

## APPLICATION FOCUS: CELLOMETER VISION

### Quantitative Measurement of GFP Transfection Rates in 60 Seconds



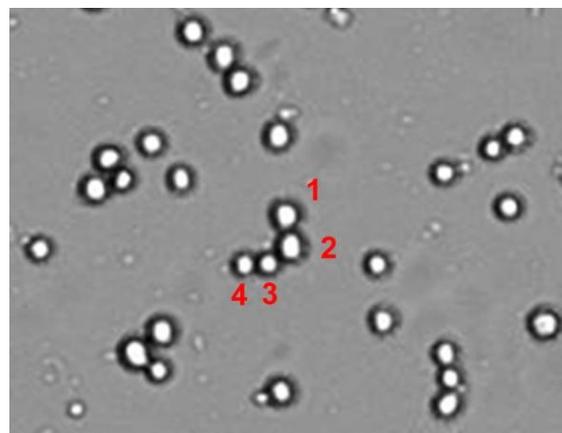
#### INTRODUCTION

Cellometer Vision incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. Cellometer Vision is an economical and faster alternative to flow cytometry for many cell population characterization assays such as rapidly determining GFP transfection rates.

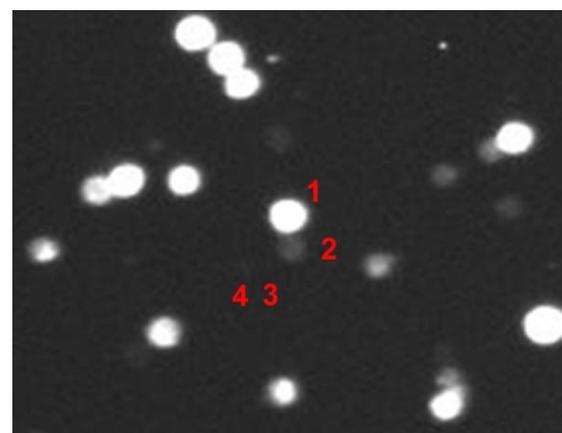
Green fluorescence protein (GFP) has been widely applied in cell based assays. The fluorescence signals are used as a readout to monitor gene functions in cell proliferation, differentiations, toxicity, motility, morphology, etc. Currently, to quantitatively analyze fluorescent cells, researchers have to utilize a calibrated flow cytometer. However, in addition to the complexity, cost and availability limitations, flow cytometry is often not the best solution for cell population analysis due to the potential of complex samples clogging the flow system. Time and materials required for analysis can also be inhibitive for rapid and routine cell characterizations such as stem cell research and clinical sample analysis.

Cellometer Vision addresses the need for simple, rapid fluorescence cell counting. By capturing both brightfield and fluorescent images from the same cell sample, Vision can determine the total cell concentration and analyze how many of those cells are expressing GFP. Therefore, using only 20µl of cell sample, researchers can now rapidly identify fluorescence positive cells from a sample, analyze individual cell fluorescence intensity, calculate cell concentration, size and determine the GFP transfection rate in minutes on the lab bench.

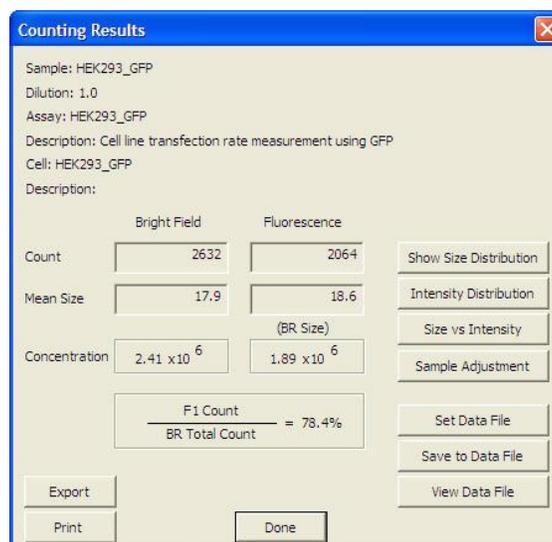
Cell images and all analysis data, including cell size distribution histograms, can be saved for documentation. Data can also be easily exported to Microsoft Excel spreadsheets for further analysis.



Brightfield Image of HEK293 Cells



Fluorescent image of HEK293 cells. White spots indicate GFP positive cells.



Counting results box displays fluorescence cell count, percentage of GFP positive cells, mean size & concentration.

## METHOD

### Running Assay:

1. Load 20 $\mu$ l of sample into the disposable counting chamber
2. Insert chamber into Cellometer Vision
3. Select Assay from drop-down menu
4. Enter Sample ID for this sample
5. Preview cell images and click 'Count' to begin analyzing sample
6. Review images and counting data.
7. Save or export images and/or report data.

## RESULTS

Total counted HEK293 cells are indicated by green circles in the brightfield image (Figure 1). GFP (fluorescent) positive, green circled cells can be seen in the fluorescence image (Figure 2). Total and fluorescence positive cells counted, concentration, mean size, and GFP positive percentage are displayed in the results box (Figure 3).

