

# GFP Expression Analysis Using the Luna-FL™ Fluorescence Cell Counter

## Introduction

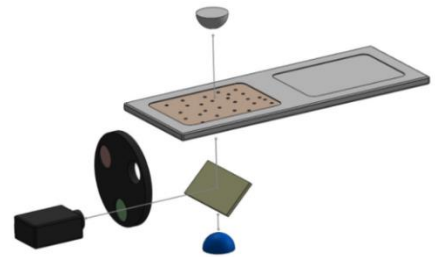
Transfection is a term to describe a process to introduce foreign genetic materials into eukaryotic cells. A number of transfection methods have been developed. Unfortunately, whatever method is used, the transfection efficiency is not 100%. Whereas this problem can be relieved to some extent by stable transfection, the variability in the transfection efficiency is inevitable in case of the transient transfection. To compare results obtained from different experiments, researchers need to determine the transfection efficiency every time they perform the transient transfection.

An easy and popular method to measure the transfection efficiency is to use a GFP gene. For example, by making a fusion protein between GFP and the protein of interest, the expression of the protein of interest can be measured by the presence of GFP protein.

## How to measure GFP transfection efficiency with the Luna-FL™

The Luna-FL™ fluorescence cell counter is equipped with an optical module for bright field signal and fluorescence signal detection. The optical specification of Luna-FL™ is listed in table 2. Fluorescence channel 1 (FL1) is adequate for exciting GFP and receiving the emitted light from GFP. It can also detect signals from Yellow Fluorescence Protein (YFP) although the excitation efficiency is lower than GFP.

Excitation	470/40nm
Dichroic (cutoff)	495nm
Emission (FL1)	530/50nm
Emission (FL2)	600LP



**Table 2.** Optical specification of Luna-FL **Fig 1.** Schematic of optical module of Luna-FL

To measure the GFP transfection efficiency with the Luna-FL™, 10 µL of negative control sample is analyzed first. The negative control can be a non-transfected control or a mock-transfected control. Through this step, the background fluorescence of the cell line is measured. Next, experimental samples are analyzed. Cells irradiating fluorescence stronger than the background level are considered as “transfected”. This strategy is just same to that of the most widely-used method, flow cytometry analysis.



## Procedure

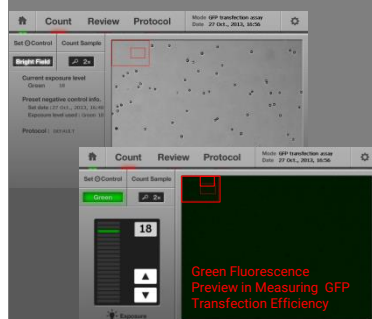
### 1 Select the "GFP Transfection Assay" button



### 2 Insert the slide containing the GFP-transfected cells



### 3 Adjusting the Focus - For the best results, use the bright field mode (2x or 4x) to adjust the focus



#### Note:

The exposure level of the green fluorescence must be started from 18 and then, depending on the signal strength, it can be adjusted.

By combining the hardware configuration of fluorescence microscopy and the algorithm of flow cytometry, Luna-FL™ has merits of both instruments: reasonable price, no need of special training, no need of a dedicated operator, no maintenance issue, high accuracy and precision, and the applicability to challenging samples.

## Experimental Procedures

1. Keep 293T cells in a healthy condition according to the instruction of the transfection method.
2. Perform a GFP transfection experiment with appropriate negative controls, eg., non-transfected control or mock-transfected control without plasmid.
3. 24 ~ 72 hours after the transfection, harvest the cells.
4. Count the number of cells. (optional)
5. Load the appropriate number of negative control cells onto the PhotonSlide™.
6. Press the "Set Negative Control" button.
7. Load the appropriate number of cells in the experimental sample onto the PhotonSlide™.
8. Press the "Count Sample" button.

## Results

Luna-FL™ calculates the concentrations of total cells and GFP positive cells in about 30 sec as shown in Fig 2. Additional information is displayed including the percentage of GFP positive cells, the numbers of total and GFP positive cells, and average size.

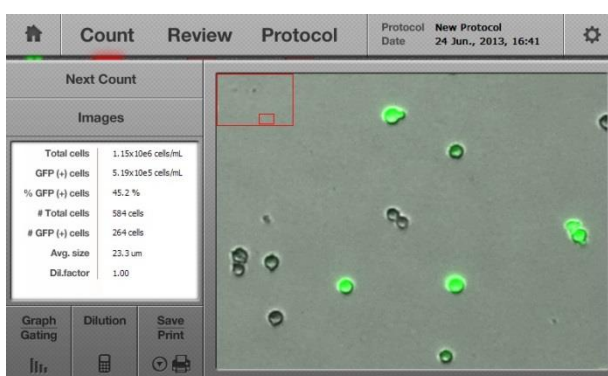
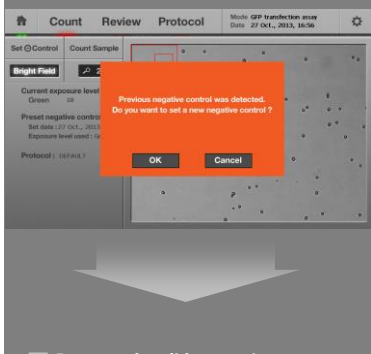


