A Cloud-based Rapid and Scalable Viral Infectivity Assay for Vaccine Development and Antiviral Screening

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ABSTRACT

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.

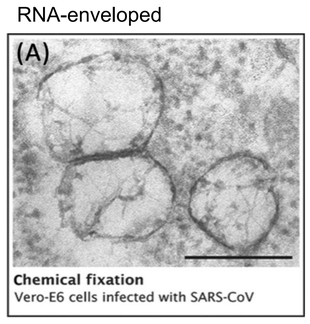
In response, ViQi, Inc. has developed AVIA™ (Automated Viral Infectivity Assay). This assay uses machine learning and brightfield microscopy to detect signs of viral infection. It does so by identifying subtle phenotypic changes within cells that are associated with viral replication. These can be detected 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.

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).

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.

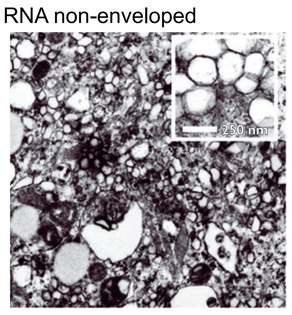
BACKGROUND

SARS-CoV2
RNA-enveloped

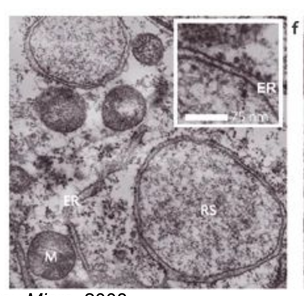


Wolff G et al. *TIBS*. 2020
doi.org/10.1016/j.tim.2020.05.009

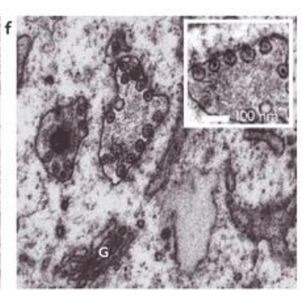
Poliovirus
RNA non-enveloped



Vaccinia virus:
DNA-enveloped



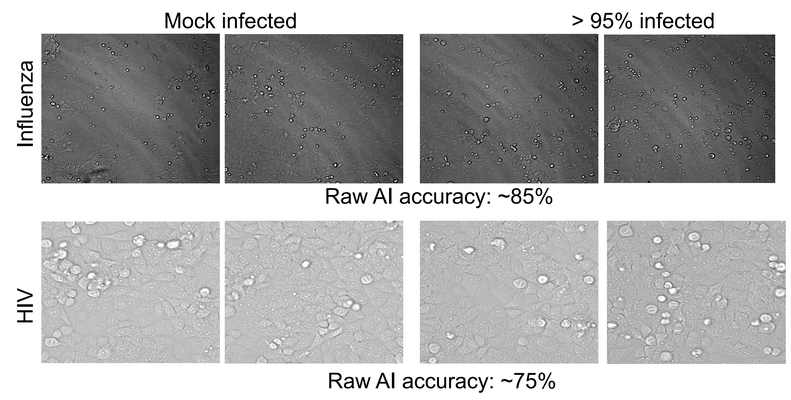
Semliki Forest Virus:
RNA-enveloped



Miller S & Krijnse-Locker J. *Nat Rev Micro.* 2008
doi.org/10.1038/nrmicro1890

Many viruses produce membraned structures ~400 nm, which should have a readout in phase contrast or brightfield

