A new way to see viruses in action: Super-resolution microscopy provides a nano-scale look

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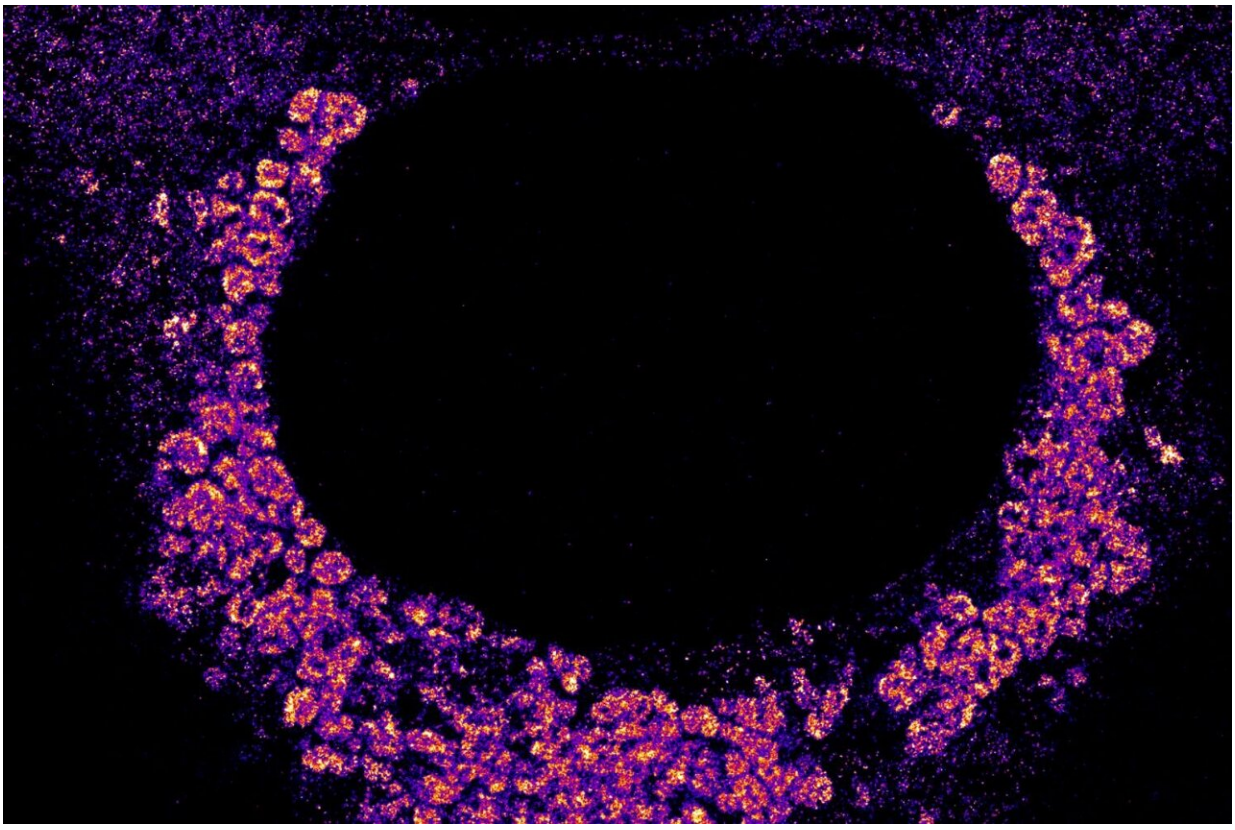

Viral RNA, labeled with a fluorescent dye, clusters around the nucleus of a cell infected with SARS-CoV-2, as captured through super-resolution microscopy. Credit: *Nature Communications* (2024). DOI: 10.1038/s41467-024-48991-x

A new, nano-scale look at how the SARS-CoV-2 virus replicates in cells may offer greater precision in drug development, a Stanford University team reports in *Nature Communications*. Using advanced microscopy techniques, the researchers produced what might be some of the most crisp images available of the virus's RNA and replication structures, which they witnessed form spherical shapes around the nucleus of the infected cell.

