Application Note

Infectious Diseases

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Quantification of Viral Infections Using AI/ML Analysis with Brightfield Imaging

A rapid viral infectivity assay using AVIA™ and the Agilent BioTek Cytation cell imaging multimode reader dramatically accelerates results, reduces costs, and increases precision

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Abstract

Measuring and quantifying viral infectivity is essential for the discovery of vaccines, the development of antiviral therapies, and the manufacturing of biologics. Current standard methods such as plaque and $TCID_{50}$ assays can take up to 14 days after multiple manual steps to confirm the presence and infection of a virus strain. However, using high-throughput, label-free brightfield microscopy and novel AI technology has been shown to reduce the effort, cost, and time required to detect viral infection while also improving reproducibility and precision. Agilent Technologies, Inc. and ViQi, Inc. describe an automated viral infectivity assay that leverages the Agilent BioTek Cytation cell imaging multimode imagers and ViQi's AI-based assay AVIA. This technology is based on sensitizing convolutional neural networks, or AIs, to specific phenotypes of infection. Therefore, this technology has potential as a rapid, broad-spectrum tool for virus characterization with demonstrated performance for viral infectivity analysis across more than 15 virus and cell line combinations. This powerful automated approach is leveraged in this application note to determine multiplicity of infection (MOI) predictions and antibody inhibition of human rhinovirus in Vero cell culture.

Introduction

Scientific discovery, research, development, and manufacturing of vaccines, antiviral therapies, and biologics depend on infectivity assays for detection and quantification of viral strains. Any improvements in the pace of measuring viral infectivity, its cost, or the reliability of the results can have profound consequences for the development of conventional, lipid-encapsulated, or viral-vectored vaccines; antiviral drugs and biocides; biological therapies that require clearance assays, and therapies that use viral vectors such as gene therapy and oncolytic therapies.

Infectivity assays are used to observe and quantify the impact of viral replication on cell culture. Standard assays such as plaque assays, $TCID_{50}$, and fluorescent focus assays (FFA) used in labs and manufacturing facilities today often require long incubation times, substantial manual intervention, cell staining or labeling, and subjective analysis. These approaches have slow turnaround times, result in higher costs and scalability challenges, and involve reproducibility challenges associated with complex manual techniques. Additionally, some techniques relying on engineered viruses or highly specific antibodies are virus specific, potentially limiting results to a single virus strain or introducing high changeover costs.

ViQi, Inc. developed an automated viral infectivity assay, AVIA, to address the common challenges with measuring and quantifying viruses in biological workflows where virus titers need to be understood or where a lab or facility needs to ensure virus infection is inhibited. AVIA uses ViQi's proprietary state-of-the-art machine learning algorithms to process brightfield images collected from cell cultures infected with virus. Brightfield imaging is an ideal method for live-cell imaging that avoids phototoxicity, minimizes both time and cost of sample preparation, and captures complex cell morphology information for AI-based analysis. Automating label-free brightfield imaging with a Cytation instrument provides a rapid and efficient solution for high-throughput image collection and downstream analysis of viral infectivity with the AVIA platform.

In this proof-of-principle application note, we detail how automated, label-free brightfield image collection on the Agilent BioTek Cytation 7 cell imaging multimode reader was paired with ViQi's AI-based assay AVIA to quantify rhinovirus infectivity through MOI determination and antibody inhibition assays.

The use of AVIA allowed rhinovirus infection detection six times faster than traditional assays and displayed a high degree of consistency across multiple biological and technical replicates. ViQi has demonstrated that the AVIA solution is flexible across a wide variety of viruses and cell types.

Experimental

Instrumentation

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.

Cell and virus models

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.[1]

AVIA for AI-based viral infectivity quantification

AVIA was built to develop AI models that rapidly detect subtle phenotypic changes in cells after viral infection. This relies on ViQi's proprietary state-of-the-art machine learning algorithms to process brightfield images. AVIA employs classification models (convolutional neural networks, a specific kind of AI) to extract image features at increasing levels of abstraction and combines them to make a binary prediction of infection (infected or uninfected). The infectivity value for each well is based on combining infection predictions from thousands of sub-image regions. Unlike an infected/uninfected score in a well of a $TCID_{50}$, this gives an infectivity estimate per well, which is a continuous value similar to a plaque count. Unlike plaque assays, AVIA does not depend on cell death, allowing for quantification of viruses that do not form plaques and providing an answer in a shorter time frame.

