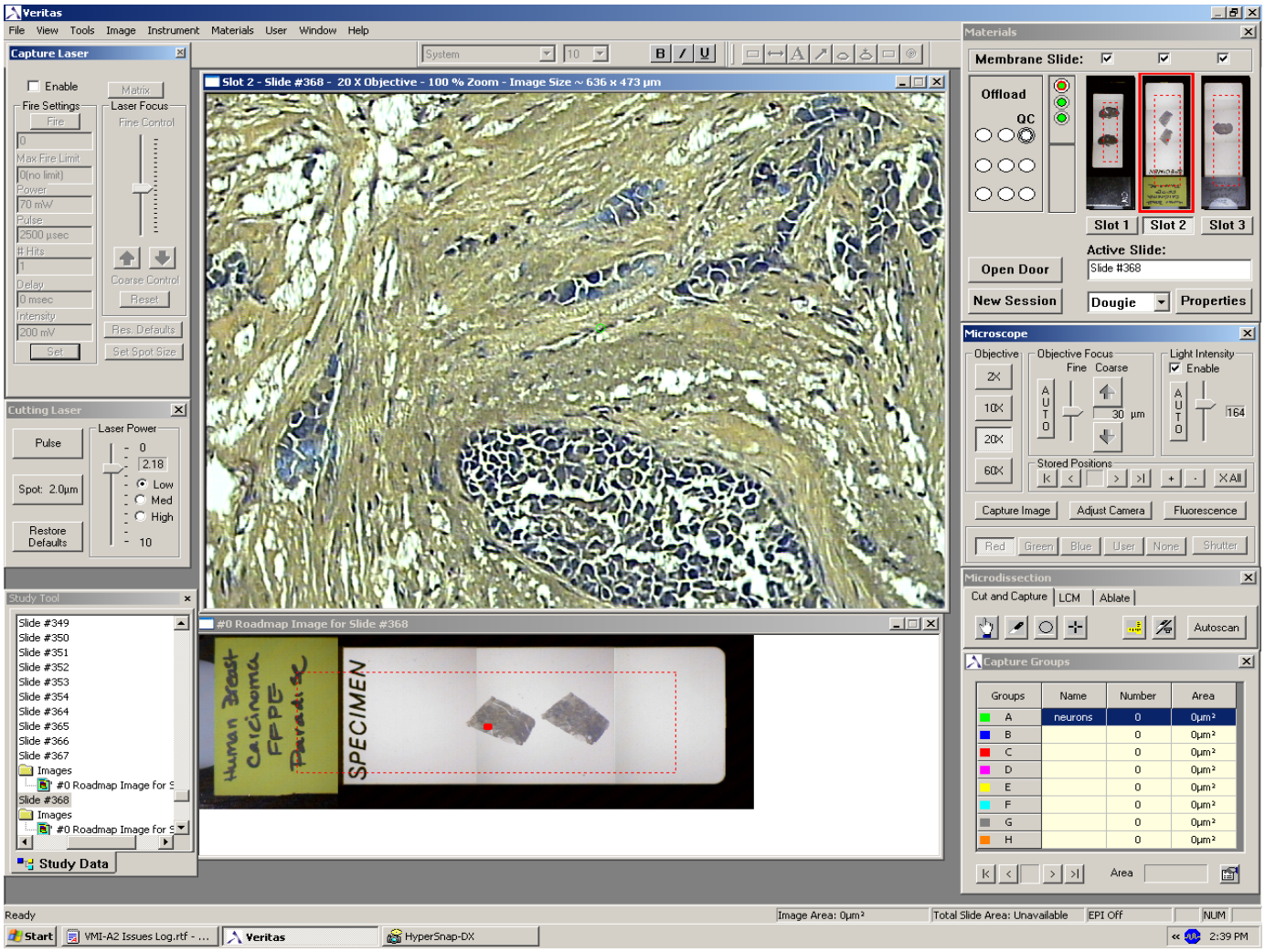


Figure 3-1. Veritas application window

Roadmap, Live Video and Static Images

There are three types of images in the Veritas application:

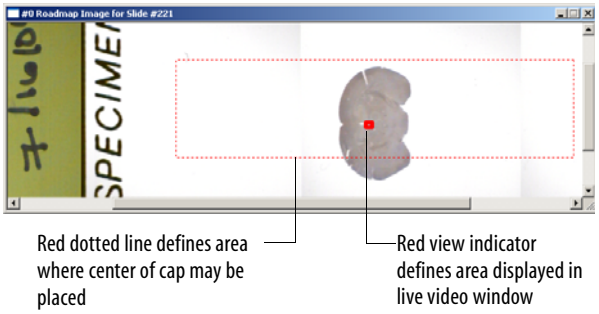
- Roadmaps
- The live video image
- Static images

Once you have finished loading materials, the system acquires a roadmap image for each slide and displays the roadmap and the live video window. A roadmap is a high-resolution scanned image of the slide. A roadmap provides a full working view of the slide and allows you to navigate around the slide. Caps may be centered on any position within the area defined by the red dotted rectangle.

The region of the slide shown in the live video corresponds to the area of the roadmap outlined by the red view indicator, a red rectangle. When the microscope stage in the instrument moves, the live video updates to show the tissue in the new location.

A static image is an image created from the live video image to reflect a particular point in time. It does not update when the stage is moved.

See "Working with Images" on page 43 for more information about working with each type of image.



Making Software Selections

There are several ways to make selections and access information within the application:

- You can right-click to open a context menu.
  Right-click on a window, the application background or a selection in a window and choose the menu item of choice.
- You can drag and drop.
  For instance, you may drag and drop caps to and from slides in the Materials Tool, or ALT-click and drag the red view indicator in the roadmap or the live video window to display a different area of the slide.
- You can choose options from pull-down menus.
  Use the menu bar at the top of the application window to make selections. For descriptions of items found in the menu bar, refer to "Software Menus" on page 63.
- You can click buttons, check boxes and radio buttons and/or click and drag other software controls.
  Dialog boxes and tools have buttons and other software controls, such as check boxes, radio buttons and sliders.

System Users

The Veritas™ Microdissection Instrument allows two types of users to access the application: users and administrators.

Users can create and access their own study data. Users may also change their own passwords.

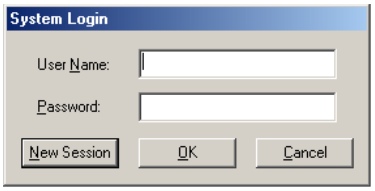
Administrators can create and access their own study data. Additionally, administrators can add users, delete users and edit user properties.

When you first log onto the system, you can log in using *administrator* as the default user name with no password. As an administrator you can then add your own user name and password and assign yourself administrator access, then add other users. Administrators cannot access study data of other users.

Logging In

All study-related menu options are enabled when you log in. The System Login dialog box appears automatically when you start the application or when you log out.

To log in, enter your user name and password, then do one of the following:



- Click **OK**.

  – or –
- Click **New Session** to display the Material Loading window and begin the session.

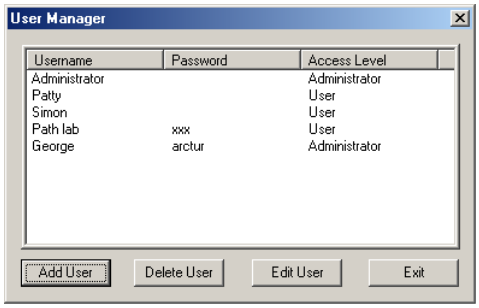
Logging Out

To log out, choose **Log Out** from the User menu. The application will open the instrument door, slide the work surface out and remind you to unload the instrument. Finally, the System Login dialog box appears, allowing another user to log in.

Managing System Users

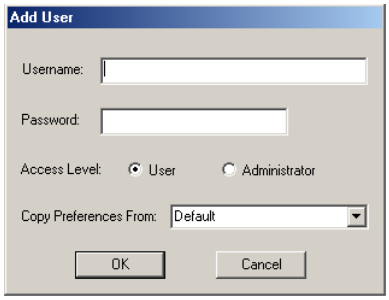
You must be an administrator to manage (add, edit, or delete) a system user.

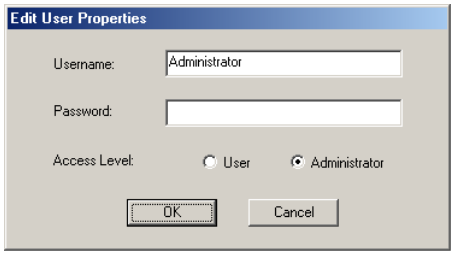
1. Choose **User Manager** from the User menu.
The User Manager window appears with a list of all users currently in the system. Scroll through the list to see all users. You may sort the list by clicking a column heading.



2. To add, edit, or delete a user, do the following:

- *To add a user,* click **Add User**. The Add User dialog box appears. Enter a user name and password. Assign an access level: **User** or **Administrator**. Copy user preferences from an existing user or select the default preferences. (See page 36 for information on user preferences.) Click **OK** to save the changes.
- *To delete a user,* select the user from the User Manager list and click **Delete User**.
- *To edit user information,* select the user from the User Manager list and click **Edit User**. The Edit User Properties dialog box appears. Make the appropriate changes to the user information. You may change the user name, password and/or access level. Click **OK** to save the changes.





3. Click **Exit** to save the changes and close the User Manager window**.**

Changing Your Password

All users can change their own passwords.

1. Choose **Change Password** from the User menu.
The Change Password dialog box appears.

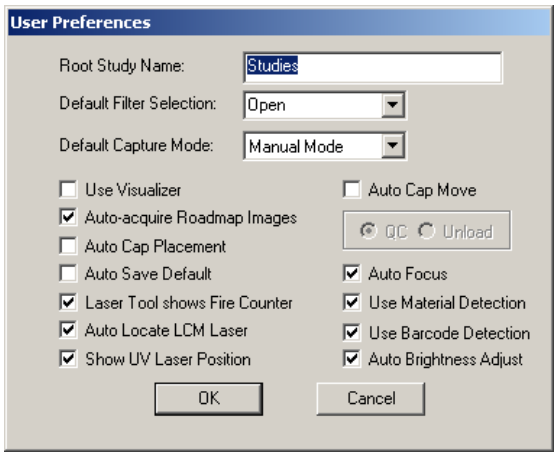
2. Enter your new password, then enter it again to verify it.
The application assumes that the person logged in is the person changing the password.

3. Click **OK**.

Setting User Preferences

The User Preferences dialog allows you to set the study parameters to settings you frequently use. The first time you log in, the user preferences default to the settings the administrator selected for you when you were added to the system.

Some options do not apply to all instrument models. Options which do not apply to your instrument are disabled (shown in gray) and cannot be selected.



1. Select **Preferences** from the User menu.

2. Enter the necessary information and change the default values according to your study needs.
   You may set unique preferences for each study you perform. You may also change these settings during your LCM session.

   Root Study Name – If this is your first study, enter a root study name. All subsequent study sessions that are created will be contained within the root study. You may change this name at any time in the future by entering a new name.

   Default Filter Selection – If you typically view samples using white light, select **Open** (no filter). If you typically view fluorescent samples, select the appropriate filter as the default.

   Default Capture Mode – Choose between **Manual Mode** and **Assisted Mode** (**Assisted Mode** is the same as **Semi-Automatic Mode**). Arcturus recommends leaving **Manual Mode** as the default.

   Use Visualizer – The visualizer is a diffuser that can be placed above the sample to increase the resolution and decrease the contrast of the live video image. When this is checked, the visualizer is on. The visualizer and the capture laser do not operate simultaneously. When you enable the capture laser, the visualizer is automatically removed from the light path.

   Auto-acquire Roadmap Images – Choose this setting to automatically acquire a roadmap image for every slide you add to a study. Each slide is scanned for the roadmap image when you click **OK** in the Material Loading window at the start of a session.

   Auto Cap Placement – Selecting this option tells the system to automatically move a cap to pick up areas targeted for capture that are outside the area under the current cap placement, or that are under portions of the cap that have already been used for capture. If this option is not selected, the system will not reposition the cap so regions outside the capture area of the cap will not be collected.

Auto Save Default – Choose this option to automatically save the current values for the Laser and Microscope Tools as the defaults for your next session. If you choose to do this, the settings will overwrite the current defaults. See "Customizing Settings for the Laser and Microscope Tools" on page 85 for more information.

NOTE: If you are using the SystemDefault settings and you are not an administrator, an error message will notify you that the settings cannot be saved.

Laser Tool shows Fire Counter – Select this option to have the Capture Laser Tools display the number of times the laser will fire to capture all selected regions of interest. If this is not checked, the system will display the total area of the regions selected.

Auto Locate LCM Laser – When this is checked, the instrument automatically locates the capture laser without requiring any user intervention. If the instrument can't locate the laser, the application displays a dialog box informing you that you must locate it manually. Locating the capture laser is described on page 50.

Show UV Laser Position – When this is checked, the instrument automatically displays the location of the cutting laser in the live video window. The position is shown as a green circular outline. If you do not have a cutting laser in your instrument (Veritas™ Microdissection Instrument Models 701 and 702 and the AutoPix 100e), this option is disabled.

Auto Cap Move – If you wish to have the cap automatically moved from the active slide after capturing selected cells, check this option and select either **QC** or **Unload** as the location where the cap will be placed. Typically, Arcturus does not recommend using this option.

Auto Focus – When this is checked, the instrument will automatically attempt to focus the microscope for each objective, the first time you choose the objective for a slide in a given session.

Use Material Detection – When this is checked, the application automatically detects the number and types of slides and caps when they are loaded (the Materials Loading window does not appear at the beginning of a session). If you need to change the properties of a detected slide or cap, right-click and choose **Properties**. This option is disabled for the AutoPix 100e.

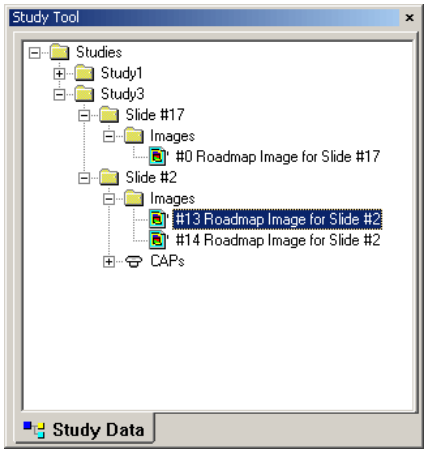
Use Barcode Detection – When this is checked, the application automatically detects a barcode on a slide, if present. The barcode becomes the name of the slide in the

Materials Tool. If a slide with the same barcode has already been read, the slide name will be appended with a number.

Auto Brightness Adjust – When this is checked, the application automatically adjusts the brightness of the image by changing the shutter speed and lamp intensity until the brightness is optimized.

Study Information

A study contains all of the information entered for the slide and cap properties, as well as the corresponding images. You can only see the study data that you created, irrespective of your user type. The name of the study at the top level is entered in the **Root Study Name** in the User Preferences.



The Study Tool window allows you to see study data and access slides, caps and images.

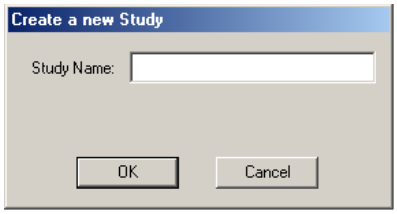
- *To open and view an image (roadmap or static)*, right-click the image and select **View** or simply double-click the image icon.
- *To delete an image*, right-click the image and select **Delete**.
- *To view the properties of a slide, cap or image*, right-click the item and select **Properties**. Properties include names and notes pertaining to the slide, cap or image.

There are other functions available for items in the Study Tool window:

- You can move a slide from one study to another; see "Moving a Slide to Another Study" on page 87.
- You can copy a slide from one study to another; see "Copying a Slide to Another Study" on page 87.
- You can archive data from a study for safekeeping; see "Archiving Study Data" on page 88.
- You can also restore archived data to a study; see "Restoring Archived Study Data" on page 88.
- You can rename a study or any item contained in a study; see "The Study Tool" on page 87.

Creating a Study

When you create a study you are the owner of that study. Only you can access the data.



1. Choose **New Study** from the File menu.
   The Create a new Study dialog box appears.
2. Enter a name for the study.
3. Click **OK**.

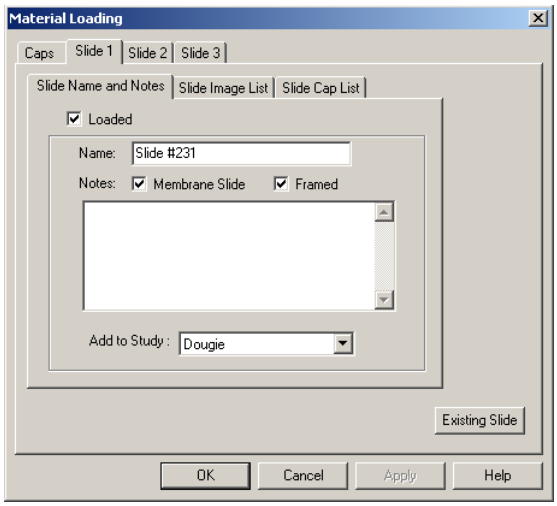
NOTE: You can also create a study at the beginning of a microdissection session by entering a new study name in the Material Loading window.

Slides

Adding a Slide to a Study

The Material Loading window appears at the beginning of each session. Click the Slide tab to enter information about the current slide. You can enter a name for the slide, select the study you wish to assign the slide to and enter any notes about the slide. You can also indicate the type of slide and whether it is a frame slide.

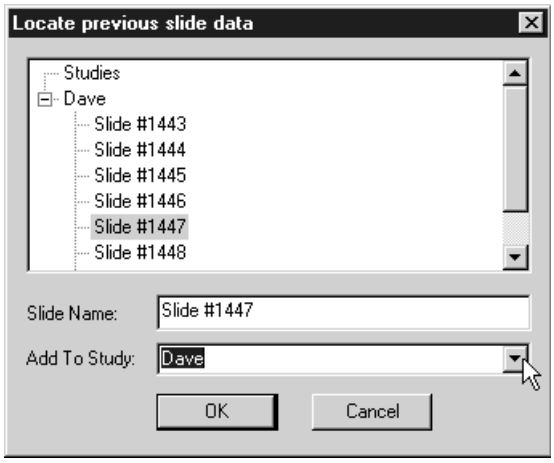
For more information on using the Material Loading window, refer to "Entering Information in the Materials Loading Window" on page 67.



Using a Slide from a Previous Study

If you would like to work with a slide from a previous study, click **Existing Slide**. A dialog box appears allowing you to select the study. Click **OK** after selecting the study.

When you use a slide from an existing study, the existing roadmap will be replaced with a new roadmap only if you save the slide to the same study. You can use the Study Tool to access any image in the study to which the image was saved.



Activating a Slide

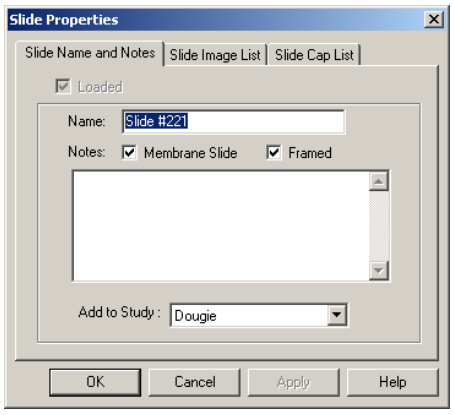
Activating a slide moves it over the objective so the camera can capture a live video image. The roadmap for the activated slide moves to the front and the active slide is outlined in red in the Materials Tool.

To activate a slide, do one of the following:

- Choose **Activate Slide** from the Materials menu, then select 1, 2 or 3.
- Double-click on the slide in the Materials Tool
- Right-click it and choose **Activate Slide**.
- Click **Slot X** in the Materials Tool, where "X" corresponds to the slide of interest.

Slide Properties

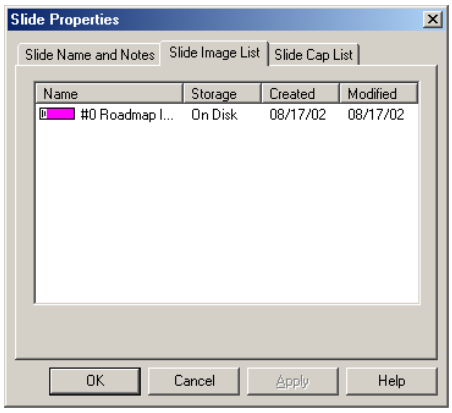
The Slide Properties window contains information about the active slide. If you are not using automatic materials detection, this is the same information you entered in the Slide tabs of the Material Loading window when you started the session.



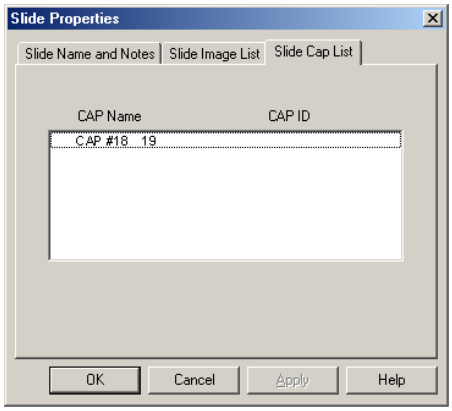
- *To access this window,* select **Active Slide Properties** from the Materials menu.

  – or –

  Right-click on a slide in the Materials Tool and select **Slide Properties**.



- *To see a list of all images that have been saved with the selected slide,* click the Slide Image List tab.



- *To see a list of all caps associated with the selected slide,* click the Slide Cap List tab.
- *To make any changes to the slide properties,* make the changes and click **OK** to dismiss the window.

Caps

Keep in mind the following rules when moving caps during a session:

- You can only have one active cap at a time. The active cap has a red circle in the Materials Tool.
- You can only place a cap such that the cap's center is within the region defined by the dotted red line on the roadmap.
- **Auto Cap Placement**, which is activated in the User Preferences (see page 36), is deactivated for the cap if the cap is moved to the QC station then placed back on a slide.

