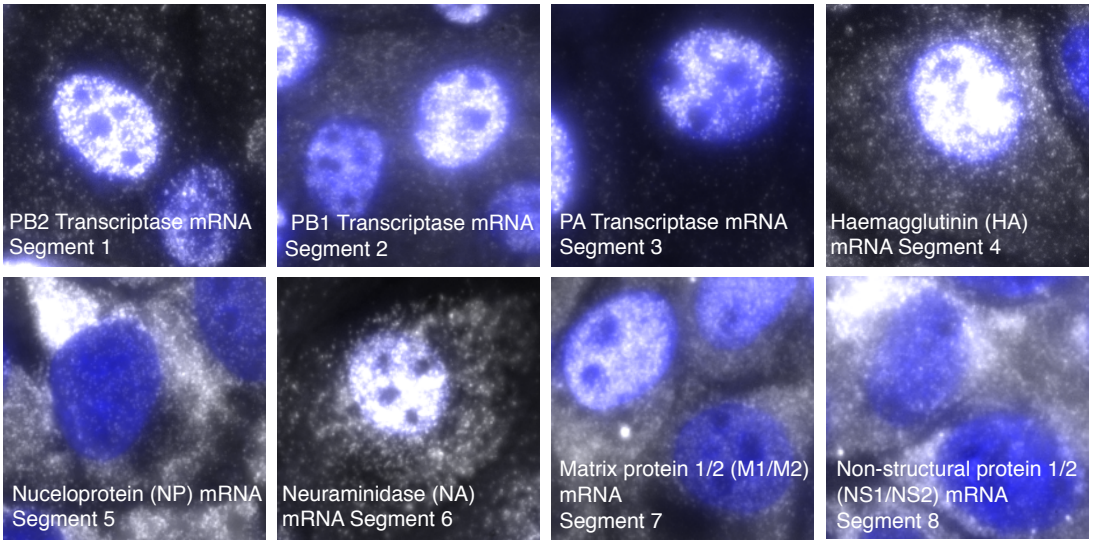
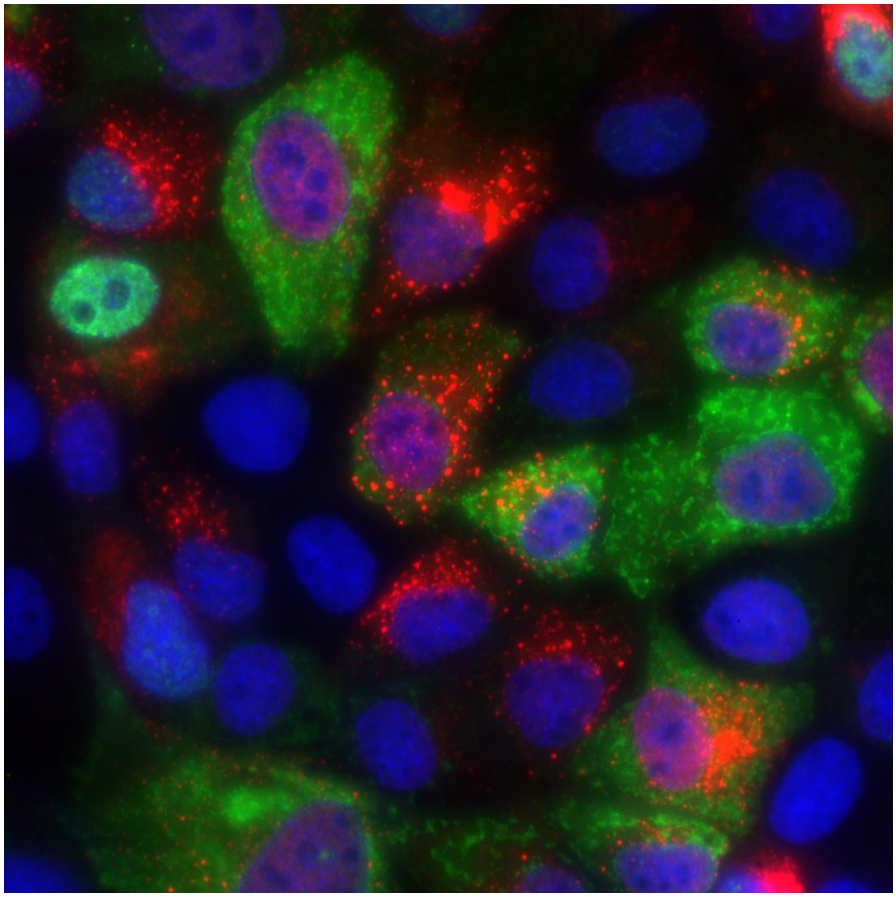
Electronic Supplementary Material (ESI) for Lab on a Chip.

