by Craig Weiss

Quantitative Cell Population Analysis

Utilizing Automated Fluorescence Imaging

The performance of cell analysis assays is a critical part of the study of any biological system, and encompasses various techniques to study and understand populations of cells. Beyond basic cell concentration and viability data, cell populations can be further characterized by determining viability using fluorescent dyes (such as propidium iodide), quantifying the presence and expression levels of fluorescent proteins such as green fluorescent protein (GFP), and detecting the presence of fluorescence-labeled surface markers to indicate which cells exhibit specific surface features such as annexin-V in apoptosis assays.

While a variety of manual and automated technologies exist for generating cell concentration and viability by trypan blue, the use of fluorescence to characterize these populations has relied on fluorescence microscopy or flow cytometry. Fluorescence microscopy is ideal for understanding complex biological structures within the cell at higher magnifications. Although using this technology to analyze a population of cells is quick and relatively simple, the results are primarily qualitative and subject to human interpretation. At best, they provide a general overview of the fluorescent properties of a population of cells and should not be relied upon for determining accurate concentration and fluorescence intensity data.

Flow cytometry, on the other hand, is incredibly powerful and capable of providing ample amounts of quantitative data such as cell sizes and fluorescence intensity, but requires a great deal of operator interaction and in many cases advanced training. This complexity, as well as the cost of instrument acquisition, daily operation, and maintenance, means that flow cytometry is often only available in a core facility setting. Use of a shared instrument in a core facility requires scheduling time in advance and transporting samples, and is generally not conducive to quick data acquisition. The advanced detection and cell sorting capabilities of a flow cytometer cannot be underestimated, but are often not needed for basic population studies such as determining which percentage of a population of cells is expressing GFP, or determining cell viability by detection of propidium iodide. Flow cytometry can be a complex, time-consuming, and expensive way to perform basic quantitative fluorescence analysis.

Figure 1 The Cellometer Vision brightfield/fluorescence imaging system consists of a compact instrument and image analysis software. Cell images, counting results, and other data are displayed on-screen.

Cellometer® Vision (**Nexcelom Bioscience**, Lawrence, MA) is a new technology that bridges the gap between traditional imaging cytometry and quantitative fluorescence analysis by providing simple, fast, imaging-based fluorescent cell analysis for cell population studies. The instrument (see *Figure 1*) combines brightfield and fluorescence microscopy and automated image analysis to generate accurate quantitative data in a compact, easy-to-use benchtop format that requires only 20 µL of sample, does not need routine maintenance, and generates data in less than 1 min.

Using automated image analysis for characterizing cell populations

By combining both brightfield and fluorescence imaging technologies, Cellometer Vision is able to generate output data such as total cell counts, concentration, viability, cell size, and fluorescence intensity data with minimal time and effort. Because the technology is image based, it also eliminates the need for large sample volumes, complex sample preparation, and system cleaning and maintenance. Other issues such as clogging and cross-contamination are also negated.

To run an assay (see *Figure 2*), samples are prepared utilizing standard protocols, and then 20 µL of sample is pipetted into a disposable counting chamber and inserted into the instrument. A user-defined assay and cell type are selected from a drop-down menu, and the "Count" button is clicked. Brightfield and fluorescent images of the sample are acquired, and the operating software uses proprietary algorithms to accurately analyze cell images to generate total cell counts, concentration, viability determination, and fluorescence intensity data. The entire process typically takes less than 1 min.

Unlike flow-based technologies, cell images are displayed on-screen with visual

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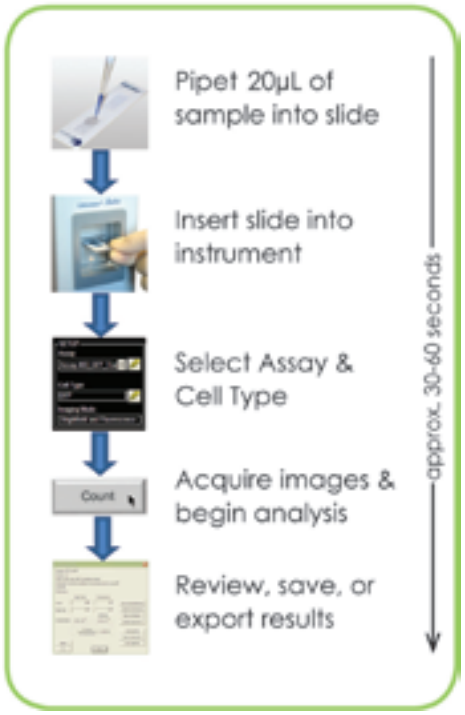

Figure 2 Steps to perform a typical cell analysis assay on Cellometer Vision.

confirmation of counting results. The user interface of the software is designed to be simple to use, and cell images and analysis data, including cell size and fluorescence intensity distribution histograms and scatter plots, may be saved for research records. All data and images can be automatically saved or easily exported to Microsoft® Excel™ (Redmond, WA) spreadsheets for further analysis.

