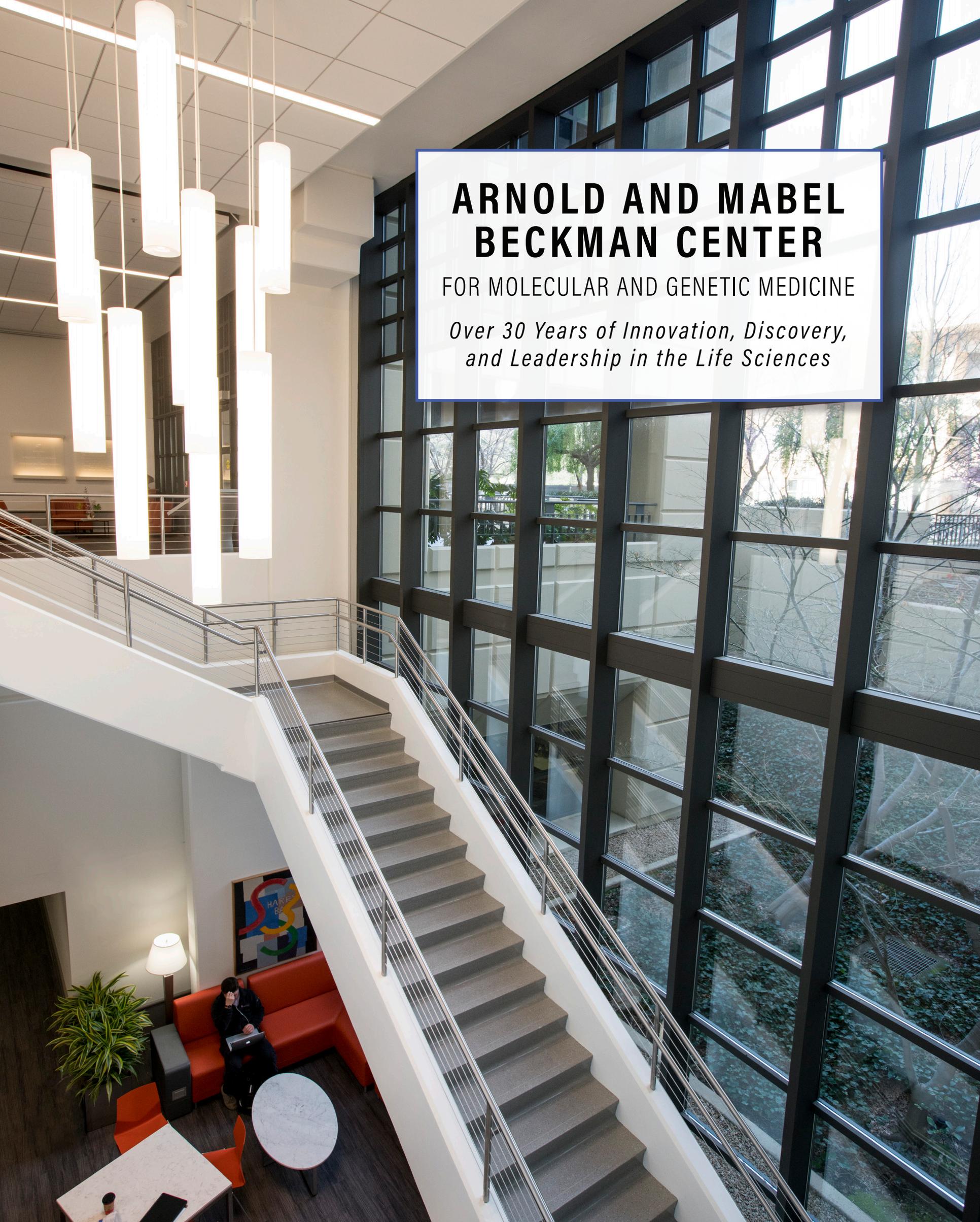


**Pandemic Preparedness**  
**2021 Annual Report**





# ARNOLD AND MABEL BECKMAN CENTER

FOR MOLECULAR AND GENETIC MEDICINE

*Over 30 Years of Innovation, Discovery,  
and Leadership in the Life Sciences*

# MESSAGE FROM THE DIRECTOR

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## *Dear Friends and Trustees,*

2020-2021 has been an enormously difficult year for the nation and the world, due to the pandemic created by SARS-CoV-2, the virus that causes COVID-19. The Arnold and Mabel Beckman Center for Molecular and Genetic Medicine has been no exception. Like many other institutions, the Beckman Center faced major disruptions to its research mission from the mounting threat of COVID-19. However, both the Beckman Center and Stanford University's School of Medicine, of which it is a part, stepped up to the many challenges in ways that were exemplary and significant, and had a positive impact.

First, the Beckman Center was able to call on an extensive and existing framework of highly skilled department chairs, administrators, and laboratory managers who rapidly came together as a team to develop a coherent and comprehensive set of policies and guidelines that laid out steps for gradual reopening of the center's research labs and service centers. These guidelines were quickly distributed and became the gold standard for policies adopted later by other centers and institutes on campus. Second, in conjunction with the School of Medicine, the center played a role in prioritizing COVID-19 research and setting safe guidelines for rapidly pursuing testing protocols, vaccines, treatments, and other research efforts vital to mounting an intervention to the pandemic.

Included in the 2020-2021 Highlights section of this report are examples of the COVID-19 research the center helped to move forward. It should be noted that the Beckman Service Centers provided significant support as well. The Protein and Nucleic Acid Facility, for example, provided critical primers and probes, when they were in short supply worldwide, for the development of Stanford University's FDA-approved COVID-19 test. The Fluorescence Activated Cell Sorting (FACS) Facility expanded into new lab space, in order to add high-end instrumentation to support Biosafety Level 2 (BSL-2) researchers analyzing the complexities of immune responses to SARS-CoV-2 infections.

Our Feature Article this year describes in more detail several of the important contributions made by Beckman Center and School of Medicine faculty in understanding SARS-CoV-2 and addressing COVID-19. Among those featured are Beckman faculty member Rhiju Das, Ph.D., associate professor of biochemistry, and his colleagues, for their work on RNA vaccines; Peter S. Kim, Ph.D., the Virginia and D.K. Ludwig Professor of Biochemistry, for his work on single-dose vaccines; and Mark A. Krasnow, M.D., Ph.D., the Paul and Mildred Berg professor and a Howard Hughes Medical Institute investigator, and his colleagues, for profiling SARS-CoV-2 infection in human lung cells. Also highlighted are Program in Molecular and Genetic Medicine (PMGM) faculty member Mark M. Davis, Ph.D., the Burt and Marion Avery Family Professor, director of the Stanford Institute for Immunity, Transplantation and Infection, and a Howard Hughes Medical Institute investigator, for his work identifying robust immune T cell determinants for SARS-CoV-2 responses; Catherine Blish, M.D., Ph.D., professor of medicine (infectious diseases) and previous recipient of the Young Investigator Award from the Arnold and Mabel Beckman Foundation, for her work tracking the immune response in patients with severe COVID-19 infections; and PMGM faculty member Daria Mochley-Rosen, Ph.D., the George D. Smith Professor in Translational Medicine, for initiating Phase 1 clinical trials for a nasal vaccine.

In addition to the contributions made to the ongoing COVID-19 research at Stanford, the Beckman Service Centers this year continued to add and offer new technologies. The FACS Facility, for example, not only expanded into new space dedicated to a High-Parameter Analysis Lab, but also leveraged both National Institutes of Health and Beckman Foundation funding provided last year for a new FACSymphony instrument to negotiate a significant discount on a 50-detector, 5-laser 6-way sorter. The new instrument, to be delivered in 2021, is capable of selecting unique populations from high-complexity cell populations and isolating them for downstream experiments and applications.

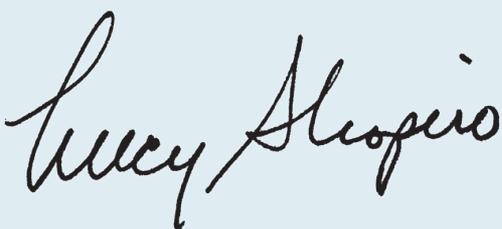
Ongoing seminars and symposia funded by the Beckman Foundation, such as the Frontiers in Biological Research Seminar Series and the Regenerative Medicine Seminar Series, were shifted to virtual formats and continue to be remarkably well attended. The FACS Facility in particular stood out in this arena by making use of the lengthy shelter-in-place period to provide nationally attended webinars on panel design and analysis that drew large audiences and contributed to demand for services.

In the fall of 2021, the center plans to fund up to five new interdisciplinary Technology Development Grants in the biomedical sciences. These seed grants are designed to support the development of new and improved instruments or devices or the development of new methodologies to be used in biomedical research. Each grant provides funding of \$100,000 per year for a two-year period. These grants have opened extraordinary opportunities for high-risk, high-reward research efforts that have led to patents and publications and have laid the foundation for more extensive grant funding.

The 30th anniversary Beckman Symposium, on the topic of "Climate Change and World Health," which was scheduled for fall 2020, has been postponed to the fall of 2021. We hope this will provide sufficient time for attention to shift away from the pandemic and back to other matters of urgent importance. The symposium will, as previously planned, be hosted jointly by the Beckman Center and the Chan Zuckerberg Biohub. Experts in the field will give presentations on the role climate change is playing in altering the geographical locations of the world's disease vectors and the spread of deadly pathogens, and the need for breakthrough technologies to respond to these threats.

This has been a difficult year for all of us, but it has also been a year in which our partnership with the Arnold and Mabel Beckman Foundation has been more valuable than ever. Foundation funds have sustained our most important research programs and continue to play a vital role in supporting state-of-the-art technology at Stanford. We are grateful for the Beckman Foundation's support and look forward to working closely with the foundation in the coming year.

Sincerely,

A handwritten signature in black ink that reads "Lucy Shapiro". The signature is written in a cursive, flowing style.

**LUCY SHAPIRO, PH.D.**

*Virginia and D.K. Ludwig Professor of Cancer Research*

*Director, Beckman Center for Molecular and Genetic Medicine*

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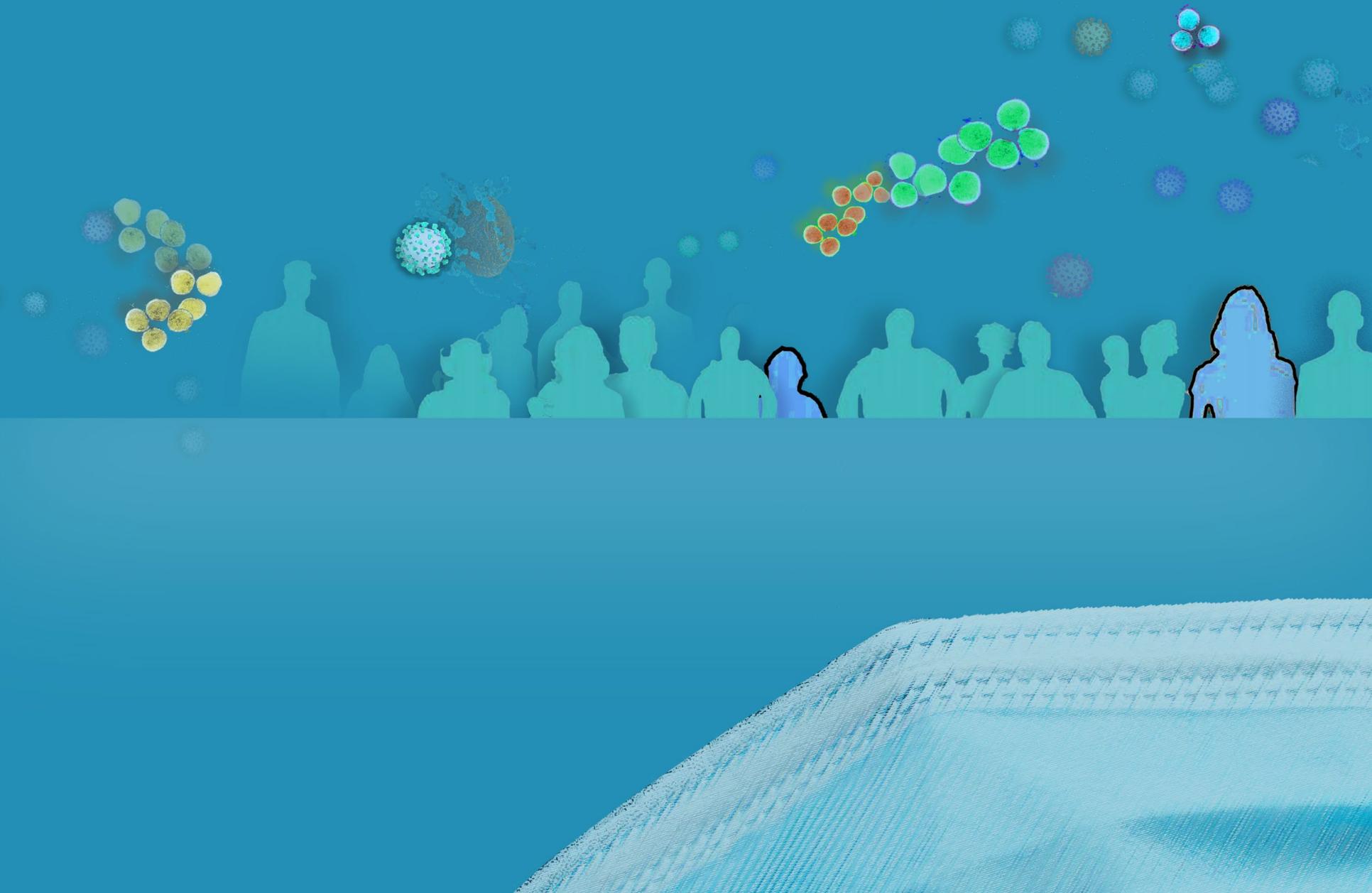
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# FEATURE ARTICLE



# SCIENTISTS WORK FURIOUSLY TO TACKLE COVID-19

BY RUTHANN RICHTER

When the SARS-CoV-2 coronavirus spread throughout the world last year, triggering a global pandemic and a university-wide shutdown, scientists across the Stanford University campus had to curtail their lab operations—and think creatively about how to carry on their research. Those at the Beckman Center for Molecular and Genetic Medicine proved nimble at adapting, with many quickly turning their attention to the new viral threat.

Indeed, over the last several months, despite the many limitations imposed by the pandemic, research on SARS-CoV-2 flourished at the Beckman Center and its affiliated labs. Many scientists pivoted their research efforts to exploring how the virus acts in the body to cause the disease known as COVID-19, and to developing innovative potential solutions.



**LUCY SHAPIRO, PH.D.**

Virginia and D.K. Ludwig Professor of Cancer Research  
Director, Beckman Center for Molecular and Genetic Medicine

“The response of the Stanford community has been one of great responsibility, as well as dedication, in very challenging times, where our labs have been downsized and people have been forced to work difficult schedules, essentially doing shift work,” said Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research and director of the Beckman Center.

“Even in the face of very difficult working conditions, the desire to help and to take projects to fruition has been incredibly impressive.”

The Beckman Center’s mission—for more than thirty years—has been to focus on basic science, with an eye to developing solutions for the betterment of humanity. In the COVID-19 pandemic, that mission became an urgent one, with the pressure of finding immediate solutions to save as many lives as possible.

“Not only do we want to understand the basic biology, but we want to understand the biology as a prelude to vaccinology, therapeutics, and community outreach,” Dr.

**“Not only do we want to understand the basic biology, but we want to understand the biology as a prelude to vaccinology, therapeutics, and community outreach. I think Stanford has rallied in a way that is pretty remarkable.”**

— *Lucy Shapiro, Ph.D.*

Shapiro said. “I think Stanford has rallied in a way that is pretty remarkable.”

The results of these concentrated SARS-CoV-2 research efforts have been exceptional. Over the last few months, Stanford scientists developed new vaccine candidates in record time, created a new model for testing vaccines, and devised a unique approach to preventing infection. They also conducted intensive studies on the impact of SARS-CoV-2 on the immune system and on the lungs, which are a major target of infection. Many of these research programs built on years of earlier basic research.

Throughout this time, the scientists have been able to take advantage of Beckman’s technology service centers, which continued to operate during the pandemic under strict safety protocols.

“The fact that we were able to continue and provide these services, again through very difficult times, is a testament to the people who direct the service centers,” Dr. Shapiro said. “They have consistently responded to every request. It’s also important to acknowledge all the people who work at the lab benches, at the lab centers, and at the service centers, as well as the principal investigators who had the vision and stamina to see where we must go, not only as a scientific community, but as an economy to help protect global health.”

## THE HUNT FOR AN IDEAL VACCINE

For decades, Peter S. Kim, Ph.D., the Virginia and D.K. Ludwig Professor of Biochemistry, has focused on creating vaccines to fight some of the world’s major scourges—HIV, Ebola, and pandemic flu. When the coronavirus struck, he paused that work and his lab immediately shifted its attention to the new foe. Dr. Kim’s experience in vaccine development suggested

this could be a huge challenge: Scientists can spend up to a decade developing a vaccine and sometimes, as with HIV, they may not succeed at all.

In a matter of months, however, Dr. Kim’s team was able to produce a vaccine prototype for SARS-CoV-2



**PETER S. KIM, PH.D.**

Virginia and D.K. Ludwig Professor of Biochemistry

that is effective in mice and has several advantages over the vaccines currently in use. He said he hopes to build a vaccine for global distribution—a simplified version that can be delivered in one dose, rather than two, that doesn’t require refrigeration, and is inexpensive to make.

“As with the Ebola vaccine, our goal is to develop a vaccine that will be a single shot, because in many places around the world, recalling somebody a month later is fraught with logistical issues,” Dr. Kim said. “We also want to create a vaccine that is stable at room temperature. Even in this country, having to deal with frozen vaccine is creating all sorts of issues. Just imagine trying to do that in low- or middle-income countries. If we want to solve this pandemic, we have to solve it on a global scale.”

Dr. Kim's prototype takes advantage of the unique crown-like spikes on the surface of the SARS-CoV-2 coronavirus (corona is Latin for "crown"). These spike proteins are crucial to the virus, as it uses them to attach to a cell and gain entry, so it can use the cell's machinery to reproduce. The spike proteins, however, can also be used as antigens—molecules that trigger an immune response in the body.

To deliver the spike proteins, the researchers relied on a ferritin-based system originally developed at the National Institutes of Health and known to be safe in humans, as it was tested in two clinical trials for a flu vaccine, Dr. Kim said. Ferritin, an iron-carrying protein, naturally self-assembles to create a nanoparticle, similar to a tiny ball, to which the researchers attached the spike protein.

The researchers then immunized mice, using the nanoparticle together with the full spike protein, as well as the nanoparticle with a truncated version that deleted an end piece of the spike. They also tested a version that contained the full and shortened versions of the protein without the nanoparticle.

In those vaccine candidates containing the nanoparticle, "We found that after a single injection, the mice produced antibodies, and those antibodies were capable of neutralizing the virus," Dr. Kim said.

Furthermore, the antibodies produced were more than twice the number of those found in the plasma of patients who've recovered from COVID-19, the researchers found. And surprisingly, the vaccine variant containing the shortened spike protein elicited the best antibody response. The scientists described their work in a January 5, 2021, paper in *ACS Central Science*.

The next step is to test the vaccine in primates to see if it generates the same level of neutralizing

antibodies found in mice, Dr. Kim said. The researchers would also have to conduct safety studies in animals before they could apply to the federal Food and Drug Administration (FDA) to begin testing in humans.

**"Our goal is to develop a vaccine that will be a single shot. In many places around the world, recalling somebody a month later is fraught with logistical issues. We also want to create a vaccine that is stable at room temperature. If we want to solve this pandemic, we have to solve it on a global scale."**

— Peter S. Kim, Ph.D.

"We are doing the planning for all of that, but we need the funding," said Dr. Kim, whose decade as president of Merck Research Laboratories has given him the perspective and experience needed to navigate the process.

While the research showed the vaccine was effective in a single dose, the team is still working to prove that it can survive without refrigeration. Dr. Kim said he also hopes it can be freeze-dried to make distribution even easier. In addition, this vaccine could be much cheaper to produce than the mRNA vaccines made by Moderna and Pfizer that are currently authorized by the FDA for emergency use. And it's not likely to have the same potential for side effects as vaccines based on viral delivery systems, he said.

In developing the prototype, Dr. Kim said he relied on collaborations with many Stanford colleagues, including those at the Stanford Linear Accelerator Center (SLAC) who used electron cryo-microscopy

to confirm the 3D structure of the spike ferritin nanoparticles, as well as colleagues at the Stanford Blood Center, who provided the plasma from recovered patients.

“It’s very exciting to be at Stanford where we have the hospital, the medical school, SLAC, all in one place; they all came to bear,” he said. “It’s the sort of collaborative interdisciplinary work that people at the Beckman Center and Chem-H (the Stanford Chemistry, Engineering & Medicine for Human Health institute) have fostered.”

Dr. Kim said it’s astounding that scientists throughout the United States have been able to respond so quickly to the epidemic with effective vaccines.

“It really has been remarkable,” he said. “There’s never been a situation where we’ve developed a vaccine against an infectious disease in less than a year. It builds upon years of work, both with mRNA vaccines as well as the viral vector vaccines. People should not underestimate what a tremendous accomplishment this was—how it builds on the investment in basic science over the decades.”

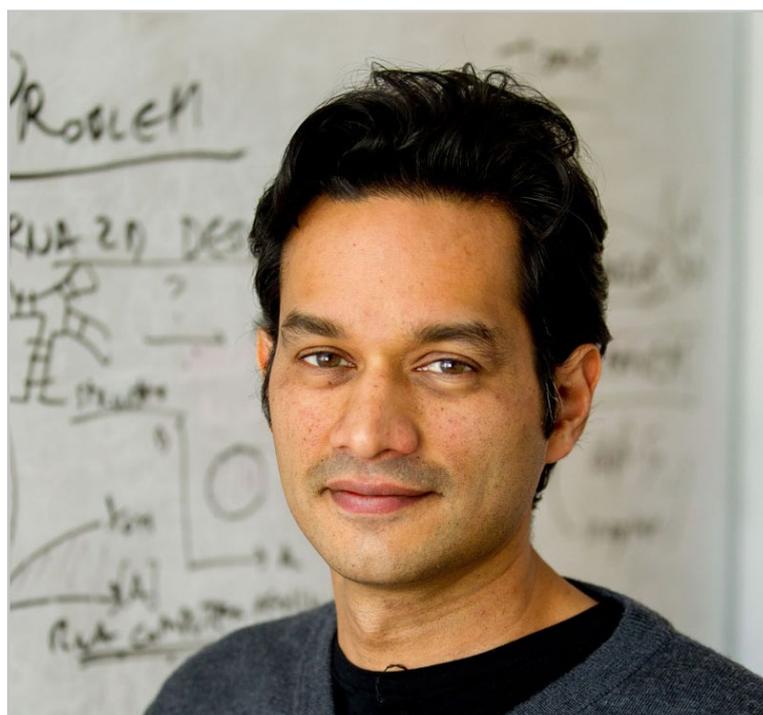
## AN INVENTIVE WAY TO BUILD A VACCINE

Like Dr. Kim, Rhiju Das, Ph.D., an associate professor of biochemistry, is working to build a COVID-19 vaccine with global potential—one that can reach billions of people quickly. That means the vaccine would have to be easy to distribute and be able to retain its potency over time at room temperature.

To accomplish that, Dr. Das is using the power of multiple minds, enlisting the help of imaginative citizen-scientists from around the world to solve what he calls a unique “molecular puzzle.”

In March 2020, Dr. Das issued an OpenVaccine challenge, a new project hosted on his decade-old gaming platform Eterna (<https://eternagame.org>), which in the past has been used to develop an inexpensive tuberculosis (TB) diagnostic and other useful medical tools. The OpenVaccine challenge called on thousands of gamers to help create a vaccine based on an RNA structure that would remain stable at various temperatures, long enough to survive a trip across the globe for shipping and distribution.

“In February, we knew RNA vaccines would be the first to be authorized, as mRNA technology was heating up,” Dr. Das said. “So the primary goal was this problem of trying to stabilize the vaccines. We’ve had some really remarkable results.”



**RHIJU DAS, PH.D.**

Associate Professor of Biochemistry

Dr. Das and his colleague Maria Barna, Ph.D., an associate professor of genetics, have discovered mRNA design rules and invented experimental technologies that can produce mRNA molecules with dramatically increased stability compared to the existing ones produced by Moderna and Pfizer. They are now testing

whether the formulated vaccine has to be frozen to remain viable, as the current mRNA vaccines do, and whether it could potentially be packaged in syringes and shipped just as the flu vaccine is now.

**“It’s really exciting. Our work has the potential to affect billions of people on a rapid time scale, as well as to lower the costs and barriers associated with future mRNA medicines.”**

*— Rhiju Das, Ph.D.*

In addition, the researchers have figured out how to rapidly redesign the RNA structure so the vaccine will be effective against some of the newer, more dangerous strains of SARS-CoV-2 that have emerged, Dr. Das said. They are now working with an

industry partner and hope to begin clinical trials soon.

“It’s really exciting for us,” said Dr. Das. “Our work has the potential to affect billions of people on a rapid time scale, as well as to lower the costs and barriers associated with future mRNA medicines.”

In the OpenVaccine challenge, participants started by tackling weekly RNA puzzles. RNA, which encodes the genetic information for proteins, folds into complex, three-dimensional shapes, which enable it to function properly. But RNA molecules tend to be “floppy,” contorting into folds that lead to the RNA cutting itself, Dr. Das said. The goal was to find designs that would remain stable.

Dr. Das said thousands of people participated in the challenge, with about 100 making fundamental contributions to the design process. He said the project also benefited from some recent computational advances he and his colleagues had

made in managing large amounts of data on RNA folding patterns on Eterna.

“Although we weren’t working on vaccines then, those large datasets ended up having a huge impact on how we tackled RNA vaccines,” he said.

The process yielded hundreds of promising designs that the researchers tested in the lab to see if they were translatable in human cells and if they maintained their stability over time. Dr. Barna developed a new technology for this purpose, using her expertise in ribosomes. There are millions of ribosomes inside each cell that are essential to the translation process, as they take encoded information from RNA to produce proteins.

Dr. Barna and her colleagues were able to determine which RNA structures would translate the best by measuring the number of ribosomes associated with them—the more ribosomes, the better the translation. They also used the technology to measure the ability of the RNAs to survive over time.

“We can take measurements by putting the RNA in the cells, then taking measurements 6, 8, 10, and 12 hours later to see how long the RNA is hanging around, as a measure of how stable it is,” Dr. Barna explained.

She said the project broke new ground by showing that putting more structure in RNA would not hamper its translational ability.

“It was a huge conceptual advance,” she said. “In the past, people thought it would never work. But we found the RNA can be more structured and still be well-translated in the cell.”

Dr. Das used crowdfunding to help support the research and has posted the resulting sequences in the public domain so any company or academic institution

can make use of them. "That's really important and something I'm really proud of," he said.

In addition to that research, Dr. Das and colleagues in the lab of Jeffrey S. Glenn, M.D., Ph.D., a professor of medicine (gastroenterology and hepatology) and of microbiology and immunology, developed an anti-viral that takes a different approach to combatting the



**MARIA BARNA, PH.D.**

Associate Professor of Genetics

coronavirus. Most anti-virals target the spike protein, the characteristic crown-like spike on the surface of the virus. This molecule targets the viral genome itself.

"We realized that there are likely really important parts of the RNA genome where, if you could design molecules that bind up those regions, you may be able to block infection and viral replication," Dr. Das said.

"What's cool about that idea is it will allow for any future pandemics, for immediate design of anti-virals on demand for any new pathogen."

The researchers collaborated with Wah Chiu, Ph.D., the Wallenberg-Bienenstock Professor, who discovered a way to visualize the elaborate, three-

dimensional structures of pieces of RNA using cryo-electron microscopy. The scientists used that technology to image pieces of the coronavirus RNA, and then looked for molecules to disrupt those structures, he said.

Dr. Das and his colleagues have had promising results with the anti-virals in cell cultures and are advancing to animal testing.

"A lot of the work has been done on the basis of experiments, technologies, and datasets uniquely available at Stanford," Dr. Das added. "At Beckman, I feel the environment and the labs we have, and the programs to defray the cost of the equipment, have been really important for setting the foundation for what we have been able to do."

Dr. Barna, who is a basic scientist, said this was her first experience in seeing her findings move into a potential clinical application.

"It's really amazing," she said. "We've worked nonstop. Even though it's been tiring, keeping an eye on the impact to humanity has pushed us to really keep going."

## A NEW TESTBED FOR VACCINES

Scientists typically test vaccine candidates in mice as a first step to introducing them into humans. But mice are notoriously unreliable test subjects, noted Mark M. Davis, Ph.D., the Burt and Marion Avery Family Professor, director of the Stanford Institute for Immunity, Transplantation and Infection, and a Howard Hughes Medical Institute investigator.

