TECHNICAL NOTE

Pre-clinical *in vivo* imaging

Well Plate Quantification

Bioluminescence and Fluorescence tomography allows a user to determine not only the depth and anatomical localization of their source(s) but also the intensity of those sources expressed as either photons/second (DLIT) or total fluorescent yield (pmol/M cm) (FLIT). It is possible to extrapolate the number of cells in a DLIT source or the number of dye molecules or cells in a FLIT source if a quantification database is available. The database is derived from an analysis of images of known serial dilutions of luminescent or fluorescent cells, dye molecules, or labeled compounds.

Note

1. Prepare your plates for imaging
   a. Serially dilute stably transfected bioluminescent/fluorescent cell lines in a black clear bottom 96 or 24 well plate.
   b. Serially dilute free dye or labeled compounds into a black clear bottom 96 or 24 well plate.

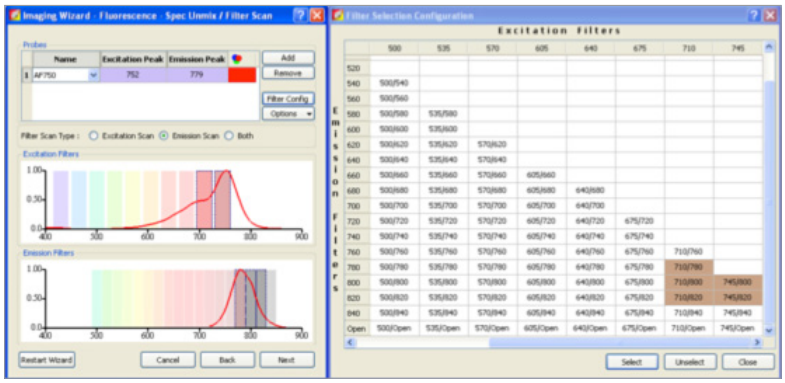

Figure 1. Use multiple pairs of excitation/emission filters when quantifying well plates for the first time

2. Take images of the plates
   a. Bioluminescent plates will be imaged with an open filter configuration
   b. Fluorescent plates will be imaged using multiple filter pairs around the peak excitation and emission for the dye, quantum dot, or fluorescent protein. Since the extinction coefficient and effective cross section of the fluorescent probes will vary depending on which excitation/emission filter pair is chosen, it is wise to take images using multiple combinations and store it away as a single database. In the example above, the peak excitation of AF750 is 752 nm and the peak emission is 77 9 nm. We have chosen excitation filers 710 and 745 nm and emission filters 780,800, and 820 nm.

3. After the images have been acquired, go to **Tools** at the top of the software and select **Well Plate Quantification.**

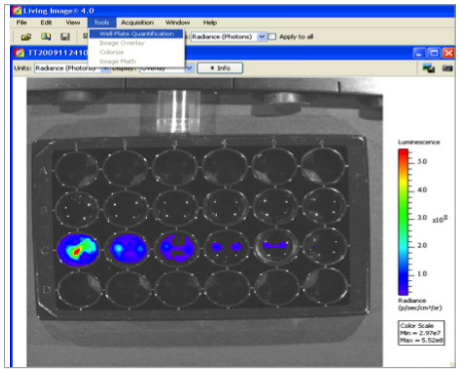

Figure 2. Well Plate Quantification option in the Tools drop-down menu

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4. The Well Plate Quantification Window will open.

5. For bioluminescent cells, choose the well plate format from the drop-down menu at the top of the window. An ROI will appear in the image. Position the ROI and enter the cell numbers corresponding to the appropriate well in the window. Select these wells and press **Set** beside the window for **Sample Wells.** Then choose wells to represent background control and press **Set** beside the window for **Background Wells.** Lastly, select Quantify to calculate photons/sec/cell.