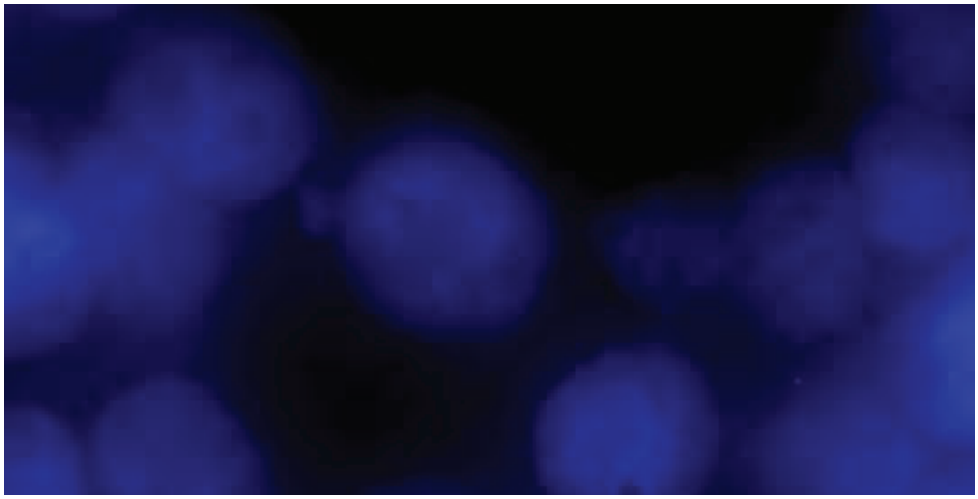


Supplementary Fig. 1: RNA FISH for each segment of influenza virus produces bright fluorescent signal. We designed RNA FISH probes to target the viral mRNA from all 8 segments of influenza A. We then infected MDCK cells with influenza A/Puerto Rico/8/1934 H1N1, fixed the cells, and performed rapid RNA FISH for the viral mRNA segments. We found that the RNA from each segment produced bright fluorescent signal. DAPI (nuclear stain) is in blue, and RNA FISH is in white. All images are 100X magnification.

