

# UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

<https://biotech.ufl.edu>

## CTAC / ICBR SOP : Recognition of the Components of the IVIS Software Tool Palette

**Title:** Recognition of the Components of the Living Image™ Tool Palette

### **Materials Required:**

Computer

Living Image™ software

### **Purpose:**

To aid the user in recognition of the component parts of 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.

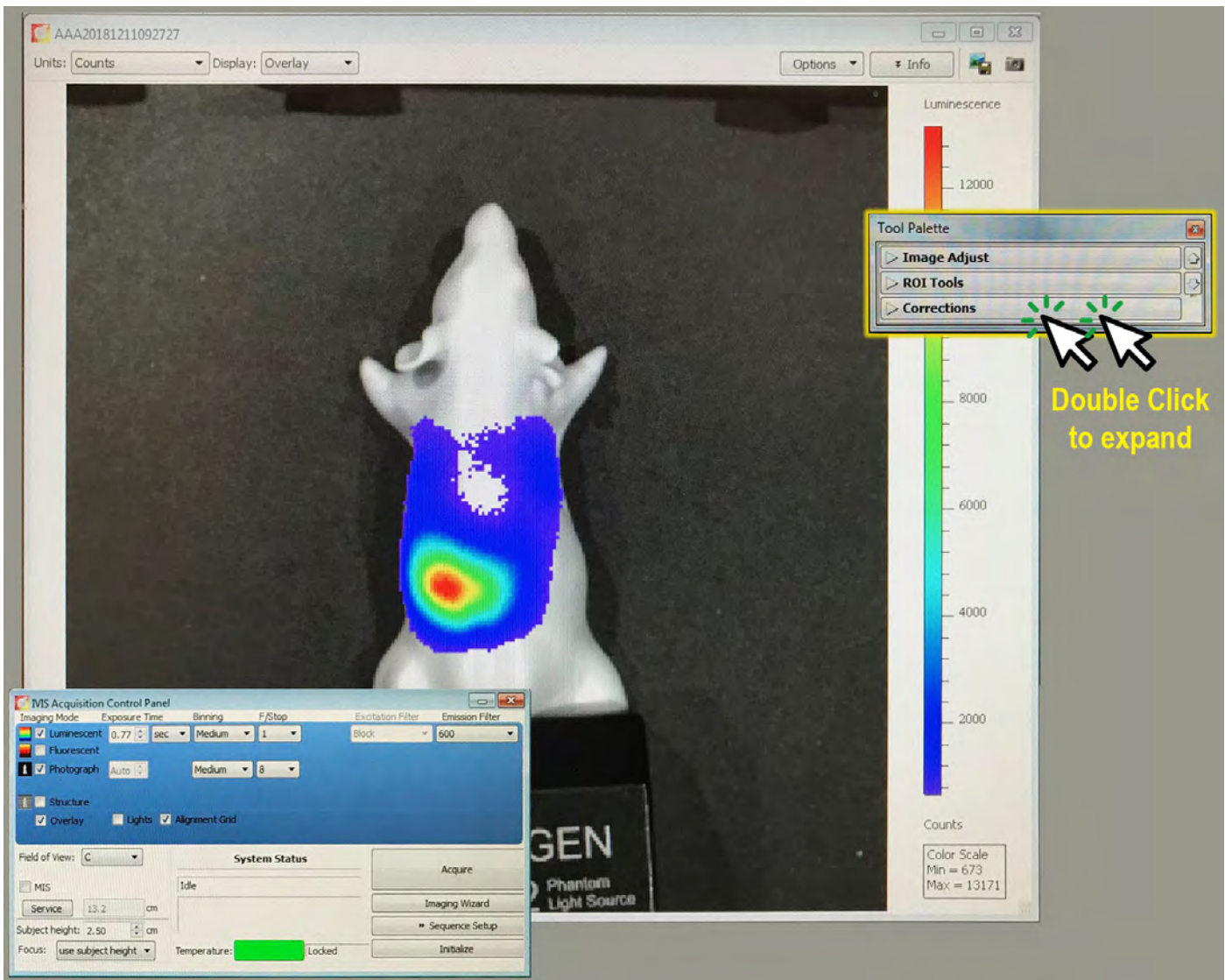
### **Tool Palette component identification:**

When conditions are input into the main instrument controls, the instrument will conduct the scan according to the set parameters and an image will be generated. When this happens, the Tool Palette will be displayed to the right side. (see figure below, yellow highlighted area).

