Stewart Wills

The Search for a Better COVID Test

Researchers are drawing on optics and photonics to improve the current "gold-standard" approach to coronavirus diagnostics—and to develop promising alternatives.

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A year and a half after the spread of COVID-19 first brought global lockdowns and social-distancing mandates, the world is itching to emerge from the pandemic's long shadow. But the much-discussed "return to normalcy" remains elusive.

Despite the amazingly rapid development of COVID vaccines, their rollout has been far from uniform from country to country. Moreover, SARS-CoV-2, the coronavirus behind the pandemic, has proved diabolically effective at churning out new, faster-spreading variants. As of this writing, one of those, the so-called Delta variant, was boosting infection rates and creating new health crises in undervaccinated areas. Its spread visibly deflated the recent Summer Olympics in Tokyo—where, amid a new COVID state of emergency, athletes found themselves competing in near-empty stadiums.

In short, while many of us understandably feel done with the coronavirus, it may not be done with us. Nor will SARS-CoV-2 be the last microbial enemy humanity will face. Indeed, the pandemic experience has underscored the importance of being able to diagnose infections fast, early and often—to track the spread of new viruses and to prevent local outbreaks from becoming global scourges.

Thus, in addition to further work on vaccines and treatments, scientists are eyeing more rapid, effective tests for SARS-CoV-2. Their research includes both schemes to improve existing diagnostics, and efforts toward innovative alternative modes for picking up COVID's traces. Many of these approaches rely at least partly on optical and photonic technologies, ranging from fluorescence to plasmonics to holography and deep learning. Here's a sample of some recent developments.

Improving the "gold standard"

Some of the new research is highlighting ways to speed up or otherwise improve what is almost universally called the "gold standard" of COVID testing, reverse-transcriptase polymerase chain reaction (RT-PCR). This method involves identifying traces of a virus' genetic signature in a patient sample—for example, from a nasal swab—by first transcribing fragments of viral RNA to DNA (the RT part) and then vastly amplifying the DNA to reach detectable levels (the PCR part).

The method's most common variety, real-time or quantitative PCR (qPCR), allows a quick optical readout of the result, by adding a fluorescently tagged probe molecule to the mix. With each round of amplification, fluorophores are released into the buffer solution, allowing the presence of the virus and the viral load to be detected, after sufficient amplification, via laser or LED excitation. (For more on the technique, see "Optics and the COVID-19 Pandemic," OPN, May 2020, p. 18.)

While optically read RT-PCR is considered the best current test for COVID-19, though, it has its drawbacks. One is that sufficiently amplifying the viral RNA takes 30 to 40 rounds of thermal cycling—heating and cooling—in bulky, specialized machines. That means completing the test takes an hour or more, and generally requires sending the samples away to third-party labs. Thus patients might wait several days to get a result. Further, as RT-PCR testing has ramped up in the face of the pandemic, some parts of the world have reportedly grappled with shortages of the chemical reagents that the technique requires.

A dash of plasmonics

Research led by Ki-Hun Jeong at the Korea Advanced Institute of Science and Technology (KAIST), Republic of Korea, has taken aim at some of these defects of RT-PCR by throwing in a dash of plasmonics. The KAIST team's work—which, Jeong told OPN, builds on efforts dating from before the pandemic—aims squarely at speeding up the thermal cycling of conventional real-time PCR, and at packing the whole testing chain into a radically smaller device.

At the heart of the system is an unusual engine for doing the required thermal cycling: a tiny array

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of row upon row of 180-nm-tall glass "nanopillars," each topped by a 40-nm-thick "nano-island" of gold. When a white LED illuminates the array, the subwavelength gold islands plasmonically confine and enhance the optical field, rapidly converting the light to highly localized heat. Turning off the LED allows similarly rapid cooling. In work published in early 2020, before the pandemic gripped the world, the team reported that the gold-topped nanopillars could execute the 30 thermal cycles required by some PCR tests in only three and one-half minutes—versus an hour or more for traditional laboratory PCR.

