Luminex

Application Note

Amnis® ImageStream®X Mk II Flow Cytometer High Gain Mode for Increased Sensitivity in the Detection of Small Particles

Haley R. Pugsley, PhD, María Gracia García-Mendoza, PhD, and Bryan R. Davidson

High Gain mode for the Amnis® ImageStream®X Mk II Flow Cytometer is designed to detect small, dim particles such as extracellular vesicles (EVs) and viruses. In High Gain mode, the time-delay integration (TDI) CCD camera at the heart of the Amnis® Technology is adjusted to a higher gain setting to maximize signal while minimally increasing the noise, allowing for increased sensitivity and increased signal from small particles. In addition to increasing the gain, the object detection thresholds and masking have been adjusted to better identify small objects like EVs and viruses. High Gain mode is designed to work at 60X and at slow speed. With the addition of a 400 mW 488 nm laser and an increase in photonic sensitivity, even more EVs and virus particles can be detected.

Example 1: Murine leukemia virus-sfGFP reference particles show an increase in object detection.

MV-M-sfGFP reference particles from ViroFlow Technologies, Inc., are inactivated murine retroviruses produced in mouse cells that express superfolder green fluorescent protein (sfGFP) on the outer surface of the viral envelope. They are small fluorescent reference particles for flow cytometry with a size of ~120 nm. In this experiment, the MV-M-sfGFP particles were reconstituted in 0.1 µm filtered water and diluted 1:400 in PBS. The samples were acquired for three minutes at four collection settings: Normal Gain 200 mW 488 nm laser power; Normal Gain 400 mW 488 nm laser power; High Gain 200 mW 488 nm laser power; and High Gain 400 mW 488 nm laser power. **Figure 1A** shows the intensity histograms for channel 2 (GFP channel) and the associated images from the mean intensity for the different gain and power settings. Note, as more dim objects are detected by the system when using increased laser power and/or High Gain, the mean intensity for the GFP+ population will not necessarily double because the dim particles that were not previously detected will lower the overall average intensity. **Figure 1B** shows an increase in GFP+ objects detected with increased laser power as well as increased camera gain.

Figure 1A.

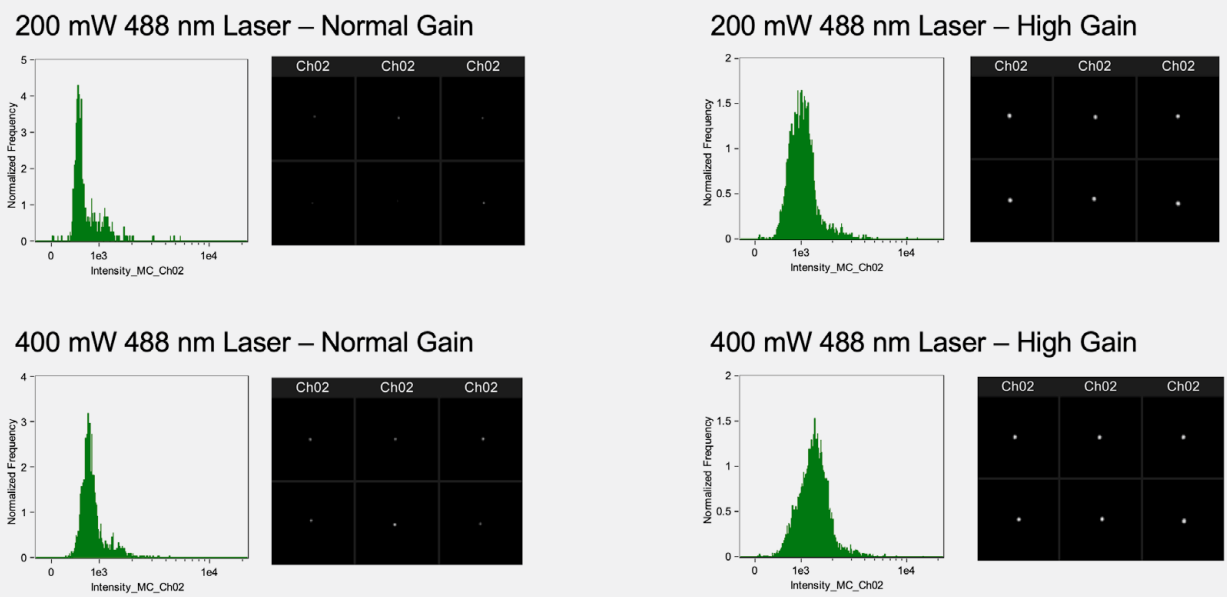

Figure 1B.