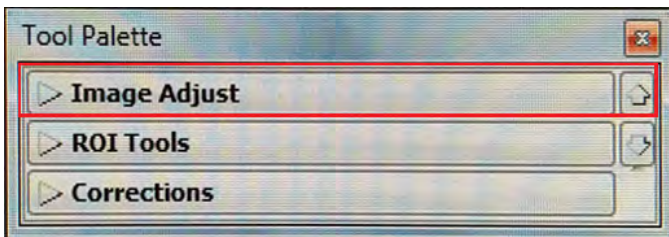
The standard Tool Palette has three different sections. These are:

1. Image Adjust
2. ROI Tools
3. Corrections

Each section is displayed as a collapsed menu – clicking on the button for each of these will expand the section downwards to allow the controls to be seen, accessed and manipulated.



## The Tool Palette

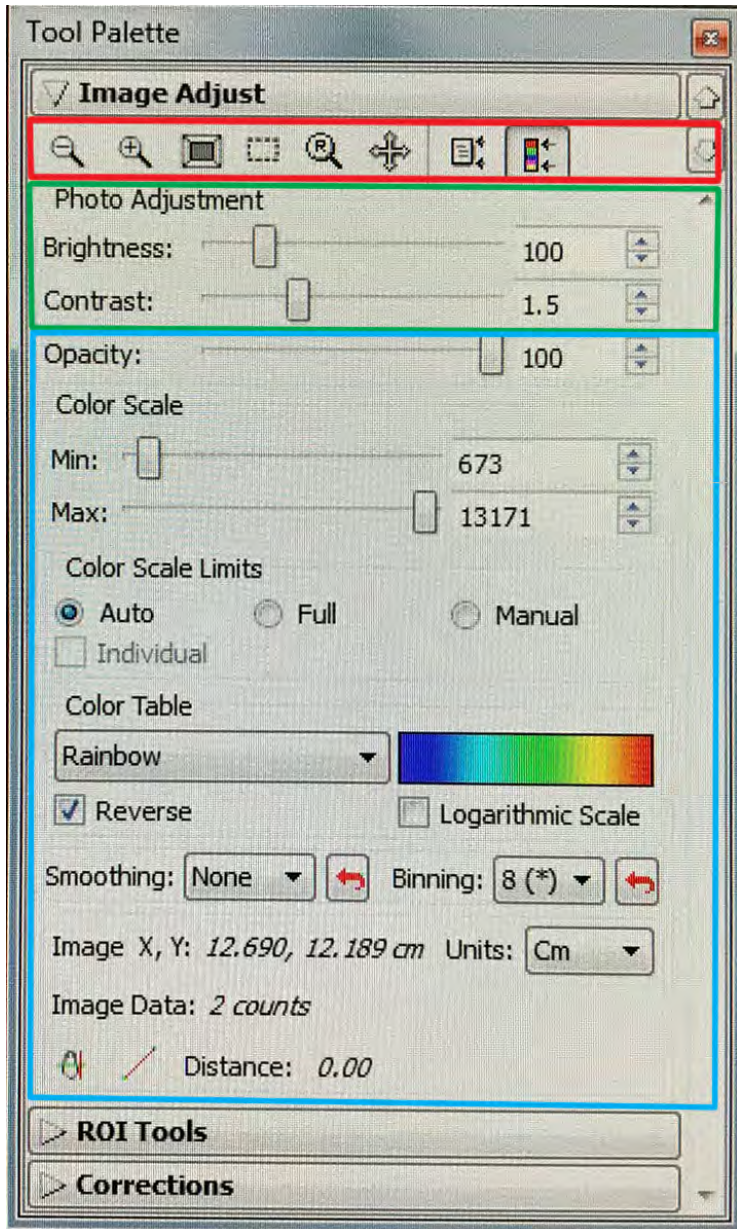


The first section in the Tool Palette is the Image Adjust section. This is comprised of three functional areas.

The first, highlighted in RED (next figure), allows general controls of the whole image, including moving it around, zooming and how it is displayed / what tools are shown.

The second area, highlighted in GREEN, allows basic photographic setting control of the black and white base image, upon which the scan will be superimposed.








The third area, highlighted in BLUE, allows control of the manner in which the scanned data set is displayed over the black and white base image.