"Mice respond to every vaccine," said Dr. Davis. "They are extremely vaccine-friendly. There are many vaccines that have worked spectacularly in mice, but not in humans. No one really knows why."

He said the recent history of vaccinology is “mostly failure,” citing TB, HIV, dengue, and malaria vaccines as examples. That’s due in part to misleading mouse models.

Now Dr. Davis has found a surprising human model for vaccine testing—one that could make vaccine development cheaper, faster, and more predictable. It’s the tonsil, an olive-sized organ in the throat that is a key part of the immune system. Dr. Davis likens the tonsil to a giant lymph node.

Each pair of tonsils contains about two billion cells—mostly B cells and T cells that comprise the body’s first line of defense against invading pathogens. Tonsils are readily available in the United States, as about half a million are discarded each year after surgery, Dr. Davis said.

In a recent study, Dr. Davis and his colleagues obtained tonsils from local patients who’d had them removed because the organs were obstructing their breathing during sleep. The researchers separated out the various cells in the tonsils and placed them in a laboratory culture. The cells then naturally reassembled into clusters that resemble the germinal centers in lymph nodes, where antibodies are produced.

Postdoctoral fellow Lisa Wagar, Ph.D., now an assistant professor at the UC Irvine School of Medicine, then introduced a flu vaccine into the mix.

“She was able to show that if you gave the tonsil a flu vaccine, the tonsil actually responded and made antibodies after a week,” Dr. Davis said. “With that, we really knew this was the way to go.” In other words, the system reacted exactly as lymph nodes do when people get a vaccine: It pumped out antibodies primed to fight off the offending pathogen.



**MARK M. DAVIS, PH.D.**

Burt and Marion Avery Family Professor  
Director, Stanford Institute for Immunity, Transplantation and Infection  
Howard Hughes Medical Institute Investigator

Dr. Davis said he had no luck in getting their study published until COVID-19 hit, when suddenly there was intense interest in vaccines. He also began working with Vaxart, Inc., a vaccine developer based in South San Francisco, which had a COVID-19 vaccine candidate in the works.

“Sean Tucker, the chief scientific officer, called me and said, ‘Would you have a use for this COVID-19 vaccine we’re developing?’” Dr. Davis recalled.

“I said, ‘Yeah, let’s put it in the tonsils.’ We got a response—a few of the subjects’ tonsils had

**“The tonsil model is ideal for studying a wide range of human immunological interactions, something that’s not been available until now.”**

*— Mark M. Davis, Ph.D.*

noticeable antibodies to the vaccine. It wasn't super robust. It wasn't as robust as the flu vaccine response, but it was detectable. It helped validate the system and showed it was something that could be broadly applied." The researchers reported their results in January 2021 in *Nature Medicine*.

Dr. Davis said the tonsil system is ideal for testing vaccines as it produces quick results, saving both time and money. Scientists could try out hundreds of candidates in a relatively short period to see which ones produce the best response.

"Say you want to make the ultimate COVID-19 vaccine," he said. "What you'd want to do is make a thousand different flavors. But if all you could do is test it in mice, it wouldn't help you very much. If you test it in tonsils, however, you'd be much more likely to find the version that works best in people."

There is a lot of variation in how people respond to vaccines. In the flu vaccine study, for instance, the tonsils from 13 children produced antibodies, but those from two others did not.

"People are different, and they respond differently," said Dr. Davis. "The goal is to optimize it to make a more effective response. This is a way to optimize, as you could test 100 or 1,000 variations and pick the one that gets the best results in the greatest variety of tonsils."

Dr. Davis said the tonsil model is ideal for studying a wide range of human immunological interactions, something that's not been available until now.

"We have a very difficult time in human work doing mechanistic experiments, as you can't slice and dice people," he said. "The better thing is to show some important function in a dish. It means you can access every cell and manipulate it to test hypotheses *in vitro*. We've not really been able to do that before."

Dr. Davis is now looking at the phenomenon of why very young children are susceptible to infection but then suddenly become more resistant after they reach age five. "No one has the slightest idea why that is. Since we can get tonsils in those age groups and in adults, it would be interesting to define what is changing, how the immune system is getting better."

He's also interested in the immune response to cancer. "Tumors appear all the time and are normally eliminated by the immune system. It's probably the rare ones that survive and end up growing and doing harm," he said. In a tonsil system, scientists could introduce cancer cells and then see what kind of response the cells engender.

The model also could open a new window on how the body is able to marshal its forces against infection, rather than be overwhelmed by harmful invaders.

"We can put live flu in the tonsils, and the virus doesn't take over," Dr. Davis said. "In fact, there is an immune response. It must be that there is enough happening to trigger the response so the infection doesn't go anywhere. It's eliminated. The ability to see that in a dish will be transformational in terms of understanding just what happens with an infection."

## HOW SARS-COV-2 ALTERS THE IMMUNE SYSTEM

Catherine Blish, M.D., Ph.D., a professor of medicine (infectious diseases), has found that the SARS-CoV-2 virus can wreak havoc on the immune system in unique and surprising ways. An infectious disease specialist, Dr. Blish has spent years stalking some major killers—HIV, Zika virus, dengue, flu, and most recently, tuberculosis. But when the 2020 lockdown forced her to abandon her TB research, she began to focus exclusively on SARS-CoV-2.

Dr. Blish's studies show that virtually all immune cells are changed in the presence of the virus. Understanding these changes is key to developing therapeutics and in furthering the development of vaccines to protect against infection.

"I want to know what a protective immune response looks like, because that's what we want to mimic with a vaccine," said Dr. Blish. "But I also want to know what inflammatory mediators we need to block to dampen severe, pathologic disease."

She began her studies using a virus obtained from the first identified patient in Seattle, Washington. She initially lacked a suitable space for her work, as Stanford did not have the needed laboratory facilities. Because the coronavirus is airborne and highly infectious, the federal Centers for Disease Control and Prevention requires a Biosafety Level 3 (BSL-3) lab for COVID-19 research.

Dr. Blish had been using a campus BSL-3 lab for her TB studies, which she shared with chemist Carolyn Bertozzi, Ph.D., the Anne T. and Robert M. Bass Professor in the School of Humanities and Sciences, the Baker Family Director of Chem-H, and a Howard Hughes Medical Institute investigator. But that lab was tiny and outdated. So Dr. Blish became the driving force in the construction of a new, 4,000-square-foot BSL-3 lab, which opened in September 2020. The state-of-the-art facility, which was completed in record time, has separate spaces for work on SARS-CoV-2 and TB. "It's really well-equipped and beautiful," she said.

Dr. Blish's initial SARS-CoV-2 research focus was on the cells that make up the front-line of defense in the immune system—neutrophils, the common white blood cells that are the first to arrive on the scene, and monocytes, which chew up harmful pathogens.

"Those first responders are just incredibly changed in the setting of coronavirus infection," she said.

Monocytes normally secrete cytokines, which help immune cells communicate and direct an attack, but they appear to become dysfunctional when the virus is present. "They are kind of paralyzed in the blood," she said. "They are not making cytokines. We know these cytokines are present, but they are not being produced by these monocytes."

Neutrophils, which are a huge part of the immune response to COVID-19, also appear to undergo unusual changes. Dr. Blish and her colleagues saw immature



**CATHERINE BLISH, M.D., PH.D.**

Professor of Medicine (Infectious Diseases)

and mature neutrophils become highly inflammatory and express markers for NETosis, a form of cell death. During this process, the cells explode and form a large net that can capture harmful bacteria. But the nets are too small to trap viruses and are highly correlated with poor outcomes during viral infection, she said.

"We think the neutrophils are causing hyper-inflammation and a huge number of problems," Dr. Blish

said. "When that neutrophil population is present, it predicts a fatal outcome 80 percent of the time."

**"It's possible that this is one of the many potential pathways to turn back this hyper-inflammatory clock."**

— Catherine Blish, M.D., Ph.D.

Dr. Blish and Dr. Bertozzi have identified a protein, called Siglec-9,

that could potentially interfere with this inflammatory response. They incubated healthy neutrophils with plasma from COVID-19 patients to induce NETosis. When they added an agonist Siglec-9 to the mix, it completely inhibited the process, they found.

"It's possible that this is one of the many potential pathways to turn back this hyper-inflammatory clock," said Dr. Blish.

Dr. Blish is also working with Calvin Kuo, M.D., Ph.D., the Maureen Lyles D'Ambrogio Professor, who has created a lung organoid, a mini ball of lung cells that arrange themselves somewhat like a human organ. The researchers infected the cells with the SARS-CoV-2 virus and found it impacted the alveolar type 2 cells, which was expected, but also club cells, which was surprising, she said. Club cells are involved in creating surfactant, a substance that lines the surface of lung cells and prevents them from collapsing during breathing.

"This could potentially explain some of the inflammatory conditions," Dr. Blish said. "We may be messing up the secretions that made the lung cells happy."

She said the researchers hope to use the organoids to test new therapeutics. "Like a mini human lung, we

can figure out which drugs have promise before we do expensive clinical trials," she said.

## SARS-COV-2'S ASSAULT ON THE LUNGS

Dr. Blish has also been collaborating with molecular biologist Mark A. Krasnow, M.D., Ph.D., the Paul and Mildred Berg Professor and a Howard Hughes Medical Institute investigator, who has been studying the structure and function of the lungs for decades.

Three years ago, Dr. Krasnow had a bittersweet experience. His friend and Beckman colleague, James Spudich, Ph.D., the Douglass M. and Nola Leishman Professor of Cardiovascular Disease, showed up at his office with the news that he had been diagnosed with lung cancer and would have a portion of his lung removed the next day. Dr. Spudich asked if Dr. Krasnow might be interested in studying his cells.

Dr. Krasnow's team sprang into action the day of the surgery, transporting tissue from the surgical suite to the lab, where they separated out cells from the healthy part of Dr. Spudich's lung. They then used single-cell RNA sequencing technology to break down the RNA and determine what genes were being turned on and off in each of the isolated cells. Dr. Krasnow's collaborator, Stephen Quake, Ph.D., the Lee Otterson Professor and co-president of the Chan Zuckerberg Biohub, helped develop the sequencing technology 10 years ago and provided access to it across Stanford as part of the Biohub.

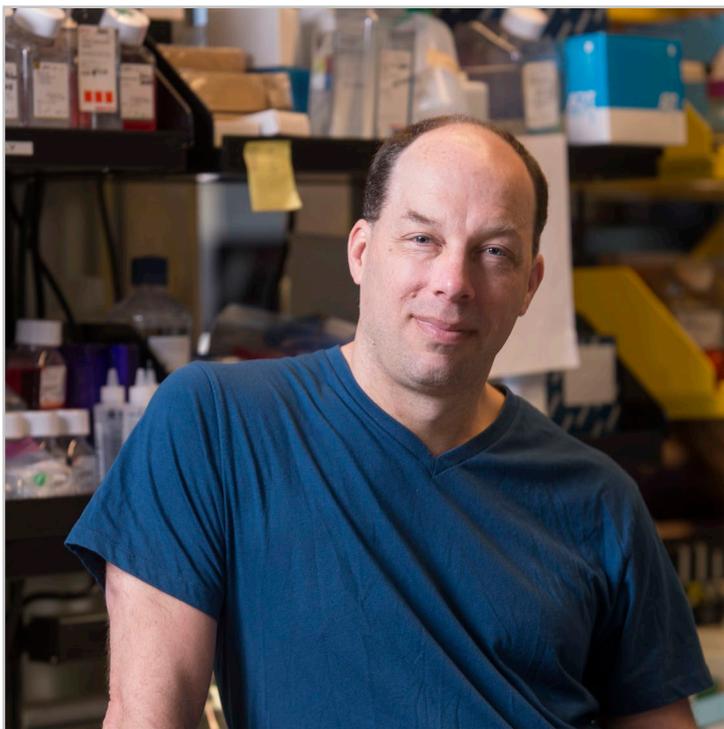
The result was a stunning accomplishment. The researchers produced a complete atlas of the human lung and its 58 types of cells, including 14 that had never been previously identified. Their findings were published in the journal *Nature*.

Then the COVID-19 pandemic struck.

“We didn’t have any inkling that these newly identified cell types were going to be at the site where the virus is doing its deadly damage and whose activity is important in preventing it from getting out of control,” Dr. Krasnow said.



**MARK A. KRASNOW, M.D., PH.D.**  
Paul and Mildred Berg Professor  
Howard Hughes Medical Institute Investigator



**STEPHEN QUAKE, PH.D.**  
Lee Otterson Professor  
Co-President, Chan Zuckerberg Biohub

Dr. Krasnow expanded his collaboration with Dr. Blish and Dr. Quake to create a system where, for the first time, scientists could study how a virus gains access to human lung tissue and observe the process of viral infection at the level of single-cell resolution. The researchers obtained additional lung tissues from donor patients and exposed exquisitely thin lung slices to the SARS-CoV-2 virus in the lab. The work was done in the new BSL-3 facility, with Dr. Quake carting his single-cell RNA sequencing instrument into the space.

“Because we have almost all of the cell types of the human lung in

that tissue, we found which cell types are infected, what the virus life cycle looks like, and how it takes over the infected cells,” Dr. Krasnow said. In studying the initial effects of the disease, he said, he hopes to find therapeutics to intervene and stop the process.

**“We didn’t have any inkling that these newly identified cell types were going to be at the site where the virus is doing its deadly damage and whose activity is important in preventing it from getting out of control.”**

— *Mark A. Krasnow, M.D., Ph.D.*

Their work has yielded some remarkable results, with the scientists effectively tracing the path of viral destruction. Dr. Krasnow said the virus targets at least half a dozen cell types, including the alveolar cells, key functional structures deep in the lung that facilitate the process of gas exchange. As these cells begin to falter, alveoli epithelial stem cells—which were newly identified by Dr. Krasnow’s team—work to replenish the dying

cells. But if the virus gains the upper hand, it can infect the underlying capillaries and stromal cells to cause leakage into the alveolar space. The alveoli then start to drown in the fluid and the barrier to the bloodstream begins to break down.

“Once the virus gets through the air-blood barrier, it spreads through the body and causes not just the devastating pneumonia but systemic disease, with all the problems in different organs patients have suffered,” Dr. Krasnow said.

In a surprising finding, the researchers discovered that alveolar macrophages also are heavily impacted by the virus, something not seen before. These are the immune cells in the alveoli that serve as sentries, constantly on the lookout for microbes so they can eat them up and destroy them. But these guardians have become traitors to the cause, giving way to the virus.

“The virus is getting in, infecting and converting these sentries into viral terrorists by helping the virus replicate,” Dr. Krasnow said. “They move around and may be spreading the disease in the lung, the exact opposite of what you want your guardians to do.”

In another striking finding, the scientists discovered that the alveolar macrophages don’t express the ACE-2 (angiotensin-converting enzyme 2) receptor, the protein on the surface of many cells through which the SARS-CoV-2 virus is thought to gain entry. Current vaccines and therapeutics are all designed around use of the ACE-2 receptor, which binds to the virus’s spike protein.

“The way it’s getting into these macrophages seems to be molecularly distinct from the way everyone believes the virus gets into other cells, like the alveolar type 2 cell,” Dr. Krasnow said.

“We’re exploring right now—what is the mechanism and the molecules that the virus uses to get into the macrophages? The question is, if the virus is using a different mechanism to get in, is that mechanism being blocked by the current antibodies and vaccines? If not, the virus might still have access to this cell reservoir deep within our alveoli, which it can infect and propagate in.”

Dr. Krasnow is now working with Dr. Kim and his colleagues, who have developed a method to block SARS-CoV-2 in macrophages, to see if this might point the way to a new therapeutic.

## A NOVEL PREVENTION METHOD

A pandemic calls for out-of-the-box solutions, and that is what Daria Mochly-Rosen, Ph.D., the George D. Smith Professor in Translational Medicine, is pursuing. Her team at Stanford’s SPARK Program in Translational Research has created a simple, cheap preventative based on an unusual approach: chicken antibodies sprayed in the nose as a temporary barrier to the virus. With one application, a user may be immediately protected for several hours, a hedge against infection that could be particularly useful in settings where a vaccine may not be available.

“It can be made locally and cheaply, so it can help stop the epidemic,” said Dr. Mochly-Rosen. “We now see how difficult it is to distribute the vaccine. There are many low- and middle-income countries where there is zero percent vaccine. If we can show this is efficacious, people in these countries will have some protection while they wait for the vaccine.”

Dr. Mochly-Rosen’s group already has tested the nasal spray in a Phase 1 trial, which showed it to be safe. She is now preparing for a Phase 2 trial to see if it’s as effective as she hopes.



**DARIA MOCHLEY-ROSEN, PH.D.**

George D. Smith Professor in Translational Medicine

The idea emerged out of SPARK, her nonprofit organization that connects academics around the world in an effort to find rapid answers to emerging medical problems. When the pandemic struck, one of her country directors in Australia, who had tested chicken antibodies in a mouse model of flu, suggested it as a possible response to SARS-CoV-2.

As she scanned the literature, Dr. Mochly-Rosen learned that chicken antibodies in protective nasal sprays had been used for a number of conditions in both animals and humans.

"It has been shown to be effective against influenza, H1N1, so I definitely think it could be used for airborne viruses," Dr. Mochly-Rosen said. "It has been shown in animals to be effective for ulcers caused by bacteria and for various coronaviruses. And it's been shown to be effective against some gut bacteria and gingivitis in humans. It's really a simple procedure that can be effective against a lot of threats, but most importantly against epidemics."

The process is straightforward. Researchers inject the spike protein of the coronavirus into the breast of hens. The animals respond by producing large quantities of antibodies, a chicken version known as immunoglobulin Y (IgY). These antibodies are found in high concentrations in chicken yolk. The researchers harvest these antibodies from eggs laid by immunized hens and then purify them into nasal drops.

The antibodies are considered safe for humans; in fact, we are exposed to them when we eat eggs, Dr. Mochly-Rosen said. "However, they are usually not snorted," she noted with a smile, which is why they need to be rigorously tested.

The approach has many advantages. For one, it provides immediate protection. "It's not like a vaccine where you have to wait three or four weeks," Dr.

Mochly-Rosen said. "Here, the moment you put in the nose drops, the antibodies capture the virus before it gets into the body."

The spray doesn't trigger an immune response, like a vaccine, because it's not injected into the bloodstream. Because it's not systemic, it has potential to be used

**"We now see how difficult it is to distribute the vaccine. There are many low- and middle-income countries where there is zero percent vaccine. If we can show this is efficacious, people in these countries will have some protection while they wait for the vaccine."**

*— Daria Mochly-Rosen, Ph.D.*



in people who are immunocompromised, in children, and in pregnant women, she said.

The protection is temporary, as the nose naturally clears material in a matter of hours. Dr. Mochly-Rosen believes users would have to reapply the nose drops every four hours when they are at risk of exposure to the virus.

But the nasal spray is easy and cheap to make. Each egg contains between 10 and 100 doses. Hens lay eggs every day, so there is a plentiful supply available. Dr. Mochly-Rosen believes the spray could be produced for as little as \$1 per daily dose.

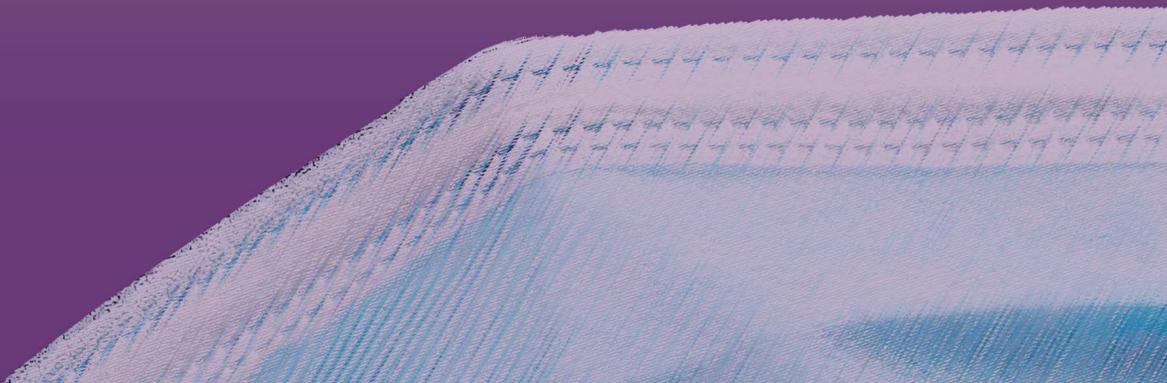
It may even be possible for people to make the spray themselves at home. She's now working with a group of Stanford undergraduates to see if it could be easily developed in a home setting. She said people anywhere could use it as an extra measure of protection in addition to a face mask.

Dr. Mochly-Rosen believes so strongly in the concept that she has used every resource she has, including most of her SPARK budget, her endowed faculty funds, and various small grants, to test the product. Members of her SPARK network around the world, including researchers in Australia, Norway, Belgium, Canada, Brazil, and South Africa, also have contributed know-how and advice to the project, she said.

Ultimately, she believes the approach will serve as a blueprint for protective nasal sprays that could be applied against other pathogens, such as Ebola. "Within three to four weeks, we could have a product produced in a country when a new epidemic begins," she said.

In that respect, Dr. Mochly-Rosen is like many Beckman scientists whose work will not only benefit human health now, but also help protect us well into the future.

# OVERVIEW & HIGHLIGHTS



# OVERVIEW & HIGHLIGHTS



## BECKMAN CENTER OVERVIEW

The breakthroughs that took place in genetic engineering, cell imaging, and genomics in the late 1970s and the 1980s had a profound impact on the field of medicine, introducing new technologies and opening up new avenues of research in genetics and molecular biology.

Recognizing the impact that this new body of knowledge would have on improving the diagnosis, prevention, and treatment of disease, Paul Berg, Ph.D., a Stanford University School of Medicine professor and Nobel Laureate, sought to establish a center at Stanford that would integrate the basic, clinical, and applied sciences. His vision was that the rapid advancements taking place in the fields of molecular biology and genetics might become more readily

available to clinical scientists, and thus hasten the translation of scientific discovery into new medical technologies and clinical applications. In 1989, with the inauguration of the Arnold and Mabel Beckman Center for Molecular and Genetic Medicine, Dr. Berg's vision became reality and Stanford ushered in a new era of rapid advancement in the field of molecular and genetic medicine.

Under the leadership of its current director, Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research in the Department of Developmental Biology, the Beckman Center continues to be at the vanguard of basic science, translational medicine, and technological discovery. The Beckman Center today houses three academic departments and the Howard Hughes Medical Institute Unit in Molecular and Genetic Medicine,

and has a world-class faculty of research scientists that includes three Nobel Laureates, 25 members of the National Academy of Sciences, and 20 Howard Hughes Medical Institute investigators.

Serving as a model of interdisciplinary collaboration at Stanford University, the Beckman Center has given rise to such forward-thinking approaches as the Stanford Bio-X program and the Department of Bioengineering, a novel joint department that spans the Schools of Medicine and Engineering.

The Beckman Center plays a central role in the School of Medicine's strategic plan to integrate the basic, applied, and clinical sciences at all levels of education and research. With the completion of the Human Genome Project and the advent of novel imaging technologies, the Beckman Center continues to influence scientific research through its support of key alliances and innovative programs.

## PROGRAMS AT A GLANCE

The Beckman Center established the Program in Molecular and Genetic Medicine (PMGM), a scientific cooperative governed by a 12-member advisory board from Stanford's School of Medicine, School of Engineering, and School of the Humanities and Sciences, to provide programmatic leadership in basic science research and education. This year, the PMGM elected to continue to support an exciting array of innovative programs, including:

**Translational Research Program**—supports early-stage research for interdisciplinary technology development projects with a translational “bench-to-bedside” emphasis.

**Faculty Recruitment Program**—helps to bring in world-class faculty in the basic sciences whose research goals are particularly well-suited to the overall mission of the Beckman Center.

**Seminars and Symposia**—funds numerous seminar series and symposia, including the annual Beckman Symposium.

**Research Technology Resources**—underwrites state-of-the-art technology development at the Beckman Service Centers, to facilitate scientific research and discovery.

**Beckman Medical Scholars**—helps to fund medical students engaged in basic science scholarly concentrations.

## 2020-2021 HIGHLIGHTS

The Arnold and Mabel Beckman Center for Molecular and Genetic Medicine enjoyed an exciting and productive year of scientific achievement, despite the limitations imposed by the COVID-19 pandemic. This year's highlights are as follows.

### **Beckman Center Director Awarded the 2020 Dickson Prize in Science**

Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research in the Department of Developmental Biology and director of the Beckman Center for Molecular and Genetic Medicine, was awarded the 2020 Dickson Prize in Science. Awarded annually since 1970 by Carnegie Mellon University, the Dickson Prize in Science recognizes substantial achievements or sustained progress in the fields of the natural sciences, engineering, computer science or mathematics. Dr. Shapiro was awarded the prize for

her work showing that the bacterial cell is controlled by an integrated genetic circuit functioning in time and space that serves as a systems engineering paradigm underlying cell differentiation and ultimately the generation of diversity in all organisms. Her work has allowed the development of novel drugs to fight emerging infectious diseases.

### **Beckman Faculty Member Discovers Defect in Pancreas Alpha Cells Linked to Diabetes**

Beckman faculty member Seung K. Kim, M.D., Ph.D., professor of developmental biology and director of the Stanford Diabetes Research Center, discovered that pancreatic alpha cells from people with diabetes release excess amounts of glucagon, a hormone important in blood sugar control. The discovery was made possible by a new mouse model of diabetes engineered by Dr. Kim and colleagues that, for the first time, permits functional studies of transplanted human alpha cells.

Previous research into the relationship between insulin and glucagon was hampered by the fact that mouse and human glucagon are identical in structure. They were impossible to distinguish from one another after human islets had been transplanted into laboratory mice. As a result, researchers could only study human glucagon production by alpha cells in test tubes or culture dishes, which does not accurately mimic what happens in the body. Dr. Kim's new technology eliminated this barrier.

The current study supports the growing realization that diabetes is likely due to defects in multiple cell types, and highlights the importance of the mouse model to simulate more accurately the complexities of the disease.

## **COVID-19 RESEARCH PROJECTS—TESTING**

### **Developing Rapid At-Home Testing for COVID-19**

PMGM faculty member William Greenleaf, Ph.D., associate professor of genetics, is leading an effort to develop new methods of COVID-19 detection at the point of care, to help address supply chain problems and allow for rapid screening. The test kit in development is meant to function directly in sample swabs and, in principle, could be done at home or in decentralized clinics, once users are taught proper swabbing techniques.

### **Tracking COVID-19 with Wearables**

PMGM faculty member Michael Snyder, Ph.D., the Stanford W. Ascherman, M.D., FACS, Professor in Genetics, and his team members, including Samuel Yang, M.D., FACEP, associate professor of emergency medicine, and Megan Mahoney, M.D., clinical professor, are using smart watches worn by patients to track biological parameters of individuals who are ill with COVID-19 or at risk for the disease. Their goal is to determine whether they can detect that the smart watch user is becoming ill based on watch measurements such as heart rate.

## **COVID-19 RESEARCH PROJECTS—VACCINATION AND TREATMENT**

### **Beckman Faculty Member Discovers Possible Drug Target for Pathogens, Including SARS-CoV-2**

Beckman faculty member Irving Weissman, M.D., the Virginia and D.K. Ludwig Professor of Clinical Investigation in Cancer Research, professor of developmental biology, and director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, along with a number of his colleagues,

discovered that cells infected by viruses or bacteria can send out a protein-based “don’t eat me” signal to avoid attack by the body’s immune system.

The researchers showed that both mouse and human cells showed increased expression of the protein CD47 on their surfaces when infected by select pathogens. SARS-CoV-2, the virus responsible for COVID-19, is one of the viruses that causes increased production of CD47.

To test whether negating the CD47 signal could boost the immune response to viruses, the scientists introduced an antibody that blocks CD47 to cells infected with a meningitis-causing virus called LCMV. They also exposed a mouse missing the gene for CD47 to tuberculosis-causing bacteria. Both CD47-suppressed and -deficient mice showed lower viral loads and significantly more resistance to infection, along with better survival.

Earlier research had shown that cancer cells also increase their expression of the protein CD47, thereby evading attack from the immune system.

The researchers hope that manipulating the CD47 response could be another tactic to fight viral and bacterial infections.

### **Evaluating the Potency and Durability of Slow-Delivery SARS-CoV-2 Vaccines via a Hydrogel**

Beckman faculty member Lingyin Li, Ph.D., assistant professor of biochemistry, in collaboration with members of the lab of Eric A. Appel, Ph.D., assistant professor of materials science and engineering, is exploring vaccination strategies that make use of a hydrogel that allows sustained, controlled release of small molecules over a period of weeks.

In studies in mice, they are testing the ability of this gel, developed in the Appel lab, to slowly release experimental vaccines against SARS-CoV-2 as well as cGAMP molecules, which have been shown to boost a vaccine’s potency. The study will also assess the potency of the vaccines when delivered this way.

