High-throughput foci counting of viral titer and antibody neutralization assays using the Celigo Image Cytometer for developing novel cross-reactive influenza vaccine

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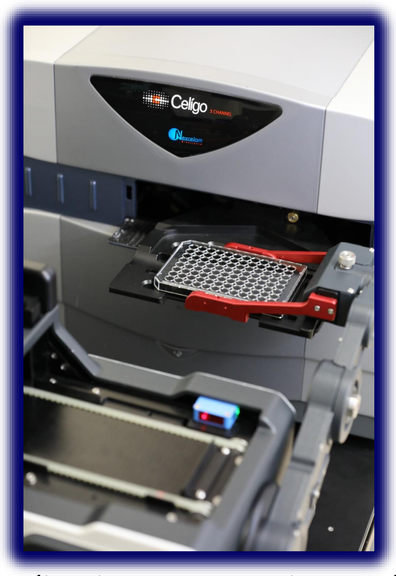
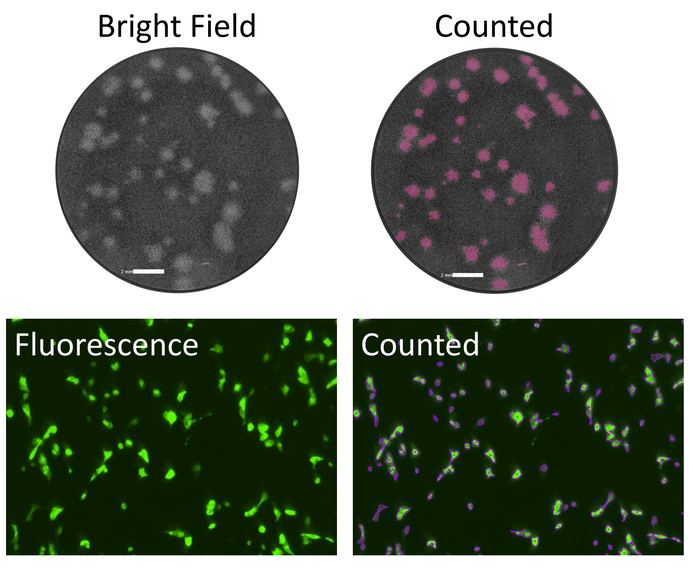
1. ABSTRACT

The study of viruses has expanded from disease research, to vaccines, antivirals, and cancer treatment. In viral vaccine research, a novel live attenuated influenza vaccine has been shown to induce serum and mucosal antibodies to cross-neutralize unmatched influenza virus strains in clinical trials. For vaccine development, viral titer and antibody neutralization assays are typically performed by manually counting viral plaques on a monolayer of host cells counter-stained with crystal violet or H&E staining. Manual counting is time-consuming and prone to operator variation. In order to increase throughput, accuracy and sensitivity, host cells can be infected on a 96-well plate and viral particles can be tagged with fluorescent proteins or antibodies for fluorescent foci analysis. However, the counting of fluorescent foci using a fluorescent microscope can be inaccurate due the limitation of analyzing a small area within a well. Therefore, high-throughput and whole well-based viral titration and antibody neutralization assays are needed for the live attenuated influenza vaccine production.

In this work, we demonstrated a high-throughput foci counting method using the Celigo Image Cytometer. First, a titration of viral particles from 1:5 to 1:512 was used to infect Vero cells in 96-well plates. Next, the cells were fixed and stained with primary antibodies against influenza A NP and secondary antibodies tagged with Dylight-488. Celigo was used to acquire bright-field and fluorescent whole well images, and directly count the foci in whole wells. In addition, antibody neutralization assays were conducted by pre-incubating a fixed amount of virus with a titrated serum sample for 1 hour. Next, cells were infected for 24h prior to the fluorescent staining and analysis. Titer calculations were determined using foci numbers from Celigo and fluorescent microscopy, and were comparable. By using the high-throughput image cytometry method, viral titer, antibody neutralization and TCID50 assays can be efficiently performed miniaturized and at earlier time points with greater accuracy and reproducibility by nature of being able to image the entire well, greatly facilitating the development of novel vaccines and antivirals.