AI training and report generation with AVIA

AVIA provides custom AI technology tailored to both the assay requirements and laboratory microscope, cell type, and virus. Brightfield images are uploaded to ViQi, and a custom AI is trained to recognize viral infection in these training images based on control conditions and a series of known viral dilutions. The trained AI is then readily applied for quantitative viral analysis in test conditions, allowing ViQi to rapidly provide a report summarizing the assay outcome.

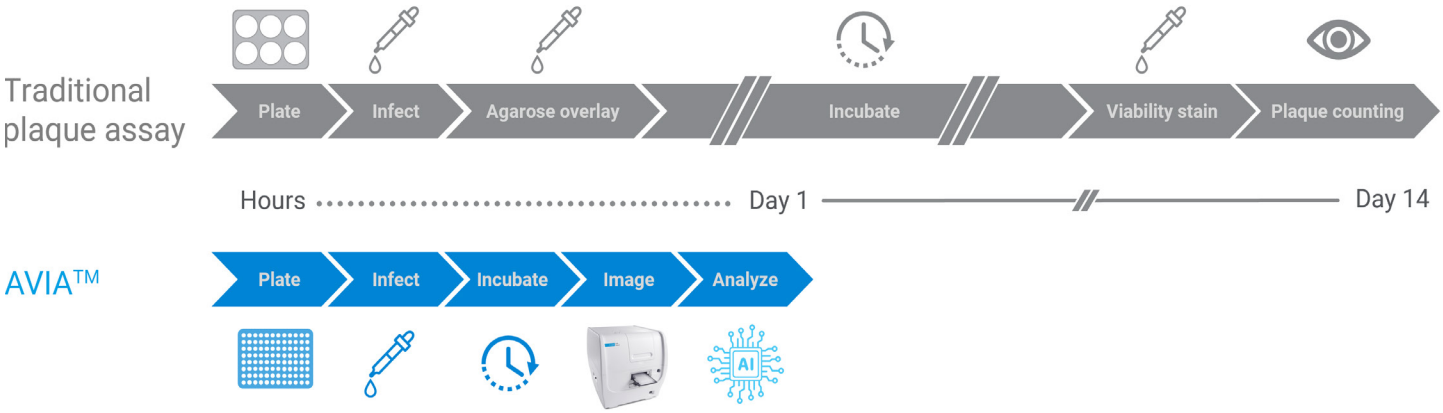

Figure 1. Workflow schematic for viral infectivity assays with automated brightfield imaging and AVIA AI-based image analysis. Traditional plaque assays, a gold standard technique for viral infectivity analysis, involves multiple sample handling and preparation steps, prolonged incubation periods, and expert-trained analysis. The approach presented here combines automated high-throughput brightfield imaging with ViQi's AI-based AVIA for comparable results in significantly less time compared to a standard plaque assay.

Results and discussion

Automated workflow for viral infectivity assays

Image-based analysis captures and analyzes cellular phenotypic changes in response to viral infection, enabling AI infectivity predictions (MOIs, IFU/mL) from brightfield images taken hours after viral introduction. The workflow diagram shown in Figure 1 presents the example experimental design and expected timing using the automated imaging and analysis approach. Cells are plated and infected with a virus: here, HRV16 infection of Vero cell culture was evaluated. Images were collected on the Cytation 7 in brightfield mode at 16 hours after infection. Initial viral infection can be detected through subtle changes in cell morphology captured through brightfield imaging, even at early time points. Brightfield image files and corresponding experimental metadata were uploaded to the ViQi platform for cloud-based analysis with AVIA.

MOI determination of rhinovirus infection

AVIA reports on the predicted infectivity values for cells exposed to virus, and the predicted infectivity is calibrated to MOI as shown in the left panel of Figure 2. Vero cells were incubated with a two-fold virus dilution series, starting with a known MOI determined using standard virus titration techniques, and uninfected control cells. Cultures were imaged at 16 hours after infection using standard brightfield mode on the Cytation 7. The left panel of Figure 2 displays the calibration curve that quantifies the relationship between the known MOI (X-axis) and the AVIA AI-based predicted infectivity score (Y-axis). Using this calibration curve, AVIA infectivity scores can accurately predict MOI for a series of unknown test MOI dilutions on a new plate of cells the AI has not been trained on, as shown in the right panel of Figure 2. AVIA demonstrates excellent agreement with experimentally determined MOIs across biological replicates, as summarized in Table 1.

