



Technical Note 195

GFP Application

Introduction

The focus of this technical note is to give a detailed description of how to optimize the GFP (Green Fluorescence Protein) application on CellDrop™ Automated Cell Counters. Accurate quantification of GFP transfection efficiency requires user input and adjustment during measurements. This technical note will provide the information needed to accurately detect and count GFP+ cells.

GFP Protocol Settings

CellDrop EasyApps® are designed to count a variety of cell lines with minimal customization. If necessary, the user has the ability to optimize count settings after a count has been taken or develop a new protocol. Protocols include several powerful settings to enable proper counting of all cells in a GFP sample while ignoring any debris and dead cells that could compromise the counts.

Developing a Protocol

There are two groups of count settings for protocols (Figure 1). On the left side are instrument settings that mainly affect sample loading volume and type. The settings on the right directly affect how a GFP cell is counted. See TechNote 189 Count Settings for additional details.

- **Dilution Factor:** The dilution factor is the ratio of total assay volume to the volume of cell suspension in the assay. Enter to calculate the correct cell density.
- **Diameter:** The diameter settings (minimum and maximum) specify the size range of objects to seek during a count. The algorithm rejects and does not process objects below the minimum or above the maximum limits.
- **Roundness:** The roundness setting identifies cells based on morphology. The higher the roundness, the more round and completely outlined a cell must be to be included in the count. Reducing the roundness setting allows more irregularly shaped cells or, cells with incomplete outlines to be counted
- **Green Fluorescence Threshold:** Allows the user to set a minimum fluorescence level for the cells to be counted.

Add New Protocol

Protocol	New Protocol Name ♥
Cell Line/Note	Default GFP Protocol

Cell Outline Option

Enhanced Outline

Circle Outline

Sample Mode

DirectPipette

Slide

Chamber Height ⓘ

400 um (low density)

100 um (standard)

50 um (high density)

Irregular Cell Mode ⓘ

Enabled

Small Cell Mode

Enabled

Dilution Factor	<u>1</u>	
Diameter (minimum)	<u>4</u>	microns
Diameter (maximum)	<u>30</u>	microns
Brightfield Roundness	<u>50</u>	(1-100)
FL Roundness	<u>1</u>	(1-20)
Green Fluorescence Threshold	<u>20</u>	(1-100)

Cancel Save

Figure 1: Protocol Settings. Each protocol has a variety of settings that can be changed to accommodate nearly any sample.

Optimizing Count Settings

Count settings may be edited after a count is performed by pressing “Optimize Settings” on the results screen. This feature allows a user to properly assess what effect a parameter change has on GFP cell count versus the previous parameter/count.

Counting GFP Cells

When counting a new sample in the GFP app, the best practice is to first load a GFP+ sample then adjust focus and exposure as necessary. Wipe this sample away, then load a control (GFP-) sample and press count using the Default Protocol. Inspect the result image to determine if cells were correctly counted. Use the Optimize Settings feature to adjust count settings until cells are correctly identified.

The GFP app also includes an intensity and diameter graph with a slider that is used to increase the accuracy of a GFP count (Figure 2).

The intensity graph shows the number of cells counted at each GFP intensity level. All non-GFP-expressing cells will have the same intensity – the background intensity. These cells will form a high peak towards the left of the graph. This is an easy indicator for where to place the intensity gate. As shown in Figures 3&4, placing the gate slightly to the right of this peak in the control sample will remove any non-GFP cells from the GFP count.

Once the settings and intensity gate have been set, load the GFP+ sample and press count. If only bright GFP cells are to be counted, readjust the intensity slider until the correct cells are counted, while excluding cells deemed to be too dim.

In the example, both the control and GFP-expressing cells have the fluorescent threshold slider set to 16. This prevents bright-field cells from being counted as GFP+ incorrectly. Because transfections can vary greatly in efficiency and fluorescent intensity, this gating procedure should be repeated each time the results of a transfection are measured on CellDrop. Result images in Figure 5 show that adjusting the slider has an impact on what cells are counted as GFP+ while the other cells are deemed to be GFP-.

