Laser Force Cytology™ Monitoring of Influenza Virus Infection in Vero Cells

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com

LumaCyte®

Laser Force Cytology™ Monitoring of Influenza Virus Infection in Vero Cells

Introduction

The quantification of viral infectivity, that is, the number of infectious viral particles per unit volume, is an essential step at various points in vaccine development, the production of virally driven recombinant protein production, and studies of the mechanism of viral infection. For each of these processes, the rapid and accurate determination of viral infectivity can yield important information about virus-cell interactions, improve yields, reduce cost, and minimize downtime.

LumaCyte's Laser Force Cytology™ (LFC) Radiance® instrument uses a combination of optical pressure and microfluidics to interrogate the biophysical and biochemical properties of individual cells. Using this data to quickly and cost effectively detect viral infection is incredibly valuable for bioprocess optimization and scale-up as well as reducing the time to results for release and other analytical assays that measure infectivity. Changes in cellular morphology, biochemistry (compositional changes in the cytoplasm, for example), or development of viral inclusion bodies in infected cells, give rise to measurable differences in cells. This capability gives LFC a distinct advantage over other detection technologies for process monitoring.

Experimental Results

In order to monitor the biophysical changes in Vero cells upon influenza infection, cells were analyzed 1 day post infection at a multiplicity of infection (MOI) of 0.02 using LumaCyte's Radiance® instrument. As each cell is exposed to the laser, its velocity in the fluid flow is decreased in proportion to its optical force or pressure. Thus, cells with a higher force will travel at a lower velocity and vice versa. The velocity histogram for both uninfected and infected cell samples is presented in **Figure 1**. Uninfected cells have a higher average velocity than the infected cells (1135 ± 28 µm/s vs. 1086 ± 93 µm/s), indicating an increase in optical force upon influenza infection. In addition to the shift to a lower velocity, the infected cell sample shows a notable broadening of the velocity

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com

distribution and a significant number of cells with lower velocity (<1,000), some as much as 15 standard deviations lower than the average velocity of the uninfected control population. Multiple images are taken of each cell, which can be used as an additional source of information about each cell as well as the overall population, though no manual image analysis is required.

Several representative cells are shown in **Figure 1**, illustrating some of the morphological changes that occur as a result of infection. Using multivariate image analysis and machine learning, LumaCyte's Illuminate™ software suite can analyze these images to look for additional metrics of interest.

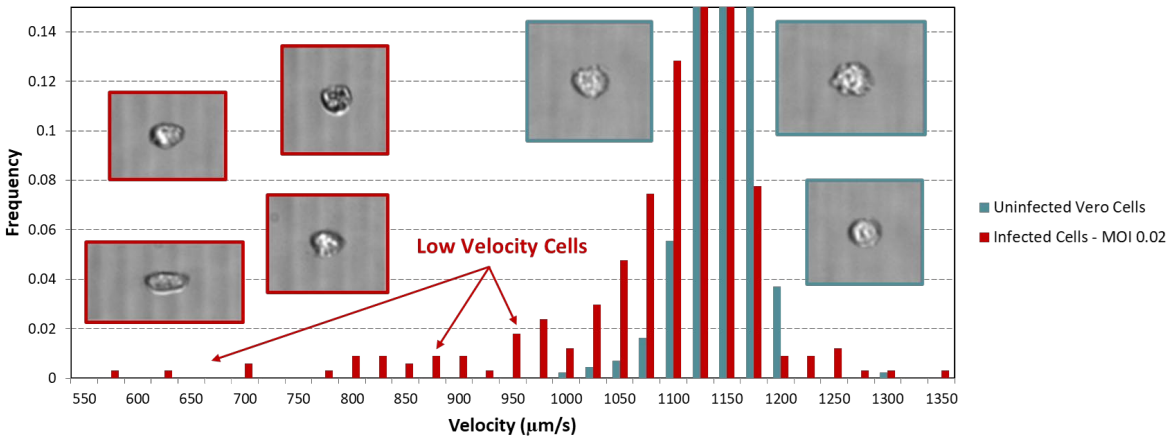

Figure 1: Velocity (laser force) comparison between uninfected and influenza infected Vero cells. Histogram comparing the velocity distribution of uninfected and infected populations of Vero cells, 1 day post infection. Velocity is inversely related to laser force, with a lower velocity representing a higher laser force, and vice versa. Inset images show representative cells from the uninfected (**green** border) and infected (**red** border) populations.

In addition to velocity, many other parameters are calculated for each cell, including size, eccentricity, and deformability. **Figure 2** shows a scatter plot of eccentricity (a measure of each cell's circularity) versus velocity. Notably, there is a group of cells within the infected population at a low velocity and high eccentricity, indicating that a change in shape occurs for many cells that have been infected with influenza.

