Expanded Capabilities with the Cytek® Guava® Muse® Cell Analyzer Open Modules

Introduction

Finding simplified analysis tools to answer cellular problems can be a challenge. Flow cytometers offer a high level of information on the single cell level, but can be very costly and complex. The Cytek® Biosciences Guava® Muse® cell analyzer is a small, easy to use, and cost-effective one-laser, three-parameter benchtop instrument that is capable of a variety of cellular applications.

Previously the Muse instrument offered optimized acquisition and analysis software modules for use with validated, dedicated reagent kits. Now, there are two simplified open software modules that allow users to acquire and analyze data from researchers' own reagents. The modules allow researchers to stain samples with their own fluorochrome-conjugated antibodies, dyes, and other reagents that are excited by a 532 nm laser. The yellow parameter uses a detection channel with 576/28 emission that can be used for the detection of fluorochromes such as Phycoerythrin (PE) and Cy3. The red parameter uses a detection channel with 680/30 emission that can be used for the detection of fluorochromes such as PE-Cy5, 7-AAD, and Propidium Iodide (PI).

The flexibility of two simplified modules allows researchers to determine the best fit for their needs. The data shown here demonstrate the application of the open modules to a variety of application questions, such as one- and two-color staining of extracellular markers, intracellular marker detection, as well as analysis of red fluorescent proteins.

Methods

Single-Color Extracellular Staining

Jurkat cells, a non-adherent human T cell line, were kept in log phase growth in complete growth medium. 1×10^5 cells in 20 µL were stained with varying concentrations of an anti-human CD45 antibody for 20 minutes at room temperature in the dark.

Following incubation, cells were washed once and incubated with 0.2 µL of a PE or PE-Cy5 goat anti-mouse IgG secondary antibody for 20 minutes at room temperature in the dark. Following incubation, cells were washed once, diluted to 200 µL with Guava® Assay Buffer BA, and acquired on the Muse cell analyzer using Open Module Yellow or Open Module Red.

Two-Color Extracellular Staining

Peripheral blood mononuclear cells (PBMCs) were separated from adult human blood via Ficoll-Paque™ layering following standard procedures.[1] PBMC samples were resuspended at a concentration of 5×10^6 cells/mL. 10 µL of adult human whole blood or PBMC samples were stained with a cocktail of either anti-CD3 PE and anti-CD20 PE-Cy5 or anti-CD3 PE-Cy5 and anti-CD4 PE for 20 minutes at room temperature in the dark. Immediately following staining, whole blood samples were lysed with Guava® Lysing Solution for 30 minutes while PBMC samples were resuspended in 180 µL of Guava Assay Buffer BA. Next, whole blood samples and PBMCs were acquired on the Muse cell analyzer using Open Module Yellow or Open Module Red.

Intracellular Staining With TUNEL Assay

Jurkat cells were kept in log phase growth in complete growth medium. Cells were then induced with staurosporine, a known protein kinase inhibitor, at 0, 0.1, 0.3, and 1 µM for 4 hours at 37°C. Post-induction, 2×10^5 cells in 200 µL were fixed with 1% paraformaldehyde (PFA) for 1 hour on ice. Following fixation, cells were washed and permeabilized with ice-cold 70% ethanol overnight at -20°C. After permeabilization, the Guava® TUNEL Assay was used and samples were stained following the manufacturer's protocol. Following staining, samples were acquired on the Muse cell analyzer using Open Module Yellow.

