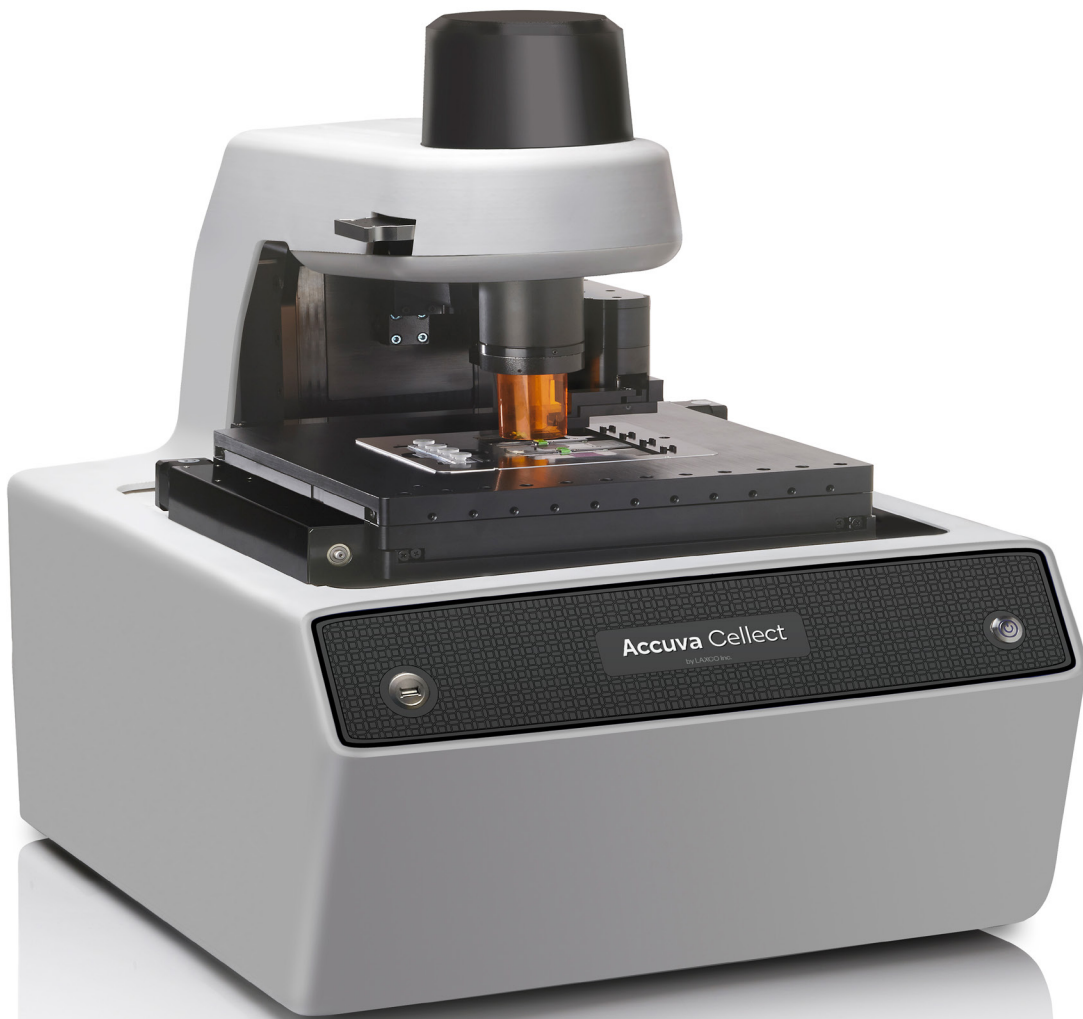




Accuva Collect



Version 1.0

User Manual

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Chapter 1 Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

Laser capture microdissection (LCM) is a method of procuring specific cell populations from specimen preparations using a low-power infrared (IR) laser and UV laser. IR laser 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 integrity of nucleic acids and proteins within the sample, and the cell morphology.

The Accuva Collect Laser Capture Microdissection System provides an automated approach to laser microdissection of individual cells or multicellular structures from slides containing tissue sections or cytological samples. The system consists of the Accuva Collect Instrument and AccuvaCollect software. For detailed specifications for the instrument, see Appendix D, “System specifications”.

To use the Accuva Collect 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.

Laser capture microdissection has been used for various research applications including tissue microdissection, forensics, live cells, neurons, live plant tissue, and single chromosomes.

Visit laxcoinc.com/lcm or contact Technical Support for information and technical resources for the Accuva Collect LCM System. See “Customer and technical support” on page 93.

Chapter 1 Product Information

Accuva Collect Laser Capture Microdissection System

The Accuva Collect Laser Capture Microdissection System is available with or without Fluorescence.

All systems contain the following components.

- Accuva Collect Instrument
- Power brick
- Touchscreen monitor
- Live cell stage insert
- Large slide stage insert
- Mouse
- Keyboard
- Trackball
- X/Y stage
- Stylus

COMPONENT	CAT. NO.
Accuva Collect PH	LCM-PH1A
Accuva Collect FL	LCM-FL1A

[1] In addition to the core Accuva Collect Laser Capture Microdissection System, the Accuva Collect FL model includes LED Epi-Fluorescence illumination.

Chapter 1 Product Information

Required materials not Supplied

Unless otherwise indicated, all materials are available through laxcoinc.com.

ITEM	SOURCE
CapSure™ Caps—Use one of the following caps	
CapSure™ LCM MicroCaps	A30154
CapSure™ HS LCM Caps	LCM0214
CapSure™ Macro LCM Caps	LCM0211
Slides and Petri dishes—Use one of the following items	
PEN membrane frame slides (50 slides)	LCM0521
PEN membrane glass slides (50 slides)	LCM0522
PEN membrane frame slides for live cell microdissections (25 slides)	LCM0531
Accuva Collect Live Cell Growth Chamber (6 dishes, sterile)	5000300
Accuva Collect Microdissection Petri Dish (6 dishes, sterile)	5000301

Chapter 1 Product Information

Recommended materials not provided

Unless otherwise indicated, all materials are available through laxcoinc.com.

Visit laxcoinc.com/lcm for a detailed list of Accuva reagent kits, including kits for staining, nucleic acid isolation, and RNA amplification and labeling.

ITEM	SOURCE
Reagent kits for processing frozen tissue samples	
HistoGene™ LCM Frozen Section Staining Kit	KIT0401
HistoGene™ LCM Immunofluorescence Staining Kit	KIT0420
PicoPure™ RNA Isolation Kit	KIT0204
PicoPure™ DNA Extraction Kit	KIT0103
RiboAmp™ PLUS Kit	KIT0521
RiboAmp™ HS PLUS Kit	KIT0521
Sample preparation kits for formalin-fixed, paraffin-embedded (FFPE) tissue samples	

Chapter 1 Product Information

ITEM	SOURCE
Paradise™ PLUS 2 Round Kit	KIT0312
Paradise™ PLUS Whole Transcript Reverse Transcription Kit (WT-RT)	KIT0315
Paradise™ PLUS RNA Extraction and Isolation Kit	KIT0312I

[1] 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.

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CAUTION! Do not place hands near the slide tracks of the modular stage insert.

Sample overview

For microdissection, you can use tissue sections that are prepared from either frozen or formalin- fixed, paraffin-embedded (FFPE) tissue. Freezing tissue helps to ensure the integrity of the biological molecules in the cells. Therefore, cells that are microdissected from frozen tissue sections provide material that is appropriate for many downstream applications. This is especially true for molecular biology applications that require intact RNA because the integrity of the RNA from formalin-fixed tissue may not be as high. Use the recommended protocols and reagents to use FFPE samples for molecular biology applications as well.

Power on the Accuva Collect Laser Capture Microdissection System

1. Flip the back power switch to the on position.
The back power switch supplies power to the whole system.
2. On the front of the instrument, press the power button to power on the computer.
The button turns blue.
3. If needed, power on the monitor.
4. Sign in to Windows™ using your Windows™ sign-in information.
The default user name and password are the same.

