Stanford | Beckman Center
for Molecular and Genetic Medicine

2023 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

Dear Friends and Trustees,

Over the past year, the Arnold and Mabel Beckman Center for Molecular and Genetic Medicine has rapidly emerged from the pandemic created by the SARS-CoV-2 virus. Among our accomplishments: we recruited several outstanding new faculty members to the center's departments, and we instituted new and expanded educational programs that promote discovery and innovation in the basic sciences. In addition, the Beckman Service Centers invested more than $2.8 million in new technologies that will greatly benefit researchers across the Stanford University campus, funded by a combination of outside grants and internal sources.

There is also some sad news to share. As you may know by now, Paul Berg, Ph.D., the founding director of the Beckman Center, died on February 15, 2023, at the age of 96. Dr. Berg's work with recombinant DNA won him the Nobel Prize in Chemistry in 1980, ushering in the field of genetic engineering and setting the stage for the construction of the Beckman Center at Stanford in 1989. Dr. Berg was also instrumental in establishing our partnership with the Arnold and Mabel Beckman Foundation, which continues to underpin all that has been

accomplished since the center opened. As founding chair of the Department of Developmental Biology at the Beckman Center, and director of the center since 2004, I worked closely with Paul. Like so many others at Stanford, I will miss him. This report is dedicated to him.

The new faculty members we welcomed this past year joined the three basic sciences departments housed in the Beckman Center.

- Alex Gao, Ph.D., joined the Department of Biochemistry in the fall of 2022. Dr. Gao did his Ph.D. with Feng Zhang, Ph.D., at the Broad Institute of MIT and Harvard. As a new assistant professor, he will be focusing his attention on understanding bacterial innate immune systems.
- Lauren Goins, Ph.D., joined the Department of Developmental Biology in January 2023. She did her Ph.D. with Dyche Mullins, Ph.D., at the University of California, San Francisco. Dr. Goins will study how multipotent stem cell lineages can maintain balance among cell fates and an ability to respond to physiological challenges imposed by environmental stressors such as infection and disease.
- Ruth Huttenhain, Ph.D., joined the Department of Molecular and Cellular Physiology in the spring of 2023. She did her Ph.D. with Ruedi Aebersold, Ph.D., in systems biology at ETH Zurich, Switzerland. Her focus will be on exploiting quantitative proteomics to capture the spatiotemporal organization of GPCR-signaling networks combined with functional genomics to study their impact on physiology.
- Nicole Martinez, Ph.D., joined the departments of Developmental Biology and Chemical and Systems Biology in January 2022. Dr. Martinez did her Ph.D. with Kristen Lynch, Ph.D., at the University of Pennsylvania. Her laboratory will focus on how modifications to both RNA-processing enzymes and RNA itself can alter the proteins encoded by that RNA and, in turn, cause disease. The lab will study RNA modifications, splicing, 3′ end processing, and co-transcriptional processing.
- Tino Pleiner, Ph.D., joined the Department of Molecular and Cellular Physiology in the spring of 2023. Dr. Pleiner did his Ph.D. at the University of Göttingen in Germany, working with Dirk Görlich, Ph.D., at the Max Planck Institute for Multidisciplinary Sciences. Dr. Pleiner will combine mechanistic cell biology, biochemistry, and protein engineering to dissect the pathways and molecular machines that mature human membrane proteins to a fully functional state.
- Florentine Rutaganira, Ph.D., joined the departments of Biochemistry and Developmental Biology in the fall of 2022. Dr. Rutaganira did her Ph.D. with Kevan Shokat, Ph.D., at the University of California, San Francisco. Her lab is studying choanoflagellates, which form complex colonial organizations, to understand how assemblies of cells signal to one another.

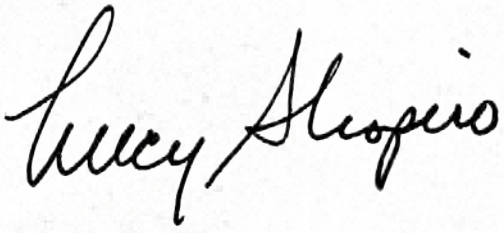
We are excited about adding these superb faculty members to Stanford's Beckman Center. The feature article in this report details the backgrounds and research interests of these outstanding new faculty members.

Providing ongoing educational opportunities to our faculty, staff, and students is a core tenet of the Beckman Center. Over the course of the pandemic, the Beckman Service Centers greatly expanded their educational programs, providing numerous opportunities to help people learn about the latest research technologies; staff members also contributed to graduate-level courses highlighting new experimental approaches and scientific breakthroughs.

In early 2023, for example, the Cell Sciences Imaging Facility (CSIF) sponsored a seminar focusing on advances in bioatomic force microscopy, and the Fluorescence Activated Cell Sorting (FACS) Facility sponsored a Get the FACS seminar that discussed controls and unmixing/spectral overlap in the cell-sorting process. Both service centers also received funding from the university's Community of Shared Research Platforms (c-ShARP) program to contribute hands-on lessons in imaging and cell-sorting technology to the Stanford Engineering course Analytical Methods in Biotechnology, which is intended for engineers who want to transition into biomedical research. The FACS Facility also received funding to provide formalized training for students in the Master of Science in Translational Research and Applied Medicine (M-TRAM) program, a new degree program that began in September 2022, with the aim of providing formalized training in translational medical sciences.

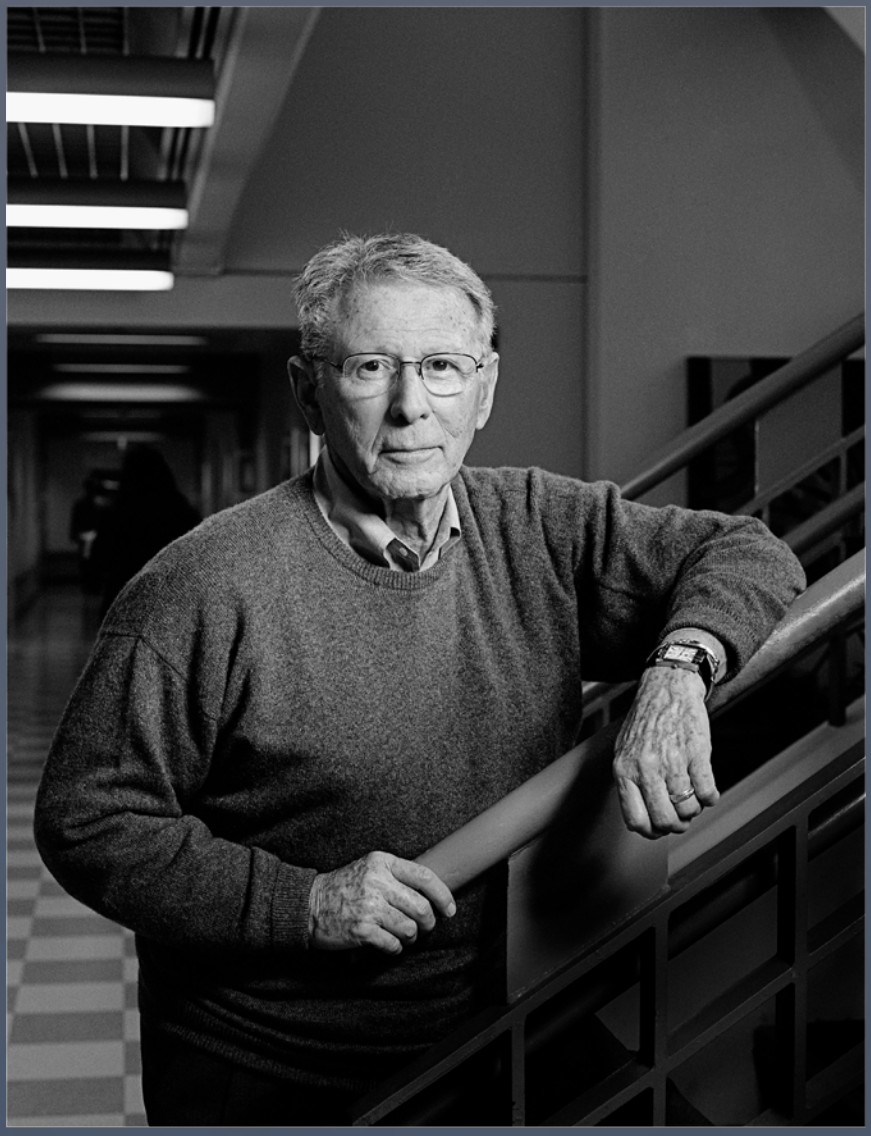
The array of new instruments and technologies acquired by the Beckman Service Centers over the past year is also remarkable. CSIF was awarded a Shared Instrumentation Grant from the National Institutes of Health for the purchase of a new Leica STELLARIS 8 DIVE microscope. Beckman Foundation funds were leveraged to provide cost-sharing for this important new instrument, a multi-photon and confocal device that is designed for deep, multi-parametric *in vivo* and *ex vivo* tissue and cell imaging. In addition, the School of Engineering provided funding, also leveraged with Beckman Foundation funds, for the purchase of several new instruments that will be housed in the CSIF satellite facility at the Shriram Center for Bioengineering and Chemical Engineering. The first of these is a Zeiss LSM 900 Airyscan 2 confocal microscope, which provides a platform that enables confocal immunofluorescence light microscopy in conjunction with atomic force microscopy. The second is a Zeiss Lattice Lightsheet 7 (LLS7) microscope; it generates an optical lattice to create an ultrathin light sheet that will facilitate live-cell imaging of a multitude of biological processes. The third is a Bruker TruLive3D light sheet microscope, which uses a Gaussian light sheet to illuminate and excite fluorescent samples. That platform is ideal for researchers who have larger samples than those that can be imaged with the LLS7.

All of these accomplishments make it clear that the Beckman Center has emerged from the pandemic period strong and with a great deal of momentum. This is largely due to our long-standing partnership with the Arnold and Mabel Beckman Foundation, which has allowed us to maintain our research programs as well as leverage foundation funds to invest in the latest state-of-the-art technologies that benefit researchers across the Stanford campus. We are honored to be hosting the foundation's annual board meeting in October 2023, and look forward to working closely with the foundation in the coming year.



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

DEDICATION



This report is dedicated to

PAUL BERG, PH.D.

Nobel Laureate

Founder of the Arnold and Mabel Beckman Center for Molecular and Genetic Medicine

June 30, 1926 - February 15, 2023

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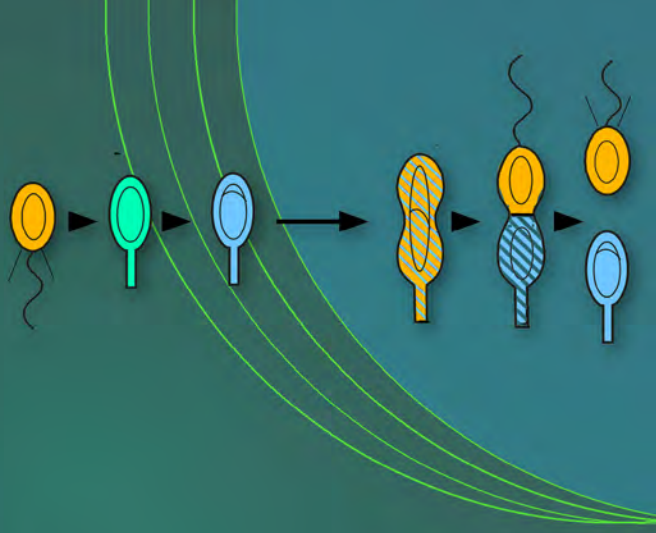
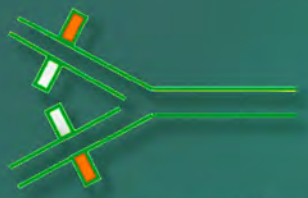
Fully Methylated

origin





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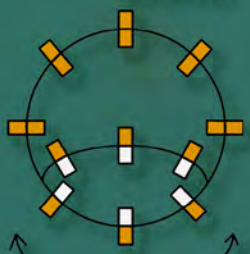

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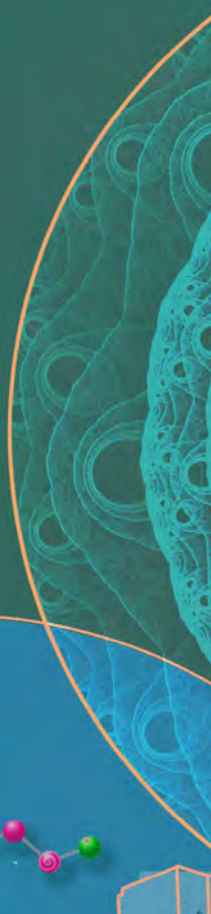
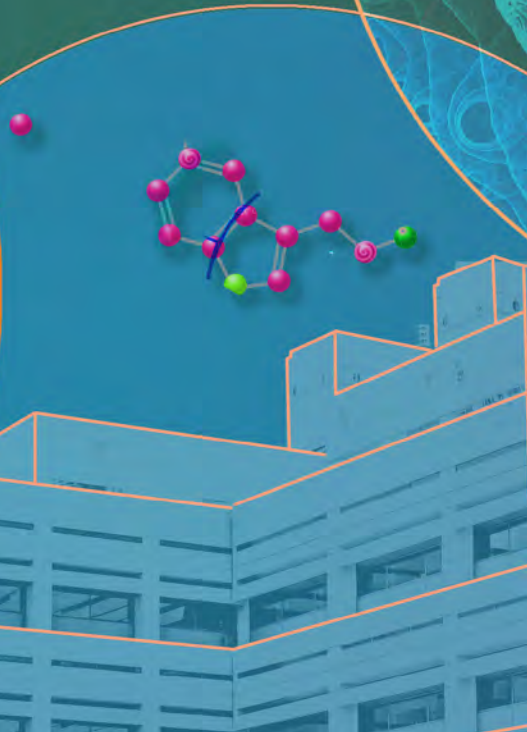




New Faculty Members Bring New Ideas to the Beckman Center

By Sarah C.P. Williams

> We are excited to welcome our extraordinary and diverse group of new faculty members, who are opening new areas of exploration and collaboration. They exemplify the interdisciplinary science and state-of-the-art technology that is the central focus of Stanford's Beckman Center.
>
> — Lucy Shapiro, Ph.D.

Scientists are the lifeblood of the Beckman Center for Molecular and Genetic Medicine. Their ideas, ingenuity, and curiosity fuel innovative research that helps to fulfill the mission of the Beckman Center: to promote discovery and innovation in the basic sciences, encourage interdisciplinary collaboration, and accelerate connections between the research bench and clinical medicine.

Over the past few months, we have been thrilled to welcome six outstanding new scientists to the Beckman Center community: Alex Gao, Ph.D., assistant professor of biochemistry; Lauren Goins, Ph.D., assistant professor of developmental biology; Ruth Huttenhain, Ph.D., assistant professor of molecular and cellular physiology; Nicole Martinez, Ph.D., assistant professor of developmental biology and of chemical and systems biology; Tino Pleiner, Ph.D., assistant professor of molecular and cellular physiology; and Florentine Rutaganira, Ph.D., assistant professor of biochemistry and developmental biology.

“We are excited to welcome our extraordinary and diverse group of new faculty members, who are opening new areas of exploration and collaboration,” says Lucy Shapiro, Ph.D., director of the Beckman Center. “They exemplify the interdisciplinary science and state-of-the-art technology that is the central focus of Stanford’s Beckman Center.”

Read on to learn more about our new colleagues and their fascinating areas of research.

FEATURE ARTICLE

> There are fascinating questions about why microbes have this amazing system. Where did it come from? What other systems might be out there? I think there's a lot of interesting molecular biology waiting to be discovered.
>
> — Alex Gao, Ph.D.

Alex Gao, Ph.D.

Assistant Professor of Biochemistry

If you were to count all the genes that exist on our planet, only a minuscule percentage would come from humans. Researchers have estimated that just across the species of bacteria that live in the human gut, there may be twenty million genes—about 1,000 times the number of genes humans have.

Alex Gao, Ph.D., a new assistant professor of biochemistry, thinks these millions of microbial genes—from the human microbiome, as well as other sources—likely hold secrets to developing new drugs and research tools.

"An enormous number of genes exist in nature, particularly in microbes," says Dr. Gao, who launched his Beckman Center lab in December 2022. "It's only in recent years that we've started to tap into the bulk of this diversity, and I think there's a lot of interesting molecular biology waiting to be discovered."

Dr. Gao grew up in a small town in Idaho, fascinated by math and science as a child. He found both beauty and mystery in the intricacies of biology and chemistry, and came to Stanford University as an undergraduate chemistry major. As part of the Bio-X Undergraduate Summer Research Program, he studied new methods for imaging proteins using fluorescent labels that were inspired by molecules found in fireflies—perhaps the first hint that Dr. Gao would devote his career to seeking out biological tools from nonhuman organisms.

But Dr. Gao also had other interests; he stayed at Stanford after his undergraduate degree to earn a co-terminal master's in electrical engineering.

"It was a complementary skill set that I wanted to learn," says Dr. Gao. "It helped expand my knowledge base in new directions, and I'm glad I had the opportunity to do that."

Dr. Gao then began to combine his engineering and life sciences skills in the Department of Biological Engineering at MIT. There, he pursued his graduate degree in the lab of Feng Zhang, Ph.D. The lab focused on the CRISPR/Cas gene editing system that researchers had co-opted from bacteria. In bacteria, the system helps fight off viruses by cutting up their genetic material; scientists had learned how to use this to specifically edit genes in the lab.

"There are fascinating questions about why microbes have this amazing system to begin with," says Dr. Gao. "Where did it come from? What other systems might be out there? I started to really look at the basic microbiology."

In 2015, Dr. Gao and his colleagues reported ways to improve the Cas9 enzyme often used in gene editing, work that was published in *Science*. Over the next few years, Dr. Gao pondered ways to systematically discover and harness genes from microbes, while continuing to develop CRISPR genome editing tools, including Cas12a. The latter part of his time at MIT—including time spent as a Junior Fellow at Harvard University, after earning his Ph.D.—was then devoted to finding new interactions between bacteria and the viruses that infect them, called bacteriophages.

"The majority of bacteria actually don't have CRISPR, so the thought was that they must have other interesting ways to defend themselves against viruses," he says.

In a 2020 *Science* paper, Dr. Gao reported 29 new types of genes important for protecting bacteria from viruses. He went on to reveal the details of how some of these genes play a role in defense. In a 2022 *Science* paper, he showed how bacteria recognize a unique section of the bacteriophage structure by wrapping around the virus; he likens it to a glove fitting onto a hand. That study was the first to reveal this kind of direct recognition of viral proteins by prokaryotes; most known bacterial defense systems detect viral DNA or RNA.

When it came time to start his own lab, returning to Stanford was an easy decision, Dr. Gao says. "I loved being here as a student and was really thrilled at the opportunity to come back."

The goal of his lab, he says, is to harness the genetic diversity of Earth's microbiome. That means developing new ways to screen countless species of bacteria and viruses for genes that could be useful. Already, bacteria have been the source of biological tools, such as restriction enzymes, recombinant DNA, and CRISPR gene editing. Dr. Gao thinks that's only the beginning.

"I'd like to understand more about the really interesting genes that are hidden out there in all these weird microbes," says Dr. Gao. "I'm excited by the potential to develop new technologies that let us study and manipulate human cells in new and better ways."

Dr. Gao's lab has deep expertise in genetics, bioinformatics, and biochemistry, all helpful in designing experiments that can pull useful information from massive sets of bacterial genomes. He thinks that with the help of emerging data mining techniques, the ability to comprehensively sequence and understand all bacterial genes will be within reach in the future. And he wants to be a part of that.

If you can't find Dr. Gao in his lab, you might follow the sounds of music; Dr. Gao is an accomplished piano player and has already drawn attention playing in White Memorial Plaza. "It's very relaxing," he says.

> It was my first taste of live microscopy and I just completely fell in love with it. I've tried lots of other things, but I always come back to microscopy.
>
> — Lauren Goins, Ph.D.

Lauren Goins, Ph.D.

Assistant Professor of Developmental Biology

When developmental biologist Lauren Goins, Ph.D., gives a seminar about her research, she often shows a cartoon of two personified stem cells: a small babyish cell and an old, wise-looking cell. "How do I decide what I'm going to be when I grow up?" the smaller cell is asking the older one.

That question is the crux of Dr. Goins' research; she wants to know how blood stem cells chose their fates—a complex process that is mediated by both their genetic identity and external molecular factors. Her research has implications for understanding how to treat infections, cancer, and other diseases.

Like the stem cells she studies, Dr. Goins' scientific career has been shaped by an amalgam of influences. She grew up in New Orleans in a struggling family. In elementary school, her father died from liver cancer; not long after, her mother became disabled with multiple chronic diseases.

"Seeing my parents face these medical predicaments really drove my passion for research and science," says Dr. Goins. "I realized, even as an elementary school student, that doctors often didn't have the tools to help people, because the research wasn't there. I wanted to become someone that helped change this."

Dr. Goins attended summer research programs in high school, excelled in her classes, and secured a spot studying biochemistry at Harvard University. The summer after her freshman year, she participated in the Stanford Summer Research Program in the Biomedical Sciences, working in the lab of Julie Theriot, Ph.D., then at the Beckman Center. Dr. Theriot was studying actin, a dynamic molecule with both structural and functional roles in cells. That summer, Dr. Goins helped image how actin in fish skin cells moved around.

"It was my first taste of live microscopy and I just completely fell in love with it," recalls Dr. Goins. "I've tried lots of other things, but I always come back to microscopy. For me, seeing something happen is believing."

During the rest of her undergraduate education, Dr. Goins took advantage of every chance to both study science and see the world—she spent summers in labs in Italy and Ireland. Back at Harvard, she worked in the lab of Ruth Ruprecht, M.D., Ph.D., studying how HIV mutations increased the ability of the virus to replicate, and working toward an HIV vaccine.

When it came time to pick a graduate school, though, Dr. Goins says she wanted to focus on what she'd first discovered at Stanford: the actin cytoskeleton. She pursued her Ph.D. at the University of California, San Francisco, working with Dyche Mullins, Ph.D., to study tropomyosin, a protein that binds to actin to regulate its organization.

"Actin is like a brick that is this simple, fundamental unit, but can be used in all kinds of elaborate structures and buildings," says Dr. Goins. "Tropomyosin helps dictate those designs."

Dr. Goins turned to isolated fruit fly cells—which have fewer kinds of tropomyosin than do human cells—to understand the details of the molecule. She discovered some surprises about how tropomyosin variants moved through cells and helped cells keep their shape. Certain mutations in tropomyosin, she found, made blebs start bubbling off the sides of cells. At the same time, Dr. Goins discovered how supportive and fun the fruit fly research community was; she knew she wanted to continue working with the model organism.

As a postdoctoral fellow in the lab of Utpal Banerjee, Ph.D., at the University of California, Los Angeles, Dr. Goins aimed to perfect live imaging of fruit fly internal organs. The lab studied how organs work together to coordinate development, immune and stress responses, and cancer. Following organs over time in living fruit flies was critical to this research. Dr. Goins had never seen a living fruit fly before, she says (she'd worked only with isolated cells in graduate school), but leapt at the opportunity to work on microscopy methods.

"People had tried so many different ways of making this kind of microscopy work, but I came up with something different," she says. "It worked incredibly quickly; within a few weeks or months of joining the lab, we were seeing things."

Dr. Goins' method for seeing inside fruit fly larva let her track stem cells in real time during development. That led to another observation: blood stem cells have a never-before-described intermediate progenitor state in between their immature and mature forms.

Now, her lab at Stanford is aiming to better understand that intermediate state. She wants to know how the balance of different intermediate stem cells dictates their eventual fate as mature blood and immune cells. Those intermediate stem cells, she hypothesizes, also could play a role in some blood cancers, like acute myeloid leukemia.

Dr. Goins' expertise in visualizing actin—which plays roles in the physical separation of cells into mother and daughter cells—will also help her probe the asymmetrical division of stem cells and how that division might impact the cells' mature states.

While her research asks basic questions, Dr. Goins says it also fulfills her desire to contribute to medical advances.

"I think with fruit flies, we can really get at the basic mechanisms of these cellular processes," she says. "Once we know what genes are involved, we can delve into how those players are implicated in disease."

> My hope is that using our quantitative proteomics approach, we can understand better what is happening inside a cell. If we understand that biology, we can use it to guide drug design.
>
> — Ruth Huttenhain, Ph.D.

Ruth Huttenhain, Ph.D.

Assistant Professor of Molecular and Cellular Physiology

The first time Ruth Huttenhain, Ph.D., ran a mass spectrometry experiment, she was hooked. At the time, she was a student in her native Germany but had flown to the United States for a short research experience at the National Institutes of Health (NIH). She was studying variants of hemoglobin—the protein in red blood cells that carries oxygen. At the NIH, she learned how mass spectrometry could differentiate these variants and help shed light on how they were modified in people with sickle cell anemia.

"I got really fascinated with the capability of the technology," recalls Dr. Huttenhain, who joined the Department of Molecular and Cellular Physiology this spring. "I realized this was a rapidly growing field and that mass spec was incredibly more powerful than most people gave it credit for."

Today, her passion for mass spectrometry hasn't faded. In her new lab, she is using the technology—coupled with other proteomics methods—to better understand a class of molecules known as G-protein-coupled receptors (GPCRs). Those receptors are the biggest class of targets for human drugs, but questions abound on how to better target them to treat conditions as wide-ranging as pain, high blood pressure, obesity, depression, cancer, and Alzheimer's disease.

