

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

(<https://biotech.ufl.edu>)

CTAC / ICBR SOP : Capturing Data Using the ROI Tools in the Tool Palette from an Acquired Image

Title: Capturing Data Using the ROI Tools in the Living Image™ Tool Palette from an Acquired Image

Materials Required:

Computer

Living Image™ software

Purpose:

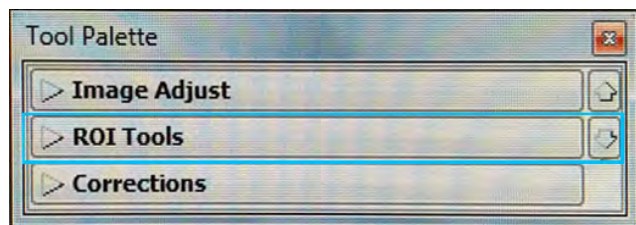
To aid the user in understanding the use and function of the ROI (Region Of Interest) tools in the image Tool Palette.

Background:

The IVIS Spectrum Imaging System is controlled by the Living Image™ software. When a scan is completed, the image (or images if a sequence scan is performed) will have its own control panel, in the form of the Tool Palette. The ROI or Region Of Interest tools allow the user to specify specific areas and shapes within their scanned images and obtain further measurements.

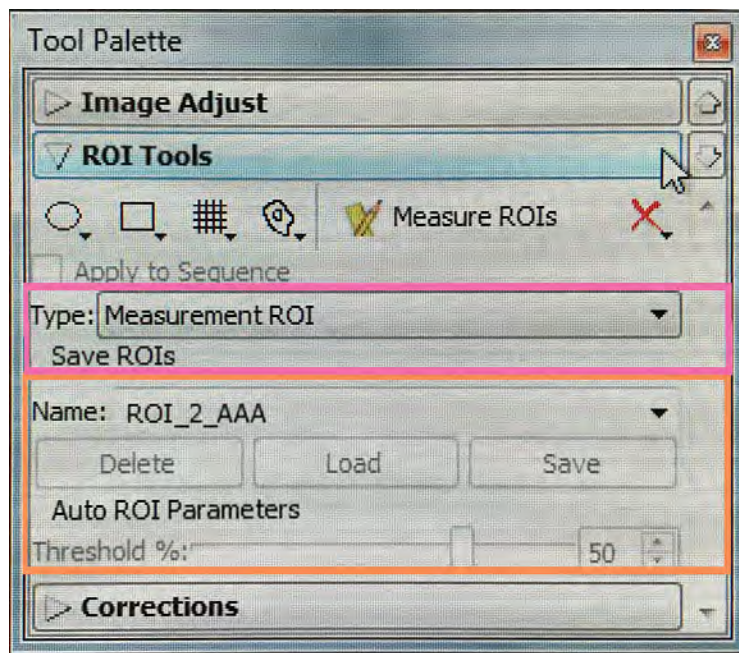
Color Scale and its use:





The ROI Tools are found in the Image Adjust section of the Tool Palette. This bar should be clicked to expand the options available.