This year (ACS Nano, doi: 10.1021/acsnano.1c02154), the KAIST team extended the work to create a prototype point-of-care device, by adding a vacuum microfluidic chamber on top of the nanopillar array. In the device—which measures only 14×22 mm in area—tiny samples from patients are sucked into the chip and shunted to a ringlet of eight microscopic reaction chambers sitting on top of the gold nanopillar arrays. The addition of microfluidic handling, Jeong explains, helps both with the sample loading and with expelling bubbles that can form in the sample owing to the rapid thermal cycling.

The team reports that the combination of quick sample handling and blazing-fast, localized plasmonic heating and cooling allowed the prototype plasmofluidic chip to accomplish the required 40 thermal cycles for PCR in as little as five minutes—and to complete a full-fledged RT-PCR test for COVID-19, from sample loading to readout using a fluorescence microscope, in as little as 10 to 13 minutes. Jeong told OPN that the team is now working on miniaturizing the system's fluorescence detection optics, to move toward a compact, integrated device for point-of-care use.

Beyond gene amplification

While methods, such as qPCR, that are based on amplifying traces of viral RNA constitute the most common way to sniff out a coronavirus infection, other approaches are possible. Numerous teams are investigating ways to detect the virus' presence more directly. These schemes commonly use antibodies to lure any viral particles roaming around in the sample to a particular spot—and then employ ultrasensitive photonic techniques to detect the virus.

A compact, 14×22-mm prototype device from the Korea Advanced Institute of Science and Technology—which includes microfluidic chambers for sample handling and white-LED-activated plasmonic "nanopillars" for rapid plasmonic heating and cooling—can reportedly slash the required thermal-cycling time for PCR-based COVID tests.
Adapted from ACS Nano, DOI: 10.1021/acsnano.1c02154 (2021

A research team led by OSA Fellow Laura Lechuga, Catalan Institute of Nanoscience and Nanotechnology, Spain, for example, is developing nanophotonic biosensors that could serve in COVID-19 diagnostics and surveillance. The work is proceeding through CONVAT, a project funded under the EU's Horizon 2020 framework funding program. Lechuga's team reported on progress in a paper earlier this year (J. Phys. Photonics, doi: 10.1088/2515-7647/abd4ee).

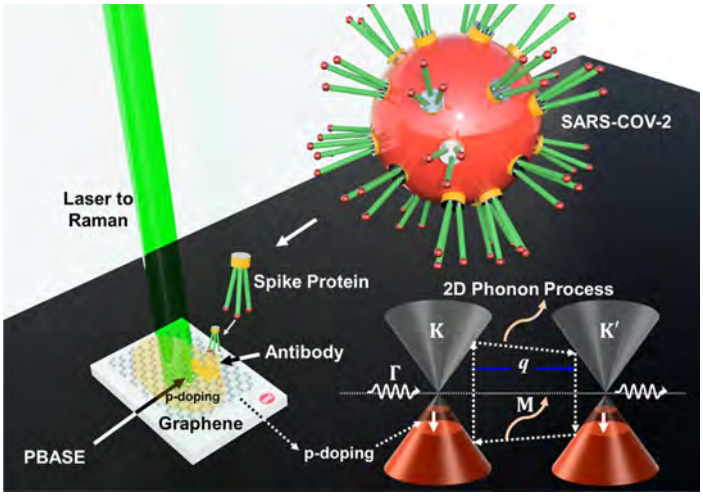
The project revolves around the creation of an integrated biosensor, centered on a bimodal-waveguide-based interferometer that's primed with COVID-19 antibodies. The antibodies fit, lock-and-key style, with antigens on the virus surface, and thus cause any viral particles present in the sample to clump along the waveguide. The clumped virus in turn alters the local refractive index, which is detected through changes in the interference pattern of evanescent

Some testing schemes under development use antibodies to lure viral particles in the sample to a particular spot—and then employ ultrasensitive photonic techniques to detect them.

waves from light passing through the bimodal waveguide. It's an approach that the lab has already demonstrated for detecting other clinical biomarkers (see "Nanophotonic Biosensors," OPN, April 2020, p. 24).