"My hope is that using our quantitative proteomics approach, we can understand better what is actually happening inside a cell when these receptors become activated," says Dr. Huttenhain. "If we understand that biology, we can use it to guide drug design in the future."

The fact that her mass spectrometry work has steered her toward drug targets, she says, brings her career full circle. As an undergraduate at the University of Bonn, Dr. Huttenhain studied to be a pharmacist; through an internship program she began working in a pharmacy.

But when an advisor mentioned the possibility of studying abroad while receiving a master's degree, Dr. Huttenhain jumped at the opportunity—leading her to the NIH and mass spec. Despite her interest in pharmacology, she decided she wanted to pursue more basic research.

Back in Europe, Dr. Huttenhain began a Ph.D. program at ETH Zurich, where she joined the lab of Ruedi Aebersold, Ph.D., one of the world leaders in proteomics technology development. There—as a graduate student and later as a postdoctoral research fellow—she worked on improving methods and workflows that researchers were using to identify cancer-related proteins.

"There had been an inherent problem with the consistency of quantitative proteomics," she says. "Data was not reproducible and consistent."

While a sample can contain hundreds of proteins, Dr. Huttenhain developed a way to home in on the most important proteins that a researcher is interested in, giving them high-quality data on that smaller set. She used the approach to screen blood samples from ovarian cancer patients and identify proteins that could be used to stratify patients by the severity of their disease. At the end of that project, though, Dr. Huttenhain says she wanted to know more about the biology of the biomarkers and how they functioned in cells.

"I got really interested in how proteins in large networks are working together inside cells and how we can use proteomics technologies to understand dynamic changes in these networks," she says.

That drive led her to the systems biology lab of Nevan Krogan, Ph.D., at the University of California, San Francisco (UCSF). In Dr. Krogan's lab, Dr. Huttenhain started out studying how HIV rewires the networks of proteins inside human immune cells. The research quickly immersed her in the new-to-her areas of virology and cell biology. While fine-tuning a method called proximity labeling, which lets researchers capture information about very short-lived protein interactions, a fellow postdoc mentioned to Dr. Huttenhain how useful the method could be to understand GPCRs.

"The signaling with GPCRs happens really fast, so capturing all of these events had been very difficult," explains Dr. Huttenhain. "With the proximity labeling I'd developed, we could for the first time track this very transient interaction."

Proximity labeling tags biomolecules in the vicinity of a specific "bait" protein, such as a GPCR. The tagged molecules can then be characterized using mass spectrometry.

"You label everything that's close by, like spray painting," says Dr. Huttenhain.

For GPCRs, Dr. Huttenhain used proximity labeling to discover new binding partners and piece together what happens when the receptors are activated. She gained enough new information that she and her colleagues could develop computational tools that let them predict the trajectory of a GPCR moving through a cell.

Dr. Huttenhain remained at UCSF as an adjunct professor to help lead a group of researchers in the Krogan lab, applying proteomics to understand how genetic mutations associated with autism can change the function of GPCRs.

More recently, Dr. Huttenhain is focusing her attention on a different GPCR, called the μ-opioid receptor (MOR). The drug morphine is known to bind MOR, making it incredibly important in drug development. If researchers can separate some of the side effects from the pain-killing properties of morphine, they could develop a safer opioid.

While she opens her new lab, Dr. Huttenhain is also staying passionate about mentoring younger scientists. She is the co-chair of the Early Career Researcher Committee for the Human Proteome Organization, and has helped organize efforts to increase the visibility of early career researchers in the field and pave the way for improving diversity, equity, and inclusion within proteomics.

"My science is important, but it's also important to work on things beyond my own science," she says. "This lets me have an even bigger impact."

> There's a lot we don't know about all the functions that RNA has, and all these points of regulation. There are a lot of exciting opportunities in this field right now.
>
> — Nicole Martinez, Ph.D.

Nicole Martinez, Ph.D.

Assistant Professor of Developmental Biology and of Chemical and Systems Biology

Ribonucleic acid, or RNA, is one of the most versatile, dynamic molecules in living cells—but for years, it was far less studied than DNA. While DNA contains the full set of genes that make up all the functional parts of cells, RNA molecules are transcribed from that DNA, like photocopied pages of an instruction manual. Recently, however, researchers have discovered that RNA is far more adaptable and critical than previously appreciated. Modifications to RNA can have drastic impacts on cells.

Nicole Martinez, Ph.D., who joined the Beckman Center in 2022, is among those leading new research into the role of RNA regulation in biology and medicine.

"There's a lot we don't know about all the functions that RNA has and all these points of regulation," she says. "We want to know how RNA is regulated in different situations, what the mechanisms are behind that, and how they are dysregulated in disease states. There are a lot of exciting opportunities in this field right now."

The Martinez lab is interested in how modifications to both RNA-processing enzymes and RNA itself can alter the proteins encoded by that RNA and, in turn, cause disease. It's a level of cellular regulation that goes beyond the genes contained in a cell's DNA.

Dr. Martinez did not always want to be a researcher. Growing up in Puerto Rico, she liked science but didn't know anyone who actually worked as a scientist.

"I figured after I got my undergraduate degree in biotechnology, I'd work in manufacturing or maybe the pharmaceutical industry, because those were the only science-related careers I'd had any exposure to," she recalls.

But while an undergraduate at the University of Puerto Rico at Mayaguez, she attended a career fair and learned about a summer research program at the Broad Institute of MIT and Harvard. She was intrigued by the opportunity, applied, and got a spot at the Broad Institute that summer. There, she worked in a lab that was trying to find new molecules to manipulate histones—the spool-like proteins around which DNA is tightly wound inside cells.

"That was the first time I identified as a scientist," Dr. Martinez says. "I loved it. I was like 'I want to do this. I want to have my own research lab.'"

Later, while in grad school at the University of Pennsylvania, Dr. Martinez took a class in immunology. She found that topic fascinating and joined a lab studying how RNA molecules changed during immune responses. It was then that she fell in love with RNA.

"It's just a fascinating macromolecule in so many ways," she says. "It has a lot of diversity and it's regulated at so many points throughout its life cycle. RNA can serve as a carrier of genetic information like DNA, but it can also fold into complex three-dimensional structures like proteins can."

In the lab of Kristen W. Lynch, Ph.D., at UPenn, Dr. Martinez probed how RNA was cut and pasted into different combinations in immune cells—a process called alternative splicing. Dr. Martinez, Dr. Lynch, and their colleagues discovered that when T cells are activated in response to a pathogen, RNA is alternatively spliced. Dr. Martinez identified the signaling pathway that connects T cell activation to RNA splicing; that research was published in *Genes & Development* in 2015.

"Understanding the biology of this pathway can potentially lead to ways of modulating it to mediate immune responses," says Dr. Martinez.

When looking for a postdoctoral fellowship, Dr. Martinez focused on other labs studying RNA processing and modifications, and landed at Yale University, where she worked with Wendy V. Gilbert, Ph.D. Dr. Martinez's project at the Gilbert lab revolved around pseudouridine, a modified version of the RNA nucleoside uridine. Pseudouridine is the most common modified nucleoside in human cells, with pseudouridine found in about five percent of all RNA molecules. Dr. Martinez showed how the structure of RNA molecules helps determine whether a uridine is converted to a pseudouridine, work that was published in *Nature Chemical Biology* in 2019.

Today, Dr. Martinez is still interested in pseudouridine, but also RNA modifications more broadly. Some RNA-binding enzymes have been found to be present in higher levels in tumor cells; others are most highly expressed in developing neurons in the brain. In both instances, Dr. Martinez wants to know why—and how RNA modifications could be linked to cancer or developmental disorders.

"We really want to understand how RNA modifications serve as a fundamental mechanism to control gene expression and how this is dysregulated in disease," she says. "When we know that, we can develop new ways to intervene."

> There's so much creative freedom with [nanobodies]. You can control lots of things about where and when they travel in the body, or how long they're active. I think they're going to become increasingly powerful in the future.
>
> — Tino Pleiner, Ph.D.

Tino Pleiner, Ph.D.

Assistant Professor of of Molecular and Cellular Physiology

What do alpacas, antibodies, and Alzheimer's disease have in common? They all play roles in the research program of assistant professor Tino Pleiner, Ph.D., who launched his Stanford lab in June. Throughout his career, Dr. Pleiner has generated new ways to answer questions about basic cell biology using small, unique "nanobodies" generated by alpacas. Now, his new lab will apply those techniques to better understand the quality control mechanisms that cells use to ensure that membrane proteins are properly folded—a process that goes awry in normal aging as well as in diseases such as Alzheimer's, Parkinson's, and cancer.

"In aging and disease, this quality control machinery basically gets weaker and weaker, and misfolded proteins accumulate," says Dr. Pleiner. "What I think we can do with nanobodies is leverage signaling pathways to turn up protein degradation and boost folding capacity."

Dr. Pleiner was born in East Germany before the fall of the Berlin Wall; when Germany reunified early in his childhood, his parents encouraged him to take advantage of the new educational opportunities that arose. He had broad interests, enjoyed school, and liked spending time in nature wondering about the inner workings of plants and animals, so when it came time to choose his undergraduate focus at nearby Leipzig University, he picked biochemistry.

In graduate school at the University of Göttingen, Dr. Pleiner was drawn to the lab of Dirk Görlich, Ph.D., who was studying how proteins were transported between the cytosol and the nuclei of cells.

"Dirk had a magnetic personality and this research fascinated me," recalls Dr. Pleiner. "How some proteins got this very selective access to the genetic material of a cell seemed to be this big enigma."

But once he joined the lab, which was housed at the Max Planck Institute for Multidisciplinary Sciences, Dr. Pleiner got caught up in another project: alpaca antibodies. Max Planck researchers had recently begun working with tiny nanobodies produced by alpacas, and a herd of the animals grazed in the fields outside the institute. Dr. Görlich wondered whether the small antibodies could be used to tag some of the proteins the lab was interested in, and Dr. Pleiner agreed to investigate.

"Nanobodies are about ten times smaller than conventional antibodies, and that means they can bring a fluorescent tag much closer to a protein of interest," says Dr. Pleiner. "That translates to seeing more detail inside a cell, and pictures that are much less blurry."

Dr. Pleiner spent the next few years developing new technology to add multiple fluorescent tags to the nanobodies. Once a nanobody was generated by an alpaca, Dr. Pleiner and his colleagues could isolate its gene and produce it in bacteria—a cheaper and more efficient alternative to typical secondary antibody generation in donkeys, sheep, and goats. Those approaches were described in *eLife* in 2015 and the *Journal of Cell Biology* in 2017.

To boost his ability to apply these new techniques to molecular and cellular problems, Dr. Pleiner accepted a postdoctoral research fellowship at the California Institute of Technology (Caltech), in the lab of Rebecca Vorhees, Ph.D., a new faculty member studying protein quality control. Dr. Pleiner was Dr. Vorhees' first hire—a move, he says now, that set him up well to launch his own lab.

At Caltech, Dr. Pleiner delved into the question of how cells mediate the complex process of creating new membrane proteins—proteins whose structure is embedded into any of the many membranes within a cell.

"These proteins have to be woven into the lipid bilayer as they're produced," he says. "It's a highly orchestrated and regulated process that oftentimes fails."

Dr. Pleiner studied the human endoplasmic reticulum membrane protein complex (EMC), a massive, nine-subunit cluster of proteins that, as a membrane protein is being synthesized, helps insert the growing strand of amino acids into the ER membrane in the correct orientation. Dr. Pleiner used an antibody-based approach to purify the EMC from human cells and then determined its cryo-electron microscopy structure—which was published in *Science* in 2020.

As he recruits graduate students for his new lab—and scouts out alpacas in the Bay Area—Dr. Pleiner is now thinking about how to use antibodies to uncover more about how membrane proteins fold and what happens when they become misfolded. He is aiming to use nanobodies to not only label proteins, but to also manipulate them in other ways, such as activating or blocking a protein. Antibodies can flip molecular switches inside cells much faster than genetic alterations, Dr. Pleiner says.

"Antibodies can block critical protein-protein interactions," he explains. "Then we can assess the immediate functional consequences without any delays. We might like to block the access of membrane proteins to a particular biogenesis factor, for instance, and see what happens."

Dr. Pleiner also imagines using nanobodies attached to nuclear localization signals to force signals into the nuclei of cells where they activate genes. Ultimately, he even thinks nanobodies capable of manipulating protein folding could be used therapeutically—to treat age-related diseases in which misfolded proteins accumulate, or in tumors where boosting levels of misfolded proteins could trigger cell death. For now, though, nanobodies are a valuable research tool to pinpoint and describe the pathways involved in these processes.

"There's so much creative freedom with this tool," Dr. Pleiner says. "You can control lots of things about where and when they travel in the body, or how long they're active. I think they're going to become increasingly powerful in the future."

> My work is driven by curiosity. My hope is that by pursuing these questions that are very basic in nature, we'll be able to understand biology more broadly.
>
> — Florentine Rutaganira, Ph.D.

Florentine Rutaganira, Ph.D.

Assistant Professor of Biochemistry and of Developmental Biology

Smaller than a grain of pollen, choanoflagellates measure just a few microns across. Under the microscope, they look like tiny tadpoles, and beat a single flagellum to propel themselves through the water, where they munch on bacteria. At first glance, choanoflagellates don't appear to have much in common with us humans. But these minuscule eukaryotes are among the closest living, single-celled relatives of animals.

Assistant professor Florentine Rutaganira, Ph.D., who joined the departments of Biochemistry and Developmental Biology in September 2022, probes how choanoflagellates use tyrosine kinases to communicate and coordinate activity across a colony. That comparatively simple signaling, she says, can shed light on how cells organized into multicellular organisms in the first place—and even how some kinases contribute to human disease.

"My work is driven by curiosity," says Dr. Rutaganira. "But I think that fundamental science so often leads to unexpected discovery, and my hope is that by pursuing these questions that are very basic in nature, we'll be able to understand biology more broadly."

Dr. Rutaganira grew up in California's San Joaquin Valley in a scientific household; her mom was a lab manager and her dad a mathematician. She started college intending to become a pharmacist, but as an undergraduate dabbled in research—working in labs studying topics as diverse as peaches, heart failure, infectious disease, and organic chemistry synthesis. After shadowing a pharmacist at the University of California, Los Angeles, one summer, she decided she found research—especially chemistry research—more exciting than the hospital.

"I realized I was a lab rat at heart," Dr. Rutaganira says.

In grad school—at the University of California, San Francisco, Chemistry and Chemical Biology Graduate Program—Dr. Rutaganira joined the lab of Kevan M. Shokat, Ph.D., studying how to inhibit kinases that triggered human disease. For her thesis, she focused on blocking calcium-dependent protein kinase 1 (CDPK1), a kinase used by the pathogen *Toxoplasma gondii* to infect human cells and replicate. Some of the most serious symptoms of toxoplasmosis—most often spread to humans through undercooked meat—involve inflammation of the brain, so Dr. Rutaganira screened compounds not only for their ability to block CDPK1, and therefore inhibit *Toxoplasma gondii,* but also for their ability to cross the blood-brain barrier. The compound she discovered was described in the *Journal of Medicinal Chemistry* in 2017; it is now being moved toward clinical testing by a commercial venture.

As she studied the parasitic kinase, though, Dr. Rutaganira had begun to wonder about the wider diversity of kinases across different organisms.

"I was starting to think more broadly about what kinases look like in organisms outside animals," she recalls. "And I became especially interested in this question of whether a tyrosine kinase was relevant for the origin of multicellularity in animals."

For years, researchers had thought that only animals had tyrosine kinases. But in 2008, Nicole King, Ph.D., of the University of California, Berkeley, sequenced the first choanoflagellate genome and discovered evidence of kinases in the single-celled organisms. The finding suggested that the enzymes might be responsible for the coordination between cells, even in single-celled organisms.

Intrigued, Dr. Rutaganira joined Dr. King's lab. Funded by a Howard Hughes Medical Institute Hanna H. Gray fellowship that gave her support to follow her own interests, Dr. Rutaganira began screening through tyrosine kinase inhibitors that had been developed in animal models, to see which ones could also inhibit choanoflagellate tyrosine kinases.

"We thought that the chemistry of these inhibitors should transfer, but nobody had tried it before," explains Dr. Rutaganira. "So it was a lot of troubleshooting and setting up new assays and probing whether these inhibitors were impacting the choanoflagellate biology at all."

As she thought about continuing this work in her own lab, Dr. Rutaganira was drawn to Stanford's Beckman Center for its convergence of cell biology, biochemistry, and developmental biology, which all come into play for studying choanoflagellate signaling.

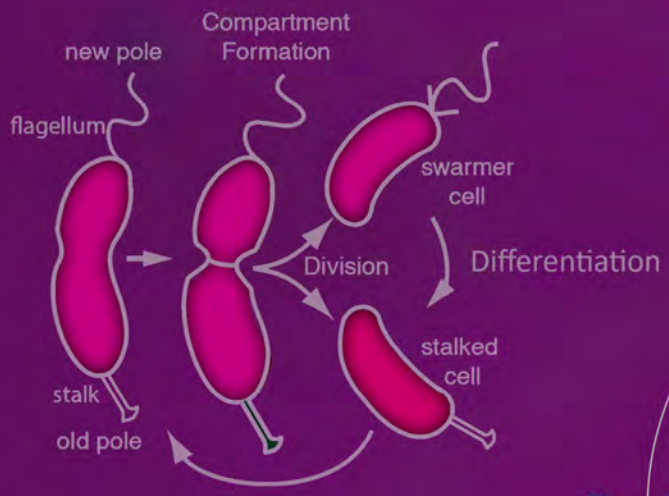
In her new lab at Stanford, Dr. Rutaganira is continuing to develop specific inhibitors for choanoflagellate kinases and use those as tools to probe how the kinases impact cell-cell communication, colony formation, and the way choanoflagellates coordinate processes like nutrient gathering or responding to nearby bacteria.

"Better understanding this symbiotic relationship between choanoflagellates and bacteria could shed light on the human gut microbiome," she says.

Because of this kind of potential link, Dr. Rutaganira is also excited about the Beckman Center's proximity to Stanford Medicine—the biology she uncovers about tyrosine kinases could have implications for human disease.

"I like knowing that there's a trajectory and support if my projects do lead to discoveries that impact human health," she says. "We know that these molecules we're studying are really important, because in animals when tyrosine kinases are misregulated, it leads to diseases, including cancer."

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 (HHMI) 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 22 HHMI 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 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.

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

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.

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

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

2022–2023 HIGHLIGHTS

The Arnold and Mabel Beckman Center for Molecular and Genetic Medicine enjoyed an exciting and productive year of scientific achievement. This year's highlights are as follows.

RESEARCH HIGHLIGHTS

Lucy Shapiro, Ph.D., Awarded the Linus Pauling Medal at Stanford University

Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research, professor of developmental biology, and director of the Beckman Center for Molecular and Genetic Medicine, was awarded the Linus Pauling Medal for Outstanding Contributions to Science and presented the 49th Annual Linus Pauling Lecture at Stanford on February 28, 2023. The award, established by the Department of Chemistry at Stanford, celebrates the life of Linus Pauling, Ph.D., who was a professor of chemistry at Stanford and who received the 1954 Nobel Prize in Chemistry "for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances."

The award is given to scientists whose research has contributed significantly to the field of chemistry and molecular biology. Titled "The Chemical Logic of Life," Dr. Shapiro's lecture elucidated her lifetime of work using *Caulobacter* as an experimental system for defining the bacterial cell as an integrated system, in which transcriptional circuitry is interwoven with the three-dimensional deployment of key regulatory and morphological proteins, and establishing systems biology as the logical basis of the chemistry of the living cell.

Beckman Center Faculty Member Develops Durable, Low-Cost COVID-19 Vaccine

Peter S. Kim, Ph.D., the Virginia and D.K. Ludwig Professor of Biochemistry, developed a low-cost, protein-based COVID-19 vaccine that offers immunity against known variants for at least one year. Researchers hope the vaccine, which can remain unrefrigerated for up to two weeks and may be especially beneficial for infants, will help alleviate the need for boosters, while improving herd immunity around the world. It could also be an alternative to mRNA vaccines, without drawbacks such as high expense and low-temperature storage requirements.

The vaccine is called Delta-C70-Ferritin-HexaPro with an aluminum adjuvant (DCFHP-alum). Unlike the bivalent Pfizer-BioNTech and Moderna mRNA vaccines, which contain the original Wuhan strain and the latest mutated strain, DCFHP-alum was developed using only the first strain of the spike protein. Such an approach dodges "variant chasing," in which researchers keep changing the booster recipe to keep pace with virus mutations.

In developing the DCFHP-alum vaccine, researchers made several changes to the spikes found on the surface of the coronavirus. First, they stabilized the spikes, making them more rigid, so they're not able to turn inside-out, which makes them a less reliable target for the immune system. Second, they fused the spikes with ferritin, a nanoparticle known to stimulate capture by immune dendritic cells. Third, the researchers deleted the last 70 amino acids of the spike closest to the membrane of the virus, to prevent antibody responses to this region, which are a distraction to the immune system and fail to neutralize the virus.

Clinical trials of the new vaccine will start within the next few months. "With this promising vaccine, if it passes clinical trials, we can target a large fraction of the world's unvaccinated population or those in need of a booster," Dr. Kim says.

Beckman Center Faculty Member Discovers Islet Cell Transplant Cures Mice of Diabetes

Seung K. Kim, M.D., Ph.D., professor of developmental biology and director of the Stanford Diabetes Research Center, has developed a technique that allows mice with diabetes to accept a transplant of unmatched islet cells and durably restore blood sugar control, without immunosuppression or graft-versus-host disease.

Prior to transplantation, the animals' immune systems were coaxed to accept the donated cells through a three-pronged process that could be easily replicated in humans, the researchers say. No immune-suppressing treatments were necessary after the transplant to prevent rejection of the foreign islet cells.

"We had a notion that we could get the bone marrow ready to accept the donor stem cells with less toxic, alternative approaches," Dr. Kim says. "We found we could reduce the radiation dose by 80 percent and replace broad-acting chemotherapy drugs with targeted antibodies. The animals rapidly gained back the weight they had lost due to the disease and were able to maintain normal blood glucose levels until the study ended after more than 100 days."

"This is exciting for many reasons," Dr. Kim says. "This approach could be applied to autoimmune diabetes, including Type 1 diabetes, and suggests that completely mismatched islet cells could be used for transplant. Beyond diabetes, it has important implications for solid organ transplants."

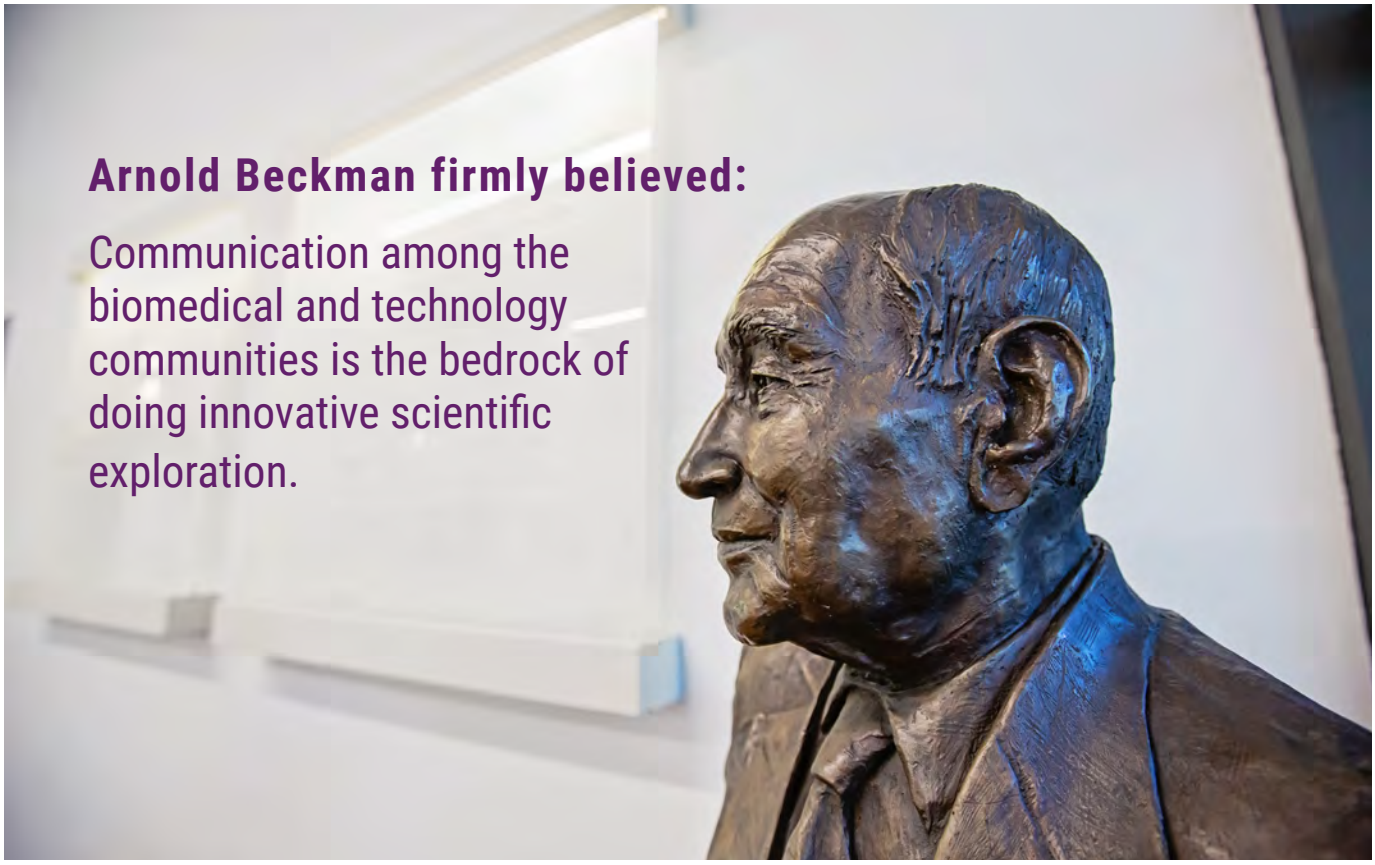
"If we are successful, we could see a future where we can treat people with diabetes at an early age to prevent or mitigate a lifetime of health problems," Dr. Kim says.

