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

 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

- 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 filters 710 and 745 nm and emission filters 780,800, and 820 nm.



- 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.





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 4. Fluorescent probe concentrations as well plate coordinates are entered



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.

r Sequence: CK20080731152638_SEQ Click CK20080731152638_012								
Well Plate Type Well Plate Type O Dye molecules Cells								
dea	feasurement							
amj	mple Wells : 3D : 3A							
2	Background V	Vells 1D:2	20		S 1			
2	Apply to Sequ	Jence						
w	ell Plate	Ouantificatio	n Plots Results					
we	Euclidation	Emission	Subscription Confi	Course Condition				
	[nm]	[nm]	Q ε [M-1cm-1] / 1000	Q or [mm ²]				
1	710	780	2.449e+00	9.354e-16				
2	710	800	1.816e+00	6.937e-16				
3	710	820	1.012e+00	3.865e-16				
4	745	800	3.640e+00	1.391e-15				
5	745	820	1.924e+00	7.351e-16				
5	equence		Databa	se				
	lame :	AF750	Name :	AF750	~			
D.			redited :	14 1 000	-			

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 6. Save the Acquisition Sequence

🛛 Well Plate	Quantification Win	dow	
r Click: TT200 # Well Plate T Measurement ample Wells : Background	091124102408_005 ype • C1 : C6 Webs A1 : A6	Click	TT20091124102406_005
Well Plate	Quantification Plots	Results	
Emission [nm]	106a1 Huz/Cell [p/s/cell] 1.507e+03		
Click Name : Delete	WPQUANT_1	Name : Delete	WPQLIANT_1

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.

▽ DLIT 3D Reconstruction						
Analyze	Properties	Results				
Tissue Properties: Muscle						
Source Spec	Source Spectrum: Firefly					
Plot: Tissue	Plot: Tissue Properties					
uminescent Calibration :						
T1-luc	T1-luc 🔽 🔲					
None	None					
T1-luc µa						
20-7 10***	~ J		_ μeff			
15-	\sim		🗧 μsp			
10-	4					
5	\sim		-			
			<u> </u>			
400	600	800	1000			
Wavelength [nm]						

Figure 8. Load the Well Plate Database

-Measured Sources Quantification: 5.44e5 cells	,		
Volume: 11.10 mm^3	Host Organ: Unknown		
Center of Mass: -1.7, 23.1, 14.3			
Export voxels	Center of mass		
Figure 9. 3D quanti	fication is reported		

as cells or pmols

 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.

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



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

PerkinElmer, Inc.

940 Winter Street Waltham, MA 02451 USA P: (800) 762-4000 or (+1) 203-925-4602 www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Copyright ©2013, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.