ROI Tools

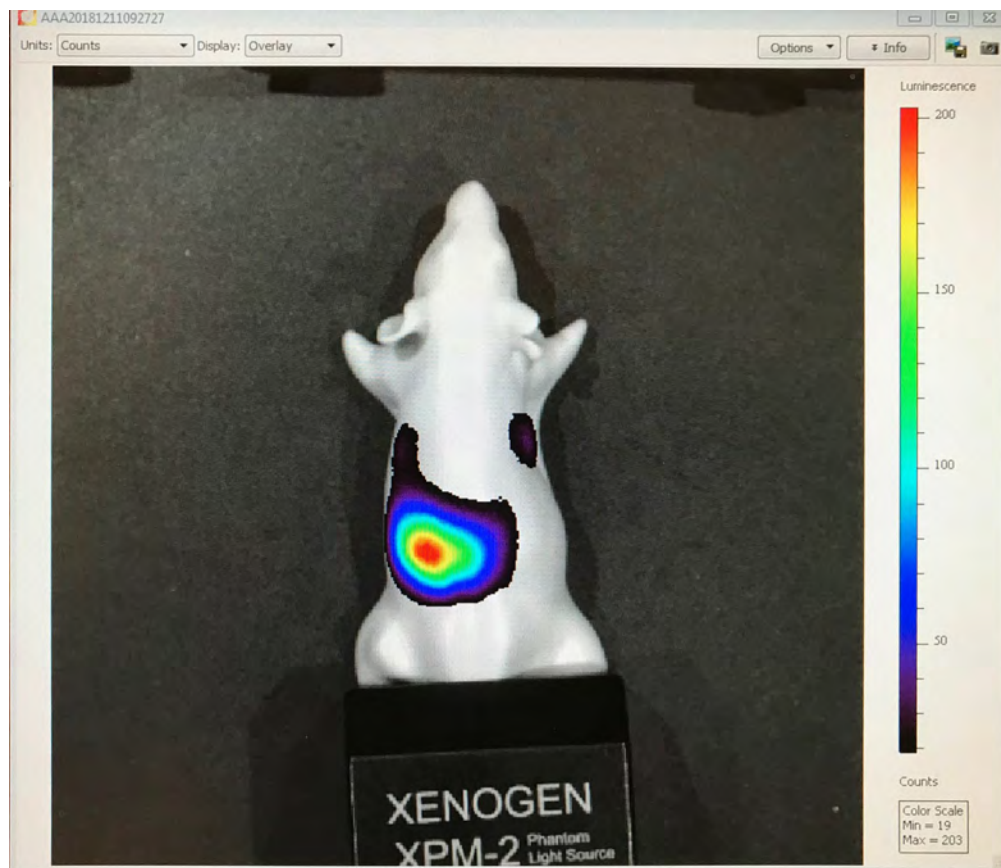
The ROI tools expand into the following dropdown.



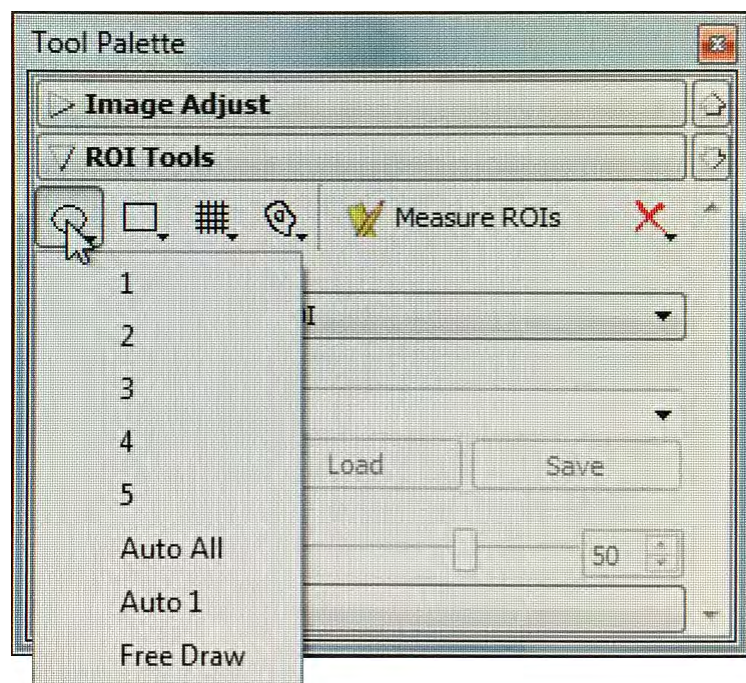
-  Create circle shape, up to 5 at a time
-  Create square shape, up to 5 at a time
-  Create different sizes of grid
-  Autodraw / Free Hand Draw
-  Measure ROIs
- Button to acquire measurements of selected areas
-  ROI Measurement Type Drop Down Menu
-  ROI Name / Load / Save and Auto ROI control

The first section (not highlighted) houses the shape selection for the Region of Interest. Circles, squares, grids, automatic or free draw options may be selected by the user, dependent on their requirements. Each of the shapes may be drawn in multiples, up to 5 objects at a time.

