2015-08-17
how to view images and quantify signal from IVIS 20150817

View images and Quantify Signal from IVIS

Computers **INVIVO-ANALYSIS** and analiza-4 are normally never shut down and are accessible on the Network from all computers in the building under **Microsoft Windows Network → Medicine**.
To access, in Windows go to Start → Run and enter **\\INVIVO-ANALYSIS \\IVIS_Data**
Please back up your data from **INVIVO-ANALYSIS** or **analiza-4** to your computer or to your Dropbox (or similar) account.

Data from **IVIS** are stored on **INVIVO-ANALYSIS** in the following location:
C:\ IVIS_Data \[IVIS year]\mm-yyyy\[PI]

Individual experiment folders saved on **IVIS** contain a PNG file (which can be opened with common imaging or system software programs) along with TXT files of experiment information and settings.
Data set folder and PNG file are named according to PI initials, acquisition date and time:
PIyyyymmddhhmmss

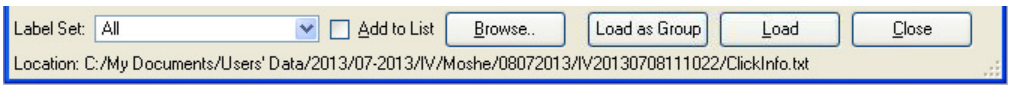
Always use locally stored file copies when you perform analysis.

Use **Living Image 3.2**, an offline software version.
When prompted to Select User use your PI's initials.

Visualization of experiments

File → Browse
You may choose a folder which contains a series of data set (experiment) folders or one data set folder.

In the ensuing table you may add to the list experiments performed on a different session by checking Add to List and pressing Browse…

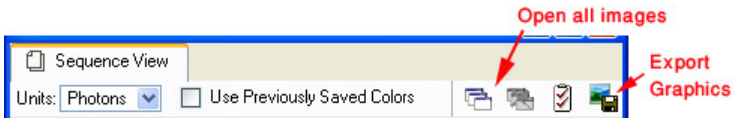


From the list, using <ctrl> or <shift>, choose individual experiments.
Press Load to open an individual window for each experiment or press Load as Group to open a sequence of experiments in one window.

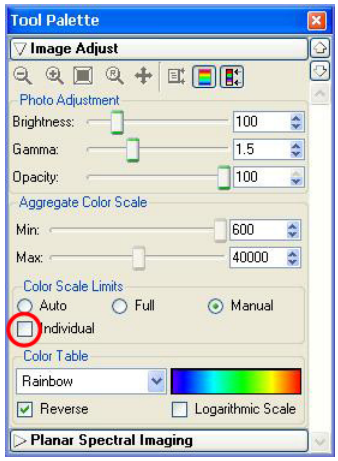
Load as Group Visualize a sequence of experiments

Press Load as group to open a group of experiments in one window.
(**Bug**: Part of the image window may be solid black. Resizing slightly by dragging the lower right corner corrects this).

Change Units: to Photons



Unify the pseudocolor scale: In the Tool Palette that appears, in Image Adjust, in Color Scale Limits uncheck Individual.
If there are great differences between the images consider checking Logarithmic Scale.



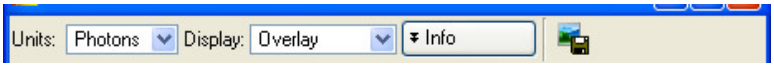
File → Save as…
The sequence is saved as a separate folder with all the sub data sets copied inside.

The image can be exported as TIFF or JPEG by pressing .
The images can be viewed individually and used for quantitation by pressing .

Load Visualize individual experiments

Use Load, or, alternatively, use File → Open and inside each experiment folder choose any file to open individual experiments side by side for visualization and/or quantitation.

In the image window change Units: to Photons.

