Lawrence Livermore National Laboratory

Science & Technology Review

June 2021

COVID

TACKLING THE COVID-19 PANDEMIC

Also in this issue:

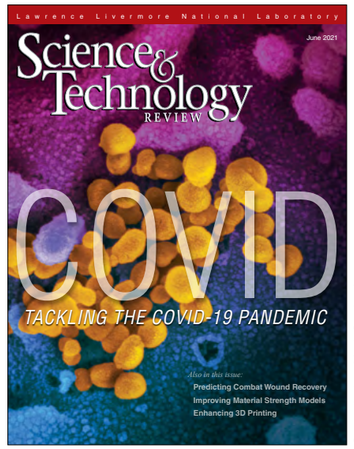
Predicting Combat Wound Recovery

Improving Material Strength Models

Enhancing 3D Printing

About the Cover

The article beginning on p. 4 describes the multidisciplinary research undertaken by Lawrence Livermore National Laboratory to meet health care challenges posed by the COVID-19 pandemic. The scanning electron microscope image on the cover shows SARS-CoV-2 (yellow)—also known as 2019-nCoV, the virus that causes COVID-19—isolated from a patient in the United States, emerging from the surface of cells (blue/pink) cultured in the lab. (Image courtesy of NIAID.)



Cover design: Mark Gartland

About *S&TR*

At Lawrence Livermore National Laboratory, we focus on science and technology research to ensure our nation's security. We also apply that expertise to solve other important national problems in energy, bioscience, and the environment. *Science & Technology Review* is published eight times a year to communicate, to a broad audience, the Laboratory's scientific and technological accomplishments in fulfilling its primary missions. The publication's goal is to help readers understand these accomplishments and appreciate their value to the individual citizen, the nation, and the world.

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Please address any correspondence (including name and address changes) to *S&TR*, Mail Stop L-664, Lawrence Livermore National Laboratory, P.O. Box 808, Livermore, California 94551, or telephone (925) 422-1266. Our e-mail address is str-mail@llnl.gov. *S&TR* is available online at str.llnl.gov.

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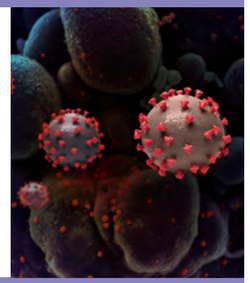
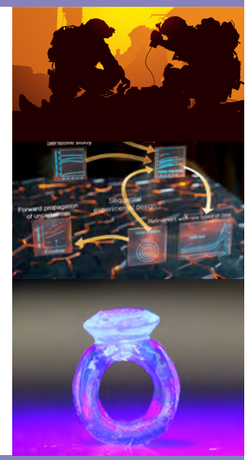


Research Highlights



Departments

Aligning Air Pollution and Drizzle

Laboratory scientists working with researchers from the Scripps Institution of Oceanography and international collaborators enhanced their understanding of rainfall's aerosol-scavenging effects on pollution through improvements to two state-of-the-art global climate models that result in better agreement with observed drizzle rates. Their findings appear in the January 11, 2021, issue of *Nature Geoscience.*

Atmospheric aerosols, which have a significant effect on the Earth's radiative energy balance and air quality, can be influenced by rainfall through wet removal processes. The research team demonstrated that more frequent, but less intense, rainfall has a disproportionate control on aerosol burden making it more effective at removing pollutants from the atmosphere than heavy rain. By improving the representation of convection in two global climate models, the Department of Energy (DOE) Energy Exascale Earth System Model version 1 and the National Science Foundation/DOE Community Atmosphere Model version 5, the relative frequency of light rain is reduced and the anticipated aerosol burden in the atmosphere increased 17 percent.

"The current models include too much light rain, washing out pollutant projections," says Shaocheng Xie, one of the Livermore authors. "In future climate projects, even if precipitation is expected to increase, its impact on modeled aerosol concentration would depend on the occurrence of light rain changes." This change to the model, according to researchers, addresses underestimation of aerosols in the atmosphere, especially over regions in tropical rain belts. In its paper, the team notes that aerosol radiative effect is a major source of uncertainty in climate change projections.

Contact: Shaocheng Xie, (925) 422-6023 (xie2@llnl.gov).

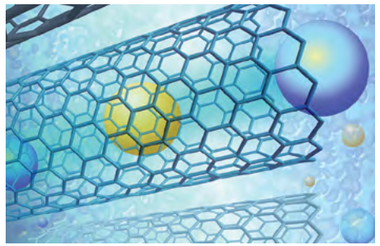
Reconstructing the Primordial Solar System

Livermore scientists have linked planetary bodies, shuffled by the gravitational effect of the formation of Jupiter and Saturn, to their initial locations by studying the compositions of meteorites originating from the asteroid belt between Mars and Jupiter. The research appears in the February 1, 2021, issue of *Earth and Planetary Science Letters*.

Tracing the source material of planetary bodies requires signatures established during planetary body formation. Isotopic anomalies of nucleosynthetic origin are powerful tools for fingerprinting the building material from which planetary bodies amassed. The asteroid belt contains a collection of materials swept up from the solar system and multiple, spectroscopically distinct asteroid families. The meteorites located there derive from at least 100 distinct parent bodies with diverse chemical and isotopic signatures. "The significant reorganization of the early solar system due to giant planet migration has hampered our understanding of where planetary bodies formed," says Jan Render, Livermore postdoc and lead author of the paper. "By looking at the makeup of meteorites from the asteroid belt, we determined that their parent bodies must have accreted from materials from very different locations in the early solar system."

The researchers measured the nucleosynthetic isotope signatures in the elements neodymium and zirconium from meteorite samples and determined that the elements were characterized by relative deficits in isotopes hosted by a certain type of presolar material. Their data correlates with nucleosynthetic signatures observed in other elements, demonstrating that the protoplanetary matter in the early solar system was distributed as a gradient according to heliocentric distance.

Contact: Gregory Brennecka, (925) 423-8502 (brennecka2@llnl.gov).



Francesco Fornasiero

Advancing Hemodialysis

Laboratory researchers have discovered that carbon nanotube membrane pores could enable ultra-rapid dialysis processes to greatly reduce treatment time for hemodialysis patients. The pores could also provide a solution to the permeability-versus-selectivity tradeoff, allowing to sieve out larger molecules without reducing the ion filtration rate. Initial results were published in the February 3, 2021, issue of *Advanced Science*.

Carbon nanotube membrane pores are graphitic cylinders with diameters thousands of times smaller than a human hair. By applying a concentration gradient across a porous membrane, ions or molecules smaller than the pore diameters can be driven from one side of the membrane to the other while blocking anything too large to fit through the pores. Small ions, such as potassium, chloride, and sodium, were found to diffuse through these tiny pores more than an order of magnitude faster than when moving in bulk solution. "The general consensus in the literature has been that diffusion rates in pores of this diameter should be equal to, or below, what we see in bulk," says Livermore scientist Steven Buchsbaum, lead author of the paper. "We did not expect this result."

Enhanced ion transport could also enable supercapacitors with high power density even at pore sizes approaching those of the ions. Livermore capabilities in computational simulation and nuclear magnetic resonance spectroscopy supported the team's study of ion movement inside carbon nanotubes. "Our findings provide a new example of exciting nanofluidic phenomena," says Francesco Fornasiero, the principal investigator.

Contact: Francesco Fornasiero, (925) 422-0089 (fornasiero1@llnl.gov).

The COVID-19 Experiment

FROM the moment COVID-19 entered our vocabulary, we have all been a part of a global science experiment. Approaches to treatments, vaccines, and ways to work and learn have been tested and fine-tuned in real time with no one sure exactly how the experiment will conclude.

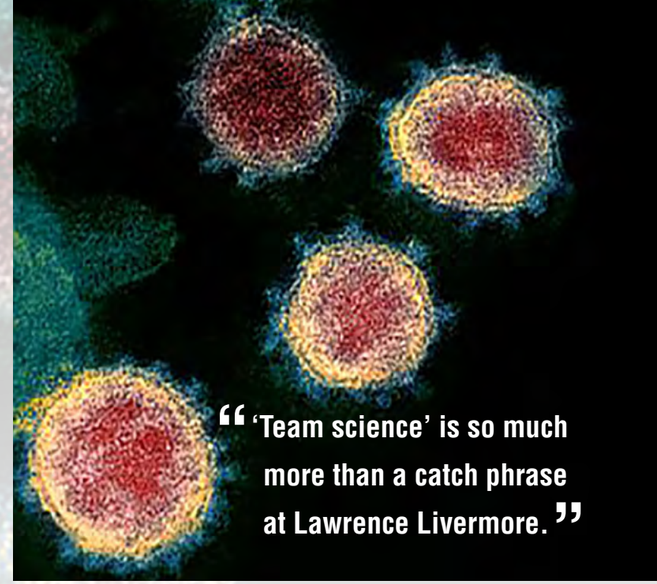
COVID-19 has affected the life of everyone on the planet. As a scientific leader at Lawrence Livermore, I am proud to introduce in this issue of *Science and Technology Review* the many ways the Laboratory research contributed to the fight to beat the pandemic. Future issues will highlight Livermore's technology transfer efforts as well as our operational adaptations and resilience.

Our COVID-19 science and engineering has been strengthened by our participation with high-performing and collaborative teams. Together with our partners in the other 16 Department of Energy (DOE) national laboratories, we worked as a part of DOE's National Virtual Biotechnology Laboratory and as a member of the COVID-19 High-performance Computing (HPC) Consortium. In addition, we benefited from close working relationships within the University of California (UC) system of ten universities, five major medical centers, and three UC-affiliated national laboratories.

For Lawrence Livermore, I knew the key to meeting the COVID-19 challenges would be our tradition of working together in multi-discipline teams to achieve tangible outcomes as well as our storied history of integrating bioengineering, biotechnology, and biocomputational tool sets. In many ways, Livermore had been readying itself for such a grand challenge by building and improving detection systems and other precise instrumentation, and developing and employing advanced computational models and HPC resources.

This month's feature, Tackling the COVID-19 Pandemic, highlights Livermore research directed at understanding the SARS-CoV-2 virus that causes COVID-19 and potential treatments for those infected. Our results are the outcome of multidisciplinary Laboratory teams in which each member is an important part of the effort. "Team science" is so much more than a catch phrase at Lawrence Livermore. It is quite simply the way we work, always. Team science is the key to our enduring success.

External research partnerships are highlighted as well. For example, rather than apply resources to designing or manufacturing unique COVID-19 testing swabs, we applied our testing and additive manufacturing expertise to confirm the best design (see p. 7). The value we place on partnerships manifests in research achievements as well as in technology transfer—translating early innovation into commercial products—for the COVID-19 fight, the focus of our next *S&TR* issue.



A transmission electron microscope image shows SARS-CoV-2, the virus that causes COVID-19, isolated from a patient in the United States. Virus particles are emerging from the surface of cells cultured in the lab. The crown-like spikes on the outer edge of the virus particles give coronaviruses their name. (Image courtesy of NIH.)