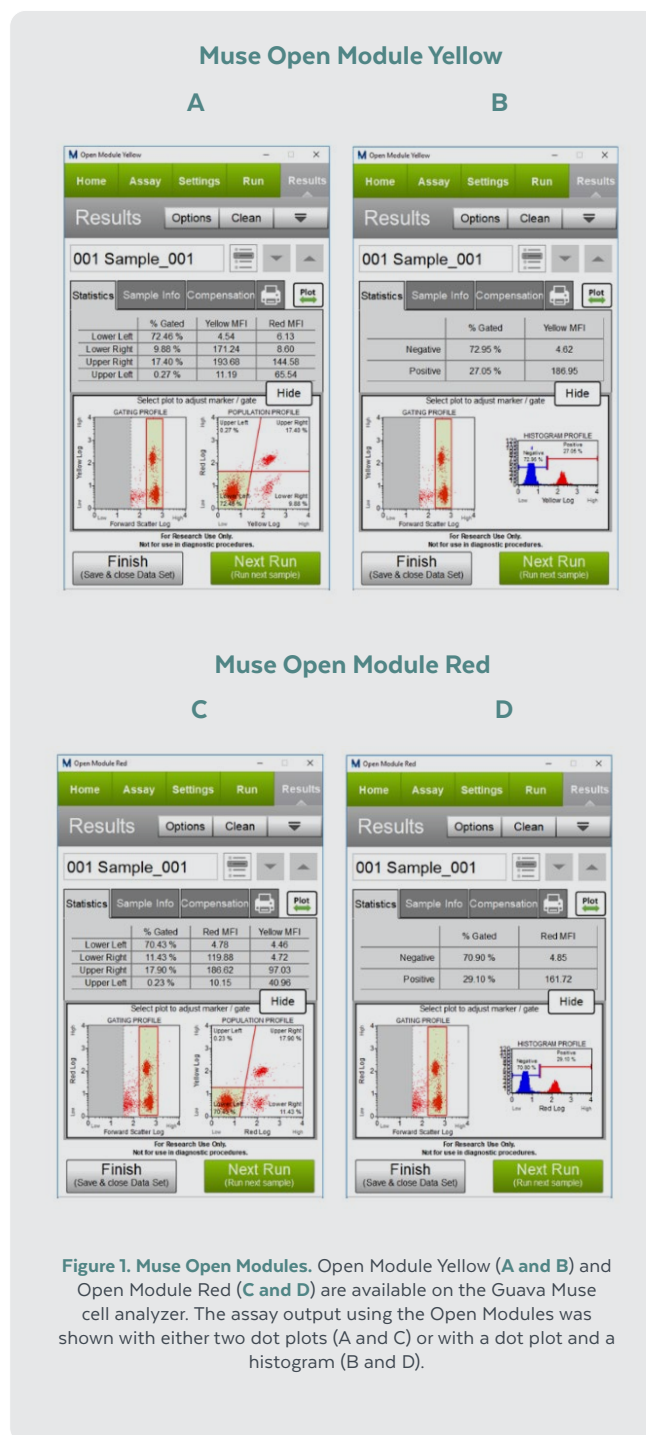## Intracellular Staining With Tyrosine Phosphorylation Assay

A431 cells, an adherent epidermoid carcinoma cell line, were kept in log phase growth in complete growth medium. Cells were synchronized by serum starvation in the growth medium without FBS for 22 hours. After serum starvation, A431 cells were stimulated with epidermal growth factor (EGF) at 0, 4, 20, and 100 ng/mL for 5 minutes at 37°C. Cells were fixed at a concentration of 2×10^5 cells in 200 µL with 4% PFA for 10 minutes at room temperature. Following fixation, cells were washed and permeabilized with 0.2% Triton® X-100 for 10 minutes at room temperature. After permeabilization, samples were washed and blocked with 2% BSA for 10 minutes at room temperature. Samples were then stained with a cocktail containing an anti-phosphotyrosine antibody (isotype IgG2b) for 30 minutes at room temperature. Control cells were stained with matching mouse IgG2b, κ isotype control. Following incubation, cells were washed and stained with PE-Cy5 goat anti-mouse IgG for 30 minutes at room temperature. Cells were washed once, diluted to 200 µL in Guava Assay Buffer BA, and acquired on the Muse cell analyzer using Open Module Red.

Red Fluorescent Protein (RFP) Analysis

HEK293 cells, an adherent human embryonic kidney 293 cell line, and RFP-transfected HEK293-RFP cells were kept in log phase growth in complete growth medium. The two cell lines were harvested and diluted to 5×10^5 cells/mL in PBS. Untransfected and transfected cells were mixed at the following ratios: 1:0, 3:1, 1:1, 1:3, and 0:1, to a total volume of 200 µL in PBS. Samples were acquired on the Muse cell analyzer using Open Module Yellow.