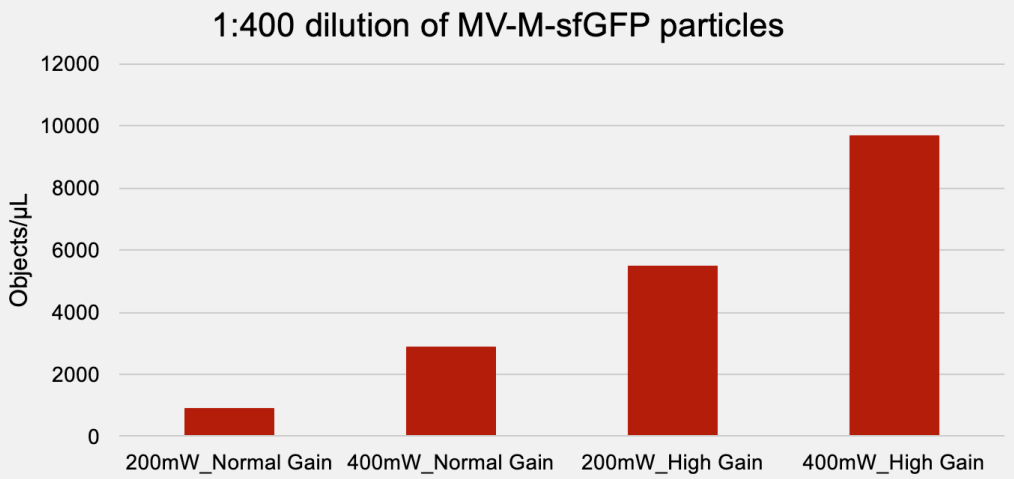

Figure 1. A) Histograms and representative images from the mean intensity for each of the collection settings. All images used the same display settings. **B)** MV-M-sfGFP diluted at 1:400 in PBS show an increase in objects per µL detected with increased laser power and increased gain settings.

Example 2: 220 nm fluorescent beads demonstrate an increase in photonic sensitivity.

To illustrate the increase in fluorescent signal using High Gain with the 400 mW 488 laser, 220 nm yellow SPHERO™ Nano Fluorescent particles were acquired on the ImageStream^X Mk II in Normal Gain and High Gain modes using the 488 nm laser at 200 mW and 400 mW. **Figure 2** shows a clear increase in the mean intensity with both High Gain and the 400 mW 488 nm laser. In this example, 2,000 bead images were collected, and the mean fluorescent intensity for channel 2 was reported.

Figure 2.

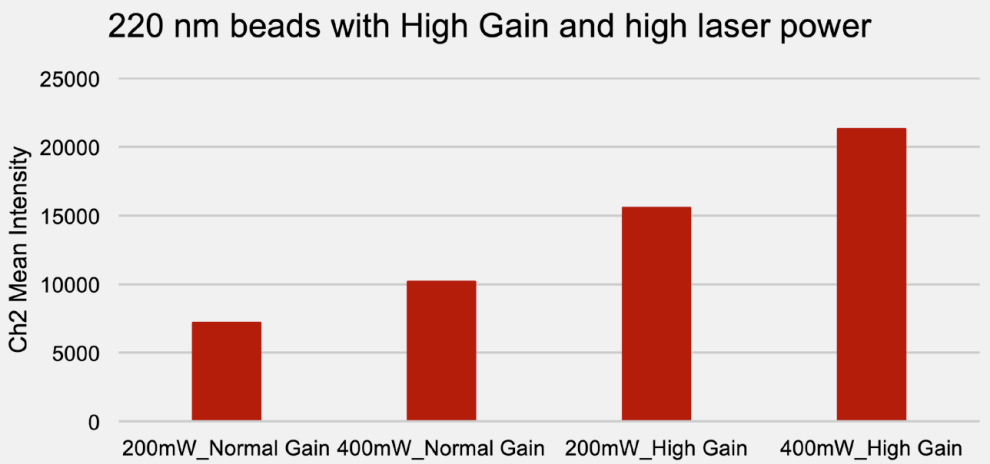

Figure 2. Increased fluorescent signal using High Gain mode and a 400 mW 488 nm laser on 220 nm yellow SPHERO™ Nano Fluorescent particles.

Example 3: HEK293-derived EVs pre-labeled with CFSE show no swarm detection.

HEK293-derived EVs from Exosomics S.p.A pre-labeled with carboxyfluorescein succinimidyl ester (CFSE) were serially diluted (1:10, 1:50, and 1:100) in PBS and data was acquired for three minutes at four collection settings: Normal Gain 200 mW 488 nm laser power; Normal Gain 400 mW 488 nm laser power; High Gain 200 mW 488 nm laser power; and High Gain 400 mW 488 nm laser power. The dilution series was performed to verify the absence of swarming (swarm detection), which happens when multiple objects such as EVs are captured as a single event. **Figure 3** summarizes the detected objects per µL for the CFSE-labeled, HEK293-derived EVs and the PBS control. These results show the dilution series concentration decreases as expected, indicating that swarm detection was not occurring. This trend held true for the different collection settings. Analysis of the various collection settings shows an increase in CFSE+ events, with both an increase in laser power and an increase in camera gain.

