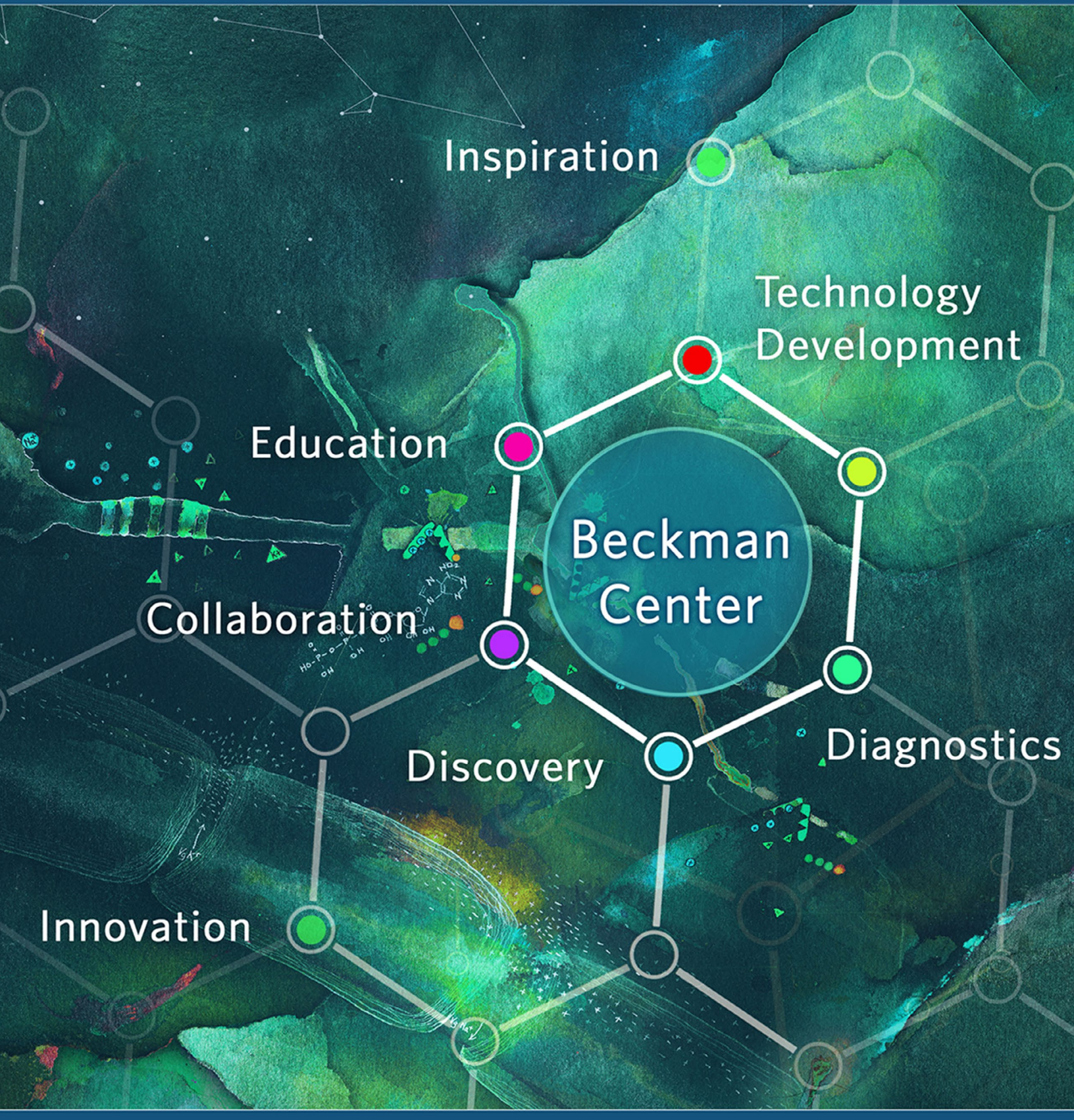
Stanford | Beckman Center
for Molecular and Genetic Medicine



2022 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,

Despite the persistence of the pandemic created by the SARS-CoV-2 virus, particularly the delta and omicron waves that hampered onsite gatherings and reduced foot traffic through the labs and service centers, 2021-2022 was nevertheless a very productive year for the Arnold and Mabel Beckman Center for Molecular and Genetic Medicine at Stanford University.