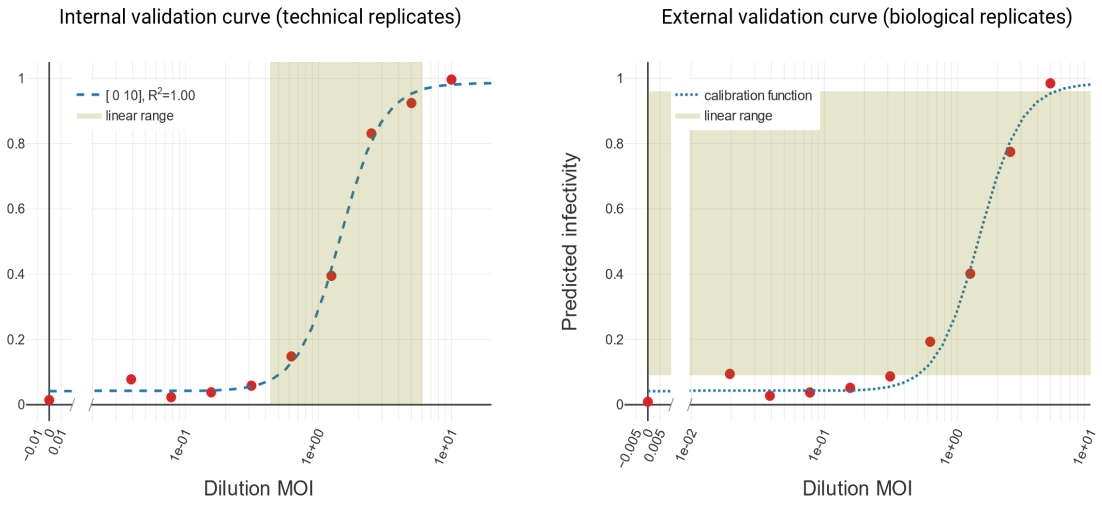

Figure 2. Internal and external validation and calibration. The chart on the left is an internal validation and calibration. The curve (blue) is a four-parameter logistic regression (4PL) between AI predictions of infectivity (Y-axis) and experimentally determined MOIs (X-axis). The chart on the right is the AI that was characterized on the left predicting an infection on a new plate. The blue curve on the right is transferred from the chart on the left. This shows strong congruence between the results from the new plate and the characterized AI.

Table 1. Summary of AVIA performance in MOI prediction from infectivity analysis.

Aggregated MOI Predictions (Biological Replicates)							
Lab MOI	Dilution Factor	Predicted Infectivity (0.09 to 0.96)	Predicted Dilution MOI (0.50 to 5.47)	Undiluted MOI (Target = 5.0)			
				Predicted	Mean	Maximum Slope	Maximum 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+			

Quantification of antibody inhibition of viral infection

Antibody-based inhibition of viral infection is a key assay to establish therapeutic efficacy. AVIA-predicted infectivity analysis was also performed in the context of inhibition of infection for antibody efficacy analysis through dose–response IC_{50} determination. At 16 hours after infection, AVIA predicted infectivity scores accurately quantified the inhibition of viral infection in Vero cells across multiple antibodies and MOIs, as summarized in Figure 3. The AVIA predicted infectivity score identified a consistent IC_{50} value at each viral MOI tested for each inhibitory antibody.