Chapter 2 Load and inspect samples

5. Double-click the AccuvaCollect software icon  to start the software.

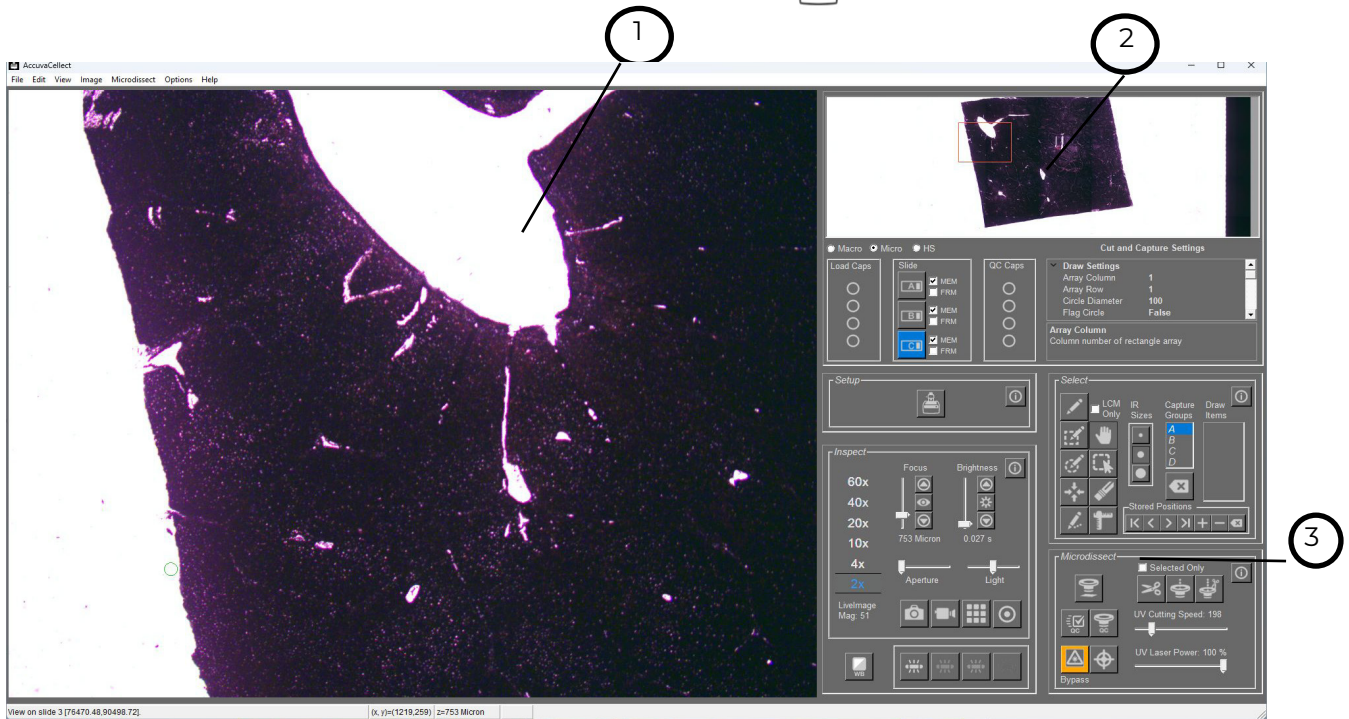


Figure 1
The Accuva Collect Instrument primary screen

① Main Image pane

③ Tool panel pane

② Slide overview image pane

Chapter 2 Load and inspect samples

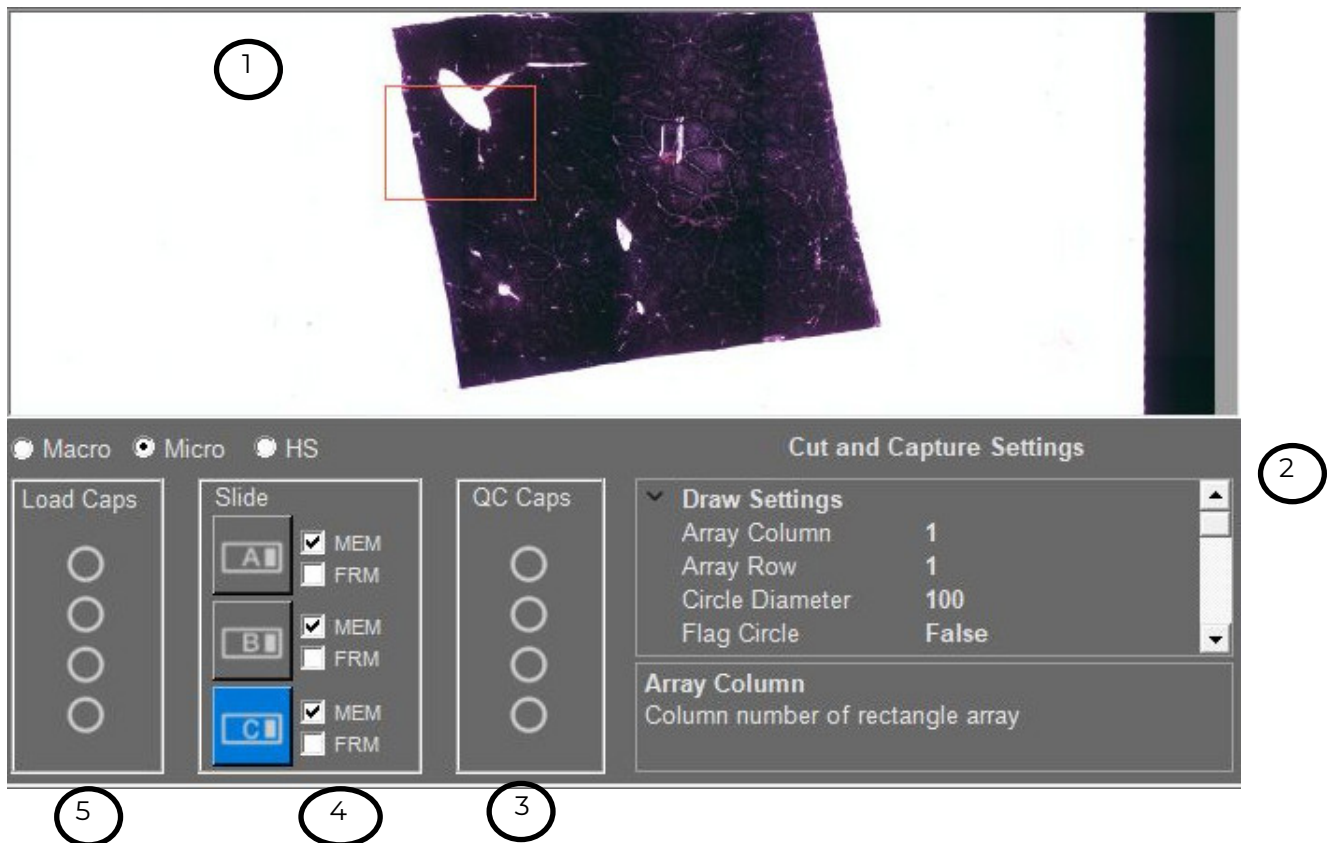


Figure 2
Slide overview image pane

- ① Slide overview image
- ② Cut and Capture Settings pane
- ③ QC Caps pane
- ④ Slide pane
- ⑤ Caps pane

Chapter 2 Load and Inspect Samples

Power on the Accuva Collect Laser Capture Microdissection System

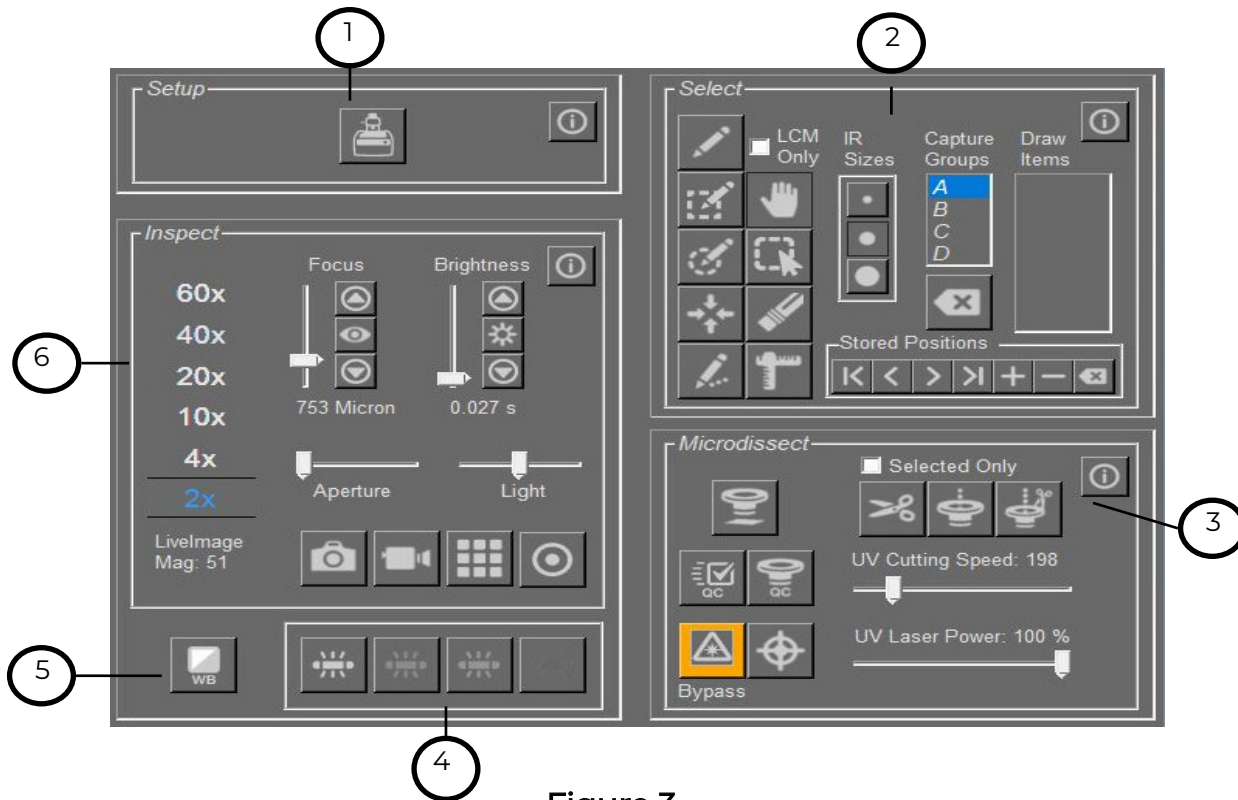



Figure 3
Tool Panel Pane

- ① Setup pane
- ② Select tool pane
- ③ Microdissect tool pane
- ④ Fluorescence tool pane
- ⑤ White balance
- ⑥ Inspect tool pane

Chapter 2 Load and inspect samples

Loading materials onto the Accuva Collect Instrument

Prepare the modular stage insert

1. In the **Setup** tool pane, click . The work surface moves forward and to the right.
2. If needed, remove any slides and CapSure™ Caps that are left on the modular stage insert.
3. For each slide, press the slide lock slider towards the back of the instrument, insert the slide, then release the slide lock slider.
4. Place a CapSure™ cassette with caps into the CapSure™ cassette slot. For every cap in the CapSure™ cassette, confirm that the corresponding cap position in the QC station is empty.

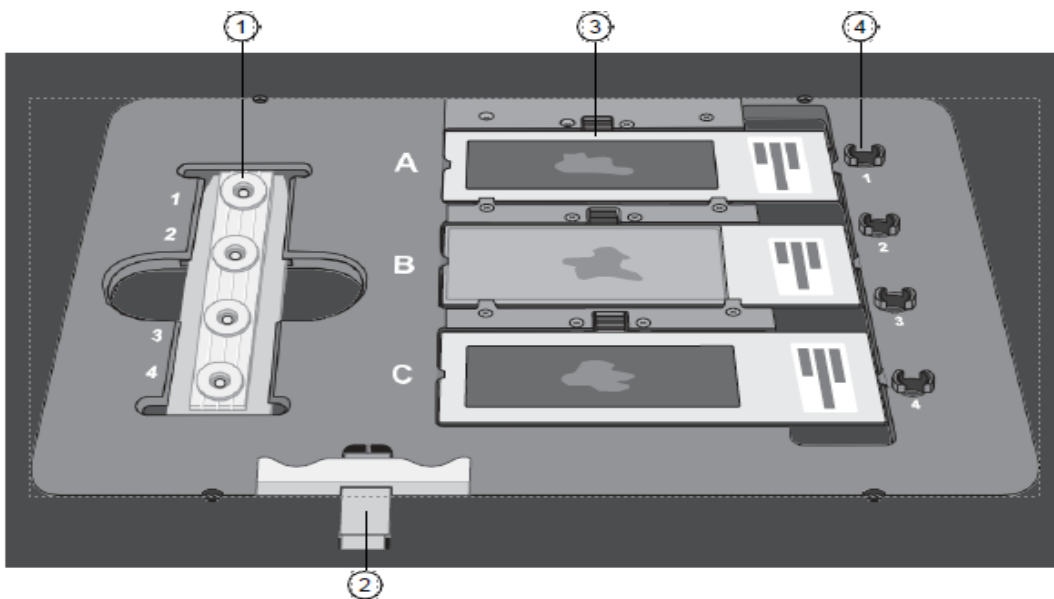


Figure 4
Modular stage insert (stage)

① CapSure™ cassette slot

③ Slide slots

② Slide lock slider

④ QC station

Chapter 2 Load and inspect samples

Loading materials onto the Accuva Collect Instrument

(Optional) Select load options for the slides

In the **Setup** tools pane, click (Information) to open the **Load Options** dialog.

1. For each slide that you are loading, select that slide by checking the **Load Slide** box. If you are loading all 3 slides, select **Load All Slides**.

For example, if you are loading a slide in position A, select the **Load Slide A** box. The positions of the slides are found in Figure 4.

2. For each slide, select the type of slide: **Glass**, **Membrane**, or **Framed**.
3. (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.

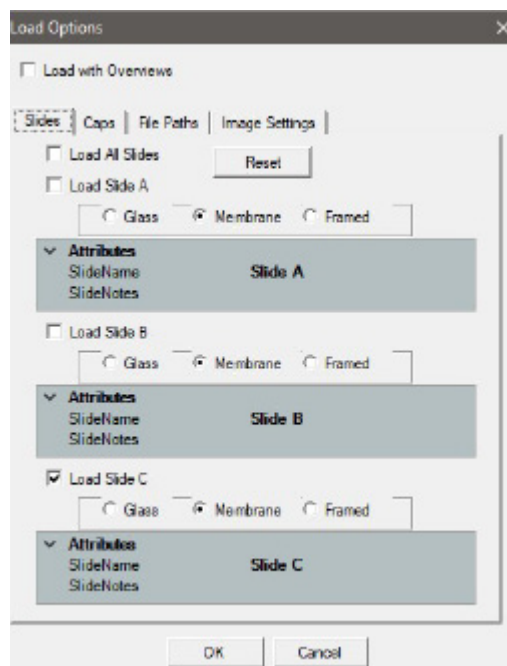


Figure 5
Load options dialog

5. (Optional) Enter any comments for each slide in the Figure 5 Load Options dialog **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 under the slide overview image.

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

Chapter 2 Load and inspect samples

Select load options for CapSure™ caps

1. In the **Setup** tools pane, select (information) to open the **Load Options** dialog box. then select the Caps tab.
2. Select the checkbox for each CapSure™ Cap that is loaded, or click **Load All Caps** if all 4 caps have been loaded. The cap positions are shown in Figure 4.
3. Select **Macro, Micro, or HS** to identify the type of caps you are loading.

Note: If you are using CapSure™ Macro LCM Caps, the maximum boundary of the capture is 4300 μM .

4. (Optional) To deselect all boxes, click **Reset**, then repeat step 2 and step 3.

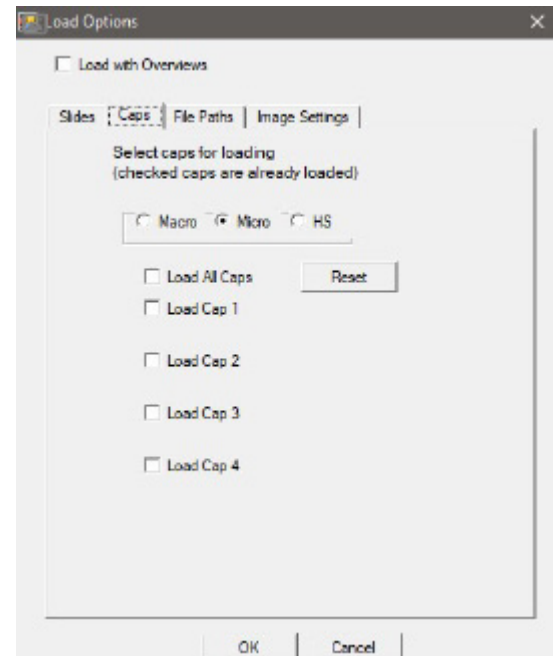



Figure 6
Load options dialog

Chapter 2 Load and inspect samples

1. In the Setup tool pane, select  (information) to open the Load Options dialog box, then click the Image Settings tab.
2. In the AutoDocument Filename Settings section, enter the following information.

