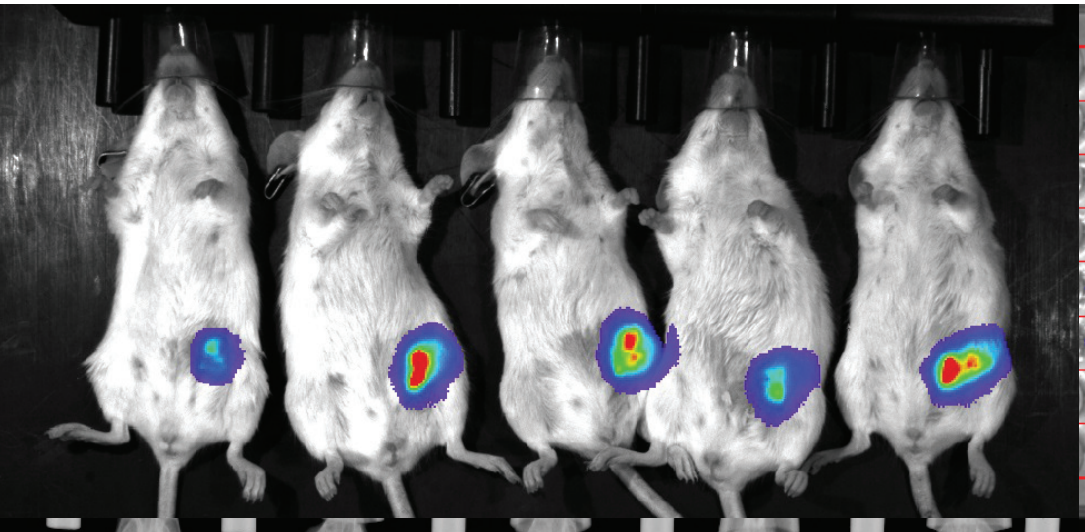
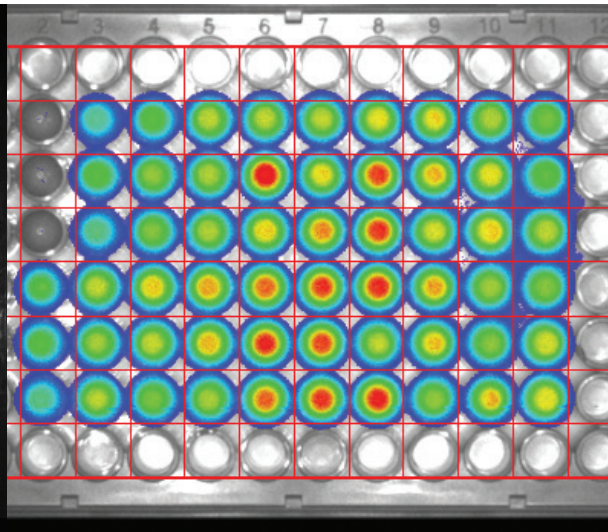
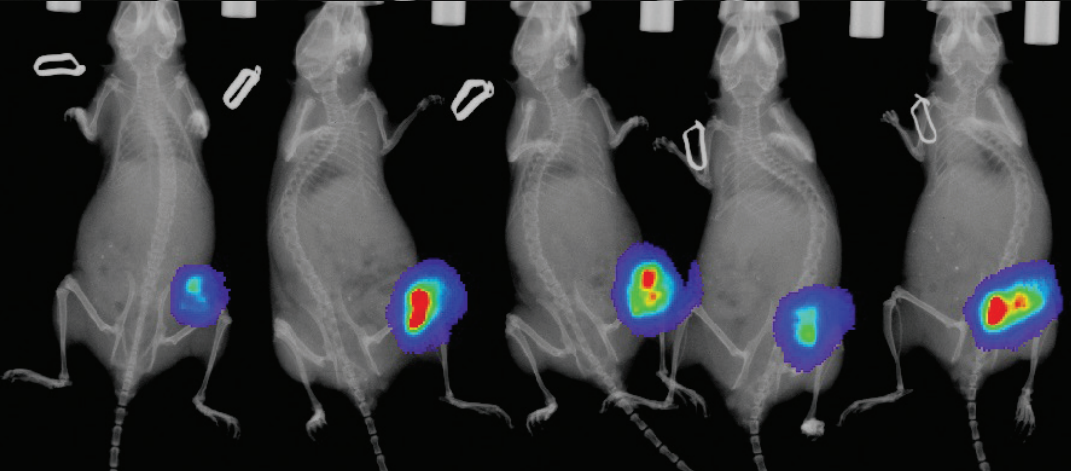
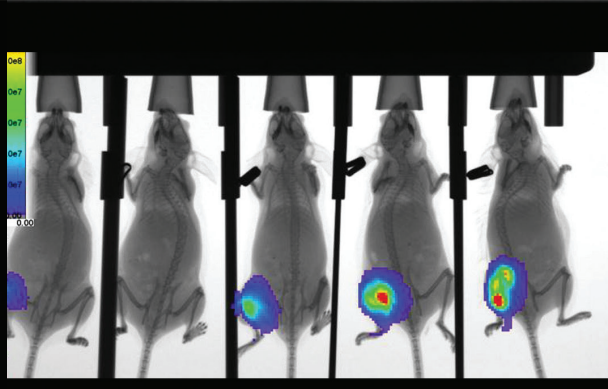
spectral instruments imaging

SPECTRAL ami HT / ami HTX
USER MANUAL









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Patents
The SPECTRAL ami HT and ami HTX imaging systems are covered by U.S. Patents 8,901,516 and D648844. Other patents pending in the U.S. and other jurisdictions.

Contents

1

Welcome

1.1 SPECTRAL ami HT and ami HTX Imaging Systems

CAUTION: Read and understand this manual in its entirety before operating the SPECTRAL ami HT or ami HTX Imaging System.

The SPECTRAL ami HT and ami HTX Imaging Systems are general-purpose imaging systems capable of fluorescence, luminescence, and photographic imaging. The ami HTX Imaging System also includes x-ray imaging capability.

The imaging system includes a:

- Control and Analysis computer with aura, an IDL-based image capture and analysis software, pre-installed.
- Spectral Instruments Imaging Calibration Device (SIICD) to help ensure accurate results over the lifetime of the instrument.

The imaging chamber features a gasket-free, light-tight design. The available horizontal fields of view for fluorescence, luminescence, x-ray (SPECTRAL ami HTX only) or photographic imaging, are 25, 20, 15, and 10 cm.

At the heart of ami HT and ami HTX instruments is a custom designed air-cooled -90°C charge coupled device (CCD) camera with a back-illuminated sensor. This camera has very low background noise from dark current and read noise, enabling accurate detection of dim light sources in samples.

These features of the SPECTRAL ami HT and ami HTX Imaging Systems (Figure 1.1) enable sensitive, quantitative imaging of luminescent sources or fluorescent reporters in subjects such as small animals, well plates, plants, bacterial colonies, electrophoresis gels, and western blots.

Image data acquired with the SPECTRAL ami HT or ami HTX Imaging System enable you to locate and measure the intensity of luminescent light sources or fluorescent reporters in a sample.

Figure 1.1 SPECTRAL ami HT and ami HTX Imaging System

SPECTRAL ami HT
Imaging capabilities:
- Luminescent
- Fluorescent
- Photographic

SPECTRAL ami HTX
Imaging capabilities:
- Luminescent
- Fluorescent
- X-ray
- Photographic

aura software provides instrument control, data acquisition, and data analysis functions for the SPECTRAL ami HT and ami HTX Imaging System. Figure 1.2 shows a typical workflow.

Figure 1.2 Example Imaging Workflow

For instructions on viewing, analyzing and managing images, please refer to the *aura Software User Manual*

1.2 About This Manual

This manual is for use with the SPECTRAL ami HT or ami HTX Imaging System, and includes:

- Instructions on how to acquire images using the aura software.
- SPECTRAL ami HT and ami HTX Imaging System specifications, requirements, and instructions on instrument safety, operation, and maintenance.

Screenshots often supplement the detailed instructions in this manual. Sometimes the screenshots may not exactly match those on your screen.

Read this manual before operating the SPECTRAL ami HT or ami HTX Imaging System. After reading, retain this manual in an easily accessible place for future use by personnel who will be operating the instrument.

Information may be presented in the following formats:

NOTE: Presents helpful information, such as best practices for instrument operation.

IMPORTANT: Presents information that is essential to safe and correct operation of the instrument or software.

CAUTION: A caution note indicates actions that may cause irreversible consequences or may cause loss of data. It advises the user to refrain from performing actions which could degrade instrument performance or damage instrument components.

WARNING! A warning indicates dangerous actions or situations that may cause severe damage to the instrument and/or serious personal injury or loss of life.

VOLTAGE! Alerts the user to situations where there is potential for exposure to high voltage.

WARNING! Informs you that X-rays are produced when the SPECTRAL ami HTX Imaging System is taking an X-ray image.

1.3 Technical Support

If you have questions about the SPECTRAL ami HT Imaging System, SPECTRAL ami HTX Imaging System, or aura software, please contact Spectral Instruments Imaging Technical Support.

IMPORTANT: Contact Spectral Instruments Imaging before making changes to the software, hardware or configuration of the ami HT or ami HTX control computer or incorporating the ami HT or ami HTX Imaging System into your network. Making changes to the hardware, software, or settings of the ami HT computer without approval and guidance from Spectral Instruments technical support will void the instrument warranty and may result in unstable operation or instrument malfunction.

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Getting Started

2.1 Starting aura Software and Initializing the Imaging System

NOTE: The ami HT or ami HTX Imaging System is powered on at installation. It is recommended to leave the instrument power on, except in some situations, for example when preparing to move the imaging system. After about 90 minutes of no image acquisitions, the system will enter a low power mode.

1. Plug the power cord and USB cable into the imaging system.
2. Press the position of the power switch located at the top and rear of the instrument, near the AC power input socket (Figure 4.9 on page 55).
3. You will hear the camera and control box fans turn on. The green button on the front control panel will illuminate. On the ami HTX Imaging System the green x-ray IDLE LED on the front control panel will also illuminate.
4. Confirm that the instrument door is closed.
5. Double-click the aura icon  on the desktop or single-click the aura icon in the taskbar.
6. Click **Click to Continue** in the IDL splash screen that appears (Figure 2.1). Alternatively, press **Enter** on the keyboard.

Figure 2.1 IDL Splash Screen

7. Enter a new or existing user name in the Spectral Instruments Imaging screen that appears (Figure 2.2) and press **Enter** on the keyboard.

NOTE: The aura user name is not password-protected and is not a security mechanism.

Figure 2.2 aura Login Screen

8. If you entered a new user name, click **Yes** in the prompt that appears (Figure 2.3). The software creates folders that are associated with the user name (Table 2.1).

Figure 2.3 New User Prompt

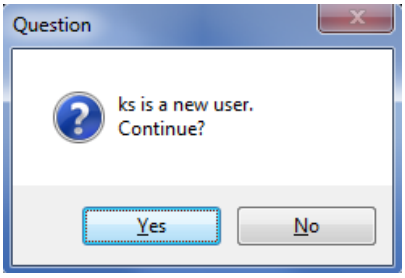


Table 2.1 Folders Associated With a User Name

Folder Location	Contents
Windows: C:\Users**Windows Username**\aura Username Macintosh: /Users/**Mac Username**/aura Username/	■ Image data (.ami) in the "data" folder. ■ Group protocols (.xml) which specify acquisition parameters for a series of multiple images in the "GroupProtocols" folder. ■ Spectral unmixing library entries (.sli) in the "spectralLibs" folder. ■ ROIs (user-defined regions of interest which specify the area(s) to measure image signal) in the "savedROIs" folder.

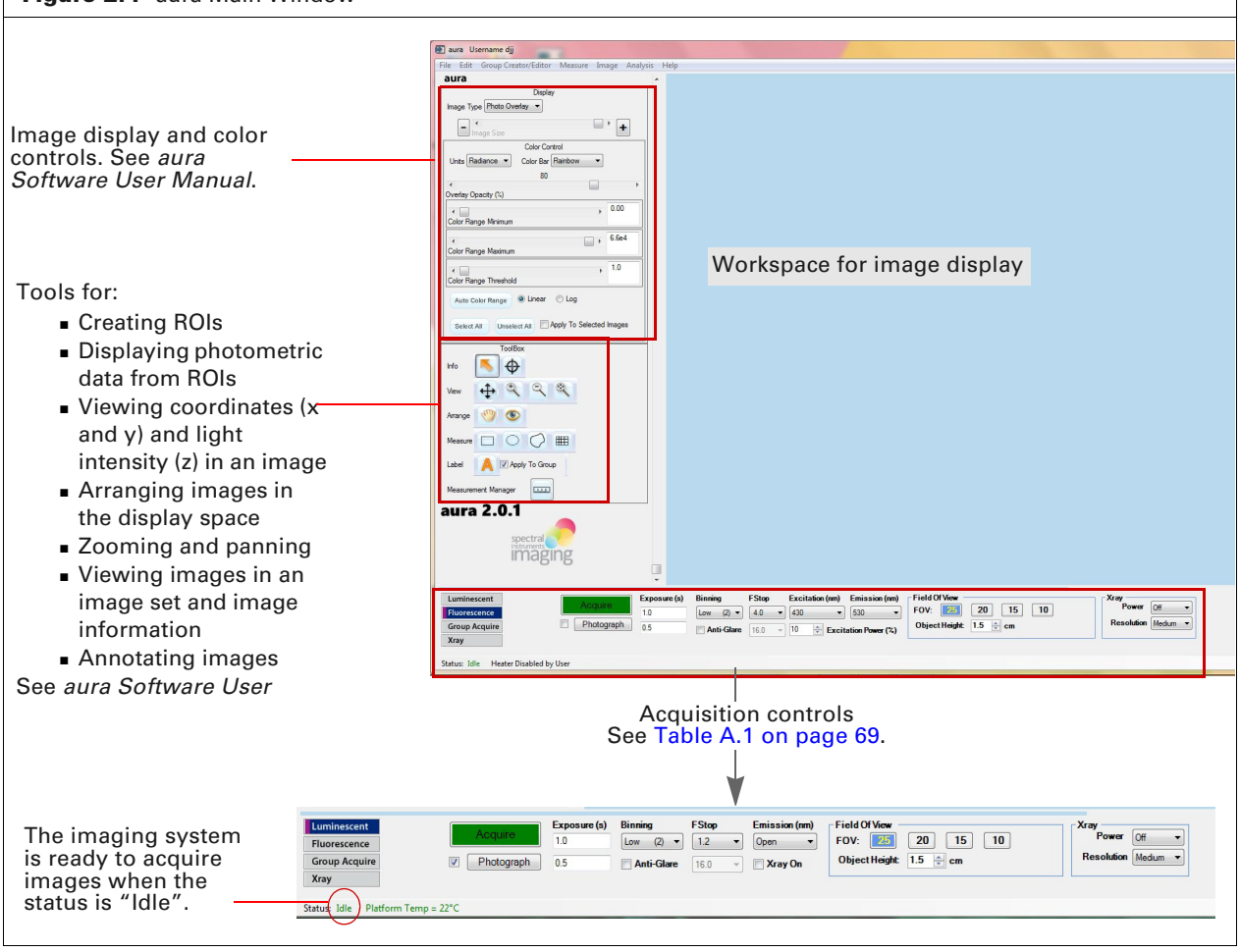
* "***Mac Username*** *and* ***Windows Username*** *are the user names used to log into that particular Mac or PC respectively.* aura Username *is the name chosen in Step 7 above.*

After entering a user name:

- The main aura window appears (Figure 2.4).
- Instrument initialization proceeds (instrument motors move to their home positions).
- The CCD camera begins cooling to operating temperature (–90°C).
- The imaging platform begins warming.

The imaging system is ready to acquire images when the status is "Idle" (Figure 2.4).

Figure 2.4 aura Main Window



2.2 Overview of Image Acquisition

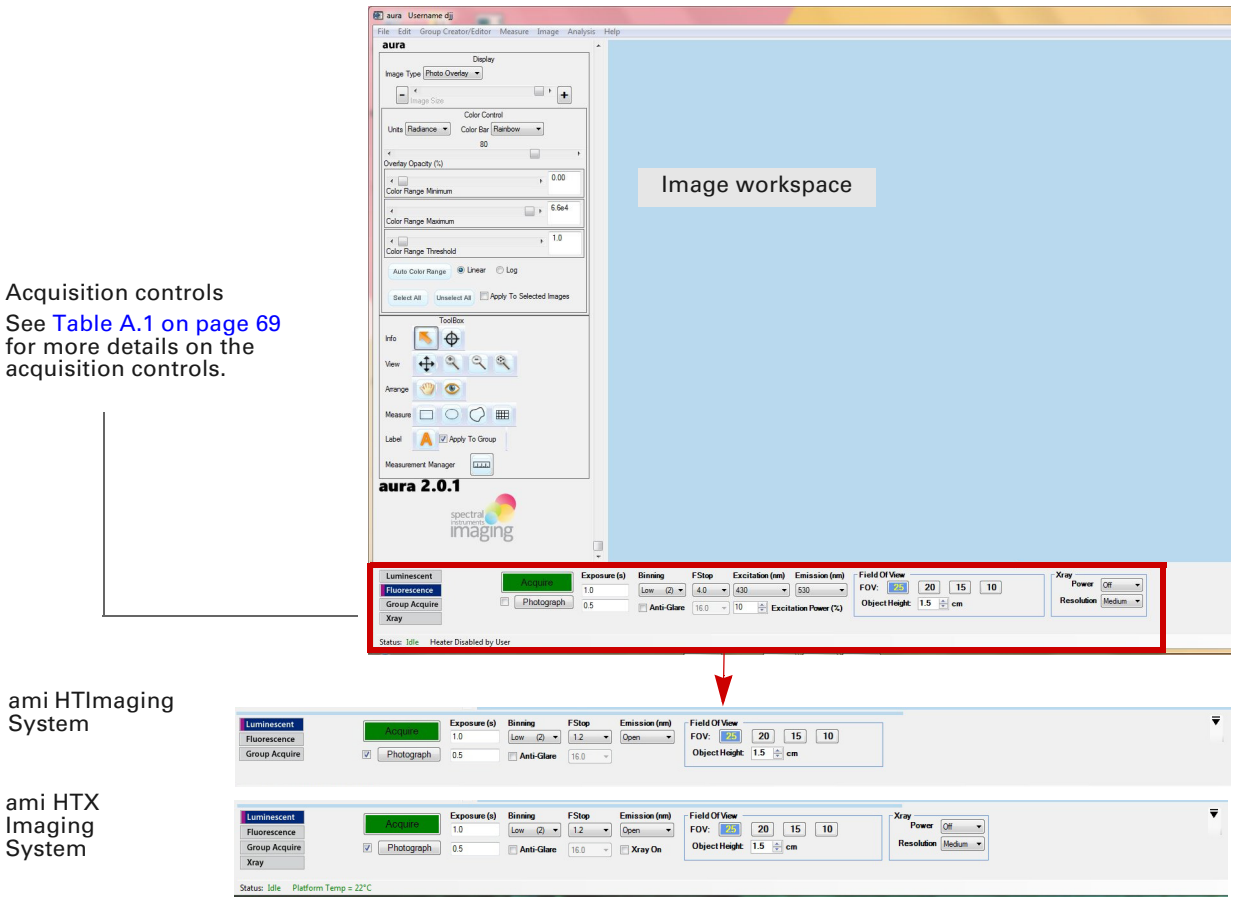
Use the acquisition controls shown in Figure 2.5 to select the imaging parameters. See:

- *Image Acquisition – Single Mode* on page 15 for detailed instructions.
- *Quick Start Guide– Single Mode Acquisition* on page 13 for a quick reference on image acquisition.
- Appendix A on page 69 for details on the acquisition parameters.