Vision incorporates optical filtering (including one brightfield and two fluorescent channels), reliable light sources, and cooled charge-coupled device (CCD) camera technologies to be used in conjunction with a wide range of commonly used fluorophores such as GFP/RFP/YFP, acridine orange, propidium iodide, ethidium bromide, and 4′,6-diamidino-2-phenylindole (DAPI).

This image-based brightfield/fluorescence analysis can be applied to many different assays. The first example is its use in assays that require total cell count and fluorescence intensity data of one or more fluorophores in each of those cells. Experiments that are ideal for this type of analysis include determining GFP expression rates, as well as detection of annexin-V in apoptosis assays.

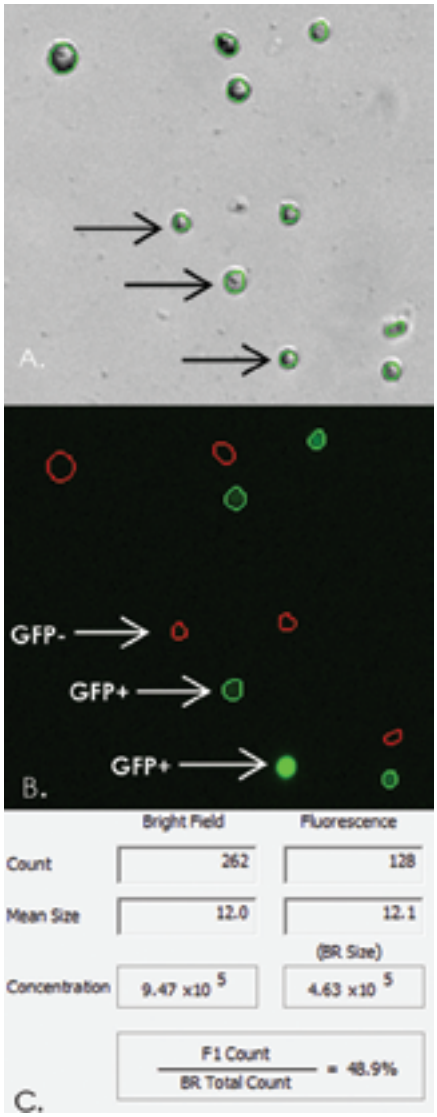

Figure 3 a) Brightfield image of cell samples. Green circles indicate cells that have been counted by the software. b) Fluorescent image of the same sample. Green circles indicate GFP-positive cells; red circles indicate GFP-negative cells. c) Results show count, size, and concentration of total and fluorescence positive cells as well as ratio.

Quantifying GFP expression levels in a population of cells

GFP is widely used in cell-based assays, and fluorescence signals can be used to monitor gene functions in cell proliferation, differentiation, toxicity, motility, and morphology. The time and effort required for analysis can be restrictive for rapid and routine use in stem cell research and clinical sample analysis. Cellometer Vision captures both brightfield and fluorescent images of the same sample and determines

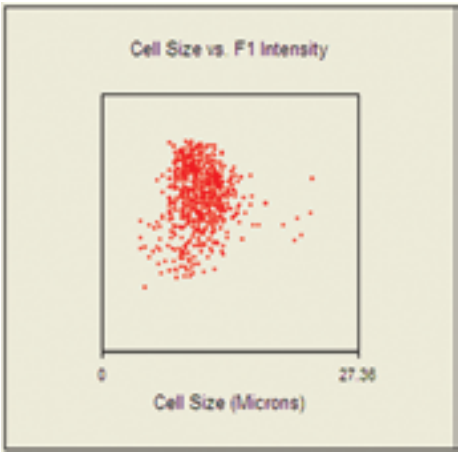

Figure 4 Scatter plot of cell size vs fluorescence intensity of annexin-V-labeled apoptotic cells.

total cell concentration, identifies fluorescence positive cells, and analyzes individual cell fluorescence intensities. Cell concentrations and GFP transfection rates are automatically calculated, and results and cell images are instantly displayed on-screen (see *Figure 3*).

Detection of apoptosis with fluorescein isothiocyanate (FITC)-conjugated annexin-V

Apoptosis, or programmed cell death, plays a critical role in organism homeostasis and tissue development. A number of in vitro and in vivo assays for detecting apoptotic cells exist, with FITC-conjugated annexin-V binding assays being one of the simplest to detect early-stage apoptosis. Like a GFP assay, total cell count is determined from brightfield images, and apoptotic cells are detected from the fluorescent images of the same sample. In addition to quickly producing total and apoptotic cell concentrations, cell size and fluorescence intensity data can be used to generate distribution histograms and scatter plots (see *Figure 4*).

