applied biosystems

Arcturus^{XT}™ Laser Capture Microdissection (LCM) System

USER GUIDE

Nikon™ Eclipse™ T*i*-E Microscope Base

for use with:
CapSure™ LCM MicroCaps
CapSure™ HS LCM Caps
Arcturus^{XT}™ Software v3.4

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Revision	Date	Description
A.0	22 September 2016	New document. Supports CapSure™ LCM MicroCaps and Arcturus^{XT}™ Software v3.4. See Pub. No. 0112-0153 for instructions for CapSure™ Macro LCM Caps and Arcturus^{XT}™ Software v3.3.

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About this guide

Purpose of this guide

This user guide is intended for use with the Applied Biosystems™ Arcturus^{XT}™ Laser Capture Microdissection (LCM) System built on the Nikon™ Eclipse™ T*i*-E microscope base. This guide provides instructions for LCM using:

- CapSure™ LCM MicroCaps or CapSure™ HS LCM Caps
- Arcturus^{XT}™ Software v3.4

See Pub. No. 0112-0153 for instructions for LCM using CapSure™ Macro LCM Caps and Arcturus^{XT}™ Software v3.3, available at **thermofisher.com/lcm**.

Prerequisites

This guide is intended for those who perform microdissection using the Arcturus^{XT}™ Instrument. Thermo Fisher Scientific is not liable for damage or injury that results from use of this manual by unauthorized or untrained parties. Instructions in this guide use conventions and terminology that assume a working knowledge of the Microsoft™ Windows™ operating system, the Internet, and Internet-based browsers.

1 System overview

Chapter contents:

About the Arcturus*XT*™ Instrument

The Arcturus*XT*™ Laser Capture Microdissection (LCM) System 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*™ LCM System consists of the Arcturus*XT*™ Instrument, a computer, and the Arcturus*XT*™ operating software. See Appendix B for detailed specifications for the instrument.

While the Arcturus*XT*™ Instrument is intended for laser capture microdissection (LCM), you can also use it for standard microscopy applications under certain circumstances. For more information, see "Using the ArcturusXT™ Instrument as a stand-alone microscope" on page 19.

CapSure™ caps and software compatibility

The Arcturus*XT*™ Instrument is compatible with the following CapSure™ caps and software.

Cap	Software	Pub. No.
CapSure™ LCM MicroCaps	Arcturus*XT*™ Software v3.4	MAN0016091 (this guide)
CapSure™ HS LCM Caps	Arcturus*XT*™ Software v3.4	MAN0016091
CapSure™ Macro LCM Caps[1]	Arcturus*XT*™ Software v3.3	0112-0153

[1] CapSure™ Macro LCM Caps users should continue to use Arcturus*XT*™ Software v3.3. Upgrade to v3.4 is not required.

About laser capture microdissection

Laser capture microdissection (LCM) is a method of procuring specific cell populations from specimen 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.

When you use the Arcturus$^{XT™}$ Instrument, specially designed CapSure™ LCM MicroCaps, CapSure™ HS LCM Caps, or CapSure™ Macro LCM Caps coated with 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 CapSure™ 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. You can then examine the captured material, and place the cap directly into a microcentrifuge tube for extracting DNA, RNA, or protein.

Types of 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” can be ablated around the region of interest and then the remaining cells can be 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.

Outline of the microdissection process

The following list of steps provides a broad outline of the microdissection process. The chapters in this guide are keyed to this list.

1. Prepare samples. See Chapter 2, "Preparing samples" on page 21.
2. Load slides and CapSure™ Caps. See Chapter 3, "Starting the system and loading samples" on page 25.
3. Locate the cells of interest. See Chapter 4, "Inspecting slides" on page 37.
4. Mark the cells and tissue for capture. See Chapter 5, "Selecting cells for microdissection" on page 53.
5. Capture the tissue. See Chapter 6, "Microdissecting cells and tissue" on page 65.
6. Unload the samples and extract the tissue. See Chapter 7, "Extracting cells and tissue" on page 79.

Using the Arcturus$^{XT™}$ operating software

To start the software, you click the Arcturus$^{XT™}$ icon on the Windows desktop (see Figure 1). The system will display the primary screen, which is shown in Figure 2. This application facilitates the microdissection workflow.

Figure 1 Arcturus$^{XT™}$ icon.

The primary screen

The most prominent feature in the primary screen is the main image window that shows the live microscope image. To the side of the screen is the tool panel, which contains tool panes arranged from top to bottom in the order of the steps for laser microdissection:

1. Setup
2. Inspect
3. Select
4. Microdissect

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

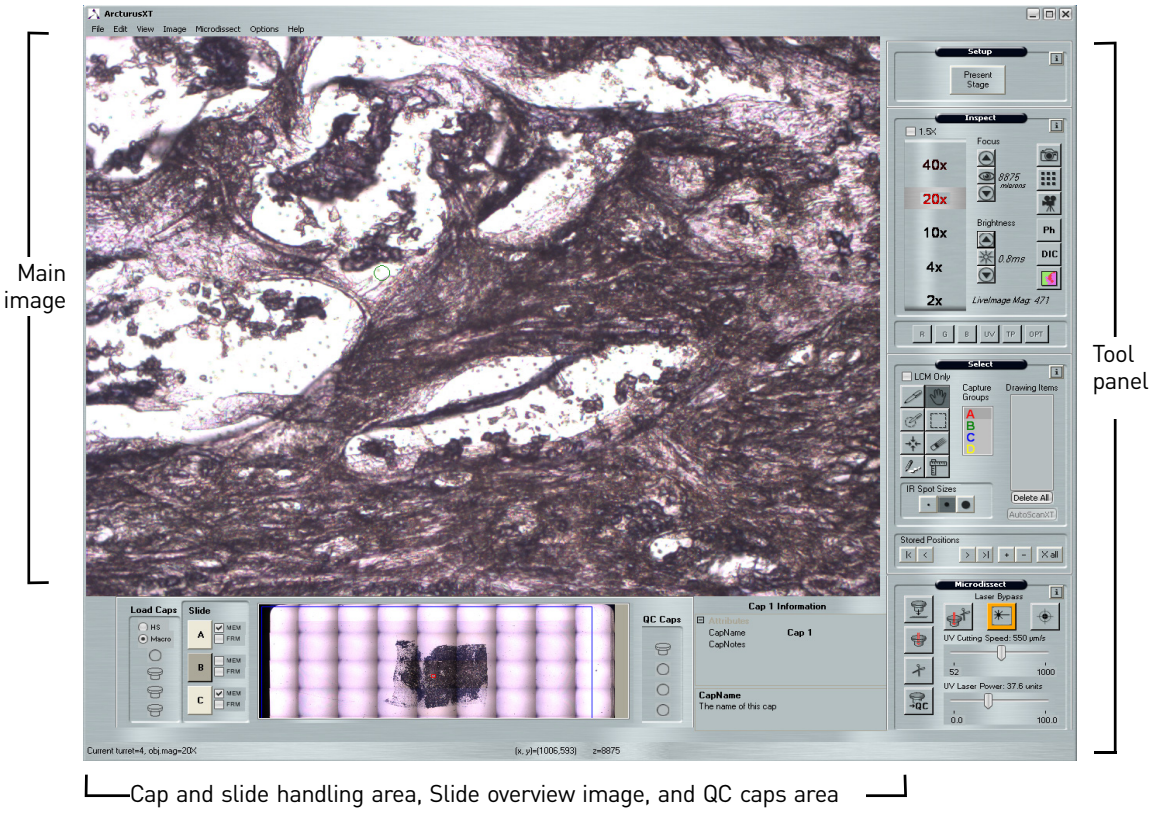


Figure 2 The Arcturus$^{XT™}$ Instrument primary screen.

At the bottom of the screen are the cap and slide handling areas, the slide overview image, and the QC caps area. To move the stage to a particular region and to view that region in the main image, you click the slide overview image in that region. The information displayed includes the properties of the currently selected object, such as a slide or a cap.

Viewing tool tips

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

To view tool tips hover the stylus or mouse over the item of interest on the screen. In the example shown in Figure 3, placing the cursor over the hand displays a message indicating that this tool moves the stage.

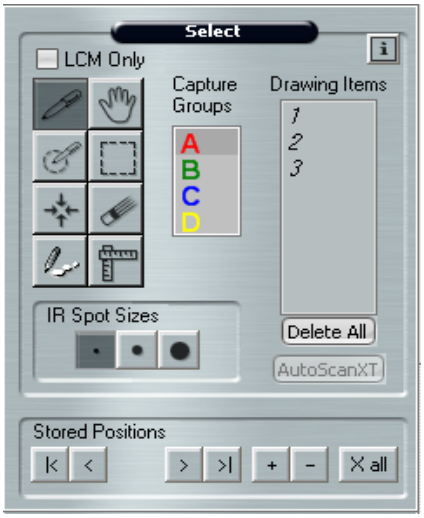


Figure 3 Select tools pane.

Making selections from pop-up menus

Some commands are available from pop-up menus in both the main image and the overview image. In either image, to view a pop-up menu:

- With the stylus, place the stylus on the image, press the lower button on the stylus, and then select from the menu.
- With the mouse, place the cursor on the image, right-click, and then select from the menu.

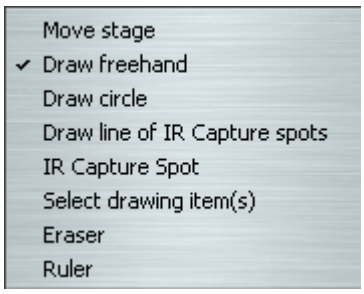


Figure 4 Main image menu.

Making menu selections from the main image

To make a menu selection from the main image, place the cursor or stylus somewhere in the image, and either press the lower stylus button, or right-click the mouse. A menu similar to the one shown in Figure 4 is displayed. When you click on your selection, a checkmark appears to the left. In the example, **Draw freehand** has been selected.

Making selections from within a drawing item in the main image

To view a menu of commands that allow you to manipulate a drawing item (object), place the stylus or cursor inside the drawing item, and then press the lower button on the stylus or right click the mouse. You will see a menu similar to the one shown in **Figure 5**. In this menu, there are no checkmarks. The chosen item is highlighted. In the example, the user has selected the first option.

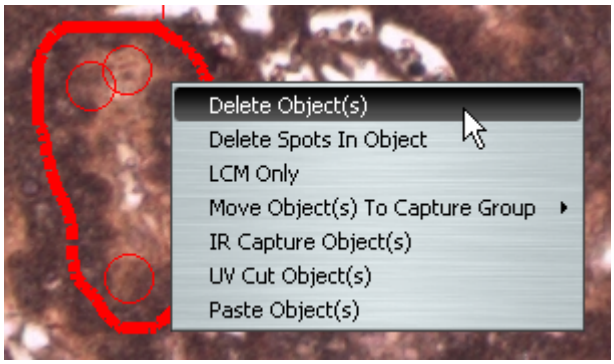


Figure 5 Drawing item menu from main image.

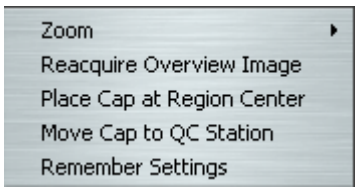


Figure 6 Menu from slide overview image.

Making selections from the slide overview image

To make a menu selection from the slide overview image, place the stylus or cursor in the image, press the lower button on the stylus or right-click the mouse, and make your selection. Note that in this menu, the first selection has an additional set of options, which you access by clicking on the arrow to the right.

Using the options dialog boxes

Each of the four tool panes in the Arcturus$^{XT™}$ software has an Options dialog box associated with it. You can use this dialog box to set properties and perform actions associated with that set of tools.

You can open an Options dialog box in one of three ways:

- Select the name of the tool pane from the Options menu.
- Click the Information button (**i**) in the upper-right corner of the tool selection pane (see **Figure 3**).
- Right-click anywhere in the tool pane.

You can close an Options dialog box in one of two ways:

- To save changes, click **OK** at the bottom of the Options dialog box.
- To exit without saving changes, click **Cancel** at the bottom of the Options dialog box or click the **X** in the upper-right corner.

Viewing informational text

There is informational text for every option in an Options dialog box. To view it, click the item of interest. The descriptive text appears in the pane below (see **Figure 7**).

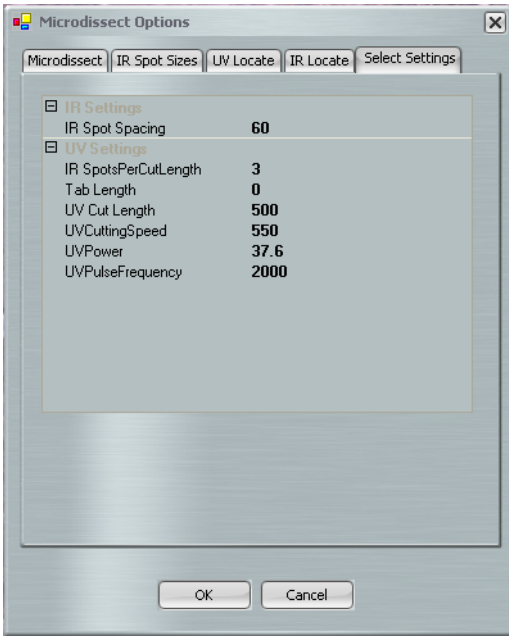


Figure 7 Options dialog box.

Selecting from a drop-down menu

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 (see **Figure 8**).

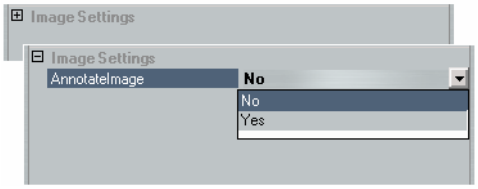


Figure 8 Drop-down options menu.

Expanding the list of options

If you do not see any options in a dialog box, the options may be minimized. To expand the list of options, click the plus sign (+) in the upper-left corner.

In the example shown in **Figure 9**, notice where the white cursor arrow is pointing. This list has just been expanded.

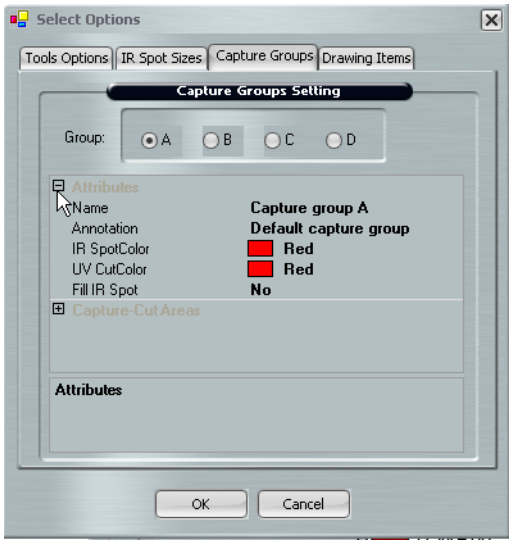


Figure 9 Expanding the list of options.

Entering text in dialog boxes

For some options in a dialog box, you can enter text in a field. To do this you click the stylus or the mouse in the field of interest, and then use the computer keyboard to type text (see **Figure 10**).

Figure 10 Typing text in dialog boxes.

Finding the version number

To find the version number, open the **About Arcturus XT** window (see **Figure 11**).

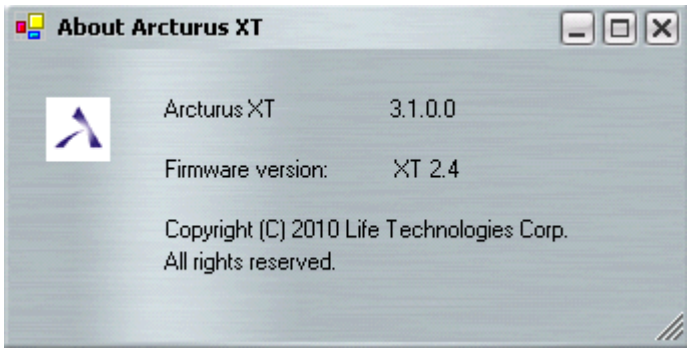


Figure 11 The About Arcturus$^{XT™}$ window.

Using menus and commands

The tables in this section explain the functions available from the menus and submenus.

File menu commands and submenus

Table 1 Functions available from the File menu.

Command	Submenu	Description
Open	Image	Opens a saved image file. The image appears in a new window.
	Markup	Opens a saved markup file.
Save	Image	Saves the image currently visible in the main image window to a file.
	Markup	Saves the current drawing item as an overlay.
Exit	—	Closes the software.

Edit menu commands and submenus

Table 2 Functions available from the 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	Allows you to choose the A, B, C, or D capture group. 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	

View menu commands and submenus

Table 3 Functions available from the View menu.

Command	Description
Scale	Displays a scale bar 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.
Zoom	Not available.

Key commands

The table below provides some keyboard shortcuts that will speed up your processing.

Table 4 Keyboard shortcuts.

Pane	Key command	Description
Primary Screen	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.

Using the Arcturus$^{XT™}$ Instrument as a stand-alone microscope

Although the Arcturus$^{XT™}$ Instrument is intended for laser capture microdissection (LCM), you can also use it for standard microscopy applications if it is equipped with the optional binocular eyepiece (Cat. No. 0200-6228).

Getting into and out of manual mode

To use the Arcturus$^{XT™}$ Instrument as a stand alone microscope, you must be in manual mode. The Arcturus$^{XT™}$ Instrument is in manual mode when it is first turned on, and it remains in this state until you initialize the Arcturus$^{XT™}$ operating software. To return to manual mode after you have initialized the software, you can shut down the software, shut down the instrument, and then restart the instrument and the software.

Using manual mode

While you are in manual mode, use the lamp intensity knob to adjust the lamp brightness and the focus control knobs to manipulate the focus. To maneuver the stage, use the trackball provided with the Arcturus$^{XT™}$ Instrument. Use the buttons on the trackball to change the objective.

Note: If you have 60X or 100X objectives installed on the Arcturus$^{XT™}$ Instrument, make sure that you have proper stage clearance before changing objectives using the trackball buttons.

2 Preparing samples

Chapter contents:

Summary of chapter topics

This chapter explains which types of slides you can use for laser microdissection, and which type to select based on your application. It also explains how to prepare tissue for laser microdissection with the suggested reagent kits.

Choosing slides and Petri dishes

With the Arcturus$^{XT™}$ Instrument, you can use glass slides, PEN membrane glass slides, PEN membrane frame slides, and Petri dishes.

- For applications that use the UV cutting laser, use either PEN membrane frame or PEN membrane glass slides.
- For live cell applications, use an untreated PEN membrane frame slide or the Arcturus$^{XT™}$ Live Cell Growth Chamber and Microdissection Petri Dish.
- For applications that use only the IR capture laser, use either plain glass or PEN membrane glass slides.

Acquiring slides and Petri dishes

PEN membrane slides and Arcturus$^{XT™}$ Live Cell Microdissection dishes are available for purchase through **thermofisher.com** (Table 5). Plain glass slides and large format slides (38 mm and 50 mm) are available from major lab suppliers.

Table 5 PEN membrane slides.

