ARCTURUS$^{XT™}$ MICRODISSECTION INSTRUMENT

User Guide

Molecular Devices

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Molecular Devices Corporation
Arcturus^{XT}™ User Guide

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1. User Safety

Please review the following precautions carefully to ensure safe and effective use of the Arcturus$^{XT™}$ Microdissection Instrument.

The Arcturus^{XT} Microdissection Instrument is classified as a Class 1 laser device. During normal operation, non-removable panels and safety interlocks limit access to laser radiation.

The Arcturus^{XT} Microdissection Instrument has one or more Class 3b lasers. The infrared beam used for capture and the ultraviolet beam used for cutting are not visible. Avoid direct skin and eye exposure to this laser radiation.

⚠**WARNING**: To minimize risk of fire, ensure the illumination tower cable is connected before the control unit is powered.

⚠ **AVERTISSEMENT**: Pour réduire le risque de feu, assurez le câble de tour d'illumination est relié avant que l'Unité de commande soit mise en marche.

⚠ **CAUTION**: To prevent damage to the instrument, turn power OFF before connecting or disconnecting cables.

⚠ **ATTENTION**: Pour empêcher endommager l'instrument, coupez le courant OFF avant de relier ou débrancher des câbles.

Do not remove or modify any of the Arcturus^{XT} optical components or subassemblies, except as described in *"User-Serviceable Parts"* on page 69.

Any modifications to the Arcturus^{XT} Microdissection Instrument may void the system warranty.

The Arcturus^{XT} Microdissection Instrument is for indoor use only.

1.1. THE SAFETY INTERLOCK SYSTEM

The Arcturus^{XT} Microdissection Instrument incorporates an interlock system that enables laser operation only when the cap is in place, the interlock switches are not defeated or bypassed, and the illumination tower is not tilted. Do not modify or override the tilt interlock.

It is possible to override the cap interlock system and operate the lasers when the cap is not in place. To do this, the interlock override key must be inserted in the instrument's

control unit and the laser must be enabled using the software controls. With the override key in place, it is possible for a reflective surface to be introduced in the space between the objective and illumination tower, which can deflect the laser beam out of the instrument and allow human exposure to hazardous laser radiation.

Please contact Molecular Devices Technical Support for information on using the interlock override key. Users should not override the interlock without adequate training to ensure safe operation. Safety measures should include the following:

→ Do not insert reflective surfaces into the beam path.

→ Wear protective eye wear that blocks 355 nm and 810 nm radiation with optical density > 2.5.

→ Post the following warning outside of the room when the instrument is being operated with the interlock overridden:

CAUTION – CLASS 3B INVISIBLE LASER RADIATION

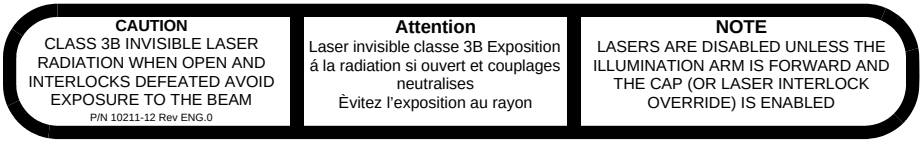
AVOID EXPOSURE TO THE BEAM

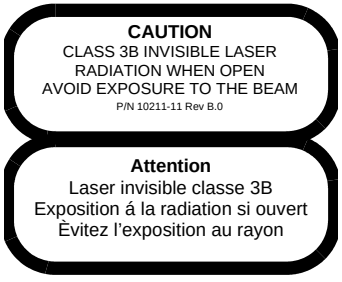
810 nm 100 mW

355 nm 4 mW (0.4 µJ/600 ms pulse at 5kHz)

1.2. WARNING LABELS AND SYMBOLS

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





⚠ **CAUTION:** Hot surface!

⚠ **CAUTION:** Possible pinching!

2. Overview

The Arcturus$^{XT™}$ Microdissection Instrument provides an automated approach to laser microdissection of individual cells or multi-cellular structures from slides containing tissue sections or cytological samples.

The Arcturus^{XT} Microdissection Instrument consists of the Arcturus^{XT} instrument, a computer, and the Arcturus^{XT} software.

See Appendix B on page 79 for detailed specifications for the instrument.

2.1. WHAT IS "LCM"?

Laser capture microdissection (LCM) is a method to quickly and easily procure specific cell populations from slide preparations, using a low-power infrared (IR) laser 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 Arcturus^{XT} Microdissection Instrument, specially designed CapSure® HS or CapSure® Macro LCM Caps that are coated with this thermoplastic film are placed on the region 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. Contact with the microdissected material is maintained throughout the entire process, ensuring the greatest possible confidence in the result. The captured material can be examined, and the cap can then be placed directly into a microcentrifuge tube for extracting DNA, RNA or protein.

2.2. WHAT IS "CUT AND CAPTURE?"

Photoablation, the volatilization of tissue by light emitted from an ultraviolet (UV) 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 IR 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 samples mounted on membrane (such as 2 µm thick polyethylene napthalate (PEN), either on glass or in a metal

frame). Here, the UV cutting laser is used to cut a narrow outline around the region of interest, after which the entire region within the outline is captured on the CapSure cap. With this method, a small number of IR capture points suffices to lift a region, making it much faster than LCM alone for microdissecting larger areas.

2.3. OUTLINE OF THE MICRODISSECTION PROCESS

The following show the steps in microdissection process.

1. Prepare samples, see Chapter 3, *"Tissue Preparation"* on page 13.
2. Load slides and caps, see Chapter 4, *"Start Up and Sample Loading"* on page 17.
3. Locate the cells of interest, see Chapter 5, *"Inspecting Slides"* on page 25.
4. Mark the cells and tissue for capture, see Chapter 6, *"Selecting Cells for Microdissection"* on page 39.
5. Capture the tissue, see Chapter 7, *"Microdissecting Cells and Tissue"* on page 51.
6. Unload the samples and extract the tissue, see Chapter 8, *"Extracting Cells and Tissue"* on page 65.

2.4. USING THE ARCTURUS^{XT}™ SOFTWARE

Start the software by tapping the Arcturus^{XT} icon on the Windows desktop.

The user interface is designed to facilitate the microdissection workflow and to allow the use of the interactive pen display. The most prominent feature is the main image window that shows the live microscope image. On the side of the window is the tool panel, containing each of the tool panes. The tool panes are arranged from top to bottom in the order of the steps for laser microdissection.

By default, the main image is in the upper left corner and the tool panel is on the right. If you prefer, you can move the tool panel to the left side by tapping Left-hand Orientation in the View menu.

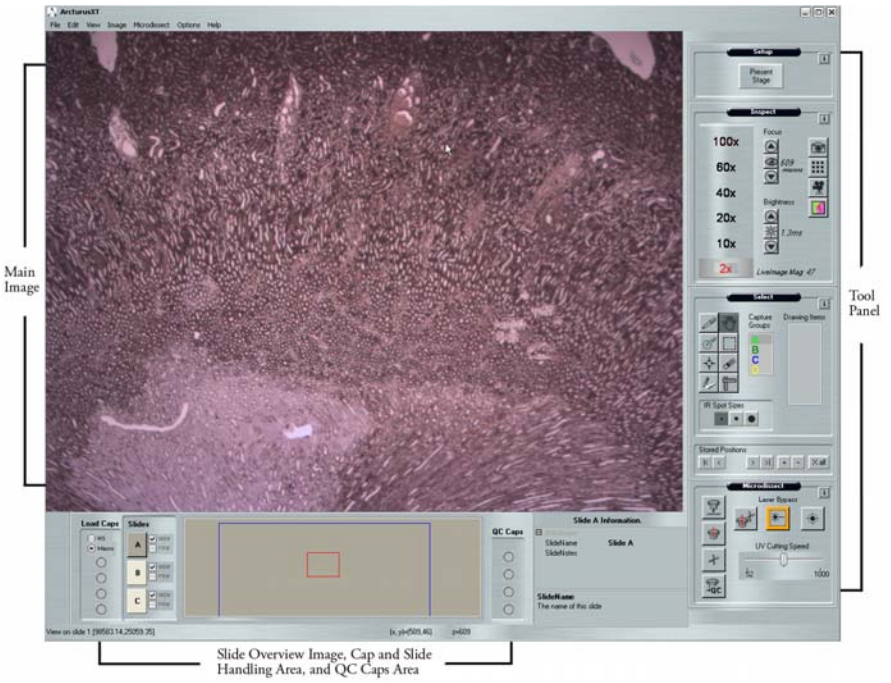


Figure 2.1: The Arcturus^{XT} software window.

At the bottom of the window are the cap and slide handling areas, the slide overview image, and the information area. You can tap the slide overview image to view a region in the main image. The information area displays properties of the currently selected object, such as a slide or a cap.

2.4.1. VIEWING TOOL TIPS

Most items in the Arcturus^{XT} software window have a tool tip associated with them. The tool tips give you information about the item.

To view tool tips:

→ Hold the stylus or mouse over the item of interest on the screen.

A tool tip will appear.

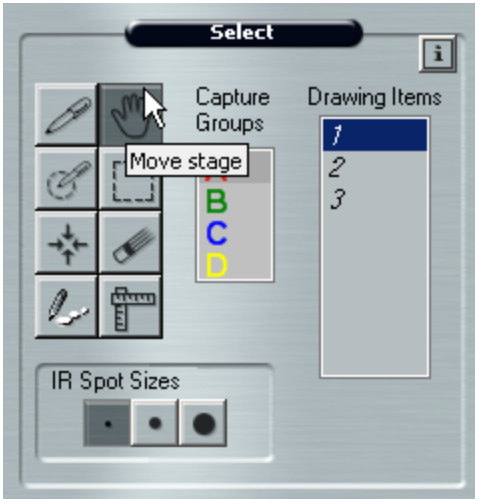


Figure 2.2: Tool tip as shown in the Select Tool Pane.

2.4.2. MAKING SELECTIONS FROM POP-UP MENUS

Some commands are available from pop-up menus in the two image windows.

To view a pop-up menu:

→ With the stylus, press the lower button on the stylus to right-click and then select from the menu, or

→ With the mouse, right-click and then select from the menu.

2.4.3. MAKING SELECTIONS IN THE MAIN IMAGE

Right-click in the main image and choose a select tool from the following menu.

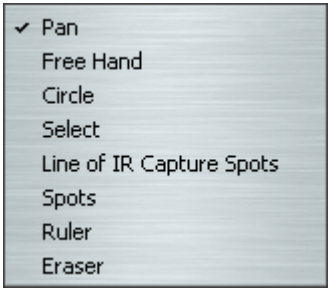


Figure 2.3: Right-click options in main image window.

2.4.4. MAKING SELECTIONS IN THE MAIN IMAGE, WITH A DRAWING ITEM SELECTED

With a drawing item selected, right-click within the selected drawing item in the main image and choose a command to manipulate the item from the following menu.

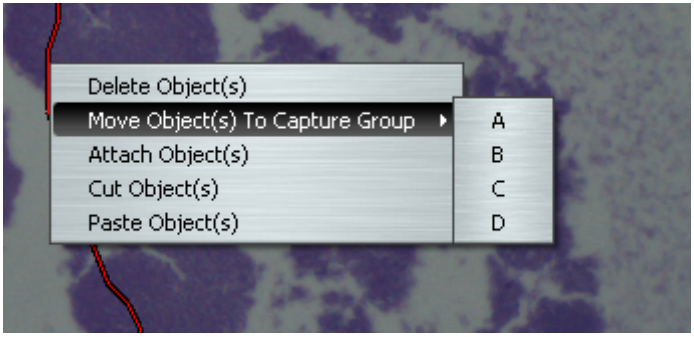


Figure 2.4: Right-click options with selected drawing item.

2.4.5. MAKING SELECTIONS IN THE SLIDE OVERVIEW IMAGE

Within the slide overview image, you may select from options in the right-click menu to manipulate the overview image or the cap.

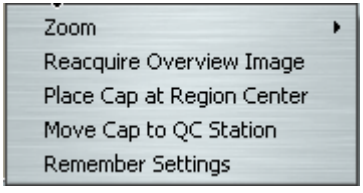


Figure 2.5: Right-click options in overview image window.

2.4.6. OPENING THE OPTIONS DIALOG BOX FOR A TOOL

Each tool pane in the Arcturus^{XT} software has an Options dialog box associated with it. You can set properties and perform actions associated with the tool in the Options dialog box.

Open the Options dialog box in one of three ways:

→ Select the name of the tool pane in the Options menu.

→ Tap the Options button in the upper-right corner of the tool pane. 

→ Right-click anywhere in the tool pane and select Options.

2.4.7. OPTIONS IN DIALOG BOXES

There is informational text for every option in a dialog box. To view it, click the line for the item of interest. The text appears in the pane below.

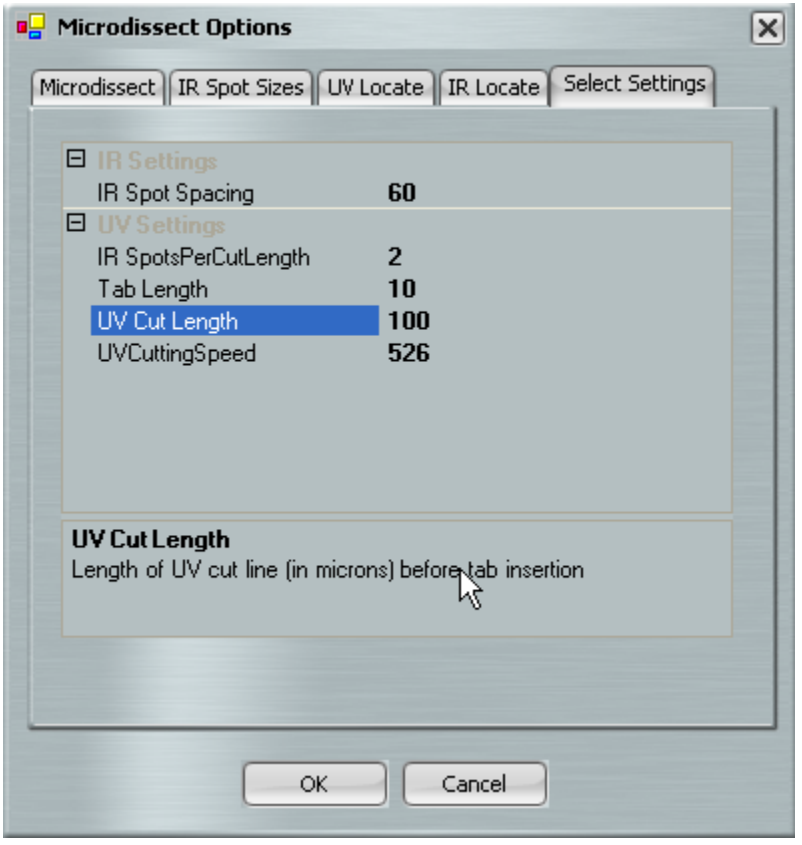


Figure 2.6: Dialog box text options.

For some options in a dialog box, you can make choices from a drop-down list. These lists are indicated by an arrow on the right side of the field.

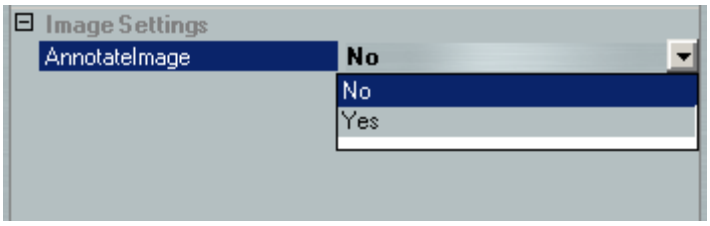


Figure 2.7: Dialog box drop down list.

If you do not see any options in a dialog box, it may be closed. To open it, tap the + in the upper left corner.