Graphene meets COVID

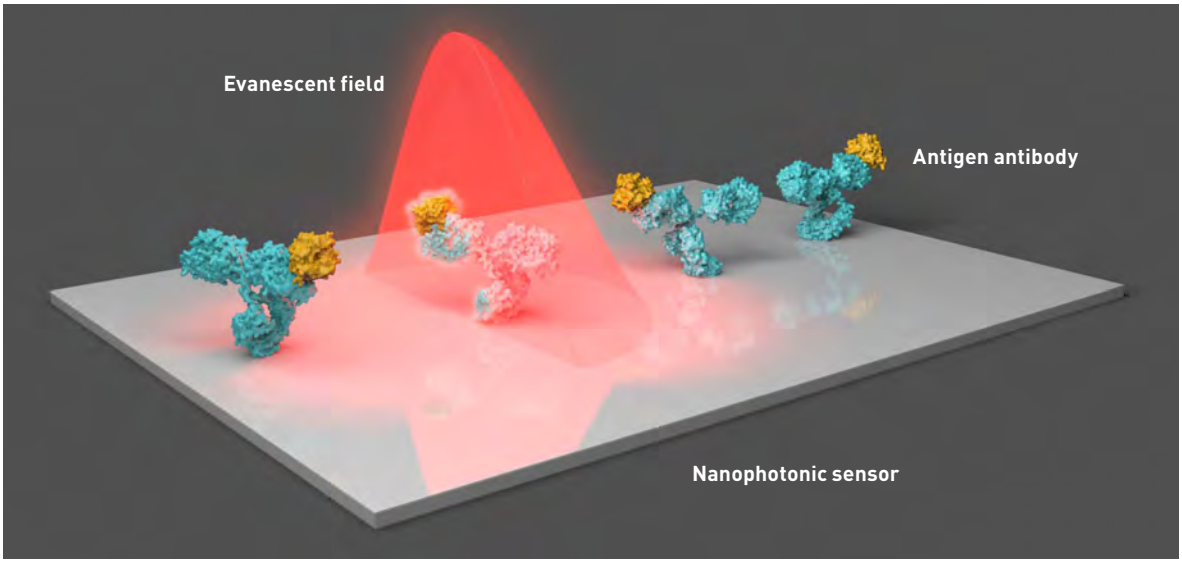
In work published this past June, researchers led by chemical engineer Vikas Berry at the University of Illinois at Chicago, USA, demonstrated antibody-based detection using another exotic substrate: the celebrated 2D material graphene (ACS Nano, doi: 10.1021/acsnano.1c02549). Berry told OPN that his team had already been working on the system "for the past five years or so" for picking up the signal of cancer cells and detecting certain neurological diseases. "When COVID came," he says, "we started to think about using this technology for [SARS-CoV-2] detection" as well.



A graphene-based detector prototype from the University of Illinois at Chicago, USA, senses the presence of the COVID spike protein through changes in the graphene's vibrational modes, picked up by laser Raman spectroscopy.

V. Berry/UIC

The team's approach depends on the specific properties of graphene—atomically thin sheets of carbon atoms, bound together in a hexagonal lattice. This



A team led by OSA Fellow Laura Lechuga is developing a fast COVID saliva test based on a bimodal waveguide spiked with SARS-CoV-2 antibodies. The signal is read from interference changes in the evanescent field around the waveguide.

M. Soler et al., Opt. Photon. News **31**(4), 24 (2020)

ultrathin structure, Berry says, makes graphene an extremely good "phonon transducer." In other words, small changes in the material's electronic properties lead to substantial, easily measured changes in its phonon properties—the vibrational modes of the elastic bonds between the atoms.

In the team's proof-of-concept, antibodies to the SARS-CoV-2 spike protein (S protein) are affixed to the graphene surface via a thin polymer interface. Then a patient sample (such as saliva) is added. S proteins from viral particles in the sample, attaching to the antibodies affixed to the graphene, suck electrons out of the 2D material, leaving an excess of "holes" behind. This change in the local electronic structure of the graphene alters the material's vibrational modes—a shift that's in turn read via Raman spectroscopy using a 532-nm laser. (In Raman spectroscopy, a small fraction of energy from a monochromatic laser source, shone on a material, is inelastically scattered by the material's vibrating molecular bonds. Shifts in the spectral peaks of the returned light can provide highly sensitive information about the vibrational modes.)