Beckman Center Faculty Member Discovers Intermittent Fasting Spurs Proliferation of Liver Cells in Mice

Cells in the adult liver were thought to divide rarely. But a study led by Roeland Nusse, Ph.D., the Reed-Hodgson Professor of Human Biology, the Virginia and Daniel K. Ludwig Professor of Cancer Research, and professor of developmental biology, in conjunction with Abby Sarkar, Ph.D., a former postdoctoral scholar, found intermittent fasting causes rapid cell division.

"One of the most defining characteristics of the adult liver has been that it is fairly stable in terms of cell turnover," Dr. Nusse says. "But we found the turnover of cells in the liver goes up dramatically after several periods of 24-hour fasting followed by refeeding. Interestingly, this type of diet mirrors the natural diet of wild animals and of early humans before the development of agriculture, when there were periods with scarce or absent food."

Further investigation identified two molecular pathways responsible for maintaining appropriate liver size in the fasted animals. One is a growth factor called fibroblast growth factor, or FGF, which is produced by the intestines and travels throughout the body; the other is a family of proteins called Wnts, which is crucial to embryonic



A bust of Arnold Beckman, who established the Beckman Foundation with his wife, Mabel Beckman, graces the halls of the Beckman Center for Molecular and Genetic Medicine.

development and the growth and maintenance of many tissues. The researchers discovered that "intermittent fasting or other changes in the food supply stimulate the production of FGF, which circulates to the liver. It wakens the liver cells from resting, then Wnt proteins give those cells near the central vein of the liver the signal to divide."

"I wouldn't recommend that people start intermittently fasting to improve their liver health," Dr. Nusse says. "But it's an exciting observation—it shows that the idea that the liver is a tissue that turns over slowly should be taken with a grain of salt."

Beckman Center Faculty Member Discovers Long-Elusive Structure of Key Cellular-Signaling Molecule

The structure of a critical cellular-signaling molecule has finally been discovered, in a study led by Christopher Garcia, Ph.D., the Younger Family Professor, professor of molecular and cellular physiology and of structural biology, and an HHMI investigator.

For more than 25 years, researchers around the world have been trying to find the structure of Janus kinases, or JAKs, a class of proteins that play a key role in cellular signaling and that have been tied to many cancers and autoimmune diseases.

The JAK protein that Dr. Garcia and his colleagues imaged has a type of mutation, called a VF mutation, that is associated with a number of myeloproliferative neoplasms. Genetic mutations can alter the structure of the protein, changing its interaction with cytokine receptors. Cytokines work by locking onto cytokine receptors on the surface of cells. "The cytokine forms a bridge between the receptors and the JAK proteins inside the cell," Dr. Garcia says.

"The VF mutation is a single amino acid change in this very large protein made up of over 1,000 amino acids," Dr. Garcia says. "But what we found is that this single amino acid change acts like a dab of glue, so that the JAK proteins and the cytokine receptor bind together

and create a positive signal inside the cell, even in the absence of a cytokine molecule."

When the VF mutation produces this molecular glue, "It's like a light switch that is always on, and the signaling can lead to the uncontrolled cell growth that characterizes cancer," Dr. Garcia says.

Now that they have a blueprint for the family of JAK proteins, Dr. Garcia says, "We'll be able to use it to look at the structures of various JAK mutations and design molecules to treat disease caused by specific mutations."

Beckman Center Faculty Member Makes Discovery Supporting the Concept of Progressive Evolution in Nature

David Kingsley, Ph.D., the Rudy J. and Daphne Donohue Munzer Professor, professor of developmental biology, and an HHMI investigator, in conjunction with graduate student Julia Wucherpfennig, conducted research that identified repeated changes in the regulation of a key developmental gene, called HOX, that increase the number and govern the length of the major defensive spines of a fish called the stickleback. New spine traits improve the fish's survival in the face of varying predators—flying in the face of a key assertion by anti-evolutionists that major changes will always leave animals unfit to survive in the wild.

Wucherpfennig collected and crossed sticklebacks from myriad North American lakes and streams, studying their genetic makeup and using CRISPR methods to confirm the effects of the HOXDB gene, a member of the family of HOX genes, on dorsal spines. She found a panel of changes in regions near the HOXDB gene, and showed they were associated with major anatomical changes that are evolving in the defensive armor of wild fish.

Wucherpfennig and her colleagues showed that repeated changes in the regulatory regions of the HOXDB gene are responsible for the recent evolution of new spine patterns in two different stickleback species they studied. They are now interested in learning whether similar changes are responsible for differences in fish that are even more distantly related.

"Scientists already know that changes in the regulation of this gene control the development of major body structures during development," says Dr. Kingsley. "What's new is that we conclusively show that mutations in this gene produce major changes in wild animals—new features that help fish thrive in natural environments. Our findings refute the common argument that these types of genes are so important, so fundamental, that animals with mutations in these regions wouldn't survive in nature—that if you play with master regulators, you're only going to make a hopeless monster."

PMGM Faculty Members and International Colleagues Determine That Tiny DNA Circles Are Key Drivers of Cancer

Howard Y. Chang, M.D., Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research, and professor of genetics, together with Paul Mischel, M.D., professor of pathology, and four international colleagues, have discovered that tiny circles of DNA, known as extrachromosomal DNA,

or ecDNA, often harbor cancer-associated genes called oncogenes. Because they can exist in large numbers in a cell, they can deliver a super-charged growth signal that can override a cell's natural programming. They also contain genes likely to dampen the immune system's response to a nascent cancer.

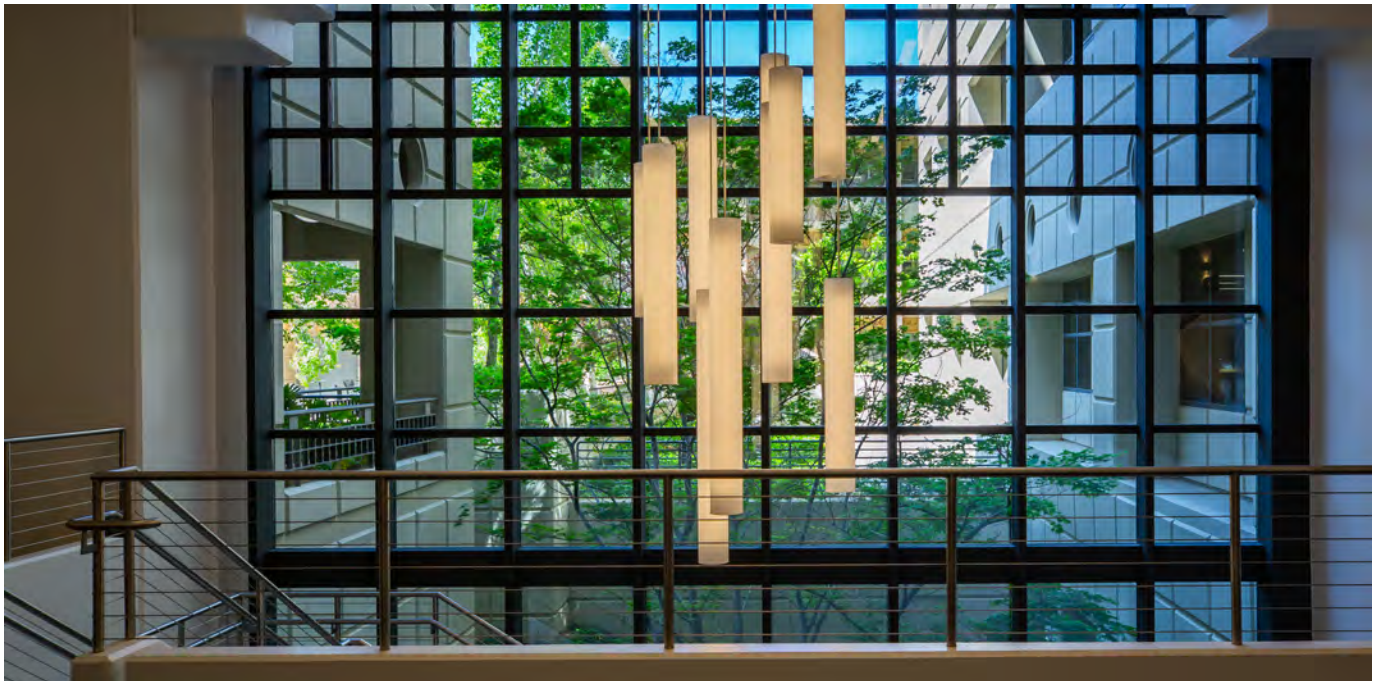
Previous research had suggested that the circles, which are widespread in human cancers but rarely found in healthy cells, primarily arise in advanced tumors as the abnormal cells increasingly botch the intricate steps required to copy their DNA before each cell division. But the new study shows that the circles can be found even in precancerous cells—and their presence jump-starts a cancerous transformation. Blocking their formation, or their effect on the cells that carry them, might stop cancers from developing, the researchers believe.

"People with ecDNA in their precancerous cells are 20 to 30 times more likely than others to develop cancer," Dr. Chang says. "This is a huge increase, and it means we really need to pay attention to this. Because we also found that some ecDNAs carry genes that affect the immune system, it suggests that they may also promote early immune escape."

"The conclusions from the study were remarkable," Dr. Mischel says. "We see that ecDNA can arise in these precancerous cells, and that if it is there, the patient is going to get cancer. We also saw the continuous formation of ecDNA as the cancer progresses, indicating that it is advantageous to cancer growth. Finally, we saw that the ecDNA can contain immune-modulatory genes, in addition to oncogenes."

PMGM Faculty Members Show How Cancer Gene Tricks Immune Cells

Cancer-associated genes called oncogenes are well known to stimulate cell growth and division—causing tumors to balloon and spread. But now, two researchers at Stanford, recent Nobel Prize winner Carolyn Bertozzi, Ph.D., the Anne T. and Robert M. Bass Professor in the School of Humanities and Sciences, the Baker Family Director of the Sarafan Chemistry, Engineering & Medicine for Human Health (ChEM-H) institute, and an HHMI investigator, and Dean Felsher, M.D., Ph.D., professor of medicine (oncology) and of pathology, have joined forces to find that one notorious oncogene, called Myc, also has a direct role in disguising growing cancers from the immune system.



Together, their labs discovered that Myc drives the production of a glycosyltransferase called ST6GalNAc4, which is necessary to make a sugar molecule called disialyl-T, which pops up in abundance on the surface of Myc-driven cancer cells. Disialyl-T binds to another molecule on the surface of macrophages, flipping these immune system cells from foe to friend.

The discovery links two seemingly unrelated previous observations: Cancer cells differ from healthy cells in the patterns of sugars on their surface, and the Myc oncogene somehow protects cancer cells from the immune system by increasing the production of specific proteins in the cells.

"It's a striking connection," says Dr. Bertozzi. "My lab had been trying to understand why cancer cells have altered patterns of sugars on their surfaces, and how these changes help the cells escape the immune system. Now we know that Myc regulates the production of proteins that make these sugar molecules that trick the immune system into ignoring the cancer cells."

"The way we see it, there are two next obvious directions of research," Dr. Bertozzi says. "First, we want to test whether other oncogenes are also affecting the sugar structures on the surface of cancer cells. Second, we want to see if we can inhibit ST6GalNAc4 to enhance the ability of macrophages to destroy the cancer cells."

SERVICE CENTER HIGHLIGHTS AND TECHNOLOGY UPGRADES

Cell Sciences Imaging Facility

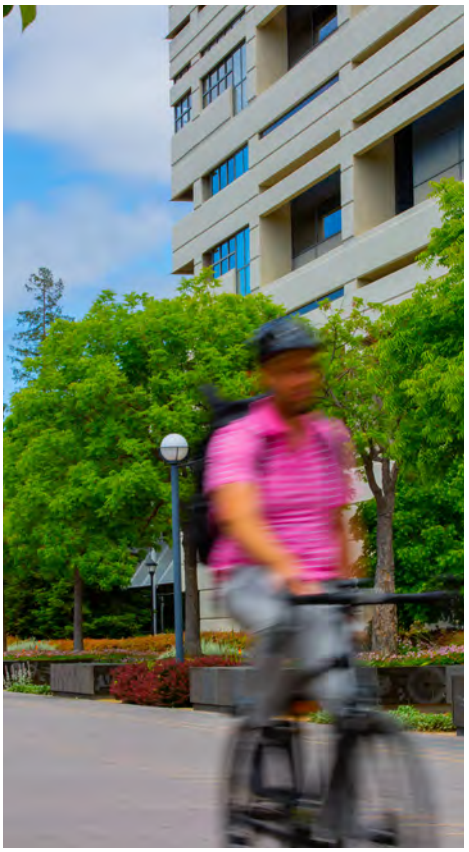
The Cell Sciences Imaging Facility (CSIF) received a combination of grant and internal funding, totaling $2,757,650, for the purchase of new cutting-edge imaging equipment that will greatly benefit researchers across the Stanford University campus. The new instruments are as follows:

- Leica STELLARIS 8 DIVE microscope—funded by a National Institutes of Health Shared Instrument Grant of $600,000, with additional cost-sharing of $252,294 leveraged from foundation funds. This microscope is a combined multi-photon and confocal, upright-configuration microscope that is designed for deep, multi-parametric *in vivo* and *ex vivo* tissue and cell imaging using both 1-photon (confocal) and 2-photon excitation for simultaneous and sequential imaging of a wide variety of expressed fluorescent proteins and endogenous signals.
- Zeiss LSM 900 Airyscan 2 confocal microscope—funded by the Stanford Department of Bioengineering and the Sarafan ChEM-H program in the amount of $405,204. This microscope is part of a combined light and atomic-force microscope platform. This platform enables

confocal immunofluorescence light microscopy (IFM) in conjunction with atomic force microscopy (AFM) for correlative IFM to AFM that is essential for advanced research in mechanobiology. Additionally, the Airyscan 2 component of the LSM 900 allows researchers to increase the resolution of the confocal light microscope beyond the diffraction limit, achieving resolutions of ~150nm.

- Zeiss Lattice Lightsheet 7 (LLS7) microscope—funded by the Department of Bioengineering and ChEM-H in the amount of $713,280 (with additional debt-financed cost-sharing of $200,000 leveraged from foundation funds). This microscope generates an optical lattice to create an ultrathin light sheet consisting of a parallel linear array of coherently interfering Bessel beams. This makes it particularly well suited for *in vivo* imaging of sub-cellular structures and molecules. Coupled with an analysis workstation for the data pipeline and a dedicated, high-speed transfer storage system, this equipment is compatible with utilization by multiple users and will facilitate live-cell imaging of a multitude of biological processes, such as genome folding, transcriptional regulation, protein interaction and aggregation, cell signaling, endocytosis, axon formation, and others.
- Bruker TruLive3D light sheet microscope—funded by the Department of Bioengineering and ChEM-H in the amount of $786,872. This microscope uses a Gaussian light sheet to illuminate and excite fluorescent samples. This light sheet microscope is optimized for larger samples than are imaged with the LLS7. For example, the TruLive3D microscope is excellent for imaging living, whole organisms and samples such as *C. elegans* and *Drosophila*. The TruLive3D has a dedicated analysis workstation and a networked data storage system.

In addition to these new technologies, the CSIF received $71,000 from the university's Community of Shared Advanced Research Platforms (c-ShARP) program, to support the teaching of courses in biological light microscopy and to add a microscopy component to the Analytical Methods in Biotechnology I course. Funding was also included for two new seminar series: Fundamentals of Biological Light Microscopy and Fundamentals of Biological Electron Microscopy. The CSIF also received $95,113 from c-ShARP to support the hire of a new bio-AFM support scientist, to develop an AFM educational program, and to train and support users on the scope.



Fluorescence Activated Cell Sorting Facility

The Fluorescence Activated Cell Sorting (FACS) Facility has been introducing engineering students to flow cytometry and sorting technologies in the Analytical Methods in Biotechnology I course. This past year, the university's c-ShARP program funded development of a follow-up course, Analytical Methods in Biotechnology II, which provided a set of lectures with hands-on flow cytometry and cell-sorting lab exercises. This provided students with the opportunity to go deeper than an overview of the technologies and get experiential learning by preparing samples, and then analyzing them and/or sorting their cells directly. Similarly, a flow cytometry analysis and a cell-sorting lab experience were provided to the inaugural class in the newly formed Master of Science in Translational Research and Applied Medicine (M-TRAM) program. These experiential learning programs provide students with an in-depth introduction to current technologies and serve as a launching point for their future research endeavors.

Protein and Nucleic Acid Facility

With the increased return of researchers to campus, the Protein and Nucleic Acid (PAN) Facility has been increasing its efforts to provide educational opportunities for different technologies. PAN staff, like those from CSIF and FACS, participated via lectures and hands-on experiments in the Analytical Methods in Biotechnology course, which helps engineering students with little or no background in genomics or proteomics to transition into research in the field of biomedicine. The lectures and hands-on experiments provided by PAN focused on SPR (Biacore) technology, which has increasingly emerged as a powerful tool for biophysical characterization of protein and small-molecule compound interactions. In the course, students characterized the binding affinity and specificity of an antibody to a target antigen.

Computer Services and Bioinformatics Facility

The Computational Services and Bioinformatics Facility (CSBF) received a School of Medicine Small Equipment Grant of $7,096, for the purchase of GPU processor boards to support the new multiplex imaging technology system installed in the CSIF.

Top to Bottom: Suraj Timilsina (FACS); Jessica Tran (PAN); Lee Kozar (CSBF); Ibanri Phanwar-Wood (CSIF).

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 from private sources made it possible to complete the center on time and under budget.

The Beckman Center houses three academic departments—Biochemistry, Developmental Biology, and Molecular and Cellular Physiology—as well as the Howard Hughes Medical Institute's local administrative offices, all dedicated to basic science research and technology development 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's 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 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 more than $5.2 million in user fees this year, continuing a level of service that sets the standard at Stanford University. 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 SEED GRANTS

In order to help initiate innovative new translational research projects, the Beckman Center conducts a highly competitive program in which two or more investigators, including basic scientists and clinical scientists, propose risky, but potentially high-payoff experiments in technology innovation. An ad hoc advisory committee evaluates 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

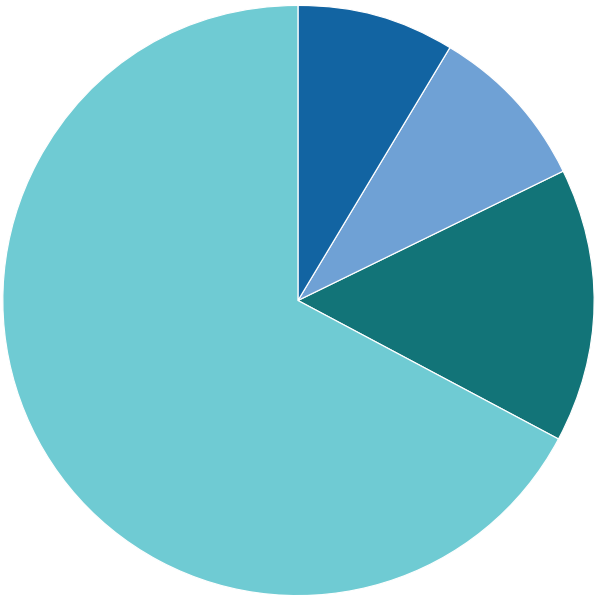
To foster the training of medical students in translational research, the center provides a stipend to selected students doing research in top-tier research labs with PMGM faculty. This is a competitive program, closely monitored by the Beckman Center.

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 sponsors a number of seminar series as well as the Beckman Symposium, which focuses on a critical area in scientific innovation. These events attract students, postdoctoral fellows, 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 Seed Grants, and technology resources. The pie chart below shows how the Beckman funds were disbursed this fiscal year.



9.0%
Research Education & Training

9.2%
Faculty Recruitment

15.3%
Technology Development Seed Grants

66.5%
Technology Resources

EXTERNAL REVIEW

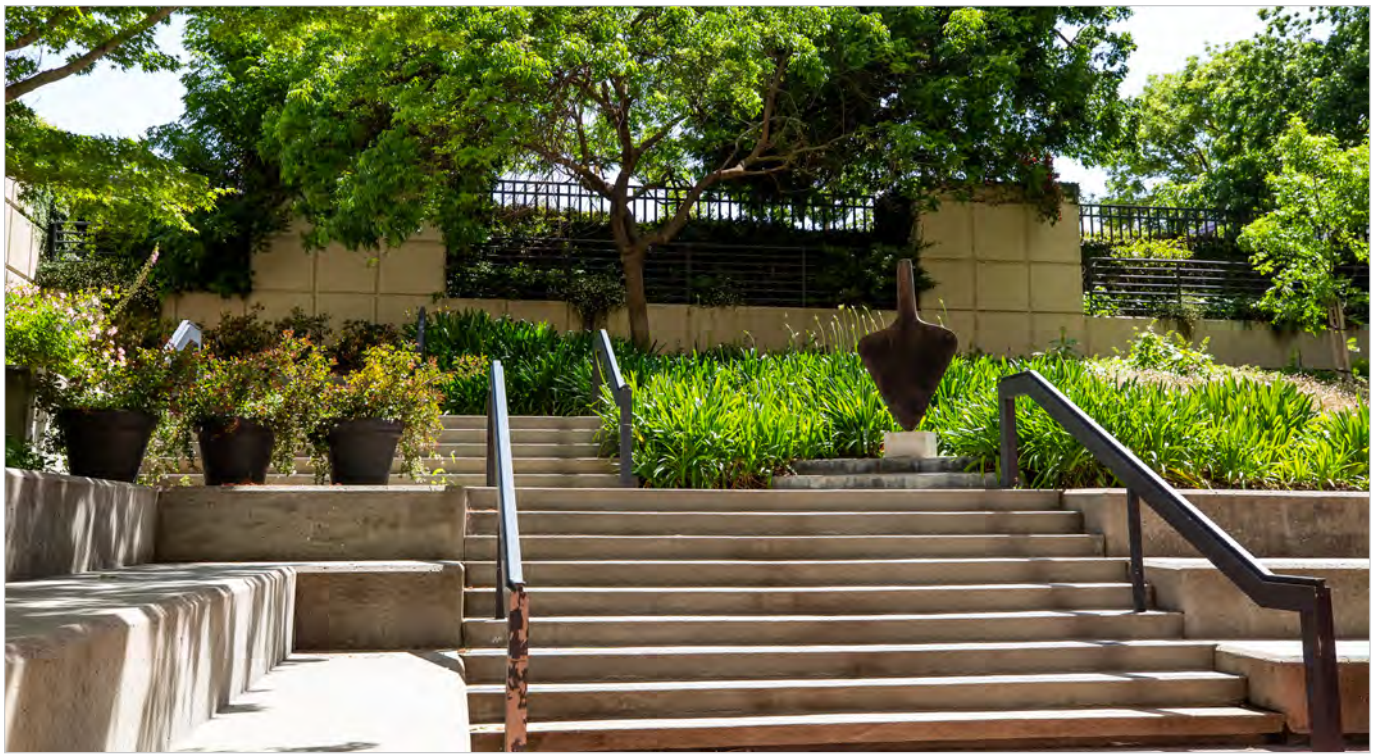
BECKMAN CENTER FIVE-YEAR REVIEW

The Beckman Center director has drafted a formal external review process for all center programs, which will take place every five years. The initial review will take place at the Beckman Center in spring 2024.

Six distinguished outside researchers—from the University of California, San Francisco, the University of California, Berkeley, and Harvard University—will be chosen to act as external reviewers. Reviewers will be presented with an overview of center research, including the contributions made to that research by each of the four Beckman Service Centers. Also reviewed will be the impact of the center's Technology Development Seed Grant program, educational activities, medical scholars training program, and symposiums. Reviewers will be invited to provide the director with feedback on center research operations, as well as suggestions for new programs or changes 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.

SERVICE CENTER OVERSIGHT COMMITTEES

The Beckman Center runs several technology resource programs—the Beckman Service Centers—that provide support for outstanding technological and scientific advances. The centers provide services in 1) state-of-the-art imaging technologies (Cell Sciences Imaging Facility); 2) protein and nucleic acid molecular analyses (Protein and Nucleic Acid Facility); 3) fluorescence activated cell sorter technologies (Fluorescence Activated Cell Sorting Facility); and 4) computer and biocomputational work (Computational Services and Bioinformatics Facility). All four service centers provide cutting-edge, high-tech resources on a fee-for-service basis. The demand for these services, as measured by the revenue generated as well as acknowledgments in peer-reviewed journals, is an important measure of their overall success and value to the scientific community at Stanford.