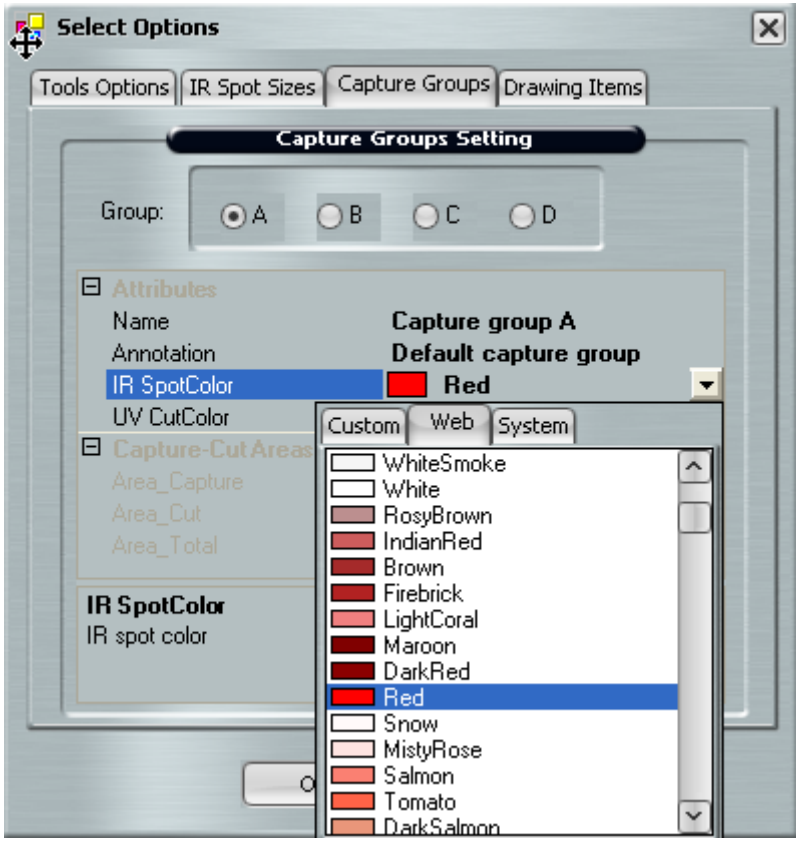


Figure 2.8: Dialog box options.

2.4.8. ENTERING TEXT IN DIALOG BOXES

For some options in a dialog box, you can enter text in a field. Tap the stylus in the field, then use the computer keyboard to type text.

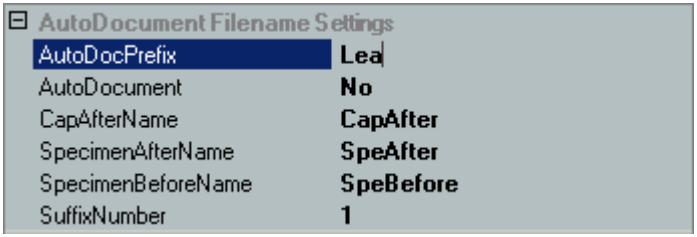


Figure 2.9: Dialog box free text options.

3. Tissue Preparation

3.1. CHAPTER OVERVIEW

This chapter explains:

→ Which types of slides you can use for laser microdissection.

→ How to prepare tissue for laser microdissection with suggested reagent kits.

3.2. SLIDES

You can use glass slides, PEN membrane glass slides and PEN membrane frame slides with the Arcturus$^{XT™}$ Microdissection Instrument.

→ For applications that use the UV cutting laser, use either PEN membrane frame or PEN membrane glass slides.

→ For live cell applications, use a PEN membrane frame slide.

→ For applications that use only the IR capture laser, use either plain glass or PEN membrane glass slides.

PEN membrane slides are available for purchase from Molecular Devices.

Table 3.1: PEN membrane slides.

Item	Catalog Number(s)
PEN membrane frame slides	LCM0521
PEN membrane glass slides	LCM0522

See Protocol #9, Optimized Protocol for Mounting Tissue Sections onto Metal-Framed PEN Membrane Slides on the Molecular Devices web site (www.moleculardevices.com) for more information.

3.3. TISSUE PREPARATION

Tissue sections prepared from either frozen or formalin-fixed paraffin-embedded tissue can be used for microdissection. Freezing tissue helps ensure the integrity of the biological molecules within the cells. Thus, cells microdissected from frozen tissue sections provide material that is suitable for many downstream applications. This is especially true for

molecular biology 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 protocols and reagents will allow the use of these samples for molecular biology applications as well.

3.3.1. FROZEN TISSUE SAMPLES

Molecular Devices 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 Molecular Devices web site.

For optimal preparation of frozen tissue samples and downstream processing, Molecular Devices recommends the following reagent kits:

Table 3.2: Reagent kits for processing frozen tissue samples.

Reagent Kit	Catalog Number
HistoGene® LCM Frozen Section Staining Kit	KIT0401
HistoGene LCM Immunofluorescence Staining Kit	KIT0420
PicoPure® RNA Extraction and Isolation Kit	KIT0204
PicoPure DNA Extraction Kit	KIT0103
RiboAmp RNA Amplification Kit	KIT0201
RiboAmp® High Sensitivity RNA Amplification Kit	KIT0205

3.3.2. FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SAMPLES

Formalin-fixed, paraffin-embedded (FFPE) tissue may also be microdissected for downstream applications. Suggested protocols based on the experience of Molecular Devices customers are available on the Molecular Devices web site. For gene expression profiling studies using FFPE tissue, Molecular Devices 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.

For optimal sample preparation of FFPE tissue samples and downstream processing, Molecular Devices recommends the following reagent systems:

Table 3.3: Sample preparation kits for formalin-fixed, paraffin-embedded tissue samples.

Reagent System	Catalog Number
Paradise® Reagent System, 1.5 rounds of amplification for use with Oligo Arrays	KIT0311
Paradise Reagent System, 2 rounds of amplification for use with cDNA arrays	KIT0312
Paradise Reagent System for Quantitative Real-Time PCR	KIT0300L

3.3.3. OTHER TYPES OF SAMPLE TISSUE

LCM has been used for a variety of research applications, aside from tissue microdissection, including forensics, live cells, neurons, live plant tissue, and single chromosomes. You can download application notes and protocols for other types of sample tissue from the Molecular Devices web site at www.moleculardevices.com.

4. Start Up and Sample Loading

4.1. CHAPTER OVERVIEW

This chapter explains:

→ How to start the Arcturus^{XT} software.

→ How to load samples on the instrument.

→ The Load Options dialog box.

4.2. START UP

The instructions below assume that you are using the interactive pen display supplied with the Arcturus^{XT} Microdissection Instrument. All of these commands are also accessible with the mouse.

To begin laser microdissection:

1 Turn on the computer.

2 Turn on the Arcturus$^{XT™}$ Microdissection Instrument.

As you face the microscope, the power button is located on the left side of the instrument.

3 Start the software by tapping the Arcturus^{XT} icon on the Windows desktop, or tap Start, point to Programs and tap Arcturus^{XT}.

The software opens to fill the screen.

4.3. LOADING MATERIALS

1 Tap Present Stage in the Setup tool pane.

Figure 4.1: Setup tools pane.

The work surface moves forward and to the right.

2 Load your slides and caps onto the work surface.

a If needed, remove any caps and slides that have been left on the work surface.

b Push the tension button in and place each slide in a slot. Release the tension button.

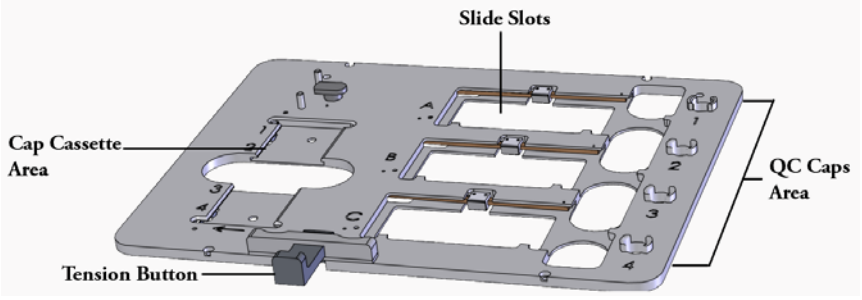


Figure 4.2: Modular stage insert.

c Place the caps, in the CapSure® cassette, into the slot on left side of the work surface.

Note: For every cap present in the cassette, make sure the corresponding cap offload position (on the right side of the work surface) is empty.

d If desired, open the Load Options dialog box and follow the steps below to enter information about your slides and caps.

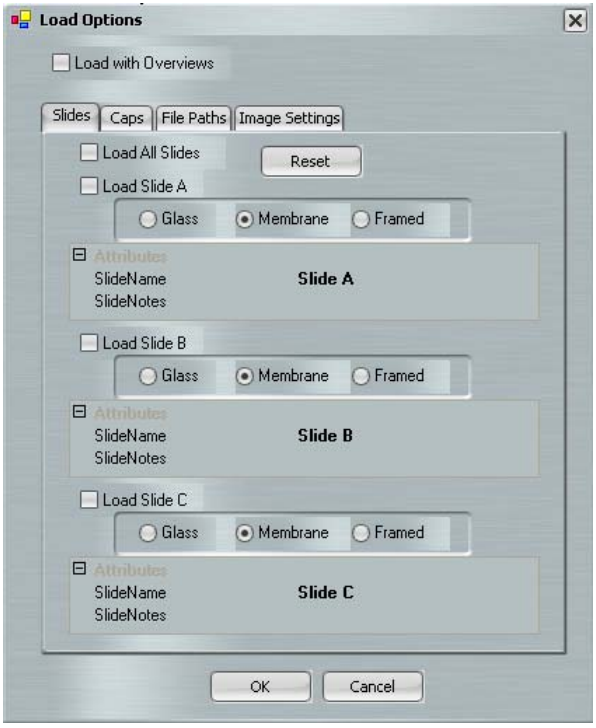


Figure 4.3: Slides tab in the Load Options dialog box.

e Check each slide that you are loading or tap Load All Slides if you are loading all slides. The slots are identified from top to bottom (A–C), based on the slot location on the stage. Slide C is the slide closest to the front of the instrument.

f Tap Load with Overviews to instruct the instrument to automatically create the slide overview image when you close this dialog box.

g Choose the type of slide: Glass, Membrane or Framed.

h Optionally:

- Enter a name to identify each slide in the SlideName field. This name is shown on the static image when you have selected "Yes" in the AnnotatedImage field in the Image Settings tab.
- Enter any comments for each slide in the SlideNotes field. These comments are saved to the cap interaction history file.

Note: You can also edit the SlideName and SlideNotes in the information area to the right of the slide overview image (see Figure 2.1 on page 7).

3 In the Caps tab:

a Tap the Caps tab, then check each cap that is loaded or tap Load All Caps to check all of the check boxes at once. Tap HS or Macro to identify the type of caps you are loading.

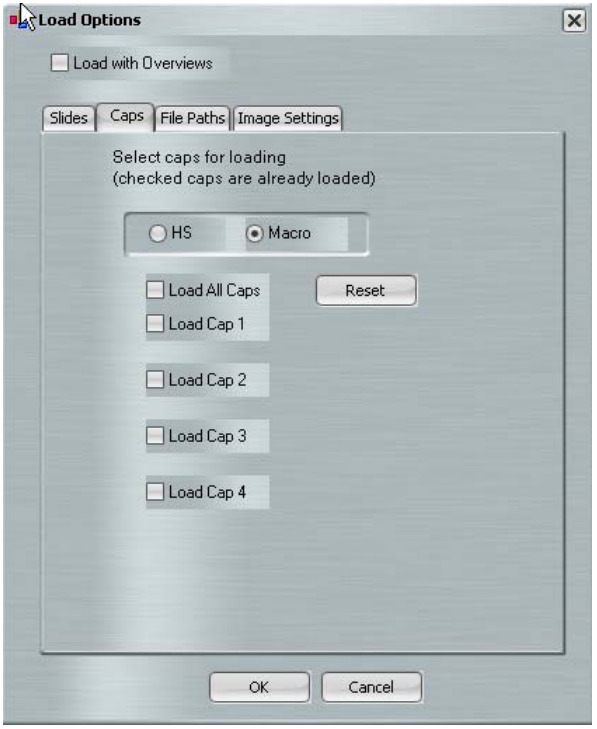


Figure 4.4: Caps tab in the Load Options dialog box.

4 Tap the File Paths tab to enter information about where image files should be saved.

The options in the File Paths tab are:

- AutomaticFilename—When this is Yes, the file name for saved images is "2007-01-23_X.tif", where X is an incrementing number. Tiled images are named "2007-01-23_002_S.tif" and videos are named "2007-01-23_X.avi". When this is No, you will be prompted for a file name when you save an image.
- StudyFolder—Enter the folder where saved images, videos and reports are to be located. Tap Browse to select the location.

- ImageSubfolder—Enter the name of the folder inside the StudyFolder where image files are to be saved.
- ReportSubfolder—Enter the name of the folder inside the StudyFolder where the cap interaction history files are to be saved. A cap interaction history is generated when you off load a cap after microdissection.
- VideoSubfolder—Enter the name of the folder inside the StudyFolder where video files are to be saved.

5 In the Image Settings tab:

a Tap the Image Settings tab, where you may also choose whether or not to automatically save images and the format for those images. See *"Saving Images Automatically"* on page 21 for detailed instructions.

6 Tap the OK button.

The instrument performs the following actions:

- Moves the work surface to the left and places the 2X objective under the first slide.
- Displays the selected slide in the main image window.
- If "Load with Overviews" was selected, it will automatically acquire and display the slide overview image for all slides loaded.

Note: If Load with Overviews is not selected as one of your Load Options, the slide overview image area will be blank. To acquire and display the slide overview, right-click in the slide overview area and tap Reacquire Overview Image.

See Chapter 5, *"Inspecting Slides"* on page 25 for the next step in microdissection.

4.4. SAVING IMAGES AUTOMATICALLY

You can choose to create and save images automatically. Three images are saved:

→ The main image before microdissection.

→ The main image after microdissection.

→ The cap after microdissection.

You can refer to these images to see how effective capture was and/or to see the context of the microdissected tissue.

To save images automatically:

1 When the Load Options dialog box is open, tap the Image Settings tab.

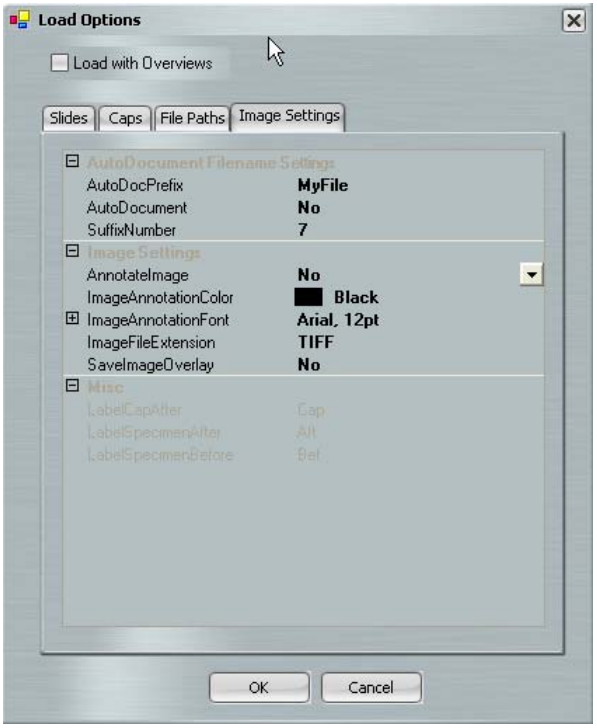


Figure 4.5: Image Settings tab in the Load Options dialog box.

2 Set the options for naming your files in the AutoDocument Filename Settings. Tap each field and use the keyboard as needed to edit the contents.

- AutoDocPrefix—Image files saved when AutoDocument is Yes begin with this text.
- AutoDocument—Tap the field to display the drop-down list and select Yes.
- SuffixNumber—This number is the last part of the file name for all images. This number increases by one each time microdissection occurs.
- LabelCapAfter—Files with images of the cap are named with the AutodocPrefix followed by the suffix and this text.
- LabelSpecimenBefore—files with images of the slide before microdissection are named with the AutoDocPrefix followed by the suffix and this text.