A critical element in Livermore's ability to quickly address COVID-19 is our Laboratory Directed Research and Development (LDRD) program. We were able to tune the direction of two ongoing LDRD projects and to solicit proposals, review them, and approve a few new targeted projects. Over time, LDRD-supported research has advanced scientific discovery in new and beneficial directions and enabled the Laboratory to amass tools and capabilities that were able to support a response to this new challenge. For example, biotechnology and biodetection research initially proposed to meet Livermore's mission to combat bioterrorism have been important components in identifying antiviral and antibody candidates for COVID-19 treatment (see p. 9).

Lawrence Livermore was built to address the most difficult and most important challenges to the nation. Stepping back for a moment from the experiment we have been living in, I am proud of the Laboratory's contributions to the fight so far. As a laboratory, we will continue to understand more and to generate more questions. And I believe that a year from now, everyone at Livermore will feel satisfaction and gratification to have been a part of the ongoing fight against this pandemic.

■ Patricia Falcone is deputy director of Science and Technology.

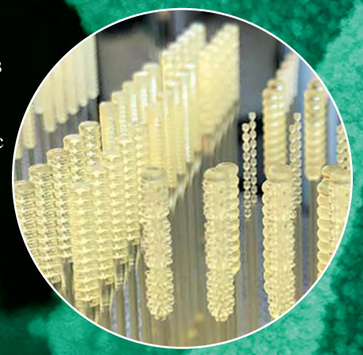
S&TR June 2021

Tackling the COVID-19 Pandemic

To help the United States fight the COVID-19 pandemic, Lawrence Livermore did what it does best: draw on existing expertise and research activities to quickly bring together interdisciplinary teams and diverse technologies to address an urgent national challenge.

LONG before the COVID-19 pandemic gripped the globe, Lawrence Livermore National Laboratory had been preparing to help the nation contend with biological threats. From its inception, Livermore has focused its scientific and technological expertise on strengthening the security of the United States. Whatever the challenge involves—enhancing the nation's defense, reducing threats from terrorism, or addressing other issues of national importance—the Laboratory responds, creating teams from different disciplines and combining diverse technologies to find answers and solutions. The approach was no different when COVID-19 swept the nation in early 2020. Senior science advisor David Rakestraw, who coordinates the Laboratory's COVID-19 technical response, says, "Decades of mission-critical research plus current programmatic work conducted at the Laboratory have helped prepare us for this moment, such that we are at the forefront of many of the technologies relevant to addressing the challenges this pandemic presents."

In late January 2020, the Laboratory was alerted to the existence of a new, highly contagious novel coronavirus that



This image shows SARS-CoV-2 (round magenta objects) emerging from the surface of cells cultured in the lab. SARS-CoV-2, also known as 2019-nCoV, is the virus that causes COVID-19. (Image courtesy of NIAID.)

HPC Efforts Put All Hands on Deck

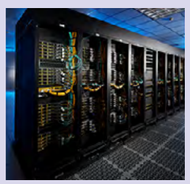
The Laboratory has long been involved in using supercomputing in the service of the nation and biological research applications. In 2013, the Laboratory established the Computational Predictive Biology initiative to address the evolution of diseases and biological threats and apply advanced high-performance computing (HPC) to biological research. The initiative became the foundation of a national collaboration—Biological Applications of Advanced Strategic Computing—of Department of Energy (DOE) laboratories, government agencies, academic institutions, and industry members.

Livermore's Deputy Associate Director for Computing Jim Brase says, "At first, we focused on simple approaches and pathways, but as system and facility capabilities grew, we started addressing the complexities of biology—efforts that required complicated computer models and enormous data sets**."** Three more collaborations formed to bring the power of HPC to human health challenges, such as cancer and infectious disease. A strategic partnership between Livermore and the American Heart Association creates computational tools to address cardiovascular disease and drug safety. Accelerating Therapeutics for Opportunities in Medicine, a consortium founded by DOE, the National Cancer Institute (NCI), and Lawrence Livermore, combines HPC, diverse biological data, and scientific expertise to accelerate discovery of cancer therapies. NCI also teamed up with Lawrence Livermore, Los Alamos, Argonne, and Oak Ridge national laboratories in a pilot program to integrate supercomputing into cancer treatments. (See *S&TR* November 2016, pp. 4–11.) The Laboratory's expertise in predictive biology is also a key element of its Biosecurity program, which focuses on detection, characterization, and mitigation to keep the world safe from ever-changing biological threats.

With the arrival of COVID-19, DOE formed the National Virtual Biotechnology Laboratory, a consortium of 17 national laboratories, including Lawrence Livermore, Los Alamos, Lawrence Berkeley, and Sandia. Supercomputing facilities at Livermore and throughout the complex are being used to address epidemiological modeling, manufacturing, molecular design for medical therapeutics, testing R&D, and viral fate and transport. In March 2020, the White House Office of Science and Technology, DOE, and IBM launched the COVID-19 HPC Consortium to provide COVID-19 researchers with access to the world's most powerful supercomputers including the Laboratory's newest supercomputer cluster, the 6-petaFLOP Ruby. The consortium currently supports nearly 100 projects around the globe.

Livermore senior science advisor David Rakestraw, who coordinates the Laboratory's COVID-19 technical response, notes, "Over the past seven years, we put a large focus on using the computational resources at the Laboratory to try to accelerate the timescales for developing a response to an emerging biological threat. We did so by using our extensive computational capabilities and developing partnerships with universities, drug companies, and tech companies. That effort put us in a position where we had the applicable tools and partnerships in place to help with the pandemic response."





Livermore's supercomputers Quartz, Lassen, and Corona (left to right) are among those leveraged in the fight against COVID-19.

was first detected in China. "We were very involved with the initial concern over this unusual virus and what it might mean if it were to spread more widely," says Rakestraw. The Laboratory was well-positioned to help, as its research areas span from the molecular level to the national-response level. When the SARS-CoV-2 novel coronavirus arrived on the nation's shores, the Laboratory had tools and people in place to begin delving into the threat and making the "unknown" known. The Laboratory's COVID-19 efforts focus on three critical science areas—medical equipment, detection, and medical countermeasures—bringing together teams to tackle a kaleidoscope of projects for each.

Equipping for the Pandemic

By March 2020, the U.S. Department of Energy (DOE) established a National Virtual Biotechnology Laboratory (NVBL) to bring all the relevant capabilities of its 17 national laboratories to bear on the science and technology challenges of the disease, such as supply chain issues of critical medical supplies and equipment. For Lawrence Livermore, one of the national laboratories tasked with addressing this challenge, the focus turned to ventilators, consumables such as testing kits, and masks and respirators.

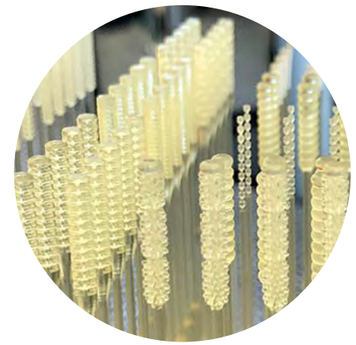
In the early phase of the pandemic, a nationwide shortage of ventilators loomed large as waves of critically ill people flooded hospitals in the Northeast United States. Immediately after the San Francisco Bay Area shelter-in-place order took effect in mid-March, a Livermore team headed by Jack Kotovsky formed with the goal of designing an easily assembled, durable mechanical ventilator using readily available parts not required by commercial ventilator manufacturers. Over long hours, the "skunk-works" team did most of their work—designing, prototyping, and testing—remotely, from home offices and garages. Within a week they had a conceptual design and

Early in the pandemic, a "skunk-works" team including (left to right) Ken Enstrom (seated), Greg Norton, and Aaron Sperry developed a production-ready ventilator built from components that do not impact the existing supply chain.

tried out components on a plywood "test stand." Two weeks later, an integrated system housed in a portable case was ready for full functionality testing. After five weeks, design, performance data, and accompanying documentation were submitted to the U.S. Food and Drug Administration (FDA) for emergency use authorization (EUA) approval. Just three months from the team's initial meetings, the Laboratory, working with industry partner BioMedInnovations, had created and tested an easily reproducible prototype and obtained EUA approval—a process that typically takes years. Kotovsky says, "When faced with global engineering challenges, like the ventilator shortage, it is hard to imagine a place better than Lawrence Livermore to find a solution. I am enormously proud of our team and grateful to the Laboratory for facilitating the work."

Around the same time, another Laboratory team formed to test prototype, nasal swabs manufactured via three-dimensional (3D) printing. When COVID-19 struck, only two companies—one in Italy and one in Maine—were manufacturing the swabs used to take samples of mucus from the nasal passages for testing. However, operations in the Italian company were severely impacted by the pandemic, and the Maine company was quickly overwhelmed with orders. Industry, academia, clinicians, and labs started a wide-scale grassroots effort to supply millions of 3D-printed COVID-19 test swabs.

Livermore's ad hoc, rapid response team of engineers including Angela Tooker, Eric Duoss, Maxim Shusteff, Razi Haque, Monica Moya, Dennis Freeman, Greg Larsen, Jack Davis, Du Nguyen, and Joshua DeOtte provided mechanical, sterilization, and other laboratory testing of hundreds of individual swabs based on more than a dozen novel designs from 3D-printing and biotech companies. Nearly six inches long, the swabs needed to be flexible enough to reach cells in the uppermost part of the throat without damaging tissue, but rigid enough to reach several inches into the nasal cavity and be rotated to collect samples. Tooker says, "We developed protocols and tested the 3D-printed swabs to provide quantitative data on swab performance, help narrow down design possibilities, and give clinicians confidence that the swabs were safe and effective to use." Mechanical tests conducted at Livermore's Advanced Manufacturing Laboratory simulated how the swabs might be used in a clinical setting.



Livermore researchers mechanically tested hundreds of 3D-printed nasal swabs based on more than a dozen designs developed through an informal consortium. The swabs pictured here were 3D-printed at Livermore from a biocompatible, surgical-grade resin and tested in Livermore's Advanced Manufacturing Laboratory.

Other Laboratory efforts to address equipment shortages included a partnership with industry and Oak Ridge National Laboratory to rapid-prototype 3D-printed vials for containing and transporting swabs used to collect samples and to examine shortages that could occur during vaccine distribution. Other efforts studied thermal methods for decontamination of N95 masks for reuse. "Along with the rest of the DOE complex, we keep analyzing the supply chain issues created by the pandemic with the goal of getting ahead of the curve and helping where and how we can," says Chris Spadaccini, division leader for Materials Engineering.