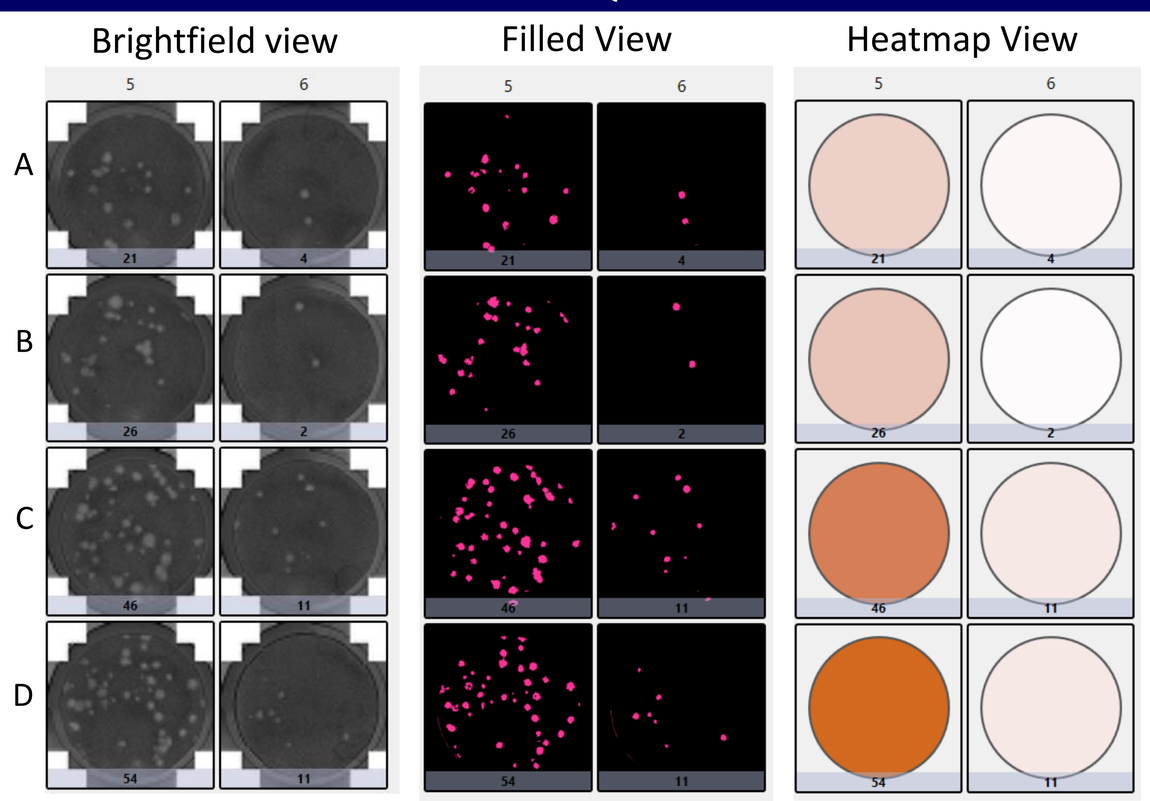
2. CELIGO IMAGING CYTOMETRY FOR VIROLOGY ASSAYS