In single image mode, multiple image types are usually acquired at the same time. For example, a photograph and a luminescence image can be taken together and saved as an image set (.ami file). Different combinations of the image types in an .ami file can be displayed together as an *overlay* (see Table 2.2 on page 8 for examples).

A series of multiple image sets can be acquired and saved as a *group*. All image sets in this group will be included in the same .ami file. Group acquisition is useful for time-dependent kinetic or longitudinal studies, fluorescence scans (acquisitions at different excitation/emission wavelengths), or acquisitions which vary an imaging parameter (for example, exposure time).

Figure 2.5 Acquisition Controls



Imaging Modes

Table 2.2 shows the imaging modes available on SPECTRAL ami HT and ami HTX Imaging Systems.

Table 2.2 Imaging Modes

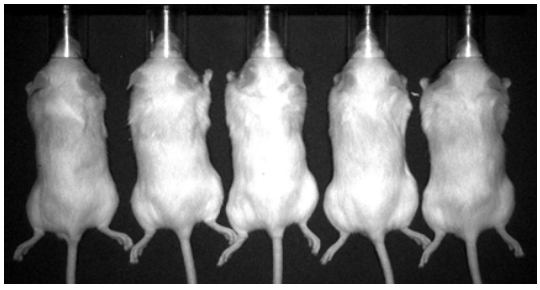
Imaging Mode	Description	Example
Photograph	An image of the white light reflected from a sample. SPECTRAL ami HT and ami HTX photographic images are displayed in grayscale (black and white). A photograph is usually acquired along with a luminescence or fluorescence image to provide a visual reference when viewing an overlay (see below).	

Table 2.2 Imaging Modes (continued)

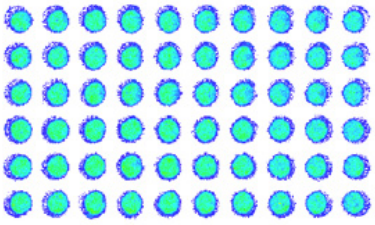
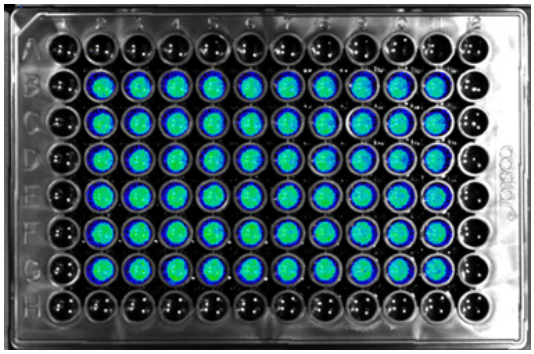
Imaging Mode	Description	Example
Luminescent	An image of light emitted from an unilluminated sample, for example, a bioluminescent source. No lights are on in the imaging chamber during acquisition. Luminescent sources are usually dim and may require exposure times of several minutes.	Luminescence image displayed using the rainbow color bar. See Appendix B on page 71 for more about image data display.  Overlay showing luminescence image on a photograph. 

Table 2.2 Imaging Modes (continued)

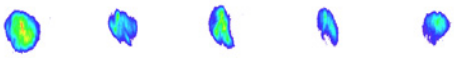
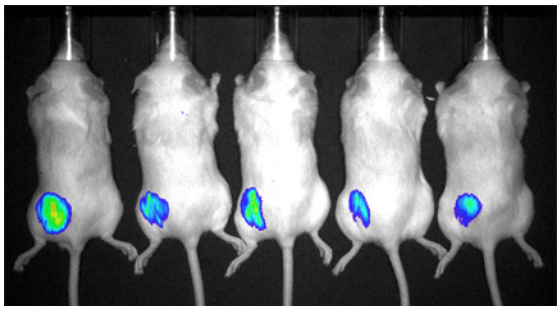
Imaging Mode	Description	Example
Fluorescent	An image of light emitted from a fluorescent reporter, at a specific wavelength when it is illuminated (excited) by a light source at a second, shorter wavelength.	Fluorescence image displayed using the rainbow color bar. See Appendix B on page 71 for more about image data display.  Overlay showing fluorescence image on photograph. 

Table 2.2 Imaging Modes (continued)

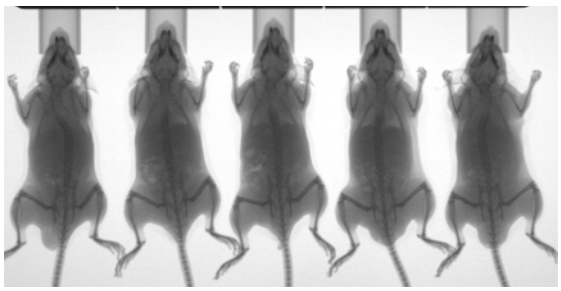
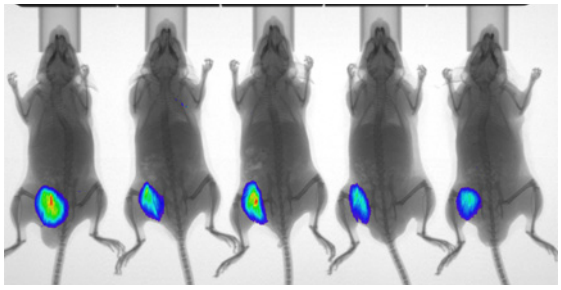
Imaging Mode	Description	Example
X-ray (only available on ami HTX Imaging System)	An image of X-ray photons transmitted through an object. ami HTX Imaging System is equipped with an X-ray source below the sample platform and an X-ray sensitive camera at the top of the imaging chamber. X-ray images provide information about internal sample structure and a visual reference when viewing an overlay.	This example image set includes a fluorescence image and an X-ray image. **Note:** X-ray imaging is only available on the SPECTRAL ami HTX Imaging System. X-ray image  Overlay of fluorescence image on X-ray image. 

Image Acquisition

3.1 Before You Begin Acquisition

IMPORTANT: Read Chapter 4, *SPECTRAL ami HT and ami HTX Imaging System on page 39* before operating the SPECTRAL ami HT or ami HTX Imaging System. Follow the safety and operating instructions in Chapter 4.

Before acquiring images, confirm that:

- The imaging system is powered on and the door is closed (see page 55).
- Imaging system safety checks have been performed (see page 57).
- aura software is running and imaging system status is “Idle” (see page 5).
- The emergency stop button is in the “Reset” position. This button’s light will not be blinking if it is in the “Reset” position. If necessary, reset this button by turning it fully clockwise (see Figure 3.10 on page 19).

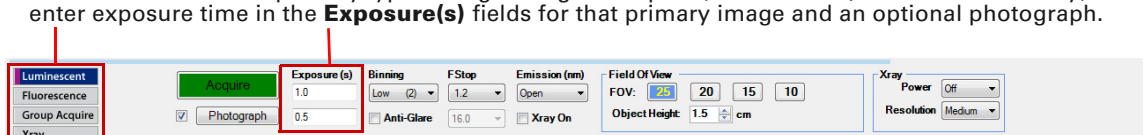
3.2 Quick Start Guide– Single Mode Acquisition

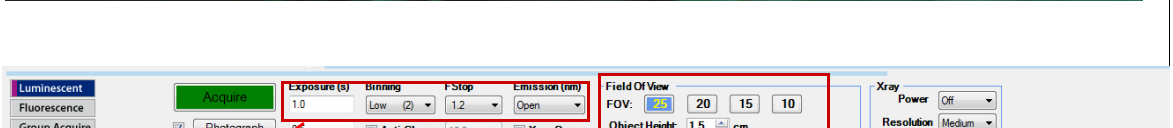
Figure 3.1 shows the basic steps to acquire a single image. See page 14 for detailed instructions.

NOTE: X-ray imaging is only available on the SPECTRAL ami HTX Imaging System.

Figure 3.1 Quick Start Guide – Acquire a Single Image

1. Click the button for the primary type of single image to acquire (Luminescent, Fluorescent or X-ray) and enter exposure time in the **Exposure(s)** fields for that primary image and an optional photograph.



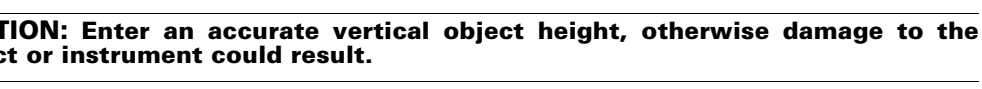


2. Set the luminescence or fluorescence imaging parameters. See Table A.1 on page 69 for details on these parameters.

3. Select a field of view (FOV). See Table A.1 on page 69 for more details on FOV.

4. Enter the object height (tallest point of the object) for accurate radiance data and proper co-registration of overlays.

CAUTION: Enter an accurate vertical object height, otherwise damage to the object or instrument could result.



5. ami HTX Imaging System Only

If acquiring an X-ray image:

- Choose a power level and resolution setting.
- If the emergency stop button (on the front panel) is blinking, reset it by turning it fully clockwise (Figure 3.10 on page 19).

6. Open the imaging system door and place the object(s) or anesthetized subject(s) on the center of the imaging platform, using the positioning fixture or anesthesia manifold if necessary. Close the door.

7. Click **Acquire** when ready to begin acquisition. The imaging platform will audibly move, the shutter will click a few times as well. As the images are taken, they will be displayed in main workspace. While the images are being acquired, the user has the option of saving the image along with identifying, searchable information ("tags"). For more details on acquiring an image, see Section 3.4, "Image Acquisition – Single Mode".

3.3 Green Mode

When the SPECTRAL ami HT or ami HTX Imaging System is powered on, but has not taken an image in 90 minutes, the system will go into Green Mode. This reduces system power consumption as well heat generated by the system. When the system is in Green Mode, the camera CCD temperature is set to approximately -50°C (see Figure 3.2 below).

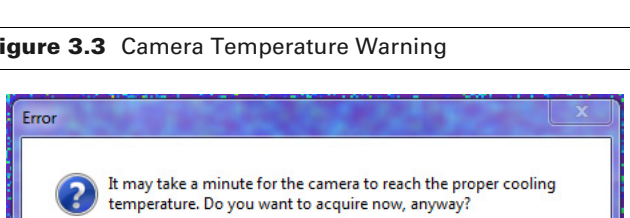
Figure 3.2 Green Mode Status

A SPECTRAL ami HT or ami HTX Imaging System which has entered Green Mode can easily be reset to normal Idle mode, cooling the CCD back down to -90°C in a matter of minutes.

Switching from Green Mode to Normal Idle Mode:

1. Click the **Acquire** button in the acquisition controls area.
2. In the warning window which appears (Figure 3.3 below), click either **Yes** or **No**. Either choice will begin cooling the CCD temperature back to the normal operating temperature of -90°C.
3. When "Idle" is displayed in green in the bottom left-hand corner of the acquisition controls area (see Figure 3.1 for example), you may operate the imaging system as usual.

Figure 3.3 Camera Temperature Warning

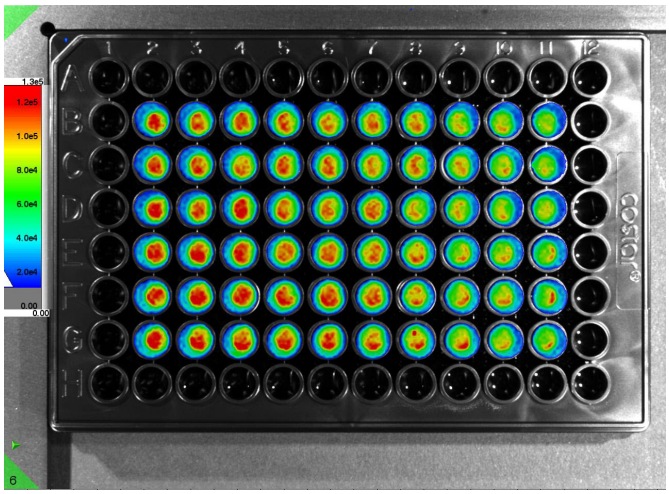


3.4 Image Acquisition – Single Mode

This section of the manual provides detailed instructions for image acquisition using the SPECTRAL ami HT or ami HTX Imaging Systems. See Appendix A on page 69 for more details on the acquisition parameters.

Acquisition in single mode usually includes multiple image types acquired at the same time, for example a photograph and fluorescent image which are saved together as an .ami file (Figure 3.4).

Figure 3.4 Example Image (.ami)
Acquisition included a fluorescent image and a photograph. The fluorescent image is displayed on top of the photograph.



To acquire images:

1. Select any one of the three (two if instrument is not a SPECTRAL ami HTX) **Single** acquisition modes: **Fluorescence**, **Luminescent** or **Xray** at the left end of the acquisition controls area which will then display mode-specific imaging options. See Figure 3.5 SPECTRAL ami HTX acquisition modes and Figure 3.6 for SPECTRAL ami HT acquisition modes.

Figure 3.5 The SPECTRAL ami HTX Single Instrument Modes and Their Options

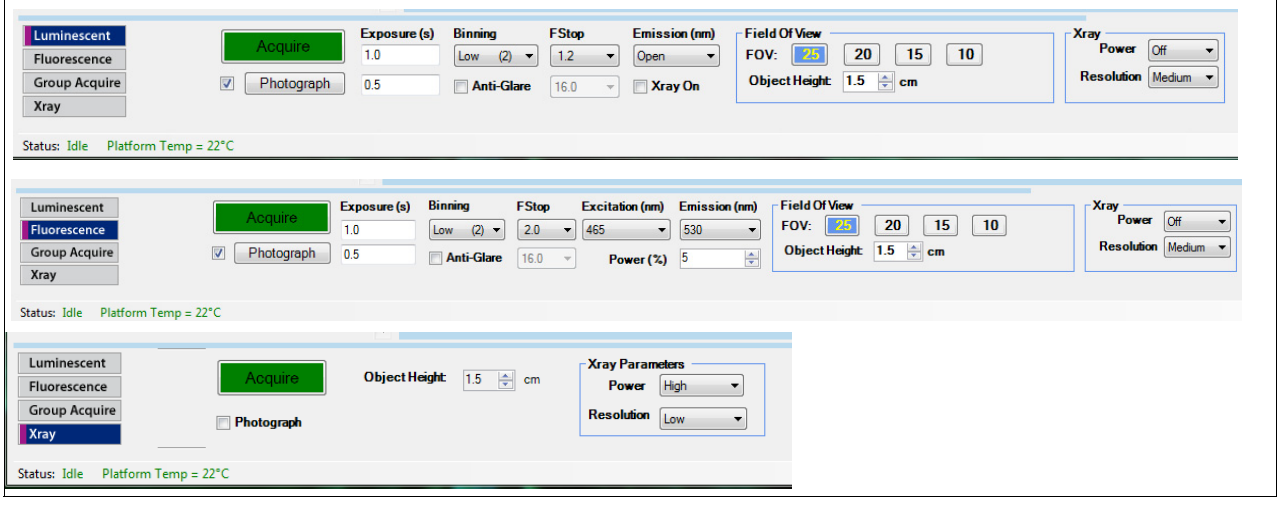
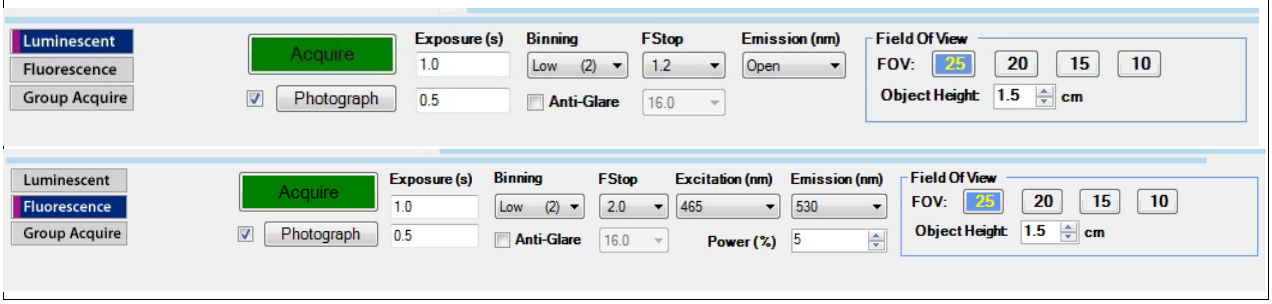


Figure 3.6 The SPECTRAL ami HT Single Instrument Modes and Their Options



2. Decide whether or not to take a photograph with your fluorescent or luminescent image:
   a. If a check mark is not already present there, click the box next **Photograph** and leave the default exposure time as 0.5 seconds. The default exposure time of 0.5 seconds should not be changed under normal circumstances.

NOTE: If photographs are saturating with the normal photograph settings (due to shiny surfaces, for example), click to check the box next to **anti-glare**. The **anti-glare** photograph mode will take about four times as long, but will result very little or no saturated pixels or blooming in the photograph. When using this special **anti-glare** photograph mode, the exposure time of the photograph usually needs to be increased to prevent grainy photographs.

   b. De-select (uncheck) **Photograph** if a photograph is not desired.
3. Enter an exposure time for the fluorescent or luminescent image in the top **Exposure** field. Dimmer light sources and reporters require longer exposures than brighter ones to be detected.
4. Select a binning level. Sensitivity increases with binning level:
   - **None** – No pixels are binned (This level is sometimes referred to as “bin 1”).
   - **Low** – 2 × 2 binning
   - **Medium** – 4 × 4 binning
   - **Heavy** – 8× 8 binning
5. Select an **f/stop** from the drop-down list. The f-stop setting determines the magnitude of the light collection vs. depth of field trade-off:
   - F/1.2 – Maximum light collection and greatest sensitivity, but smallest depth of field
   - F/16 – Minimum light collection and least sensitivity but greatest depth of field

NOTE: F/1.2 is not an option for fluorescent imaging.

6. For luminescent imaging only (and only available on the SPECTRAL ami HTX): select (check) **Xray on** in order to illuminate the object(s) on the platform with x-rays during a luminescent exposure (i.e. x-ray induced optical luminescence image).
7. For fluorescent imaging only:
   a. Select an appropriate wavelength for your fluorescent probe from the **Excitation** drop-down list. The **Excitation** light is the light which the object is illuminated with.
   b. Enter a power level (100% or less) for the excitation light source from the **Power** (%) drop-down list.
8. Select an appropriate wavelength from the **Emission** drop-down list.

Emission is the light produced by the imaged object, either on its own (via bioluminescence) or when excited by light of a shorter wavelength (i.e. fluorescence). An emission filter must be used for fluorescence imaging and may be used for luminescence imaging.

The **Open** option–available only for luminescence–allows all the wavelengths of the emitted light to be detected by the CCD camera, because no emission filter is used.

NOTE: For fluorescence imaging, the aura software requires an **Emission** wavelength at least 35nm greater than the **Excitation** wavelength.

9. Select a field of view (**FOV**, size of the area to be imaged) (see Table A.1 on page 69).

Figure 3.7 FOV Options for Horizontal Field of View (cm)

10. Enter the object height in cm (see Figure 3.8 below).

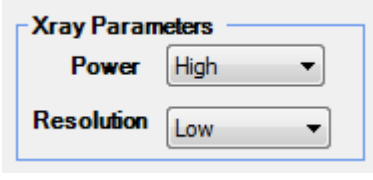
Figure 3.8 Object Height

IMPORTANT: Measure the tallest vertical object height as accurately as possible. The factory-set absolute radiance calibration of each instrument corrects for the stage position, and includes an adjustment for the height of the object being imaged. Additionally, X-ray images have their own FOV setting which positions the imaging platform very close to the top of the imaging chamber.