The Virus Hunt: Detection and Diagnosis

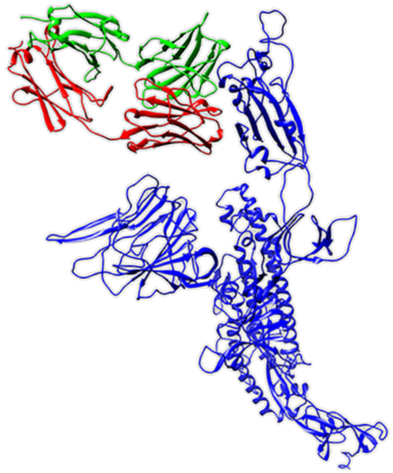
Livermore researchers support national efforts to detect the SARS-CoV-2 virus, developing rapid and accurate diagnostic technologies building on previous genomics work and existing research that supports the military. Biologist Crystal Jaing belongs to an NVBL working group tasked with developing new approaches for improved diagnostic testing. "One of the first projects we took on for the NVBL was to identify ways to extract viral RNA from nasal swabs that didn't use the chemical reagents employed by commercially available kits," says Jaing. In addition to Jaing, the Livermore team includes Jessica Wollard, Aubree Hinckley, James Thissen, Michael Morrison, and Nisha Mulakken.

Kit reagents break down the cells from the nasal swab samples to release DNA and RNA, so COVID-19 diagnostics tests can be performed. Most diagnostic tests use polymerase chain reaction (PCR),

a fast, highly sensitive, DNA-based technology, to identify the pathogens. The surge in demand for PCR-based COVID-19 tests had a rattle-down effect, leading to a shortage of reagents used in RNA extraction kits and a subsequent shortage of the kits themselves. Jaing and her team developed RNA extraction methods based on different reagents and tested them on archived COVID-19 samples provided by the California Department of Public Health (CDPH). The team compared their PCR results to results CDPH obtained from an FDA-approved SARS-CoV-2 test kit. Two of the Livermore-developed reagent formulations performed well and are now with CDPH, being validated on a larger sample size. Laboratory researchers also developed a rapid PCR diagnostic that showed promise for at-home use.

For another task, Livermore built on its decades of analysis of DNA and identification of pathogens, specifically by extending the capabilities of the Lawrence Livermore Microbial Detection Array (LLMDA). Developed at the Laboratory

Livermore's James Thissen (left) and Crystal Jaing (right) examine a microarray slide for the Lawrence Livermore Microbial Detection Array (LLMDA). In a single test, the LLMDA can detect up to 12,000 microbial species, including the COVID-19 virus and the viruses that cause co-infections, such as Influenza A, Human metapneumovirus, Human parvovirus, and Haemophilus influenzae.



A Livermore-designed machine-learning platform generated computer models showing the 3D structure of an antibody candidate (green and red) alongside the spike protein of SARS-CoV-2 (blue). The antibody, which binds with SARS-CoV-1 but not SARS-CoV-2, was the starting point for designing an antibody to the virus that causes COVID-19.

in 2008 by a team of biologists and bioinformatics specialists, the LLMDA simultaneously identifies up to 12,000 microbial species in a single test within 24 hours. (See *S&TR* April/May 2018, pp. 16-17.) In spring 2020, Jaing's team set out to design a COVID-19 "signature" that could be used by the LLMDA to detect the presence of the SARS-CoV-2 virus. Jaing explains, "First, we had to identify regions in the genome of the SARS-CoV-2 virus that were unique from other viruses, were conserved—that is, can be detected in the sequences of many variants—and were short in length." The SARS-CoV-2 genome has about 30,000 base pairs, a typical length for a coronavirus but long for viruses in general. For the array, the team had to identify regions about 60 bases long. Using the Laboratory's bioinformatics capabilities, the team analyzed more than 41,000 SARS-CoV-2 genomes in just a few months. They identified 78 conserved regions, designed signatures corresponding to those regions, and incorporated them into the array. "With this many signatures from conserved regions, we should still be able to detect and identify the virus as it mutates and evolves over time," says Jaing.

Jaing's team was also tasked with identifying additional pathogens in COVID-19 samples to ascertain what other infections were commonly found with the disease and whether there was a pattern. CDPH provided 200 samples taken from nasal and nose-and-throat swabs, evenly divided between those samples that had tested positive for COVID-19 and those that had not. After analyzing the samples with the LLMDA, the team found some of the COVID-19-positive samples also had co-infections, such as Influenza A and Streptococcus pneumoniae. "It will be interesting to compare our results to the patient data and see whether the more severe COVID-19 cases have more than one infection," says Jaing.

Designing Medical Countermeasures

Identifying that the virus is present is only the beginning. Fighting the virus requires medical countermeasures: vaccines to prevent or mitigate infection, and antibodies and antivirals to treat an infection. In the medical countermeasure arena, Livermore researchers combined artificial intelligence, machine learning, bioinformatics, and supercomputing to uncover candidates for new antibodies and antivirals that could fight the novel coronavirus. The Laboratory was well-positioned for the task, given its mission focus on biosecurity and use of high-performance supercomputing to support projects and partnerships to combat disease and aid human health. (See the box on p. 6.)

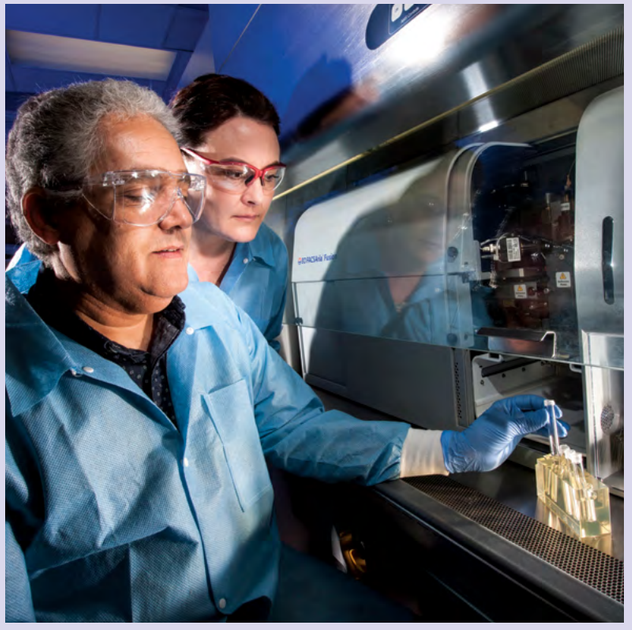
Initial work on sequencing the SARS-CoV-2 viral proteins began in late January 2020. Felice Lightstone, Livermore's technical lead for medical countermeasure R&D, notes, "The pandemic required the Laboratory to pivot quickly to meet this

Laboratory Directed Research and Development (LDRD) Program Invests in COVID-19 Research

Livermore's LDRD program invests in high-risk, potentially high-payoff research to meet some of the nation's most difficult challenges. As a key source of internally directed research funds, the program enabled the Laboratory to rapidly launch five projects related to COVID-19 early in 2020.

According to Doug Rotman, who leads the LDRD program at Livermore, "We had several scientists contact us with ideas regarding how they could help our Laboratory respond to the national COVID challenge, including emerging needs related to therapeutic targets and designs. Working closely with science leaders across the Laboratory, the LDRD program rigorously and quickly evaluated proposals and approved funding needed to start work." The LDRD-sponsored projects focus on leveraging Livermore's capabilities in high-performance computing, simulation, and data science—combined with Livermore's expertise in bioscience and bioengineering—to model, identify, and validate possible antiviral and antibody candidates. Computational scientists are also applying machine-learning tools to existing medical data sets to identify risk factors for COVID-19 patients.

In early 2021, the LDRD program funded research aimed at antibody screening technology to significantly reduce the time needed to develop countermeasures. The team includes experts in biophysics, bioengineering, synthetic biology, and molecular biology, who anticipate that their approach will enable researchers to rapidly identify and validate antibodies capable of neutralizing the virus.



Laboratory Directed Research and Development investments enabled Livermore scientists, including (left) biologist Matt Coleman and immunologist Amy Rasley, to explore how nanolipoprotein technology can be used to develop new vaccines. Laboratory biologists (top) Jessica Wollard and James Thissen use molecular-based technologies, such as polymerase chain reaction, microarray and genomic sequencing, to characterize microbes and pathogens in samples. (Above) Nick Fischer works with the LLMDA. (Photos by Randy Wong.)

national emergency." Lightstone points out that sequencing genomes for medical countermeasures research differs from traditional genome sequencing, requiring the creation of 3D models of the key viral proteins. In early February 2020, Livermore's Adam Zemla published a preliminary set of predictive 3D protein structures based on the genomic sequence of the SARS-CoV-2 virus and the known structure of a protein found in the original SARS virus. Starting with these preliminary protein models and a few antibodies known to bind and neutralize SARS-CoV-1, a team led by Daniel Faissol and Thomas Desautels used the Mammoth and Catalyst HPC clusters to screen for antibodies capable of binding to the SARS-CoV-2 spike protein.

The team employed a modeling platform that integrated experimental data, structural biology, bioinformatic modeling and molecular simulations—all driven by a machine-learning algorithm. The platform was used to identify potential high value modifications to the antibodies from the 2002 SARS virus so that the antibodies would bind and, therefore, neutralize SARS-CoV-2. By mid-March 2020, Lawrence Livermore researchers had identified about 20 promising antibody designs after simulating 90,000 antibodies chosen by the machine-learning model from a total of 10^{40} possible candidates. Although none of the 20 antibody designs turned out to bind strongly to the virus, subsequent design iterations yielded significantly improved candidates, several of which have been experimentally validated to neutralize SARS-CoV-2 while maintaining neutralization of SARS-CoV-1. The team is working with external partners to redesign existing antibody drug products to achieve binding and neutralization to SARS-CoV-2 escape variants of concern as well as beginning to work toward antigen design for a pan-coronavirus vaccine. 3D models of the SARS-CoV-2 proteins and the antibody

> "Here at the Lab, we were ready and able to quickly draw upon our deep technical expertise, ongoing research to improve the nation's biosecurity, and expansive infrastructure to offer help and solutions to a wide range of science and engineering issues."

structures are accessible through a public database, covid19drugscreen.llnl.gov.

In addition to groundbreaking work on antibodies, Livermore researchers focused attention on antivirals. Once infection sets in, antivirals—therapeutic drugs that treat the infection—can also be employed. "Antiviral drugs can sometimes be more

Livermore virologist Monica Borucki, along with other experimentalists from the Microbiology and Immunology Group, collaborated with Lawrence Livermore computational teams on projects focused on virtual antibody design and virtual screening of small molecule antivirals.

effective than therapeutic antibodies in treatment," explains Lightstone. Antiviral drugs frequently target the viral replication proteins, which are less likely to mutate. As a first step in developing new antiviral drug therapies to fight COVID-19 infection, a group led by Lightstone and Jonathan Allen used Livermore's Quartz, Corona, Lassen, and Ruby supercomputer clusters to screen small molecules against two COVID-19 proteins. Small molecules and drug therapies resulting from them need to be carefully targeted so that they only bind to viral proteins and not to similar proteins that exist in patients. Both physics-based and machine-learning methods were used to screen existing molecules. To suggest new compounds, a Livermore-developed machine-learning model was used to train the model on the 1.6 billion known molecules and 1 million additional compounds that looked promising for treating COVID-19. The model, which reduced training time from 24 hours to 23 minutes, was named a finalist for the prestigious Gordon Bell Special Prize for High Performance Computing-Based COVID-19 Research. The model is being added to the active learning loop that suggests novel molecules with improved properties and efficacy.