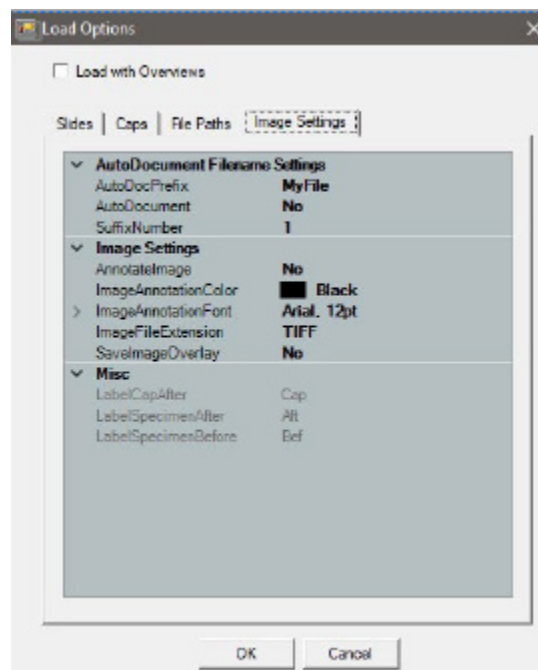


Figure 7
Load Options dialog

FIELD	ACTION
AutoDocPrefix	Enter the prefix that you want to use at the beginning of the name of each image file saved.
AutoDocument	(Optional) To automatically save images, select Yes from the dropdown.
SuffixNumber	Enter the number for the first image. Note: This number is the last part of the file name for all images. It increases by one each time microdissection occurs.

Chapter 2 Load and Inspect Samples

3. In the **Image Settings** section, enter the following information.

FIELD	ACTION
AnnotateImage	<ul style="list-style-type: none">• Select Yes to include the text from the SlideName and SlideNotes fields and the objective that was used in the upper-left corner of all image files.• Enter the color for the annotation in the ImageAnnotationColor field.• Enter the font for the saved annotation in the ImageAnnotationFont field. Click the plus sign to view the formatting options.
ImageFileExtension	Choose the file format for saved images. You can choose either JPEG (.jpg) or TIFF (.tif).
SaveImageOverlay	To save the drawing items when you save images, enter Yes. Otherwise, enter No.

Chapter 2 Load and Inspect Samples

4. In the **Misc** section, enter the following information.

FIELD	ACTION
LabelCapAfter	Enter the text that you want to add after the AutoDocPrefix and the SuffixNumber of each image of the CapSure™ Cap.
LabelSpecimenBefore	Enter the text that you want to add after the AutoDocPrefix and the SuffixNumber for each image of the slide before microdissection.
LabelSpecimenAfter	Enter the text that you want to add after the AutoDocPrefix and the SuffixNumber for each image of the slide after microdissection.

For example, the second image of a slide before LCM, with the AutoDocPrefix = "LeafStudy", the **LabelSpecimenBefore** = "Before", and the **SuffixNumber** "10", the file is named LeafStudyBefore10.

Implement your selections

When you have finished making your choices, click **OK**. The instrument then performs the following actions.

- Moves the modular stage insert 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** in the Load Options dialog box (see Figure 5), the instrument 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 is blank. To acquire and display the slide overview, right-click in the slide overview area, then click **Reacquire Overview Image**.

Chapter 2 Load and Inspect Samples

Inspect Slides

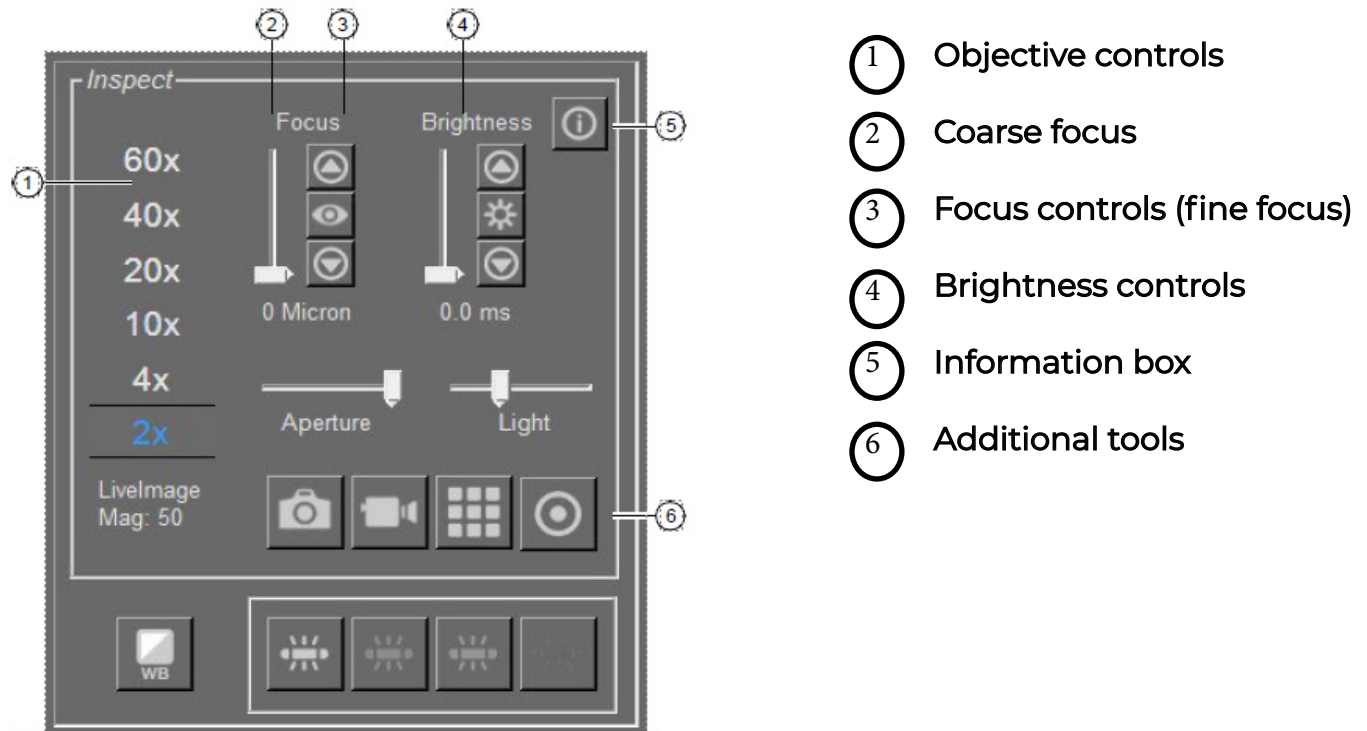



Figure 8
The Inspect tools pane

View slides

1. Move the stage to display an area of interest in the main image in one of three ways.
 - In the **Select** tools pane, click  (**Move Stage**) (see Figure 14). Press the stylus on the main image window, then drag the stylus to move the stage. You can also use the trackball to move the image or 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** (A, B, or C) button for the slide of interest. The stage moves the selected slide over the objective and the slide overview updates to show the new slide.
3. (Optional) Change the objective by tapping or clicking the label corresponding to the selected objective. The selected objective is blue (see Figure 8).

Chapter 2 Load and Inspect Samples

View the slide properties

You can view the slide type in side pane of the slide overview image pane (see Figure 2).

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 deselected.

You can view properties of a slide (its name and any notes) in the information area under the slide overview image (see Figure 9).

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

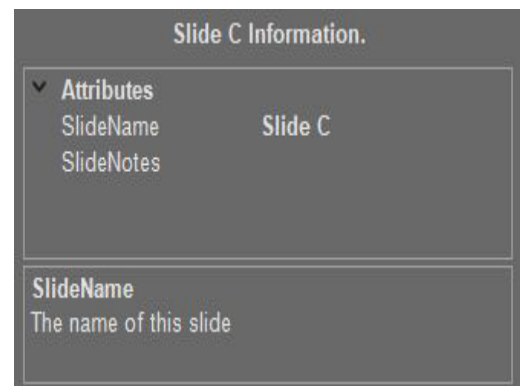





Figure 9
Slide information area


(Optional) Adjust the brightness

Control the brightness using the software buttons in the **Inspect** tools pane (see Figure 8).

- To increase the brightness, click or tap. 
- To decrease the brightness, click or tap. 

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



- To adjust the brightness automatically, click  (**Autobrightness**).
- To set a different level of brightness for the Autobrightness button, see “Change the Autobrightness setting and save as the default” on page 23.

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 that is needed for the main image window to refresh after you move the stage can be slow. If so, in the **Inspect Options**  **Illumination** tab, adjust the Intensity and Camera Gain to set the brightness value lower. (See Figure 12.)

Chapter 2 Load and Inspect Samples

Focus the image

Control the image focus using the software buttons in the **Inspect** tools pane (see Figure 8).

- To move the objective closer to the slide, click or tap. .
- To move the objective farther from the slide, click or tap. .

Note: Using the stylus, if you hold the arrow buttons, the software adjusts the focus in larger steps

To adjust the focus automatically, click  (**Autofocus**).

Chapter 2 Load and Inspect Samples

Set the automatic focus

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

1. In the Inspect tools pane, click (Information) to open the Inspect Options dialog box, then click the Focus tab (see Figure 8).
2. Select Tracking Autofocus On, then click OK.

Display a different slide

1. In slide overview image pane, in the Slides section, click or tap the slide of interest (see Figure 2). The stage moves 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, then select Reacquire Overview Image.

Locate the lasers

Locate or relocate the UV cutting laser and IR capture laser at the beginning of each session. In addition, relocate the lasers during a session if you perform any of the following actions.

- 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 Capture laser is not firing at the desired location.

Place cap on slide

Click (Place Cap) to place a CapSure™ Cap on the slide. The instrument places a cap at the center of the field of view designated 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.

Chapter 2 Load and inspect samples

Locate the UV cutting laser

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

1. In the Microdissection tools pane, click (Information) to open the Microdissect Options dialogbox.
2. Click the **UV Locate** tab (see Figure 10), then follow the steps in the dialog box.
3. Click **Locate UV**. The UV cutting laser is fired.
4. In the main image window, place the cursor in the center of the UV laser spot, then click the spot.
5. Click **OK** (top button).
6. If the laser spot is not visible in the main imagewindow, relocate the UV cutting laser.
 - a. Select UV Current On.
 - b. When the laser spot becomes visible, deselect UVCurrent On.
 - c. Repeat step 3 through step 5.
7. Click **OK** (bottom button).
A green circle appears on the live image at the location of the UV cutting laser.

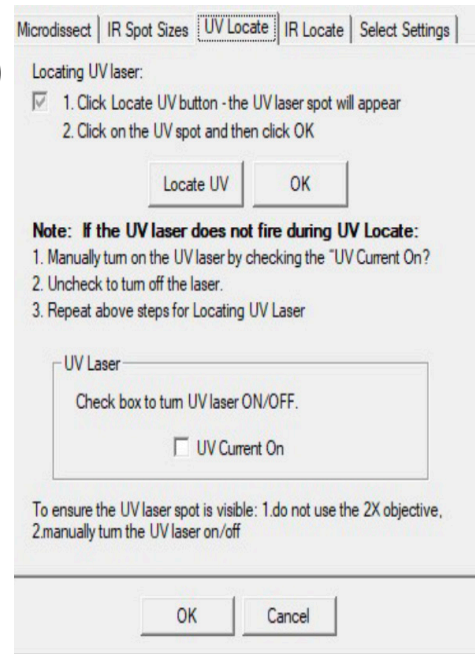


Figure 10
UV Locate tab

Locate the IR Capture laser

1. In the **Microdissection** tools pane, click (Information) to open the **Microdissect Options** dialogbox



Figure 11
IR locate tab

Chapter 2 Load and Inspect Samples

2. Click the **IR Locate** tab.
3. Locate the IR Capture laser.

METHOD	DESCRIPTION
Locate manually	<ol style="list-style-type: none"> 1. Click Locate IR. The live image darkens and the IR laser guide light is visible. If the IR laser guide light is not visible, enter a larger value in the Show Current (Bias) field until the IR laser guide light becomes visible. A value ≥ 3 is required. If needed, increase the Brightness settings that are located in the Inspect tools pane until the IR laser guide light is visible. 2. Center the cursor on the IR laser guide light, then left-click to save the location. 3. Click OK next to the Locate IR button.
Locate automatically	<ol style="list-style-type: none"> 1. Click Auto Locate IR. The AccuvaCollect software powers on the IR capture laser at low power and tries to find the laser. If the laser is successfully found, the system draws a blue plus sign on the main image where the laser is located. 2. To confirm the IR laser location, in the microdissect tools pane, click IR Capture Test Spot and perform a test fire. 3. Look at the main image to see if the laser spot is in the circle. <ul style="list-style-type: none"> • If the blue plus sign is in the LCM spot, click OK at the bottom of the dialog to close the dialog box and save the changes. • If the blue plus sign does not fall in the center of the LCM spot, follow the steps to manually find the IR Capture laser.
Locate from the primary screen	<ol style="list-style-type: none"> 1. Place the blue plus sign in a non-tissue area. 2. In the Microdissect tool pane, click IR Capture Test Spot to fire the IR laser. 3. Place the cursor at the center of the wetted IR spot, right-click, then select IR Laser Capture Laser Here. The blue plus sign relocates itself to the center of the LCM spot.