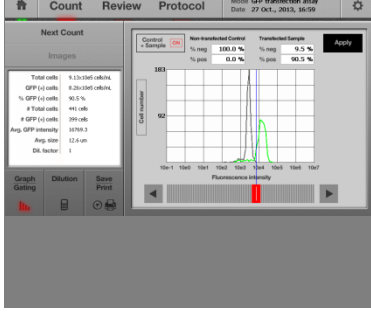
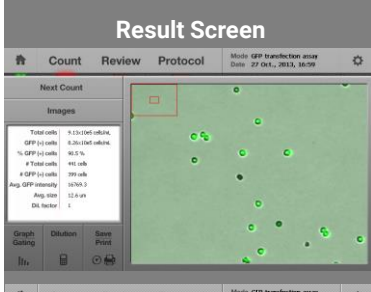
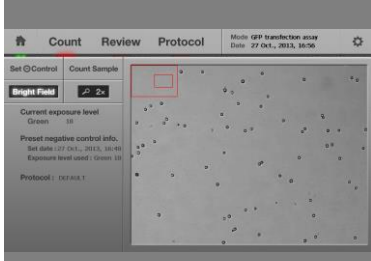
Fig 2. A result screen of a typical GFP transfection assay.

The accuracy of the GFP transfection assay can be verified on the Luna-FL™ instrument itself by using the "tag" function. With this function on, cells considered as GFP-positive cells by a Luna-FL™ software module are circled in green (Fig 3). By comparing the green fluorescence intensity of cells in

**4** Remove the slide containing the transfected sample and insert a slide containing the negative control. Press the "Set (-) control" button



**5** Remove the slide containing the negative control sample and insert a slide containing the GFP-transfected sample again and press the "Count Sample" button



the image and the presence of the green circle, users can easily verify the accuracy of the Luna-FL™ software module. Users are also able to enhance the image quality by adjusting bright-field and green fluorescence images.

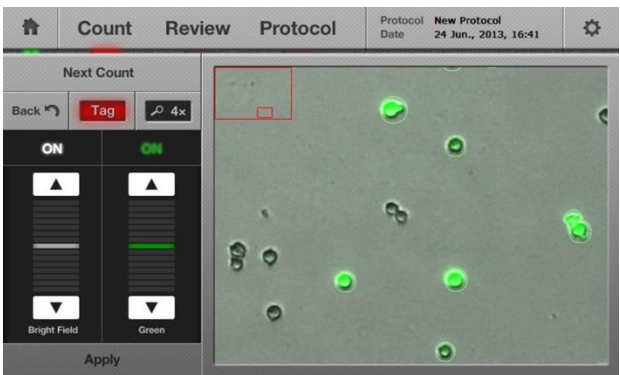


Fig 3. A result screen of a typical GFP transfection assay.

The distribution of GFP intensity and the cell number can be presented in a histogram format as flow cytometry (Fig 4). The graphs of a control and a sample are plotted, and the threshold is determined automatically. Users can manually change the threshold, and the percentage of GFP positive and negative cells will be reflected accordingly.

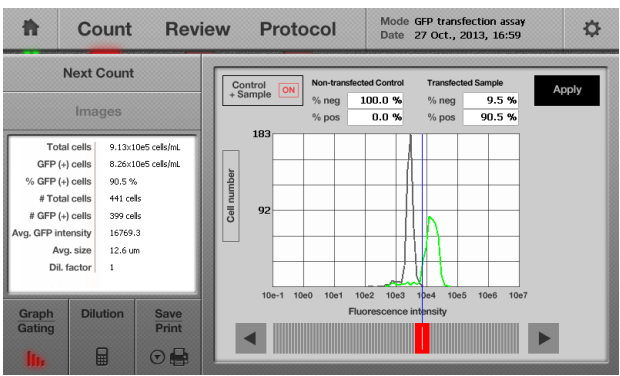


Fig 4. An analysis screen.

## Conclusion

In addition to the cell counter function, Luna-FL™ provides with cytometry function at no additional cost. Advantages of fluorescence microscopy and flow cytometry have successfully been combined to generate an ideal system to measure GFP transfection efficiency.

To learn more about the Luna-FL™ fluorescence cell counter, go to [www.logosbio.com](http://www.logosbio.com).

- **LUNA-FL™ Specifications**

Performance	Sample volume	10 µL
	Cell counting time	30 sec
	Cell concentration range	5X10 <sup>4</sup> ~ 1X10 <sup>7</sup> cells/ml
Hardware	Excitation wavelength	470 +/- 20 nm
	Emission wavelength	530 +/- 25 nm (Green) 600 nm LP (Red)
	Light Source	LED
	Image Resolution	5 MP
	LCD	7 inch
	Computer	on board
	Dimension (W x D x H)	22 x 21 x 9 cm (8.6 x 8.3 x 3.5 inch)

- **Ordering Information**

Cat #	Description	Size
L20001	LUNA-FL™ Fluorescence Cell Counter	each
L12005	PhotonSlide™, 50 slides (100 counts)	Box
L12006	PhotonSlide™, 500 slides (1,000 counts)	
L12007	PhotonSlide™, 1,000 slides (2,000 counts)	
P10001	LUNA™ printer	each

