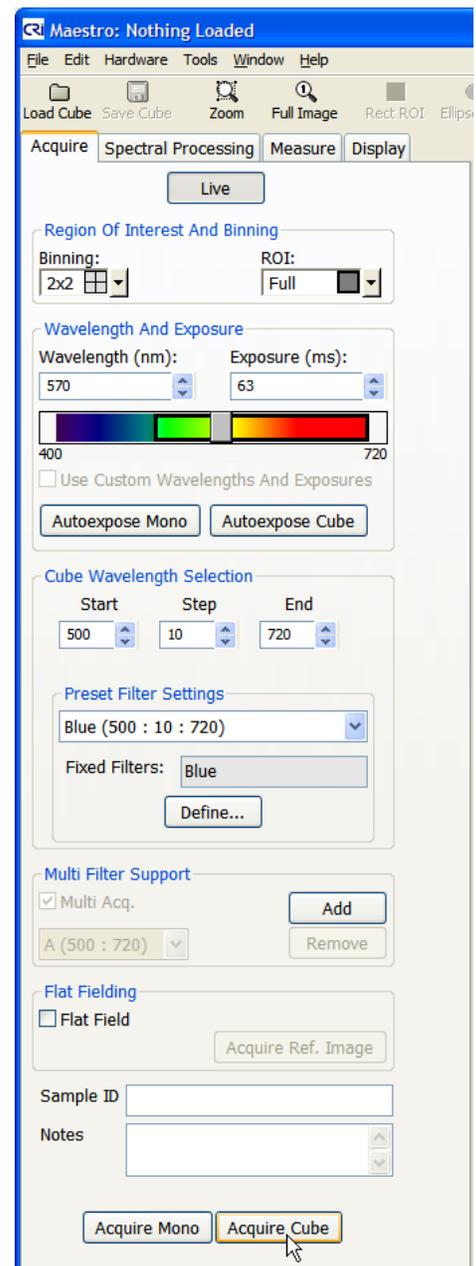# Acquire Images

⚠ This guide presumes that the Maestro *In-Vivo* Imaging System is properly installed and configured.

1. Install the excitation and emission filters for the fluorescence you wish to see. (Refer to the Filter Selection Guide in the *"Maestro User's Manual"*.)

2. Switch the main system power **ON** using the switch on the back of the imaging module.

3. With the **SHUTTER** switch in the **CLOSED** position, switch the illumination module **ON** using the switch on the lower right exterior of the imaging module.

4. Switch the interior lights **ON** using the switches on the upper right side of the imaging module.

⚠ Remember that if you choose the infrared (**IR**) lights, you will not see any noticeable illumination.

5. Power up the computer and start the **Maestro** application.

6. Place your specimen onto the specimen stage and set the stage and illuminator arms to the heights appropriate for the size of your specimen. Use the field of view markings on the stage surface as a guide to determine the stage and illuminator arm settings.

7. In the Maestro application, select the **Acquire** tab.

8. Click the **Live** button to see live streaming video of the image.

9. Select **Binning** and **Region-Of-Interest (ROI)** options according to the desired image quality. The default is full-field and 2x2 binning. Image files will be smaller with smaller ROI and increased binning. Binning increases sensitivity to light but reduces image resolution.

10. In the **Wavelength and Exposure** box, enter the wavelength at which you expect to see an image of your specimen. This should correspond to the installed emission filter.

11. Click the **Autoexpose Mono** button to improve the live image.

12. Open the door and focus the lens to get a sharp image of your specimen. (If the live image becomes saturated with light when the door is open, it may be difficult to focus the image properly. Try autoexposing the image again with the door open.)

13. Close the imaging module door. Autoexpose the image again if you had to change the exposure setting while focusing.

14. In the **Cube Wavelength Selection** box, select the **Preset Filter Setting** that corresponds to the installed filters. If you don't find a preset that works for your specimen, you can edit the **Start**, **Step**, and **End** settings manually. Refer to the *"Maestro User's Manual"* for instructions.

15. Switch all interior lights **OFF** and **OPEN** the excitation light shutter to illuminate the specimen with the excitation light.

16. Click the **Autoexpose Cube** button to automatically calculate the exposure settings for the image cube. You can also adjust the exposure time manually:

    a. If the image is too dark, increase the exposure time in the **Time (milliseconds)** box.

    b. If the image is too bright or saturated (indicated by solid red pixels), reduce the exposure time.

17. Click the **Acquire Cube** button to generate an image cube.

18. When Maestro has finished generating the image cube, a color representation of the cube displays in the image viewing area.

19. Click the **Save Cube** button on the toolbar to save the cube. Select a location and enter a file name. (File name format suggestion: *project_sample_operator_ datetime*).

20. Select a cube type option:

    a. **Image Cubes** saves the cube in CRi format, which includes hardware and display settings for the cube.

    b. **TIFF Cubes** saves the cube as a series of TIFF images with the assigned file name plus an appended number indicating the wavelength for each image in the cube.