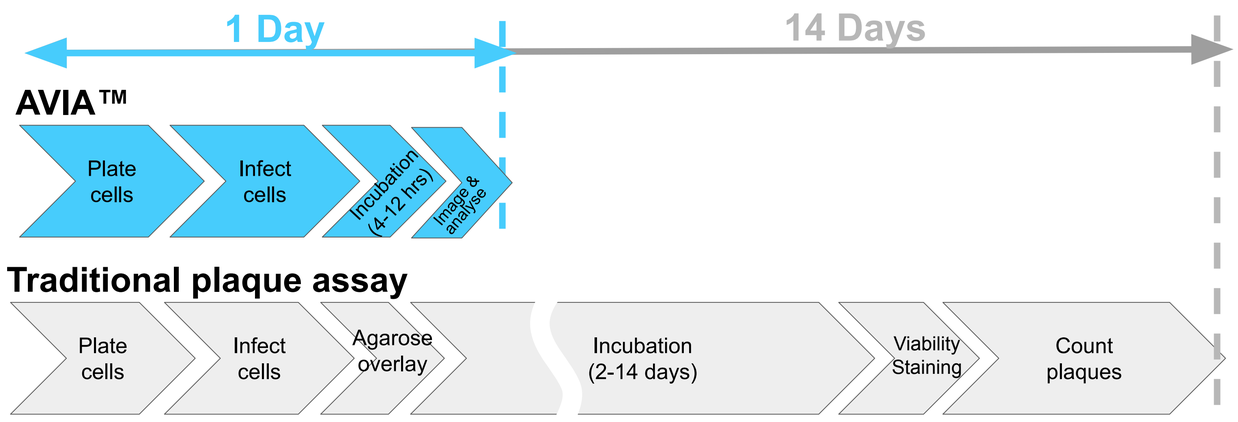
AVIA IS TRAINABLE WITHOUT VISIBLE CPEs

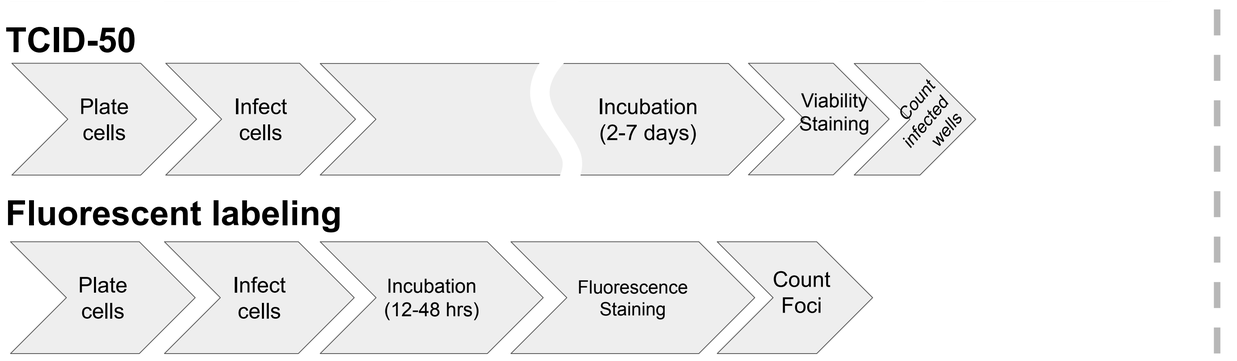


AI accuracy per-image sample (~2-5 cells) is high without visible CPEs.

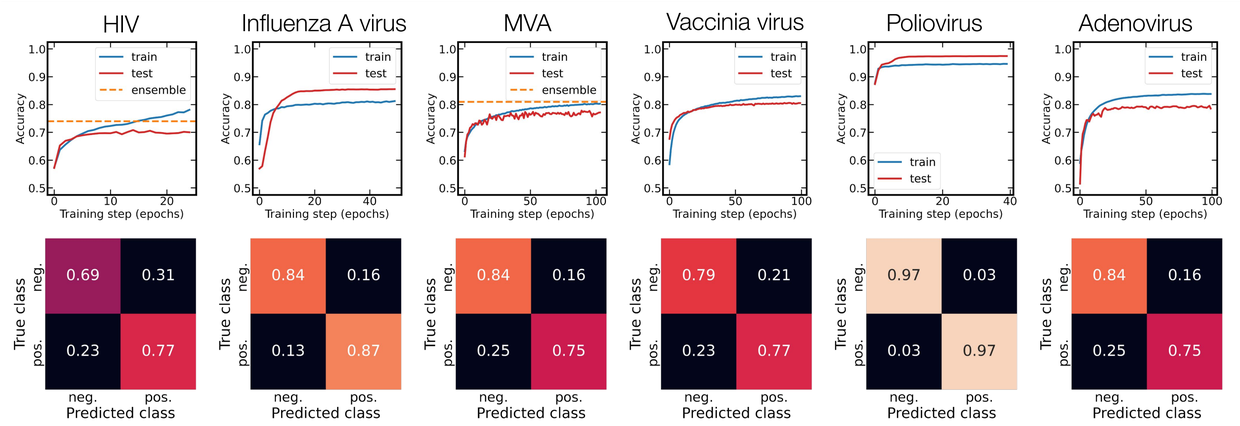
- Imaging with 20x objective in brightfield: ~0.5 µm/pixel.
- AI accuracy > 75% is sufficient to produce accurate dilution curves: thousands of samples per MOI prediction.
- Infection phenotypes are visible to the AI in the first round of infection (8-16 hpi).