Among the many notable achievements, the center awarded five new Technology Development Seed Grants, recruited an outstanding new faculty member (and is in the final stages of recruitment for several more), and purchased new cutting-edge technologies for the service centers that will greatly advance research endeavors at Stanford. We have also renewed and expanded our educational programs, including a quarterly Beckman Center newsletter and two newly revived seminar series that provide consultation and hands-on training to young investigators in the application and use of the latest research technologies.

The five new Technology Development Seed Grants, awarded in the fall of 2021, drew from a pool of outstanding applicants from across the schools of Medicine, Engineering, and Humanities and Sciences at Stanford. The grants support the exploration of novel research ideas that have enormous potential for developing new and improved instruments or devices, or the development of new methodologies to be used in biomedical research and diagnostics. The grant recipients are:

- A team of researchers developing an internal systemic light source that would allow optogenetic screening of the gastrointestinal (GI) nervous system, thus revealing how different neuron subtypes contribute to GI functions
- A group developing an automated milli-fluidic system that would allow automated dissociation of tumor tissues into viable single cells that can be analyzed to gain insight into the pathophysiology of cancers
- A set of researchers using membrane protein biology and computational genomics to establish a new technology that enables efficient, scalable, and low-cost discovery for biologics that target membrane proteins
- A team of researchers developing a biofabricated pulmonary vascular tree graft that could offer vastly improved treatments for pediatric patients with aortopulmonary diseases
- A group using 3D fluorescence lifetime imaging microscopy (FLIM) to detect and characterize mitochondria containing extracellular vesicles, as a first step in designing novel heart failure therapies

Our new faculty member, Florentine Rutaganira, Ph.D., joined the Beckman Center in the fall of 2022 as an assistant professor of biochemistry and of developmental biology. Dr. Rutaganira did her Ph.D. in chemical biology working with Kevan Shokat, Ph.D., at the University of California, San Francisco, and her postdoctoral work with Nicole King, Ph.D., at the University of California, Berkeley. To understand how assemblies of cells signal to one another, Dr. Rutaganira is studying choanoflagellates (the closest living single-celled relatives to animals), which form complex colonial organizations through intercellular signaling. Using this experimentally tractable system, Dr. Rutaganira will apply chemical, genetic, and cell biological tools to probe the mechanisms that these cells use to communicate. The coordinated assembly of choanoflagellate colonies is thought to represent a precursor to animal multicellularity, and thus her work has wide-ranging implications for cell communication and organismal evolution. Dr. Rutaganira has also played an important role in advocacy for women and underrepresented minorities in STEM fields and is recognized for her excellence in mentorship.

The Beckman Service Centers have used the pandemic period to evaluate the need for new research technologies and to purchase and install new cutting-edge instruments. These new technologies, which might otherwise be too costly for individual researchers to acquire and operate, are now available and easily accessible to all investigators on the Stanford campus. The Cell Sciences Imaging Facility (CSIF) was awarded a Stanford Community of Shared Advanced Research Platforms (c-ShARP) grant totaling $295,000 for the purchase of a second CODEX microfluidics highly multiplexed imaging platform and related training costs. The CODEX multiplex imaging platform relies on antibodies conjugated to DNA barcodes, rather than directly to fluorescent markers. The barcodes can be successively bound and washed off, allowing multiple rounds of imaging—and the visualization of up to 40 or 50 different markers—in one single tissue. The technology is a boon to anyone who wants to study the spatial organization of molecules, including immunologists, cancer researchers, and neuroscientists. The Computational Services and Bioinformatics Facility received an anonymous grant of $17,000 to purchase a new server to support the new imaging system at CSIF. The Fluorescence Activated Cell Sorting Facility (FACS) purchased two additional 5-laser analyzers for its new High-Parameter Analysis Lab. A 5-laser Agilent Penteon with Auto-Sampler ($259,719) provides a second workhorse

instrument for screen assay from plates/tubes. A 5-laser, 29-color FACSymphony ($367,882) is a match for the Symphony purchased in 2020 with National Institutes of Health grant funds, which has already reached capacity. And lastly, a 50-parameter FACSymphony S6 spectral sorter ($814,340), with a biosafety hood, provides much-needed support for clinical, COVID-19, infectious disease, and other Biosafety Level 2 sorts. The Protein and Nucleic Acid Facility has likewise expanded its Surface Plasmon Resonance capabilities with the acquisition of a $368,984 Biacore T200 instrument, which offers researchers the opportunity to work confidently at the limits of kinetic, molecular weight, and concentration ranges, bringing improvements in data quality to a wide range of applications.

