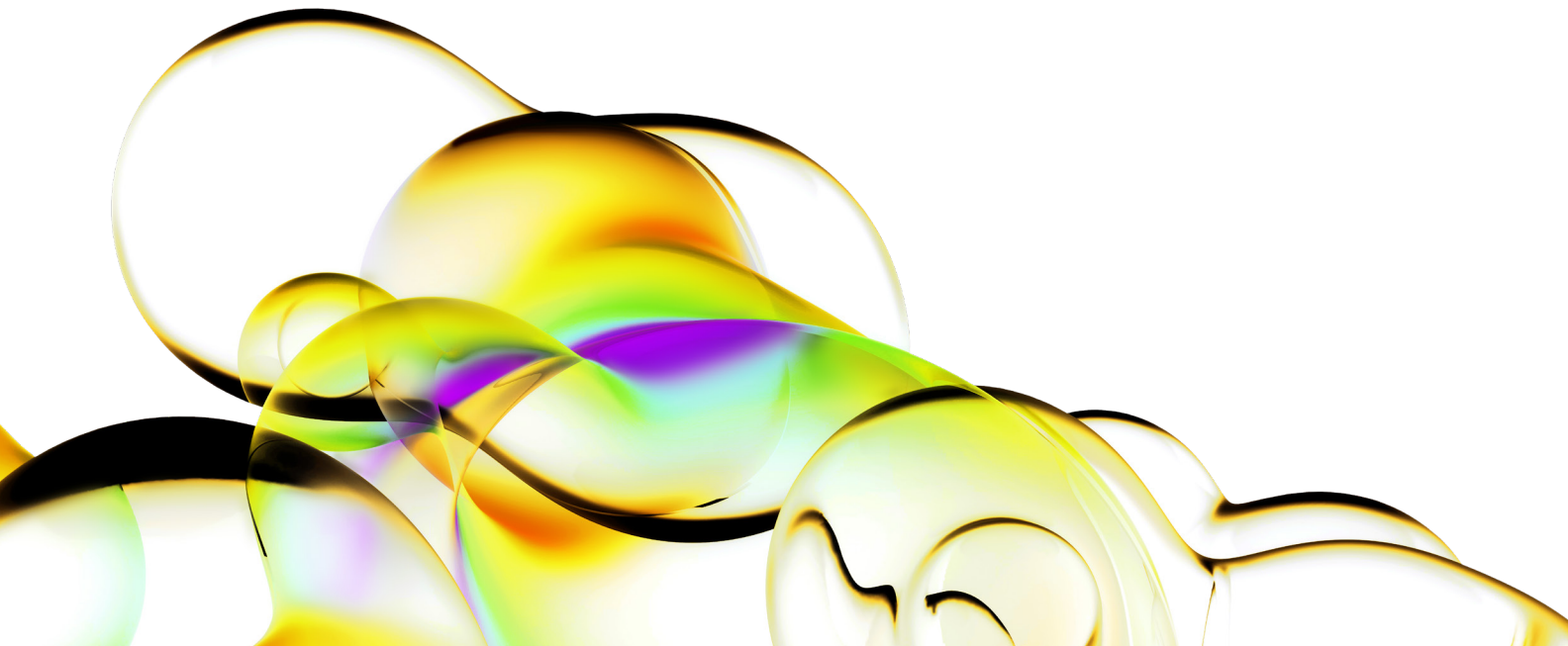


# A modern approach to traditional virology research.

The drastic rise in global Coronavirus cases highlights the urgency to uncover more about the pathogenesis of respiratory viruses and how they interact with host cells. More research and faster exploration of the mechanisms of interaction between the virus and the host cells will undoubtedly aid in the discovery of revolutionary infection control strategies and drug candidates. Traditional research methods can be limited by issues with accuracy, sensitivity, and most often throughput. The Revvity team has been actively supporting scientists by developing novel methods and assays for vaccine development, pathogenesis investigation, and discovering new or repurposed antiviral therapies.

## Traditional approaches for conducting virology assays

The traditional plaque assay, focus formation assays, cytopathic effect, fluorescent infectivity assays, and antibody neutralization assays are all common experiments conducted in virology laboratories. But the traditional ways of analyzing these assays can be problematic. Imaging by manual microscopy is extremely slow with an unavoidable operator to operator variation. Flow cytometry requires trypsinization and typically takes between one and two hours to run a 96-well plate. This method also requires extra maintenance and a larger sample volume for testing. Plate readers provide fast results but do not provide sensitive cell level analysis. Many labs are now using image cytometry as a modern alternative. It offers rapid results with increased accuracy and fit-for-purpose sensitivity to improve the efficiency of the anti-viral and vaccine candidate screening processes.