- Adjustment controls for manipulation of the whole image
- Adjustment controls for the underlying black and white photograph of the subject(s)
- Adjustment controls for the scanned image(s) generated by the IVIS Spectrum

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**Whole Image Controls**

-  Zoom Out
-  Zoom In
-  Expand to / contract from Full Screen
-  Set image selection area
-  Reset changes to start
-  Drag / move image X or Y axis
-  Display / Hide Image Min. / Max. Information
-  Display / Hide Color Scale Min. / Max. Information

Controls for the Photo Adjustment are basic, where only lightness / darkness and contrast are changeable.

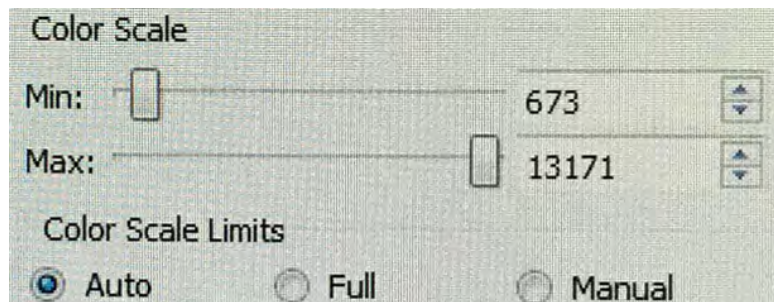
The next series of controls are those for adjustments of the scanned image(s).

**Opacity**



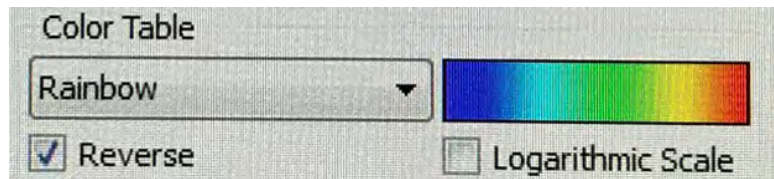
This control changes the degree of opacity of the colored data set superimposed upon the black and white base image(s). 100% is full opacity, and moving the slider left to reduce the percentage value makes the data set more and more transparent.

## Color Scale

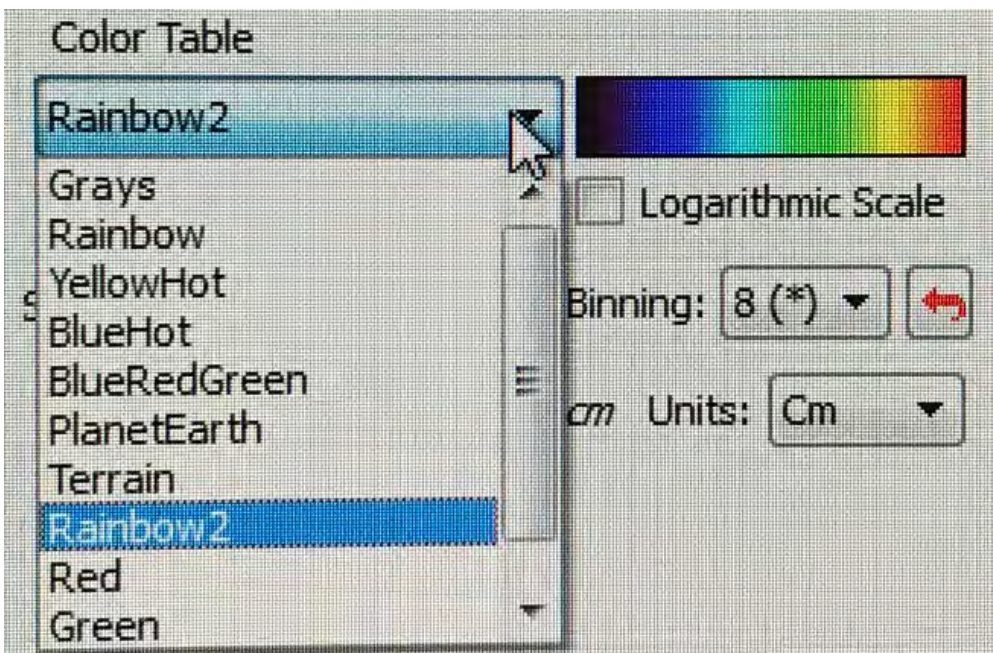


This control provides an automatic gating of the dataset to show default minimum and maximum thresholds (default, Automatic check box enabled), or allows the user to gate the data set to given values under Manual control. This threshold gating control is a powerful tool. It can be used to discount low background signal and better refine the signal-to-noise, and can change the maximum thresholds to more accurately acquire high values without compromising the fidelity of lower values. It may be used to normalize data gathered at different times or conditions to create larger data pools.