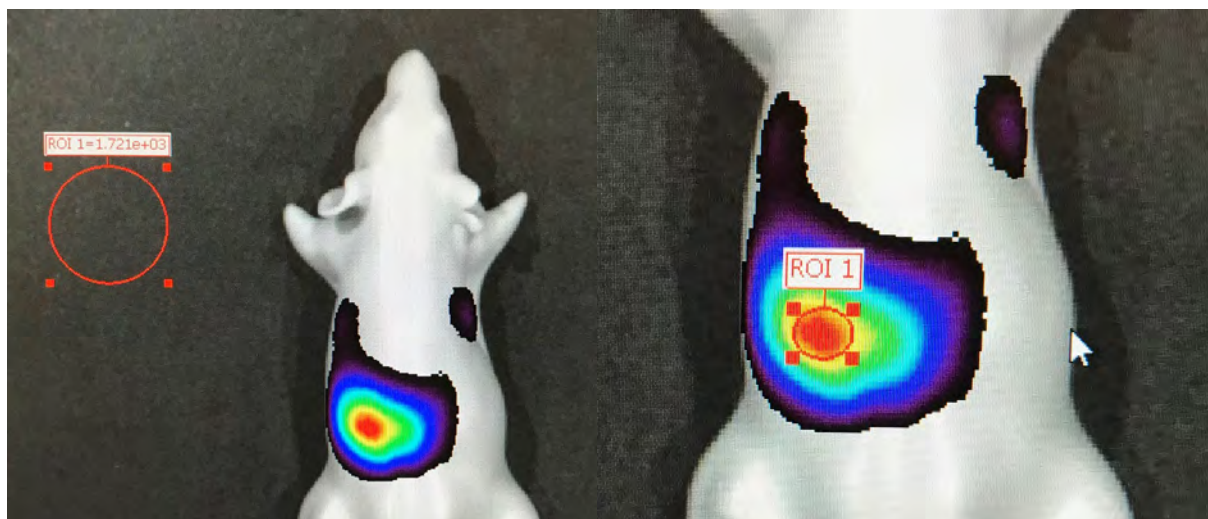
A simple ROI may be conducted as follows. First, a scan of the subject is required.



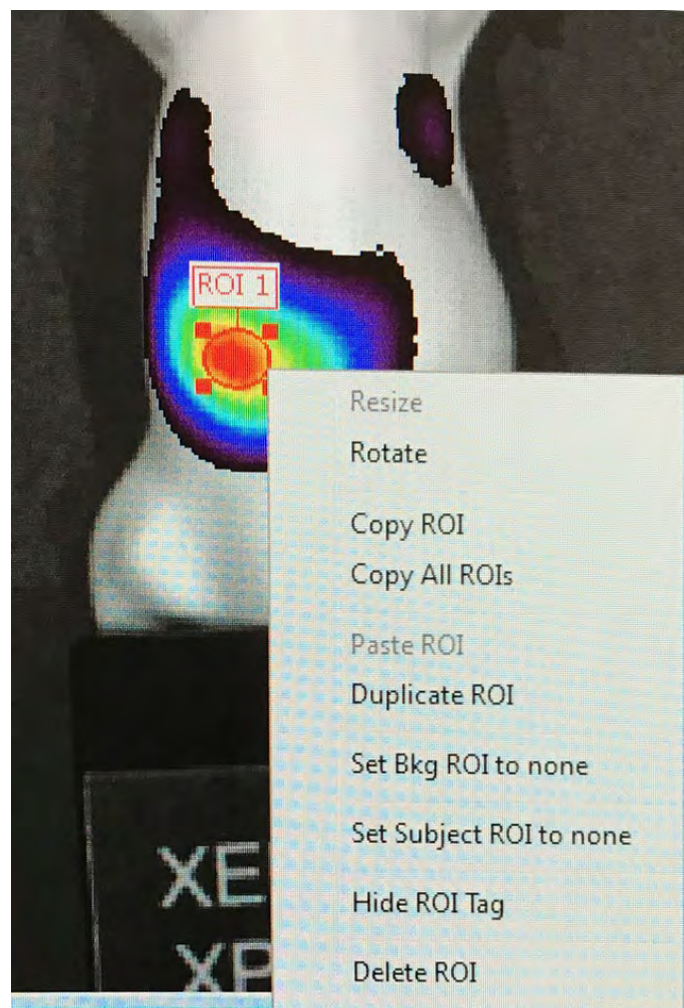
Further pictures will zoom in on the area providing detectable signal. For this example, the user will pick a single circle to be the ROI boundary. The user selects the circle tool, and then the dropdown number 1 to make one circle shape in the image.



A circle shape is propagated in the image. This shows the ROI number, and the value ascribed to the photons detected within its area. In the shown position, there are only background level photons (1721 for an area approximately 1.5cm in diameter).



The ROI circle may be pulled at the "corners" indicated by the 4 red squares to adjust its size and whether it is circular or ellipsoid in final shape, using the computer mouse.