Placing a Cap on a Slide

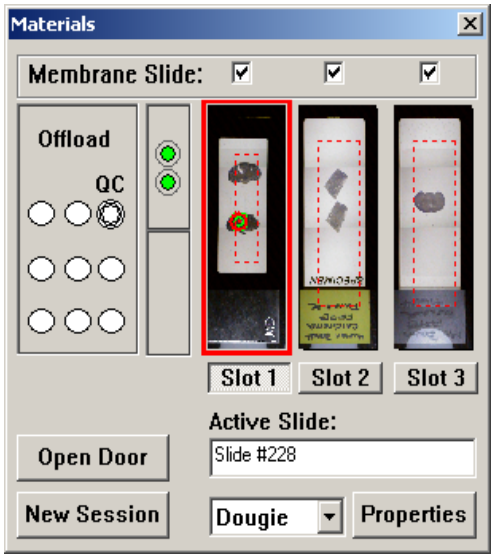
The **Go Capture** tool in the Microdissection Tools automatically places a cap as the first step in the cut and capture process.

To manually place a cap on a slide, do one of the following:

- Right-click the live video and choose **Place Cap at Region Center**.
- In the Materials Tool, right-click the active cap and choose **Move To**, then select **Slot 1, 2** or **3**.
- In the Materials Tool, click and drag the active cap to the slide. The cap cannot be placed outside the rectangle defined by the red dotted line.
- Choose **Place Cap at Region Center** from the Materials menu.

The cap will be placed on the slide at the location of the live video image. The Capture Laser Tools are automatically displayed when a cap is placed (unless the tools were previously closed).

The Cap Properties window appears where you can enter information about the cap (see "Cap Properties" on page 43).

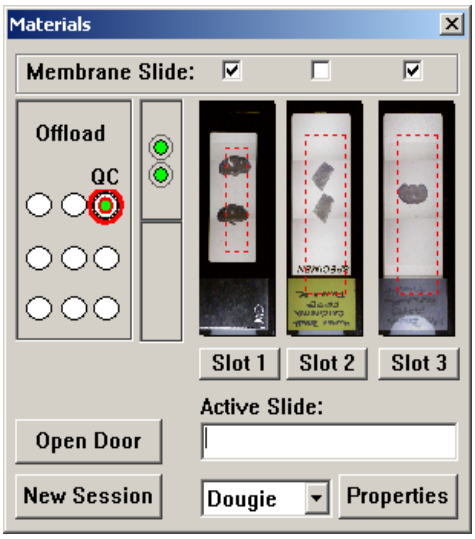


Moving a Cap to the QC Station

To move the cap to the QC station after capture is complete, do one of the following:

- Right-click the live video and choose **Move To**, then select **QC**.
- In the Materials Tool, right-click the cap on the active slide and choose **Move To**, then select **QC**.
- In the Materials Tool, click and drag the cap from the slide to the QC station.
- Choose **Move Cap To** from the Materials menu, then select **QC**.

When the cap has been moved to the QC station, the application will display the cap in the live video window, where you may inspect the captured tissue and, if you have a cutting laser (Models 703 and 704), ablate undesired tissue from the cap.



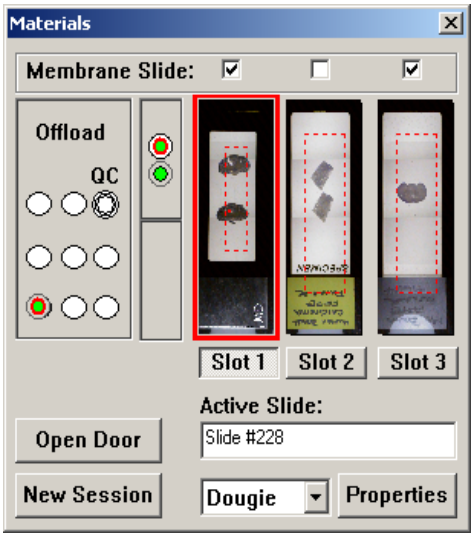
Moving the Cap to the Unload Tray

You can move the cap from the QC station or from the active slide to the unload tray.

To move a cap to the unload tray, do one of the following:

- If the cap is on the active slide or in the QC station, right-click the live video and choose **Move To**, then select **Unload**.
- In the Materials Tool, right-click the cap on the active slide or QC station and choose **Move To**, then select **Unload**.
- In the Materials Tool, click and drag the cap from the slide or QC station to the unload tray.
- Choose **Move Cap To** from the Materials menu, then select **Unload**.

If a cap is left on a slide or in the QC station when you log out or exit the application, the instrument will automatically move the cap to the unload tray.

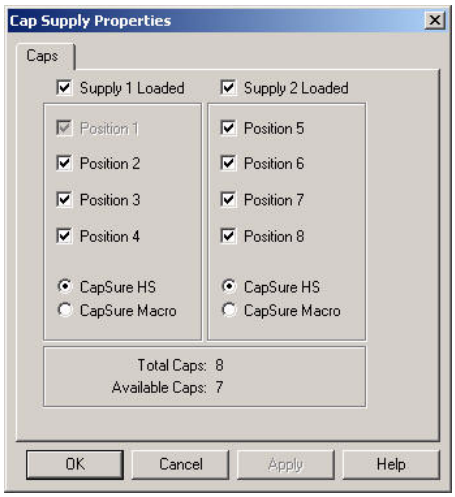


Cap Supply Properties

The Cap Supply Properties window contains information about the loaded cap cassettes.

To access this window, right-click on the Materials Tool and select **Cap Supply Properties**.

If any information is incorrect, you may change it. Click **OK** to make the changes.



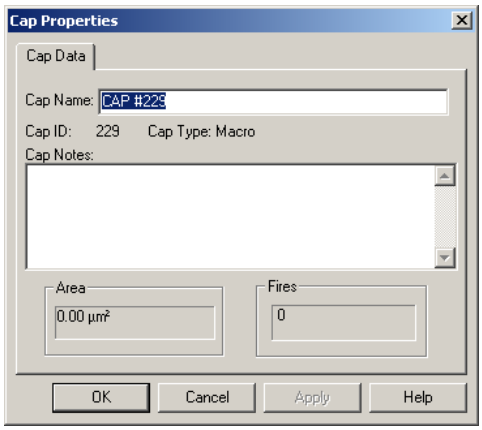
Cap Properties

The Cap Properties window contains information about the selected cap.

To access this window, choose **Active Cap Properties** from the Materials menu, or right-click on any cap and select **Cap Properties**.

In addition to displaying the name and any notes entered when the cap was placed on the slide, the Cap Properties window displays the total area and total number of laser fires for all regions captured on a cap.

You may rename the cap by editing the **Cap Name** field.



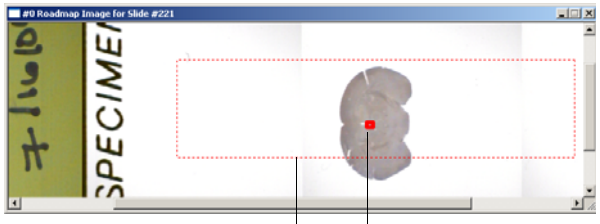
Working with Images

The Roadmap Image

A roadmap is a series of tiled static images that the system creates after scanning a slide. A roadmap provides a full working view of the slide and allows you to select an region of interest from which to capture. A roadmap image is created for each slide in the current session.

If you selected an existing slide from a previous study, the new roadmap acquired for the current session will overwrite the roadmap image from the previous study. Any images saved for the existing slide will be saved to the previous study. If you acquire a roadmap image that is not optimal, you can adjust the light intensity and reacquire the image. The last roadmap image acquired will be saved.

- *To automatically acquire a roadmap for each new slide*, choose **Auto-acquire Roadmap Images** in your user preferences, as described on page 36.



- *To manually acquire a roadmap image,* right-click on the slide in the Materials Tool and select **Acquire Roadmap Image**.

  – or –

  Activate the slide and choose **Reacquire Roadmap Image** from the Image menu.

- *To zoom in on a roadmap*, right-click the roadmap and choose **Zoom**, then select the desired zoom setting.

  – or –

  Click on the roadmap image to make it active and select **Zoom** from the Image menu.

The Live Video Image

The live video image displays the area of the slide that corresponds to the area in the roadmap bounded by the red view indicator, a red rectangle.

You cannot resize the live video window, but you can minimize it. You can also close it.

- *To reopen the live video window,* select **Activate Live Video** from the View menu.
- *To display a different area of the slide in the live video image*, you must move the stage. You can move the stage from the roadmap, the live video image or the Materials Tool, as described below. In all cases, the red view indicators in the roadmap and the Materials Tool move as needed to indicate the area in the live video.

  NOTE: The red view indicators on the roadmap and the Materials Tool only approximate the area shown in the live video.

- *To zoom in on the live video*, right-click the live video and choose **Zoom**, then select the desired zoom setting.

  – or –

  Click on the live video window to make it active and select **Zoom** from the Image menu.

Moving the Stage from the Live Video Window

There are several methods to move the stage to display a new region of the slide in the live video window. (The actual movement will vary based on the objective currently in use.)

To move the stage from the live video image window, do one of the following:

- By default, when the live video window is active, the cursor changes to a hand. Click and drag to move the stage.
- Press the UP, DOWN, LEFT and RIGHT arrow keys to move the stage.
- Hold down the CONTROL key while pressing an arrow key to move one half of the field of view at a time.

- Use the Navigation tool, as described on page 80.

Moving the Stage from the Roadmap Window or the Materials Tool
You can also move the stage from the roadmap or the Materials Tool and thus update the live video. As before, the red view indicators in move as needed to indicate the current area shown in the live video.

To move the stage from the roadmap window or the Materials Tool, do one of the following:

- Double-click on the position of interest.
- ALT-click on the red view indicator to select it, and then drag it to a new area of the slide.
- Press the UP, DOWN, LEFT and RIGHT arrow keys to move the stage.
- Hold down the CONTROL key while pressing an arrow key to move one half of the field of view at a time.

Static Images

A static image is a picture of the live video image at the current magnification. You can mark the areas of interest that you wish to capture on a static image. You can also use static images to create a training file for the Auto Scan feature.

Acquiring a Static Image
There are several ways of acquiring a static image:

- *To acquire a static image that shows the same area as the live video image*, right-click the live video and select **Acquire Static Image**.

  – or –

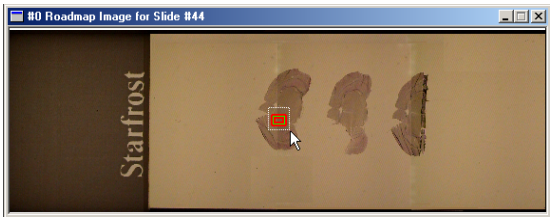
  Select **Acquire this Region as New Image** from the Image menu.

  – or –

  Click the **Capture Image** button in the Microscope Tools.

- *To acquire a static image for a region of any size*, zoom in on the roadmap as necessary, select the Region of Interest tool from the Static Annotations Toolbar, and then click and drag to draw a rectangle on the roadmap. Double-click to turn off the Region of Interest tool, then right-click the region and select **Acquire Region**.
  The system creates a series of tiled images. The advantage of a tiled image is that you have a much larger area from which to capture.

  NOTE: The size of the region you can draw is limited by the computer's memory. The larger the region, the more memory required to generate a static image. Additionally, for a specific selected area, the higher the magnification (i.e. the larger the objective), the more memory required to generate



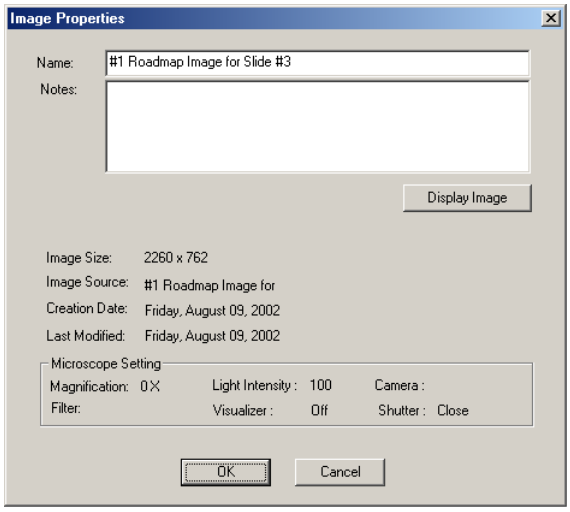
Acquiring a static image using the Region of Interest drawing tool.

an image. If you have trouble generating a static image due to memory limitations, either reduce the size of the area you are drawing or use a lower magnification.

- *To acquire a static image that covers the area of the entire cap*, right-click the live video and select **Acquire Static Image of full Cap Area**. The system creates a series of tiled images representing the area within the cap. This option is only available at 2x.

Image Properties

The **Image Properties** window contains information about the image, including the source of the image, the date it was created and the microscope settings that were used when the image was saved.



1. To open the Image Properties window, choose **Image Properties** from the Image menu.

   – or –

   Right-click the image in the Study Tool and select **Properties**.

2. You can change the name of the image and enter notes.

3. Click **Display Image** to view the image.

4. Click **OK** to close the window and save your changes.

Saving an Image

The application automatically saves any images generated this session. You can also manually save any image. Images are saved as JPEGs to the study directory. Images include:

- roadmap image of a slide
- static image of the live video image
- a series of tiled images of an area larger than the live video image
- static image of cap in the QC station, after cells are captured

NOTE: If you wish to save the image to a separate storage device for your own use, export the image (see "Exporting an Image" on page 48).

To manually save an image:

1. Activate an image window.

2. Choose **Save Image** from the Image menu.
   The Image Properties window appears.

3. Enter a name for the image and notes, if you wish.

4. Click **OK**.

Opening an Image

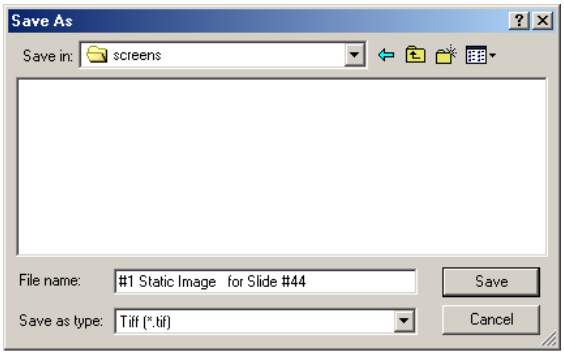
You can open and view any image after a study. Images are accessible from the Study Tool in one of the following ways:

- Locate and double-click the image in the Study Tool.
- Right-click it and select **View**.
- If the Image Properties window is open, click **Display Image**.

Exporting an Image

Any annotations are exported along with the image. Images can be exported in the following file formats.

- TIFF (*.tif)
- JPEG (*.jpg)
- PNG (*.png)
- Windows bitmap (*.bmp)



1. Choose **Export Image** from the Image menu.
   A standard Save As dialog box appears.
2. Choose the location where you wish to save the file.
3. Enter a file name and select the file type to save.
4. Click **Save**.

Deleting an Image

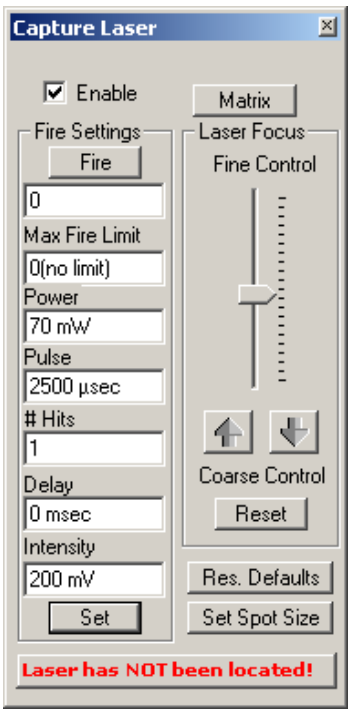
1. Locate the image in the Study Tool.
2. Right-click the image and select **Delete**.
   The image and its associated files are deleted.

4. Additional Procedures for Microdissection

Working with the Capture Laser

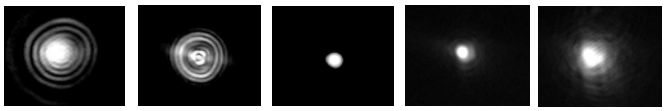
Manually Focusing the Capture Laser Beam

The Veritas™ Microdissection Instrument incorporates an automatic focus lock for the capture laser beam. In most cases, this will be sufficient in order to maintain proper focus. If not, you may manually focus the capture laser as described below.



1. Focus the capture laser beam.

- Ensure **Enable** is selected in the Capture Laser Tools.
- Enter **Power**, **Pulse**, **# Hits**, **Delay** and **Intensity**. The default settings may be sufficient for starting out.
- Move the beam to an area where there is no tissue or the tissue is very thin and light. If you are using a CapSure HS Cap, move to an area outside the black circle.
- Focus the beam by adjusting the coarse and fine settings. The beam spot should be a discrete point. The halo around the spot should be as close as possible to the focused centroid point of the laser.



Unfocused beam Focused beam Unfocused beam

NOTE: If you are having trouble seeing the beam, decrease the lamp intensity using the **Light Intensity** slider in the Microscope Tools. If the laser is difficult to see even at low light intensity, reduce the camera shutter speed to 1/50. See "Camera Control Dialog" on page 68 for instructions on setting the shutter speed.

- Click **Set** to apply the changes.

For detailed information on using the Capture Laser Tools and recommended settings, refer to "The Capture Laser Tools" on page 76.

2. Locate the capture laser beam.

When **Auto Locate LCM Laser** is selected in your User Preferences, the system automatically locates the capture laser. Locating the laser beam establishes a connection between the cursor and the laser beam so that when you click to fire or target the beam, the laser accurately fires on the cells you have selected.

When the capture laser has been captured, the button in the Capture Laser Tools says "Laser has been located" in blue

text and the live video window will show a blue + at the laser's location.

You may also locate the laser manually; see "Manually Locating the Capture Laser" on page 50.

Manually Locating the Capture Laser

The Veritas™ Microdissection Instrument will normally automatically locate the capture laser for you. If not, you may assist the instrument by moving the stage so that a clear spot appears in the center of the live video window and then clicking the button in the Microscope Tool which corresponds to the current objective. The instrument will attempt to locate the capture laser. If it fails to do so (the button at the bottom of the Capture Laser Tools says "Laser has NOT been located"), you may manually locate the capture laser as described below.

In the live video, place the point of the cursor directly at the center of the laser spot. Then, right-click and select **CAPTURE Laser Is Here**.

NOTE: It may be helpful to zoom in on the image –right-click on the image and choose a zoom setting – to more accurately mark the laser location.

When the capture laser has been captured, the button in the Capture Laser Tools says "Laser has been located" in blue text and the live video window will show a blue + at the laser's location.

Test Firing the Capture Laser

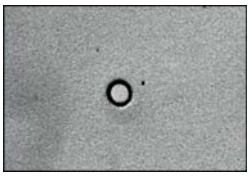
Wetting refers to melting the polymer on the cap so that it fuses adequately to the tissue or cells when the laser fires. Perform this step each time a new cap is placed on a slide.

Until you can confidently recognize proper wetting on tissue, fire on an area outside the tissue where you can see a clear outline.

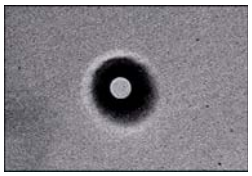
1. Click **Fire** in the Capture Laser Tools to fire the laser. Move the cursor away from the area to examine the wetted spot. When the film is visible as a dark ring fused to the slide and the center of the ring is clear, the wetting is adequate.

2. If wetting is not adequate, adjust the laser parameters. The laser **Power** and **Pulse** settings affect the size of the wetted spot. (For information on adjusting the spot size, refer to "Optimizing the Capture Laser Spot Size" on page 77.)

NOTE: You may also set the laser parameters using a calibration matrix; see "Using a Calibration Matrix to Determine Capture Laser Settings" on page 78.



CapSure Macro Cap

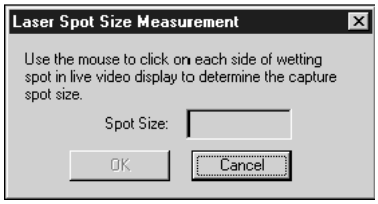


CapSure HS Cap

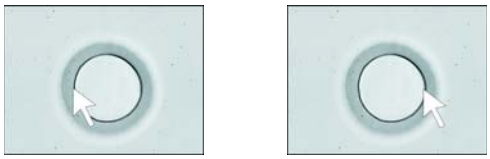
Manually Measuring the Capture Laser Spot Size

The application uses the spot size to control the movement of the stage during capture and to calculate the area of tissue that will be captured.

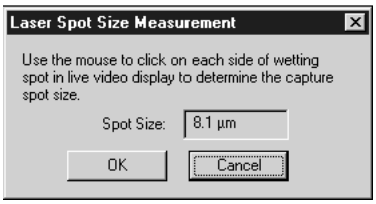
1. To measure the laser spot size, click the **Set Spot Size** button located in the Capture Laser Tools. The Laser Spot Size Measurement dialog box appears.



2. Measure the diameter of the center of the laser spot by clicking on one side of the spot, then clicking on the opposite side.



- The application calculates the diameter of the spot and displays the value.
- Click **OK**. The spot size value replaces the word "Small" in the Capture Laser Tools.



NOTE: The measured spot size applies only to the current laser parameters. If you change the parameters, you must remeasure the laser spot size.

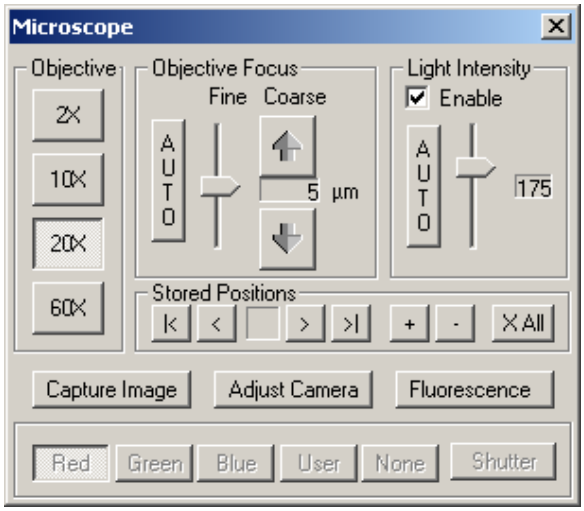
Working with the Live Video Image

Manually Focusing the Live Video

If you have **Auto Focus** selected in your User Preferences, the system automatically focuses the live video image. Likewise, if you have **Auto Brightness Adjust** selected, the system automatically sets the image brightness.

