

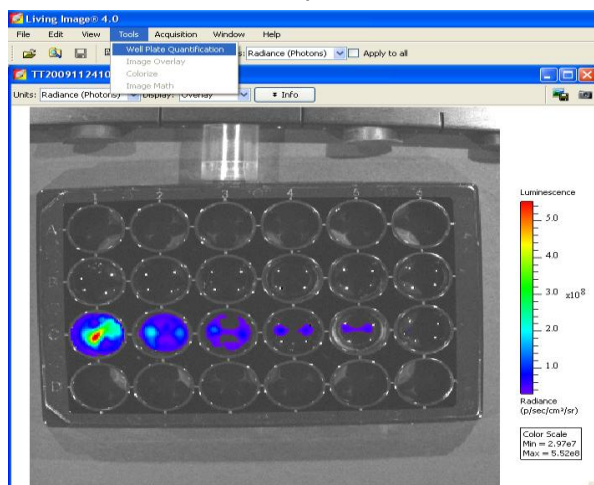
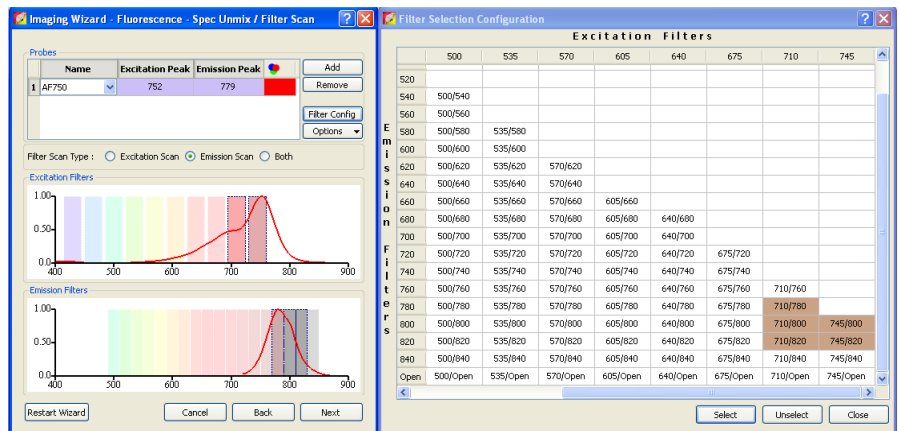


# Well Plate Quantification

**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:** Please refer to the **Bioluminescence Tomography Tech Note Series 4** and/or the **Fluorescence Tomography Tech Note Series 6** for more information on 3D reconstructions needed for unit conversions.

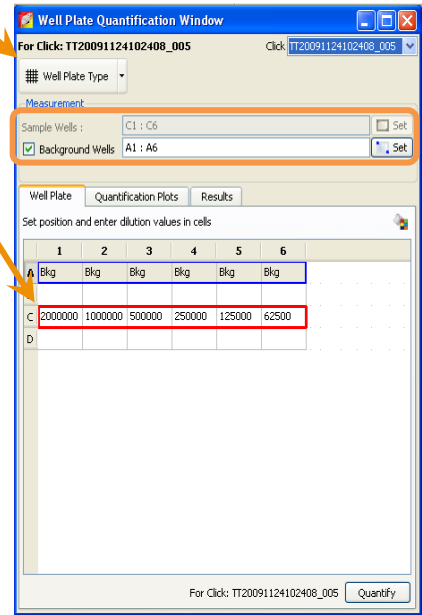
1. Prepare your plates for imaging
  - a. Serially dilute stably transfected bioluminescent/fluorescent cell lines in a black clear bottom 96 or 24 well plate.
  - b. Serially dilute free dye or labeled compounds into a black clear bottom 96 or 24 well plate.
2. Take images of the plates
  - a. Bioluminescent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



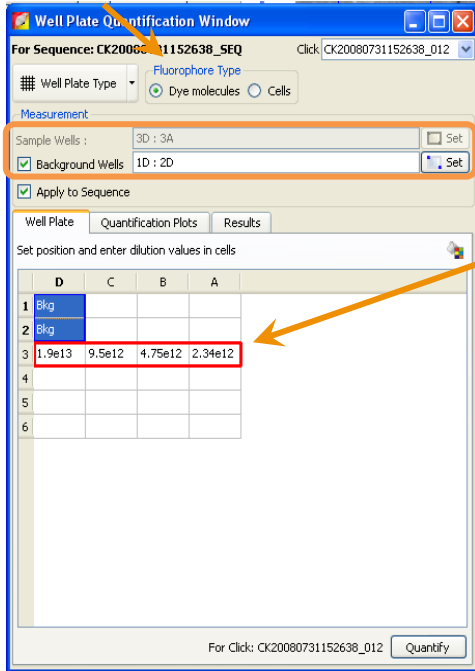
Since the extinction coefficient and effective cross section of the fluorescent probes will vary depending on which excitation/emission filter pair is chosen, it is wise to take images using multiple combinations and store it away as a single database. In the example above, the peak excitation of AF750 is 752nm and the peak emission is 779nm. We have chosen excitation filters 710 and 745 nm and emission filters 780,800, and 820nm.

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

- The Well Plate Quantification Window will open.
- For bioluminescent cells, choose the well plate format from the dropdown 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.