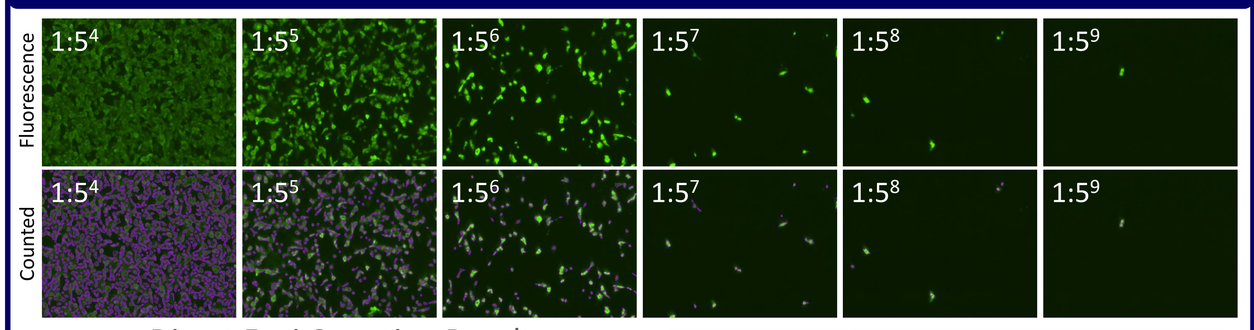
Celigo is an automation-ready, plate-based image cytometer capable of scanning entire wells in bright-field and 4 fluorescence channels in high-throughput. Images are automatically analyzed by Celigo Virology Module to quantify count, size, morphology, and fluorescent intensity. Reports are generated for cell proliferation, fluorescence expression, cytotoxicity, foci counting for viral titer and antibody neutralization assays.

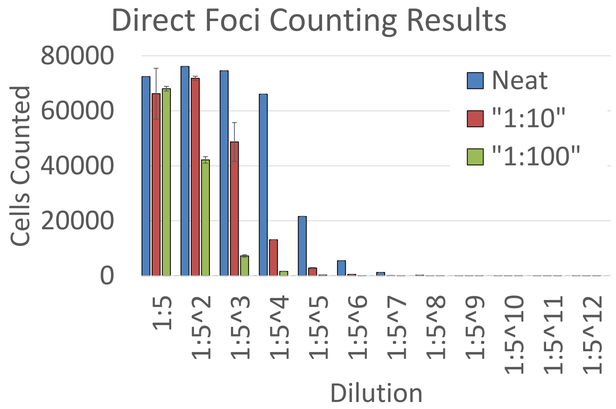
3. DIRECTLY COUNTING CYTOLYTIC PLAQUES - IMAGE CYTOMETRY



Celigo was used to image the whole well in bright-field at 1µm resolution. Celigo software de-clustered, identified (filled view) and reported (heatmap view) cytolytic plaques in bright-field. The number, morphology, and size of the plaques was automatically quantified.