Once promising small molecules were identified, Livermore virologists and researchers, including virologist Monica Borucki, moved into the experimental phase of the task, investigating efficacy and safety in a laboratory setting. But one phase does not simply leave the other behind. "We iterate, folding the experimental results back into our calculations, to improve our predictions," says Lightstone. "The closer we make our predictions match the experimental data, the faster we can arrive at an effective drug therapy."

A Twist in the Plot

By their very nature, viruses mutate frequently, and the novel coronavirus is no exception. Most of these mutations

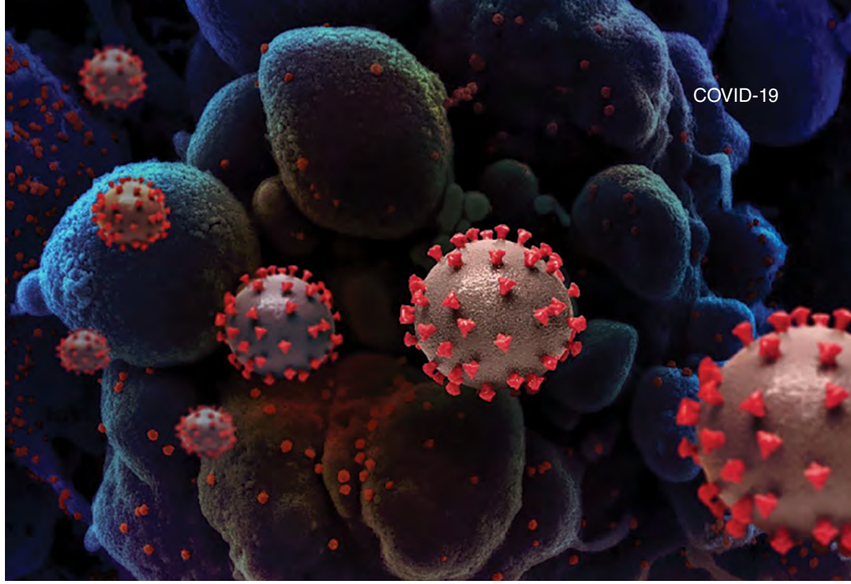
have been minor; however, in fall 2020, reports surfaced of a new variant with 23 mutations, some of which could enable COVID-19 to spread faster and easier than before. Borucki, who has studied other emerging pandemics including the Middle East Respiratory Syndrome and Zika virus, notes, "We expect new variants of the novel coronavirus will continue to emerge. As they do, we in the scientific community will need to deepen our understanding of the mutations and how they spread, adjust our detection technologies accordingly, and continue to develop countermeasures."

Borucki adds that the increased transmissibility of variants highlights the importance of fighting pandemic fatigue. "We must continue our efforts to stop viral transmission chains and continue to distribute vaccines to contain the pandemic," she says.

The Story Continues

The Laboratory's work on COVID-19 is far from over. In one ongoing effort that supports the COVID-19 research community, the Laboratory is creating a diagnostic-testing knowledge base

Environmental scientist Staci Kane collects a sample from a door on the Laboratory's main site for a research study to evaluate how environmental sampling might be used as an added tool in COVID-19 prevention and response efforts.



The RNA-based coronavirus responsible for COVID-19, SARS-CoV-2, is covered with a crown or "corona" of spike proteins. These proteins bind to ACE2, a protein found on the surfaces of many human cells, allowing cell and virus membranes to fuse, providing a pathway for the virus to enter the cell. The viral RNA then hijacks the cell's protein-making machinery to make new copies of the virus. The SARS-CoV-2 is about 80 nanometers in diameter, nearly 1,000 times smaller than the cells it infects.

that includes information regarding the microbes present in clinical samples obtained from COVID-19 patients, as well as genomic data regarding virus mutations. Even with effective vaccines in place, new tests are needed to monitor susceptibility, infection, and immunity. The Laboratory is also evaluating clinical and environmental tests that could help the Laboratory and other organizations determine how, when, and where to remove restrictions and open safely, without causing new infections.

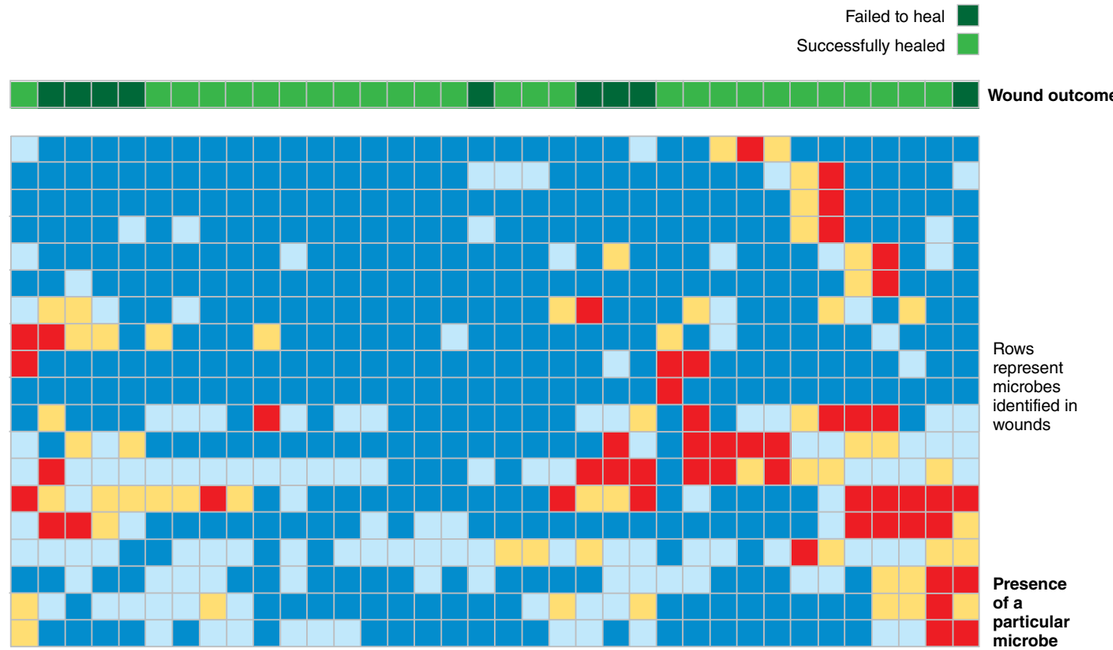
"The COVID-19 pandemic has been one of the most significant challenges to the safety and security of our nation in the past century," says Rakestraw. "Here at Lawrence Livermore we were ready and able to quickly draw upon our deep technical expertise, ongoing research to improve the nation's biosecurity, and expansive infrastructure to offer help and solutions to a wide range of science and engineering issues." These contributions drew on specific capabilities developed in anticipation of a large-scale biological event and on the rapid application of broad capabilities to solve emerging challenges difficult to anticipate. "We are still living the story of the pandemic," says Rakestraw. "Whatever happens next, and then after that, the Laboratory is ready to help the nation face the future."

–Ann Parker

Key Words: Accelerating Therapeutics for Opportunities in Medicine, antibodies, antivirals, Biological Applications of Advanced Strategic Computing, biosecurity, Computational Predictive Biology initiative, COVID-19, detection, diagnosis, high-performance computing (HPC), Laboratory Directed Research and Development (LDRD), Lawrence Livermore Microbial Detection Array (LLMDA), medical countermeasures, medical equipment, nasal swabs, National Virtual Biotechnology Laboratory (NVBL), novel coronavirus pandemic, polymerase chain reaction (PCR), RNA, severe acute respiratory syndrome (SARS), SARS-CoV-2, small molecules, supply chain, vaccine, ventilator, virology.

For further information contact David Rakestraw (925) 422-3694 (rakestraw1@llnl.gov).

Predicting Combat Wound Recovery



Whole metagenome DNA sequencing was performed to identify the microbial bioburden of multiple combat wound samples.

RECENT advances in body armor and other battlefield damage control strategies have increased the proportion of survivable blast-related combat injuries. Over the last few decades, treatment of warfighters surviving increasingly severe injuries has created new challenges across the Department of Defense's Military Health System. The nature and extent of trauma from improvised explosive device blast injuries and high-energy ballistics, including traumatic amputation and open fracture, are fundamentally distinct from civilian wounds such as a gunshot or soft tissue injury caused by a car accident, and push the limits of physiological recovery and existing technology to facilitate healing. Moreover, the extent and size of these wounds, with large surface areas of vulnerable open tissue, extended wound care procedures, and the presence of environmental contaminants embedded in tissue and bone, contribute to a high likelihood of microbial contamination, colonization, infection, and increased morbidity.

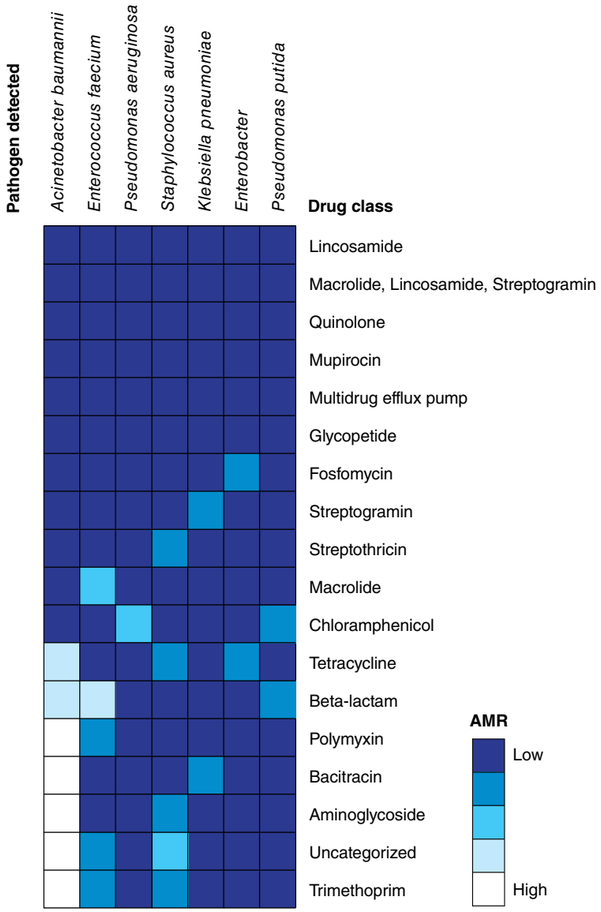
"In today's combat environments, soldiers are surviving unthinkable injuries. Clinicians work hard to treat wounds our human bodies just haven't evolved to handle," says Nicholas Be, Livermore molecular biologist. Traditional, culture-based assessments of wound colonization underestimate the foreign microbes in a wound, but Be's previous research suggested that having a healthy mixture of microbes could assist the healing process. (See *S&TR*, October/November 2015, pp. 16–19.) "We thought, 'What if we could help clinicians know the exact microbiome of a wound?' Then they would be able to provide personalized, precision medicine that would remove clinical guesswork, save time, avoid unnecessary treatments, and hopefully save lives," says Be.