The Veritas™ Microdissection Instrument is calibrated so the objectives are parfocal. If you focus at 40x or 60x and then change the objective, the image should remain in focus.

NOTE: You **must** focus at the highest objective (either 40x or 60x) to take advantage of the parfocality. The depth of field for the 40x (or 60x) object is narrower than for the lower power objectives.



If the live video is not in focus once the auto-focus completes, change the light intensity and click **AUTO** in the Objective Focus section of the Microscope Tools. The software will repeat the auto-focus procedure.

If needed, you may manually focus the live video image, as follows:

1. On the Instrument menu, choose **Filter** then **None**, to open the filter.

2. Select the **40x Objective** (or 60x if your instrument is so equipped). Focus using the **Coarse Control** arrows and the **Fine Control** slider.

3. Check **Enable** under **Light Intensity** to turn the lamp on. Either click **AUTO** or use the slider to adjust the lamp intensity.

   NOTE: If you are performing fluorescence LCM, turn on the fluorescence lamp by clicking **Fluorescence.** Click **Shutter** to in the Microscope Tools to expose the sample to fluorescence excitation. Click **Shutter** again to block the excitation light source to avoid photo-bleaching. The light is blocked when the button appears "not pressed"; the sample is exposed to the light when the button appears depressed. You can change the color of the illumination by selecting a filter in the Microscope Tools.

4. If you plan to use another objective later, you may check its focus now. The instrument's parfocality should ensure that the microscope is still in focus after you change the objective. If not, adjust it as needed.
   The application remembers the focus settings for each objective used for a given slide.

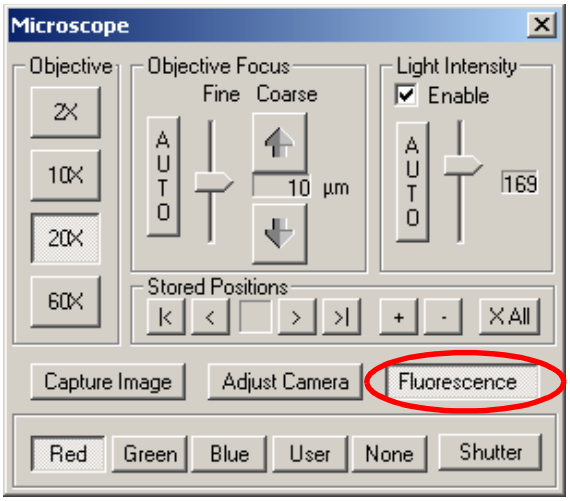
For detailed information on using the Microscope Tools, refer to "The Microscope Tools" on page 83.

Microdissection of Fluorescent Samples

This section describes tips for working with fluorescent samples. In general, the procedures outlined in Chapter 2, "Easy Access to Microdissection" on page 17, apply to fluorescent samples.

NOTE: The **Fluorescence** button is enabled when your instrument is equipped for epi-fluorescence (Models 702 and 704). When you click **Fluorescence**, the fluorescence lamp is turned on and the controls for the fluorescent filters and shutter are enabled.

- The fluorescence lamp requires several minutes to warm up in order to reach its maximum intensity. Turn it on by clicking the **Fluorescence** button in the Microscope Tools. When the lamp is ready, "EPI ready" appears in the lower right corner of the screen.
- Once the lamp is on, you must click **Fluorescence** again to turn it off. If you turn off the fluorescence lamp, it must cool down sufficiently before the lamp controller will turn it on again. This may take as long as five minutes. (If you turn the lamp on before the lamp has cooled, the lamp will not illuminate until cooling and the subsequent warm-up have completed.)



Microscope Tools for EPI fluorescence-equipped systems

- If you are not viewing the sample, you should close the shutter to avoid photo-bleaching. To do this, click **Shutter**, in the Microscope Tools or uncheck **Shutter** in the **EPI Filter/Shutter** item on the Instrument menu.
- For the AutoPix 100e instrument, you may select a neutral density filter from the **EPI Filter/Shutter** item on the Instrument menu. Filter choices are 100%, 25%, 10% and 1%.
- To view fluorescent-labeled samples on a dark field, turn off the white light by unchecking **Enable** in the Microscope Tools, then open the shutter by depressing the Shutter button.
- You may need to change **Integration** and/or **AGC** (automatic gain control) in the Exposure tab of the Camera Control dialog if you are having a hard time seeing your sample. See "Camera Control Dialog" on page 68 for details.
- Also in the Camera Control dialog, you may need to change **Gamma**, located on the Color tab.
- If you regularly work with both fluorescent and non-fluorescent samples, you should consider creating default settings for the Laser and Microscope Tools for each type of work, as described on page 85. You can save time by loading the appropriate settings file at the beginning of your session and eliminating much of the time needed to set up your system.

Overview of Additional Microdissection Methods

In addition to the microdissection process described in Chapter 3, the Veritas™ Microdissection Instrument offers the following additional modes for capturing cells or tissue:

- **Live Point-and-Shoot Capture of Single Cells** – You may capture cells while viewing the live video image for the slide. Simply navigate to a cell of interest, then double-click on it to fire the laser and capture the cell. See below for more information.
- **Mark and Capture Cells or Regions on a Static Image** – With the Microdissection Tools, you can mark the desired cells and/or regions on a static image and then the system will capture each cell or area, until all selected tissue is captured.
  See page 54 for more information.
- **Auto Scan on a Static Image** – Use a representative tissue slide to "train" the application to recognize cells and areas of interest and saves the information to a training file. Once a training file is created, you can use the file again to quickly scan a similar slide and automatically capture the areas of interest. See page 55 for more information.

Live Point-and-Shoot Capture of Single Cells

1. Prepare your samples, load your slides and caps and focus the microscope as described in Chapter 2, "Easy Access to Microdissection", on page 17.

2. Select **Manual Capture** from the **Capture Mode** submenu on the Instrument menu.

   NOTE: You can mark images for automatic capture and perform point-and-shoot capture on the live video in Manual Capture mode. The **Semi-Automatic Capture** option prevents you from performing point-and-shoot capture on the live video and should only be used if accidental point-and-shoot firing is a concern.

3. Double-click on each cell you wish to capture within the live video image.
   Each time you double-click on a cell, the capture laser fires and captures the cell.

   To navigate to other areas, use any of the methods described in "Moving the Stage from the Live Video Window" on page 44.

4. You may check the slide and the contents of the cap to verify that the capture was successful. Refer to "Inspecting Captured Material" on page 25.

Mark and Capture Areas on a Static Image

1. Prepare your samples, load your slides and caps and focus the microscope as described in Chapter 2, "Easy Access to Microdissection" on page 17.

2. Acquire a static image. Right-click on the live video and choose **Acquire Static Image**.

   – or –

   Click the **Capture Image** button in the Microscope Tools.

   – or –

   Use the **Region of Interest** tool from the Drawing Toolbar to outline a static image of a region from the roadmap, then right-click and choose **Acquire Image**. (For more information on creating a static image, refer to "Acquiring a Static Image" on page 45.)

3. Mark the image for selection.
   Use the Microdissection Tools to mark cells or regions to capture. (Refer to page 80 for information on using the Microdissection Tools.) Stay within the confines of the cap as you move around the image to mark cells and tissue.

Any dissection marks you add to the image are also shown on the roadmap and the Materials Tool (though you may not see them, depending upon the magnification).

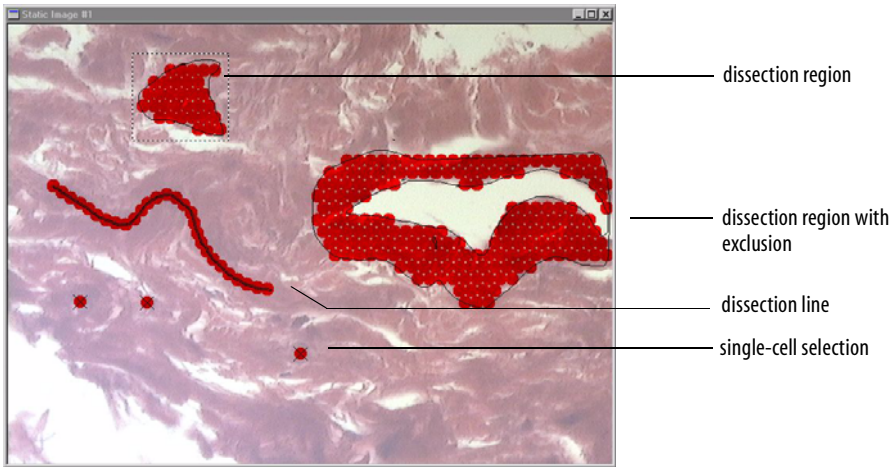


Figure 4-1. Static image marked up for capture, using the Microdissection Tools.

4. If desired, use the Drawing Toolbar to add annotations to the image. (See "The Static Image Annotations Toolbar" on page 90 for details.)

5. Capture the selected cells by doing one of the following:
   - Clicking **Go Capture**.
   - Right-click on the static image away from the selected cells and choose **LCM Capture Selected Cells**. This starts the collection process.
   - If you've chosen to ablate before capturing, right-click and chose **Cut Selected Cells**, then right-click and choose **LCM Capture Selected Cells**.

6. You may check the slide and the contents of the cap to verify that the capture was successful. Refer to "Inspecting Captured Material" on page 25.

Introduction to Auto Scan on a Static Image

The Auto Scan feature allows you to "train" the system to capture cells of interest for a particular tissue type. You define the capture criteria by indicating what areas of the tissue are of interest and what areas are background.

NOTE: This feature operates *only* on a static image.

The information is saved to a file, which you can use later on other slides with the same or similar tissue. You can also combine multiple files to create a new file. Refer to "Combining Auto Scan Training Files" on page 61.

The application provides three levels of training. You can apply as many levels as is necessary to achieve the most desirable results, but you must start with and perform at least the Pixel Analysis. For many samples, Pixel Analysis is sufficient.

- **Pixel Analysis** differentiates regions based on pixel color. It is best suited for slides that have a high contrast or definite differences in color between the regions of interest and background.
- **Texture Analysis** differentiates tissue based on textural and topological characteristics. It can be used after Pixel Analysis to further discriminate regions of similar color by analyzing groups of pixels for differentiating characteristics.
- **Morphology Analysis** differentiates the shape of regions of interest from targeted background areas.

Microdissection Using Auto Scan

1. Prepare your samples, load your slides and caps and focus the microscope as described in Chapter 2, "Easy Access to Microdissection", on page 17.

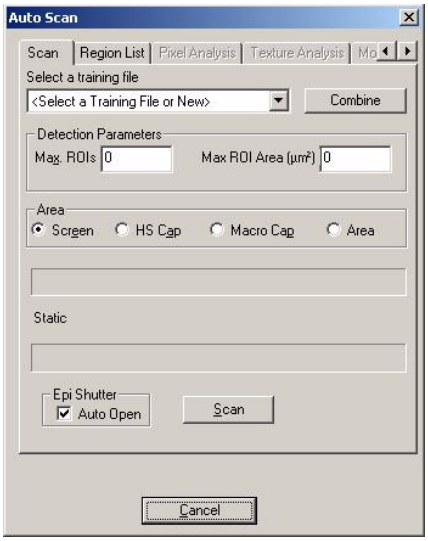
NOTE: If you are creating a training file, you can create the file and scan the slide before placing a cap and locating and measuring the laser. A default laser spot size of 10 µm will be used until you measure the spot size.

2. Right-click the live video image and select **Auto-Scan**

– or –

Click the **Auto-Scan** button on the Capture Groups Tool.

The Auto Scan window appears and a static image is acquired in a new window.



3. Select **<New>** to create a new training file.

– or –

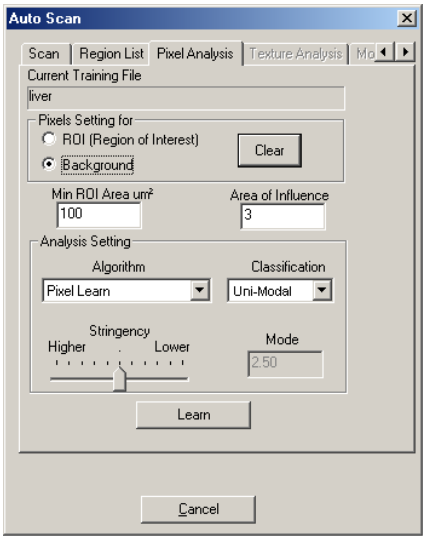
Select an existing training file from the drop-down list

If you choose an existing training file, proceed to step 8. to select an area and scan.

When you create a new file, the Pixel Analysis tab becomes active, allowing you to define the training criteria.

NOTE: Do not click the **Scan** button until you have finished training the system. Clicking the **Scan** button saves the training file.

NOTE: If you are performing fluorescence imaging, check **Auto Open** in the **Epi Shutter** section of the Auto Scan dialog box. This will only open the shutter briefly while performing the scan, to prevent photo-bleaching of the sample. If you do not check **Auto Open**, the shutter remains in the state set in the Microscope Tools.



4. Click the Pixel Analysis tab.
Use this first level of training to select representative pixels of regions of interest (ROIs) and representative pixels of background.

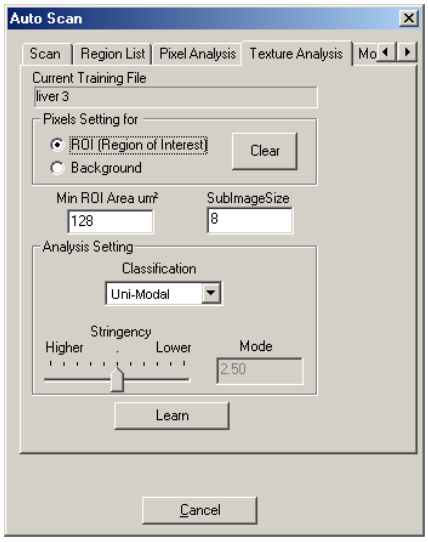
- Click the **ROI (Region of Interest)** button, then click on areas you wish to capture. Select samples of ROIs representative of all color variations in which you are interested.
- Click the **Background** button, then click on areas of background. Select samples of background representative of all color variations you wish to exclude.
- The **Clear** button removes all selected areas of either ROI or background (depending on which is selected), allowing you to start the selection process over.
- Enter a value for the **Min ROI Area µm^2**.
  This value defines the minimum size for an area to be considered for analysis by this algorithm. For most applications, the default value of 100 is suitable.
- Enter a value for the **Area of Influence**.
  This value defines an area of pixels surrounding the point that you select. This area will be averaged and used by the algorithm to differentiate ROI from background. The default value of 3 is suitable for most samples.
- Select the **Algorithm**.
  Pixel Learn – a basic algorithm used for standard tissue using the color camera.
  Pixel Learn Advanced – used for tissue with more complex coloring or intensity variation, where Pixel Learn does not provide adequate results.
  Pixel Detect (gray scale) – an algorithm for black and white differences.
  Pixel DetectS (convex hull) – a fast algorithm for black and white differences. Useful for scanning large areas on cytology samples.
- Select the **Classification**.
  Uni-Modal – used for samples where there is sharp contrast between background and ROI. If you select **Uni-Modal**, you can adjust the stringency of the ROI selections. A lower stringency favors ROI detection but increases false positives, while a higher stringency results in less false positives, at the risk of missing some ROIs (higher ROI purity).
  Multi-Modal – used for samples where the background has a number of color variations. The classification number entered indicates the number of color patterns. The number after the decimal point indicates the level of accuracy. For example, 2.50 indicates an analysis using a total of four distinct color patterns—two for ROI and

two for background—with a probability threshold of 0.5. Decreasing the probability threshold (for example, 0.2) will favor ROI detection and increase false positives, while increasing the threshold will result in less false positives, at the risk of missing some ROIs.

- Click **Learn**.
  The application analyzes the area within the image and marks ROI areas.
- The next step in the process depends on your level of satisfaction:
  If you are satisfied with the results, proceed to step 8. to scan the selected area.
  If you are not satisfied with the results, you can repeat the selection of ROIs and background to provide the application with more information. You may also change the settings within the Pixel Analysis tab to modify the results.
  To refine the selections, you may continue with further training by proceeding to texture analysis in step 5. and/or morphology analysis in step 7.

NOTE: Using texture and morphology analysis in addition to pixel analysis will result in longer sample scanning times.

5. To train the system for texture analysis, click the Texture Analysis tab and enter the appropriate information.
Use this second level of training to select areas of ROIs and areas of background to perform an analysis based on textural differences.



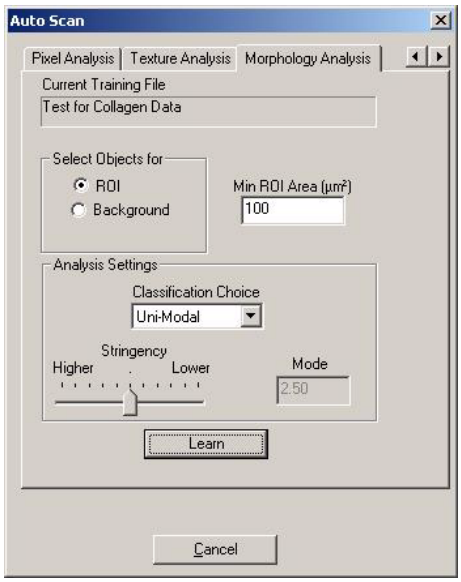
- Click the **ROI (Region of Interest)** button, then click to create regions around areas of interest with typical texture or topology. Double-click to close the region.
- Click the **Background** button, then click to create regions around areas of background texture or topology not found in areas of interest. Double-click to close the region.
- Enter a value for the **Min ROI Area µm^2**.
  This value defines the minimum size for an area to be considered for analysis by this algorithm.
- Enter a value for the **SubImage Size**.
  This value defines the number of sections into which the region areas previously defined are divided. The texture features are derived from these sub-images.
- Select the **Classification**.
  Uni-Modal – used for samples where there is sharp difference in texture between background and ROI.
  Multi-Modal – used for samples where the background has a number of texture variations. The classification number entered indicates the number of texture patterns.
- Click **Learn**.

The application analyzes the area within the image, applies the new information and marks ROI areas.

6. The next step in the process depends on your level of satisfaction:

- *If you are satisfied with the results*, proceed to step 8. to scan the selected area.
- *If you are not satisfied with the results,* repeat the pixel level analysis. You may also change the settings within the Texture Analysis tab to modify the results.
- *To refine the selections*, you may continue with further training by proceeding to morphology analysis in step 7.

7. To add a final morphology screening, click the Morphology Analysis tab and enter the appropriate information.
This tab provides a final screen allowing you to verify the areas to be captured and to eliminate any false-positive areas that have been selected. Use this level of learning only if you see background areas that were selected as ROIs.



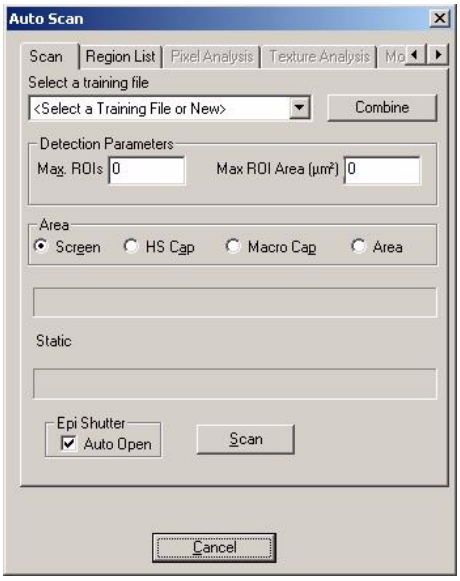
- Click **ROI**, then click to select at least two representative areas of ROI (a blue outline appears).
- If you see a background area that is selected for capture, click the background button, then click on at least two areas to change them to background (a green outline appears).
- Select the **Classification**.
  Uni-Modal – used for samples where there is sharp difference in morphology between background and ROI.
  Multi-Modal – used for samples where the background has a number of morphology variations. The classification number entered indicates the number of morphology patterns.
- Click **Learn**.
  The application analyzes the area within the image, applies the new information and marks ROI areas.

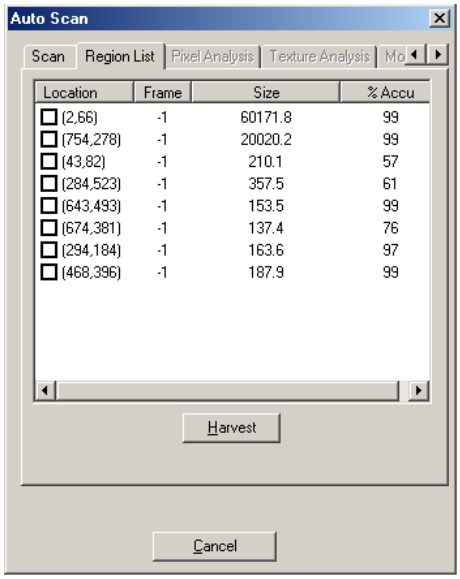
8. Click the Scan tab and enter the appropriate information. Once you click the Scan tab, the training is complete and the file is saved. The file can no longer be edited; however, it can be overwritten.

- Enter the **Max ROIs** to capture.
  This the maximum number of regions to be captured.
- Enter the **Max ROI Area µm^2**.
  This value defines the maximum size for an area to be considered for capture.

NOTE: Enter a value of 0 for **Max ROI** and **Max ROI Area µm^2** to indicate no limits.

- Select the area to capture.
  Screen scans the area within the live video image. Locate an area of interest, then scan and capture. Continue locating areas and scanning, while staying within the confines of the cap.
  HS Cap scans an area approximately the size of the capture area of an CapSure™ HS Cap (3 mm x 3 mm). This option assumes the current position in live video is the center of the cap. If necessary, the cap will be moved during the capture process to collect all targeted areas.
  Macro Cap scans an area approximately the size of the area of a CapSure Macro Cap (6 mm x 6 mm). This option assumes the current position in live video is the center of the cap. If necessary, the instrument will move the cap during the capture process to collect all targeted areas.
  Area scans an area 20 mm x 20 mm starting from the region center. To prevent the system from attempting to place the cap off the edge of the slide, place the live video in the center of the slide. If necessary, the cap will be moved during the capture process to collect all targeted areas. Use only for samples where tissue is sparse to avoid capturing cells onto an area of the cap that already contains tissue.
- Click **Scan**.
  The application scans the selected area for ROIs using the information it has learned and creates a list of them in the Region List tab.