"We have not seen COVID infecting cells at this high resolution and known what we are looking at before," said Stanley Qi, Stanford associate professor of bioengineering in the Schools of Engineering and of Medicine and co-senior author of the paper. "Being able to know what you are looking at with this high resolution over time is fundamentally helpful to virology and future virus research, including antiviral drug development."

Blinking RNA

The work illuminates molecular-scale details of the virus' activity inside host cells. In order to spread, viruses essentially take over cells and transform them into virus-producing factories, complete with special replication organelles. Within this factory, the viral RNA needs to duplicate itself over and over until enough genetic material is gathered up to move out and infect new cells and start the process over again.

The Stanford scientists sought to reveal this replication step in the sharpest detail to date. To do so, they first labeled the viral RNA and replication-associated proteins with fluorescent molecules of different colors. But imaging glowing RNA alone would result in fuzzy blobs in a

conventional microscope. So they added a chemical that temporarily suppresses the fluorescence. The molecules would then blink back on at random times, and only a few lit up at a time. That made it easier to pinpoint the flashes, revealing the locations of the individual molecules.

Using a setup that included lasers, powerful microscopes, and a camera snapping photos every 10 milliseconds, the researchers gathered snapshots of the blinking molecules. When they combined sets of these images, they were able to create finely detailed photos showing the viral RNA and replication structures in the cells.

"We have highly sensitive and specific methods and also high resolution," said Leonid Andronov, co-lead author and Stanford chemistry postdoctoral scholar. "You can see one viral molecule inside the cell."

The resulting images, with a resolution of 10 nanometers, reveal what might be the most detailed view yet of how the virus replicates itself inside of a cell. The images show magenta RNA forming clumps around the nucleus of the cell, which accumulate into a large repeating pattern. "We are the first to find that viral genomic RNA forms distinct globular structures at high resolution," said Mengting Han, co-lead author and Stanford bioengineering postdoctoral scholar.

The clusters help show how the virus evades the cell's defenses, said W. E. Moerner, the paper's co-senior author and Harry S. Mosher Professor of Chemistry in the School of Humanities and Sciences. "They're collected together inside a membrane that sequesters them from the rest of the cell, so that they're not attacked by the rest of the cell."

Nanoscale drug testing

Compared to using an electron microscope, the new imaging technique

can allow researchers to know with greater certainty where virus components are in a cell thanks to the blinking fluorescent labels. It can also provide nanoscale details of cell processes that are invisible in medical research conducted through biochemical assays.

The conventional techniques "are completely different from these spatial recordings of where the objects actually are in the cell, down to this much higher resolution," said Moerner. "We have an advantage based on the fluorescent labeling because we know where our light is coming from."

Seeing exactly how the virus stages its infection holds promise for medicine. Observing how different viruses take over cells may help answer questions such as why some pathogens produce mild symptoms while others are life-threatening. The super-resolution microscopy can also benefit drug development. "This nanoscale structure of the replication organelles can provide some new therapeutic targets for us," said Han. "We can use this method to screen different drugs and see its influence on the nanoscale structure."

Indeed, that's what the team plans to do. They will repeat the experiment and see how the viral structures shift in the presence of drugs like Paxlovid or remdesivir. If a candidate drug can suppress the viral replication step, that suggests the drug is effective at inhibiting the pathogen and making it easier for the host to fight the infection.

The researchers also plan to map all 29 proteins that make up SARS-CoV-2 and see what those proteins do across the span of an infection. "We hope that we will be prepared to really use these methods for the next challenge to quickly see what's going on inside and better understand it," said Qi.

More information: Leonid Andronov et al, Nanoscale cellular

organization of viral RNA and proteins in SARS-CoV-2 replication organelles, *Nature Communications* (2024). DOI: 10.1038/s41467-024-48991-x