Chapter 2 Load and Inspect Samples

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

Select imaging parameters

Proceed to one of the following sections.

- For bright field imaging, proceed to “Working in bright field mode”
- For fluorescence imaging, proceed to “View fluorescently labeled samples”

Working in bright field mode

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

Adjust the bright field lamp

1. In the Inspect tools pane, click (Information) (see Figure 8) to open the Inspect Options dialog box.
2. Click the Illumination tab.
3. Optimize the image using a combination of the Intensity and Camera Gain controls, and the Brightness controls in the Inspect tools pane.

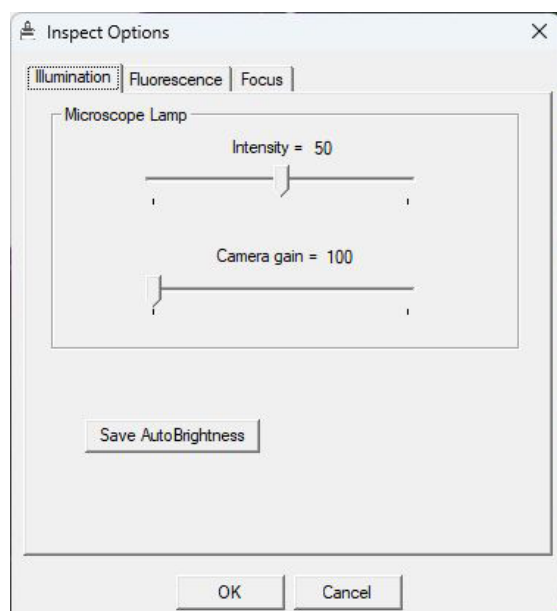


Figure 12
The illumination tab

Chapter 2 Load and Inspect Samples

CONTROLLER	DESCRIPTION
Intensity	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..
Camera Gain	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
Brightness	If needed, in the Inspect pane (see Figure 8), adjust the Brightness controls. A recommended brightness setting at 2X is 2.0 ms to 8.0 ms.


4. Adjust the white balance within the camera settings, see “Solving problems with image quality”.
5. Tap or click OK to close the dialog box and save your changes.

Change the Autobrightness setting and save as the default

The AccuvaCollect software automatically sets the brightness when you tap or click (Autobrightness) on the Inspect tools pane (see Figure 8). If the default autobrightness is not appropriate for your sample, you can adjust the value manually and save it as the default.


1. Adjust the brightness in the main image window as desired.
2. Click (Information) (see Figure 8) to open the Inspect Options dialog box.

Chapter 2 Load and Inspect Samples

3. Click the **Illumination** tab (see Figure 12).
4. Click **Save AutoBrightness**, then click **OK** to close the dialog box and save your changes. The next time you click  (Autobrightness) button in the Inspect tools pane, the illumination is set to this value.

Returning to the original setting

1. Click (Information) (see Figure 8) 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  (Autobrightness) button in the Inspect tools pane, the illumination is set to the default value.

View fluorescently labeled samples



1. Click a **Fluorescence** icon in the fluorescence tool pane of the tool panel pane (see Figure 3.)

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



2. Open the Inspect Options dialog box. The Fluorescence tab is selected automatically

Chapter 2 Load and Inspect Samples

3. Locate the fluorescence signal.
 - a. Toggle the microscope lamp off.
 - b. Set the **Fluorescence Lamp** Intensity to 100% (see Figure 13).
 - c. Set the Camera Gain to the maximum setting (see Figure 13).
 - d. Adjust the brightness setting (exposure time) in the Inspect tool pane using the brightness controls ( and ) until a fluorescence signal is seen (see Figure 8).
 - e. In the Focus tab, focus the image.

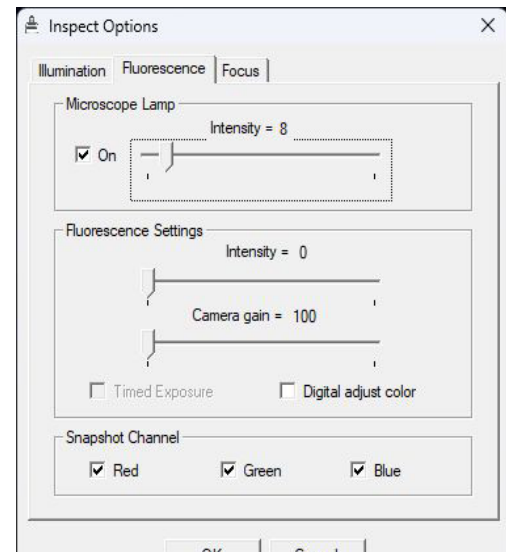


Figure 13
Fluorescence tab

4. After the sample has been located and focused, to optimize the image, perform the following adjustments.
 - a. Adjust the Camera Gain (see Figure 13) to the lowest possible value that still allows sufficient signal. (Higher values increase image pixelation.)
 - b. Set the Brightness (exposure time) (see Figure 8) to 1 second 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 13)

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 13) for optimal fluorescence image.
2. Adjust the Microscope Lamp Intensity (see Figure 13). A good starting point for the Microscope Lamp Intensity setting is 30. Adjust from this point to get enough light.

Chapter 2 Load and inspect samples

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 photobleaching of 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 13) only with the Timed Exposure feature. Adjusting this setting outside of Timed Exposure does not result in a change.

Perform fluorescence timed exposure

When working with the Fluorescence lamp, samples can become photobleached 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 AccuvaCollect software.

1. To work with a static image instead of the main image, click Timed Exposure in the lower-left corner of the Inspect Options dialog.

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

2. Click **OK** to close the dialog box, then save your changes. The Accuvas Collect Instrument uses the lamp and camera settings you have set for the fluorescence mode.
3. Click (Camera) to capture a static image for tissue selection.

Chapter 2 Load and Inspect Samples

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) again to acquire another snapshot of the new area.

(Optional) Capture and save images

To save images, proceed to one of the following sections.

- Tiled Images—See “Capture tiled images” .
- Static Images—See “Capture static images” .

Capture 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.

1. Find the area for the tiled image.
2. Select the objective for the desired magnification.
3. Adjust the image using the tools in the Inspect tools pane.
4. Click in the Inspect tools pane (see Figure 8).
5. In the slide overview image, tap or click, 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, then 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.

Chapter 2 Load and Inspect Samples

6. 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, then repeat step 5.
7. To close the tiled image, tap the close box in the upper-right corner of the static image window. The stitched image is saved in the folder that is specified in the File Paths tab of the Load Options dialog box. It is saved in the format that is specified in the Image Setting tab of the Load Options dialog box.

Proceed to “Mark cells for microdissection” .

Note: The tiled image is used similarly to the main image to identify and mark the tissue for microdissection.

Capture 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.

Note: When taking a static image using fluorescence, a total of 4 images are generated: one image in each channel (3 total) and one overlay 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 if needed.

Chapter 2 Load and Inspect Samples

3. Click in the Inspect tool pane.
The static image appears in a new window and is saved in the folder specified in the File Paths tab of the Load Options dialog box. It is saved in the format that is specified in the Image Setting tab of the Load Options dialog box.
4. When you are done viewing and/or working with the image, click or tap close (upper-right corner of the static image window).

Open an image

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

Chapter 3 Select cells for microdissection

- Mark cells for microdissection .
- Edit the area for microdissection .
- Measure distances and objects
- Working with overlays
- Working with stored positions

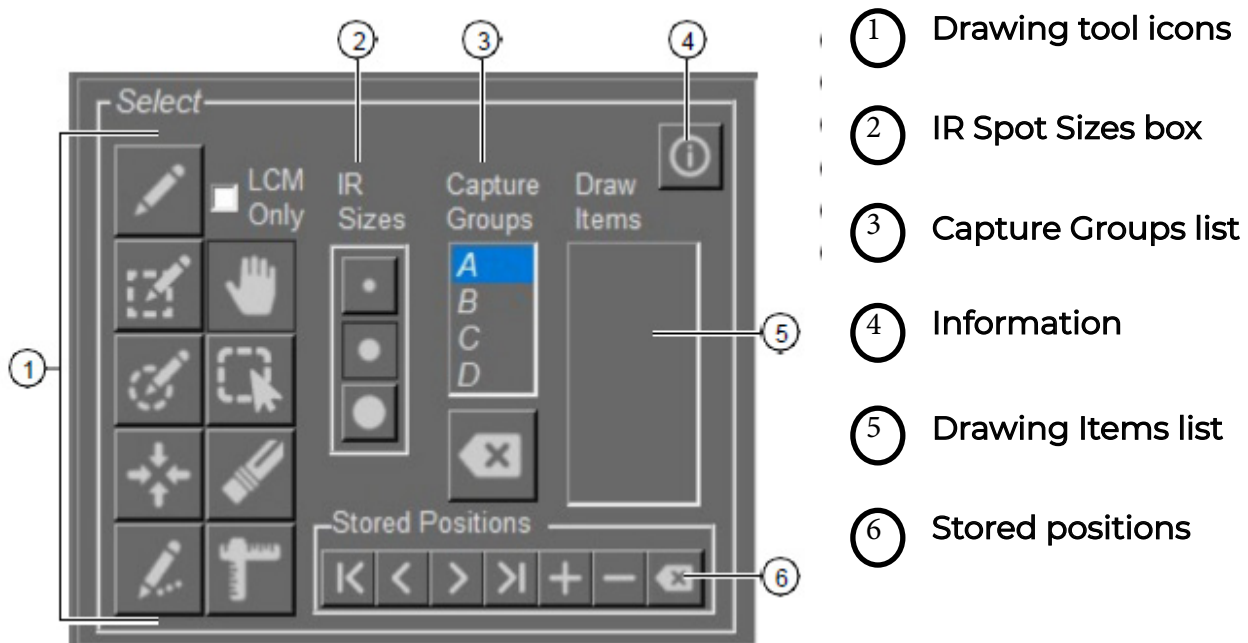


Figure 14
Select tools pane

Mark cells for microdissection

Note: We recommend that you mark your cells and perform microdissection using the same objective. This ensures that the UV cutting laser accurately follows the dissection marks.

Chapter 3 Select cells for microdissection

The following table describes the placement of IR capture spots, when you are using the Freehand and Defined Circle Area drawing tools.

SLIDE TYPE	DESCRIPTION
Glass	Drawing items using the Freehand and Defined Circle Area tools are filled in with IR capture spots. The UV laser is not used.
Glass or Frame membrane	Drawing items using the Freehand and Defined Circle Area tools mark the perimeter to define the UV cut line. IR capture spots are placed automatically, serving only to attach the sample to the capture.

1. Select the relative size of the IR capture spots. In the Select tools pane, click the desired size (small, medium, or large) in the IR Spot Sizes box (see Figure 14).

IMPORTANT! The IR capture spot size is one of the factors that determines where the IR capture spots are placed in a drawing. The IR capture spots identify where the IR capture laser is fired.

To set IR capture spot size, see “Set the IR capture spot size” on page 59.






2. Select the capture group.
The user can set and edit 4 capture groups to manage the capture items.
3. Using the drawing tools, draw the capture area. The tool remains active until you tap or click another tool.
 - a. To activate a drawing tool, tap or click the tool (see Table 1). The tool remains active until you tap or click another tool.
 - b. To turn off a drawing tool, tap or click (Move Stage, Figure 14).

Chapter 3 Select cells for microdissection

When check the LCM only box, draw the items filled in with IR Capture spots. The UV laser is not used.

When uncheck the LCM Only box, drawing items to define the UV cut line. IR capture spots are placed automatically, serving only to attach the sample to the capture

Table 1 Drawing tools

ICON	TOOL	PURPOSE
	Freehand drawing	Drawing items using the Freehand.
	Define Rectangle Area	Drawing items using the rectangle.
	Define Circle Area	Used to select the circle area for each capture. Note: The software draws IR capture spots inside the circle. To set a fixed size for the circle area, see "Set drawing tool options" on page 65.
	Single IR Spot	Used to capture individual cells without using the UV laser.
	IR Spot Line	Used to draw a series of IR spots along a tissue feature such as tumor–stroma border. Note: The IR Spot Line Tool can be useful if using plain glass slides or when trying to capture different cell types that are right next to each other.

For each item that you draw, a number appears in the **Drawing Items** list (see Figure 14).

4. (Optional) To designate cells and tissue for a second capture group, tap or click a different capture group in the Capture Groups list (Figure 14), then repeat step 3. For example, drawing items that belong to capture group B are not collected on the same CapSure™ Cap as capture groups A, C, and D.
5. (Optional) To mark more cells for microdissection, move the stage to display other area in the main image window.