9. After scanning, click the Region List tab and manually select all of the regions that you wish to capture.
The regions appear highlighted in the static image.

- *To see a region*, double-click the entry in the tab (a green outline appears around the region).
- *To select a region for capture*, click to place a check mark in the box (a blue outline appears around the region). Select multiple regions by holding down the SHIFT key to highlight the entries. Press the space bar to select them.
- *To modify regions on the static image prior to capture*, use the Microdissection Tools.

10. Click **Harvest** to capture all the regions with "X" next to them in the list.
If you have not placed a cap, you will be prompted to do so. Drag a cap in the Materials Tool to the active slide and adjust the laser parameters as described in "Working with the Capture Laser" on page 49.

You can capture selected areas on the static image by right-clicking and selecting **LCM Capture Selected Cells** from the menu.

11. You may check the slide and the contents of the cap to verify that the capture was successful. Refer to "Inspecting Captured Material" on page 25.

12. For subsequent slides using the same training file, perform steps 1. and 2., then proceed to step 8. to scan the selected area.

Combining Auto Scan Training Files

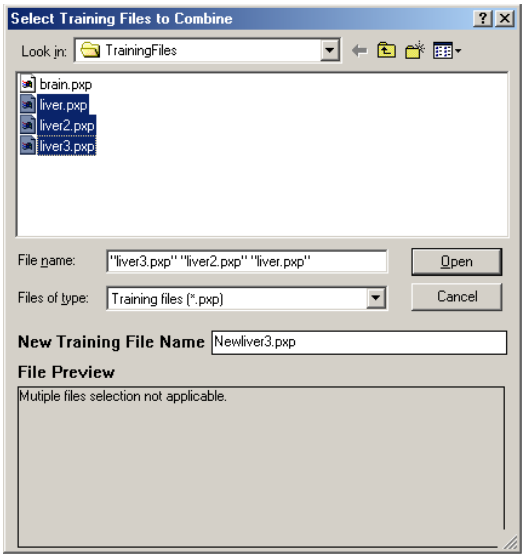
To create a training file that you can use to scan different slides of the same tissue type, create multiple training files for slides with different staining intensities, tissue thickness, as well as various light intensities, then combine the files. The resulting file incorporates all of the information from each of the files used to create it.

NOTE: You can combine only files that use the same algorithms. You cannot edit a training file.

1. Create as many individual training files as you wish. For each file, you may want to use a different slide of the same tissue type.

2. When you are ready to combine the files, right-click on the live video image and select **Auto-Scan**.

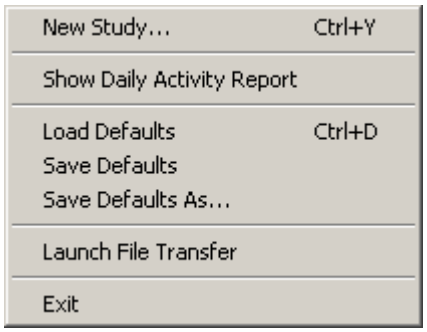
3. Click **Combine** to display a list of training files.
4. Click to select the training files you want to combine. Hold the SHIFT key to select multiple files.
5. Enter a file name in the **New Training File Name** field, or use the default name.
6. Click **Open**.
   The system creates the file and adds it to the **Select a Training File** drop-down list in the Auto Scan window.
7. To use the file, select it from the list and click **Scan**.



5. Software Menus

This section lists the Veritas™ application menus and provides a brief description of each option.

File Menu



New Study – Allows you to create a new study. A study contains cap and slide properties and any images that are saved during the session.

Show Daily Activity Report – This will display a report in Notepad, showing all of the Cap Interaction Histories for the day. The file is a text file named with the date (YYYY-MM-DD-Activity.txt) and is saved in the C:\Veritas Images\DailyActivityReports folder.

Load Defaults – Allows you to load a laser and microscope settings file. The system will initially load the settings file that was selected in your User Preferences when your account was created. If your account was not assigned a specific default setting file, the SystemDefault settings are automatically loaded. See page 36 for more information on saving other types of settings as your defaults.

Save Defaults – Allows you to save the current laser and microscope settings to a file that you can recall and use later for another session.

Save Defaults As – Allows you to save the current laser and microscope settings under another name. If you are a user and you attempt to save changes to the SystemDefaults settings, you will not be allowed to do so. Instead, you must use the **Save Defaults As** option. Only an administrator can change and save the SystemDefault settings.

Launch File Transfer – This option should only be used when instructed to do so by Arcturus service personnel. It is only accessible by an administrator.

Exit – Logs you out and closes the Veritas™ application. The application will prompt you to remove and caps and slides from the instrument and save any images created during the session.

View Menu

Toggle Laser Tool – Selects what is shown in the Laser Tool. Choose **Area Capture** to show the area of targeted regions or **Fire Capture** to show the number of laser fires required to cover targeted regions.

Activate Live Video – Choose this to open the live video window if it has been closed.

Tools Menu

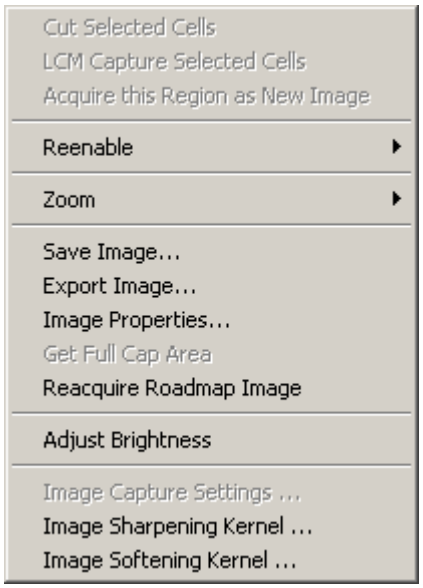
The Tools menu allows you to show or hide the tools and toolbars. A check mark indicates that the toolbar is displayed. To hide the toolbar, choose the option again to remove the check mark.

You can also display the Tools menu by right-clicking in the background of the application window.

Image Menu

The Image menu allows you to work with the current image. You can use the cutting laser and capture laser as well as zoom in on the active roadmap or static image.

See "Working with Images" on page 43 for detailed information on images.



Cut Selected Cells – If one or more regions have been created using the Cut and Capture Tools and/or the Ablate Tools, this option will cut the region(s). If you do not have a cutting laser (Models 701 and 702), this option is not available.

LCM Capture Selected Cells – If you have identified a region or cells or tissue to capture, this command operates the capture laser and fuses the tissue to the cap. If a cap has not already been placed, the system will first place a cap, then capture.

Acquire this Region as New Image – Allows you to reacquire the current static image without removing any annotations or dissection marks on the image. Use this if the initial microscope settings were unsatisfactory and/or the image is not as clear as you'd like.

Reenable – Enables the system so you may repeat any cuts or captures. (Ordinarily, the Veritas™ Microdissection Instrument does not allow you to cut or capture any area a second time.) You can set this option for a single dissection mark, as well, by right-clicking on the dissection mark and selecting **Reenable**.

There are three choices for **Reenable**:

- **Cut All Selected Cells** – If you select this, the next time you choose **Cut Selected Cells,** any area marked with one of the Cut and Capture or Ablation Microdissection tools will be cut, irrespective of whether it was previously cut.
- **LCM Capture All Selected Cells** – If you select this, the next time you choose **LCM Capture Selected Cells**, any area marked to capture will be captured, irrespective of whether it was previously captured.

- **Cut + LCM All Selected Cells** – If you select this, the next time you choose either **Cut Selected Cells** or **LCM Capture Selected Cells**, any area marked to cut or capture will be cut or captured, even if it was previously cut or captured.

NOTE: If you do not have a cutting laser (Models 701 and 702), only **LCM Capture All Selected Cells** is available on this submenu.

Zoom – Enlarges the active roadmap, live video window or static image. Choose the desired percentage zoom.

Save Image – Allows you to save an image. The image is saved to the study directory and can be opened using the Study Tool. See "Saving an Image" on page 47.

Export Image – Allows you to save an image as a bitmap, jpeg, or tiff file. See "Exporting an Image" on page 48.

Image Properties – Opens the image properties window. See "Image Properties" on page 47.

Get Full Cap Area – This will cut and capture a region the same size as the cap. The application first draws the dissection marks on the area and then performs the cut and capture.

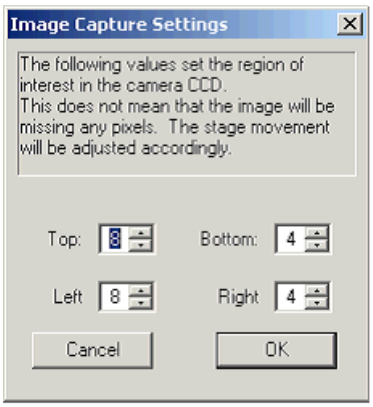
NOTE: If you do not have a cutting laser (Models 701 and 702), this option is not available.

Reacquire Roadmap Image – Allows you to acquire a roadmap image for a selected slide. Use this command if **Auto-acquire Roadmap Images** is not selected in the User Preferences, or if you want to reacquire a roadmap with a different light intensity. For details on roadmaps, see "The Roadmap Image" on page 43.

Adjust Brightness – Performs the same adjustments to the shutter and lamp intensity to optimize brightness as the **Auto** button in the Light Intensity section of the Microscope Tools.

Image Capture Settings – Opens the Image Capture Settings dialog where you may set a margin of a given width around the live video image. See the "Image Capture Settings Dialog" on page 66, for details.

Image Capture Settings Dialog



These settings can be used to crop pixels from the edge of the image frame. By default, the maximum area of the camera's CCD is used. It is common for CCDs to have lower response at the extreme edge. If there are noticeable dark lines in the stitched image, you may wish to crop the edges using the Image Capture Settings dialog.

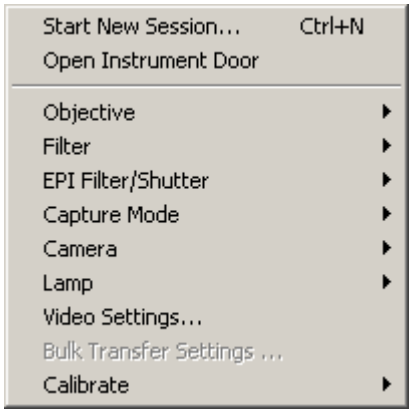
You may only change these settings when there is no active session.

1. Choose **Log Out** from the User menu. The application will open the instrument door, slide the work surface out and remind you to unload the instrument. Finally, the System Login dialog box appears, allowing you to log in.
2. Enter your name and password and click **OK** to log in without starting a new session.
3. Choose **Image Capture Settings** from the Instrument menu.
4. Enter the values, in pixels, for each margin around the image.
5. Click **OK** to save the changes.
6. The application will display a message informing you that the changes will take effect after the application is restarted.
7. Dismiss the message and then the application will exit.
8. Restart the application, log in and begin a new session to use the new settings.

Image Sharpening Kernel – This option is unavailable.

Image Softening Kernel – This option is unavailable.

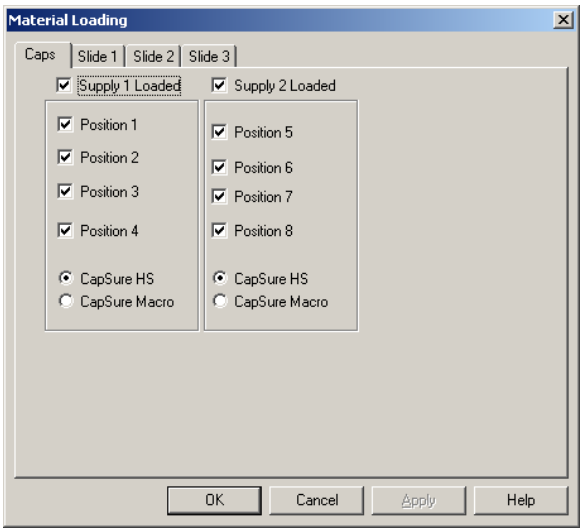
Instrument Menu



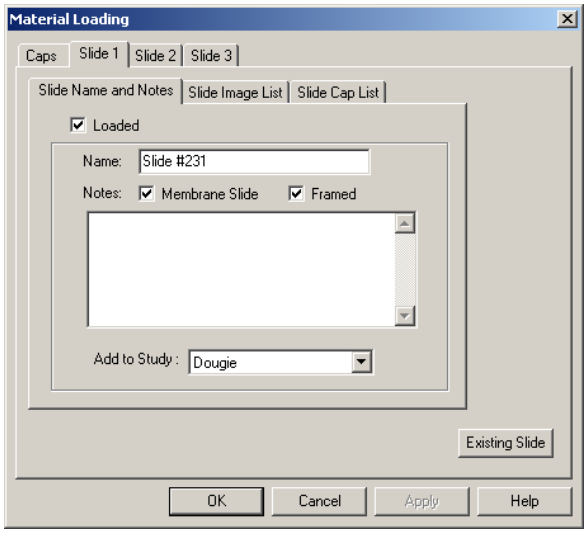
The Instrument menu allows you to make certain adjustments to the microscope, as well as perform other instrument-related functions. You can also use the Microscope Tools (see "The Microscope Tools" on page 83) to make these adjustments.

Start New Session – Opens the instrument door and displays the Material Loading window, where you specify the materials for the new session (see "Entering Information in the Materials Loading Window", below). If you are currently running a session, a dialog box appears informing you that if you start a new session, the current session will end.

Entering Information in the Materials Loading Window



1. On the Caps tab, uncheck any positions where there are no loaded caps.
2. Choose the type of cap you are using, either CapSure™ HS or CapSure Macro.



3. Click the Slide 1 tab. Check Loaded, if needed. Enter a name for the slide (or use the default name) and any notes you wish to save with the slide data.
4. Select a study from the **Add to Study** drop-down list, or create a new study by choosing **New Study** and entering a study name.
   - If you are using a membrane slide, check **Membrane**.
   - If you are using a membrane frame slide, check **Membrane** and then **Frame**.
5. Repeat for Slides 2 and 3, as needed.
6. Click **OK** in the Material Loading window when you are finished entering information about the loaded materials.

Open Instrument Door – Allows you to open the Veritas instrument door to unload and remove caps from the unload tray and/or add or remove slides. When you are finished, click **OK** to close the door. This option is available after you log in.

NOTE: Do not add new materials to the instrument using this option; instead use the button on the Materials Tool.

Objective – Allows you to choose an objective (**2x**, **10x**, **20x**, **40x**, or **60x** if your instrument is so configured). Choose **Restore Focus** to move the objective back to the default focus position.

Filter – Allows you to choose a fluorescence filter. Choose **None** (for no filter) or the appropriate filter for microdissection.

EPI Filter/Shutter – Allows you to open and close the shutter. The shutter is open when **Shutter** has a check next to it.

For the AutoPix 100e instrument, you may select a neutral density filter. Filter choices are **100%, 25%, 10%** and **1%.**

Capture Mode – Allows you to choose between **Manual Capture** and **Semi-Automatic Capture**.

- **Manual Capture** allows you to scan the slide while you point and fire the laser to capture cells. In Manual mode you can also mark up a static image of the slide to select cells and regions of interest before you fire the laser. Typically, the system can be left in the Manual mode.
- **Semi-Automatic Capture** allows you only to mark up a static image of the slide to select all cells and regions of interest before you fire the laser. This mode prevents you from firing the laser on the live video image.

Camera – There are two option on the submenu: **Color Camera** and **Camera Control**.

- The **Color Camera** option is checked to indicate the presence of a color camera in the instrument.
- Choose **Camera Control** to view the Camera Control dialog box, where you may set various camera settings. The settings in the dialog box are described below.

Camera Control Dialog

The following settings are available from the two tabs in the Camera Control dialog.

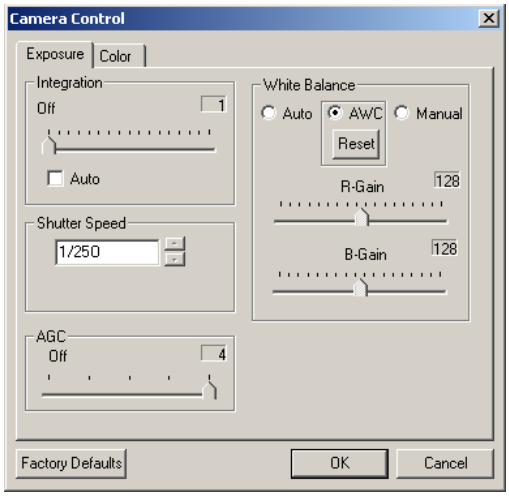
On the Exposure tab:

Integration – Turns on camera integration for the specified number of frames. Use this in case of insufficient illumination, such as particularly darkly stained tissue or fluorescent illumination. When **Auto** is selected in this mode, the camera will integrate up to the specified number of frames to obtain sufficient illumination; the default is **Off**.

NOTE: Long integration times introduce substantial time lag in the video image, making it difficult to position or focus the video.

Shutter Speed – Choose the desired speed. Under manual control, the application will no longer change shutter speed to compensate for differing illumination levels due to objective and visualizer selection. This means that light intensity levels will need to change over a wider dynamic range and there will be more variation in the color temperature of the lighting.

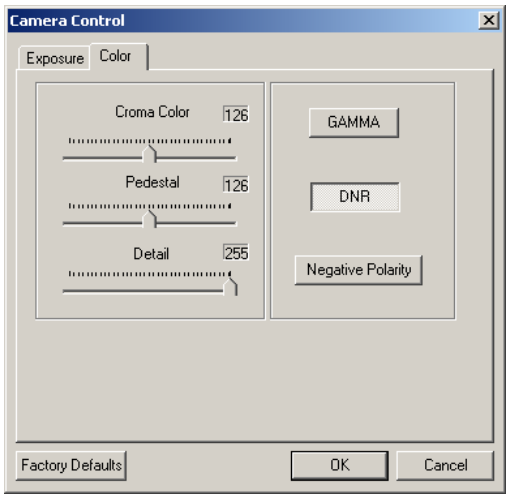
AGC – Automatic gain control; allows the camera to select electronic gain up to the maximum specified. Electronic gain will introduce some additional noise into the video images. The default is **Off**. For fluorescence imaging, you may obtain better results with this turned on.



White Balance – There are three modes for controlling white balance, **Auto, AWC** and **Manual**, described below. **AWC** is the default mode.

- **Auto** – The camera constantly adjusts for optimal white balance. Under normal conditions, this adjustment will not be noticeable. However, if lighting conditions change quickly, users may notice this camera adjustment (which can take up to ten seconds). Additionally, the **Auto** setting can lead to a "patchwork quilt" pattern on your images.
- **AWC** – In this mode, white balance is adjusted based on the current video image and then not changed until **Reset** is pushed. For optimal adjustment, find a scene in which white fills the majority of the screen. It takes approximately ten seconds for this white balance adjustment.
- **Manual** – In this mode, you can set the red (**R-Gain)** and blue gain (**B-Gain)** manually.

On the Color tab, the following options are available:



Chroma Color, Pedestal and **Detail** – Optimum levels have been pre-set at the factory. Use the sliders for manual control of these settings.

Gamma – Click the button, to depress it, for gamma correction. The default is off. For fluorescence imaging, **Gamma** should be on.

DNR – Click the button, to depress it, to choose digital noise reduction. The default is on.

Negative Polarity – Click the button to depress it, to create a "negative" video image. The default is off**.**

Factory Defaults – Click this button to restore the factory default settings.

To save your changes, click **OK**. To close the dialog without saving changes, click **Cancel.**

Lamp – Allows you to choose between **White Light** and **Activate EPI Lamp** to illuminate the sample. You may have both lights on simultaneously.

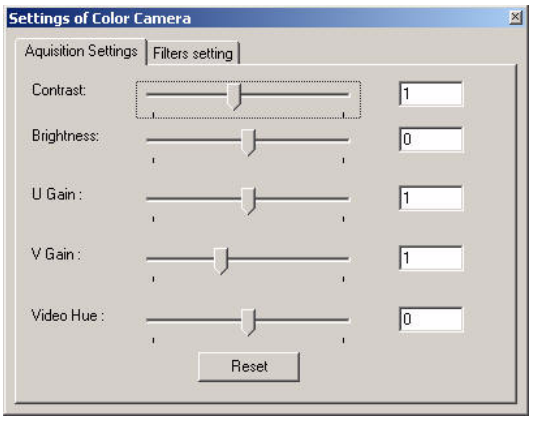
- **White Light** is available after materials have been loaded.
- **Activate EPI Lamp** turns on and off the EPI lamp used for fluorescence applications. The EPI lamp requires several minutes to warm up once it is turned on and before it can be selected. When the lamp is ready, "EPI ready" appears in the lower-right corner of the screen. Once the EPI lamp is turned off, it must cool down sufficiently before the lamp controller will turn it on again. This may take as long as five minutes.
  To view fluorescent labeled samples on a dark field, turn off the white light by clicking **Enable** in the Microscope Tools and open the EPI lamp shutter.

NOTE: This option is only available if your instrument is equipped with an EPI lamp (Models 702 and 704).

Video Settings – Allows you to adjust the camera settings for the live video (for example, brightness and contrast), as described in "Color Camera Dialog", below. You can also access this dialog box by right-clicking in the live video image. In most cases, you will not need to make any changes to these settings.

Color Camera Dialog

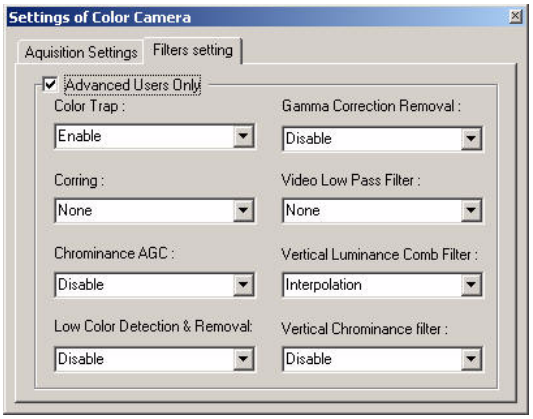
This dialog box allow you to adjust the camera settings.