### **Assessing Remdesivir in Hospitalized Adults With COVID-19**

PMGM faculty member Kari Nadeau, M.D., Ph.D., the Naddisy Foundation Endowed Professor of Medicine and Pediatrics, and director of the Sean N. Parker Center for Allergy and Asthma Research, and Neera Ahuja, M.D., clinical professor of medicine, are conducting a randomized, double-blind, placebo-controlled Phase 2 clinical trial to evaluate the safety and efficacy of the Ebola drug remdesivir in hospitalized adult patients diagnosed with COVID-19. The study, sponsored by the National Institutes of Health, is a multicenter trial that will be conducted at as many as 50 sites globally.

### **Defining the Therapeutic Potential of Host-Targeted Approaches for Combating COVID-19**

PMGM faculty member Shirit Einav, M.D., a Beckman faculty recruit and associate professor of medicine (infectious diseases) and of microbiology and immunology, is exploring repurposed and novel anti-viral approaches targeting two cellular kinases that act as Achilles’ heels of multiple unrelated RNA viruses. Her data shows that a combination of two already-approved anti-cancer drugs that act against these kinases inhibits replication of multiple viruses *in vitro* and reduces mortality in mice infected with the dengue or Ebola viruses. Her lab has also been developing more selective compounds that target these cellular kinases and have potent activity against multiple RNA viruses, including flaviviruses and coronaviruses, both *in vitro* and in human primary cells.

### **Developing Proofs-of-Concept for Two Novel COVID-19 Drugs to Begin Clinical Trials**

Beckman faculty member Rhiju Das, Ph.D., associate professor of biochemistry, and Jeffrey S. Glenn, M.D., Ph.D., professor of medicine (gastroenterology and hepatology) and of microbiology and immunology, are preparing two potential COVID-19 drugs for clinical trials: a lipid kinase inhibitor that could be used to combat SARS-CoV-2 and other viruses, and a locked nucleic acid, similar to one that was successfully developed earlier to target every known strain of the flu and that could potentially be used during any flu pandemic. Both of these drugs could treat multiple viruses and strains, have high barriers to resistance, and in some cases, could be used in nonviral applications.

### **Crowdsourcing Solutions to Better Understand Molecular Structures of SARS-CoV-2**

Beckman faculty member Rhiju Das, Ph.D., associate professor of biochemistry, in collaboration with members of the lab of Howard Y. Chang, M.D., Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research, is engaging participants of the online gaming platform Eterna to help scientists understand how the RNA genome of the SARS-CoV-2 virus might change during the pandemic. Eterna educates and enables its players to “be the virus,” simulating how it shifts its sequence over time to evade diagnostics, therapeutics, and vaccines being developed to fight COVID-19. The project seeks to provide RNA-structure-informed predictions and analyses that are not available through other computational efforts, while engaging and educating millions of citizens worldwide through compelling puzzles.

## **COVID-19 RESEARCH PROJECTS—EPIDEMIOLOGY**

### **Determining Pathogenicity of Various Variants and Strains of COVID-19**

PMGM faculty member Michael Snyder, Ph.D., the Stanford W. Ascherman, M.D., FACS, Professor of Genetics, and members of the lab of Benjamin Pinsky, Ph.D., associate professor of pathology and of medicine (infectious diseases), plan to track different variants and strains of COVID-19 and quantitatively determine which ones correlate with different phenotypes, such as levels of pathogenicity. Dr. Snyder’s team also plans to study the false negative rate of COVID-19, seeking to better understand how many people receive a false negative test result during early stages of the disease.

## **COVID-19 RESEARCH PROJECTS—IMMUNOLOGY**

### **Exploring Comparative Pathology and Pathogenesis of COVID-19 Infections in Humans and Animal Models**

PMGM faculty member Garry Nolan, Ph.D., the Rachford and Carlota A. Harris Professor, postdoctoral fellows David McIlwain, Ph.D., and Sizun Jiang, Ph.D., and Brice Gaudilliere, M.D., Ph.D., associate professor of anesthesiology, perioperative and pain medicine (adult-MSD), and other collaborators, are applying multiplexed technologies to achieve in-depth profiling of immune responses to SARS-CoV-2 in nonclinical and clinical studies. Their goal is to identify immune markers associated with outcomes for COVID-19 and to understand the arrangement of immune cells relative to lung tissue cells during infection.

## TECHNOLOGY RESOURCES HIGHLIGHTS

### Protein and Nucleic Acid Facility

As the COVID-19 pandemic crisis intensified, materials needed for COVID-19 testing were found to be in critically short supply. The Protein and Nucleic Acid (PAN) Facility was called on to rapidly produce the primers and probes that would be used to amplify the coronavirus genetic material in patient samples in diagnostic tests, which were being developed by Benjamin Pinsky, M.D., Ph.D., associate professor of pathology and of medicine (infectious diseases), and his team at the Stanford Clinical Virology Laboratory. The FDA-approved test that was developed as a result of this work greatly benefited Stanford patients and communities across the region and could not have been done without the critically important help of the PAN Facility.

### Cell Sciences Imaging Facility

The Cell Sciences Imaging Facility (CSIF) received funding in the amount of \$196,083 from the National Institutes of Health (NIH) Shared Instrumentation Grant Program (PI, Jonathan Mulholland, director of the CSIF) for a new Gatan, Inc. OneView scintillator-coupled sCMOS transmission electron microscope (TEM) camera, and a OneView 16bit computer running Gatan Microscopy Suite V3 software. The camera represents a new class of state-of-the-art, fast and sensitive sCMOS scintillator-coupled cameras that offer a large field of view and real-time drift correction. The availability of this camera to Stanford's researchers and microscopists greatly advances Stanford's biomedical electron microscopy-dependent research. Importantly, having the new OneView TEM

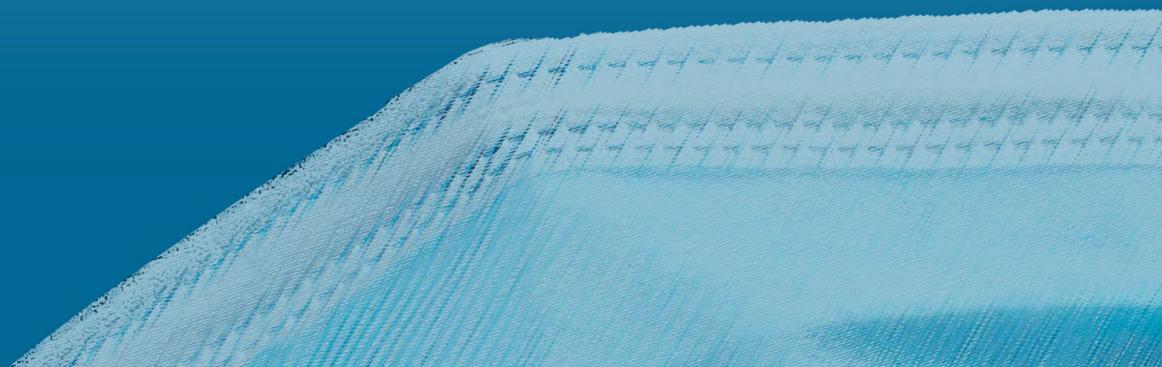
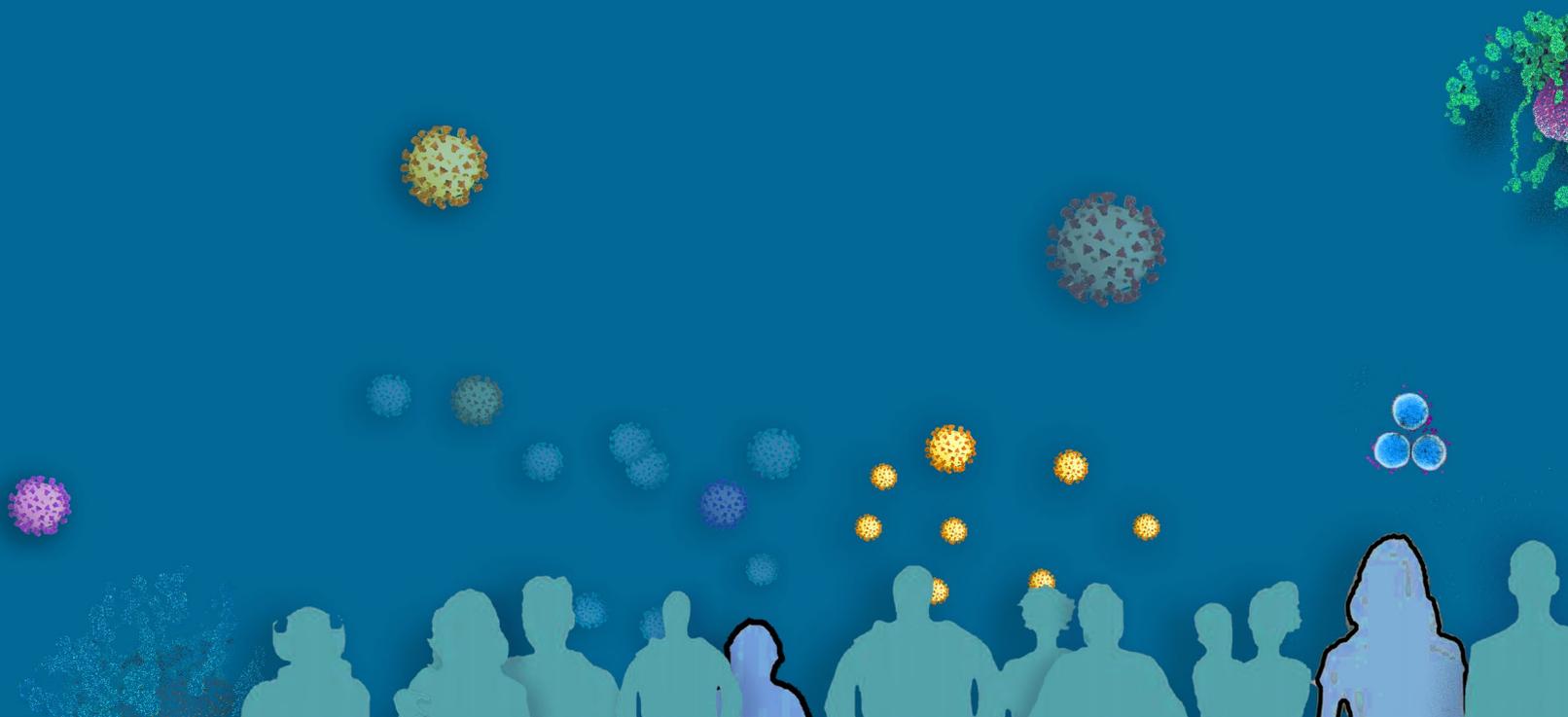
camera managed and supported by the CSIF will enhance and expand Stanford's openly accessible biological and biomedical electron microscopy resources.

### Fluorescence Activated Cell Sorting Facility

The Fluorescence Activated Cell Sorting (FACS) Facility used the lengthy shelter-in-place period prompted by the pandemic to provide nationally attended webinars on panel design and analysis. Later, when re-launching services, FACS was able to adapt additional lab space to support Biosafety Level 2 (BSL-2) research and opened its High-Parameter Analysis Lab, which houses four analyzers capable of 20-40 parameter analyses, as well as a spectral analysis remote-access workstation.

In the fall of 2020, the facility was awarded a National Institutes of Health Shared Instrumentation Grant totaling \$510,431, which was supplemented with Beckman Foundation funds to purchase a FACSymphony 5-laser, 30-parameter instrument. The new equipment was placed in the new High-Parameter Analysis Lab to support researchers analyzing the complexities of immune responses in health and disease. The facility also made use of the NIH and Beckman Foundation funding for the FACSymphony to negotiate a substantial discount on another new 50-detector, 5-laser 6-way sorter, to be delivered in 2021. The new instrument is capable of selecting unique populations from high-complexity populations and isolating them for downstream experiments and applications.

# EXPENDITURES



# EXPENDITURES

## FOUNDATION FUNDS IN THE CONTEXT OF CENTER OPERATIONS

The Arnold and Mabel Beckman Center for Molecular and Genetic Medicine officially opened in 1989 with an initial gift from the Arnold and Mabel Beckman Foundation of \$12 million. Another \$50 million in funding from private sources made it possible to complete the center on time and under budget.

The Beckman Center houses three academic departments—Molecular and Cellular Physiology, Developmental Biology, and Biochemistry—as well as the Howard Hughes Medical Institute Unit in Molecular and Genetic Medicine, all dedicated to basic sciences research and the teaching and training of medical students, graduate students, and postdoctoral fellows.

The center plays an important role in Stanford's scientific community by providing funding that would not otherwise be available for interdisciplinary research, for technology development, and for securing cutting-edge resources and services for the research community. The center's programs and initiatives serve to complement and enhance the research efforts of the resident departments, the Program in Molecular and Genetic Medicine (PMGM) faculty, and the broader research community of the university.

Without the Beckman Foundation support, many of our highly successful programs simply would not exist.

In recognition of the unique role the center plays with respect to the basic sciences, the Stanford University

School of Medicine Office of the Dean provides an annual operating budget to the Beckman Center to cover the cost of administering the programs funded by the center. In addition, the School of Medicine recently funded a complete overhaul of the Beckman Center building.

The four Beckman Service Centers—the Cell Sciences Imaging Facility (CSIF), the Protein and Nucleic Acid (PAN) Facility, the Fluorescence Activated Cell Sorting (FACS) Facility, and the Computational Services and Bioinformatics Facility (CSBF), which are used by scientists throughout the campus and are managed by the Beckman Center—are expected to generate nearly \$4.1 million in user fees this year (despite severe setbacks due to the COVID-19 pandemic), continuing a level of service that sets the standard at Stanford University. One-time emergency support, in the amount of \$1,236,088, was provided by the School of Medicine to cover COVID-related revenue losses resulting from mandatory service center restrictions. The service centers normally operate at or close to break-even each year.

## THE IMPORTANCE OF FOUNDATION FUNDS TO STANFORD'S MISSION AND GOALS

### SERVICE CENTERS

Major advances in new imaging, bioinformatics, and genomics technologies are having a remarkable impact on our ability to translate basic research into medical applications. These new technologies are very expensive and many investigators find themselves unable to purchase state-of-the-art

instrumentation. We have created service centers that provide these instruments and technologies on a fee-for-service basis, underwritten and administered by the Beckman Center.

An important component of these service centers is technology development. The Beckman Center enables the design and implementation of leading-edge technologies that are then made available to the Beckman research labs, using Beckman funds to leverage scientific discovery.

### TECHNOLOGY DEVELOPMENT GRANTS

In order to help initiate innovative new translational research projects, the Beckman Center conducts a highly competitive program in which pairs of investigators (one a basic scientist and the other a clinician scientist) propose risky, but potentially high-pay-off experiments in technology innovation. PMGM advisory board members evaluate the proposals and the center provides \$100,000 a year (for projects of two years duration) to the best proposals. This program has been highly successful and has leveraged a large multiple of funding from both federal and private sources for many of the seeded proposals.

### MEDICAL SCHOLARS PROGRAM

To foster the training of medical students in translational research, the center provides a stipend to selected students doing research in top-tier research labs with PMGM faculty. This is a competitive program, closely monitored by the Beckman Center.

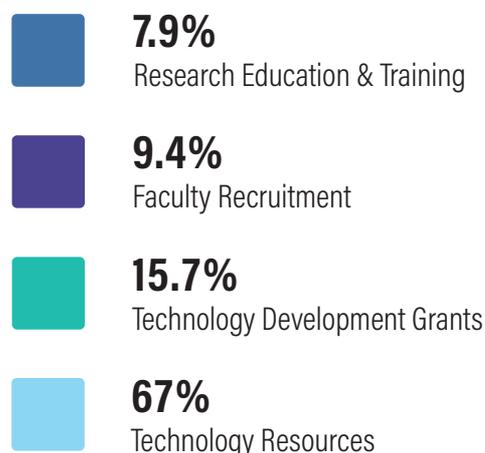
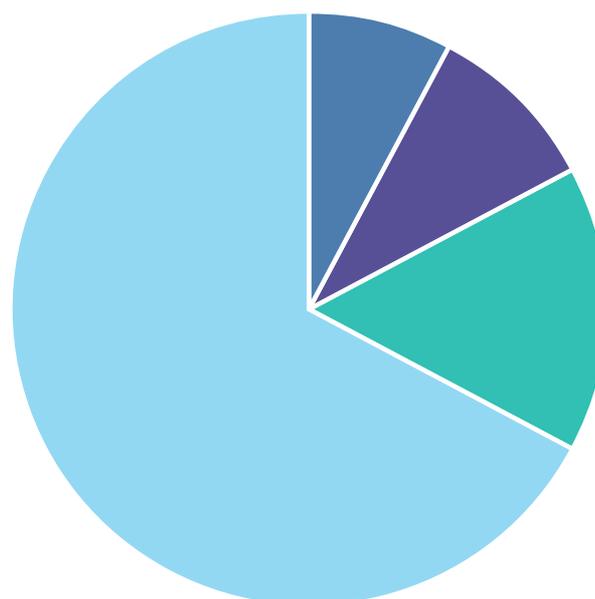
### RESEARCH COMMUNICATION AND EDUCATION

Communication among the biomedical and technology communities is, as Arnold Beckman firmly

believed, the bedrock of doing innovative scientific exploration. Accordingly, the center supports the Frontiers in Science seminar series and the annual Beckman Symposium on a critical area in scientific innovation. These symposia attract students and faculty, as well as the lay community.

## EXPENDITURE OF DIRECTOR FUNDS

The Beckman Center receives an annual gift from the Beckman Foundation that is disbursed to its programs in research education and training, faculty recruitment, technology development grants, and technology resources. The pie chart below shows how the Beckman funds were disbursed this fiscal year.



## EXTERNAL REVIEW

The Beckman Center director is initiating a formal external review process for all center programs, which will take place every five years. Given the exigencies of the current COVID-19 pandemic response, the first such review will take place in the spring/summer of 2022. Reviewers from both within and outside the university will be chosen by the director and presented with an overview of center research, including the contributions made to that research by each of the four service centers operated by the Beckman Center. Also reviewed will be the impact of the center's seed grant program, educational activities, medical scholars training program, and symposiums held over the preceding years. Reviewers will be invited to provide the director with their expert feedback on center research operations, along with suggestions they may have for new programs and/or changes and improvements to existing programs. The results of this review, along with an appraisal by the center director, will be provided under separate cover to the Beckman Foundation.

The Beckman Center runs several technology resource programs—the Beckman Service Centers—that provide support for outstanding technological and scientific advances. These service centers require ongoing monitoring, review, and assessment. The centers provide services in 1) state-of-the-art imaging technologies (CSIF); 2) protein and nucleic acid molecular analyses (PAN Facility); 3) fluorescence activated cell sorter technologies (FACS Facility); and 4) computer and biocomputational work (CSBF). All four service centers provide cutting-edge, high-tech resources to scientists on a fee-for-service basis. The

demand for these services, as measured by the revenue generated as well as acknowledgments of the work done by these facilities in peer-reviewed journals, is an important measure of their overall success and value to the scientific community at Stanford.

Each service center is under the oversight of two committees: an advisory committee of prominent users tailored to each service center, and a Cores Advisory Board that oversees and evaluates all service centers at the School of Medicine. One important role of these advisory committees is to review revenues and expenses and determine which services should be continued or discontinued.

The primary goals of the advisory committees tailored to each service center are:

- Inform the service center directors about the research tools and methods that are most needed by users of the facility
- Provide feedback to the director about the effectiveness of the services being provided
- Assess the quality of those services
- Assess the timeliness of the work being done by service center staff
- Evaluate the level of training provided to graduate students, postdocs, and other research staff
- Assess the service center staff's input and advice related to sample preparation, experimental design, and data analysis

The Cores Advisory Board meets at least once a year, and includes faculty members from the School of Medicine (often chairs of departments),

appointed by the senior associate dean for research. The board's goals are to:

- Review and approve detailed business plans for proposed new service centers
- Invite existing service center directors, in rotation, to present their budgets, revenues/expenses, and lists of users
- Analyze overall subsidies required to operate each facility, including the cost-to-income ratio of each service being provided
- Evaluate the overall demand for services in a given facility over time
- Review the list of users for each facility and the dollar volume of activity per user, in order to determine the scope of demand for those services
- Assess the degree of duplication of services between service centers across the campus
- Evaluate which high-cost technologies should be subsidized
- Determine the need for new services or new service centers
- Evaluate whether or not certain services have outlived their usefulness, are readily available outside the university, or should be discontinued

The board's recommendations are summarized and relayed to the Beckman Center director for consideration. This evaluation provides important feedback that allows the Beckman Center director to consider changes (expansion or elimination) to the services provided by the four service centers.

In addition to these review committees, Stanford University's Department of Audit, Compliance,

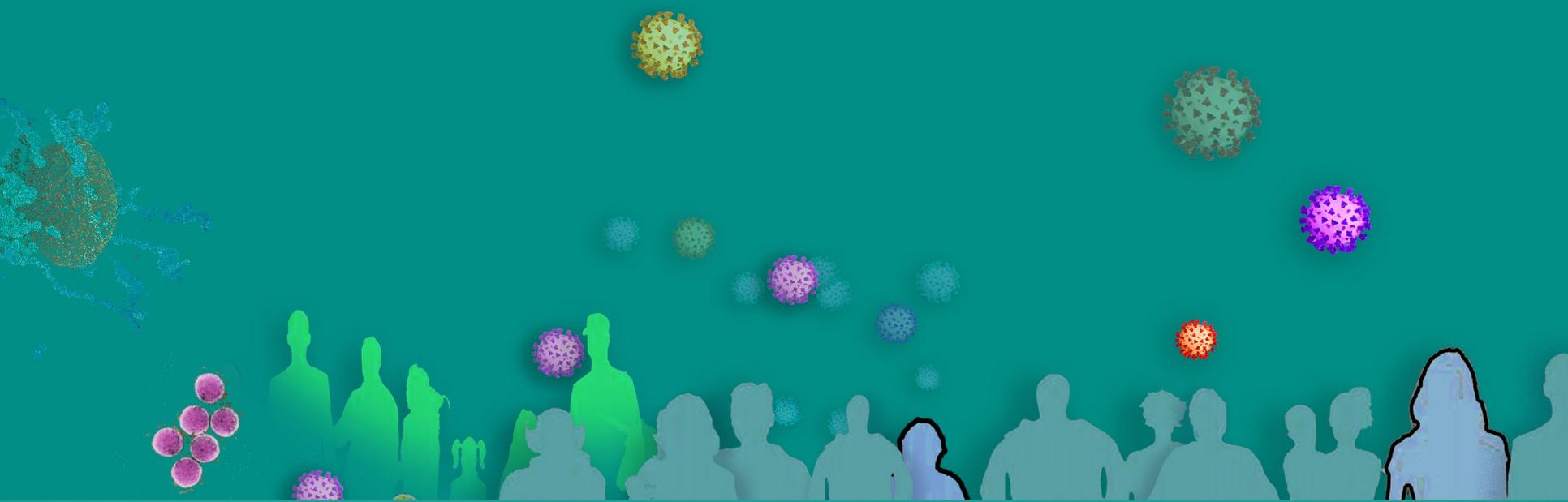
and Privacy conducts internal financial audits of the facilities, and the Office of Research Administration oversees compliance of the facilities with the university's cognizant agency, the Office of Naval Research.

Additional external review is provided by the 12-member board that advises the scientific cooperative established by the Beckman Center, the Program in Molecular and Genetic Medicine. Members of the PMGM advisory board review seed grant applications for highly innovative work in interdisciplinary technology development, aiming to ensure that awards are made equitably and on the basis of outstanding merit. In addition, the PMGM advisory board advises the Beckman Center director on matters of faculty recruitment and the need for new or revised programming.

## **ENDOWMENT FUNDS**

In lieu of endowment funding from the Beckman Foundation, the Beckman Center received an initial \$12 million gift from the foundation to partially defray the cost of building construction and the center receives an annual gift to cover operational expenses.

# PROGRAMS



# PROGRAMS

## TECHNOLOGY DEVELOPMENT GRANTS

Advances in our knowledge of basic biology, together with a rapid increase in our understanding of molecular genetics, are providing unprecedented opportunities to develop new approaches to the diagnosis and treatment of human disease. As part of the Beckman Center's emphasis on translational medicine, the Program in Molecular and Genetic Medicine (PMGM) established the Interdisciplinary Translational Research Program (ITRP).

The ITRP awards a number of Technology Development Grants. The primary goal of this seed grant program is to stimulate collaborations across multiple disciplines and forge meaningful interfaces between basic, applied, and clinical sciences, so that laboratory research and discovery can be "translated" into new diagnostic and therapeutic applications. The grant program also seeks to engage trainees—including medical students, graduate students, clinical fellows, and postdoctoral fellows—in groundbreaking collaborative research. Projects funded under the program represent innovation in a broad array of scientific disciplines, with teams composed of two or more researchers, including combinations of physician investigators, basic scientists, applied scientists, and trainees.

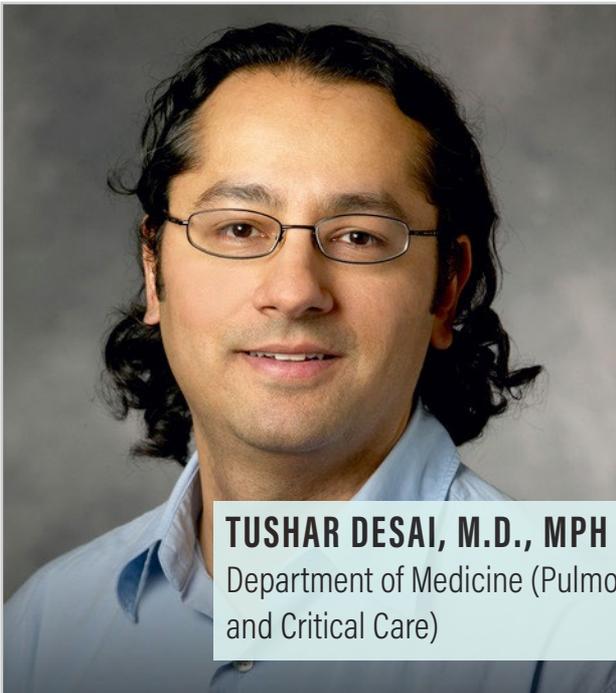
In November 2021, the Beckman Center plans to award up to five new Technology Development Grants that are geared toward supporting innovative research in the biomedical sciences. Each Technology Development Grant will provide funding of \$100,000 per year for a two-year period.

Applicants are encouraged to submit proposals to support research geared toward 1) the development of new and improved instruments or devices, or 2) the development of new methodologies to be used in biomedical research.

Preference will be given to applications that have a disease focus, are truly innovative, and meet the interdisciplinary and translational criteria of the ITRP grant program. Part of the selection process for the Technology Development Grants will be based on an assessment of the likelihood that the pilot research project will attract new or additional extramural funding.

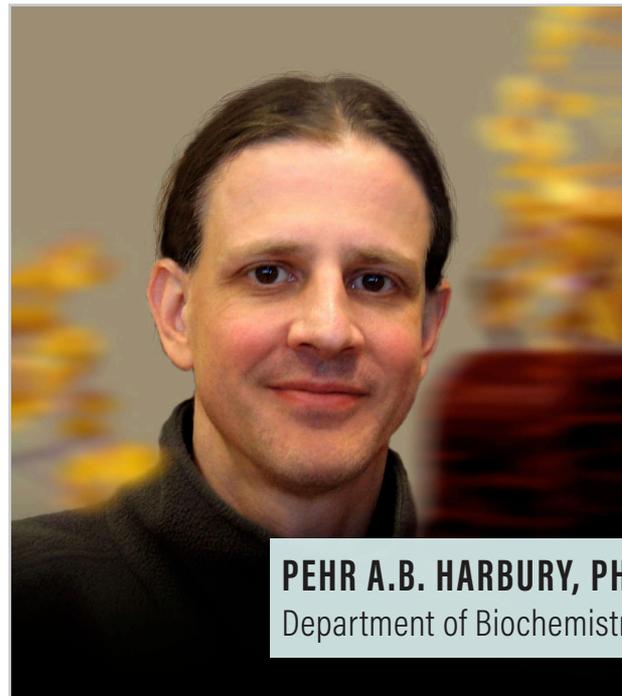
All of the grant recipients chosen in October 2018 have now completed their two-year projects. Progress reports from each group follow.

## A NEXT-GENERATION IMAGING TECHNOLOGY FOR HUMAN TISSUE ATLASES



**TUSHAR DESAI, M.D., MPH**  
Department of Medicine (Pulmonary and Critical Care)

Many human diseases cannot be modeled in animals and must therefore be studied in human subjects—requiring direct molecular analysis of cells and tissues from patients. The strategy of using humans as their own “model organism” has been greatly advanced in the last decade by high-throughput sequencing technology. An ongoing phase of this revolution is the introduction of single-cell molecular profiling methods, which enable high-dimensional, quantitative analysis of the body’s fundamental unit—the isolated cell. In animals, single-cell profiles must be synthesized into a systems-level picture of multicellular structures. Importantly, current single-cell sequencing approaches operate on dissociated tissues, and lack information about the 3D organization and interactions of the cells. This missing information



**PEHR A.B. HARBURY, PH.D.**  
Department of Biochemistry

is essential for moving beyond cell classification to an understanding of the global programs that control animal biology. Mapping technologies that molecularly profile single cells in intact tissues are therefore needed. It is already clear that *in situ* mapping will uncover cell classes and functional specializations that are invisible to dissociative sequencing approaches. If *in situ* mapping could be implemented on a genome-wide scale and in fully intact biological structures, it could rival sequencing as an upfront discovery tool.

