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Digital infectivity assay using drop-based microfluidics

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The plaque assay has been used for decades to measure virus infectivity, but the assay is slow, labor intensive, uses excessive reagents, and is hard to automate. Moreover, since plaque assays use logarithmic dilution series as samples, they provide logarithmic precision, and can err more than 100%.1 Using drop-based microfluidics, we developed a high throughput single-virus-host system and apply it to perform for the first time a digital infectivity assay. Wecompartmentalize single virus and host cells in picoliterdrops, and culture the virus for one infection cycle before amplifying the target viral genome in each drop. By counting the number of fluorescing drops we measure the number of infection events per sample, and calculate the infectivity of the virus. By sorting fluorescing drops and sequencing their viral contents, we can also retrieve the genotype of infective progenies. Digital infectivity assays require just one viral cycle, reduce labor, minimize consumption of reagents, and most importantly provide absolute count of infection events, with theoretical precision that is only limited by the number of drops measured. We use this new method to assess the neutralization effect of a known antibody on different viral strains, as well as to characterize unknown novel viral genomes that escaped the neutralization effect of the antibody.Thus, digital infectivity assay is an important tool for characterizing phenotype and genotype of abundant and rare viral progenies, and should assist greatly in viral research .

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