# AI-Powered Label-Free Imaging Assay for Rapid Viral Infectivity Detection

Rebecca Mongeon[1], Joe Clayton[1], Rebecca Winfree[2], Teresa Findley[2], Ilya Goldberg[2]

[1]Agilent Technologies, Inc., [2]ViQi Inc.

SBI2
POSTER # 26

## Introduction

Infectivity assays are essential for vaccine development and antiviral drug discovery. They are used to assess attenuation in live virus vaccines, titer inactivating antibodies produced by vaccine candidates, and monitor an immunized population for continued resistance to emerging virus variants. Traditional assays like plaque and $TCID_{50}$ rely on cell death as an endpoint, which requires several rounds of infection to occur. This results in long incubation periods that can take up to 15 days. Alternatives, like fluorescent focus assay (FFA), require antibodies and extensive sample preparation or GFP-labeled viruses. Further, automated image analysis tools for interpreting FFA require manual parameter selection, which can make the assay subjective. Returning rapid and unbiased results, automation of these assays holds the key to rapid development of new vaccines and antiviral reagents.

In response, ViQi, Inc. has developed AVIA™ (Automated Viral Infectivity Assay). This assay uses machine learning and brightfield microscopy to detect signs of viral infection. It does so by identifying subtle phenotypic changes within cells that are associated with viral replication. These can be detected by the AI long before they can be seen by manual inspection. Infection phenotypes can be identified within a few hours of exposure to the virus and can be detected in live cells without any sample preparation or fluorescence imaging. The output of this assay is an infectivity measurement similar to a multiplicity of infection (MOI). The assay does not require any parameter tuning by the user, ensuring objectivity and ease of use.

AVIA is deployed on ViQi, a cloud-based analysis platform with integrated workflow management, input and output traceability, and a suite of data visualization tools. Together, this system provides researchers with a scalable and reproducible analytic tool for measuring infectivity in automated screens.

## Experimental

### Instrumentation and infection model

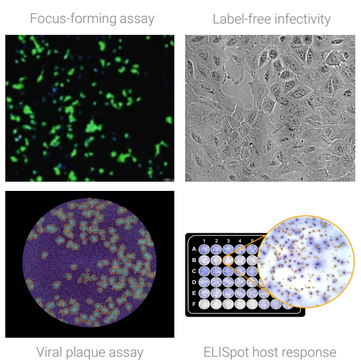
Agilent BioTek cell imaging multimode readers combine automated microscopy and traditional microplate detection technology in a configurable and upgradable platform. Live-cell imaging is supported across multiple label-free and fluorescence imaging modes, with built-in environmental controls and gas controller options. Here, the Cytation 7 cell imaging multimode reader provided automated, high- quality, and rapid brightfield imaging in multiwell microplates formats required for high-throughput, AI-based viral infectivity analysis. Images were acquired using a 20x objective and a 5 x 5 grid of nonoverlapping fields of view in each well. The integrated laser autofocus was used to maintain target focus settings. In this example study, Vero cells were plated in 96-well microplates for image-based analysis of infectivity with human rhinovirus (HRV16). Vero cell cultures were incubated for 16 hours at various MOI dilutions and then imaged using the Cytation 7. AVIA has demonstrated effective quantification of infectivity of over 15 viral strains including DNA, RNA, enveloped and nonenveloped viruses. Importantly, AVIA has successfully identified infection for viruses that do not reliably result in quantifiable cytopathic effects, including HIV and adenovirus.

### AVIA™ for AI-based viral infectivity quantification

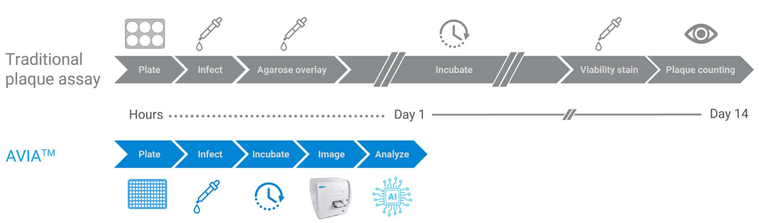
A machine learning model is trained for each virus, cell line, and imaging instrument within a laboratory. An initial assessment is done on a single 96-well plate which includes wells with a saturated synchronized infection at high MOI, uninfected wells, and wells containing virus dilutions. This initial model is tested for reproducibility across cell passage number, viral stock, and other day to day variation using duplicate plates. These are added to the model's training set to ensure reproducibility. Once established, this AI can then process assay plates containing various experimental conditions, such as cells exposed to attenuated virus or cells exposed to live virus and inactivating antibodies from vaccine candidates or patient serum. The assay reports a quantitative result for each well as an infection rate within the linear range of the assay. Initial training reports are typically returned within a day, and assay reports are emailed back in under an hour. Thus far, our machine learning models have been successfully trained on ten viruses including DNA, RNA, enveloped, and non-enveloped virus types. This includes viruses that do not reliably have manually observable cytopathic effects, such as human immunodeficiency virus (HIV).