- LabelSpecimenAfter—Files with images of th eslide after microdissection are named with the AutoDocPrefix followed by the suffix and this text.

For example, for the second image of a slide before LCM, with the AutoDocPrefix "LeafStudy", the LabelSpecimenBefore "Before," and the SuffixNumber "10", the file is named LeafStudyBefore0.

3 Set the other options for your saved image files. Tap each field and use the keyboard as needed to edit the contents.

- AnnotateImage—If this is Yes, when you save images, the SlideName and SlideNotes (from the Slides tab) and the objective in use are saved in the upper left corner of all image files.
- ImageAnnotationColor—This is the color for the annotation saved if AnnotateImage is Yes.
- ImageAnnotationFont—This is the font for the annotation saved if AnnotateImage is Yes. Tap the  to view other options for formatting annotation text.
- ImageFile Extension—Sets the file format for saved images. Choose between JPEG (.jpg) and TIFF (.tif).
- SaveImageOverlay—If this is Yes, when you save images, any drawing items are saved as part of the image.

5. Inspecting Slides

5.1. CHAPTER OVERVIEW

This chapter explains:

→ How to move around a slide to view the sample.

→ How to work with the microscope.

→ How to work with the fluorescence lamp.

→ How to capture static images, tiled images, and movies.

5.2. USING THE INSPECT TOOLS

After you have loaded the slides and caps, use the tools in the Inspect tools pane to inspect the slides and to identify the cells you want to microdissect. These tools allow you to adjust the microscope, turn on the fluorescence lamp and work with images.

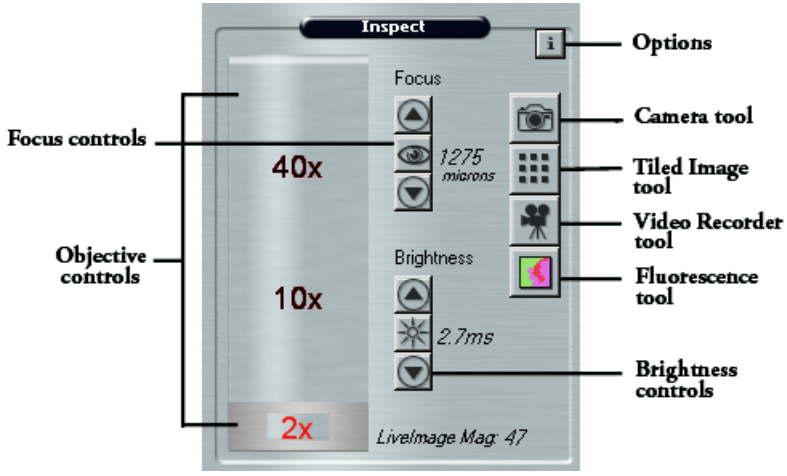


Figure 5.1: The Inspect tools pane.

Note: Depending upon your instrument configuration, you may see additional objectives in the objective controls.

To inspect your slides:

1 Move the stage to display an area of interest in the main image in one of three ways:

- Move the trackball.
- Tap the Move Stage tool in the Select tools pane and then press the stylus on the main image window. Drag the stylus to move the stage. 
- In the slide overview image, tap the stylus at the location of interest. The stage moves to that location and the main image updates, centered on the location where you tapped.

2 To view a different slide, tap the Slide button for the slide of interest. 

The stage will move the selected slide over the objective and the slide overview will update to show the new slide.

3 Change the objective as needed by tapping the label corresponding to the objective of choice. The selected objective is red.

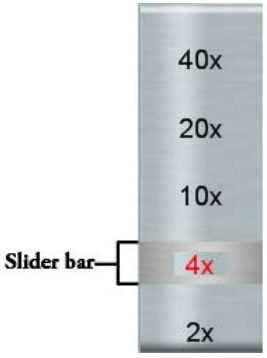


Figure 5.2: Objective control.

If you drag the slider bar up, between objectives, the Arcturus^{XT} Microdissection Instrument will zoom the image digitally.

4 Adjust the Brightness controls as needed to illuminate the sample.

Figure 5.3: Brightness controls.

- Tap or press the up arrow to increase the brightness.
- Tap or press the down arrow to decrease the brightness.

  Note: If you press the stylus down on the arrow buttons, the software adjusts the brightness in larger steps.
- Tap the Autobrightness button in the middle to adjust the brightness automatically.

  You can set a different level brightness for the Autobrightness button, see *"Working with the Autobrightness Settings"* on page 30.

If the value in the brightness control is > 0.5 seconds, the time needed for the main image window to refresh after you move the stage may be slow. If you feel this is the case, adjust the Intensity and Camera gain in the Illumination tab in the Inspect Options dialog box (see page 27) so that you can set the Brightness value lower.

5 Use the Focus controls to change the focus manually.

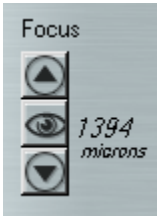


Figure 5.4: Focus controls.

- Tap or press the up arrow to move the objective closer to the slide.
- Tap or press the down arrow to move the objective farther from the slide.

  Note: When you press the stylus down on the arrow buttons, the software adjusts the focus in larger steps.
- Tap the Autofocus button in the middle to set the focus automatically.

6 To use the microscope's 1.5X feature, see *"Using 1.5X Magnification"* on page 30.

7 To work with fluorescent samples, see *"Working with Fluorescence"* on page 32.

See Chapter 6, *"Selecting Cells for Microdissection"* on page 39, for the next step in microdissection.

5.3. WORKING WITH THE BRIGHT FIELD LAMP

To adjust the bright field lamp:

1 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog.

2 Tap the Illumination tab.

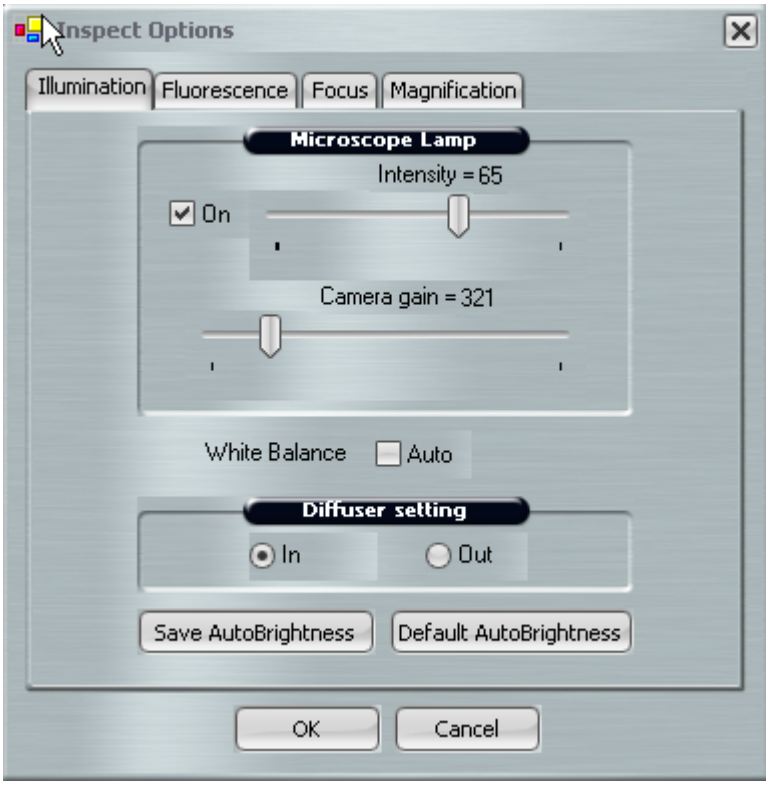


Figure 5.5: Illumination tab in the Inspect Options dialog box.

3 If needed, tap On to turn on the bright field lamp.

4 If desired, choose Diffuser. The diffuser diffuses the white light so the slide will look more similar to a slide with a cover slip. Choose In or Out.

5 Work with the Intensity and Camera Gain controls, here, and the Brightness controls, in the Inspect tools pane, to optimize the image.

- Use the Intensity slider to change the bright field lamp intensity. Slide the control to the right to increase intensity and slide the control to the left to decrease the intensity.
- Use the Camera Gain slider to adjust the camera gain. The camera gain amplifies the signal from the video camera. Slide the control to the right to increase intensity and slide the control to the left to decrease the intensity.
- Adjust the Brightness controls in the Inspect Pane as needed, until the sample is illuminated to your liking.

6 Go to the next section to set the white balance within the camera settings or tap OK to close the dialog box and save your changes.

5.3.1. ADJUSTING THE VIDEO CAMERA PROPERTIES

Very rarely, you may need to adjust the video camera. See *"The Camera Properties Dialog Box"* on page 87 for more information.

5.3.2. SETTING THE WHITE BALANCE

You may need to adjust the white balance to achieve the proper color representation in your image and for optimal visualization for microdissection.

To set the white balance automatically:

1 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog.

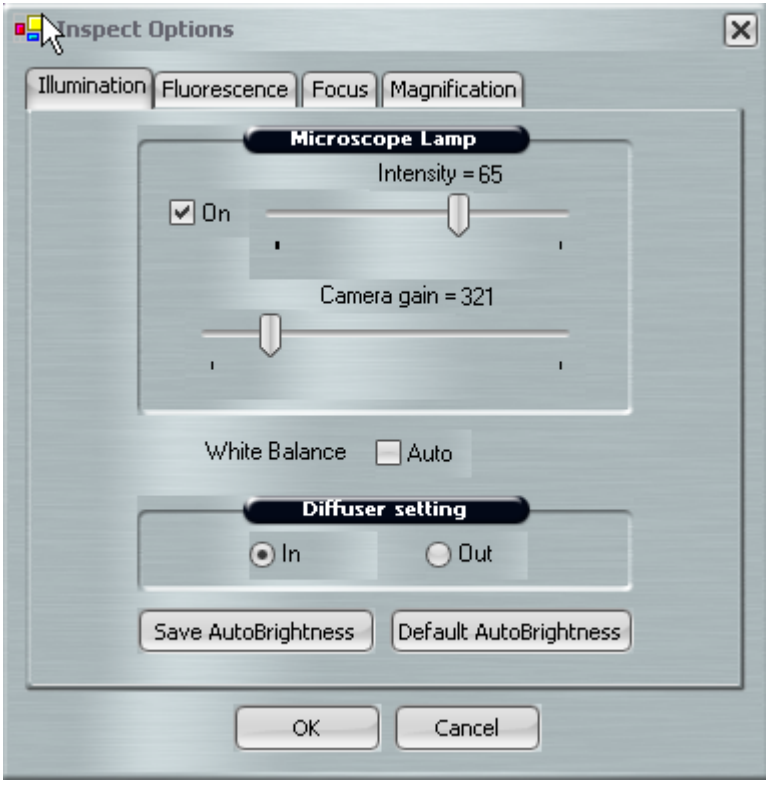


Figure 5.6: White balance setting in the Inspect Options dialog box.

2 Tap the Illumination tab.

3 Check White Balance Auto to automatically set the white balance.

4 Go to the next section to work with autobrightness or tap OK to close the dialog box and save your changes.

5.3.3. WORKING WITH THE AUTOBRIGHTNESS SETTINGS

The Arcturus^{XT} software automatically sets the brightness when you tap the Autobrightness button. 

You may find that the default brightness is not appropriate for your sample. You can adjust the brightness value manually to the appropriate level and save it as the default.

To save the current brightness as the default for the Autobrightness button:

1 Adjust the brightness in the main image window as desired.

2 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog. 

3 Tap the Illumination tab.

4 Tap Save AutoBrightness, then tap OK to close the dialog box and save your changes.

The next time you tap the Autobrightness button in the Inspect tools pane, the illumination is set to this value.

To return to the original autobrightness setting:

1 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog. 

2 Tap the Illumination tab.

3 Tap Default Autobrightness, then tap OK to close the dialog box and save your changes.

The next time you tap the Autobrightness button in the Inspect tools pane, the illumination is set to the default value.

5.4. USING 1.5X MAGNIFICATION

The microscope has an option that allows you to increase the magnification of the current 1.0 objective to 1.5X. The dial for this setting is located on the right side of the instrument, above the manual focus knobs. You must tell the Arcturus^{XT} software you are using this feature on your microscope for the instrument to work correctly.

To use the 1.5X magnification feature:

1 On the right side of the microscope, turn the magnification knob to use 1.5X.

2 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog. 

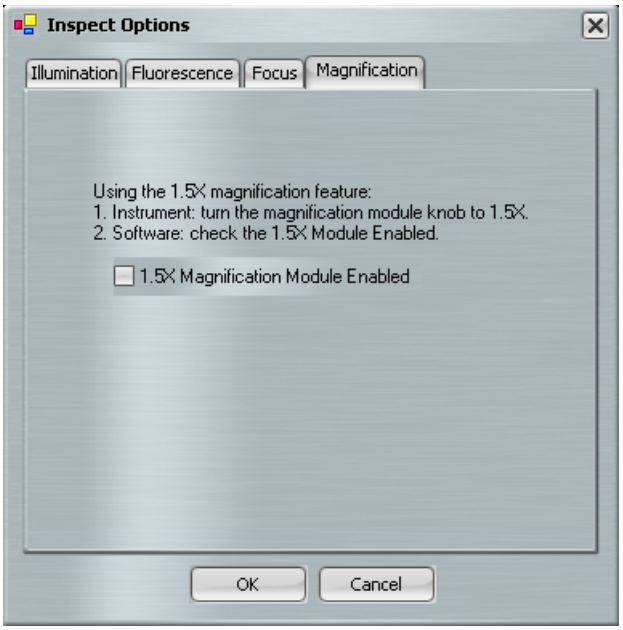


Figure 5.7: Enabling 1.5X magnification in the Inspect Options dialog box.

3 Tap the Magnification tab.

4 Check 1.5X Magnification Module Enabled.

This tells the Arcturus^{XT} software that the microscope is using the 1.5X feature, so images and other measurements will be correctly scaled.

5 Tap OK to close the dialog box and save your changes.

5.5. AUTOMATICALLY FOCUSING WHEN THE OBJECTIVE CHANGES

The instrument can automatically focus each time you change the objective.

To set up automatic focusing:

1 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog. 

2 Tap the Focus tab.

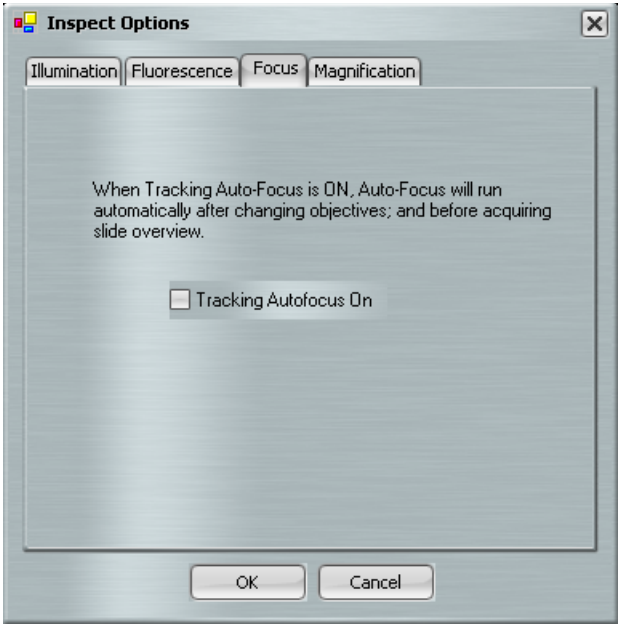


Figure 5.8: Tracking autofocus in the Inspect Options dialog box.

3 Check Tracking Autofocus On to instruct the Arcturus^{XT} software to automatically focus each time the objective is changed and before acquiring slide overview images.

4 Tap OK to close the dialog box and save your changes.

5.6. WORKING WITH FLUORESCENCE

When you work with the fluorescence lamp, your sample can be photo-bleached if it is exposed to the light source for too long. The procedure below describes how to use the Arcturus^{XT} software to take timed exposures so that your sample's exposure to the fluorescence lamp is limited.