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

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

- Inform the service center directors about research tools and methods most needed by users of the facility
- Provide feedback to the director about the effectiveness of the services provided
- Assess the quality of those services
- Assess the timeliness of work done by service center staff
- Evaluate training provided to graduate students, postdocs, and others
- Assess the service center staff's input 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, appointed by the senior associate dean for research. The board's goals are to:

- Review and approve business plans for new service centers
- Invite service center directors 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
- Evaluate the overall demand for services in each facility
- Review the list of users for each facility and the dollar volume of activity per user, to determine the demand for services
- Assess the degree of duplication of services between service centers across campus
- Evaluate which technologies should be subsidized
- Determine the need for new services or service centers
- Evaluate whether certain services have outlived their usefulness, are readily available outside the university, or should be discontinued

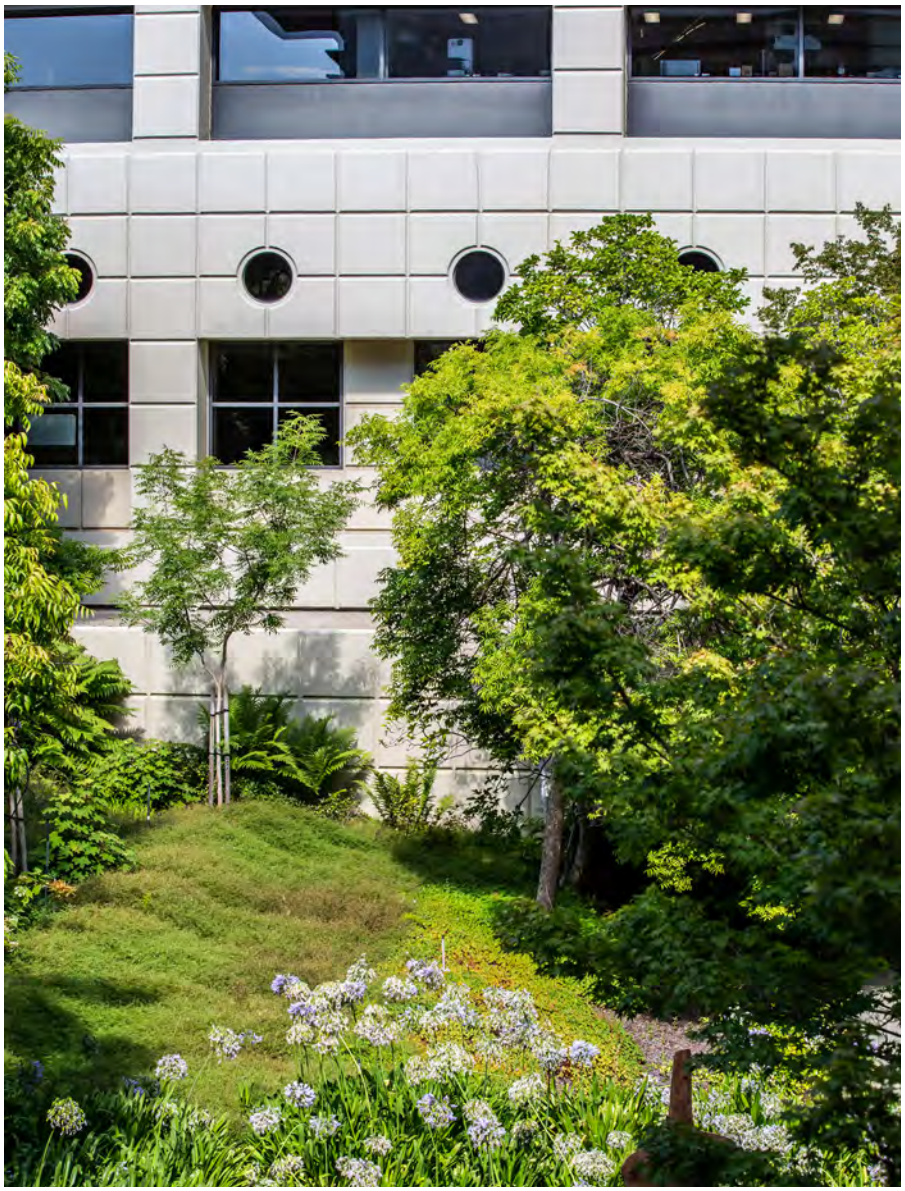
The board's recommendations are summarized and relayed to the Beckman Center director. This feedback allows the director to consider changes to the services provided.

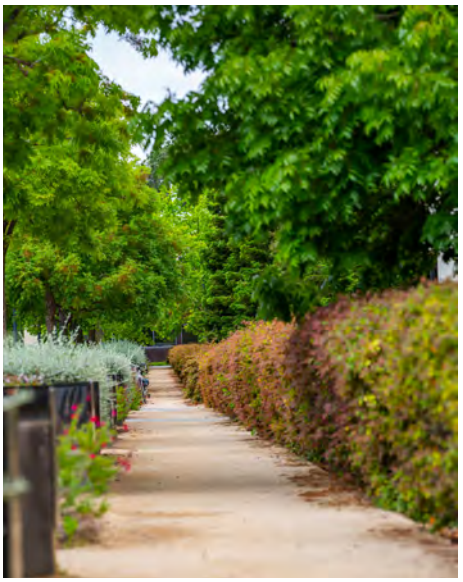
INTERNAL AUDIT

In addition, Stanford's Department of Audit, Compliance, and Privacy conducts financial audits of the facilities, and the Office of Research Administration oversees compliance with the university's cognizant agency, the Office of Naval Research. Additional review is provided by the ad hoc advisory committee that advises PMGM, the scientific cooperative established by the Beckman Center. Members of that committee review seed grant applications for innovative work in interdisciplinary technology development, aiming to ensure that awards are made equitably and based on merit. In addition, that committee advises the Beckman Center director on faculty recruitment and the need for new or revised programming.

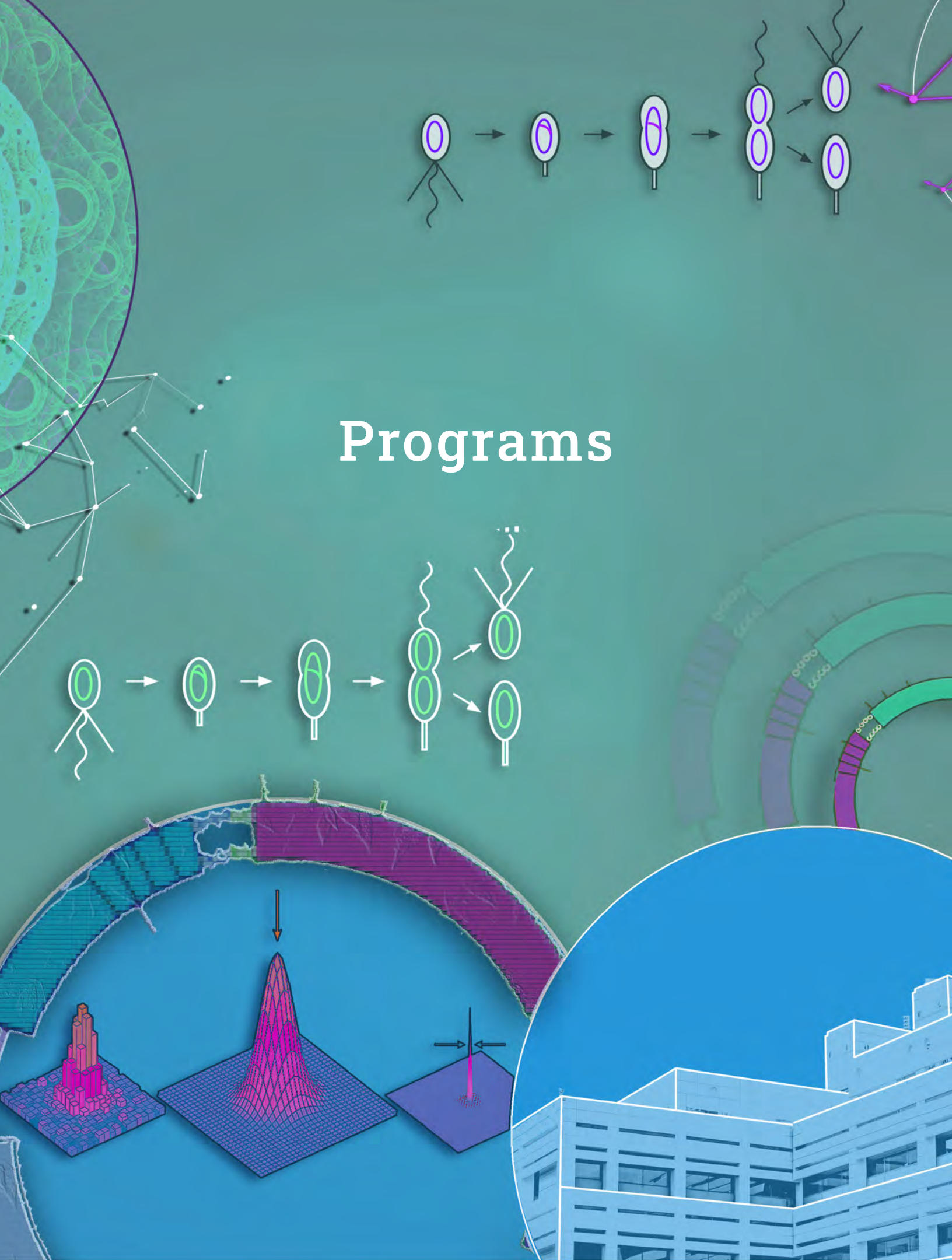
ENDOWMENT FUNDS

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 SEED 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 Seed Grants. The primary goal of this 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.

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

In November 2021, the Beckman Center awarded five new Technology Development Seed Grants that are geared toward supporting innovative research in the biomedical sciences. Each grant provides funding of $100,000 per year, for a two-year period. Updates from those projects follow.

A SYSTEMIC LIGHT SOURCE FOR OPTOGENETIC SCREENING OF ENTERIC NERVOUS SYSTEM FUNCTIONS

GUOSONG HONG, PH.D.
Department of Materials Science and Engineering

JULIA KALTSCHMIDT, PH.D.
Department of Neurosurgery

In this project, we aim to transform the conventional paradigm of *in vivo* light delivery with an internal systemic light source, thus enabling selective neural activation in the enteric nervous system (ENS) with region-specific illumination patterns in live mice.

In Aim 1, we aim to develop this internal systemic light source by leveraging the optically stimulated luminance (OSL) of intravenously injected nanotransducers at 470nm. In Aim 2, we aim to demonstrate noninvasive optogenetic modulation of opsin-expressing neurons in the mouse gastrointestinal (GI) tract with the internal systemic light source developed in Aim 1. This project combines the latest advances in materials science (OSL nanotransducers) and the neurobiology in the enteric nervous system (neuron-type specific activation in the gut to drive motility *in vivo*).

We have accomplished the following goals specified in our original proposal.

To accomplish the goals set for Aim 1 of the project, we have synthesized bright, 470nm emitting OSL nanotransducers using a biomineral-inspired suppressed dissolution approach, which was published in *Science Advances* and the *Journal of the American Chemical Society* (*Sci. Adv.* 2022, 8, eabo6743; *J. Am. Chem. Soc.* 2022, 144, 18406-18418). These nanotransducers have been found to have both OSL and mechanoluminescence properties, making them excitable to yield 470nm light emission under both 1064nm infrared light and focused ultrasound. We have also tested their repeated recharging and emission properties in an artificial circulatory system, as proposed in Aim 1 of the proposal. We are continuing the research specified in Aim 1 by quantifying the power density of 470nm emission in the gut under transdermal 1064nm illumination that does not cause excessive heating in the tissue.

To accomplish the goals set for Aim 2, we have determined the variability in position and accessibility of different gut regions, which will enable us to accurately and reliably stimulate these gut regions with transdermal illumination of 1064nm near-infrared lasers that produce local 470nm emission therein. In addition, we are in the process of performing retro-orbital injections of the optically stimulated luminescence nanoparticles (OSLNPs) into a mouse expressing channelrhodopsin in the gut, applying transdermal 1064nm illumination at specific regions, and performing immunohistochemistry for c-fos, a marker of neuronal activity. Once the efficacy of this approach is confirmed, we will file a patent application for using this intra-abdominal light source as a noninvasive GI pacemaker to potentially alleviate issues such as constipation or other GI dysfunctions in humans.

AN INTEGRATED MILLI-FLUIDIC SYSTEM FOR AUTOMATED TISSUE DISSOCIATION INTO SINGLE CELLS

JAMES D. BROOKS, M.D.
Department of Urology - Divisions

SINDY KAM-YAN TANG, PH.D.
Department of Mechanical Engineering

Next-generation sequencing and high-throughput single-cell assays have shown significant promise in gaining insight into the pathophysiology of cancers.

The first step to single-cell sequencing of solid tumors is the dissociation of tumor tissues into single-cell suspensions. Standard dissociation methods involving multiple manual steps are laborious, time consuming, and highly variable in cell recovery. The lengthy process can also introduce cellular stress and lead to cell death. Importantly, the recovered cells may be biased toward certain subtypes and fail to accurately mirror the tissue of origin.

The goal of this project is to address this challenge by developing an automated milli-fluidic system to dissociate tissues into single cells, consisting of multiple stages of mechanical cutting, enzymatic digestion, and sorting, operated in a closed-loop manner.

In the past year, we have focused on three main activities: 1) developing technologies for mechanical cutting of tissues; 2) developing technologies for improving the efficiency of enzymatic digestion; and 3) learning the conventional single-cell dissociation protocol using manual dissociation.

Optimize µDicer Blade Geometry and Process Flow

Our original plan was to fabricate µDicers out of silicon, using the Plasma Therm Versaline LL ICP Deep Silicon Etcher (PT-DSE) at the Stanford Nanofabrication Facility. However, the PT-DSE has been having technical issues and was down for maintenance for a significant amount of time. Therefore, while waiting for the PT-DSE to be repaired, we explored alternative fabrication methods using stainless steel, which is a common material for medical devices.

We designed and tested a grater-like device, “µGrater,” in stainless steel to have slicing holes smaller than 500 microns. The grater is made from chemically etched stainless steel sheets, which results in sharp edges. We tested the µGraters using fresh mouse tumor tissue loaded into a carrier barrel, using a plunger to manually press the tissue down in the barrel while passing the sample over the grater blades. The tissue started out at 6mm in diameter and was dissociated into pieces smaller than 500µm. We found the process to be effective and efficient; the 6mm sample was dissociated in approximately 15 seconds of grating. We are continuing to test viability of the tissue after grating, to ensure the process does not have a harsh effect on the cells. We are currently developing the device further to improve blade shape, as well as automate the grating to control for translation speed and pressure on the tissue while grating.

Develop a Microfluidic Enzyme Digestion Device ("μDigester")

After the mechanical tissue dissociation, the remaining cell clumps are chemically dissociated into single cells by the enzyme liberase. We introduced a herringbone flow mixer to promote the reaction between the enzyme and the cell clumps. The key challenge in the chemical dissociator is that the flow rate mismatch between the tissue sample and enzyme results in backflow into the enzyme inlet. To overcome this, we proposed to introduce a check valve at the junction between the channels containing the tissue sample and the enzyme solution, to minimize the backflow. Furthermore, we performed numerical and experimental investigations to optimize our microfluidic check valve design.

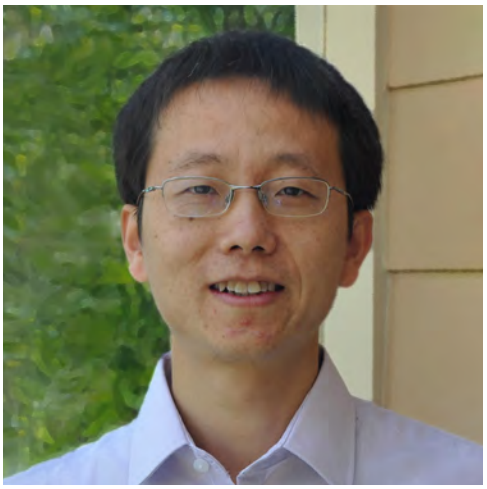
The preliminary results with swine kidney tissue were promising. The mechanically dissociated kidney cell clumps were introduced at the inlet of the mixer, and a number of single cells were found at the outlet, indicating the chemical dissociation by the enzyme liberase. Currently, we are further optimizing the design and experimental parameters for the herringbone mixer and the check valve. Finally, we will integrate the chemical dissociation module with the upstream mechanical module.

Learn the Manual Tissue Dissociation Protocol

Trainees from the Tang lab in the Department of Mechanical Engineering have learned the conventional tissue dissociation protocol from researchers in the Brooks lab. This ensures that the trainees are familiar with the critical steps and pain points in the conventional process flow that they aim to improve. This collaboration highlights the interdisciplinary nature of this project.

BREAKING THE BARRIERS TO DISCOVERING BIOLOGICS AGAINST MEMBRANE PROTEINS

LE CONG, PH.D.
Departments of Pathology and Genetics



LIANG FENG, PH.D.
Department of Molecular and Cellular Physiology

Membrane proteins are central to many disease processes and account for a majority of drug targets, yet their biochemical properties have largely excluded them from the "antibody revolution" in the pharma industry in recent years.

In this project, we are developing a platform technology that can make antibody discovery against membrane proteins substantially more feasible, efficient, and scalable. Highly specific antibodies for membrane proteins will support R&D by providing much-needed tools to probe their physiological functions and regulation and to explore their therapeutic potential.

Ultimately, the technology that we are developing will deliver novel therapeutics that address unmet medical needs for diverse diseases and conditions.

We have leveraged knowledge and expertise in membrane protein biochemistry, protein engineering, and computational genomics to overcome challenges associated with the conventional method for developing antibodies that recognize membrane proteins.

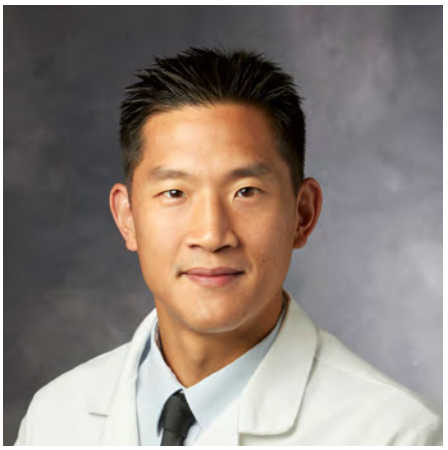
We have made significant progress on multiple fronts during the initial year:

- By comparing different cell types, we have identified a scalable human cell system that can allow us to identify antibodies in the context of native cell membrane.
- We have completed preparation of a panel of high-value membrane protein targets, with corresponding cell systems established for single-cell-based antibody discovery. Moreover, we have obtained several antibodies that can specifically recognize target proteins and the corresponding target-expressing cells. These critical positive controls enable us to optimize our downstream strategies and benchmark our technology.
- We have quantitatively analyzed our synthetic antibody library, validating the library's quality, setting the baseline of antibodies' abundance before selection, and establishing the suitable library size for downstream single-cell assays.
- We have optimized a high-throughput sequencing strategy, which allows us to capture all the sequence diversity that contributes to antibody specificity.
- We have designed and completed the preparation of a multi-target, single-cell sequencing workflow, which allows us to capture and quantify the antibody and the target membrane protein sequences simultaneously from the same cell. This paves the way to pinpoint the specifically enriched antibodies against the target protein.

Our progress puts us in an excellent position to fully develop our proposed technology, which we expect to finish in the second year. We also plan to prepare a National Institutes of Health SBIR/STTR or R01 proposal to continue technology development based on the results of this seed grant.

DEVELOPMENT OF A PHOTO-ENZYMATIC 3D BIOPRINTER FOR PEDIATRIC TISSUE ENGINEERED VASCULAR GRAFTS

STEVEN G. BOXER, PH.D.
Department of Chemistry



MICHAEL MA, M.D.
Department of Cardiothoracic Surgery

MARK A. SKYLAR-SCOTT, PH.D.
Department of Bioengineering

Background

Our proposal is to develop an entirely new light-driven 3D-printing system that uses light-activatable enzyme systems to catalyze the formation of wholly natural scaffolds. While applications for this technology are broad, our goal is to derive complex 3D-printed vascular systems with translational potential for patients with pulmonary atresia.

Patent Filing

We have filed an international patent application: PCT/US22/80871: PHOTO-ENZYMATIC PRINTING.

System Recipe Optimization

Using assays of clotting kinetics, we have fine-tuned the concentrations of system components. We have optimized concentrations of fibrinogen, thrombin, Itelo, Razo, calcium, potassium, sodium, phosphate, and tris to maximize on:off kinetic contrast, clotting speed, cell viability, and light penetration depth.

Vessel Printing and Perfusion

We have created a simple fluidic chip in which we have printed fibrin containing vessel-like channels that can be perfused with cell culture media by a pump. Fibroblast cells are included in the fibrin as a preliminary cell type, with cardiomyocytes and endothelial cells to be additionally included in the future. Perfusion maintained cell viability over several days and the fibrin structure remained well intact with patent channels for the duration of perfusion.

Fluidic Chip Multilayer Printing

Regulation of fibrinogen clotting by the Razo + Itelo system yields beautiful single-layer prints of fibrin, but cannot print many layers serially in a traditional stereolithography setup due to limited on:off contrast.

To side-step this limitation, we are developing a fluidic chip and associated fluidic systems, which integrates mixing of the thrombin and fibrinogen sides of the reaction with an enclosed printing chamber that may be washed after printing of each layer, to supply fresh solution. This washing removes any material that was not meant to polymerize, resetting the background buildup of polymerization, and should allow printing of multiple layers in series without excessive buildup of background polymerization. At the enclosed printing chamber, a thin PDMS membrane on one side can be deflected by a motorized stage to function as the build platform. The internal side of the PDMS membrane, which faces the fibrin, has salt mixed in when cast, which is subsequently dissolved to yield an interconnected porous network at

the surface of the PDMS in which the first layer of fibrin can polymerize throughout to anchor it to the otherwise slippery PDMS.

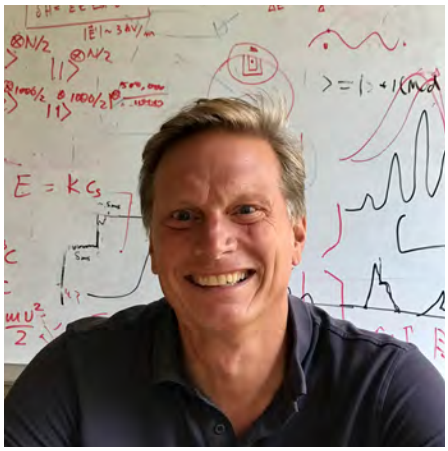
Volumetric Printing

We have developed an optimal set of concentrations of the system for a volumetric printing format, and tested printing by a custom designed and built volumetric printing adaption to our custom designed and built stereolithography printer. We have validated the printer function with traditional materials. We have established the need for a viscosifying agent such as xanthan gum to limit diffusion throughout the volume while printing fibrin.

Next Steps

Upcoming is further testing and development of the fluidic chip for multi-layer fibrin printing. We aim to use the chip to print a five-layer fibrin construct containing perfusing vessel-like channels. In following experiments, fibroblasts and cardiomyocytes will be included in the bulk of the fibrin, while endothelial cells will be lined along the channels. Such a construct would be used for publication and to apply to a major grant such as an R01.

FLUORESCENT LIFETIME IMAGING MICROSCOPY OF MITOCHONDRIA-RICH EXTRACELLULAR VESICLES FOR DIRECT AUGMENTATION OF MYOCARDIAL BIOENERGETICS



MARK A. KASEVICH, PH.D.
Departments of Physics and Applied Physics

SOICHI WAKATSUKI, PH.D.
Departments of SLAC National Accelerator Laboratory, Structural Biology, and Energy Sciences

PHILLIP C. YANG, M.D.
Department of Medicine - Med/ Cardiovascular Medicine

In year 1, we focused on the optics development of our fluorescent lifetime imaging microscope (FLIM) and preparation of the laboratory space in Varian Physics for Biosafety Level 1 and 2 for sample measurement and storage from mitochondria-enriched extracellular vesicles (M-EVs). Preliminary time-resolved cryo-electron tomography data from another organism, gram-negative bacteria, upon acidification captured budding and release of extracellular vesicle-like particles. We plan to apply this approach to further characterize export and import of M-EVs from cardiomyocytes.

FLIM Optics Development

Optics for multidimensional EO-FLIM have been constructed and tested on a variety of benchmark samples, including single-molecule imaging. Additionally, a versatile excitation laser source has been developed to allow excitation over a broad range of wavelengths, from ultraviolet to visible, to characterize M-EV and cardiomyocyte samples. High-speed acquisition up to 1kHz frame-rate is possible for imaging samples in-flow and also for functional imaging of calcium and/or voltage activity in cardiomyocytes.

Preliminary Data Collection on Isolated M-EVs

We were able to visualize mitochondria in M-EV samples prepared from iPS cells-derived cardiomyocytes (iCMs). Briefly, we used isolated M-EVs stained with MitoTracker Green where the isolation method for M-EVs was based on a differential ultracentrifugation method from iCM-conditioned medium. A total of $1x10^8$ M-EVs were resuspended in PBS and stained with 100nmol/l MitoTracker Green for ten minutes at room temperature and protected from light before imaging. Potential areas to reduce observed background signal, including implementing wash steps or purification after straining, are currently being explored.

Time-Resolved Cryo-Electron Tomography

We obtained a snapshot of a gram-negative bacterium, *Caulobactor crescentus*, shedding extracellular vesicles from the outer membrane at 0.75 sec after mixing the bacteria with pH2 solution. This was achieved by using a time-resolved method S. Wakatsuki and P. Dahlberg developed. While unexpected, this provides a strong basis for our M-EV project to use the microfluidics system currently being developed to visualize the biogenesis of M-EVs derived from cardiomyocytes as well as the fusion of exogenously added M-EVs in damaged cardiomyocytes.