Figure 3.

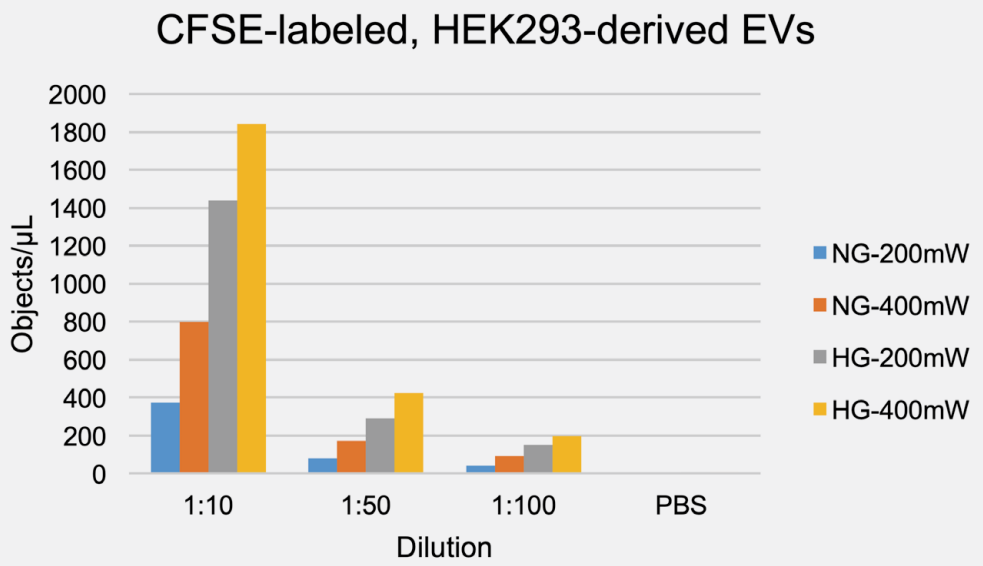

Figure 3. CFSE-labeled, HEK293-derived EVs show increased objects per µL when detected at High Gain with 400 mW 488 nm laser power. CFSE-labeled, HEK293-derived EVs dilution series and collection settings: Normal Gain (NG) with 200 mW 488 nm laser power; Normal Gain (NG) with 400 mW 488 nm laser power; High Gain (HG) with 200 mW 488 nm laser power; and High Gain (HG) with 400 mW 488 nm laser power.

Example 4: RBC-derived EVs labeled with anti-CD235ab-PE show how antibodies can be used to immunophenotype EVs.

EVs derived from red blood cells (RBCs) purchased from Cellarcus Biosciences were labeled for one hour at room temperature with anti-CD235ab-PE (BioLegend). After labeling, the samples were serially diluted (1:15, 1:30, and 1:60) in the Cellarcus Biosciences vFC™ Staining Buffer. The PE-labeled EV samples were acquired for three minutes at four collection settings: Normal Gain 200 mW 488 nm laser power; Normal Gain 400 mW 488 nm laser power; High Gain 200 mW 488 nm laser power; and High Gain 400 mW 488 nm laser power. Control samples for antibody-only, buffer-only, and detergent controls were similarly diluted in vFC™ Staining Buffer and acquired in the same manner as the EV samples. Labeled EVs were incubated in 0.1% Triton™ X-100 for 10 minutes to break down the EVs. **Figure 4A** summarizes the objects per µL for the PE-labeled, RBC-derived EVs. The detected objects per µL linearly decreased as expected for the dilutions, indicating there was no swarm detection occurring, and there is a clear increase of PE+ objects detected with both increased laser power and increased gain settings. The control samples for the antibody-only and detergent controls are shown in **Figures 4B** and **4C**, respectively. The vFC™ Staining Buffer control had zero PE+ objects per µL detected (data not shown). While the antibody-only controls and detergent controls show the same trend as the labeled EVs, there is a clear difference in the number of objects per µL compared to the PE-labeled EV samples, validating the EV population.

Figure 4.