Note: Turn on fluorescence EXFO X-Cite™ 120 PC control prior to launching Arcturus^{XT} software to ensure proper system communication.

To set up to work with fluorescence:

1 Turn on the EXFO X-Cite™ 120 PC fluorescence illumination source. The fluorescence lamp needs several minutes to warm up to reach its maximum light intensity.

Note: The instrument will not open the shutter if the lamp is hot. If you have recently turned the lamp off, there may be a delay of several minutes before the shutter can be opened again. To avoid waiting for the lamp to cool, turn off the lamp only when it is no longer needed.

2 Tap the Fluorescence button in the Inspect tools pane to open the shutter between the instrument and the fluorescence lamp. 

When the shutter is open, the button changes to look like this:  .

3 On the microscope:

- Manually open the shutter. The shutter is located on the fluorescence cube turret.
- Manually rotate the fluorescence cube turret to select the appropriate filter cube.

4 Tap the Options button in the upper right corner of the Inspect tools pane to open the Inspect Options dialog. 

5 The Fluorescence tab will open by default when the fluorescence has been selected (Step 5.6.2).

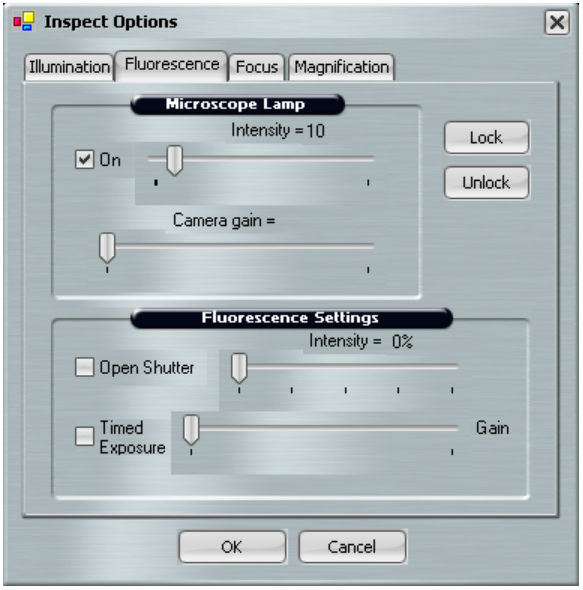


Figure 5.9: Fluorescence controls in the Inspect Options dialog box.

6 Tap Lock to lock the controls on the EXFO control box so that all control of the lamp is done by the Arcturus^{XT} software.

7 Adjust the Microscope Lamp controls in the Fluorescence tab. These settings are only used when the fluorescence lamp is on, otherwise the settings in the Illumination tab are in use.

- Tap On to turn on the bright field lamp to use it when the fluorescence lamp is on. Otherwise, uncheck it.
- Use the Intensity slider to change the bright field lamp intensity. To increase the intensity, slide the control to the right. To decrease the intensity, slide the control to the left.
- Use the Camera Gain slider to set the camera gain. The camera gain amplifies the signal from the video camera. Slide this to the right to increase the camera gain; slide it to the left to decrease the gain.

8 Adjust the Fluorescence Settings controls to optimize live image:

a Tap Open Shutter to open the shutter. The shutter remains open until you uncheck Open Shutter.

b Use the Intensity slider to change the fluorescence lamp intensity until it is appropriate.

c Use the Camera Gain slider to change the camera gain.

d Use Brightness controls in the Inspect Tool Pane until appropriate.

9 Tap Open Shutter to close the shutter so your sample is not photo-bleached while you make the rest of the changes to the settings.

10 Tap Timed Exposure to work with a static image rather than the main image.

When Timed Exposure is selected, use the Camera button to capture a static image while the shutter is briefly open. You perform all tissue selection on this static image, rather than leaving the shutter open to bleach the sample (see *"More About Timed Exposures"* on page 34).

Note: If you want to control the shutter manually, do not check Timed Exposure. The shutter will open when you tap the Fluorescence button. Each time you want to open or close the shutter, you will need to open the Inspect Options dialog box and tap Open Shutter in the Fluorescence tab. Otherwise, the shutter will only close when you revert back to bright field illumination.

11 Tap OK to close the dialog box and save your changes.

The Arcturus$^{XT™}$ Microdissection Instrument will use the lamp and camera settings you have set for the fluorescence mode. If you had selected Time Exposure, the fluorescence shutter will be closed.

5.6.1. MORE ABOUT TIMED EXPOSURES

When Timed Exposure is checked, the instrument only opens the shutter briefly to illuminate the slide when a snapshot is taken to prevent photo-bleaching of the sample. Tap the Camera button to take the snapshot/static image (see "Capturing and Saving Images" on page 36). Draw on the snapshot to indicate areas for capture.

When you have identified another area for a timed exposure, tap the Camera button again to acquire another snapshot of the new area. 

5.7. WORKING WITH SLIDES

5.7.1. DISPLAYING A DIFFERENT SLIDE

To display a different slide:

1. To the left of the slide overview image, tap the Slide button for the slide of interest in the cap and slide handling area at the bottom of the screen. 

   The stage will move the slide over the objective. The slide overview and the main image update to show this slide.

2. If the slide overview does not update, right-click in the slide overview and select Reacquire Overview Image.

5.7.2. VIEWING SLIDE PROPERTIES

You can view the slide type in the cap and slide handling area to the left of the slide overview image. If you did not select the correct slide type when you loaded your slides, you can change it here, at any time except when a cap is on the slide. For plain glass slides, leave MEM and FRM unchecked.

Figure 5.10: Cap and slide handling area.

You can view properties of a slide (its name and any notes) in the information area to the right of the slide overview image. If you want, you can edit the SlideName and/or SlideNotes here. Editing here is the same as entering the information in the Load Options dialog box, as described on page 19.

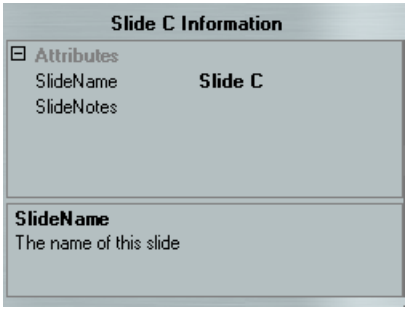


Figure 5.11: Information area, showing slide information.

5.8. WORKING WITH IMAGES AND VIDEOS

5.8.1. CAPTURING AND SAVING IMAGES

You can capture and save images for later viewing. There are two kinds of images:

→ Tiled images—A tiled image is a snapshot of a selected region of the slide. The software captures as many images as needed to span the selected region and stitches them together into one image. A tiled image can show a region larger than a static image.

Note: The available computer memory and degree of magnification limits the size of the tiled image.

→ Static images—A static image is a snapshot of the area visible in the main image window. You select the image file format and other options for static images in the Image Settings tab of the Load Options dialog box (see page 21). You set the location for the file in the File Paths tab of the Load Options dialog box (see page 21).

To capture a tiled image:

1 In the main image window:

a Locate the area for the tiled image.

b Select the objective for the desired magnification.

c Adjust the image using the tools in the Inspect tools pane.

2 Tap the Tiled Image button in the Inspect tools pane. 

3 In the slide overview image, tap and then drag the cursor to outline the area for the tiled image.

The selected area is outlined with a dashed red line and a pop-up menu appears.

→ If this area is not correct, select Cancel. To try again, repeat steps 2 and 3.

→ If this area is correct, select Take Tiled Image of Selected Area.

A tiled image of the selected region is acquired and opens in a new window.

If the software cannot capture a tiled image (due to limited memory), select a smaller region on the slide overview and/or change to a lower magnification objective and try again.

4 Work with the tiled image as you would with the main image to identify and mark tissue for microdissection (see Chapter 6, *"Selecting Cells for Microdissection"* on page 39).

5 To close the tiled image, tap the close box in the upper right corner of the static image window. 

6 The stitched image is saved in the folder specified in the File Paths tab of the Load Options dialog box. They are saved in the format specified in the Image Setting tab of the Load Options dialog box.

To capture a static image:

1 Move the stage so the main image window displays the area that you want to save as an image.

Note: The static image consists of only the area visible in the main image window.

2 Adjust the image as needed using the Inspect tools.

3 Tap Camera Button. 

The static image appears in a new window and will be saved in the folder specified in the File Paths tab of the Load Options dialog box. They are saved in the format specified in the Image Setting tab of the Load Options dialog box.

4 When you are done viewing and/or working with the image, tap the close box in the upper right corner of the static image window. 

5.8.2. OPENING AN IMAGE

To open an image:

1 Choose Open Image from the File menu.

2 In the resulting dialog box, tap the name of the image you want to open and tap Open.

The image opens in a window.

3 When you are done viewing the image, tap the close box in the upper right corner of the window. 

5.8.3. CAPTURING, SAVING, AND VIEWING VIDEOS

To capture and save a video:

1 Tap the Video Recorder button in the Inspect tools to begin recording a video. 

2 The camera turns red while the camera is recording.

3 Tap Video Recorder again to stop recording. 

The camera turns black when recording stops.

The video is saved as an .avi file in the folder specified in the Video File Path setting in the Image Settings tab of the Load Options dialog. The file is named "2007-01-23_X.avi", where "X" is a number.

To view a video:

→ Use Windows Media Player or a similar program.

6. Selecting Cells for Microdissection

6.1. CHAPTER OVERVIEW

This chapter explains:

→ How to indicate the cells and tissue you want to microdissect.

→ How to set the size of the IR capture spots inside the items you want to microdissect.

→ How to measure a distance or object size in the main image window.

→ How to delete a drawing item.

→ How to find out more information about a drawing item.

→ What a capture group is and how to work with a capture group.

→ How to save and use an overlay image.

6.2. SELECTING THE CELLS FOR MICRODISSECTION

Use the Select tools pane to mark the cells for microdissection. These tools also enable you to designate "capture groups"—cells and tissue to be collected on the same cap. For example, if you want to collect two different types of cells from one slide, you can utilize two capture groups. You can have a maximum of four capture groups.

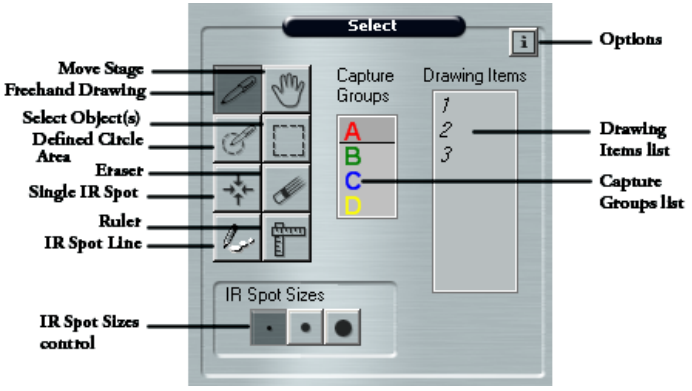


Figure 6.1: The Select tools pane.

To mark cells for microdissection:

Note: If your instrument is equipped with a UV cutting laser, you should mark your cells and perform microdissection using the same objective. This ensures the UV cutting laser will most accurately follow the dissection marks.

1 Tap the desired size (small, medium or large) in the IR Spot Size control in the Select tools pane to choose the relative size of the IR capture spots.

Before you create any drawing items to select cells or tissue, set the physical size of each of these spots in the IR Spot Sizes tab of the Select Options dialog box (see *"Setting the IR Capture Spot Size"* on page 45).

Figure 6.2: IR spot size selection.

The IR capture spot size is one of the factors that determines how the IR capture spots are placed in a drawing item to identify where the IR capture laser will be fired.

2 Tap any of the four drawing tools to activate it. The tool remains active until you tap another tool.

- Use the Freehand Drawing tool to draw a free-form shape indicating the cells to be microdissected. 

  The software will automatically close the drawing item if the ends are "close enough" together and draw IR capture spots inside the shape. You can set the distance at which the software will automatically close the object in the Tools Options tab of the Select Options dialog box (see *"Setting Drawing Tools Options"* on page 44).

- Use the Defined Circle Area tool to indicate circular areas for microdissection. 

  The software will draw IR capture spots inside the circle. You may also set a fixed size for the Defined Circle Area tool in the Tools Options tab of the Select Options dialog box (see *"Setting Drawing Tools Options"* on page 44).

- Use the IR Spot Line tool to indicate a line along which you want to place IR capture spots. 
- Use the Single IR Spot tool to indicate single cells for capture. 

  A number for each drawing item that you draw appears in the Drawing Items list, located to the right of the Capture Groups list (see Figure 6.1 on page 39).

3 When you are done marking cells for microdissection, tap the Move Stage tool to turn off the drawing tool.

4 As needed, move the stage to display other areas in the main image window so you can mark more cells for microdissection.

5 If you want to designate cells and tissue for a second capture group, tap B in the Capture Groups list and then mark those cells with the desired tool as described above. Drawing items belong to capture group B and will not be collected on the same cap as capture group A.

6 Once you have one or more drawing items drawn, you may:

- Use the Eraser tool to remove any marks made by the drawing tools. 
- Select all drawing items that fall inside a region. Draw a rectangular region with the Select Object(s) tool to select all the drawing items that fall inside the region. 

  Once selected, you can move the drawing items, delete them, move the them to a different capture group, or view information about the them (see *"Working with Drawing Items"* on page 41).

- Add more IR capture spots to any item. Tap the Single IR Spot tool and then tap inside any of the drawing items. 

See Chapter 7, *"Microdissecting Cells and Tissue"* on page 51, for the next step in microdissection.

6.3. WORKING WITH DRAWING ITEMS

6.3.1. MOVING DRAWING ITEMS

To move a drawing item:

1 Tap the Select Object(s) tool, then in the main image window:

- Tap a single drawing item to select it, or
- Drag the stylus to select more than one drawing item.

Tap the cursor within the selected object(s) and the cursor changes to a four headed arrow: 

- In the main image window, drag the stylus to the new location for the drawing item(s) and release it.

  The drawing item(s) move to the new location.

6.3.2. MOVING DRAWING ITEMS TO A DIFFERENT CAPTURE GROUP

To move a drawing item:

1 Tap the Select Object(s) tool, then, in the main image window:

- Tap a single drawing item to select it, or
- Drag the stylus to select more than one drawing item.

2 Right-click within the selected object(s). A pop-up window will appear., tap Move Selected Object(s) to Group and select the desired capture group.

The selected drawing items move to the new capture group.

6.3.3. DELETING DRAWING ITEMS

To delete a single drawing item (or a group of drawing items):

1 Do any of the following:

- In the Drawing Items list, tap the number of the drawing item, then right-click the stylus button and select Delete Object.
- Tap the Select Object(s) tool, tap the item, right-click and select Delete Object(s) from the pop-up menu.
- Select a group of drawing items by tapping and dragging with the Select Object(s) tool, tap within one of the selected objects and right-click. Then choose Delete Object(s) from the pop-up menu.

The selected drawing items are deleted.

To delete all drawing items on the slide:

1 To select all drawing items on the slide, type Ctrl-A on the keyboard.

2 Do one of the following:

- Type Ctrl-X on the key board, or
- Right-click anywhere on one of the drawing objects and select Delete Object(s).

All drawing items are deleted.

6.3.4. DELETING IR CAPTURE SPOTS FROM A DRAWING ITEM

To delete IR capture spots from a drawing item:

1 Place cursor on an IR capture spot, right-click and tap the IR spot.

Figure 6.3: Deleting IR capture spots.

2 In the pop-up menu that is displayed, select either:

- Delete Spot, to delete the selected IR capture spot.
- Delete Spots in Object, to delete all the IR capture spots in the drawing item.

The specified IR capture spots are deleted.

6.3.5. VIEWING INFORMATION ABOUT A DRAWING ITEM

You can see the properties, such as the number of IR capture spots or capture area, for any of the drawing items you have drawn.