The method can reportedly detect the spike protein to levels of femtograms (10^{-15} g) per milliliter of solution, which Berry views as "pretty competitive in resolution" with other antigen-based detectors. And, he says, the detection itself "happens in milliseconds," making it extremely fast. The team is now working on a microfluidic add-on for handling of saliva samples, and on engineering the detector's electronics and optics to bring it closer to a commercial device.

Infrared and TR-FRET detection

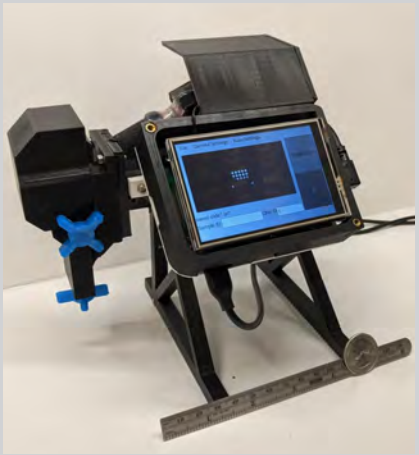
Still other groups have put their own optical spin on direct detection of viral particles, biomarkers or antibodies. Researchers from Monash University and the Peter Doherty Institute for Infection and Immunity, Australia, for instance, have reported a proof-of-concept of a simple, infrared-based approach that screens for COVID-19 biomarkers in saliva samples (Angew. Chem. Intl. Ed., doi: 10.1002/anie.202104453).

In this approach, a patient with COVID-19 symptoms "dribbles out" a stream of saliva onto an infrared transflection substrate. The sample is dried, and its infrared spectra are read using an off-the-shelf portable infrared spectrometer (modded to allow for contactless, high-throughput scanning). The spectra—which, according to the authors, constitute "a chemical snapshot of the entire saliva chemistry, including COVID-19 markers"—then

Detecting COVID antibodies

In addition to fast virus detection in patients who may show no symptoms, the pandemic presents another testing challenge: serology—checking the blood of known COVID patients for SARS-CoV-2 antibodies, to measure the patient's immune response. Rapid, accurate serology is crucial in assessing vaccine effectiveness, and can help determine how well a patient might respond to specific treatments. Unfortunately, best-of-breed serology tests sometimes require expensive, heavy and complex instruments.

A team at Duke University, USA, is working toward a more straightforward, user-friendly and sensitive point-of care approach for COVID serology (Sci. Adv., doi: 10.1126/sciadv.abg4901). The system is built on a platform demonstrated at Duke in 2017. Called D4, the system works by adding patient blood to a chip with fluorescently tagged antigens for target antibodies, which attach to those antibodies if they're present in the sample. Then, a second set of "capture" antigens grab onto the now-tagged antibodies and hold them at a specific, fixed point on a polymer-brush substrate—where laser-induced fluorescence reveals their presence.



The Duke team's "D4Scope" for COVID serology embeds laser, optics, camera and control circuitry in lightweight setup that fits in a small backpack.
J. Heggestad/Duke University

When the pandemic hit, Ph.D. students Jake Heggestad, David Kinnamon and Jason Liu worked with other Duke scientists to adapt the D4 system to a simple, portable setup for COVID serology. Heggestad developed SARS-CoV-2-specific assays for the D4 chip. Kinnamon devised an ingenious microfluidic add-on that automates the system's complex fluid-handling requirements, with no external power needed. And Liu—who describes himself as "a bit of a hardware hacker"—found a way to pack together off-the-shelf components including a CMOS camera, a laser, control circuitry, filter and lens into a battery-powered detector that can fit into a small backpack.

According to the team, the system can detect the antibody response against multiple antigens in a single test—at a price tag orders of magnitude below lab-scale alternatives. Another plus is its adaptability, which could enable it to test not only for the immune response to new COVID variants, but for unknown future microbial scourges.