Next Steps

In year 2, we will finalize the FLIM hardware and data analysis software and collect a number of purified

M-EV images using MitoTracker and autofluorescent molecules such as NADPH and FAD. We will also develop a microfluidics chip and integrate it to the FLIM setup for fast purification of M-EV using NADPH/FAD signals as indication of active mitochondria enrichment in EVs collected from cardiomyocytes. Finally, we will visualize biogenesis and fusion of M-EVs using time-resolved cryo-ET.

Related grants obtained after the launch of our Beckman Technology Development Seed Grant project:

- "Optical and X-ray multimodal-hybrid microscope systems for live imaging of plant stress responses and microbial interactions," DOE, Biology and Environmental Research, S. Wakatsuki (PI), M. Kasevich (coPI), M.B. Mudget (Stanford Biology, coPI), and F. Brandizzi (Michigan State Univ., coPI), (grand total of $3.9M for 3 years).
- "Time-resolved cryogenic electron tomography studies enabled by LCLS mixing-injector technology," P. Dahlberg (PI, SLAC), S. Wakatsuki (coPI), M. Hunter (SLAC, coPI). Lab Director Research and Development (2022-2024, $350K for 2 years).
- "Mitochondria-rich microvesicles for restoration of intracellular bioenergetics," NIH R01-HL156945-01A1. Yang (PI), K. Svensson (Co-I), YP. Yang (Co-I), R. Zhao (Co-I) (2023-2027, $1M for 4 years: pending Academic Council decision).

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 engage in dialogue with Stanford faculty, students, and postdoctoral investigators.

Top: Get the FACS seminar, September 2022;
Bottom: Munzer Auditorium.

During the COVID-19 pandemic, when many researchers were working from home, Beckman Center program leaders redesigned several programs as online webinars and video tutorials, allowing vital scientific interactions and training to continue. Several of those programs have now been restarted and expanded, and the program leaders are holding in-person sessions on campus once again.

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 Beckman Symposium, a major event that draws scientists from around the world, has been postponed since 2020 due to the pandemic. We are looking forward to possibly soon rescheduling the program that was planned for 2020.

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 through 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 by scientists from 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 BIOLOGY SEMINAR SERIES

The Cancer 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.

Get the FACS seminar, September 2022.

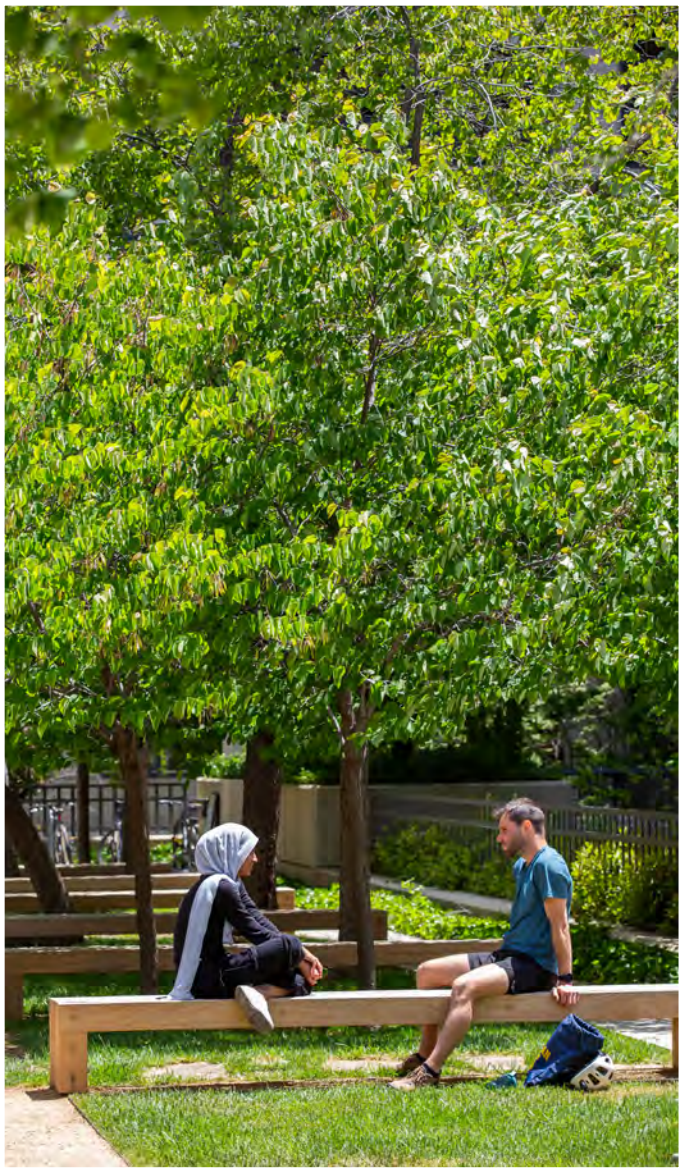
FACULTY RECRUITMENT PROGRAM

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.

During the past year, the Beckman Center recruited six new assistant professors:

- Alex Gao, Ph.D., joined the Department of Biochemistry. He did his Ph.D. with Feng Zhang, Ph.D., at the Broad Institute of MIT and Harvard. Dr. Gao will be focusing on understanding bacterial innate immune systems.
- Lauren Goins, Ph.D., joined the Department of Developmental Biology. She did her Ph.D. with Dyche Mullins, Ph.D., at the University of California, San Francisco. Dr. Goins will study how multipotent stem cell lineages can maintain balance among cell fates and an ability to respond to physiological challenges imposed by environmental stressors such as infection and disease.
- Ruth Huttenhain, Ph.D., joined the Department of Molecular and Cellular Physiology. She did her Ph.D. with Ruedi Aebersold, Ph.D., in systems biology at ETH Zurich, Switzerland. Her focus will be on exploiting quantitative proteomics to capture the spatiotemporal organization of GPCR-signaling networks combined with functional genomics to study their impact on physiology.
- Nicole Martinez, Ph.D., joined the departments of Developmental Biology and Chemical and Systems Biology. She did her Ph.D. with Kristen Lynch, Ph.D., at the University of Pennsylvania. Her laboratory will focus on how modifications to both RNA-processing enzymes and RNA itself can alter the proteins encoded by that RNA and, in turn, cause disease.
- Tino Pleiner, Ph.D., joined the Department of Molecular and Cellular Physiology. He did his Ph.D. at the University of Göttingen, working with Dirk Görlich, Ph.D., at the Max Planck Institute for Multidisciplinary Sciences. He will combine mechanistic cell biology, biochemistry, and protein engineering to dissect the pathways and molecular machines that mature human membrane proteins to a fully functional state.
- Florentine Rutaganira, Ph.D., joined the departments of Biochemistry and Developmental Biology. She did her Ph.D. with Kevan Shokat, Ph.D., at the University of California, San Francisco. Her lab is studying choanoflagellates, which form complex colonial organizations, to understand how assemblies of cells signal to one another.

The Beckman Center is now recruiting an additional faculty member to join the Department of Developmental Biology. An announcement concerning this new faculty member will be made in an upcoming issue of *The Beckman Center News*.



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. 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 Medical Scholars.

GUAN QIANG LI

Academic Year: 2023-2024

Year at Stanford Medical School: 3

Undergrad Education: Yale University; Molecular, Cellular, and Developmental Biology, BS

Hometown: Oakland, California

Title of Medical Scholars Project: Elucidating the Role of Resistin in the Immune Microenvironment of Acute Myeloid Leukemia and in Hematopoietic Stem and Progenitor Cell Homeostasis

Research Description

Acute myeloid leukemia (AML) is an aggressive blood cancer, resulting in uncontrolled proliferation and accumulation of abnormal myeloid progenitors in the bone marrow (BM) and peripheral blood. Despite advances in new treatments, the 5-year overall survival rate is 20-30 percent.

Patients with AML often succumb to the disease primarily due to progressive BM failure. Traditionally, BM failure in AML is thought to be a result of overcrowding of the BM space, however many patients with low leukemic burden (20-30 percent) and incomplete involvement of the BM compartment show signs of BM failure. These observations suggest that additional mechanisms must be involved in AML-induced BM failure. Several studies have investigated the effects of AML on normal progenitors and the BM niche, implicating production of AML-secreted factors as potential mechanisms driving niche dysfunction. AML cells have been shown to scavenge critical niche factors, such as thrombopoietin (TPO), and to cause differentiation arrest of residual hematopoietic stem cells. AML blasts can produce inflammatory cytokines including granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-1β (IL-1β), tumor necrosis factor–α (TNFα), IL-6, C-X-C motif chemokine 12 (CXCL12), and C-C motif chemokine ligand 3 (CCL3), which affects normal hematopoietic stem cell function. Recently, proteomic and transcriptomic studies by Zhang, et al., showed that paracrine section of IL-6 from AML suppresses erythroid differentiation and anti-IL-6 blocking antibody improved overall survival in a mouse model. Additional proteomic analysis from the study revealed a significant decrease in human AML blast secretion of resistin, a proinflammatory cytokine, compared to normal human hematopoietic stem and progenitor cells (HSPCs). Surprisingly, little is known about the role of resistin in human AML pathogenesis and in normal human HSPC homeostasis.

In this study, the researchers aim to investigate the role resistin plays in human HSPC homeostasis and to understand the effect of resistin on human AML blast and its immune microenvironment. This study represents the first known studies to characterize resistin effects on human HSPCs and how it might affect malignant hematopoiesis and aid in AML progression. We anticipate that this study will provide a better understanding of how resistin may play a role in AML pathogenesis and AML-induced BM failure (HSPCs loss), thereby potentially offering a novel therapeutic target against one of the most devastating leukemias.

CARMEL G. MCCULLOUGH

Academic Year: 2023-2024

Year at Stanford Medical School: Berg Scholar

Undergrad Education: University of Southern California; Neuroscience, BS

Hometown: Phoenix, Arizona

Title of Medical Scholars Project: GMP Manufacturing of Autologous Esophageal Epithelial Cells for the Prevention of Esophageal Strictures

Research Description

Epidermolysis bullosa (EB) is a family of inherited skin disorders that are characterized by blister formation within the skin and internal epithelium, with recessive dystrophic epidermolysis bullosa (RDEB) as the most phenotypically severe form of EB resulting from biallelic COL7A1 mutations. COL7A1 is critical in encoding for type VII collagen, which serves to anchor the epithelium to the underlying stromal tissues. Without type VII collagen, the stratified epithelium that comprises the skin and esophagus is disorganized and results in painful blisters and erosions. With repeated abrasions, RDEB patients experience chronic skin wounds and narrowing of the esophagus, also called strictures. 90-100 percent of RDEB patients have gastrointestinal abnormalities, including esophageal blisters and strictures. Esophageal strictures are associated with dysphagia and microstomia, which limit patients in their oral intake, leading to malnutrition and vitamin/mineral deficiencies.

While there have been massive strides in addressing the skin presentation of EB, there is still an unmet need for esophageal symptoms. Current therapy for esophageal strictures is far from definitive—it currently involves palliative balloon dilation to increase esophageal diameter and patency. This can be incredibly uncomfortable for the patient and leads to progression of esophageal blistering and scarring.

Previous work in the Oro laboratory has capitalized on the promise of induced pluripotent stem cell (iPSC) tissue regeneration for skin blistering in RDEB. We hypothesize that we can modify our cGMP cell manufacturing platform, which produces autologous genetically corrected tissue stem cells, to treat esophageal blisters and prevent stricture formation. Originally, this platform was designed to manufacture COL7A1-corrected keratinocyte (iKC) grafts, as part of RDEB cell therapy (DEBCT) currently being submitted as a pre-IND package to the FDA. This manufacturing platform is flexible and can use the same corrected patient-iPSCs to make other tissues. In addition, this is an attractive method to translate to the esophagus, because both tissues are stratified epithelial surfaces and rely on type VII collagen for adhesion. Our collaborator has already demonstrated the ability to produce pluripotent cell-derived esophageal-like tissues, and we have since modified this method to generate a reagent-defined and cGMP-compliant manufacturing protocol to increase efficiency of esophageal differentiation. The development of CRISPR-corrected, iPSC-derived esophageal epithelial cell therapy (indESOCET) and a clinically compatible cell delivery method will address an enormous unmet need with first-in-human esophageal therapy for RDEB patients.

KATHERINE PROTHRO

Academic Year: 2023-2024

Year at Stanford Medical School: 4

Undergrad Education: Wake Forest University; Biology, BS

Hometown: Bedford, New Hampshire

Title of Medical Scholars Project: Pericytes: A Cell of Origin in Pulmonary Hypertension

Research Description

Pulmonary arterial hypertension (PAH) is a disease defined by elevated blood pressure within the vasculature of the lungs. Despite diverse and often unknown etiologies, PAH has widely been accepted as a disease of the small muscular arteries of the lung. However, recent work in our lab suggests that at least one type of PAH may instead arise from pericyte dysfunction, challenging the current framework of PAH etiology.

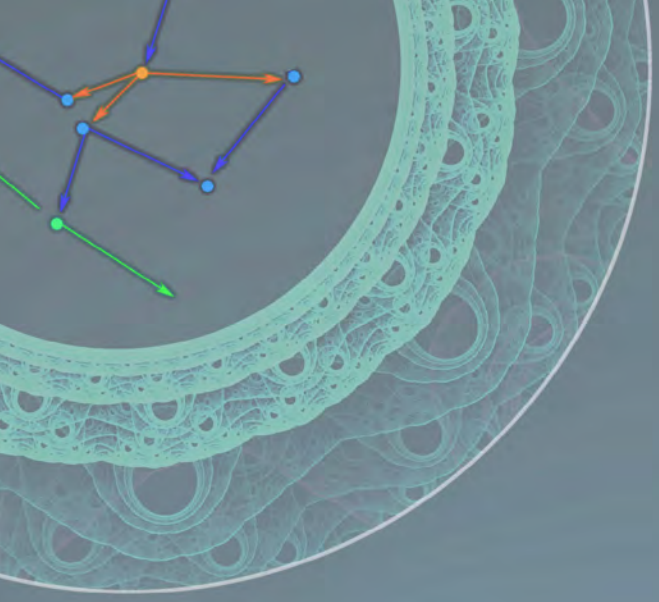
Clinically, PAH is characterized by elevated pulmonary arterial pressure and remodeling of pulmonary vessels that lead to the narrowing of pulmonary blood vessels. Some forms of PAH are caused by heritable, familial mutations, one of which is in KCNK3. This gene, which encodes an outward rectifying potassium channel, plays an essential role in setting the resting membrane potential of excitable cells. The current working hypothesis is that a loss of KCNK3 primarily affects resting membrane potential of pulmonary vascular smooth muscle cells, causing hypercontractility that initiates pulmonary arterial remodeling and ultimately PAH.

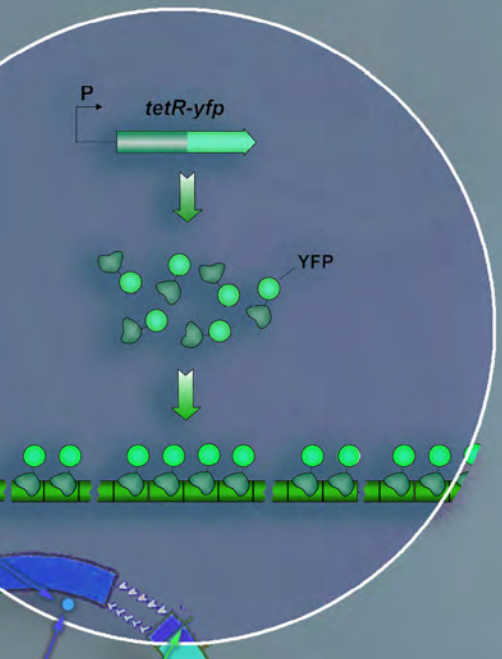
Yet, using the power of our comprehensive molecular cell atlas of the human lung, we looked at gene expression across all lung cell types and found that the most prominent site of KCNK3 expression is in pericytes, not vascular smooth muscle. This suggested that PAH might be a disease of lung pericytes, with the disease initiating from loss of KCNK3 function in pericytes, rather than in pulmonary arterial cells. Under this new pathogenesis model, the pulmonary arterial pathology and other clinical manifestations of PAH arise as a secondary consequence of a primary defect in pericytes.

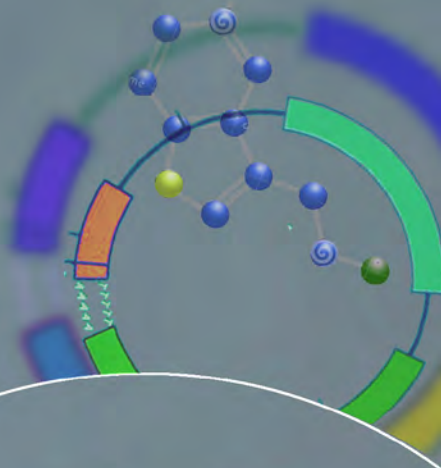
To investigate this hypothesis, mice were genetically edited and bred to generate a conditional gene knockout line (referred to as $KCNK^{CKO}$) that 1) labels pericytes, and 2) allows for induction of KCNK3 deletion specifically in pericytes. Using this system, we intend to measure pulmonary pressure and right heart function in $KCNK3^{CKO}$ animals to evaluate whether the loss of KCNK3 in pericytes can recapitulate the hallmarks of human disease. If our work proves to identify pericytes as the source of disease, KCNK3-mediated PAH would become the first documented “pericytopathy.”

Furthermore, by employing genetically driven, cell-specific labeling of pericytes, we hope to gain better insights into the normal behavior of pericytes within the lung, such as their proliferation and regeneration capacity. Fully characterizing the role of pericytes, as well as their relationship to other specialized cell types, will be crucial in determining the factors governing proper gas exchange in the lung, thus having implications for many respiratory diseases and potentially pericytophathies in other tissues.

Technology Resources







Technology Resources

THE BECKMAN SERVICE CENTERS

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

In continuous operation since 1989, these core service centers are currently among the most successful service centers at Stanford. They typically generate more than $4.5 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 are:

- 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 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 centers' operations, their importance to the Stanford research community, and how they used center funds.

CELL SCIENCES IMAGING FACILITY

ADVISORY COMMITTEE

Lucy Shapiro, Ph.D. (Committee Chair)
Alexander Dunn, Ph.D.
Margaret Fuller, Ph.D.
Emma Lundberg, Ph.D.
Beverly Mitchell, M.D., SCI
Lucy O'Brien, Ph.D.
Mark Shepard, MPH
Georgios Skiniotis, Ph.D.
Aaron Straight, Ph.D.

OVERVIEW

The Cell Sciences Imaging Facility (microscopy.stanford.edu) fosters high-impact research by providing researchers with access to training, education, and support on high-resolution, state-of-the-art imaging technologies and visualization and analysis software for multidimensional analysis 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, and live-cell imaging, as well as spatial proteomics, atomic-force measurements, immuno-electron microscopy, and high-pressure freezing.

The CSIF is organized into three interdependent imaging cores: 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 provides full wet-lab services; and the Spatial Multiplexing Core (SMC), which provides highly multiplexed spatial proteomics imaging (CODEX) 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.

JON MULHOLLAND
Director

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 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 (spatial proteomics, CODEX), thus creating the Multiplexing and Array Tomography Core. This lab is now known as the Spatial Multiplexing Core.

CELL SCIENCES IMAGING FACILITY
Front Row, Left to Right: Yuanyuan Li, Kitty Lee, Ibanri Phanwar-Wood, Ruth Yamawaki, John Perrino
Back Row, Left to Right: Jon Muholland, David Lenzi

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 technologies. While these technologies have evolved substantially over the last 29 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 nine-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 researchers from the Beckman Center, the Stanford Cancer Institute, and Stanford's School of Medicine, School of Engineering, and School of Humanities and Sciences.

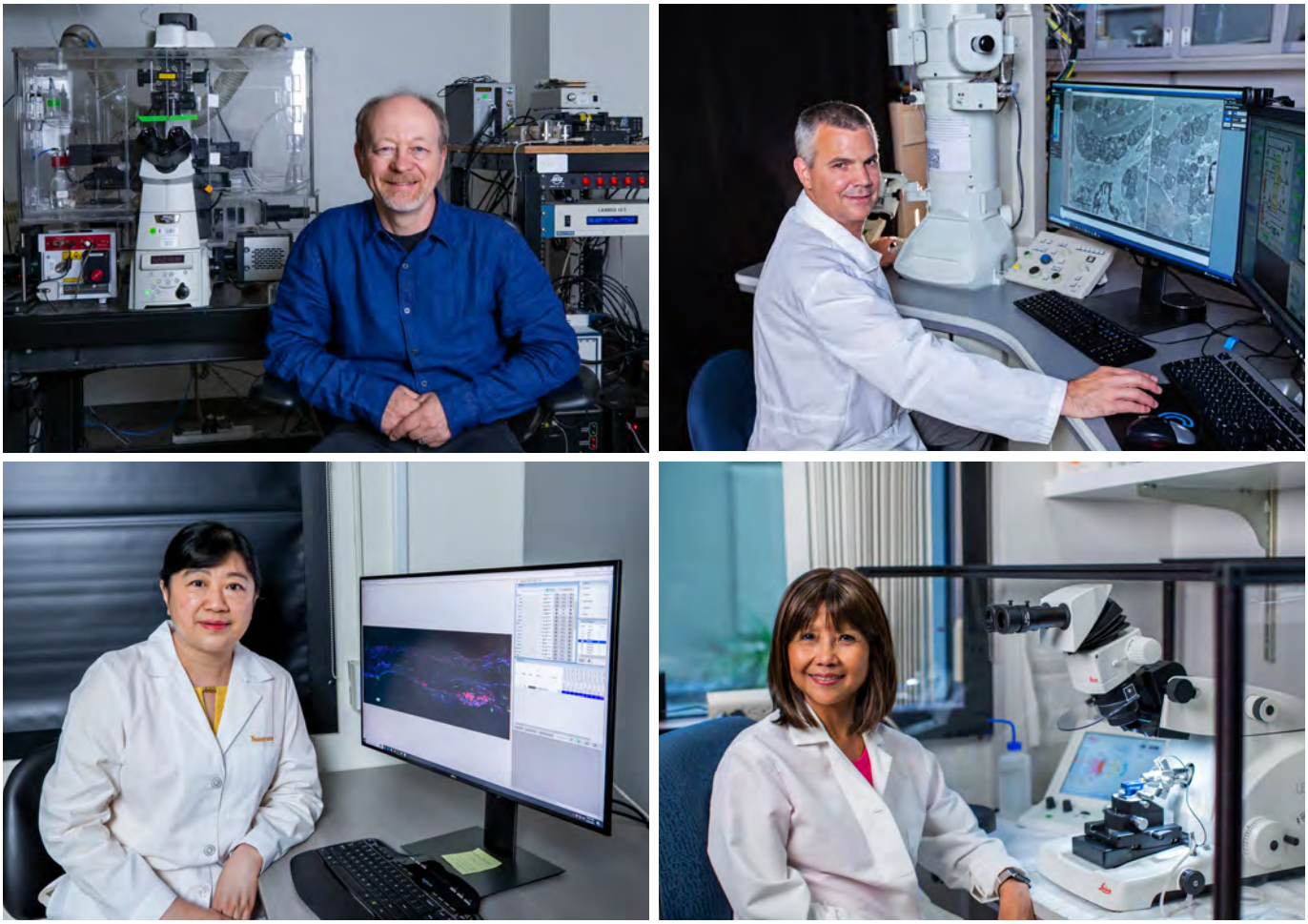
The CSIF is staffed by its director, Jon Mulholland, who has led the facility for 23 years, as well as several full-time research professionals who have expertise and training in electron and light microscopy.

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, Zeiss Airyscan)
- Laser scanning confocal (Zeiss LSM 880, LSM 780, Leica SP8, Leica STELLARIS 8 DIVE)
- Spinning disk confocal (Nikon-Yokogawa)
- Deconvolution (API OMX Delta Vision)
- 2-photon (Zeiss LSM 780, Leica DIVE, each with Spectra Physics DeepSee laser)
- Lattice light sheet microscope (Zeiss LLS7, Bruker TruLive3D)
- Fluorescence lifetime imaging (FLIM) light microscopy technologies
- Bio-atomic force microscopy (Bio-AFM, Bruker Resolve BioScope)



Clockwise from Top Right: John Perrino with the Gatan OneView TEM microscope and OneView 16bit computer; Ibanri Phanwar-Wood with the Leica Ultracut UC7; Yuanyuan Li with CODEX technology; David Lenzi with the Inverted Nikon Spinning Disk Confocal Microscope.

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 spectra and intensity. Bio-AFM enables innovative live-cell experiments that provide high-resolution force measurements and mapping over the surface of cells and other biological materials. 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 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). Most recently, CSIF added VisioPharm AI software for spatial proteomics analysis.

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 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.