To view information about drawing items:

1 Tap the Options button in the upper right corner of the Select tools pane to open the Select Options dialog box.

2 Tap the Drawing Items tab to view the properties of interest.

There is a row for each drawing item in the selected capture group. You can view the area of the item, its status and other information.

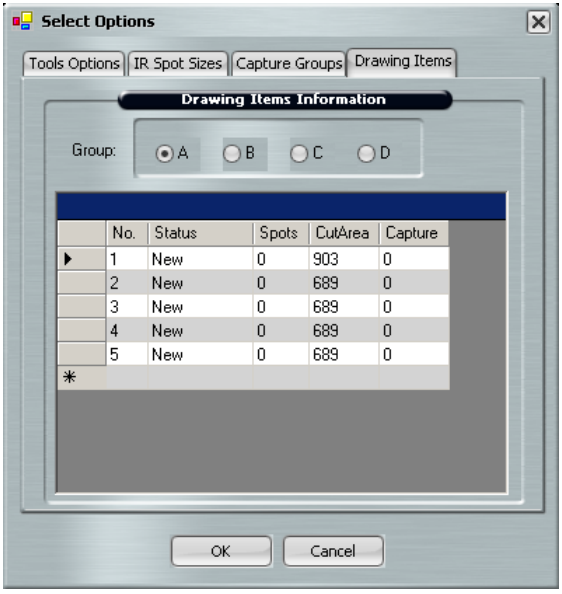


Figure 6.4: Drawing item information.

3 To view information for another capture group, tap the radio button for that group.

4 Tap OK.

Note: This information is also available in the information box located to the right of the slide overview. Tap on a drawing item in the Select Tool pane to display the information.

6.4. SETTING DRAWING TOOLS OPTIONS

To set options for the Eraser, Freehand Drawing, and Defined Circle Area tools:

1 Tap the Options button in the upper right corner of the Select tools pane to open the Select Options dialog box.

2 Tap the Tools Options tab.

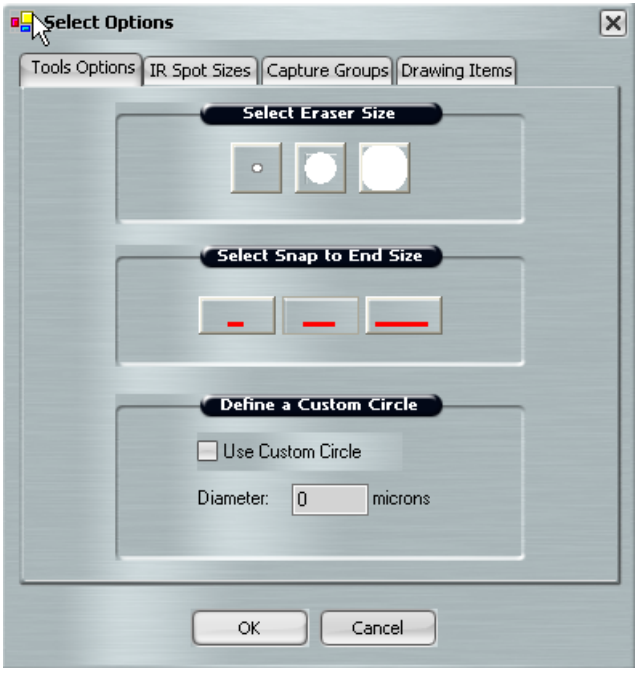


Figure 6.5: Tools Options tab in the Select Options dialog box.

3 In the Tools Options tab, set the following options as desired.

- Select Eraser Size—Tap the button corresponding to the size for the Eraser tool.
- Select Snap to End Size—Tap the button to indicate the distance considered "close enough" for the two ends of a shape drawn by the Freehand tool to automatically snap together and close the drawing item. When the distance is the specified length or less, the software creates an enclosed shape.
- Define a Custom Circle—Check Use Custom Circle to set the Circle tool to draw a circle of a fixed diameter. In the Diameter field, enter the diameter for the custom circle, in microns. To draw a custom circle, tap the Circle tool and then tap the main image window where the circle should be placed.

4 Tap OK to close the dialog box and save your changes.

6.5. SETTING THE IR CAPTURE SPOT SIZE

In the IR Spot Sizes tab, you can set the size for each of the three sizes of IR capture spots. When you create a drawing item, you choose the size of the capture spots associated with the drawing item. If you know you are only going to use one of the three spot sizes, only follow the procedure below for that size.

This procedure involves adjusting the laser power and duration so that the laser spot in the main image window is the same diameter as set in the Select Options dialog box. If you do not set the spot size, you risk incomplete capture of your tissue.

Note: You should set the IR capture spot size before creating any drawing items. The select IR spot size will be placed in all drawn/marked items. IR laser power and duration are stored with the drawing item and will be used for IR capture.

To set the IR capture spot size:

1. Place cap onto slide and move to a clear (non-tissue) area on the slide.
2. Tap the Options button in the upper right corner of the Select tools pane to open the Select Options dialog box. 
3. Tap the IR Spot Sizes tab.

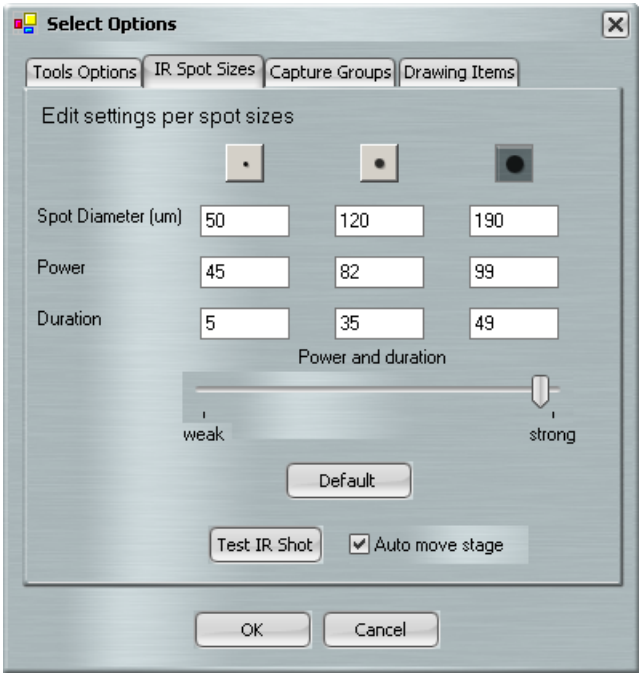


Figure 6.6: IR Spot Sizes tab in the Select Options dialog box.

4 Tap the button under Edit settings per spot sizes for the size of spot (small, medium or large) for which you want to set the parameters.

5 In the Spot Diameter (microns) field, enter the desired diameter for the IR capture spot or tap Default to use the default values.

Note: The Default button resets the values for all of the IR capture spots, not just the currently selected spot.

6 Check Auto move stage to move the stage each time the laser is test fired.

7 Tap Test IR Shot.

The software will fire the IR capture laser.

Note: A CapSure Cap must be placed prior to test IR shot.

8 In the main image window, measure the diameter of the spot created by the laser.

a Tap the Ruler tool in the Select tools pane. 

b Tap the left side of the spot created by the IR capture laser then drag the stylus to the other side of the spot. The software draws a line and a label showing the diameter of the spot.

c Tap the Move Stage tool in the Select tools pane to turn off the Ruler tool. 

9 If the diameter is the same as the value in Spot Diameter field, you are done.

If the diameter is not the same, adjust the laser power and duration with the Power and duration slider.

- To make the spot larger, move the slider to the right.
- To make the spot smaller, move the slider to the left.

10 Repeat steps 6 through 8 until the diameter in the main image window and the Spot Diameter field are the same.

11 If desired, repeat the procedure for the other spot sizes.

6.6. MEASURING DISTANCES AND OBJECTS

You can measure distances or objects in the main image window with the Ruler tool.

To measure with the Ruler tool:

1 Tap the Ruler tool. 

2 In the main image window, drag the stylus from one edge to the other across the distance or object you want to measure.

The software draws a line and a label showing the length of the line.

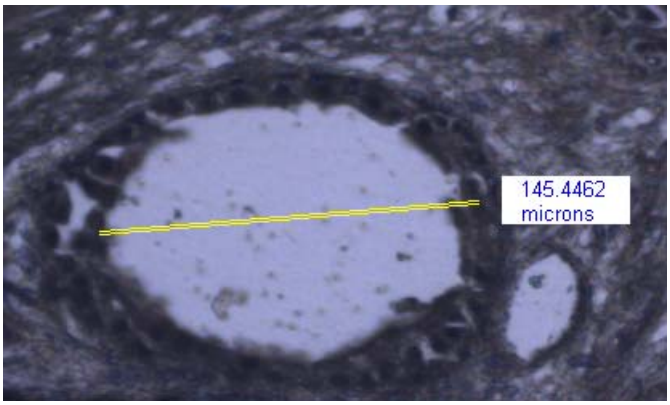


Figure 6.7: Measuring distances and objects with ruler tool.

3 Measure other objects as desired.

To remove the measuring lines and labels, tap the Move Stage tool. 

6.7. WORKING WITH OVERLAYS

You can save the drawing items to a separate file, then open the file and use it to capture identical regions from the same slide.

To save an overlay:

1 Load your slides and identify the tissue you want to capture with the drawing tools.

2 Choose Save > Markup from the File menu.

3 Enter a file name in the dialog box and tap Save.

Note: Do not use any of the following file names for the markup file: capturegroupattributes.bin, containers.bin, cutncapture.bin, drawingitems.bin, imagesettings.bin and observesettings.bin. These names are reserved for system files required by the Arcturus^{XT} software.

The overlay is saved as a .bin file in the C:\Program Files\Molecular Devices/ArcturusXT folder. The overlay file contains the drawing items and their locations.

To use a saved overlay:

1 Choose Open > Markup from the File menu.

2 Navigate to the .bin file containing the overlay and tap Open.

The drawing items appear in the main image window in the original locations. Any drawing items in the main image window remain there. You can microdissect at this stage or you can work with the drawing items as needed.

6.8. WORKING WITH CAPTURE GROUPS

To move the stage to view a specific drawing item in a capture group:

1 In the Select tools pane, select the drawing item:

a Tap the letter (A, B, C, or D) in the Capture Groups list for the capture group of interest.

b In the Drawing Items list, tap the number of the item you want to see.

The stage moves so that the selected item is highlighted and centered in the main image window.

To set formatting properties of a capture group:

1 Tap the Options button in the upper right corner of the Select tools pane to open the Select Options dialog box. 

2 Tap the Capture Groups tab.

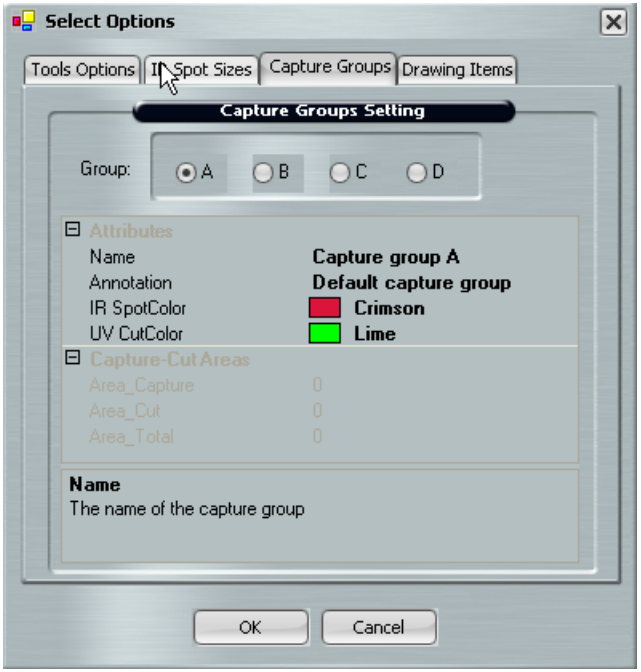


Figure 6.8: Capture Groups tab in the Select Options dialog box.

3 Tap the radio button for the capture group of interest.

4. In the Attributes area, set options for the drawing items within the current capture group. Information supplied in this field appears in the cap interaction history. If use "Default capture group" then:
   - Name—The name of the capture group. This appears in the cap interaction history.
   - Annotation—This appears in the cap interaction history.
   - IR SpotColor—The color of the IR capture spots (in the main image).
   - UV CutColor—The color of the drawing line (in the main image).
5. If desired, repeat steps 3 and 4 for any other capture groups.
6. Tap OK to close the dialog box and save your changes.

   Note: These settings are also available in the Information area to the right of the slide overview image (see Figure 2.1 on page 39). Tap on a capture group in the Select tools pane to display the information and make any desired changes.

7. Microdissecting Cells and Tissue

7.1. CHAPTER OVERVIEW

This chapter explains:

→ How to place a cap and microdissect cells and tissue.

→ How to inspect caps and slides after microdissection.

→ How to unload caps and slides.

→ How to locate each of the lasers.

→ How to set parameters for IR capture and UV cutting.

→ How to view the cap interaction history file.

→ How to use the laser bypass button.

7.2. CAPTURING CELLS BY MICRODISSECTION

Use the tools in the Microdissect tools pane to microdissect the selected cells.

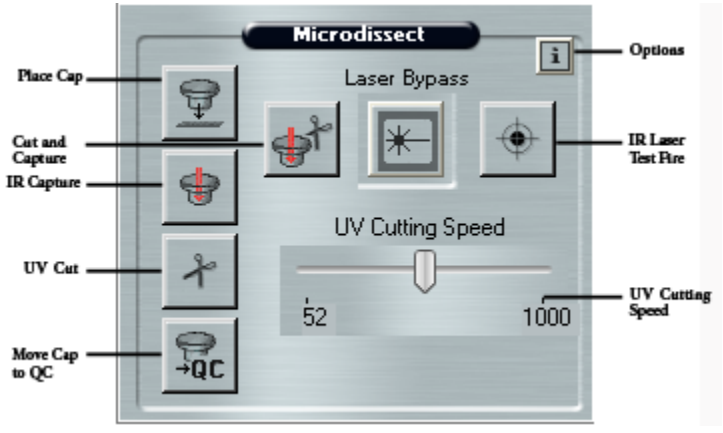


Figure 7.1: The Microdissect tools pane.

To capture cells automatically:

1 If desired, review the drawing items in each capture group.

a In the Select Tools pane, tap the letter (A, B, C, or D) in the Capture Groups list for the capture group of interest.

b Tap each number in the Drawing Items list.

The stage moves as needed to display the drawing item at the center of the main image.

2 If you want to add more IR capture spots to any item:

a If needed, tap the IR Spot Size control to select a different spot size.

b Tap the IR Spot tool. 

c Tap inside any of the items in the main image.

A spot is drawn where you tapped.

d Tap the Move Stage tool to deactivate the IR Spot tool. 

3 Locate the laser(s) as appropriate:

- See *"Locating the UV Cutting Laser"* on page 56.
- See *"Locating the IR Capture Laser"* on page 58.

4 When you are ready to microdissect, tap the Cut and Capture button. 

The order of the two procedures, cut and capture, depends upon a setting in the Microdissect tab in the Microdissect Options dialog box (see *"Setting the Cut and Capture Order"* on page 59).

The section below describes the default order: IR capture followed by UV cutting.

a If a cap is not present on the slide, then the instrument automatically picks up a cap from the load area and places it on the slide, at the center of the main image window. If a cap is already present on the slide, as necessary, the instrument will pick up the cap and move it to the area represented in the main image. The cap location is outlined in green in the slide overview image.

b The instrument fires the IR capture laser at the position of each IR capture spot, to fuse the cells to the cap.

c After IR capture is completed, the instrument automatically continues with UV cutting. 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.
- For membrane glass slides and membrane frame slides—The instrument cuts around the region of interest, leaving tabs. Tabs are short stretches where the UV 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. You can set the number of

tabs, their size and spacing (see *"Setting Properties for Cut and Capture"* on page 60).

5 Follow the instructions in *"Inspecting Microdissected Material"* on page 54 to inspect the cap and slide.

To manually perform IR capture and UV cutting:

1 If desired, review the drawing items in each capture group.

a In the Select Tools pane, tap the letter (A, B, C, or D) in the Capture Groups list for the capture group of interest.

b Tap each number in the Drawing Items list.