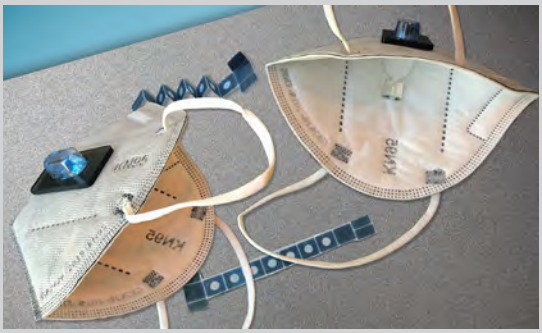
Toward a COVID "breathalyzer"

A holy grail of COVID testing would be a simple breath test. Researchers at the Harvard University Wyss Institute for Biologically Inspired Engineering and the Massachusetts Institute of Technology, USA, have now prototyped such a system: a facemask that reportedly can detect the presence of SARS-CoV-2 in breath droplets in 90 minutes or less (Nat. Biotechnol., doi: 10.1038/s41587-021-00950-3).

The process begins by pressing a button on the mask, which releases a dose of water that dissolves a set of freeze-dried, shelf-stable biological elements embedded in the fabric. Those elements then go to work on any SARS-CoV-2 virus in the wearer's breath droplets: popping open, or "lysing," the virus to extract its RNA; amplifying the genetic material through a room-temperature technique called RT-RPA; and then detecting the amplified genes through a previously designed system, dubbed SHERLOCK, that leverages the CRISPR gene-editing platform. The result is read in a simple signal that, according to co-lead author Peter Nguyen of the Wyss Institute, is "similar to what you see in [an at-home] pregnancy test."

The COVID facemask effort grew out of work the team was already doing to embed so-called synthetic-biology systems into wearables, for detection not only of viruses but of other hazards such as nerve agents and chemical toxins. Some of the systems (such as the COVID facemask) signal a positive result colorimetrically; others report on their molecular targets via fluorescent or luminescent outputs from optical fibers embedded in the wearable fabric.

While the COVID-detecting facemask for general use—which, Nguyen says, costs only US$5 to make—will likely be the first such wearable to come to market, the team sees other potential uses in more specialized settings. One possible example: a lab coat for scientists that changes color or gives a fluorescent signal when exposed to a specific toxin.



Prototype of the Harvard–MIT team's facemask-based COVID detection system.

F. Frankel and MIT News Office

pass to a computer for statistical analysis, to infer the likelihood of a coronavirus infection. The team writes that, using this approach, it was able to identify the signature of SARS-CoV-2 in 27 of 29 infected patients that it tested.

In yet another take on optical detection of SARS-CoV-2, researchers affiliated with the University of Helsinki, Finland, have developed an antigen-based test for the virus that they say can be conducted in as little as 10 minutes (mBio, doi: 10.1128/mBio.00902-21). The approach uses the optical technique known as time-resolved Förster resonance energy transfer (TR-FRET).

TR-FRET works because pairs of different fluorophores—called donors and acceptors—can transfer energy to one another when they're in close enough proximity, an exchange that leads to the emission of a photon at a distinct wavelength. In the Finnish team's system, SARS-CoV-2 antibodies that are labeled with such donor and acceptor fluorophores are added into the sample to be tested. The presence of virus in the sample will cause the labeled antibodies to bind simultaneously against the virus, bringing the donor and acceptor fluorophores into close contact, triggering the donor–acceptor energy transfer and causing measurable light emissions.

The team reportedly found high specificity and sensitivity in the tests it conducted. And the researchers believe that "the test format could easily be adapted to high-throughput testing" and could "be applicable to a wide variety of infectious and perhaps also non-infectious diseases."