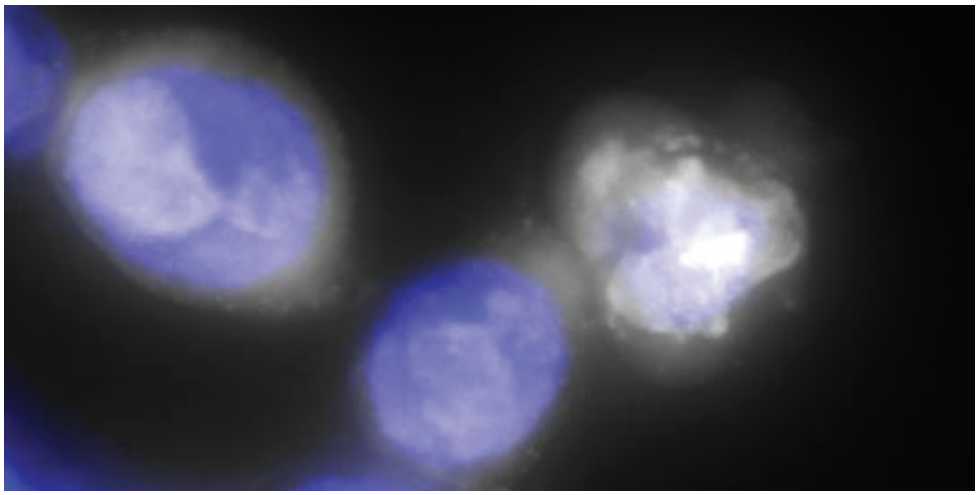


Supplementary Fig. 2: RNA FISH for genomic and messenger viral RNA produces bright fluorescent signal. We designed RNA FISH probes to target the viral mRNA and genomic RNA of influenza A. We then infected MDCK cells with influenza A/California/7/2009 H1N1, fixed the cells, and performed rapid RNA FISH for the different RNA. We found that the viral RNA produced bright fluorescent signal in a different spatial distribution from the mRNA. DAPI (nuclear stain) is in blue, genomic viral RNA is in red, and viral mRNA is in green. All images are 100X magnification.