To assist clinicians with critical guidance for wound infection care, Be and his team work with the Uniformed Services University (USU), the nation's federal health professions academy dedicated to cutting-edge, military-relevant research. Together, they have leveraged a multidisciplinary approach, integrating experimental and computational analyses, to detect and identify genetic markers for virulence and antimicrobial resistance (AMR) in microbes, and to predict a wound's likely healing outcome using machine learning and statistical analysis based on these genetic markers.

Using the Fewest Lures

For the project, USU provided tissue and wound effluent samples from combat-injured patients, including wounds that successfully healed and those that did not. The samples were subjected to whole metagenome and targeted antimicrobial resistance gene sequencing, yielding a comprehensive portrait of the bioburden across the samples. "One of the challenges of this project," says Be, "is identifying the sheer breadth of microbial species that could be present in a wound. We needed more than just taxonomic information. We also needed to know what those microbes are capable of doing. So, we focused on identifying the genetic markers that signal resistance to antibiotics, as well as virulence, meaning how likely the bacteria will successfully colonize and cause infection. Once we found the most common, relevant genetic sequences that indicate AMR and virulence, we could optimize the parameters for identifying those factors and, in essence, catch the most fish using the fewest lures."

Nisha Mulakken, bioinformatics software engineer at Lawrence Livermore, designed the target enrichment panel and performed data analysis of the experiment's results. "Before creating this gene panel, we only had a targeted amplicon sequencing panel containing primers for detecting AMR genes from bacteria," says Mulakken. "The enrichment panel designed for this wound project contained an updated set of AMR genes from the Naval Research Laboratory as well as additional types of probes for organism detection and gene virulence." To create the organism detection probes, Mulakken extracted about 11,900 fungal and bacterial probes from the Lawrence Livermore Microbial Detection Array and selected a subset based on organisms important to the project, such as hospital-acquired, or nosocomial, bacteria. To minimize the number of probes needed to detect about 3,000 virulence genes



The results of the study's logistic regression models indicate resistance to drug classes and detection of nosocomial pathogens. The lighter the box, the stronger the evidence for a correlation between the presence of antimicrobial resistance (AMR) and pathogen species abundance.

across 32 pathogens, she identified well-conserved regions common to all available sequences for each gene.

Paradoxical Data Challenge

The small number of genetic samples for this project presented a paradoxical challenge for the Livermore scientists. "The project only included data from 56 patients, but we were also working with extremely high-dimensional data: patient immune biomarkers, inflammation, patient blood loss, wound size and location, treatments administered, and *then* synthesizing that clinical data with tens of thousands of microbial genomic data points," says Mulakken. "The disadvantage of having too many variables is also the advantage of this project. Rarely are we able to look at so many different aspects of the patients' health in one study. The fact that the patients were also all physically fit, in about the same age group, and living under the same conditions before getting injured, provides some semblance of controlled factors, and makes the project unique."

Once the metagenomic and targeted functional identification had been performed, the first step in the data analysis was to reduce the number of variables by looking for highly correlated variables and selecting one variable per correlated group. "We began by identifying the bacteria and fungi in a wound and then determined which resistance genes were associated with a particular healing outcome based on the clinical data. That's where machine learning comes in," says Livermore chem-bio data engineer Aram Avila-Herrera, who provided exploratory data analysis for the project. The team used random decision forest analysis to get an idea of the important variables associated with healing results. They also grouped genes into higher-level classes, organizing antimicrobial genes according to the drugs they interfere with and virulence genes with mechanisms and pathways. "In an iterative feature selection process such as this, something important pops up early on," says Mulakken. "Then you remove correlated features that don't add insight, group genes by function, and down select important features." The team used a battery of machine-learning approaches including logistic regression, neural networks, support vector machines, and penalized logistic regression to sift through and examine potential predictors such as the onset of pneumonia, infection, wound closure timing, blood clots, and abnormal bone growth. This created an overall characterization of the bioburden, clinical variables, and wound healing or failure across the samples that could be investigated for possible medical interventions to improve healing outcome.

"As with many projects using big data and data science, interpreting results and applying techniques to cut through the statistical noise common with biological data sets presented a challenge," says Seth Schobel, scientific director for the Surgical Critical Care Initiative at USU and collaborator in the project. "We put together a team of experts who developed a set of methods to demonstrate the value of a targeted sequencing panel sensitive enough to detect the same types of microbes commonly seen in wound infections from cultures, but also capable of identifying antimicrobial resistance and virulence factors associated with wound healing. Lawrence Livermore has access to great computational resources unavailable to USU, as well as incredible expertise in microbial genomics, which helped drive this project forward."

Association of antibiotic resistance gene detection (top hemisphere) with antibiotics administered (bottom hemisphere) in wounds that failed to heal. A larger number of connections (represented by bands of different colors) indicates a larger number of associations for a given gene with a given antibiotic (with different genes/antibiotics shown in different colors).

A Nice Surprise

"We weren't expecting to get as much information as we did," says Be, "But given the depth of data we have, it's a nice surprise." The team's analysis suggests that wound success or failure doesn't come down to one common microbial profile. Adding a molecular microbial layer to the information typically collected for these wounds offers value, and using both microbial and non-microbial factors, the results of the study indicate that a majority of wound failures could potentially be predicted from these variables. The project uncovered patterns like the association of multiple genes conferring resistance to the same administered antibiotic in failed wounds. The team also observed distinctions across separate wounds at comparable progression points in the healing process, revealing that predictive values may shift over time.

The project is in the early, proof-of-concept stage due to its small sample size, but if the data is found to be representative in the next research stage, the project could lay the groundwork for a clinical diagnostic tool. The team hopes that their findings will motivate funding for larger studies to validate and further explore this potentially lifesaving work. "We're working with a complicated data set," says Be, "Biomedical data can be extremely difficult to harmonize. We hope to zoom in on a few key components and patterns and design specific experiments to see what holds up. Then we can understand what microbial factors trigger mechanisms relevant to wound healing or what microbial genetic signatures suggest that healing will be impeded."

—Genevieve Sexton

Key Words: antimicrobial resistance, bioburden, clinical decision support, combat injury, Lawrence Livermore Microbial Detection Array, machine learning, metagenomics, microbiome, military medicine, precision medicine, Uniformed Services University (USU), wound infection.

For further information contact Nicholas Be (925) 423-1612 (be1@llnl.gov).

Building Confidence in Materials Modeling Using Statistics

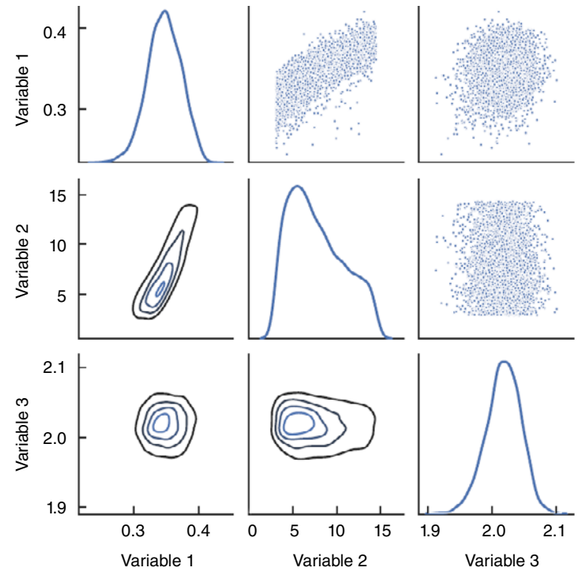
The Livermore-developed statistical framework is intended to assess sources of uncertainty in strength model input, recommend new experiments to reduce those sources of uncertainty, and evaluate how existing sources of uncertainty in strength models impact physics codes that rely on those models. (Illustration by Jacob Long.)

THE best way to understand how a material behaves under certain conditions is to test it. For instance, an engineering project may rely on understanding a material's strength—its resistance to permanent deformation—across a range of temperatures and strain rates. Gathering all relevant experimental data could be challenging or even impossible due to the time and cost required, material availability, or the difficulty of recreating certain conditions in a laboratory setting. Researchers, therefore, rely on models informed by available data to predict the performance of materials at untested conditions.

Lawrence Livermore National Laboratory has a vested interest in understanding the accuracy of these models and the data that feed them. Material property models play a foundational role in a range of Livermore's science and engineering research endeavors including stockpile stewardship, the National Nuclear Security Administration's program to ensure the safety and reliability of the nation's nuclear stockpile. Materials modeler Nathan Barton explains, "As we shift manufacturing and design approaches to more modern methods, we need to quantify uncertainty to maintain confidence in our nuclear stockpile and our stockpile modernization activities. Understanding the uncertainties gives us increased confidence in the experimental results and the models informed by the experimental data."

Led by Livermore materials scientist Jeff Florando and supported by the Laboratory Directed Research and Development (LDRD) program, Barton and other Laboratory statisticians, computational modelers, and materials scientists have been developing a statistical framework for researchers to better assess the relationship between model uncertainties and experimental data. In an earlier effort, Florando helped build the Material Implementation, Database, and Analysis Source (MIDAS), a central repository for material strength-related data and models. (See *S&TR*, January/February 2012, pp. 19–22.) "My role in developing MIDAS helped me realize we needed to do a better job understanding uncertainties in material strength research," says Florando. "MIDAS helps us create material strength model parameterizations, but the simulations are deterministic—they give us an answer that is based on the parameters we put in them."

The latest framework, based on Bayesian methodology, allows for uncertainties to be updated as new and different types of strength data become available and can be used to determine the future experiment with the greatest potential to reduce uncertainty. Methods developed by the team have informed experimental planning efforts within the Laboratory's Weapons and Complex Integration (WCI) organization as well as research ventures exploring how materials evolve and degrade.

