

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

<https://biotech.ufl.edu>

CTAC / ICBR SOP In Vitro Bioluminescence Imaging Using the IVIS

Materials Required:

Luciferase substrate (D-Luciferin, Firefly), 150mg/mL

IVIS instrument & Living Image™ software

Support media of choice

Luciferase transfected cells

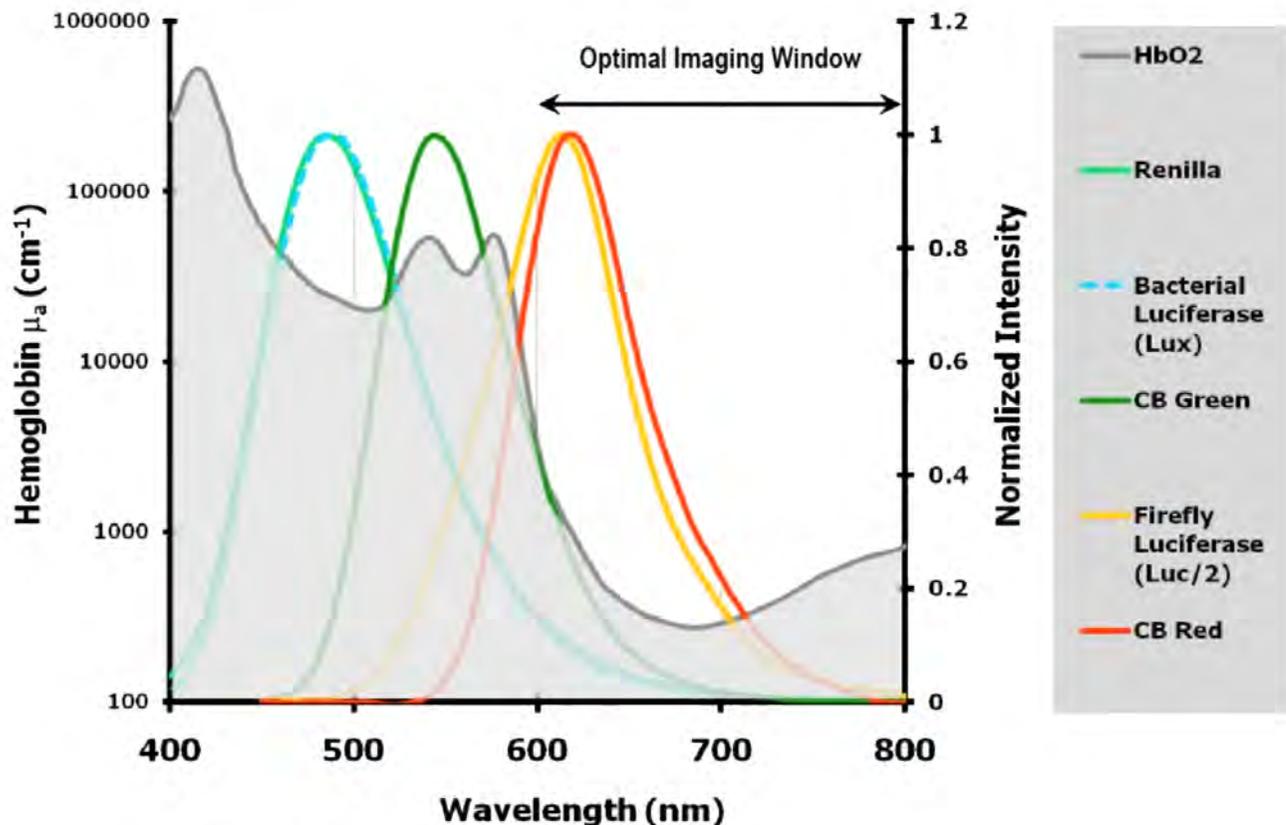
Black 96 well plate (Optiplate-96 Black Opaque Microplate, Perkin Elmer, Cat. # 6005270 recommended)

Purpose:

To inform the user of the process necessary for quantifying bioluminescent cells for the model used / experiment.

Background

Firefly / Luciferin is a preferred product to conduct bioluminescent imaging, as it's emission peak avoids most of the autofluorescence associated with hemoglobin in animal tissues.