CAUTION: Ensure that accurate subject height is entered in the control panel, otherwise damage to both the instrument and the object could result.

11. If acquiring an X-ray image either by itself or with together with a luminescent or fluorescent image (refer to Figure 3.9):

Figure 3.9 X-Ray Options



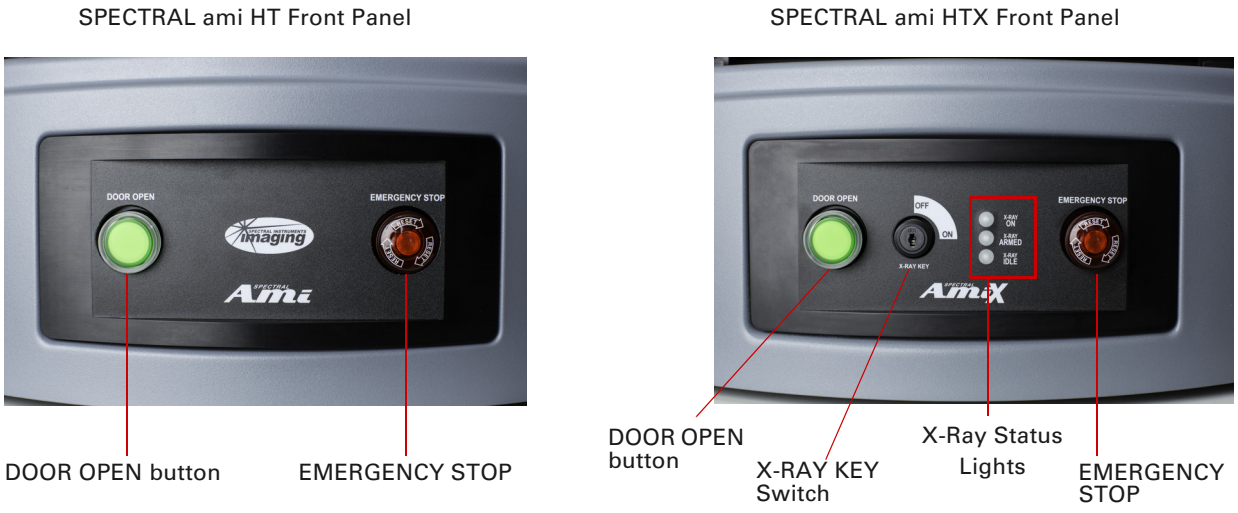
   a. Select the **High** or **Low** power.
   b. Select **High** (no binning) or **Low** (2 × 2 binning) resolution.
   c. Make sure that the X-ray key is turned to the ON position and the emergency stop button is set to the ready position. The emergency stop button light will be continuously lit while in the RESET position and blinking while depressed. (Figure 3.10).

NOTE: X-ray imaging is only available on the SPECTRAL ami HTX.

12. Open the imaging system door by pushing the illuminated green DOOR OPEN button on the front panel (Figure 3.10).

NOTE: See Table 4.11 on page 49 for more information on the front control panel.

Figure 3.10 ami HT and ami HTX Front Control Panels



13. Place object(s) or anesthetized animal(s) on the imaging platform and close the imaging system door.
14. Click **Acquire** in the acquisition controls when ready to begin acquisition.

    The workspace displays the image when acquisition is finished (Figure 3.12). While the image is being taken, a small window outlined in red (Figure 3.11) will appear in the bottom-right corner with options to save user-defined information about the image. When the image acquisition is finished, this window will change to a green border and shift to the bottom-left corner of the screen (as seen in Figure 3.12).

Figure 3.11 Enter Tag Information During Acquisition

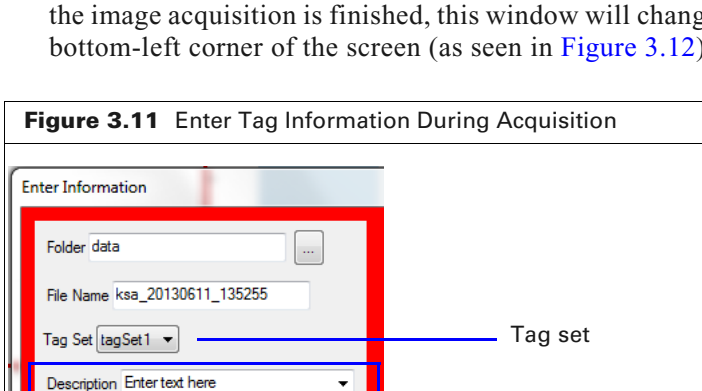
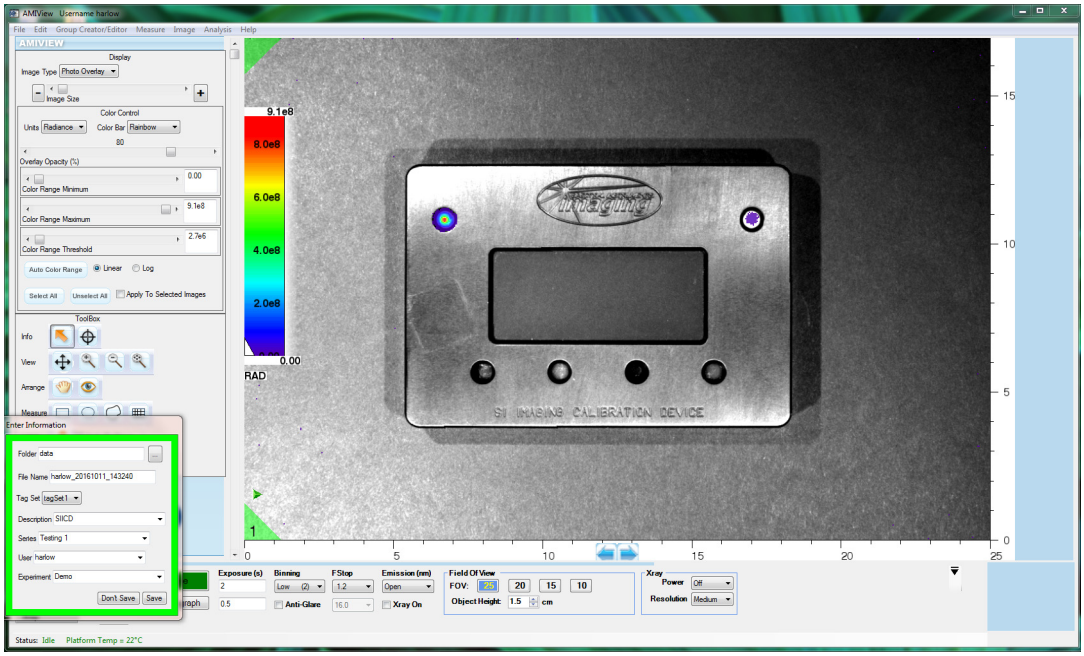


Figure 3.12 Example Image – Spectral Instruments Imaging Calibration Device



NOTE: Entering tag information about the image is optional, but recommended since the Image Manager uses tags to organize and search image data. See page 41 for more information on the Image Manager.

15. Click **Save** or **Don't Save** in the Enter Information box after acquisition is finished.

NOTE: If you choose **Don't Save**, then after acquisition decide to save the image, choose **File** → **Save** on the menu bar. The image will be saved with the tag set and values which were in the green box when **Don't Save** was clicked. Also, aura automatically asks the user whether or not to save an unsaved image before another one is acquired.

Table 3.1 Enter Information Box

Item	Description
Folder	The folder name where image data will be saved. The default folder is the "data" folder that was created for the user name (C:\Users*Windows User**aura user name*\data). Click the ... button to select a different folder. **Note:** It is recommended to create subfolders in the "data" folder in order to organize saved images and to decrease the time Image Manager spends cataloging the images when it is launched. See page 41 for more information on the Image Manager.
File Name	The file name (with an extension of .ami) generated by aura software which contains the date and time of the acquisition. **Note:** Changing the default file name is not recommended because identifying information in the file name is not searchable. Instead use tag values to create identifying information. If necessary, create custom tag sets (see *Creating a Tag Set* on page 30).

Table 3.1 Enter Information Box

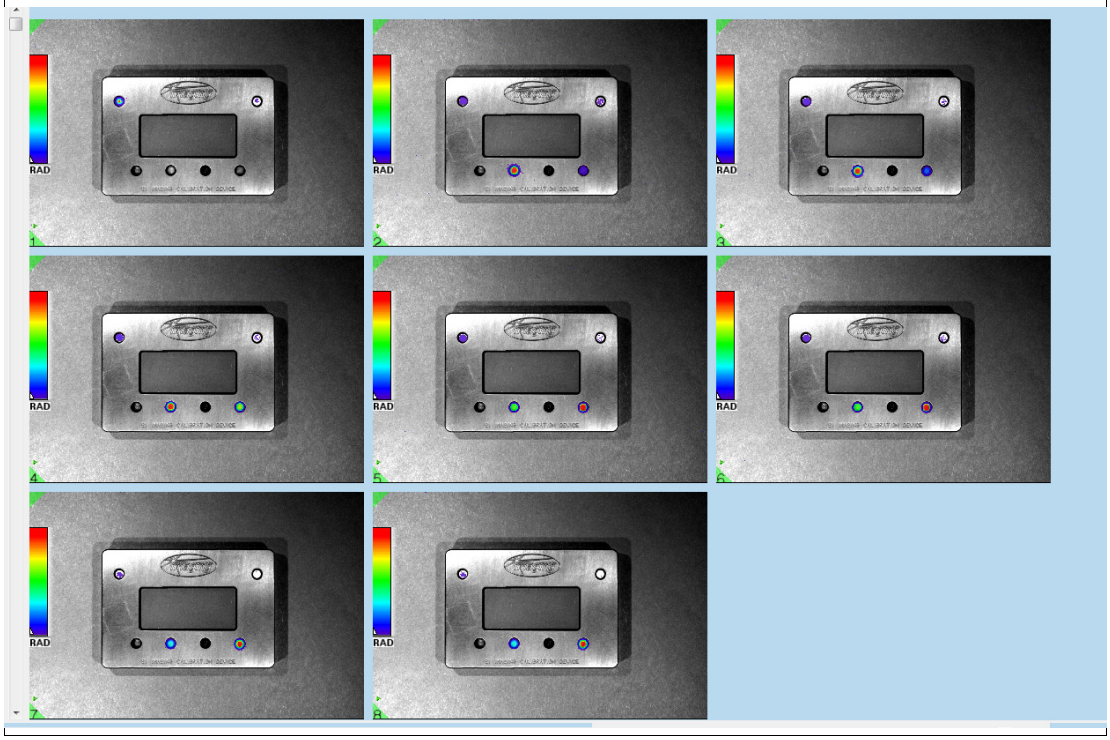
Item	Description
Tag Set	A set of tags (descriptive identifiers) with user-defined values. Figure 3.11 shows the default tag set "tagSet1" which includes the following tags: ■ Description – Specific image conditions (e.g. control or variable). ■ Series – General topic of research. ■ User – Your user name. ■ Experiment – Question or area of study. Apply tag values to the image data by entering information for each tag or making a selection from the from the drop-down lists. See page 28 for instructions on creating and managing tag sets.

3.5 Image Acquisition – Group Mode

Multiple images can be acquired, one after the other, as a "group". Grouped images are saved together in one .ami file. All of the images are displayed when the group is loaded (Figure 3.13).

To acquire a group image, first define a group protocol which specifies the imaging modes and imaging parameters for each image in the group, then acquire the group using the protocol.

Figure 3.13 Example Group Image



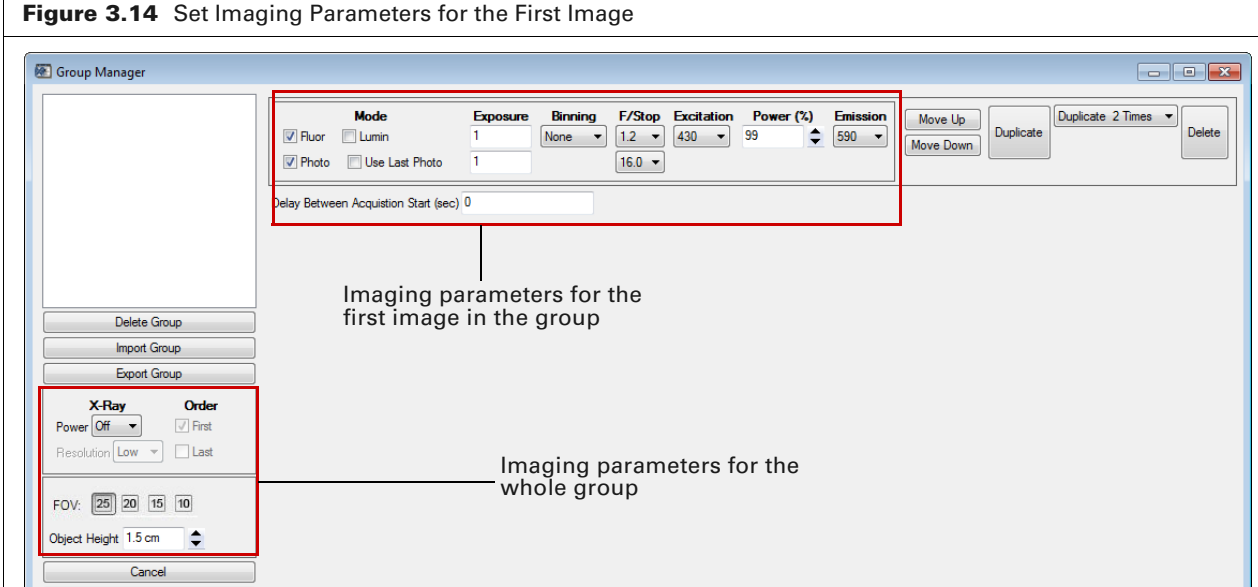
Creating a Group Protocol

This section explains how to create a group protocol which specifies the imaging parameters for each image in a group.

You can also create a protocol by editing a protocol and saving it to a new name. See page 24 for instructions.

1. Select **Group Creator/Editor → Group Creator/Editor** on the menu bar.
   The Group Manager opens (Figure 3.14).

Figure 3.14 Set Imaging Parameters for the First Image



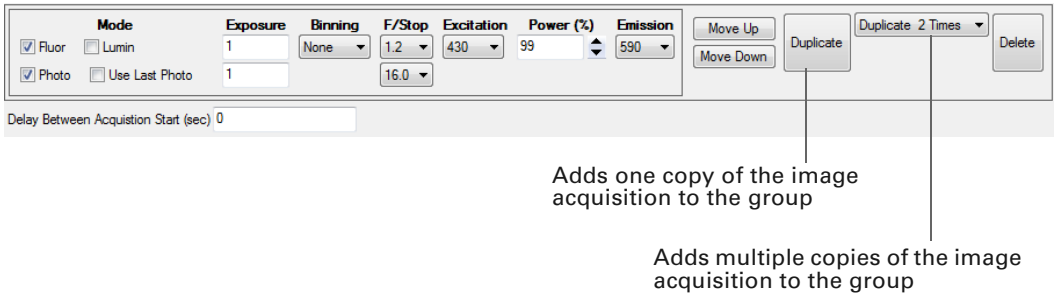
2. Set up the first image acquisition in the Group Manager by following step 2 to step 12 on page 17 to page 19. See Appendix A on page 69 for details on the acquisition parameters.
3. If acquiring an X-ray image, choose an **Order** option:
   - **First** – The X-ray image will be the first image acquired.
   - **Last** – The X-ray image will be the last image acquired.

> **NOTE:** X-ray imaging is only available on the SPECTRAL ami HTX. Only one X-ray image can be acquired per group protocol.

4. If acquiring an X-ray image, choose an option for **Power** and an option for **Binning**:
   - see the "X-ray" section of Table A.1 on page 69 for more information about these X-ray settings.
5. Optional: Enter a time delay (in seconds) between the start of the first image acquisition and the start of the second acquisition into the **Delay Between Acquisition Start** field. The default delay time is zero seconds (second acquisition begins immediately). The actual delay will be the value entered here minus the acquisition and readout times of the previous image. For example, if a 20 second delay is entered, the actual delay between the end of the readout of the previous image and the start of the next acquisition will only be about 13 seconds, depending on exposure time and binning level.
6. To add images to the group, copy the first image acquisition and edit the imaging parameters. Do either of the following to add image acquisition(s) to the group:
   - Click **Duplicate** to copy and add one image acquisition (Figure 3.15).
   - Make a selection from the **Duplicate 2 Times** drop-down list to add multiple copies (2 to 11 copies) of the image acquisition to the group.

> **NOTE:** If an image includes a photograph (usually the first image in a group), the same photograph can be used for visual reference in the subsequent images of the group. Choose **Use Last Photo** for the images that follow the image which includes a photograph. Re-using the same photo image shortens the total acquisition time of the group and results in a smaller size for the saved .ami file.

Figure 3.15 Duplicate the Image Acquisition



7. Click **Move Up** or **Move Down** to reorder an image acquisition in the group.
8. Save the group protocol when setup is finished.
   a. Click **Save**.
   b. Enter a group name in the box that appears and click **OK** (Figure 3.16). The protocol appears in the Group Manager directory list (Figure 3.17).

Figure 3.16 Enter a Group Protocol Name

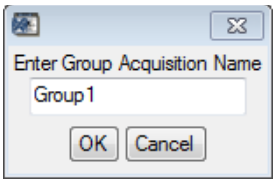
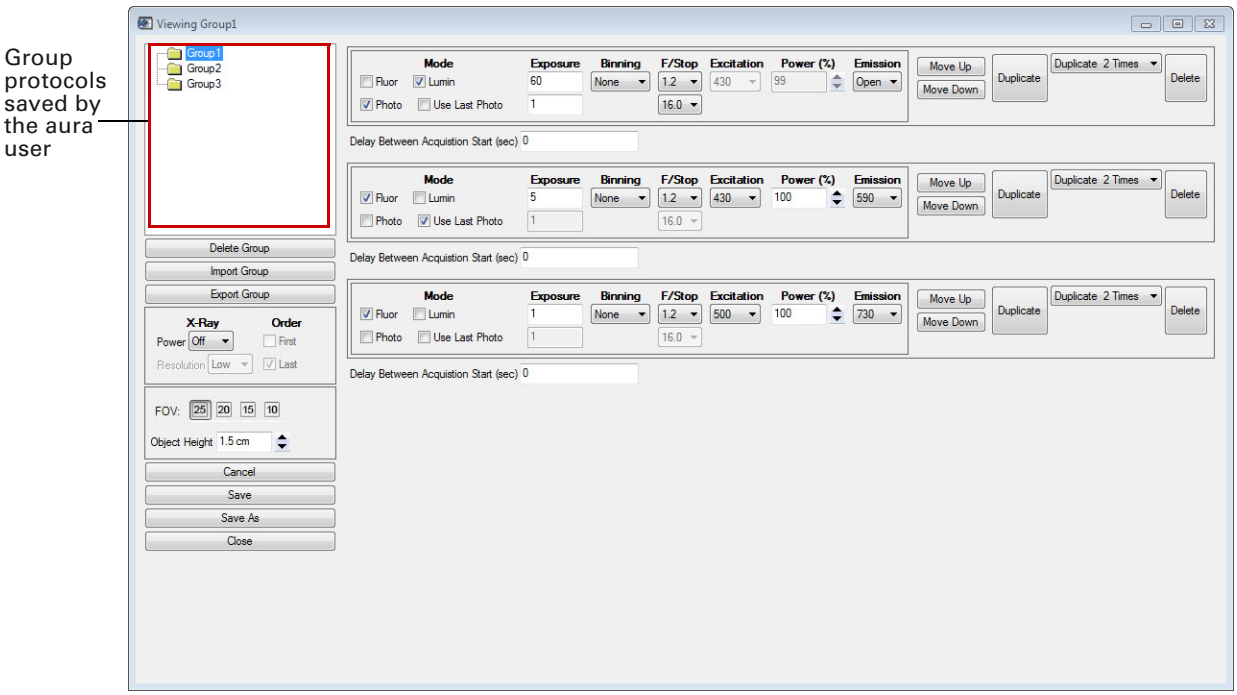


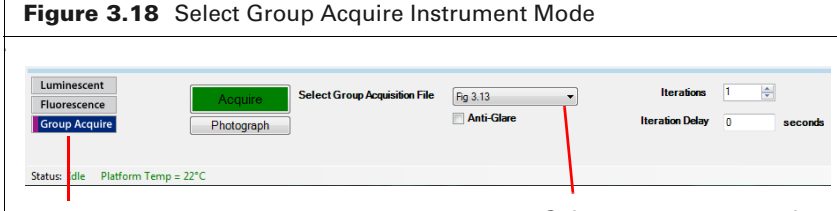
Figure 3.17 Group Manager Directory List



Group Acquisition

1. Select **Group Acquire** instrument mode in the acquisition controls (Figure 3.18).
2. Make a selection from the **Protocol** drop-down list.