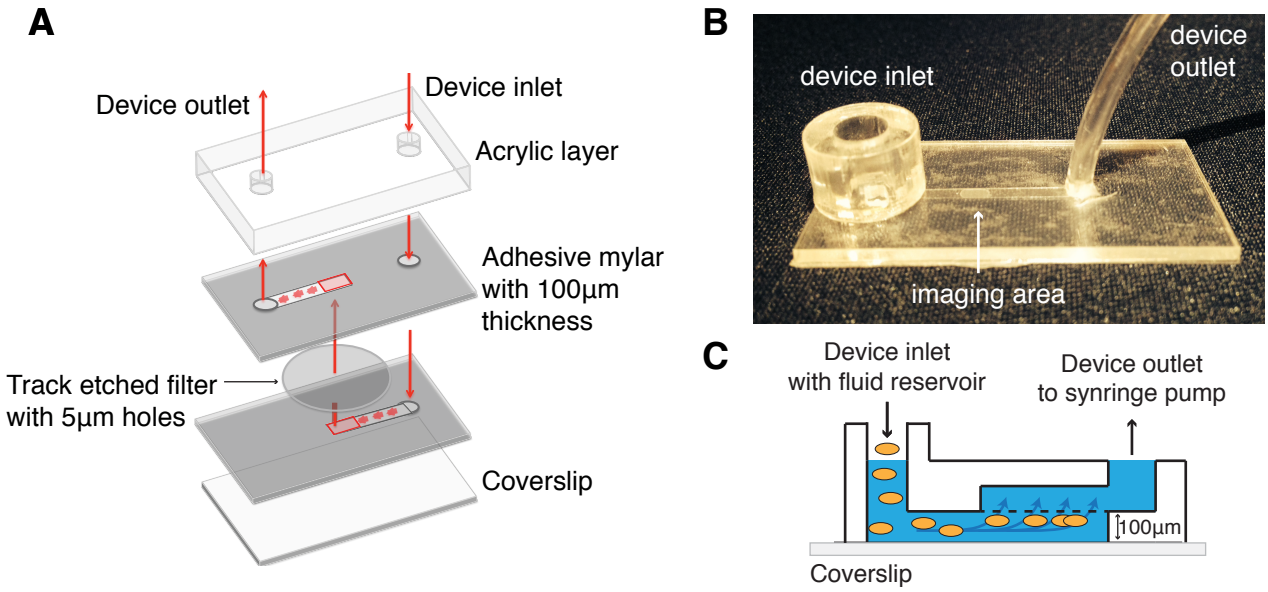
Uninfected



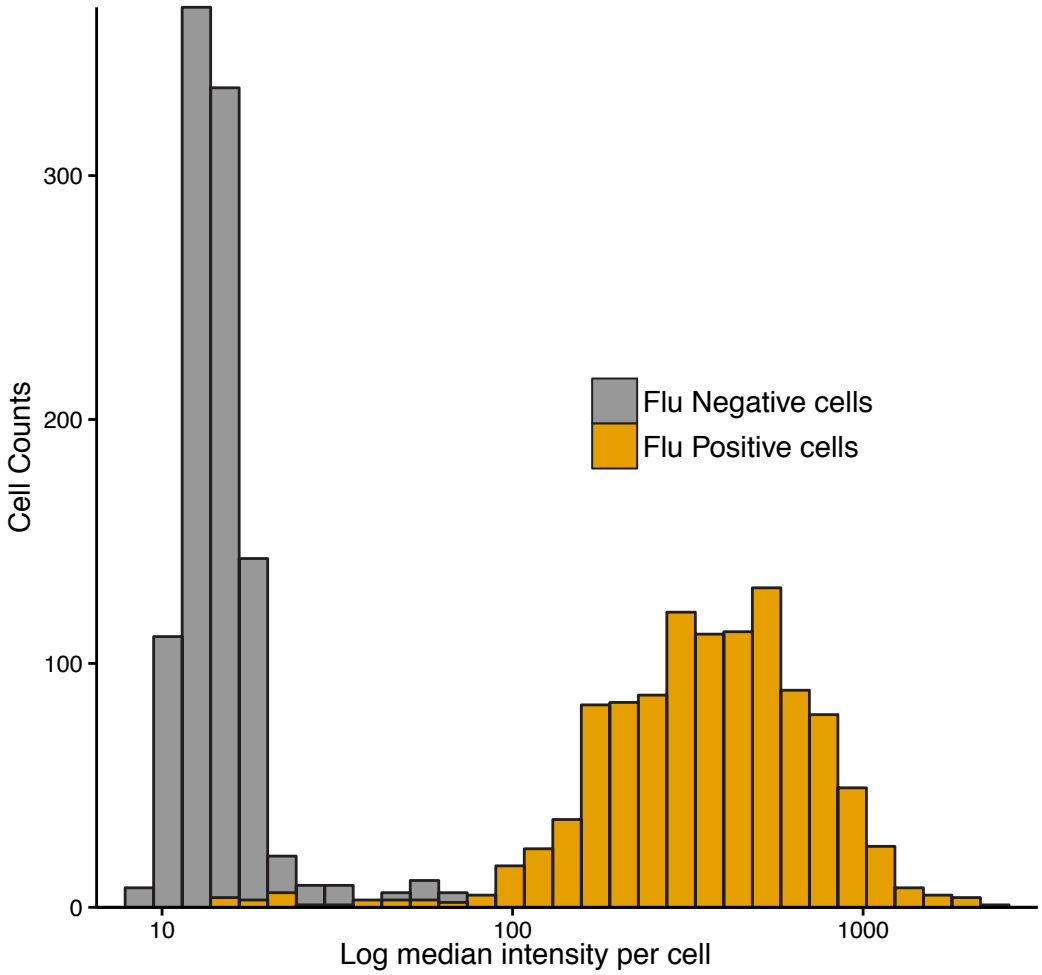
Adenovirus infected



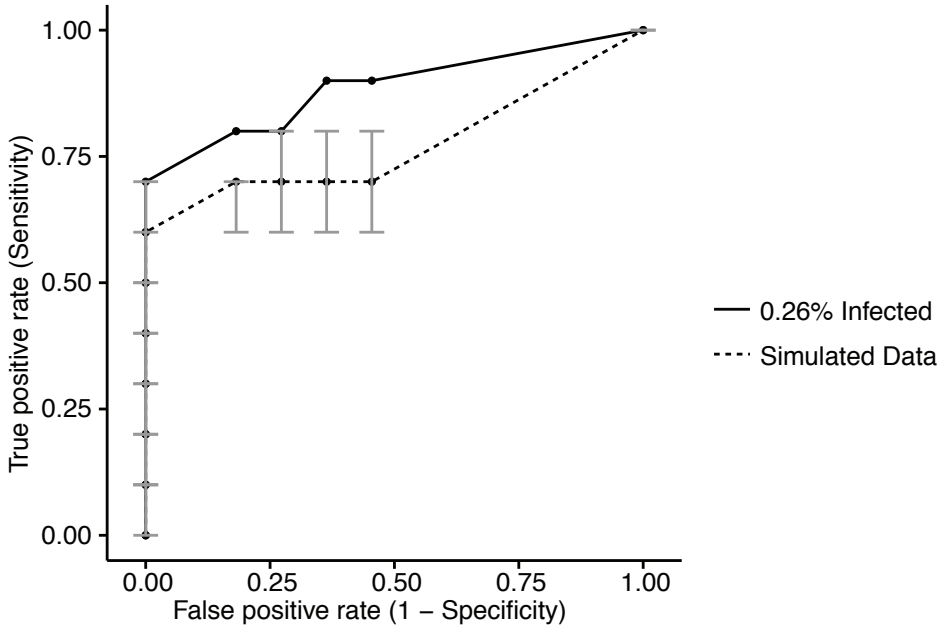
Supplementary Fig. 3: Adenovirus pan-probe labels adenovirus infected cells. We designed adenovirus pan-probes to cover adenovirus serotypes 1-7. We then infected 293T cells with adenovirus Ad5ΔE1-GFP, fixed the cells with methanol, and performed rapid RNA FISH for the virus. Infected cells had bright signal from the RNA FISH probes while uninfected cells remained dark. DAPI (nuclear stain) is in blue, and RNA FISH is in white. All images are 100X magnification.