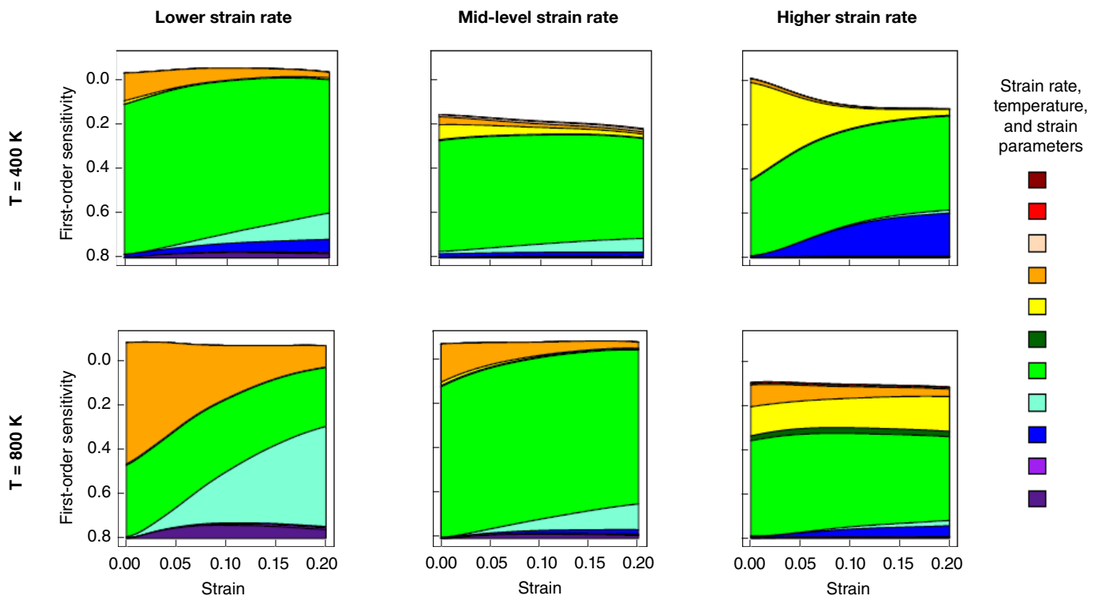


The posterior distribution—the outcome of Bayesian calibration—quantifies the state of knowledge about the model parameters given the experimental data and the choice of the prior distribution. In these plots of posterior distribution based on dynamic tantalum experiments, the probability distributions on the diagonal represent the marginal posterior distributions for each parameter: the narrower the distribution, the more certain researchers can be of the parameter value. The off-diagonal pairwise plots, shown with two different plotting types above and below the diagonal, illustrate the correlations between the variables. In this example, Variables 1 and 2 have a stronger correlation compared to the other pairings as observed with the linear-like grouping with a positive slope in the corresponding off-diagonal plots, which indicates a positive correlation.

Rigorous Model Comparison

The first step in the statistical framework—sensitivity analysis—determines which inputs to a given strength model most strongly influence the output of the model under a specific set of conditions, highlighting the most significant variables and those with little effect that can remain fixed. Shrinking the number of variables from the dozens or even hundreds involved in a strength model significantly reduces the computational cost, paving the way for the next step: calibration.

After using sensitivity analysis to determine which parameters to vary, researchers apply Bayesian calibration, training several models on a subset of experimental data and quantifying how



These example plots show how the sensitivity of strength parameters, represented by different colors, changes as a function of strain rate, temperature, and strain. Parameters with low sensitivity across the conditions of interest may be fixed rather than estimated in subsequent analysis, effectively reducing the dimension of the problem.

well the trained models predict data from elsewhere in the data set. Each model is tested hundreds or thousands of times, predicting results across a range of conditions, and the models are ranked based on their overall prediction error. In this flexible framework, Florando and colleagues could, for example, combine the data from two experiments—one at low strain rates and the other at high—on the deformation of tantalum, a material of interest due to its stable crystal structure over a wide range of pressure and temperature conditions, in their initial demonstration to ensure sufficient coverage for model cross-validation.

To reduce computational demand, Livermore researchers created surrogate versions of the finite element models that only emulate behavior under a subset of conditions. "Often we are trying to replicate complex physics, which requires computationally expensive 3D simulations," explains Florando. "So, we create a surrogate model that matches the physics data of the full model in a very narrow regime but runs much more cheaply. We can use statistical tools to do tens of thousands of runs with the surrogate model to explore the parameter space in this regime."

The calibration and cross-validation element of the framework enables researchers to identify which of the tested strength models provides the most accurate predictions overall, as well as under specific experimental conditions. For instance, while all strength models in one study appeared to provide similarly accurate strength predictions for very high temperature conditions, one of the models was significantly less accurate than the others for room-temperature conditions. Such insights into the relative strengths and weaknesses of a model help researchers assess how confident they can be in the accuracy of that model's output. Most importantly, however, the comparison helps engineers and scientists anticipate the accuracy of future predictions—that is, how well a given model will generate new strength projections in a certain pressure, temperature, and strain rate regime—aiding researchers in selecting the optimal model for a given project.

Experimental Design Enhancement

The sensitivity analysis and calibration elements of the statistical framework not only uncover sources of uncertainty in the strength models, they are also intended to help guide and optimize experimental design decisions. Having determined which input parameters have the greatest influence on model results, researchers can shape future experiments to decrease uncertainty in a given parameter. Further, if model cross-validation reveals that a model predicts poorly for a certain range of conditions, experiments could focus on collecting more data at those conditions to improve model performance. Results of the analysis might also suggest that the model form needs to be refined to better capture experimental observations. Another round of Bayesian calibration and cross-validation incorporating the additional data (or an update of the model) could help determine which models provide the best predictions and under which conditions, given the new information.

The team has incorporated statistical methods into the framework for evaluating, based on previous experimental data and model performance, which experiment will give the greatest reduction in parameter uncertainty. Florando says, "We can use the framework to help inform two important questions: If I had data in a different phase space, how would that change the answer? And given these choices of experiments, which one best reduces the overall uncertainty? Working with stress–strain curves in which we had confidence, we used this approach to pick the strain rate experiment that would best help lower the uncertainty." In the future, Florando would like to see this approach used to discriminate among a more heterogeneous set of experiments. For instance, would it be better to run a higher strain rate test or a higher temperature test in a given context?

The team notes that while these methods will likely help researchers gather more useful data more efficiently, they may not, necessarily, need to do fewer experiments. Minor differences in experimental setup and some random variation in the results always exist. Their findings do foster a fresh outlook on experimental design. Statistician Ana Kupresanin explains, "Incorporating statistical rigor into your approaches requires changing methodologies and ways of thinking and questioning every data set you work with, even if the data comes from another scientist. Different parameters and materials with slightly different properties are involved in each experiment. If the goal in the experiment is to characterize variability, you need to collect enough, and representative enough, samples to make the method work. The experimental setup must be in line with the methodology."

> "By improving the accuracy of our models of these different materials, we are creating the building blocks for larger and more complex models."

Building Block for Big Codes

Material strength models are typically incorporated into larger, more complex, and more computationally intensive physics codes. Understanding how uncertainty in the input parameters of the strength models can affect the output of these larger codes is a difficult task, and researchers are still evaluating the best path forward, making the statistical framework foundational, according to the team. Says statistician Katie Schmidt, "By improving the accuracy of our models of these different materials, we are creating the building blocks for larger and more complex models."

Uncertainty quantification is a growing field within the stockpile stewardship program, and efforts are already underway to apply the tools and methods developed in this LDRD project to specific problems within the program as well as to other national security-relevant materials science projects. "We have seen a real and healthy continuation of this work in the program space, which is gratifying," observes Barton. "Ideas from our project have helped inform WCI strategic planning, including experimental choices." The team is also looking to incorporate some of their statistics tools into MIDAS.

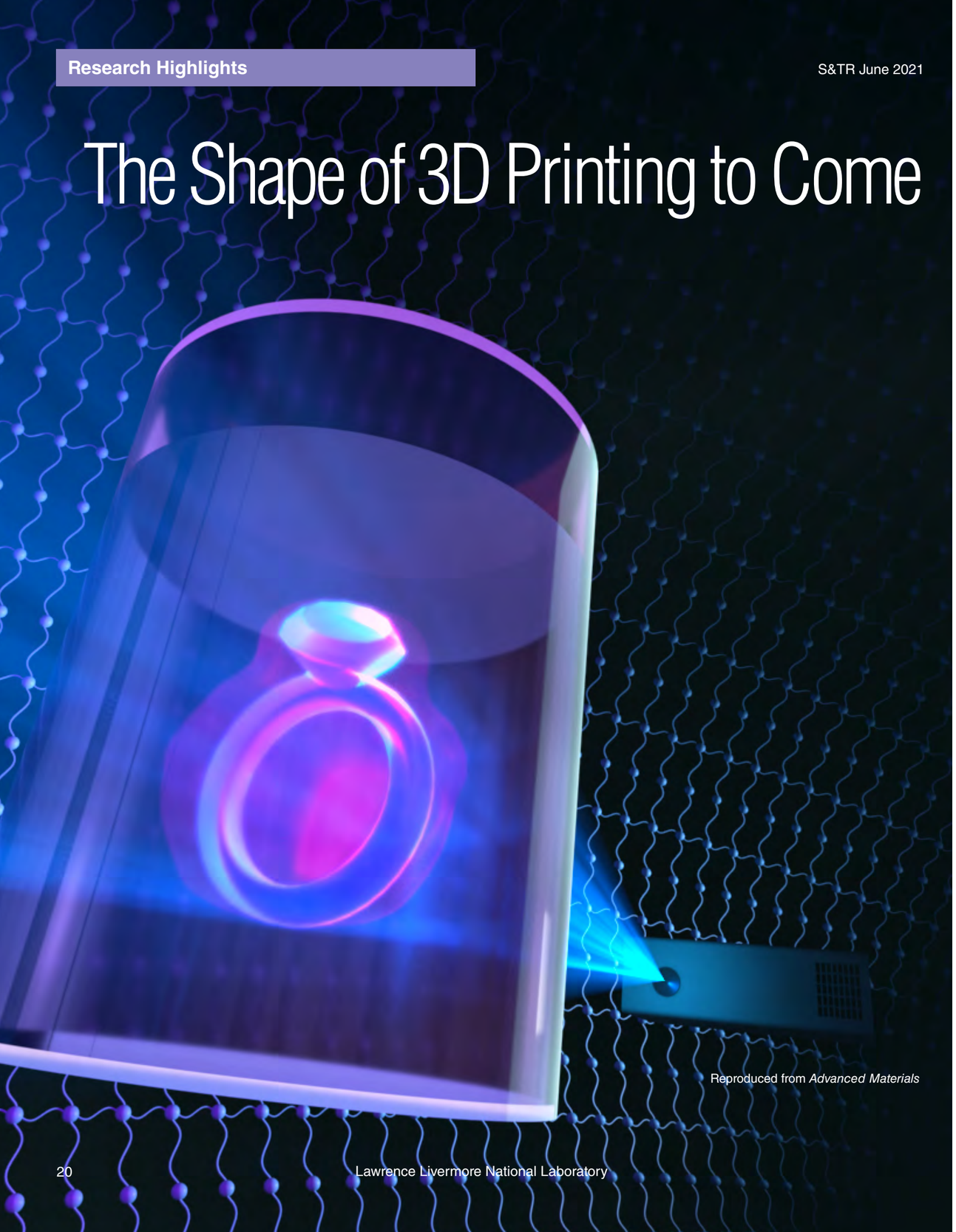
A rewarding part of the project for the team was bringing together researchers from diverse disciplines—computational modeling, statistics, and materials science—and all career stages—from postdoctoral scholar to senior scientist. Says Schmidt, "This was my first project as a postdoctoral scholar at Lawrence Livermore. I knew nothing about materials science coming in, but I was able to absorb a lot. Now I'm working on other materials science projects." In addition to Schmidt, postdocs engaged in the project include Jason Bernstein, David Rivera, Amanda Muyskens, Matthew Nelms, and William Schill. Florando adds, "This project gave me a deeper appreciation for statisticians and statistics. I'm thinking of ways to incorporate statistics into my other projects."