At the Beckman Center, we are dedicated to educational programs that allow leading scientists to share their cutting-edge research; we sponsor seminars and symposia on the latest research topics, and host seminars that disseminate and provide training in advanced research technologies. Our Feature Article this year describes some of these educational programs in more detail.

While some major onsite events have been postponed during the pandemic, the center has moved forward with new outreach programs, including a quarterly newsletter for occupants of the Beckman Center and other interested parties across campus, which aims to foster cohesiveness during this period of disruption. The first issue of the *Beckman Center News* was published in the fall of 2021. The newsletter provides updates on the status of all Beckman Center programs, along with topics of broad interest to all faculty, staff, students, and postdoctoral fellows at the Beckman Center. In addition, two of the Beckman Service Centers are in the process of restarting and expanding their training programs to researchers across campus in the application and use of our latest research technologies. CSIF will resume its What's the Scope? seminar series on advanced imaging technologies and FACS will resume its Get the FACS series on flow cytometry. We predict that these efforts to foster academic collaboration will extend to more normal times.

The pandemic years have been difficult for everyone. We are fortunate that our partnership with the Arnold and Mabel Beckman Foundation has allowed us to not only maintain our research programs, but to invest in the latest state-of-the-art technologies for the benefit of all participants. Without the foundation's support for our most challenging and innovative programs, we simply would not be able to sustain our consistent discovery and technological advances. We are grateful that the Beckman Foundation supports our and Dr. Arnold Beckman's vision of breakthrough science and look forward to working closely with the foundation in the coming year.