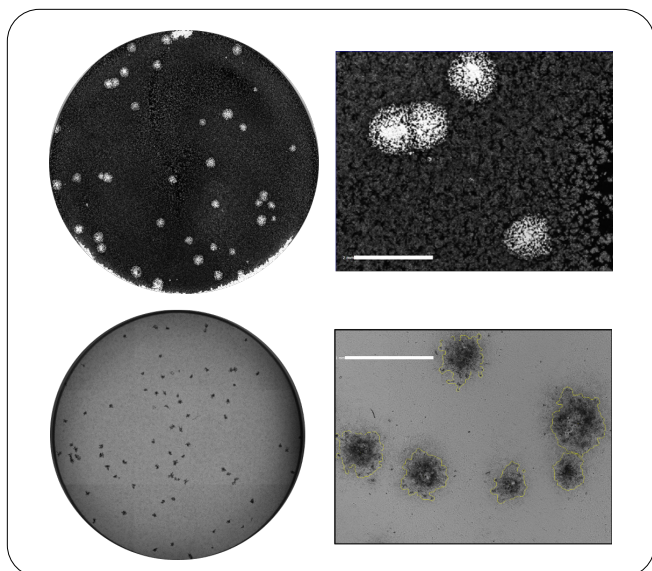


Figure 1: Lytic plaque assay (top) and focus formation assay (bottom)

## Image cytometry offers a novel approach for virology research

The Celigo™ image cytometer is a multichannel, fluorescent, and brightfield system that rapidly captures autofocused, whole-well images from any SBS format plate and automatically analyzes them to produce quantitative data. The high-throughput benchtop image cytometer enables simultaneous imaging and robust analysis of both adherent and suspension cells to sort various cell types, within the same well, by differences in size, shape, or fluorescent marker. It can also easily identify and analyze colonies, plaques, and foci.

Table 1: Times shown are for whole-well imaging of the entire plate

Plate type	Images/well	Typical time (min)
384-well	4	< 5 min
96-well	16	< 3.5 min
6-well	276	~ 6 min

## The Celigo applications for virology advancement

Many leading labs in the field of virology have used a Celigo plate-based image system to directly measure multiplex cell-based assays in real-time. The Celigo offers scientists the ability to perform many tasks with ease:

- Measure viral infectivity or titer by plaque assay, cytopathic effect assay, or infectivity/transduction assays.
- Determine TCID<sub>50</sub>, cytotoxicity, and cell-based antibody binding inhibition.
- Perform neutralization assays to detect viral infection and host cell protection at the single-cell level

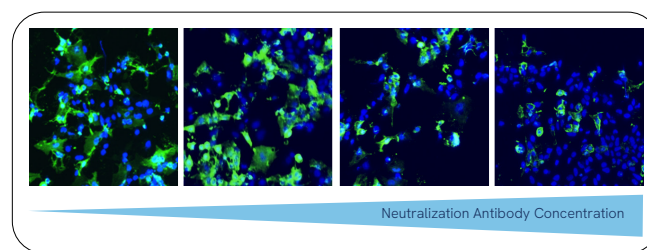


Figure 2: Infectivity assay

- Monitor host cell responses to vaccines and antivirals with morphological quantification
- Create customizable projects utilizing up to 4 fluorescent channels per assay
- Use less virus, host cells, antibody serum, and reagents for testing
- Analyze settings and produce results directly from experimental plates of any size
- Obtain data up to 20 times faster than traditional methods
- Seamless workflow integration including security and logging add-ons

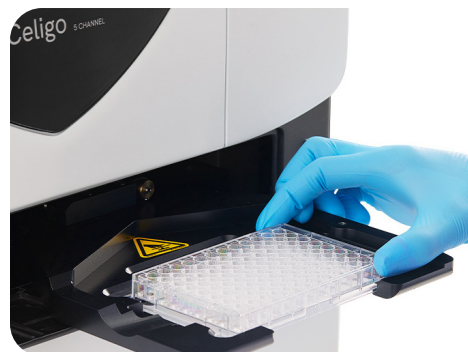


Figure 3: Celigo plate-based imaging system

## Measuring cytopathic effect

Cytopathic effects are morphological changes in size, shape, and structure presenting after virus incubation (Figure 4). The Celigo allows for determination of monolayer depletion while providing critical information on cell death, cell morphology, and syncytia.