## Color Table

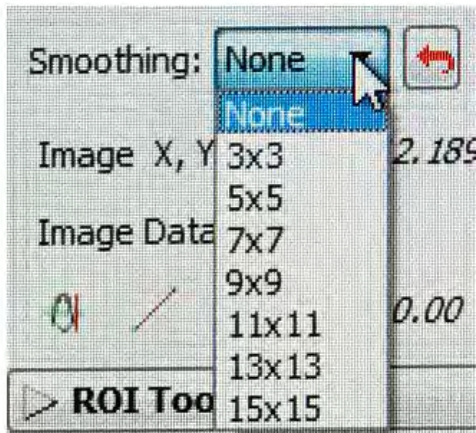
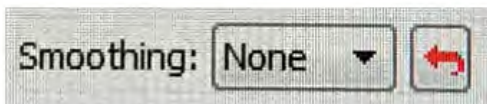


The Color Table allows the user to select different ways of representing the acquired data that is superimposed upon the black and white picture. The default for Luminescent scans is the Rainbow color table, which is a standard 5 color rainbow. The default for Fluorescent scans is YellowHot, which is a 2 color gradient. There are many different choices for the user to select from. The color table used may also be reversed using the Reverse checkbox toggle. The scale may also be toggled from linear (default) to logarithmic by checking the Logarithmic Scale checkbox.



## Smoothing

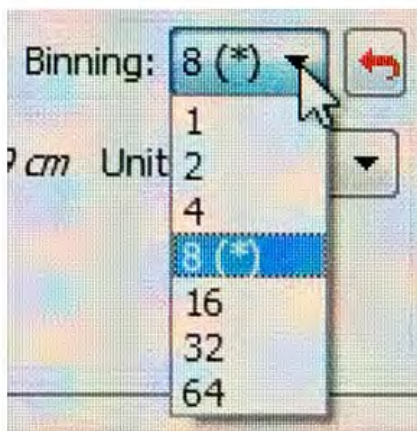
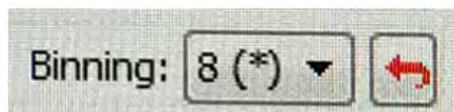
This control reduces the degree of pixelation by averaging the values over different areas. The normal (default) display shows the scan pixel values at 1 x 1 (no averaging, marked None in the control dropdown). The user may select 3 x 3 averaging, or 5 x 5, etc. There will be a consequent drop in scan resolution as the smoothing is increased.



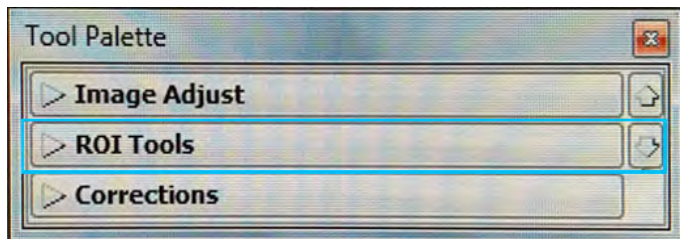
## Binning

This selection represents Binning control post acquisition, rather than during it. Binning, unlike averaging, is based upon a compression algorithm. Increased binning, or application of the algorithm, results in compression of the image size. However, the more binning that occurs, the lower the

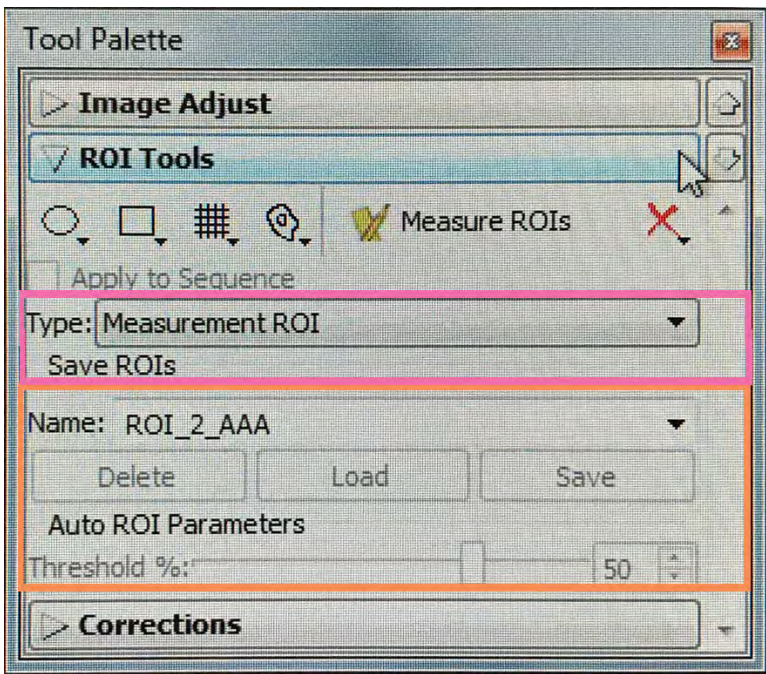
effective resolution will be. The algorithm is written in such a way that when all pixels around a center have the same value, within the tolerances programmed, this will be compressed to a single value for all 9 (e.g. 3 x 3 pixels). This effect is similar visibly to smoothing, but occurs by a different process.