Sincerely,

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

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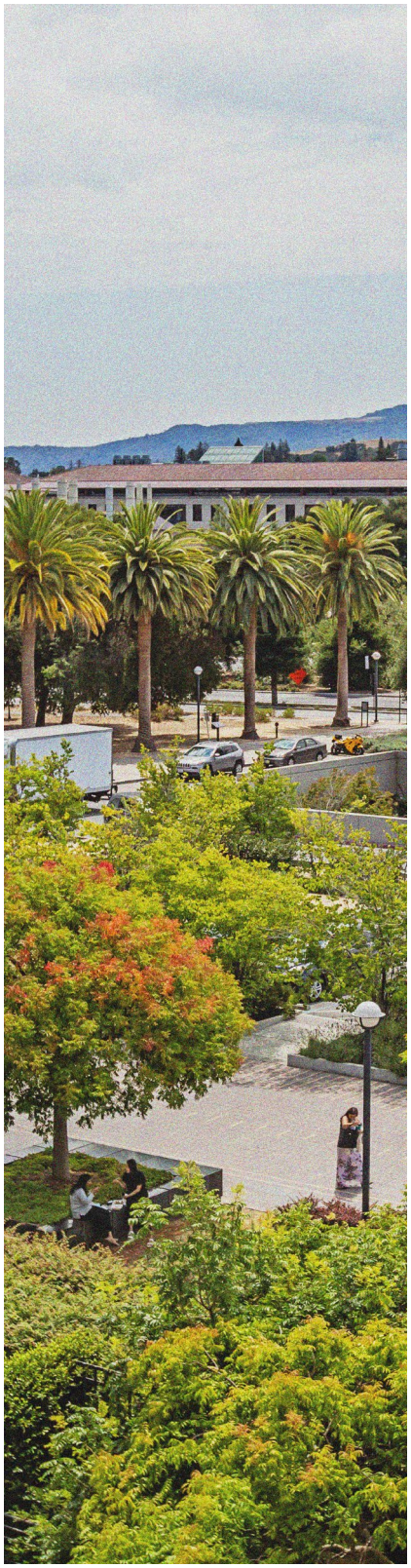
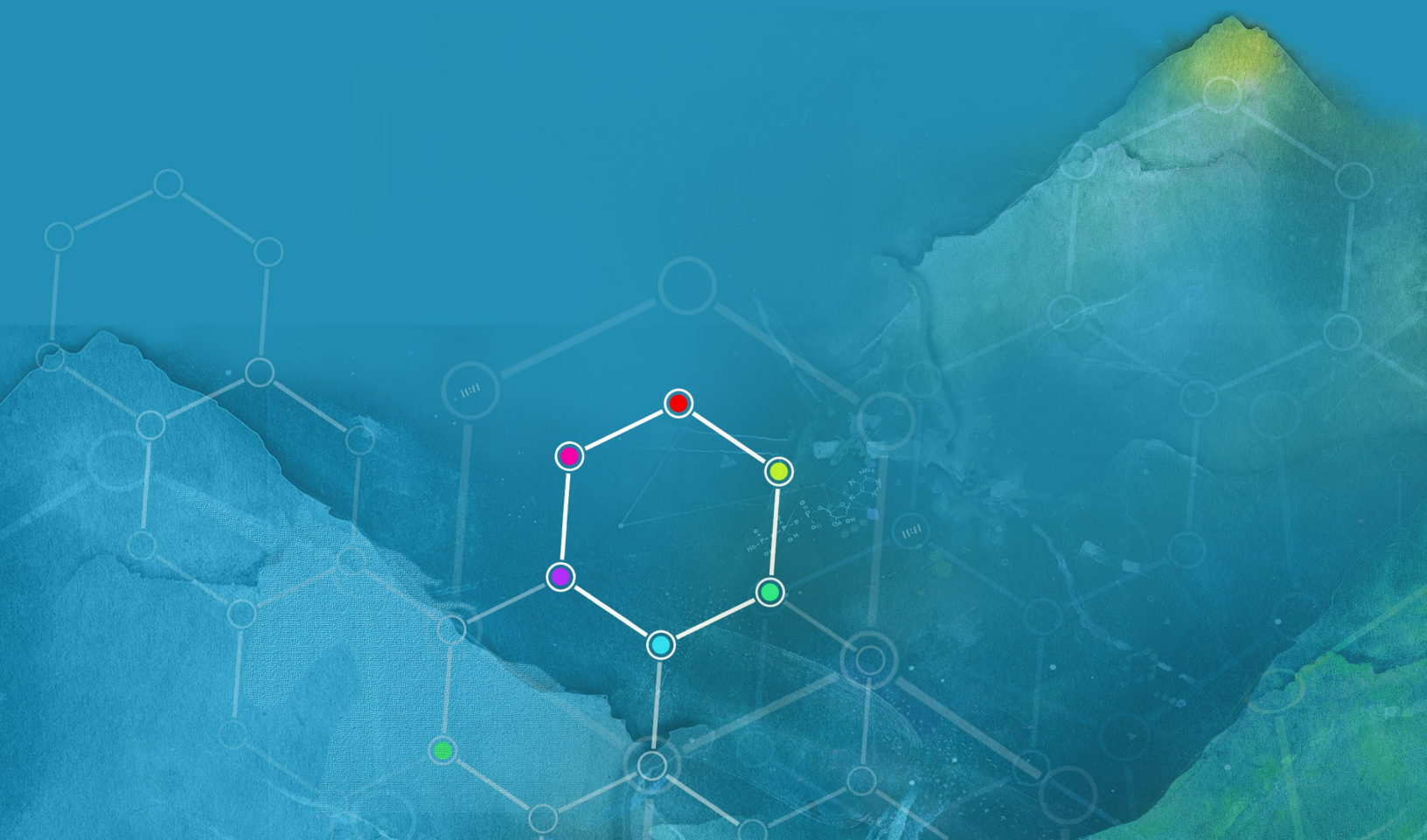


FEATURE ARTICLE



TO EDUCATE, TO LEARN, TO INSPIRE: BECKMAN CENTER EDUCATIONAL PROGRAMS HELP SCIENTISTS EXPLORE NEW IDEAS

By Sarah C.P. Williams

As they probe how the universe works, from the tiniest atoms to the vast reaches of outer space or the complexities of human cognition, scientists are quintessential lifelong learners.

Scientists have chosen a profession that lets them straddle the known and the unknown; to stay current in their fields they must, in many ways, never leave the classroom, never stop learning. And yet, for any one scientist to stay on the forefront of all scientific advances is impossible. So the best scientists don't just read manuscripts focused on their own areas of study—they turn to colleagues, mentors, and their own departments and institutions to continually explore new ideas across many disciplines.