—Rose Hansen

Key Words: Bayesian statistics, Laboratory Directed Research and Development (LDRD) program, Material Implementation, Database, and Analysis Source (MIDAS), material strength, stockpile stewardship, uncertainty quantification.

For further information contact Jeff Florando (925) 422-0698 (florando1@llnl.gov).

The Shape of 3D Printing to Come



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ADDITIVE manufacturing has improved the strength and reliability of parts for the aerospace and automotive industries and the ease of tailoring prosthetic parts for the medical field. Yet on-demand three-dimensional (3D) printing, for the everyday consumer has remained out of reach due to limited material options and poor product quality. New research at Lawrence Livermore, however, has broadened the range of materials available for 3D printing. "The promise of 3D printing has long been touted, but low-quality materials have held back widespread implementation," says Maxim Shusteff, principal investigator for the research effort. "Making more material classes available for 3D printing is a big step."

Unlike acrylate resins that yield brittle, fragile objects, Livermore researchers have demonstrated that tough and tunable thiol-ene resins achieve the material properties required to 3D print reliable biomaterials as well as functional materials—materials with innate, controllable properties for applications in electronics, among other uses. Further, the research team successfully printed 3D structures in thiol-ene resin using Computed Axial Lithography (CAL), an additive manufacturing technology co-developed with the University of California, Berkeley. The team's efforts also uncovered reference points for controlled 3D fabrication that can be applied to printing with other high-performance polymers, informing next-generation 3D printing.

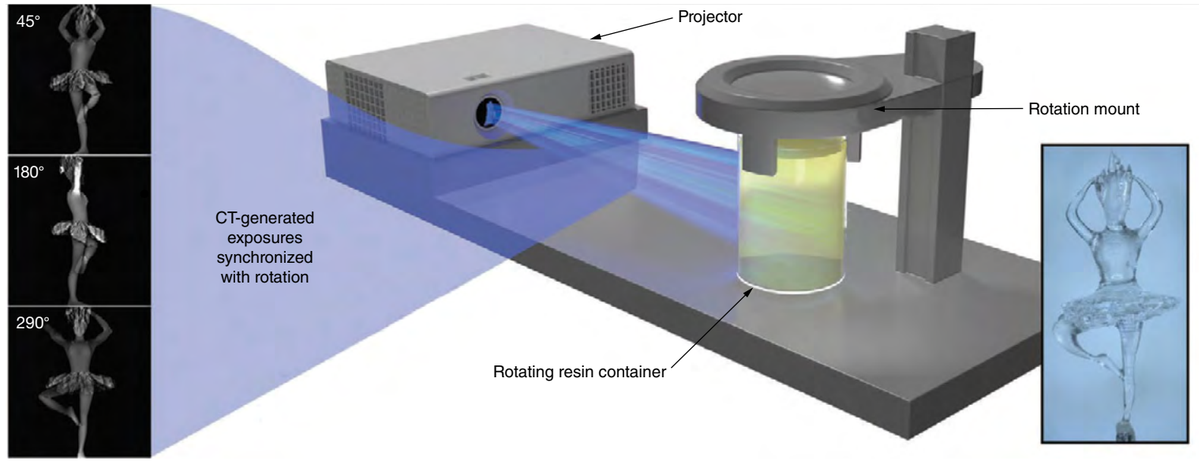
Volume Versus Layers

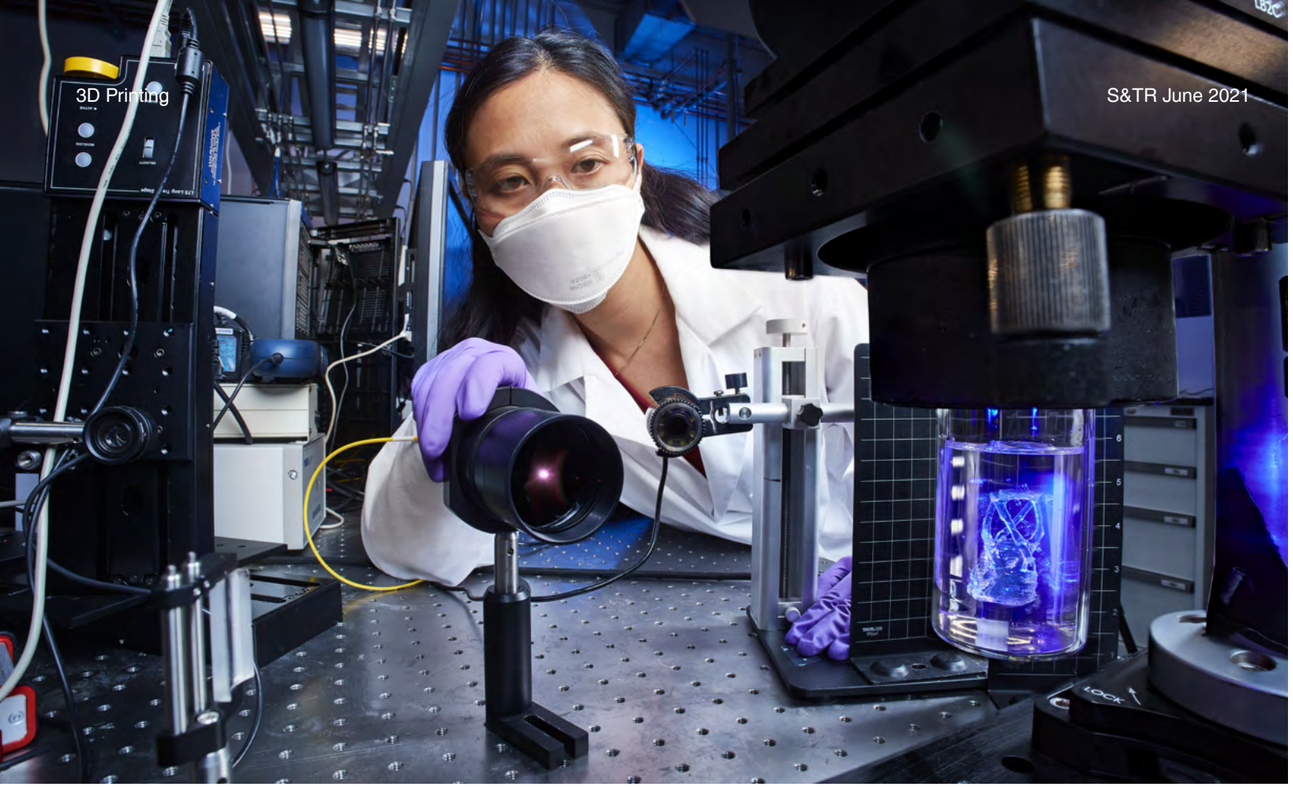
CAL produces objects by delivering 3D-patterned light energy from multiple angles into a spinning vial of photosensitive resin. The resin has an energy threshold below which the material will not polymerize (cure). As the vial spins, the light creates a 3D energy pattern within the resin to cure the liquid into a solid at designated points. The uncured resin is drained, leaving behind a printed object in a matter of seconds, significantly faster than layer-by-layer assembly, which can take from 30 minutes up to days. The uncured material can also be re-used, reducing material waste. (See *S&TR,* March 2018, pp. 13–15.)

Compared to traditional, layer-by-layer additive manufacturing, CAL is a volumetric additive manufacturing (VAM) technology that operates more like a glass blower transforming molten material into a vase than a carpenter building a container board by board. As a result, the one-step process creates a smoother finished product.

CAL uses standard parts for a lower operating cost than earlier VAM technologies. The ability to control the projected image into a print feedstock and the rotation of the resin vial enables faster production of complex and curved shapes. CAL can overprint as well, meaning a polymer object can be printed over an existing object. In an early example, the team printed a handle for a metal screwdriver. More recently, the researchers printed hollow channels for conveying liquids within an object's inlets and outlets. Given these advantages, in combination with the research team's recent material discoveries, CAL can enable faster 3D printing of higher quality parts than those made from commercially available 3D printable materials, removing barriers to 3D printing at scale.

Rather than printing objects layer by layer, the Computed Axial Lithography (CAL) projects beams of 3D-patterned light into a vial of photosensitive resin. As the vial spins, the light cures the resin into the desired shape with a smoother surface than other additive manufacturing techniques. (Illustration by Jacob Long.)





Livermore scientist Erika Fong demonstrates how CAL creates a three-dimensional, solid part within a container of resin. (Photo by Garry McLeod.)

A Different Chemistry

Additive manufacturing techniques that apply material layer by layer require fast-acting chemical reactions to solidify the part as it prints. Acrylates, which undergo rapid-chain growth reaction kinetics during curing, meet this need and have been the standard choice for 3D printing. Yet, acrylates yield tough, brittle printed parts. Shusteff's team understood that a material with a better-ordered molecular structure than the structure of acrylates, if successfully used in 3D printing, would yield a more durable product able to withstand greater mechanical loads.

The team's research identified thiol-ene-based polymers, a class of materials with controllable (tunable) properties. Supported by a Laboratory Directed Research and Development project in advanced photopolymer materials development, Shusteff, his Livermore colleagues, and academic collaborators from the University of Colorado developed thiol-ene resins with the kinetics needed for VAM technologies such as CAL. Unlike oxygen inhibition that contributes to fast curing in acrylate, thiol-ene photopolymerization reactions see negligible oxygen inhibition. Therefore, the team incorporated an inhibitor, 2,2,6,6-tetromethyl-1-piperidinyloxy, also known as "TEMPO," to generate the required threshold behavior. From that point, the team formulated resins from specific combinations of monomers to create a tunable mechanical response.

Mechanical testing of the resin samples revealed a range of qualities from inflexible to rubbery, tough to soft. "We needed to build in curing kinetics with a threshold response for this new set of chemicals to be successful in VAM," explains materials engineer Caitlyn Cook. "The result is a much more versatile material that can be hard, tough, elastic, or everything in between for use in functional materials and biomaterials."

The Right Dose

The next step was designing the dose of light CAL delivers in the thiol-ene resin to successfully print a 3D structure, establishing a reference for other resin systems yielding high-performance printed engineering polymers. Studying how the resin behaves at different light dosages aligns the experimental data with computational models that apply predicted photochemical behavior to predict the success of building more 3D-printed structures. "We created a common reference scale for controlled 3D fabrication that we can use to understand which polymers react to light and how the reactions impact different material properties," says Livermore researcher Erika Fong. "Greater knowledge of material properties combined with our understanding of how to tailor the energy dose make us confident we can successfully 3D print with other chemistries, even chemicals on the market today."