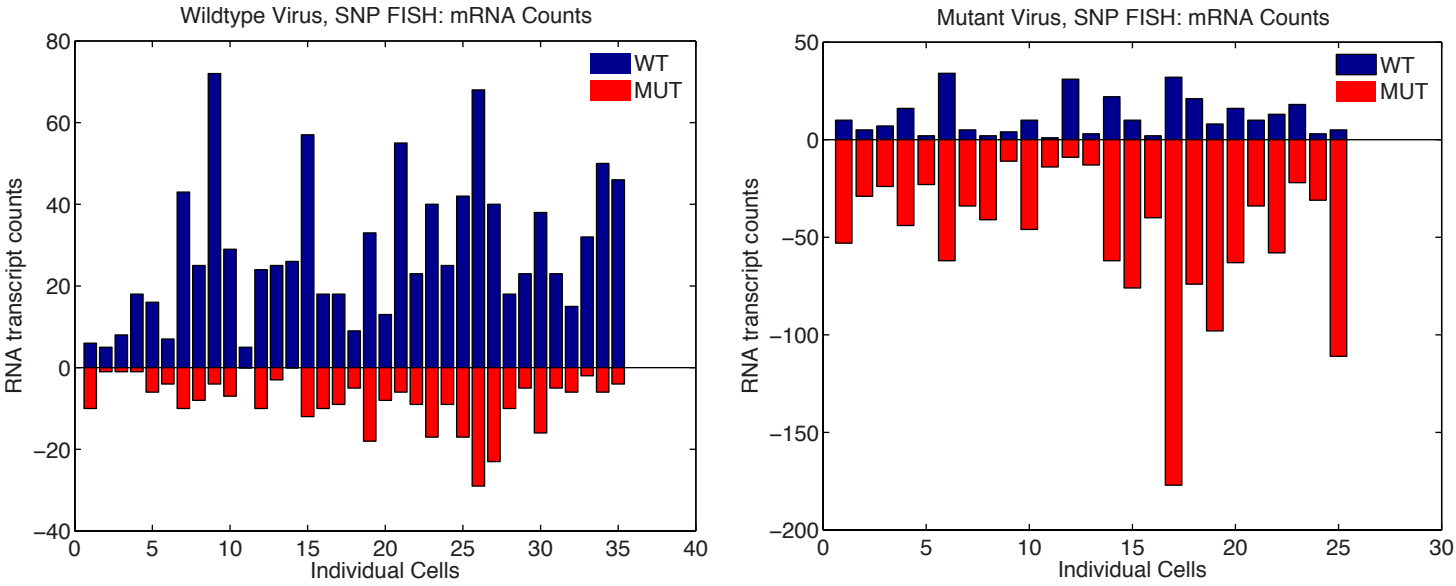
Supplementary Fig. 4: Overview of device construction. We constructed the device from laser cut pieces of acrylic, double sided adhesive mylar (thickness 100μm, 3M company), a polycarbonate track-etched filter (pore diameter = 5 μm, Whatman, Nuclepore), and a number 1 coverslip. A) We assembled the device by layering the pieces as shown. B) Photograph of the assembled device. C) Schematic of the device with cells trapped under the filter.