In this SOP we use this product to generate quantifiable data on the cell line assayed. The uses of this data are diverse. The most common are:

Quantification of cells to be used later in *in vivo* experiments

Quantification of transfection / expression efficiency

Quality control across operators

Quality control across multiple passages

Procedure description:

Assessment of transfection efficiency and light output from cells

1. Prepare serial dilutions of your cells in their support media, starting with 10^6 cells / 100m
2. Pipette 100ml of each dilution into wells on a 96 well black plate. Ensure that controls are plated that include 100ml of the support media and unlabeled cells at 10^6 cells / 100m
3. Add 100ml of luciferase substrate to each well.
4. Incubate for 5 minutes at 37°
5. Take the microplate to the IVIS Spectrum, enter the software under your user account and initialize the system by clicking the "Initialize" button on the bottom right side of the control panel.
6. When the system is ready, select the folder you wish to save your acquisition to by using the top menu bar and selecting ":Acquisition", then "Auto-Save to..."
7. The Field of View, or shelf position height, should be set to "C" to image one microplate. Position "D" may be used to image multiple plates.
8. The microplate should be positioned using the Field of View laser guide projected inside the lightproof imaging chamber.
9. Close the door and on the Control Panel click "Acquire" to begin the scan.
10. The "Edit Image Labels" window will appear. This will prompt for identification for the experiment to be conducted. It is recommended that you use this to thoroughly label your image in order to facilitate easier search, sorting and loading during analysis.
11. The system will use an internal LED light to acquire a black and white digital picture of the plate, which will turn off and then the luminescence scan will begin. Once the software displays the acquired image, the black and white picture will have a photon density map from the light output of the cells overlaid onto the photographic image of the plate.

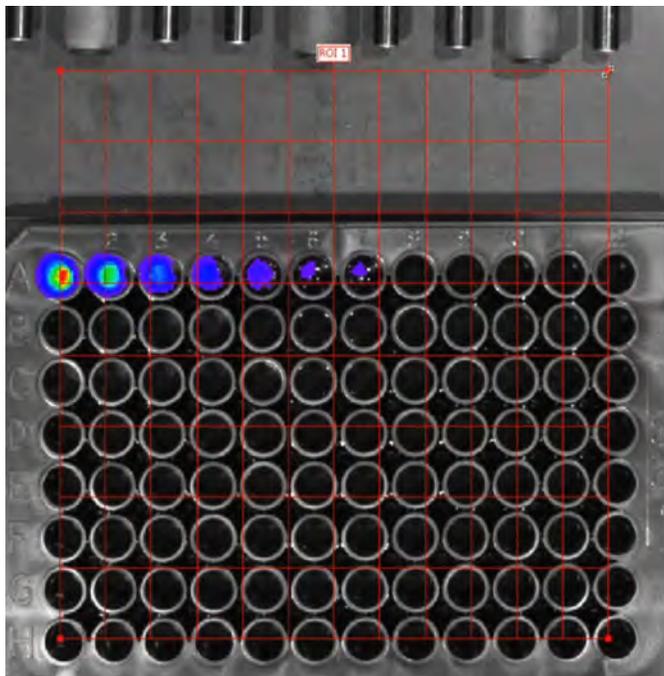
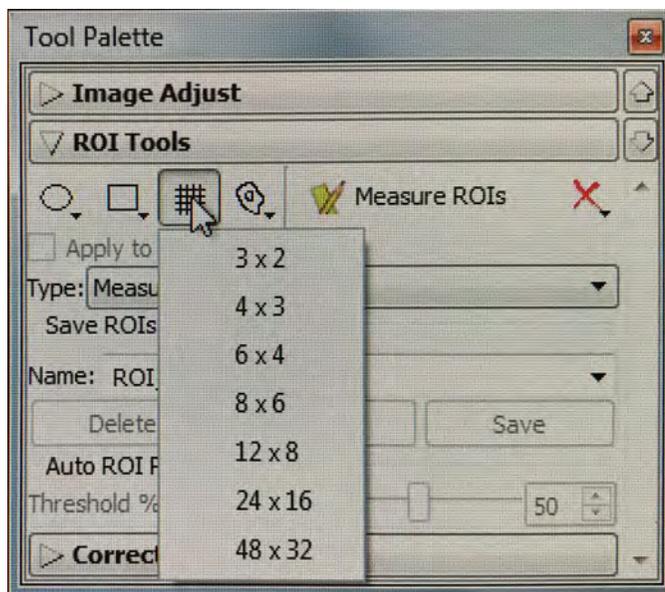
Quantification of signal to assess the optical activity of our cells

Note: Any image adjustments do not change the quantitative output.