For all the parameters on the Acquisition Settings tab, use the slider or enter a value in the text field. These parameters are: **Contrast**, **Brightness**, **U Gain**, **V Gain**, and **Video Hue**.

Reset – Click this to return to the default values for these parameters.

On the Filters Settings tab, you may set the following parameters:



Advanced Users Only – Check this to enable the other controls. Once the controls are enabled, choose the desired option from the list.

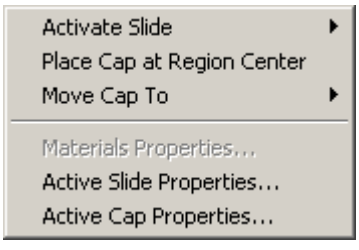
The following options are available: **Color Trap**, **Corring**, **Chrominance AGC**, **Low Color Detection & Removal**, **Gamma Correction Removal**, **Video Low Pass Filter**, **Vertical Luminance Comb Filter**, and **Vertical Chrominance Filter**.

Bulk Transfer Settings - This option is unavailable.

Calibrate - This option is unavailable.

Materials Menu

Use the commands on the Materials menu to move caps and slides from one area to another on the work surface.



Activate Slide – Allows you to choose a specific slide for analysis. This option is available once materials have been loaded.

Place Cap At Region Center – Moves the current cap to the selected location of the active slide. This option is available when a slide is active (an active slide has a red border around it).

Move Cap To – Moves the current cap to the QC station, slot (slide) 1, 2, 3, or the unload tray. This option is available when a cap is active (an active cap has red circle in the center).

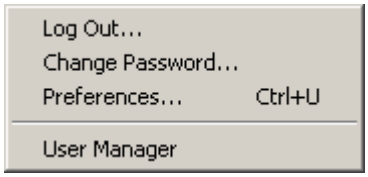
Materials Properties – This option is unavailable.

Active Slide Properties – Displays the properties for the active slide. This option is available when a slide is active. See "Slide Properties" on page 40 for more information.

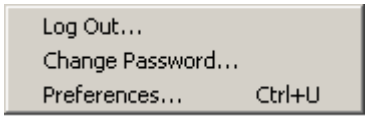
Active Cap Properties – Displays the properties for the active cap. This option is available when a cap is active. See "Cap Properties" on page 43 for more information.

User Menu

The User menu options depend on whether a user or an administrator is logged in.



Administrator



User

Log Out – Allows you to log out of the system.

Change Password – Allows you to change your password.

Preferences – Displays the user preferences. See "Setting User Preferences" on page 36 for more information.

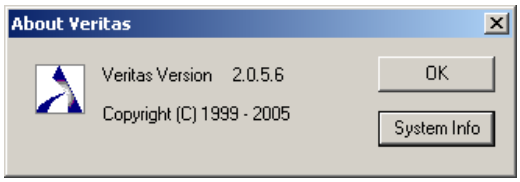
User Manager – Allows an administrator to see a list of all users in the system, and to add, delete, or edit user information. Available to administrators only.

Window Menu

The Windows menu displays a list of all windows currently open in the application. Choose a window from the list to make it the active window and bring it to the front.

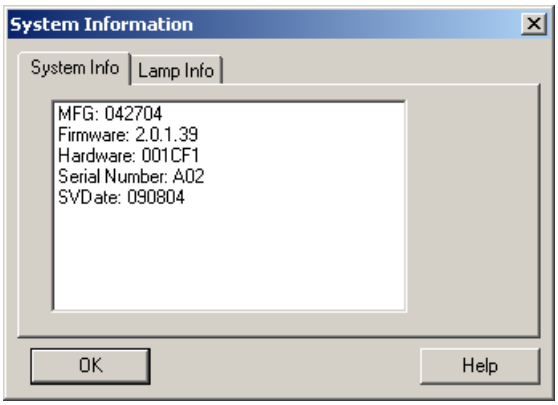
Help Menu

About Veritas – Displays the About Veritas dialog showing the application version number. Click **OK** to dismiss the dialog box.



Displaying System Info

Click **System Info** in the About Veritas dialog to show information about the system. You can see information about the system and the lamp.



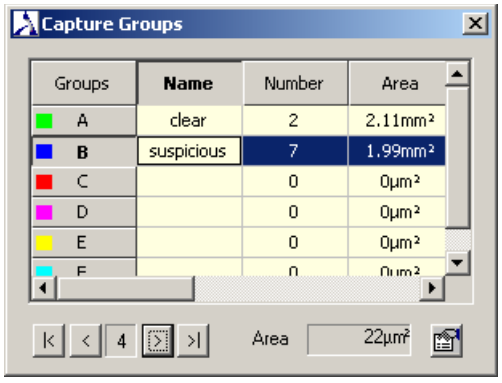
6. Software Tools and Toolbars

This section describes the Veritas™ application tools and toolbars.

There are eight tools, listed below in alphabetical order. Each tool is described in more detail on the corresponding page.

There are two toolbars in the application:

The Capture Groups Tool

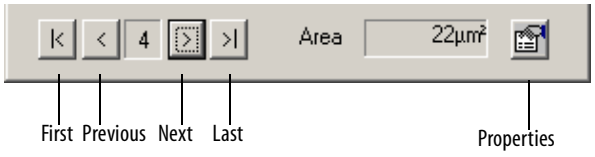


The Capture Groups Tool allows you to mark different tissue targets, containing the same cell types, to be collected together on the same cap. For instance, you may have two different types of cells on one slide, both of which you are interested in assaying separately. You can create two groups, one for each type of cell. When you capture, you can capture the first group on one cap and the second group on another cap.

Each group is displayed in a different color on the screen, making it easy to distinguish between them. You can assign a name to each capture group. The number of items in the capture group is shown in the **Number** column and the total area for each group is shown in **Area** column.

To name a capture group, click in the **Name** cell for the group and type the name.

There area three controls at the bottom of the Capture Groups Tool.



- The controls at the bottom left of the tool allow you to view all items in the capture group.
- The **Area** field updates to show the area of the currently selected item.
- The **Properties** button allows you to set the properties of the capture group, as described below.

Adjusting Capture Group Properties

You can set the properties for each capture group, including details of the cutting and capture laser and the color in which the group will be displayed on the screen. These changes apply to any *new* dissection marks in this group. The properties of existing dissection marks in this group will not change.

Only the properties relevant to the type of dissection marks you create will apply. For instance, if you only use the LCM Microdissection Tools to mark cells for capture, then the Cut Properties will not be used.

NOTE: You may wish change the properties of a *single* dissection mark; to do so, see "Adjusting Dissection Mark Properties" on page 83.

1. Select the group by clicking on the letter next to the group in the Capture Groups tool.

   – or –

   Use the UP and DOWN arrow keys to scroll through the list of groups.

2. Right-click and choose **Properties** from the menu.

   – or –

   Click **Properties** in the Capture Groups Tool.

3. The Cut Properties tab has parameters which pertain to the cutting laser. If you do not have a cutting laser, this tab is not shown. Depending upon the type of slide you are using, different parameters are used by the system.

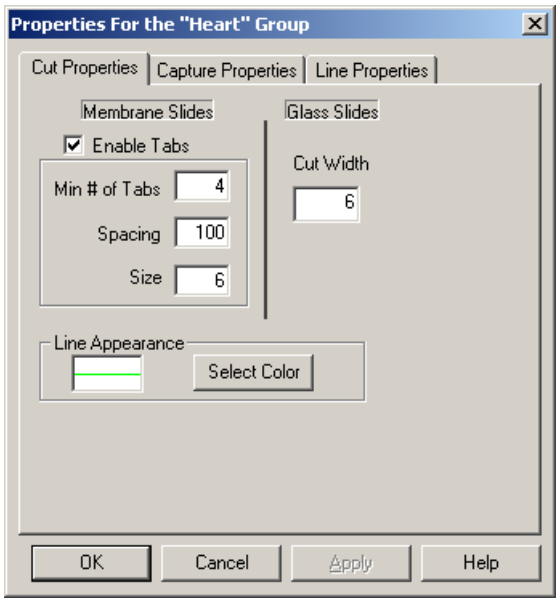
   - *To change the color of the line representing the path of the cutting laser*, click **Select Color**, choose a color from the Color dialog box, and click **OK**.

You may set the following parameters for membrane slides:

- *To disable tabs*, uncheck **Enable Tabs**. Tabs are short regions that are not cut and which prevent the tissue from curling off the slide before the capture laser can attach the CapSure ™ film to the tissue. If this is not checked, no tabs will be generated and the cutting laser will cut entirely around the region.
- *To change the number of tabs and other tab parameters*, enter the following information: **Min. # Tabs** is the number of tabs in the region. **Spacing** is the distance between the tabs, in µm. **Size** is the length of the tab, in µm.

You may set the following parameters for glass slides:

- *To change the width of the cutting laser*, enter a number in µm, in the **Cut Width** field. If the **Cut Width** is larger than the **Cutting Laser Spot Size**, the Veritas™ application will round down until the **Cut Width** can be evenly divided by the **Cutting Laser Spot Size**. (For example, if the **Cut With** is 7 µm and the **Cutting Laser Spot Size** is 2 µm, then the application will adjust the **Cut Width** to 6 µm.)



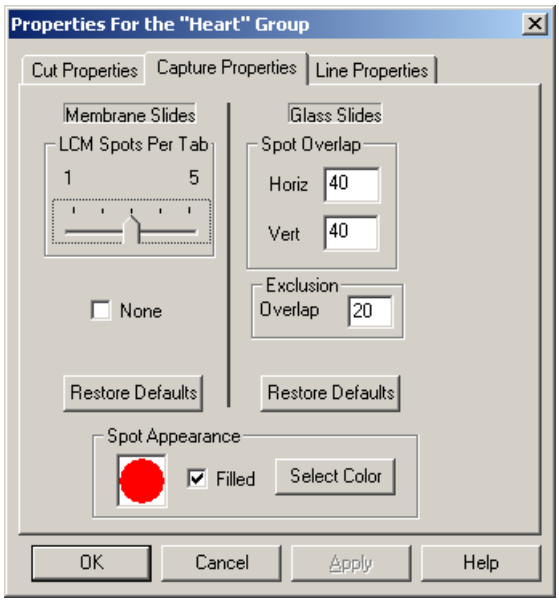
4. In the Capture Properties tab, you may set the parameters pertaining to the capture laser. As before, there are different parameters for membrane and glass slides.
For membrane slides:

- Set **LCM Spots Per Tab**. The capture laser spots are used to fuse the tissue to the cap.
- Check **None** if you don't wish to place any capture laser spots. Use this option when you are planning to mechanically extract the tissue; the system will use the cutting laser to cut around the region but will not use the capture laser.
- Click **Restore Defaults** to return to the default values.

For glass slides, the capture laser spots are arranged in parallel rows, covering the entire area to be captured.

The following parameters influence the position of the spots:

- *To control how much the capture laser spots will overlap each other*, change the **Horizontal** and **Vertical** fields in Spot Overlap. **Horizontal** controls how much two adjacent spots on the same line cover each other; **Vertical** controls how much a spot will over lap a spot on the next line. The greater the values, the more the spots will be situated on top of each other. Enter a number from 0 to 99.
- *To adjust the degree to which the laser target spots for the area of interest extend into an excluded region*, change the **Overlap** (in the Exclusion area of the dialog box). For example, if the

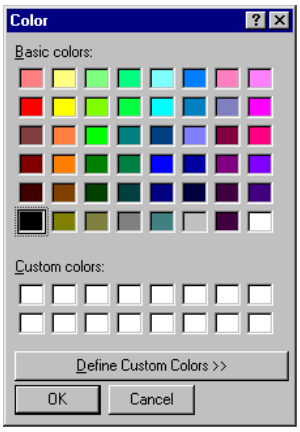


Dissection Exclusion Region tool is used to designate an area to be excluded from capture, some of the capture laser spots may extend past the border into the excluded region. The **Overlap** defines the degree to which these targeting spots extend into the excluded region. Spots with a higher percentage of their area overlapping the excluded region than is defined by **Overlap** will be deleted.

- Click **Restore Defaults** to return to the default values.

If desired, change how the capture laser spot is displayed on the screen.

- Change the capture laser spot fill. You can fill the spot or display it as an outline. Check **Filled** to fill the spot; remove the check to display an outline.
- Change the capture laser spot color. Click **Select Color**, choose a color from the Color dialog box, and click **OK**.



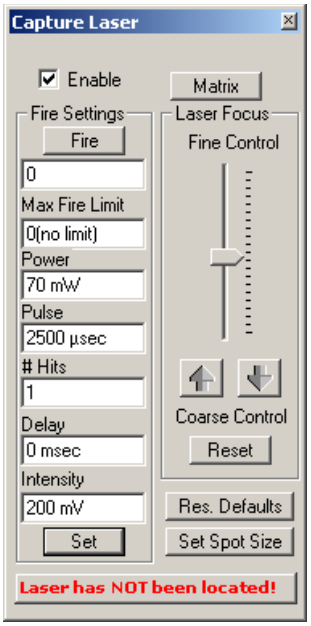
5. Click **OK** to save the changes. These changes apply to any new dissection marks in this group. The properties of existing dissection marks in this group will not change.

The Capture Laser Tools

The Capture Laser Tools allow you to make adjustments to the capture laser. The Capture Laser Tools are not available until you place a cap on the slide or choose **Capture Laser** from the Tools menu.

The Capture Laser Tools have two modes. In one mode, the number of laser fires to cover the area selected is shown. In the second mode, the total area of the selected region is shown in square microns. See "Setting User Preferences" on page 36 for instructions on choosing the mode.

NOTE: The cutting laser, if installed, is adjusted using the Cutting Laser Tools, described on page 78.



Capture Laser Tools set to show the number of fires to cover the area (Fire Counter selected on Toggle Laser Tool menu)

Enable – Turns on the laser and allows you to adjust the settings.

Matrix – Allows you to set up a test firing pattern to determine the appropriate laser settings to adequately wet the film. For detailed information on using a matrix, see "Using a Calibration Matrix to Determine Capture Laser Settings" on page 78.

Fire/Capture – Pulses the laser according to the specified **# Hits** and **Delay**. For example, if the **# Hits** is 4 and the **Delay** is 10 msec, the laser will pulse 4 times with a 10 msec pause between each hit. A counter keeps track of how many times you fire. You can reset the counter at any time by entering 0 in the text box.

NOTE: The counter considers the value in the **# Hits** field as a single fire. For example, if you enter 4 in **# Hits**, although the laser pulses 4 times, the counter registers 1 fire.

Max Fire Limit/Max Area Limit – Allows you to enter a maximum number of laser fires or the maximum area to capture, for a static image. When the **Max Fire Limit/Max Are Limit** is reached, the capture stops.

Power – Sets the laser power between 3 mW–100 mW.

Pulse – Defines how long the laser fires. Enter a value from 100 µsec to 1,000,000 µsec.

 # Hits – Determines the number of times the laser actually fires when you click the **Fire** button. For some tissue, multiple pulses may be necessary to achieve proper wetting.

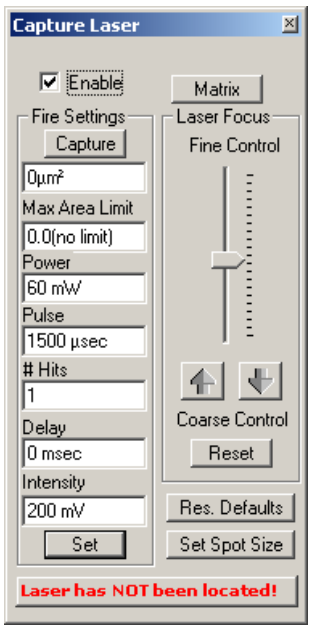
Delay – Sets the pause between each laser fire.

Intensity – Sets the degree of laser light for the targeting beam. If you can't see the laser beam when focusing, set **Intensity** to 200 mV, then turn it down once you've focused the beam. Click **Set** after entering the **Intensity** value.

Laser Focus – Allows you to use a fine control and coarse control to focus the laser beam. Click **Reset** to return the fine and coarse controls to their default values. For information on focusing the laser, refer to "Manually Focusing the Capture Laser Beam" on page 49.

Set Spot Size – Allows you to measure the spot size of the capture laser. The application uses this to control the movement of the stage during capture and to calculate the area of tissue that will be captured. For information on optimizing the laser spot size, refer to the following section, "Optimizing the Capture Laser Spot Size".

Laser Status – Click the **Laser has NOT been located** button to locate the capture laser. If the location of the capture laser is known, the text changes to "Laser has been located."



Capture Laser Tools set to show total area of regions to capture (Area Capture selected on Toggle Laser Tool menu)

Optimizing the Capture Laser Spot Size

To optimize the spot size for the capture laser, start with the following initial settings and test fire the laser to ensure adequate wetting (See "Manually Measuring the Capture Laser Spot Size" on page 51 for instructions on wetting the film).

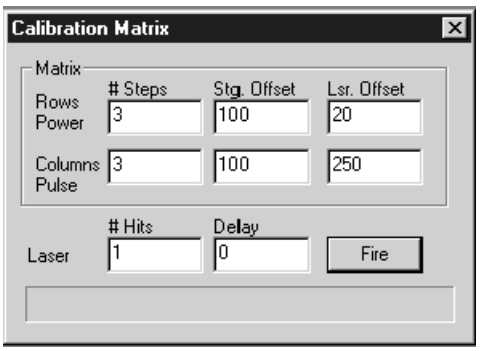
- For CapSure™ HS Caps start with **Power** at 60 mW and **Pulse** at 1500 µsec.
- For CapSure Macro Caps start with **Power** at 50 mW and **Pulse** at 1000 µsec.

For dissecting individual cells, you may want to use a very small spot size.

- *To minimize the spot size*, use the lowest **Power** setting that produces an adequate wetted spot. Sometimes it is easier to obtain smaller spots by using very low **Power** and **Pulse** settings and increasing the **# Hits** setting.
- *To increase the spot size*, start by increasing only the **Pulse** setting. Then, if necessary, increase the **Power**.

Using a Calibration Matrix to Determine Capture Laser Settings

The laser matrix option allows you to set up a test firing pattern to help you determine the appropriate settings for the capture laser to adequately wet the film. The settings in the Capture Laser Tools are used as a starting point, then modified based on the values you enter in the Calibration Matrix window.

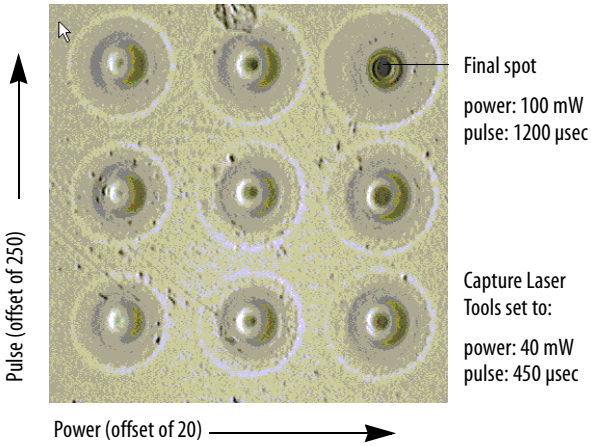


 # Steps sets the number of intervals (rows and columns) for the pulse and power parameters.

Stage Offset sets the distance (from center to center) between spots.

Laser Offset allows you to set the values at which the power and pulse will increment each step.

For the example shown in the Calibration Matrix window, if you set the **Lsr. Offset** for **Power** to 20, and the **Power** in the Capture Laser Tools is set to 40 mW, at three steps, you will get a laser hit at 60, 80 and 100 mW. If you set the **Lsr. Offset** for **Pulse** at 250, and **Pulse** in the Capture Laser Tools is set to 450 µsec, at three steps, you will get a laser hit at 700, 950 and 1200 µsec.



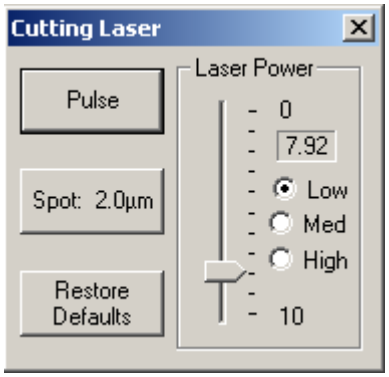
NOTE: You can use the **# Hits** and **Delay** in the Calibration Matrix window to override the settings in the Capture Laser Tools.

Enter the appropriate values, then click **Fire**. The laser will fire in a matrix pattern with three columns of increasing pulse (bottom to top) and three rows of increasing power (left to right).

Choose the **Power** and **Pulse** values that give the best wetting in the matrix and enter them into the Capture Laser Tools.

The Cutting Laser Tools

The Cutting Laser Tools allow you to make adjustments to the cutting laser, if your instrument is so equipped. Choose **Cutting Laser** from the Tools menu to view the tool.



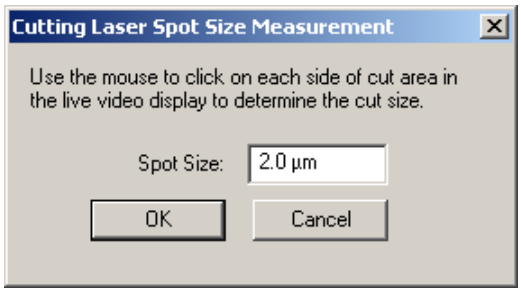
Pulse – Click this button to pulse the cutting laser.

Spot – Allows you to measure the spot size for the cutting laser; see "Measuring the Spot Size for the Cutting Laser", below. Once you have measured the spot size, the size is shown on the button.

Restore Defaults – Click this to restore the power and spot size to their default values.

Laser Power – Choose between **Low**, **Medium** or **High**. Use the slider for finer control of the laser power at the selected setting. The exact power is shown in the field above the radio buttons.

Measuring the Spot Size for the Cutting Laser



1. To measure the spot size, click **Spot** and enter a value for the spot size.

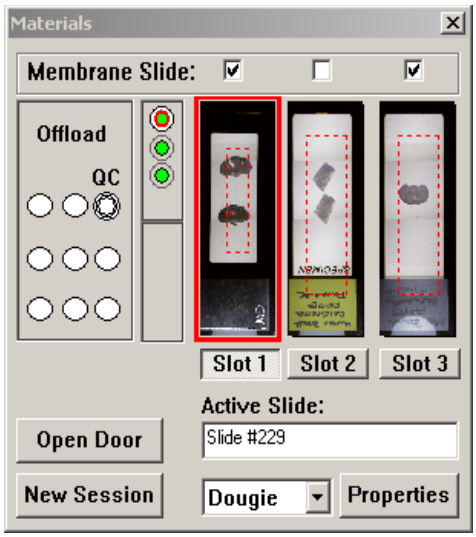
   – or –

   - Perform a cut.
   - Use the Ruler tool to measure the width of the cut. (Change to a higher objective if you need to see the cut more clearly.)
   - Click **Spot** and enter the width you measured with the ruler.

