Stain-free, rapid, and automated viral plaque assay using time-lapse holographic imaging and deep learning

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Abstract: We report a rapid and automated viral plaque assay using time-lapse holographic imaging and deep learning, significantly reducing the detection time needed for traditional viral plaque assays and entirely eliminating staining and manual counting procedures.

1. Introduction

To combat the long-existing global threat of viral infections [1], various techniques have been developed for virus detection and quantification, aiding the advancement of vaccines and antiviral drugs. These techniques include the viral plaque assay, polymerase chain reaction (PCR) [2], immunofluorescence focal forming assays (FFA) [3], etc. Among these, the viral plaque assay is the gold-standard method due to its unique ability to assess *virus infectivity* cost-effectively by observing the formation of plaques caused by viral infections on a cell monolayer. However, the traditional viral plaque assay necessitates experts to manually count the crystal-violet stained plaque-forming units (PFUs) in infected samples after an incubation period of 2-14 days [4]. This process is time-consuming and susceptible to staining artifacts and human counting errors.

Here, we present a stain-free, rapid, and automated viral plaque assay technique using deep learning and time-lapse holographic imaging, which could significantly reduce the time needed for PFU detection and entirely circumvent the staining and manual counting processes [5]. To show the efficacy of our system, Vero E6 cells were cultured and then infected by the vesicular stomatitis virus (VSV). Using the presented technique, >90% of the VSV PFUs were detected without any false positives at the 20th hour of incubation, saving >24 hours compared to the traditional plaque assay which takes 48 hours of sample incubation. Moreover, our method was demonstrated to easily generalize to new types of viruses by reducing the incubation time for the herpes simplex virus type-1 (HSV-1) and the encephalomyocarditis virus (EMCV), saving us on average ~48 and ~20 hours, respectively. In addition, the presented system could handle viral samples with ~10-fold higher virus concentrations by identifying individual PFUs during their early growth stage before the formation of the PFU clusters. This deep learning-powered label-free PFU detection system can be widely used in virological research and related clinical applications.

2. Methods

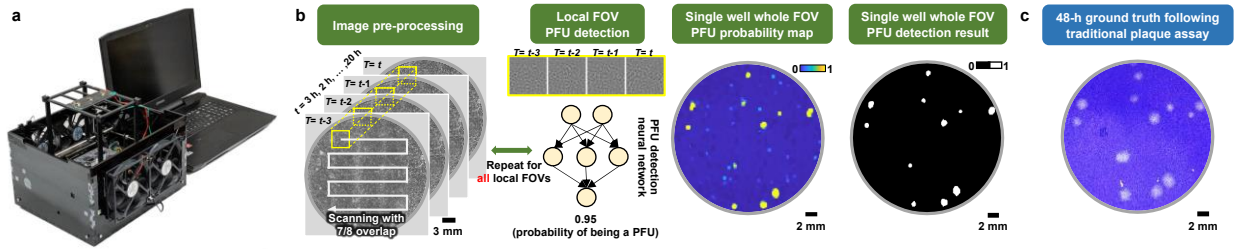

Fig. 1. (a) Automated and label-free PFU detection system with a data processing laptop. (b) Deep learning-based image processing workflow of our system. (c) 48-h ground truth following the standard viral plaque assay protocol and staining [5].

Each sample in the presented work was prepared following the standard viral plaque assay protocol [5]. A monolayer of Vero E6 cells was achieved on the 6-well plate and then infected by the VSV, HSV-1, or EMCV. After the VSV sample was prepared, it was first placed onto our lens-free imaging device (Fig. 1a) to perform a holographic time-lapse imaging experiment for 20 hours (72 hours for HSV-1 and 60 hours for EMCV) with an imaging time interval of 1 hour (2 hours for HSV-1 and 1 hour for EMCV). Then, the same sample was further incubated up to 48 hours

(120 hours for HSV-1 and 72 hours for EMCV), fixed, and stained to get the ground truth images (Fig. 1c) to compare with our results. After the imaging, a deep learning-based PFU classifier was applied in a scanning manner to convert the reconstructed phase images from four registered consecutive frames to a PFU probability map, where each pixel represents the probability of a local area (0.8×0.8 mm^2) centered at this pixel having a PFU. Finally, by setting an unbiased decision threshold of 0.5, this probability map was converted into a binary detection mask that indicates the sizes and locations of the PFUs [5].