1. First, ensure that the "Image Adjust" Tab on the Tool Palette is within the linear range of the CCD camera. This should be between the values 600 to 60,000 log counts and can be adjusted using the "Color Scale". The CCD will tend to oversaturate the image if it is set to much above this range, which would necessitate the image needing to be retaken.

The default capture settings will display this image with "Counts" as the units used. This is a raw data setting, and is very useful to determine whether there is enough actual signal to accurately quantify and for overall image quality. To compare across data sets you must account for acquisition adjustments, including exposure time, binning, F-stop and Field of View (FoV). The calibrated unit "Radiance (Photon)", measured as $p/sec/cm^2/sr$ (photons per **second** per **cm²** (squared) per **steradian** accounts for these changes and normalizes these images for comparison, even when settings have changed. This facilitates long time courses and longitudinal analyses.

2. Go to the Tool Palette, and open the "ROI Tools" tab.
3. Select the "Grid" measurement tool. There will be a dropdown menu displaying several common microplate dimensions and formats. For a 96 well plate, "12 x 8" should be selected



4. A grid pattern appropriate for a 96 well plate will be superimposed on your acquired image. Using the mouse, align the grid to the 96 well pattern. For help with ROI controls, please see [CTAC ICBR SOP: Operation of the ROI Tool Palette \(https://ctac.mbi.ufl.edu/ivis-spectrum-how-to-documents-and-sops/ctac-icbr-sop-capturing-data-using-the-roi-tools-in-the-tool-palette-from-an-acquired-image/\)](https://ctac.mbi.ufl.edu/ivis-spectrum-how-to-documents-and-sops/ctac-icbr-sop-capturing-data-using-the-roi-tools-in-the-tool-palette-from-an-acquired-image/).
5. Click on the “Measure ROIs” button. The ROI Measurements window will now appear.
6. Ensure that “Radiance (Photons)” is selected as your “Measurement Type”. Click the tab on the top left to show the ‘Grid ROI Measurements’.
7. A grid or table is displayed with values associated with each well of your plate.

Living Image® 4.4 (64-bit) - [ROI Measurements]