Cell size distribution histograms (Figure 4), fluorescent intensity vs. size scatter plots and data files can be instantly generated. All experimental data is easily exported to an Excel spreadsheet or saved in a data table.

Cellometer Vision can also be used to detect RFP or YFP transfection rates in a similar manner.

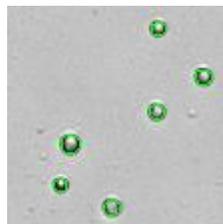


Figure 1. Total counted cells are indicated in the brightfield image by green circles

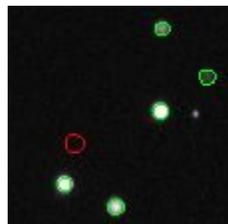


Figure 2. GFP positive cells are indicated in the fluorescent image by green circles

	Bright Field	Fluorescence
Count	2547	1998
Mean Size	16.7	17.0 (BR Size)
Concentration	$2.33 \times 10^6$	$1.83 \times 10^6$
$\frac{\text{F1 Count}}{\text{BR Total Count}} = 78.4\%$		

Figure 3. Total concentration (Brightfield), GFP positive cell concentration (Fluorescence) and transfection rate percentage are displayed immediately after image analysis

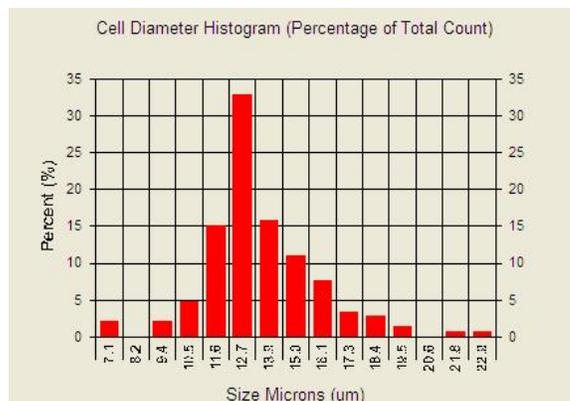


Figure 4. Cell size histograms are generated instantly

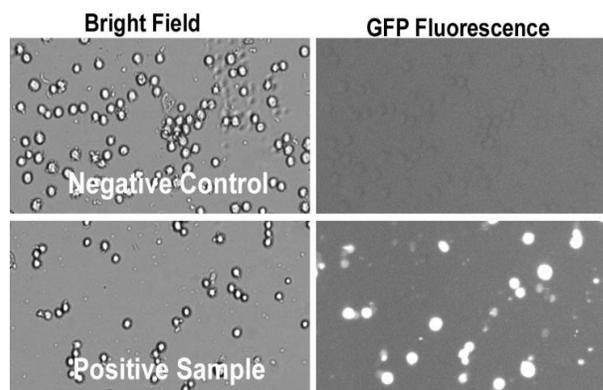


Figure 5. Comparison of GFP negative control sample and GFP positive sample.

Sample	GFP + Rate	Cell Concentration	GFP+ Concentration
HCT116	58.6%	$9.3 \times 10^5$ /mL	$5.4 \times 10^5$ /mL
Hela	71.9%	$7.0 \times 10^5$	$5.0 \times 10^5$
K562-1	50.5%	$7.0 \times 10^5$	$3.5 \times 10^5$
K562-2	70.0%	$4.6 \times 10^5$	$3.3 \times 10^5$
HEK293-1	23.4%	$5.0 \times 10^5$	$1.6 \times 10^5$
HEK293-2	4.3%	$6.3 \times 10^6$	$2.7 \times 10^5$
HEL293-3	25.7%	$4.4 \times 10^6$	$1.1 \times 10^6$
HEK293-4	20.2%	$7.0 \times 10^5$	$1.4 \times 10^5$
6020	21.1%	$4.9 \times 10^5$	$1.0 \times 10^5$
LNCAP	12.1%	$3.9 \times 10^6$	$4.7 \times 10^5$
A20	13.5%	$5.1 \times 10^6$	$6.9 \times 10^5$
ES (YFP)	95.2%	$3.3 \times 10^5$	$3.1 \times 10^5$
CHO (EGFP)-1	1%	$4.6 \times 10^6$	$4.7 \times 10^4$
CHO (EGFP)-2	42%	$5.5 \times 10^5$	$2.3 \times 10^5$
RPE	31.3%	$1.1 \times 10^6$	$3.5 \times 10^5$

Figure 6. GFP Transfection rates of various samples run on Cellometer Vision.

## CELLOMETER Vision Trio SPECIFICATIONS:

**Imaging Modes:** Brightfield & 2 Fluorescence Channels

Filter Set 101: Excitation/Emission Peak: 475nm/535nm

Filter Set 202: Excitation/Emission Peak: 525nm /595nm

**Dimensions:** 6" x 8.5" x 14" (15cm x 22cm x 36cm)

**Weight:** 25lbs (11kg)

**PC Specs:** WinXP/1.8+GHz/1GB RAM/laptop included