2. Click **OK** to save the changes and close the dialog box**.**

The Materials Tool

The Materials Tool allow you to see the locations and types of slides and caps on the work surface. Use the Materials Tool to activate slides and to move caps to the slides, the QC station or the unload tray. You can also open and close the instrument's door, rename slides, and change the study.



In the Materials Tool:

- A white slide represents a slide present but for which no roadmap has been acquired.
- A thumbnail of the slide represents a slide present for which a roadmap has been acquired.
- The active slide has a red border drawn around it.
- The boundary of the region where caps may be placed is shown by a red dotted rectangle. You cannot place the center of a cap beyond the dotted line.
- *To change the slide type,* check or uncheck **Membrane Slide** as appropriate.
- *To change the current study for the active slide,* choose the study of choice from the drop-down menu.
- *To open the instrument door,* click **Open Door**.
- *To start a new session*, click **New Session.** The instrument door will open, allowing you to remove any materials. Once you have done this click **OK** on the dialog box. The door will close, then open again. Load the new slides and/or caps, then click **OK** in the dialog box. The door will close and the Material Loading window will appear.
- *To change the properties the active slide,* click **Properties**. The Slide Properties dialog for that slide will appear. Make any changes and click **OK** to close the dialog. (See "Slide Properties" on page 40 for details.)

The Microdissection Tools

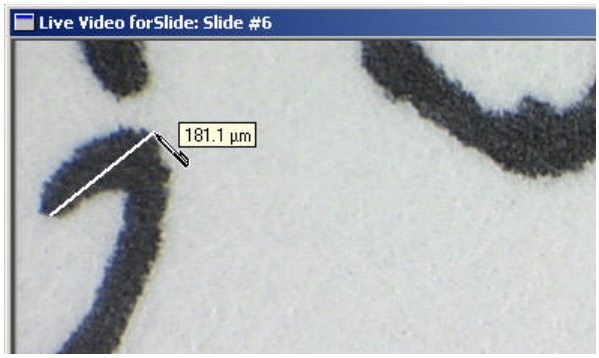
The Microdissection Tools allow you to designate cells for capture and initiate the capture process. Use these tools on either the live video image or a static image. If you are marking cells on a static image and save it, these dissection marks are also saved.

Common Tools

The following tools are common to all of the tabs: the **Hand**, the **Ruler**, **Go Capture** and **Autoscan**.

Hand – Allows you to move the stage by dragging in the live video window. Click on the tool and the cursor will change to a hand. in the live video window, click and drag. The stage will move as needed and the live video image will update to show the new area of the slide.

Ruler – Allows you to measure the length of an item on the screen. Click once at the end of the item, then drag the mouse to the other end of the item. The application draws a line on the screen and the length of the line is shown in a tool tip. Click again to reset the beginning of the line to the current location. When you deactivate the tool, the line disappears. This tool operates on the roadmap, the live video or a static image.



Go Capture – Allows you to cut and capture all the marked cells in one step. This button is enabled once you have a dissection mark on the live video image or a static image.

Autoscan– Allows you to train the system to automatically identify cells for capture. See "Introduction to Auto Scan on a Static Image" on page 55 for detailed instructions on this feature.

Using the Tools

- *To activate a microdissection tool*, click on the image then click on the tool to select it.
- *To deactivate the tool when you are finished*, click the tool again.
- *To switch between tabs*, use the arrow keys or click on the tab of interest.

The Cut and Capture Tab

If your system is equipped with a cutting laser (Models 703 and 704), the Cut and Capture tab is present. The tools in this tab allow you to mark areas where you wish to use the cutting laser to outline a region for capture.

Free-Form Cut and Capture Region – Allows you to outline a region to be captured. Click and drag to outline a region. When you release the mouse button, the region is automatically closed. The application displays an outline of the region to be captured as

well as any tabs (regions where the tissue won't be cut) and the positions of the capture laser spots.

To capture a region the same size as the cap, use the **Get Full Cap Area** command on the Image menu; for more details see page 64.

Circular Cut and Capture Region – Allows you to outline a circular region to be captured. Click and drag to outline a circular region. The application displays an outline of the region to be captured as well as any tabs (regions where the tissue won't be cut) and the positions of the capture laser spots.

See "Adjusting Capture Group Properties" on page 74 for more information on setting the tabs and the capture laser spots for dissection marks created using these tools.

The LCM Tab

The tools on this tab are used to choose which cells the capture laser will capture. Use these tools if you have only a few cells to capture.

These tools operate on both the live video image and a static image.

The dissection marks are displayed as spots, using the spot size of the capture laser. The diameter of the targeted point represents the diameter of the wetted area on the film, once the capture laser has fired. If you did not measure the spot size, the application uses 10 µm. (See "Manually Measuring the Capture Laser Spot Size" on page 51 for instructions on manually measuring the spot size.)

For all of the LCM Microdissection tools, you can adjust the overlap and shift of the capture laser spots (See "Adjusting Capture Group Properties" on page 74).

Single Point Dissection – Allows you to target cells individually for capture. Click to mark individual points on the image.

Dissection Line – Allows you to draw a line on the image to capture a layer or line of cells, for example, to capture an epithelial cell layer. Click and drag to draw the line. The line represents the cells that will be captured by the laser as it fires at each spot on the line.

Free Form Dissection Region – Allows you to draw an area on the image to identify an area to be captured. Click and drag to draw the region. Release the mouse to close the region.

Dissection Exclusion Region – Allows you to deselect a specific area that you don't want to capture within a region. Before creating the area of exclusion, first define the region to be captured with the Free-Form Dissection Region tool. ALT-click on the region to select it, then click on the tool and click and drag to draw the exclusion region inside the region.

The Ablation Tab

If your system is equipped with a cutting laser (Models 703 and 704), you will see the Ablation tab. These tools allow you to mark areas where you wish to ablate tissue using the cutting laser.

For all of the Ablation tools, the width of the lines is based upon the **Cut Width**, set in the Capture Group Properties dialog box, (See "Adjusting Capture Group Properties" on page 74).

Free Form Ablation Region – Allows you to outline a free-form region to be ablated. Click and drag to draw the region. When you release the mouse button, the region is automatically closed. The application displays lines inside the region to indicate the path of the cutting laser. (Depending upon the magnification, the region may appear filled.)

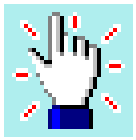
Ablation Exclusion Region – You can exclude cells or tissue from ablation within a region defined by the **Free Form Ablation Region** tool. To do this, first mark the region to be ablated. Then ALT-click on the region to select it, and then click on the tool and click and drag with the **Ablation Exclusion Region** tool to mark the area to be excluded from ablation.

Working with Dissection Marks

Once you create a dissection mark, you can move, hide, or delete it You can also show its properties.

- *To select a dissection mark*, hold down the ALT key and click the dissection mark.
- *To move a dissection mark*, ALT-click on it to select it, then drag it to a new location.
- *To hide a dissection mark*, ALT-click on it to select it, then right-click and select **Hide**. When the cursor is over a hidden annotation, it changes to a hand with red lines radiating from it. You can redisplay the dissection mark by right-clicking the area where the dissection mark is hidden and selecting **UnHide**.

  NOTE: Although you may hide a dissection mark from view, the selected cells will still be captured.



- *To delete a dissection mark*, right-click the dissection mark and select **Delete**. You cannot delete a dissection mark if you have already captured the cells.

  NOTE: To delete dissection marks created by the **Dissection Exclusion Region** and the **Ablation Exclusion Region** tools, you must delete *both* dissection marks.

- *To show the properties of a dissection mark*, right-click the dissection mark to select it and select **Properties** to view the Annotations Properties dialog box. See "Adjusting Dissection Mark Properties", below, for more information.

Adjusting Dissection Mark Properties

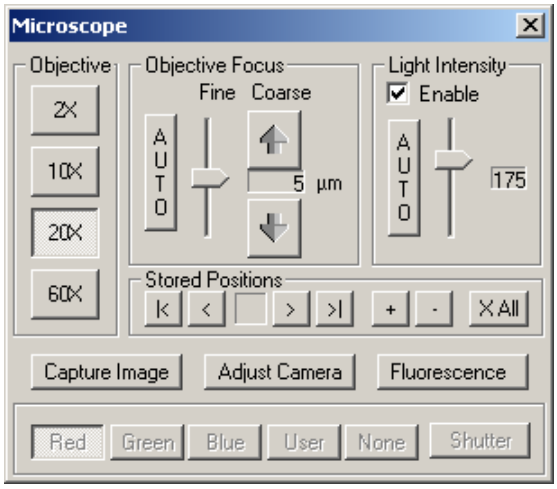
You can adjust various properties of a dissection mark. The properties of a dissection mark are a subset of the Capture Group properties, based upon the type of dissection mark you have selected.

For example, if you have created a dissection mark using one of the LCM tools, the properties of the cutting laser won't be shown because the cutting laser won't be used during microdissection.

See "Adjusting Capture Group Properties" on page 74 for information on the various properties for a dissection mark.

The Microscope Tools

The Microscope Tools allow you to adjust the image you see in the live video image. After adjusting the settings, you can save the microscope settings to a file by selecting **Save Defaults** from the File menu.



Set Objective – Allows you to select from the four objectives: **2x**, **10x**, **20x**, and **40x** (or **60x** if your instrument is so configured). You can also select the objective by choosing **Objective** from the Instrument menu and then the objective from the submenu.

The Veritas™ Microdissection Instrument is calibrated so the objectives are parfocal. If you focus at 40x (or 60x if your instrument is so configured) and then change the objective, the image should remain in focus.

NOTE: You **must** focus at the highest objective (40x or 60x) to take advantage of the parfocality. The depth of field for the higher objectives is narrower than for the lower power objectives.

Objective Focus – Provides auto focus, coarse, and fine adjustments. Click **AUTO** to automatically focus the microscope at the selected objective. When the fine control is active, you can also use the UP and DOWN arrow keys to make fine adjustments. The height of the objective above the slide is shown between the arrows for the coarse controls.

Light Intensity – Allows you to adjust the amount of light emitted by the white lamp. Check **Enable** to turn the light on and enable the slider. When the slider is active, you can use the UP and DOWN arrow keys on the keyboard to adjust the light intensity. When **Enable** is unchecked, the light is off and the live video image will appear black. Click **AUTO** to automatically set the brightness.

Stored Positions – Use these controls to store the locations of any areas of interest. Once you have stored the positions, you can easily navigate through them at whatever magnification you choose. See "Saving Positions of Interest" on page 84 for more information.

Capture Image – Click this to create a static image from the current live video image. The instrument will capture a static image and display it on the screen. The application draws a red box on the roadmap at the location of the static image.

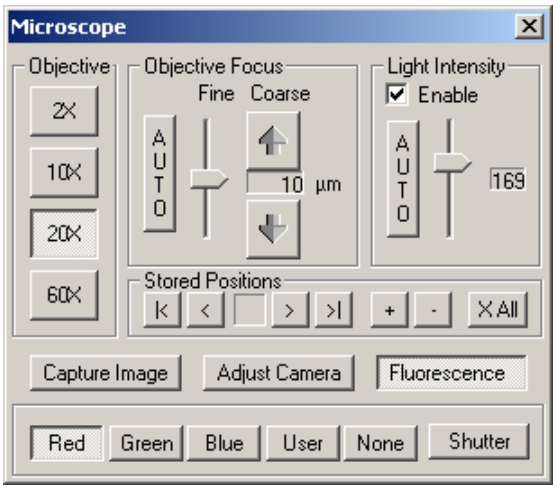
Adjust Camera – Click this to open the Camera Control dialog where you can override the default settings for the camera (see "Camera Control Dialog" on page 68). For most situations, you will not need to make changes to these controls.

Fluorescence – Click this to turn on the fluorescence lamp and enable the controls specific to epi-fluorescence. These controls are described below. If your system is not equipped for epi-fluorescence, this button is not enabled.

NOTE: It takes several minutes for the fluorescence lamp to warm up. When the lamp is ready, "EPI ready" appears in the lower right corner of the screen. If you turn off the fluorescence lamp, it must cool down sufficiently before the lamp controller will turn it on again. This may take as long as five minutes.

Additional Controls for Epi-Fluorescence

These controls are only available on Model 702 and Model 704 instruments and are only enabled when the **Fluorescence** button has been clicked. See "Microdissection of Fluorescent Samples" on page 52 for more information.



Microscope Tools for EPI-fluorescence

Filter buttons – Allow you to select between the red, blue, or green filter when using the fluorescence lamp. Choose **None** (no filter) to view the sample under white light. Choose the appropriate excitation filter when performing fluorescence LCM. If you have a UV filter installed, the **User** button will say UV.

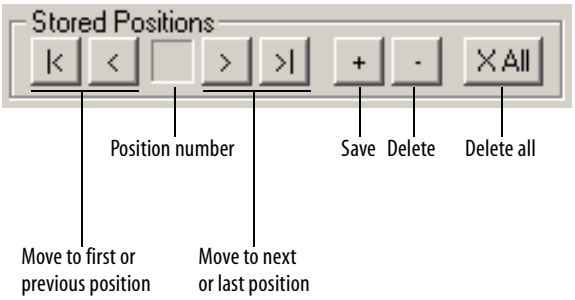
Shutter – Use this to open and close the shutter on the fluorescence lamp. When the button is depressed, the shutter is open. To avoid photo-bleaching the sample, close the shutter except when you are viewing the sample. When you are not viewing the sample, for example, after you have acquired a static image, click **Shutter** to close the shutter.

Saving Positions of Interest

The Microscope Tools allow you to save locations on the slide that are of interest at one magnification, and then easily find them at any other magnification. This operates only on the live video image.

Storing Positions

1. Navigate to a location of interest in the live video.
2. Click the **+** button in the Microscope Tools. The location is now saved.



Moving to Stored Positions

1. To move to the next position in the list, click the **>** button.

   – or –

   To move to the previous position, click the **<** button.

   – or –

   To move to either end of the list, click either **|<** or **>|**.

   The stage will move as needed to place the center of the saved location in the center of the live video image.

Deleting a Stored Position

1. Use the controls to move the stage to the saved location that you wish to delete.

2. Click the **–** button to remove the location from the list.

   – or –

   Click the **X All** button to delete all the stored locations in the list.

   The next time you save a location it is added to the end of the list.

Customizing Settings for the Laser and Microscope Tools

Each time you log in, the application automatically sets the parameters for the Laser and Microscope Tools to the values in your default settings file. If you have never saved the settings, the default file (named SystemDefault), is automatically loaded.

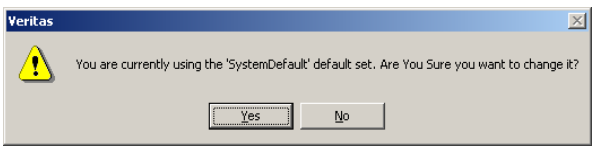
If you wish to change the settings for either or both tools, and make them the new defaults, you may do so. You may also wish to create settings files for each type of tissue, or for fluorescence and non-fluorescence work or for some other reason. Using a saved settings file lets you quickly set up your system for an LCM session.

NOTE: The settings file in use when you log out becomes the new default file for your next LCM session.

Saving Default Settings

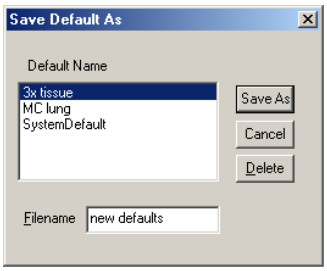
1. Choose **Save Defaults** from the File menu when you want to save the laser and microscope settings.
   The settings file that is currently loaded is automatically updated with the current settings.

   - *If you are an administrator*, and you are currently using the SystemDefault settings, an error message appears asking if you want to overwrite the file. (This message also appears if you have selected the **Auto Save Default** option in User Preferences; see "Setting User Preferences" on page 36.) If you choose **Yes**, the new laser and microscope settings will be used as the SystemDefaults for the entire system.



To avoid overwriting the SystemDefaults, choose **No** and then choose **Save Defaults As** from the File menu. Enter a file name and click **Save As**.

- *If you are not an administrator*, you will not be allowed to overwrite the SystemDefaults file. If you want to save the settings, choose **Save Defaults As** from the File menu. Enter a file name and click **Saves As**.

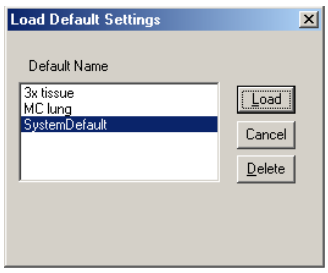


Loading Saved Settings from a File

If you wish to use a previously saved settings file, rather than the default file, load that settings file. You might wish to do this, for instance, if you have different settings for each type of tissue you study.

1. Choose **Load Defaults** from the File menu at the beginning of your LCM session.

2. Choose the file withe the laser and microscope settings you want to load and click **Load**.
   If you log out without loading another settings file, this file will become the default. (The settings file last in use, before you log out, become the default for your next session.)

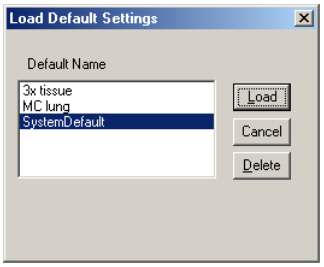


Deleting Default Settings

1. Choose **Save Defaults As** from the File menu.

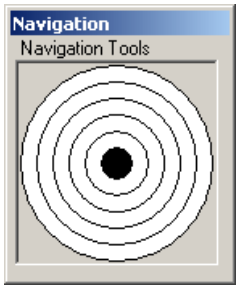
2. Choose the settings you want to delete and click **Delete**.

3. Click **Yes** in the confirmation dialog box to delete the settings.



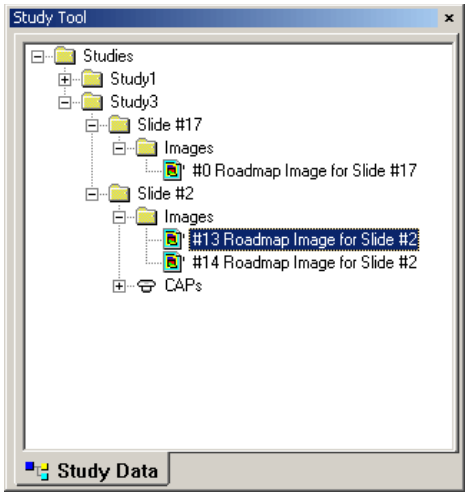
The Navigation Tool

The Navigation Tool provides another way to move the live video image to look for areas to capture. Click the black dot in the center of the bull's-eye and drag in any direction towards the edge. Each time you move the black dot, the stage moves. The speed of the movement is proportional to the distance the black dot is pulled away from the center of the Navigation Tool. When you release the mouse button, the black dot snaps back to the center point and the work surface movement stops.



The Study Tool

The Study Tool allows you to see how study data is organized and saved, and to access study, slide, cap and image data. You can see and access only the data for studies that you created.



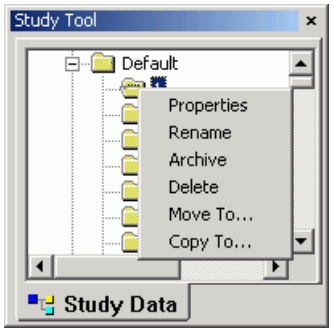
- *To activate the window*, click in the white area where the tree diagram appears.
- *To display the properties for a slide, an image or a cap*, right-click on the item and choose **Properties**.
- *To open an image*, double-click the name or right-click and choose **View**.
- *To delete a study, a slide or an image*, right-click the object and choose **Delete**.
- *To rename a study or a slide*, right-click the object and choose **Rename**.

  NOTE: You cannot rename the active slide.

In the Study Tool window, you can manage study data in various ways, described below.

Moving a Slide to Another Study

To move a slide from one study to another:



1. Right-click on the slide you wish to move and choose **Move to**.
   A dialog box appears, allowing you to choose a study.
2. Click on the study of choice to select it and click **OK.**
   The slide is moved to the selected study.

   If a slide already exists with this name, a dialog box appears giving you the chance to rename the slide. Enter a new name and click **OK**.

Copying a Slide to Another Study

To copy a slide from one study to another:

1. Right-click on the slide you wish to move and choose **Copy to**.
   A dialog box appears, allowing you to choose the study where you wish to move the slide.
2. Click on the study of choice to select it and click **OK**.
   A copy of the slide is made and placed in the selected study.

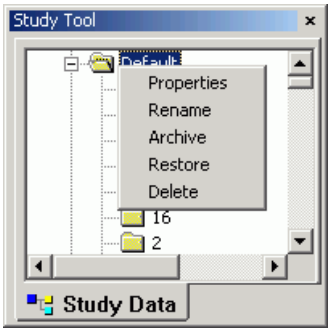
   If a slide already exists with this name, a dialog box appears giving you the chance to rename the slide. Enter a new name and click **OK**.

Archiving Study Data

You may wish to archive study data to a another place. You may archive all studies, a selection of studies, a slide or a selection of slides.

1. Click on the item you wish to archive.
   To select more than one item at a time, use CONTROL-click when selecting.

2. Right-click and choose **Archive**.
   A dialog box will appear where you can select the destination for the archive.



3. Click **OK** to save the archive.

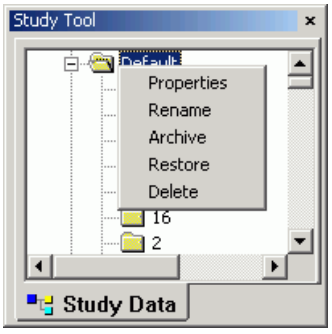
   The application will display a message when the archiving process is complete.