21. Unmix the cube as explained on the reverse side of this Quick Start Guide.

Unmix Images

1. In the Maestro application, select the **Spectral Processing** tab.

2. Open or acquire an image cube. To open a cube, click the **Load Cube** button on the toolbar. Cube format types include CRi format (*.im3) cubes and TIFF (*.tif) cubes. An RGB color representation of the cube will display in the Maestro image viewing area.

3. If a **Live Stream** window is still open, you may close it by clicking its **Close** [x] box.

4. If desired, select a spectral display option from the **Scaling** combo box.

5. Click the **Real Component Analysis (RCA)** button near the bottom of the **Spectral Processing** panel. The RCA function automatically detects and separates each of the component signals in the cube.

💡 If you prefer to unmix the cube by manually identifying the component signals, populate the color library with spectra and click the **Manual Compute Spectra** button. Refer to the *"Maestro User's Manual"* for instructions.

6. In the **RCA** dialog box, you have the option of manually sampling the autofluorescence. Click the **Sample Spectrum** button and use the mouse pointer to draw a sampling line on an area of the specimen that contains only autofluorescence. (Hold down the left mouse button and drag the pointer to draw.)

7. Click the **Find Component Images** button. Maestro identifies and displays the autofluorescence and fluorescence signals.

8. Select the signals you want to unmix:

   a. The autofluorescence is displayed in the upper left image. Click two times anywhere in the image to select it as the autofluorescence. Set its pseudo color to white.

   b. Single-click on the other component image(s) you want to unmix, and select a pseudo color for each.

9. Extract the pure fluorescence signal(s) from the autofluorescence by clicking the **Find Spectra** button. The component signals display in the spectral graph.

💡 Occasionally, for weak signals, there may be too much baseline offset in the data. If you did not get the desired unmixing results, select the **Fit Offset** option and see if the unmixing results improve.
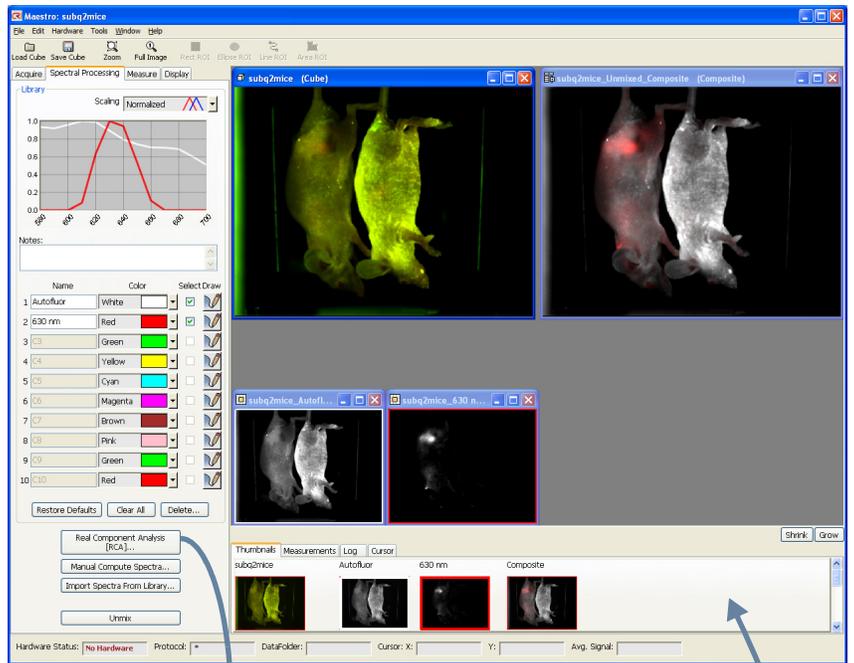
10. Use the controls in the **Computed Spectra** box to specify each spectrum's name, pseudo color, and location within the spectral library.

11. When finished, click the **Transfer to Library, Unmix and Close** button. This adds the spectra to the spectral library and unmixes the spectra in the cube. (If a library is open already, you may be asked if you want to overwrite the existing spectra.)

12. New images will display in the image viewing area:

    a. There is a small component image for each selected spectrum, each with a colored border that corresponds to the pseudo color of the spectrum.

    b. There is also a larger pseudo-colored composite image displayed beside the original RGB image of the cube. The colors used to create this image are library pseudo colors. The result should be a clear display of the specific fluorescence signal, with obvious differentiation from the autofluorescence.

13. Save the resulting images:

    a. You can save all images as displayed by selecting **File > Save Image > Save All (As Displayed)**. Select or create a folder in the same directory as the original image cube. Images are saved as TIFF images that can be opened in a variety of image display programs.

    b. You can save all images as unscaled data by selecting **File > Save Image > Save All Images (As Unscaled Data)**. See the *"Maestro User's Manual"* for more about saving cubes and images.

    c. You can also save the entire workspace by selecting **Save Result Set** from the **File** menu. Enter a file name to save all images and results in a single file.

14. Save the Protocol and/or Spectral Library for use throughout your experiment:

    a. You can save the complete Maestro protocol, which includes the current spectral library, by selecting **Save Protocol** from the **File** menu.

    b. If you want to save the spectral library as a separate file, select **Save Spectral Library** from the **File** menu.