Results

The Muse cell analyzer comes equipped with dedicated modules for pre-optimized assays covering applications that range from cell health analysis, signaling, and immunophenotyping. As shown in **Figure 1,** two types of open modules are available on the Muse software menu: Open Module Yellow and Open Module Red. Each module allows for the choice of dot plot or histogram outputs. The choice of module for an experiment is dictated by the reagents being used. Modules include standard features such as compensation, histogram overlays, choice of gain settings, and flow rates. Statistics include population percentages, mean and median fluorescent

Muse Open Module Yellow

Muse Open Module Red

Figure 1. Muse Open Modules. Open Module Yellow (**A and B**) and Open Module Red (**C and D**) are available on the Guava Muse cell analyzer. The assay output using the Open Modules was shown with either two dot plots (A and C) or with a dot plot and a histogram (B and D).

intensities, and percent CV for all populations of interest. These newly introduced Open Module Yellow and Open Module Red modules provide researchers with additional flexibility to evaluate questions with their own optimized one- and two- color assays.

Here, we show the application of the Muse Open Modules to a variety of different analytical experiments of interest and the results that can be obtained using these approaches with ease.

Application To Extracellular Staining

The study of cell surface markers is important in many cellular expression studies. Often, only primary antibodies are available for the marker of interest, and they must be paired with a secondary fluorescently-labeled detection antibody. Additionally, antibodies must be titered for the optimal conditions for simplified use. The Muse Open Modules allow for the study of extracellular markers and optimization of detection conditions with ease.