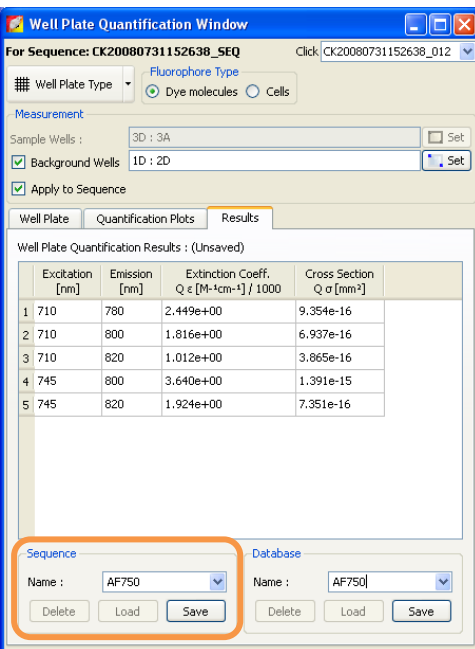


- For fluorescent reporters, choose what you are measuring – cells or dye/quantum dots. Choose the well plate format from the dropdown menu at the top of the window. An ROI will appear in the image. Position the ROI and enter the cell numbers, number of quantum dots, or dye molecules corresponding to 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.

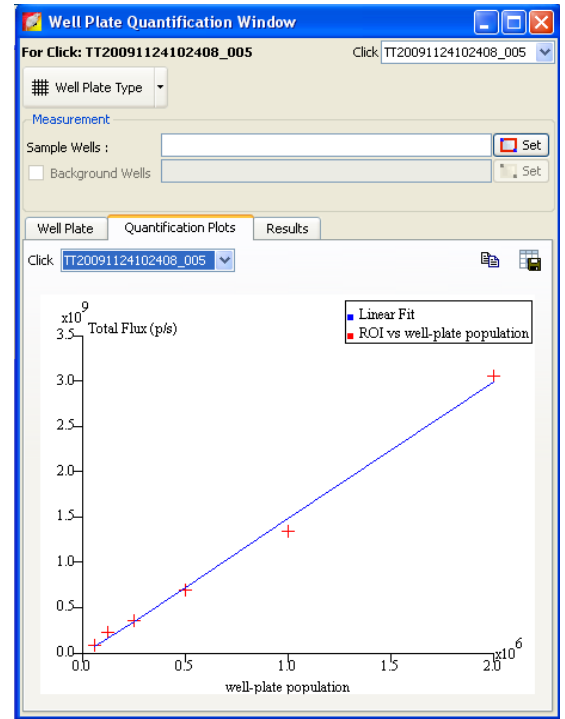
coefficient and cross section for the fluorophore at each of the filter pairs imaged.

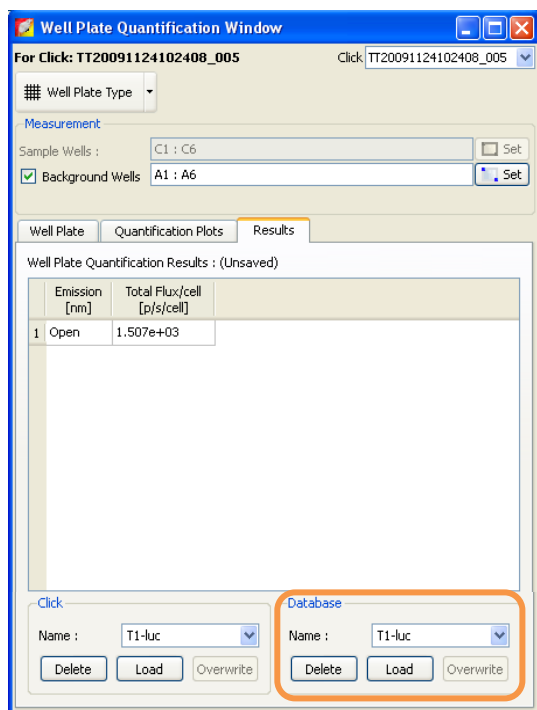


7. The **Quantification Plots** tab will show you the linear fit of the calculated values to the measured ROIs.

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**.





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

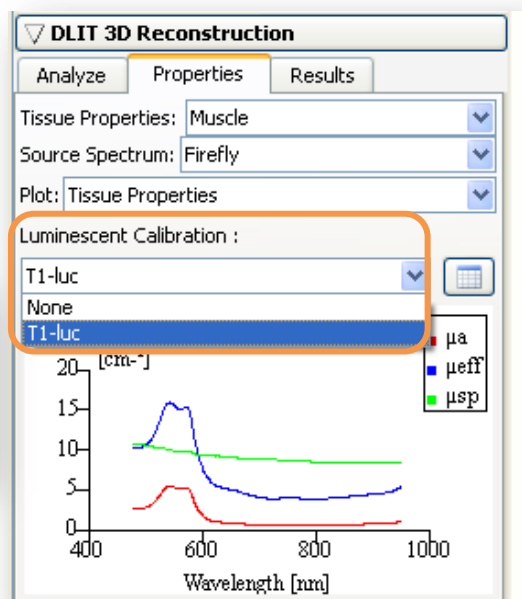
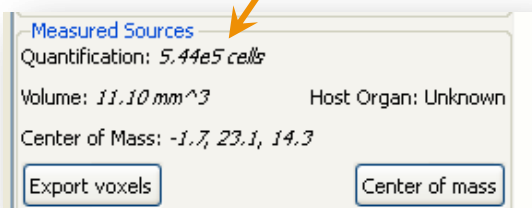
**Note:** Databases stay with a system and are not user specific. To transfer a database, copy the image sequence to the computer you would like to create the database on. **Load** the results using the Well Plate Quantification Window and save a copy of the database on the new system.

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 or 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**.

11. When measuring voxels,

results will be displayed as cells or pmol.



$5.44 \times 10^5$  cells

$2.93 \times 10^4$  cells

