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Analysis of Viral Particles on Amnis® Flow Cytometers

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Introduction

Until recently, the analysis of viruses and viral particles by flow cytometry was limited due to the range of detection (300-500 nm)[1] and the low signal-to-noise ratio of traditional flow cytometers in the lower size range. However, the development of camera-based systems in flow cytometry now allows for the analysis of viruses and viral particles (VPs). Due to the high sensitivity of the time-delay integration (TDI) and CCD-camera technology that is unique to Amnis® systems, the Amnis® CellStream® and ImageStream® flow cytometers can be used to study viruses and VPs. These flow cytometers also offer a low signal-to-noise ratio, which further increases their ability to resolve particles 100 nm or smaller.

The recent coronavirus pandemic has demonstrated that flow cytometry can be used as a high-throughput tool for studying viruses and their effects on cells. The research presented here demonstrates the ability of two Amnis systems to analyze VPs. The VPs (MV-M-sfGFP) used in these studies were developed by ViroFlow Technologies, Inc.[2] MV-M-sfGFP are inactivated murine retroviruses produced in mouse cells that express superfolder green fluorescent protein (sfGFP) on the outer surface of the viral envelope and have a size of ~120 nm.

First, we analyzed the viral particles on the CellStream Flow Cytometer, which is a compact, camera-based benchtop system that uses TDI-CCD technology to deliver highly sensitive small particle resolution with low background noise. Then, we analyzed the viral particles on the ImageStream®^{X} Mk II Imaging Flow Cytometer, which obtains image data in addition to flow cytometric data. The new High Gain mode for ImageStream was used for these experiments in conjunction with a high-powered 400 mW 488 nm laser for increased detection of viral particle signal.

In conclusion, both the Amnis CellStream Flow Cytometer and the Amnis ImageStream^{X} Mk II Imaging Flow Cytometer are excellent choices for flow virometry.

Materials and Methods

Acquisition and Analysis of VPs on CellStream
The lyophilized VPs were resuspended in 200 µL of 0.1 µm filtered dH_2O. The resuspended VPs were then diluted in PBS at 1:400, 1:800, 1:1,600, and 1:3,200. Samples were run in triplicate for 3 minutes on the Amnis CellStream System in Small Particle Detection mode. Detection was triggered off of all channels with the 488 nm laser power set to 100%. Results were analyzed using CellStream analysis software.

Acquisition and Analysis of VPs on ImageStream^{X} Mk II
The MV-M-sfGFP particles were reconstituted in 0.1 µm filtered dH_2O and diluted 1:400 in PBS. Samples were acquired for 3 minutes at 4 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. Results were analyzed using IDEAS® 6.3 image analysis software.

Results

CellStream®

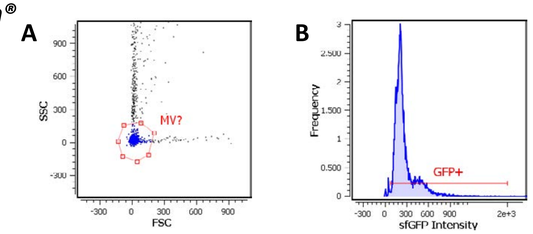
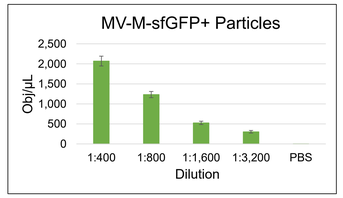


Figure 1: Gating strategy for identifying MV-M-sfGFP on the CellStream®. **A)** Dot plot of FSC vs. SSC shows the potential MV population. **B)** Histogram of GFP intensity.

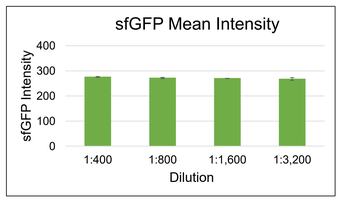
A



B

MV-sfGFP+ Obj/µL			
Dilution	Run 1	Run 2	Run 3
1:400	2,210	2,010	1,990
1:800	1,321	1,200	1,178
1:1,600	574	504	502
1:3,200	341	296	275
PBS	0.7	0.5	1.4

C



D

sfGFP Mean Intensity			
Dilution	Run 1	Run 2	Run 3
1:400	273	277	277
1:800	270	271	274
1:1,600	270	270	271
1:3,200	262	269	273

Figure 2: MV-M-sfGFP concentration and mean fluorescent intensity of dilution controls on the CellStream®. **A)** Linear decrease of VP concentration with sample dilution. **B)** Concentration measurements were obtained with high precision. **C)** Mean fluorescent intensity is consistent at each dilution. **D)** Dilution controls indicate single objects (no "swarming").

ImageStream®

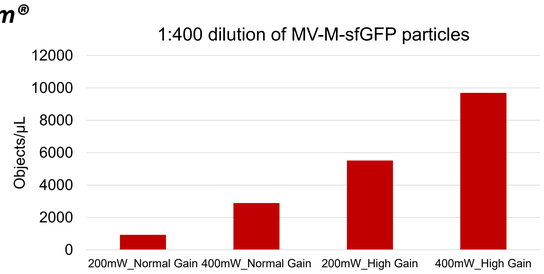


Figure 3: There is an increase in GFP+ objects detected with increased laser power, as well as increased camera gain when analyzing viral particles on the Amnis® ImageStream®^{X} Mk II.

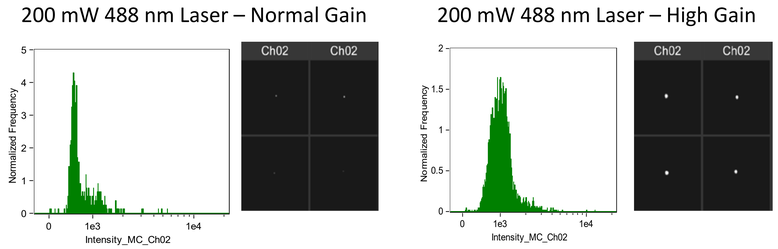


Figure 4: Histograms and images of VPs analyzed with either the 200 mW or 400 mW 488 nm laser and High Gain or Normal Gain mode. Channel 2 (Ch02) is the detection channel on the camera for the 488 nm laser. There is an increase in GFP+ objects detected with increased laser power, as well as increased camera gain when analyzing viral particles on the Amnis® ImageStream®^{X} Mk II. Note: the same display settings were used for all VP images.

Conclusions

- Viruses and viral particles, such as the MV-M-sfGFPs used in this study, are easily detected and analyzed using Amnis flow cytometers with TDI-CCD camera technology.
- The CellStream Flow Cytometer is a compact, benchtop system that is easy to use, has high sensitivity, and offers precise and accurate detection of viral particles.
- The CellStream study demonstrated that the dilution control concentrations show a linear decrease with a stable mean intensity, validating the detection of single VPs.
- The ImageStream^{X} Mk II Imaging Flow Cytometer offers high-end detection of viral particles, which includes acquisition of image data.
- The new High Gain mode for the ImageStream, in parallel with high-powered lasers, enables increased sensitivity for the detection of small particles.

References and Acknowledgements

[1]Lippé, Roger. 2018. Flow Virometry: A Powerful Tool To Functionally Characterize Viruses. J Virol. 2018 Feb 1; 92(3): e01765-17.

[2]The MV-M-sfGFP particles were obtained from ViroFlow Technologies, Inc.