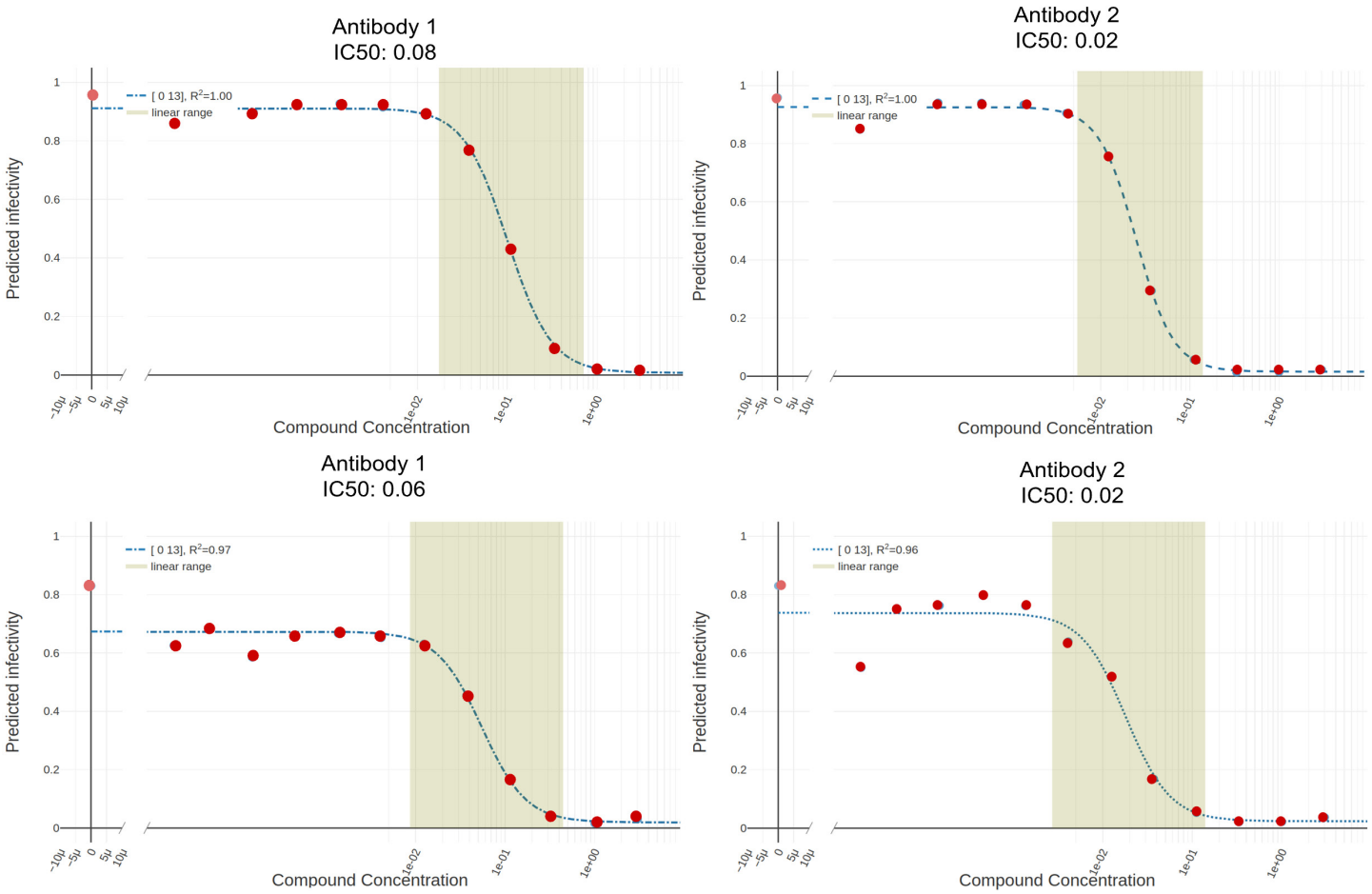

Figure 3. AVIA quantification of antibody inhibition of viral infection. Dose-response curves for two antibodies (left and right) inhibiting an infection at MOI 2.0 (top) and MOI 1.0 (bottom) for HRV16 in Vero cells at a 16-hour time point, using the AI trained and characterized in Figure 2. The charts 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) and the half maximal inhibitory concentration (IC_{50}). Antibody IC_{50} values displayed in the graph are consistent across MOIs.

Conclusion

The automated approach presented here leverages high-throughput, automated imaging and AVIA technology for AI-based viral infectivity analysis in label-free brightfield images. The proof-of-concept experiments quantified human rhinovirus infection in unlabeled Vero cell cultures by 16 hours after infection, with MOI predictions comparable to labor-intensive traditional methods. This represents a substantial advancement in viral infectivity analysis that decreases time to results, simplifies the experimental process, lowers reagent and consumables dependencies, and ultimately recovers research time.

AVIA detection and quantification of viral infectivity has several advantages over the current standard assays:

- **High throughput:** AVIA uses fewer reagents and consumables, requires less incubation time, and offers higher confidence with fewer replicates. These advantages mean more samples can be run on shorter timelines compared to other methods.
- **High precision:** Automated analysis using AIs ensures the results are objective and reproducible. This approach, in tandem with the Agilent BioTek Cytation automated imaging capabilities, enables reproducible, automated workflows from start to finish.
- **Flexibility and accessibility:** The proposed workflow is as simple as capturing images early after infection, uploading the images and a plate map to the ViQi platform, and then receiving an automated AVIA report within an hour. This reduces the amount of required training and expertise for lab personnel in determining infection of cells or tissue.

The live-cell brightfield imaging capabilities of the Cytation instrument line are complemented by a full range of automated microscopy techniques, including fluorescence and color brightfield imaging modes. Flexible automated imaging supports both emerging techniques for AI-based infectivity analysis as well as traditional plaque and fluorescent focus-forming assay analyses. This combination of features results in a powerful single-instrument solution with added value across virology applications and beyond.

The AVIA quantification approach can be applied to increase scale and accelerate time to results across a spectrum of viral detection applications. These include determining infectivity of stock viruses over the course of purification steps; inactivation assays accessing efficacy of antiviral, inactivating antibody, and biocide treatments; as well as validating viral vectors for vaccines, gene therapies, or other therapeutics during initial screening and manufacturing.

References

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