Chapter 3 Select cells for microdissection

6. (Optional) To remove any marks made by the drawing tools, select (Eraser tool).

Note: You can also remove marks made by the drawing tools using tools from the drawing items list. See “Delete a drawing item” on page 37.

7. (Optional) To add more IR capture spots to any drawing item, click the Single IR Spot tool, then tap or click inside any of the drawing items.

Note: IR spot sizes cannot exceed the draw item.

Edit the area for microdissection

All edits are applied only to the active Capture Group.

Move a drawing item

1. Click (Select Object(s)) tool (see Figure 14).
2. In the main image window, click a single drawing item to select it, or drag the stylus to select more than one drawing item.
3. Hold down the cursor in 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), then release it.
The drawing item(s) move to the new location

Move a drawing item to a different capture group

1. Click (Select Object(s)) tool (see Figure 14).
2. In the main image window, click a single drawing item to select it, or drag the stylus to select more than one drawing item.
3. Right-click in the selected object(s), select Move Selected Object(s) to Group, then select the desired capture group.
The selected drawing items move to the new capture group.

Chapter 3 Select cells for microdissection

Delete a drawing item

- 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.
- In the live image, tap the item, right-click and select Delete Object(s) from the pop-up list.
- To delete multiple drawing items.
- Select a group of drawing items by tapping and dragging with the Select Object(s) tool, then tap in one of the selected objects. Right-click the selected object, then select Delete Object(s) from the pop-up list.
- Ctrl+click each object to be deleted, then right-click in a selected object and select Delete Objects(s) from the pop-up list.
- To delete all drawing items from the active capture group. Press Ctrl+A on the keyboard, then press Ctrl+X on the keyboard.

Delete IR capture spots from a drawing item

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

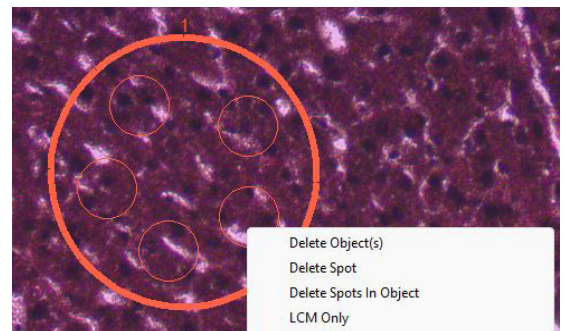


Figure 15
Delete IR capture spots.

The specified IR Capture Spots are deleted.

Chapter 3 Select cells for microdissection

Change the microdissection properties of a drawing

- To change from LCM (IR Capture) only to UV Cut and IR Capture.
 - a. Right-click on the drawing item, then select LCM Only in the pop-up window. The check mark disappears next to LCM Only. The LCM spots remain in the object, but the perimeter line changes to the UVCutColor (designated in the Capture Group settings window). The IR and UV lasers will both fire for this object.
- To change from UV Cut and IR Capture to LCM Only.
 - a. Right-click on the drawing item, then select LCM Only in the pop-up window. A check mark appears next to LCM Only. The object is filled in with LCM spots and the outline of the object changes to the IR SpotColor (designated in the Capture Group settings window). Only the IR laser will fire for this object.
 - b. (If needed) Reduce the number of LCM spots in the object. Right-click in the object, select Delete Spots in Object, then manually place as many LCM spots as desired to attach the area to the CapSure™ Cap.

View drawing item information

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.

1. In the Select tools pane, click (Information) to open the Select Options dialog.
2. Click the Drawing Items tab to view the properties of interest. There is a row for each drawing item in the selected capture group. You can view the area, status, and other information of the item.

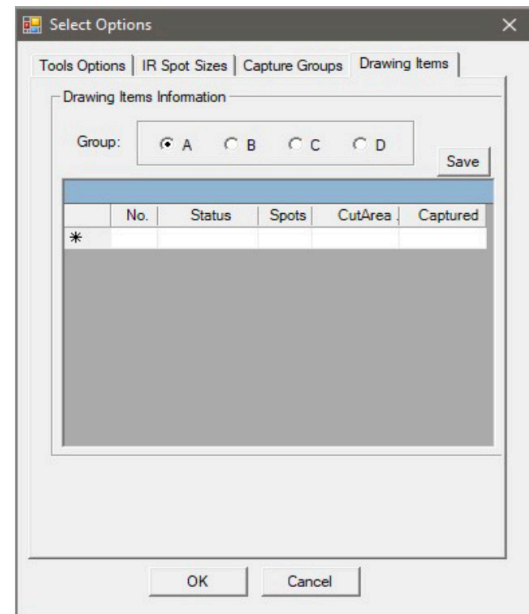


Figure 16
Drawing Item Information


Chapter 3 Select cells for microdissection

View drawing item information (continued)

3. To view information for another capture group, select the radio button for that group.
4. To export this data chart, click Save, then save the CSV file to the appropriate location.
5. Click **OK**.
This information is also available in the information box that is found under the slide overview. Click a drawing item in the Select tool pane to display the information.

Measure distances and objects

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

1. Click  (**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.

Chapter 3 Select cells for microdissection

3. The software draws a line and displays a label showing the length of the line (see Figure 17)

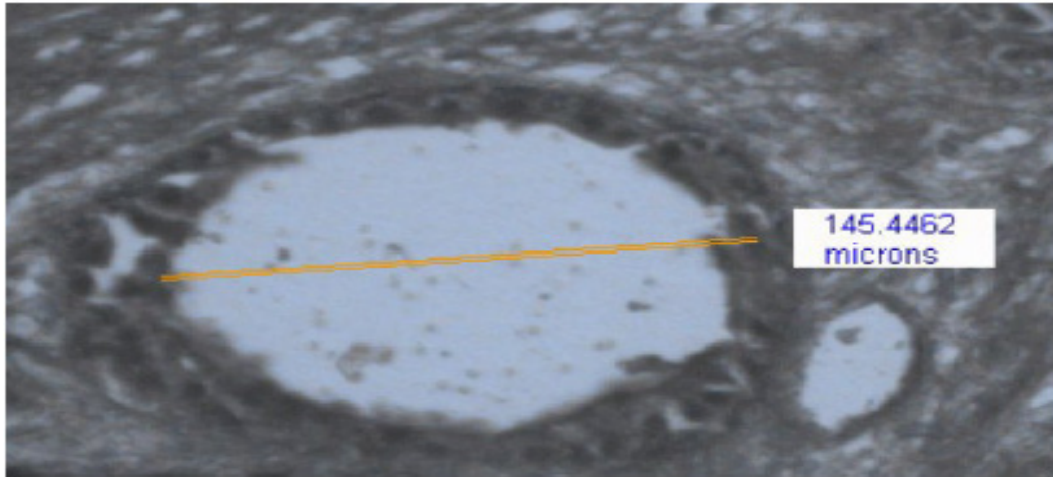


Figure 17
Ruler measurement

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

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”.

Save an overlay

1. Load your slides, then identify the tissue that you want to capture with the drawing tools.
2. Click **File** ► **Save** ► **Markup**.

Chapter 3 Select cells for microdissection

3. Enter a file name in the dialog box, then click **Save**.

Note: Do not save it to any subfolder in the programs data folder such as c:\ProgramData

Use a saved overlay

1. Click **File** ► **Open** ► **Markup**.
2. Navigate to the .bin file containing the overlay, then click **Open**.
The drawing items appear in the main image window in the original locations. Any drawing items already in the main image window remain there. You can microdissect at this stage or you can work with the drawing items if needed.

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.

Store positions on the slide

- To add a position.
 - a. Move the stage to a desired location on the slide.
 - b. Click or tap on the + button to add that location as a stored position.

The stored position is given a number, starting with 1 and increasing with each next stored position.


- To review stored positions, click the forward and back arrows to scroll through the stored locations. The stage moves to the location of the stored position and centers it in the live image.
- To delete the displayed stored position, click – (minus symbol).
- To delete all stored positions, click 



Figure 18
Stored Positions pane

Chapter 4 Microdissect and analyze cells and tissue

- Capture cells by microdissection
- Analyze microdissected samples

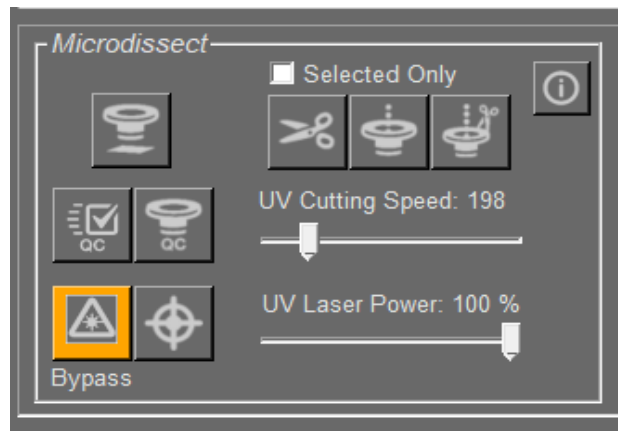



Figure 19
Microdissect tools pane

1. Laser Bypass button—See “Inspect the microdissected material” .
2. Quick QC button—See “(Optional) Check cut tissue”
3. Place Cap button—See “Place cap on slide”
4. QC button—See “Inspect the microdissected material” .
5. Selected Only checkbox—Enables actions to be performed on selected areas for microdissection.
6. Cut and capture button—See “Microdissect the sample” .
7. Information button—See “Select preferences for cut and capture”.
8. UV Cutting Speed slide bar —See “Set the cut and capture properties”
9. UV Laser Power slidebar —See “Set the cut and capture properties” .
10. IR Capture button—See “Microdissect the sample” .
11. UV Cut button—See “Microdissect the sample” .
12. IR spot button—Used to test fire the IR laser. Contact support for details.

Capture cells by microdissection



Place cap on slide

Click  (Place Cap) to place a CapSure™ Cap on the slide.

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

Chapter 4 Microdissect and analyze cells and tissue

(Optional) Review cells selected for microdissection

1. 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 a number in the **Drawing Items** list.
The stage moves to display the drawing item at the center of the main image.
2. Add more IR capture spots.
 - a. In the **Select** tools pane, click the **IR Spot Sizes** control to select a different spot size.
 - b. Click  (IR Spot) tool.
 - c. Click inside any of the items in the main image.
A spot is drawn where you clicked.
 - d. Click  (Move Stage) to deactivate the **IR Spot Sizes** tool.

Test fire the lasers

IMPORTANT! Test firing of the lasers should be performed outside of the tissue.



1. Locate the lasers.
 - a. See “Locate the UV cutting laser” on page 24.
 - b. See “Locate the IR Capture laser” on page 25.
2. Move the stage so the CapSure™ Cap is on the slide on an area away from the tissue.
3. Click **IR Laser Test Fire** to test the capture laser.
4. Inspect the laser spot.
You should see a dark ring with a clear center, as shown in Figure 24.
5. If you do not see a dark ring with a clear center, as shown in Figure 24, open the MicrodissectOptions dialog, then adjust the Power and duration slider in the **IR Spot Sizes** tab.

Chapter 4 Microdissect and analyze cells and tissue

Microdissect the sample

Select one of the following options.

Table 2

OPTION	DESCRIPTION
Capture cells in one step	<p>Click the Cut and capture button (see figure 19).</p> <p>Note:</p> <ul style="list-style-type: none"> • The order of the two procedures, cut and capture, depends on a setting in the Microdissect Options dialog. The default order is IR capture followed by UV cutting. • To change the order of the cut and capture procedures, see “Select preferences for cut and capture” on page 66. • 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.
Capture cells using the capture and cutting tools separately	<ol style="list-style-type: none"> 1. Click  (UV Cut) to perform all cuts. 2. Click  (IR Capture) to perform all captures.

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

Chapter 4 Microdissect and analyze cells and tissue


The instrument fires the IR Capture laser at the position of each IR capture spot, to fuse the cells to the cap. After the IR capture laser completes firing, the instrument automatically continues cutting with the UV cutting laser. Depending on the type of slide you have loaded, the details of the cutting vary.

- Glass slides—The instrument fires only the IR laser, unless you have deselected **LCM Only** for this drawing item.
- 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 does not cut. Tabs keep the tissue from curling up from the surface of the slide before the IR Capture laser can fuse it to the cap.
- You can set the number of tabs, their size, and spacing (see “Set the cut and capture properties” on page 67).

Analyze microdissected samples

(Optional) Check cut tissue



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

1. Click  in the **Microdissect** tool pane. The cap is moved up and to the right. The stage does not move. The cap is shown in the slide overview image pane (Figure 2).
2. View the tissue. The hole in the tissue should be visible.