Figure 3.18 Select Group Acquire Instrument Mode



3. If acquiring an X-ray image:
   a. Verify that the X-ray key is in the ON position (Figure 3.19).
   b. Reset the emergency stop button to the ready position if the button is blinking.

NOTE: X-ray imaging is only available on SPECTRAL ami HTX Imaging System.

Figure 3.19 SPECTRAL ami HTX X-Ray Key On

4. Follow Step 12 on page 19 to Step 15 on page 20.
   Images are displayed as they are acquired.

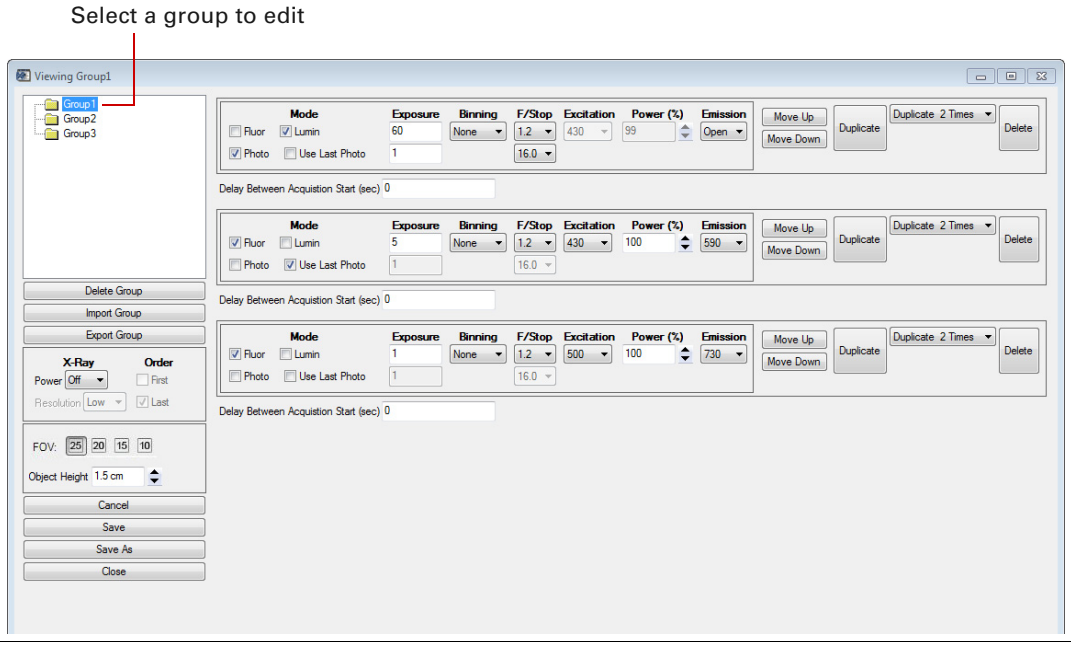
Editing a Group Protocol

Group protocols may be edited. Editing a protocol and saving it to a new name is convenient way to create a new protocol.

To edit a protocol:

1. Open the Group Manager by selecting **Group Creator/Editor → Group Creator/Editor** on the menu bar.
2. Click the protocol in the directory tree that you want to edit.
   The imaging parameter settings are displayed (Figure 3.20).

Figure 3.20 Group Editor



3. Edit the acquisition parameters following Step 2 on page 22 to Step 7 on page 23.
4. Do either of the following:
   - To overwrite the protocol, click **Save**.
   - To save as a new protocol, click **Save As**, enter a new protocol name in the box that appears, and click **OK**.

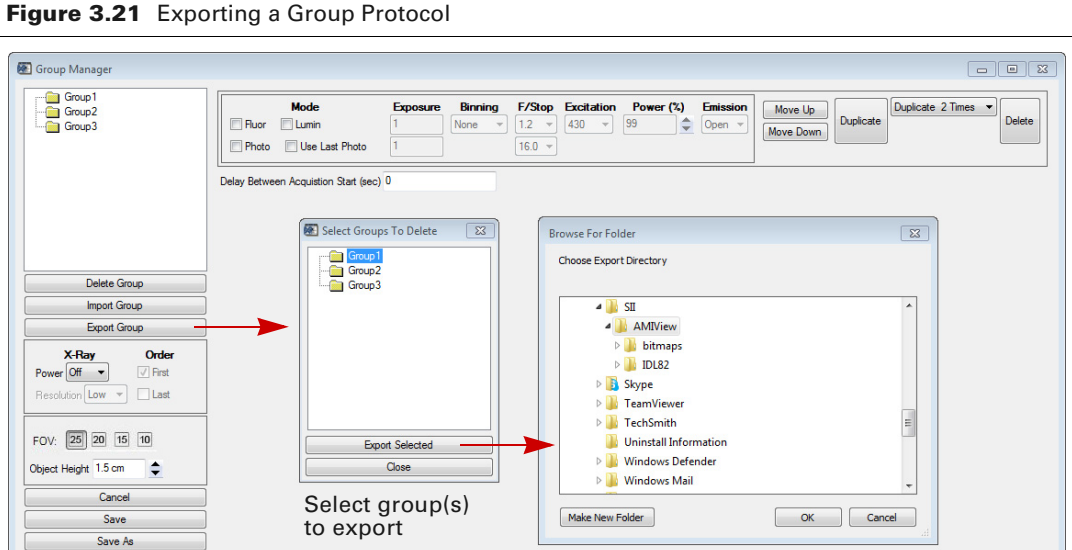
3.6 Managing Group Protocols

Exporting or Importing Protocols

Exporting and importing group protocols provides a convenient way to share protocols.

1. Open the Group Manager by selecting **Group Creator/Editor → Group Creator/Editor** on the menu bar.
2. To export a protocol:
   a. Click **Export Group** (Figure 3.21).
   b. Select the group(s) for export in the box that appears and click **Export Selected**.
   c. Choose the export directory in the Browse for Folder dialog box that appears and click **OK**.
   d. Click **Close** in the Select Groups To Export dialog box.

Figure 3.21 Exporting a Group Protocol



3. To import a protocol:

   a. Click **Import Group** in the Group Manager (Figure 3.22).

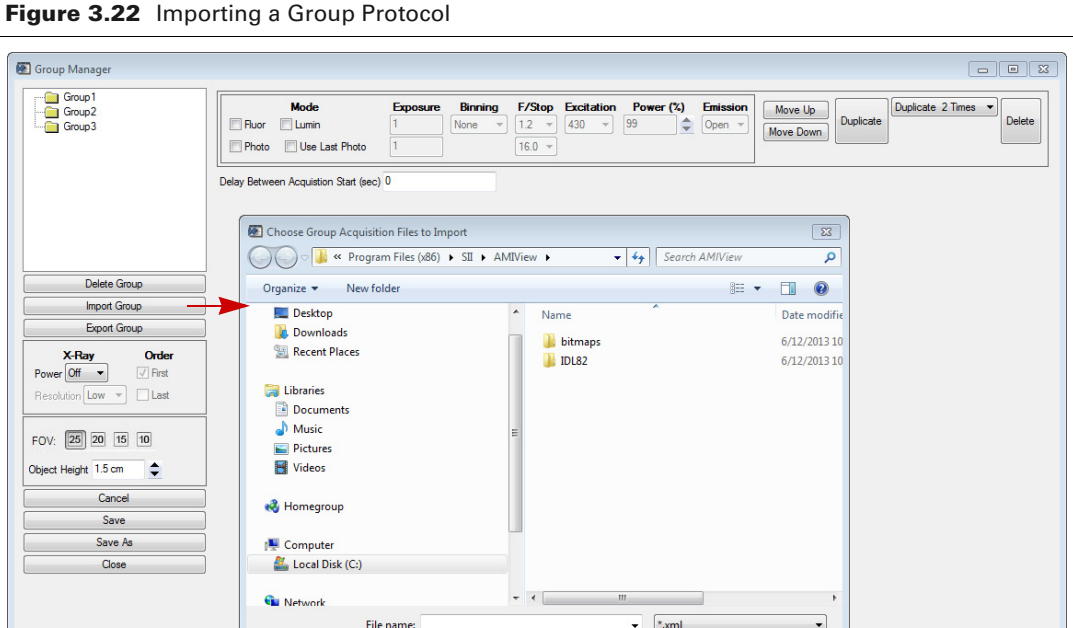
   b. In the dialog box that appears, navigate to the directory that contains the protocol (.xml) to be imported, and click **Open**.

      The protocol is copied to the folder that is associated with your user name (see Table 2.1 on page 6).

> **NOTE:** If there is a protocol with the same name in the destination folder, the user will be asked whether or not to overwrite the existing file.

   c. Close the dialog box.

Figure 3.22 Importing a Group Protocol

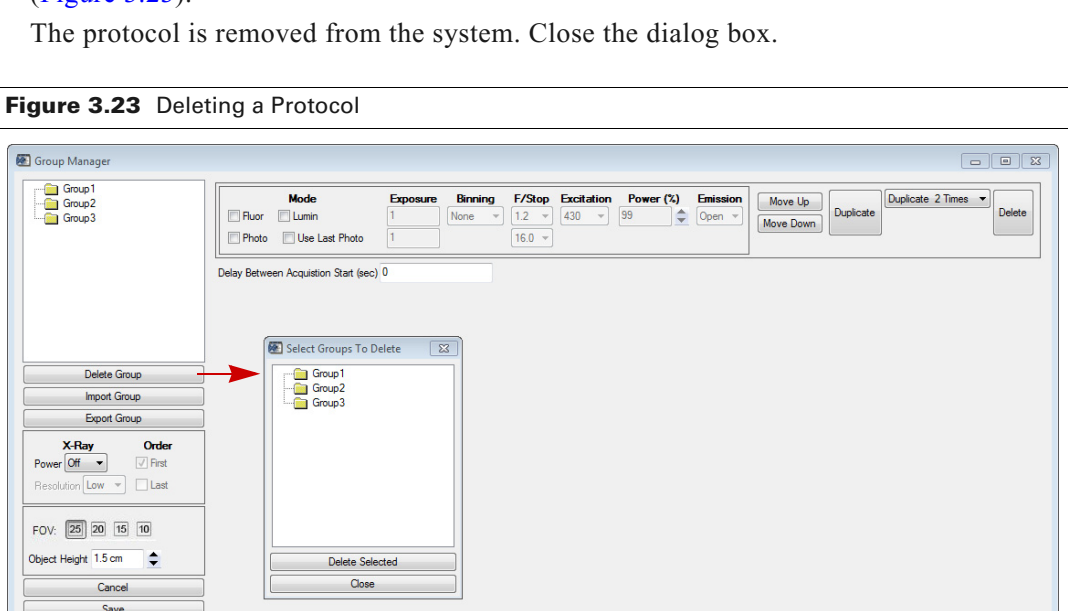


Deleting a Protocol

1. Click **Delete Group** in the Group Manager.
2. Select a group to delete in the dialog box that appears and click **Delete Selected** (Figure 3.23).

   The protocol is removed from the system. Close the dialog box.

Figure 3.23 Deleting a Protocol



Iterations of Group Protocols

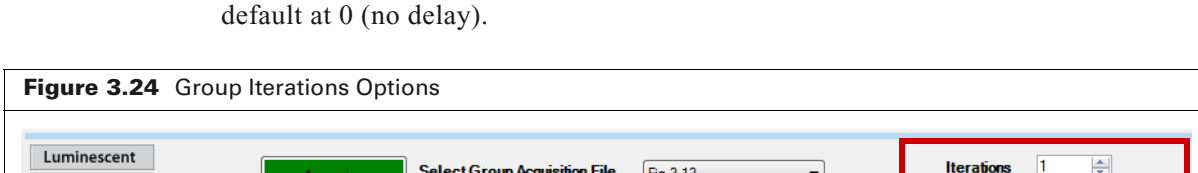
The same group protocol can be acquired multiple times and automatically saved in a separate file after each iteration. Users can insert delays between each iteration of a group just as easily as they can insert delays between images in a group protocol. Iterations of group protocols are necessary when attempting to acquire and analyze more images than is possible in a single group due to software memory limits. This is because users can acquire many group images composed of a few images rather than one single group of many images. Since group iterations allow experimenters to acquire a practically unlimited number of single images, overnight and other multi-hour imaging sessions with many time points are easily accomplished.

Since each group image is saved to disk immediately after iteration, iterations of groups of one are useful for acquiring images that are very important (i.e. impractical to retake) or very long. For example, if an experimenter starts acquiring 10 iterations of a group protocol of one image and a power blackout occurs during the sixth iteration, the previous five images are not lost. In contrast, if a power blackout or other failure occurs before a group completes, no images are saved.

Acquiring Multiple Iterations of a Group Protocol

1. Click the **Group Acquire** button on the left hand side of the acquisition section in order to switch to group mode. The iteration options will be at the right hand side of the acquisition controls area when in **Group Acquire** mode (See red box in Figure 3.24 below.
2. Select a group protocol to acquire.
3. Change the value in the **Iterations** field from 1 (the default) to the desired number of iterations.
4. Enter a delay (in seconds) between iterations in the **Iteration Delay** field or leave the default at 0 (no delay).

Figure 3.24 Group Iterations Options



5. Enter user information during or after all iterations complete.
6. Click **Save** or **Don't Save** after all iterations complete.

NOTE: After each iteration of the group is done acquiring, that group image is saved to disk. If **Don't Save** is clicked at the end of the last iteration and the user declines again to save the image(s) in the window which appears before the next acquisition starts, all those previously saved files will be erased.

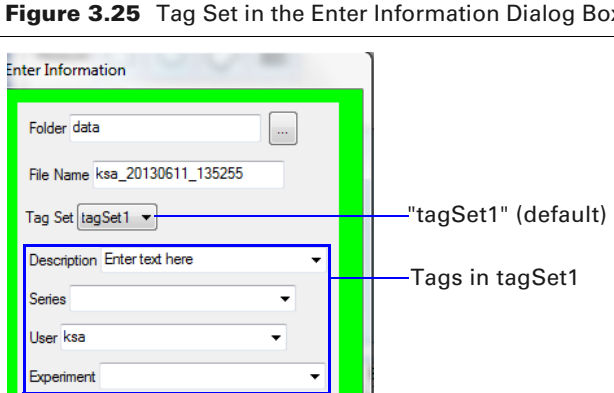
3.7 Tag Sets

A tag set is a set of descriptive identifiers for an image or group (Figure 3.25). Tag values are specified at the time of acquisition and are saved with the image data (.ami), and "tagSet1" is the default tag set (see Table 3.1 on page 20).

The Image Manager uses tag information to organize, filter and search image data. See Chapter 4, *Viewing Images on page 41* for more information on the Image Manager.

This section of the manual explains how to create and manage tag sets.

Figure 3.25 Tag Set in the Enter Information Dialog Box

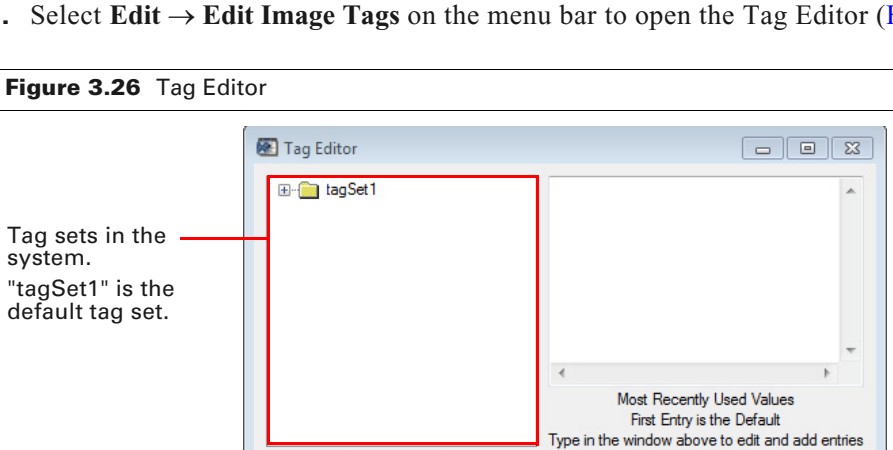


About the Tag Editor

Use the Tag Editor to view, create, or manage tag sets.

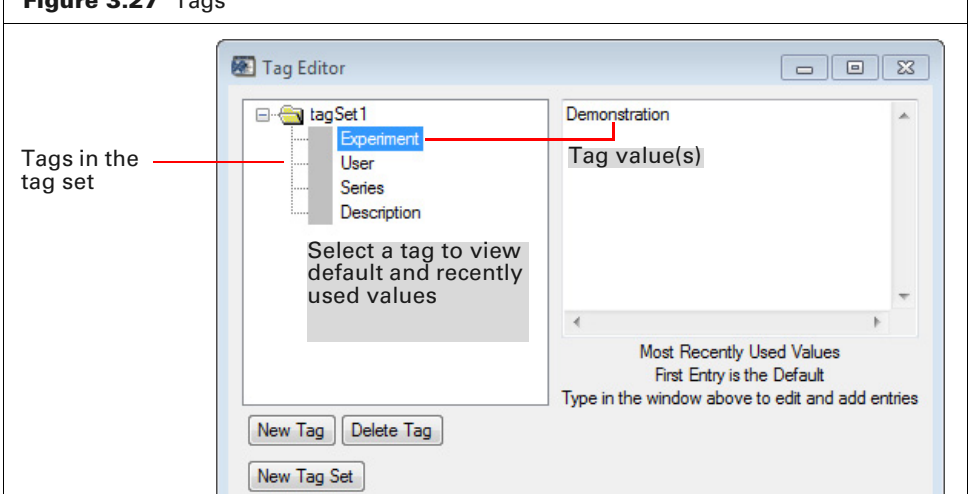
1. Select **Edit** → **Edit Image Tags** on the menu bar to open the Tag Editor (Figure 3.26).