The Arnold and Mabel Beckman Center for Molecular and Genetic Medicine plays a crucial role in such explorations for the scientists and students at Stanford University. Indeed, the mission of the Beckman Center is to "promote discovery and innovation in the basic sciences," as well as inspire and encourage interdisciplinary collaboration and accelerate the connections between the research bench and the patient bedside. To fulfill that mission, the Beckman Center has developed a diverse array of educational programs that help scientists and students stay on the leading edge of discovery.

"The purpose of our educational programs is to keep our faculty, staff, students, and postdocs aware of the most exciting new experimental approaches and scientific breakthroughs, and to enable them to use all of that to make their own research better," says Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research and director of the Beckman Center.

Through seminars, symposia, courses, and sponsored scholarships, the Beckman Center gives Stanford researchers and students a vast number of opportunities to learn about what is happening in the worldwide scientific community, from the latest experimental instruments and technologies to the most cutting-edge scientific theories. Each lesson they learn has the potential to enhance how they carry out their own research, making them better, more inspired scientists.

"Science is taking a lot of bricks and putting them on top of each other," says Dr. Shapiro. "Each nugget you take away from a talk or a class helps you build up and strengthen your own research, even if it's not obvious right away."

BECKMAN SERVICE CENTERS AS CLASSROOMS

For researchers across the Stanford community, some of the most valuable assets of the Beckman Center are its service centers, four specialized facilities that provide scientists with shared, state-of-the-art technologies. The 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)—offer equipment, software, expertise, and training. The expertise and training services take many forms: one-on-one consultations with staff scientists, training before a researcher uses new equipment for the first time, classes and lectures for Stanford students, and regularly scheduled seminars and speakers.

"With our service centers, we don't want to just provide a product for someone; we don't want to just hand them something they ordered and have that be the end of the conversation," says Dr. Shapiro. "We want them to become educated about what they need and why they need it, and how to design an experiment to get answers to the questions they're asking."

Researchers, of course, can turn to textbooks, colleagues, or the internet to learn the basics of an experimental approach, but the service centers offer something unique—in-depth, targeted, and personalized education on how to integrate the latest technology into one's own research.

"There are numerous YouTube videos out there," says Lisa Nichols, Ph.D., director of the FACS Facility. "If you want that, you know how to find it. That's not what we're trying to recreate."

Two seminar series sponsored by the service centers—Get the FACS and What's the Scope?—are especially focused on helping scientists make the best use of the advanced technologies at the facilities, as well as learn from other researchers.

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>
> — Lucy Shapiro, Ph.D.

GET THE FACS SEMINAR SERIES

Fluorescence activated cell sorting (FACS) lets researchers purify subsets of cells from a mixture, based on the cells' fluorescent labels—or the lack thereof. Getting useful and pure populations of cells requires not only an understanding of how the physical cell-sorting machinery works, but also how to design the best combinations of fluorescent tags and prepare cells for sorting. This complexity is why Dr. Nichols launched the Get the FACS Seminar Series.

Get the FACS rotates between a set of basic repeating topics that new FACS Facility users frequently need training in, plus other, more advanced, one-time sessions. But even the most basic "Intro to Flow" seminar can provide new insight for experienced FACS Facility users.

"We have had some of our very experienced scientists come listen to 'Intro to Flow' and learn something new that changes how they think about their experiments," says Dr. Nichols.

In one instance, she recalls, an advanced FACS Facility user did not recognize how using cells too large for the selected stream nozzle affected the sort until they heard it discussed in a seminar. When the scientist learned the symptoms, they realized that some larger cells had likely been disrupting the stream stability and droplet formation during their experiments, impacting their results. Dr. Nichols was able to explain how to fix the problem—a solution as easy as adjusting nozzle selection on the machine.