File Edit View Tools Window Help

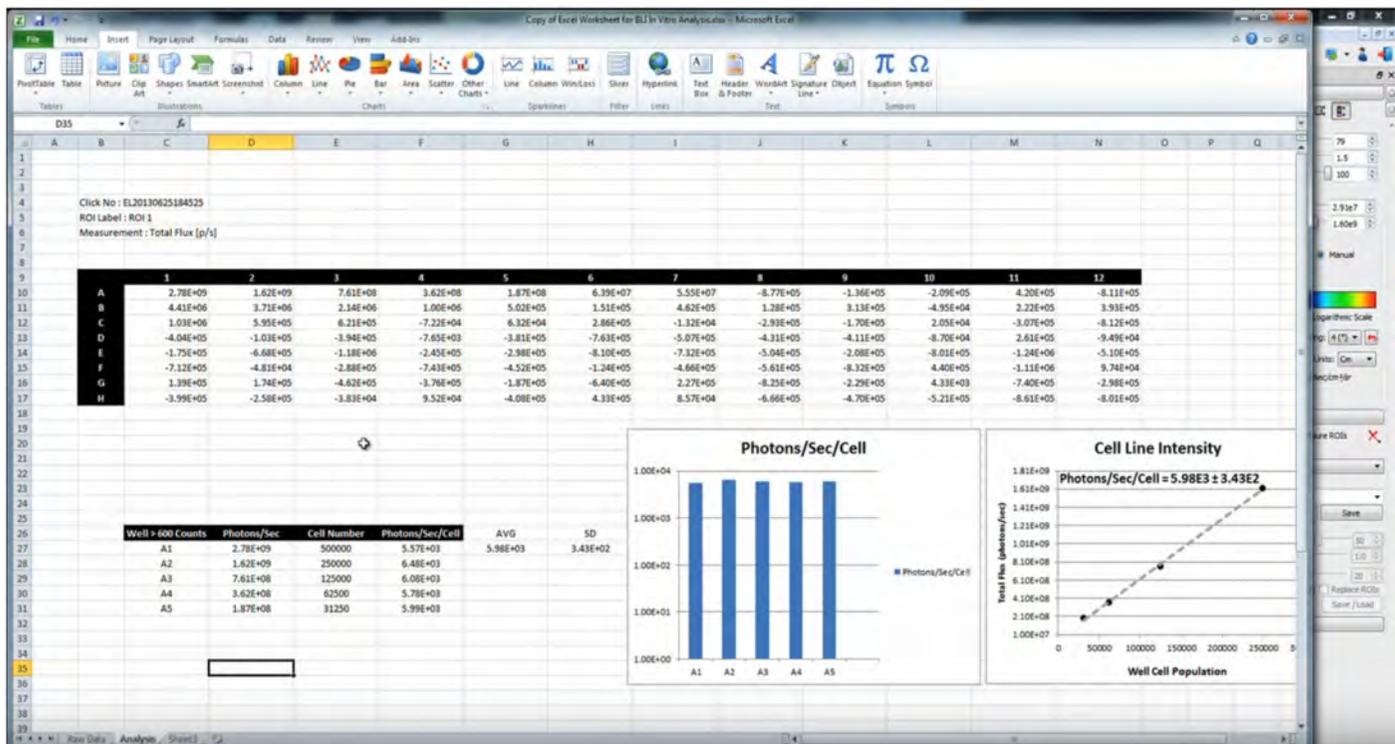
Units: Radiance (Photons) Apply to All

ROI Measurements Grid ROI Measurements

Click: EL20130625184525 ROI: ROI 1 Measurement: Total Flux [p/s]

	1	2	3	4	5	6	7	8	9	10	11	12
1	2.765e+09	1.608e+09	7.527e+08	3.638e+08	1.867e+08	6.364e+07	5.530e+07	-7.354e+05	-4.323e+05	-2.294e+05	5.241e+05	-8.035e+05
2	4.215e+06	3.533e+06	2.295e+06	9.308e+05	5.661e+05	8.681e+04	4.260e+05	2.464e+05	1.931e+05	-1.354e+05	2.967e+05	1.640e+05
3	8.636e+05	5.557e+05	5.079e+05	-1.685e+05	-1.011e+05	3.223e+05	1.007e+05	-4.850e+05	-5.016e+04	5.378e+04	-1.484e+05	-6.702e+05
4	-4.039e+05	-1.032e+05	-3.620e+05	2.379e+04	-2.479e+05	-6.582e+05	-3.925e+05	-4.907e+05	-4.014e+05	-1.029e+05	2.157e+05	-2.423e+05
5	-1.753e+05	-6.676e+05	-1.013e+06	-2.052e+05	-2.464e+05	-9.951e+05	-6.123e+05	-5.256e+05	-2.549e+05	-8.608e+05	-1.303e+06	-3.240e+05
6	-7.117e+05	-4.805e+04	-4.904e+05	-5.766e+05	-3.838e+05	-1.643e+05	-5.818e+05	-5.240e+05	-5.924e+05	2.608e+05	-6.397e+05	1.445e+05
7	1.388e+05	1.742e+05	-3.531e+05	-4.475e+05	-1.748e+05	-5.415e+05	3.354e+05	-8.787e+05	-3.772e+05	-1.033e+05	-8.967e+05	-5.588e+05
8	-4.767e+05	-3.529e+05	-6.475e+03	6.372e+04	-3.946e+05	2.338e+05	-1.169e+04	-5.759e+05	-4.288e+05	-6.445e+05	-6.823e+05	-4.325e+05

8. This may be exported into common spreadsheet applications, such as MS Excel or Apple's Pages by clicking "Select All" in the bottom right and then "Copy", which will place the data on the Clipboard for downloading into your preferred application.



This data may be used to generate graphs of Photons per Second per Cell, which should demonstrate a linear relationship between cell number and photon emission (flux, or p/sec). The value obtained for determining the cell line intensity provides guidance as to the sensitivity of the cells when they may be used in *in vivo* applications. This may range from single digits to several thousands of photons per second. Brighter cells are simply more sensitive, and allow earlier detection with fewer cells.

The IVIS may be used in *in vitro* applications to allow direct comparison of different cell lines directly, such that the best may be selected for the purpose. Additionally, it may be used to develop excellent internal quality controls for consistency, by tracking the phenotypic performance of cell lines across operators, facilities and passage numbers.