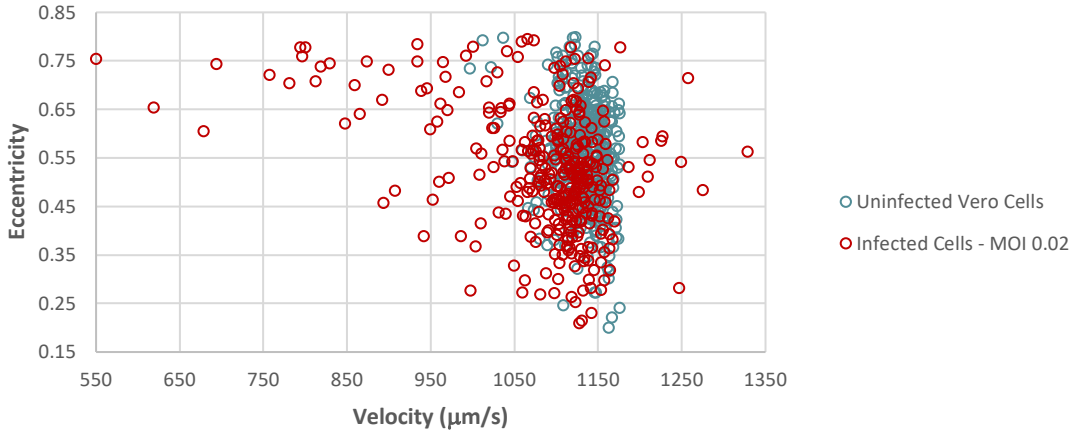

Figure 2: Eccentricity versus Velocity. Scatter plot comparing the eccentricity and velocity of uninfected Vero cells to influenza infected cells 1 day post infection. Eccentricity is a measure of the cell's circularity, with 0 being a circle and 1 being a straight line.

Changes were also seen in the size and velocity of infected cells. This is illustrated in **Figure 3**, a scatter plot of size versus velocity for cells infected at an MOI of 0.02 and analyzed 2 days post infection.

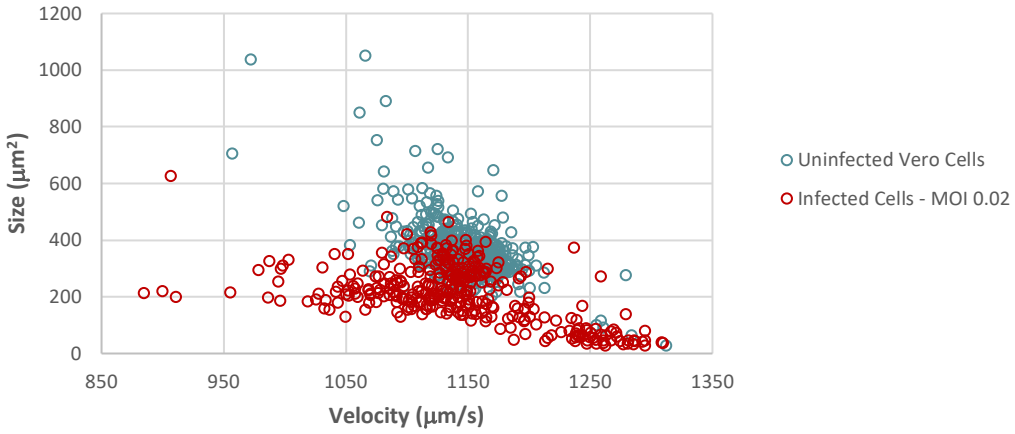

Figure 3: Size versus Velocity. Scatter plot comparing the size and velocity of uninfected Vero cells to influenza infected cells 2 days post infection.

Specifically, infected cells have a larger distribution of velocities and lower size when compared to uninfected cells. There are populations of cells with low velocity (high optical force) and low size, as well as high velocity (low optical force) and low size. These populations likely represent cells at different stages of infection that could be used to help quantify the infectivity of the sample or other aspects of the cell culture.

In conclusion, changes were seen in both the velocity (optical force) and eccentricity (shape) of Vero cells infected with influenza. The magnitude of the shift in velocity is related to the viral load, as cells infected at a lower MOI or at the same MOI for a shorter duration had a reduced shift in velocity and fewer low velocity cells (data not shown).

Importantly, the number of low velocity cells relative to the control population has been shown to correlate directly with the viral titer[1]. This indicates the utility for using LFC as a rapid, quantitative method to assess viral infectivity, for use in real-time bioreactor monitoring and other applications including neutralization assays[2].

Cell Culture, Infection, and Harvest

Vero cells (ATCC# CCL-81) were maintained in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum and grown at 37° C in a humidified incubator supplemented with 5% CO2. Influenza viral stocks (PR8) were titered prior to experimentation and stored in the vapor phase of liquid nitrogen. Cells were seeded 1 day prior to infection and analyzed 1-2 days post infection using Radiance®, LumaCyte's LFC instrument. Minimal sample processing was required: cells were detached using a cell dissociation buffer, diluted to a concentration of approximately 500,000 cells/mL in LumaCyte Sample Fluid, and analyzed with a Radiance® instrument.

References

1. Hebert, C.G., et al., *Rapid quantification of vesicular stomatitis virus in Vero cells using Laser Force Cytology*. Vaccine, 2018. **36**(41): p. 6061-6069.
2. *Hebert, C.G., et al., Viral Infectivity Quantification and Neutralization Assays Using Laser Force Cytology, in Vaccine Delivery Technology: Methods and Protocols,* B.A. Pfeifer and A. Hill, Editors. 2021, Springer US: New York, NY. p. 575-585.

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com