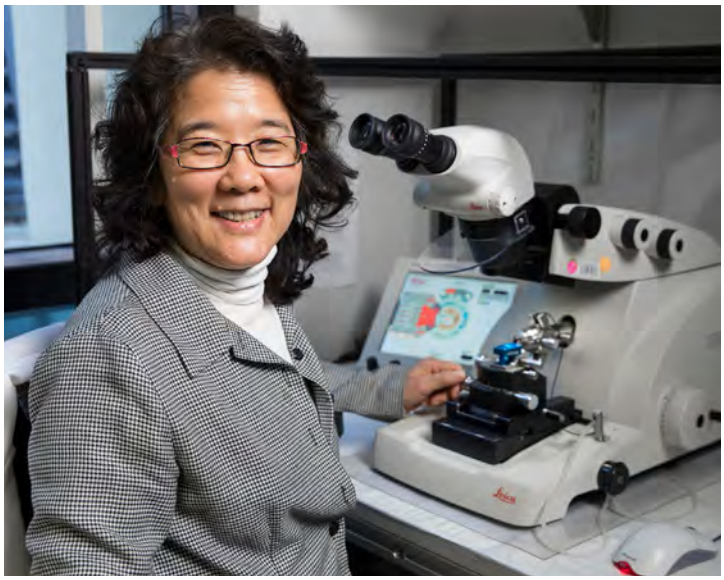
Ancillary equipment includes four ultramicrotomes for cutting ultrathin sample sections (less than 100nm), all equipment necessary for sample preparation, and computers for image analysis. Additionally, the EMC houses a state-of-the-art Leica EM ICE high-pressure freezing machine. High-pressure freezing 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.

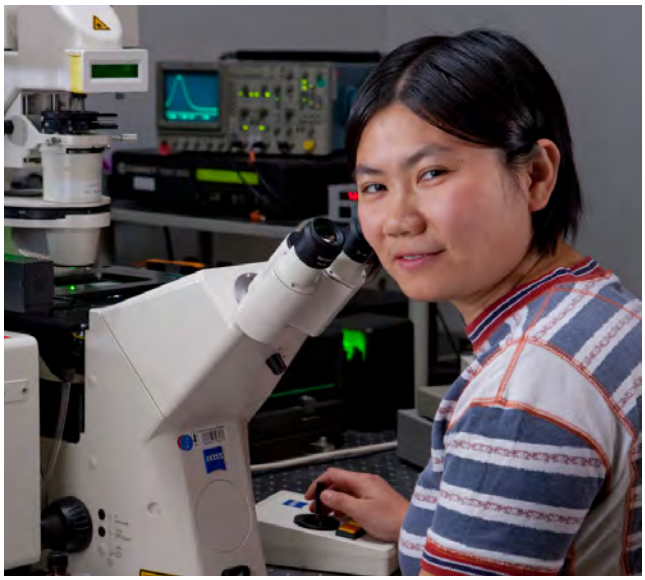
Spatial Proteomics Services

The facility's Spatial Multiplexing 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

David Lenzi with the Bruker TruLive3D Imager.





Clockwise from Top: Yuanyuan Li with CODEX technology; Kitty Lee; Ruth Yamawaki.

their spatial context. The facility 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. The array tomography methodology enables acquisition of electron microscopic images in register with immunofluorescence. Array tomography thus provides an opportunity to explore the 3D molecular architectures of tissue at an unprecedented level of detail.

RECENT DEVELOPMENTS

Experiential Learning

Stanford University's c-ShARP (Community of Shared Advanced Research Platforms) initiative is providing funding to support experiential learning in Stanford

The School of Engineering's Shriram Center houses a satellite CSIF microscopy lab.

facilities. CSIF has again been awarded funds (approximately $15,000) to support the following courses.

- MCP222, BIO152: Imaging: Biological Light Microscopy. This is an eight-week "short course," co-taught by Richard S. Lewis, Ph.D., professor of molecular and cellular physiology, and Gordon Wang, Ph.D., director of the imaging facility at the Wu Tsai Neurosciences Institute. The fall quarter 2022 marked the sixth year of this course.
- EE235A: Analytical Methods in Biotechnology I. The goal of this course is to enable engineering students with little or no background in molecular biology to transition into research in the field of biomedicine.

Instrument Upgrades

The National Institutes of Health's (NIH) Shared Instrumentation Grant Program awarded the CSIF funding to purchase a Leica STELLARIS 8 DIVE confocal-multiphoton *in vivo* and *ex vivo*, as well as *in vitro*, imaging microscope. The new microscope was installed in January 2023; it replaces and upgrades the facility's 12-year-old SP5 upright confocal-2P microscope. CSIF received $600,000 in NIH funds, plus additional funding from the Beckman Center, for a total of $852,294.

In collaboration with the School of Engineering's Department of Bioengineering, the CSIF's Shriram Center facility has added three new microscopes.

- A Zeiss LSM 900 Airyscan 2 confocal microscope has been installed to upgrade the facility's combined light and atomic-force microscope platform. This platform enables confocal immunofluorescence light microscopy (IFM) in conjunction with atomic force microscopy (AFM), for correlative IFM to AFM, which is essential for advanced research in mechanobiology. Additionally, the Airyscan 2 component of the LSM 900 allows researchers to increase the resolution of the confocal light microscope beyond the diffraction limit, achieving resolution of approximately 150nm.
- A Zeiss Lattice Lightsheet 7 (LLS7) microscope, which generates an optical lattice to create an ultrathin light sheet consisting of a parallel linear array of coherently interfering Bessel beams, is the second new microscope. The resulting light sheet is exceptionally thin (0.4 um) and flat, with unparalleled optical sectioning and essentially no out-of-focus light. Coupled with an analysis workstation for the data pipeline and a dedicated, high-speed transfer storage system, this equipment is compatible with utilization by multiple users and will facilitate live-cell imaging of a multitude of biological processes, such as genome folding, transcriptional regulation, protein interaction and aggregation, cell signaling, endocytosis, axon formation, and others.
- In contrast to the Zeiss LLS7, the third new instrument, a Bruker TruLive3D light sheet microscope, uses a Gaussian light sheet to illuminate and excite fluorescent samples. This light sheet microscope is optimized for larger samples than are imaged with the LLS7. For example, the TruLive3D microscope is excellent for imaging living, whole organisms and samples, such as *C. elegans* and *Drosophila*. The TruLive3D has a dedicated analysis workstation and a networked data storage system.

FUTURE VISION

Several new programs and services are now in development.

- 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.
- The CSIF will continue to develop its educational program. This year, CSIF was funded by Stanford's c-ShARP initiative to develop a series of lectures focused on the fundamentals of biological electron microscopy and the fundamentals of biological light microscopy. These lectures will be developed and presented over the coming year.

PROTEIN AND NUCLEIC ACID FACILITY

ADVISORY COMMITTEE

Lucy Shapiro, Ph.D. (Committee Chair)
Laura M.K. Dassama, Ph.D.
Jeffrey S. Glenn, M.D., Ph.D.
Ron Kopito, Ph.D.
Mark Krasnow, M.D., Ph.D.
Mark Shepard, MPH
Tom Wandless, Ph.D.
Joanna Wysocka, Ph.D.

OVERVIEW

The Protein and Nucleic Acid Facility (pan.stanford.edu) is a key part of the Beckman Center's shared technology resources. The PAN Facility's mission is to be adaptable and responsive to the changing needs of biomedical research by providing researchers, faculty, and students across various disciplines with continued access to key research tools, applications, and specialized expertise in an efficient and cost-effective manner.

With the growing complexity of biomedical research, PAN remains 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, which 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

MICHAEL ECKART, PH.D.
Director

of the Beckman Center, and includes researchers from the Beckman Center, the Stanford Cancer Institute, and Stanford's School of Medicine and School of Humanities and Sciences.

The PAN Facility is staffed, in addition to its director, by five full-time, experienced research professionals who possess extensive knowledge and expertise in all the services provided. The PAN Facility is organized into several interdependent services, as listed below.

SERVICES

The PAN Facility offers a number of interdependent services:

- Gene expression analysis
- Microarrays
- Real-time PCR

PROTEIN AND NUCLEIC ACID FACILITY

Left to Right: Michael Eckart, Jessica Tran, Yen Tran, Jennifer Okamoto, Katia Alvarez, Brandon Lee

- Pyrosequencing
- Nucleic acid QC
- Single-cell genomics
- Spatial transcriptomics
- Next-generation sequencing
- DNA sequencing
- Synthetic nucleic acid synthesis
- Biomolecular interaction analysis (surface plasmon resonance)
- Peptide synthesis
- Mass spectrometry

Shared Services

The PAN Facility contributes to resource optimization within Stanford University by consolidating expensive equipment, maintenance costs, and technical expertise to ensure efficient utilization of resources. Rather than duplicating equipment and services across multiple departments or research groups, service centers such as PAN centralize these resources, reducing expenses and enhancing overall productivity. This consolidation allows Stanford's biomedical research community to invest in a broader range of cutting-edge technologies and expand the scope of research projects undertaken, leading to greater scientific impact.

The core strength of the PAN Facility comes from its talented, highly experienced, and dedicated scientific staff. PAN staff members are specialists in their respective areas of expertise and have also cross-trained in the operation of multiple instruments and applications; thus, they serve as valuable resources for researchers, offering guidance on experimental design, protocol optimization, data analysis, and troubleshooting.

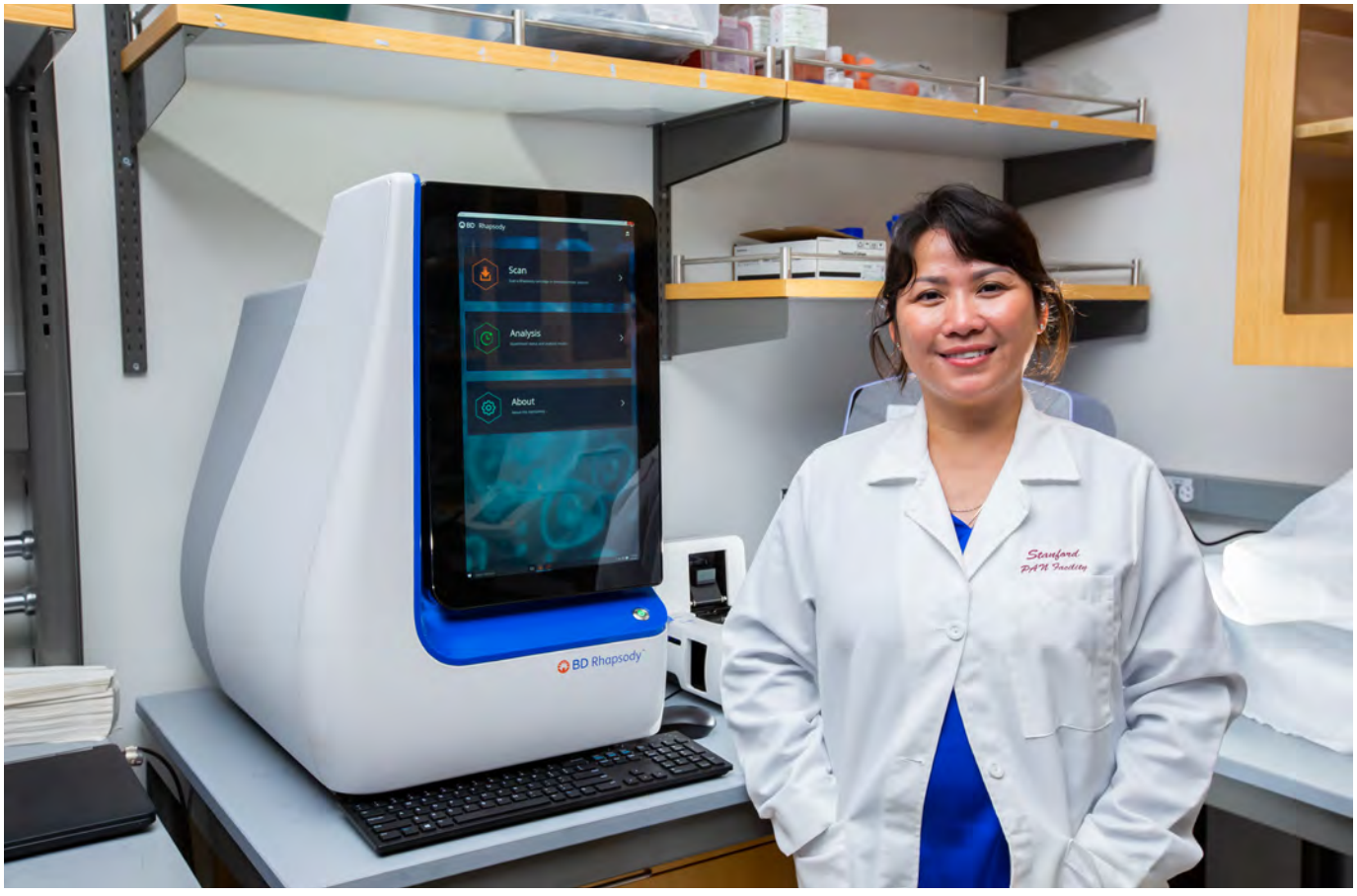
The PAN Facility often serves as a central meeting point for scientists from diverse backgrounds, thereby fostering interdisciplinary collaboration by promoting the exchange of ideas, expertise, and methodologies. By working together and combining complementary skill sets, PAN staff and researchers can address complex scientific questions that can lead to an acceleration in scientific progress. Throughout, PAN staff maintain high quality standards and follow strict quality control measures to ensure the accuracy, reproducibility, and

reliability of the research data generated within the PAN Facility. This applies not only to existing standard protocols, but also to the adaptation of existing assays or creation of new assays to address a specific research question. The results of these efforts are often highlighted in publications to which PAN scientists have made contributions.

In addition to supporting research projects, the PAN Facility provides a learning environment that integrates theory with practical applications. 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. In addition, PAN, often with suppliers of different technology platforms, offers training programs and workshops that educate researchers and students on the proper use of equipment and techniques, thereby promoting skill development and empowering them to tackle complex scientific challenges. For example, PAN staff recently participated via lectures and hands-on experiments in a course for engineering students, with the goal of enabling students with little or no background in genomics or proteomics to transition into research in the field of biomedicine.

Genomic Resources

Genomic resources play a crucial role in advancing scientific research and understanding the complexities of biological systems. The PAN Facility provides researchers with access to a wide range of genomic services and expertise, including various DNA sequencing technologies, such as Sanger sequencing, next-generation sequencing (NGS), and single-cell analyses.



Yen Tran with the BD Rhapsody Single-Cell Analysis System.

Top: PAN equipment; ***Bottom***: Jessica Tran with the Biacore T200.

These techniques enable the determination of DNA sequences, identification of genetic variations, and exploration of gene expression patterns.

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, 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 genomics technologies have revolutionized the understanding of biological systems by enabling the analysis of individual cells at a molecular level. In contrast to traditional bulk genomics methods that average the genetic information from a population of cells, potentially masking crucial cellular heterogeneity, single-cell genomics allows the characterization of cellular diversity, identifying rare cell types and uncovering cell-to-cell variations within populations.

Single-cell RNA sequencing (scRNA-seq) is one of the most widely used techniques in providing transcriptomic profiles of individual 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.

PAN works closely with the Beckman Fluorescence Activated Cell Sorting Facility, which performs high-throughput isolation of single cells from the biological system of interest. The PAN single-cell genomics resource features single-cell capture microfluidic technology, such as the ddSEQ Single-Cell Isolator instrument (BioRad), which performs rapid single-cell isolation using droplet

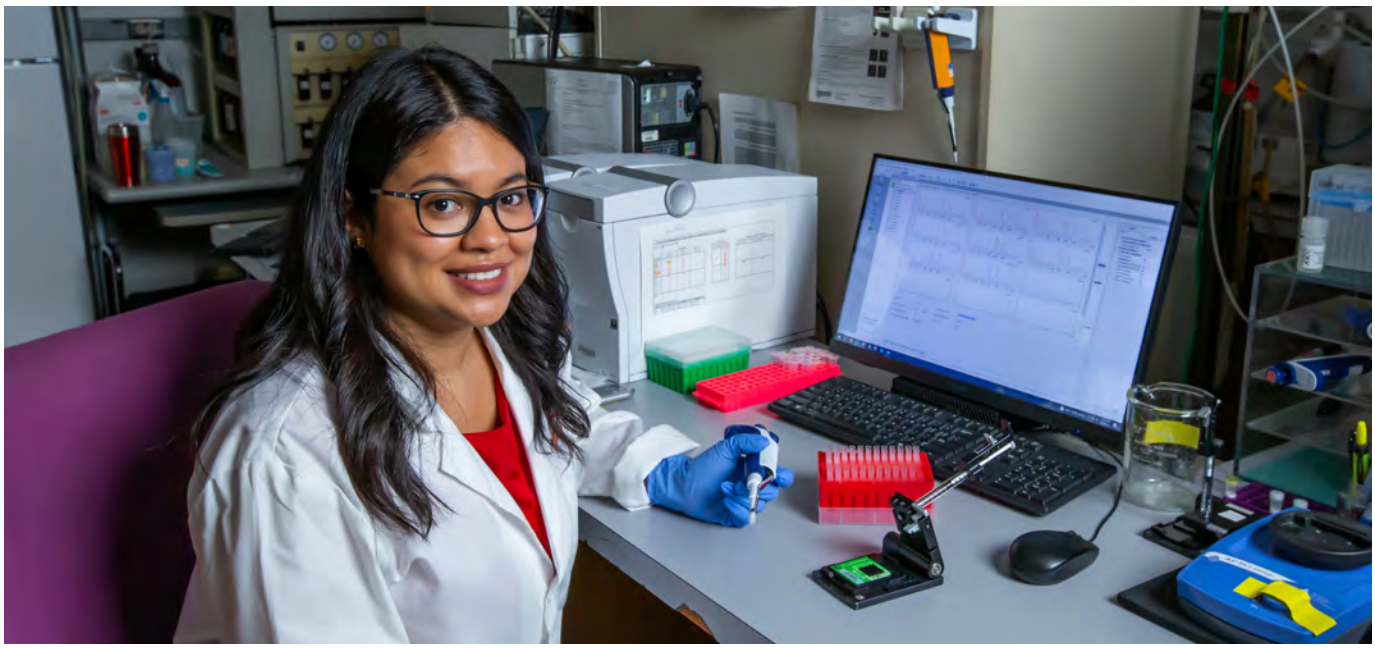
partitioning technology. Cell acquisition is confirmed via an EVOS Cell Imaging System.

The PAN Facility also utilizes the BD Rhapsody Single-Cell Analysis System, which enables simultaneous measurement of surface proteins and mRNA expression, thereby facilitating the identification of distinct subsets of cells. This system includes the BD Rhapsody cartridge, sample loading station, and scanner. The system enables single-cell capture and barcoding of hundreds to thousands of single cells for analysis of genomic and proteomic information, using proprietary, gentle, robust microwell-based single-cell partitioning technology. The BD Rhapsody Scanner, by direct imaging, is designed to visualize all steps in the single-cell capture workflow and provide detailed quality control metrics at every step, enabling the user to make key decisions throughout the workflow, including troubleshooting, before submitting the samples for expensive downstream next-generation sequencing.

Although omics techniques such as scRNA-seq provide valuable insights into the molecular composition of cells or tissues, they often overlook the spatial organization of cells within complex tissue microenvironments. Spatial-omics approaches aim to bridge this gap by providing spatially resolved molecular information. This is achieved through the combination of high-resolution imaging technologies and spatial transcriptomics that enable the simultaneous visualization of gene expression patterns and transcriptomic analysis at a single-cell resolution.

Currently, PAN offers the Visium platform provided by 10X Genomics that combines traditional histology with high-throughput 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.

PAN processes the templates generated from individual cells using the above technologies for analysis by next-generation sequencing. The conversion to next-generation sequencing libraries is accomplished using automated liquid handling instruments. The Mosquito HTS Nanoliter Liquid Handler (STP 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.



Katia Alvarez

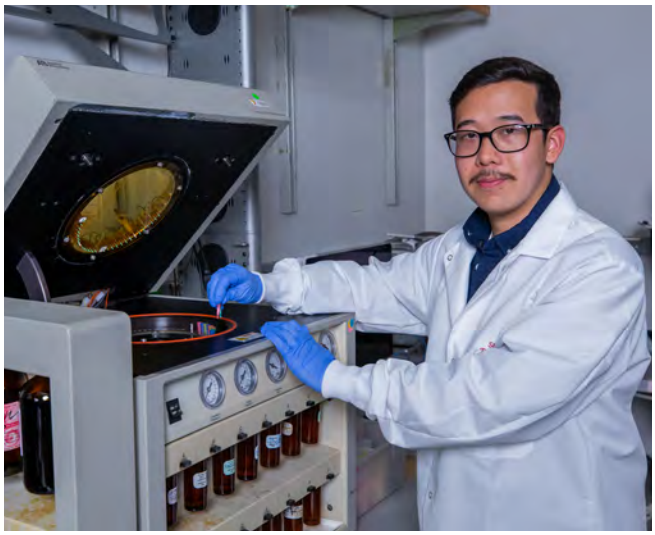
Top: Yen Tran; ***Middle***: Jennifer Okamoto; ***Bottom***: Katia Alvarez

The future of spatial-omics holds great promise, but the field is still evolving. Thus, the PAN Facility is continuously evaluating and implementing different spatial transcriptomics technologies. Spatial-omics is expanding beyond transcriptomics by incorporating other omics technologies, such as proteomics and metabolomics. To achieve the goal of integrating the different technologies, PAN is working closely with the Beckman Cell Sciences Imaging Facility, which has implemented the CODEX (CO-Detection by indEXing) technology, on the identification and implementation of multi-modal spatial technology platforms.

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.

Among the gene expression analysis technologies, PAN continues to provide Affymetrix microarray technology. 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.

PAN's portfolio of genomic technologies also includes those required for the validation of genes identified in large-scale genomic studies. We believe that the need for such validation technologies will continue to grow, as they are key to demonstrating how genetic or proteomic differences have effects in a specific disease. Quantitative-PCR continues to be a popular



Top: Brandon Lee

technique to validate array and NGS 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, is also in demand. 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 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 continue to see an increase in the demand for fragment analysis in STR analysis workflow that uses the capillary electrophoresis technology in our DNA sequencer, since it is a simple, economical method, and the gold standard for establishing the identity of human samples.

Protein Analytical Resources

Protein analytical resources encompass 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.

The ability of PAN to provide peptide synthesis services and expertise has had a significant impact by enabling researchers to design peptides, often with unique modifications, to develop peptide-based therapeutics, or to use as tools to uncover the complexities of protein structure and function.

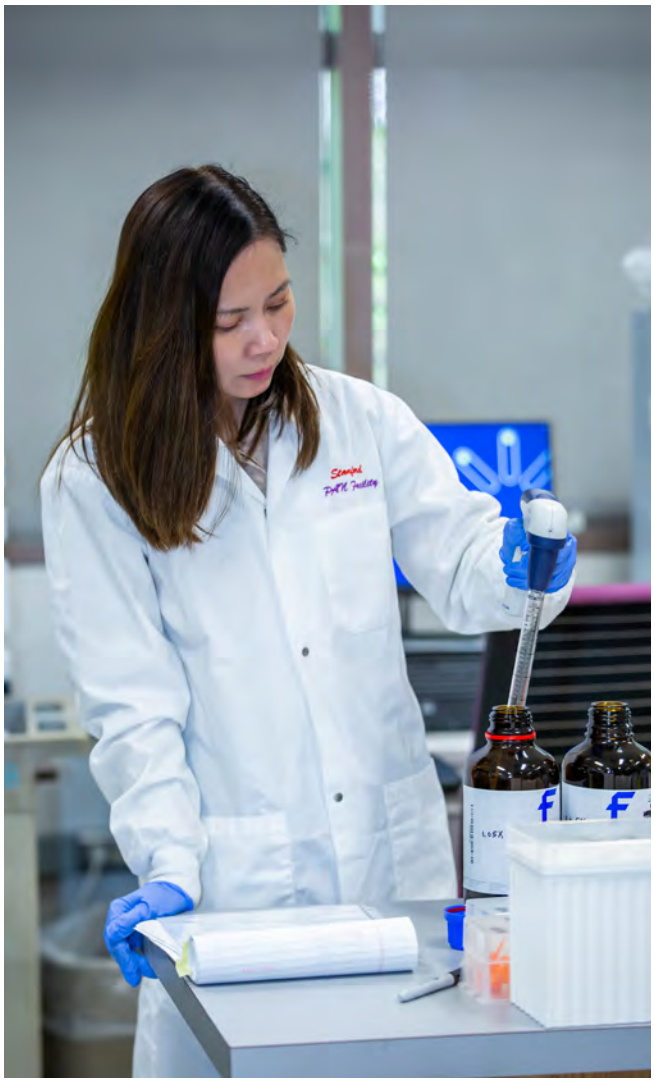
SPR is an optical technique used to study protein-protein and protein-ligand interactions in real-time; it is a key technology in support of our efforts to meet the post-genomic biological challenge of understanding the complex networks of interacting genes, proteins, and small molecules that give rise to biological form and function. Demand for biophysical characterization of protein and small molecule compound interactions continues to increase, and SPR technology has emerged as a powerful tool for hit identification, hit validation, and lead optimization. The technology is used extensively by researchers in drug discovery programs, such as Stanford's Innovative Medicines Accelerator program, centered in the School of Medicine, and in interdisciplinary life sciences institutes, such as the Sarafan Chemistry, Engineering & Medicine for Human Health (ChEM-H) institute.

Hit identification is achieved by screening libraries of chemical compounds to evaluate their binding affinity, specificity, and kinetics with target molecules, such as receptors, enzymes, or antibodies, using the two Biacore T200 instrument systems. Hit validation using SPR refers to the verification of the compound-target interaction, whether identified using SPR in the PAN Facility or in high-throughput screening campaigns. Lead optimization studies usually require in-depth binding characterizations, especially the measurement of kinetic parameters (kon and koff). Molecules with identical affinities for a target may display considerably different kon and koff values, which would probably be overlooked by traditional end-point assays and may ultimately influence the therapeutic performance *in vivo*. SPR technology also enables the identification of covalent or allosteric binders and is suitable for studying competitive inhibition as well. In addition to drug discovery, PAN's Biacore T200 instrument systems are also used extensively to understand protein-protein interactions for deciphering various cellular processes, as well as biotherapeutic antibody discovery and development.