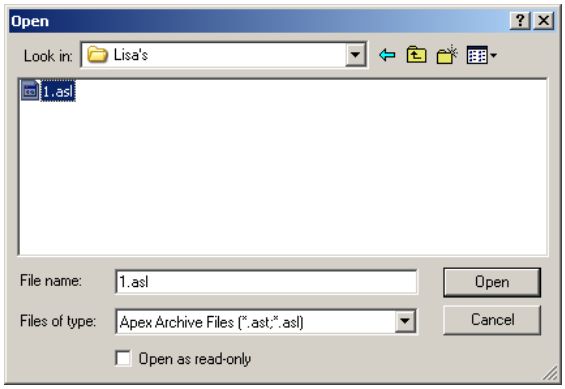
Restoring Archived Study Data

You may wish to restore study data that has been archived. You may restore all studies, a selection of studies, a slide or a selection of slides.

1. In the Study Tool, click on the study where you wish to add the restored data.

2. Right-click and choose **Restore**.
   A dialog box will appear where you can choose the archived study data that you wish to restore. Studies have the file extension ".ast" and slides have the extension ".asl".





3. Choose the data to be restored and click **Open**.
If you are restoring a study and the name of any study you are restoring is the same as an existing study, you will see a dialog box informing you of the conflicting names.

Choose one of the following options and then click **OK** to begin restoration:

- *To add the contents of the study to the study of the same name,* choose **Merge**. If any names in the two studies are the same, the names of the archived items are made unique by adding the date and time after the name, e.g. Slide #37 becomes Slide #37 [20040401 15.58.07.0363].
- *To overwrite the items in the study with the items from the archive,* choose **Overwrite**.

  NOTE: This option will replace the newer item with the item from the archive.

- *To add the archived items and make their names unique*, choose **Make Unique**. The names are made unique by adding the date and time after the name, as above.

4. If you are restoring a slide or have chosen to merge a study, you may have slides with the same names. As before, the application will display a dialog box informing you of this. Choose one of the following options and click **OK**.

- *To add all the slides to the existing study,* choose **Merge**. If any names are the same, the names of the archived items are made unique by adding the date and time after the name, e.g. Slide #37 becomes Slide #37 [20040401 15.58.07.0363].
- *To overwrite the slides in the study with the slides from the archive,* choose **Overwrite**. As above, this option will replace the newer item in the study with the archived version.
- *To add the slides from the archive and make their names unique,* choose **Make Unique**. The names are made unique by adding the date and time after the name, as above.

The application will display a message when the restoration is complete.

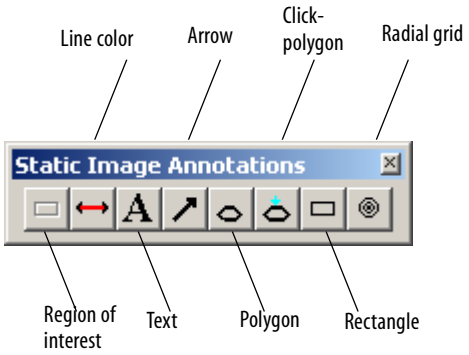
Viewing and Arranging Tools

You can show or hide the tools by selecting the appropriate item from the Tools menu.

Arrange the tools the way you want them on the desktop. The next time you log into the application, the tools will appear where you left them when you last logged out.

The Static Image Annotations Toolbar

The Static Image Annotations Toolbar contains tools that allow you to add text and graphics to a static image (these are referred to as *annotations*). If you save the static image, the annotations are also saved.



Region of Interest – Used to define the area of interest on the roadmap when creating a static image. Click and drag to define the region of interest. See "Acquiring a Static Image" on page 45 for information on using this tool.

Line Color – Allows you to change the line color of static annotations for anything drawn this session. You can also change the color for a specific static annotation by right-clicking it and selecting **Properties**. In the resulting dialog box, click the Line Properties tab and make the changes.

Text – Allows you to type text on the static image. Click and drag from the upper left to the lower right to draw a text box, then click again on the Text tool to turn it off. Finally, click in the box to type the text. Use the Annotations Font Toolbar to change the font, its size and style; see "The Annotations Font Toolbar" on page 93.

Arrow – Allows you to draw an arrow on the static image. Click to create the head of the arrow, then drag in any direction to create the other end of the arrow. You can adjust the position of the arrow by right-clicking it, selecting **Adjust Arrow**, then clicking and dragging the end you want to move.

Polygon– Allows you to draw a closed region on the static image. Click and drag to draw the region then release the mouse button to close the region.

Click-Polygon– Allows you to draw a closed region on the static image. Click at the starting point, then click to form each side of the region then double-click the mouse button to close the region.

Rectangle – Use this tool to draw a rectangle on the static image. Click to place the upper left corner, then drag to the lower right to form the rectangle.

Radial Grid – Places a radial grid on the static image. Click once to place a radial grid on the static image. After creating the radial grid, you can change the line width and color, number of rings and the spacing between rings to use the radial grid as a scale (see "Adjusting Radial Grid Properties" on page 92).

Moving the Static Image Annotations Toolbar

The toolbar floats in the application window as do all the other tools. You can drag it directly under the menu bar to dock it, if desired.

- *To move the toolbar*, click on the border and then drag it to the desired location.

- *To change the toolbar's dimensions*, hold the mouse over the edge of the toolbar's border until the cursor changes to a double-headed arrow, then click and drag to change the width.

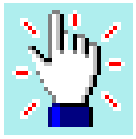
Adding Annotations to a Static Image

1. Click on the static image to activate the Static Annotations Toolbar.
2. Click on the individual tool to activate it.
3. Draw on the image as desired.
4. Click the tool again to turn it off.

Working with Annotations

Once you create an annotation, you can move, hide, or delete it You can also show its properties.

- *To move an annotation*, move the mouse over the annotation, hold down the ALT key and the left mouse button then drag it to a new location on the static image.
- *To hide an arrow annotation*, right-click it and select **Hide**. When the cursor is over a hidden annotation, it changes to a hand with red lines radiating from it. You can redisplay it by right-clicking the area where the hidden annotation is and select **UnHide**.



- *To delete an annotation*, right-click the annotation and select **Delete**.
- *To show the properties of an annotation*, right-click the annotation and select **Properties** to view the Annotations Properties dialog box. See "Adjusting Annotation Properties", below, for information on changing the annotation properties.

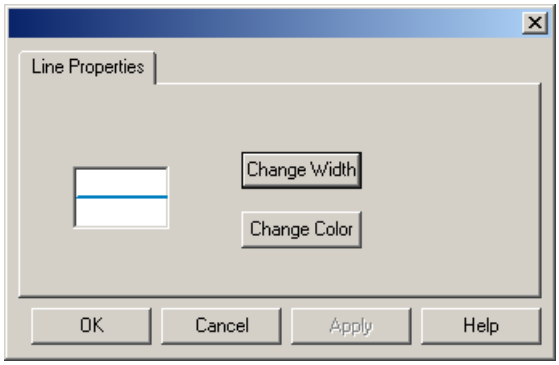
Adjusting Annotation Properties

You can adjust various attributes of an annotation.

Adjusting Color and Line Width

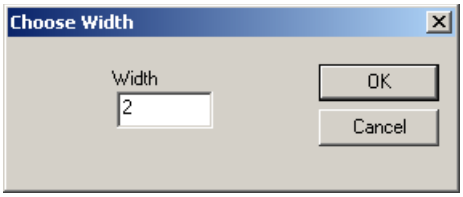
You can change the color and line width of the line, rectangle, region and radial grid annotations.

1. Right-click the annotation and choose **Properties**. Depending on the annotation you selected, the dialog box will vary.
2. If necessary, click the Line Properties tab.

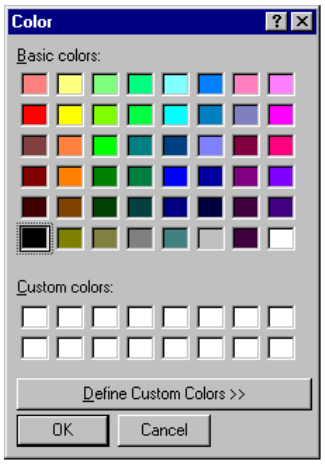


3. You can make the following line adjustments:

- *To change the line width*, click **Change Width**, enter a number from 1 to 10, and click **OK**. Click **OK** in the Line Properties dialog box.



- *To change the line color*, click **Change Color**, select a color in the color dialog box, and click **OK**. Click **OK** in the Annotations Properties dialog box.



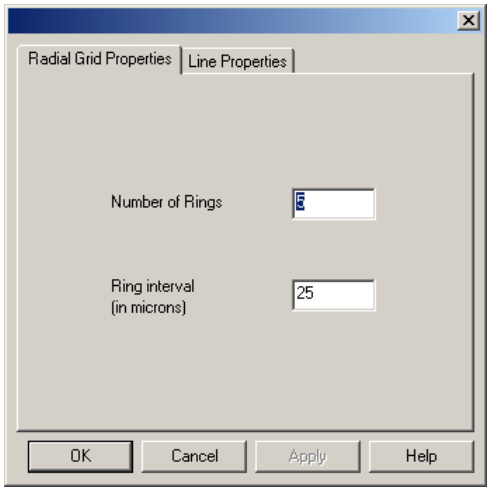
Adjusting Radial Grid Properties

You can change the number of rings, and their interval, for radial grid annotations. You can also change the line width and color of the rings.

1. Right-click a radial grid annotation and choose **Properties** from the menu.

2. You can make the following adjustments to the radial grid:

- *To change the number of rings*, enter a number, then click **Apply** or **OK**.
- *To change the distance between rings*, enter the distance (in microns) between rings, then click **Apply** or **OK**.
- *To change the Line Properties*, click on the Line Properties tab and make changes as described in "Adjusting Color and Line Width", above.



3. Click **OK** to close the dialog and save changes.

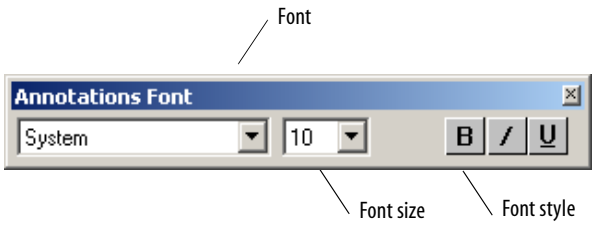
The Annotations Font Toolbar

You may change the font, style, size and/or color for a text annotation by selecting the corresponding option in the Annotations Font Toolbar.

To make font changes, do one of the following:

- Click the font text box on the annotation, then select the appropriate font feature from the Annotations Font Toolbar.
- Right-click on the annotation text and select **Change Font** to display the standard Windows font dialog box, which allows you to make all of the available font changes, including color.

As with the Static Image Annotations Toolbar, the Annotations Font Toolbar can be dragged from its default location to be docked under the menu bar, if so desired.



7. Maintenance and Troubleshooting

▲**CAUTION:** Do not attempt to remove the covers from the instrument except as specified for the user-serviceable parts described on page 100. If the instrument requires service or repair contact Arcturus Bioscience at 1 (888) 446-7911.

Cleaning the Veritas Instrument

- Clean the outside of the instrument using a damp cloth. Do not use any solvents or abrasives.
- Clean the work surface, as necessary, by wiping it with a cloth moistened with ethanol.

Troubleshooting

If you encounter a problem that you cannot resolve using this troubleshooting section, contact Arcturus Bioscience in North America at 1 (888) 446-7911, or in Europe at [00] (800) 2728-8787. You can also send email to techsupport@arctur.com or contact your local distributor.

To minimize the time to diagnose and correct your problem, you may be asked to perform all or a portion of the Operational Qualification Procedure by Arcturus Bioscience technical support. This procedure is found in document #13801-00 on the Veritas installation CD.

Starting the Application

Database errors given.

- Upon starting the program, the application creates backup copies of the application's databases (where the images and user information are stored) for safekeeping. After the copy, the application compresses the databases. This is done to improve performance during the session.

  If the compression fails, the application will display the message "An Error Occurred While Compressing Database. You Can Continue With Normal Operations.". The system will then attempt to automatically restore the databases from the backup copies.

  If the restoration fails, the application will display the message "SERIOUS FAULT: The database [Database File Name] does not exist. You Can NOT Continue With Normal Operations, the application will exit after you click OK. CONTACT TECHNICAL SUPPORT". In this case, you will need to manually restore the databases.

  To do this, exit the Veritas application. Locate the backup databases in the C:\APEX_Databases folder. They will have the ".bak" file extension. Copy the backup files to a different

location on your hard drive. Rename the files in the C:\APEX_Databases folder by removing the ".bak" from the name. Launch the Veritas application.

Images

Roadmap image appears white.

- The lamp intensity it set too high. Reacquire the roadmap image by right-clicking on the slide in the Materials Tool. Select **Acquire Roadmap Image**, then click **Yes** to readjust the light intensity.

The live video image appears white.

- The area of interest you are viewing is clear glass and not tissue. Click and drag the red view indicator on the roadmap to an area of tissue.

The live video image appears black.

- The lamp intensity is set too low. Use the Microscope Tools to turn up the lamp intensity.
- The objective turret failed to index properly. Open the door and manually rotate the objective to the set objective position.

Roadmap and live video windows are blank.

- The computer was in "Hibernate" or "Standby" mode, due to a lack of activity. Exit from the application and restart the computer. To prevent this in the future, disable **Hibernate** in the Power Options Properties control panel and change **System standby**.

  To make these changes to your system, log in to an account with Administrator privileges. Right-click on the desktop and select **Properties -> Screen Saver -> Power**. Click the **Hibernate** tab. In the tab, uncheck **Enable hibernate support**. Click on the **Power Schemes** tab and set **Select System standby** to Never. Click **OK** to close the dialog box.

Capture

Cells do not adhere to the film.

- Ensure wetting is adequate. Check the wetting (See "Test Firing the Capture Laser" on page 50).
- The cap is not seated properly on the tissue. Try firing the laser at four points around the edge of the capture area of the cap (up, down, right, left) to see if the wetting is consistent. If necessary, move the cap to a new location.
- Ensure your tissue preparation technique is correct (see "Tissue Preparation" on page 17). If necessary, dehydrate the sample in 100% EtOH for 1 minute, followed by xylene for 5 minutes.

Cap is moved randomly.

- Turn off **Auto Cap Placement** in the User Preferences (see "Setting User Preferences" on page 36) and use a new cap.

Capture Laser

Cannot find the capture laser targeting beam.

- A cap has not been placed on the slide. You must place a cap before you can see the laser beam.
- You may need to adjust the shutter speed and dim the background lighting to see the laser-targeting beam. Use the slider control under Lamp Intensity in the Microscope Tools to lower the lamp intensity. If the laser is difficult to see even at low light intensity, reduce the camera shutter speed to 1/50. See "Camera Control Dialog" on page 68 for instructions on setting the shutter speed.
- Change to a lower objective to see a larger field of view and re-focus the laser.
- Tissue is very thick or dark. Move to thinner tissue or an empty area of the slide.
- Laser is out of focus. Focus the laser beam (See "Manually Focusing the Capture Laser Beam" on page 49).
- Laser targeting beam is too weak to see. Turn the white light off. Increase the Laser Intensity on the Capture Laser Tools and click **Set**.
- You can try firing the laser to locate the beam. Fire within the area of the cap—away from tissue if using a Macro Cap, or outside the circle if using an HS Cap.

"Laser has not been located" message appears.

- Locate the laser beam as described in "Manually Locating the Capture Laser" on page 50.

Capture laser beam is fuzzy.

- The laser is not focused. See "Manually Focusing the Capture Laser Beam" on page 49.
- A cap was not placed. Place a cap.
- The cap may be damaged. Try placing another cap.

Capture laser will not fire.

- The laser is not enabled. Click the **Enabled** check box at the top of the Capture Laser Tools window.

  NOTE: You may not always see the flashes from the laser beam as it fires since they are shorter than many of the shutter settings used. If the laser has been properly focused you will see the laser wetting. If in doubt, use the **Light Intensity** slider in the Microscope Tools to darken the background and move to a position on the slide where there

is no tissue. (If the laser is difficult to see even at low light intensity, reduce the camera shutter speed to 1/50. See "Camera Control Dialog" on page 68 for instructions on setting the shutter speed.) Fire the laser again, then adjust the light intensity to the previous illumination.

Capture laser fires off target.

- The laser beam location was set inaccurately or the objective was changed. Relocate the laser beam as described in "Manually Locating the Capture Laser" on page 50.
- Measure the spot size, then adjust the size, if necessary.

Cannot achieve proper wetting when capture laser is fired.

- Ensure the laser settings are correct (See "Manually Focusing the Capture Laser Beam" on page 49.).
- The cap is not seated properly on the tissue. Try firing the laser at four points around the edge of the capture area of the cap (up, down, right, left) to see if the wetting is consistent. If necessary, move the cap to a new location.
- Tissue may have folds or an uneven surface. Make sure the slide has been treated with a PrepStrip. If the problem occurs, move to a different section of tissue. If the problem occurs regularly, check the sample quality, microtome blade, and sectioning techniques.
- The cap may be damaged. Try another cap.

Cutting Laser

Laser does not cut.

- Select the Cutting Laser Tools from the Tools menu. Increase the power and click **Pulse** to fire the laser. You should see a small (1-5 microns) hole in the tissue in the center of the live video window. If not, increase the power again.

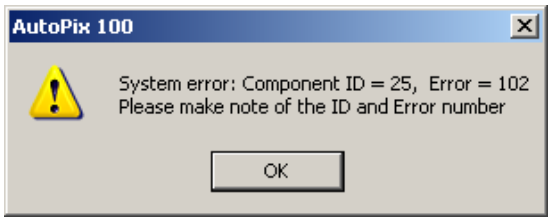
Auto Scan

Application worked when training but not on new tissue sections.

- Application needs more training information. Create multiple training files using various tissue sections. Combine the files to create a file that incorporates all of the information.

System Error

- If you see a system error, please make a note of the ID and error numbers. (In the example, ID = 25 and Error = 102.) Arcturus Technical Support can use this information to determine the severity of the problem. A system error is typically followed by an instrument error message. The type of message indicates the degree of severity, as discussed below.

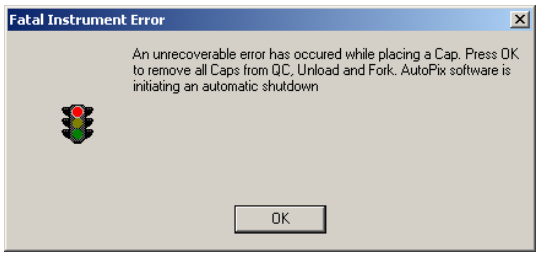


Fatal Instrument Error

A fatal instrument error may indicate a serious issue. However, it may just mean that a cap was dropped during handling. Please note the two example messages to the right and the discussion below.

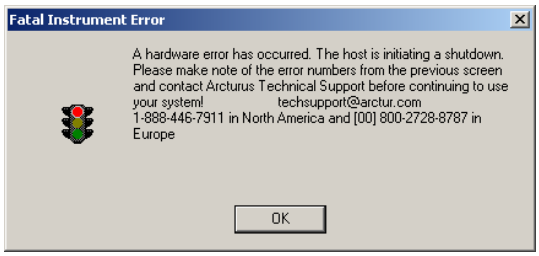
Cap Handling Error

- See the message to the right. In this case, the instrument door will open once you click **OK**. Please remove all caps from the QC and the unload tray and check the work surface for any loose caps. You can immediately restart the application and continue working.
  Contact Arcturus Technical Support if this persists.



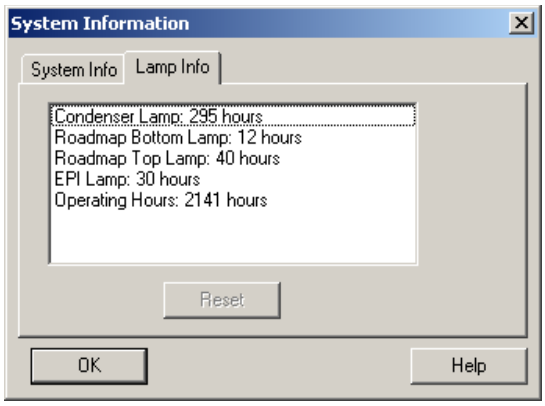
Other Fatal Instrument Errors

- See the message to the right. If you see this message, please contact Arcturus Technical Support before continuing to use the Veritas Microdissection Instrument.



Checking Lamp Hours

Each of the lamps in the instrument has a finite lifetime. Check the accumulated hours as follows:



1. Select **System Info** from the Help menu to display the Veritas About Box.
2. Click **System Info**. In the System Information dialog, click **Lamp Info**.
   The accumulated hours for each lamp is shown in the dialog box.
   - The fluorescence lamp's typical lifetime is 1500 hours. At 4000 hours, the lamp will not strike.
   - The bright-field illumination lamp (listed here as "Condenser Lamp") typical lifetime is 100 hours.
3. Click **OK** to close the dialog.
4. Click **OK** to close the Veritas About Box.

User Serviceable Parts

The Veritas™ Microdissection Instrument has only four user-serviceable parts:

- the bright-field illumination lamp
- the user-interchangeable fluorescence filter cube, for Model 702 and Model 704 instruments
- the fluorescence lamp, for Model 702 and Model 704 instruments
- the fuse

Other than replacing these components, and for transport, unpacking or operational qualification as per Arcturus Bioscience instructions, the covers should not be removed from the instrument unless authorized by a qualified Arcturus Bioscience service representative.

Replacing the Bright-Field Illumination Lamp

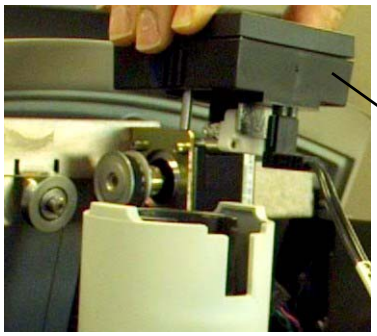
After replacing the lamp, you must also reset the counter for the lamp hours. Follow the directions below.

You will need the replacement lamp, a #2 Phillips screwdriver and clean, powder-free gloves for this procedure.

1. If needed, exit the Veritas application. Turn the instrument off and unplug it. (When you are facing the instrument door, the switch and plug are located on the left side, near the back.)
2. Facing the back of the instrument, remove the shaped top cover of the instrument. Near the center of the instrument, loosen the two screws that hold the cover. Slide the cover

towards you a few inches, then lift it up and off. Place the cover in a safe location.