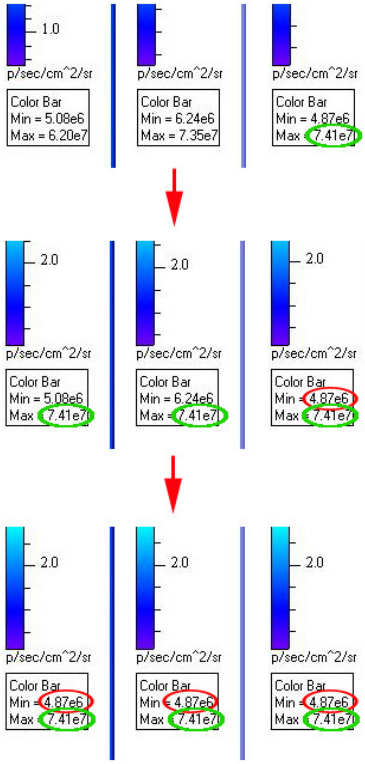


Unify the pseudocolor scale:
Press Info to see the experiment parameters.
You can edit info by Edit → Image Labels

In Tool Palette, Image Adjust, Color Scale, select Manual.

From the Color Bar legends of the images (legend below the pseudocolor bar to the right of each image) note the lowest minimum and the highest maximum of the group.

In Tool Palette, Image Adjust, Color Scale, copy-paste these values at the Min: and Max: sliders of each image.



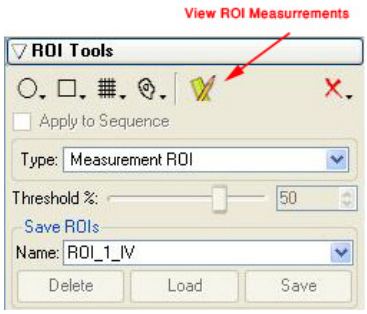
Quantitation of Regions of Interest (ROI):

Luminescent signal:

When the active window is a single experiment, the tool palette is extended.

Manually defined ROIs:

In Tool Palette, ROI Tools, choose circle, rectangle or contour to define one ROI at the first image (if you choose a number greater than 1 you will not be able to resize equally).

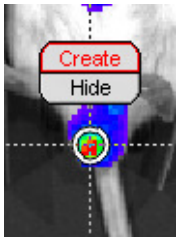


Resize or change proportions by dragging ROI handles (↘), reposition by panning (✣), rotate by <right-click> on the ROI, choosing Rotate, and dragging ROI handles (↻).

<Right-click> Duplicate ROI.
Reposition/manipulate duplicates.
Copy ROIs including their position by <right-click> Copy all ROIs.
In the other experiment windows <right-click> Paste ROIs.

Automatically defined ROIs:

To automatically draw ROIs detected by the software, in Tool Palette, ROI Tools, in one of the circle/square/contour choose Auto All.



To automatically draw one ROI at a user-specified location choose Auto 1.
Use the ring that appears to position it.

Press Create.

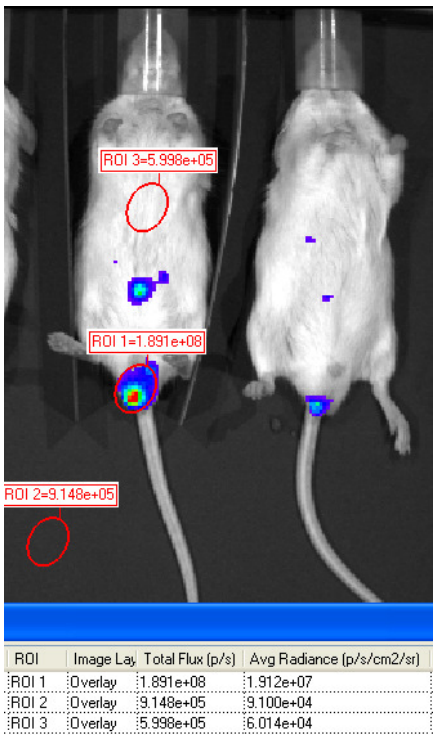
For automatically generated ROIs define the minimum % of peak pixel intensity to be included automatically by the Threshold% slider in ROI Tools.
Rule of thumb: Use 20% threshold.