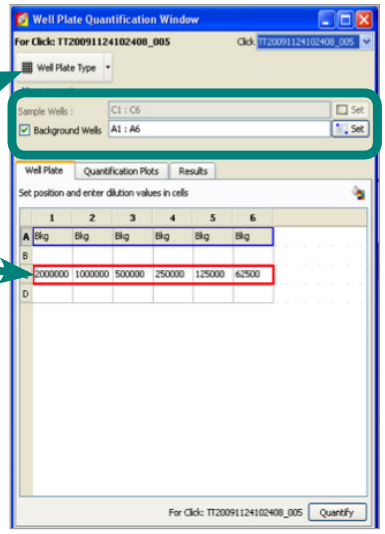

Figure 3. Bioluminescent cell quantifications and well plate coordinates are entered

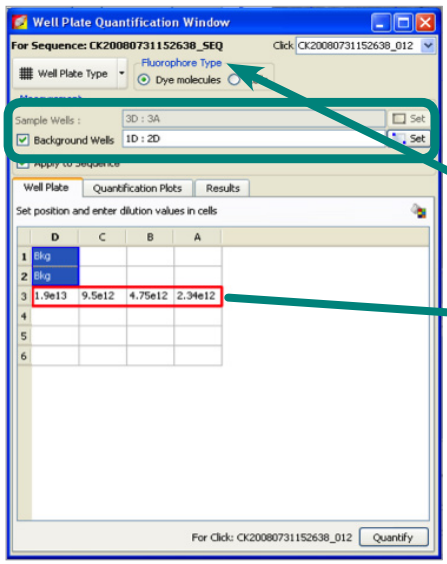

Figure 4. Fluorescent probe concentrations as well plate coordinates are entered

6. For fluorescent reporters, choose what you are measuring – cells or dye/quantum dots. Choose the well plate format from the drop-down menu at the top of the window. An ROI will appear in the image. Position the ROI and enter the cell numbers, number of quantum dots, or dye molecules corresponding to the appropriate well in the window. Quantification can also be performed with a labeled compound (drug, antibody, etc) and must be entered as number of molecules of compound per well if you would like the output to be expressed as pmol of your compound. Select these wells and press **Set** beside the window for **Sample Wells.** Then choose wells to represent background control and press **Set** beside the window for **Background Wells.** Select **Apply to Sequence** to make the calculation for every filter pair in the sequence. Lastly, select **Quantify** to calculate the extinction coefficient and cross section for the fluorophore at each of the filter pairs imaged.

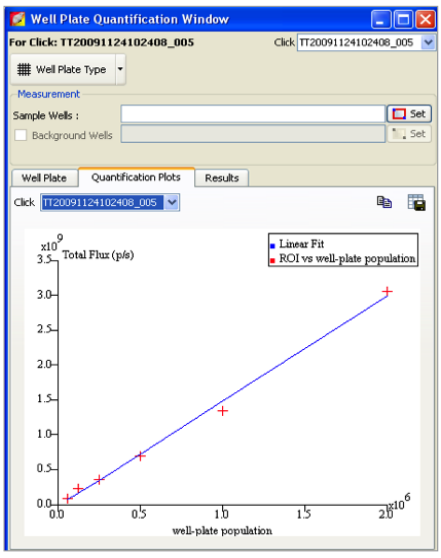

Figure 5. Linear fit between dilutions and signal intensity is verified

7. The **Quantification Plots** tab will show you the linear fit of the calculated values to the measured ROIs.

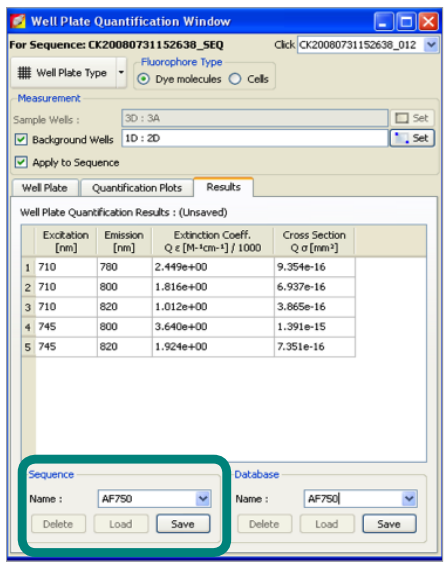

Figure 6. Save the Acquisition Sequence

8. The results tab displays the calculated values for each image that was included in the analysis. To save the data to the image **Sequence** either type a name or use the default name and press **Save.**