3. Results and Discussion

Our VSV detection results show a high level of agreement with the stained ground truths obtained by the traditional viral plaque assay, as shown in Fig. 2a. By testing on five 6-well plates (containing a total of 335 VSV PFUs and five negative control wells), our label-free system could achieve an average VSV PFU detection rate of 90.3% at 17 hours of incubation (Fig. 2b), reducing >24 hours compared with the 48-h stained ground truth. In addition, our method successfully generalized to new types of viruses, HSV-1 and EMCV, through transfer learning [5]. Blindly tested on two 6-well plates (containing in total 214 HSV-1 PFUs and two negative control wells), it achieved a 90.4% HSV-1 detection rate at 72 hours (Fig. 2d), reducing 48 hours of incubation time compared with the 120 hours required by the traditional HSV-1 plaque assay. Furthermore, an EMCV detection rate of 90.8% was obtained at 52 hours of incubation (Fig. 2e) when tested on two 6-well plates (containing in total 249 EMCV PFUs and two negative control wells), achieving 20 hours of incubation time saving compared with the 72-h ground truth for the traditional EMCV plaque assay [5]. Note that *no false positives* were detected for all the test wells across all time points.

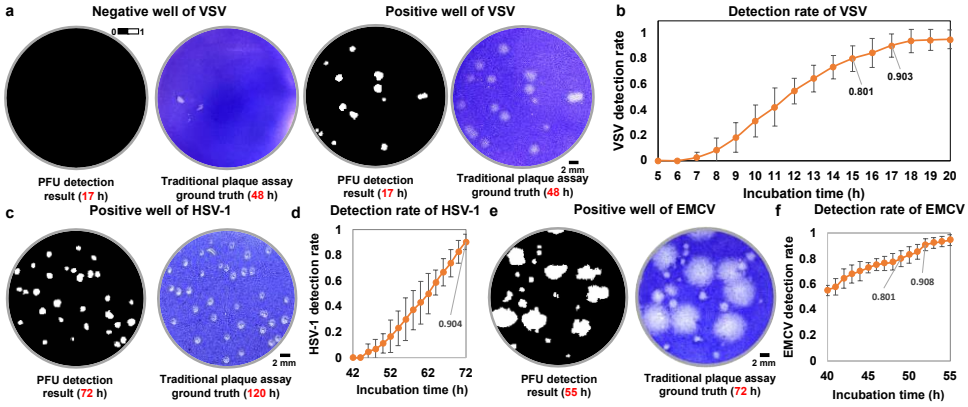

Fig. 2. (a) VSV PFU detection result at 17-h of incubation and its 48-h ground truth after staining for one example negative well and one example positive well. (b) Average VSV detection rate vs. incubation time across five test 6-well plates, where the error bar shows the standard deviation across five test plates. (c) HSV-1 PFU detection results at 72-h of incubation and its stained 120-h ground truth. (d) Average HSV-1 detection rate vs. incubation time across two test 6-well plates. (e) EMCV PFU detection results at 55-h of incubation and its stained 72-h ground truth. (f) Average EMCV detection rate vs. incubation time across two test 6-well plates.[5]

Moreover, our system could handle viral samples covering a larger dynamic range of virus concentrations. Compared with the 48-h ground truth PFU assay (Fig. 3b), which was not suitable for counting, our system could identify individual PFUs at their early growth stage before the formation of the PFU clusters (Fig. 3a) [5].

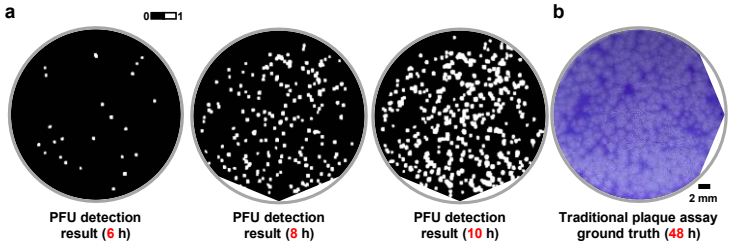

Fig. 3. (a) Label-free PFU detection results for one example test well with high virus concentration in its early growth stage. (b) 48-h stained ground truth for the same dense well in (a). [5]

4. References

[1] L. Singh, *et al. Ther Adv Infect Dis*, vol. 4, no. 4, pp. 105–131, Jul. 2017.
[2] S. D. Gallaher, *et al. J Virol Methods* vol. 192, no. 1-2, pp. 28–38, Sep. 2013.
[3] D. J. M. Cruz, *et al. J Virol Methods* vol 145, no. 1, pp. 56–61, Oct. 2007.
[4] A. Baer, *et al. J Vis* e. 52065, Nov. 2014.
[5] T. Liu, *et al. Nature Biomedical Engineering* DOI: 10.1038/s41551-023-01057-7 (2023)