In **Figure 2**, detection of varying levels of CD45 antigen expression on Jurkat cells was evaluated. The Open Module Yellow was used for PE detection and the Open Module Red was used to detect the PE-Cy5 stained cells. Cells stained with varying concentrations of a primary antibody demonstrate an improved signal-to-noise ratio that was obtained with increasing amounts of primary antibody. Antibody titration using this method can be used to find the ideal concentration of primary and secondary antibodies for optimal performance.
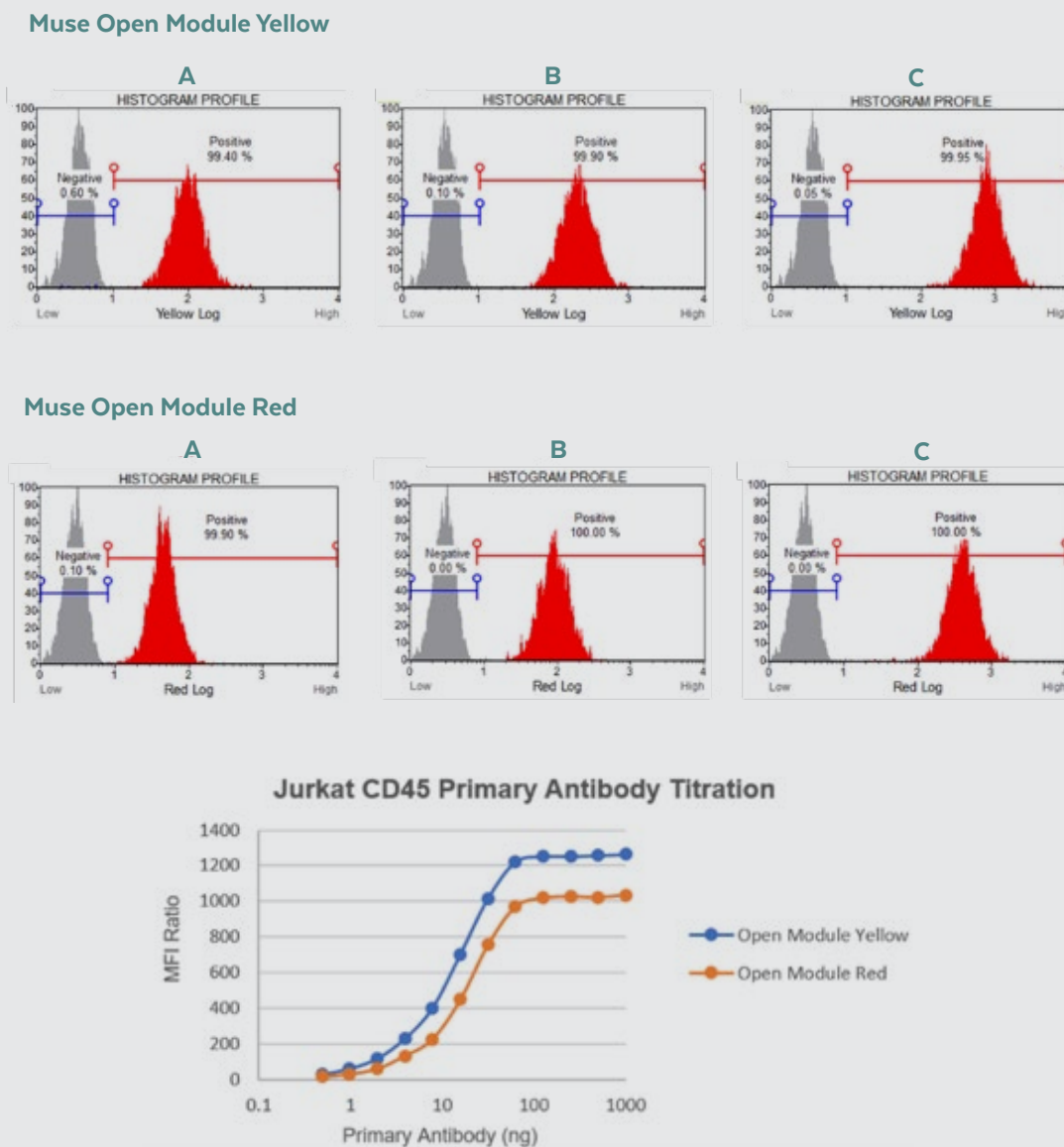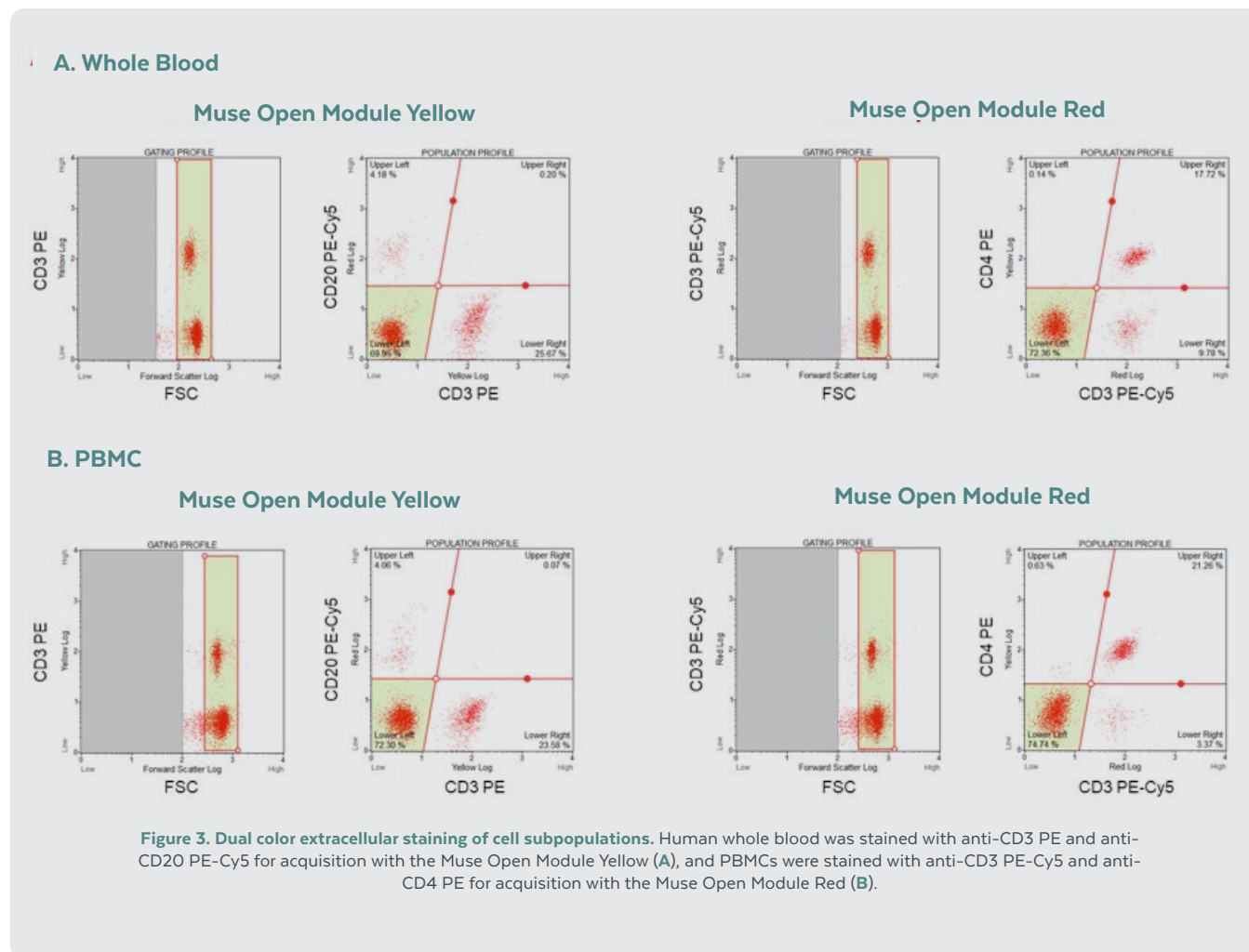