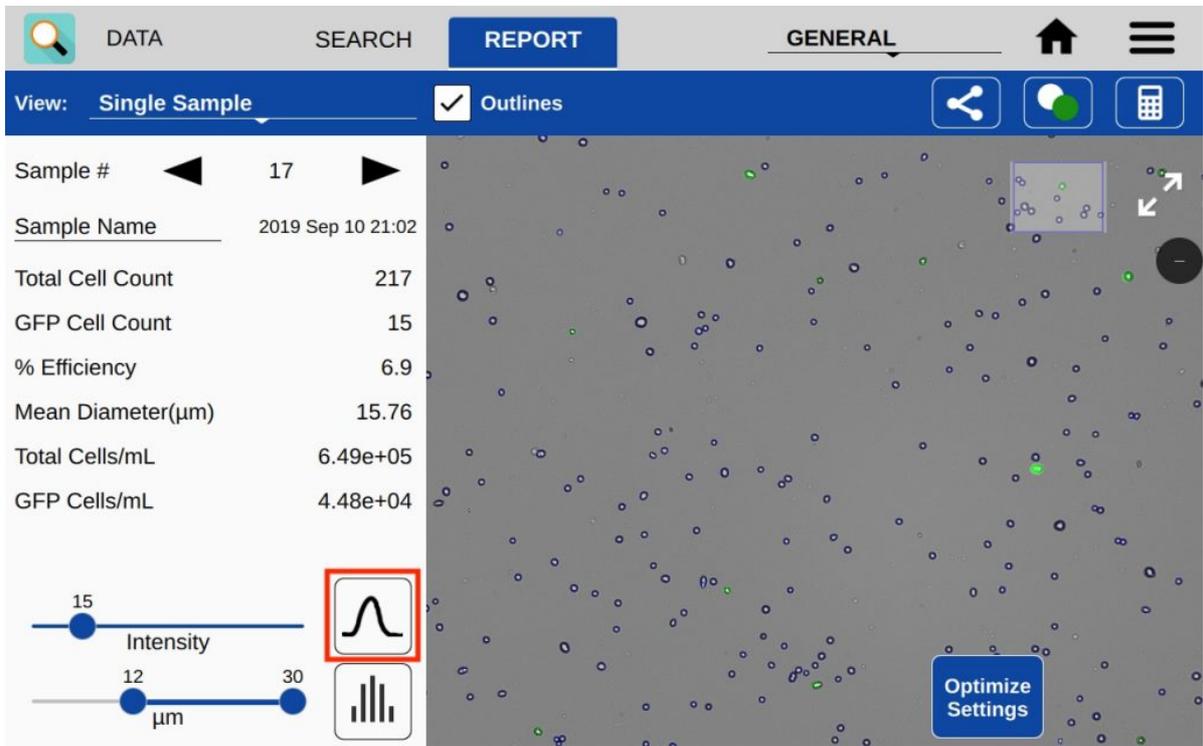


Figure 2: GFP Intensity Slider. The red box highlights the GFP intensity graph. The adjustment slider is located to the left.