> "We have had some of our very experienced scientists come listen to 'Intro to Flow' and learn something new that changes how they think about their experiments."
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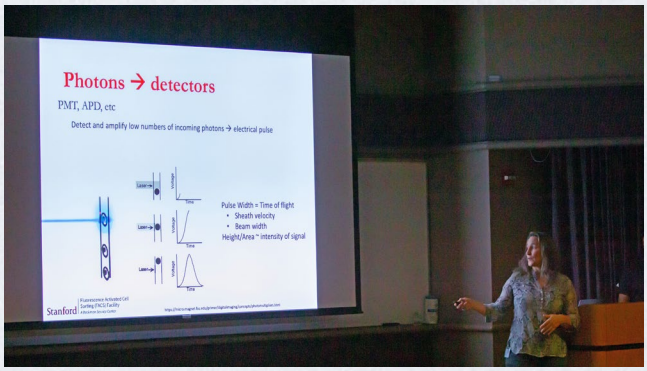

Top: Lisa Nichols; ***Bottom:*** Get the FACS seminar, September 2022.

All above: Attendees at a reception for a Get the FACS seminar, September 2022.

More advanced Get the FACS seminars sometimes delve into complex tools that push the limits of current cell-sorting technology. Most FACS experiments, for example, use several fluorescent markers to differentiate cell types. If a scientist wants to obtain more in-depth information, such as function, they often run multiple sets of sequential experiments. With the current cytometers available, however, such steps are often unnecessary. Dr. Nichols and her staff can teach advanced users how to analyze/sort using more than 30 independent measurements—simultaneously.

"That kind of experiment might give someone the same information, but save a lot of time and resources," she says.

The Get the FACS seminars also serve to bring together scientists tackling similar issues, which can lead to new solutions, collaboration, and comradery, Dr. Nichols says. "It's really been a great opportunity for users to not only network with us, but network with each other," she says.

Valuable conversations often emerge after the formal seminar presentation is completed, notes Dr. Nichols. At each event, scientists have an opportunity to ask questions that have been niggling at them or chat with others about how the FACS technology is or isn't working well for them. Often, they discover that other seminar participants have faced the same challenges or have tips on how to improve their experiments.

Those connections and conversations are why Dr. Nichols put the seminars on hold during the COVID-19 pandemic; so much of the value of the seminars is their in-person nature and the casual receptions that follow them, she says. For the last two years, prerecorded training videos and seminar slides were still available, and the FACS Facility staff members were available to answer questions. This fall, however, Dr. Nichols was happy to once again offer in-person seminars that can foster connections.

Dr. Nichols also plans to add new topics to the slate of lectures.

"We want to get to a point where our users are more comfortable working out all the kinks in not only their data collection, but their data analysis," says Dr. Nichols. "Right now, we often help users collect data, but then they disappear and there's no connecting back to see if their experiments worked or they need more help."

To that end, Dr. Nichols is hoping to launch small, advanced workshops on the analysis of FACS data; she also wants to collect more detailed information from scientists about other topics they want to delve into. She has also invited industry representatives to give educational talks about their FACS analysis software—the workgroups will provide data sets or have users bring their own data, and will walk them through analysis workflows.

To round out their educational mission—and reach students who may not yet be regular users of the center—the FACS Facility also supports two hands-on graduate courses that introduce science and engineering students to cell-sorting technology. Through those classes, Dr. Nichols says, she has a chance to pique students' interest about how FACS might help them in the future; she hopes they'll return later to learn more.

WHAT'S THE SCOPE? SEMINAR SERIES

At the Cell Sciences Imaging Facility, the What's the Scope? Seminar Series, similar to Get the FACS, rotates monthly through several topics that users of the facility might want to explore.

CSIF director Jon Mulholland echoes Dr. Nichols' thoughts on the high value of in-person seminars. Each seminar includes plenty of time for questions, as well as a meet-and-greet reception.

"We do have lots of website pages that people can go to if they want to learn about our different technologies, but the interactive part of our in-person lectures are really valuable," says Mulholland.

After a hiatus during the COVID-19 pandemic, Mulholland says he's gearing up to restart the What's the Scope? series. In the meantime, he's focused his attention on boosting other educational efforts at CSIF. In early 2022, he won funding through Stanford's Community of Shared Advanced Research Platforms (c-ShARP) program to not only buy new microscopes for CSIF, but also to cover the cost of graduate courses that use the facility. In the fall of 2022, Mulholland and his staff are teaching Biological Light Microscopy—in collaboration with Richard Lewis, Ph.D., professor of molecular and cellular physiology, and Gordon Wang, Ph.D., director of the Neuroscience Microscopy Service at Stanford's Wu Tsai Neurosciences Institute—as well as giving guest lectures in two electrical engineering courses.

