

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

<https://biotech.ufl.edu>

CTAC / ICBR SOP : Using the Color Scale to Manipulate an Acquired Image in the Tool Palette

Title: Using the Color Scale to Manipulate an Acquired Image in the Living Image™ Tool Palette

Materials Required:

Computer

Living Image™ software

Purpose:

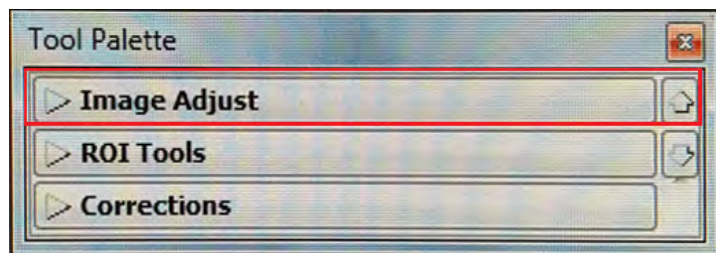
To aid the user in understanding the function of the Color Scale in the image Tool Palette.

Background:

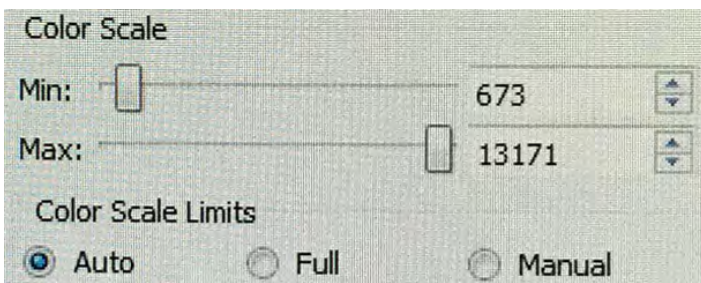
The IVIS Spectrum Imaging System is controlled by the Living Image™ software. When a scan is completed, the image (or images if a sequence scan is performed) will have its own control panel, in the form of the Tool Palette. The Color Scale is a powerful tool that can be used to gate the signal to perform signal-to-noise reduction and increase image fidelity.

Color Scale and its use:

The Color Scale is found in the Image Adjust section of the Tool Palette. This bar should be clicked to expand the options available.

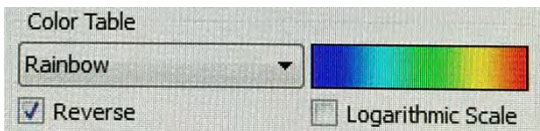


Color Scale

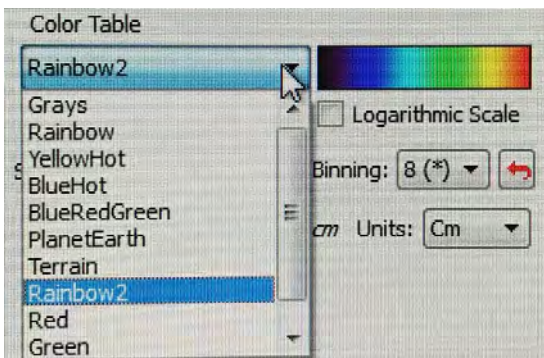


This control provides an automatic gating of the dataset to show default minimum and maximum thresholds (default, Automatic check box enabled), or allows the user to gate the data set to given values under Manual control. This threshold gating control is a powerful tool. It can be used to discount low 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. The representation of the dataset shown within these minimum and maximum thresholds is controlled through the use of the Color Table.