The present state of *in situ* mapping is similar to the early days of high-throughput sequencing. The transition from being a scientific curiosity to a game-changing technology will require performance advances, specifically in data quality, information throughput, and the range of applications. This Beckman-funded project addresses two severe limitations of current 3D volumetric mapping methods: reliance on traditional fluorescence microscopy for imaging,

and reliance on passive transport for delivery of imaging agents. The operational consequences are 1) poor detection of low-abundance molecular features in tissue due to autofluorescence background and non-specific retention of labeling reagents—a particularly vexing problem in human tissue; 2) low information throughput due to the small number of spectrally-resolvable fluorescence data channels; and 3) a minimal 3D mapping capability in large, anatomically-intact tissue structures due to slow reagent penetration.

With Beckman funding, these researchers are developing a novel optical imaging modality (LRET) with ultrasensitive detection and an order-of-magnitude increased data throughput. They are also developing a tool for rapid, serial staining and erasing of molecular features in large (10 mm length scale) volumetric tissue blocks. These tools are enabling the construction of 3D atlases of the human body at single-cell resolution. The atlases localize 100s of distinct molecular features, and provide comprehensive assignments of cellular identity.

### Specific Aims

Aim 1. Create and benchmark a library of chemical probes that populate 20 data channels of an LRET imaging system. The probes will span five decades in luminescence lifetime and cover the entire visible spectrum.

Aim 2. Build and validate an open-access, luminescence light-sheet microscope for LRET imaging. This light-sheet microscope will be designed to retrofit conventional inverted microscope stands.

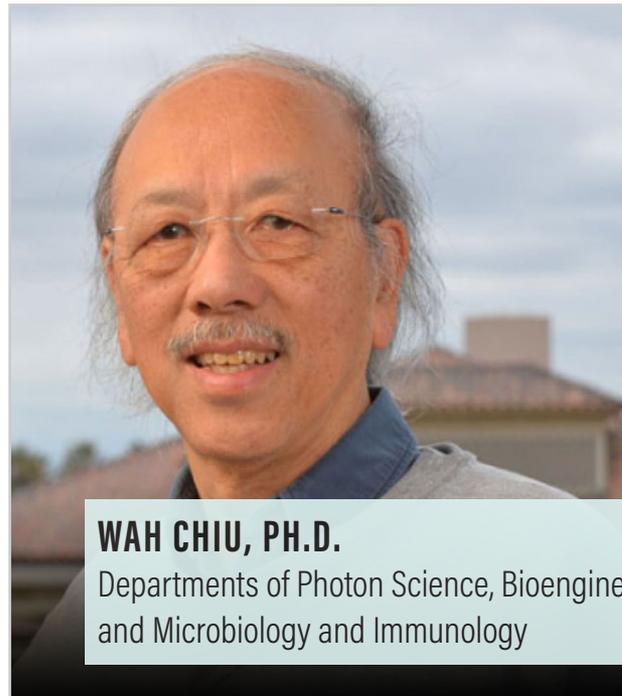
Aim 3. Engineer disposable electrophoretic imaging flow cells for ultra-fast and automated exchange of luminescent oligonucleotides and oligonucleotide-tagged antibodies.

## TARGETING THE INFLUENZA MATRIX LAYER THROUGH HYPERSTABILIZATION



**KARLA KIRKEGAARD, PH.D.**  
Departments of Genetics, and Microbiology  
and Immunology

Influenza viruses have been endemic in human populations for thousands of years. Due to a high mutation frequency and the ability to exchange chromosomes with other influenza viruses, available vaccines and drugs are not universally effective, and the drugs available to inhibit influenza virus are compromised by the rapid selection of viruses that are resistant to this inhibition. The researchers have pursued the development of the influenza matrix protein as an antiviral target because its oligomeric character is predicted to make inhibitors less susceptible to the development of drug resistance. Many other viruses, including coronaviruses, make matrix layers as well, and they plan to adapt this line of research accordingly.



**WAH CHIU, PH.D.**  
Departments of Photon Science, Bioengineering,  
and Microbiology and Immunology

Matrix proteins are highly oligomeric and must change shape during viral infection to convert between the infectious form and the uncoated form within the cell. The researchers reasoned that hyperstabilization of one form or the other would create a powerful antiviral-inhibiting virus formation, virus disassembly, or both. They pursued two complementary approaches to build their platform to identify and characterize hyperstabilizers of the matrix.

The virion-like state of assembled, full-length M1 protein was so regular that they were able to solve the structure by cryo-electron microscopy to 3.4 angstrom resolution (*PLOS Biology*, <https://doi.org/10.1371/journal.pbio.3000827>). The assembled form of this structure features a single protein molecule that can be seen to make multiple contacts with its neighbors, creating many binding sites for potentially stabilizing small molecules.

The researchers found that the structure of even a single molecule of M1 protein is more stable at low pH than at neutral pH, probably due to its naturally extended structure at low pH. This provided the basis for a high-throughput screen for hyperstabilizing molecules. When this screen was performed with a small Stanford library of compounds known to work in humans, it was gratifying that several known (but weak) inhibitors of influenza virus growth were found. A much larger screen of known, diverse, drug-like molecules revealed multiple compounds that stabilized the different forms indicated. Those that are commercially available, or could be synthesized at a relatively low cost, have been retested individually, yielding a collection of 21 compounds for further testing and optimization. Not all have been tested yet, but one of the low-pH stabilizers has already shown, as predicted, to inhibit M1 oligomerization at neutral pH.

The researchers look forward to further experiments, and thank the Beckman Foundation for funding these studies, which have transformed the influenza virus matrix protein from a difficult-to-purify protein to a viable drug target with a known, and beautiful, three-dimensional structure.

**DEVELOPING FFPE-OPTIMIZED  
CODEX TO REVEAL THE CUTANEOUS  
T CELL LYMPHOMA TUMOR  
MICROENVIRONMENT IN RESPONSE TO  
IMMUNOTHERAPY**



**GARRY NOLAN, PH.D.**  
Department of Pathology

Cutaneous T cell lymphoma (CTCL) is a potentially fatal malignancy of the skin for which current treatments are inadequate. Anti-PD-1 immunotherapy has revolutionized cancer treatment and is a promising option for CTCL. A recently reported clinical trial of pembrolizumab in advanced CTCL showed that 38% of patients achieved clinical response, while an equal proportion experienced rapid disease progression (Khodadoust, MS, et al. *J Clin Oncol* 2020; PMID: 31532724). No predictive biomarkers of pembrolizumab response were identified as part of this clinical trial, underscoring the need for new experimental approaches capable



**YOUN H. KIM, M.D.**  
Department of Dermatology

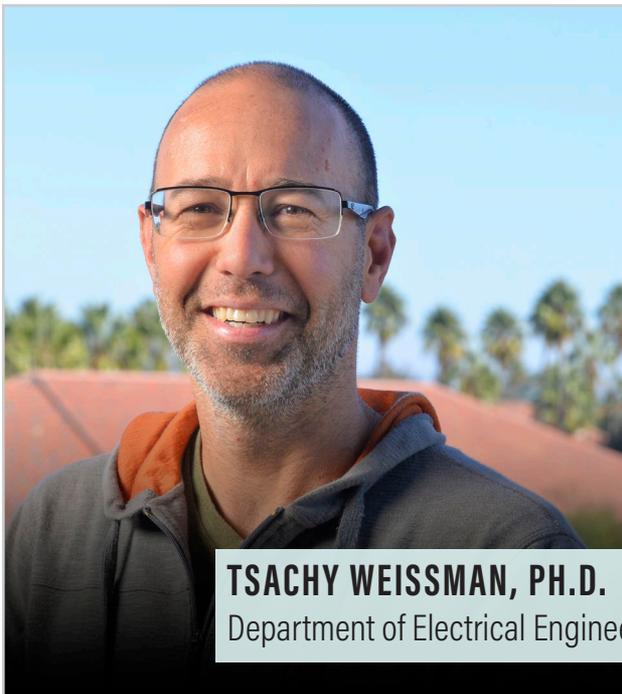
of guiding the clinical use of immunotherapy in cancer patients.

The current project used innovative technologies to advance biomedical discovery and benefit clinical practice. Specifically, the researchers combined CODEX multiplexed tissue imaging and RNA sequencing to gain insight into the drivers and resistors of PD-1 blockade. The key contribution of this work was the discovery of a simplified spatial biomarker predictive of pembrolizumab response in CTCL. Termed the SpatialScore, this biomarker computes the physical distance ratio of each PD-1<sup>+</sup>CD4<sup>+</sup> T cell and its nearest tumor cell relative to its nearest Treg. Responders have a lower SpatialScore (i.e., PD-1<sup>+</sup>CD4<sup>+</sup> T cells are closer to tumor cells, reflecting increased T cell effector activity), whereas non-responders have a higher SpatialScore (i.e., PD-1<sup>+</sup>CD4<sup>+</sup> T cells are closer to Tregs, reflecting increased T cell suppressive activity). Importantly, while the SpatialScore

was discovered using a 55-marker antibody panel and the next-generation CODEX platform, the researchers validated it using a 7-marker antibody panel and Vectra, a widely used clinical imaging system. Finally, they used RNA-sequencing data to identify a chemoattractant mechanism that explains how effector PD-1<sup>+</sup>CD4<sup>+</sup> T cells are brought into contact with tumor cells, facilitating the positive and sustained response to pembrolizumab therapy observed in responders.

In summary, this study shows that the balance between T cell effector and suppressive activity in the tumor microenvironment (TME) is associated with distinct spatial cellular patterns predictive of PD-1 blockade in CTCL. The researchers utilized these concepts to provide a novel, spatially based approach for identifying biomarkers that predict immunotherapy response. As such, they anticipate that dissemination of these results and concepts will lead to improved leveraging of immunotherapy and better outcomes for cancer patients. A manuscript detailing this work is currently in preparation for *Nature Medicine*.

## SCALABLE LONG-TERM DNA STORAGE WITH ERROR CORRECTION AND RANDOM-ACCESS RETRIEVAL



**TSACHY WEISSMAN, PH.D.**  
Department of Electrical Engineering

With the amount of data being stored increasing rapidly, there is significant interest in exploring alternative storage technologies. In this context, DNA-based storage systems can offer significantly higher storage densities (petabytes/gram) and durability (thousands of years) than current technologies. Recent advances in DNA sequencing and synthesis pipelines have made DNA-based storage a promising candidate for the storage technology of the future. However, the synthesis and sequencing processes are error-prone, leading to a need for error-correction coding. Furthermore, significant work needs to be done on the automation and scalability of the pipeline to achieve a practical storage system.

Part of this research group's work has focused on the development on novel error-correction



**HANLEE P. JI, M.D.**  
Department of Medicine (Oncology)

schemes optimized for DNA storage. In their work on Illumina sequencing-based DNA storage, they studied the tradeoff between the writing and reading costs involved in DNA-based storage and proposed a practical scheme to achieve an improved tradeoff between these quantities. Unfortunately, state-of-the-art Illumina sequencing is too costly, slow, and bulky to deliver on the promises of DNA-based storage. Meanwhile, third-generation nanopore sequencing offers affordable high-throughput sequencing using devices as small as a USB thumb drive. But as of now, error rates remain high. The researchers' proposed approach uses additional information present in the raw current signal as distilled by machine-learning-based basecallers. This provides around 3X reduction in sequencing cost over previous state-of-the-art systems. The work on error-correction techniques for Illumina and nanopore sequencing-based DNA storage was published in conferences. More recently, by employing advances in basecaller research, the researchers

were able to further reduce the sequencing costs for nanopore-based systems by more than 2X, while simultaneously improving the synthesis cost and computational requirements.

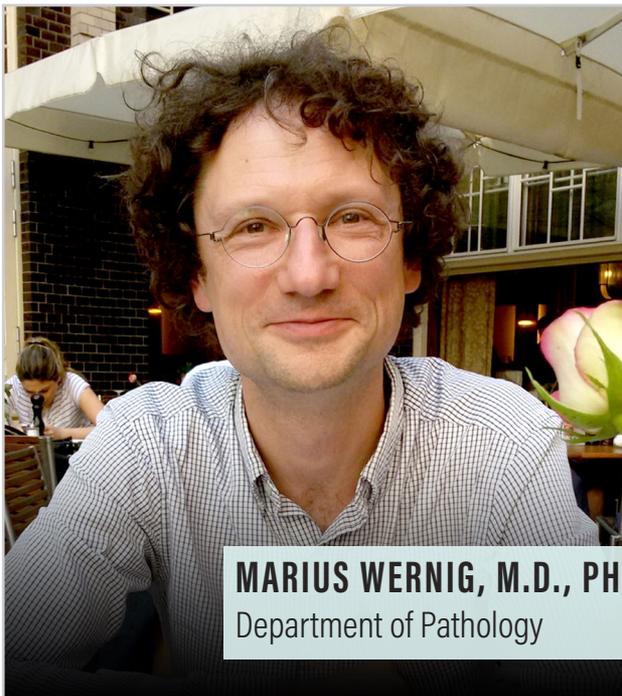
Beyond optimized error correction coding schemes, the researchers have recently demonstrated other crucial aspects of a practical storage system, such as random access to parts of the stored data and the ability to repeatedly read the data without depleting the material. They use a hierarchical random-access strategy, enabling them to address millions of separate “files” without the need to synthesize unique addressing DNA primers for each file. For the repeated reading of data, they attach the DNA storage sequences to a bead, enabling repeated reading without depleting the original library or introducing bias due to amplification. These advances are part of a manuscript currently in preparation, although certain aspects of these technologies were previously published in non-DNA storage contexts.

Finally, they are exploring automation of the entire pipeline in collaboration with Miroculus and alternative synthesis strategies, which can be significantly cheaper and scalable. Some preliminary work in this direction was presented as a poster at the Advances in Genome Biology and Technology (AGBT) 2020 General Meeting.

The potential of DNA storage and the researchers’ work on this were prominently featured at the 2019 Stanford Compression Workshop, in an invited talk by Hanlee P. Ji, M.D.

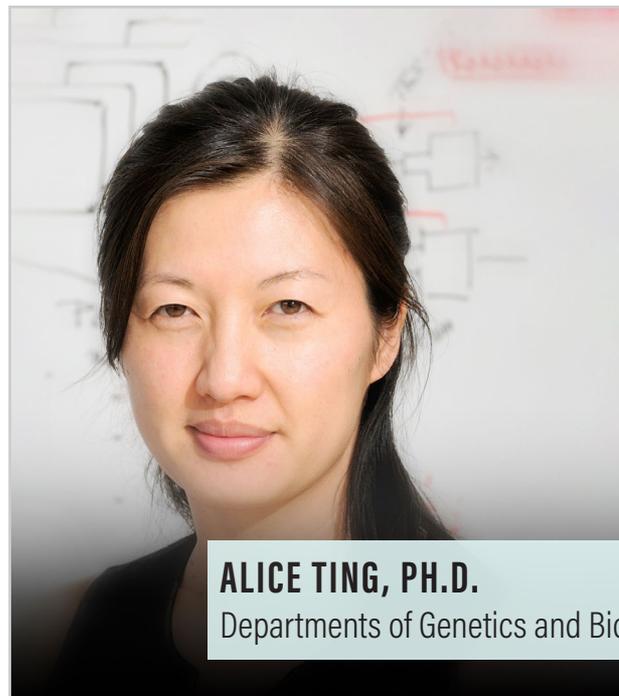
The error correction aspect was presented at the Intelligent Systems for Molecular Biology/European Conference on Computational Biology (ISMB/ECCB) 2019, by graduate student Shubham Chandak.

## OPTICAL TOOLS TO ASSESS NEURONAL FUNCTION IN HUMAN STEM CELL-BASED DISEASE MODELS



**MARIUS WERNIG, M.D., PH.D.**  
Department of Pathology

The first goal of this research project was to evaluate the already existing FLARE tool ( $\text{Ca}^{2+}$ -light coincidence detector) in human neurons. The researchers reported that they ran into a few unexpected problems. They tested the AAV constructs already available and verified to work in mouse cultures and in mouse brains *in vivo* and infected human neuron cultures derived from human iPS cells using the Ngn2 method. Unfortunately, they could not detect any  $\text{Ca}^{2+}$ /light-dependent activity of the transcriptional reporter. They then investigated the expression levels of the three components needed for the system to work, and essentially found no expression. After a few replicates and assurance that no reagents were mixed up, and continued lack of expression, they then tested other AAV constructs with GFP reporters and made the surprising discovery that



**ALICE TING, PH.D.**  
Departments of Genetics and Biology

human iPS-cell derived Ngn2-neurons cannot be infected with AAV viruses. This was completely unexpected since undifferentiated human iPS cells are readily infectable and AAV vectors targeting the CNS are even in clinical trials with verified expression.

The researchers then resorted to lentiviral delivery. They cloned the three main components into a standard lentiviral vector that they successfully use routinely in their cultures. They delivered the three FLARE components in these lentiviruses together with the Ngn2 virus needed to convert iPS cells into neurons. Of note, they needed to delay infection with the tetO-mCherry FLARE readout reporter because they needed the doxycycline-inducible system to express Ngn2. The researchers therefore first needed to test various timings to make sure that there was no interference with the doxycycline needed to differentiate the cells and the FLARE readout. Indeed, following some optimization of viral delivery time points of the tetO-mCherry

reporter, they did find a “beautiful” response to electrical stimulation that was both  $\text{Ca}^{2+}$  and light dependent.

The group then moved on to test this system in an iPS cell line carrying a conditional disease-causing mutation. As proposed in their original plan, they used conditional *Fmrp1* KO cells. They applied the optimized protocol to these cells. In addition, they needed to infect the cells with Cre and a control virus to establish the two experimental groups (KO and WT). Again, the reporter worked in the tested condition, but only very rare cells could be found.

Thus, even though the lentiviral approach does work in principle, the expression is still low and variable and does not work robustly among multiple cell lines. Moreover, the doxycycline interference is less than ideal. For these reasons, the researchers are now moving on to piggybac transposition-mediated gene delivery of a modified FLARE system, which is based on another transcription factor, called LexA. They assume that the expression will be higher and more consistent using piggybac insertion. Moreover, there is no size limit for piggybac gene delivery, which will further benefit as the lentiviral titers decrease with increased insert length.

In addition, during this reporting period, the group made significant progress with regard to further technological development—more specifically, the development of an optical integrative activity-history detector. Starting with their original FLARE tool, they have made several modifications and improvements to adapt it for

the proposed application in stem cells. First, they used directed evolution to improve the catalytic efficiency of the TEV protease component; this improved the temporal resolution of the tool to just one minute. Second, they improved the LOV domain by combining mutations in eLOV with mutations in iLID to improve the dark-state blockage of the TEV cleavage site (TEVcs). Third, they developed a single-chain version of FLARE (scFLARE), which is easier to use and reduces expression level variability (FLARE is a two-component tool and its performance varies a great deal, depending on component expression levels and relative stoichiometry of the two components). The improved performance of scFLARE at high- and low-expression levels is now being prepared for submission to *Nature Chemical Biology*.

## SEMINARS AND SYMPOSIA

The Beckman Center has become a vital source of support for faculty leaders seeking to promote broad-based scientific interaction and training through speaking events. Support from the Program in Molecular and Genetic Medicine for seminar series, conferences, and symposia has allowed departments to bring leading scientists to Stanford to share their cutting-edge research and also engage in dialogue with Stanford faculty, students, and postdoctoral investigators.

This past year, in response to the COVID-19 pandemic, which led to many researchers working from home, Beckman Center program leaders quickly redesigned several programs as online webinars and video tutorials, allowing vital scientific interactions and training to continue throughout the pandemic.

The Beckman Center has provided funding for a number of seminar series, conferences, and symposia that are primarily interdisciplinary in nature, such as those listed below.

### BECKMAN SYMPOSIUM

The upcoming Beckman Symposium, "Climate Change and World Health," will provide a major venue for discussion of the significant role climate change plays in shifting the geographical location of many of the global disease vectors, such as mosquitos, bats, and ticks, that carry serious pathogens. The response to this very real threat to world health must be global and based on breakthrough technology. The symposium will be a joint venture between the Beckman Center and the Chan Zuckerberg Biohub, and will feature prominent speakers with expertise in climate change and global health.



## GET THE FACS SEMINAR SERIES

“Get the FACS” is held throughout the calendar year and features lectures from staff from the Fluorescence Activated Cell Sorting (FACS) Facility as well as outside institutions. The seminars progress throughout the year from basic to advanced flow cytometry topics. These seminar topics help improve the flow cytometry knowledge of the Stanford community.

## WHAT'S THE SCOPE? SEMINAR SERIES

“What’s the SCOPE?” is held every other month and features talks from scientists in the Cell Sciences Imaging Facility (CSIF) as well as guest speakers. The series focuses on in-depth presentations of new and existing advanced imaging technologies available in the CSIF. The aim is to increase knowledge of the advanced light and electron microscopy imaging options that are available to Stanford’s research community.

## FRONTIERS IN BIOLOGICAL RESEARCH SEMINAR SERIES

The Frontiers in Biological Research Seminar Series focuses on cutting-edge research involving interdisciplinary approaches to bioscience and biotechnology. Leading investigators from Stanford and throughout the world speak on a broad set of scientific and technical themes related to interdisciplinary approaches to important issues in bioengineering and medicine as well as the chemical, physical, and biological sciences. The series also gives students the opportunity to meet informally with seminar speakers to discuss their research and future directions.

Support for the Frontiers in Biological Research Seminar Series spans several basic science departments in the School of Medicine.

## CANCER AND TUMOR BIOLOGY SEMINAR SERIES

The Cancer and Tumor Biology Seminar Series features guest lecturers from Stanford and peer institutions who discuss the molecular, genetic, cellular, and pathobiological aspects of cancer, as well as the current state of clinical diagnosis and treatment of human cancers.

## REGENERATIVE MEDICINE SEMINAR SERIES

Three Stanford programs, the Beckman Center, the Bio-X program, and the Institute for Stem Cell Biology and Regenerative Medicine jointly sponsor weekly seminars on regenerative medicine topics.

The seminars bring together students, postdocs, faculty, and trainees from diverse Stanford disciplines, including bioengineering, engineering, medicine, and the biological sciences to hear about and discuss work in progress. The seminars have been a tremendous help in making the Stanford research community aware of the broad range of research being carried out on campus.

## FACULTY RECRUITMENT PROGRAM

The Faculty Recruitment Program helps persuade outstanding faculty candidates, whose research goals are particularly well-suited to the overall mission of the Beckman Center, to join the Stanford University faculty. Competition for the most outstanding researchers is keen, and the innovative services and technologies provided by the Beckman Center offer a strong incentive to join the scientific community at Stanford. The Beckman Center is currently recruiting faculty for several departments.



## BECKMAN CENTER MEDICAL SCHOLARS PROGRAM

The Beckman Center Medical Scholars Program was established in 1997 to create a source of funds to provide financial stipends to medical students doing translational biomedical research under the direction of a Program in Molecular and Genetic Medicine faculty member. This support is critical to the success of the work of the Beckman Center, and is aligned with the center's goal of ensuring that the results of basic and applied sciences are made broadly available for clinical use and practical application.

The program targets medical students engaged in projects appropriate to the Beckman Center's mission, and selection is made through the Stanford Medical Scholars Program by the Medical Scholars Committee, which is composed of leading PMGM faculty members drawn from the basic and clinical sciences in the School of Medicine. Applications are reviewed on a quarterly basis. Student awardees are required to make an oral presentation of project results to an audience of their faculty advisor and others with expertise in the field, and must also prepare a written summary of their project results.

This year the Beckman Center is supporting the research of three Beckman Center Medical Scholars.

## KHRISTIAN ERICH BAUER-ROWE RAMOS

**Academic Year:** 2020-2021

**Year at Stanford Medical School:** 3

**Undergrad Education:** Massachusetts Institute of Technology; Mathematics, BS

**Hometown:** Stamford, CT

**Title of Medical Scholars Project:**  
Identifying the Role of Mesenteric Adipocyte  
Remodeling in Intestinal Fibrosis



### Research Description

Inflammatory bowel disease (IBD) is a set of autoimmune diseases characterized by recurrent and destructive episodes of inflammation in the digestive tract; it affects more than three million people in the United States alone. Repeated cycles of inflammation cause the formation of scar tissue in the bowel, a process called intestinal fibrosis. Intestinal fibrosis eventually leads to the formation of a stricture, a circumferential scar that narrows the bowel lumen and can cause a bowel obstruction. In Crohn's disease, a type of IBD, 30% of patients eventually require a bowel resection to manage strictures, and recurrence rates are estimated to be 50-70%.

Currently, there are no anti-fibrotic treatments, and anti-inflammatory therapies often fail to mitigate fibrosis. Consequently, there is a significant clinical need to understand the pathophysiology of intestinal fibrosis to improve clinical management.

Surgeons have known for decades that fat wraps around stricture sites, in a process called creeping

fat. Creeping fat derives from the mesentery, a membrane that attaches the bowel to the posterior abdominal wall and contains cells such as adipocytes and fibroblasts, which are activated upon injury and deposit collagen. Whether the mesentery is a passive bystander or an active player in stricture formation is unknown. An intriguing hypothesis is that creeping fat formation promotes stricture formation by contributing to the pool of fibroblasts.

This project explores how creeping fat-derived fibroblasts promote stricture formation. Specifically, it leverages a murine surgical model that recapitulates key characteristics of IBD fibrosis in conjunction with lineage tracing to determine the cellular origins of bowel wall fibroblasts. By understanding the sources of intestinal fibrosis, this project may eventually provide novel anti-fibrotic therapeutic targets.

## EKATERINA (KATIA) TKACHENKO

**Academic Year:** 2020-2021

**Year at Stanford Medical School:** 3

**Undergrad Education:** University of California, Berkeley;  
Chemical Biology, BS

**Hometown:** Tyumen, Western Siberia region, Russia

**Title of Medical Scholars Project:**  
Molecular Profiling of the Viral and Host Reactivity to  
SARS-CoV-2 and SARS-CoV-2 Vaccine in Adult Population



### Research Description

As of April 20, 2021, there have been almost 150 million COVID-19 cases globally, and almost 82 million people have recovered from the disease. Patient outcomes vary greatly and there are many reasons for such variety. For example, the patient's immune response and overall health seems to greatly affect disease phenotype. This leads to the following questions: Why do some patients have such strong adverse reactions while others are asymptomatic? Why do some patients go on to develop an autoimmune disease, tissue damage, or other persistent symptoms, becoming so-called "COVID-19 long-haulers?"

Due to the extreme heterogeneity of COVID-19 infection and many unknowns around predictors and mechanisms of disease severity, reinfection, and post-recovery complications, with up to 46% of asymptomatic infection cases, there is a need for studies that collect comprehensive molecular data on COVID-19-positive individuals over time in a large cohort. According to multiple published studies, as many as one-third of recovered COVID-19 patients continue to experience impaired mental and physical health for weeks or months after their illness. If this

finding holds true, about 28 million people in the world are still struggling with persistent symptoms affecting their well-being and quality of life. In addition, as many countries are rolling out their vaccination programs, the immune response and potential health consequences of those vaccines are largely unknown.

My projects aim to identify signature correlates of viral infection, using global COVID-19 protoarray with spike peptide tiling after natural SARS-CoV-2 infection and COVID-19 vaccine. Additionally, host reactivity in "long-haulers" and vaccinated individuals is being assessed using a unique HuProt Human Proteome Microarray. This will allow us to identify both viral and auto-antibody signatures, which will be correlated to demographic and clinical data (e.g., disease severity/morbidity) as well as integrated with metabolomics, lipidomics, and cytokine datasets. We hope to uncover pathophysiologic mechanisms potentially responsible for the adverse reactions and the multitude of symptoms COVID-19 patients and a subset of COVID-19 vaccinated individuals are experiencing.

## DEBORAH DEIJA TSAO

**Academic Year:** 2020-2021

**Year at Stanford Medical School:** 3

**Undergrad Education:** McGill University;  
Honours Physiology

**Hometown:** Winnipeg, Manitoba, Canada

**Title of Medical Scholars Project:**  
Discovering Insulin-Resistance and Drug Target Gene  
Function using *Drosophila* Genetic Screens

### Research Description

The incidence of diabetes mellitus has risen rapidly in the past half-century, reaching epidemic proportions in many areas of the world. This increasing incidence, and the recognition that diabetes mellitus is a genetic disease, has generated intensive effort in deciphering the genetic basis of diabetes. Human genome-wide association studies (GWAS) have revealed thousands of polymorphic loci linked to hundreds of genes that may be associated with diabetes risk. The volume of information generated from GWAS has proved to be a challenge for investigators aiming to understand the function of these “diabetes risk” genes, as genetic experiments with organisms such as mice and other vertebrates are time-consuming and expensive. Thus, the development of efficient *in vivo* systems to assess tissue-specific gene function could transform the scale of our understanding of diabetes risk genetics.

