

## IVIS Cheat Sheet

Open Living Image (on Desktop)

Click “Initialize” button on lower right

Click temperature indicator bar – set stage temp. to desired value (default is 37° C for animals)

Shave/Nair a wide area of your animals around the target zone for best results (not needed for nude animals)

For luminescence - dose luciferin according to relevant protocols

Select desired image types and acquisition settings, or go through the wizard for settings

Field of view is A, B, C, or D when the expander lens is removed. It will be fixed as “E” if the lens is in place. When the lens is in place “XFOV24” **must** be checked. When the lens is removed, “XFOV24” **must** be unchecked. If the checkbox does not match the hardware, the image will be very out of focus.

For acquisition– look at Counts (under Units), max should be 600-60,000, otherwise change your acquisition settings (exposure time is simplest to change) – simple approach, click down arrow on exposure time past zero it will say “auto” for the exposure.

For fluorescence, take a fluorescence background for each set of settings you use with the sample removed. Acquisition->Measure and Replace fluorescence background, or once you have measured it once Acquisition->Add or Replace Fluorescence Background, for each selected image. Once you measure the background for a given setting, it will be applied automatically to subsequent measurements using the same exact settings. Auto exposure is less desirable for fluorescence because the fluorescence background needs to be measured for every unique setting used, we suggest using 2 or 3 different exposure times that you can determine based on your samples, then at the beginning or end of each experiment, you can just measure the background for these few settings.

For analysis, switch units to “Radiant Efficiency” for fluorescence; switch to “Radiance” for bioluminescence and use the ROI tools to quantify.

Contact facility staff with questions: <http://sites.duke.edu/imaging/contacts/>