The sum of animal non luminescent background and system background signals should be subtracted from the signal.

To this end, the ROI of interest should be duplicated twice:
1) on a non luminescent place on the animal and
2) on a black surface in the image.

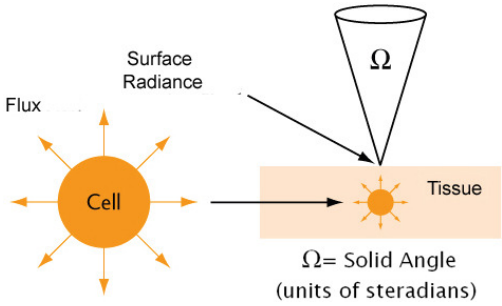
Positions of automatically created ROIs are locked.
<Right-click> Unlock positions to unlock and move.

View → ROI measurements (or click on the icon in ROI Tools to obtain a table of measurements).
(You may choose to order the list by clicking on the ROI column to facilitate comparison between different experiments on the same animal.)



Average Radiance, in photons/second/cm²/steradian is a commonly used measurement of interest in luminescence.

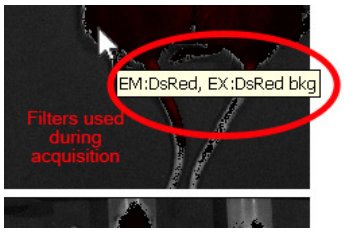
The table can be exported by pressing Export and choosing CSV which is opened by EXCEL.



Fluorescence:

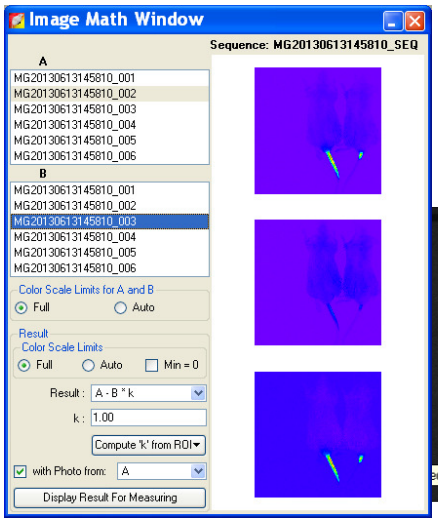
Browse to open images to create a sequence (see "visualization of experiments" above for details).

File → Save as…
The sequence is saved as a separate folder which includes all the sub data sets.

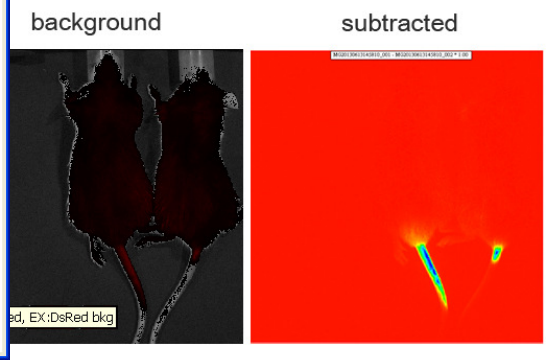


A tool tip with the excitation and emission filters used for acquisition appears when you point to the different images.

With the saved sequence file open we can use Tools → Image Math on **PIyyyymmddhhmmss_SEQ**.



We can e.g., subtract the signal acquired with the **DsRed ex. DsRed em. Bkgd**. from the one acquired with **DsRed ex. DsRed em**.



The subtracted image can be analyzed and quantified like luminescence experiments.

Online manual of Living Image 4.0

http://www.perkinelmer.com/CMSResources/Images/44-135288BRO_LIV-BR-01-5832.pdf