4. DETERMINING VIRAL TITER - FLUORESCENT FOCI COUNTS

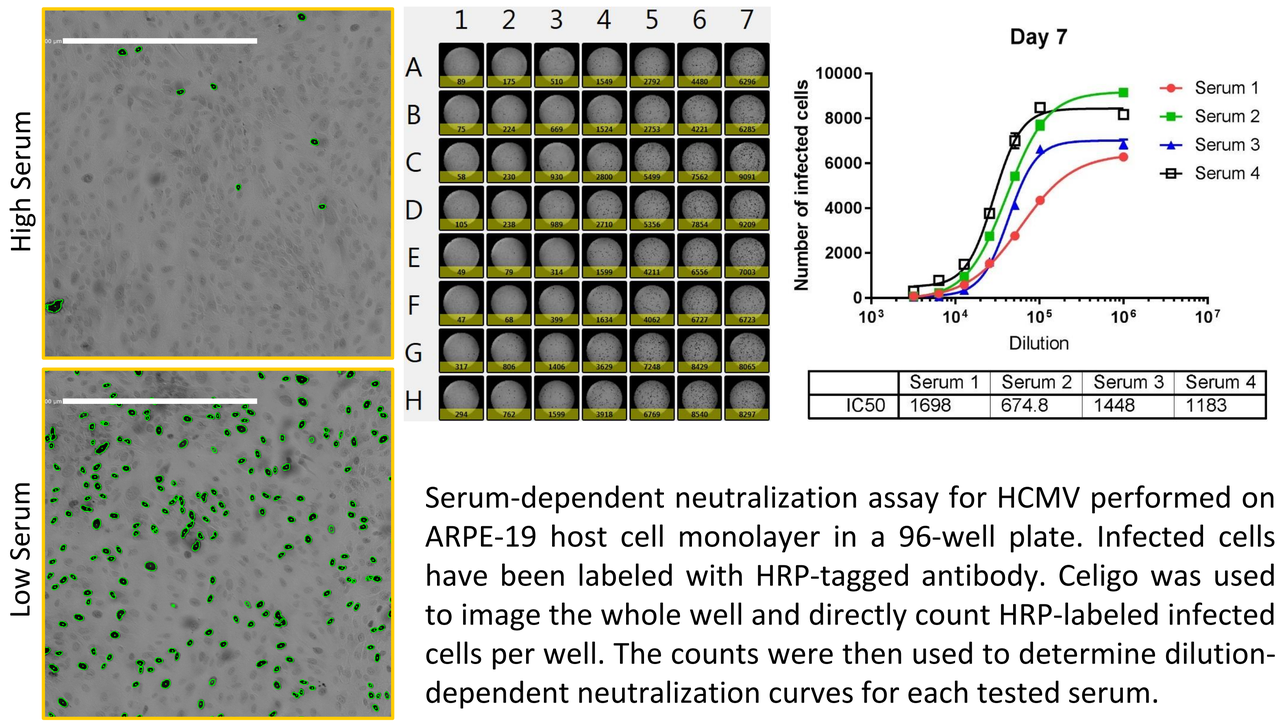




Comparison	Log10 (FFU/mL)	
	Celigo	Manual
Neat	8.97	9.03
1:10	8.05	8.03
1:100	6.97	6.99

Celigo was used to count Dylight positive foci at different viral titer concentrations. The entire plate was whole-well scanned and analyzed in less than 10 minutes.

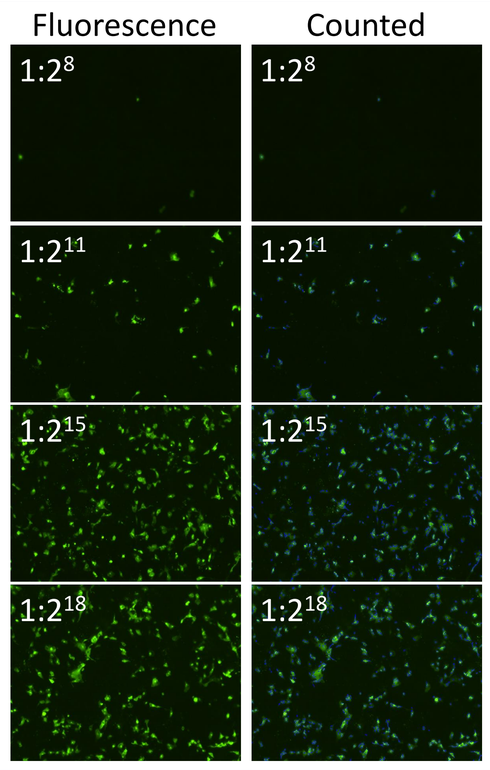
5. SERUM-DEPENDENT NEUTRALIZATION ASSAY - BRIGHT FIELD COUNTS