- Resize
- Rotate
- Copy ROI
- Copy All ROIs
- Paste ROI
- Duplicate ROI
- Set Bkg ROI to none
- Set Subject ROI to none
- Hide ROI Tag
- Delete ROI
- Delete All ROIs
- Properties...
- Lock Position
- Lock Size

ROI Properties

ROI: ROI 1
Label: ROI 1
Shape: Circle
Type: Manual

Background ROI Subject ROI

Image Number
AAA20181211092727

ROI _none_
ID
Label

☐ Lock Position
Xc (pix) 891.33211
Yc (pix) 1167.73962
Angle (deg) 0.0000

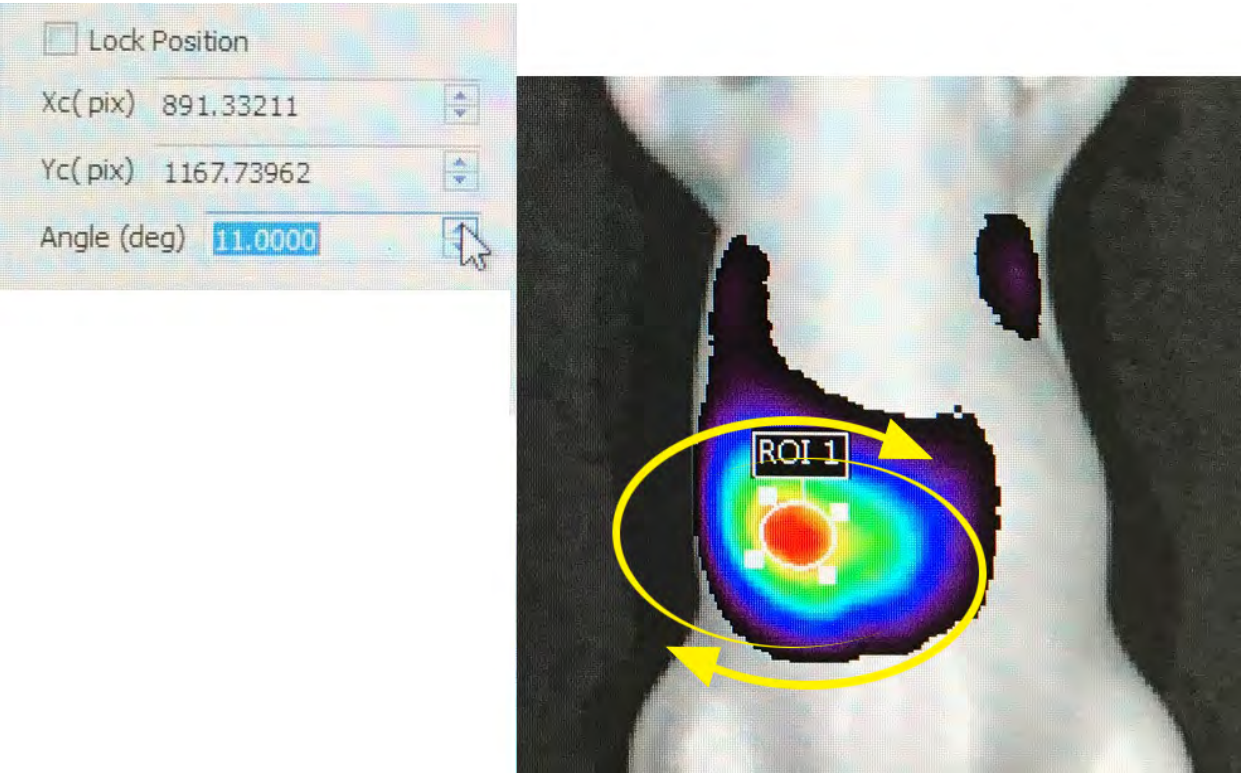
☐ Lock Size
Width (pix) 77.15491
Height (pix) 66.35322