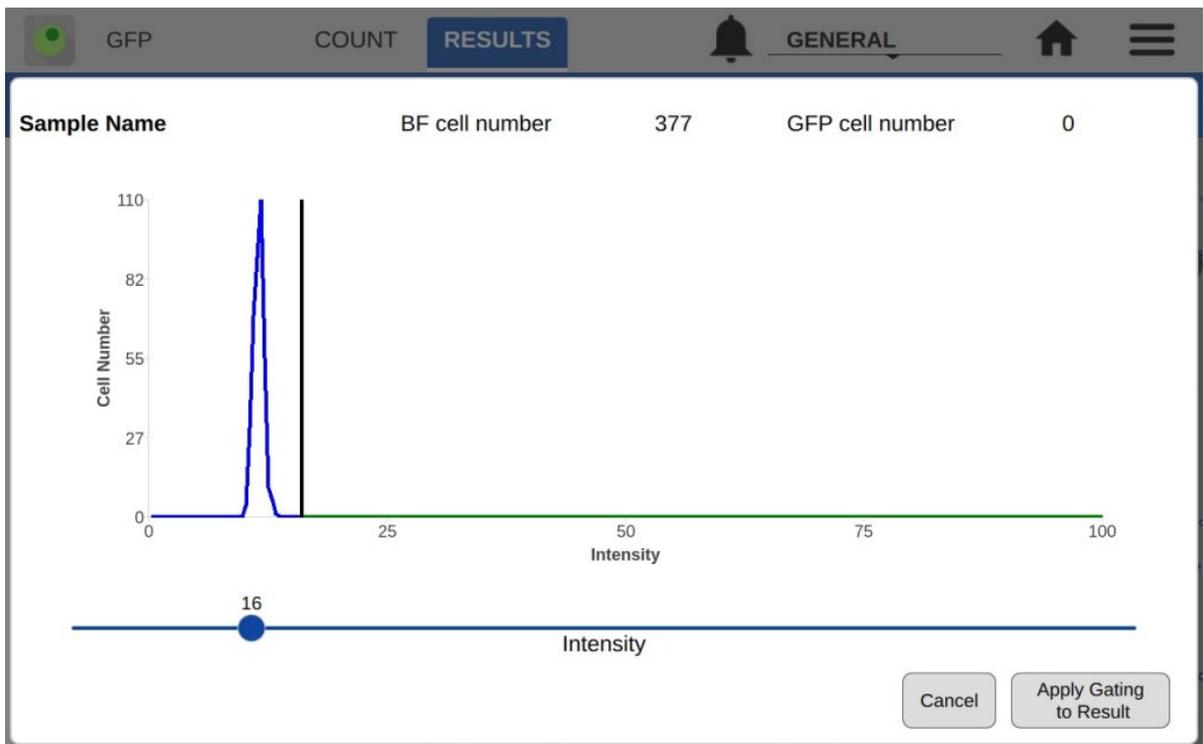


Figure 3: Setting GFP Baseline. To set a baseline for detection GFP+ cells, a control sample should first be counted using the GFP app. The slider, representing the Fluorescent Threshold, should be set at a value just above the tall peak representing non-GFP cells.