Item	Cat. No.
PEN membrane frame slides	LCM0521 (50 slides)
PEN membrane glass slides	LCM0522 (50 slides)
PEN membrane frame slides for live cell microdissections	LCM0531 (25 slides)
Arcturus$^{XT™}$ Live Cell Growth Chamber	5000300 (6 dishes, sterile)
Arcturus$^{XT™}$ Microdissection Petri Dish	5000301 (6 dishes, sterile)

For information about using these products

Visit **thermofisher.com/lcm** for information and technical resources for the Arcturus$^{XT™}$ Laser Capture Microdissection System, including:

- *Protocol #9: Optimized Protocol for Mounting Tissue Sections onto Metal-Framed PEN Membrane Slides*
- *Application Note #11: Applied Biosystems™ Arcturus$^{XT™}$ Microdissection Systems: Optimized Protocol for Laser Microdissection of Living In Vitro Cells*
- *Application Note #13: Isolation of Living In Vitro Cells Using the Applied Biosystems™ Arcturus$^{XT™}$ Microdissection Instrument*

Preparing tissue samples

For microdissection, you can use tissue sections prepared from either frozen or formalin-fixed, paraffin-embedded (FFPE) tissue. Freezing tissue helps to 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 you to use these samples for molecular biology applications as well.

Using frozen tissue samples

For optimal preparation and processing of frozen tissue samples, we recommend the reagent kits listed in Table 6.

Table 6 Reagent kits for processing frozen tissue samples.

Reagent kit	Cat. No.
HistoGene™ LCM Frozen Section Staining Kit	KIT0401
HistoGene™ LCM Immunofluorescence Staining Kit	KIT0420
PicoPure™ RNA Isolation Kit	KIT0204
PicoPure™ DNA Extraction Kit	KIT0103
Arcturus™ RiboAmp™ PLUS Kit	KIT0521
Arcturus™ RiboAmp™ HS PLUS Kit	KIT0525

Using formalin-fixed, paraffin-embedded tissue samples

Formalin-fixed, paraffin-embedded tissue can also be microdissected for downstream applications. Visit **thermofisher.com/lcm** for more information.

For gene expression profiling studies using FFPE tissue, we recommend using the Paradise™ PLUS Reagent System. This system provides all of the reagents for sample preparation, RNA extraction and isolation, reverse transcription and linear amplification of the RNA.

For optimal sample preparation of FFPE tissue samples and downstream processing, we recommend the reagent systems listed in Table 7.

Table 7 Sample preparation kits for formalin-fixed, paraffin-embedded tissue samples.

Reagent system	Cat. No.
Arcturus™ Paradise™ PLUS 2 Round Kit	KIT0312
Arcturus™ Paradise™ PLUS Whole Transcript Reverse Transcription Kit (WT-RT)	KIT0315

Note: For a complete list of Arcturus$^{XT™}$ microgenomics reagents, see Appendix D.

Using other types of samples

Laser capture microdissection has been used for a variety of research applications, besides tissue microdissection, including forensics, live cells, neurons, live plant tissue, and single chromosomes. Visit **thermofisher.com/lcm**, or contact Technical Support, to download application notes and protocols related to the use of other types of samples.

3 Starting the system and loading samples

Chapter contents:

Summary of chapter topics

This chapter explains how to use the three operation panels on the Nikon™ Eclipse™ T*i*-E microscope: the front panel, the right panel, and the left panel. It explains which controls will be disabled when the Arcturus*XT*™ operating software application is running during an LCM session. This chapter also provides instructions for starting the Arcturus*XT*™ operating software, and for loading samples on the Arcturus*XT*™ Instrument. Finally, it lists the steps for saving images automatically.

Introduction to the Nikon™ Eclipse™ T*i*-E microscope controls

The Arcturus*XT*™ Instrument is built around a Nikon™ Eclipse™ T*i*-E research microscope. There are three operation panels located on the microscope base, each containing switches, knobs, and buttons, which you use to control various microscope functions. This section explains how to use these panels in general, and also provides instructions for using them during an LCM session on the Arcturus*XT*™ Instrument.

Note: For complete details on the Nikon™ Eclipse™ T*i*-E microscope, please refer to the manufacturer's product user manual.

Using the front operation panel

The front operation panel has a display area, a magnification knob, and several sets of buttons, all of which are described below, and shown in Figure 12.

Viewing status from the Status Display window

The status display window displays the microscope status, including the Z-position. You can use the Display buttons directly below the window to select the format of the display.

Selecting the display format with the Display buttons

Press the up and down Display buttons to select the display format that you want to use. The display formats are described in the Nikon™ user manual.

Controlling illumination with the Brightness button

Press the **Brightness** button to change the illumination of the Status Display window and the LEDs on the operation panels.

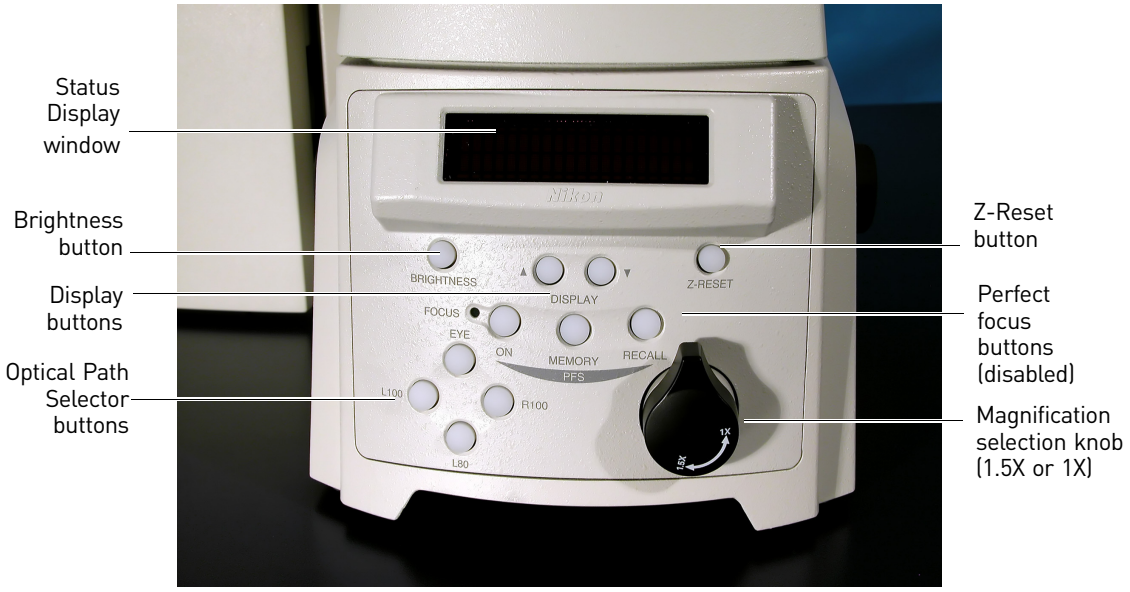


Figure 12 Nikon™ Eclipse™ T*i*-E front operation panel.

Changing image output with Optical Path Selector buttons

Press the Optical Path Selector buttons to change the ouput for the image. For details, refer to Table 8.

Table 8 Optical Path Selector options.

Optical path position	Light distribution	Use on Arcturus*XT*™ Instrument	Details of suggested use
EYE	Eyepiece port 100%	Standard microscopy	When you want to use the microscope in manual mode without initiating the Arcturus*XT*™ operating software, select the EYE position. You can also switch to the EYE position if you want to view the sample through the eyepieces during an LCM session, but in this position you will not see the live video image on the monitor. **Note:** To use the EYE position, you must also have the optional Microscope Binoculars (Cat. No. 0200-6228).
L100	Left side port 100%	Microdissection	The live video camera for the Arcturus*XT*™ Instrument is installed in the left microscope port. To view the image using the operating software, you must select either the L100 or L80 optical paths. The Arcturus*XT*™ operating software will default to the L100 position when initiated.

Optical path position	Light distribution	Use on Arcturus$^{XT™}$ Instrument	Details of suggested use
L80	Left Side Port 80% / Eyepiece Port 20%	Microdissection	The live video camera for the Arcturus$^{XT™}$ Instrument is installed in the left microscope port. To view the image using the operating software, you must select either the L100 or L80 optical paths. The Arcturus$^{XT™}$ operating software will default to the L100 position when initiated. **Note:** To use the L80 position, you must also have the optional Microscope Binoculars (Cat. No. 0200-6228). Use this selection to view the sample through the eyepieces while simultaneously displaying it through the operating software.
R100	Right Side Port 100%	High Resolution Imaging	If you have installed the optional High Resolution Second Camera for the Arcturus$^{XT™}$ Instrument (Cat. No. 14379-00) it is installed in the right microscope port. To use this optional camera for high-resolution imaging, select the R100 position. **Note:** The optional High Resolution Second Camera is intended only for imaging and is not available for microdissection. Microdissection can only be performed using the integrated Arcturus$^{XT™}$ Instrument camera mounted in the left port (position L100 or L80).

Reseting the Z-position with the Z-reset button

Press the Z-Reset button to reset the Z-position on the Status Display window to zero. The Z-axis position display will be increased or decreased in conjunction with movement of the nosepiece, with the zero position as the new reference point.

The Arcturus$^{XT™}$ operating software recognizes the absolute Z-position of the objective nosepiece. If you press the Z-Reset button, the focus position on the Arcturus$^{XT™}$ software interface will no longer match the Z-position read-out on the Status Display window. If you want to realign the values between the Arcturus$^{XT™}$ software interface and the Status Display window, you must turn the instrument off and then on again, and restart the operating software.

Disabling the Perfect Focus buttons (PFS)

The Perfect Focus buttons (marked PFS on the panel) are disabled when you are using the Arcturus$^{XT™}$ Instrument. These controls are used for the Perfect Focus System option, which is not available for the Arcturus$^{XT™}$ Instrument.

Selecting the magnification level with the Magnification Selection knob

Use the Magnification Selection knob to change between 1X and 1.5X objective magnification. Apply this setting for all microscope output ports.

Using the left operation panel

The left operation panel contains a large focus knob, a smaller brightness knob, focus selection and objective switchs, and a lamp ON/OFF button. These controls are described below, and shown in Figure 13.

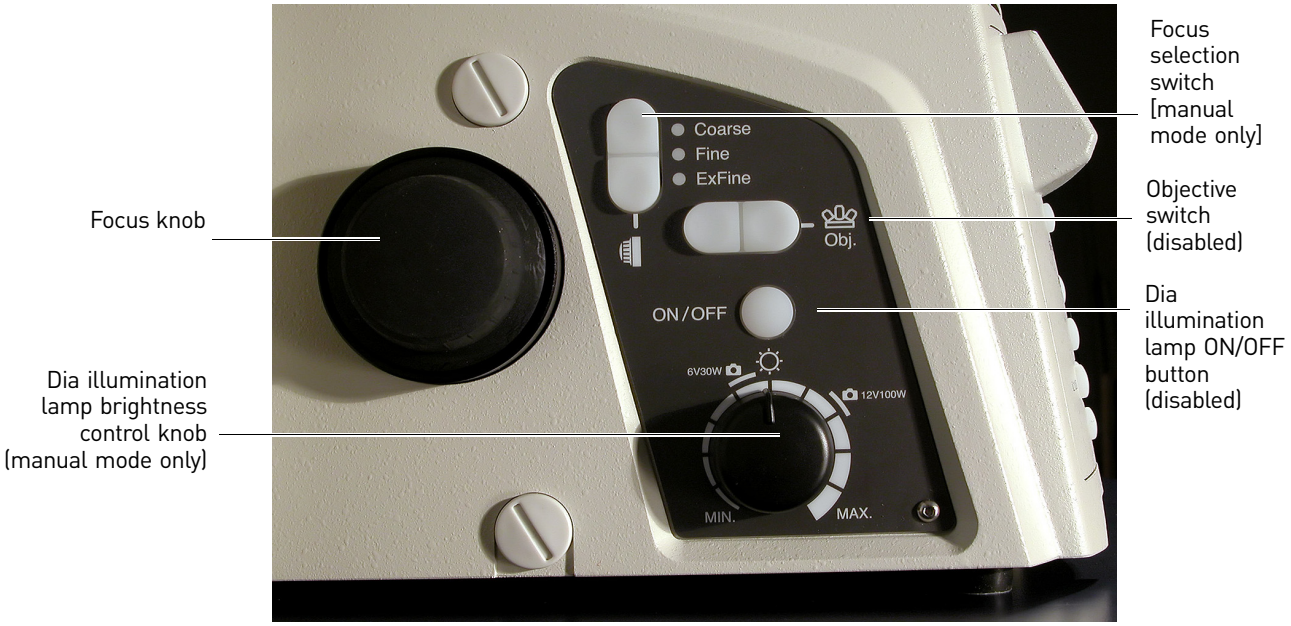


Figure 13 Nikon Eclipse T*i*-E left operation panel.

Selecting the resolution for vertical movement with the Focus Selection switch

Use the Focus Selection switch to select the resolution for the vertical movement of the nosepiece when using the focus knobs on the instrument. Press the switches up and down to toggle between coarse, fine, and extra fine resolutions. The indicator will light up next to the selected resolution.

Note: The Coarse/Fine/Extra Fine Focus Selection switch is only relevant when you are manually adjusting focus using the knobs on the instrument. The Arcturus$^{XT™}$ operating software uses its own Z-step settings. When you use the focus buttons in the Arcturus$^{XT™}$ operating software, only those Z-step settings are applied, regardless of the focus resolution chosen on the operation panel.

Moving the nosepiece using the Focus knob

Use the Focus knob to move the nosepiece up and down for focus adjustment. If you use the Focus knob while using the Arcturus$^{XT™}$, the software will track the position. The numbers displayed in the Arcturus$^{XT™}$ software interface will continue to match the numbers displayed in the Status Display window in the front operation panel.

Changing objectives without the Objective switch

Whenever you use the Arcturus$^{XT™}$ Instrument, the Objective switch is disabled. To change objectives, you use the Arcturus$^{XT™}$ operating software. If you are operating the microscope in manual mode (i.e., without the Arcturus$^{XT™}$ operating software) you click the toggle button on the Arcturus$^{XT™}$ track ball to change objectives.

Turning the lamp On/Off through software controls

The Dia Illumination button is disabled when you are using the Arcturus$^{XT™}$ Instrument. You control the illumination lamp through theArcturus$^{XT™}$ Instrument control box. To turn on the illumination lamp, you must first turn on the Eclipse™ T*i*-E microscope base, and then turn on the Arcturus$^{XT™}$ Instrument.

Adjusting the lamp brightness through software controls

When you use the Arcturus$^{XT™}$ operating software, the Lamp Brightness Control knob is disabled. You adjust the lamp brightness using the Arcturus$^{XT™}$ software controls. However, if you are in manual mode (i.e., not using the Arcturus$^{XT™}$ operating software) you can use the Brightness Control knob to increase or decrease microscope illumination intensity.

Using the right operation panel

The right operation panel contains a large Focus knob, a Focus Selection switch, Escape and Refocus buttons, and several controls that are not used with the Arcturus$^{XT™}$ Instrument. These features are described below, and shown in Figure 14.

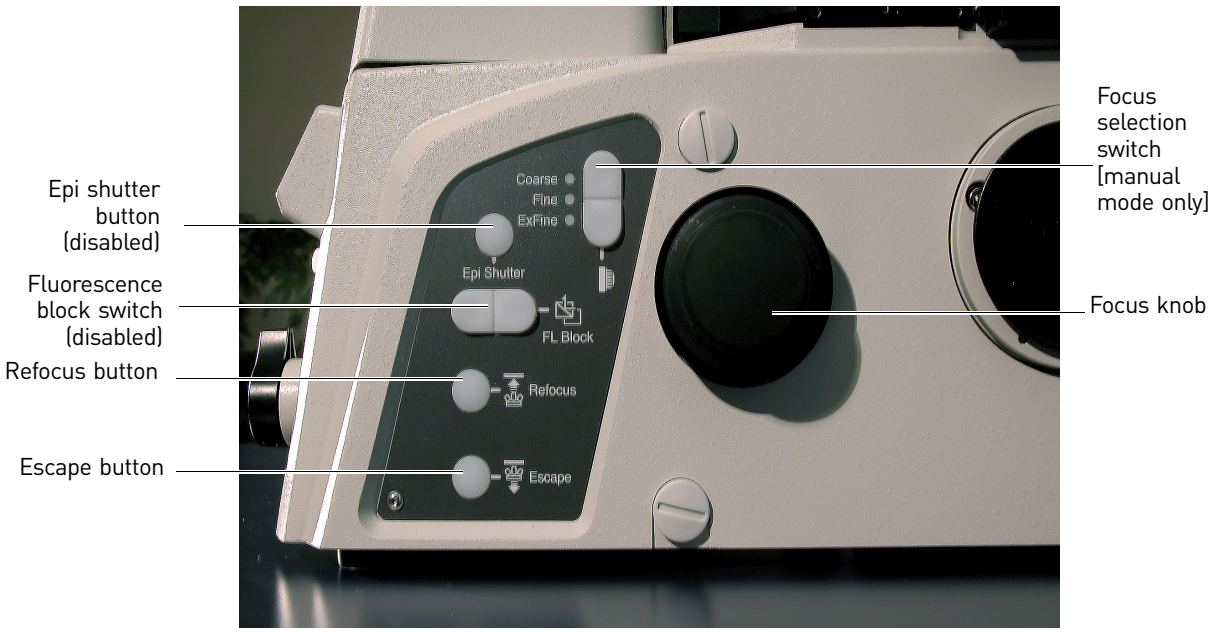


Figure 14 Nikon™ Eclipse™ T*i*-E right operation panel.

Selecting the resolution for vertical movement with the Focus Selection switch

You use the Focus Selection switch to select the resolution for the vertical movement of the nosepiece when using the focus knobs on the instrument. Press the switches up and down to toggle between Coarse, Fine, and Extra Fine resolutions. The indicator will light up next to the selected resolution.

Note: The Coarse/Fine/Extra Fine Focus Selection switch is only relevant when you are manually adjusting focus using the knobs on the instrument. The Arcturus$^{XT™}$ operating software uses its own Z-step settings. When you use the focus buttons in the Arcturus$^{XT™}$ operating software, only those Z-step settings are applied, regardless of the focus resolution chosen on the operation panel.

Controlling the lamp through the software or through the control box

The Epi Shutter button is disabled on the Arcturus$^{XT™}$ Instrument. The Arcturus$^{XT™}$ Instrument uses an external fluorescence lamp, which you control through either the Arcturus$^{XT™}$ operating software or the fluorescence control box. For more information about using Epi-fluorescence with the Arcturus$^{XT™}$ Instrument, see "Working with fluorescently labeled samples" on page 48.

Rotating the fluorescence filter turret by hand

The Fluorescence Block switch is disabled on the Arcturus$^{XT™}$ Instrument. The Arcturus$^{XT™}$ Instrument has a manual fluorescence filter turret that you rotate by hand.

Moving the nosepiece using the Focus knob

Use the Focus knob to move the nosepiece up and down for focus adjustment. If you use the Focus knob while using the Arcturus$^{XT™}$ Instrument, the software will track the position. The numbers displayed in the Arcturus$^{XT™}$ software interface will continue to match the numbers displayed in the Status Display window in the front operation panel.

Returning to the original position with the Refocus button

Press the **Refocus** button to move the nosepiece and objective back to its original position after you have pressed the **Escape** button. After you have pressed the **Refocus** button, you can use the Focus knob for manual focus control.

Moving to the retracted position with the Escape button

Press the **Escape** button to move the nosepiece and objective to the retracted position, which is approximately 2 mm below the reference position. When you press the **Escape** button, the current position is recorded so that the nosepiece can return to this position when you press the **Refocus** button.

Starting the Arcturus$^{XT™}$ operating software

To follow these instructions you can use the interactive pen display supplied with the Arcturus$^{XT™}$ Instrument, or you can use the mouse.