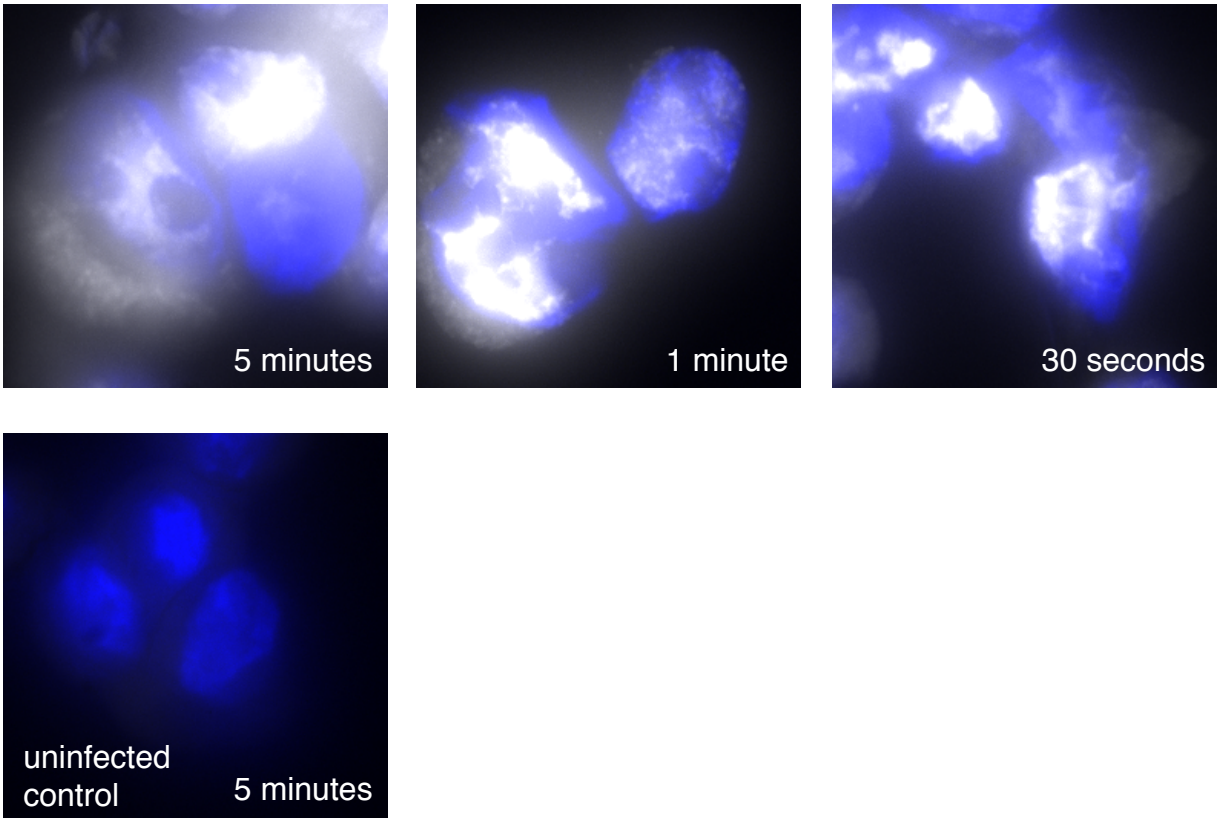
Supplementary Fig. 5: The intensity profiles for infected and uninfected cells are reproducible between biological replicates. We loaded the device with either influenza A/Puerto Rico/8/1934 H1N1 infected or uninfected MDCK cells and performed RNA FISH for the virus. We ran our image processing pipeline to extract the fluorescence intensity signal for the viral RNA FISH in each cell and calculated the median intensity. As expected, we found that the median intensity in most infected cells was higher than the median intensity in most of the uninfected cells. In this biological replicate, our intensity cutoff of 330 was again sufficient to discriminate uninfected and infected cells.