### Flexible, high-throughput microscopy with the Agilent BioTek Cytation 7 automated imagers supports a range of virology applications

Agilent BioTek Cytation 7 cell imaging multimode reader.




### One day viral infectivity assay–AVIA™




## Experimental

### AVIA™ assay development pipeline

**Stage 1: Training**

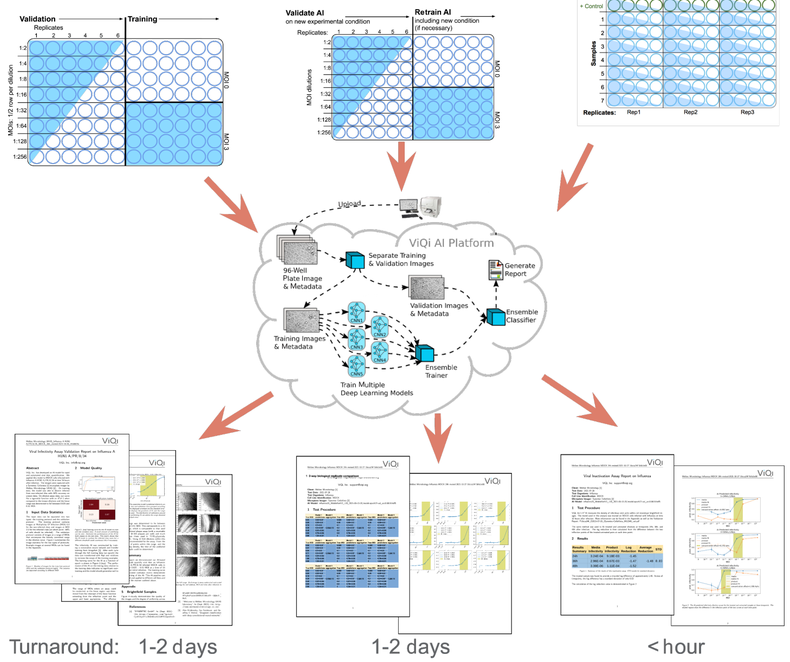
- Train on a single plate with MOI dilutions and time points
- Determine optimal assay time point, linear range of the assay

**Stage 2: Reproducibility**

- Cross validate models trained on plates with different viral stocks, strains, cell passages
- Compare against $TCID_{50}$ or plaque assay.
- Retrain AI to add new experimental conditions
- One plate per round of validation & retraining