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.

FUTURE VISION

The PAN facility will continue to support interdisciplinary scientific research within the Stanford scientific community, through its different technology platforms, technical expertise, and collaborative environment. This includes working closely with the other Beckman Center shared technology resources to advance the application of different technologies in the multi-omic sciences through investments in shared instrumentation and data resources. Along with support and access to the different technology platforms themselves, the PAN Facility will increase training by equipping Stanford researchers with scientific knowledge and practical skills, thereby investing in education, publications, and securing of grants, increasing collaborations and future scientific innovation.



Top: Jessica Tran; ***Bottom***: Brandon Lee

FLUORESCENCE ACTIVATED CELL SORTING FACILITY

ADVISORY COMMITTEE

Garry Nolan, Ph.D. (Committee Chair)
Mark Davis, Ph.D.
David Lewis, M.D.
Holden Maecker, Ph.D.
Lucy Shapiro, Ph.D.
Melody Smith, M.D.

OVERVIEW

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

In this technology, biological cells or particles are labeled with one or more fluorescent reagents, often antibodies, that detect specific molecules inside cells or on their surfaces. The cells are streamed through a sequence of laser beams and the resulting fluorescence intensities are measured on a per-cell basis. Flow cytometers can interrogate up to 35,000 cells per second and measure up to 40 parameters simultaneously. 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 simultaneously from a mixed population. In addition to the typical applications using mammalian cells, FACS is also valuable for work with yeast, bacteria, plankton, and other small particles.

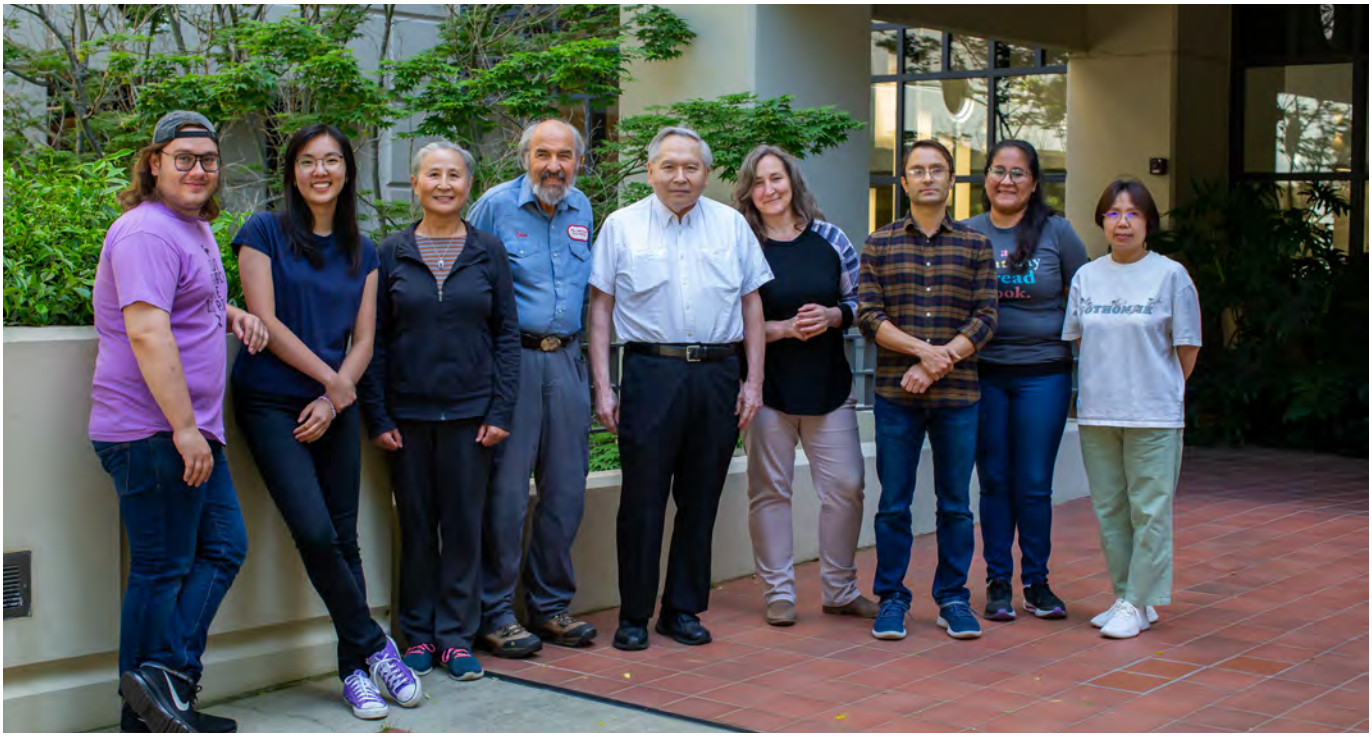
The Fluorescence Activated Cell Sorting Facility (facs.stanford.edu) in the Beckman Center has provided these

LISA NICHOLS, PH.D.
Director

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.

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



FLUORESCENCE ACTIVATED CELL SORTING FACILITY
From Left: Rudy Wycallis, Melody Wang, Cindy Jiang, Dave Parks, Tom Nozaki, Lisa Nichols, Suraj Timilsina, Bianca Gomez, Yanrong Zhang

education and provides training for users who want to become self-operators of the 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 20 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, experiment design, 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. The flow cytometry analyzers support sample acquisition from individual tubes, 96-well plates, or even 384-well plates.

The FACS Facility currently provides eight analyzers at the main campus facility, plus an additional instrument

at the Page Mill Road satellite facility to facilitate research at the School of Medicine Technology & Innovation Park. The full flow cytometer lineup includes instrumentation suitable for high-throughput screening assays, providing low-cost multi-plate assays, as well as multiple high-parameter analyzers. The High-Parameter Analysis Lab houses three 30-color, 5-laser cytometers and a spectral flow analyzer capable of 40+ parameter analysis. These state-of-the-art flow cytometers readily interrogate more than 30,000 cells per second, providing in-depth information to dissect the phenotypic and functional properties of complex cell populations.

Facility staff provide sample drop-off service for collection of data and basic analysis by the support staff, as well as technical training on the instruments for researcher-independent operation. The latter option enables round-the-clock instrument accessibility for experienced researchers.

Cell Sorting

Cell-sorting services include BSL-1 and BSL-2 sample handling, aseptic sorting, single-cell sorting into 96-well and 384-well plates (for cloning or for use in downstream single-cell RNA sequencing), and measurement and sorting using from one to more than 30 simultaneous fluorochromes to identify populations. Current sorters support sorting of up to six populations simultaneously. Sorting is either operator-supported during normal business hours or self-operated 24/7 upon completion of training. Eight sorters are available, each with different capabilities.

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. Instructional training videos and a protocols webpage are available as educational resources.

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.

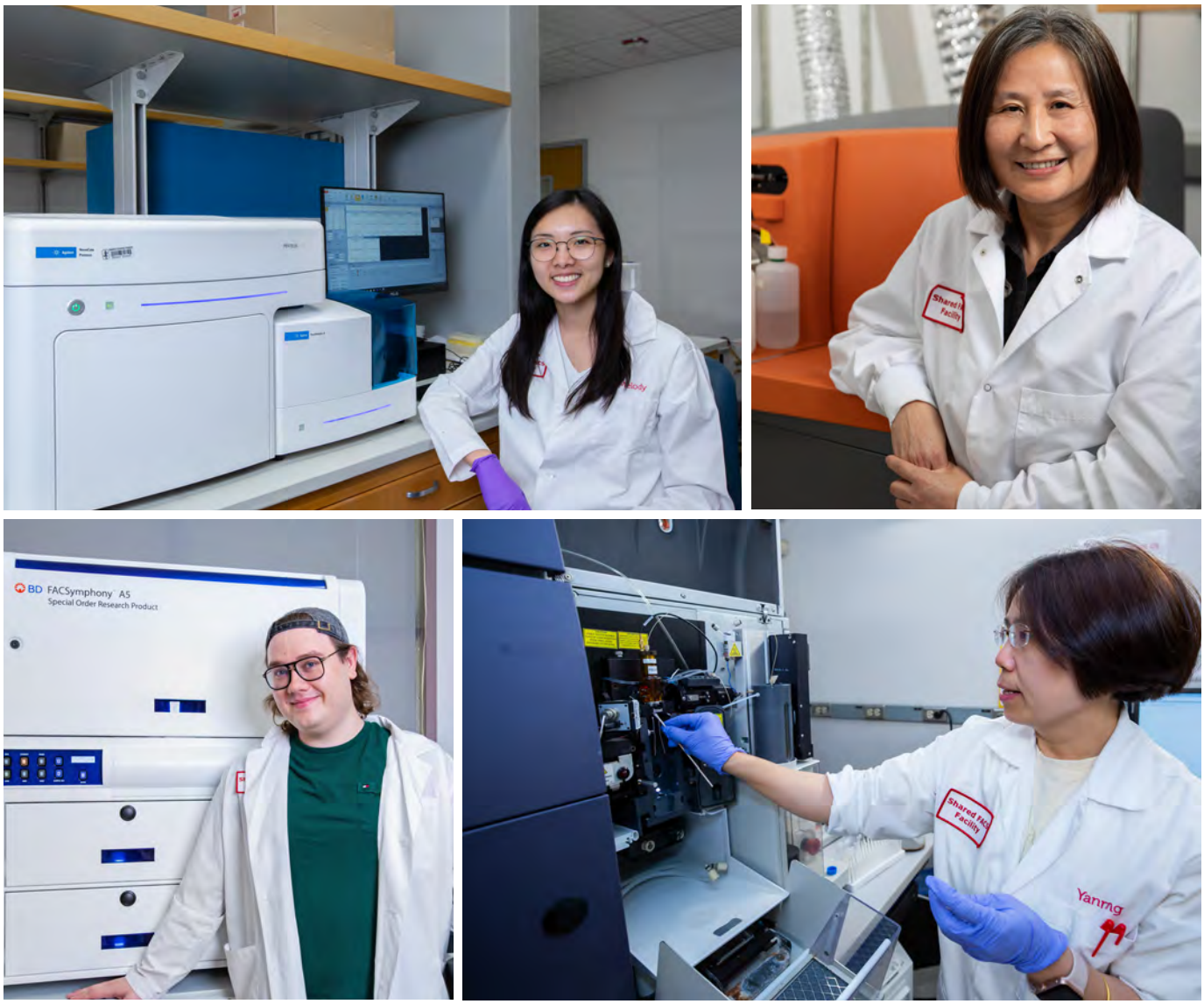
Data Management Services

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 also utilizes 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.

Guests attending a Get the FACS seminar.



Clockwise from Top Right: Cindy Jiang; Yanrong Zhang; Rudy Wycallis with the 5-laser, 29-color FACSymphony; Melody Wang with the 5-laser Agilent Penteon.

RECENT DEVELOPMENTS

This past year, the FACS Facility has continued to focus on education. This includes support for a new master's degree program in translational research and applied medicine, spearheaded by Dean Felsher, M.D., Ph.D., professor of medicine (oncology) and of pathology, and Joanna E. Liliental, Ph.D., director of the new master's program. The FACS facility provided the inaugural class with lectures on flow cytometry as well as cell sorting, and had students in two lab sessions to get hands-on experience with the instrumentation, as well as data evaluation and analysis.

Additionally, several educational seminars introducing cytometry basics to a broad university audience were provided during the year. These lectures provided an opportunity to not only teach, but also to gather the flow cytometry community at Stanford in an environment where common questions, obstacles, and solutions can be discussed in an collaborative environment. This seminar series, known as Get the FACS, has continued to be well received and plans are to increase frequency in the upcoming year.

In the interim, we have continued to support research efforts across the many labs that utilize the service center. In the High-Parameter Analysis Lab, we have collaborated

with research groups to push cytometry assay development to more than 35 color assays, including a 40-color optimized panel (under review). Additional large panels developed for the Symphony and Aurora analyzers are under development.

To support these advanced studies, the FACS Facility has welcomed two new staff members. Yanrong Zhang, Ph.D., is using her laboratory experience and knowledge to guide researchers in the development of their projects, and Suraj Timilsina, Ph.D., is focusing on creating educational materials to help researchers get up to speed quickly on technologies.

FUTURE VISION

Flow cytometry is a key technology for many areas of research, and the FACS Facility continues to serve more than 200 labs annually. We continue to research and test all new technologies as they emerge in the field. With a strong focus on expanding our educational and technical support outreach, the upcoming year will include added educational seminars. We look forward to increasing our in-person engagement with the research community at Stanford and the surrounding community, and continuing to provide a wide range of support.

Clockwise from Top Right: Suraj Timilsina; Melody Wang; Bianca Gomez; Yanrong Zhang

COMPUTATIONAL SERVICES AND BIOINFORMATICS FACILITY

ADVISORY COMMITTEE

Lucy Shapiro, Ph.D. (Committee Chair)
Onn Brandman, Ph.D.
J. Michael Cherry, Ph.D.
Michael Eckart, Ph.D.
Joshua Elias, Ph.D.
Jonathan Mulholland, MS
Mark Shepard, MPH

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 research labs and 36 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 at 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.

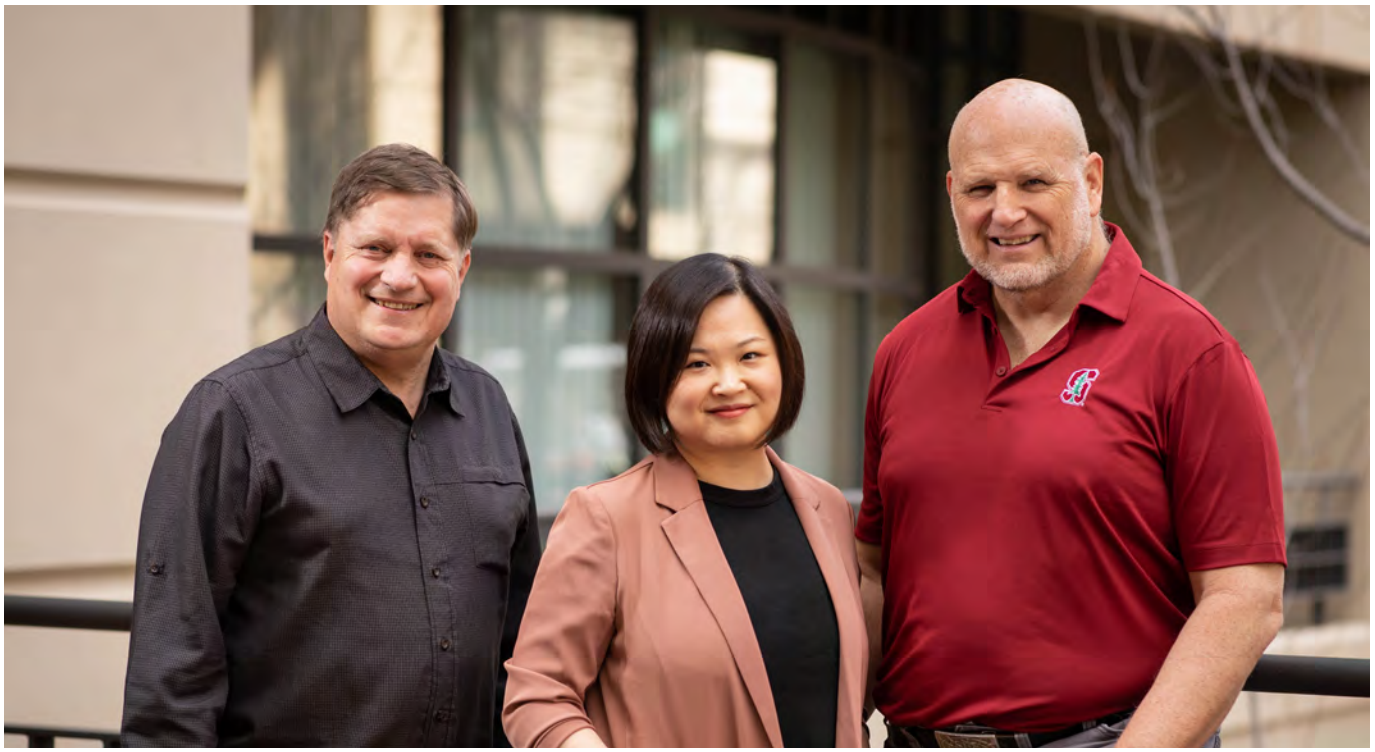
LEE KOZAR
Director

SERVICES

Available Software

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

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.



COMPUTATIONAL SERVICES AND BIOINFORMATICS FACILITY
Left to Right: Lee Kozar, Ling Xie, Alan Herbert

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 when a lab can afford to purchase its 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 (Partek)
- Genomics analysis (Geneious, Partek, iPathwayGuide)
- Mass spectrometry (Mascot, PEAKS, ProteinMetrics)
- Database (FileMaker, EndNote, Paperpile)
- Statistical and mathematical analysis (SPSS, Matlab, Systat, Mathematica, SigmaPlot, GraphPad)
- Graphics (Adobe Creative Cloud, BioRender)
- Microscope imaging (Volocity, Imaris)
- 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 in recent years, as many people had to work remotely due to the COVID-19 pandemic. A full list of the software offered by the CSBF can be seen at 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 often 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

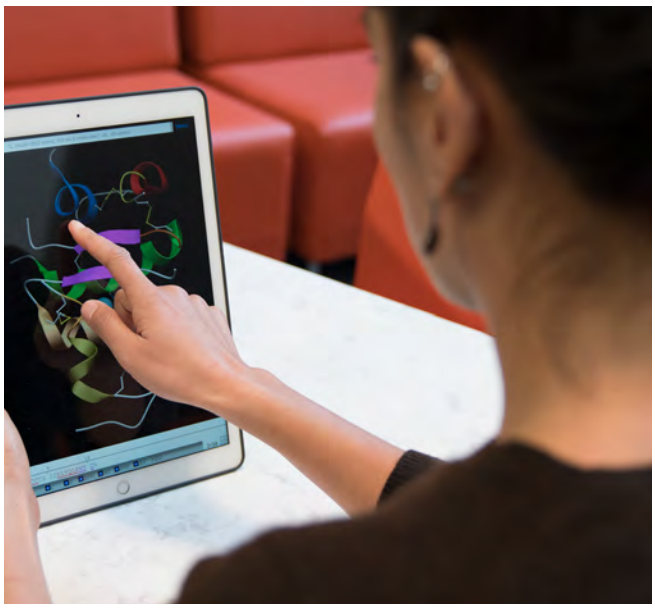
To access CSBF software, researchers must first obtain a CSBF membership. This can be done at csbf.stanford.edu/membership.

The CSBF has two levels of membership:

- A Level 1 membership gives everyone in a 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 lab access to all CSBF software, including the more expensive software packages such as iPathwayGuide, Imaris, Volocity, Partek, and others.

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 online at csbf.stanford.edu/membership/Level1.html and 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.



Top: Alan Hebert, facility user; ***Bottom:*** Ling Xie, Alan Hebert

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 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 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 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.

Lee Kozar in a CSBF server room.

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.

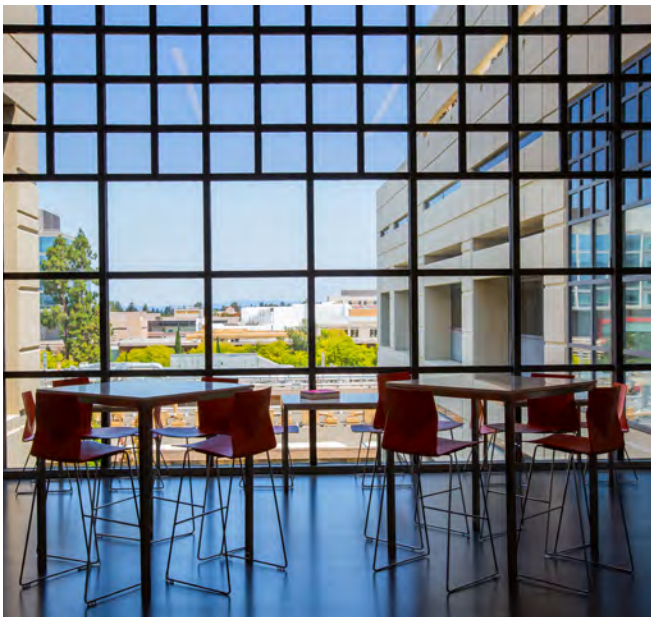
Two features of the department are especially noteworthy. First, members of the department share all of the space and major equipment. Thus, students and postdocs from different groups are intermixed. This enhances interaction at all levels and guarantees equality in terms of access to all resources 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.

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

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. **Alex Gao**'s lab is focused on understanding bacterial innate immune systems. **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. **Florentine Rutaganira**'s lab uses choanoflagellates (the closest living single-celled relatives to animals) and applies chemical, genetic, and cell biological tools to probe the mechanisms that these cells use to communicate.



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.

2022-2023 FACULTY HONORS, AWARDS AND APPOINTMENTS

Daniel Herschlag—professor of biochemistry, member of Stanford Bio-X and the Stanford Cancer Institute, and faculty fellow at the Sarafan Chemistry, Engineering & Medicine for Human Health (ChEM-H) institute, was elected to the American Academy of Arts and Sciences, which honors exceptional scholars who discover and advance knowledge and who apply knowledge to the problems of society.

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.

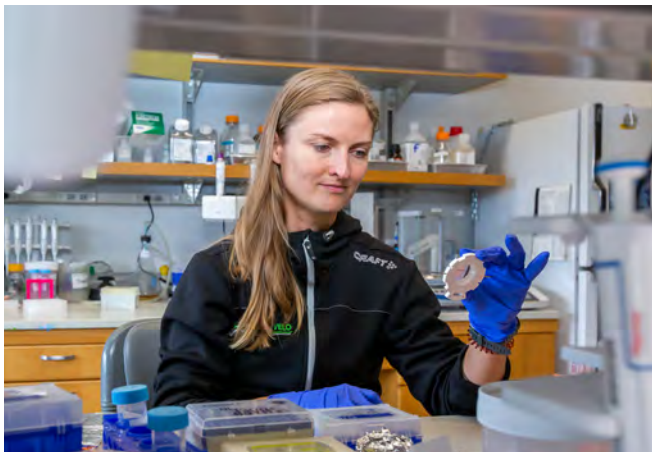
FACULTY RESEARCH

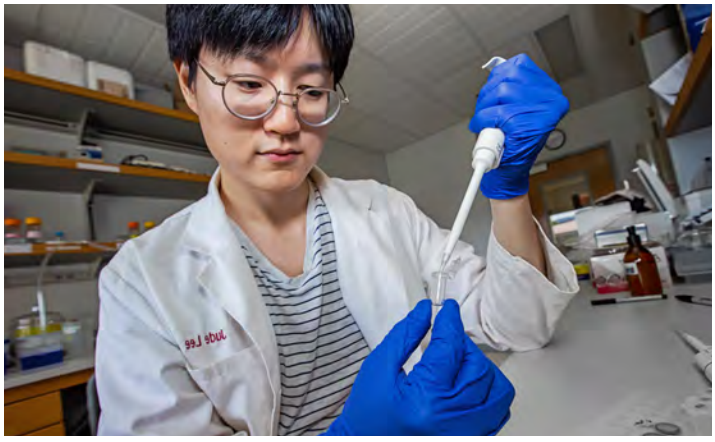
Philip Beachy's group studies the function of hedgehog proteins and other extracellular signals in injury repair and regeneration, primarily through effects on stem cell physiology. They also study abnormal signaling and perturbed stem cell physiology as it occurs in tissue disorder and in the formation and expansion of cancer stem cells. The members of **Gill Bejerano**'s lab focus on a fundamental question in humans genomics: the relationship between geno(me) type and phenotype. The group studies genome function in humans 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

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

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. **Lauren Goins** studies how multipotent stem cell lineages can maintain balance among cell fates and an ability to respond to physiological challenges imposed by environmental stressors such as infection and disease. **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

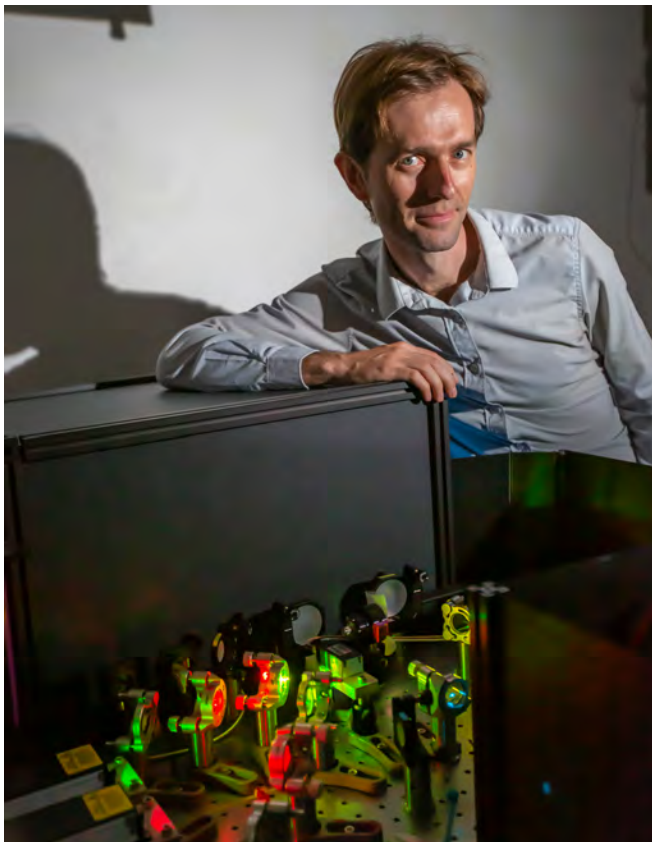
prevent Type 2 diabetes, and generate new therapeutic strategies for endocrine or exocrine pancreas cancers. **David Kingsley** is using a combination of genetic and genomic approaches to identify the detailed molecular mechanisms that control evolutionary change in vertebrates. **Kyle M. Loh**'s lab aspires to understand how different human cell types form from stem cells, and how developing tissues incipiently take shape and form. **Nicole M. Martinez** focuses on how modifications to both RNA-processing enzymes and RNA itself can alter the proteins encoded by that RNA and, in turn, cause





LAB OF ALISTAIR BOETTIGER, PH.D.
Clockwise from Top: Tonia Hafner; William Roman; Aleena Patel; Jude Lee; Sedona Murphy

disease. The lab studies RNA modifications, splicing, 3' end processing, and co-transcriptional processing. **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. **Florentine Rutaganira**'s lab uses choanoflagellates (the closest living single-celled relatives to animals) and applies



Left and Right: Alistair Boettiger

chemical, genetic, and cell biological tools to probe the mechanisms that these cells use to communicate. **Lucy Shapiro**'s laboratory studies the mechanisms used to generate the three-dimensional organization of a cell from a one-dimensional genetic code. The 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, remolding, 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.