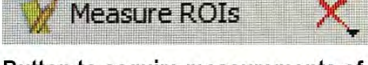





The second dropdown area in the Tool Palette is the ROI Tools. ROI stands for Region Of Interest, and represents 1 or more selected areas on the image targeted by the user to acquire specific data sets within the scanned image.



Clicking this center bar will expand the tools to show all options under this heading.



-  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 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.

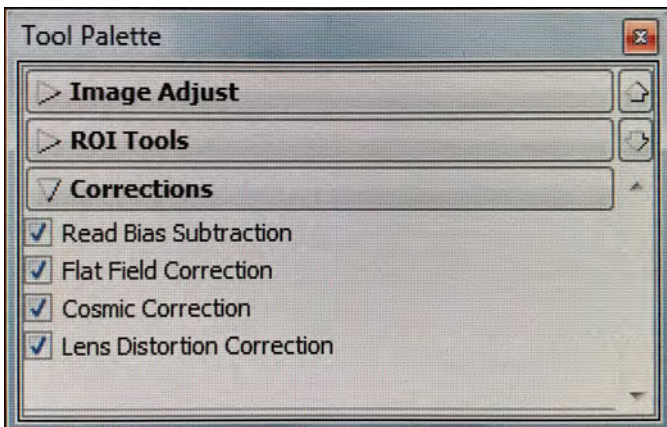
Once the region(s) of interest are selected, the user can click the Measure ROIs button and a new pane will appear containing the data for each on a separate line in the table displayed.

For more details on specifics involved in the acquisition of data from ROI selected areas, please refer to [CTAC / ICBR SOP Capturing Data Using the ROI Tools in the Tool Palette from an Acquired Image](https://ctac.mbi.ufl.edu/ivis-spectrum-how-to-documents-and-sops/ctac-icbr-sop-capturing-data-using-the-roi-tools-in-the-tool-palette-from-an-acquired-image/) (<https://ctac.mbi.ufl.edu/ivis-spectrum-how-to-documents-and-sops/ctac-icbr-sop-capturing-data-using-the-roi-tools-in-the-tool-palette-from-an-acquired-image/>)

The area highlighted in PINK is the control to specify the measurement type.

The ORANGE area contains the controls to save ROIs of specific shapes, sizes or orientations.

The last area in the Tool Palette is the **Corrections** area. By default, all corrections are on.



**Read bias subtraction** – This is an electronic offset that exists on every pixel. This means that the zero photon level in the readout is not actually zero, but is typically a few hundred counts per pixel. The read bias offset is reproducible within errors defined by the read noise, another quantity that must be determined for quantitative image analysis.

**Flat Field Correction** – Flat fielding refers to the uniformity of light collected across the field of view (FOV). A lens usually collects more light from the center of the FOV than at the edges. The Living Image software provides

a correction algorithm to compensate for the variation in the collection efficiency of the lens. This enables uniform quantitation of ROI measurements across the entire FOV.

**Cosmic Correction** – Cosmic rays are extraterrestrial high-energy particles that register a false signal on a CCD detector. Cosmic rays as well as other sources of ionizing radiation cause infrequent interactions (a few per

minute) on the CCD. These interactions result in large signals that are usually isolated to a single pixel, making them easy to correct. The Living Image software searches for isolated, high amplitude hot pixels and replaces them with a collective average of surrounding pixels. The Cosmic Correction option should always be selected for in vivo image data because hot pixels can significantly affect an ROI measurement.

**Lens Distortion Correction** – This option is selected to correct for distortion at the perimeter of an image due to curvature of the CCD lens. Lens distortion correction is available for data acquired by Living Image® software version 4.3 and higher