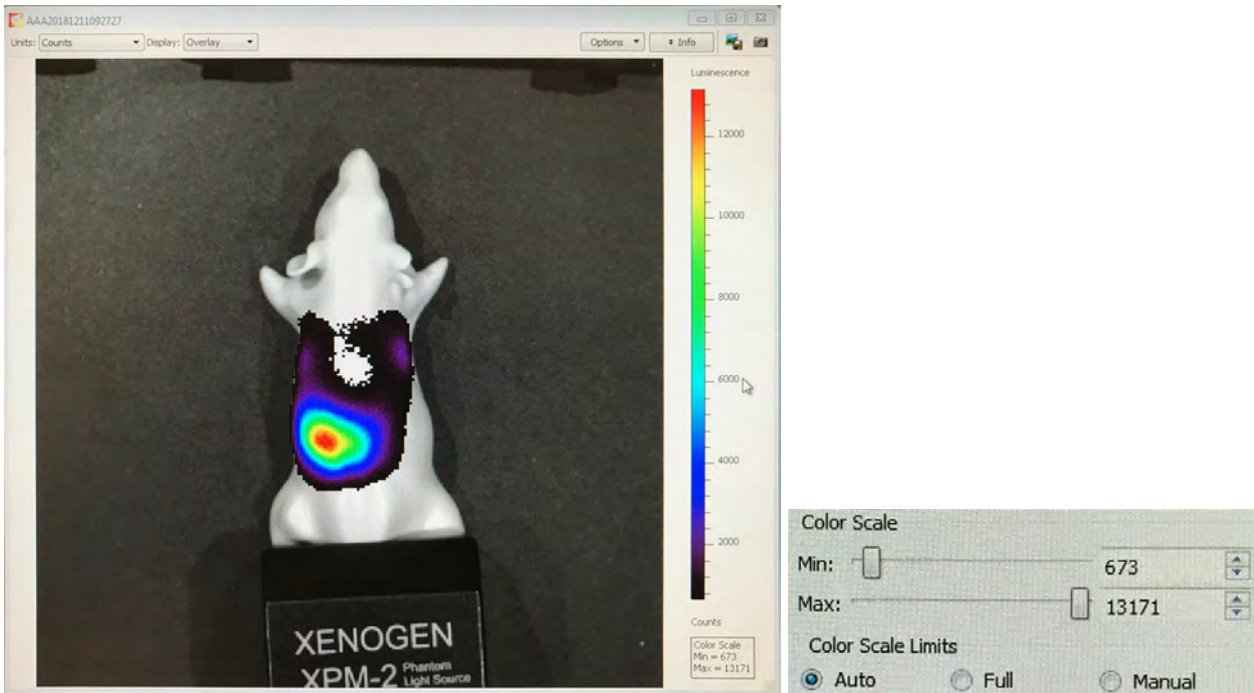
Color Table



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.



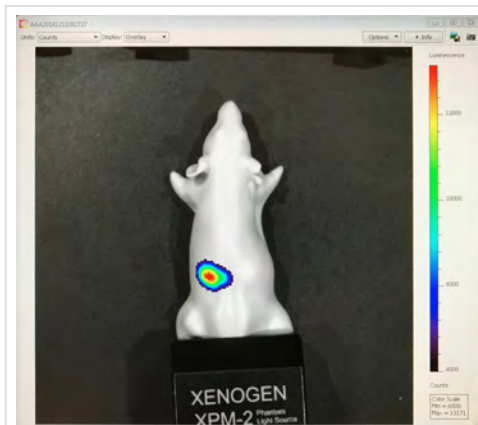
When we scan a subject, the software automatically assigns minimum and maximum threshold values. Data beneath the minimum is not displayed on the image. Data above the threshold is not represented by any further intensity.



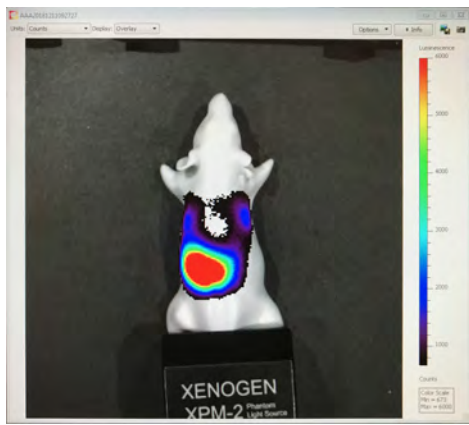
The IVIS software typically sets the gates at a minimum of 5% of signal and a maximum of 95% of signal.

This is a powerful tool to assist in optimizing your signal. However, care must be taken to use this setting to normalize all your subjects to either known positive and negative controls, or to derive the overall minimum and maximum for a heterogeneous experimental group.

As data is typically acquired across many day, weeks or months, all data acquired can be processed post-acquisition, so that experimental groups may be normalized to each other at that time. This is particularly useful when there is an unexpected observation or variable that may result in a change in the overall assessments of positivity or negativity.



Same scan with the maximum gate value reduced to 6000 from an automatic value of 13171.



Same scan with the minimum gate value increased to 6000 from a automatic value of 13171.