The stage moves as needed to display the drawing item at the center of the main image.

2 If you want to add more IR capture spots to any item:

a If needed, tap the IR Spot Size control to select a different spot size.

b Tap the IR Spot tool. 

c Tap inside any of the items in the main image.

A spot is drawn where you tapped.

d Tap the Move Stage tool to deactivate the IR Spot tool. 

3 Tap the Place Cap tool to place a cap on the slide.

The instrument places a cap at the center of the area bordered by the red box in the slide overview image. The cap location is outlined in green. The entire area inside the circle is available for capture.

4 Move the stage so the cap is on an area away from the tissue but still within the cap. Tap the IR Laser Test Fire button to test the capture laser. 

Inspect the laser spot. You should see a dark ring fused to the slide with a clear center, as shown below.

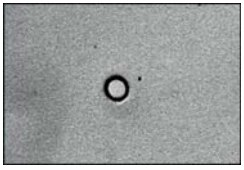


Figure 7.2: Properly wetted spot for a CapSure® Macro LCM Cap.

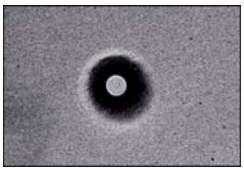


Figure 7.3: Properly wetted spot for a CapSure® HS Cap.

If you do not see this, open the Select Options dialog box, adjust the Power and duration slider in the IR Spot Sizes tab (see *"Setting the IR Capture Spot Size"* on page 45), then tap the IR Laser Test Fire button again.

5 Repeat step 4 until you see proper wetting with the IR capture laser.

6 As desired:

- Tap the UV Cut button to perform all cuts. 
- Tap the IR Capture button to perform all captures. 

7 If the microdissection was not complete, you can repeat the process. Select one of the following from the Microdissect menu:

- UV Cut > Selected Items or Current Capture Group.
- IR Capture > Selected Items or Current Capture Group.
- IR Capture and UV Cut > Selected Items or Current Capture Group.

8 Follow the instructions in *"Inspecting Microdissected Material"* on page 54 to inspect the cap and slide.

7.3. INSPECTING MICRODISSECTED MATERIAL

Following microdissection, you may inspect the slide and the cap to verify that the collection was successful. If microdissection was incomplete, you can repeat the cut and/or capture steps.

1 Tap the Move Cap to QC station button in the Microdissect Tool pane. Alternatively, right-click on the slide overview and select Move Cap to QC station from pop-up menu. 

2 The cap is moved to the QC position and the stage moves the QC position over the objective. The cap is shown in the main image window.

The Arcturus^{XT} software creates the cap interaction history file. This file is named "CapReport-YYMMDD-HHMM.html," where YYMMDD is the date and HHMM is the time when the file was created. The file is saved to the location specified in the ReportSubfolder in the Load Options dialog box.

Figure 7.4: QC cap position.

3 Inspect the tissue on the cap.

You can capture a static image at this point, if desired. See page 37 for directions on capturing a static image.

4 To inspect the slide, tap the slide overview image at the position you want to see in the main image window, or

Tap any of the items in the Drawing Items list in the Select Tools pane.

The stage will move as needed to display the item in the main image window.

5 If any drawing item was not captured during microdissection, do the following:

a Move the cap from the QC station back to the slide by placing the icon on the cap and right-clicking on the cap in the QC area to the right of the slide overview image. Then select Replace Cap on Slide from the pop-up menu.

b Select the items to re-cut or re-capture using either the Select Object(s) tool or by tapping the number of the item in the Drawing Items list (in the Select tools pane).

c Select one of the following from the Microdissect menu:

- UV Cut > Selected Items or Current Capture Group.
- IR Capture > Selected Items or Current Capture Group.
- IR Capture and UV Cut > Selected Items or Current Capture Group.

6 To capture using another capture group, select the desired Capture Group (A, B, C or D) in the Select Tools pane and proceed following the steps detailed above.

7.4. UNLOADING MATERIALS

To unload materials:

1 Tap Present Stage in the Setup tools pane.

The work surface presents itself.

2 Slide the cap insertion tool onto the work surface. Make sure the open end of the insertion tool faces the cap in the QC station and slides over the empty cap receptacles.

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

4 Remove the insertion tool from the QC position with the cap attached to it.

Note: Be sure you do not touch the polymer surface that holds the microdissected cells as you remove the caps from the unload stations.

5 Repeat steps 2, 3, and 4 for each cap in the QC Cap area.

6 Press the tension button in to release the springs holding the slides in place and then lift each slide out of its slot.

7 Tap Present Stage again to move the work surface to the left.

Microdissected cells are now available for extraction of nucleic acids and proteins. The final step in microdissection is extracting cells and tissue from the caps; see Chapter 8, *"Extracting Cells and Tissue"* on page 65 for instructions.

7.5. LOCATING THE UV CUTTING LASER

You should locate the UV cutting laser for each objective at the beginning of each session to ensure that the laser will cut accurately. If you notice that the UV laser is not fired at the desired location, you should repeat this procedure.

To locate the UV laser:

1 Tap the Options button in the upper right corner of the Microdissection tools pane to open the Microdissect Options dialog box. 

2 Tap the UVLocate tab.

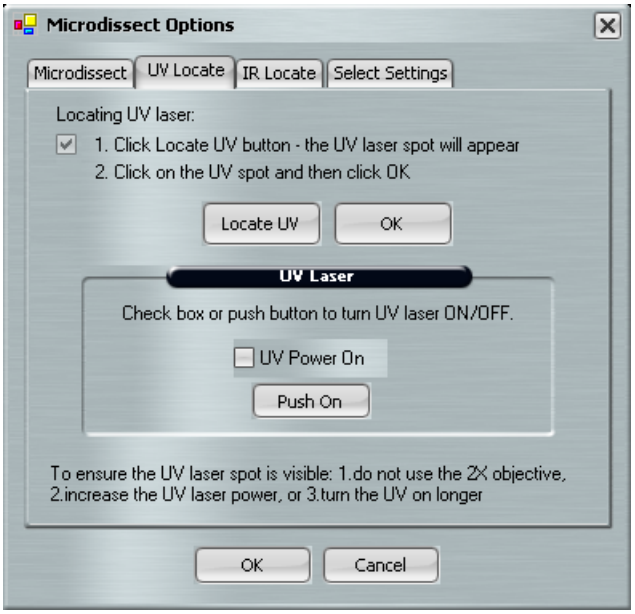


Figure 7.5: UV Locate tab in the Microdissect Options dialog box.

3 Follow the steps in the dialog box. The check mark indicates the current step in the procedure.

a Tap the Locate UV button.

The Arcturus$^{XT™}$ Microdissection Instrument fires the cutting laser.

b In the main image window, tap the location of the UV laser spot, then tap OK in the dialog box.

c If the laser spot is not visible in the main image window, tap Push On to manually turn on the UV cutting laser until the laser spot is visible.

4 Tap OK to close the dialog box.

7.6. LOCATING THE IR CAPTURE LASER

You should locate the IR capture laser at the beginning of each session. You should also relocate the IR capture laser during a session:

→ If you change objectives between IR captures.

→ If you notice that the IR laser is not firing at the desired location.

To locate the IR capture laser:

1 Tap the Options button in the upper right corner of the Microdissection tools pane to open the Microdissect Options dialog box.

2 Tap the IR Locate tab.

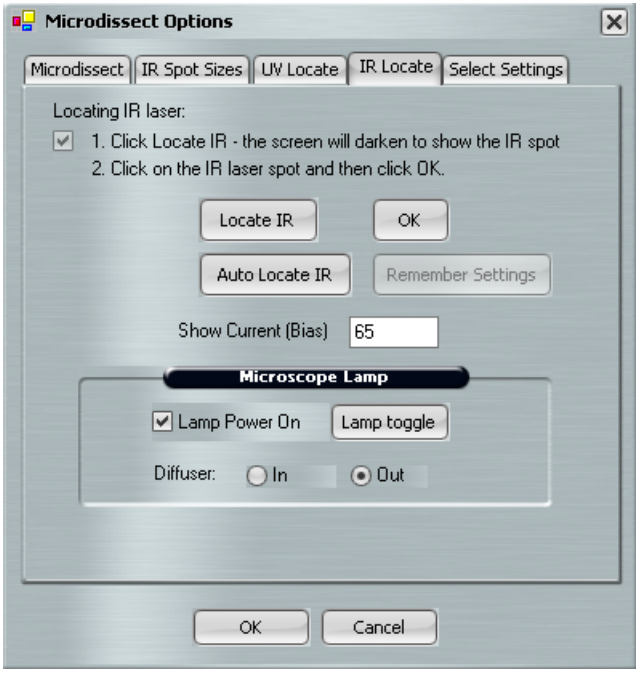


Figure 7.6: IR Locate tab in the Microdissect Options dialog box.

3 To manually locate the IR laser:

- The live image should darken and the IR laser should be visible.
- If the diffuser is in use, tap Out to remove it.
- Tap the Show Current (Bias) field and enter a value until the IR laser becomes visible. Typically a value greater than 50 is required in such instances. If necessary increase the Brightness settings located in the Inspect Tool pane until the IR laser is visible. Also, if necessary adjust the brightness settings in the Inspect Tool pane until the IR laser becomes more visible.
- Click on the center of the IR laser beam and then click the OK button in the Microdissect Option dialog.

4 To automatically locate the IR laser, tap Auto Locate IR.

The Arcturus^{XT} software turns on the IR capture laser at low power and tries to locate the laser. If the laser is successfully located, the Arcturus^{XT} software draws a circle on the main image where the laser is located.

5 Look at the main image and see if the laser spot is located within the circle.

- If the spot is within the circle, you are done. Tap OK, at the bottom of the dialog, to close it and save the changes.
- If the spot is not located within the circle, follow the steps to manually locate the capture laser.

6 Tap OK to close the dialog box and save the changes.

7.7. SETTING THE CUT AND CAPTURE ORDER

By default, the Arcturus$^{XT™}$ Microdissection Instrument performs the IR capture first, followed by UV cutting. For HS Caps, which are farther from the surface of the tissue, or when performing live cell applications, it is better to complete the capture process first, before cutting around the drawing item.

To set the order of cutting and capture:

1 Tap the Options button in the upper right corner of the Microdissection tools pane. 

2 Tap the Microdissect tab.

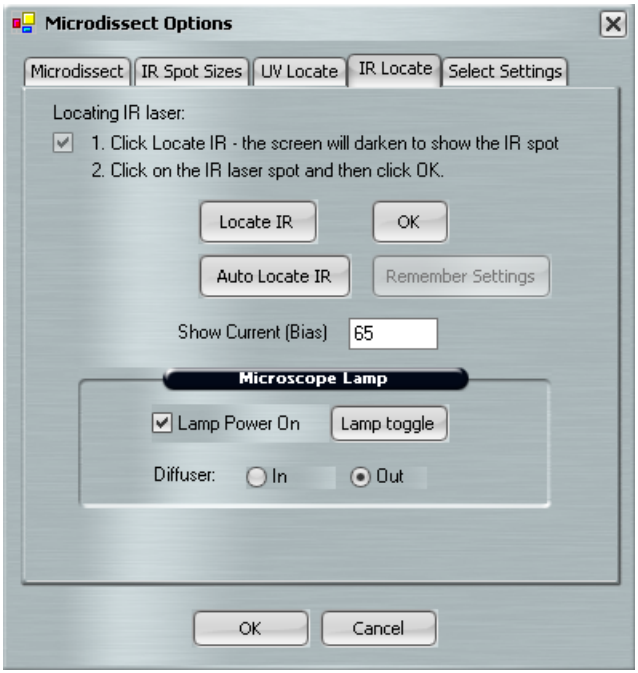


Figure 7.7: Microdissect tab in the Microdissect Options dialog box.

3 Tap IR Capture first or UV-Cut first to set the order.

4 Tap OK to close the dialog box and save your changes.

7.8. SETTING PROPERTIES FOR CUT AND CAPTURE

The settings in this tab allow you to set the properties for cutting and capture.

To set cutting and capture options:

1 Tap the Options button in the upper right corner of the Microdissection tools pane.

2 Tap the Select Settings tab.

3 As needed, tap IRSpotSpacing and enter a value. This is the spacing between IR capture spots, as a percentage of the spot diameter.

If IRSpotSpacing is:

- < 100%, the IR capture spots will overlap.
- 100%, all the IR capture spots will touch but not overlap.

- > 100%, there will be spaces between the IR capture spots.

With overlap, there is tighter IR capture of the cells. If your cells are loosely aggregated, less overlap might be preferable.

4 As needed, set the UV Settings:

The settings here include details of 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 tissue to the CapSure® Caps.

- IR SpotsPerCutLength—Number of IR spots provided per each UV cut length.
- Tab Length—Distance (in microns) of space in between each UV cut length.
- UV CutLength—Length of UV cut line (in microns) before tab insertion.
- UVCuttingSpeed—This is the speed of the UV cutting laser. Increase this to cut more quickly. If this value is too high, UV cutting may be less accurate.

5 Tap OK to close the dialog box and save your changes.

All changes you make here apply only to new any drawing items, except DashedCutLength and DashedSkipLength which apply retroactively to existing drawing items.

Note: These settings are also available in the Information area to the right of the slide overview (see Figure 2.1 on page 7). To display these settings in the Information area, tap in the UV Cutting Speed in the Microdissect tools pane.

7.9. WORKING WITH CAPS

7.9.1. VIEWING A DIFFERENT CAP IN THE QC AREA

To view a different cap:

1 To the right of the slide overview image, place cursor, right-click and tap on the Cap button for the cap of interest in the QC cap position. Then select View Cap at QC station from pop-up menu.

Figure 7.8: QC cap position.

The stage will move the cap over the objective. You can view the cap and any microdissected material on the cap. You may also capture static images of the cap, if desired.

7.9.2. VIEWING CAP PROPERTIES

You can view the cap type in the cap and slide handling area to the left of the slide overview image. If you did not select the correct cap type when you loaded your caps, you can change it here.

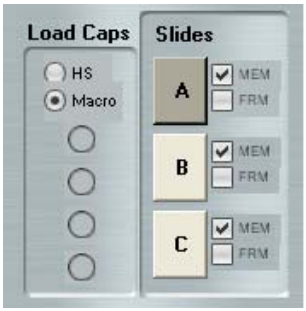


Figure 7.9: Cap and slide handling area.

In addition, you can add caps to the loaded caps area by tapping a cap in the Load area here. This shortcut lets you load caps without opening the Load Options dialog box.

Note: In order to use this shortcut, the corresponding QC station must be empty.

You can view properties of a cap (its name and any notes) in the information area to the right of the slide overview image. If you want, you can edit the CapName and/or CapNotes here. Editing here is the same as entering the information in the Load Options dialog box (see page 19).

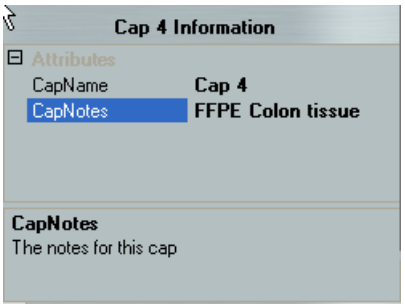


Figure 7.10: Information area, showing cap information.

7.9.3. VIEWING THE CAP INTERACTION HISTORY

The Arcturus^{XT} software creates the cap interaction history file the first time a cap is off-loaded and updates it throughout the session. This file contains information about the caps, the slides, and the capture groups, as well as the total area of microdissected tissue.

To view the cap interaction history:

1. Tap outside of the Arcturus^{XT} software so you can see the desktop.
2. Navigate to the StudyFolder\ReportSubfolder that you set in the Load Options dialog box.
3. Double-click on the CapReport-YYMMDD-HHMM.html file.

   The cap interaction history opens in an Internet Explorer window.