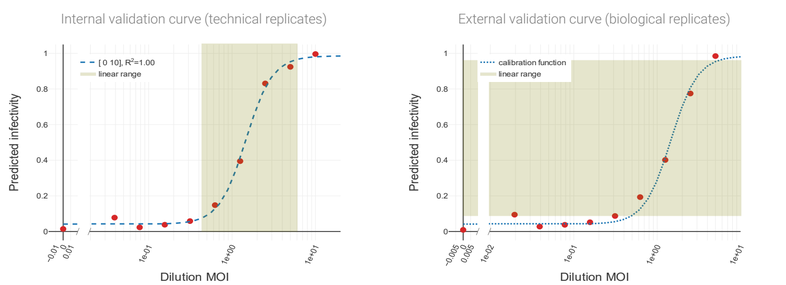
**Stage 3: Testing unknowns**

- User-trained AI from stages 1 and 2
- Single time point, 8·16 samples/plate




## Results

### AVIA™ achieves consistent precision across biological and technical replicates



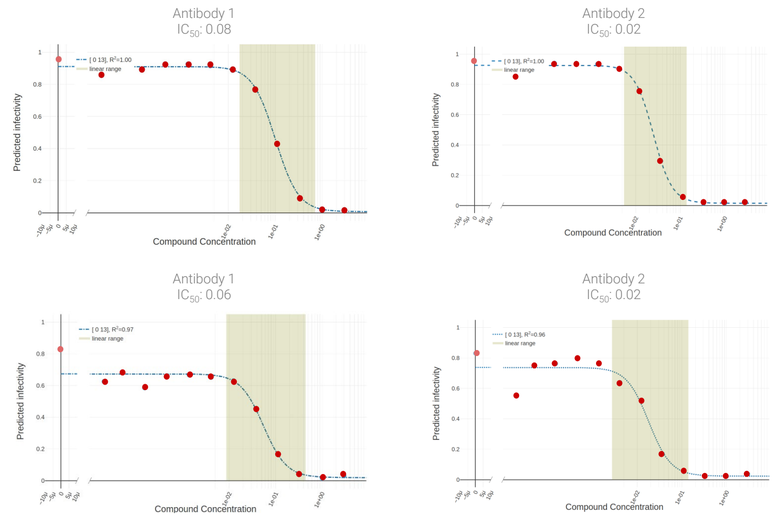

**Internal and external validation and calibration.** Predicted infectivity values across MOI dilutions for human rhinovirus (HRV16) in Vero cells at a 16-hour time point and imaged on the Agilent BioTek Cytation 7 cell imaging multimode reader. Results show good agreement with experimentally determined MOIs (< 5% difference). The chart on the left is an internal validation and calibration where predictions are made on wells from the same plate as used in training. The curve (blue) is a four-parameter logistic regression (4PL) between AI predictions (y axis) and experimentally-determined MOIs (x axis). The chart on the right is the AI that was trained and characterized with the plate on the left predicting an infection on a new plate. The blue curve on the right is transferred from the chart on the left. The titer predictions are made using all the points in the assay range (green shading) corrected from the known dilution (mean in Table 1).

## Results

**Table 1.** Summary of titer predictions are made using all the points in the assay range corrected from the known dilution (mean in table), or the single point closest to the maximum slope of the calibration curve (Max Slope), or the Max Slope including neighboring points that are within 20% of the Max Slope prediction (Max Slope+).

| Aggregated MOI Predictions (Biological Replicates) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lab MOI | Dilution Factor | Predicted Infectivity (0.09-0.96) | Predicted Dilution MOI (0.50-5.47) | Undiluted MOI (target = 5.0) | | | |
| | | | | Predicted | Mean | Max Slope | Max Slope+ |
| 0.62 | 1:8 | 0.19 | 0.8 | 6.38 | 5.30 ∓0.77 | 4.90 | 4.76∓0.10 |
| 1.25 | 1:4 | 0.4 | 1.23 | 4.9* | | | |
| 2.5 | 1:2 | 0.77 | 2.31 | 4.62+ | | | |

### Rhinovirus inactivation results demonstrate multi-application usage of AVIA™




**Antibody inhibition results**. Dose-response curves for two antibodies (left and right) inhibiting an infection at MOI 2.0 (top) and MOI 1.0 (bottom) for human rhinovirus (HRV16) in Vero cells at a 16-hour time point, using the AI trained and characterized above. The charts show the relationship between AI-predicted infectivity values on the y axis relative to inhibitor concentrations on the x axis. The blue curve represents a fit to the four-parameter logistic regression (4PL), which determines the linear range (green shading) and the half maximal inhibitory concentration ($IC_{50}$). Antibody $IC_{50}$ values displayed in the graph are consistent across MOIs.

## Conclusions

- The Agilent BioTek Cytation 7 cell imaging multimode reader provides high-quality label-free images of live-cell cultures required for rapid AI-based analysis
- AVIA successfully detects infection by human rhinovirus (HRV16) in Vero cells as early as 16 hours
- Both antibodies tested demonstrate viral inhibition as measured by AVIA at 16 hours and infectivity predictions fall within < 5% of experimentally validated MOI concentrations
- These results demonstrate the Agilent BioTek Cytation and AVIA analysis platform accelerates results, reduces costs and increases precision for infectivity assays

## Acknowledgments

Special thanks to Tong-Ming Fu for advising.

 RA251014.522