Chapter 4 Microdissect and analyze cells and tissue

Inspect the microdissected material

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

1. Click  in the **Microdissect** tool pane. Alternatively, right-click on the slide overview, then 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 slide overview image pane (Figure 2). 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 theyear/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** box.
2. Inspect the tissue on the cap.
3. (Optional) To capture a static image, click  in the Inspect tools pane.
4. To inspect the slide, click the slide overview image at the position you want to see in the main image pane. Alternatively, click an item number in the **Drawing Items** list in the **Select** tools pane (Figure 14).

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

The stage moves to display the item in the main image pane.

5. If any drawing item was not captured during microdissection, repeat the microdissection.
 - a. Move the cap from the QC station back to the slide.
 - b. Place the cursor on the cap in the **QC Caps** box (Figure 2).
 - c. Right-click, then select **Replace Cap on Slide** from the pop-up menu.

Chapter 4 Microdissect and analyze cells and tissue

- d. If no new drawing items have been placed and the CapSure™ Cap has not been moved from its original position, repeat the two-step microdissection procedure (“Microdissect the sample” on page 46.
- e. If the microdissection was not complete or if new drawing items were placed after performing the microdissection, highlight the desired drawing items to be repeated, then 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.

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.

View a CapSure™ cap in the QC area

1. In the **QC Caps** area, right-click the Cap to be viewed (see Figure 20).
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. To inspect the material on the cap with a higher objective, move the cap to a clean place on the slide, then follow standard protocols for inspection.

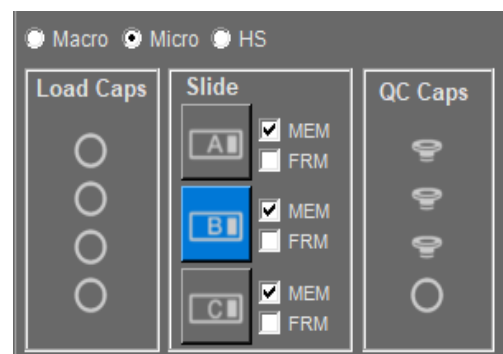


Figure 20
Cap and slide handling area

Chapter 4 Microdissect and analyze cells and tissue

View and update cap properties

You can view the CapSure™ Cap type in the cap and slide handling area. 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.

You can also add CapSure™ Caps to the Load Caps area by clicking a cap icon in the Load Caps area (see Figure 20).

This shortcut lets you load caps without opening the Load Options dialog box. However, the corresponding QC Caps position must be empty to use this shortcut.

1. Remove any caps whose positions have been cleared in the QC caps area.

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

Chapter 4 Microdissect and analyze cells and tissue

2. To view cap information. In the Cap Information area, click **CapName** or **CapNotes** (see Figure 21).
3. To edit cap information. In the Cap Information area, click **CapName** or **CapNotes**, then edit the information in the adjacent field. Alternatively, enter the information in the **Load Options** dialog box.

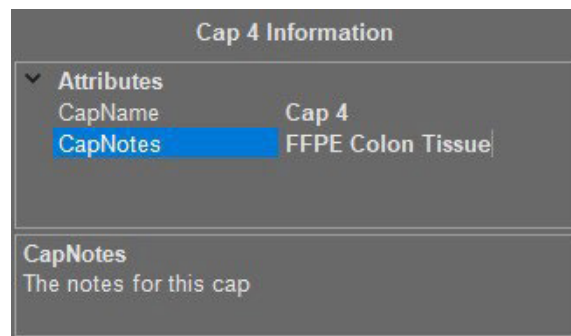



Figure 21
Cap information area

View the cap interaction history

The AccuvaCollect software creates the cap interaction history file the first time a CapSure™ Cap is off-loaded. The software then updates this file throughout the session. The cap interaction history file contains information about the caps, the slides, capture groups, and the total area of microdissected tissue.

1. Click outside of the Accuva Collect 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 a browser window.

Unload materials from the instrument

1. In the **Setup** tools pane, click .
The work surface is displayed.
2. Using a gloved hand, carefully remove each cap from the QC station.

IMPORTANT! Do not touch the polymer surface that holds the microdissected cells as you remove the caps from the unload stations.

3. Press the tension button in to release the springs holding the slides in place, then lift each slide out of its slot.
4. Load new slides and caps to continue microdissection, or close the Accuva Collect software to end your session.

Chapter 4 Microdissect and analyze cells and tissue

If you plan to continue microdissection and must load new caps, you must reset the software.

IMPORTANT! Ensure 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.

5. To clear caps from the QC position.
 - Click **Reset** in the **Load Options** dialog box.
 - Click each CapSure™ Cap icon in the QC Caps area. The icon disappears, indicating that you have removed the cap from the position on the stage off load area.

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

Power off the Accuva Collect Instrument

1. Sign out of AccuvaCollect software.
2. Power off the computer using Windows.
3. Ensure that the blue power button on the front panel of the Accuva Collect Instrument is off.
4. Power off WACOM monitor.
5. Power off the instrument by switching off the power switch on the back panel.

CHAPTER 5 Extract biomolecules from captured cells & tissue

- Select an extraction kit
- Extracting tissue from CapSure™ LCM MicroCap caps
- Extracting tissue from CapSure™ HS LCM caps

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.

Select an extraction kit

See “Required materials not Supplied” on page 7 for extraction kits designed to work with the CapSure™ LCM Sample Preparation System. For additional information about related instruments and Accuva Collect Microgenomics reagents kits, see laxcoinc.com/lcm.

Extracting tissue from CapSure™ LCM MicroCap caps

CapSure™ LCM MicroCap caps can capture a diameter of up to 4300 µm and are compatible with reaction tubes (both 0.2- and 0.5-mL) and 96-well plates for nucleic acid isolation after LCM.

The procedure for extracting tissue with a CapSure™ LCM MicroCap varies depending on the nucleic acid that is extracted and the starting tissue type.

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.

CHAPTER 5 Extract biomolecules from captured cells & tissue

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 22).

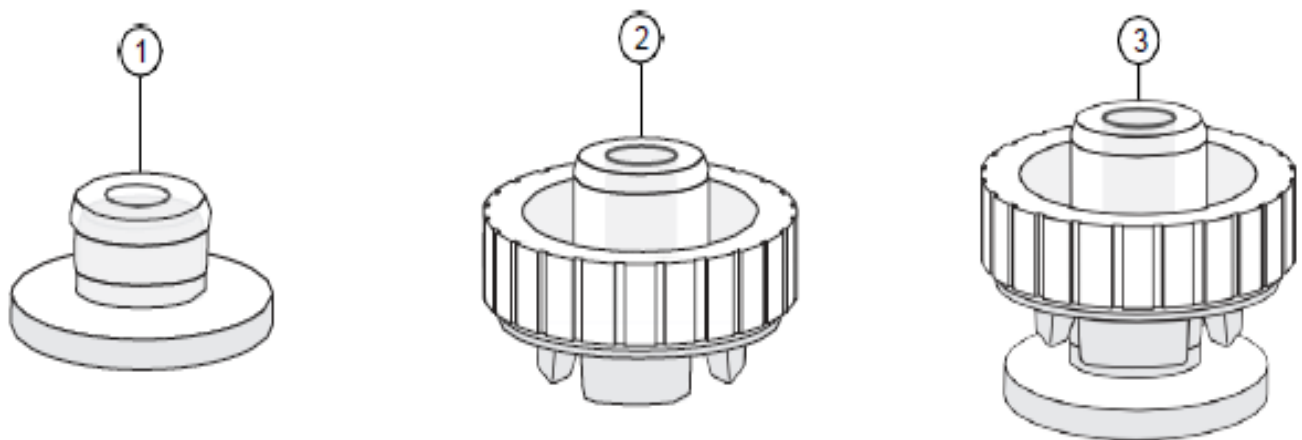


Figure 22
CapSure™ HS LCM cap and ExtracSure™ device

- (1) CapSure™ HS LCM Cap
- (2) ExtracSure™ Sample Extraction Device
- (3) CapSure™ HS LCM Cap sealed with ExtracSure™ Sample Extraction Device

CHAPTER 5 Extract biomolecules from captured cells & tissue

Using the ExtracSure™ device during extraction

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

1. Using a gloved hand, carefully remove the cap from the QC station. Ensure that 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.
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.
6. Place the tube into a microcentrifuge and centrifuge briefly to bring the buffer to the bottom of the tube and complete the extraction procedure as described in the appropriate user guide.



APPENDIX A Troubleshooting

Troubleshooting

If you encounter a problem that you cannot resolve using this troubleshooting section, contact your Laxco, Inc. service representative.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
Microdissection (UV or IR) is not aligned to markings of drawing item(s)	UV or IR laser is not located properly	Use the IR or UV locate options found in the microdissection dialog box.

Solving problems with IR laser capture (LCM)

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
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 bigger than 3. 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	<ol style="list-style-type: none"> 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. <p>If this doesn't work, the IR laser location may need adjustment. Contact Technical Support to arrange for service.</p>
	Tissue is thick or dark	Move to a thinner tissue area or a clear area on the slide.
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.

APPENDIX A Troubleshooting

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
Cells do not adhere to the CapSure™ HS LCM Cap (continued)	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.
IR Capture laser fires off target	IR laser location is set incorrectly	Relocate the IR laser.

Solving problems with UV laser cutting

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
UV Cutting is inefficient	Cutting speed	Reduce the UV cutting speed using the slide bar located in the Microdissect Tool pane.
UV Laser does not cut	Laser power is too low	Increase laser power or reduce cutting speed.

APPENDIX A Troubleshooting

Solving problems with image quality

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
Background of live image is not clear or white	White balance is off	Reestablish white balance in camera properties dialog box, located under View in the pull-down menu.
Long lag time when updating the live image	Light intensity or camera gain settings are not optimized	<ol style="list-style-type: none">1. Check the Brightness setting located in the Microscope tool panel. It should be ≥ 0.200 ms.2. Open the Select dialog box and readjust intensity or camera gain to achieve an appropriate brightness setting.
Slide overview image too dark or too bright	Settings are not optimized	<ol style="list-style-type: none">1. Change magnification to 2x and adjust the brightness setting.2. Right-click in the slide overview area, then select Remember Settings in the pop-up window.3. Select Yes to save for all future overviews.4. Select Require Overview.

APPENDIX B Settings

Settings

- Set the IR capture spot size
- Select file path options
- Set capture group formatting properties
- Set drawing tool options
- Select preferences for cut and capture

Set the IR capture spot size

IMPORTANT! You should set the IR capture spot size before creating any drawing items. The selected IR spot size is placed in all drawn items. IR laser power and duration are stored with the drawing item and used for IR laser capture.

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


We recommend the following guidelines for spot sizes.

- Small spot size: less than 15 μm in diameter
- Medium spot size: 15–45 μm in diameter
- Large spot size: greater than 45 μm in diameter

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.

Test fire the IR Capture laser

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

1. Place a CapSure™ Cap onto a clear (non-tissue) area of the slide.
2. In the **Select** tools pane, click  (Information) to open the **Select Options** dialog box.

APPENDIX B Settings

3. Click the **IR Spot Sizes** tab, then select the size of spot (small, medium, or large) under Edit settings per spot sizes for which you want to set the parameters (see Figure 23).
4. Select **Auto move stage** (the modular stage insert moves each time the laser is test-fired), then click **Test IR Shot**. The software fires the IR capture laser.
5. Ensure that you obtain a properly wetted IR Capture Spot. You should see a dark ring with a clear center, as shown in Figure 24

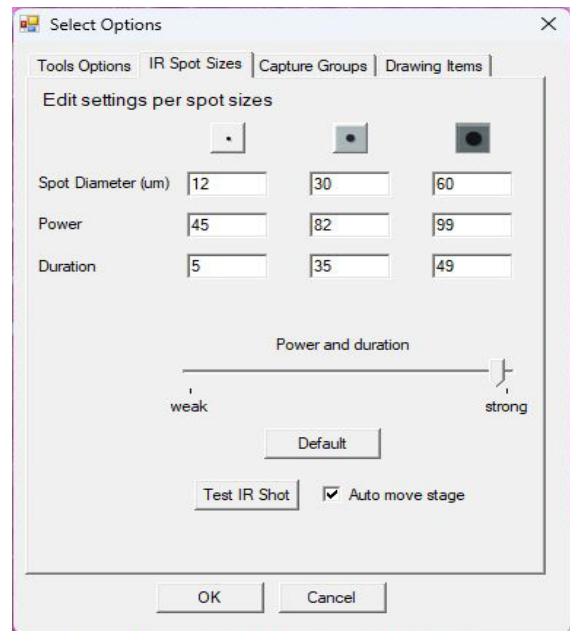


Figure 23
IR spot sizes tab

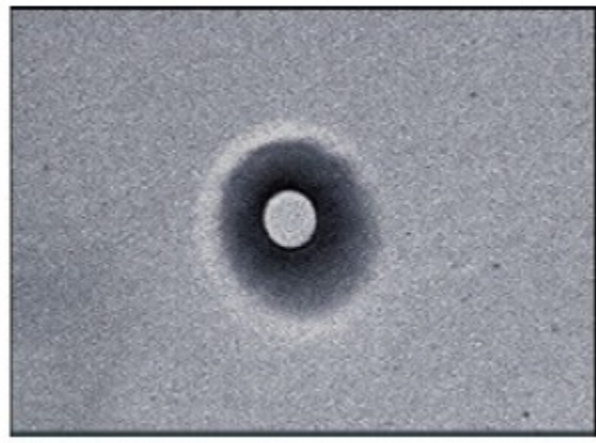
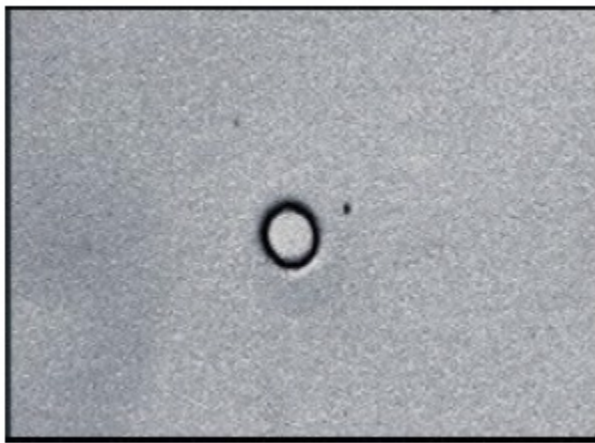





Figure 24
Variation in properly wetted spots

If you do not see a properly wetted spot, adjust the **Power and duration slider**, then tap or click **Test IR Shot** again.