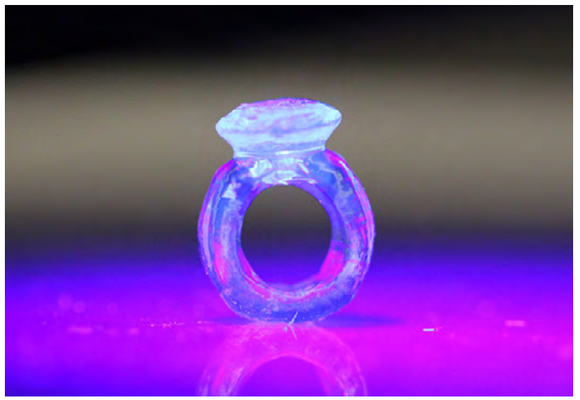
Shusteff adds that the approach of studying absorbed energy dose in resin can be applied for more accurate predictions of resin curing in layer-by-layer printing as well. The absorbed energy dose paints a picture of printing parameters such as light intensity, exposure time, photoinitiator absorbance, and light penetration depth—all relevant factors for 3D printing techniques using photosensitive polymers, even layer-by-layer additive manufacturing technologies. Next, the team plans to apply their research to the silicone materials class used for contact lens material and weather-tolerant caulking, a material group Shusteff describes as "notoriously difficult to adapt to 3D printing."

Scaling for Industry

Enabling a greater range of materials in faster, single-step VAM technologies makes a technology such as CAL even more promising. "Industry partners see CAL's speed as game changing, particularly for bio-related applications," says Shusteff. "Speeds greater than those offered by traditional techniques are needed to maintain the viability of biomaterial cells."

In the near term, the team is working with Livermore's Innovation and Partnerships Office (IPO) to demonstrate CAL's value in rapid prototyping. "The Laboratory has built a very strong intellectual property portfolio in 3D printing that attracts collaborative research partners and licensing interest," says Genaro Mempin, a business development executive in IPO. "But industry demands proof. So, we've demonstrated this technology at Livermore's Advanced Manufacturing Laboratory."

The Advanced Manufacturing Laboratory, located outside the Livermore gate, helps accelerate strategic partnerships by presenting how innovations can meet specific operational needs. The facility helps partners speed material evaluation and characterization by offering state-of-the art 3D printing equipment and analytical instruments too expensive for most companies to purchase for their own use.



Livermore researchers can quickly 3D print stronger, more flexible objects from thiol-ene resins using CAL, a volumetric additive manufacturing (VAM) technology. (Photo by Maxim Shusteff.)

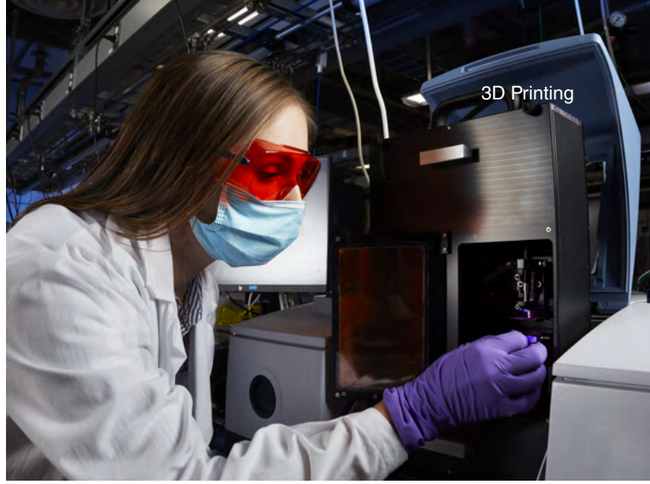
So far, Shusteff's team has printed small parts. Mempin can see CAL's potential for car parts, household goods, toys—anything where plastic is used—with the long-term goal of placing an affordable 3D printer in retail stores, even dental offices. Shusteff adds, "Tissue printing and bioprinting are still to be proven, but we can see the possibilities more clearly. Currently, the choice of materials with the right properties for bioprinting—generally polymer hydrogels—is much narrower than for other applications, but the VAM framework is yielding insights into designing new and better biocompatible hydrogels."

Recent material developments include successful printing of silicon photopolymers to increase functionality in VAM-printed parts. The team has printed in glass and a glass-precursor material. Livermore's research points the way to more possibilities in 3D printing. Says Cook, "Laser-based systems and new VAM approaches will continue to enhance print resolution and improve overall part performance, moving industry closer to on-demand, 3D-printed parts for consumers."

—Suzanne Storar

Key Words: 3D printing, additive manufacturing, Advanced Manufacturing Laboratory, Computed Axial Lithography (CAL), Laboratory Directed Research and Development program, photopolymerization, resins, thiol-ene-based polymers, volumetric additive manufacturing (VAM).

For further information contact Maxim Shusteff (925) 423-0733 (shusteff1@llnl.gov).



Materials engineer Caitlyn Cook tests how fast a material reacts to light in VAM technologies to characterize the kinetics and apply her findings to other materials considered for 3D printing. (Photo by Garry McLeod.)

In this section, we list recent patents issued to and awards received by Laboratory employees. Our goal is to showcase the distinguished scientific and technical achievements of our employees as well as to indicate the scale and scope of the work done at the Laboratory. For the full text of a patent, enter the seven- or eight-digit number in the search box at the U.S. Patent and Trademark Office's website (uspto.gov).

Patents

Surface Modification of Polymer Foams Using Plasma
Landon Nash, Duncan Maitland, Nicole Docherty, Thomas Wilson, Ward Small IV, Jason Ortega, Pooja Singhal
10,781,294 B2
September 22, 2020

Pulsed Start-Up System for Electrostatic Generators
Richard Post
10,804,819 B2
October 13, 2020

Stent with Expandable Foam
Thomas Wilson, Duncan Maitland, Ward Small IV, Patrick Buckley, Jonathan Hartman, William Benett, David Saloner
10,806,561 B2
October 20, 2020

High Temperature Additive Manufacturing Print Head
Kevin Kramer, Andrew Bayramian, James DeMuth, Bassem El-dasher
10,807,273 B2
October 20, 2020

Hybrid Indirect-Drive/Direct-Drive Target for Inertial Confinement Fusion
John Lindsay
10,818,400 B2
October 27, 2020

Halbach-Array Levitating Passive Magnetic Bearing Configuration
Richard Post
10,830,278 B2
November 10, 2020

Adsorption Cooling System Using Metal Organic Frameworks
Theodore Baumann, Joe Satcher, Jr., Joseph Farmer
10,830,504 B2
November 10, 2020

Dual-Core Fiber Amplifier for Separation of Thermal and Nonlinear Effects
Derrek Drachenberg, Paul Pax
10,838,149 B2
November 17, 2020

Laser Gain Media Fabricated via Direct Ink Writing and Ceramic Processing
Stephen Payne, Nerine Cherepy, Eric Duoss, Ivy Jones, Zachary Seeley, Cheng Zhu
10,840,668 B2
November 17, 2020

Cathode System for Electrodeposition of Metals on Microspheres
Thomas Bunn, Corie Horwood
10,844,507 B2
November 24, 2020

Systems and Methods for Micromechanical Displacement-Based Logic Circuits
Robert Panas, Logan Bekker, Julie Mancini, Andrew Pascall, Jonathan Hopkins, Amin Farzaneh
10,855,259 B2
December 1, 2020

Intrinsic Use Control for System and Use Controlled Component Security
Mark Hart
10,867,079 B2
December 15, 2020

Laser-Driven Hydrothermal Processing
Raymond Mariella, Jr., Alexander Rubenchik, Mary Norton
10,870,173 B2
December 20, 2020

System and Method for High Efficiency Electrochemical Desalination
Steven Hawks, Michael Stadermann, Juan Santiago, Ashwin Ramachandran
10,875,792 B2
December 29, 2020

Awards

Texas A&M's Department of Nuclear Engineering honored Livermore physicist **Kelli Humbird** with its **2020–21 Young Former Student award** for her work combining machine learning with inertial confinement fusion (ICF) research at the Laboratory. Humbird graduated from Texas A&M with a Ph.D. in nuclear engineering in 2019. Since joining Livermore as an intern in 2016, she has made key contributions to the ICF program such as creating a widely used neural network algorithm—DJINN (Deep Jointly Informed Neural Networks)—to help produce higher performing implosions and applying a technique called "transfer learning" to create a more predictive model of ICF experiments.

A paper co-authored by Livermore computer scientist **Rushil Anirudh** received the **Best Paper Honorable Mention award** at the **2021 IEEE Winter Conference on Applications of Computer Vision**. In "Generative Patch Priors for Practical Compressive Image Recovery," Anirudh and co-authors from Mitsubishi Electric Research Laboratories and Arizona State University demonstrated a new type of prior that can outperform common techniques in compressive sensing and compressive phase retrieval tasks. The authors determined that generative patch priors are more broadly applicable to a wide variety of images than competitors, and they proposed a technique enabling the model to automatically calibrate itself against real world sensor distortions and corruptions.

U.S. Government Printing Office: 2021/570-075-20014

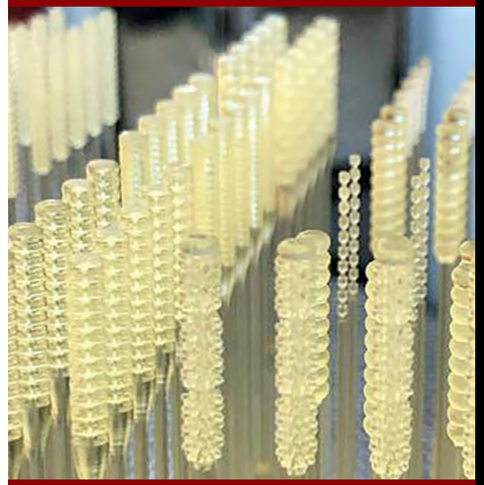
Abstract

Tackling the COVID-19 Pandemic

When the COVID-19 pandemic hit, Lawrence Livermore National Laboratory scientists came together, leveraging many different disciplines and technologies to address this global challenge. Livermore focused on three areas of research: creating alternatives for medical equipment in short supply, such as ventilators and nasal swabs; developing methods to help detect the SARS-CoV-2 virus responsible for COVID-19; and designing medical countermeasures such as antibodies and antivirals to combat the disease.

Contact: David Rakestraw (925) 422-3694 (rakestraw1@llnl.gov).

Tech Transfer in the Fight Against COVID



Transferring Livermore technology to industry partners played an important role in addressing unexpected needs during the COVID-19 pandemic.

Also in the next issue:

- *New breathable materials protect first responders from chemical and biological threats without sacrificing comfort.*
- *Next generation laser science at Livermore's National Ignition Facility brings together machine learning, big data, target fabrication, and advances in short pulse laser technology.*
- *Versatile Cold Spray, an award-winning device, harvests industrial waste heat and maximizes energy resources.*

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