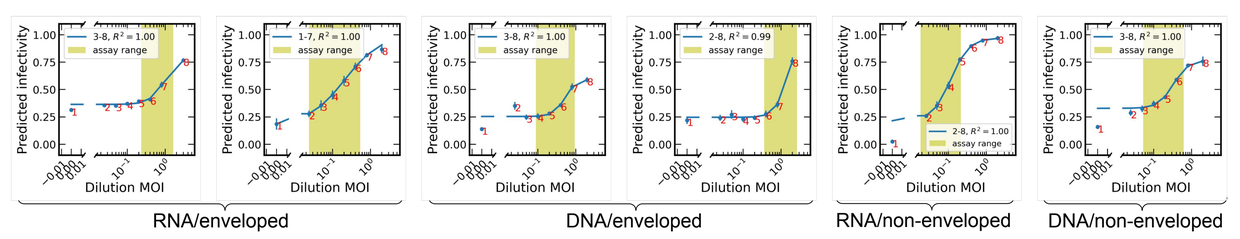
ONE DAY VIRAL INFECTIVITY ASSAY WITH AVIA™





AVIA IS TRAINABLE ON ALL FOUR MAJOR TYPES OF VIRUS





- Training curve shows AI performance on training data (infected vs. uninfected)
- 2x2 matrix shows false positive vs false negatives
- Validation of each AI on serial dilutions
- Green box (■) is linear range determined from sigmoid fit

Also verified on:

- Zika
- Dengue
- VEEV
- Rhinovirus
- MHV
- TMEV
- HSV
- MVM
- Coronavirus 229E

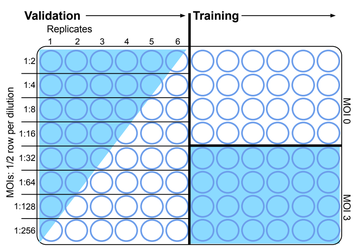
Ongoing work:

- SARS-CoV-2
- AAV (replication competent systems)
- Hepatitis B
- … and others

FULL ASSAY DEVELOPMENT

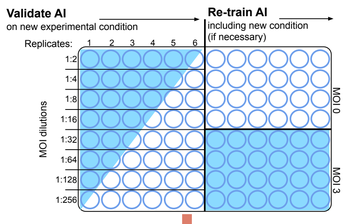
Stage 1: Training

- Train on a single plate with MOI dilutions and timepoints
- Determine optimal assay timepoint, linear range of the assay



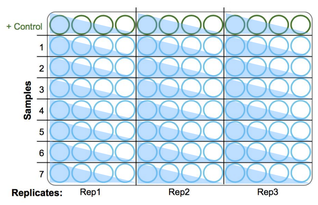
Stage 2: Reproducibility

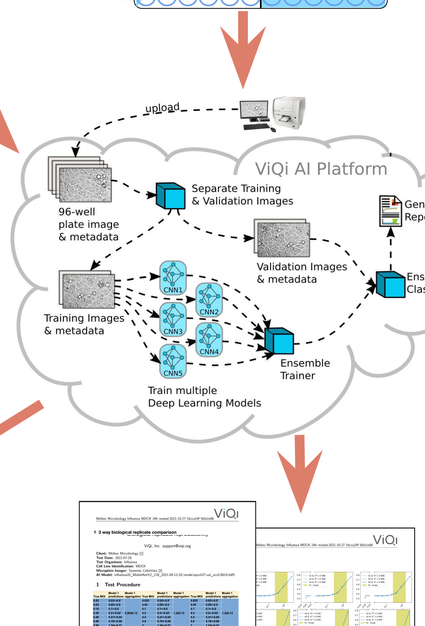
- Cross validate models trained on plates with different viral stocks, strains, cell passages
- Compare against $TCID_{50}$ or plaque assay.
- Re-train AI to add new experimental conditions
- One plate per round of validation & re-training