To begin laser microdissection:

1. Turn on the computer.
2. Turn on the Eclipse™ T*i*-E microscope base using the switch located on the back of the microscope.
3. Turn on the Arcturus$^{XT™}$ Instrument.
   As you face the microscope, the power button is located on the left side of the instrument.
4. Start the software by clicking the Arcturus$^{XT™}$ icon on the Windows™ desktop, or click **Start**, point to **Programs,** and click the Arcturus$^{XT™}$ option.
   The software opens to fill the screen.

Loading materials onto the Arcturus$^{XT™}$ Instrument

To begin loading materials onto the Arcturus$^{XT™}$ Instrument:

Prepare the work surface

1. In the **Setup** tool pane, click **Present Stage** (see Figure 15).

   The work surface moves forward and to the right.

Figure 15 Present stage from Setup panel.

2. If needed, remove any slides and CapSure™ Caps left on the work surface.
3. Load your slides and CapSure™ Caps onto the work surface (see Figure 16).
4. Push the tension button in and place each slide in a slot.
5. Release the tension button.
6. Place the caps in the CapSure™ cassette into the slot on the left side of the work surface.

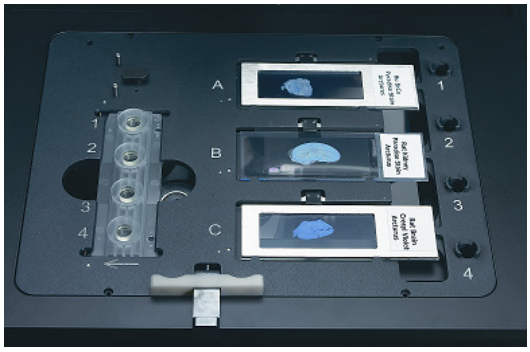


Figure 16 Modular stage insert.

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

(Optional) selecting load options for slides

Open the Load Options dialog box and follow the steps below to enter information about your slides (see Figure 17).

1. Check each slide that you are loading or, if you are loading all slides, click **Load 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.

2. Click **Load with Overviews** (at the top) to instruct the instrument to automatically create the slide overview image when you close this dialog box.

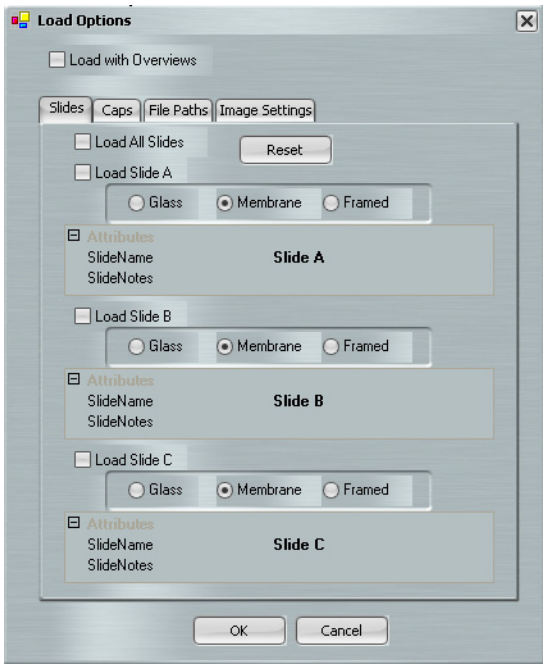


Figure 17 Load options: slides.

3. For each slide, choose the type of slide: **Glass**, **Membrane,** or **Framed.**
4. (Optional) 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 Annotated Image field in the Image Settings tab.
5. (Optional) 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 the SlideNotes in the information area to the right of the slide overview image.

Selecting load options for CapSure™ Caps

To enter information about the CapSure™ Caps:

1. Click the **Caps** tab.
2. Click the checkbox for each CapSure™ Cap that is loaded, or click **Load All Caps** to check all of the check boxes at once (see Figure 18).
3. Click **HS** or **Micro** to identify the type of caps you are loading.

   If you are using CapSure™ Macro LCM Caps and v3.4 software, you can select **Micro**, but the maximum boundary of the capture will be 4300 µM.

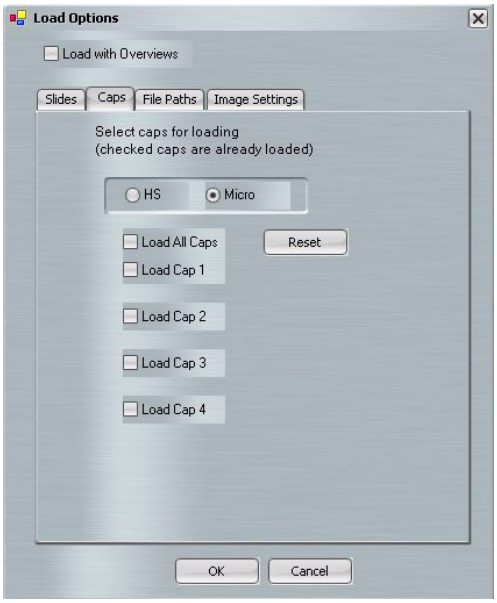


Figure 18 Load options for Capsure™ Caps (software v3.4).

Selecting file path options

1. To enter information about where image files should be saved, click the **File Paths** tab (see Figure 19).
2. In the AutomaticFilename field, enter **Yes** if you want the file name:
   - For saved images to be the date and a number, e.g., "2007-01-23_X.tif", where X is an incrementing number.
   - For tiled images to be a date and a number, e.g., "2007-01-23_002_S.tif.
   - For videos to be a date and a number, e.g., "2007-01-23_X.avi".

   Enter **No** if you want to be prompted for a file name when you save an image.

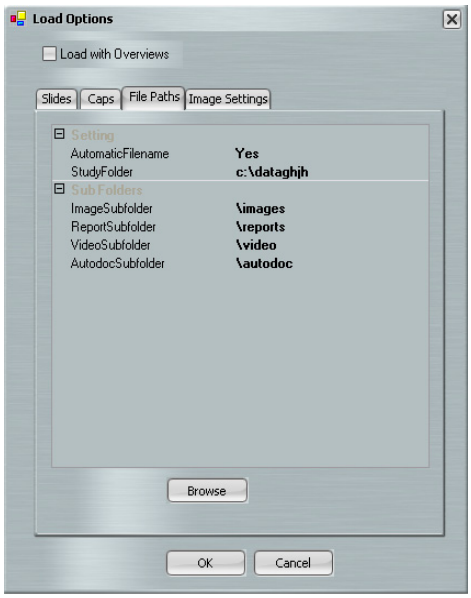


Figure 19 File path options.

3. In the StudyFolder field, enter the name of the folder where saved images, videos, and reports are to be located. Click **Browse** to select the location.
4. In the ImageSubfolder, enter the name of the folder inside the StudyFolder where image files are to be saved.
5. In the 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.
6. In the VideoSubfolder field, enter the name of the folder inside the StudyFolder where video files are to be saved.

Selecting static image options

To enter information related to static images:

1. Click the **Image Settings** tab.
2. In the first panel, choose the AutoDocument Filename Settings that you want to use.
3. In the AnnotateImage field, if you want the SlideName and SlideNotes (from the Slides tab) and the objective in use to be saved in the upper-left corner of all image files, enter **Yes**. Otherwise, enter **No**.
   a. If you entered **Yes** in the AnnotateImage field, enter the color for the annotation in the ImageAnnotationColor field.

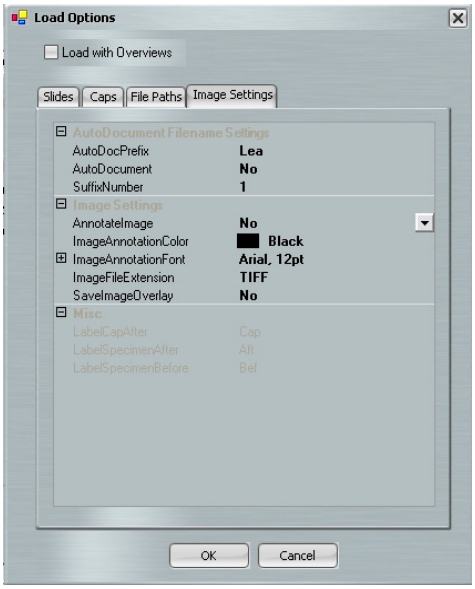


Figure 20 Image settings.

b. If you entered **Yes** in the AnnotateImage field, enter the font for the saved annotation in the ImageAnnotationFont field.

   Click the plus sign to view the formatting options.

4. In the ImageFile Extension field, choose the file format for saved images. You can choose either JPEG (.jpg) or TIFF (.tif).
5. In the SaveImageOverlay field, if you want to save drawing items when you save images, enter **Yes**. Otherwise, enter **No**.

Implementing your selections

When you have finished making your choices, click the **OK** button. The instrument then performs these 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 you selected **Load with Overviews**, automatically acquires and displays the slide overview image for all slides loaded.

Note: If you did not select **Load with Overviews**, the slide overview image area will be blank. To acquire and display the slide overview, right-click in the slide overview area, and click **Reacquire Overview Image.**

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 CapSure™ 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. Open the **Load Options** dialog box.
2. Click the **Image Settings** tab (see Figure 20).
3. In the AutoDocPrefix field, enter the prefix that you want to use at the beginning of the name of each image file saved.
4. In the AutoDocument field, click the field to display the drop-down list and select **Yes**.
5. In the SuffixNumber field, enter the number for the first image. This number is the last part of the file name for all images. It increases by one each time microdissection occurs.
6. In the LabelCapAfter field, enter the text that you want to add after the AutodocPrefix and the SuffixNumber of each image of the CapSure™ Cap.
7. In the LabelSpecimenBefore field, enter the text that you want to add after the AutodocPrefix and the SuffixNumber for each image of the slide before microdissection.

8. In the LabelSpecimenAfter field, enter the text that you want to add after the AutodocPrefix and the SuffixNumber for each image of the slide after microdissection.

   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 LeafStudyBefore10.

4 Inspecting slides

Chapter contents:

Summary of chapter topics

This chapter explains how to move around a slide to view the sample, and how to work with the microscope and the fluorescence lamp. It also explains how to capture static images, tiled images, and movies.

Using the Inspect tools

After you have loaded the slides and CapSure™ Caps, you use the tools in the Inspect tools pane to view the slides and to identify the cells you want to microdissect. These tools allow you to adjust the microscope, to control the fluorescence lamp, and to work with images.

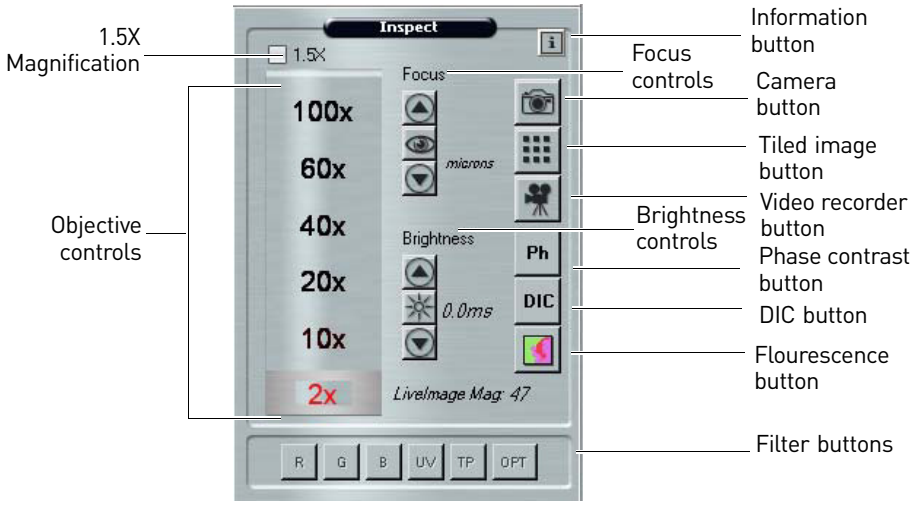


Figure 21 The Inspect tools pane.

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

Viewing the slides

To view the slides:

1. Move the stage to display an area of interest in the main image in one of three ways:
   - Move the trackball.
   - Click 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 or click 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 or clicking the label corresponding to the chosen objective. The selected objective is red (see Figure 22).

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

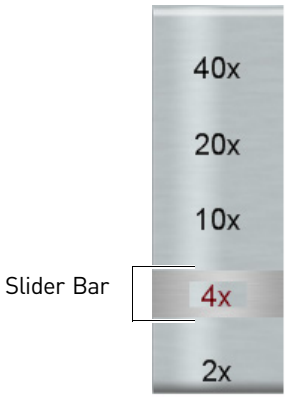


Figure 22 Objective control.

Adjusting the brightness

The brightness knob on the left operation panel of the microscope is disabled when the Arcturus$^{XT™}$ software is running. You control brightness using the software buttons shown in Figure 23.

Figure 23 Brightness controls.

To adjust the brightness as needed to illuminate the sample:

- Click or tap the up arrow to increase the brightness.
- Click or tap 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.
- Click the Autobrightness button in the middle to adjust the brightness automatically.

  To set a different level of brightness for the Autobrightness button, see "Changing the autobrightness settings" on page 42.

Note: The brightness value corresponds to the shutter speed/exposure time of the camera. 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 this is the case, adjust the Intensity and Camera Gain in the Illumination tab in the Inspect Options dialog box (see Figure 26) so that you can set the brightness value lower.

Focusing the image

Use the Focus controls to focus the live image::

- Click or tap the up arrow to move the objective closer to the slide.
- Click or tap the down arrow to move the objective farther from the slide.

  Note: If you press the stylus down on the arrow buttons, the software adjusts the focus in larger steps.

- Click the Autofocus button in the middle to adjust the focus automatically.

Figure 24 Focus controls.

Matching the focus positions

If the Z-Reset button (located on the front panel of the microscope) has been pushed, the focus position indicated on the LED readout will not match the focus position on the Arcturus$^{XT™}$ software. To have the numbers coincide once again, you must turn off the Eclipse™ T*i*-E microscope, then turn it on again and restart the Arcturus$^{XT™}$ software.

Setting up automatic focus

You can set up the instrument to automatically focus the live image each time you change the objective. To set up automatic focusing:

1. Click the Information (**i**) button in the upper-right corner of the Inspect tools pane (see Figure 21) to open the Inspect Options dialog box.
2. Click the **Focus** tab (see Figure 25).
3. Check **Tracking Autofocus On**.
4. Click **OK** to close the dialog box and save your changes.

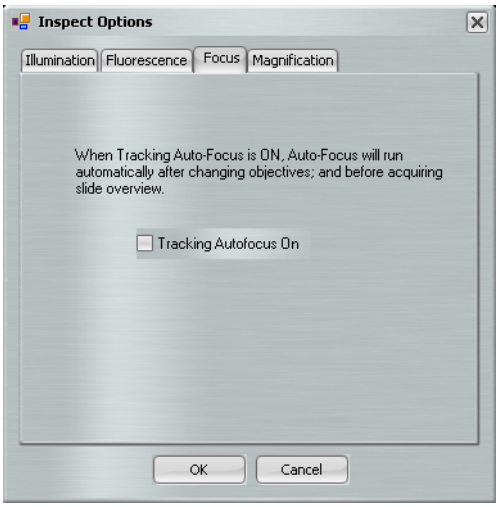


Figure 25 Autofocus.

Magnifying the image

The microscope has an option that allows you to increase the magnification of the current 1.0X objective to 1.5X. The dial for this setting is located on the front of the instrument. For the instrument and the software to properly align, you must indicate in the Arcturus$^{XT™}$ software that you are using this feature. To use the 1.5X magnification feature:

1. On the front panel of the microscope, turn the magnification knob to the 1.5X position.
2. Click on the 1.5X box in the Inspect tools pane (see Figure 21) to indicate that this feature has been selected.

Note: You can also indicate that you have enabled the 1.5X magnifier in the Inspect Options dialog box on the Magnification tab.

Working with the Bright Field lamp

This section explains how to adjust the settings on the bright field lamp, how to adjust the properties of the video camera, and how to work with the autobrightness settings.

Adjusting the Bright Field lamp

To adjust the bright field lamp:

1. Click the Information (**i**) button in the upper-right corner of the Inspect tools pane (see Figure 21) to open the Inspect Options dialog box.
2. Click the **Illumination** tab.
3. If needed, click **On** to turn on the bright field lamp.
4. If you want to disperse the white light so that it is spread evenly across the image, causing the slide to appear more like a slide with a cover slip, use the Diffuser Setting. Choose **In** or **Out** depending on your preference and application needs.

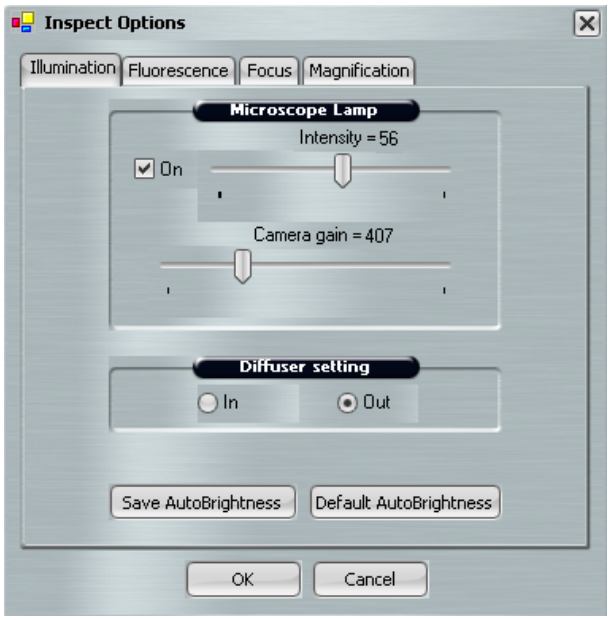


Figure 26 The illumination tab.

5. Work with the Intensity and Camera Gain controls here, and the Brightness controls in the Inspect tools pane, to optimize the image. These tips may be useful:
   - Use the Intensity slider to change the bright field lamp intensity. Slide the control to the right to increase intensity or 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 the gain or to the left to decrease the gain.
   - Adjust the Brightness controls in the Inspect pane as needed. A recommended brightness setting at 2X is 5.0 ms to 8.0 ms.
6. To adjust the white balance within the camera settings, see "Adjusting the video camera properties" on page 41.
7. Tap or click **OK** to close the dialog box and save your changes.

Adjusting the video camera properties

You probably won't need to adjust the video camera often, but in case you do, this section describes how to work with the video camera properties and set the white balance.

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

To set the white balance:

1. To open the Camera Properties dialog box, click **Camera Properties** in the View drop-down menu.
2. Move the stage to a position on the slide that has both tissue and blank slide areas visible in the field of view.
3. Adjust the focus and brightness as needed.
4. Check the **White Balance Auto** check box.
5. Uncheck the **White Balance Auto** check box.
6. Click the **One Push** button. The system will automatically adjust the White Balance Blue and Red for the slide and specimen in the field of view.

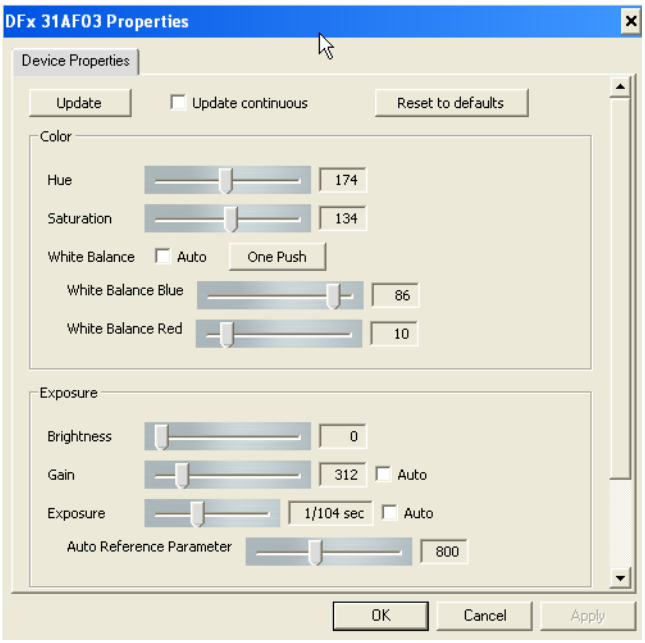


Figure 27 Camera properties.