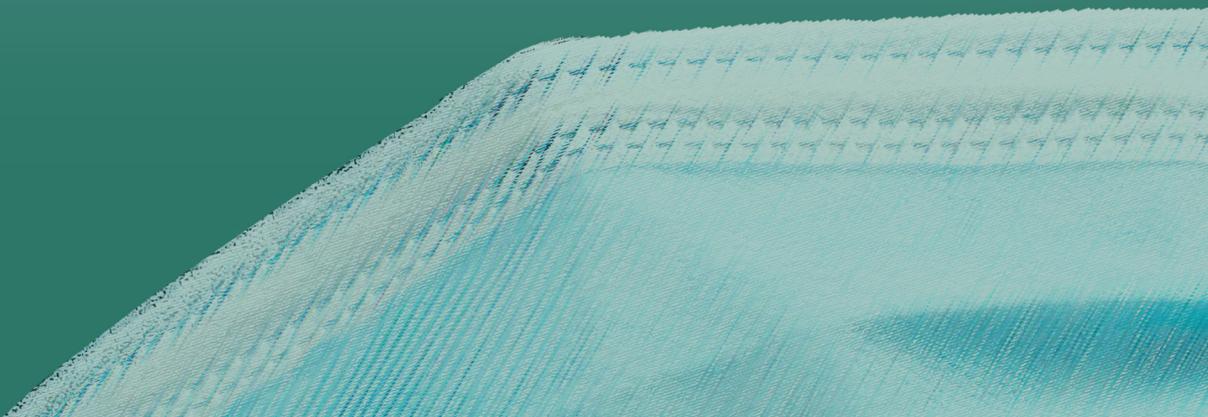
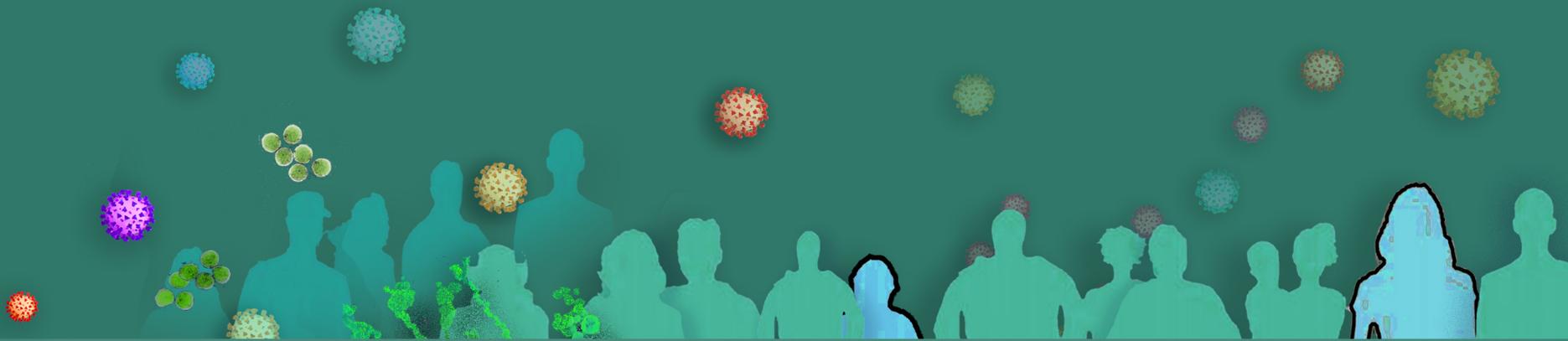
Working in the lab of Seung K. Kim, M.D., Ph.D., professor of developmental biology, researchers used the fruit fly, *Drosophila melanogaster*, as an ideal model organism to conduct these studies. Crucially, *Drosophila* researchers have 1) demonstrated hormone regulation of fruit fly metabolism astonishingly similar



to that of mammals, and 2) built a powerful genetic toolkit housed in a public repository that enables efficient, high-throughput screens to identify gene function.

The project is currently working on developing assays for peripheral glucose uptake in *Drosophila* that can be used to screen for genes involved in insulin resistance. Specifically, it is targeting three key signaling events along the insulin-mediated glucose uptake pathway: AS160 phosphorylation, translocation of glucose transporter 4 (GLUT4) to the plasma membrane, and finally, increased intracellular glucose levels after glucose uptake. It is also exploring novel potential targets for metformin, one of the most widely prescribed diabetes drugs in the world. The study is starting with a focus on ATM and SLC2A2—two genes identified with human GWA studies to be associated with altered metformin responsiveness. Ultimately, the hope is to develop a genetic model to enable efficient and high-throughput study of genes involved in both diabetes risk and response to treatment.

# TECHNOLOGY RESOURCES



# TECHNOLOGY RESOURCES

The Beckman Center's shared technology resources include four highly specialized scientific facilities that serve departments and laboratories throughout Stanford University.

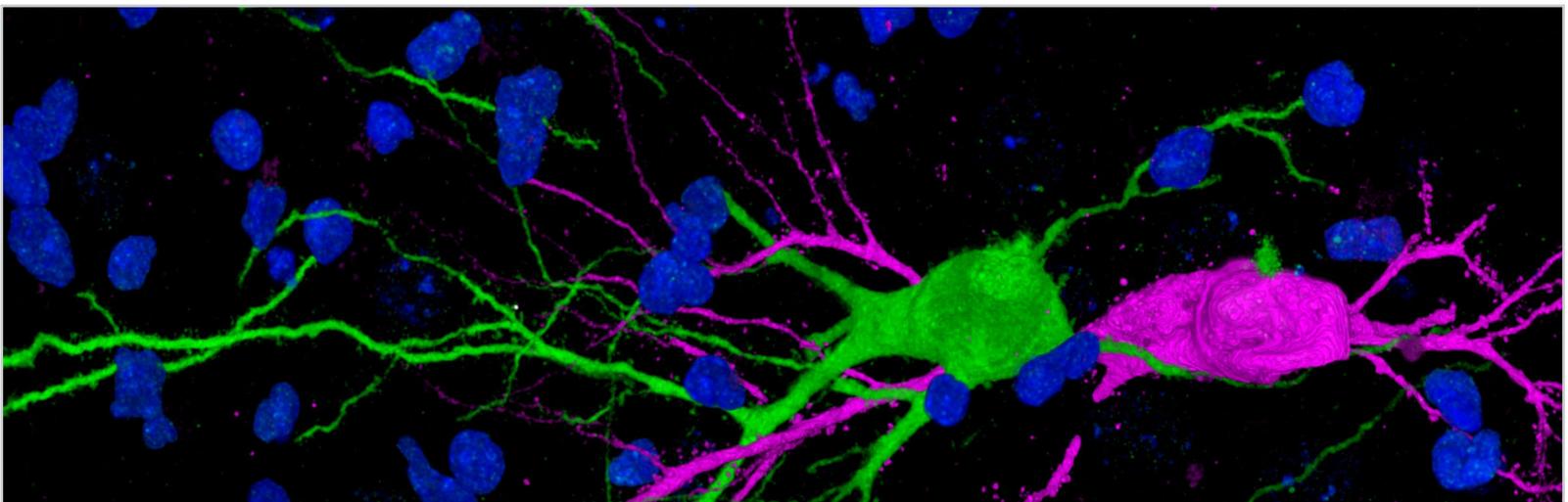
In continuous operation since 1989, these core service centers are currently among the most successful service centers at Stanford. They typically generate more than \$5 million in annual revenues from faculty, postdoctoral fellow, and graduate student users campus-wide, as well as from the broader scientific community. This allows the service centers to operate at or close to break-even. The service centers include:

- Cell Sciences Imaging Facility (CSIF)
- Protein and Nucleic Acid (PAN) Facility
- Fluorescence Activated Cell Sorting (FACS) Facility
- Computational Services and Bioinformatics Facility (CSBF)

The ability to keep these services available and

viable is dependent on user fees that reimburse general operating costs, labor, and overhead. Rates are structured by the Beckman Center, with review and consultation by service center managers. Rate-setting decisions are made annually, based on a review of needs for labor, equipment updates, and other unusual operating costs. Stanford University's Office of Research Administration audits the rate-setting process on an annual basis, certifying to the campus community and the university's cognizant federal agency that service center rates are reasonable and therefore appropriate to charge to sponsored project funds.

In order for the facilities to remain competitive within the academic community, and to avail Beckman-affiliated scientists of the use of state-of-the-art scientific technologies, the Beckman Center provides funding as needed to underwrite new technologies employed by the service centers that cannot be recovered through fee structures. The goal is to keep the rates as low as possible in order to encourage the use of services housed in the Beckman Center. This year, the Beckman Center provided supplemental funds to all four service centers. Provided in this section are detailed descriptions of the four service center's operations, their importance to the Stanford research community, and how they used center funds.



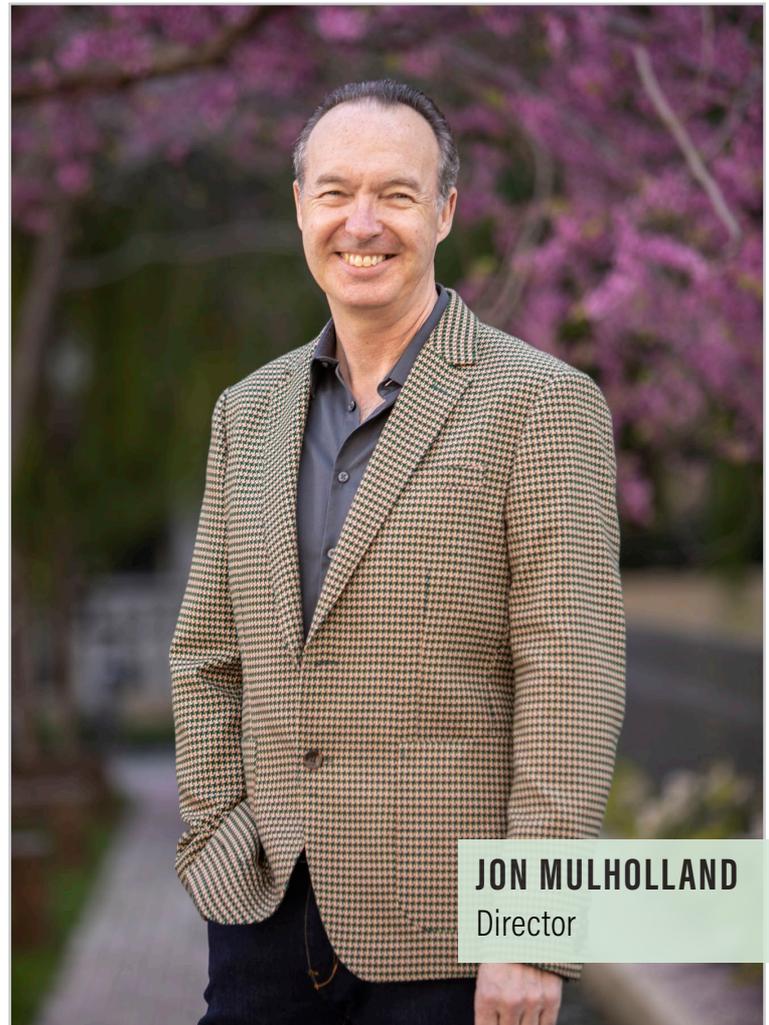
# CELL SCIENCES IMAGING FACILITY

## OVERVIEW

The Cell Sciences Imaging Facility (<https://microscopy.stanford.edu>) provides high-resolution, state-of-the-art technologies for imaging and analyzing the molecular and structural organization of cells and tissues, as well as bioengineered materials. The facility offers sophisticated and demanding microscopy techniques to Stanford University and industry researchers, including super-resolution, confocal, FLIM, FRET, FRAP, 2-photon live cell imaging, photo-activation and uncaging, array tomography, atomic-force measurements, immunoelectron microscopy, and high-pressure freezing.

The CSIF is organized into three interdependent imaging labs: the Fluorescence Microscopy Core (FMC), which houses multi-photon, confocal, super-resolution, fluorescence lifetime and deconvolution microscopes, as well as image analysis software; the Electron Microscopy Core (EMC), which houses high-resolution scanning and transmission electron microscopes; and the Multiplexing and Array Tomography Core (M-ATC), which provides multiplexed marker imaging and array tomography services.

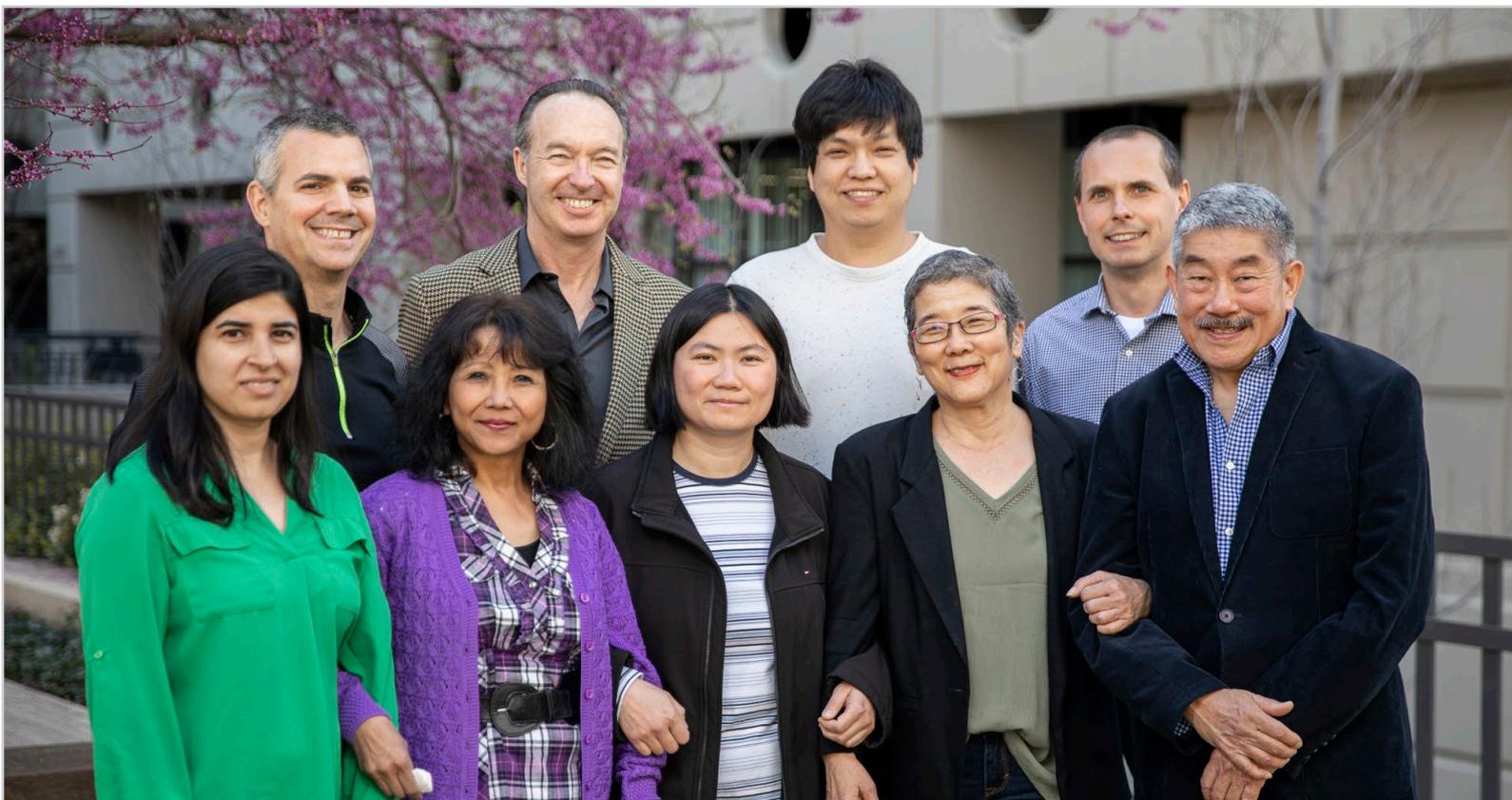
The CSIF was founded in 1994 to address the Stanford biomedical research community's growing need for advanced light microscopy expertise, services, and equipment. In 2002, in response to many researchers' need for state-of-the-art electron microscopy imaging services, the CSIF established its integrated electron microscopy core. In 2006, the CSIF joined Stanford University School of Medicine's successful effort to establish a National Cancer Institute-designated



Comprehensive Cancer Center, and is now a member of the resulting Stanford Cancer Institute, supporting cancer research. In 2008, with support from the Beckman Center and Stanford's Bio-X program, the CSIF's Array Tomography Core was created.

In 2014, in a collaborative effort with the Stanford School of Engineering (SOE), the CSIF opened a satellite light microscopy facility in the SOE's Shriram Center. This new facility brings biological imaging instrumentation and expertise to the departments of Bioengineering and Chemical Engineering. More recently, in 2019 the CSIF added highly multiplexed antibody marker fluorescence imaging, thus creating the Multiplexing and Array Tomography Core.

Today, the CSIF's mission remains the same as when it was first established: to provide access to and training in high-resolution, state-of-the-art imaging



### **Cell Sciences Imaging Facility**

**Front Row, Left to Right:** Anum Khan, Ibanri Phanwar-Wood, Kitty Lee, Ruth Yamawaki, Philip Huie, Jr.

**Back Row, Left to Right:** John Perrino, Jon Mulholland, Youngbin Lim, Marcin Walkiewicz

technologies. While these technologies have evolved substantially over the last 25-plus years, they remain essential, basic tools for studying molecular, sub-cellular, and cellular biology and disease. A major element of the CSIF's commitment to its mission is the continuous and ongoing process of upgrading technologies, equipment, and expertise, to remain at the forefront of cell sciences imaging.

### **EXPERTISE**

A ten-member advisory committee provides leadership and direction for the CSIF. The committee is chaired by the Beckman Center director, Lucy Shapiro, Ph.D., and includes nine other researchers from the Beckman Center, the Stanford Cancer Institute, and Stanford's School of Medicine, School of Engineering, and School of the Humanities and Sciences.

The CSIF is staffed by its director, John Mulholland, as well as several full-time research professionals who have expertise and training in electron and light microscopy.

### **SERVICES**

#### **Fluorescence Microscopy Services**

The CSIF's Fluorescence Microscopy Core provides training and consultation in the application of numerous microscopy technologies:

- Super-resolution (API OMX-SIM, STORM, Leica SP8-gSTED, Zeiss AiryScan)
- Laser scanning confocal (Zeiss LSM880, LSM780, Leica SP8, Leica SP5)
- Spinning disk confocal (Nikon-Yakogawa)
- Deconvolution (API OMX Delta Vision)
- 2-photon (Zeiss LSM780, Leica SP5, each with Spectra Physics DeepSee laser)
- Lattice light sheet microscope (3i Inc., LLSM V2)

- Fluorescence lifetime imaging (FLIM) light microscopy technologies
- Bio-atomic force microscopy (Bio-AFM, Bruker Resolve BioScope)

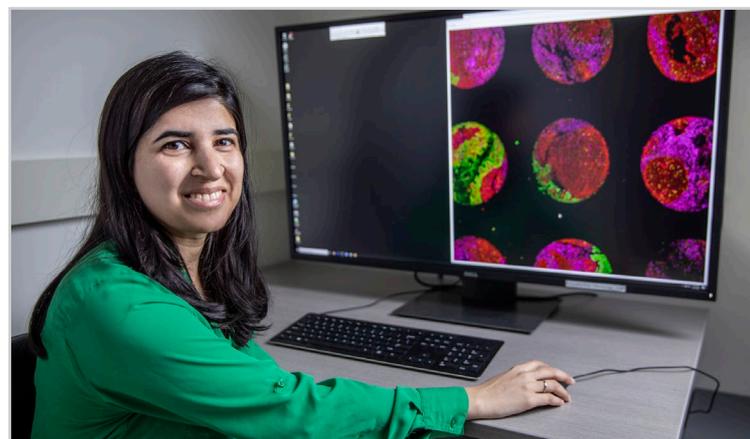
Super-resolution technologies allow researchers to exceed the diffraction-limited resolution limits of conventional light microscopy (<200nm). This allows researchers to image and resolve structures and cellular dynamics that were previously unresolvable with other optical technologies. Two-photon, confocal, and deconvolution technologies allow optical sectioning while eliminating out-of-focus fluorescence. This makes the precise 3D localization of fluorescently labeled proteins within the cell or tissue possible. Lattice light sheet microscopy is the standard for fast live-cell imaging, with low phototoxicity. FLIM allows researchers to measure changes in a molecule or protein's fluorescence lifetime, in addition to its fluorescence intensity. Bio-AFM enables innovative live-cell experiments that provide high-resolution force measurements and mapping over the surface of soft materials, cells, and other biological material. Using epifluorescence, brightfield, and phase contrast optical imaging, these measurements can be directly correlated with macromolecules, proteins, and subcellular structures, as cells sense and respond to mechanical cues and environmental changes. The CSIF also has capabilities for total internal reflection microscopy (TIRF) and super-fast, wide-field, live-cell imaging.

Additionally, time-lapse software allows 3D localization of labeled proteins over time, thus providing 4D data sets. The CSIF also provides advanced software resources for 3D, 4D interactive, volume imaging (Improvision Volocity, Bitplane Imaris) of data sets, as well as advanced deconvolution software packages (SoftWoRx and SVI Huygens).

## Electron Microscopy Services

The facility's Electron Microscopy Core is a full-service lab that offers sample preparation, training, and consultation for both transmission and scanning electron microscopy technologies.

The EMC houses a transmission electron microscope (TEM) equipped with a high-resolution, cooled sCMOS camera for digital acquisition of images (JEOL



**Top:** Ibanri Phanwar-Wood, **Middle:** Anum Khan  
**Bottom:** Marcin Walkiewicz

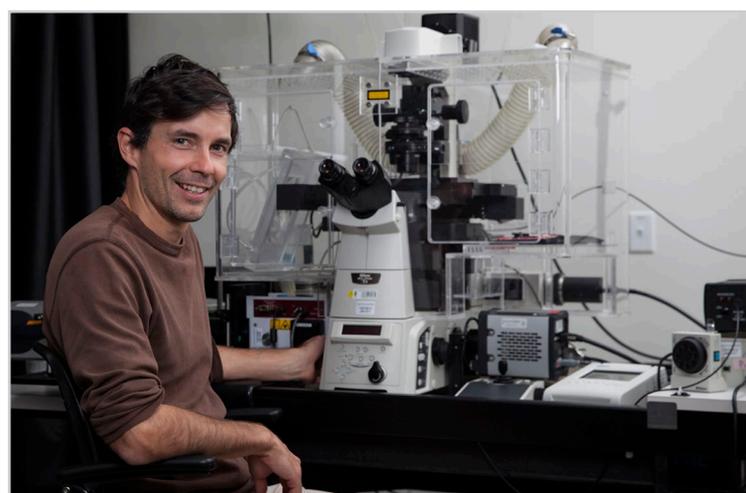
1400-TEM). The CSIF's TEM can produce a resolution of two angstroms, thus making it possible to image and study isolated macromolecules and subcellular structures. TEMs are also fitted with a high-contrast, biological objective lens, making them ideal for imaging thin, immuno-localized samples used for the determination of a protein's subcellular location. The facility is also equipped with a field emission scanning electron microscope (FE-SEM, Zeiss Sigma), for high-resolution study of specimen structure and topology.

Ancillary equipment includes four ultramicrotomes for cutting ultra-thin sample sections (less than 100nm), a cryo-ultramicrotome for sectioning ultra-thin frozen sections, all equipment necessary for sample preparation, and computers for image analysis. Additionally, the EMC houses a new state-of-the-art Leica ICE high-pressure freezing machine. High-pressure freezing is the gold standard for fixation of biological microscopy samples; in the numerous studies where it has been applied, high-pressure freezing has extended our understanding of the structural and molecular organization of cells and tissues.

### Multiplexed and Array Tomography Services

The facility's Multiplexing and Array Tomography Core provides complete multiplexing epitope localization (CODEX) and array tomography (AT) services.

CODEX, a highly multiplexed imaging platform, allows automated, multiplexed, antibody localizations of a potentially unlimited number of proteins on tissue sections or tissue arrays, with cellular-level resolution. The CODEX instrument provides greatly increased throughput and analysis of multiple cancer, neurological, and other tissue-specific markers, which allows phenotypic cluster analysis of different cell types within their spatial context. The facility provides



*Top: Ruth Yamawaki, Bottom: Cedric Espenel*

image analysis and pipeline development support, and also develops and validates antibody panels for research groups.

The AT imaging method was invented at the Beckman Center in the Department of Molecular and Cellular Physiology by neuroscientists Stephen J. Smith, Ph.D., emeritus professor of molecular and cellular physiology, and Kristina D. Micheva, Ph.D. Compared to previous microscopic methods for 3D imaging of fixed tissue, array tomography offers increased resolution (z resolution of 200-50nm), quantitative reliability, antibody multiplexing capacity and throughput and volume (automated image acquisition). Array tomography also complements live, whole animal, or tissue explant imaging studies,

providing higher-resolution 3D data with many more molecular markers, which can extend the molecular interpretation of *in vivo* dynamics. Array tomography permits easy acquisition of electron microscopic images in register with immunofluorescence. Array tomography thus promises an opportunity to explore the 3D molecular architectures of tissue at an unprecedented level of detail. This methodology is applied by many Stanford researchers and provides insights into structural organization and protein location in tissue of numerous organisms and disease models.



**Top Left:** Kitty Lee, **Bottom Left:** John Perrino

## RECENT DEVELOPMENTS

Despite the 2020 COVID-19 pandemic, CSIF continues to collaborate with the Protein and Nucleic Acid Facility to correlate, in fresh-frozen tissue slices, protein-localization specific cell phenotypes with RNA expression patterns, using data from multiplexed, epitope-based tissue imaging (CODEX, Akoya, Inc.) combined with spatial transcriptomics (Visium, 10X Genomics, Inc.) technologies. This correlative approach is done using tissue slices taken from both cancer and normal patient samples, using the CSIF's validated marker panels and the PAN Facility's next-generation sequencing (NGS) methodologies.



Correlating highly multiplexed tissue imaging to deep spatial transcriptomics is expected to identify new phenotypic classifications, based on the protein-RNA expression correlation maps, which will in turn lead to the identification of new molecular subtypes of cancer with identifiable treatment targets. It is expected that this combined proteomic and transcriptomic spatial analysis will provide more precise diagnoses of cancer types and stages and subsequently, earlier diagnosis and therapeutic interventions.

### Instrument Upgrades

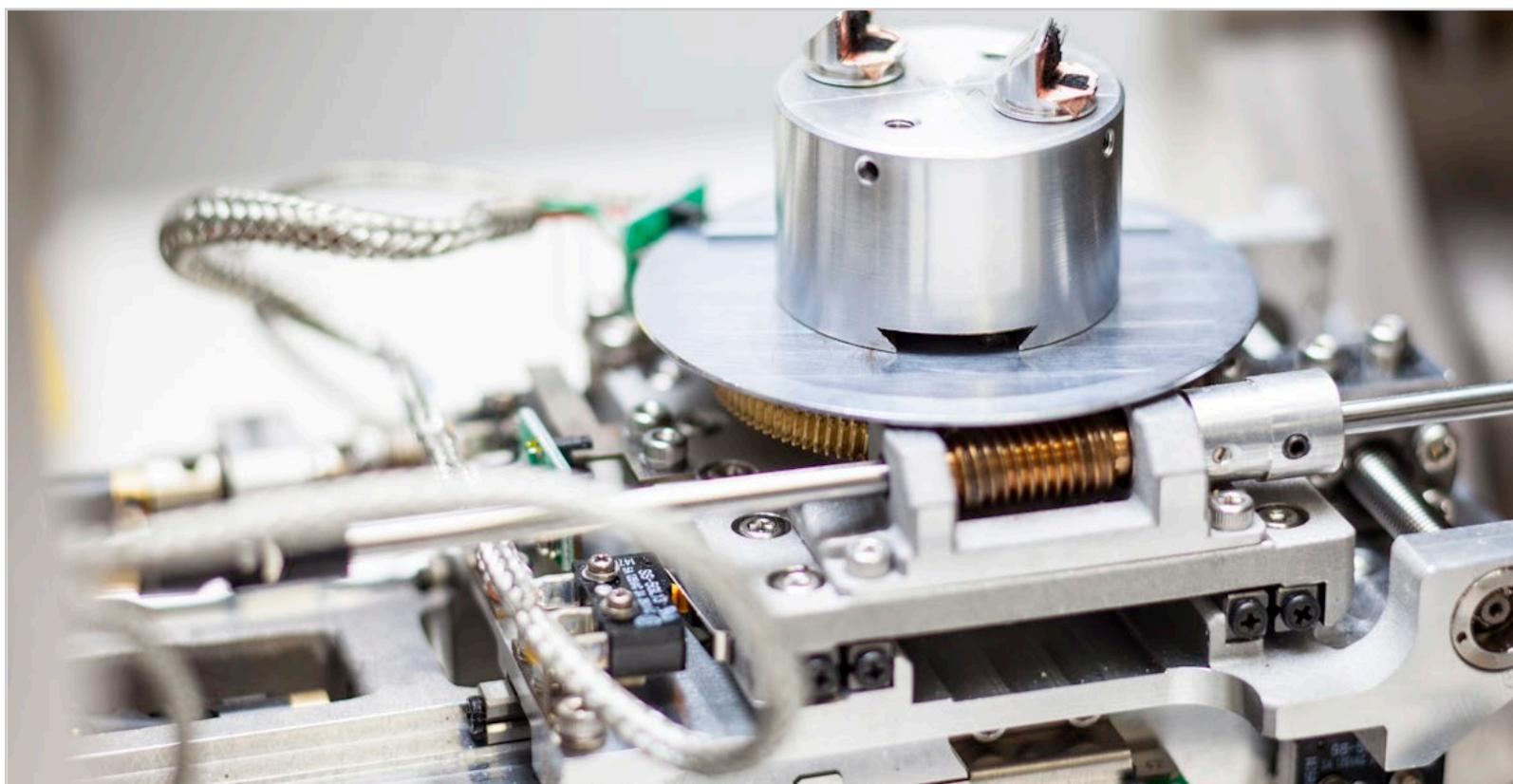
In 2020, the CSIF received funding from the National Institutes of Health's (NIH) Shared Instrumentation Grant (SIG) Program to replace and upgrade the Electron Microscopy Core's aging and obsolete CCD TEM camera. The new equipment, a OneView sCMOS TEM camera, has now been installed on the EMC's transmission electron microscope and is being used to digitally record TEM images of biomedical samples. It will be used in both high- and low-electron-dose TEM imaging applications and will be used to correct, in

real time, the inherent drift of TEM samples. The new sCMOS camera will significantly advance the research projects of 15 different facility users, including two Nobel Laureates.