3. Pull the black lamp housing vertically up and out. Pull the used bulb vertically out of the socket.



▲CAUTION: Hot Surface!

The lamp housing may be hot.

▲CAUTION: Possible Pinching!

Keep fingers clear of the door mechanism.

4. Wearing clean, powder-free gloves, open the new lamp. Plug the lamp into the socket and replace the lamp housing into the instrument.

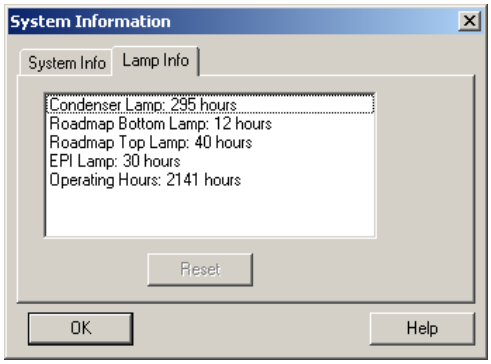
5. Replace the shaped top cover, sliding it forward to seat it into the front cover, then tighten the screws.

6. Plug the instrument in and turn it on.

7. Restart the Veritas application and log in.

8. Select **System Info** from the Help menu to display the Veritas About Box.

9. Click **System Info**. In the System Information dialog, click **Lamp Info**.



10. Double-click on the row that says "Condenser Lamp" to select it, then click **Reset**.
This resets the lamp hours to 0.

NOTE: Be careful to select the correct row. If you reset the wrong lamp, the counter for that lamp will be incorrect.

11. Click **OK** to close the dialog box.

12. Click **OK** to close the Veritas About Box.

Interchanging Fluorescence Filter Cubes

When selecting alternate fluorescence filter cubes, you should ensure the dichroic and emission filters have at least 65% transmission at 810nm. Compatible filter holders include the

Chroma #91018 Olympus Filter Cube and the Omega XC113 Olympus Cube.

You will need a 2 mm hex key and the filter cube of choice for this procedure.

1. If needed, exit the Veritas application. Turn the instrument off and unplug it. (When you are facing the instrument door, the switch and plug are located on the left side, near the back.)
2. Slide the door up to open it. (You must manually hold the door open for the rest of the procedure.)
3. Unscrew the two screws on either side of the black sheet metal dust shield. Lift the dust cover straight up, then out of the instrument. Place the cover in a safe location.
4. Loosen the set screw for the filter you wish to remove with the 2 mm hex key. Slide the filter cube straight up.
5. Slide the new filter down until it is seated. Check the orientation of the filter to be sure it matches the other filters, then tighten the set screw until it is snug.
6. Replace the dust shield, tightening the screws by hand until snug. The door will close when you release it.
7. Plug the instrument in and turn it on.

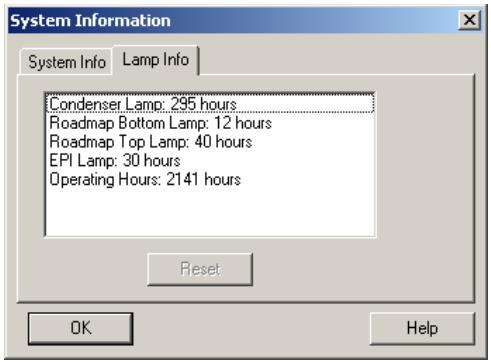
Replacing the Fluorescence Lamp

You must first remove the access panel before you can replace the lamp. Once you have replaced the lamp, you will need to reset the counter for the lamp hours.

You will need the new fluorescence lamp, a 3mm hex key (provided with the instrument) and a slotted screwdriver for this procedure.

1. If needed, exit the Veritas application. Turn the instrument off and unplug it. (When you are facing the instrument door, the switch and plug are located on the left side, near the back.)
2. Facing the back of the instrument, unscrew the two thumbscrews on the upper right side to remove the access cover. (You may need to use the slotted screwdriver.) Pull the cover back and remove it.
3. Remove the 3mm hex key from the clip in the access cover. Place the cover in a safe location.
4. Follow the steps in "Installing the Lamp Module" on page 112 to replace the lamp itself.

5. Replace the access cover and tighten the screws.
6. Plug in the instrument and turn it on.
7. Restart the Veritas application and log in.
8. Select **System Info** from the Help menu to display the Veritas About Box.
9. Click **System Info**. In the System Information dialog, click **Lamp Info**.
10. Click on the row that says "EPI Lamp" to select it, then click **Reset**.
    This resets the lamp hours to 0.
    NOTE: Be careful to select the correct row. If you reset the wrong lamp, the counter for that lamp will be incorrect.
11. Click **OK** to close the dialog box.
12. Click **OK** to close the Veritas About Box.



Replacing the Fuse

You will need the new fuse (6A, time delay, 5 x 20 mm) and a slotted screwdriver for this procedure.

1. If needed, exit the Veritas application. Turn the instrument off and unplug it. (When you are facing the instrument door, the switch and plug are located on the left side, near the back.)
2. The fuse is located on the left side of the instrument, between the switch and the power cord. Use the slotted screwdriver to open the fuse holder.
3. Remove the fuse and replace it with the new one. As you are facing the switch, the fuse goes into the right side of the holder. Slide the fuse holder into the instrument and flip the cover up.
4. Plug in the instrument and turn it on.

Specifications

Veritas™ Instrument

Electrical Supply:	100–240 VAC, 50–60 Hz, 600W (voltage fluctuations not to exceed ±10% of nominal supply voltage)
Fuse:	6A, time delay, 5 x 20 mm
Capture Laser:	laser diode, 810 nm
Cutting Laser: (Models 703 & 704 only)	Diode-pumped solid-state UV laser, 349 nm

Filters: (Models 702 and 704 only)	Color	Excitation	Emission
	Red:	570–630 nm	>655 nm
	Green:	503–548 nm	>565 nm
	Blue:	455–495 nm	>510 nm
	Optional		
	UV:	340–390 nm	>410 nm

Bright Field Illumination:	120W, 1500 hour metal halide fluorescence lamp (Arcturus catalog number 10124-00)
Fluorescence Light Source: (Models 702 & 704, only)	EXFO X-Cite™ 120 fluorescence illumination system
Operating Temperature:	18°–30°C
Operating Humidity:	≤60% relative humidity (noncondensing)
Dimensions:	Height: 30 in. (76 cm) Width: 36 in. (92 cm) Depth: 27 in. (69 cm)
Weight:	265 lb (120 kg)
Work Surface Requirements:	38 in. x 72 in. (97 cm x 180 cm) with 32 in. (80 cm) vertical clearance
Altitude:	For use up to 6600 ft. (2000 m)

The Veritas instrument complies with the following standards:

- IEC 664 – Installation Category II
- IEC 664 – Pollution Degree 2

- EN 60825-1 and IEC 60825-1 – Safety of laser products – Part 1: Equipment classification, requirements, and user's guide, Section Two-Manufacturing requirements
- EN 61326-04:1997+A106:1998+A2:2001 (Emissions and Immunity) – Electrical equipment for measurement, control, and laboratory use – EMC requirements
- EN 61010-1 and IEC 61010-1 – Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General Requirements.

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Computer

- 2.8 GHz Pentium 4 processor (minimum)
- 40 GB hard drive (minimum)
- Windows® XP Professional operating system
- Read/write CD drive
- Flat panel LCD monitor, 1280 x 1024 pixels, true color

Appendix A - Keyboard Commands

The following table lists the keyboard commands in the Veritas application.

Window	Keys	Notes
Microscope Tool	Arrow keys (UP, DOWN, RIGHT and LEFT)	Adjusts Fine Focus
Live Video	Arrow keys	Move stage in the direction of the arrow key
	CTRL+arrow key	Move stage 1/2 frame in the direction of the arrow key
	ALT+CLICK	Select dissection mark or annotation
	ALT+X	Turn cutting laser on and off
	CTRL+SHIFT	Turn cutting laser on while keys are depressed
Any editable text field	CTRL+C	Copy text
	CTRL+X	Cut text
	CTRL+V	Paste text
	CTRL+U	Undo last text edit
	ESC	Exit text field with no changes
Capture Groups Tool	UP and DOWN arrow keys	Move through list of capture groups and make group active
	RIGHT and LEFT arrow keys	Move cursor through capture group columns
Roadmap	Arrow keys	Move stage in the direction of the arrow key
	CTRL+arrow key	Move stage 1/2 frame in the direction of the arrow key

Window	Keys	Notes
Cutting Laser Tool	Arrow keys (UP, DOWN, RIGHT and LEFT)	Change cursor position
Any	F1 or SHIFT-F1	Opens PDF version of this manual
	CTRL+N	Start new session
	CTRL+D	Load default settings
	CTRL+Y	Create new study
	CTRL+U	Open User Preferences dialog
	CTRL+S	Show all tools (unless active window is a tool)
	CTRL+H	Hide all tools (unless active window is a tool)
Any tool or the roadmap	CTRL+P	Prints 1”x 3” version of roadmap
A static image	CTRL+P	Prints a thumbnail (1” x 1 1/4”) of the static image

Appendix B - Fluorescence Lamp Information

This appendix provides information about the EXFO X-Cite 120 fluorescence lamp supplied as with Model 702 and Model 704 instruments, including instructions on changing the lamp.

Safety Precautions

The EXFO X-Cite 120 is equipped with two safety sensors to protect the user from accidental UV exposure. In addition, please observe the following precautions during use. This series of cautions, warnings and dangers relate to the operation and maintenance of the EXFO X-Cite 120. They are also presented throughout this chapter where necessary.

Warning

Eye damage may result from directly viewing the light produced by the lamp used in this product. Always use protective eye wear and turn the lamp off before removing cover.

Caution

Never look into the light emitting end of the light guide. The light could severely damage the cornea and retina of the eye if the light is observed directly. Eye shielding must be used at all times as well as clothing to protect exposed skin.

Warning

Always make sure the light guide is properly inserted into the EXFO X-Cite 120 and the microscope prior to turning on power to the unit. This will minimize the risk of exposure to the UV light.

Warning

To reduce the risk of fire or shock, always replace the fuses with the same type and rating.

Warning

Disconnecting of main supply source is only possible by unplugging the power cord.

Danger

This unit contains HIGH VOLTAGE components. It is recommended that ONLY QUALIFIED TECHNICAL PERSONNEL perform any testing or repairs described in this manual. Disconnect the AC power cord from the unit before opening the cover of this unit. All cover screws must be replaced prior to applying power to the unit, or safety of the unit will be impaired.

Monitoring the unit during manual operation

The level of UV energy supplied by the EXFO X-Cite 120 is sufficient to ignite flammable substances. During manual operation, the unit must be attended at all times by a qualified operator. The unit must not be left unattended while turned on. If an operator leaves the work area of the unit, the lamp power switch must be turned off.

Monitoring the unit during automated operation

The level of UV energy supplied by the EXFO X-Cite 120 is sufficient to ignite flammable substances. Therefore, when the unit is operated unattended in an automated environment, an alarm function must be provided by the user to indicate a malfunction in the associated equipment used.

Warning

Hg – LAMP CONTAINS MERCURY!

Manage in Accord with Disposal Laws, see: http://www.lamprecycle.org or 1-800-668-8752.

Danger

When unpacking or installing the lamp, always wear protective clothing and a face mask. Operate lamp only in the EXFO X-Cite 120 lamp housing. This prevents direct viewing of the arc and in the case of lamp bursting, contains lamp particles. In the rare instance in which a lamp bursting occurs, and the mercury content is released, the following safety precautions are recommended: All personnel should be immediately evacuated from the area to prevent inhalation of the mercury vapor. The area should be well ventilated for a minimum of 30 minutes. After the lamp housing elements have cooled, the mercury residue should be collected with the use of a special absorbing agent available from laboratory equipment suppliers.

Warning

Should this EXFO X-Cite 120 unit be used in a manner not specified by EXFO, the protection provided by the equipment may be impaired.

Caution

The lamp module's operational life can be significantly shortened if it is handled incorrectly. Do not touch the bulb's glass envelope or the inside surface of the reflector. Skin oils can cause the lamp module to fail prematurely.

Caution

Prior to opening the unit and handling the lamp module, allow the lamp module to cool down completely.

Caution

Any electronic equipment connected to the EXFO X-Cite 120 must be IEC950 certified.

Cleaning

Clean exterior of the unit with a water dampened cloth and simple detergent only.

EXFO X-CITE 120 Message Reference

The table below lists various messages you may see on the lamp display (inside the Veritas™ Microdissection Instrument; these are not visible when the instrument's covers are in place).

Symbol	Message	Description
XXXX.	Lamp Hours	Displays the accumulated hours the lamp has been on.
XXX.X	Exposure Time	Displays the time in seconds the shutter will remain open when activated.
XXX	Iris Setting	Displays the iris setting as a percentage of maximum.
Flashing **XXX.X**	Flashing display	The lamp is warming up. Warm up time is approximately 90 sec.
bulb	Bulb Error	Lamp installed incorrectly / Lamp did not strike or extinguished after striking.
cool	Cool Warning	Lamp is too hot to strike. The lamp will automatically strike when it has reached optimum striking temperature.
old/bulb	Alternating "Old" then "Bulb"	The lamp has accumulated over 2000 hours. Lamp may be near end of life.
end/ bulb	Alternating "End" then "Bulb"	The lamp has accumulated over 4000 hours. The lamp will not strike.
LOC	Adjustment Locked	The UP/DOWN adjustment buttons have been locked. No changes can be made to the exposure time or iris setting.
ULOC	Adjustment Unlocked	The UP/DOWN adjustment buttons have been unlocked. Changes can be made to the exposure time and iris setting.
SFI	Shutter Failure	The shutter has failed. Unit should be restarted. If the error repeats unit should be serviced.

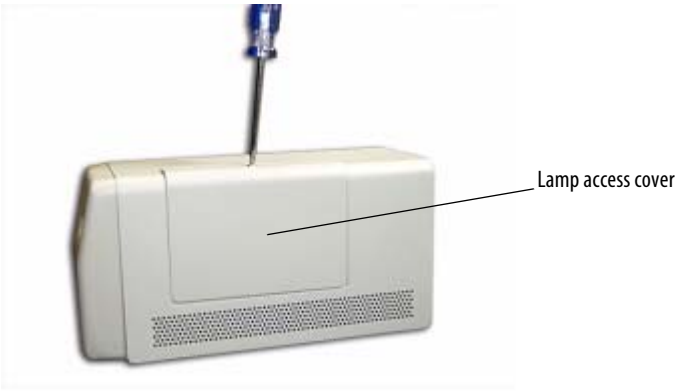
Installing the Lamp Module

You will need the new fluorescence lamp, a 3mm hex key (provided with the instrument) and a slotted screwdriver for this procedure.

1. Be sure the instrument is turned off and the power cord is disconnected from the instrument. (When you are facing the instrument door, the switch and plug are located on the left side, near the back.)

2. Remove the access cover from the Veritas™ Microdissection Instrument as directed in "Replacing the Fluorescence Lamp" on page 102.

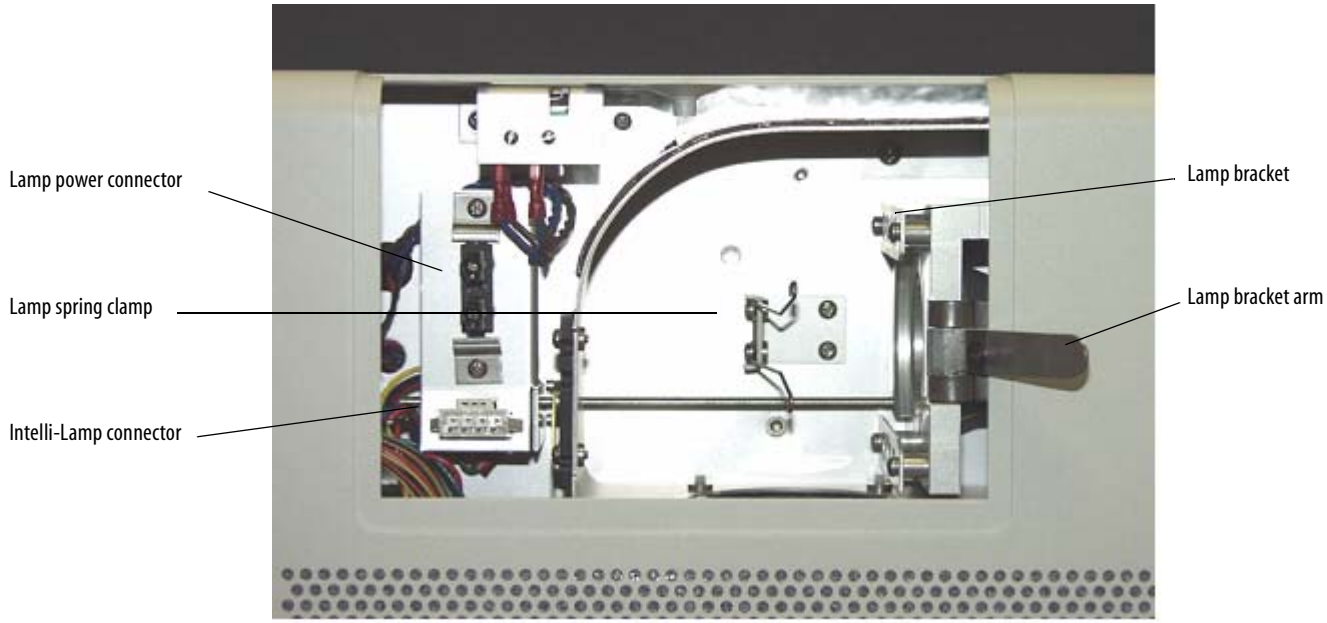
3. Using the 3mm hex key located in the clip in the instrument access cover, remove the lamp access panel from the unit cover.



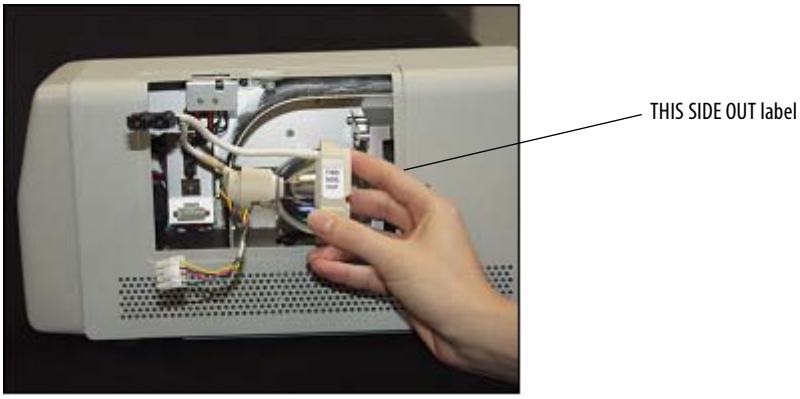
4. Carefully remove the lamp module from its container, holding only the ceramic components.
   Caution: Handle bulb carefully!

   The lamp module's operational life can be significantly shortened if handled incorrectly. Be sure only to handle the ceramic surfaces. Do not touch the bulb's glass envelope or the inside surface of the reflector. Skin oils can cause the lamp module to fail prematurely.

5. Open the lamp bracket arm by pulling towards you and to the right.



6. Position the lamp so that the two leading edges of the lamp ceramic mount slide into the groove of the lamp bracket. The middle of the lamp should be in position to fit into the spring clamp.



Tip: Make sure that the "This side out" label is facing outwards before trying to insert the lamp as illustrated below.

7. Slide the lamp all the way in so that both leading edges of the lamp ceramic mount are in the groove of the lamp bracket. The middle of the lamp will snap into the spring clamp. Close the lamp bracket arm.

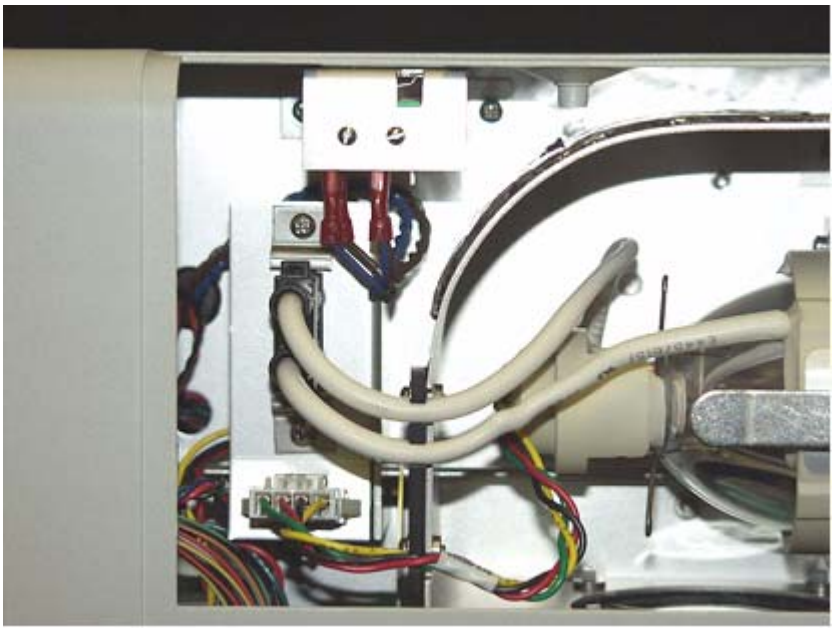
8. Locate the 4-pin Intelli-Lamp sensor connector (c/w multi-colored wiring harness) at the rear of the lamp module and connect it to its mate located on the lamp-housing wall.

Tip: The Intelli-Lamp connector will only attach in the correct orientation. If you are having difficulty attaching the connector, try rotating it by 180º.

9. Attach the 2-pin lamp power connector to its mate on the lamp-housing wall.

Tip: The 2-pin lamp power connector will only attach in the correct orientation. If you are having difficulty attaching the connector, try rotating it by 180º.

10. Ensure the Intelli-Lamp sensor and the lamp power leads are secured into the appropriate alignment grooves of the lamp housing as illustrated below.



11. Replace the lamp access side panel and tighten the fastening screw.

NOTE: If the lamp module has been installed incorrectly the message "bulb" will appear on the display and a continuous audible beep will be heard after the 90 second warm-up cycle has been completed.

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