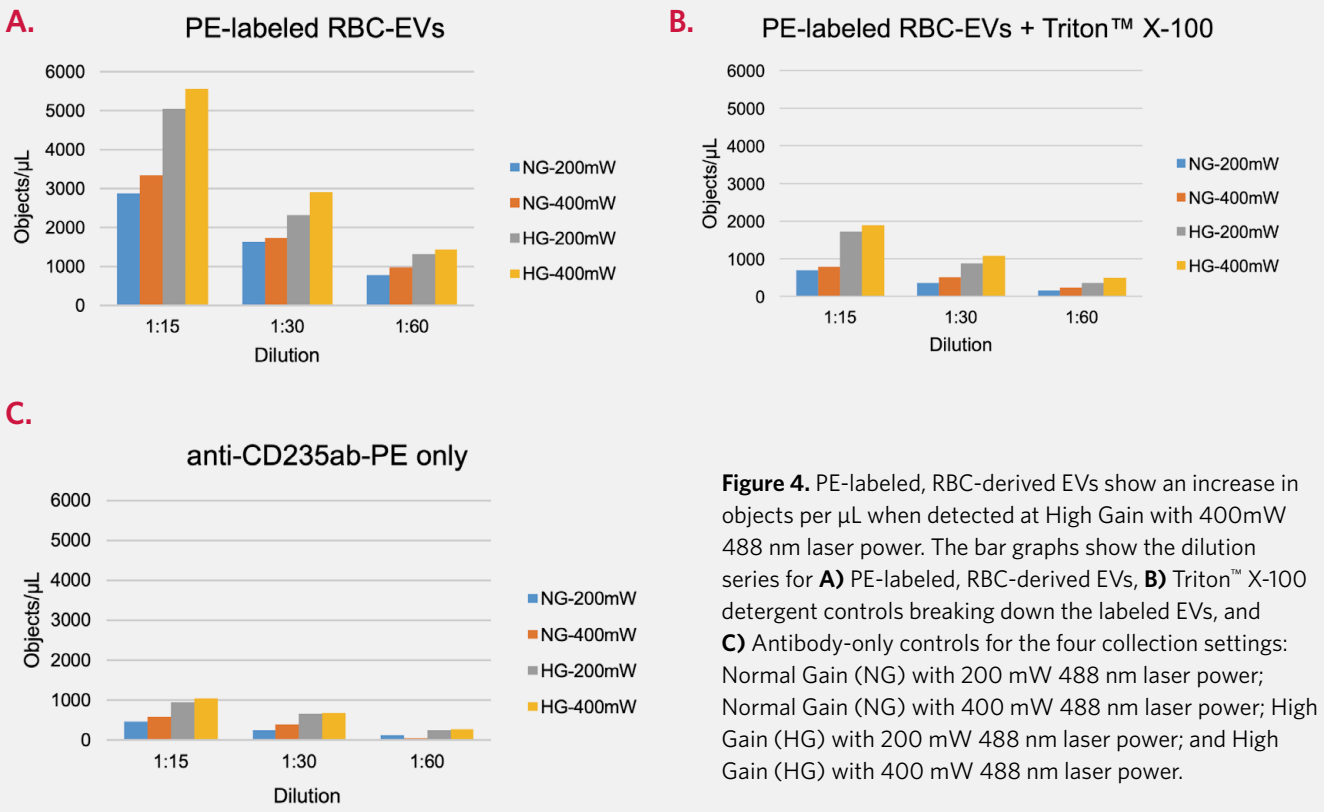

Figure 4. PE-labeled, RBC-derived EVs show an increase in objects per µL when detected at High Gain with 400mW 488 nm laser power. The bar graphs show the dilution series for **A)** PE-labeled, RBC-derived EVs, **B)** Triton™ X-100 detergent controls breaking down the labeled EVs, and **C)** Antibody-only controls for the four collection settings: Normal Gain (NG) with 200 mW 488 nm laser power; Normal Gain (NG) with 400 mW 488 nm laser power; High Gain (HG) with 200 mW 488 nm laser power; and High Gain (HG) with 400 mW 488 nm laser power.

Summary

These four examples demonstrate the use of High Gain mode and the high-powered 488 nm laser on the ImageStream^X Mk II. The 220 nm yellow fluorescent beads showed a consistent increase in signal intensity with increased laser power and increased gain. The EV and virus examples all showed an increase in positive objects detected for both increased 488 nm laser power and increased gain settings. Using serial dilutions, we were able to verify the absence of swarming. The RBC-EV data demonstrates the ability to use antibodies that specifically identify the type of EV, with the potential to use multiple fluorochromes to characterize the EVs further, or measure multiple EV types in a single sample.

Acknowledgments

Thank you to ViroFlow Technologies, Inc. for the MV-M-sfGFP particles, Exosomics S.p.A. for the HEK293-derived EVs labeled with CFSE, and Cellarcus Biosciences for the RBC-derived EVs.

Luminex®
complexity simplified.

For more information, please visit luminexcorp.com/imagestreamx-mk-ii/

luminexcorp.com

HEADQUARTERS
UNITED STATES
+1.512.219.8020
info@luminexcorp.com

EUROPE
+31.73.800.1900
europe@luminexcorp.com

CANADA
+1.416.593.4323
info@luminexcorp.com

CHINA
+86.21.8036.9888
infocn@luminexcorp.com

JAPAN
+81.3.5545.7440
infojp@luminexcorp.com

FL255437