7. Once the system has completed the auto adjustment, click **Apply** and then **OK** to close the window.
8. If further adjustment is needed, repeat these steps.

Note: If the automatic routine is insufficient for specific requirements, you can also adjust the White Balance Blue and Red settings manually by moving each slider until you achieve the desired color balance.

Changing the autobrightness settings

The Arcturus$^{XT™}$ software automatically sets the brightness when you tap or click the Autobrightness button on the Inspect tools pane (see Figure 21). If the default autobrightness is not appropriate for your sample, you can adjust the value manually and save it as the default.

Saving the current setting

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

1. Adjust the brightness in the main image window as desired.
2. Click the Information button in the upper-right corner of the Inspect tools pane to open the Inspect Options dialog box.
3. Click the **Illumination** tab (see Figure 26).
4. Click **Save AutoBrightness**, then click **OK** to close the dialog box and save your changes.

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

Returning to the original setting

To return to the original autobrightness setting:

1. Click the Information button (**i**) in the upper-right corner of the Inspect tools pane to open the Inspect Options dialog.
2. Click the **Illumination** tab.
3. Click **Default Autobrightness**, then tap or click **OK** to close the dialog box and save your changes.

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

Performing phase contrast and DIC imaging

This section explains how to work with both Phase Contrast imaging and Differential Interference Contrast (DIC) imaging.

Using phase contrast imaging

Figure 28 shows the phase contrast components for a T-DH diailluminator 100W and LHS-H100P-1 120V100W lamphouse.

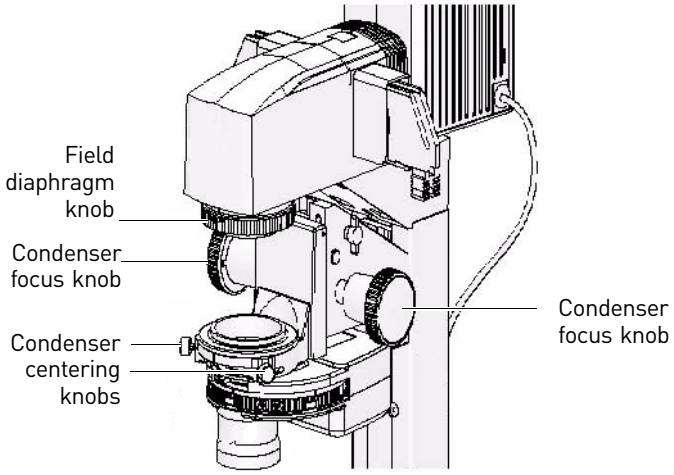


Figure 28 Phase contrast components.

Note: Proper set-up requires the Arcturus$^{XT™}$ Binoculars option (Cat. No. 0200-6228).

Getting set up for phase contrast imaging

For optimal phase contrast image quality, ensure that all components are properly aligned. Prior to starting an experiment using Phase Contrast:

1. If necessary, center the field aperture.

   IMPORTANT! The condenser cannot be focused completely. To focus the condenser, you would need to drop it lower than is allowable due to the IR laser assembly.

2. Click on the **Ph** icon in the Inspect tools pane (see Figure 21). The diffuser will move to the Out position.

3. Select the 10X objective, and focus the live image.

4. Close down the field aperture diaphragm, located in the illumination tower (see Figure 28), until the field diaphragm appears in the field of view.

   The diaphragm edges will not be in focus.

5. Turn the two condenser-centering screws to move the field diaphragm to the center of the field of view (see Figure 29).

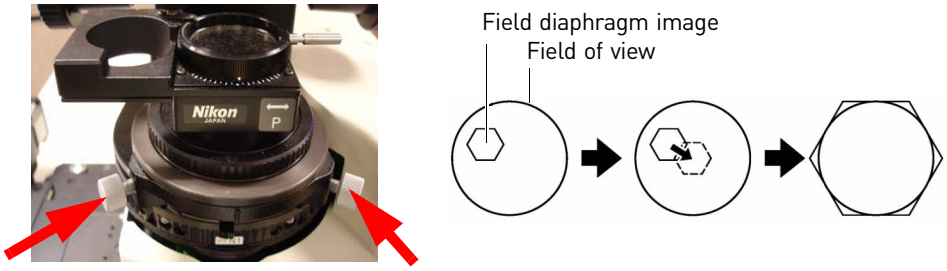


Figure 29 Condenser-centering screws and condenser allignment.

Working with phase contrast imaging

1. If you haven't done so already, click on the **Ph** icon in the Inspect tools pane (see Figure 21). The diffuser must be in the Out position for proper phase contrast and DIC imaging.
2. Ensure that both the field aperture and the condenser aperature are fully open.
3. Rotate the Bertrand Lens (B) on the binoculars, located in the eyepiece turret of the binoculars.
4. View the phase plate (black ring) through the microscope oculars.

   If needed, focus the ring by rotating the screw located on the eyepiece turret to the right of the Bertrand lens selection (see Figure 30).

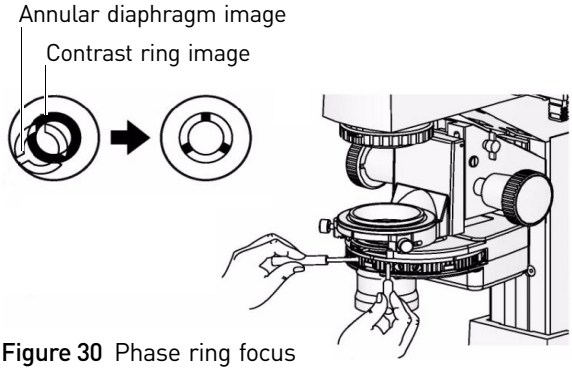


Figure 30 Phase ring focus and annular diaphragm.

5. Rotate into the light path the appropriate phase annulus plate to match the objective in use. Phase plates are located in the condenser turret.

   Note: If you have the motorized condenser, the appropriate annulus plate will automatically move into position based on the selected objective when you click the **Ph** icon.

   These are the plates and objectives:

   - PhL: 4X
   - Ph1: 10X, 20X
   - Ph2: 40X, 60X

6. If necessary, center the annular diaphragm.

   Using 2 mm hex head screwdrivers, adjust the two set screws associated with phase insert until the phase annulus coincides with the phase plate (see Figure 31).

7. Focus the image and adjust the brightness for the optimal image.

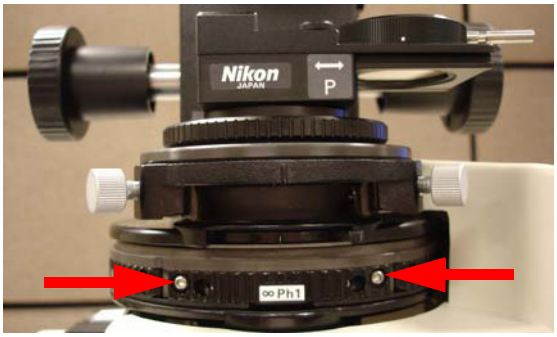


Figure 31 Annular Diaphragm adjustment.

8. To return to brightfield illumination, click on the **Ph** icon.

   The Diffuser will return to the original position.

9. For bright field imaging, rotate the condenser to the A position.

   Note: If you have a motorized condenser, the phase annulus will automatically return to the A position.

Using differential interference contrast (DIC) imaging

This section explains how to prepare for and perform DIC imaging.

Getting set up for DIC imaging

For optimal DIC image quality, ensure that all components are properly aligned. Prior to starting an experiment using DIC:

1. Click on the **DIC** icon in the Inspect tool pane.

   The diffuser will move to the Out position. The diffuser must be in the Out position for proper DIC imaging.

2. Ensure that both the field aperture and the condenser aperture are fully open.

3. Remove the DIC slider from beneath the objective (see Figure 32).

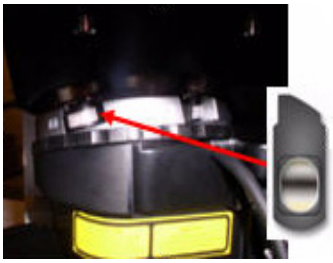


Figure 32 DIC slider/prism.

4. Push in the analyzer (beneath the objective nosepiece/fluorescence turret).

5. Make sure the analyzer is set at 0 as shown in Figure 33.

   It should be locked in place.

   Note: If you have purchased the DIC Analyzer Cube in conjunction with the motorized fluorescence filter turret, when you click on the DIC button, the DIC Analyzer Cube moves into position automatically. If you are using the DIC Analyzer Cube, you do not need to use the sliding analyzer.


Figure 33 DIC analyzer.

6. Push the polarizer into the light path, above the condenser turret, and set the index mark to 0 as shown in Figure 34.
7. Loosen the condenser turret set screw and rotate until the darkest image (lowest brightness) is achieved.

   This is the “extinction point”.
8. Tighten the screws to set the condenser to this position.
9. Place the DIC slider back into place in the objective.

Figure 34 DIC polarizer in position.

Working with DIC imaging

1. If you haven’t done so already, click on the **DIC** icon in the Inspect tool pane to move the diffuser to the Out position.
2. Ensure that the DIC slider is in place in the objective.
3. Rotate the condenser turret to the DIC N1 position.

   Note: If you have purchased the motorized condenser, the condenser will automatically rotate into position when you click the **DIC** icon.
4. Focus the image and adjust the brightness setting.
5. Push in the analyzer, located beneath the objective nosepiece/fluorescent turret (should be set at 0).

   Note: If you have purchased the DIC Analyzer Cube in conjunction with the motorized fluorescence filter turret, when you click on the DIC button, the DIC Analyzer Cube moves into position automatically. If you are using the DIC Analyzer Cube, you do not need to use the sliding analyzer.
6. Push in the polarizer, located above the condenser turret, and rotate until the best image is achieved.
7. Sharpen the image if necessary by adjusting (closing down) the condenser diaphragm.

Working with fluorescence

This section explains how to set up and use fluorescence.

Before you begin

To control the EXFO fluorescence illumination box through the Arcturus$^{XT™}$ software, turn on the EXFO box prior to initiating the software. If you turn on the EXFO box after initiating the software, you will be prompted with a pop-up window asking if you would like the Arcturus$^{XT™}$ Instrument to take control of the illumination source. Click **Yes** or **No** and proceed with the steps below.

Getting set up for fluorescence

Prior to starting a fluorescence experiment:

1. Make sure that the fiber optic cable is fully inserted into the EXFO cone attached to the scope, and is fully inserted into the back of the EXFO box.
2. Make sure that the EXFO cone is properly seated into the insert attachment on the scope.
3. Make sure that the shutter located in the fluorescence turret is in the Open O position (see Figure 35).
4. Make sure that the Analyzer and Polarizer (used with DIC) are in the OUT position.
5. Make sure the DIC prisms are removed.

   When the DIC option is installed, the DIC prisms are beneath the 10X, 20X, 40X, and 60X objectives.

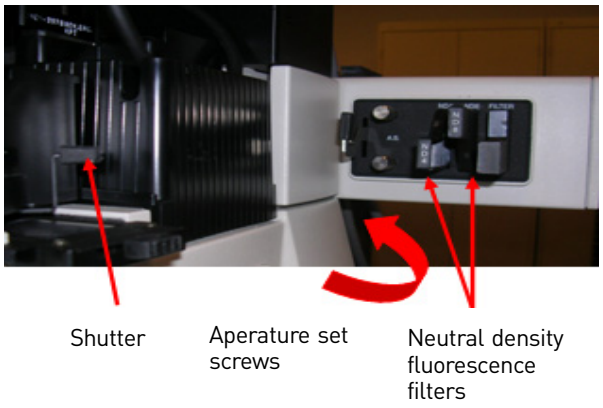


Figure 35 Fluorescence components.

6. Make sure that the fluorescence aperture is open (pulled out) and centered.

   Center the aperture using the 2 set screws (see Figure 35).

7. Make sure that the two neutral density fluorescence filters located to the left of the EXFO cone are in their OUT position (see Figure 35).

Working with fluorescently labeled samples

1. Click on the **Fluorescence** icon on the right side of the Inspect tool pane (see Figure 21.)

   Note: If you have the motorized fluorescence filter turret in place, the filter selection buttons will become active when the fluorescence module is turned on.

2. Open the Inspect Options dialog box. The Fluorescence tab is selected automatically.
3. Make sure that the Microscope Lamp Intensity is turned off (unchecked) (see Figure 36).
4. Manually rotate the fluorescence filter turret to the desired filter cube.

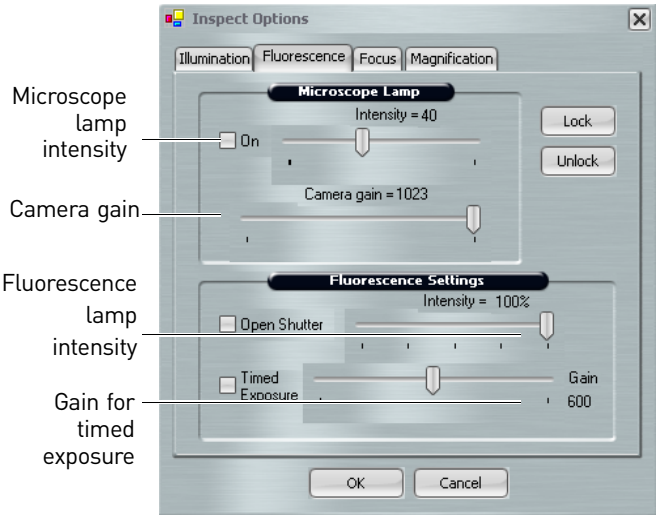


Figure 36 Fluorescence tab.

Note: If you have the motorized fluorescence filter turret in place, click on the appropriate filter button (see Figure 37) at the bottom of the Inspect pane (see Figure 21) to rotate the filter turret.

Figure 37 Filter buttons [active].

5. Locate the fluorescence signal:
   a. Set the Fluorescence Lamp Intensity to 100% (see Figure 36).
   b. Set the Camera Gain to the maximum setting (see Figure 36).
   c. Adjust the brightness setting (exposure time) in the Inspect panel until a fluorescence signal is seen (see Figure 38).
   d. Focus the image.
   e. If needed, check the Microscope Lamp on (see Figure 36) and adjust the intensity to allow minimal brightfield light.

Figure 38 Brightness (exposure time).

6. Once the sample has been located and focused, make these adjustments to optimize the image:
   a. Adjust the Camera Gain (see Figure 36) to the lowest possible value to still allow sufficient signal. (Higher values increase image pixilation.)
   b. Set the Brightness (exposure time) (see Figure 36) to 1s or less. (Higher values result in significant delay in live image updates.)
   c. If the live image displays with very bright fluorescence intensity, lower the Fluorescence lamp intensity (see Figure 36).

Toggling between fluorescence and Bright Field illumination

If you need to toggle between Fluorescence and Bright Field illumination, for example when performing IR test fires, use the camera gain and microscope intensity settings on the Fluorescence tab, not the Illumination tab.

1. Set Camera Gain (see Figure 36) for optimal fluorescence image.
2. Adjust the Microscope Lamp Intensity (see Figure 36).

   A good starting point for the Microscope Lamp Intensity setting is 30. Adjust from this point to get enough light.

Interactions between Fluorescence and Illumination tab settings

This section explains how the Fluorescence and Illumination tabs work.

- The Microscope Lamp Intensity and Camera Gain settings in the Fluorescence tab are independent of those in the Illumination tab. Changes to these settings apply only while using fluorescence illumination. Once you switch off the fluorescence, the settings in the Illumination tab are applied.
- Camera Gain in the fluorescence tab controls the gain of both brightfield and fluorescence illumination.
- The Microscope Lamp Intensity in the Fluorescent tab can contribute to the photobleaching of the sample even at minimal settings. Ensure that the Microscope Lamp is OFF (unchecked) when fluorescence is not in use.
- Use the Gain setting next to Timed Exposure (see Figure 36) only with the Timed Exposure feature. Adjusting this setting outside of Timed Exposure will not result in a change.

Working with Fluorescence timed exposure

When working with the Fluorescence lamp, samples can become photo-bleached if they are exposed for too long to the light source. You can limit a sample's exposure to the fluorescence lamp by using timed exposures in the Arcturus$^{XT™}$ software.

To set up for timed exposure:

1. To work with a static image rather than the main image, click **Timed Exposure** in the lower-left corner of the Inspect Options screen .

   Note: The instrument opens the shutter only briefly to illuminate the slide when a snapshot is taken to prevent photo-bleaching of the sample. The snapshot is then used to indicate areas for capture.

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

   The Arcturus$^{XT™}$ Instrument will use the lamp and camera settings you have set for the fluorescence mode.

3. Click the **Camera** button to capture a static image for tissue selection.
4. Draw on the snapshot to indicate areas for capture.
5. When you have identified another area for a timed exposure, tap or click the **Camera** button again to acquire another snapshot of the new area.

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 or click **Open Shutter** in the Fluorescence tab. Otherwise, the shutter will only close when you revert back to bright field illumination.

Working with slides

This section provides information about selecting and viewing slides.

Displaying a different slide

To display a different slide:

1. To the left of the slide overview image, in the cap and slide handling area at the bottom of the screen, tap the **Slide** button (see Figure 39) for the slide of interest.

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

2. If there is not already a slide overview image, right-click in the slide overview and select **Reacquire Overview Image**.

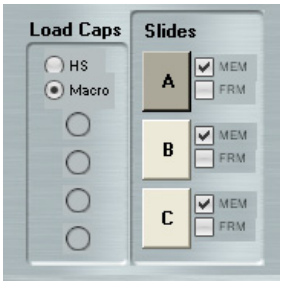


Figure 39 Cap and slide handling area.

Viewing slide properties

You can view the slide type in the cap and slide handling area to the left of the slide overview image (see Figure 39).

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 CapSure™ Cap is on the slide. For plain glass slides, leave MEM and FRM unchecked.

You can view properties of a slide (its name and any notes) in the information area to the right of the slide overview image (see Figure 40).

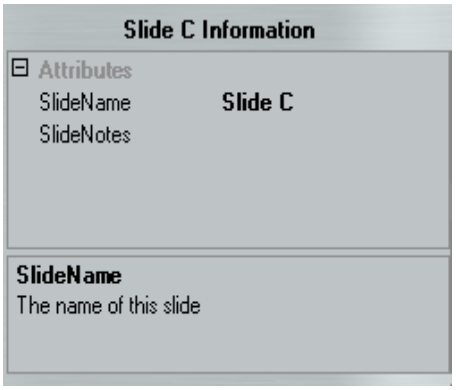


Figure 40 Slide information area.

You can edit the SlideName and/or SlideNotes here. Editing here is the same as entering the information in the Load Options dialog box.

Working with images and videos

This section dicusses capturing, saving, and viewing tiled and static images and videos.

Capturing and saving images

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

- Tiled Images
- Static Images

Capturing 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 larger region than a static image.

The size of the tiled image is limited by the available computer memory and the degree of magnification.

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. Click the **Tiled Image** button in the Inspect tools pane.
3. In the slide overview image, tap or click and then drag the cursor to outline the area for the tiled image.
   The selected area is outlined with a solid green line and a pop-up menu appears.
   - If this area is not correct, select **Cancel**, and 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.