Figure 3.26 Tag Editor



2. Expand a tag set node to view the tags in the set and recently used tag values.

Figure 3.27 Tags



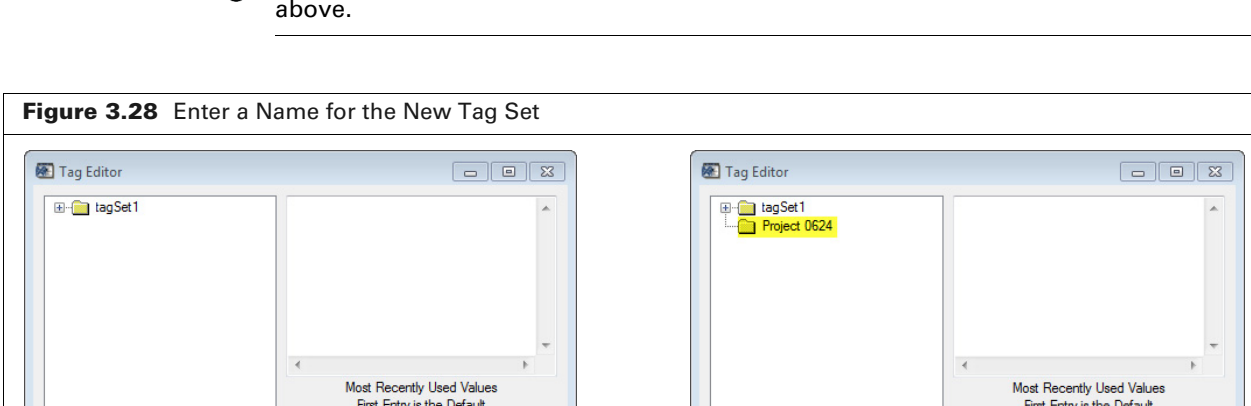
Creating a Tag Set

1. Open the Tag Editor (select **Edit** → **Edit Image Tags** on the menu bar).
2. Click **New Tag Set** in the Tag Editor, enter a name for the tag set in the box that appears, and click **OK** (Figure 3.28).

   The Tag Editor shows the new tag set. The tag set will be available in the **Enter Information** dialog box (Figure 3.25 on page 29).

NOTE: Tag set names cannot be edited in the Tag Editor. If you need to change the tag set name, delete the tag set name (select the tag set and click **Delete**), and repeat step 2 above.

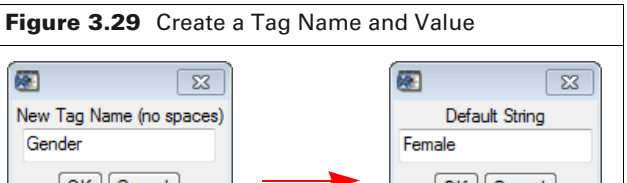
Figure 3.28 Enter a Name for the New Tag Set



3. Add a tag to the tag set.
   a. Select a tag set and click **New Tag**.

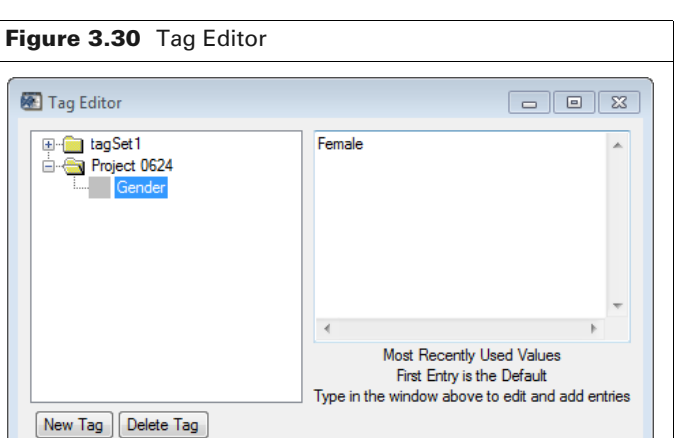
b. Enter the tag name in the box that appears and click **OK** (Figure 3.29).

Figure 3.29 Create a Tag Name and Value



c. Enter a tag value in the next box that appears and click **OK** (Figure 3.29). The tag name and default value appear in the Tag Editor (Figure 3.30).

Figure 3.30 Tag Editor



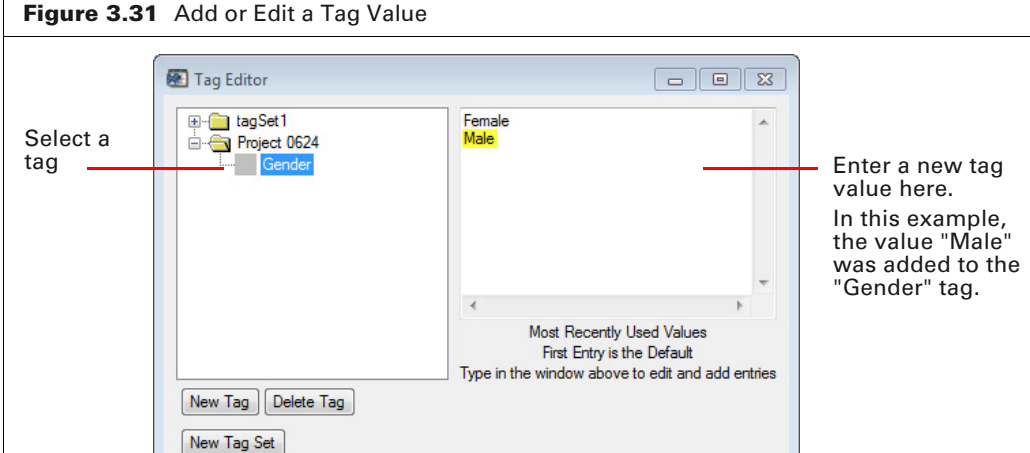
d. To add another tag value:

1) Select the tag name (Figure 3.31).

2) Enter a new value in the right-hand pane (Figure 3.31). Repeat this step to add another value.

NOTE: Tag values can be edited in the right-hand pane.

Figure 3.31 Add or Edit a Tag Value



4. Repeat step 3 to add tags and values to the tag set.

   The tags will appear in the **Enter Information** dialog box when the appropriate tag set is selected, and the values will be available in the tag drop-down lists (Figure 3.25 on page 29).

5. Click **Accept** when you are done adding tags and values to the tag set.

   The new tag set (including tags and tag values) are saved to the system. The Tag Editor closes.

NOTE: If you close the Tag Editor by clicking **Cancel** or the button, the tag set will not be saved to the system.

Managing Tag Sets and Tags

NOTE: Click Accept in the Tag Editor when you finish making changes. If you close the Tag Editor by clicking **Cancel** or the button, the changes will not be saved to the system.

Tag Sets

Tag sets can be deleted from the system. However, a tag set name cannot be edited.

To delete a tag set:

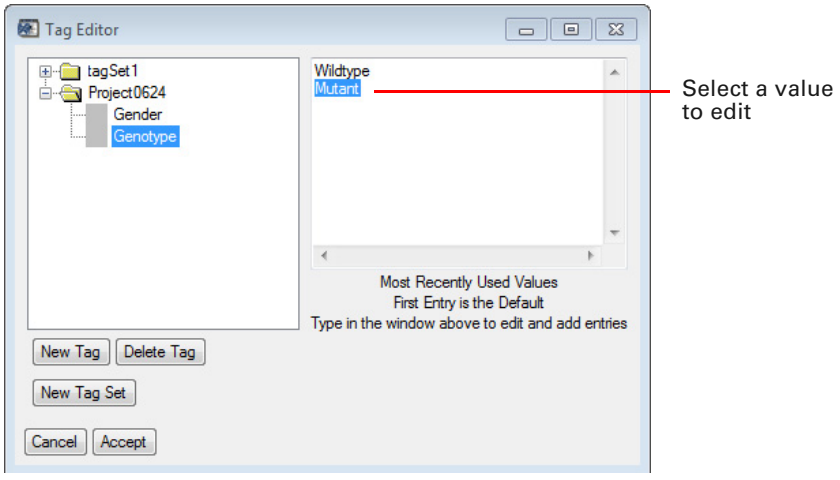
1. Select the tag set in the Tag Editor.
2. Click **Delete Tag**.
3. Click **Accept**.

Tags

To edit a tag value:

1. Select the tag name in the Tag Editor (Figure 3.32).
2. Select a tag value in the right-hand pane by highlighting it with the text cursor (click and drag over the text). Type in the new tag value.

Figure 3.32 Editing a Tag Value

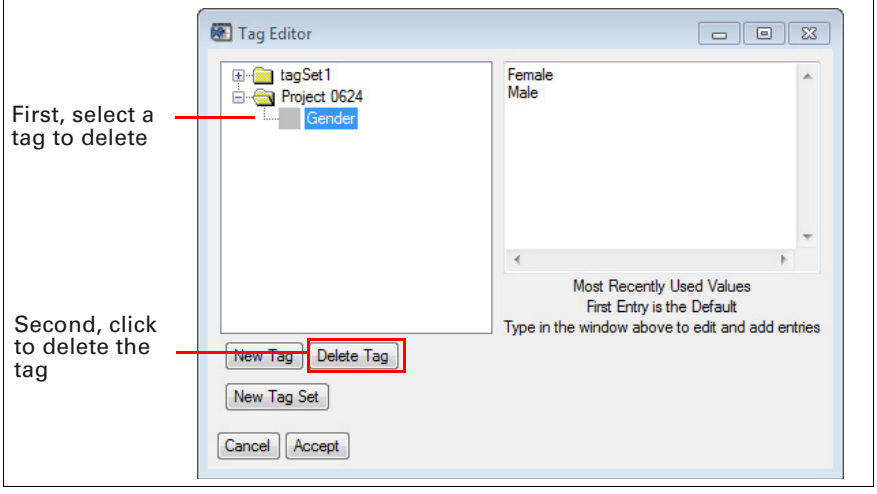


3. Click **Accept**.

To delete a tag:

1. Select a tag in the Tag Editor and click **Delete Tag** (Figure 3.33).
2. Click **Accept**.

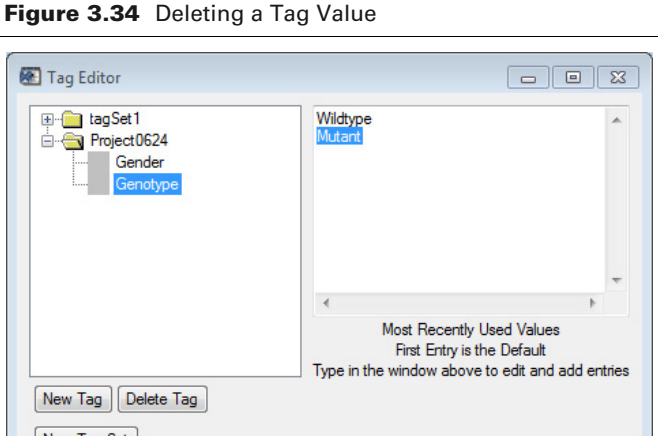
Figure 3.33 Deleting a Tag



To delete a tag value:

1. Select a tag name and tag value in the Tag Editor (Figure 3.34).
2. Press the **Delete** key on the keyboard.
3. Click **Accept**.

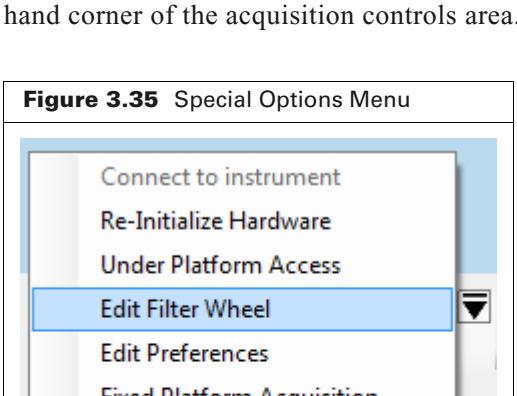
Figure 3.34 Deleting a Tag Value



3.8 User Acquisition Preferences

aura users can change many of the default acquisition parameters for the single image acquisition modes. The **Edit Preferences** window is accessible through the Special Options menu (Figure 3.35)accessed by clicking on the downward arrow ▼ located in the top right-hand corner of the acquisition controls area.

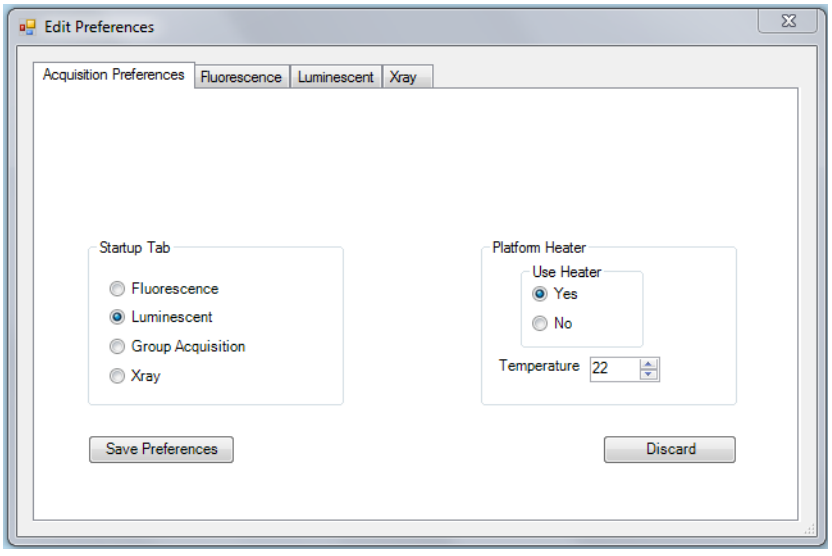
Figure 3.35 Special Options Menu



Platform Heater Control

By default, the platform is heated to stabilize the body temperature of anesthetized living subjects. To disable platform heating or to adjust the platform surface temperature, do the following once the **Edit Preferences** window appears (Figure 3.36):

Figure 3.36 Edit Preferences Window



1. Click the tab labeled **Acquisition Preferences**.
2. In the Platform Heater section, Click **Yes** under **Use Heater** if you wish to turn on platform heating. Select **No** to leave the platform at room temperature.
3. If you selected **Yes** in the previous step, enter the desired platform surface temperature into the **Temperature** field. The default for all new users is 37°C
4. Click the **Save Preferences** button to save changes and close the **Edit Preferences** window.

Imaging Preferences

In addition to the platform heater settings (see above) the **Edit Preferences** window allows users to change and save defaults for image acquisitions.

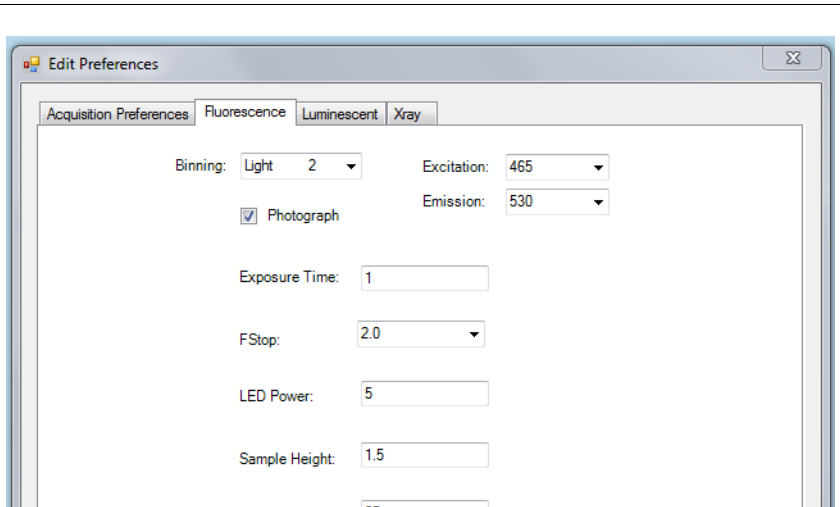
Changing the Default Imaging Mode

1. Click either the **Fluorescent**, **Luminescent**, **Group Acquisition** or **Xray** options under the **Startup Tab** section of the **Acquisition Preferences** tab (see Figure 3.36 above). The **Xray** option will only be displayed if the instrument is an SPECTRAL ami HTX.
2. Click the **Save Preferences** button to save changes and close the **Edit Preferences** window.

Changing Fluorescence Imaging Defaults

1. Click the **Fluorescence** tab.
2. Make your changes to fluorescent imaging default parameters (Figure 3.37).

Figure 3.37 Fluorescence Imaging Defaults

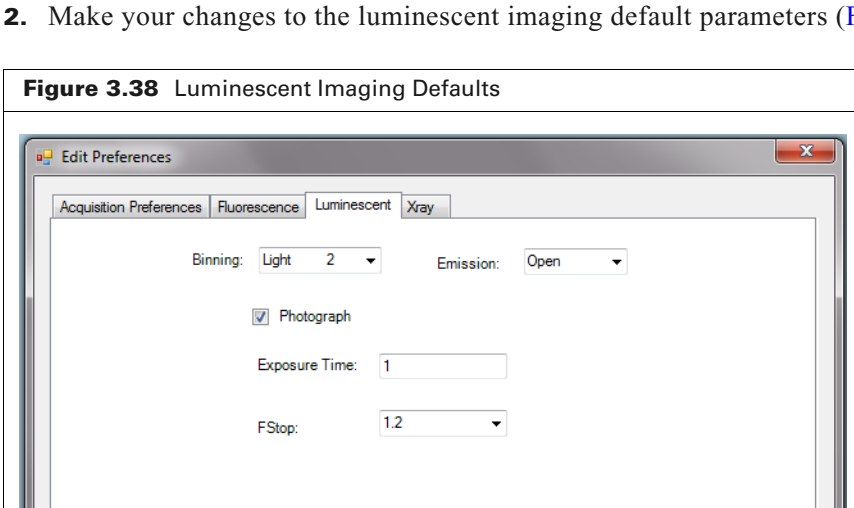


3. Click on the **Acquisition Preferences** tab.
4. Click the **Save Preferences** button to save changes and close the **Edit Preferences** window.

Changing Luminescent Imaging Defaults

1. Click the **Luminescent** tab.
2. Make your changes to the luminescent imaging default parameters (Figure 3.38)

Figure 3.38 Luminescent Imaging Defaults



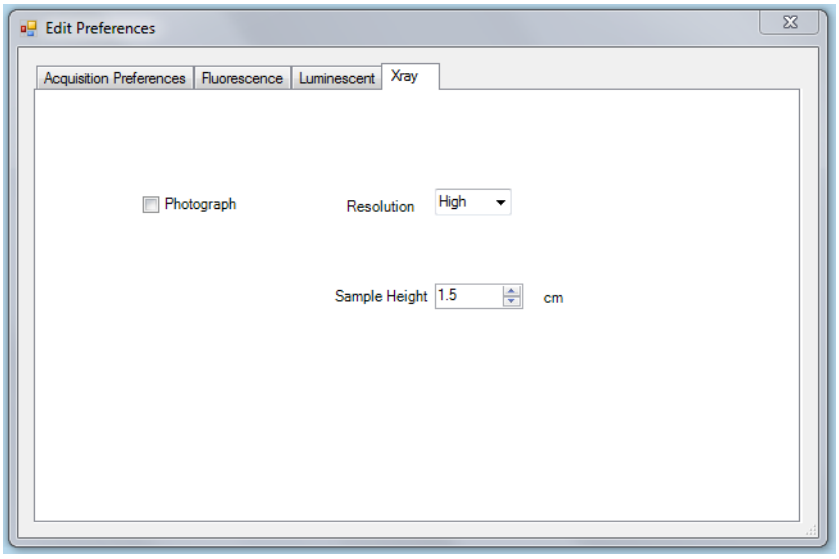
3. Click on the **Acquisition Preferences** tab.
4. Click the **Save Preferences** button to save changes and close the **Edit Preferences** window.