Line Size 2
Line Color

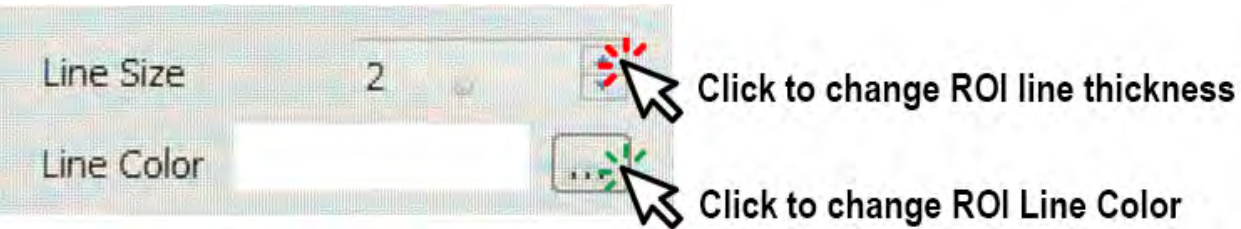
Done

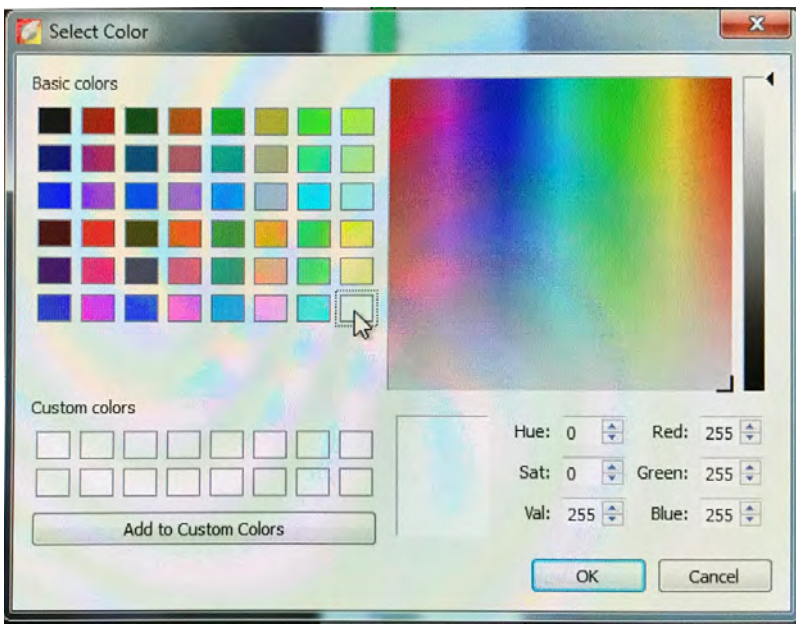
Further adjustments may be made by revealing the ROI menu. This requires using the computer mouse to right click on the actual circle (or other shape). Once this is done more menu options will be visible. This menu provides simple controls, and access to the ROI properties. Click on the Properties menu option to access these.

Shape Angle (deg.) change – use up/down controls to rotate shape



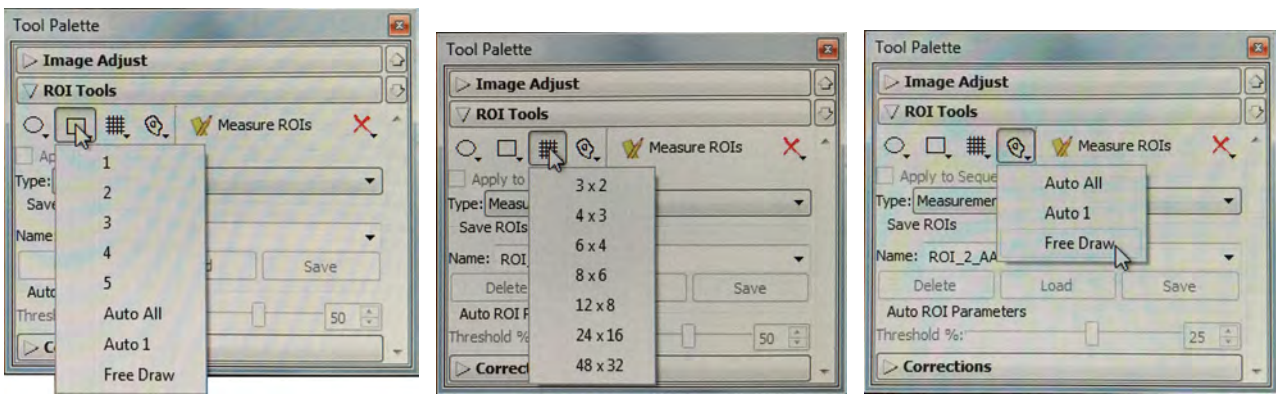
Line thickness and color change – click button to the right of the Line Color to access the color chart.