### FUTURE VISION

Several new programs and services are now in development.

- The CSIF is collaborating with the imaging facility at Stanford's Wu Tsai Neurosciences Institute to further develop and expand the university's light sheet microscope (LSM) imaging program. A significant aspect of this development is seeking funding to fill a major gap in LSM instrumentation and provide support for expanded data science services.
- The CSIF will be working with the PAN Facility to establish standardized validation protocols for the antibody-probe conjugation chemistries being used for CODEX multiplexing.



# PROTEIN AND NUCLEIC ACID FACILITY

## OVERVIEW

The mission of the Protein and Nucleic Acid Facility (<http://pan.stanford.edu>) is to be adaptable and responsive to the changing needs of biomedical research, by providing the Stanford scientific community with continued access to key research tools and applications in an efficient and cost-effective manner. The PAN Facility is committed to providing a diverse array of instrumentation and technical capabilities in molecular genetics and protein analytics, with the goal of benefiting investigators in their biomedical research projects and helping them succeed in relevant grant applications.

The advancement and expansion of the PAN Facility's services, since its inception in 1989, has been driven by a collaborative spirit between the Beckman Center administration and PAN Facility staff that has supported an increasing variety of Stanford research programs, leading to innovation and biomedical advances.

## EXPERTISE

An eight-member advisory committee provides oversight, leadership, and direction for the PAN Facility. The committee is chaired by Lucy Shapiro, Ph.D., director of the Beckman Center, and includes Michael Eckart, Ph.D., director of the PAN Facility, as well as researchers from the Beckman Center, the Stanford Cancer Institute, and Stanford's School of Medicine and School of the Humanities and Sciences.

The PAN Facility is staffed, in addition to its director, by six full-time, experienced research professionals who



**MICHAEL ECKART, PH.D.**  
Director

have been trained in all the services provided and who also offer expertise in specific service areas. The PAN Facility is organized into number of interdependent services, as listed below.

## SERVICES

The PAN Facility offers a number of interdependent services:

- Gene expression analysis
- Microarrays
- Real-time PCR
- Pyrosequencing
- Nucleic acid QC
- Single-cell genomics
- Spatial transcriptomics
- Next-generation sequencing
- DNA sequencing
- Synthetic nucleic acid synthesis



### **Protein and Nucleic Acid Facility**

**Front Row, Left to Right:** Ian Anderson, Nghi Cat Bao Le, Jessica Tran

**Back Row, Left to Right:** Jennifer Okamoto, Kyle Fukui, Yen Tran, Michael Eckart

- Biomolecular interaction analysis (surface plasmon resonance)
- Peptide synthesis
- Mass spectrometry

### **Shared Services**

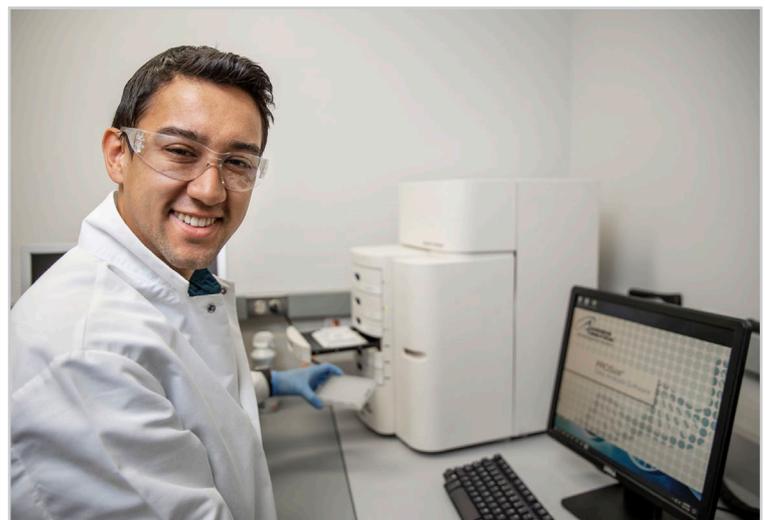
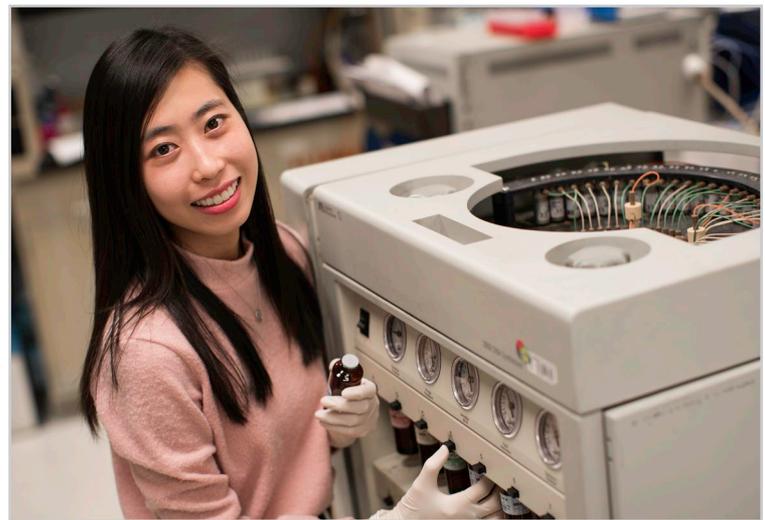
The core services offered by the PAN Facility enable and facilitate efficient and economical biomedical research by providing users with technology, without the necessity of major capital or staffing expense. With the organized and efficient infrastructure that shared resources such as the PAN Facility provide, researchers are able to investigate complex research questions. The PAN Facility also enables education, methods development, and new applications development, all designed to meet the needs of Stanford's biomedical research community.

The core strength of the PAN Facility comes from its talented, highly experienced, and dedicated scientific staff. The dedication of PAN staff in advancing our mission of education and research was very evident this year, as they adapted and accomplished their work under extraordinary circumstances during the COVID-19 pandemic. They rallied to rapidly produce the primers and probes used in diagnostic tests—developed by Benjamin Pinsky, M.D., Ph.D., associate professor of pathology and medicine (infectious diseases), and medical director of the Stanford Clinical Virology Laboratory, and his team—to amplify the coronavirus genetic material in patient samples, when those test components were in short supply. The ability of the Clinical Virology Laboratory to develop the FDA-approved test benefited Stanford patients and communities across the region, who were facing a largely unknown threat at the time. According to James L. Zehnder, M.D., professor of pathology

(research) and of medicine (hematology), and director of Coagulation and Molecular Pathology Laboratories, “We were not going to be able to do any testing if we didn’t get this.”

The PAN staff members are specialists in their respective areas of expertise and have also cross-trained in the operation of multiple instruments and applications; they are able to provide the best possible comprehensive research support, including participating in training the next generation of scientists. The PAN Facility can provide researchers with as much assistance as needed, from initial study design to all procedures needed for an experiment, as well as final interpretation and analysis of data. Services include standard assays as well as customized services. The PAN staff members are always interested in developing new assays or adapting existing, established assays to address a specific research question.

The training and professional development of PAN staff is a top priority, to ensure both personal and research project success. This is often achieved through an active, open exchange of ideas between PAN Facility staff and researchers that enables leveraging the full potential of the available technologies. Development and implementation of new applications and technologies at Stanford are often achieved when a research group and the PAN staff engage in a joint project, with all contributing their individual strengths. The results of these efforts are often highlighted in publications to which PAN scientists have made contributions. Indeed, the consultation provided by PAN staff is often as important as the data obtained, since biomedical researchers not trained in a specific technique or field can find it difficult to interpret specialized data without help from PAN scientists.



*Top: Nghi Cat Bao Le, Bottom: Kyle Fukui*

### Single-Cell Genomics

In the past few years, science has become increasingly interdisciplinary; as a shared resource that aims to be at the forefront of new developments and ideas, we see the PAN Facility as part of this progression. This is especially true in the field of single-cell genomics, which is the application of genomic technologies to understanding biology at the level of an individual cell, rather than an entire population of cells. There is currently strong enthusiasm to pursue activities that will develop and implement a suite of integrative multimodal technologies that simultaneously measure multiple types of information from the same cell. This will enable researchers to achieve a better understanding of the cellular and molecular mechanisms governing cellular function in healthy, developmental, and disease states.

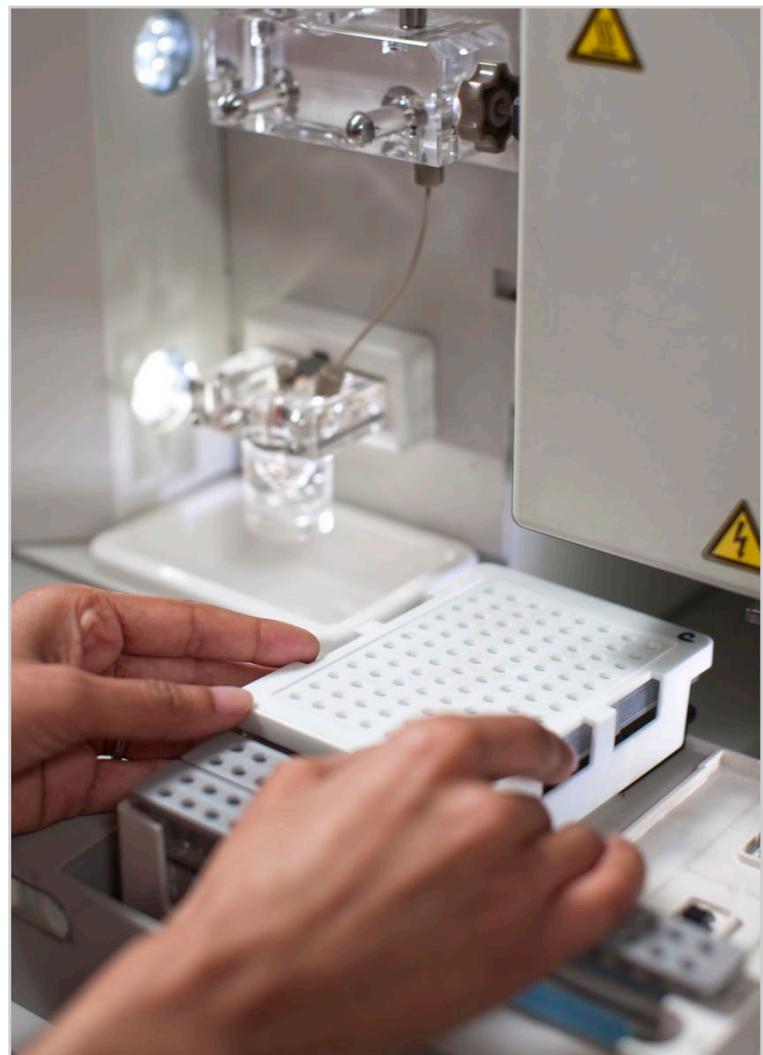
Single-cell genomics has revealed how much variation there is between individual cells at the molecular level in, for example, tumor tissue, stem cells, or rare subpopulations of immune cells. However, analyzing genomic DNA or RNA at the single-cell level may provide only genome, methylome, chromatin, or transcriptome information. Although these individual sets of information are valuable, by themselves they do not provide a full understanding of all the genomic, transcriptomic, epigenomic, and proteomic activities of individual cells. Thus, the goal is to apply a multi-omics approach whereby all the different techniques, genomic and proteomic, are applied to specific individual single cells. Single-cell multi-omics is not straightforward and will require the modification of existing single-cell protocols, in addition to the development of novel techniques, so that different types of both genetic and protein molecules can be analyzed simultaneously.

The PAN Single-Cell Genomics Laboratory, which was established by the Beckman Center together with a group of research programs in cancer, stem cells, and immunology, remains committed to supporting novel and high-impact work from across the many different disciplines, particularly technologies that will have a major impact in future years. In addition, despite enormous advances, many approaches remain far from achieving the low costs, high quality, and rapid-time-to-results that are PAN's mission, and to generate comprehensive genomic and proteomic information in many single-cell applications.

Many of the existing single-cell methods work well for human and mammalian cells, but do not work for bacteria and viruses. The COVID-19 pandemic has the potential to spur increased interest in applying single-cell techniques to problems in infectious disease, immunology, and microbiology. Thus, as better technology is developed, we anticipate costs will be

reduced, resulting in a significant increase in single-cell research.

Currently, the PAN Facility provides a full range of services aimed at advancing discoveries and the development of methods to analyze genomes and transcriptomes in single cells. Single-cell sequencing is performed in three major steps: cell isolation, whole genome/transcriptome library construction, and high-throughput sequencing. The first step, the successful, rapid isolation of single cells for genomic analysis, is a critical step for obtaining meaningful results. It can be achieved by using, for example, fluorescence activated cell sorting, by simple micromanipulation, or by capture using microfluidic technology. The PAN single-cell genomics resource features single-cell capture microfluidic technology, the C1 Single Cell Auto Prep instrument (Fluidigm), which processes 96



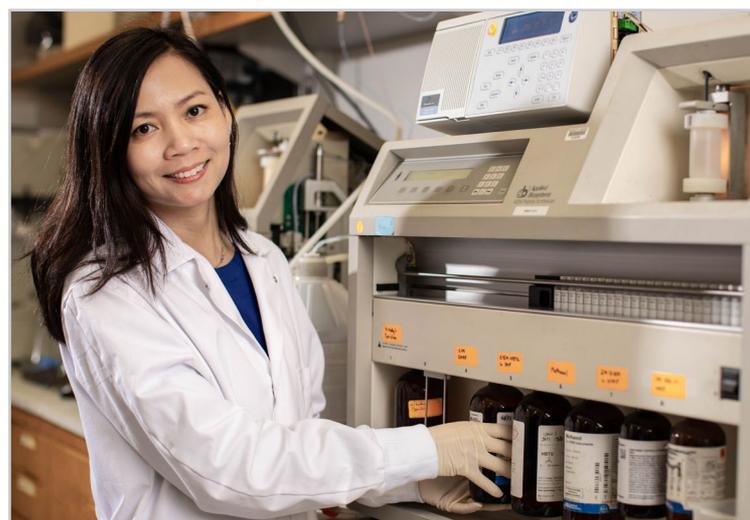
or 800 single cells, and the ddSEQ Single-Cell Isolator instrument (BioRad), which performs rapid single-cell isolation using droplet partitioning technology.

PAN also works closely with the Fluorescence Activated Cell Sorting Facility to perform high-throughput isolation of single cells from the biological system of interest. Cell acquisition is confirmed via an EVOS Cell Imaging System. Once isolated, the cells are automatically lysed and a nucleic acid template is generated on the microfluidic chip.

Subsequently, PAN processes the templates generated from individual cells for analysis by next-generation sequencing. The conversion to next-generation sequencing libraries is accomplished using automated liquid handling instruments. The nanoliter Mosquito HTS liquid handler (TTP Labtech) allows us to significantly decrease library preparation costs and increase throughput. To ensure quality control at different steps in all the workflows, a fragment analyzer instrument is used to perform nucleic acid quality control.

### Spatial Transcriptomics

Currently, single cells are collected from suspensions of dissociated tissue, in which spatial information has been lost. Spatial resolution of gene expression enables gene expression events to be pinpointed to a specific location in biological tissue. The ability to not only determine the gene expression within a cell, but also how the cells are organized in relation to one another, offers invaluable insight into understanding disease states in oncology, neurology, and immunology, as well as organism development. Spatially resolved gene expression in tissue sections is traditionally analyzed using immunohistochemistry (IHC) or *in situ* hybridization (ISH); however, these technologies, aside from being laborious and challenging, are low-throughput and nonquantitative.



**Top:** Ian Anderson, **Bottom:** Jessica Tran

To overcome these limitations, the PAN Facility is implementing a technology known as spatial transcriptomics. This technology combines traditional histology with high-throughput, single-cell RNA sequencing (scRNA-seq), whereby intact tissue sections are captured on an array containing spatially barcoded, complementary DNA primers for the capture of either full-transcriptome or transcript subsets. Subsequent RNA library generation for next-generation sequencing of a single intact tissue sample utilizes the existing instrumentation in PAN's Single-Cell Genomics Laboratory. The spatial transcriptomics workflow bridges new microscopy techniques and RNA sequencing to generate protein biomarker and transcriptome data, respectively, from a single intact tissue sample. To accomplish this, the PAN Facility

is working closely with the Cell Sciences Imaging Facility, which has implemented the CODEX (CO-Detection by indEXing) technology; that technology enables the analysis of at least 50 different protein biomarkers.

The collaboration of PAN with different research programs and technologies in other shared resources (FACS and CSIF), in accordance with our mission, adapts and takes advantage of single-cell tools, protocols, and technologies, including equipment acquisition, as they become available, so that scientists and clinicians within the Stanford scientific community remain on the cutting edge of scientific research. It is anticipated that advances made using PAN's scientific resources will enable researchers

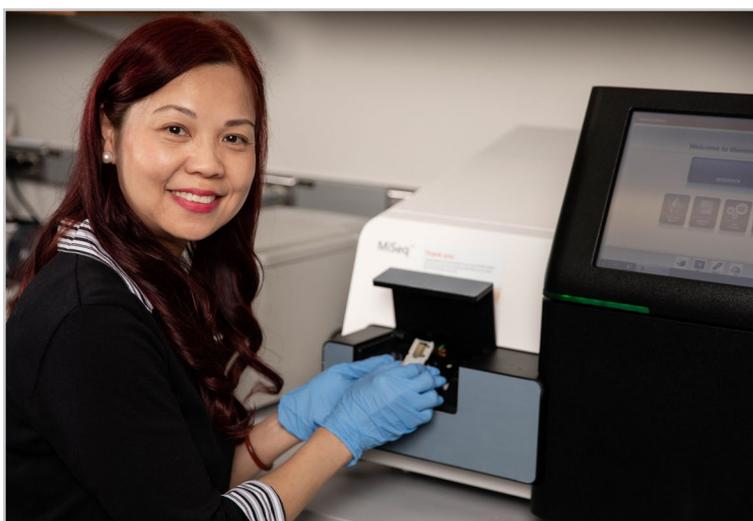
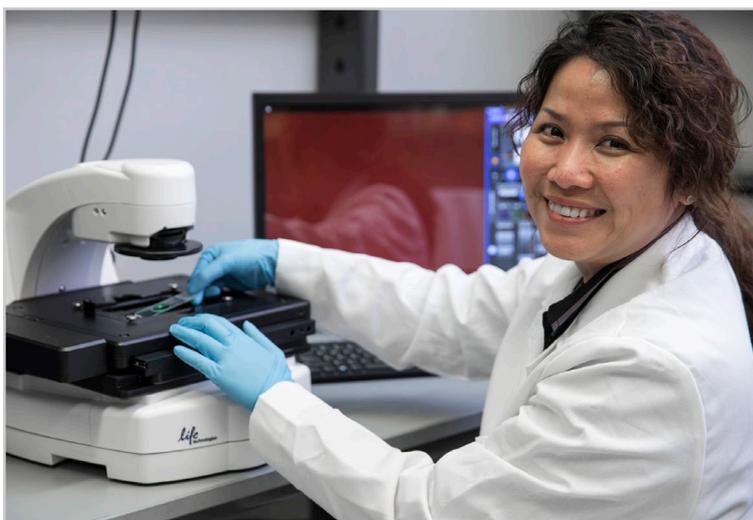
to obtain a deeper understanding of the underlying causes of diseases such as cancer and immune disorders, and the differentiation of stem cells, which have the promise of developing diagnostics and therapeutics in the different areas.

### Other Technologies

The PAN Facility continues to provide Affymetrix microarray technology for gene expression analysis. Besides a cost and time differential between the NGS and microarray platforms, with microarrays being less expensive and faster, the PAN Facility continues to provide both technologies in a manner that is most effective, most informative, and carefully tailored to the scientific questions and the biological systems that are being addressed by researchers.

With the next-generation Affymetrix GeneChip Clariom microarrays, a highly detailed view of the transcriptome is achieved that rapidly leads to actionable results. A comparison of array and RNA-Seq profiling technologies, in terms of throughput and performance, found that the Clariom arrays outperformed RNA-Seq in most all parameters when detecting exonic changes implicated in human disease and genetic disorders. A cost-free, easy-to-use Transcriptome Analysis Console (TAC) software program is available for Affymetrix microarray data analysis and visualization, to allow easy interpretation of significant gene expression changes. Overall, PAN scientists continue to work closely with stem cell and cancer researchers to develop both NGS and microarray methods for genomic profiling of single cells.

PAN's portfolio of technologies also includes those required for the validation of genes and proteins identified in large-scale genomic and proteomic studies. We believe that the need for such validation technologies will continue to grow, as they are key to



**Top:** Yen Tran, **Bottom:** Jennifer Okamoto



**Top:** Ian Anderson, Nghi Cat Bao Le

**Middle:** Nghi Cat Bao Le, **Bottom:** Ian Anderson

demonstrating how genetic or proteomic differences have effects in a specific diseases. Quantitative-PCR (Q-PCR) continues to be a popular technique to validate array study data. The use of pyrosequencing,

using the Qiagen PyroMark Q24 instrument for real-time, sequence-based detection for quantification of sequence variants (SNPs/mutation detection) and epigenetic methylation, has also increased. The validation of methylation events identified by microarray and high-throughput, massively parallel sequencing technologies has been the main driver in pyrosequencing services.

Sample identification and verification is essential to research that interrogates and compares specific regions of the human genome, called short tandem repeats (STR). Short tandem repeat genotyping is an important tool in verification of authenticity of human cell lines and quality control of stored human tissues and fluids. Cells grown *in vitro* can be misidentified or become contaminated with other unrelated cell lines. Misidentification of cell lines produces misleading results and has a significant negative impact on research costs. Journals and funding agencies now require proof that the cell lines being used are authentic and have remained so over the course of a study. We have seen an increase in the demand for fragment analysis in STR analysis workflow that uses the capillary electrophoresis (CE) technology in our DNA sequencer, since it is a simple, economical method, and the gold standard for establishing the identity of human samples.

Other research phases involve the use of technologies such as peptide synthesis, mass spectrometry, and surface plasmon resonance (SPR) to facilitate a more detailed and more comprehensive molecular study focusing on the complex of proteins expressed in biological systems, their structures, interactions, and post-translational modifications. SPR is a key technology in support of our efforts to meet the post-genomic

biological challenge of understanding the complex networks of interacting genes, proteins, and small molecules that give rise to biological form and function. PAN's Biacore T200 instrument offers researchers the opportunity to work confidently at the limits of kinetic, molecular weight, and concentration ranges, bringing improvements in data quality to a wide range of new applications. Using the capabilities of the T200 instrument, PAN scientists have and will continue to work with investigators to perform fragment-based lead discovery (FBLD) to discover small-molecule drug candidates for a variety of drug targets in different disease indications.

The coupling of existing genomic and protein analysis tools within the PAN Facility significantly extends the understanding of many research questions and helps to further accelerate research programs. For example, by applying and combining a multi-omics (single-cell genomics, epigenomics, proteomics) approach, researchers are discovering the variation that exists between genetically identical cells within a tissue in response to various physiological and pathophysiological stimuli.

The PAN Facility environment allows the Stanford research community to bridge the technical diversity gap and encourages collaborations that apply different technologies to biomedical research. PAN Facility scientists reach out for new technical opportunities to broaden horizons by working closely with scientists from different disciplines in implementing scientific breakthroughs and associated methodologies in genomics and proteomics. This enables researchers to make connections between basic and clinical research



that will benefit the field of translational medicine. PAN Facility scientists have made significant contributions to many different scientific programs in the form of publications in peer-reviewed journals and patents.

## FUTURE VISION

The PAN Facility will continue, in an ever-changing scientific environment that encompasses a wide range of biological, chemical, engineering, and physical sciences, to focus on providing solutions to the scientific technological needs that confront researchers. With the existing strengths and expertise in the different areas of the PAN Facility, the collaborative efforts between the different Beckman Center shared technology resources will enable multidisciplinary innovation and strategies that will broaden the application of different technologies to the multi-omic sciences. Overall, despite the tremendous challenges and changes during the COVID-19 pandemic this past year, PAN's work has never been more urgent, and we will continue to play a role in supporting and ensuring that Stanford researchers have access to technological capabilities to perform their research and a brighter future ahead.

# FLUORESCENCE ACTIVATED CELL SORTING FACILITY

## OVERVIEW

Fluorescence activated cell sorting (FACS), also known as flow cytometry, is a high-throughput technique for measuring, classifying, and sorting single cells.

In this technology, biological cells are labeled with one or more fluorescent reagents, often antibodies, that detect specific molecules inside cells or on their surfaces. These labeled cells are then streamed at a high rate through a sequence of laser beams, and the resulting fluorescence is measured on a per-cell basis. Quantitative evaluation of multiple reagents on each cell enables resolution and analysis of complex mixtures of cell types, such as tumor and bone marrow cells. Cell sorters, an advanced subset of flow cytometers, utilize the quantitative criteria provided by the fluorescent tags for selection, and then physically isolate those subsets at a high rate for further studies. Particular strengths of FACS technology are the flexibility of the selection criteria (e.g., high for label A, but low for labels B and C) and the ability to isolate up to six specified live cell types at once. In addition to the typical applications using mammalian cells, FACS is also valuable for work with yeast, bacteria, plankton, and other small particles.

The Fluorescence Activated Cell Sorting Facility (<https://facs.stanford.edu>) in the Beckman Center has provided these technologies of cell analysis and sorting to Beckman researchers, other Stanford University research groups, and the regional biotechnology community since the opening of the Beckman Center in 1989.



**LISA NICHOLS, PH.D.**  
Director

A team led by the late Leonard Herzenberg, Ph.D., a Stanford professor of genetics, was one of the main developers of FACS instrumentation and techniques in the late 1960s and early 1970s, and the Herzenberg laboratory continued to be a major source of innovation in the field throughout the subsequent years. Dr. Herzenberg initiated the precursor to the current facility in the mid-1980s and joined the Beckman Center when it opened. The FACS Facility, which was then part of the Herzenberg group, also moved to the Beckman Center at that time and was reorganized into a service center.

Today, the FACS Facility, in addition to providing access to FACS technologies, acts as a hub for general FACS education and provides training for users who want to become self-operators of the



### **Fluorescence Activated Cell Sorting Facility**

**Front Row, Left to Right:** Meredith Weglarz, Tom Nozaki, Bianca Gomez, Cindy Jiang, Lisa Nichols, Dave Parks

**Back Row, Left to Right:** Edgar Henriquez, Tim Knaak, Kenneth Quayle, Ricardo Zermeno

facility instruments. The FACS Facility director, Lisa Nichols, Ph.D., and her staff members have decades of experience in flow cytometry, and are available to assist with experimental design and data analysis. In addition to the more routine instrument maintenance and operational support, staff members work on evaluation and development of advanced applications and instrumentation.

### **EXPERTISE**

The FACS Facility is under the general oversight of a faculty advisory committee chaired by Garry Nolan, Ph.D., the Rachford and Carlotta A. Harris Professor, who did graduate work in the Herzenberg lab. Dr. Nolan's current research is groundbreaking in its use of cell sorting technology to measure intracellular phosphorylation signaling networks in single cells.