Figure 2. Extracellular staining of CD45 on Jurkat cells. Jurkat cells were stained with multiple concentrations of unlabeled primary anti-CD45 antibody, examples at 0.49 ng (**A**), 0.98 ng (**B**), and 3.9 ng (**C**) are shown above, and then with 0.2 ng of PE (upper panel) or PE-Cy5 goat anti-mouse IgG (lower panel). Data was acquired with the Muse Open Module Yellow for PE conjugated IgG and with the Muse Open Module Red for PE-Cy5 conjugated IgG. The figures show stained cells in red with an optional histogram overlay of unstained cells in grey. Complete titration profiles are shown on the right. Optimal conditions for detection can be determined using the Muse Open Modules.

Two-Color Staining

The Muse Open Modules allow for easy analysis of two-color staining experiments to distinguish multiple cell populations and obtain more enriched information. The modules can be used for phenotyping whole blood and PBMCs as shown in the example below, and also used to determine the percentages of populations of interest. Absolute count information can be obtained for whole blood staining with no washes. In **Figure 3**, whole blood (**Figure A**) and PBMCs (**Figure B**) were stained, lysed, and analyzed using both Muse Open Module Yellow and Open Module Red. As shown, the Open Module Yellow allowed for the clear distinction of B and T cells in the samples stained with CD3 PE and CD20 PE-Cy5. Similarly, in the Open Module Red, CD4 T cells were distinguished by CD3 PE-Cy5 and CD4 PE staining.

A. Whole Blood

Muse Open Module Yellow   **Muse Open Module Red**

B. PBMC

Muse Open Module Yellow   **Muse Open Module Red**

Figure 3. Dual color extracellular staining of cell subpopulations. Human whole blood was stained with anti-CD3 PE and anti-CD20 PE-Cy5 for acquisition with the Muse Open Module Yellow (**A**), and PBMCs were stained with anti-CD3 PE-Cy5 and anti-CD4 PE for acquisition with the Muse Open Module Red (**B**).