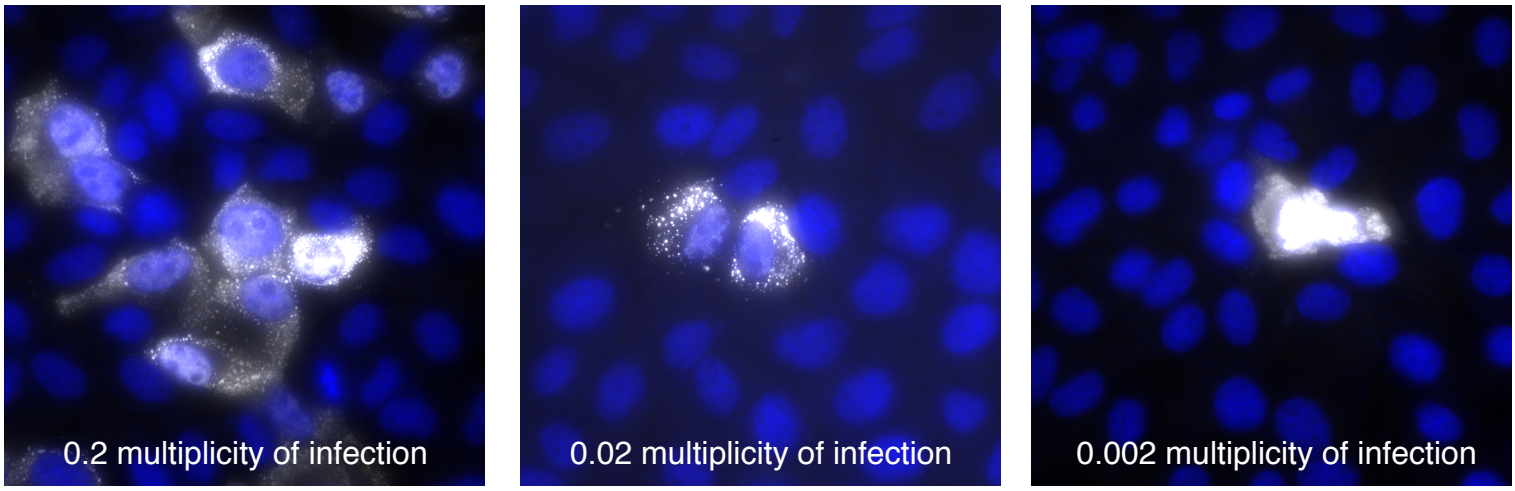
Supplementary Fig. 6: Classification using our pipeline approaches the expected classification limit of sampling from small numbers of cells. Simulated data of percent infected cells was generated by sampling 25,000 times from a binomial distribution with probability of trial success equal to 0.26% and number of observations equal to the experimentally determined number of cells in each device with the 0.26% infected sample. For each of the 25,000 simulations, we generated a receiver operating characteristic curve using this simulated data for infected devices and experimental data for uninfected devices. Dotted line represents the median and error bars in gray represent the upper and lower quartile at each percent infected threshold of the receiver operating characteristic curves. Solid black line represents experimental data from uninfected devices and devices with 0.26% infected cells, as shown in Fig. 3B.