2022-2023 FACULTY HONORS, AWARDS AND APPOINTMENTS

Lucy Shapiro—the Virginia and D.K. Ludwig Professor of Cancer Research, professor of developmental biology, and director of the Beckman Center for Molecular and Genetic Medicine, was awarded the Linus Pauling Medal for Outstanding Contributions to Science and presented the 49th Annual Linus Pauling Lecture at Stanford on February 28, 2023. The award is given to scientists whose research has contributed significantly to the field of chemistry and molecular biology. Dr. Shapiro's lecture, titled "The Chemical Logic of Life," elucidated her lifetime of work using *Caulobacter* as an experimental system for defining the bacterial cell as an integrated system.

Irving Weissman—professor of pathology and of developmental biology, and the Virginia and D.K. Ludwig Professor of Clinical Investigation in Cancer Research, was honored with the 2022 Wallace H. Coulter Award for Lifetime Achievement in Hematology from the American Society of Hematology. Dr. Weissman, the society noted, has made "monumental contributions to hematology over the past 56 years," and is best known for his work on hematopoiesis, leukemia, and hematopoietic stem cells.

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 members conduct 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/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

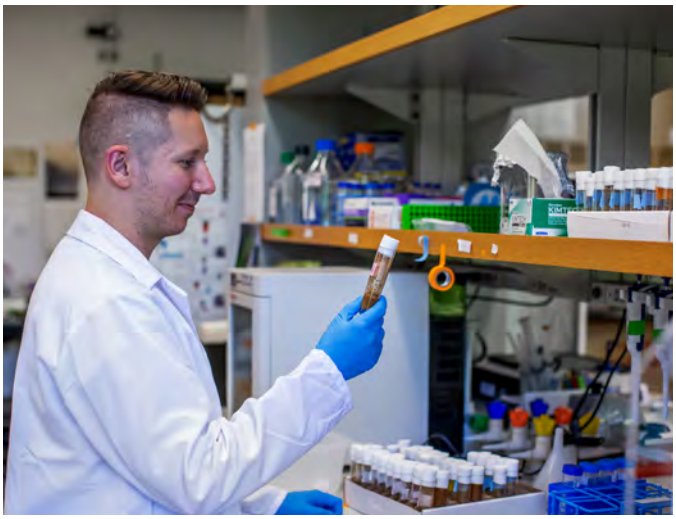
MIRIAM B. GOODMAN, PH.D.
Professor and Chair of Molecular and Cellular Physiology

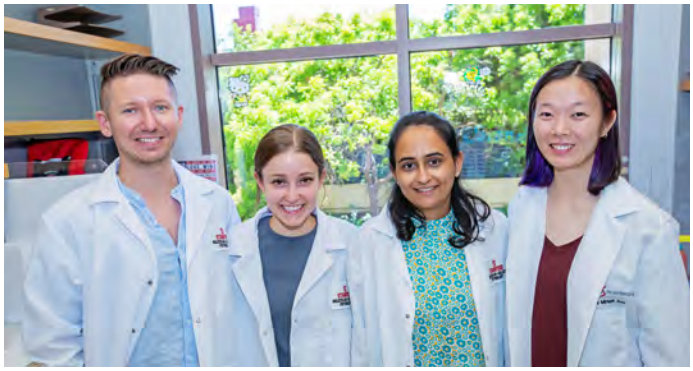
the structure, dynamics, and function of eukaryotic transport proteins that mediate ions and major nutrients across the membrane, the kinetics and regulation of transport processes, the catalytic mechanism of membrane-embedded enzymes, and the development of small molecule modulations based on the structure 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. **Ruth Huttenhain**'s lab focuses on exploiting quantitative proteomics to capture the spatiotemporal organization of GPCR-signaling

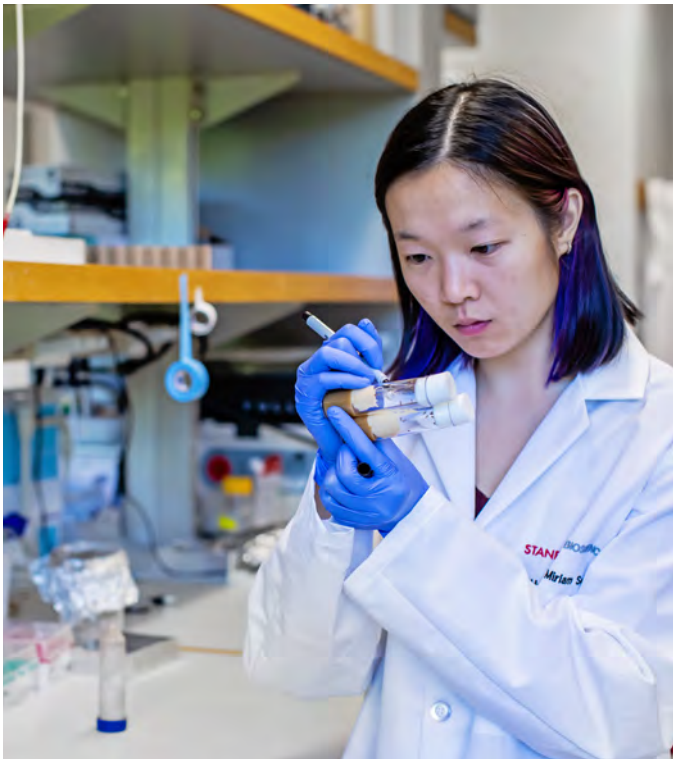
LAB OF BRIAN KOBILKA, M.D.
Clockwise from Top Right: Betsy White; Brian Kobilka; John Janetzo; Marina Casiraghi

networks combined with functional genomics to study their impact on physiology. **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. **Tino Pleiner** combines mechanistic cell biology, biochemistry, and protein engineering to dissect the pathways and molecular machines that mature human membrane proteins to a fully functional state. **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 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.







LAB OF LUCY O'BRIEN, PH.D.

Clockwise from Top Right: Jason Millington; Hsuan-Te (Miriam) Sun; Elsa Su; Aparna Sherlekar; Lauren Perry; (l-r) Jason Millington, Lauren Perry, Aparna Sherlekar, Hsuan-Te (Miriam) Sun

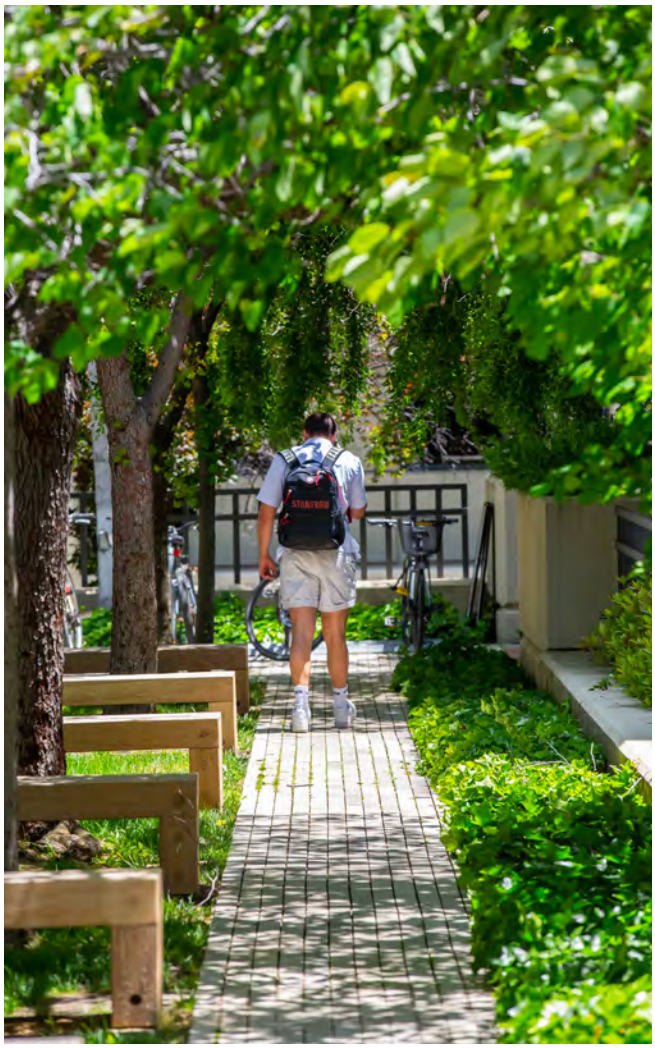
PROGRAM IN MOLECULAR AND GENETIC MEDICINE

2022-2023 FACULTY HONORS, AWARDS AND APPOINTMENTS

Carolyn Bertozzi—the Anne T. and Robert M. Bass Professor in the School of Humanities and Sciences, the Baker Family Director of the Sarafan Chemistry, Engineering & Medicine for Human Health (ChEM-H) institute, professor of chemistry, and a Howard Hughes Medical Institute investigator, was awarded the 2022 Nobel Prize in Chemistry. She shared the prize with Morten Meldal, a professor at the University of Copenhagen, and Karl Barry Sharpless, a professor at Scripps Research. The Nobel Prize in Chemistry is awarded by the Royal Swedish Academy of Sciences. Dr. Bertozzi was recognized for founding the field of bioorthogonal chemistry, a set of chemical reactions that allow researchers to study molecules and their interactions in living things without interfering with natural biological processes. Dr. Bertozzi's lab first developed these methods in the late 1990s and early 2000s. Since then, her lab and others have used them to answer fundamental questions about the role of sugars in biology, to solve practical problems, such as developing better tests for infectious diseases, and to create a new biological pharmaceutical that can better target tumors, which is now being tested in clinical trials.

Anne Brunet—professor of genetics and the Michele and Timothy Barakett Endowed Professor, won the 2022 Lurie Prize in Biomedical Sciences for her pioneering research in the mechanisms of aging. The Lurie Prize, awarded annually for the past ten years, recognizes researchers who are 52 or younger. The award, which comes with an honorarium of $50,000, will support Dr. Brunet's investigation into age-related diseases and the mystery of why we age.

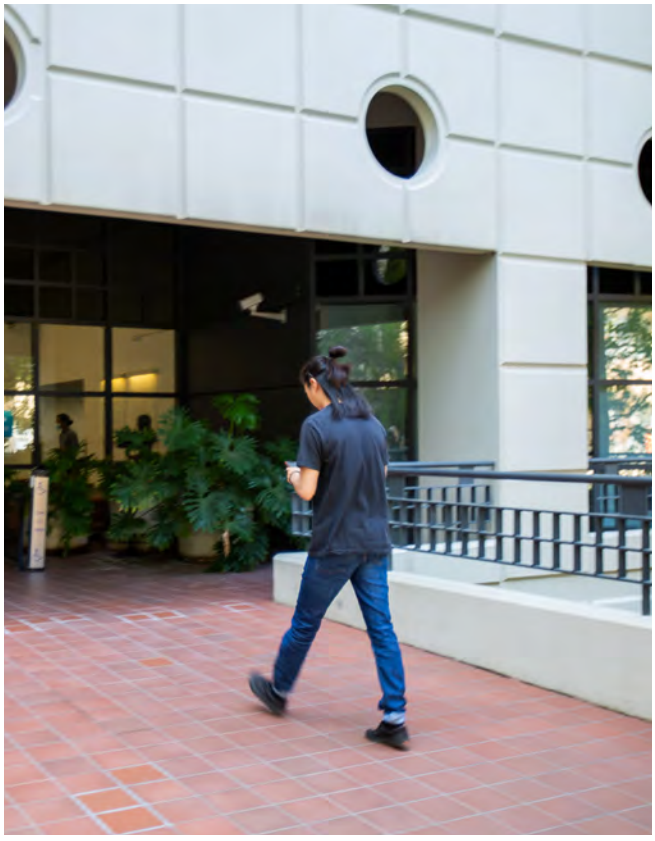
Karl Deisseroth—the D.H. Chen Professor, and professor of bioengineering and of psychiatry and behavioral sciences, was awarded the Japan Prize in



the field of Life Sciences for developing methods that use genetically addressable light-sensitive membrane proteins to unravel neural circuit function. The award honors scientists and researchers worldwide who have contributed significantly to the peace and prosperity of humankind through original and outstanding achievements.

Dr. Deisseroth was also named a recipient of the 2022 Louisa Gross Horwitz Prize for his foundational contributions to the advancement of optogenetics, a technology that has transformed neuroscientific research. The Horwitz Prize has been awarded annually by Columbia University since 1967, for groundbreaking work in medical science. Of the 108 previous Horwitz Prize winners, 51 have gone on to receive Nobel Prizes.

Eric Kool—the George A. and Hilda M. Daubert Professor of Chemistry, faculty fellow at Sarafan ChEM-H, and member of Bio-X, the Maternal & Child Health Research Institute, the Stanford Cancer Institute, and the Wu Tsai Neurosciences Institute, was elected to the National Academy of Arts and Sciences, which honors exceptional scholars who discover and advance knowledge and who apply knowledge to the problems of society.



Crystal Mackall—the Ernest and Amelia Gallo professor and professor of pediatrics-hematology and oncology, and of medicine, received the Edward Netter Leadership Award from the Alliance for Cancer Gene Therapy. The award recognizes an individual who has made unparalleled and groundbreaking research contributions to cell and gene therapy for cancer. Dr. Mackall was recognized for her role as a leader in research and development of gene-edited cellular therapies for cancer, and her lab's efforts to translate their use into solid tumors.

Dr. Mackall was also elected to the National Academy of Medicine for "pioneering immune therapies for children's cancers and for discovering fundamental principles of human immunology and translating these insights into cutting-edge engineered cell therapies for cancer."

Yvonne (Bonnie) Maldonado—senior associate dean for faculty development and diversity, the Taube Professor of Global Health and Infectious Diseases, and professor of pediatrics and of epidemiology and population health, was elected to membership in the American Academy of Arts and Sciences, which honors exceptional scholars who discover and advance knowledge and who apply knowledge to the problems of society.

Robert Malenka—the Nancy Friend Pritzker Professor of Psychiatry and Behavioral Sciences and the deputy director of the Wu Tsai Neurosciences Institute, was awarded the Peter Seeburg Integrative Neuroscience Prize for his work in identifying the mechanically distinct forms of synaptic plasticity present in the human brain.

Emmanuel Mignot—the Craig Reynolds Professor of Sleep Medicine, won a 2023 Breakthrough Prize in Life Sciences. He shared the prize with Masashi Yanagisawa of the University of Tsukuba, for discovering the causes of narcolepsy and paving the way for new treatments for sleep disorders.

Paul Mischel—professor of pathology and vice chair for research in the Department of Pathology, was elected to the National Academy of Medicine for "his paradigm-shifting research on extrachromosomal DNA, which has opened a new field in cancer biology with profound implications for non-Mendelian disease genetics and the impact of altered genome architecture." The academy noted that "his pioneering research has provided seminal insight into the molecular pathogenesis of brain cancer, revealing a landscape of actionable drug targets."

Michelle Monje—professor of neurology and neurological sciences, received the Richard Lounsbery Award for advancing understanding of pediatric brain cancers and the neurological effects of cancer treatments. The prize is given in alternate years by the National Academy of Sciences and the French Académie des Sciences to young French and American scientists, to recognize extraordinary scientific achievement in biology and medicine and to stimulate research and scientific exchanges between the U.S. and France.

Kari Nadeau—professor of medicine and of pediatrics, director of the Sean N. Parker Center for Allergy & Asthma Research, and the Naddisy Foundation Endowed Professor of Medicine and Pediatrics, was elected to the National Academy of Medicine "for leadership in studies of climate change and health, drawing on expertise in immunology, genetics, environmental sciences, allergy, and asthma." The academy noted that "her pioneering research that environmental exposures modify immune cell genes linked to health effects is leading to new policies as well as therapeutic and prevention strategies."

Anthony Oro—the Eugene and Gloria Bauer Professor of Dermatology, and co-director of the Stanford Center for Definitive and Curative Medicine and the Stanford Maternal & Child Health Research Institute, was elected to the National Academy of Medicine for "solidifying the first link between Hedgehog signaling and human cancer and building chromatin maps identifying how environmental factors drive tumor epigenetic plasticity and drug-resistance." The academy noted that "he built developmental chromatin maps to uncover disease mechanisms and enable clinical manufacturing of pluripotent cell-derived tissues for incurable skin diseases."

Lei (Stanley) Qi—associate professor of bioengineering and Institute Scholar of Sarafan ChEM-H, was inducted into the 2023 Class of the American Institute for Medical and Biological Engineering College of Fellows. Election to the college is among the highest professional distinctions accorded to a medical and biological engineer. Dr. Qi was nominated, reviewed, and elected by peers and members of the college "for contributions to the development and dissemination of genome editing techniques."

David Relman—the Thomas C. and Joan M. Merigan Professor and a professor of infectious diseases and of microbiology and immunology, was elected to membership in the American Academy of Arts and Sciences. Dr. Relman pioneered the modern study of the complex microbial community residing in and on the human body. A past president of the Infectious Diseases Society of America, he has advised the U.S. government on emerging infectious diseases, human-microbe interactions, and future biological threats. He is a member of Stanford Bio-X, the Stanford Cancer

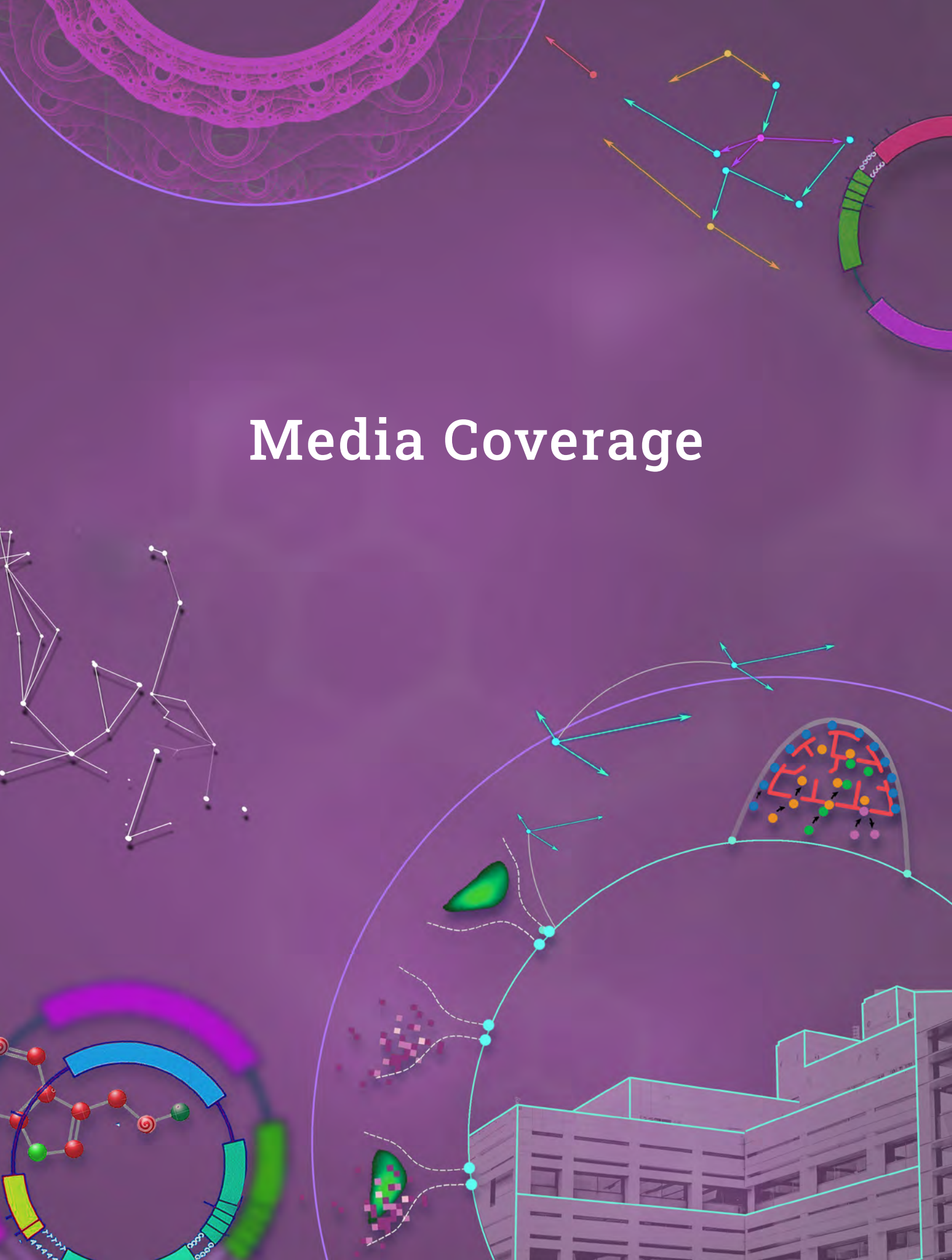
Institute, and the Stanford Child & Maternal Health Research Institute, and is a senior fellow of Stanford's Center for International Security and Cooperation.

Krishna Shenoy—professor of electrical engineering and the Hong Seh and Vivian W.M. Lim Professor of Engineering, was elected to the National Academy of Medicine for "making seminal contributions both to basic neuroscience and to translational and clinical research." The academy noted that "his work has shown how networks of motor cortical neurons operate as dynamical systems, and he has developed new technologies to provide new means of restoring movement and communication to people with paralysis."

Alice Ting—professor of genetics and of biology, was elected to membership in the prestigious National Academy of Sciences, at the academy's 160th annual meeting in April 2023. Dr. Ting creates technologies to map out cells and parse the signals and circuits that support cellular function. She draws from a variety of protein engineering techniques to identify, quantify, and manipulate molecules that may play critical roles in cell and animal behavior. She is developing methods to understand how different organ systems communicate with one another to affect different biological functions, and is exploring signaling in mitochondria and in the mammalian brain.

Joseph Wu—director of the Stanford Cardiovascular Institute, the Simon H. Stertzer, M.D., Professor, and professor of cardiovascular medicine and of radiology, was one of 169 distinguished inventors to be inducted into the 2022 National Academy of Inventors Class of Fellows. Election as an NAI Fellow is the highest professional distinction awarded to academic inventors.

Media Coverage



Media Coverage

The following articles were referenced in the 2022-2023 Highlights section.

"Durable, low-cost COVID-19 vaccine could help fill in gaps around the world"
Stanford Medicine News Center
April 17, 2023
https://med.stanford.edu/news/all-news/2023/04/covid-vaccine.html

"'Gentle' islet cell transplant cures mice of diabetes with few side effects, Stanford Medicine researchers say"
Stanford Medicine News Center
November 8, 2022
https://med.stanford.edu/news/all-news/2022/11/islet-transplant-diabetes.html

"Intermittent fasting spurs proliferation of liver cells in lab mice, Stanford Medicine-led study finds"
Stanford Medicine News Center
February 1, 2023
https://med.stanford.edu/news/all-news/2023/02/liver-intermittent-fasting.html

"Scientists find long-elusive structure of key cellular-signaling molecule"
Stanford Medicine News Center
March 10, 2022
https://med.stanford.edu/news/all-news/2022/03/signaling-molecule-structure.html

"Wild fish thrive despite 'hopeless monster' mutations, according to Stanford-led study"
Stanford Medicine News Center
September 1, 2022
https://med.stanford.edu/news/all-news/2022/09/stickleback-evolution.html

"Tiny DNA circles are key drivers of cancer, Stanford Medicine-led international study finds"
Stanford Medicine News Center
April 12, 2023
https://med.stanford.edu/news/all-news/2023/04/ecDNA-cancer.html

"Going to the dark(er) side: Stanford Medicine study shows how cancer gene tricks immune cells"
Stanford Medicine News Center
March 22, 2023
https://med.stanford.edu/news/all-news/2023/03/myc-cancer-bertozzi-felsher.html

CREDITS

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Photos provided by each grantee: Photos on pages 41, 42, 44, 45, & 47
Justin Lewis: Photos on pages 58, 62 (Photos of Kitty Lee and Ruth Yamawaki), 64, 69 (Photo of Jennifer Okamoto), 70 (Photo of PAN equipment), 72, 76 (Photo of Bianca Gomez), 77-79, 82, 84, & 87

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