7.10. LASER BYPASS

For safety reasons, the lasers are disabled when outside of the CapSure LCM Cap area. However, there may be applications for which the cap is not desired or required. In such instances, it is necessary to bypass the instrument laser safety settings.

To do so, the user must insert the laser bypass key into the back of the instrument. At such point the user must depress the Laser Bypass button on the user interface (see Figure 7.1). The laser bypass is activated and the laser is ready to fire when both the bypass key is in position and the bypass button has been depressed. For a full description of laser status indicators, as observed by color changes of the Laser Bypass button, refer to Appendix A.

8. Extracting Cells and Tissue

8.1. CHAPTER OVERVIEW

This chapter contains:

→ Instructions for extracting microdissected tissue from the CapSure® Macro LCM Caps and CapSure® HS Caps.

→ Suggestions for extraction kits.

8.2. EXTRACTING TISSUE FROM THE CAPS

After you have microdissected the cells of interest, you may extract the DNA or RNA from the samples. Depending upon the type of cap you used, the procedure varies.

Molecular Devices offers the PicoPure® RNA Isolation Kit (cat.# KIT0202) and the PicoPure 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 Molecular Devices, is designed specifically for extracting RNA from formalin-fixed paraffin-embedded tissue.

8.2.1. EXTRACTING FROM CAPSURE MACRO LCM CAPS

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

Detailed steps are listed below.

1 Do one of the following:

- If you are using the PicoPure RNA Isolation Kit or the PicoPure DNA Extraction Kit, follow the instructions in the user guide, or
- Place at least 40 µL of extraction buffer into a 0.5 mL microcentrifuge tube.

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

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

3 Press down firmly and rotate the insertion tool to ensure the Macro LCM 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 microdissected cells on the cap surface. If necessary, lightly tap the microcentrifuge tube.

5 Incubate the sample as described in the appropriate extraction procedure, then place the tube into a microcentrifuge and briefly spin (5–10 seconds) to bring the buffer to the bottom of the tube.

8.2.2. EXTRACTING FROM CAPSURE HS CAPS

Following microdissection, 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 cells in a small volume of extraction buffer.

Note: Due to the manner in which 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.

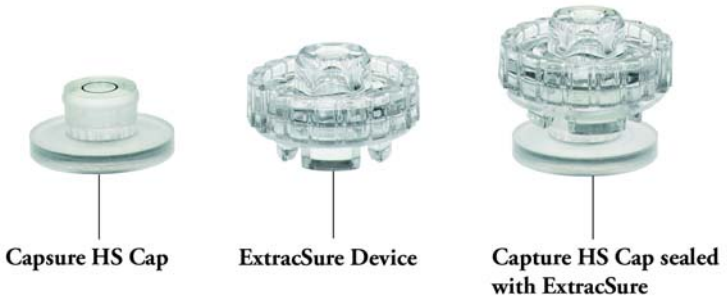


Figure 8.1: CapSure HS cap and ExtracSure device.

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.
4. At this point, the ExtracSure Device should be firmly sealed to the CapSure HS Cap.
5. You may add extraction buffer to the device. Do not remove the assembled ExtracSure and CapSure HS Device from the alignment try until incubation is completed.
6. 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.
7. 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.

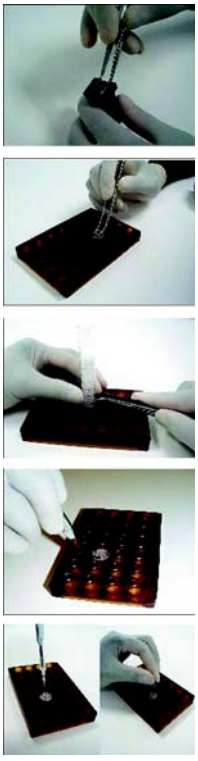


Figure 8.2: Placing assembled CapSure HS Cap and Extracsure Device in the Alignment Tray.

8.3. MOLECULAR DEVICES SYSTEMS FOR MICROGENOMICS

Molecular Devices offers two extraction kits specifically designed to work with the CapSure® LCM Sample Preparation System:

→ The PicoPure® RNA Isolation Kit (cat.# KIT0202)

→ The PicoPure® DNA Extraction Kit (cat.# KIT0103)

These kits provide detailed step-by-step protocols for extracting DNA and RNA from frozen cells.

The Paradise® Reagent System, also from Molecular Devices, is designed specifically for extracting RNA from formalin-fixed paraffin-embedded tissue.

9. Maintenance and Troubleshooting

⚠ **CAUTION:** Do not attempt to remove the covers from the instrument except as specified for the user-serviceable parts listed below. If the instrument requires service or repair contact Molecular Devices at +1-800-635-5577.

9.1. CHAPTER OVERVIEW

This chapter contains:

→ Instructions for cleaning the Arcturus$^{XT™}$ Microdissection Instrument.

→ Information about user-serviceable parts.

→ Tips for troubleshooting.

9.2. CLEANING THE ARCTURUS^{XT} MICRODISSECTION 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.

9.3. USER-SERVICEABLE PARTS

The Arcturus^{XT} Microdissection Instrument has only four user-serviceable parts:

→ The bright field illumination lamp.

→ The interchangeable fluorescence filter cube, for instruments so equipped.

→ The fluorescent lamp, for instruments so equipped.

→ The fuse.

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

9.4. REPLACING THE BRIGHT FIELD ILLUMINATION LAMP

Depending upon the model of instrument you have purchased, there are different instructions for replacing the bright field illumination lamp.

9.4.1. FOR INSTRUMENTS WITH THE 100 W HALOGEN LAMP

You will need the replacement lamp, a 2mm hex driver, a slotted screw driver, clean, powder-free gloves and the instructions from the microscope manufacturer for this procedure.

Follow the instructions provided with the instrument to replace the lamp.

9.4.2. FOR INSTRUMENTS WITH THE HIGH INTENSITY LED

You will need the replacement LED, a #2 Phillips screwdriver, a 2.5 mm hex key and clean, powder-free gloves for this procedure.

To replace the bright field illumination lamp:

1. If needed, exit the Arcturus^{XT} software. Turn the instrument off and unplug it. (When you are facing the instrument, the switch is located on the left side. The plug is on the back of the instrument.)
2. Remove the top cover of the instrument. There are four screws on the top of the cover and one screw on the left side. Unscrew the cover and lift it up and off. Place the cover in a safe location.
3. Use the hex key to unscrew the two screws holding the LED.
4. Unplug the LED cable (it has a white connector) and remove the LED.
5. Replace the LED, connecting the cable and replacing the two screws.
6. Replace the top cover and then tighten the screws.
7. Plug the instrument in and turn it on.
8. Restart the Arcturus^{XT} software.

9.4.3. INTERCHANGING FLUORESCENCE FILTER CUBES

When selecting alternate fluorescence filter cubes, you should ensure the dichroic and emission filters have at least 65% transmission at 810 nm, otherwise the automatic laser location feature will fail when the filter is in the optical path. (If you want to use a filter with lower transmission, you can manually locate the laser.) Filters should be compatible with the Nikon TE2000 microscope.

To replace a fluorescence cube:

1. If needed, exit the Arcturus^{XT} software. Turn the instrument off and unplug it. (When you are facing the instrument, the switch is located on the left side. The plug is on the back of the instrument.)

2 Remove the cover and select the desired filter position from the carousel below the microscope's objective turret.

3 Remove the existing filter cube (if present) by pulling straight out.

4 Insert the new filter cube by pushing straight in.

5 Replace the cover.

9.4.4. REPLACING THE FLUORESCENCE LAMP

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

Follow the instructions provided with the lamp.

9.4.5. REPLACING THE FUSE

You will need the new fuse (2A, time delay, 5 x 20 mm) and a slotted screwdriver for this procedure.

To replace the fuse:

1 If needed, exit the Arcturus^{XT} software. Turn the instrument off and unplug it. (When you are facing the instrument, the switch is located on the left side. The plug is on the back of the instrument.)

2 The fuse is located in the plug socket. Use the slotted screwdriver to open the fuse holder.

3 Remove the fuse and replace it with the new one.

4 Plug in the instrument and turn it on.

9.5. TROUBLESHOOTING

If you encounter a problem that you cannot resolve using this troubleshooting section, contact Molecular Devices +1-800-635-5577. You can also send email to support@moldev.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 Molecular Devices Technical Support. This procedure is found in the 14601-00, Arcturus^{XT} Adjustment, Calibration and Test Procedure document located in the C:\Program Files\Molecular Devices\ArcturusXT folder (it is also available from Technical Support).

9.5.1. CAPTURE

Symptom	Remedy
Cells do not adhere to the film	• Ensure wetting is adequate. If necessary, adjust the laser settings to achieve proper wetting before proceeding (see *"Capturing Cells by Microdissection"* on page 51). • 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 Chapter 3, *"Tissue Preparation"* on page 13). If necessary, dehydrate the sample in 100% ethanol for 1 minute, followed by xylene for 5 minutes.

9.5.2. UV CUTTING LASER

Symptom	Remedy
Laser does not cut	• Select Microdissect from the Options menu. In the UV Locate tab press "Push ON" under UV Laser to fire the UV laser. You should see a small (1–5 microns) hole in the tissue in the center of the main image window. If not, check the Neutral Density Filter settings under the UV cover. Remove one or more filters from the light path to increase the intensity.

9.5.3. IR CAPTURE LASER

Symptom	Remedy
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 Illumination controls in the Inspect tools pane 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. • Change to a lower objective to see a larger field of view and refocus 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. • Laser targeting beam is too weak to see. Turn the white light off and increase the laser intensity. • You can try firing the laser to locate the beam. Fire within the area of the cap—away from tissue if using a CapSure® Macro LCM Cap, or outside the circle if using a CapSure® HS Cap.
Capture laser does not fire	• The laser is not enabled. • 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. Use the Illumination controls in the Inspect tools pane to lower the lamp intensity 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.) 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. • Measure the spot size, then adjust the size, if necessary.

Symptom	Remedy
Cannot achieve proper wetting when capture laser is fired	• Ensure the laser settings are correct. • 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.

A. Appendix: Laser Safety

A.1. LASER BYPASS

For safety reasons, the lasers are disabled when outside of the CapSure LCM Cap area. However, there may be applications for which the cap is not desired or required. In such instances, it is necessary to bypass the instrument laser safety settings.

To do so, the user must insert the laser bypass key into the back of the instrument. At such point the user must depress the Laser Bypass button on the user interface (see Figure 7.1). The laser bypass is activated and the laser is ready to fire when both the bypass key is in position and the bypass button has been depressed.

The table below details possible status scenarios, showing action combinations and resulting system response. For example, Line 4 indicates that when the laser bypass button has been depressed and a CapSure cap is in place, but the laser bypass key has NOT been inserted, the laser status is "Standby". The user must turn the key into position for the laser to be ready to fire.

Actions											
State	**Laser Bypass Button Depressed**	**Laser Bypass Key in Position**	**CapSure Cap in Place**	**Laser State**	**To State**	**SetLaser**	**Color**	**Pop-up**	**Cut/ Capture**	**Others**	**Tool Tip Messages**
0				Standby		Standby	U.Red		Stop		tt = 'Laser disabled because cap is out of beam path and override is off.'
1				ON	0	Standby	U.Red	x	Stop		tt = 'Laser disabled because cap is out of beam path and override is off.' popup = 'Lasers have been disabled because cap is out of beam path and override is off. Change cap placement or enable override to continue.'
2			x	Standby		Standby	U.Yellow		Stop	Clean obj status	tt = 'Laser ready to fire. Cap placed in beam path.'

Actions											
State	Laser Bypass Button Depressed	Laser Bypass Key in Position	CapSure Cap in Place	Laser State	To State	SetLaser	Color	Pop-up	Cut/ Capture	Others	Tool Tip Messages
3			x	ON		Power=p	U.Green				tt = 'Laser is firing. Cap placed in beam path.'
4	x		Standby		Stand by	U.Orange		Stop	Clean obj status		tt = 'Laser disabled because cap is out of beam path and override is off.'
5		x		On	4	Standby	U.Orange	x	Stop	Clean obj status	tt = 'Laser disabled because cap is out of beam path and override is off.' popup = 'Lasers have been disabled because cap is out of beam path and override is off. Change cap placement or enable override to continue.'
6		x	x	Standby		Standby	U.Yellow		Stop	Clean obj status	tt = 'Laser ready to fire. Cap placed in beam path.'
7		x	x	ON		Power=p	Green				tt = 'Laser is firing. Cap placed in beam path.'
8	x			Standby	0	Standby	U.red	x	Stop	Clean obj status	tt = 'Laser disabled because cap is out of beam path and override is off.' popup = 'Lasers cannot be enabled because the hardware bypass key is absent.'
9	x			ON	0	Standby	U.Red	x	Stop	Clean obj status	tt = 'Laser disabled because cap is out of beam path and override is off.' popup = 'Lasers cannot be enabled because the hardware bypass key is absent.'
10											tt = 'Laser ready to fire. Cap placed in beam path.'

Actions											
State	**Laser Bypass Button Depressed**	**Laser Bypass Key in Position**	**CapSure Cap in Place**	**Laser State**	**To State**	**SetLaser**	**Color**	**Pop-up**	**Cut/ Capture**	**Others**	**Tool Tip Messages**
11	x		x	ON	3	Power=p	U.Green	x			tt = 'Laser is firing. Cap placed in beam path.' popup = 'Laser interlock cannot be bypassed because the hardware key is not detected.'
12	x	x		Standby		Standby	PF.Yellow		Stop	Clean Obj. Status	tt = 'Laser ready to fire. Cap overridden and not placed in beam path.'
13	x	x		ON		Power=p	PF.Green				tt = 'Laser is firing. Cap overridden and not detected in beam path.'
14	x	x	x	Standby		Standby	P.Yellow		Stop	Clean obj status	tt = 'Laser ready to fire. Cap overridden but detected in beam path.'
15	x	x	x	ON		Power=p	P.Green				tt = 'Laser is firing. Cap overridden but detected in beam path.'

C. Appendix: Software Reference

C.1. MENUS

C.1.1. FILE MENU

Command	Submenu	Description
Open	Image	Allows you to open a saved image file. The image appears in a new window.
	Markup	Allows you to open a saved markup file.
Save	Image	Allows you to save the image currently visible in the main image window to a file.
	Markup	Allows you to save the current drawing items as an overlay.
Exit		Closes the software.

C.1.2. EDIT MENU

Command	Submenu	Description
Paste Selected Object(s)		Pastes the currently selected drawing items.
Delete Selected Object(s)		Deletes the currently selected drawing items.
Select Objects	In Current Capture Group	Selects all drawing items in the current capture group.
	In Capture Group	Choice of A, B, C, or D from a sub-submenu. Selects all drawing items in the specified capture group.
	In All Capture Groups	Selects all drawing items, irrespective of their capture group.
Move Selected Object(s) to Group	A	Moves the selected object(s) to the capture group in the submenu.
	B	
	C	
	D	

C.1.3. VIEW MENU

Command	Description
Scale	Displays a scale on the main image. 
Change to Left-hand Orientation	Moves the tools pane from the right side of the software to the left side.
Camera Properties	Opens the Camera Properties dialog box and allows you to set up the video camera (see image below).
Zoom	Not available.

C.1.4. THE CAMERA PROPERTIES DIALOG BOX

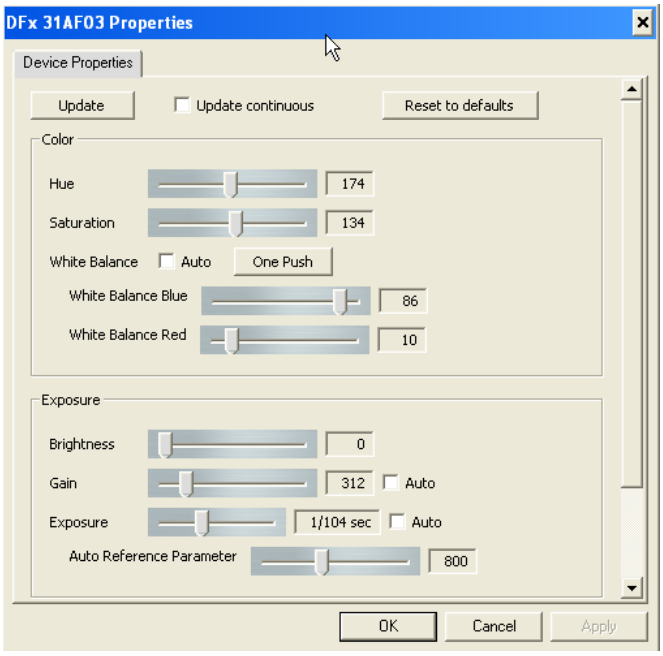


Figure C.1: Camera properties dialog box.