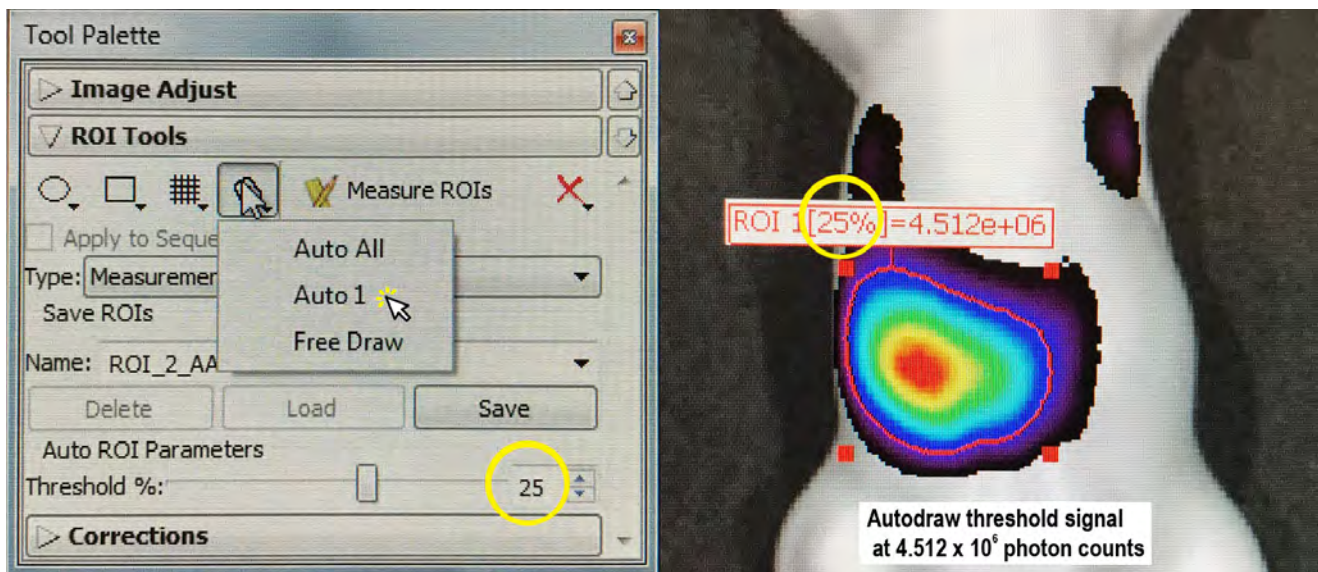
These properties allow the locking of the position and the size of the ROI, change of the line color and line weight (thickness), and provides information regarding the ROI and its parent image.

Similar options are available for the other tools.

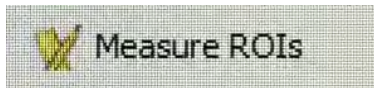


Other than the grid selection, there are options in each category to auto draw (software makes the best determination of the ROI) or to free draw the selected shape, where the user may use the mouse to draw around the area within the scanned image. Free drawing is the preferred option when the area to be defined as an ROI is irregular or complex in shape.

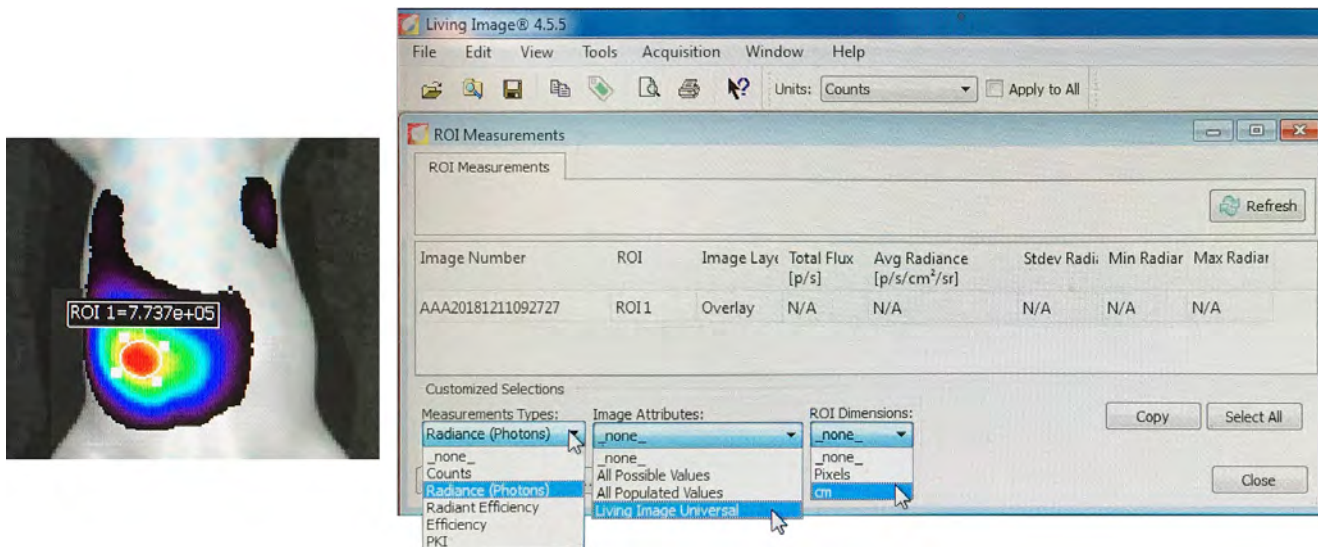
When using Autodraw, the software draws a line shape discounting a percentage of the low signal as the ROI. This percentage is set by the user using the slider control.



