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Expanded Analysis Capability of Guava® Muse® Cell Analyzer with New Open Modules

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Introduction

Flow cytometers offer a wealth of information on the single cell level, but can be very costly and complex. The Muse® Cell Analyzer is a simple, easy-to-use, and cost-effective three-parameter benchtop instrument that is capable of a variety of cellular applications. Previously, the acquisition and analysis software modules for the Muse Instrument were optimized for use with dedicated reagent kits validated by Luminex. Here, we present two new software modules for the Muse System that preserve the simplicity of the guided software, while offering increased options for applications of the Muse Instrument by providing flexibility in choice of reagents, plots, and settings. Our studies demonstrate how the Muse Open Modules can be applied to a variety of cellular investigations, providing expanded utility for researchers using Muse Cell Analyzers.

Background

- Two new modules have been introduced on the Muse Cell Analyzer that provide greater experimental flexibility to researchers: Muse Open Module Yellow and Muse Open Module Red. These modules provide researchers with flexibility to evaluate questions with their own optimized one- or two- color reagents. The choice of module is dictated by the reagents being used.
- The modules guide users through acquisition, enable users to choose to view their data in dot plots or histograms, and provide options for pre- or post-acquisition compensation and statistical outputs.

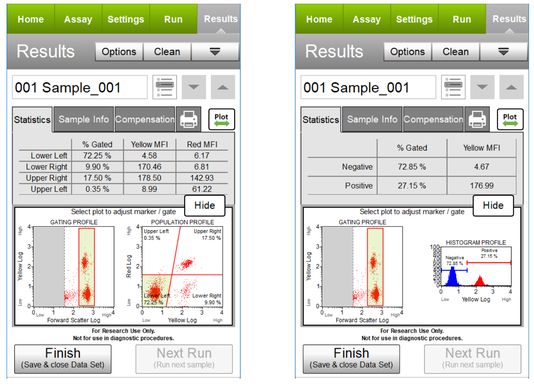

Figure 1. Muse® Open Modules. Assay outputs using the Muse® Open Module Software shown with either dot plots or with a histogram plot. The software populates the statistics displayed based on user selection.

Results

Optimizing Conditions for Extracellular Markers

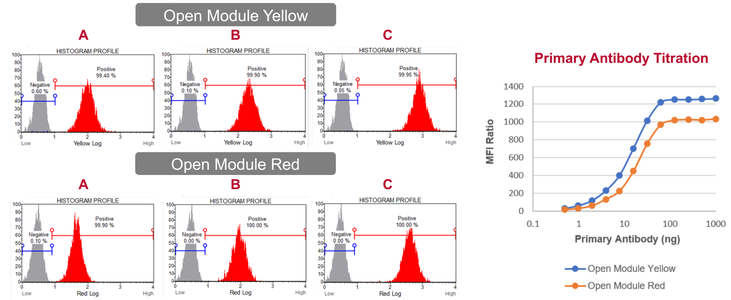

Figure 2. Extracellular staining of CD45 on Jurkat cells. The Muse® Open Modules can be used for optimizing detection conditions for extracellular markers. In figure 2, Jurkat cells (1 x 10^5 cells) were stained with multiple concentrations of unlabeled primary anti-CD45 antibody. Examples at 0.49 ng (A), 0.98 ng (B), and 3.9 ng (C) are shown above, with 0.2 µg of PE (upper panel) or PE-Cy5 goat anti-mouse IgG (lower panel) for 30 min each. 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 the optional histogram overlay of unstained cells in grey. Complete titration profiles are shown on the right. Optimal conditions for antigen detection can be determined using the Muse Open Modules.

Dual Color Extracellular Staining

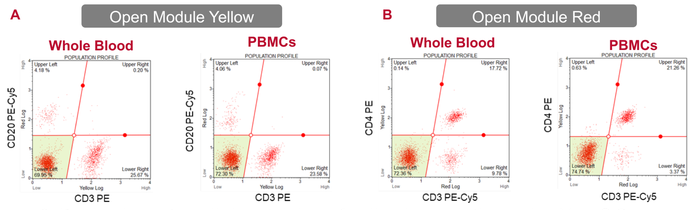

Figure 3. Dual color extracellular staining of cell subpopulations. The Muse® Open Modules can be used for dual color extracellular staining of cell populations to provide more enriched information. Human whole blood (10 µL) or PBMCs (5 x 10^4 cells) were stained with anti-CD3 PE and anti-CD20 PE-Cy5 antibodies (A), or anti-CD3 PE-Cy5 and anti-CD4 PE antibodies (B) for 20 min. Blood was lysed for 30 min. Data was acquired with the Muse Open Module Yellow (A) or Open Module Red (B) on the Muse Cell Analyzer. The modules allow for phenotyping and quantitation of populations of interest.