Changing the X-Ray Imaging Defaults

NOTE: This tab will only appear if the instrument is a SPECTRAL ami HTX

1. Click the **Xray** tab.
2. Make your changes to the luminescent imaging default parameters (Figure 3.39).

Figure 3.39 X-Ray Imaging Defaults



3. Click on the **Acquisition Preferences** tab.
4. Click the **Save Preferences** button to save changes and close the **Edit Preferences** window.

4

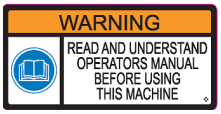
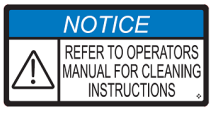
SPECTRAL ami HT and ami HTX Imaging System

4.1 Safety Information

Instrument Safety Labels

Table 4.1 shows the safety labels found on the SPECTRAL ami HT or ami HTX Imaging System. Follow the instructions on all safety labels for maximum personal safety and optimal instrument performance.

Table 4.1 Instrument Safety Labels

Label	Description
	Do not use flammable anesthesia.
	Read and understand this manual before operating the instrument.
	Do not attempt to open the instrument door when x-rays are being produced.
	Keep fingers and hands clear while closing the instrument door.
	See *Cleaning the Instrument*, page 57 for detailed cleaning instructions. Contact Spectral Instruments Imaging for further information (see page 4).

Instrument Symbols

Table 4.2 Instrument Symbols

Symbol	Description
	Power switch OFF symbol. Push this side of the AC input switch to turn off power to the imaging system.
	Power switch ON symbol. Push this side of the AC input switch to turn on power to the imaging system.
	This symbol identifies the protective ground.

About These Instructions

CAUTION: Only personnel who have read this manual in its entirety should attempt to operate the ami HT or ami HTX Imaging System.

Follow the instructions exactly as stated in this manual to avoid degrading instrument performance, damaging the instrument, or personal injury.

IMPORTANT: Operating the ami HT or ami HTX Imaging System in a manner other than that specified in this manual will void the warranty.

Safety Standards Compliance

Spectral Instruments Imaging, LLC declares that the ami HT and ami HTX imaging systems have been certified to or are compliant with the following:

IEC/EN 61010-1:2010 (Third Edition) *Safety requirements for electrical equipment for measurement, control and laboratory use*

CLASS 8721 06 - *Laboratory Electrical Equipment*

CAN/CSA-C22.2 No. 61010-1-12, UL Std. No 61010-1 (3rd Edition) *Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use*

CLASS 8721 86 - *Laboratory Electrical Equipment (Certified to U.S. Standards)* 21 CFR 1020.40, *Cabinet X-Ray Systems*

RoHS2 Directive 2011/65/EU

X-Ray Safety (ami HTX Imaging System Only)

WARNING! X-rays are produced when this equipment is energized during x-ray imaging.

The ami HTX Imaging System includes x-ray imaging capability. The instrument's steel enclosure blocks all x-rays from leaving the instrument, and all instruments must pass an x-ray leakage test before factory release to verify that no x-rays escape the imaging chamber.

Hardware interlocks prevent accidental exposure to x-rays. Check with your organization's radiation control policies as well as local, state, and national regulations before energizing the x-ray equipment. Installation site regulations may require periodic radiation surveys.

CAUTION: Mechanical shock or impact to the ami HTX Imaging System may compromise the instrument's radiation shielding. If this occurs, contact Spectral Instruments Imaging immediately (see page 4) so that the instrument can be serviced and radiation leakage checks performed.

Environmental Requirements

The ami HT or ami HTX Imaging System includes the instrument and several other components. Verify that the following environmental requirements are met before installing the imaging system.

- Ambient temperature of 10 – 35°C.
- No dripping or puddling of water on floor or walls.
- Relative humidity between 20% – 80% non-condensing.
- No excessive vibrations.
- No flammable or corrosive gases.
- Low amounts of dust.
- No sparks or open flames.
- Bench for the imaging system capable of supporting 154 kg (339 lbs).

NOTE: The bench height should allow all users to reach the door handle when the instrument is installed on the bench and the door is open.

- At least six inches of space between the fan vents on the back of imaging system and any wall.

NOTE: Do not install the imaging system in a location where the AC power ON/OFF switch is inaccessible. At least one foot (1′) of space is required on the left side of the instrument (when viewed from the front of the imaging system door).

Moving the Imaging System

Verify that the new location meets the Environmental Requirements listed (see *Environmental Requirements* above)

CAUTION: The instrument's back is heavier than the front. Take precautions to ensure that the unit does not fall backward while being moved.

Power off and unplug all system components before attempting to move the instrument,. At least two people are required to lift the instrument using the side handles at the bottom of the unit.

Electrical Power Considerations

Use only a power cord which meets all applicable safety standards for the local mains voltage. It is recommended to plug the instrument power cord into the surge protector sockets of an uninterruptible power supply (UPS), but not the sockets which provide battery backup.

The UPS must be plugged into an electrical outlet that meets the current requirements of the imaging system and the connected devices (i.e. computer and monitor). Using a dedicated circuit for the imaging system is strongly recommended.

Route power cords so that they won't be tripped over, stepped on, spilled on, rolled over, pinched, cut, or pulled. The imaging system is designed to be powered on continuously, however if the system experiences a power outage, power on the instrument first. Next, power on the computer and monitor. Finally, start the aura software which will re-initialize all motors and cool the CCD camera back down to operating temperature.

System Connectors

Table 4.3, Table 4.4 and Table 4.5 list of the user-accessible connectors for the ami HT or ami HTX Imaging System monitor, computer and instrument respectively.

Table 4.3 Monitor Connectors

Connector	Description
AC Power	Input, 100 – 240 VAC, 50 or 60 Hz
Video	DVI and VGA inputs (DisplayPort available on some units)
USB	2.0, type B female
USB	two to four (depending on unit) USB 2.0, type A female ports

Table 4.4 ami HT and ami HTX Control Computer Connectors

Component	Description
USB	One USB 3.0 and three USB 2.0 in the front. Three USB 3.0 and three USB 2.0 in the rear.
Audio	one output and one microphone input
Ethernet	RJ45, 10/100/1000 Mbps
Video	Output, DisplayPort (and DVI on some systems).
AC Power	Input, 100 – 240 VAC, 50 or 60 Hz
Serial	9 pin D-shaped connector
PS/2	Input, one each for keyboard and mouse, 6 pin mini-DIN in rear

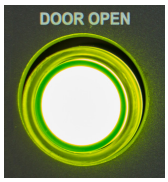
Table 4.5 ami HT and ami HTX Connectors

Component	Description
USB	2.0, type B female
AC input	ON/OFF switch, filtered. Input 100-240 VAC, 50 or 60 Hz
Anesthesia	Two ¼" double barb brass fittings on rear. One each for input and output.
Anesthesia	One 1/8" double barb plastic fitting for input and one ¼" double barb fitting for output on imaging system platform.

Operating Controls

Table 4.6 lists the user-accessible operating controls on the ami HT or ami HTX Imaging System.

Table 4.6 User-Accessible Operating Controls

Operating Control		Description
DOOR OPEN		Illuminated green push button located on the lower front of the imaging system. Push the button when it is illuminated to open the door. The button does not illuminate during image acquisition.
X-RAY KEY ami HTX Imaging System only		The X-RAY KEY switch is located to the right of the door open button and must be turned to the ON position using the x-ray key to enable X-rays. The key must be turned to the OFF position before it can be removed.
EMERGENCY STOP		Illuminated red button on the lower front of the imaging system. Pressing this button turns off power to the X-ray source (ami HTX Imaging System only) and all motors. It must be reset by turning the button fully clockwise. If the EMERGENCY STOP button is pressed while aura software is running, the software must be restarted before imaging can resume. When the ami HT or ami HTX Imaging System is powered on, the EMERGENCY STOP button will blink if it was pressed and reset is required.
ON/OFF power switch		Located at the top rear of the ami HT or ami HTX Imaging System (see Figure 4.9 on page 55). Energizes or de-energizes the imaging system. This switch controls power for the ami HT or ami HTX only. The computer, monitor, and anesthesia pump system (if applicable) each have their own ON/OFF power switch.

Servicing

The ami HT or ami HTX Imaging System has no user-serviceable parts, except for the replaceable emission filters and camera fan. If instrument requires service, contact Spectral Instruments Imaging technical support (see page 4).

4.2 Warnings

Electrical Safety

The ami HT and ami HTX Imaging System meet or exceed applicable standards for electrical safety. It is strongly recommended to connect the imaging system to the surge protected outlets on an uninterruptible power supply (UPS).

NOTE: Due to the high current draw of the instrument, it is recommended to plug the instrument AC power cord into a UPS outlet which provides only surge protection and not battery backup, leaving the battery capacity of the UPS outlet for the computer and monitor (if using a UPS).

VOLTAGE! Do not attempt to insert anything into or take the cover off of the electronics control box located on the top of the unit. Hazardous voltages are inside.

CAUTION: Do not allow any object, especially small pieces of metal to enter the unit through the fan vents. Doing so would damage the unit and customer's equipment, and possibly cause bodily harm.

WARNING! Do not attempt to remove cover of the control circuitry. Doing so will expose line AC voltages as well as connectors carrying large amounts of current.

CAUTION: Adequate ventilation is essential to the proper and safe performance of the ami HT or ami HTX Imaging System. Do not block any vents.

NOTE: There are no user-replaceable fuses in the ami HT or ami HTX Imaging System, computer, or monitor.

X-Ray Safety Considerations (ami HTX Imaging System Only)

This unit is capable of producing x-rays with a maximum energy of 40kV. The instrument's steel enclosure blocks all x-rays from leaving the instrument by design, and all units must pass an x-ray leakage test before leaving the factory to verify that no x-rays escape the imaging chamber.

Hardware safety interlocks prevent the x-ray source from being turned on when:

- The imaging system door is open
- The x-ray key is not in the lock or is turned to the “OFF” position
- The emergency stop button is depressed
- The cable access port cover is not installed (on systems which have the optional cable access port feature)

No x-rays will be produced under any of the above conditions.

Thus, in order to acquire an x-ray image, the door must be closed, the x-ray key must be turned to the ON position, the red emergency stop button must be disengaged (the button must be in the “reset” position by turning the button as indicated in Figure 4.1.) and if the SPECTRAL ami HTX has a cable access port, the port cover must be installed.

NOTE: Once depressed, the Emergency Stop button will continue to flash on and off until it is reset

Figure 4.1 Emergency Stop Button – Reset Position

WARNING! Do not attempt to defeat any of the safety interlocks.

NOTE: If the ami HTX Imaging System will not acquire x-ray images, check all of the safety interlocks.

A red "X-RAY" ON LED at the center of the instrument front panel is illuminated when x-rays are being generated. Pressing the red emergency stop button (Figure 4.1) immediately turns off all motors and the x-ray source.

The front door cannot be opened by pressing the green button when x-rays are being produced, preventing accidental x-ray exposure. Even if the door is forced open, a safety interlock will turn off all power to the x-ray source.

Mechanical Safety

The ami HT and ami HTX Imaging Systems are designed to be mechanically safe to operate. The vertically opening door saves laboratory space requirements and eliminates the risk of injury from accidentally hitting or bumping into an open hinged door.

It is recommended to place both hands on the door handle when closing the door to help keep hands away from possible pinch hazards. The smooth molded plastic covers and curved handle design eliminate sharp edges and thus scraping or cutting hazards.

The instrument's wide footing greatly reduces the risk of the unit tipping over. The door slowly slides open on its own when the green button is pressed (Figure 4.1). There are no pinch hazards during opening.

CAUTION: Ensure that accurate object height is entered in the aura object height field before acquiring an image, otherwise damage to both the instrument and the object could result.

CAUTION: Do not place anything on top of the instrument.

CAUTION: Always close the door using the door handle, not the plastic door cover, after removing hands from inside the unit.

Chemical and Biological Safety

Decontamination is required before requesting service from Spectral Instruments Imaging. Please follow proper laboratory practices and common sense when handling samples or solutions which are toxic, pathogenic, or contain substances which emit ionizing radiation.

4.3 Pre-Installation Checklist

Confirm that the components in Table 4.7 are included in the imaging system shipment.

Table 4.7 ami HT Imaging System

Quantity	Item Description
1	ami HT or ami HTX Imaging System
1	Top Dome Cover
1	Computer
1	Flat Panel Monitor
1	Keyboard
1	USB Optical Mouse
3	Power cables for: ■ Monitor ■ Computer ■ ami HT or ami HTX Imaging System
3	Signal cables (including adapter) for: ■ Monitor ■ ami HT or ami HTX Imaging System (Long USB cable)
1	X-ray Key (for ami HTX Imaging System only)
1	SPECTRAL ami HT and ami HTX Manual

4.4 Optical System

All of the major components of the ami HT or ami HTX optical system are mounted a special assembly which serves as a stable optical rail, keeping the camera, lens filter wheel, excitation LEDs, and imaging platform in precise optical alignment.

The camera which captures images is a custom built air-cooled charged coupled device (CCD) camera that operates at -90°C. The imaging sensor is 1152 pixels x 770 pixels.

Table 4.8 CCD Camera Specifications

Item	Description
Camera Sensor	Back-illuminated, cooled CCD sensor.
Pixel Dimensions	1152 x 770
Pixel Size	22.5 x 22.5 µm

Table 4.8 CCD Camera Specifications (continued)

Item	Description
CCD Size	25.9 x 17.3 mm
Quantum Efficiency	>85% from 500-650 nm, >30% from 400-850 nm
CCD Operating Temperature	– 90°C, air cooled
Dark Current	<100e-/cm^2/second
Binning	1x1, 2x2, 4x4, 8x8
Lens	50 mm, F/1.2 min, F/16 max
Maximum Imaging Field of View	25 x 17cm
Fluorescence Excitations LEDs	10
Fluorescence Emission Filter Slots	10
Available Emission Filters	20

A filter wheel below the camera positions a user-selected filter directly under the CCD sensor. The filter wheel has 10 available filter slots and one slot which is kept open (no filter). The open filter position is useful for luminescence imaging where no attenuation is desired because the emitted light is usually very dim.

The instrument ships with the filter configuration specified by the customer at the time of the sales order. If the user needs change after the instrument is installed, it is possible for the user to replace one or more filters (see *Replacing an Emission Filter*, page 63). The available emission filter wavelengths are: 490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, and 870 nm. Each emission filter has a 20 nm transmission window, thus a 670 nm emission filter transmits all light from 660 to 680 nm.

An electronically controlled 50 mm lens under the filter wheel provides apertures from F/1.2 to F/16. This range of apertures provides a way to control the amount of light during imaging to help avoid saturation of the camera when using the excitation LEDs or very bright luminescent sources (see Table 4.9). The software-controlled lens also moves vertically to keep the sample in focus at different platform positions.

Table 4.9 F-Stop and Light Attenuation

F-stop	Approximate Light Attenuation (Relative to F/1.2)
2	1/2
4	1/8
8	1/32
16	1/128

Pairs of excitation LEDs positioned around the lens provide the excitation energy necessary for fluorescence imaging. The LED light is diffused and filtered to give uniform illumination at 430, 465, 500, 535, 570, 605, 640, 675, 710, and 745 nm. The full spectral width of an excitation LED source is 20 nm, so for example, when 570 nm excitation light is selected, the emitted wavelengths will include 560 -580 nm.

4.5 X-Ray System (ami HTX Imaging System Only)

The ami HTX Imaging System includes an x-ray source at the bottom of the instrument. X-rays are emitted from the source through an x-ray transparent window in the metal enclosure. A time delay integration (TDI) x-ray scanner with integrated scintillator plate is located near the top of the instrument. During x-ray imaging, the scanner sweeps above the platform while x-rays are generated for approximately 10 to 15 seconds.

Table 4.10 X-Ray System Specifications

Item	Description
X-Ray Energy	10 – 40 keV
X-Ray Camera	TDI line scanning imaging system
X-Ray Field of View	24.8 x 14.6cm
X-Ray Source Current	0 – 200 µA
X-Ray Scanner CCD Pixel Dimensions	1520 x 128
X-Ray Scanner CCD Pixel Size	48 x 48 µm
X-Ray Scanner A/D Converter Resolution	12 bit

4.6 Front Panel Controls and Indicators

The ami HT and ami HTX Imaging Systems have a control panel located near the bottom front of the instrument (Figure 4.2 and Figure 4.3). See Table 4.11 for a description of the control panel items.

Figure 4.2 Front Control Panel of ami HT Imaging System

Figure 4.3 Front Control Panel of ami HTX Imaging System

Table 4.11 Imaging System Front Control Panel

Item	Description
DOOR OPEN button	Illuminated button – Indicates the imaging system is idle. Pushing the illuminated button opens the imaging system door. Unilluminated button – Indicates acquisition in progress.
X-RAY KEY switch (ami HTX Imaging System only)	Enables or disables the x-ray source. The key can only be removed when it is the "OFF" position.
X-ray status indicators (ami HTX Imaging System only)	X-RAY ON (Red) – X-ray source is enabled and x-rays are being generated. X-RAY ARMED (Yellow) – X-ray source is enabled, but no x-rays are being generated. X-RAY IDLE (Green) – X-ray source is not enabled.
EMERGENCY STOP button	Illuminated button - Indicates the EMERGENCY STOP button is in the ready position. Pushing the button immediately disables power to all motors and on the ami HTX Imaging System, also turns off x-rays. Blinking button – Indicates the button has been pushed to the stopped position. Rotate the button clockwise to reset the emergency stop.

4.7 Imaging Chamber

The imaging chamber is a light-tight metal enclosure which includes an imaging platform and the optical system components (see *Optical System*, page 46). The imaging chamber has a custom gasket-free design which prevents light leaks over the entire lifetime because deteriorating gasket materials are eliminated. This design also eliminates the particulate debris caused by degrading gasket material. The imaging chamber prevents all x-rays produced by the ami HTX Imaging System from escaping.

Table 4.12 Imaging Chamber Specifications

Item	Description
Power requirements	5.3 A max at 90 – 240VAC 50 – 60 Hz
Dimensions	58 cm wide x 68 cm deep Height with door closed is 122 cm, 131cm with door open.
Weight	256 lbs (116 kg)

NOTE: Do not touch, move, or adjust anything inside the imaging chamber except for what is necessary to place or remove samples from the imaging platform.

Imaging Platform

The samples to be imaged are placed on a platform inside the imaging chamber. The platform is software controlled and can move vertically to the position which corresponds to the user-selected field of view in the aura software. Tubing connects the anesthesia gas inlet and outlet on the back of the instrument with connectors on the platform that supply and remove gas from an anesthesia manifold.

Under Platform Access

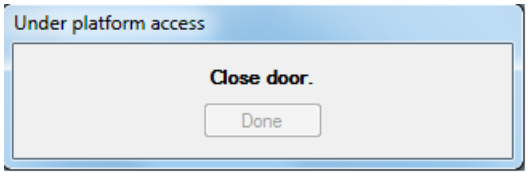
In the rare event that an object falls under the imaging platform, perform the following steps to raise it so that the space underneath is accessible:

1. Click the downward arrow ▼ in the upper right-hand corner of the acquisition controls (Figure 4.4) for the Special Options.

Figure 4.4 Special Options

2. In the menu which appears, click **Under Platform Access**.
3. Wait for a window to appear saying that the door can be opened.

Figure 4.5 Under Platform Access Ready Window



4. Open door and retrieve object.
5. Close door and click **Done** (see Figure 4.5). The platform will then move back down to the load position.