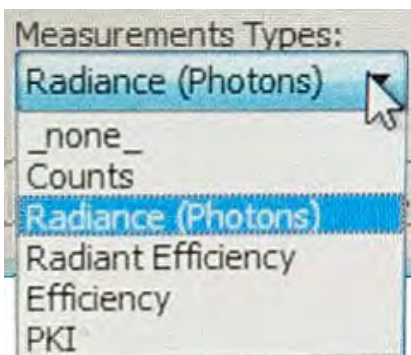
Once the region(s) of interest are selected, the user can click the Measure ROIs button and a new pane will appear containing the data for each on a separate line in the table displayed.



The new pane will require some modification by the user.



The above pane is shown with dropdowns. The user must make some selections to allow effective data capture.



Available Selections

none –

Excludes ROI measurements from the table.

Counts (most often used for LUMINESCENCE) –

Includes Total Counts, Avg Counts, Stdev Counts, Min Counts, and Max Counts in the table.

Total Counts = the sum of all counts for all pixels inside the ROI.

Avg Counts = Total Counts/Number of pixels or super pixels.

Stdev Counts = standard deviation of the pixel counts inside the ROI

Min Counts = lowest number of counts in a pixel inside the ROI.

Max counts = highest number of counts in a pixel inside the ROI.

Radiance (Photons) (most often used for FLUORESCENCE) –

Total Flux = the radiance (photons/sec) in each pixel summed or integrated over the ROI area (cm^2) x 4p.

Average Radiance = the sum of the radiance from each pixel inside the

ROI/number of pixels or super pixels (photons/sec/ cm^2/sr).

Stdev Radiance = standard deviation of the pixel radiance inside the ROI

Min Radiance = lowest radiance for a pixel inside the ROI.

Max Radiance = highest radiance for a pixel inside the ROI.

Radiant Efficiency (most often used for FLUORESCENCE) –

Epi-fluorescence – Fluorescence emission radiance per incident excitation irradiance: $\text{p/sec/cm}^2/\text{sr}/\mu\text{W/cm}^2$

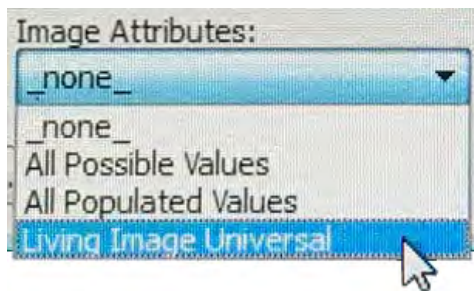
Transillumination fluorescence – Fluorescence emission radiance per incident excitation power: $\text{p/sec/cm}^2/\text{sr/mW}$

Efficiency (most often used for EPI-FLUORESCENCE) –

Fluorescent emission yield normalized to the incident excitation intensity

(radiance of the subject/illumination intensity)

PKI – Perkin Elmer saved configuration – not for client use.

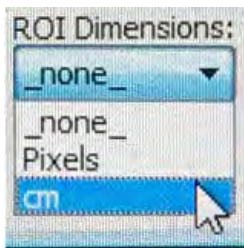


none – Excludes image attributes from the table.

All Possible Values – Includes all of the image attributes (for example, label name settings and camera settings) in the table.

All Populated Value – Includes only the image attributes with values in the table.

Living Image Universal – Includes all Living Image Universal label name settings in the table.