Intracellular Analysis - TUNEL Assay

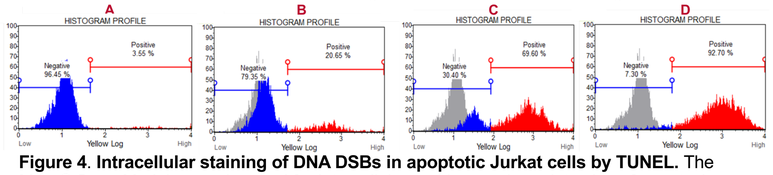

Figure 4. Intracellular staining of DNA DSBs in apoptotic Jurkat cells by TUNEL. The Muse® Open Modules can be used for intracellular probing, such as detection of apoptosis-induced DNA fragmentation using the TUNEL assay. Figure 4 shows intracellular staining of DNA DSBs in apoptotic Jurkat cells by TUNEL assay. Jurkat cells were induced with staurosporine at 0 µM (A), 0.1 µM (B), 0.3 µM (C), and 1 µM (D) for 4 hours at 37 °C. 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.

Study of Phosphotyrosine Expression

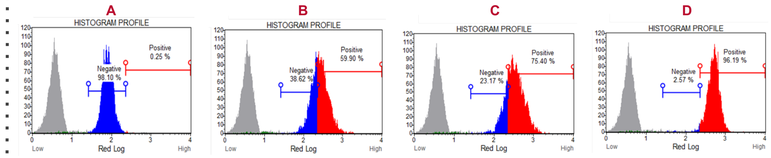

Figure 5. Detection of phosphotyrosine in EGF-treated A431 cells. Phosphotyrosine expression under different stimulation conditions can be studied using the Muse® Open Modules. Figure 5 shows detection of phosphotyrosine in EGF-treated A431 cells. A431 cells were stimulated with EGF at 0 ng.mL (A), 4 ng/mL (B), 20 ng/mL (C), and 100 ng/mL (D) for 5 minutes at 37 °C. Cells were fixed, permeabilized, and stained with an unlabeled anti-phosphotyrosine antibody and then 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, κ Isotype control in grey. The data shows increasing expression of phosphotyrosine with higher EGF concentrations.

Red Fluorescent Protein (RFP) Analysis

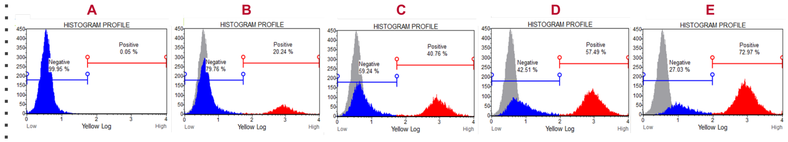

Figure 6. RFP analysis. The percentage of cells exhibiting RFP expression can be detected using the Muse® Open Module Yellow. In figure 6, untransfected and RFP transfected HEK293 cells were mixed at 1:0 (A), 3:1 (B), 1:1 (C), 1:3 (D), and 0:1 (E) ratios. Data was acquired with the Muse Open Module Yellow. The figure 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 in B-E. Muse Open Module Yellow can distinguish and provide population percentages for cells that have undergone transfection versus those that have not.

Conclusions

- The Muse Open Module Yellow and Open Module Red are flexible modules for data acquisition and analysis on the Muse Cell Analyzer—a touchscreen-based cytometer that uses microcapillary cytometry. The modules allow researchers to stain samples with a variety of fluorochrome-conjugated antibodies, dyes, or other reagents compatible with the platform, and run one- or two-color assays.
- In this study, we demonstrate the application of the Muse Open Modules to a variety of cellular experiments, such as the study of one- and two-color extracellular markers, detection of intracellular markers, and expression of red fluorescent proteins.
- The flexible features of the Muse Open Modules have transformed the Muse Cell Analyzer into an affordable cytometer with amplified capabilities to study an increased variety of cellular problems, while still maintaining the simplicity of a guided software interface.

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