Cable Access Port

The cable access port is an optional feature which allows user-supplied electrical or fiber optic cables to be brought into the imaging chamber without ambient light entering in or x-rays escaping out. The user could, for example, illuminate the objects on the platform with laser light from an external source carried by a glass fiber. This port is located on the right side of the instrument when viewed from the front (see Figure 4.6). For the mechanical specifications of the cable access port, see Table 4.13.

Figure 4.6 SPECTRAL ami HTX with Cable Access Port

Table 4.13 Cable Access Port Specifications

Item	Description
Hole diameter	3.8cm
Cable channel width	1.27cm
Cable channel depth	1.6cm
Port cover dimensions	15.8cm x 15.8cm

Using the Cable Access Port

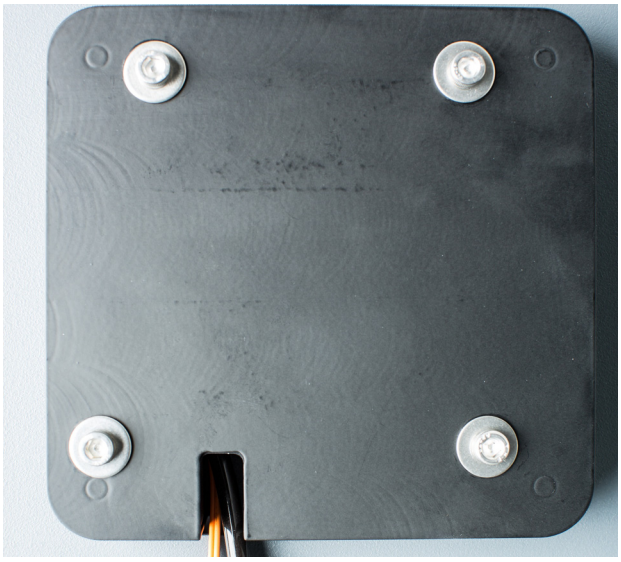
Any cabling intended to pass through the cable access port must meet the following criteria:

- Long enough to pass through the spiral channel inside the cable access port, reach inside the imaging chamber as well as reach any needed equipment on the outside of the imaging chamber.
- Sufficiently flexible for the tight bend inside the port.

CAUTION: If one or both of these criteria are not met, damage could result to the user's equipment.

1. Remove the four bolts which secure the cover of the access port (see Figure 4.7).

Figure 4.7 Cable Access Port Cover

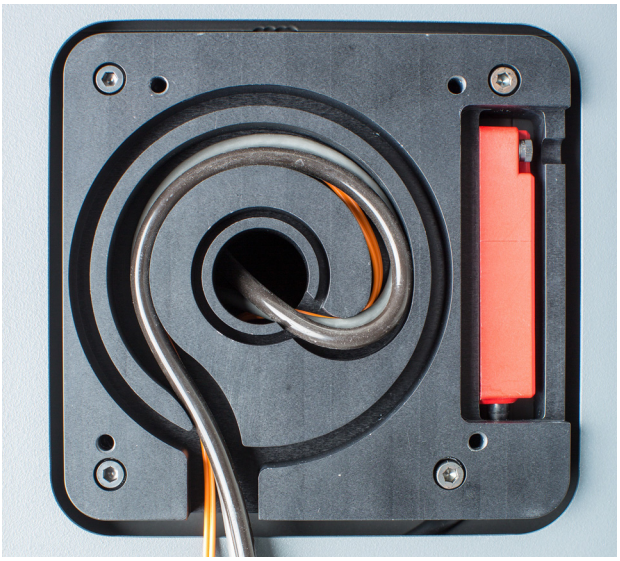


2. Remove the cable access port cover.
3. Feed the cable through the hole in the middle of the port until the end of the cable reaches the desired location inside the imaging chamber.

CAUTION: Ensure that any cabling inserted into the imaging chamber will not be pinched or trapped as the platform moves up and down or damage could result to both the ami HT or ami HTX and the user's equipment.

4. On the outside of the imaging chamber, lay the cable into the spiral channel of the cable access port (see Figure 4.8).

Figure 4.8 Cable Access Port Cabling Channel



5. Place the port cover back onto the cable access port.
6. Insert the four bolts back into the cover and tighten.

NOTE: If an ami HTX is built with an access port, no x-rays will ever be generated if the port cover is not installed. Attempting to take an x-ray image with the cover removed will disconnect the x-ray source power and the aura software will show an error.

NOTE: Since the imaging chamber is not light tight without the cover, it is the user's responsibility to ensure that the cover is installed before attempting fluorescent or luminescent imaging.

4.8 Control and Analysis Computer

IMPORTANT: Contact Spectral Instruments Imaging before making changes to the factory standard configuration or incorporating the ami HT or ami HTX Imaging System into your network. Making changes to the hardware, software, or settings of the ami HT computer without approval and guidance from Spectral Instruments technical support will void the instrument warranty and may result in unstable operation or instrument malfunction.

Factory Computer Configuration

Each ami HT or ami HTX Imaging System includes a computer with factory installed aura software. The computer has been configured and tested with the instrument.

The imaging systems are fully functional in a stand-alone (i.e. not networked) mode, allowing installation at locations without a network connection to a local area network (LAN) or a wide area network (WAN) such as the Internet. Quantitative analysis of the captured images can be performed using aura software immediately after images are acquired, and high quality data can be recorded in official laboratory notebooks.

The control computer inside the imaging system features a stable, standard operating system and application configuration which has been tested and validated to perform reliably. Also, maintaining only one software configuration in the field prevents crashes due to regressions in updated drivers, libraries, or operating system patches.

Computer Customization

Although the SPECTRAL ami HT/HTX imaging system, including computer, can function in a standalone mode (i.e. not connected to a network), the computer can be connected to the customer's local area network (LAN) by attaching a Category 5, 5e or 6 network cable to a network jack (computer is Gigabit Ethernet capable) on one end and to the control computer's Ethernet port on the other end. This allows image files and data to be easily backed up, shared, and stored on a shared network resource such as a server.

Attaching the instrument's computer to the LAN also enables remote administration of the computer per your information technology policies and procedures (such as automated backup, virus scans, operating system critical updates, review of log files, user account setup for access control, network authentication). The SPECTRAL ami HTX/ami HT computer does not come with anti-virus software installed or configured.

Users may also want to customize the standard factory configuration (for example, installation of security software, backup programs, firewall configuration if connected to network) to maintain security and integrity of image data.

User Account Management

All SPECTRAL ami HT/HTX instruments are shipped with a standard administrator-level user account. Spectral Instruments Imaging strongly suggests creating at least one non-administrator (i.e. non-privileged) user account for the regular use of acquiring and analyzing images. Running the aura software as a non-administrator user prevents overwriting critical configuration information for the SPECTRAL ami HT/HTX as well as for the operating system.

It is also possible to join the SPECTRAL ami HT/HTX computer to a managed domain network. If domain logins are implemented, do not give most users Administrator-level access to the SPECTRAL ami HT/HTX control computer.

NOTE: Administrator-level access is required to replace an emission filter.

Please consult your Information Technology department and Spectral Instruments Imaging before making these or any other changes to the SPECTRAL ami HT/HTX computer.

Table 4.14 Computer Specifications

Item	Description
Power requirements	2 A at 115 VAC, 3 A at 230 VAC; 50/60 Hz
Height	44.8 cm (17.6 inches)
Width	17.25 cm (6.8 inches)
Depth	46.8 cm (18.4 inches)
Weight	17.3 kg (38.0 lbs)

Table 4.15 Monitor Specifications

Item	Description
Maximum resolution	1920 x 1200 or 1920 x 1080 at 60 Hz
AC power requirements	100 to 240 VAC/ 50 or 60 Hz ± 3 Hz/1.5A (Maximum)
Height	369.0mm to 500.0mm (14.53 inches to 19.69 inches)
Width	556.0 mm to 568.0mm (21.89 inches to 22.36 inches)
Depth	180 mm (7.09 inches)
Weight (panel only)	3.51 kg to 4.04 kg (7.74 lbs to 8.91 lbs)
Operating temperature	0 to 40°C (32 to 104 °F)
Storage temperature	–20 to 60°C (–4 to 140 °F)
Relative humidity	20% – 80% non-condensing
Operating altitude	0 – 3048 m (0 – 10000ft)
Storage altitude	0 – 10,668 m (0 – 35,000 ft)
Pollution degree	Control Level 2

4.9 Operating the ami HT or ami HTX Imaging System

Powering On the Imaging System

1. Plug the power cord and USB cable into the imaging system.
2. Press the  position of the power switch located at the top and rear of the instrument, near the AC power input socket (Figure 4.9).

   You will hear the camera and control box fans turn on. The green button and green LED on the front control panel of the ami HTX Imaging System will illuminate.

Figure 4.9 Imaging System – Rear View

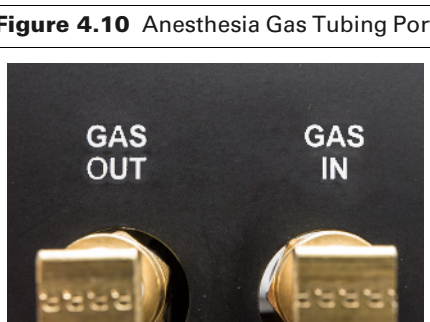
3. Turn on the computer and monitor.

Anesthesia Gas Plumbing

WARNING! Do not use flammable anesthesia.

Two anesthesia gas ports are located near the top rear of the imaging system. Port direction is indicated by the label located above the ports (Figure 4.10).

Figure 4.10 Anesthesia Gas Tubing Ports



The ports are ¼ inch barbed brass fittings that will accept a ¼ internal diameter tube. The ports are connected by tubing to the gas ports on the imaging system. If the imaging system will be used with an anesthesia system, consult the anesthesia system user manual for information on proper tubing size and materials, and instructions for connecting the anesthesia system to the imaging system.

Door Operation

The front door of the ami HT or ami HTX Imaging System slides smoothly up and down without binding. To open the door while unit is powered on, press the illuminated green button on the front control panel. To close door while the instrument is powered on, pull the door handle all the way down until the door stops. Electromagnetic locks will then engage to keep the door closed.

NOTE: If the imaging system is not powered on, the door will not stay closed.

Shutting Down the Imaging System

It is recommended to leave the instrument powered on, except in some situations, such as preparing to move the instrument.

1. Close the aura software.
2. Press the ○ position of the power switch located at the top, rear of the instrument, near the AC power input socket.

   CAUTION: The imaging system door will slide open if it was closed when the instrument was powered off.

3. Turn off the computer and monitor.

4.10 Care and Maintenance

Safety Checks

Perform the following checks at the start of each imaging session to ensure safe functioning of the imaging system. Ensure that:

- The imaging system is resting level on the bench.
- There is no visible damage to the covers, front panels, power cords, monitor cable or USB cables.
- The door slides smoothly up and down without excessive effort.
- The imaging system successfully powers on (as described in *Powering On the Imaging System*, page 55), including fans and the illuminated green button and green LED on the front control panel.
- When the ami HTX Imaging System is powered on, the door stays shut except when the lighted green button is pressed.
- The X-ray key on the ami HTX Imaging System is only removable in the **OFF** position.
- You can hear the motors initialize when aura software is started.

Cleaning the Instrument

IMPORTANT: Do not clean the ceiling of the ami HT or ami HTX without contacting Spectral Instruments Imaging first as extreme care must be taken to avoid damaging optical components.

Clean the outside surfaces of the ami HT or ami HTX using a lint-free wipe and soap and water or any of these approved cleaning solutions:

- 70% alcohol (ethyl or methyl)/30 deionized water
- Clidox®-S
- Sporicidin®
- Trifectant® Disinfectant

To clean the inside the ami HT or ami HTX, do not spray or pour liquids directly into the imaging chamber. Saturate a lint-free wipe first then gently clean the platform or walls. Due to potential fluorescence properties of some cleaning solutions it is recommended to wipe surfaces with deionized water afterwards taking care not to allow water to pool on the platform.

IMPORTANT: Refer to the manufacturer's literature for information regarding the applicability of the cleaning agent for the organism of interest. Spectral Instruments Imaging cannot make any claims as to the sterility of the imaging system after use. For immunodeficient animals, use a dedicated system to reduce the risk of contamination.

X-Ray Radiation Survey (ami HTX Imaging System Only)

Perform periodic radiation surveys according to your organization's policies, as well as local, state, and national radiation control laws to ensure that all generated x-rays are contained within the instrument. If the ami HTX Imaging System sustains any shock or impact, a radiation survey must be performed.

Verifying Correct Operation of the ami HT/HTX Imaging System

To verify the imaging system operation, acquire images of the Spectral Instruments Imaging Calibration Device (SIICD) (Figure 4.11) using the imaging settings in Table 4.16.

Figure 4.11 Spectral Instruments Imaging Calibration Device (SIICD)

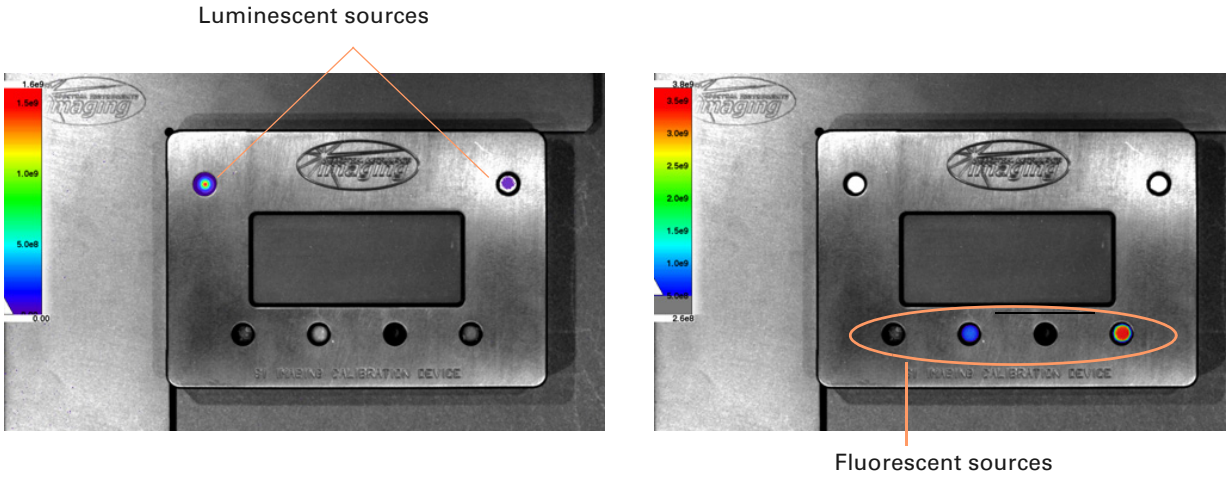


Table 4.16 Imaging Settings for Verifying ami HT or ami HTX Imaging Capability Using the SIICD

Imaging Setting	**Type of Imaging**			
	Luminescent	**Fluorescent**	**X-ray**	**Photograph (f/16)**
Exposure	4 seconds	0.5 seconds	~10-15 seconds (not user-adjustable)	0.5 seconds (default)
Binning	None	None	Low or High	None
F-Stop	1.2	1.2	N/A	1.2
Excitation	N/A	430 nm	N/A	N/A
X-ray settings Power Resolution	Off	Off	 High High	Off
Emission	Open	Choose a filter close to 430 nm	N/A	N/A
FOV	25 cm	25 cm	25 cm	25 cm
Object height	1.5 cm	1.5 cm	1.5 cm	1.5 cm

Verifying Imaging Capability

To verify ami HT or ami HTX imaging capability, acquire images as indicated in Table 4.17 using the settings in Table 4.16.

Table 4.17 Verifying ami HT or ami HTX Imaging Capability

To Verify:	Use the Settings in Table 4.16 to Acquire:	Expected Results
Luminescent imaging	Luminescent image of SIICD	Signal from both luminescent sources in the upper left and right corners of the SIICD should be visible (Figure 4.11). Put an ROI around each luminescent source. The factory set luminescent sources have either a factor of 10 or 100 difference in radiance.
Fluorescent imaging	Fluorescent image of the SIICD	Signal from at least one of the fluorescent plastics should be seen in the image (Figure 4.11).
X-ray imaging (ami HTX Imaging System only)	X-ray image of the SIICD	The step-wedge and the Spectral Instruments Imaging logo should be visible in the image (Figure 4.12).
Photographic imaging	Photograph of the SIICD	A crisp black and white photograph of the SIICD.

Figure 4.12 X-ray Image of SIICD

Verifying Co-registration

To verify co-registration of overlays, acquire images as indicated in Table 4.18 using the settings in Table 4.16 on page 58.

Table 4.18 Verifying Co-registration

Type of Images Acquired	Expected Overlay
X-ray and luminescent image	Both luminescent sources are visible, one in each upper corner of the overlay.
X-ray and fluorescent image	Any fluorescing plastics, depending on the emission filter, appear in a row at the bottom of the SIICD. Depending on the emission filter and other settings chosen for the fluorescent image, one or both of the luminescent sources may also appear in the fluorescent image.
Photograph and luminescent image	Both luminescent sources are visible and appear exactly in the circular corner locations of the SIICD seen in the photograph.
Photograph and fluorescent image	Any fluorescing plastics, depending on the emission filter, appear exactly in the circular bottom locations of the SIICD seen in the photograph.

4.11 Troubleshooting

Table 4.19 Troubleshooting Guide

Issue	Possible Cause	Possible Remedy
Camera not detected	USB to Ethernet adapter settings are incorrect.	■ To check the network settings, open a web browser (such as Internet Explorer) and type in the following address: http://192.168.0.175. If the web browser reports the web page times out, or otherwise can't be displayed, restart the control and analysis computer while leaving the imaging system powered on. ■ Verify that the USB to Ethernet adapter ("USB3GIG") has the correct settings (IPv4 only, static IP address, blank DNS): **1. Control Panel>System>Network and Sharing Center>Network Connections**. Find "USB3GIG". Double click on that interface. **2.** Click once to highlight **Internet Protocol Version 4 (TCP/IPv4)** **3.** Click **Properties>Use the following IP address:** (192.168.0.2, 255.255.255.0 subnet mask, blank default gateway) **4. Use the following DNS server addresses:** (both addresses are blank).
	USB, camera, or firmware issue.	Power cycle the imaging system and restart the control and analysis computer.
	Windows firewall settings changed.	Make sure both WinSiia and IDL programs are allowed through the Windows firewall (ask your IT department for help in checking this setting).
Fuzzy or bad images.	High dark noise due to being warmer than background images.	Wait until aura software displays the "Idle, ready" message in green at the bottom right-hand corner of the main window.
	■ Lens out of position. ■ Condensation on CCD window. Take a photograph of a large piece of blank white paper. Is the photo uniformly white? Any circular shadows/blurry parts present?	Take a photograph of a piece of paper with some text and/or images on it. If the text or images are not crisp and legible, contact Spectral Instruments Imaging technical support (see page 4). Take a photograph of a large piece of blank white paper. If the photograph is not uniformly white or there are circular shadows/blurry parts present, contact Spectral Instruments Imaging technical support (see page 4).
Stall errors		■ Contact Spectral Instruments Imaging technical support (see page 4).
No x-ray image acquired.	X-ray key is not turned to the ON position.	Before taking an x-ray image, verify that the: ■ X-ray key is inserted into the key switch on the front panel, and turned to the ON position. The x-ray key cannot be removed when it is turned to the ON position. ■ Emergency Stop button is in the "Reset" position by rotating it clockwise.
	Imaging system door not closed.	Confirm that the imaging system door is properly closed.