Note: 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.
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. It is saved in the format specified in the Image Setting tab of the Load Options dialog box.

Capturing 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. You set the location for the file in the File Paths 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.
   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. Click the **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. It is 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.

Opening an image

To open an image:

1. Choose **Open Image** from the File menu.
2. In the resulting dialog box, tap or click the name of the image you want to open and tap or click **Open**.
   The image opens in a window.
3. When you have finished viewing the image, tap the close box in the upper-right corner of the window.

Capturing, saving, and viewing videos

TCapturing and saving a video

Figure 41 Video camera icons.

1. To begin recording a video, click the **Video Recorder** button in the Inspect tools panel.
   The camera icon turns red while the camera is recording.
2. To stop the recording, click the Video recorder button again to stop recording.
   The camera icon 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 with the date and a number.

Viewing a video

To view a video use Windows Media Player or a similar program that can handle .avi files.

Note: To capture a video, make sure AutoDocument is turned off in the SetUp option dialog box. If you have turned on the AutoDocument feature, the video feature will not work, as the live image feed is frozen when the capture is moved to the QC station.

5 Selecting cells for microdissection

Chapter contents:

Summary of chapter topics

This chapter explains how to indicate the cells and tissue you want to microdissect, how to use the drawing tools, how to measure a distance or object size in the main image window, and how to set the size of the IR capture spots inside your samples. It also explains how to use overlays, capture groups, and stored positions. You will access all of these features from the Select tools pane (see Figure 42).

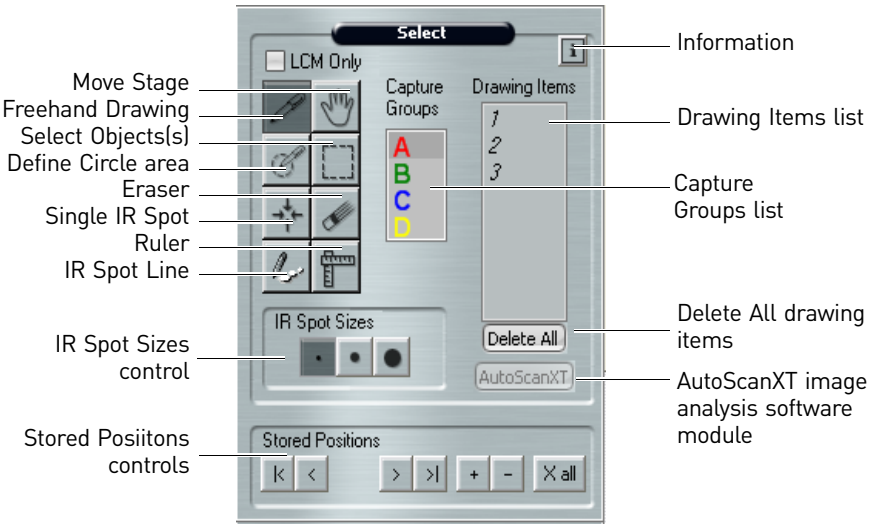


Figure 42 The Select tools pane.

Marking cells for microdissection

You use the Select tools pane (see Figure 42) to mark the 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 that the UV cutting laser will most accurately follow the dissection marks.

To mark cells for microdissection:

1. Click the desired size (small, medium, or large) in the **IR Spot Sizes** control in the Select tools pane (see Figure 42) to choose the relative size of the IR capture spots.

   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 or click any drawing tool to activate it. The tool remains active until you tap or click another tool. For information on each of these tools see Table 9.

   Note: When you are using the Freehand and Defined Circle Area drawing tools, the system will place IR capture spots depending on the slide type selected during set up. If you are using a glass slide, drawing items using these tools will be filled in with IR capture spots and the UV laser will not be used. If you are using glass or frame membrane slides, the Freehand and Defined Circle Area tools mark the perimeter to define the UV cut line, and IR capture spots will be placed automatically, serving only to attach the sample to the capture.

   To use LCM spots on a membrane slide (glass or frame) as for glass slides, check the **LCM Only** button in the Select tool pane, above the drawing tool icons.

Table 9 Drawing tools.

Tool	Suggested use
	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 will 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.
	Use the Defined Circle Area tool to indicate circular areas for microdissection. The software will draw IR capture spots inside the circle. You can also set a fixed size for the Defined Circle Area tool in the Tools Options tab of the Select Options dialog box.
	Use the IR Spot Line tool to indicate a line along which you want to place the IR capture spot.
	Use the Single IR Spot tool to indicate single cells for capture.

Tool	Suggested use
	Use the Eraser tool to remove any unwanted marks made by the drawing tools.
	Use the Select Object(s) tool to draw a rectangle that includes all drawing items that fall inside a region.
	Use the Ruler tool to measure distances or objects in the main image window.

3. When you are done marking cells for microdissection, tap or click the **Move Stage** tool (Figure 42) to turn off the drawing tool.

   For each item that you draw, a number appears in the Drawing Items list located to the right of the Capture Groups list (see Figure 42).

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 or click **B** in the **Capture Groups** list and then mark those cells with the desired tool as described above.

   These drawing items belong to capture group B and will not be collected on the same CapSure™ Cap as capture group A.

6. Once you have one or more drawing items drawn, you can:
   - Use the Eraser tool to remove any marks made by the drawing tools.
   - Use the Select Object(s) tool to draw a rectangle that includes all drawing items that fall inside a region.
   - Once selected, you can move the drawing items, copy and paste them, delete them, move them to a different capture group, or view information about the them.
   - Add more IR capture spots to any item. Click the Single IR Spot tool and then tap or click inside any of the drawing items.

Working with drawing items

When working with drawing items, all actions apply *only* to the active Capture Group.

Moving drawing items

To move a drawing item:

1. Click the **Select Object(s)** tool (see Figure 42), then click in the main image window.
2. Click a single drawing item to select it, or drag the stylus to select more than one drawing item.
3. Hold down the cursor within the selected object(s).
   The cursor changes to a four-headed arrow.
4. 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.

Moving drawing items to a different Capture Group

To move a drawing item to a different capture group:

1. Click the **Select Object(s)** tool (see Figure 42), then click in the main image window:
2. Click a single drawing item to select it, or drag the stylus to select more than one drawing item.
3. Right-click within the selected object(s).
   A pop-up window will appear. Select **Move Selected Object(s) to Group** and select the desired capture group.
   The selected drawing items move to the new capture group.

Deleting drawing items

To delete a single drawing item:

- In the Drawing Items list, tap or click the number of the drawing item, then right-click the stylus button or mouse and select **Delete Object.**
  or
- In the live image, tap the item, right-click and select **Delete Object(s)** from the pop-up menu.

To delete multiple drawing items:

- 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.
  or
- Hold down the **Ctrl** button on the keyboard and tap on multiple objects, tap on one of the selected drawing items, and right-click. Then choose **Delete Objects(s)** from the pop-up menu.

To delete all drawing items from the active capture group:

- Press Ctrl+A on the keyboard and then press Ctrl+X on the keyboard.

  or
- Click on the **Delete All** button, located in the Select tool panel below the drawing items list.

Deleting IR Capture Spots from a drawing item

To delete IR capture spots from a drawing item:

1. Place the cursor on an IR Capture Spot and right-click the IR spot.
2. In the pop-up menu, select either **Delete Spot**, to delete the selected IR capture spot or **Delete Spots in Object**, to delete all the IR capture spots in the drawing item.

   The specified IR Capture Spots are deleted.

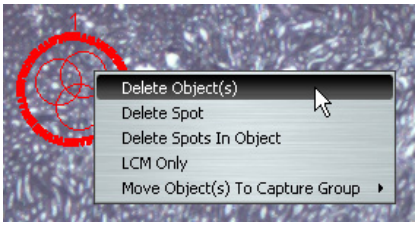


Figure 43 Deleting IR Capture Spots.

Changing the microdissection properties of drawing items

To change from LCM (IR Capture) only to UV Cut and IR Capture:

1. Place the cursor on the drawing item and right-click.
2. Select **LCM Only** in the pop-up window.
   - The check mark will disappear next to LCM Only.
   - The LCM spots will remain in the object, but the perimeter line will change to the UVCutColor (designated in the Capture Group settings window).
   - The IR and UV lasers will both fire for this object.

Note: To reduce the number of LCM spots in the object, right-click within the object and select **Delete Spots in Object** and then manually place as many LCM spots as desired to attach the area to the CapSure™ Cap.

To change from UV Cut and IR Capture to LCM Only:

1. Place the cursor on the drawing item, and right-click.
2. Select **LCM Only** in the pop-up window.
   - A check mark will appear next to LCM Only.
   - The object will be filled in with LCM spots and the outline of the object will change to the IRSpotColor designated in the Capture Group settings window.
   - Only the IR laser will fire for this object.

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. Click the Information button (**i**) in the upper-right corner of the Select tools pane to open the Select Options dialog box (see Figure 44).
2. Click the **Drawing Items t**ab 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.
3. To view information for another capture group, select the button for that group.
4. To export this data chart, click the **Copy** button and save the .csv file to the appropriate location.
5. Click **OK.**

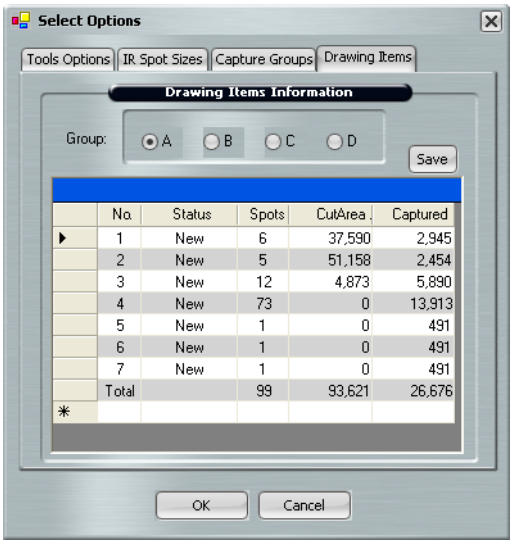


Figure 44 Drawing item information.

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

Setting drawing tools options

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

1. Click the Information button (**i**) in the upper-right corner of the Select tools pane to open the Select Options dialog box.
2. Click the **Tools Options** tab.
3. In the Tools Options tab, set the following options as desired:
   - Select the Eraser size – Click the button corresponding to the size for the Eraser tool.
   - Select the Snap to End Size – Click the button to indicate the distance for the two ends of a shape drawn by the Freehand tool to automatically snap together and close the drawing item.

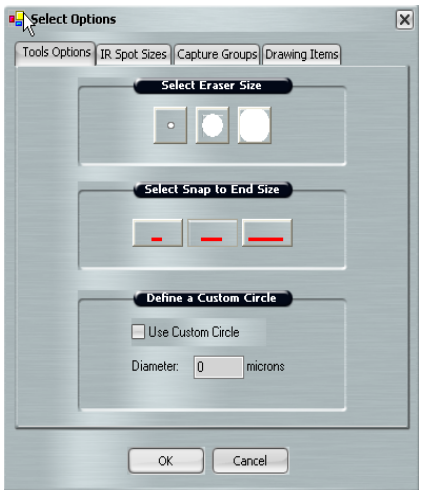


Figure 45 Tools options tab.

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

Measuring distances and objects

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

1. Tap or click 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.
3. The software draws a line and displays a label showing the length of the line (see Figure 46).

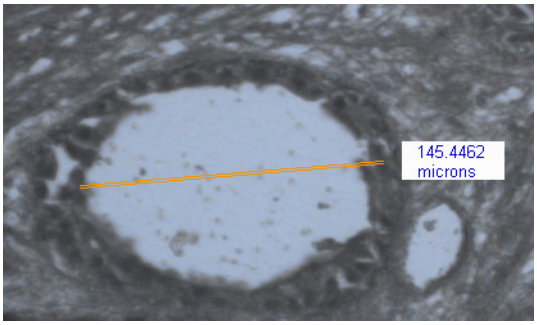


Figure 46 Ruler measurement.

4. To remove the measuring lines and labels, tap or click the **Move Stage** tool.

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, follow the procedure below only 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 selected IR spot size will be placed in all drawn or marked items. IR laser power and duration are stored with the drawing item and will be used for IR capture.

Setting up the Test Fire

To get set up for the Test Fire:

Note: Prior to setting the IR Capture Spot size, ensure that you have located the IR laser.

1. Place a CapSure™ Cap onto the slide and move to a clear (non-tissue) area on the slide.
2. Click the Information button (**i**) in the upper right-corner of the Select tools pane to open the Select Options dialog box.

3. Click the **IR Spot Sizes** tab.
4. Click 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 (see Figure 47).
5. Check **Auto move stage** to move the stage each time the laser is test-fired.
6. Click **Test IR Shot**.

   The software will fire the IR capture laser.

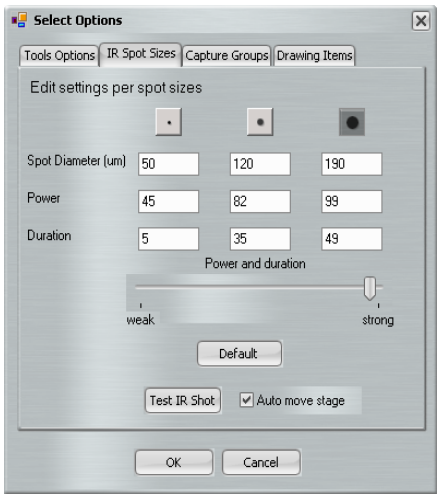


Figure 47 IR Spot Sizes tab.

7. Ensure that you obtain a properly wetted IR Capture Spot.

   You should see a dark ring with a clear center, as shown in Figure 48.

   If you do not see this, adjust the Power and duration slider, and then tap the **IR Laser Test Fire** button again.

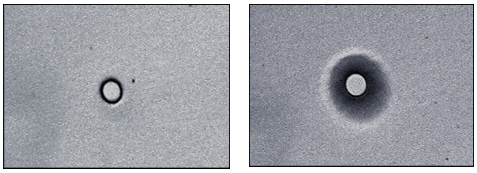


Figure 48 Properly wetted spots.

Checking the spot diameter

To check the diameter of the properly wetted spot:

1. Measure the diameter of the spot:
   a. In the main window, tap the **Ruler** tool in the Select tools pane.
   b. Click the left side of the spot created by the IR capture laser, then drag the stylus to the other side of the spot until the system draws a line and displays a label showing the diameter of the spot.
   c. Click the **Move Stage** tool in the Select tools pane to turn off the Ruler tool.
2. If the diameter is not of the desired size, adjust both the laser power and duration settings simultaneously 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.
   - You can also enter values for both the power and duration setting fields manually.
3. Once you have achieved the desired spot size, enter the diameter for the IR capture spot in the **Spot Diameter (µm)** field.
4. If necessary, repeat the procedure for the other spot sizes.
5. Click **OK** to save the adjusted IR capture spot settings.

IMPORTANT! If you click the X to close the dialog box, the new settings will not be saved. The Default button resets the values for all of the IR capture spots, not just the currently selected spot.

Working with overlays

You can save drawing items to a separate file, then open the file and use it to capture identical regions from the same slide. The drawn object that is laid over the tissue image is called an "overlay".

Saving an overlay

To save an overlay:

1. Load your slides and identify the tissue you want to capture with the drawing tools.
2. Choose **Save** and then **Markup** from the File menu.
3. Enter a file name in the dialog box and click **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\Life Technologies\ArcturusXT folder. The overlay file contains the drawing items and their locations.

Using a saved overlay

To use a saved overlay:

1. Choose **Open** and then **Markup** from the File menu.
2. Navigate to the .bin file containing the overlay, and click **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.

Working with Capture Groups

Tools in the Select tools pane enable you to designate "capture groups, " (i.e., 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 or other slides, you can use two capture groups. You can have a maximum of four capture groups.

Viewing a capture group

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

1. In the Select tools pane, click the letter (A, B, C, or D) in the Capture Groups list for the capture group you want to view.
2. In the Drawing Items list, tap or click 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.

Setting formatting properties for a Capture Group

To set formatting properties of a capture group:

1. Click the Information button in the upper-right corner of the Select tools pane to open the Select Options dialog box.
2. Click the **Capture Groups** tab (see Figure 49).
3. Click 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 you use the Default capture group, then enter these fields:

   a. Name – The name of the capture group. This appears in the cap interaction history.

   b. Annotation – This appears in the cap interaction history.

   c. IR SpotColor – The color of the IR capture spots (in the main image).

   d. UV CutColor – The color of the UV cut line (in the main image).

   e. Fill IR Spo – If this is set to "Yes", the IR Capture spot will be filled with the IR SpotColor.
5. Repeat steps 3 and 4 for any other capture groups.
6. Click **OK** to close the dialog box and save your changes.

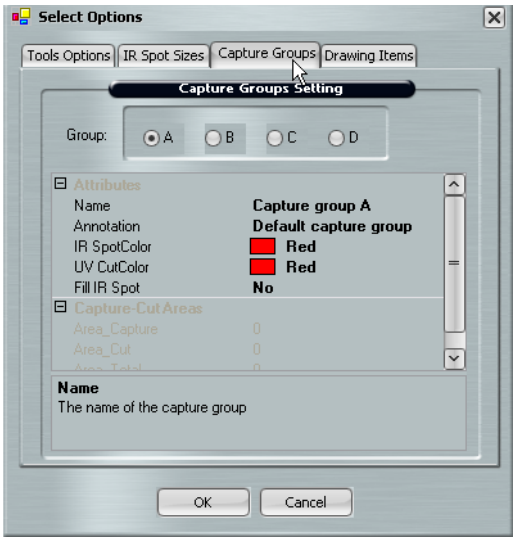


Figure 49 Capture Groups tab.

Note: These settings are also available in the Information area to the right of the slide overview image. Click on a capture group in the Select tools pane to display the information and make any desired changes.

Working with stored positions

Sometimes you find a location on the slide that is interesting, but you are unsure if you want to mark it up for microdissection. In these instances you can store positions and then come back and review them before you mark them up for final microdissection.

To use stored positions:

- To add a position, move the stage to a desired location on the slide and tap on the **+** button to add that location as a stored position. The stored position will be given a number, starting with 1 and increasing with each next stored position.
- To review stored positions, click on the forward and back arrows to scroll through the stored locations. The stage will move to the location of the stored position and bring it to the center of the live image.
- To delete the displayed stored position, click on the minus symbol (**–**).
- To delete all stored positions, click on the **X All** button.

6 Microdissecting cells and tissue

Chapter contents:

Summary of chapter topics

This chapter explains how to microdissect cells and tissue, inspect CapSure™ Caps and slides after microdissection, and unload the caps and slides. It also tells you how to locate both the IR Capture Laser and the UV Cutting Laser, and how to set parameters for IR capture and for UV cutting. It covers setting the order and selecting properties for cut and capture, as well as viewing and updating CapSure™ Cap information. It explains how and when to use the laser bypass feature. You access all of these features from the Microdissect tools pane (see Figure 50).

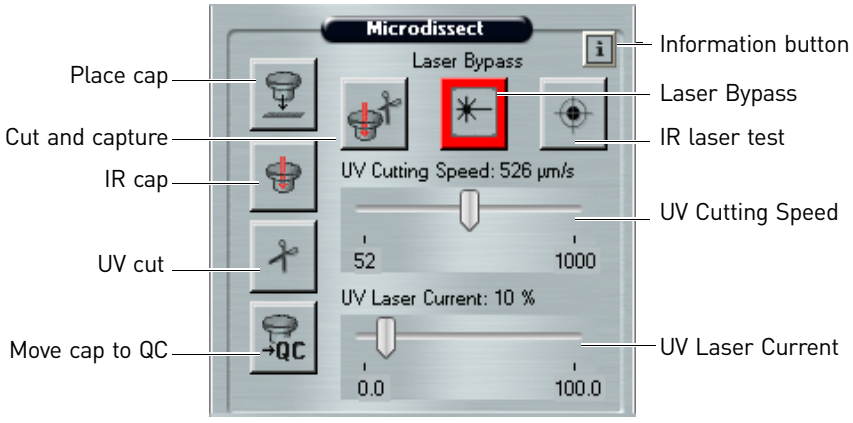


Figure 50 The Microdissect tools pane.