Application To Intracellular Staining With The TUNEL Assay

Apoptosis is a process of controlled intracellular programmed cell death that is marked by distinct biochemical and morphological changes within a cell that eventually lead to cell death. Studying the mechanisms of apoptosis plays an important role in research; as a result, multiple assays are used to characterize apoptosis and cell death. A hallmark of late apoptosis is the fragmentation of DNA that generates DNA double-strand breaks (DSBs) with exposed 3'-hydroxyl ends. The Guava TUNEL Assay detects apoptosis-induced DNA fragmentation through a quantitative fluorescence assay. The assay evaluates terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) by using TdT to catalyze the incorporation of bromo-deoxyuridine (BrdU) residues into the fragmented nuclear DNA at the 3'-hydroxyl ends. These fragments are then detected using a TRITC-conjugated anti-BrdU antibody.

Figure 4 shows the application of the TUNEL assay to stauropsorine-treated Jurkat cells. As the dose of staurosporine treatment increases, there is an increase in TUNEL-responsive cells, demonstrating progress towards DNA fragmentation and late apoptosis, even under four hours of treatment.

The data demonstrates the ease of studying additional cell health markers on the Muse system using the TUNEL assay to obtain more enriched information.
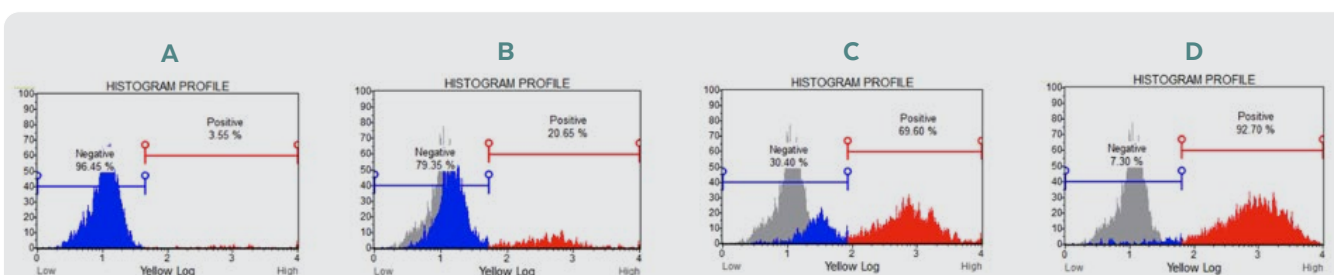

Figure 4. Intracellular staining of DNA DSBs in apoptotic Jurkat cells by TUNEL. Jurkat cells were induced with staurosporine at 0 (**A**), 0.1 µM (**B**), 0.3 µM (**C**), and 1 µM (**D**) for 4 hours at 37°C. The TUNEL assay was performed and data was acquired with the Muse Open Module Yellow. The figure shows TUNEL-negative cells in blue and TUNEL-positive cells in red, with the optional histogram overlay of untreated cells in grey.

Application To Signaling Studies

Flow cytometry is a powerful way to study tyrosine phosphorylation under different induction conditions. Compared to traditional methods, microcapillary cytometry on the Muse system allows this to be studied using a low number of cells. When coupled with analysis using the Muse Open Module, this results in a simple method to evaluate tyrosine phosphorylation.

Figure 5, shows the response of A431 cells to treatment with Epidermal Growth Factor (EGF). The results demonstrate that the combination of this staining method and analysis on the Muse system allowed for the rapid and easy determination of the percentage of cells that exhibit phosphotyrosine expression, as well as the response to different concentrations of EGF. The Muse Open Module was able to distinguish the dose-dependent shift of signals.
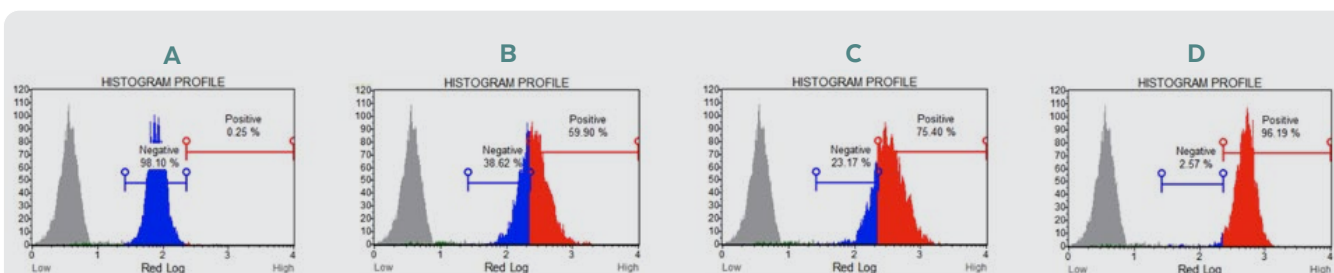

