Empowering drug discovery by evaluating antivirals in thousands of single cells

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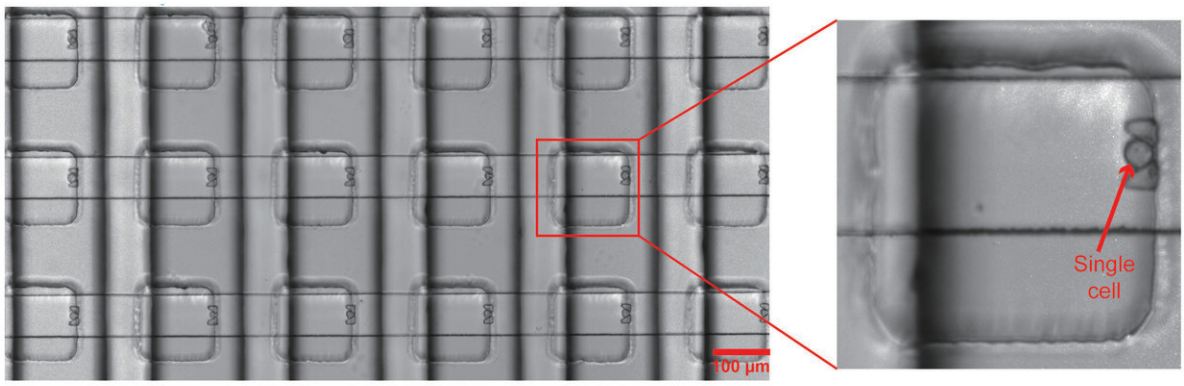


An enhanced microfluidic device allows researchers to simultaneously observe thousands of individual cells that are infected with virus. A close-up of the device shows 18 wells, each containing a single cell. Credit: Cameron Lab, Penn State

A new enhancement to a lab-on-a-chip device allows researchers to simultaneously observe thousands of individual cells that are infected with virus, providing important information about infection dynamics not available using traditional methods.

A team of researchers from Penn State and the University of Texas at Austin have enhanced an older version of a microfluidic device they developed, greatly increasing the number of individual cells that can be

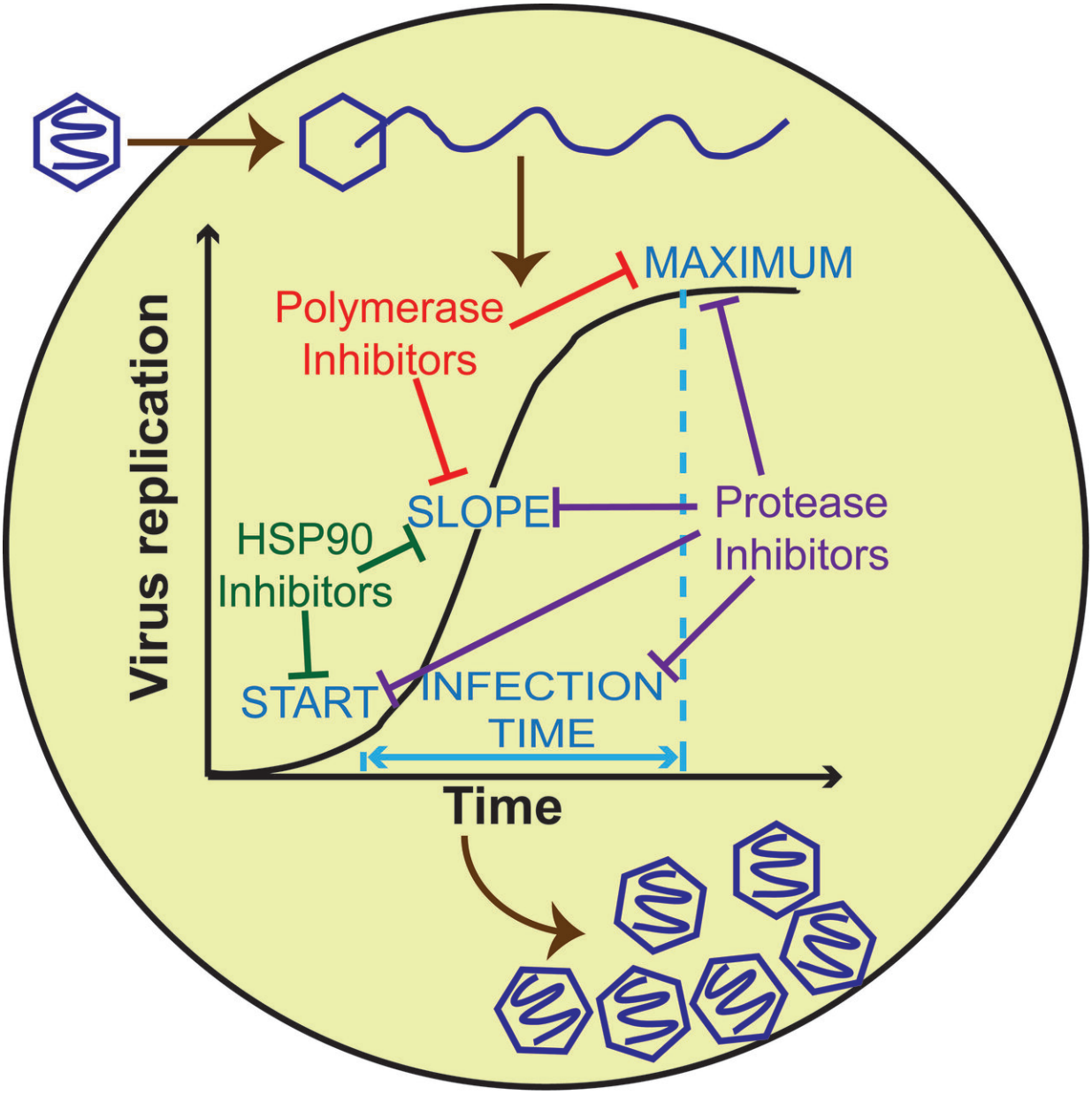
observed at one time and making this historically laborious single-cell approach viable for drug screening. A paper describing the device, which also provides insight into how the antivirals work and whether a virus is likely to develop resistance, appears online on October 30, 2019 in the journal *Science Advances*.