Capturing cells by microdissection

This section explains how to capture cells in one step, how to capture cells in two steps, and how to repeat a microdissection.

Capturing cells in one step

1. (Optional) Review the drawing items in each capture group.
   a. In the Select tools pane, click the letter (A, B, C, or D) in the Capture Groups list for the capture group of interest.
   b. Click 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. (Optional) If you want to add more IR capture spots to any item:
   a. In the Select tools pane, click the **IR Spot Size** control to select a different spot size.
   b. Click the **IR Spot** tool.
   c. Click inside any of the items in the main image.
      A spot is drawn where you clicked.
   d. Click the **Move Stage** tool to deactivate the IR Spot tool.

Preparing for Microdissection

1. Locate the laser(s) as appropriate:
   a. See "Locating the UV cutting laser" on page 71.
   b. See "Locating the IR capture laser" on page 72.
2. Move the stage so the CapSure™ Cap is on an area away from the tissue but still within the cap.
3. Click the **IR Laser Test Fire** button to test the capture laser.
4. Inspect the laser spot.
   You should see a dark ring with a clear center, as shown in Figure 48. If you do not see this, open the Microdissect Options dialog box, adjust the **Power and duration** slider in the IR Spot Sizes tab.

Microdisecting the Sample

Note: The order of the two procedures, cut and capture, depends upon a setting in the Microdissect tab in the Microdissect Options dialog box. The section below describes the default order, which is IR capture followed by UV cutting.

When you are ready to microdissect:

1. Click the **Cut and Capture** button (see Figure 50).
   Note: If you are using DIC, the DIC prism/slider will need to be removed before proceeding. Follow the instructions provided in the pop-up window.

- If you did not place a CapSure™ Cap on the slide, 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 you did place a cap on the slide, the instrument picks up the cap and moves it to the area represented in the main image. The cap location is outlined in green in the slide overview image.
- The instrument fires the IR capture laser at the position of each IR capture spot, to fuse the cells to the cap.
- 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 will fire only the IR laser, unless you have unchecked **LCM Only** for this drawing item.
  - For membrane glass slides and membrane frame slides, the instrument cuts around the region of interest, leaving tabs if designated in the Cut and Capture Settings.

    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 74).

2. Inspect the cap and slide. (See "Inspecting microdissected material" on page 69).

Capturing cells using the capture and cutting tools separately

This explains how to capture cells using the IR Capture and UV Cutting tools separately.

1. (Optional) Review the drawing items in each capture group.
   a. In the Select tools pane, click the letter (A, B, C, or D) in the Capture Groups list for the capture group of interest.
   b. Click 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. (Optional) If you want to add more IR capture spots to any item:
   a. In the Select tools pane, click the **IR Spot Size** control to select a different spot size.
   b. Click the IR Spot tool.
   c. Click inside any of the items in the main image.

   A spot is drawn where you clicked.
   d. Click the **Move Stage** tool to deactivate the IR Spot tool.

Preparing for Microdissection

1. Click the **Place Cap** tool to place a CapSure™ Cap on the slide.

   The instrument places a cap at the center of the field of view disignated 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.

2. Locate the laser(s) as appropriate:
   a. See "Locating the UV cutting laser" on page 71.
   b. See "Locating the IR capture laser" on page 72.
3. Move the stage so the cap is on an area away from the tissue but still within the cap.
4. Click the **IR Laser Test Fire** button to test the capture laser.
5. Inspect the laser spot.

   You should see a dark ring with a clear center, as shown in Figure 48. If you do not see this, open the Microdissect Options dialog box, adjust the Power and duration slider in the IR Spot Sizes tab.

Microdisecting the Sample

1. Click the **UV Cut** button to perform all cuts.
2. Click the **IR Capture** button to perform all captures.

   Note: If you are using DIC, the DIC prism/slider will need to be removed before proceeding. Follow the instructions provided in the pop-up window.
3. Inspect the cap and slide. (See "Inspecting microdissected material" on page 69.)

Repeating microdissection

If you want to repeat the microdissection, if no new drawing items have been placed and the CapSure™ Cap has not been moved from its original position:

1. Click the **UV Cut** button to repeat all cuts.
2. Click the **IR Capture** button to repeat all captures.
3. If the microdissection was not complete or if new drawing items were placed after performing microdissection, highlight the desired drawing items to be repeated and 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

   Note: An asterix appears next to a drawing item number in the Drawing Items list once IR Capture or UV cut have been performed.

Inspecting microdissected material

After you have completed the microdissection, you can inspect the slide and the CapSure™ Cap to verify that the collection was successful. If microdissection was incomplete, you can repeat the cut and/or capture steps.

To inspect the microdissected material:

1. Click 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 the pop-up menu.

   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 system automatically creates the cap interaction history file when the cap is moved to the QC position. This file is named "CapReport-YYMMDD-HHMM.htm", where YYMMDD is the year/month/day and HHMM is the hour/minute when the file was created. The file is saved to the location specified in the ReportSubfolder in the Load Options dialog box.

Figure 51 QC cap position.

2. Inspect the tissue on the cap.
3. (Optional) You can capture a static image at this point.

   The highest objective allowable at the QC position is 20X. If you want to inspect the cap with a higher objective, move the cap to a clean place on the slide and follow standard protocols for inspection.
4. To inspect the slide, click the slide overview image at the position you want to see in the main image window, or click an item number 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:
   a. Move the cap from the QC station back to the slide by placing the cursor on the cap in the QC Caps area to the right of the overview image. Right-click and select **Replace Cap on Slide** from the pop-up menu.
   b. Follow the steps outlined in "Repeating microdissection" on page 68 to recut the drawing items.

Unloading materials

To unload materials:

1. Click **Present Stage** in the Setup tools pane.
   The work surface is displayed.
2. Slide the LCM Cap Insertion Tool onto the work surface.
   Make sure the open end of the insertion tool faces the CapSure™ Cap in the QC station.
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.
   IMPORTANT! 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. Load new slides and caps to continue microdissection, or close the Arcturus*XT*™ software to end your session.
   If you plan to continue microdissection and need to load new caps, you must reset the software.
   To clear caps from the QC position do one of the following:
   - Click the **Reset** button in the Load Options dialog box.
   - Click on each CapSure™ Cap icon in the QC Caps area, next to the Slide overview image.
     When you click on the cap icon it will disappear, indicating that you have removed the cap from the position on the stage off load area

   IMPORTANT! Make sure that you remove any caps whose positions have been cleared in the QC caps area. Failure to do so can result in a new cap running into the one remaining.

Microdissected cells are now available for extraction of nucleic acids and proteins. The final step in microdissection is extracting biomolecules from the CapSure™ Cap.

Locating the lasers

This section explains how to locate both the the UV Cutting Laser IR and the IR Capture Laser.

Locating the UV cutting laser

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

To locate the UV laser:

1. Click the Information button (**i**) in the upper-right corner of the Microdissection tools pane to open the Microdissect Options dialog box.
2. Click the **UV Locate** tab (see Figure 52).
3. Follow the steps in the dialog box. The check mark indicates the current step in the procedure.
4. Click the **Locate UV** button.
   The Arcturus$^{XT™}$ Instrument fires the cutting laser.
5. In the main image window, place the cursor in the center of the UV laser spot and click the spot.
6. Click **OK** in the dialog box.
   If the laser spot is not visible in the main image window, check **UV Power On** to manually turn on the UV cutting laser until the laser spot is visible. Once the laser spot becomes visible, uncheck **UV Power On** and repeat steps 3–6.
7. Click **OK** to close the dialog box.
   A green circle will appear on the live image at the location of the UV laser.

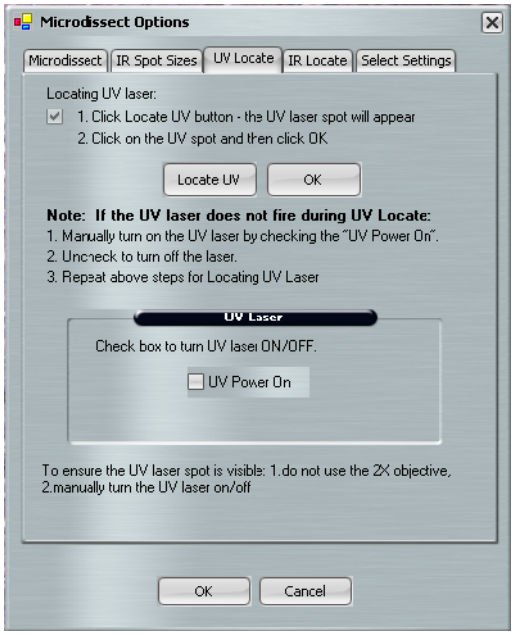


Figure 52 UV Locate tab.

Locating the IR capture laser

Locate or relocate the IR Capture Laser at the beginning of each session. You should relocate the IR Capture Laser during a session if you:

- Place a new CapSure™ Cap onto a slide.
- Move the cap to a different position on the same slide or onto a new slide.
- Change objectives between IR captures.
- Notice that the IR laser is not firing at the desired location.

Locating the IR laser from the Microdissect options pane

To locate the IR capture laser from the Microdissect options dialog box:

1. Open the Microdissect Options dialog box by clicking the Information (**i**) button in the upper-right corner of the Microdissection tools pane.
2. Click the **IR Locate** tab and locate the IR Laser either manually or automatically.

   To locate the IR laser manually:

   a. Click **Locate IR**.

   The live image darkens and the IR laser guide light is visible.

   If the IR laser guide light is not visible, click the **Show Current (Bias)** field and enter a value until the IR laser guide light becomes visible.

   Typically a value greater than 50 is required. If necessary, increase the Brightness settings located in the Inspect tools pane until the IR laser guide light is visible.

   b. To save the IR laser location, place the cursor on the center of the IR laser guide light, and click.

   c. Click the **OK** button next to the Locate IR button in the Microdissect Options dialog box.

   A blue plus sign will appear on the live image at the new location of the IR laser.

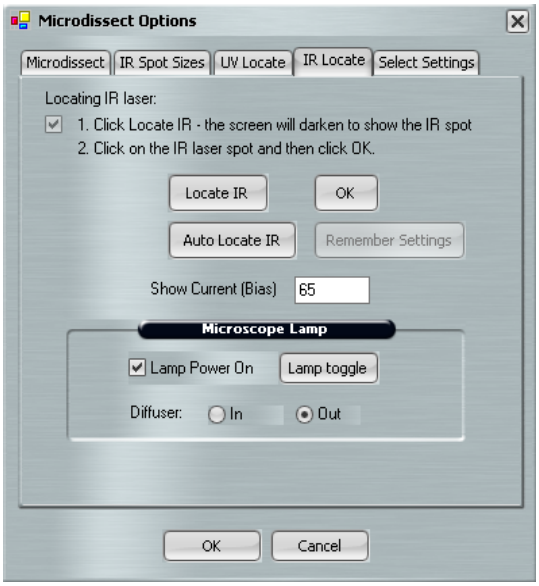


Figure 53 IR Locate tab.

To locate the IR laser automatically:

d. Click **Auto Locate IR**.

   TheArcturus$^{XT™™}$ software turns on the IR capture laser at low power and tries to locate the laser. If the laser is successfully located, the system draws a blue plus sign on the main image where the laser is located.

e. To verify the IR laser location, click the **IR Capture Test Spot** icon and perform a test fire.

f. Look at the main image and see if the laser spot is located within the circle.
   - If the blue plus sign is within the LCM spot, click **OK** at the bottom of the dialog to close the dialog box and and save the changes.
   - If the blue plus sign does not fall in center of the LCM spot, follow the steps to manually locate the capture laser.

Note: If you do not click the **OK** at the bottom of the dialog box, the changes will not be saved.

Locating the IR Laser from the Primary screen

You can also locate the IR laser on the top level of the software user interface. To locate the IR laser:

1. Place the blue plus sign in a non-tissue area.
2. Fire the IR laser using the **IR Capture Test Spot** icon located in the Microdissect tool pane.
3. Place the cursor at the center of the wetted IR spot, right-click, and select **IR Laser Capture Laser Here**.

   The blue plus sign will relocate itself to the center of the LCM spot.

Selecting preferences for cut and capture

This section explains how to switch the order of the cut and capture tasks, and how to set up the properties for the cut and capture.

Changing the cut and capture order

By default, the Arcturus$^{XT™}$ Instrument performs an IR capture first, followed by UV cutting. Performing an IR Capture first can be beneficial, such as when using CapSure™ HS LCM Caps, which are farther from the surface of the tissue, or when performing live cell applications using frame membrane slides. After the IR laser has adhered the cells of interest to the CapSure™ Cap, the UV laser can be fired to cut around the areas of interest. For some applications, you may want to fire the UV laser first. You can choose the order in the Arcturus$^{XT™}$ software.

To set the order of cutting and capture:

1. Click the Information button in the upper-right corner of the Microdissection tools pane.
2. Click **IR Capture first** or **UV-Cut first** to set the order (see Figure 54).
3. Click **OK** at the bottom of the dialog box to close the box and save your changes.

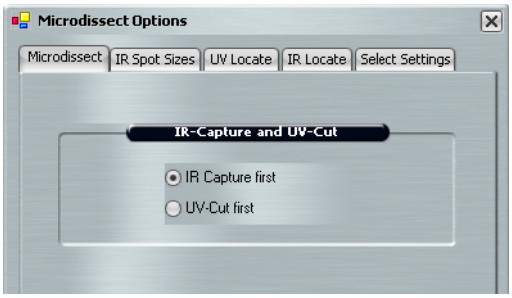


Figure 54 Microdissect tab.

Setting properties for cut and capture

To set properties for cut and capture:

1. Click the Information button (**i**) in the upper-right corner of the Microdissection tools pane.
2. Click the **Select Settings** tab (see Figure 55).
3. If needed, click **IR SpotSpacing** and enter a value.

   This value is the spacing between IR capture spots, as a percentage of the spot diameter.

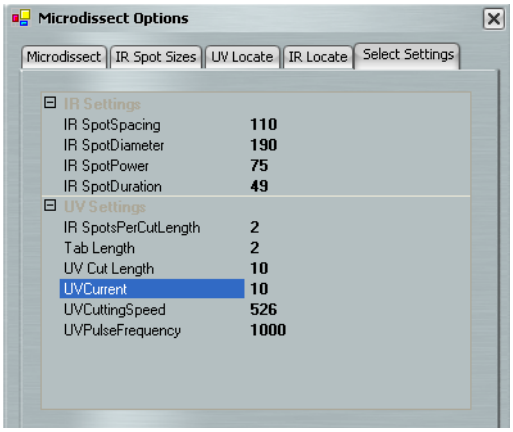


Figure 55 Select Settings tab.

If IRSpotSpacing is:

- <100%, the IR capture spots will overlap.

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

- 100%, all the IR capture spots will be adjacent without overlap.
- >100%, there will be spaces between the IR capture spots.

Note: Changes to IR SpotSpacing will only take effect on new drawing items. Changes are not retroactive on existing drawing items

4. If needed, modify the UV Settings.
   - IR SpotsPerCutLength is the number of IR spots provided per each UV cut length, and between tabs.

     Note: 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. The use of tabs is important only when you are performing the UV cut before the IR capture.

   - Tab Length is the distance (in microns) of tab/space in between each UV cut length.
   - UV CutLength is the length of UV cut line (in microns) between tab insertion.

- UVCuttingSpeed is the speed of the stage movement, which affects the speed of UV cutting.

  Note: Increase this value to cut more quickly; however, if this value is too high, UV cutting can be less accurate. This value corresponds to the UV Cutting Speed slider in the Microdissect tool pane.

- UVCurrent is the current of the UV cutting laser.

  Note: Increase this value to apply more current to the line of ablation. As this value increases, the cutting width will increase. This value corresponds to the UV Current slider in the Microdissect tool pane.

- UVPulseFrequency is the frequency/repetition rate of the UV cutting laser.

  Note: Pulse frequency above 1000 Hz will attenuate the UV laser current.

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

Note: All the changes you make to the IR SpotsPerCutLength, Tab Length, UV Cut Length, UVCuttingSpeed, UVPower, and UVPulseFrequency will be applied retroactively to all drawing items.

These settings are also available in the Information area to the right of the slide overview. To display these settings in the Information area, click in the **UV Cutting Speed** in the Microdissect tools pane or on the **IR Spot Size** in the Select tools pane.

Working with CapSure™ Caps

This section explains how to view CapSure™ Caps in the QC area, how to view and update cap properties, and how to view the Cap Interaction History.

Viewing a CapSure™ Cap In the QC area

To view a CapSure™ Cap in the QC area:

1. Place the cursor to the right of the slide overview image, right-click on the **Cap** button for the cap of interest in the QC cap position (see Figure 56).
2. Select **View Cap at QC Station** from the pop-up menu.

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

Note: The highest objective allowable at the QC position is 20X. If you want to inspect the material on the cap with a higher objective, move the cap to a clean place on the slide and follow standard protocols for inspection.

Viewing and updating CapSure™ Cap properties

You can view the CapSure™ Cap type in the cap and slide handling area to the left of the slide overview image. If you did not select the correct CapSure™ Cap type in the Load Options dialog box when you loaded your cap, you can change it here.

If you are using CapSure™ Macro LCM Caps and v3.4 software, you can select **Micro**, but the maximum diameter of the capture will be 4300 µM.

You can also add CapSure™ Caps to the Load Caps area by clicking a cap in the Load area here (see Figure 56). This shortcut lets you load caps without opening the Load Options dialog box, but to use this shortcut, the corresponding QC Caps position must be empty.

Figure 56 Cap and slide handling area (v3.4 software).

IMPORTANT! Make sure that you remove any caps whose positions have been cleared in the QC caps area. Failure to do so can result in a new cap running into the one remaining.

You can also view Cap Information (name and notes) in the information area to the right of the slide overview image. You can edit the CapName or CapNotes here (see Figure 57). Editing here is the same as entering the information in the Load Options dialog box.

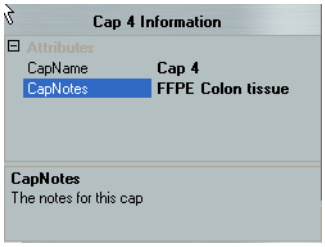


Figure 57 Cap information area.

Viewing the cap interaction history

The Arcturus$^{XT™}$ software creates the cap interaction history file the first time a CapSure™ Cap is off-loaded. It then updates this file 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. Click outside of the Arcturus$^{XT™}$ application so that 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.

Using the Laser Bypass feature

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

In order to bypass the laser safety mechanism, the laser bypass key must be in place in the back of the instrument. You must then depress the **Laser Bypass** button (see Figure 50), activating the UV laser. For a full description of laser status indicators, as observed by color changes of the Laser Bypass button, see "Laser safety scenarios" on page 110.

7 Extracting cells and tissue

Chapter contents:

Summary of chapter topics

After you have microdissected cells, you can extract the biomolecules from the samples. Depending on the type of CapSure™ Cap you used, the procedure varies. This chapter contains suggestions for appropriate extraction kits, and instructions for extracting microdissected tissue from CapSure™ LCM MicroCaps or CapSure™ HS LCM Caps. See Pub. No. 0112-0153 for instructions for CapSure™ Macro LCM Caps.