The facility director, Dr. Nichols, is a flow cytometry expert with more than 15 years of experience, as well as a scientist with expertise in T cell immunology and cancer immunotherapy. Many of the staff members have similarly long histories in flow cytometry, and contribute a level of expertise available at no other site, enabling researchers to perform innovative and top-quality work using flow cytometry.

### **SERVICES**

The services offered by the FACS Facility include cell analysis, cell sorting, instrument training, and more.

#### **Cell Analysis**

Cell analysis services include analysis of both Biosafety Level 1 and 2 (BSL-1 and BSL-2) samples. These analyses run the gamut from high-throughput screening assays to complex experiments collecting

measurements of up to 40 simultaneous fluorochromes. Instrumentation supports sample acquisition from either individual tubes or 96-well plates. Users have the option to either drop off samples for collection of data and basic analysis by the support staff, or to receive technical training on the instruments (with instrumentation accessible 24/7 for experienced researchers).

FACS is currently operating six analyzers at the main campus facility, plus an additional instrument at the Page Mill Road satellite facility to support researchers based at the School of Medicine Technology & Innovation Park. The main campus instruments include one analyzer operated by staff for contact-free, sample drop-off analysis service, plus five additional flow cytometers available for either staff-assisted or independent operation. This lineup includes the 30-parameter, 5-laser analyzer purchased in late 2020 with funds from an NIH Shared Instrumentation Grant and the Beckman Foundation.

### Cell Sorting

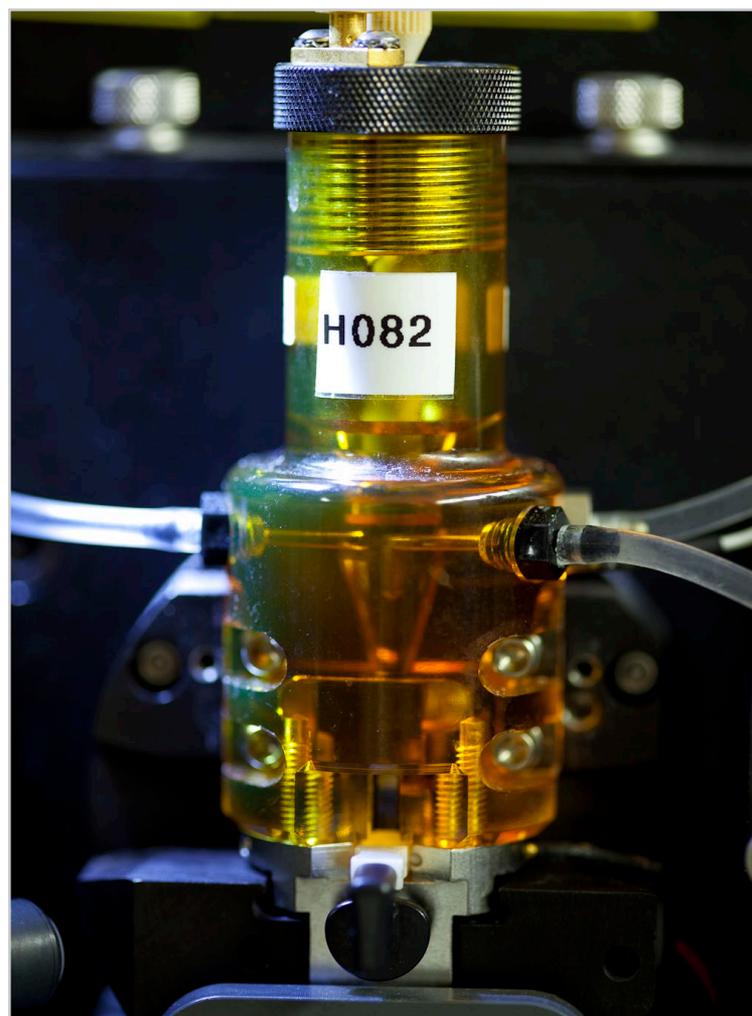
Cell sorting services include BSL-1 and BSL-2 samples, aseptic sorting, single cell sorting into 96-well and 384-well plates (cloning), and measurement and sorting using up to 18 simultaneous fluorochromes. Sorting is either operator-supported during normal business hours or self-operated 24/7 upon completion of training. Nine sorters are available, each with different capabilities. This year, a biosafety cabinet was added to the newest 5-laser instrument and three sorters were moved to single occupancy rooms, to provide enhanced biosafety and support for sorting of clinical specimens, including those needed for critical COVID-19 studies.

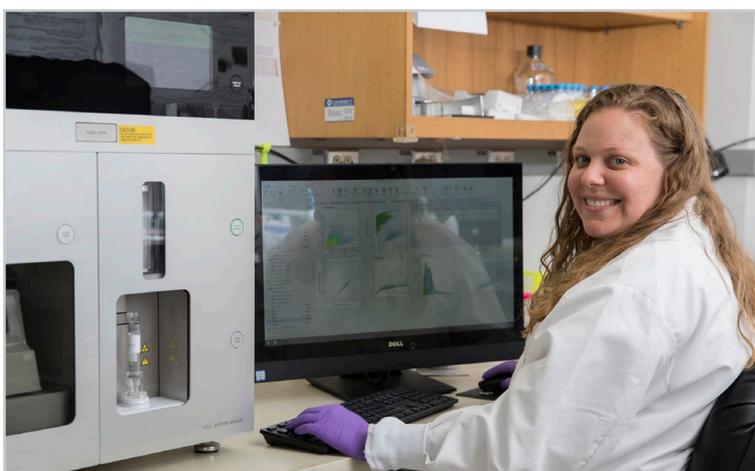
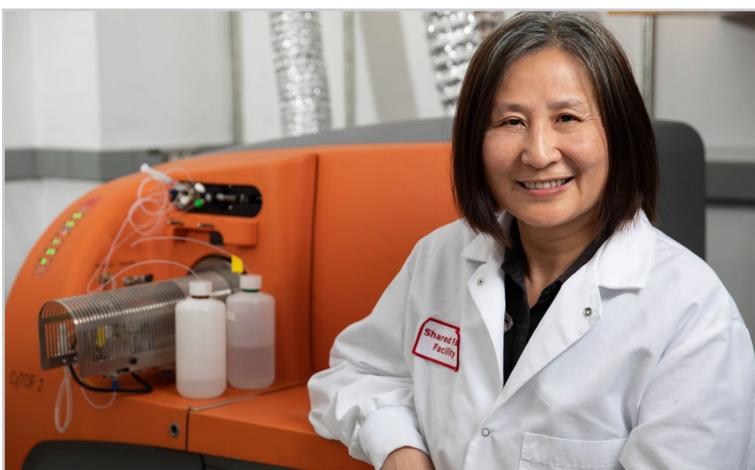
### Mass Cytometry

Mass cytometry services include analysis of samples using Time of Flight (TOF) mass spectrometry, measuring up to 100 different parameters with proteins conjugated to metal ions. Training and operator support are also available.

### Instrument Training

Intensive instrument training is provided to users of the facility and is tailored to each user's needs and experimental goals. All training programs were recently re-tooled with video, as well as remote- and video-teaching technologies, to allow researchers to continue to receive both theory and hands-on training while maintaining social distancing and personnel safety.





**Top:** Cindy Jiang, **Middle:** Meredith Weglarz  
**Bottom:** Bianca Gomez

### Consulting

Staff expertise is available to aid researchers in experimental design and data analysis. An educational seminar series continued this year, featuring topics ranging from basic cytometry techniques to advanced or specialized cytometry applications.

### Software Support

The facility manages a site license for FlowJo data analysis software. This license provides Stanford researchers with a discount of approximately 60 percent off the cost of an individual license. Additionally, the facility administers the FlowJo SeqGeq license to support high-parameter single-cell analysis.

### Data Management Services

Data collected in the facility is stored and archived in a secure, highly redundant system, and made available over the internet. This service is available to the entire Stanford community. The Stanford Institute for Stem Cell Biology and Regenerative Medicine and the Canary Center at Stanford for Cancer Early Detection utilize this service for their flow cytometry data.

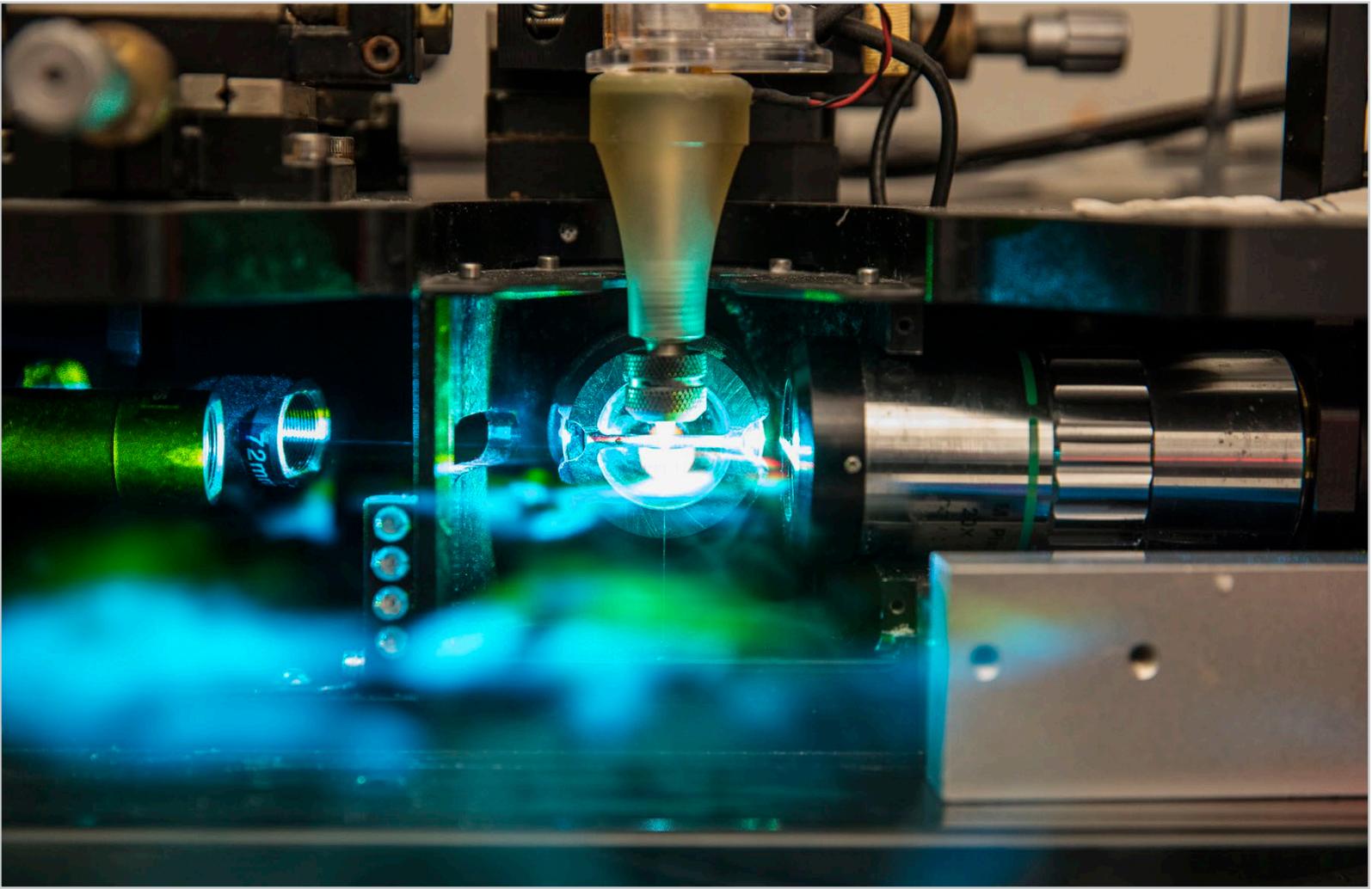
### Page Mill Road Satellite Facility

The FACS Facility continues to support the instrumentation at the Page Mill Road satellite facility. This location houses both a sorter and an analyzer, to support the research efforts of those with laboratories at the School of Medicine Technology & Innovation Park.

## RECENT DEVELOPMENTS

2020 was a dynamic year, marked by a series of adaptations and new approaches to accommodate a socially distanced year in scientific research, in response to the COVID-19 pandemic. With flow cytometry now a critical tool for many aspects of biomedical research, the FACS Facility rapidly reconfigured labs and training to provide the highest level of support to researchers in a changing workplace landscape.

A major update this year was the creation of a high-parameter cytometry lab. High-parameter cytometry



requires strong staff support, to provide an efficient mechanism for researchers to design and carry out complex experiments. To provide room for instrument access, training, and protocol testing, all in the context of a pandemic-safe workspace, the FACS Facility adapted a new lab space in the Beckman Center. The new High-Parameter Analysis Lab provides more than 500 square feet of additional BSL-2-approved lab space, and houses the upgraded spectral analyzer, a spectral analysis workstation, the 25+ parameter Acea Quanteon and, new in 2020, a 30-parameter, 5-laser BD FACSymphony analyzer. The latter instrument was purchased using funds from an NIH Shared Instrumentation Grant submitted in collaboration with Dr. Garry Nolan, and from the Beckman Foundation.

In response to the pandemic, the facility quickly implemented contact-free, sample drop-off services,

with remote monitoring available to researchers, to continue to promote high-level research and support essential research for the more than 200 labs that use the FACS Facility. This allowed critical experiments to continue, even with strict occupancy limits on campus and in the surrounding community. In addition, new training programs and techniques, including video sessions and remote guidance, allowed the re-implementation of user training for both the Symphony and the Aurora analyzers, as well as continued sample drop-off analysis support on the Quanteon. These three instruments are the core of the high-parameter, single-cell analysis lab, and use the latest technologies to provide data for researchers across a broad range of disciplines.

To support sorting applications, a modified training program with video tutorials and remote support

of hands-on training was developed; this allowed a re-launch in early fall 2020 of technical training for researchers who needed to use this critical research tool. In addition, our post-shelter-in-place re-opening featured enhanced biosafety in response to the pandemic, with increased distance between instrument operators, three sorters located in single-occupancy lab spaces, and a new biosafety hood and containment system for our newest 5-laser, 4-way sorter. The latter improvements provide enhanced biosafety for support of clinical specimen analysis. The biosafety enclosure also allows the FACS Facility to directly support researchers who refocused their experiments to investigate diagnostics and clinical outcomes in COVID-19 studies and vaccination trials.

Through all of the changes in the past year, we have maintained a dedication to continued education and teaching for the biomedical research community. The early months of shelter-in-place work provided an opportunity for staff to step back from day-to-day processes and spend more time learning specialized applications and analysis approaches. We then shared that knowledge with our users. We moved our educational seminars to a virtual space and opened them to national audiences, and saw high turnout for the panel design webinar in particular.

## **FUTURE VISION**

The FACS Facility continues to be heavily used by a large number of laboratories in the university and the community. As we adapt to a more remote workplace, the facility continues to adapt and to seek new ways to automate and facilitate user experiments.

In the next several months, we will continue to expand our video and remote training capabilities to the lesser-used instrumentation, such as the mass cytometer, so incoming users can gain access.

Importantly, research has begun to rebound, so we can restart our trajectory for the expansion and updating of older equipment. This began in earnest three years ago, with the purchase of the high-parameter instrumentation. We will continue this progress in 2021.

We are anticipating the arrival of an advanced 6-way sorter, complete with biocontainment, in 2021. This 5-laser, 50-detector BD S6 sorter will provide the latest technology and will be capable of supporting traditional data collection using fluorochrome emission peak filters, as well as live spectral analysis and sorting. Thus, this instrument will complement the capabilities of the new analyzers in our High-Parameter Analysis Lab, by providing a mechanism for researchers to transfer their high-parameter analysis panels directly to the sorter, so populations identified can be sorted and used in downstream applications.

With excellent staffing levels and a commitment to both education and updating to the latest technologies, the FACS Facility is positioned to provide users with a wide range of support. We look forward to another productive year supporting more than 200 labs both at Stanford and in the surrounding Bay Area community.

# COMPUTATIONAL SERVICES AND BIOINFORMATICS FACILITY

## OVERVIEW

Under the direction of Lee Kozar, the Computational Services and Bioinformatics Facility provides computer software support for more than 5,000 people in over 300 different research labs and 36 different departments at Stanford University. Both commercial and public domain software for sequence analysis, molecular modeling, and mathematical and statistical analysis are available from the facility. A full description of the facility and its services can be seen on the facility's website: <https://csbf.stanford.edu>.

## EXPERTISE

The CSBF staff members have many years of experience in providing computer support to biomedical researchers, and most have also worked in laboratories at some point in their careers. They are intimately familiar with the CSBF software and the needs of the scientific research community.

The CSBF works closely with other service centers at the Beckman Center to ensure that the CSBF has the necessary hardware and software for analyzing the wide variety of data that is generated by the different facilities. In essence, the other service centers provide the instrumentation for generating data, and the CSBF provides the computer hardware and software for analyzing the data flowing out of these facilities.

## SERVICES

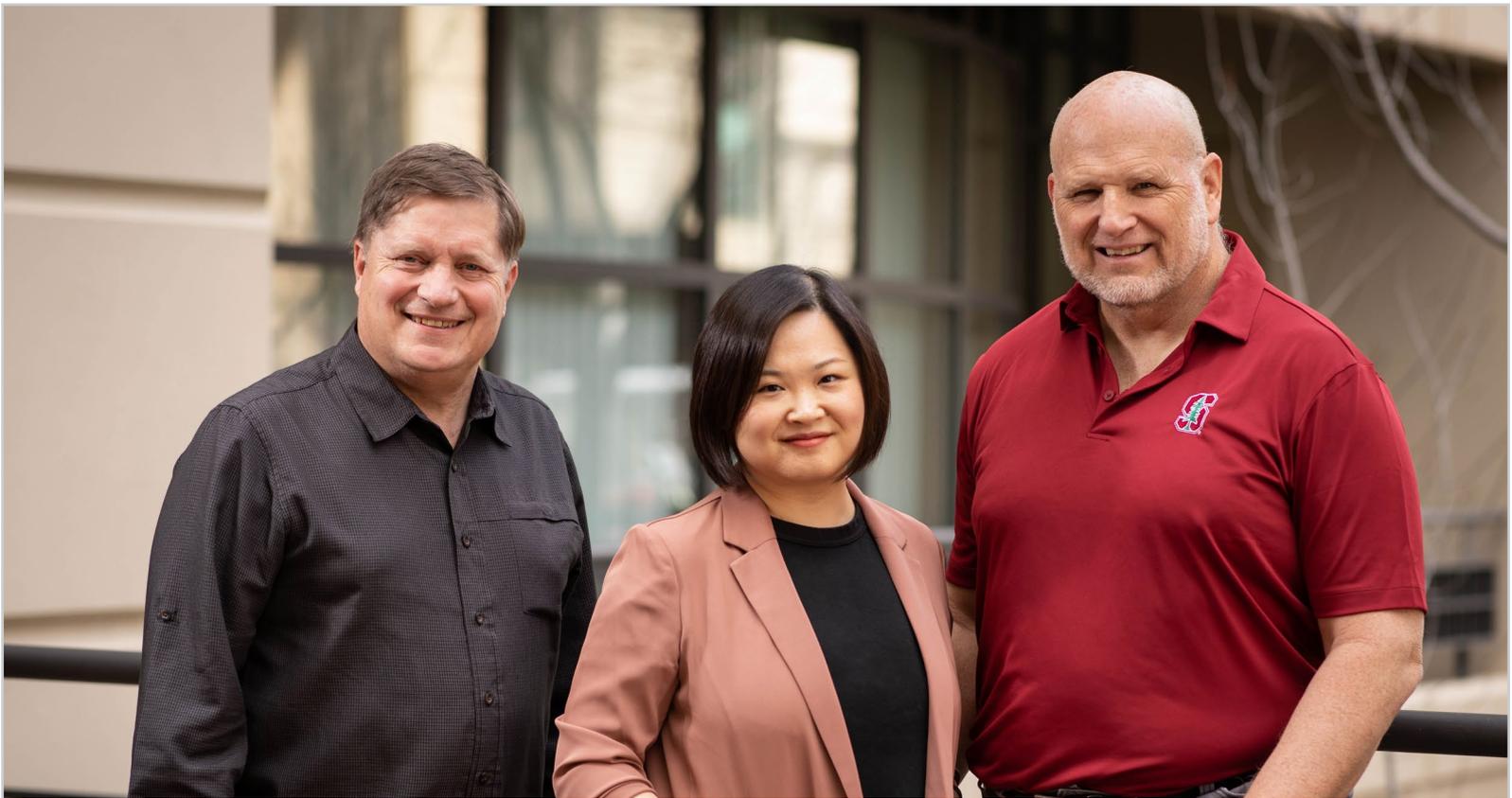
### Available Software

The CSBF provides a variety of Macintosh, Windows, and Linux software for scientific research and general administrative use.



**LEE KOZAR**  
Director

The CSBF obtains concurrent network licenses that work under the control of a software license manager. This allows the facility to purchase a limited number of copies of expensive software, but distribute the software widely within the Stanford network, thus providing substantial savings to individual researchers. For example, one of CSBF's most popular software packages costs more than \$20,000 per license per year, which makes it prohibitively expensive for many labs. Other software packages cost hundreds or thousands of dollars per license. With a membership in the CSBF, researchers can gain access to these software products at a significantly lower cost. This gives even small labs access to software tools that previously only large, well-funded labs could afford. The CSBF also shoulders the hidden cost of installing and managing the licenses and license servers, making a membership in the CSBF attractive even



**Computational Services and Bioinformatics Facility** *Left to Right: Lee Kozar, Ling Xie, Alan Hebert*

when a lab can afford to purchase their own software.

While there are many public domain software packages available for doing scientific research, the CSBF has focused on providing access to commercial software because, in most cases, commercial scientific software has significant advantages over its public domain counterparts. Commercial software offers technical support, is usually easier to install and run, is updated more frequently, and is less prone to errors.

In addition to providing a full range of popular software programs, such as Microsoft and Adobe products, the facility offers software in the following categories:

- Sequence analysis (DNASTAR, SnapGene, MacVector, Sequencher, Geneious, CLCBio)
- Microarray analysis (GeneSpring, Partek)
- Genomics analysis (Geneious, Golden Helix, Partek, JMP Genomics, iPathwayGuide)

- Mass spectrometry (Mascot, PEAKS, ProteinMetrics)
- Database (FileMaker, EndNote, Paperpile)
- Statistical and mathematical analysis (SPSS, Matlab, Mathematica, SigmaPlot, GraphPad)
- Graphics (Illustrator, Photoshop, BioRender)
- Microscope imaging (Volocity, Imaris, Metamorph)
- Gel electrophoresis imaging (Nonlinear Dynamics)
- Electronic lab notebooks (LabArchives, Benchling)

These software programs are repackaged by the CSBF so they can be easily downloaded from the facility's website and installed, already configured for use within the Stanford network. Many of these software programs can be used off-campus; special licensing arrangements can be made so the software will work even when not connected to a network. That means that Stanford researchers have access to the software they need no matter where they are.

This has been especially useful this year, as most everyone had to work remotely at home due to the COVID-19 pandemic. A full list of the software offered by the CSBF can be seen at <http://csbf.stanford.edu/software>.

The CSBF depends on our research community to alert us to software titles that may be of value to their research. Researchers often request that specific titles be added to the software library. The CSBF is frequently able to negotiate a concurrent network license with the vendor so newly acquired software can be shared with other users of the CSBF software library.

The quantity and quality of software available through the CSBF is unmatched by any other university. Most other bioinformatics service centers provide only open source, free software. While the CSBF does provide a wide variety of public domain software, we also attempt to obtain the very best commercial software for biomedical research. Very few other universities provide even one of the commercial packages that the CSBF makes available to Stanford researchers.

### CSBF Membership

To access CSBF software, researchers must first obtain a CSBF membership. This can be done online at <http://csbf.stanford.edu/membership>.

The CSBF has two levels of membership:

- A Level 1 membership gives everyone in a specific lab access to the bioinformatics computer facilities, including the large library of commonly used Mac, PC, and UNIX software packages.
- A Level 2 membership gives a specific lab access to all CSBF software, including the more expensive software packages such as GeneSpring, iPathwayGuide, Imaris, Volocity, Partek, and others.



*Middle: Ling Xie, Bottom: Alan Hebert*



It is possible to join at Level 1 and upgrade to Level 2 at a later date with a prorated charge. More information about the different levels of software is available at <http://csbf.stanford.edu/membership/Level1.html> and <http://csbf.stanford.edu/membership/Level2.html>.

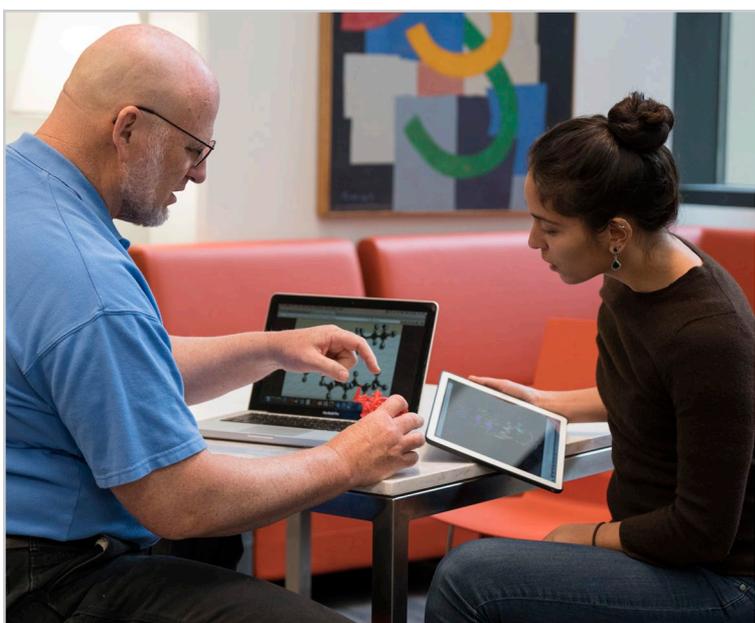
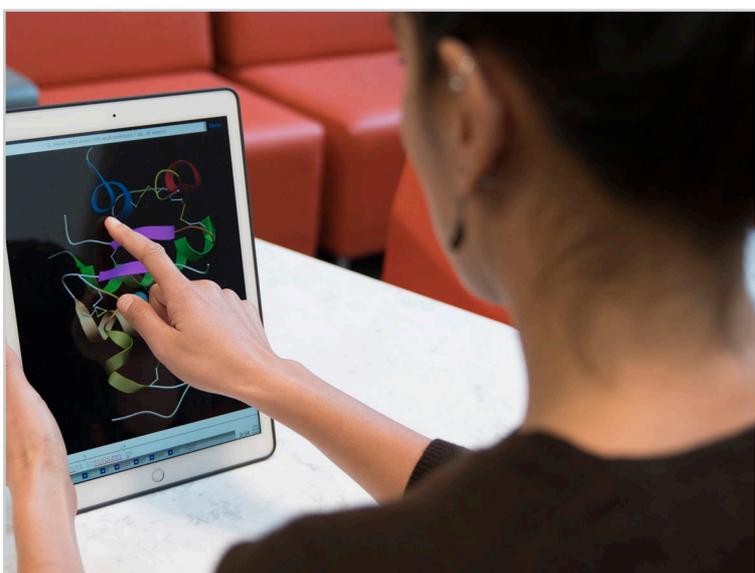
In the past year, more than 300 labs from 36 different departments have had memberships in the CSBF. On average, more than 5,000 computers per month utilize the software library; at peak usage, over 500 individual software licenses are checked out. The CSBF software library has become an indispensable asset to researchers in the Beckman Center, as well as to the broader research community at Stanford and those working from home around the world.

### Additional Services

In addition to the software library, the CSBF provides a variety of other services for CSBF members, including website hosting and hands-on computer support. The CSBF moved most of its hardware and storage to the Stanford cloud as a cost-saving measure. The CSBF web server is the primary route for distributing software to users at Stanford; it also hosts many lab and departmental websites. The CSBF also has a large Linux system that hosts proteomics and genomics software.

The CSBF offers desktop computer support for Beckman Center researchers. Services such as software installation, troubleshooting, data recovery, and minor computer repairs are routinely provided through phone calls, email, online chat, and personal visits. We also recently installed a stereolithographic 3D printer, which we use to create physical models of molecular structures or laboratory equipment. These models are useful to help visualize the structure of biological molecules.