RBCs, holography and deep learning

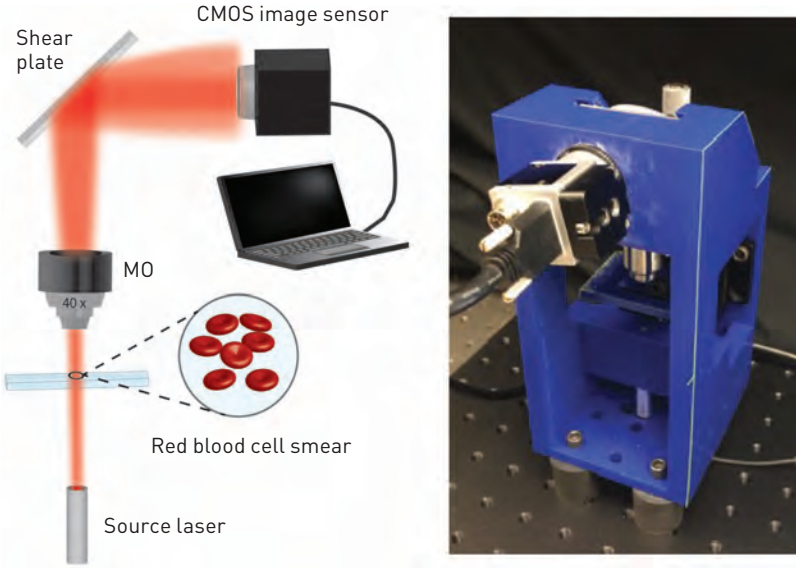
An intriguing and very different approach to coronavirus detection was proposed in early May by a research group headed by OSA Fellow Bahram Javidi, University of Connecticut, USA (Opt. Lett., doi: 10.1364/OL.426152). This approach hinges not on detecting amplified viral RNA or antibody-snagged viral particles, but on picking up COVID-19's impact on a patient's red blood cells (RBCs).

Previous research has shown that, particularly in severe cases, COVID-19 can cause measurable changes in laboratory parameters such as the RBC distribution width (an indicator of RBC volume and size variation), as well as other aspects of RBC morphology. Javidi's group has tested out a system to detect these changes in blood smears from patients in a compact, field-ready setup, through

The many coronavirus testing improvements now being trialed point to the continued role of optical techniques in addressing this and other global problems.

a combination of digital-holographic microscopy (DHM) and deep learning.

In the team's 3D-printable prototype device, an RBC smear from a patient—such as one might get from a finger prick in a doctor's office—is placed on a microscope slide in a DHM setup. A 635-nm diode laser beam passes through the sample and is magnified through a microscope objective, after which the transmitted beam strikes an angled glass plate. The front and back surfaces of the glass create two reflected beams, separated by a lateral shear, that then self-interfere. The resulting time-varying interference patterns—digital holograms—are captured by a CCD camera in 10-second, 20-fps videos.



A 3D-printable device from the lab of OSA Fellow Bahram Javidi uses a field-portable shearing digital-holography microscope and deep learning to detect COVID-19 in patients through the disease's effects on their red blood cells.

T. O'Connor et al., Opt. Lett. **46**, 2344 (2021)

Next, computer Fourier analysis extracts the phase profiles of the RBCs from the video hologram data. Features pulled from the phase profiles are then piped into a deep-learning recurrent neural network that's been trained to classify samples as COVID-positive or COVID-negative based on RBC morphology. In preliminary tests with 24 separate blood samples, the team found that the setup correctly identified eight of ten patients known to be COVID-positive (80% sensitivity), and also correctly identified 13 of 14 individuals known to be COVID-negative (92.86% specificity).

While the researchers see distinct advantages to their DHM system in "cost, accessibility and time to results," they acknowledge that it does have some drawbacks. In particular, it's unclear whether the RBC morphology effects found in moderate and severe COVID cases, such as those studied in the proof-of-concept, can also be used to root out asymptomatic or mild cases. The team plans to continue working on validating the system's effectiveness in detecting milder cases, and on exploring the use of "different cell types such as white blood cells for COVID classification."

Optics' continued role

Like many researchers working to find better, faster COVID-19 tests, Javidi hopes that the work may particularly benefit areas that have struggled with resources to fight the pandemic. "Countries with under-resourced health care systems may benefit from this low-cost, rapid testing," he told OPN.

And the many COVID-19 testing improvements now being trialed—only a small fraction of which are sampled here—point to the continued role of optical techniques, such as the holography at the center of Javidi's work, in addressing this and other global problems.

"This year is the 50th anniversary of [Dennis] Gabor's Nobel Prize for holography," noted Javidi. "It is great to see that holography continues to be applied in new and important ways." OPN

Stewart Wills is the senior editor of OPN.