Choosing an extraction kit

See Appendix D for extraction kits specifically designed to work with the CapSure™ LCM Sample Preparation System. For additional information about related instruments and Arcturus™ Microgenomics reagents kits, see **thermofisher.com/lcm**.

Extracting tissue from CapSure™ LCM MicroCaps

CapSure™ LCM MicroCaps can capture a diameter of up to 4300 µm and are compatible with both 0.2- and 0.5-mL reaction tubes for nucleic acid isolation after LCM.

The procedure for extracting tissue with a CapSure™ LCM MicroCap varies depending on the nucleic acid that will be extracted and the starting tissue type. See the *Arcturus™ CapSure™ LCM MicroCaps User Bulletin* (Pub. No. MAN0014607) for detailed protocols.

Extracting tissue from CapSure™ HS LCM Caps

Following microdissection, place the ExtracSure™ Extraction Devices onto the CapSure™ HS LCM Caps containing the microdissected cells. The ExtracSure™ Device seals around the perimeter of the CapSure™ HS LCM 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.

Performing LCM captures with CapSure™ HS LCM Caps

Due to the way in which the ExtracSure™ Device fits onto the CapSure™ HS LCM Cap, you should perform LCM captures in the center of the CapSure™ HS LCM Cap, within the black capture ring (see Figure 58).

Figure 58 CapSure™ HS LCM Cap and ExtracSure™ Device.

Using the ExtracSure™ Device during extraction

Assemble an ExtracSure™ Device with the CapSure™ HS LCM Cap before starting the extraction procedure:

1. Use clean tweezers to remove the CapSure™ HS LCM Cap from the LCM Cap Insertion Tool and place the cap with the sample facing up into the alignment tray.

   Make sure the CapSure™ HS LCM Cap is properly seated in the alignment tray following the directions in the CapSure™ HS LCM Caps manual.

2. Use clean tweezers to remove and position the ExtracSure™ Device over the CapSure™ HS LCM Cap.

   The fill port on the ExtracSure™ Device should be facing up.

3. Use tweezers to firmly push down the ExtracSure™ Device onto the cap.

   The ExtracSure™ Device should fit securely into place.

   At this point, the ExtracSure™ Device should be firmly sealed to the cap.

4. Add extraction buffer to the device, as specified in the user guide for your extraction kit.

   Do not remove the assembled ExtracSure™ Device and CapSure™ HS LCM Cap from the alignment tray until incubation is completed.

5. Place a 0.5-mL microcentrifuge tube over the fill port and allow the samples to incubate as described in the appropriate extraction procedure.

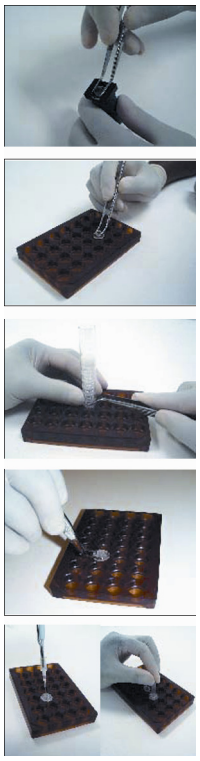


Figure 59 CapSure™ HS LCM Cap and ExtracSure™ Device in alignment tray.

6. Place the tube into a microcentrifuge and spin briefly to bring the buffer to the bottom of the tube and complete the extraction procedure as described in the appropriate user guide.

A Maintenance and troubleshooting

Chapter contents:

Summary of chapter topics

This chapter contains instructions for cleaning the Arcturus$^{XT™}$ Instrument, information about parts that you can service yourself, and tips for troubleshooting.

IMPORTANT! Do not attempt to remove the covers from the instrument except as specified for the user-serviceable parts. If the instrument requires service, contact your Thermo Fisher Scientific service representative.

Cleaning the Arcturus$^{XT™}$ Instrument

To keep the instrument clean:

- 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.

Replacing user-serviceable parts

The Arcturus$^{XT™}$ Instrument has only four parts that you can service yourself:

- Bright field illumination lamp
- Interchangeable fluorescence filter cube (if your instrument has one)
- Fluorescent lamp (if your instrument has one)
- Fuse

Instructions for replacing these components are provided here. For other service needs, contact your Thermo Fisher Scientific service representative.

Replacing the bright field illumination lamp

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

Replacing the 100 W halogen lamp

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

Follow the Nikon™ microscope instructions provided with the Arcturus*XT*™ Instrument to replace the lamp.

Replacing 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 necessary, exit the Arcturus*XT*™ software.
2. 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.
3. 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.
4. Use the hex key to unscrew the two screws holding the LED.
5. Unplug the LED cable (it has a white connector) and remove the LED.
6. Replace the LED, connecting the cable and replacing the two screws.
7. Replace the top cover and then tighten the screws.
8. Plug the instrument in and turn it on.
9. Restart the Arcturus*XT*™ software.

Replacing fluorescence filter cubes

When selecting alternate fluorescence filter cubes, be sure that 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™ Eclipse™ T*i*-E microscope.

To replace a fluorescence cube:

1. Remove the cover and select the desired filter position from the carousel below the objective turret of the microscope.
2. Remove the existing filter cube (if present) by pulling it straight out.
3. Insert the new filter cube by pushing it straight in.
4. Replace the cover.

Replacing the fluorescence lamp

For this procedure, 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.

Follow the instructions provided with the lamp.

Replacing the fuse

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

To replace the fuse:

1. If needed, exit the Arcturus$^{XT™}$ software.
2. 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.
3. Use the slotted screwdriver to open the fuse holder.
   The fuse is located in the plug socket.
4. Remove the fuse and replace it with the new one.
5. Plug in the instrument and turn it on.

Troubleshooting tips

If you encounter a problem that you cannot resolve using this troubleshooting section, contact your Thermo Fisher Scientific service representative.

Solving problems with IR Laser Capture (LCM)

Table 10 Possible Problems with IR Laser Capture (LCM).

Observation	Possible cause	Recommended action
Cells do not adhere to the CapSure™ HS LCM Cap	Inadequate IR laser power or duration settings	Open the IR Settings tab in the Microdissect dialog box and adjust the power and duration settings until proper wetting is achieved.
	Tissue preparation	Folds may be present: Position the CapSure™ HS LCM Cap such that it is not placed on the fold. Alternatively, increase IR laser power and duration settings to achieve proper wetting. Tissue not dehydrated: Ensure that the section is properly dehydrated. Proper dehydration is 100% ethanol for 1 minute, followed by Xylenes for 5 minutes, and then air dry for 5 minutes.
	Cap	The CapSure™ HS LCM Cap may be damaged. Try another CapSure™ HS LCM Cap.
Cannot locate IR Laser	No CapSure™ Cap on slide	Place a CapSure™ Cap on the slide.
	Bias or brightness settings are too low	Open the IR locate tab in the Microdissect dialog box. The Bias setting should be at 60. You may also need to adjust the Brightness setting, in the Inspect tool pane, to darken the field of view and visualize the IR laser guide light.
	IR laser is out of the field of view	1. Fire IR laser using the test fire button located in the Microdissect tool pane. 2. Move around under the CapSure™ Cap to locate a wetted spot. 3. Right-click in the center of the spot and select the IR spot located from the pop up window. If this doesn't work, the IR laser location may need adjustment. Contact Technical Support to arrange for service.
	Tissue is thick or dark	Move to a thinner tissue area or a clear area on the slide.
IR Capture laser fires off target	IR laser location is set incorrectly	Relocate the IR laser.

Solving problems with UV Laser cutting

Table 11 Possible problems with UV Laser cutting.

Observation	Possible cause	Recommended action
UV Laser does not cut	Neutral density filters may be in place	Verify that the ND filters are in the out position (levers tilted to the right). The ND filters are located behind the front cover of the UV laser housing, on the left side of the instrument. **Note:** Instruments with the Enhanced UV Laser do not have neutral density filters. They have software controlled UV Laser Current and UV Pulse Frequency control.
	Tissue is thick or fibrous	Reduce the UV cutting speed, or for systems with the Enhanced UV Laser, increase UV Laser Current (located in the Microdissect tool pane).
UV Cutting is inefficient	Cutting speed	Reduce the UV cutting speed using the slide bar located in the Microdissect Tool pane.
Double UV Cut line appears	DIC prism/slider is in place	Remove the DIC prism/slider from beneath the objective prior to firing the UV laser. **Note:** The DIC prisms/sliders should only be in place during DIC visualization. For optimal visualization using brightfield, fluorescence, or phase contrast, and for optimal UV cutting, ensure that the sliders/prisms have been removed from the objectives.

Solving problems with image quality

Table 12 Possible problems with image quality.

Observation	Possible cause	Recommended action
Long lag time when updating the live image	Light intensity or camera gain settings are not optimized	1. Check the Brightness setting located in the Microscope tool panel. It should be ≥0.200s. 2. Open the **Select** dialog box and re-adjust intensity or camera gain to achieve an appropriate brightness setting.
Brightfield image is fully or partially blocked	Apertures: Field or Condenser	1. If you have the Nikon™ phase contrast illumination tower, check the field and condenser apertures to make sure they are both fully open. 2. Check that the filter sliders found in the illumination tower are not partially pushed in.
	Field aperture is not centered	Center the field aperture.
	Fluorescent cube turret	Check to ensure that the position is fully clicked in.
	Magnification Tube	Check to ensure that the position is fully clicked in.
Poor image quality at 40x and 60x	DIC sliders	Remove the DIC sliders located below each objective. **Note:** Replace any empty slot with a cover to prevent dust from entering.
Background of live image is not clear or white	White balance is off	Re-establish white balance in camera properties dialog box, located under View in the pull-down menu.
	Fluorescence cube in place	Check the fluorescence turret. If using brightfield illumination, it should be in a position not containing a filter cube.
Slide overview image too dark or too bright	Settings are not optimized	1. Change magnification to 2x and adjust the brightness setting. 2. Right-click in the slide overview area and select **Remember Settings** in the pop-up window. 3. Select **Yes** to save for all future overviews. 4. Select **Require Overview**.

Solving problems with fluorescence

Table 13 Possible problems with fluorescence.

Observation	Possible cause	Recommended action
Fluorescence Image is fully or partially blocked	Fluorescent Field Aperture, Fluorescence ND Filters	1. Ensure that the fluorescence field aperture is centered. 2. Ensure that the Fluorescence ND filters are in the OUT positions. 3. Ensure that shutter on the fluorescence turret is open (0 position).
Fluorescence signal is suboptimal (long exposure time is required)	Fluorescent ND Filters	ND fluorescent filters should be in the OUT positions.
	DIC or Phase Optics	The DIC analyzer and the polarizer should both be in the OUT positions. The Condenser position should be set at A.
	Fiber Optic Cable	1. Check the fluorescence adapter cone to ensure that it has been attached properly. 2. Ensure that the fiber optic cable is fully inserted into the cone and the EXFO control box.

Solving problems with phase contrast/DIC

Table 14 Possible problems with phase contrast/DIC.

Observation	Possible cause	Recommended action
The phase contrast image does not appear optimal	The diffuser	Ensure that the diffuser is in the OUT position in the Select Options dialog box.
	Phase annulus	Ensure that the proper phase annulus is chosen for the objective in use. PhL = 4x; Ph1 = 10x and 20x; Ph2 = 40x and 60x.
	Annular diaphragm	Ensure that the annular diaphragm is centered.
The DIC image does not appear optimal	The diffuser	Ensure that the diffuser is in the OUT position in the Select Options dialog box.
	Condenser turret position	Ensure that the condenser is turned to the DIC N1 position.
	Polarizer and analyzer	Ensure that the polarizer and analyzer are both fully pushed into the IN positions.
	Condenser alignment	Ensure that the condenser is properly centered and focused.
	DIC Slider	Ensure that the DIC Slider has been inserted beneath the objective in use.

Note: For more information on Phase Contrast and DIC, as well as complete instructions for use, please refer to the Nikon™ user guides provided with the Arcturus^{XT}™ Instrument, or visit **www.microscopyu.com**.

Solving general problems

Table 15 Possible problems with the instrument in general.

Observation	Possible cause	Recommended action
Microdissection (UV or IR) is not aligned to markings of drawing item(s)	Magnification of image does not match the software operation	Check the position of the Intermediate magnification dial and compare against the magnification tab located in the microscope dialog box. Both of these items should match at 1X or 1.5X.
	UV or IR laser is not located properly	Use the IR or UV locate options found in the microdissection dialog box.
Brightfield light is flashing (either slow or fast)	Stage bump	1. Re-align the stage so that all front edges of the stage are parallel. 2. Close down the software and turn off the instrument. 3. Restart the instrument and re-initiate the software.
	Limit for the cap robot arm has been exceeded	1. Lower the cap fork by turning the lead-screw counter-clockwise (3-mm slotted screwdriver) until the motor takes hold. 2. Close down the software and turn off the instrument. 3. Restart the instrument and re-initiate the operating software.
Microdissection process does not initiate within AutoScanXT by using the Harvest button	Live image was moved away from where static image was taken	Move the CapSure™ Cap to the area containing the items for microdissection and click the **Harvest** button again.

B System specifications

This appendix provides the specifications for the Arcturus$^{XT™}$ Laser Capture Microdissection (LCM) System with a Nikon™ Eclipse™ T*i*-E microscope base. Depending on the configuration of your system, not all options listed below may be included with your instrument.

Instrument specifications

Table 16 Description of instrument specifications.

Item	Description
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	Solid-state, near IR laser-state, 810 nm
UV Cutting Laser	Diode-pumped solid-state UV laser, 349 nm. Adjustable UV Laser Current (0-100%), Pulse Frequency (10 - 5000 Hz) and Cutting Speed.
Microscope Stage	Computer and trackball controlled. Range 155 x 125 mm, repeatability 2 µm.
Bright Field Illumination Source	100 W halogen lamp or High intensity LED illumination system.
Fluorescent lamp	EXFO X-Cite™ 120 PC metal halide fluorescence illumination system. Lamp life = 2000 hours.
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 Optional Triple Dichroic: Excitation: 385 - 400 / 475 - 493 / 545 - 565 nm Emission: 450 - 465 / 503 - 533 / 582 622 nm
Operating Temperature	18–30°C
Operating Humidity	60% relative humidity (noncondensing)
Base Unit Dimensions	Height: 28 in. (71 cm) Width: 22 in. (56 cm) Depth: 30 in. (76 cm)

Item	Description
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)

Computer specifications

The following specifications apply to the computer used with the Arcturus$^{XT™}$ Instrument.

- 2.8 GHz Pentium 4 processor (minimum)
- 2 GB RAM (minimum) 40 GB hard drive (minimum)
- For Arcturus$^{XT™}$ Software v3.4 (required for LCM with CapSure™ LCM MicroCaps): Windows™ 7 Professional operating system
- For Arcturus$^{XT™}$ Software v3.3 or earlier: Windows™ XP Professional operating system with SP 2
- Read/write DVD drive
- Interactive pen display, 17" diagonal LCD, 1280 x 1024 (SXGA)

Available instrument configurations

You can choose the options for your Arcturus$^{XT™}$ Instrument. These options are pictured below and described in the following pages. In Figure 60 the photo on the left is an Arcturus$^{XT™}$ Instrument with an LED illuminiation tower; the instrument on the right has a Nikon illumination tower.

Figure 60 Arcturus$^{XT™}$ Instrument with LED tower and with Nikon™ tower.

Base station

There are three base station configurations for the Arcturus$^{XT™}$ Instrument. Each instrument includes IR laser capture, an operating system and software, three objectives (2X, 10X, 40X), and a standard 0.7 MP video camera. You may select from three illumination options to complete a base system configuration.

Note: The catalog numbers listed here are not included on the website, as these items cannot be ordered online.

Illumination tower options

Table 17 Illumination tower options.

Description	Cat. No.
Arcturus$^{XT™}$ LED Illumination Tower	0310-5537
Arcturus$^{XT™}$ Phase Contrast Nikon Illumination Tower (manual)	0310-5535
Arcturus^{XT} Phase Contrast Nikon Illumination Tower (motorized)	0310-5761

Additional options

Table 18 Additional upgrades and options.

Description	Cat. No.
UV Cutting	
Arcturus^{XT} UV Cutting Option	0310-5538
Arcturus^{XT} Enhanced UV Cutting Option	0310-5950
Fluorescence	
Arcturus$^{XT™}$ Fluorescence Option. Includes fluorescence cubes for excitation with emission at Red, Blue and Green.	0310-5504 (manual) 0310-5749 (motorized)
Differential Interference Contrast option for Nikon Illumination Tower **Note:** We recommend that you purchase Arcturus$^{XT™}$ Binoculars along with the Phase Contrast Illumination Tower.	
DIC Base Includes 10X and 40X objective sliders **Note:** Optional objective sliders can be purchased in conjunction with the selection of the respective optional objectives	14423-00
• Differential Interference Contrast–20X Slider	6550-0118
• Differential Interference Contrast–60X Slider	14468-00
DIC Analyzer Cube **Note:** Requires purchase of motorized fluorescence filter turret (Cat. No. 0310-5749)	9000-1055
Optional objective upgrades	
4X	14676-00
20X	14658-00
60X	14659-00
100X Dry	14662-00

Description	Cat. No.
100X Oil	14661-00
Binoculars	
Microscope Binoculars **Note:** We recommend that you purchase Arcturus^{XT}™ Binoculars along with the Phase Contrast Illumination Tower.	0200-6228
Second Camera	
High Resolution 5 MegaPixel Camera with MetaVue™ Imaging System **Note:** We recommend that you purchase a second monitor when you purchase a high-resolution camera. This way you can view the camera output on one monitor, and use the other to run the operating system software.	14379-00
Monitor	
LCD Flat screen monitor for dual monitor operation	10904-00
UV Cube	
Excitation 340–390 nm, emission >410 nm	9000-1034
Triple Dichroic Filter Set. DAPI/FITC/TRITC	
Excitation 385-400/475-493/545-565 Emission 450-465/503-533/582-622	6530-0056
AutoScan^{XT} Image Analysis Software Module	
AutoScan^{XT} Image Analysis Software Module	9050-0005
Modular Stage Inserts	
Large Format Slide Stage Insert	0310-5401
Petri Dish Stage Insert	0310-5631

C Installation instructions

Instructions for lifting and carrying the instrument

The Arcturus^{XT}™ Instrument is shipped from the factory in two or more boxes, depending upon the configuration you have ordered. Use proper lifting techniques when unpacking and installing the instrument. Improper lifting can 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 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.

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.
- Up to six power receptacles, depending on system configuration. The base system (IR laser only) requires four power receptacles; 1)Arcturus^{XT}, 2) Eclipse T*i*-E 3) Computer, 4) Monitor.
- Temperature 18–30°C, relative humidity <60%.
- Indoors, pollution degree 2.
- Installation Category II.
- Altitude requirements specified in Appendix B, “System specifications”.

General unpacking and installation instructions

CAUTION! Failure to correctly install the instrument can result in damage that is not covered by the warranty.

A qualified Thermo Fisher Scientific service professional will install your Arcturus$^{XT™}$ Instrument. This will assure optimum performance and minimize risk of damage to the instrument.

Note: Any damage encountered as a result of self installation may not be covered under instrument warranty, or may result in voiding of the warranty. Please contact us for full details.