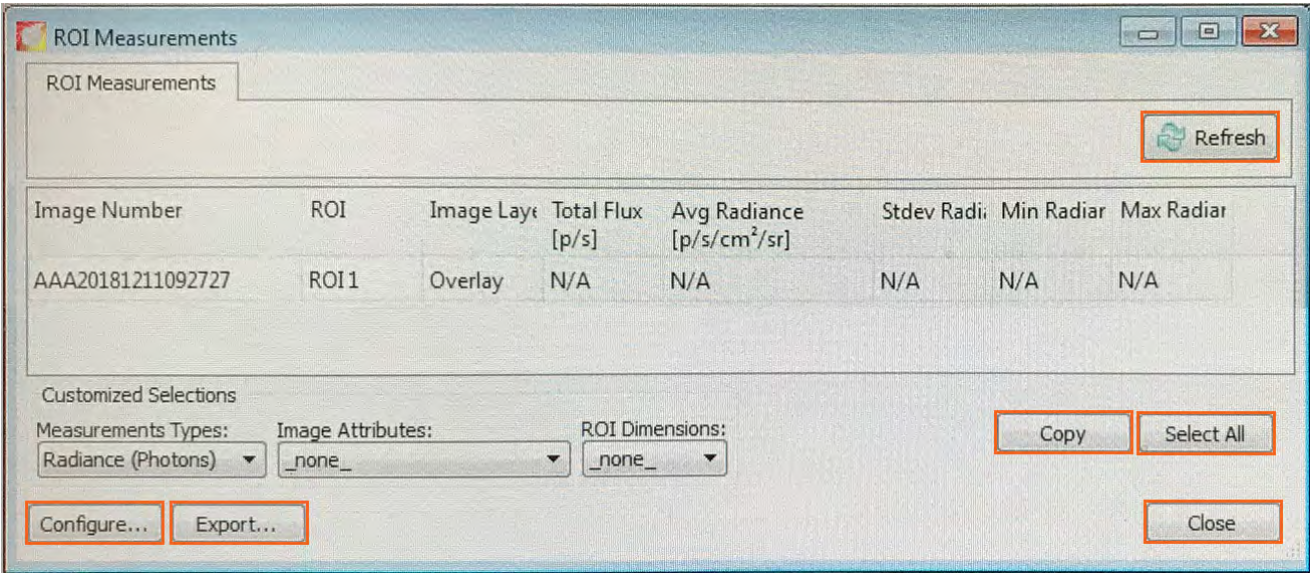


none – Excludes the ROI area, x,y-coordinates, and dimensions from the table.

Pixels – Includes ROI area, x,y-coordinates, and dimensions (in pixels) in the table.

cm – Includes ROI area, x,y-coordinates, and dimensions (in cm) in the table.

More Pane controls



Configure...

(https://ctac.mbi.ufl.edu/wordpress/files/2019/02/ROIPane_Configure.jpg) Displays the Configure Measurements box that enables you to specify and organize the data categories (column headers) for the table.

Export...

Displays the Save Measurements box so that the data can be saved to a .txt or .csv file. Note: Grid ROI measurements exported to a .csv file can be opened in a spreadsheet application like Microsoft® Excel®.

Copy

Copies the selected row(s) in the table to the system clipboard.

Refresh

Updates the ROI Measurements table (for example, after you draw new ROIs, move an ROI, and close or open image data).

Select All

Copies all rows in the table to the system clipboard.

Close

Closes the ROI Measurements table.

Once the user configures the ROI Pane to use, many more data columns will appear.

ROI Measurements

Refresh

Image Number	ROI	Image Layer	Total Flux [p/s]	Avg Radiance [p/s/cm ² /sr]	Stdev Radi	Min Radiat	Max Radiat	Area [cm ²]	Xc [cm]	Yc [cm]	Width [cm]	Height [cm]	Threshold	User ID	Analysis Ut	Sequence I	Date and T	Binning	Exposure	Field of Vie	f-Sto
AAA20181211092727	ROI 1	Overlay	N/A	N/A	N/A	N/A	N/A	1.902e-01	6.128e+00	8.028e+00	5.304e-01	4.562e-01	_none_	AAA			12/11/20...	1	0.77	13.20	1.00

Customized Selections

Measurements Types:
Radiance (Photons)

Image Attributes:
Living Image Universal

ROI Dimensions:
cm

Copy

Select All

Configure...

Export...

Close