Supplementary Fig. 7: Viral RNA molecules in cells infected with wild-type and resistant mutant virus are classified by SNP FISH. We infected MDCK cells with wild-type influenza A/California/07/2009 H1N1 and with A/California/07/2009 H1N1 engineered to possess the H274Y (C823T nucleotide) NA mutation. We used SNP RNA FISH to classify individual transcripts as wild-type or resistant mutant RNA. For each cell, we quantified the amounts of each RNA as shown by the bar graphs. Cells infected with wild-type virus had more wild-type RNA, and cells infected with the resistant mutant virus had more mutant RNA.



Supplementary Fig. 8: Hybridization of RNA FISH probes requires as little as 30 seconds. We infected MDCK cells with influenza A/Puerto Rico/8/1934 H1N1 and fixed the cells 18 hours after infection. We then performed rapid RNA FISH using hybridizations times of 5 minutes, 1 minute, and 30 seconds. For each sample, we used probe sets for the haemagglutinin (HA) and nucleoprotein (NP) segments of influenza A/Puerto Rico/8/1934 H1N1. With each of the hybridization times tested, we observed bright fluorescent signal in infected cells. DAPI (nuclear stain) is in blue, and RNA FISH is in white. All images are 100X magnification.



Supplementary Fig. 9: At low levels of infection with influenza, individual cells still have bright signal by RNA FISH, but are at low frequency. We infected MDCK cells with influenza A/Puerto Rico/8/1934 H1N1 at multiplicity of infection 0.2, 0.02, and 0.002. Eighteen hours after infection, we fixed the cells and then performed rapid RNA FISH using probes for all 8 segments of influenza A/Puerto Rico/8/1934 H1N1. In the samples with lower multiplicity of infection, we observed fewer infected cells overall, but noted that the individual infected cells in each samples had comparable fluorescence intensity. DAPI (nuclear stain) is in blue, and RNA FISH is in white. All images are 100X magnification.

Phase	Solution	Flow Rate (uL/min)	Time (sec)	Total Volume (uL)
Trap Cells	Methanol Fixed cells diluted in 2xSSC	100 uL/min	120 sec	200 uL
Pause	-	-	10 sec	-
Pre-Wash	Wash Buffer	200 uL/min	60 sec	200 uL
Pause	-	-	10 sec	-
Hybridization	Probe	100 uL/min	12 sec	20 uL
Pause	-	-	10 sec	-
Hybridization	Probe	100 uL/min	2.4 sec	4 uL
Pause	-	-	60 sec	-
Hybridization	Probe	100 uL/min	2.4 sec	4 uL
Pause	-	-	60 sec	-
Hybridization	Probe	100 uL/min	2.4 sec	4 uL
Pause	-	-	60 sec	-
Hybridization	Probe	100 uL/min	2.4 sec	4 uL
Pause	-	-	60 sec	-
Hybridization	Probe	100 uL/min	2.4 sec	4 uL
Pause	-	-	60 sec	-
Wash	Wash Buffer	200 uL/min	60 sec	200 uL
Pause	-	-	10 sec	-
Wash	Wash Buffer	200 uL/min	60 sec	200 uL
Pause	-	-	10 sec	-
Wash	Wash Buffer	200 uL/min	60 sec	200 uL

Pause	-	-	10 sec	-
Final	2xSSC	200 uL/min	60 sec	200 uL

Supplementary Fig. 10: Overview of program for automatic fluid handling on the device with a syringe pump. We programmed the syringe pump to automatically pull fluids through the device at fixed speeds. The program includes many steps, and this table outlines the details for each step. The “phase” column explains the purpose of each step. The “solution” column indicates what fluid is pumped by each step. The flow rate, time, and total volume show the parameters for the syringe pump operation.