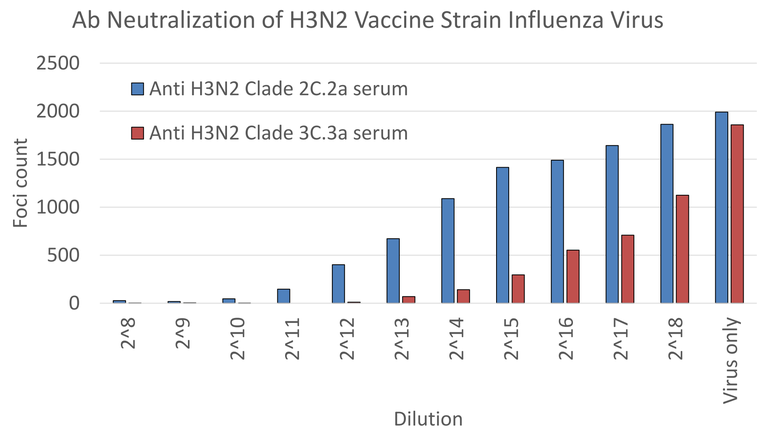


	Serum 1	Serum 2	Serum 3	Serum 4
IC50	1698	674.8	1448	1183

Serum-dependent neutralization assay for HCMV performed on ARPE-19 host cell monolayer in a 96-well plate. Infected cells have been labeled with HRP-tagged antibody. Celigo was used to image the whole well and directly count HRP-labeled infected cells per well. The counts were then used to determine dilution-dependent neutralization curves for each tested serum.

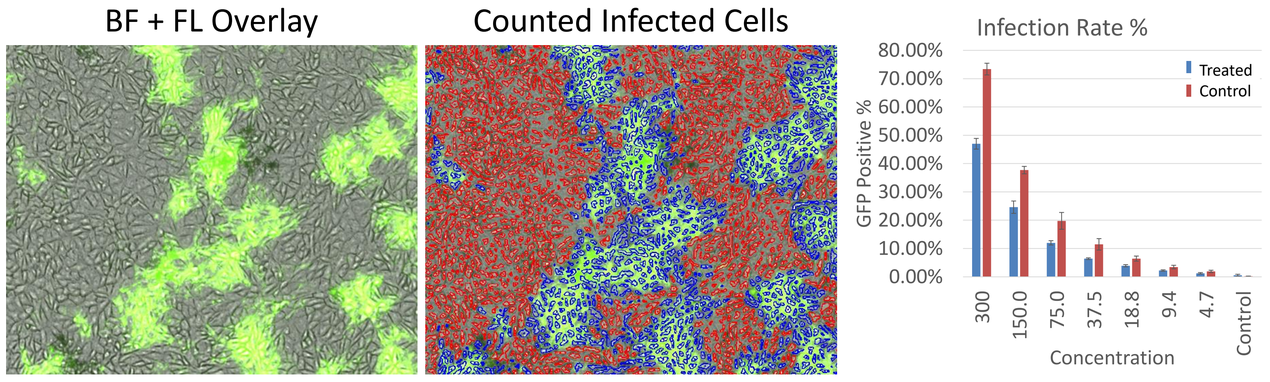
6. ANTIBODY NEUTRALIZATION ASSAY – FLUORESCENT CELL COUNTS





Celigo was used to analyze antibody neutralization of Anti H3N2 Clade 3C.2a serum and Anti H3N2 Clade 3C.3a serum. The sera were used to neutralize 14 different viruses at different titers. Celigo counted Foci in each well, imaging and analysis took ~10 minutes per plate. The results showed that the 2 sera displayed different levels of neutralizations for the 14 different viruses.

7. TCID50 ASSAY – BRIGHTFIELD AND GFP POSITIVE CELL COUNTS



Cells are labeled with a GFP reporter carrying virus and imaged on Celigo (left side). Total number of cells (bright-field) and infected cells (green) were detected. Data was gated to determine the percentages of GFP positive (blue) vs negative (red) cells, indicating the infection rate under treatment / control. Data can be used for quick TCID50 assessment.

8. SUMMARY AND CONCLUSION

- Celigo performs high-throughput imaging of the whole well and bright-field / fluorescent foci counting in less than 10 min per 96-well plate and is compatible with automation robotics.
- Assays classically performed in 6-well format and manually counted can be miniaturized to save material (96- or 384-well) and automatically evaluated after shorter incubation period.
- The proposed method can vastly improve the accuracy and efficiency of performing standard virology assays for vaccine development or production while reducing material cost.