"Traditional methods to study the effect of an antiviral on infected cells focus on a population of many infected cells," said Craig Cameron, professor and holder of the Eberly Family Chair in Biochemistry and Molecular Biology at Penn State at the time of the research and senior author of the paper.

"When you apply an antiviral at a particular dosage to a population, you can see how many infected cells are killed, or the efficacy of the antiviral. But individual cells may respond differently to a drug, which can have important implications for infection outcome and drug resistance. We previously developed a way to study individual infected cells, and here we adapted the technique to increase tenfold the number of single cells we can study at one time."

The team uses a microfluidic device—a chip etched with tiny channels—with about 5700 individual wells, each of which can be filled with single, infected cells. Their first generation device relied on a method that left most of the wells empty. Now, the team has developed a physical trap that they incorporated into one of the layers of the device, improving occupancy so they can now fill about 90 percent of the wells.

"Other people have tried to study infections in single cells, but they have to manually add cells to 96-well or 384-well plates," said Wu Liu, a postdoctoral researcher at Penn State at the time of the research who developed the trap. "This is tedious and time-consuming. With our trap and microfluidic device, we can observe more than 5000 single cells at one time."



Single-cell analysis of antiviral candidates reveals efficacy, mechanism of action, and, perhaps, the likelihood for development of resistance. Credit: Wu Liu, Craig E. Cameron

To test the updated device, the team infected cells with a modified

version of poliovirus that produces a green-fluorescent protein and monitored the amount of fluorescence, which increases as a virus replicates in a cell, over time. They also applied one of three antiviral compounds to infected cells. These compounds are known to be effective in treating viral infections and operate via different mechanisms, targeting different parts of the virus or the host to prevent virus replication.

The research team measured five parameters to describe the course of the infection—including when the virus began to replicate, how quickly it replicated, and the maximum amount of virus growth—that together provide a signature of the antiviral compound's effect. Each of the three compounds had a different signature, which supports the idea that compounds with different signatures may operate in different ways. Comparing a compound's signature to those of known drugs could help narrow down the drug's target—information that takes considerable follow-up research after a population-based study.

"During drug development, we might create a number of different compounds that are structurally similar to a promising antiviral drug candidate," said Cameron. "Now, by comparing signatures, we can determine if these analogs act on the same targets and compare them to drugs known to be safe."

Using the device, the researchers can also tell if particular members of a viral population are susceptible to treatment and in which stage of the virus's lifecycle the treatment acts. For example, one class of drugs seems to target the strongest members of the virus population, which reduces the likelihood of the virus developing resistance to a treatment.

"This single-cell approach could also be useful for studying combinations of antivirals, as we can now see effects other than just the overall amount of killing," said Cameron. "For example, a drug

combination might slow the speed of virus replication, which might give the host's immune system time to clear the infection. You wouldn't see that from a population analysis."

Because they can quickly provide so much information, the team hopes that the single-cell approach will complement, or perhaps even replace, early screens for drug candidates. The approach can also be used to screen drugs for any disease for which a cell-based assay exists that monitors a change in fluorescence.

"The device is currently challenging to manufacture," said Cameron, "so we are working to make it more accessible so that it can be used by anyone."

More information: W. Liu el al., "More than efficacy revealed by single-cell analysis of antiviral therapeutics," *Science Advances* (2019). DOI: 10.1126/sciadv.aax4761 , advances.sciencemag.org/content/5/10/eaax4761

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