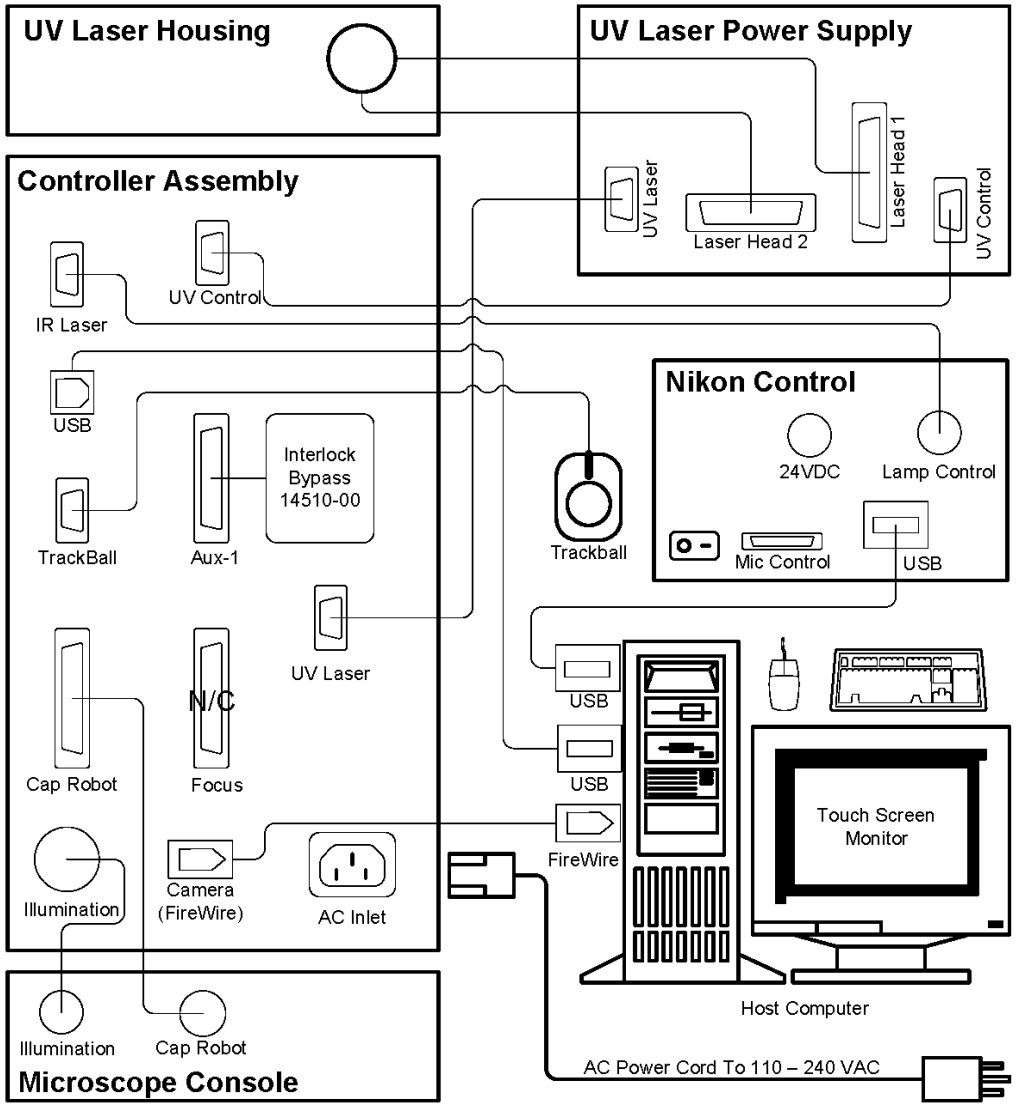


Figure 61 Connections between components of the Arcturus$^{XT™™}$ System.

Installing software upgrades

Each version of the software is accompanied by detailed instructions for updating an existing installation. Follow the update instructions distributed with the software. The latest version of Arcturus$^{XT™}$ operating software is available at **thermofisher.com/lcm ▸ Arcturus$^{XT™}$ LCM Software**.

D Arcturus™ reagent kits

Note: Only the most frequently used kits are listed here. Additional kit configurations are available depending on individual research needs. For more information, visit **thermofisher.com/lcm.**

Kit	Cat. No.	Description
HistoGene™ LCM Frozen Section Staining Kit	KIT0401 – 72 slides	The HistoGene™ LCM Frozen Section Staining Kit is used to process tissue sections for LCM in order to maximize the quality and yield of RNA from the LCM cells. The kit comes with all dehydration and staining reagents, disposable staining jars, specially treated slides, and a detailed protocol and troubleshooting guide.
HistoGene™ LCM Immunofluorescence Staining Kit	KIT0420 – 32 slides	The HistoGene™ LCM Immunofluorescence Staining Kit is designed to enable retrieval of high-quality RNA from immunofluorescently stained frozen tissue. It enables convenient and reliable staining, dehydration, and LCM of tissue sections. The kit's protocols are streamlined and optimized for efficient LCM capture while maintaining RNA quality for downstream applications that require intact RNA, such as microarray analysis and RTPCR.
PicoPure™ RNA Isolation Kit	KIT0204 – 40 isolations	The PicoPure™ RNA Isolation Kit is used for the extraction and isolation of total RNA from small samples, particularly LCM cells. The PicoPure RNA Kit comes with optimized buffers, MiraCol™ Purification Columns and an easy-to-use protocol to maximize recovery of high-quality total cellular RNA, ready for amplification with the Arcturus™ RiboAmp™ Plus RNA Amplification Kits.
PicoPure™ DNA Extraction Kit	KIT0103 CapSure™ LCM MicroCap: 30 extractions (50 µL) to 150 extractions (10 µL) CapSure™ HS LCM Cap: 150 extractions CapSure™ Macro LCM Cap: 30 extractions Tissue scrapes: 10 extractions	The PicoPure™ DNA Extraction Kit is optimized to maximize the recovery of genomic DNA from 10 or more cells captured by LCM. The kit comes with reagents and protocols tested to ensure complete extraction of DNA from LCM samples prepared with any standard tissue preparation procedure. DNA prepared using the kit is PCR-ready and needs no additional purification to perform amplification.

Kit	Cat. No.	Description
Paradise™ PLUS RNA Extraction and Isolation Kit	KIT0312I – 12 samples	The Paradise™ PLUS RNA Extraction and Isolation Kit is designed for extraction of RNA from formalin-fixed paraffin-embedded (FFPE) tissue samples. The kit includes Proteinasae K, Proteinase K buffer, purification columns, column conditioning, binding, and elution buffers, and microcentrifuge tubes.
Paradise™ PLUS FFPE Kits	KIT0312 – 12 samples KIT0312B – 12 samples with biotin labeling KIT0312C – 12 samples with Cy®3 labeling KIT0312D – 12 samples with Cy®5 labeling	The Paradise™ PLUS Reagent System is designed to enable gene expression studies using formalin-fixed paraffin-embedded (FFPE) tissue samples. Components include sample preparation and staining reagents, RNA extraction and isolation reagents, RNA amplification reagents and a comprehensive user guide.
Paradise™ PLUS FFPE WT-RT Kit	KIT0315 – 12 Samples	The Paradise™ PLUS Whole Transcript Reverse Transcription (WT-RT) Reagent System Kit enables QRT-PCR using formalin-fixed, paraffin-embedded (FFPE) tissue samples. The kit was developed specifically to overcome obstacles often associated with formalin-fixed tissue, such as chemical modification and RNA fragmentation. The kit provides RNA isolation and reverse transcription reagents optimized for use with archived FFPE samples at small sample input amounts, and delivers unparalleled yield, fidelity, and representation. The kit was designed with exon-spanning primers at varying distances from the 3' end of the transcript, and allows the study of splice variants in archived or degraded samples. The Paradise™ WT-RT system also allows the use of gene-specific primers for reverse transcription, to suit specific assay requirements.
RiboAmp™ PLUS RNA Amplification Kits	KIT0521 RiboAmp™ PLUS Kit – (12) 1-round amplifications or (6) 2-round amplifications KIT0525 RiboAmp™ HS PLUS Kit – (6) 2-round amplifications	The RiboAmp™ PLUS RNA Amplification Kit enables the production of microgram quantities of antisense RNA (aRNA) from as little as picogram amounts of total cellular RNA. Amplified RNA produced using the kit is suitable for labeling and use on expression microarrays. The kit achieves 1,000- to 3,000-fold amplifications in one round of amplification, and up to 1,000,000-fold in two rounds. The kits include microarray labeling options for biotin, fluorescent dyes and amino allyls. Kits are available in two sensitivity options, RiboAmp™ PLUS (5–40 ng input) and a high- sensitivity version RiboAmp™ HS PLUS (0.1– to 5–ng input).

Kit	Cat. No.	Description
Turbo Labeling™ Kits	KIT0608 – Biotin – 12 samples KIT0609 – Cy®3 – 12 samples KIT0610 – Cy®5 – 12 samples	The Turbo Labeling™ Kits provide a proprietary, non-enzymatic technology for the labeling of unmodified aRNA for gene expression profiling. The unmodified aRNA is labeled post-amplification, thereby avoiding the need to incorporate modified nucleotides. The use of natural nucleotides in the amplification step results in unmodified aRNA with higher yields and longer aRNA fragments, thus providing better representation of the mRNA transcript for downstream analysis.

E

Safety

Instrument safety

Symbols on instruments

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Electrical symbols on instruments

The table below describes the electrical symbols that may be displayed on Applied Biosystems™ instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.

Symbol	Description
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems™ instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the SDSs before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**WARNING!** Hot lamp.	**AVERTISSEMENT!** Lampe brûlante.
	WARNING! Hot. Do not remove lamp until **15** minutes after disconnecting supply.	**AVERTISSEMENT!** Lampe brûlante, après avoir déconnecté le câble d'alimentation de l'appareil, attendre environ **15** minutes avant d'effectuer un remplacement de la lampe.
	WARNING! Hot. Replace lamp with an Applied Biosystems™ lamp.	**AVERTISSEMENT!** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems™.
	CAUTION! Hot surface.	**ATTENTION!** Surface brûlante.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems™ qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Applied Biosystems™.
	**CAUTION!** Class 2(II) visible and/or invisible LED radiation present when open. Do not stare directly into the beam or view directly with optical instruments.	**ATTENTION!** Rayonnement visible ou invisible d'un faisceau LED de Classe 2(II) en cas d'ouverture. Ne pas regarder le faisceau directement ou au travers d'un instrument optique.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

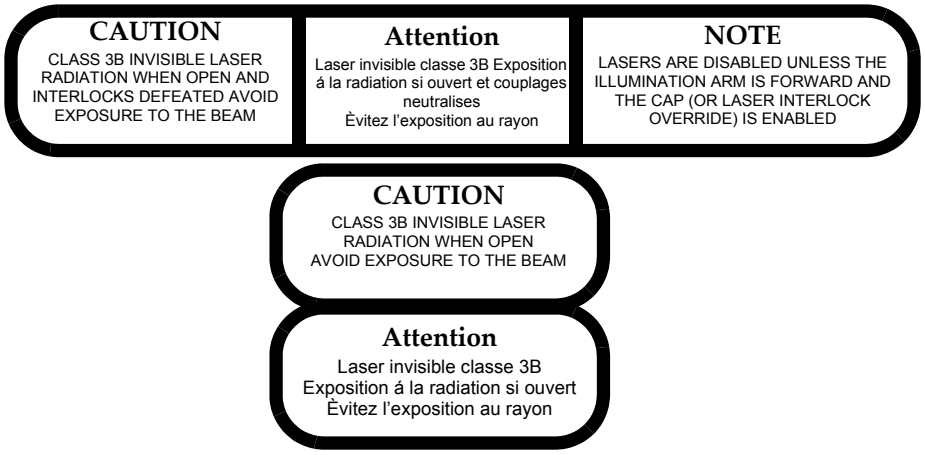
Environmental symbols on instruments

The following symbol applies to all Applied Biosystems™ electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Customer Service office for equipment pick-up and recycling. Visit **thermofisher.com/contactus** for a list of customer service offices in the European Union.

Safety labels on instruments

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



General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by us may result in personal injury or damage to the instrument.

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor 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.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs).

Cleaning or decontaminating the instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Ultraviolet light

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

Moving Parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

WARNING! PHYSICAL INJURY HAZARD. Do not operate the instrument without the arm shield in place. Keep hands out of the deck area when the instrument is spotting.

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected. Plug the system into a properly grounded receptacle with adequate current capacity.

WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

Laser safety

Laser classification

The Arcturus$^{XT™}$ 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$^{XT™}$ 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.

Laser safety requirements

To ensure safe laser operation:

- The system must be installed and maintained by an Thermo Fisher Scientific Technical Representative.

- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating (during service with safety interlocks disabled), you may be exposed to laser emissions.
- Do not remove safety labels or disable safety interlocks.

Safety interlock system

The Arcturus$^{XT™}$ LCM System 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.

WARNING! Contact 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 349 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
349 nm, 60 uJ, variable pulse frequency (10 - 5000 Hz)

Additional laser safety information

Refer to the user documentation provided with the laser for additional information on government and industry safety regulations.

WARNING! LASER HAZARD. Lasers can burn the retina, causing permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Do not remove the instrument top or front panels. Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the top or front panels are removed for service.

WARNING! LASER BURN HAZARD. An overheated laser can cause severe burns if it comes in contact with the skin.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European Safety and EMC standards
- Australian EMC standards

U.S. and Canadian safety standards

The instrument has been tested to and complies with standard:

- UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."
- FDA "Radiation Control for Health and Safety Act of 1968 Performance Standard 21 CFR 1040.10 and 1040.11," as applicable.

Canadian EMC standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European Safety and EMC standards

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards:

- EN 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."
- EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide.

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class A), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Product-specific warnings

Please review the following precautions carefully to ensure safe and effective use of the Arcturus$^{XT™}$ Laser Capture Microdissection (LCM) System, which consists of the Arcturus$^{XT™}$ Instrument, a computer, and the Arcturus$^{XT™}$ operating software.

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

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.

IMPORTANT! 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$^{XT™}$ Instrument optical components or subassemblies. Any modifications to the Arcturus$^{XT™}$ Instrument may void the system warranty.

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

Laser safety scenarios

For safety reasons, the lasers are disabled when they are outside of the CapSure™ LCM Cap area. However, there may be applications for which the CapSure™ Cap is not required. In such instances, you can bypass the instrument laser safety settings.

In order to bypass the laser safety mechanism, the laser bypass key must be in place in the back of the instrument. You must then depress the Laser Bypass button, activating the UV laser. The laser bypass key should be in place when the instrument is received. If the bypass key is not present, contact customer support.

The table below details possible status scenarios, showing action combinations and the resulting system responses. For example, Line 4 indicates that when the laser bypass button has been pressed and a CapSure™ Cap is in place, but the laser bypass key has *not* been inserted, the laser status is "Standby". You must insert the key into position for the laser to be ready to fire.

Table 19 Laser Safety Actions.

State	Laser Bypass Button Pressed	Laser Bypass Key in Place	CapSure™ Cap in Place	Laser State	To State	Set Laser	Color	Pop-up	Cut Capture	Other	Tool Tip Messages
0				Stand by		Stand by	U. Red		Stop		tt = 'Laser disabled because cap is out of beam path and override is off.'
1				ON	0	Stand by	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	Stand by		Stand by	U. Yellow		Stop	Clean obj status	tt = 'Laser ready to fire. Cap placed in beam path.'
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	Stand-by	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	Stand-by		Stand-by	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			Stand-by	0	Stand-by	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.'

State	Laser Bypass Button Pressed	Laser Bypass Key in Place	CapSure ™ Cap in Place	Laser State	To State	Set Laser	Color	Pop-up	Cut Capture	Other	Tool Tip Messages
9	x			ON	0	Stand-by	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.'
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		Stand-by		Stand-by	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	Stand-by		Stand-by	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.'

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: **http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf**
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**

Documentation and support

Related documentation

Visit **thermofisher.com/lcm** for information and resources for the Arcturus$^{XT™}$ Laser Capture Microdissection (LCM) System and related products:

Document	Pub. No.	Description
Instruments		
Arcturus$^{XT™}$ Instrument Quick Reference	5003009	Provides a concise set of instructions for using the Arcturus$^{XT™}$ Instrument.
Arcturus$^{XT™}$ Instrument Large Format Slide Stage Insert Installation Guide	5000313	Provides instructions for installing the optional Large Format Slide Stage Insert. This document is shipped with the product.
Arcturus$^{XT™}$ Instrument Petri Dish Stage Insert Installation Guide	5000554	Provides instructions for installing the optional Petri Dish Stage Insert. This document is shpped with the product.
Arcturus$^{XT™}$ Instrument AutoScanXT Software Module User Manual	4458765	Provides instructions for using the AutoScanXT software. This document is embedded in the online help for this product.
Arcturus$^{XT™}$ Instrument Software Installation Instructions	4458766	Provides instructions for installing the Arcturus$^{XT™}$ software. This document accompanies each new release of the software.
Arcturus$^{XT™}$ Instrument Tip Sheet – Using the 100X Objective	4458768	Provides tips for microdissecting at 100X using the Arcturus$^{XT™}$ Instrument.
Arcturus$^{XT™}$ Instrument Tip Sheet – Fluorescence Imaging Optimization (Ti)	4458767	Provides tips for working with fluorescently labeled samples using the Arcturus$^{XT™}$ Instrument.
Arcturus$^{XT™}$ Instrument Tip Sheet – Phase Contrast and DIC	4458769	Provides tips for setting up and viewing phase contrast and DIC images.
Arcturus$^{XT™}$ Instrument Troubleshooting Guide	4458770	Provides tips and recommendations for handling problems encountered while using the Arcturus$^{XT™}$ Instrument.

Document	Pub. No.	Description
Reagents		
Arcturus™ CapSure™ LCM MicroCaps User Bulletin	MAN0014607	Provides instructions for DNA and RNA isolation using CapSure™ LCM MicroCaps.
Arcturus™ Paradise™ PLUS Reagent System Kit User Guide	12872-00	Provides instructions for using the Arcturus™ Paradise™ PLUS Reagent System Kit.
Arcturus™ PicoPure™ DNA Extraction Kit User Guide	12637-00	Provides instructions for using the Arcturus™ PicoPure™ DNA Extraction Kit.
Arcturus™ PicoPure™ RNA Isolation Kit User Guide	12682-00	Provides instructions for using the Arcturus™ PicoPure™ RNA Isolation Kit.
Arcturus™ RiboAmp™ HS PLUS Kit User Guide	12672-00	Provides instructions for using the Arcturus™ RiboAmp™ HS PLUS Kit.
Arcturus™ Turbo Labeling™ Kit User Guide	14827-00	Provides instructions for using the Arcturus™ Turbo Labeling Kit with Biotin, Cy®3, and Cy®5 dyes.
Arcturus™ Paradise™ Whole Transcript RT (WT-RT) Reagent System Kit User Guide	14360-00	Provides instructions for using the Arcturus™ Paradise™ PLUS WT-RT Kit.
Arcturus™ HistoGene™ Frozen Section Staining Kit User Guide	12294-00	Provides instructions for using the Arcturus™ HistoGene™ Frozen Section Staining Kit.
Arcturus™ HistoGene™ Immunofluorescence Staining Kit User Guide	12653-00	Provides instructions for using the Arcturus™ HistoGene™ Immunofluorescence Staining Kit.

Note: To open the documentation included on the Installation CD, use the Adobe Acrobat Reader software available at **www.adobe.com**.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support

• Product documentation, including:

  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation, part of Thermo Fisher Scientific Inc., and/or its affiliate(s) warrant their products as set forth in the Terms and Conditions of Sale and/or Services, found at **www.thermoscientific.com/su-tcs.**

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