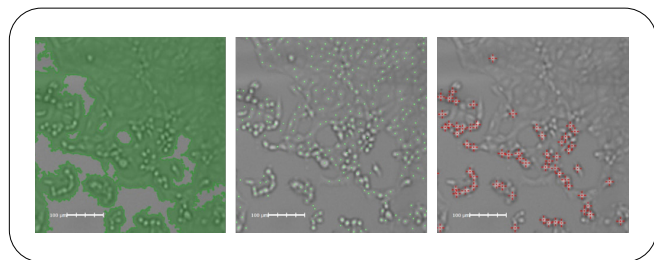


Figure 4: Cytopathic effect being measured by: host cell monolayer depletion (left), number of cell remaining (middle), and cell morphology (right)

## Using a multi-channel approach to gain more information from a single scan

The opportunity to scan a full well in five distinct channels enables the qualification of more data from a single assay. This example shows a multiplex fluorescent plaque assay utilizing all four fluorescent channels. Three antibodies which all bind to different regions of the virus were used and fluoresce in the green, red, and far-red spectra. This process quantifies the number and size of the plaques, as well as determines if mutations occur as the virus spreads. Three antibodies were tested in unison for binding to the virus and the presence of all three fluorescent antibodies throughout the plaques suggests the virus is not mutating. If one region of the virus is lost or mutated then only two of the fluorescent antibodies are present. DNA-binding DAPI staining solution was used to determine total host cell concentration (Figure 5).

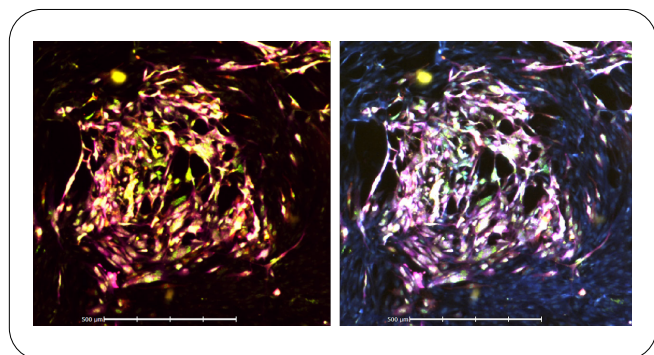


Figure 5: Fluorescent plaque analysis assay - 3 viral antibodies: merged image (left) and 3 viral antibodies + DAPI: merged image (right)

## Collect entire transwell images and measure virus infectivity

The Icahn School of Medicine at Mount Sinai in New York published a paper on innate immune responses as they relate to the respiratory pathogen Influenza A virus (IAV)<sup>1</sup>. The researchers performed experiments using the Celigo image cytometer to quantify viral titer and hemoglobin donation concerning IAV. Investigating the innate immune response to IAV at the single-cell level offers a better understanding of how respiratory epithelial cells respond to and counteract virus infection.

Primary normal human bronchial epithelial (NHBE) cells were cultured in transwell filters in the presence of the influenza virus and infectivity was measured by hemagglutinin (HA) staining. The Celigo collected entire transwell images and quantified the percentage of infected cells within the total population, counterstained by DAPI (Figure 6).

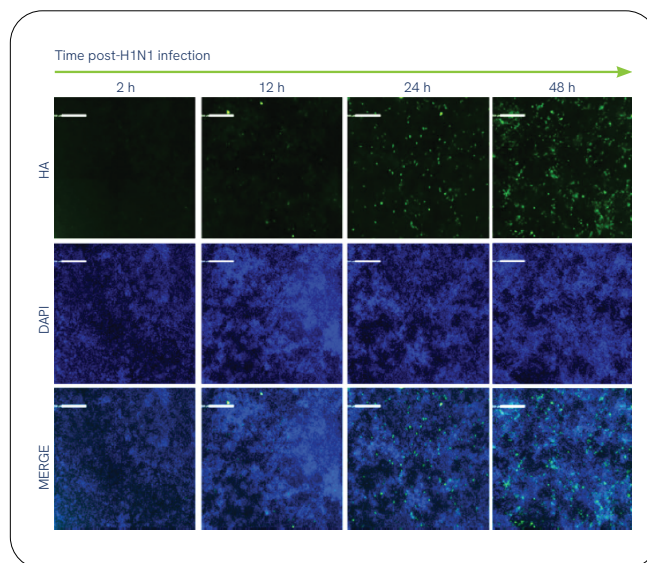


Figure 6:

Image cytometry combines the speed offered by a plate reader and the cell-level sensitivity of a flow cytometer to provide high-throughput imaging-based viral titration and neutralization assays. This increase in speed while maintaining reliability is critical to future developments against rapidly spreading and devastating viruses such as the infamous Coronavirus.

## References

1. Innate Immune Response to Influenza Virus at Single-Cell Resolution in Human Epithelial Cells Revealed Paracrine Induction of Interferon Lambda 1; Irene Ramos, et al.; Journal of Virology Sep 2019, 93 (20) e00559-19; DOI: 10.1128/JVI.00559-19



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