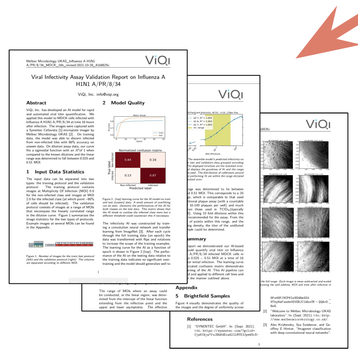


Stage 3: Testing unknowns

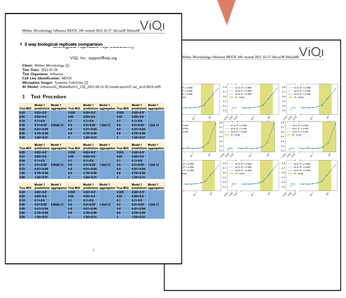
- Use trained AI from stages 1 & 2
- Single timepoint, 8-16 samples/plate



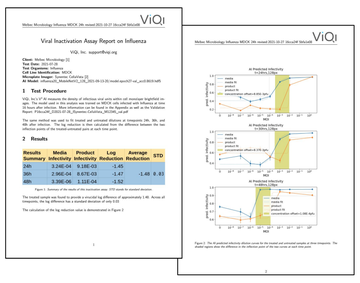




turnaround: 1-2 days

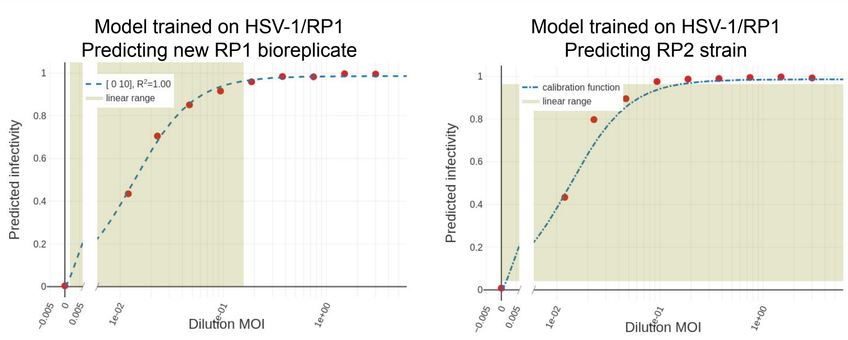


1-2 days



< hour

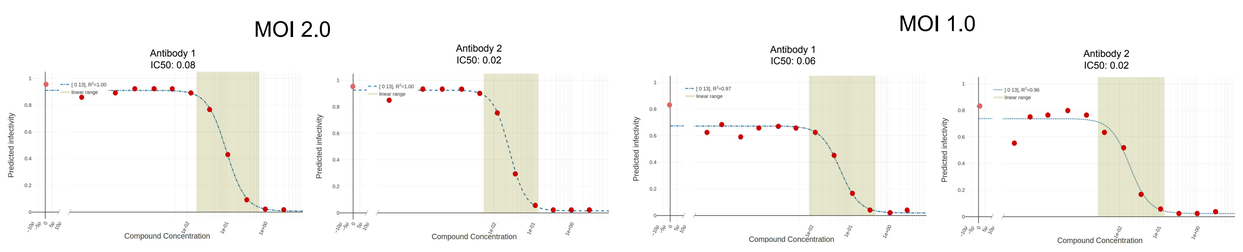
AVIA REPRODUCIBILITY: BIOREPLICATES & VIRUS STRAINS



Blue calibration curve from fitting model training data superimposed on dilution data for new bioreplicate of HSV-1/RP1 (left), and HSV-1/RP1 (right).

- Models can be trained on multiple cell passages or strains to increase their predictive power across experimental conditions.
- A model trained on one strain can (HSV-1/RP1) can often be used to accurately assay closely related strains (HSV-1/RP2)
- Prediction reproducibility is typically better than 10%.

EXAMPLE ANTIBODY INACTIVATION ASSAY



- Rhinovirus infection at MOI 2.0 and MOI 1.0, with increasing concentrations of two inactivating antibodies.
- Consistent IC_{50}s under different infection conditions.

CONCLUSION & NEXT STEPS…

ViQi has developed AVIA, an AI-based viral infectivity assay that:

- Uses brightfield imaging, works with live cells, minimizes reagents and sample preparation
- Is quantitative across a broad range of infectivities
- Has a much faster turnaround time than traditional assays
- Has been shown to work across all four major virus categories

We are looking for collaborators to both train on new virus-cell lines and test existing models

Learn More at www.viqiai.com/avia