Figure 5. Detection of phosphotyrosine in EGF treated A431 cells. A431 cells were stimulated with EGF at 0 (**A**), 4 ng/mL (**B**), 20 ng/mL (**C**), and 100 ng/mL (**D**) for 5 minutes at 37°C. Cells were stained with an unlabled anti-phosphotyrosine antibody and then a PE-Cy5 goat anti-mouse IgG secondary antibody. Data was acquired with the Muse Open Module Red. The figure above shows unphosphorylated cells in blue and phosphorylated cells in red, with the optional histogram overlay of matching IgG2b,　Isotype control in grey.

Application To Fluorescent Protein Detection With RFP Analysis

Expression of a fluorescent protein tag in combination with proteins or biomarkers of interest has greatly transformed our understanding of cellular biology, protein expression levels, protein interactions, and a host of biological phenomena. RFP emission can be detected using the Muse Open Module Yellow. **Figure 6** shows untransfected and RFP-transfected HEK293 cells mixed at different ratios. This highlights the ability of the Open Module Yellow to distinguish populations and provide the respective percentages of cells that have undergone transfection versus those that have not.
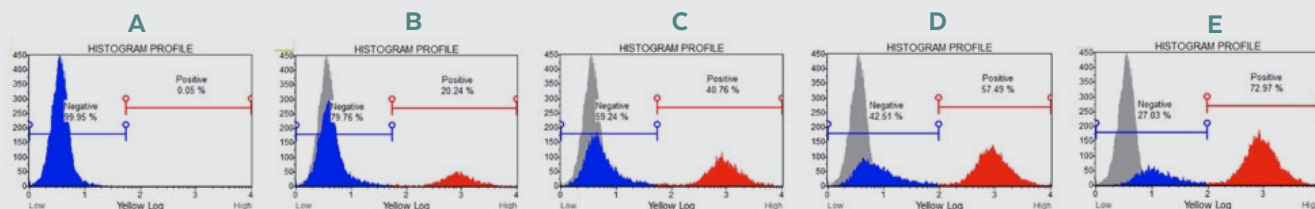

Figure 6. RFP analysis. Untransfected and RFP transfected HEK293 cells were mixed at 1:0 (**A**), 3:1 (**B**), 1:1 (**C**), 1:3 (**D**), and 0:1 (**E**) ratios. Data was acquired with the Muse Open Module Yellow. The figure above shows untransfected HEK293 cells in blue and RFP transfected HEK-RFP cells in red, with the optional histogram overlay of 100% untransfected HEK293 cell sample in grey (**B-E**).

Summary

The Muse Open Modules provide additional flexibility and versatility to researchers for a variety of research problems. We have shown how existing Guava® reagents, as well as other commercially available reagents, may be utilized along with the Yellow or Red Open Modules to expand the applications of the Muse system. The flexible features of the Muse Open Modules have transformed the Muse cell analyzer into an affordable cytometer with amplified capability for researchers to study an increased variety of cellular problems while still maintaining the simplicity of a guided software interface.

REFERENCES

1. Ficoll-Paque™ PLUS Intended Use. GE Healthcare (PDF). Cited 2019 May. Available from: https://cdn.gelifesciences.com/dmm3bwsv3/AssetStream. aspx?mediaformatid=10061&destinationid=10016&assetid=12637.

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