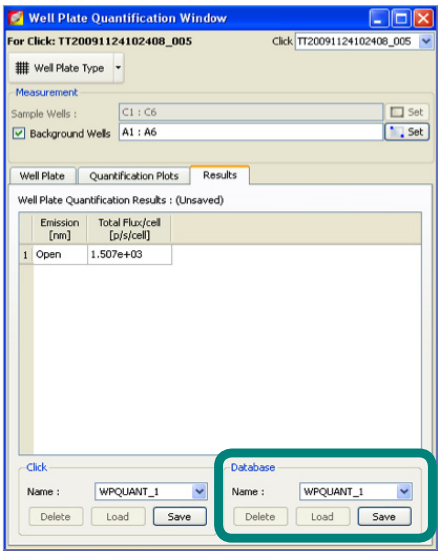

Figure 7. Save the newly created Well Plate Database

9. To create a database for use on the system you are working with, enter a name and select **Save.**

   Note: Databases stay with a system and are not user specific. To transfer a database, copy the image sequence to the computer you would like to create the database on. **Load** the results using the Well Plate Quantification Window and save a copy of the database on the new system.

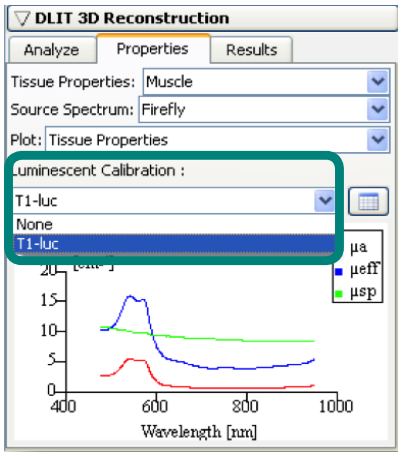

Figure 8. Load the Well Plate Database

10. To use this database to calculate cell numbers or pmol when analyzing DLIT and FLIT results, select the database from the drop down menu below **Luminescent** or **Fluorescent Calibration** under the **Properties** tab in **DLIT 3D Reconstruction** in the **Tool Palette.**

Note: For more information on setting parameters for DLIT of FLIT analysis, please see the Bioluminescence Tomography – Setup and Sequence Acquisition Tech Note 4a, or the Fluorescence Tomography – Setup and Sequence Acquisition Tech Note 6a.

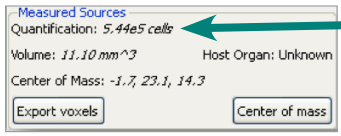

Figure 9. 3D quantification is reported as cells or pmols

11. When measuring voxels, results will be displayed as cells or pmol.

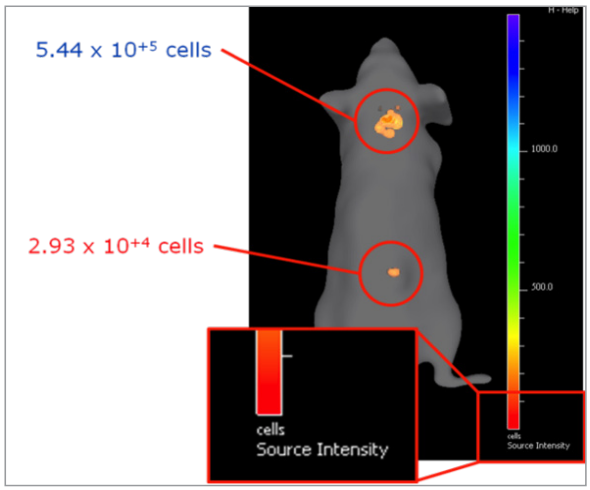

Figure 10. 3D quantification is noted on the color map.

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