Check the spot diameter

1. Measure the diameter of the spot.
 - a. In the main window, click  (**Ruler**) in the **Select** tools pane.
 - b. Click the left side of the spot that is created by the IR capture laser, then drag  the stylus to the other side of the spot. The system draws a line and displays a label showing the diameter of the spot.
 - c. Click  (**Move Stage**) to deselect the Ruler tool.

APPENDIX B Settings

2. If the diameter of the IR spot is not 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. Click **Test IR Shot** to create a new wetted spot, then repeat step 1 and step 2 if needed.
4. After you have achieved the desired spot size, enter the diameter for the IR capture spot in the **Spot Diameter (µm)** field.
5. (**If needed**) Repeat the procedure for the other spot sizes.
6. 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.

Select file path options

1. In the **Setup** tool pane, select to open the **Load Options** dialog, then click the **File Paths** tab. 

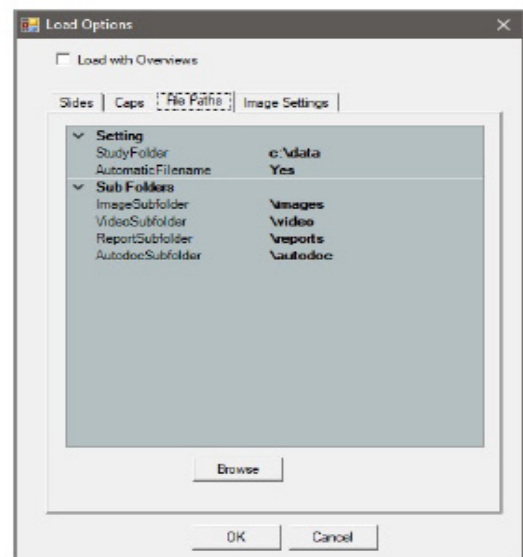


Figure 25
Load Options dialog

APPENDIX B Settings


2. Select one of the following options.

OPTION	ACTIONS
<p>Select the file name for the saved images</p>	<ol style="list-style-type: none"> 1. Enter No in the AutomaticFilename field. 2. In the StudyFolder field, enter the name of the folder where saved images, videos, and reports are to be found. Click Browse to select the location. 3. In the ImageSubfolder field, enter the name of the folder where image files are to be saved. 4. In the ReportSubfolder field, enter the name of the folder where the cap interaction history files are to be saved. <p>Note: A cap interaction history is generated when you off-load a cap after microdissection.</p> <ol style="list-style-type: none"> 5. In the VideoSubfolder field, enter the name of the folder where video files are to be saved.
<p>Use the default naming convention</p>	<p>Enter Yes in the AutomaticFilename field.</p> <p>Note: All image file names contain the date and an integer number. See the following list for examples.</p> <ul style="list-style-type: none"> • For saved images: 2023-07-23_X.tif • For tiled images: 2023-07-23_002_S.tif • For Videos: 2023-07-23_X.avi

APPENDIX B Settings

Set capture group formatting properties

Tools in the **Select** tools pane enable you to designate "capture groups," (that is, cells and tissue to be collected on the same cap). For example, to collect two different types of cells from one or more slides, you can use two capture groups. You can have a maximum of four capture groups.

1. In the Select tools pane, click  (Information) to open the **Select Options** dialog box.
2. Click the **Capture Groups** tab (see Figure 26).
3. Select the radio button for the capture group of interest.
4. In the Attributes section, enter or select the relevant information for the drawing items in the current capture group.

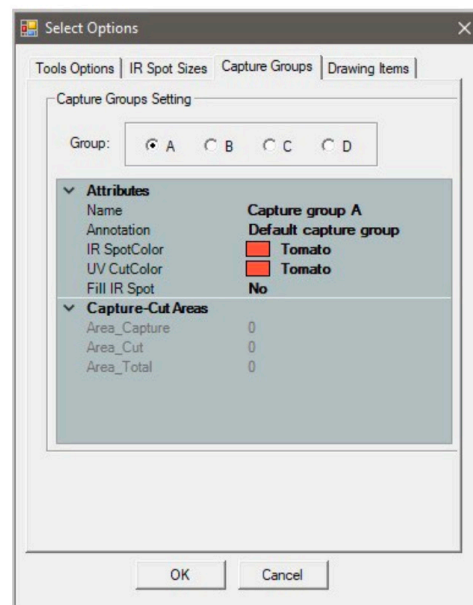


Figure 26
Capture groups tab

FIELD	DESCRIPTION
Name	The name of the capture group. This information appears in the cap interaction history.
Annotation	This information appears in the cap interaction history.
IR SpotColor	The color of the IR capture spots in the main image.
UV CutColor	The color of the UV cut line in the main image.
Fill IR Spot	If set to "Yes", the IR Capture spot is filled with the IR SpotColor.

APPENDIX B Settings

Information that is supplied in this section appears in the cap interaction history


5. Repeat step 3 and step 4 for any other capture groups.
6. (Optional) View a capture group
 - a. In the Select tools pane, select the capture group (A, B, C, or D) you want to view.
 - b. In the Drawing Items list, tap or click the number of the item you want to view. The stage moves so that the selected item is highlighted and centered in the main image window.
7. Click OK to chose the dialog box and save your changes.

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

APPENDIX B Settings

SET DRAWING TOOL OPTIONS

In the **Tools Options** tab, you can set parameters of the Eraser, Freehand Drawing tools, and Defined Circle Area Tools.

1. In the **Select** tools pane, click  (information) 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 Eraser Size—Click the size for the **Eraser** tool.
- Select Snap to End Size—Click the distance for the two ends of a shape drawn by the **Freehand** tool to automatically snap together and close the drawing item.
- Define a Custom Circle—Select the Use Custom Circle checkbox 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, then tap the main image window.
- Define a Custom Rectangle—Select the Use Custom Rectangle checkbox to set the Rectangle tool to draw a rectangle of a fixed size. Enter the Base and Height for the custom rectangle. To draw a custom rectangle, tap the Rectangle tool, then tap the main image window.

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

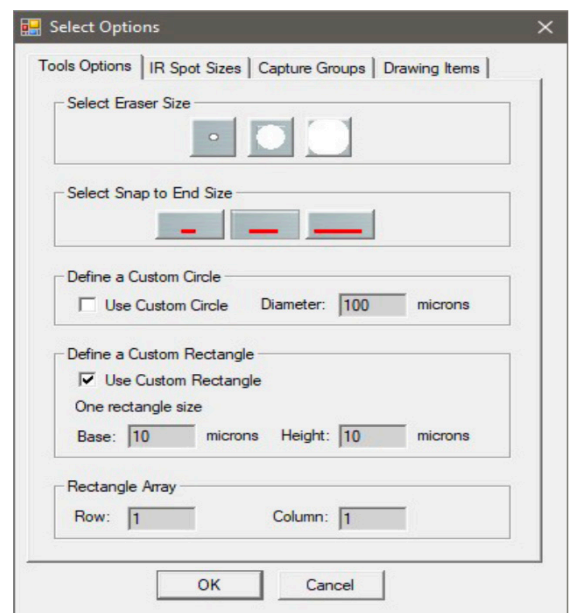


Figure 27
Tools options tab

APPENDIX B Settings

SELECT 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

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- μ m thick polyethylene naphthalate [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.

CHANGE THE CUT AND CAPTURE ORDER

By default, the Accuva Collect Instrument performs an IRcapture 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 select the order in the AccuvaCollect software.

To set the order of cutting and capture:

1. Click **i** (Information) in the upper-right corner of the **Microdissection** tools pane.
2. Select **IR Capture first** or **UV-Cut first** to set the order (see Figure 28)
3. Click **OK**.

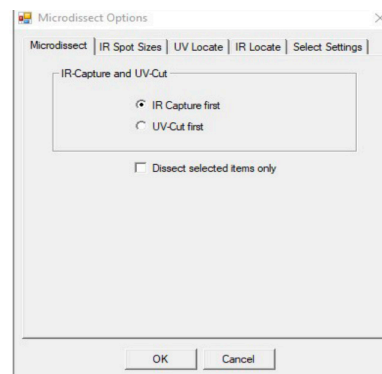



Figure 28
Microdissect tab

APPENDIX B Settings

SET THE CUT AND CAPTURE PROPERTIES

1. Click  (Information) in the upper-right corner of the **Microdissection** tools pane.
2. Click the Select Settings tab (see Figure 29).
3. If needed, click IR Spot Spacing, then enter a value. This value is the spacing between IR capture spots, as a percentage of the spot diameter. If IR Spot Spacing is:

- <100%, the IR capture spots 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 are adjacent without overlap.

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

4. If needed, modify the UV Settings

- IR SpotsPerCutLength is the number of IR spots that are 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.
- UV CuttingSpeed is the speed of the stage movement, which affects the speed of UV cutting.

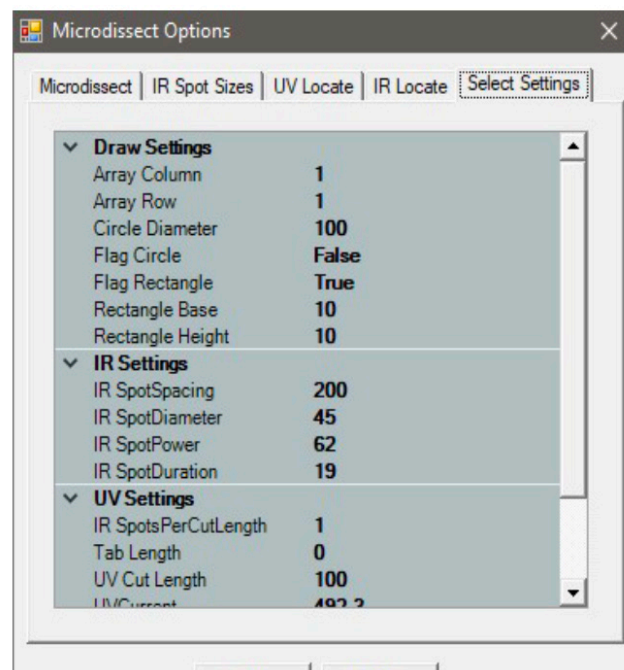


Figure 29
Select settings tab

APPENDIX B Settings

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.

- UV Current is the current of the UV cutting laser.

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

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

Note: Recommended UV pulse UV pulse frequency is 3,000Hz.

5. Click **OK**.

Note: All the changes that you make to the IR Spots Per Cut Length, Tab Length, UV Cut Length, UV Cutting Speed, UV Power, and UV Pulse Frequency are applied retroactively to all drawing items.