The CSBF houses most of its computer equipment in a dedicated server room in the Beckman Center, as well as



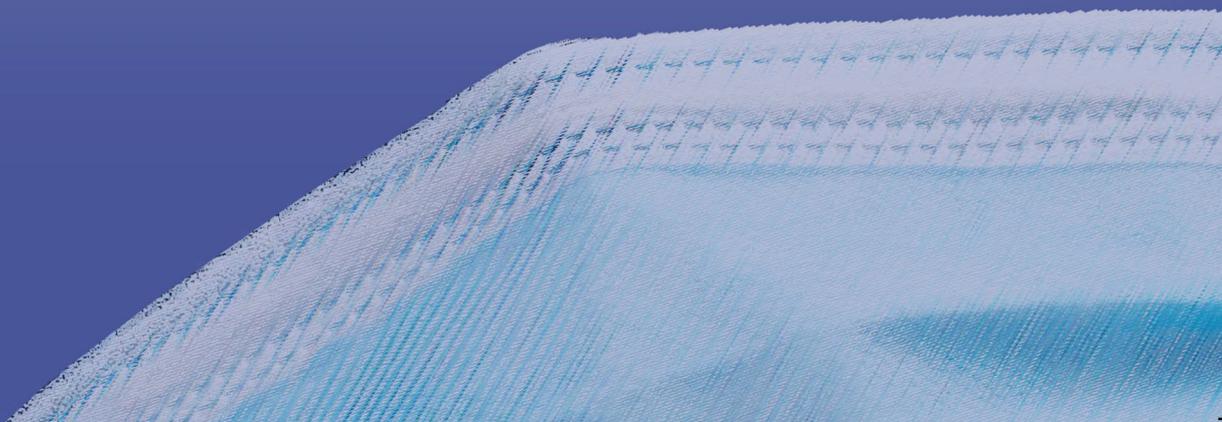
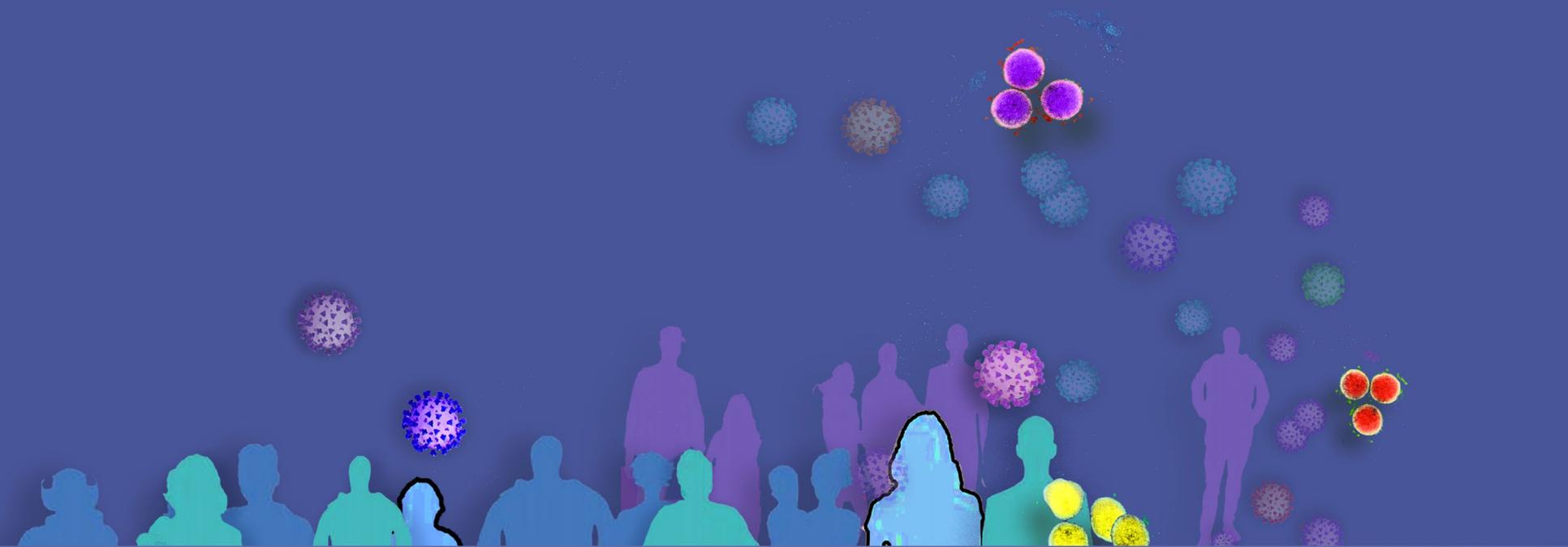
**Top:** Alan Hebert, Ling Xie, **Middle:** Facility User  
**Bottom:** Alan Hebert, Facility User

at the main Stanford server farm. This special room in the Beckman Center is controlled for temperature and humidity; a regulated power source has been installed to control power spikes, which could damage equipment. The room has been earthquake retrofitted and is also protected by a Halon™ fire suppression system. The server room also houses computer equipment from other labs and service centers in the Beckman Center, providing a secure location to store important computer hardware and research data. The server room is equipped with a variety of environmental monitors and CSBF staff members are alerted by email or text message if there is a problem in the room.

There is a significant amount of institutional knowledge in the CSBF that is critical to the functioning of this core facility. While it is important to back up computer data, it is also important to back up the knowledge that each member of the CSBF has acquired over time. To accomplish this, the CSBF has set up two Wiki sites: one public and one private. The public Wiki site has information that can help users of the CSBF better utilize the available software and hardware. The private Wiki can be accessed only by members of the CSBF and contains important information regarding policies, procedures, license codes, troubleshooting techniques, and other information that the CSBF team deems important to record.



# ACADEMIC DEPARTMENTS



# ACADEMIC DEPARTMENTS

## DEPARTMENT OF BIOCHEMISTRY

Under the leadership of department chair Aaron Straight, Ph.D., research in the Department of Biochemistry encompasses very diverse questions and uses a wide variety of approaches, experimental systems, and techniques. Nevertheless, what bonds members of the department is an interest in understanding fundamental biological questions at the level of how molecules act and interact to accomplish highly complex, intra- and intercellular processes. The diversity of the department enriches the intellectual environment and provides an incredibly broad spectrum of expertise that benefits everyone, as members of the department tackle a wide variety of important questions.

All researchers in the department study molecules (proteins, RNA, DNA, and polyphosphate) and analyze their synthesis, structure, actions, and interactions. They use physical techniques such as spectroscopy, laser light traps, and crystallography, cell biological techniques such as light microscopy and cell fractionation, and biochemical techniques such as enzyme purification and characterization, as well as molecular biological techniques and genetics. By attacking problems using these complementary approaches, departmental researchers are best suited to pave the way toward solving the questions at hand.



**AARON STRAIGHT, PH.D.**  
Professor and Chair  
of Biochemistry

Two features of the department are especially noteworthy. First, members of the department share all of the space and major equipment. Thus, students and postdocs from different groups are intermixed. This enhances interaction at all levels and guarantees equality in terms of access to all resources and equipment. Second, everyone works hard to maintain a collegial, cooperative, and supportive environment. All faculty are engaged in the operation and mission of the department, and share and uphold philosophies of operation and community spirit that all members hold dear.

## FACULTY RESEARCH

**Steve Artandi's** lab is interested in unraveling the molecular and cellular mechanisms with which telomeres and telomerase modulate stem cell function and carcinogenesis. **Onn Brandman's** lab studies how cells ensure protein quality and how they signal stress. The lab uses an integrated set of techniques, including single cell analysis of proteotoxic stress pathways, structural studies, *in vitro* translation, and full genome screens. **Gil Chu's** laboratory studies cellular responses to damaged DNA. The group focuses on pathways for the repair of UV-damaged DNA and the repair of DNA double-strand breaks induced by ionizing radiation and V(D) J recombination in order to understand the mechanisms that generate immunological diversity. **Rhiju Das's** research group strives to predict how RNA sequence determines the folding properties of proteins, nucleic acids, and heteropolymers and establishes their ultimate structure. **Ron Davis** is using *Saccharomyces cerevisiae* and human DNA to conduct whole genome analysis projects. The **James Ferrell** lab has been studying the system of regulatory proteins that drives the cell cycle, through a combination of quantitative experimental approaches, computational modeling, and the theory of nonlinear dynamics. **Pehr Harbury** aims to measure and understand dynamic structural changes in proteins, and their role in the functional biology of macromolecular machines. **Dan Herschlag's** laboratory is aimed at understanding the chemical and physical behavior underlying biological macromolecules and systems, behaviors that define the capabilities and limitations of biology. **Peter Kim** studies the process by which proteins cause viral membranes

to fuse with cells, designs molecules that stop membrane fusion by HIV, and pioneers efforts to develop vaccines based on similar principles. **Silvana Konermann's** lab is applying multiple modes of targeted transcriptional perturbations to understand genetic interactions of APOE in late onset Alzheimer's disease. The research in **Mark Krasnow's** laboratory is focused on understanding lung development, stem cells, and diseases, including cancer, and the neural circuits that control lung function, including breathing and speaking. **Lingyin Li** uses chemical biology to uncover biochemical mechanisms in innate immunity and, in parallel, develop therapeutic hypotheses and lead compounds. **Suzanne Pfeffer's** group is investigating the molecular mechanisms by which proteins are targeted to specific membrane compartments. They seek to understand how transport vesicles select their contents, bud, translocate through cytoplasm, and then fuse with their targets, as well as other similar processes. **Rajat Rohatgi's** lab is working to elucidate the biochemical and cell biological principles that govern signaling pathways that sit at the intersection between developmental biology and cancer. **Julia Salzman's** research group develops statistical and experimental tools to construct a high dimensional picture of gene regulation, including cis and trans control of the full repertoire of RNAs expressed by cells. The broad research interest of the **James Spudich** lab is the molecular basis of cell motility. Research interests include the molecular basis of energy transduction that leads to ATP-driven myosin movement on actin, the biochemical basis of regulation of actin and myosin interaction and their assembly states, and the roles these proteins play *in vivo*, in cell

movement and changes in cell shape. The **Aaron Straight** group studies the process of cell division in eukaryotes focusing on the mechanisms of chromosome segregation. **Ellen Yeh**'s research goal is the elucidation of apicoplast biology, function, and role in pathogenesis, with the ultimate goal of realizing the potential of the apicoplast as a therapeutic target.

## 2020-2021 FACULTY HONORS, AWARDS AND APPOINTMENTS

**Rhiju Das**—associate professor of biochemistry, was featured on the PBS NOVA program, "Decoding COVID-19: Scientists race to understand and defeat the coronavirus behind the COVID-19 pandemic," for his work on COVID-19 treatments and vaccines.

**Lingyin Li**—assistant professor of biochemistry, was highlighted by *Chemical & Engineering News* as one of a dozen rising young stars who are using chemical know-how to change the world. Dr. Li was noted for her work focused on harnessing the immune system to fight cancer.

**Suzanne Pfeffer**—the Emma Pfeiffer Merner Professor of Medical Sciences and professor of biochemistry, and Monther Abu-Remaileh, assistant professor of chemical engineering, received an award from the Aligning Science Across Parkinson's initiative. The award, given in collaboration with colleagues at the University of Dundee in Scotland, is for further understanding Parkinson's disease and developing therapies for it.

## DEPARTMENT OF DEVELOPMENTAL BIOLOGY

Researchers in the Department of Developmental Biology, under department chair Anne Villeneuve, Ph.D., are working at the forefront of basic science research to understand the molecular mechanisms that generate and maintain diverse cell types during development. The research groups use a variety of innovative approaches, including genomics, computation, biochemistry, and advanced imaging, and study organisms ranging from microbes to humans, with a primary interest in the evolution of these organisms. This work has connections to many areas of human health and disease, including stem cell biology, aging, cancer, diabetes, and novel strategies for stimulating repair or regeneration of body tissues. The department is a dynamic, interactive research community situated in one of the world's best environments for biomedical research.

### FACULTY RESEARCH

**Maria Barna** is investigating ribosome-mediated control of gene expression in space and time during cellular differentiation and organismal development. Her research group is also employing state-of-the-art live cell imaging to visualize cell signaling and cellular control of organogenesis. **Philip Beachy's** group studies the function of hedgehog proteins and other extracellular signals in injury repair and regeneration, primarily through effects on stem cell physiology. They also study abnormal signaling and perturbed stem cell physiology as it occurs in tissue disorder and in the formation and expansion of cancer stem cells. The members of **Gill Bejerano's** lab focus on a fundamental question in human



**ANNE VILLENEUVE, PH.D.**  
Professor and Chair of  
Developmental Biology

genomics: the relationship between geno(me) type and phenotype. The group studies genome function in human and related species by mapping genome sequence (variation) to phenotype (differences) and extracting specific genetic insights from deep sequencing measurements. **Alistair Boettiger's** lab aims to understand how long-range interactions between nonconsecutive parts of the genome are regulated to control gene expression. **James Chen's** group integrates synthetic chemistry and developmental biology to interrogate the molecular mechanisms that control embryonic patterning, tissue regeneration, and oncogenesis. The focus of research in the **Gerald Crabtree** laboratory is the role of chromatin regulation in development and human cancer. **Margaret Fuller's** research group seeks to understand the mechanisms that

regulate stem cell behavior and in particular the mechanisms that regulate and mediate cellular differentiation during male gametogenesis, using spermatogenesis in *Drosophila* as a powerful genetic model system. **Daniel Jarosz's** lab aims to gain insight into the interplay among genetic variation, phenotypic diversity, and environmental fluctuations in complex cellular systems. **Seung K. Kim's** lab has created unprecedented opportunities for harnessing knowledge about the molecular and cellular basis of pancreatic development and growth to restore pancreas islet function and to diagnose pancreas cancers. They trust their discoveries will provide the tools and expertise needed to produce islet regeneration therapies for type 1 diabetes, improve treatments and tests to mitigate or prevent type 2 diabetes, and generate new therapeutic strategies for endocrine or exocrine pancreas cancers. **David Kingsley** is using a combination of genetic and genomic approaches to identify the detailed molecular mechanisms that control evolutionary change in vertebrates. **Kyle M. Loh's** lab aspires to understand how different human cell types form from stem cells, and how developing tissues incipiently take shape and form. **Roeland Nusse's** laboratory is interested in the growth, development, and integrity of animal tissues. The group studies multiple different organs, trying to identify common principles and extend these investigations to cancer and injury repair. The laboratory has a long-standing interest in the activity of Wnt proteins during embryogenesis and other processes. **Lucy Shapiro's** laboratory studies the mechanisms used to generate the three-dimensional organization of a cell from a one-dimensional genetic code. Their goal is to

define the complete genetic circuitry that regulates cell cycle progression in time and space. **Will Talbot's** lab focuses on the development and function of glial cells in the vertebrate nervous system. **Anne Villeneuve's** lab group is interested in elucidating the events required for the orderly segregation of homologous chromosomes during meiosis, the crucial process by which diploid germ cells generate haploid gametes. **Bo Wang's** research group is working at the interface between statistical physics, developmental biology, and bioengineering. They seek to understand, quantitatively, the fundamental rules that control stem cell collective behavior to optimize tissue regeneration, remodeling, and adaptation. **Irving Weissman's** lab studies the phylogeny and developmental biology of the cells that make up the blood-forming and immune systems. The focus of the research in **Joanna Wysocka's** lab is to understand how regulatory information encoded by the genome is integrated with the transcriptional machinery and chromatin context to allow for emergence of form and function during human embryogenesis and evolution, and how perturbations in this process lead to disease.

## 2020-2021 FACULTY HONORS, AWARDS AND APPOINTMENTS

**Seung K. Kim**—professor of developmental biology, and director of the Stanford Diabetes Research Center, was elected to membership in the Association of American Physicians.

**Lucy Shapiro**—the Virginia and D.K. Ludwig Professor of Cancer Research, and the director of the Beckman Center for Molecular and Genetic Medicine, was awarded the 2020 Dickson

Prize in Science from Carnegie Mellon University. The prize recognized her work in understanding how a one-dimensional genetic code generates three-dimensional cellular architecture. Dr. Shapiro established that the cell is an integrated network operating in time and space, with implications for computer science networks and systems biology. Her research has informed the development of medications to fight infectious diseases.

Dr. Shapiro also received an honorary doctorate from the Rockefeller University.

**William Talbot**—professor of developmental biology and former senior associate dean for graduate education and postdoctoral affairs, won a 2020 Kenneth M. Cuthbertson Award for “exceptional contribution” to Stanford University. He was recognized for his support of postdoctoral candidates from underrepresented groups and his pursuit of racial justice in the biosciences.

## DEPARTMENT OF MOLECULAR AND CELLULAR PHYSIOLOGY

The Department of Molecular and Cellular Physiology (MCP), under department chair Miriam B. Goodman, Ph.D., seeks to understand how cells communicate, interact, and enable complex physiological function. MCP labs take an interdisciplinary approach, with an emphasis on quantitative and structural approaches drawn from multiple scientific disciplines, including structural biology, biophysics, cell biology, immunology, and neuroscience.

By uncovering molecular and cellular processes, MCP scientists have established new paradigms in the biology of signaling and communication, such as the relationship between the structure and function of G-protein-coupled receptors (GPCRs), and the presynaptic molecular mechanisms underlying neuronal communication. Key research areas include understanding how cell signaling occurs and enables complex physiological function and response to the environment. The department conducts studies at every level of life, ranging from atoms and molecules to macromolecular assemblies, cells and cellular networks, organ systems, and entire organisms. They have established new paradigms in the biology of signaling and communication by practicing across multiple scientific disciplines, including structural biology, biophysics, cell biology, and neuroscience.

### FACULTY RESEARCH

The goal of research in **Axel Brunger's** lab is to understand the molecular mechanism of synaptic neurotransmission by conducting single-molecule/



**MIRIAM B. GOODMAN, PH.D.**  
Professor and Chair of Molecular and Cellular Physiology

particle reconstitution and imaging experiments, combined with high-resolution structural studies (by X-ray crystallography and electron cryo-microscopy) of the synaptic vesicle fusion machinery. Other interests include the development of advanced methods for biomolecular structure determination. **Steven Chu's** areas of research include tests of fundamental theories in physics, atom interferometry, the study of polymers and biological systems at the single molecule level, and biomedical research. **Liang Feng** is interested in the structure, dynamics, and function of eukaryotic transport proteins that mediate ions and major nutrients across the membrane, the kinetics and regulation of transport processes, the catalytic mechanism of membrane-embedded enzymes, and the development of small molecule modulations based on the structure and function of membrane

proteins. **Christopher Garcia's** group focuses on structural and functional studies of transmembrane receptor interactions with their ligands in systems relevant to human health and disease, primarily in immunity, infection, and neurobiology. **Miriam B. Goodman's** research investigates the biophysics and mechanics of touch sensation by combining *in vivo* electrophysiology with genetics and novel tools for mechanical stimulation, through quantitative behavioral studies, light and electron microscopy. **Brian Kobilka's** laboratory investigates the molecular mechanisms of G-protein-coupled receptor signaling. G-protein-coupled receptors are responsible for the majority of cellular responses to hormones and neurotransmitters, as well as the senses of sight, olfaction, and taste. The laboratory of **Richard Lewis** investigates calcium signaling mechanisms and their consequences for cell behavior, with a focus on store-operated calcium channels. **Daniel Madison's** laboratory uses electrophysiological techniques to study the mechanisms of synaptic transmission and plasticity in the mammalian hippocampus. A major focus of the lab is the study of long-term potentiation and mechanisms underlying memory formation in the central nervous system. The goal of research in **Merritt Maduke's** lab is to determine the molecular mechanisms of chloride selective ion channels and transporters. These membrane proteins are ubiquitously expressed in humans and are necessary for proper cardiovascular, muscular, neuronal, and epithelial function. **Lucy O'Brien's** lab uses a stem cell-based *Drosophila* epithelium, the intestinal lining of the adult midgut, as a system to explore the regulatory interface of stem cell and epithelial tissue biology. **Georgios Skiniotis** and his research group are using electron cryo-microscopy (cryoEM) to study the mechanisms of transmembrane signal instigation with a particular focus on G-protein-

coupled receptors and cytokine receptors. **Thomas Südhof's** laboratory studies how synapses form in the brain, how synapses work at a molecular level and change during synaptic plasticity, and how synapses become dysfunctional in diseases such as autism and other neuropsychiatric disorders. **William Weis's** research group studies molecular interactions that underlie the establishment and maintenance of cell and tissue structure, including cadherin-based adhesion and its interaction with the cytoskeleton, the relationship between cell-cell junction formation and generation of cell polarity, and the Wnt signaling pathway.

## 2020-2021 FACULTY HONORS, AWARDS AND APPOINTMENTS

**Axel Brunger**—professor of molecular and cellular physiology and of neurology, and professor of photon science at Stanford and SLAC, was elected to membership in the American Academy of Arts and Sciences. The Academy honors exceptional scholars, leaders, artists, and innovators engaged in advancing the public good.

## PROGRAM IN MOLECULAR AND GENETIC MEDICINE

### 2020-2021 FACULTY HONORS, AWARDS AND APPOINTMENTS

**Charles K.F. Chan**—assistant professor of surgery, has been appointed an investigator of the Heritage Medical Research Institute. The institute will support Dr. Chan's study of skeletal stem cells' ability to regenerate cartilage damaged by injury or disease.

**Wah Chiu and Serena Yeung**—Dr. Chiu, the Wallenberg-Bienenstock Professor, and a professor of bioengineering and of microbiology and immunology, and Dr. Yeung, assistant professor of biomedical data science, received a Chan Zuckerberg Initiative Neurodegeneration Challenge Network award. The award will be used to study neurons using cryo-electron tomograms and a computer vision method with the goal of learning about the cellular structure and pathology of Huntington's disease and other neurodegenerative diseases.

**Mark M. Davis**—the Burt and Marion Avery Family Professor, professor of microbiology and immunology, and director, Stanford Institute for Immunity, Transplantation and Infection, was awarded the Szent-Györgyi Prize for Progress in Cancer Research from the National Foundation for Cancer Research. The award, shared with Tak Mak, Ph.D., at the University of Toronto, is for discoveries about the genetic basis of T cell recognition. Those discoveries have led to treatments for blood cancers and other diseases.

**Dean Felsher**—professor of medicine and of pathology, received a 2020 Outstanding Investigator Award from the National Cancer Institute. The award will be used to target oncogene pathways that could be blocked as a treatment for cancer.

**Judith Frydman**—professor of biology and of genetics, and the Donald Kennedy Chair in the School of Humanities and Sciences, was elected to membership in the prestigious National Academy of Sciences, at the academy's 158th annual meeting in April 2021. Scholars are elected in recognition of their outstanding contributions to research. The National Academy of Sciences is a private organization, created in 1863 to advise the nation on issues related to science and technology. Dr. Frydman uses a multidisciplinary approach to address fundamental questions about protein folding and degradation, and molecular chaperones, which help facilitate protein folding.

**Jeffrey S. Glenn**—professor of gastroenterology and of microbiology and immunology, received a Harrington Discovery Institute Scholar Award for Coronavirus. Dr. Glenn, whose research focus is molecular virology, received the award for his work on a single-dose antiviral therapeutic for COVID-19.

**Paul A. Khavari**—the Carl J. Herzog Professor in Dermatology, received the Stephen Rothman Memorial Award from the Society for Investigative Dermatology. The award is given to dermatologists who have made outstanding contributions to the field in research as well as teaching.

**Michelle Monje and Sergiu P. Pasca**—Dr. Monje, associate professor of neurology, and Dr. Pasca, associate professor of psychiatry and behavioral sciences and the Bonnie Uytensu and Family Director of the Stanford Brain Organogenesis Program, were winners in the life sciences category of the 2020 Falling Walls Science Breakthroughs competition. Dr. Monje was recognized for her discovery that certain brain cancers interact with normal neurons to help the malignant tumor grow, uncovering potential therapeutic strategies for

lethal brain cancers. Dr. Pasca was recognized for developing 3D brain spheroids from pluripotent stem cells from patients, allowing the study of human neural circuits.

**Tirin Moore**—professor of neurobiology, received the 2021 Pradel Research Award from the National Academy of Sciences. The prize recognizes mid-career neuroscientists whose work is making major contributions to the understanding of the nervous system. Dr. Moore’s research demonstrates how neural activity in motor regions of the brain influences visual representation in other regions. His work has established a deeper understanding of brain mechanisms underlying spatial attention and is relevant to our understanding of attention disorders.

Dr. Moore was also elected to membership in the prestigious National Academy of Sciences, at the academy’s 158th annual meeting in April 2021. Scholars are elected in recognition of their outstanding contributions to research. The National Academy of Sciences is a private organization, created in 1863 to advise the nation on issues related to science and technology.

In addition, Dr. Moore was elected to membership in the American Academy of Arts and Sciences. The Academy honors exceptional scholars, leaders, artists, and innovators engaged in advancing the public good.

**Sergiu P. Pasca**—associate professor of psychiatry and behavioral sciences and the Bonnie Uytengsu and Family Director of the Stanford Brain Organogenesis Program, received the Basic Research Award from the Schizophrenia International Research Society. Dr. Pasca was recognized for pioneering methods of assembling patient-derived neural cells into three-dimensional organoids and

using them to uncover the molecular mechanisms of genetic forms of neuropsychiatric disease.

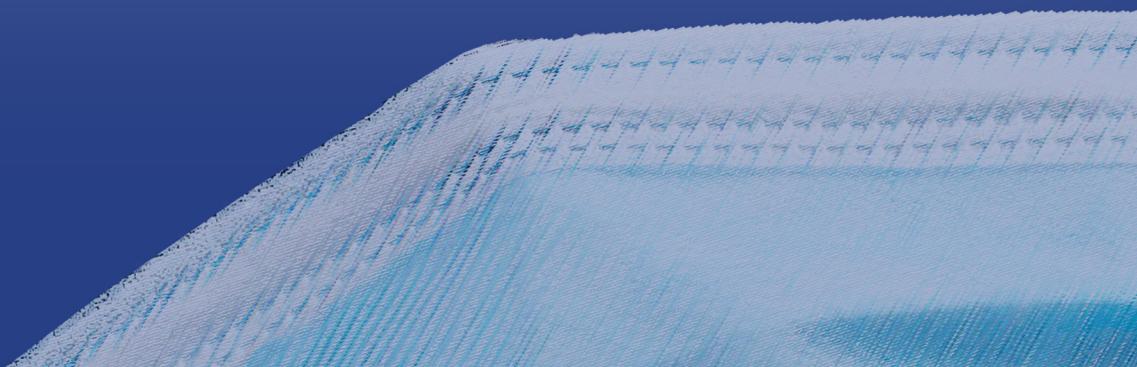
**Manu Prakash**—associate professor of bioengineering, received the Rotary Club’s Humanitarian STAR (Science, Technology, Aerospace, Robotics) Award for knowledge-sharing. His team invents novel technologies to aid health workers in resource-poor settings.

**David Relman**—the Thomas C. and Joan M. Merigan Professor, and professor of medicine and microbiology and immunology, received the Walsh McDermott Medal from the National Academy of Medicine. The medal is given to members of the academy who have provided distinguished service throughout their careers. Dr. Relman has served on 15 committees or forums within the academy since 2002. He is working with members of the academy to investigate mystery illnesses affecting U.S. State Department employees overseas, as well as SARS-CoV-2, the virus that causes COVID-19.

**Gary K. Steinberg**—the Bernard and Ronni Lacroute-William Randolph Hearst Professor in Neurosurgery and Neurosciences, received the 2020 Ralph G. Dacey, Jr. Medal for Outstanding Cerebrovascular Research from the American Association of Neurological Surgeons and the Congress of Neurological Surgeons. The award recognizes neurological surgeons who have made many contributions to researching and treating cerebrovascular disease.

**Bo Wang**—assistant professor of bioengineering, was included in *Science News*’ “SN 10: Scientists to Watch” for 2020. His research focuses on tissue regeneration, adaptation, and evolution. The *Science News* list includes scientists under 40 who have made significant contributions in their fields.

# MEDIA COVERAGE



# MEDIA COVERAGE

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Included in the appendix are the following articles referenced in the 2020-2021 Highlights section.

**“The Dickson Prize in Science 2020 Recipient:  
Lucy Shapiro”**

Carnegie Mellon University  
January 27, 2021

**“Defect in Pancreas Alpha Cells Linked to  
Diabetes, Stanford Medicine Study Shows”**

Stanford Medicine News Center  
June 24, 2020

**“Development of Rapid At-Home Testing for  
COVID-19”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Tracking COVID-19 with Wearables”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Pathogens Suppress Immune Response with  
Molecule, a Possible Drug Target, Stanford  
Researchers Find”**

Stanford Medicine News Center  
July 14, 2020

**“Evaluating the Potency and Durability of Slow-  
Delivery SARS-CoV-2 Vaccines via a Hydrogel”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Clinical Trial of Remdesivir in Hospitalized  
Adults with COVID-19”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Defining the Therapeutic Potential of Host-  
Targeted Approaches for Combating COVID-19”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Developing Proofs-of-Concept for Two Novel  
COVID-19 Drugs to Begin Clinical Trials”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Crowdsourcing Solutions to Better Understand  
Molecular Structures of SARS-CoV-2”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Determining Pathogenicity of Various Variants  
and Strains of COVID-19”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

**“Comparing Pathology and Pathogenesis of  
COVID-19 Infections in Humans and Animal  
Models”**

Stanford Medicine COVID-19 Research  
<https://med.stanford.edu/covid19/research.html>

## **CREDITS**

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