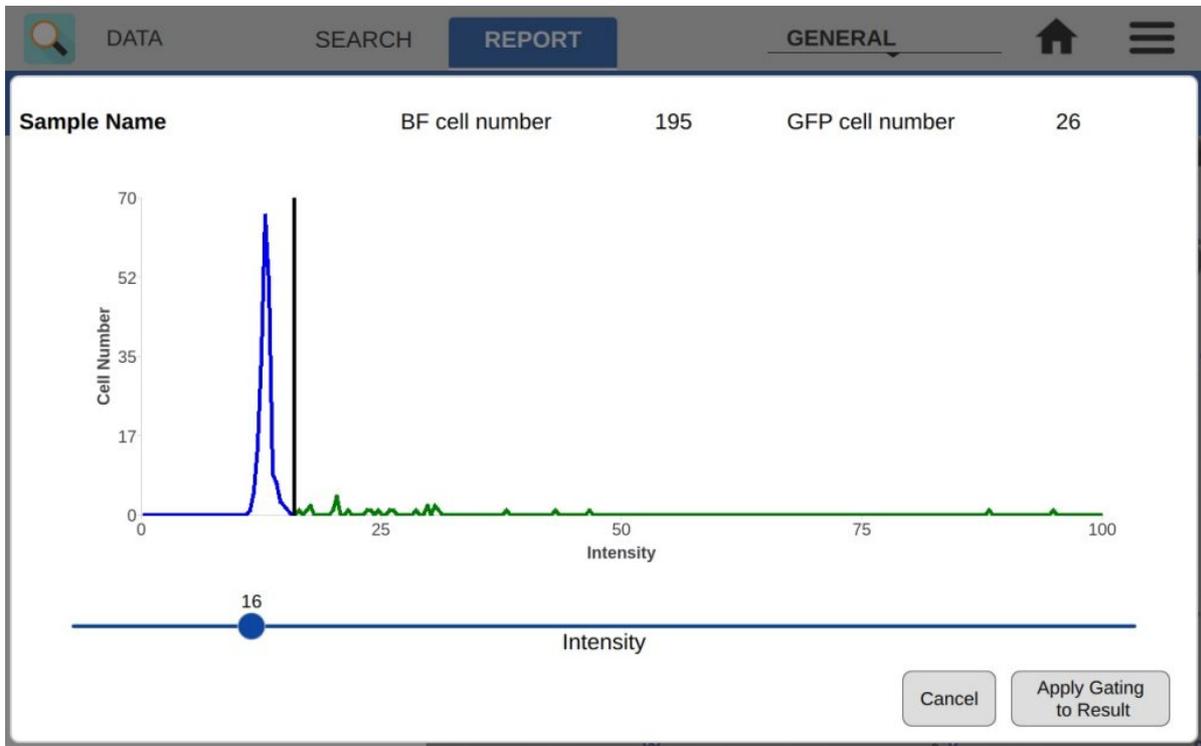


Figure 4: GFP Samples Measured After Setting Baseline. The graph shows the number of cells at each measured intensity. The slider is set at a value just above the high peak of the non-GFP cells from the count.

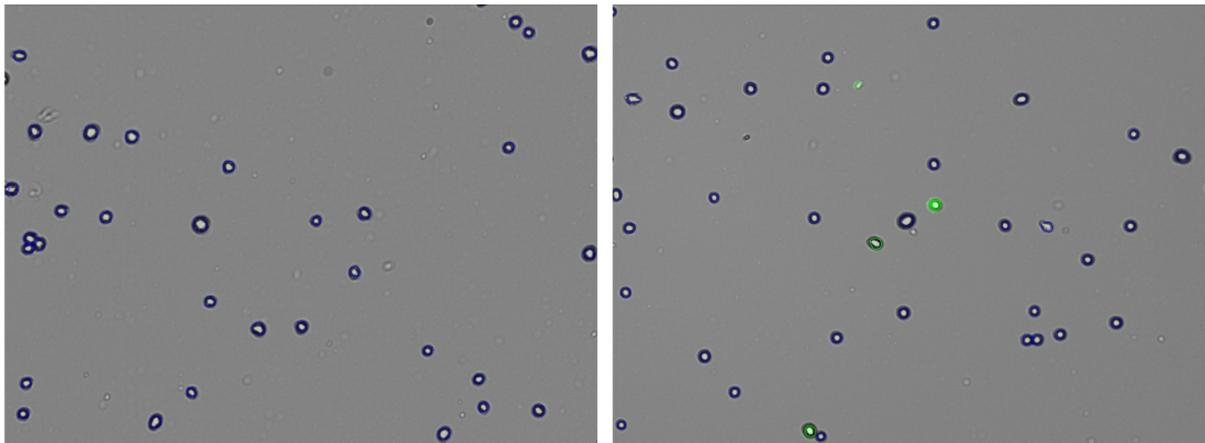


Figure 5: Non-GFP vs. GFP+ Samples. Counting a sample with no GFP expression (left) in the GFP app and properly setting the intensity results in no cells identified as GFP+. A properly set intensity slider results in GFP+ cells of varying intensities to be properly identified as GFP+ (right).

Summary

The CellDrop GFP App functions best when user knowledge and input is used to enhance the results of a count. The CellDrop has powerful counting algorithms and default settings designed to cover a broad number of cell lines. The CellDrop is designed to provide rapid results with settings that can be easily adjusted to enhance the accuracy of a count. The software includes intensity and diameter graphs as well as counted result images to help researchers correctly count the GFP cells. The app ensures that the count is accurate and acceptable to each researcher.

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