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

APPENDIX C Supplemental Procedures

- Cleaning the Accuva Collect Instrument
- Change stage insert
- Capturing, saving, and viewing videos
- Use of the laser bypass feature

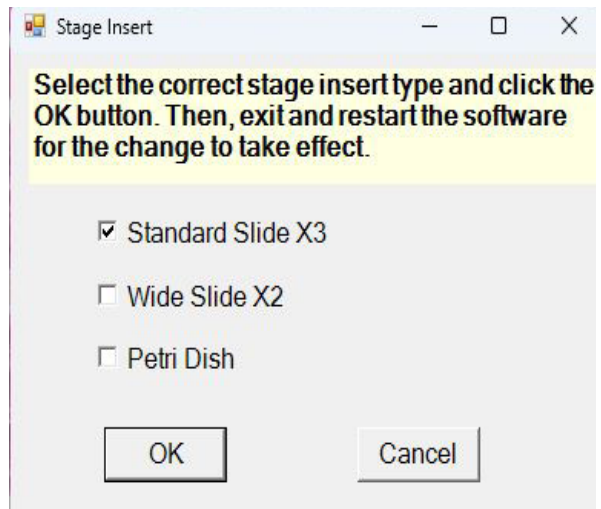
Cleaning the Accuva Collect Instrument

- Clean the outside of the instrument using a damp cloth. Do not use any solvents or abrasives.
- Clean the work surface as necessary, by wiping it with a cloth moistened with 70% ethanol.

IMPORTANT! Do not attempt to remove the covers from the instrument. If the instrument requires service, contact your Laxco, Inc. service representative.

Change stage insert

1. Go to and click the menu Options → System → Change Stage Insert
2. Select the stage insert type and click the button “OK”.
3. Exit and restart the AccuvaCollect software.



APPENDIX C Supplemental Procedures

Capturing, saving, and viewing videos

Capturing and saving a video

1. To start recording a video, in the Inspect tools panel, Click the  (**VideoRecorder**). The camera icon turns red while the camera is recording.
2. To stop the recording, click  (**VideoRecorder**) 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.

Use of the laser bypass feature

For safety reasons, the lasers are disabled when outside of the CapSure™ LCM Cap area. However, there can 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.



WARNING! LASER. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

APPENDIX D System specifications

This appendix provides the specifications for the Accuva Collect LCM System. Depending on the configuration of your system, not all options listed below may be included with your instrument.

Instrument specifications

Table 3 - Description of instrument specifications.

ITEM	DESCRIPTION												
Electrical supply from External Power Supply	100–240 VAC, 50–60 Hz, 240 W (Voltage fluctuations not to exceed $\pm 10\%$ of nominal supply voltage.).												
Capture laser	Diode, near IR laser, 808 nm.												
UV Cutting laser	<ul style="list-style-type: none"> • Solid-state UV laser, 355 nm. • Adjustable UV Laser Current 0–99%. 												
Microscope Stage	<ul style="list-style-type: none"> • Computer controlled. • Range 155 x 125 mm, repeatability 2 μm. 												
Bright Field Illumination source	High intensity LED illumination system.												
Fluorescent Illumination source	LED												
Objectives	2X, 4X, 10X, 20X, 40X, 60X												
Filters	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 33%;">Color</th> <th style="width: 33%;">Excitation</th> <th style="width: 33%;">Emission</th> </tr> </thead> <tbody> <tr> <td>DAPI</td> <td>375-28 nm</td> <td>460-50 nm</td> </tr> <tr> <td>FITC</td> <td>480-50 nm</td> <td>535-40 nm</td> </tr> <tr> <td>Texas Red</td> <td>635-60 nm</td> <td>560-40 nm</td> </tr> </tbody> </table>	Color	Excitation	Emission	DAPI	375-28 nm	460-50 nm	FITC	480-50 nm	535-40 nm	Texas Red	635-60 nm	560-40 nm
Color	Excitation	Emission											
DAPI	375-28 nm	460-50 nm											
FITC	480-50 nm	535-40 nm											
Texas Red	635-60 nm	560-40 nm											
Operating Temperature	18-30 ^o C												
Base unit dimensions	Height: 24 in (61 cm) Width: 18.25 in (46 cm) Depth: 22.5 in (57 cm)												

APPENDIX D System specifications

Table 3 - Description of instrument specifications (Continued)

ITEM	DESCRIPTION
Weight	108 lb (49 kg)
Work surface requirements	62 in. X 40 in. (158 cm X 100 cm) with vertical clearance of 32 in. (80 cm)

APPENDIX E Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on).

Symbols on this instrument



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.

Standard safety symbols

SYMBOL AND DESCRIPTION	
	CAUTION! Risk of danger. Consult the manual for further safety information

APPENDIX E Safety

SYMBOL AND DESCRIPTION	
	CAUTION! Risk of electrical shock.
	CAUTION! Ultraviolet light.

SYMBOLE ET DESCRIPTION	
	MISE EN GARDE! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité
	MISE EN GARDE! Risque de choc électrique.
	MISE EN GARDE! Rayonnement ultraviolet.

Location of safety labels

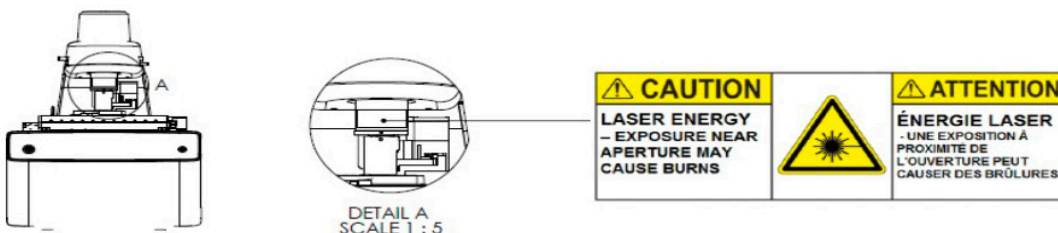


Figure 30
Front of Instrument

APPENDIX E Safety

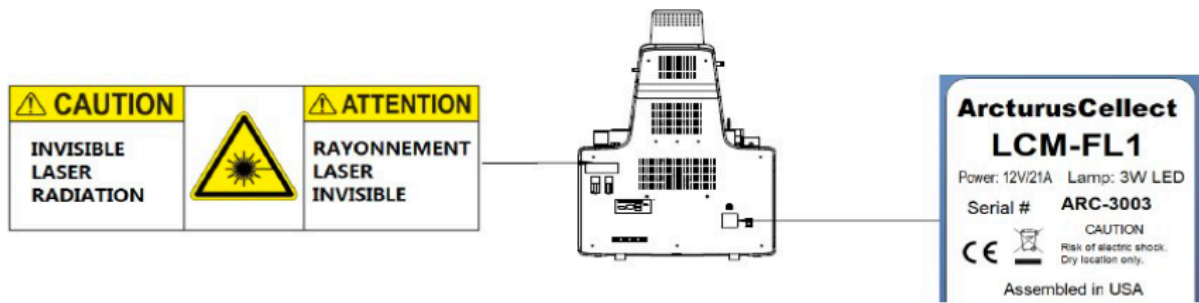


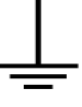

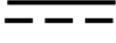






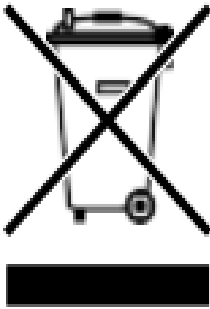
Figure 31
Bank panel of instrument

Control and connection symbols

SYMBOLS AND DESCRIPTIONS	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Direct current
	Alternating current
	Both direct and alternating current

APPENDIX E Safety

Conformity symbols

COMFORMITY MARK	DESCRIPTION
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with China RoHS requirements.
	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with the WEEE Directive 2012/19/EU. CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contract customer service for information about responsible disposal options.

APPENDIX E Safety

Safety information for instruments not manufactured by Laxco Inc.

Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General



CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.



CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

APPENDIX E Safety

Physical injury



CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- 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.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.



CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

APPENDIX E Safety

Electrical safety



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.



WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.



AVERTISSEMENT ! Cordons d'alimentation électrique et approuvés pour raccorder l'instrument au circuit électrique du site.



WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

APPENDIX E Safety

Cleaning and decontamination



CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.



MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas

APPENDIX E Safety

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Laser safety





WARNING! LASER HAZARD. Under normal operating conditions, the is categorized as a Class 1 laser product. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class . Lasers can burn the retina, causing permanent blind spots. To ensure safe laser operation:

- Never look directly into the laser beam.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Laxco Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others
- Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing
- DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.

APPENDIX E Safety

The following table lists laser safety symbols and alerts that may be present on the instrument.

ALERT	
	DANGER! Class 4 (IV) visible and/or invisible radiation present when open. Avoid eye or skin exposure to direct or scattered radiation.
	DANGER ! Rayonnement visible ou invisible de classe 4 (IV) présent en position ouverte . Éviter toute exposition des yeux ou de la peau au rayonnement direct ou diffusé.

Laser safety scenarios



WARNING! LASER. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

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 cap is not required. In such instances, you can bypass the instrument laser safety settings.

To bypass the laser safety mechanism, depress the Laser Bypass button to activate the UV laser.

APPENDIX E Safety

The following table details possible status scenarios, showing action combinations, and the resulting system responses. For example, row 4 indicates that when the laser bypass button hasn't been pressed and a CapSure™ LCM Cap is out of place. You must press the laser bypass button down or move the cap in place.

Table 4 - Laser Scenarios (continued)

State	Laser Bypass Button Pressed	CapSure™ LCM Cap in Place	Laser State	To State	Pop-up	Cut Capture	Tool Tip Messages
4[1,6]			Standby			Stop	tt='Laser disabled because cap is out of beam path and override is off.'
5 [1,6]			ON	4	X	Stop	tt='Laser disabled because cap is out of beam path and override is off. 'pop up='UV Laser cannot be powered on. Ensure the cap is present OR both hardware-software bypass are on.'

APPENDIX E Safety

Table 4 - Laser Scenarios (continued)

State	Laser Bypass Button Pressed	CapSure™ LCM Cap in Place	Laser State	To State	Pop-up	Cut Capture	Tool Tip Messages
6[1,3]		X	Standby			Stop	tt='Laser ready to fire. Cap placed in beam path.'
7[4,5]		X	ON				tt='Laser is firing. Cap placed in beam path.'
12 [1, 7]	X		Standby			Stop	Laser is firing. Cap detected in beam path.
13 [4.2]	X		ON				tt='Laser is firing. Cap overridden and not detected in beam path.'

APPENDIX E Safety

Table 4 - Laser Scenarios (continued)

State	Laser Bypass Button Pressed	Cap-Sure™ LCM Cap in Place	Laser State	To State	Pop-up	Cut Capture	Tool Tip Messages
14 [1, 9]	X	X	Standby			Stop	tt='Laser ready to fire. Cap overridden but detected in beam path.'
15[4,10]	X	X	ON				tt='Laser is firing. Cap overridden but detected in beam path.'

- [1] Set Laser: Standby
- [2] Color: U. Red
- [3] Color: U. Yellow
- [4] Set Laser: Power =p
- [5] Color: U. Green
- [6] Color: U. Orange
- [7] Color: PF. Yellow
- [8] Color: PF. Green
- [9] Color: P. Yellow
- [10] Color: P. Green

APPENDIX E Safety

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

REFERENCE	DESCRIPTION
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 62020-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 NO. 61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
IEC 61010-2-081	Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-081: Particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes
IEC 60825-1: 2014 EN 60825-1: 2014	Safety of laser products - Part : Equipment classification and requirements
21 CFR 1040.10 and 1040.11 as applicable	U.S. FDA Health and Human Services (HHS) “Radiological health performance standards for laser products” and “Radiological health performance standards for specific purpose laser products”

EMC Standards

REFERENCE	DESCRIPTION
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1	Electrical Equipment for Measurement, Control and Laboratory Use - EMC Requirements Part 1: General Requirements
ICES-003, Issue 6	Industrial, Scientific and Medical (ISM) Radio Frequency Generators

APPENDIX E Safety

REFERENCE	DESCRIPTION
<p>FCC Part 15 Subpart B (47 CFR)</p>	<p>U.S. Standard Radio Frequency Devices</p> <p>This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.</p> <p>This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:</p> <ul style="list-style-type: none"> • Reorient or relocate the receiving antenna. • Increase the separation between the equipment and receiver. • Connect the equipment into an outlet on a circuit different from that to which the receiver is connected. • Consult the dealer or an experienced radio/TV technician for help. <p>This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference.</p> <p>Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshield intentional RF sources), as these can interfere with the proper operation.</p>

APPENDIX E Safety

Environmental Design Standards

REFERENCE	DESCRIPTION
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products

APPENDIX E Safety

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

APPENDIX E Safety



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.

Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.

APPENDIX E Safety

- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

APPENDIX E Safety

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! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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:
https://www.cdc.gov/labs/pdf/CDC-Biosafety_microbiological_Biomedical_Laboratories-2020-P.pdf
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/publications/i/item/9789240011311

Documentation and Support

Customer and technical support

Visit <https://www.laxcoinc.com> for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - 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

Laxco warrant <https://www.laxcoinc.com/lcm/warranty>. If you have any questions, please contact <https://www.laxcoinc.com>



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