C.1.5. IMAGE MENU

Command	Submenu	Description
Acquire Overview		Acquires the slide overview image for the currently selected slide.

C.1.6. MICRODISSECT MENU

Command	Submenu	Description
Place Cap		Places a cap in the center of the screen. (Identical to the Place Cap button in the Microdissect tools pane.)
Offload Cap		Moves the currently loaded cap to the offload station. (Identical to the Offload button.)
UV Cut	Selected Objects	Cuts all of the currently selected drawing items.
	Current Capture Group	Cuts all drawing items in the current capture group.
	Capture Group	Choice of A, B, C, or D from a sub-submenu. Cuts all drawing items in the specified capture group.
IR Capture	Selected Objects	Attaches all of the currently selected drawing items.
	Current Capture Group	Attaches all drawing items in the current capture group.
	Capture Group	Choice of A, B, C, or D from a sub-submenu. Attaches all drawing items in the specified capture group.
IR Capture and UV Cut	Selected Objects	Attaches and cuts all of the currently selected drawing items.
	Current Capture Group	Attaches and cuts all drawing items in the current capture group.
	Capture Group	Choice of A, B, C, or D from a sub-submenu. Attaches and cuts all drawing items in the specified capture group.

C.1.7. OPTIONS MENU

Command	Submenu	Description
Load		Displays the Load Options dialog box.
Inspect		Displays the Inspect Options dialog box.
Select		Displays the Select Options dialog box.
Microdissect		Displays the Microdissect Options dialog box.
System	Calibrate	Displays the Calibrate dialog box.
	Setup Stylus	Displays a dialog box where you can set the properties of the stylus and calibrate the display.
	Trackball	Turns the trackball, when present, on or off.

C.1.8. SETTING TABLET AND STYLUS DISPLAY PARAMETERS

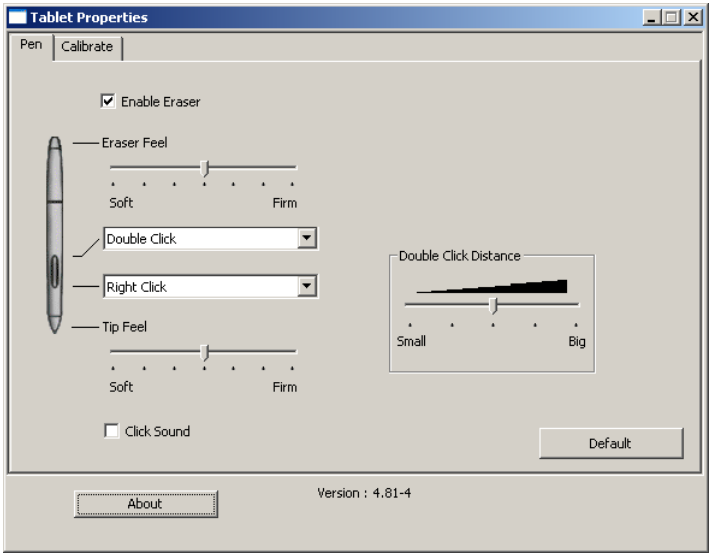


Figure C.2: The Pen tab in the Tablet Properties dialog box.

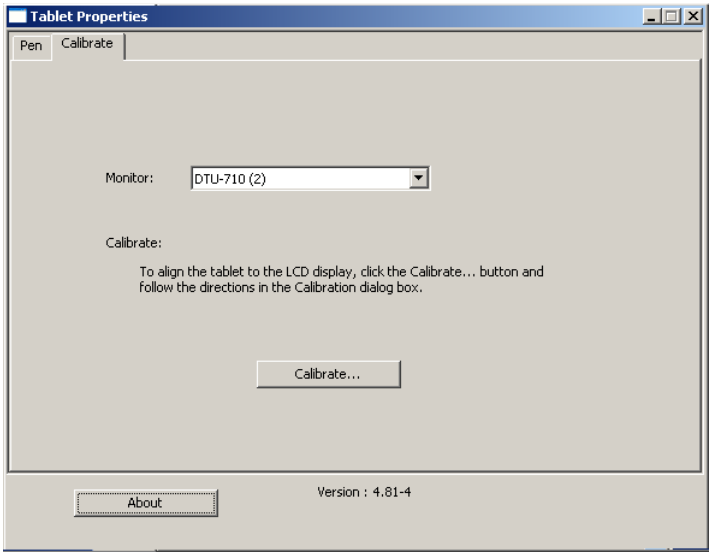


Figure C.3: The Calibrate tab in the Tablet Properties dialog box.

C.1.9. HELP MENU

Command	Description
About Box	Displays the software About Box.
Users Guide	Opens the user guide in Adobe Reader.

C.1.10. THE ABOUT BOX

The software version number is shown here.

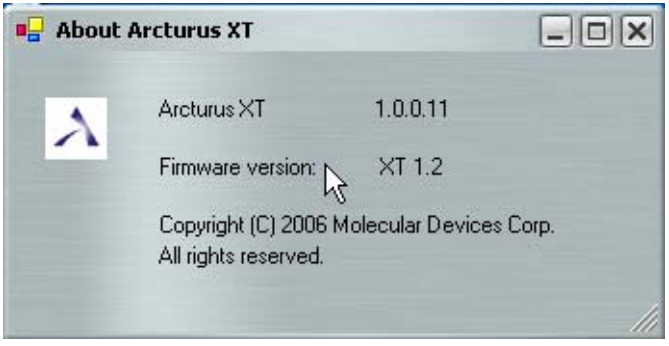


Figure C.4: About Arcturus^{XT} window.

C.2. KEY COMMANDS

Pane	Command	Notes
Main Image window	Ctrl-A	Selects all drawing items in the main image window.
	Ctrl-V	Pastes selected drawing items in the main image window.
	Ctrl-X	Deletes selected drawing items.

B. Appendix: Specifications

B.1. ARCTURUS^{XT}™ MICRODISSECTION INSTRUMENT

Depending upon the configuration of the instrument you have purchased, not all options listed below may be present on your instrument.

Electrical Supply	100–240 VAC, 50–60 Hz, 250 W (Voltage fluctuations not to exceed ±10% of nominal supply voltage.)
Fuse	2A, time delay, 5 x 20 mm
Capture Laser	Laser diode, 810 nm
UV Cutting Laser	Diode-pumped solid-state UV laser, 355 nm
Microscope Stage	Computer and trackball controlled Range 150 x 100 mm, repeatability 2 mm
Filters	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 Source	100 W halogen lamp or High intensity LED illumination system
Fluorescence Light Source	EXFO X-Cite™ 120 PC metal halide fluorescence illumination system
Operating Temperature	18°–30°C
Operating Humidity	< 60% relative humidity (noncondensing)
Dimensions	Height: 28 in. (71 cm) Width: 22 in. (56 cm) Depth: 30 in. (76 cm)
Weight	110 lb (50 kg)
Work Surface Requirements	36 in. x 72 in. (92 cm x 180 cm) with vertical clearance of 32 in. (80 cm)
Altitude	For use up to 6600 ft. (2000 m)

B.1.1. SAFETY AND ELECTROMAGNETIC COMPATIBILITY (EMC) STANDARDS

The Arcturus$^{XT™}$ Microdissection 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.

B.2. COMPUTER

→ 2.8 GHz Pentium 4 processor (minimum)

→ 2 GB RAM (minimum) 40 GB hard drive (minimum)

→ Windows® XP Professional operating system with SP 2

→ Read/write DVD drive

→ Interactive pen display, 17" diagonal LCD, 1280 x 1024 (SXGA)

B.3. AVAILABLE INSTRUMENT CONFIGURATIONS

You can choose the options for your Arcturus^{XT} Microdissection Instrument to give you the most flexible, customized system for microdissection.

Figure B.1: The Arcturus^{XT} Microdissection Instrument with LED illumination tower.

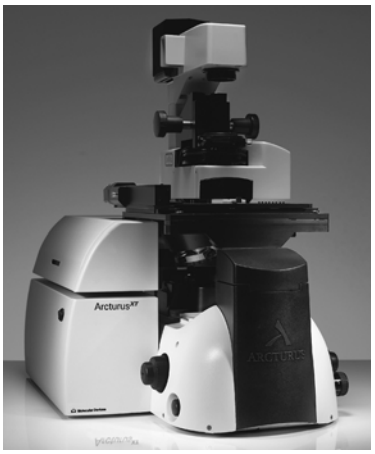


Figure B.2: The Arcturus^{XT} Microdissection Instrument with Nikon illumination tower.

B.3.1. BASE STATION

There are three base station configurations for the Arcturus^{XT} Microdissection Instrument. Each comes with three objectives (2X, 10X, 40X) and a standard 0.7MP video camera.

Table B.1: Base station configurations.

Base Station Configurations	Catalog Number
Arcturus^{XT} w/LCM	Arcturus^{XT}
Arcturus^{XT} UV Cutting Option	13796-00
Arcturus^{XT} Fluorescence Option Fluorescence cubes for excitation with emission at Red, Blue and Green	13846-00

B.3.2. ILLUMINATION TOWER OPTIONS

Table B.2: Illumination tower options.

Description	Catalog Number
Arcturus^{XT} LED Illumination Tower	14267-00
Arcturus^{XT} Phase Contrast Nikon Illumination Tower	14266-00
Differential Interference Contrast option for Nikon Illumination Tower *Note: It is recommended to purchase Arcturus^{XT} Binoculars along with the Phase Contrast Illumination Tower.*	
DIC Base Includes 10X and 40X objective sliders	14423-00
Optional objective sliders. May be purchased in conjuction with the selection of the respective optional objectives.	
•Differential Interference Contrast–20X Slider	14466-00
•Differential Interference Contrast–60X Slider	14468-00

B.3.3. ADDITIONAL OPTIONS

Table B.3: Additional upgrades and options.

Description	Catalog Number
Optional objective upgrades	
4X	14676-00
20X	14658-00
60X	14659-00
100X Dry	14662-00
100X Oil	14661-00
Binoculars	
Microscope Binoculars *Note: It is recommended to purchase Arcturus^{XT} Binoculars along with the Phase Contrast Illumination Tower.*	0200-6201
Camera	
High Resolution 5 MegaPixel Camera *Note: It is recommended to purchase the second monitor with purchase of the high resolution camera. See 10904-00.*	14379-00
Monitor	
LCD Flat screen monitor for dual monitor operation	10904-00
UV Cube	
Excitation 340–390 nm, emission > 410 nm	14776-00

D. Appendix: Installation

D.1. INSTRUCTIONS FOR LIFTING AND CARRYING THE INSTRUMENT

The Arcturus$^{XT™}$ Microdissection Instrument is shipped from the factory in two or more boxes, depending upon the configuration you have purchased. Use proper lifting techniques when unpacking and installing the instrument. Improper lifting may cause painful and permanent back injury.

Keep the following points in mind while lifting:

→ Make sure that you have a secure, comfortable grip when lifting.

→ Make sure that the path from where the object is to where it is being moved is clear of obstructions.

→ Do not lift an object and twist your torso at the same time.

→ Keep your spine in a good neutral position while lifting with your legs.

→ Share the load. Use the lifting handles that were in place when the instrument was shipped and lift with four people.

D.2. PREPARING FOR INSTALLATION

The location for the instrument must meet the following requirements:

→ Stable laboratory bench capable of supporting 400 lb. (200 kg).

→ Work surface 36 in. x 72 in. (92 cm x 180 cm) with 32 in. (80 cm) vertical clearance.

→ Electrical Supply: 100–240 VAC, 50–60 Hz, 500 W, voltage fluctuations not to exceed ±10% of nominal supply voltage.

→ Temperature 18°–30°C, relative humidity < 60%.

D.3. GENERAL UNPACKING AND INSTALLATION INSTRUCTIONS

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

It is recommended that you schedule installation with a Molecular Devices service professional. This will assure optimum performance and minimize risk of damage to the instrument.

If you choose to install the instrument yourself, refer to the following documents, included in the C:\Program Files\Molecular Devices\ArcturusXT folder:

→ Arcturus^{XT} Packing and Unpacking Procedure (14600-18).

→ Arcturus^{XT} Adjustment, Calibration, and Test Procedure (14601-00).

Detailed installation instructions are provided with the instrument. Any tools required for the installation are supplied with the instrument.

If you encounter any difficulties, contact Technical Support at Molecular Devices at +1-800-635-5577. You can also send email to support@moldev.com.

To unpack and install the Arcturus$^{XT™}$ Microdissection Instrument:

Note: Please read "Instructions for Lifting and Carrying the Instrument" on page 93 before beginning.

1. Remove the top of the shipping crate.
2. Release the straps.
3. Lift the instrument to the benchtop.
4. Remove the shipping handles, the three lock-down screws from the microscope stage and the green tape from the moving parts.
5. Set up the PC.
6. Connect the PC to the instrument control unit with USB and FireWire cables.
7. Connect the trackball to the instrument control unit.
8. Install any optional components using the installation procedures supplied with them.
9. Complete the IQ/OQ checklist in the Arcturus^{XT} Adjustment, Calibration, and Test Procedure (14601-00).

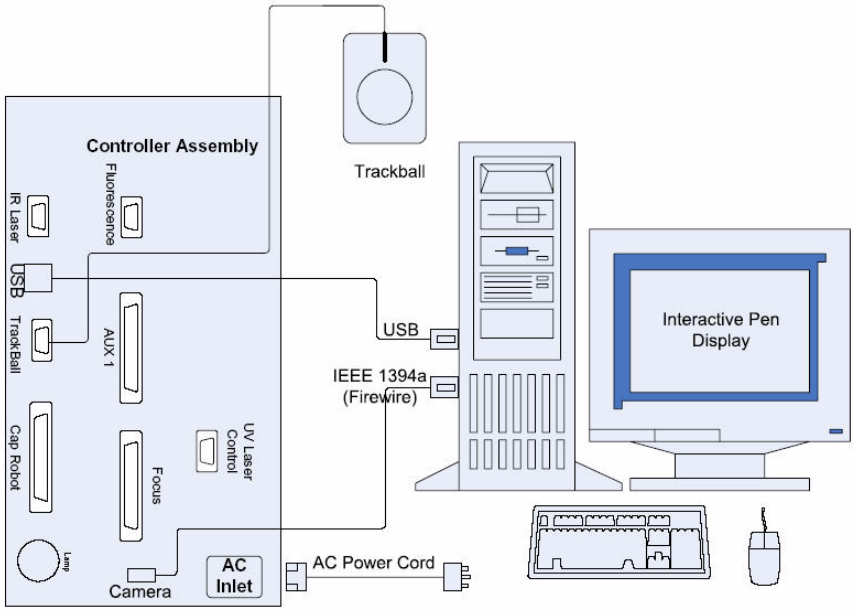


Figure D.1: Connections between the components of the Arcturus$^{XT™}$ Microdissection Instrument.

D.4. INSTALLATION QUALIFICATION

Complete the IQ checklist in the Arcturus^{XT} Adjustment, Calibration and Test Procedure (14601-00) document. This document is located in C:\Program Files\Molecular Devices\ArcturusXT folder.

D.5. OPERATIONAL QUALIFICATION

Complete the OQ checklist in the Arcturus^{XT} Adjustment, Calibration and Test Procedure (14801-00) document. This document is located in C:\Program Files\Molecular Devices\ArcturusXT folder.

D.6. 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.

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