This technology can also be applied to basic cell counting assays, such as determining viability by the presence of propidium iodide (PI) in the cell nucleus of dead cells, or to positively and accurately identify the presence of specific cells in a heterogeneous sample, such as acridine orange-stained white blood cells in whole blood samples.

Determining viability using PI

Cell viability is one of the most routinely tested parameters in cell population analy-

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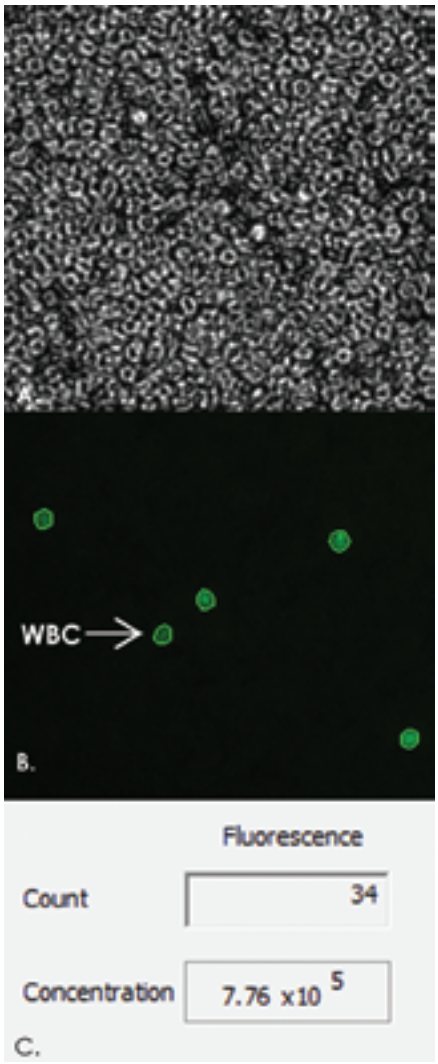

Figure 5 a) Brightfield image of whole blood. RBCs and platelets obscure WBCs. b) Fluorescent image of the same sample. Green circles indicate AO-stained WBCs. c) Results show count and concentration of AO-stained WBCs.

sis, and is used in cell proliferation, cytotoxicity, and tumor-killing activity assays. Viability is typically determined by the trypan blue exclusion method in conjunction with manual counting. Cellometer Vision can be used to determine viability automatically by this method, and can also be used to automatically determine the percentage of dead/dying cells by detecting PI, a fluorescent dye that can be used to stain dead cell nuclei.

Counting white blood cells (WBCs) in peripheral blood without lyses

White blood cell concentrations in whole blood are routinely assessed in the study of bacterial and viral infections, leukemia, and leukopenia. Conventional methods to count WBCs require lysing red blood cells (RBCs), a tedious process that can also lyse WBCs. By mixing a cell membrane permeable DNA dye, such as acridine orange, with diluted whole blood, WBCs can be specifically identified and counted in the fluorescent image of the sample, ignoring RBCs, platelets, and other cells lacking a nucleus (see *Figure 5*) to rapidly generate cell count and concentration data. This technique can also be used to count cells in samples that may contain debris or multiple cell types, such as bronchoalveolar lavage fluid, or distinguishing peripheral blood mononuclear cells (PBMCs) from platelets or residual RBCs.

In addition to the above applications, fluorescent dyes can be used to improve analysis of samples that traditionally cannot be counted in automated systems due to cell size or morphology. Examples of this include hepatocytes, which can clog flow cytometers and are difficult to count and determine viability manually, and adipocytes, which are difficult to distinguish from lipid droplets and are often too large for manual or automated analysis.

Cellometer Vision's brightfield imaging mode can be used by itself to determine basic cell count, concentration, and viability via trypan blue, as an alternative to manual cell counting with a hemacytometer. Benefits of using automated imaging technology to count cells are numerous, and include increased throughput; cell count accuracy; and generation of cell size data, not to mention freeing researchers from a monotonous, tedious process.

A typical research laboratory can be expected to utilize the versatility of this imaging technology in each of these application areas on a daily basis. A good example of this would be performing a transfection assay. Prior to transfection, an accurate cell count is critical in determining the proper conditions for optimum results. Post-transfection success can be confirmed by determining the percentage of GFP-positive cells, and additional analysis such as correlating expression levels with cell sizes can also be performed.

Conclusion

As the use of cell-based assays and fluorescence technologies continues to increase, new tools will be required to enable researchers to generate these data in a simple and quick manner. Use of multimode imaging analysis systems such as Cellometer Vision permits the generation of cell count, cell size, viability, and fluorescence intensity data in a compact, easy-to-use system for a variety of assays. The simplicity and speed make quantitative data analysis available at the bench level to more researchers, increasing the overall rate of discovery in fields such as immunology, cancer research, and stem cells.

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