Table 4.19 Troubleshooting Guide (continued)

Issue	Possible Cause	Possible Remedy
Door does not open when the illuminated DOOR OPEN button is pushed.	aura is acquiring an image.	■ Wait for imaging to complete.
	Door has been closed for a long period of time.	■ Give the door a little push upward while the DOOR OPEN button is depressed. ■ Cycle the imaging system power.
aura controls will not respond.	Open window behind aura window.	Minimize the aura window and close the other window.
Camera status not idle.	Imaging system or aura recently restarted and camera hasn't cooled down to –90°C.	Wait until "Idle, ready" is displayed in green at the bottom left corner of the aura window.
	No camera present.	Cycle the imaging system power and restart aura.
	Camera temperature stable and displaying a temp not -90C	Contact Spectral Instruments Imaging technical support (see page 4).
No photographic reference image.	Photograph check box not selected.	Put a check mark in the Photograph check box.
	Shutter is sticking (blank images).	Cycle the imaging system power and restart aura. If this does not resolve the issue, contact Spectral Instruments Imaging technical support (see page 4).
Images of microplate wells not constrained to the wells.	Using a clear plastic microplate.	Use black plastic microplates.
	Saturated images.	■ Reduce imaging time or excitation power. ■ Increase f-stop.
Not connected to instrument.	USB cable not connected between ami HT/HTX and computer.	Ensure that the USB cable is connected to the control and analysis computer on one end and the imaging system on the other.
	Computer failure or error.	■ Restart aura and cycle the imaging system power. ■ Restart the computer. If this does not resolve the issue, contact Spectral Instruments Imaging technical support (see page 4).
Saturated image warning.	Parts of the image are too bright.	■ Reduce imaging time. ■ Reduce excitation power. ■ Increase f/stop or switch to a larger FOV.
One or more sections of X-ray image is significantly darker.	Unbalanced sensors in the x-ray camera.	Contact Spectral Instruments Imaging technical support (see page 4).
Expected signal in fluorescent image is not visible.	Wrong excitation/emission filter pair.	Ensure that: ■ Excitation/emission filters are properly selected. ■ Excitation power is not set to zero. ■ Fluor mode is selected.
Expected signal in luminescent image is not visible.	Very low signal.	■ Increase exposure time. ■ Increase binning. ■ Ensure that an emission filter is not selected.
Filter wheel error message.	Filter wheel positioning failed.	■ Re-initialize hardware from the ▼ drop-down menu in the acquisition controls. ■ Cycle the imaging system power. If this does not resolve the issue, contact Spectral Instruments Imaging technical support (see page 4).

Table 4.19 Troubleshooting Guide (continued)

Issue	Possible Cause	Possible Remedy
Random IDL errors.	Various.	Clear error. Click **OK**. If problem persists, contact Spectral Instruments Imaging technical support and send screenshot with description of process.

4.12 Replacing an Emission Filter

NOTE: Administrator-level access is required to update aura's list of installed filters. Login as an administrator in Windows before attempting to replace any emission filters.

1. Remove the top plastic dome of the imaging system.
   The filter wheel access cover with four thumbscrews should now be visible.

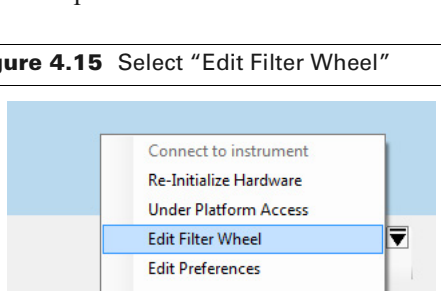
Figure 4.13 Filter Wheel Access Cover

2. Unscrew the four thumbscrews which secure the filter wheel access cover and remove the cover.
   A filter in the filter wheel should now be visible (Figure 4.14).

Figure 4.14 Exposed Filter

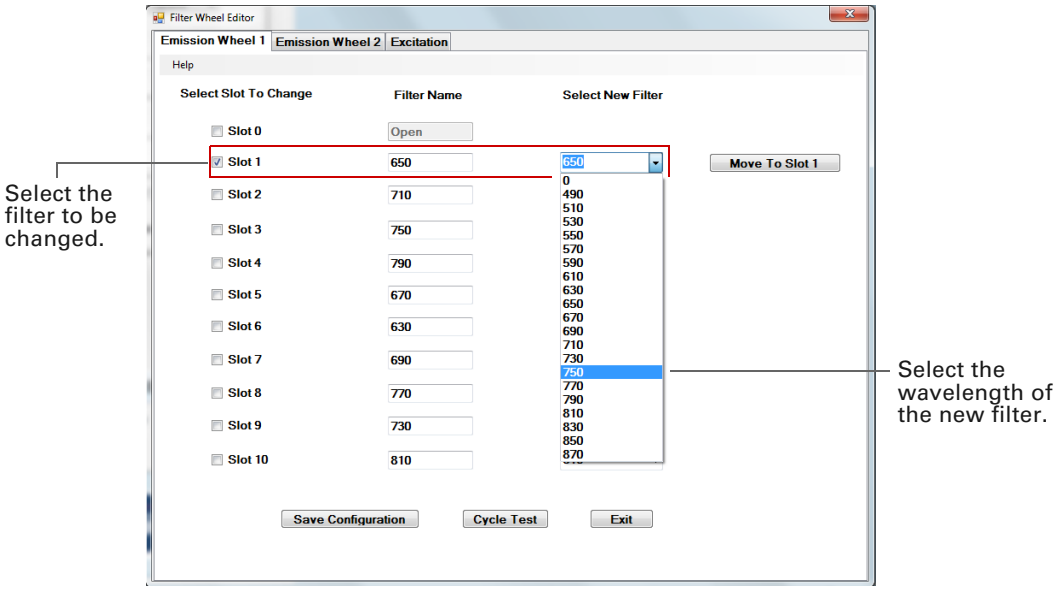
3. Click the ▼ arrow in the Acquisition Controls (Figure 4.15). Select Edit Filter Wheel from the drop-down menu.

Figure 4.15 Select "Edit Filter Wheel"



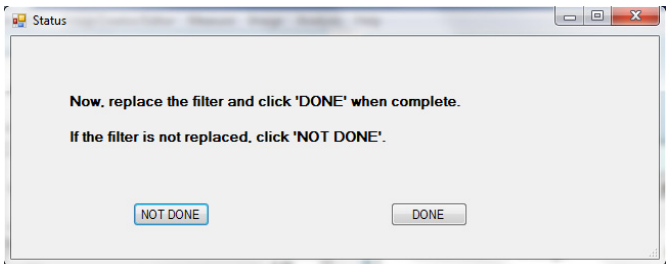
4. In the Filter Wheel Editor that appears (Figure 4.16):
   a. Put a check mark next to the slot number of the filter that you want to change in the **Emission Wheel 1** tab.
   b. Select the wavelength of the new filter from the drop-down list.

Figure 4.16 Filter Wheel Editor



5. Click **Move to Slot xx** ("xx" is the selected slot number) (Figure 4.16)).
   You will hear the filter wheel move and the Status dialog box appears (Figure 4.17).

Figure 4.17 Status Dialog Box



6. Put the filter tool with the knurled knob end up into the filter wheel access opening. Firmly place it directly on top of the filter that you want to replace.

Figure 4.18 Filter Tool

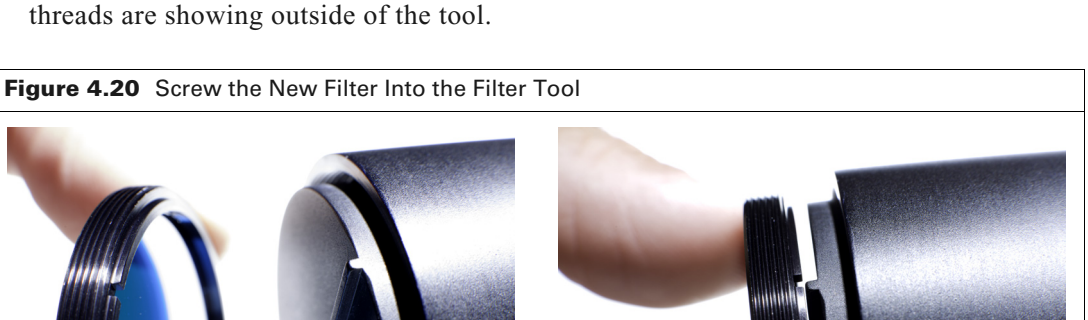
Figure 4.19 Inserting the Filter Tool Into the Filter Wheel Access Opening

7. Firmly hold down the filter tool and rotate the knob clockwise until it engages the notches in the filter retaining ring.

   You may hear a "click" sound when the filter tool engages. The knob will not rotate once the tool is engaged in the retaining ring.

8. Firmly hold down the filter tool and rotate the knob counter-clockwise until the filter and retaining ring are completely out of the filter wheel. The knob will stop rotating once this happens.
9. Carefully unscrew the filter from filter tool without touching the glass surfaces. Store the filter in a safe, dust-free place.
10. Without touching the filter glass surfaces, remove the new filter from its packaging and carefully screw the filter and retaining ring into the filter tool so that about half of the threads are showing outside of the tool.

Figure 4.20 Screw the New Filter Into the Filter Tool

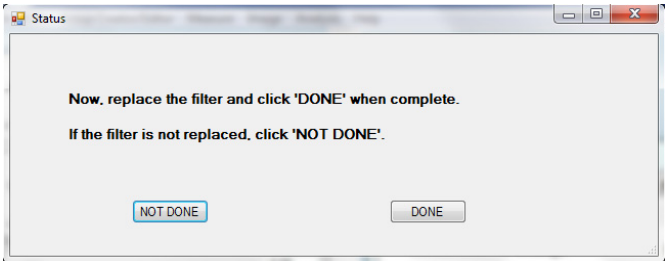


11. Place the end of the tool with the replacement filter through the filter wheel access opening and onto the empty hole in the filter wheel.
12. Rotate the knob clockwise into the hole until snug.

NOTE: If the knob stops rotating before the filter is completely installed (i.e. you are unable to lift the filter tool out), rotate the body of the filter tool ¼ turn counter-clockwise, then turn the knob clockwise until the filter is completely installed.

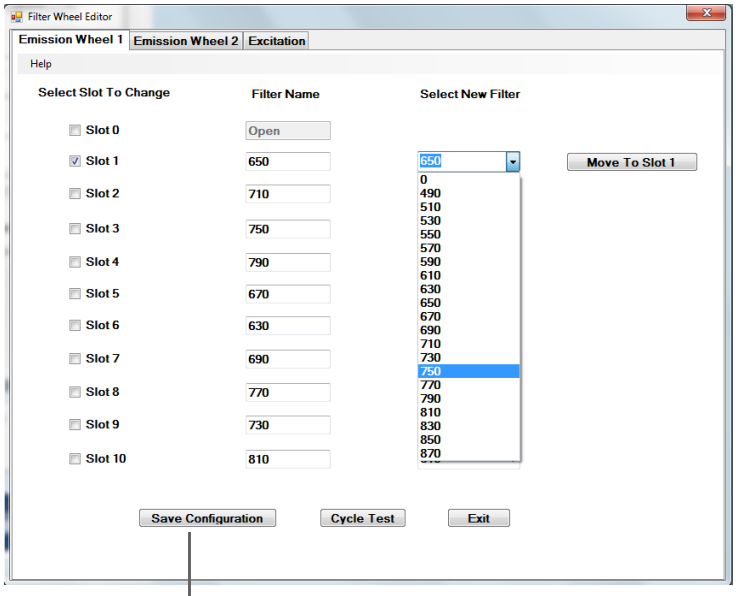
13. Remove the filter tool from the filter wheel access opening.
14. Click **DONE** in the Status dialog box (Figure 4.21).

Figure 4.21 Status Dialog Box



15. If replacing more filters, repeat step 4 to step 14 for each filter.
16. Click **Save Configuration** and **Exit** in the Filter Wheel Editor when finished replacing filters (Figure 4.22).

Figure 4.22 Filter Wheel Editor



17. Re-install the cover over the filter wheel access opening with the four thumbscrews removed in step 2.
18. Reinstall the top plastic dome.
19. Restart aura and confirm that the new filters are included in the emission filter drop-down list.

A

Acquisition Parameters

Table A.1 Acquisition Parameters

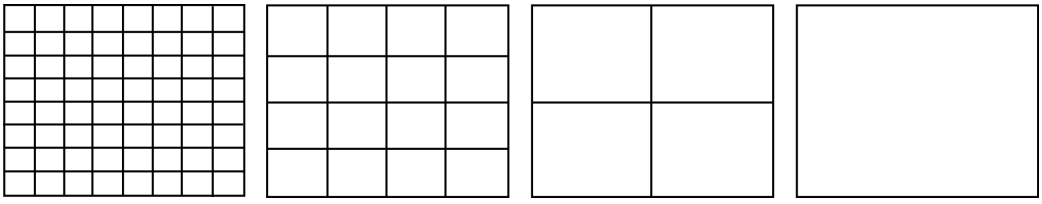
Item	Description
Acquire	Begins acquisition.
Mode	The types of image acquisition: ■ **Fluorescence** – Fluorescent mode acquires an image of light emitted from a fluorescent source at a specific wavelength when the subject is illuminated using a different (shorter) light source at a specific wavelength. ■ **Luminescent** – Luminescent mode acquires an image of light emitted from an Unilluminated sample, for example, bioluminescent emission from plants, animals, or *in vitro* sources such a cells in microplate wells. There are no lights on inside the imaging chamber during acquisition. ■ **Photograph** – Black and white image illuminated with white light.
Exposure (s)	The amount of time (seconds) the shutter is open during image acquisition. Longer exposure times result in higher sensitivity.
Binning	Sets the pixel size of the CCD camera. A higher level of binning increases sensitivity, but reduces spatial resolution. Higher sensitivity is due to a higher signal-to-noise ratio resulting from binning. A certain amount of read noise (the random number of electrons added during readout) is added to each pixel. If there is no binning, the read noise is added to each pixel. When individual pixels are binned to create a "super pixel", read noise is spread over the entire super pixel area, which reduces the read noise. For example, 8 x 8 binning results in read noise that is 1/64 the read noise with no binning. Thus, faint signals have a much better chance of rising above the noise. Also, binning increases signal. An 8 x 8 binning level produces a super pixel that is 64 times as large with 64 times the signal compared to a non-binned pixel.  No binning Pixels in a small area of the CCD shown with no binning. Binning = 2 Combines four (2 x 2) pixels. Increases signal four times. Quadruples pixel size. Binning = 4 Combines 16 (4 x 4) pixels. Increases signal 16 times. Increases pixel size by a factor of 16. Binning = 8 Combines 64 (8 x 8) pixels. Increases signal 64 times. Increases pixel size by a factor of 64.
FStop	Setting that determines the size of the lens aperture. F-stop 1.2 is the largest aperture setting, and provides maximum sensitivity, but the smallest depth of field (the range over which the image is in focus). As the **FStop** number increases, the aperture size decreases and the depth of field increases.
Excitation (nm)	Excitation light wavelength.

Table A.1 Acquisition Parameters (continued)

Item	Description
Power (%)	Power level of the excitation light source.
Emission (nm)	Emission filter center wavelength. Emission filters will allow light from 10nm shorter than this center wavelength to 10nm longer, thus a total transmission window of 20nm.
Xray	Power – Power level of the X-ray source. ■ Low Power: 35kV tube voltage and 175uA tube current. ■ High Power: 40kV tube voltage and 200uA tube current. Resolution – Binning level for the X-ray image. High resolution = no binning, low resolution = 2 x 2 binning.
FOV	Field of view is the width of the area to be imaged. It is good practice to set the FOV no larger than is necessary to include the subject(s) because sensitivity decreases as the FOV increases. This is because the imaging platform moves farther from the camera as the FOV increases.
Object height	Object height entered by user (the highest vertical point of the object).

B

Image Data

B.1 Numerical Images

The charge coupled device (CCD) camera in an ami HT or ami HTX Imaging System is an integrated circuit etched onto a silicon surface and consists of light-sensitive elements called *pixels*. During image acquisition, photons incident on the CCD surface generate charge which is electronically read and converted into a two-dimensional array of numbers. Each number in the array represents the light intensity recorded by a pixel. The numerical value of a pixel is proportional to the intensity of the light it recorded. See Spectral Instruments online for more detailed information about CCDs and pixels.

aura software displays luminescent or fluorescent numerical images as a *pseudocolor image* (Figure B.1) which is generated by:

- Mapping pixel intensity values to colors defined by a color bar.
- Displaying each pixel filled with the color assigned to the pixel's intensity value (Figure B.1).

NOTE: The appearance of a pseudocolor image does not affect the numerical image data.

Image data is independent of the color bar, the minimum and maximum of the color bar range, or the color bar scale (linear or log) used to display the data. The color bar only affects data display, not the underlying pixel numerical values. This means that ROI measurements will give the same result, regardless of the color bar settings.

B.2 About ami HT and ami HTX Images

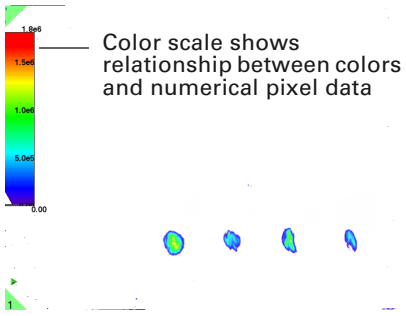
More than one type of image is usually acquired during acquisition, for example:

- A luminescent image and a photograph.
- A fluorescent image, x-ray image, and a photograph (if the imaging system has x-ray imaging capability).

aura software saves images and image information from an acquisition in one file (.ami). Load saved aura image files in the workspace to display and analyze images. The numerical image data in the file can also be exported (TIFF and DICOM) for use with other image processing software such as MATLAB, Python, Origin, or ImageJ. For steps on how to export the numerical image data, see Exporting Image Data on page 92

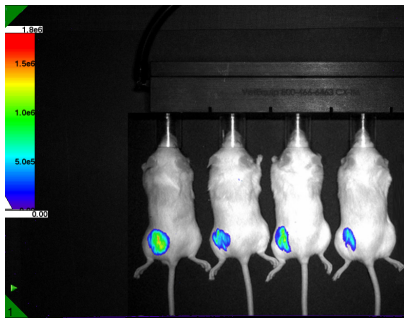
ami HT and ami HTX Imaging Systems are absolutely calibrated. As a result, the numerical pixel values are related to a *standard* (a physical measurement of the photons per second emitted from a pixel area that is the same for the standard and image). Therefore, a measurement in radiance units has physical meaning and can be quantitatively compared to measurements made on any other absolutely calibrated instrument. In addition, the measurements are independent of instrument settings. If a subject emits twice as many photons compared to another, the radiance value will be twice as much, independent of the exposure time.

Figure B.1 Example Fluorescent Image, Photograph, and Overlay



Numerical image (fluorescent data) displayed as a pseudocolor image using a rainbow color bar

Photograph



Overlay of fluorescent image on photograph

B.3 Graphic Images

A photograph captured by the ami HT or ami HTX Imaging System CCD camera is an image of the light reflected from a subject when it is illuminated using the white lights near the top of the imaging system. aura software displays photographs using a grayscale color bar that assigns white to the largest pixel value and black to the smallest value, and shades of gray to the values in between (Figure B.1).

The reflected light in a photograph isn't calibrated against any standard; therefore, a photograph cannot be analyzed using ROI measurements. Refer to Saving Graphic Files on page 92 for how to export photographs.

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X