"This is really essential for training the next generation of scientists, in terms of how to use the technology and how it's being applied to research," says Mulholland. "When they go off and start their own labs, I want them to be able to use microscopes appropriately for their experimental goals."

In the future, Mulholland plans to keep adding educational material to the CSIF calendar and website—microscopy is such a rapidly changing field that researchers must continually learn about new offerings, he says.

"It's evolving and changing so quickly; it's hard to keep the most cutting-edge instrumentation in the facility and it's certainly hard for researchers to stay current," says Mulholland.

John Perrino with the Leica EM ICE.

> "This is really essential for training the next generation of scientists, in terms of how to use the technology and how it's being applied to research. When they go off and start their own labs, I want them to be able to use microscopes appropriately for their experimental goals."
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> — Jon Mulholland

Jon Mulholland and Lucy O'Brien.

> You can accomplish a tremendous amount just by having fantastic researchers come together and become aware of what each other is working on.
>
> — Margaret T. Fuller, Ph.D.

SEMINARS BRIDGE FIELDS, SPARK IDEAS

To launch collaborative, translational research projects, scientists need more than just training in the latest instrumentation. They need to learn how to think like a scientist, as well as how to stay on top of what new approaches to scientific thinking are emerging, and what the latest breakthroughs are that might change their own focus. They need to explore beyond their own disciplines.

"More and more of the work in biomedicine is based on physics and chemistry, and involves large databases requiring innovative new ways of working out problems," says Dr. Shapiro. "At the Beckman Center, we want to help researchers have an interdisciplinary vision of the most important questions in the living world and how to tackle those questions."

To pave the way for this kind of vision, the Beckman Center sponsors three seminar series that bring researchers together to hear scientists—from Stanford and from around the world—describe their latest findings. The Frontiers in Biological Research Seminar Series, the Cancer Biology Seminar Series, and the Regenerative Medicine Seminar Series each feature lectures that fall under large umbrellas in terms of research topics. The sessions are purposefully wide-ranging, with the goal of teaching scientists about ideas and discoveries outside of their own realms of expertise.

"You can accomplish a tremendous amount just by having fantastic researchers come together and become aware of what each other is working on," says Margaret T. Fuller, Ph.D., the Reed-Hodgson Professor of Human Biology and professor of developmental biology and genetics, and an organizer of the Regenerative Medicine Seminar Series.

FRONTIERS IN BIOLOGICAL RESEARCH SEMINAR SERIES

> A lot of our faculty think the habit of going to talks is really something to cultivate. There are several senior members of our community who set a wonderful tone of lifelong learning.
>
> — Anne Villeneuve, Ph.D.

Stanford graduate students in the departments of Biochemistry, Developmental Biology, and Genetics have diverse academic coursework and research backgrounds, but they all share many things; not only do their scientific interests and techniques tend to overlap, they all must develop the same basic skills in analyzing literature, designing original research, and thinking critically. That's why first-year students in the three departments convene for a seminar-based course, Frontiers in Biological Research. The students—as well as interested members of the entire Stanford community—attend weekly seminars that bring speakers from across all three disciplines, and around the world, to share their work.

"Students come in and are exposed to a broad range of science, and this can help them think more broadly about what labs they might like to join or what approaches they want to take in their research," says Anne Villeneuve, Ph.D., professor of developmental biology and of genetics, and chair of the Department of Developmental Biology.

The afternoon before each seminar, students in the course meet to discuss a related manuscript—often a paper from the laboratory of the speaker, or a foundational paper in the field. In addition to making the students better prepared to learn from the talk the next day, Dr. Villeneuve says the class discussions impart valuable lessons on how to digest papers.

"Getting the students to learn how to critically read scientific literature is a really important educational goal," she says. "It doesn't necessarily have to be coupled to something like a seminar series, but we've found that it works really well for us and it keeps the material fresh for those of us who have been teaching the course for many years."

The pre-seminar discussions, Dr. Villeneuve adds, help the students prepare questions they might want to ask the speakers, either during the seminars or at the small discussion sessions that follow.

In recent years, Frontiers seminars have covered topics such as stickleback adaptation, protein-DNA interactions, membrane dynamics, and CRISPR genome editing—to name just a few of the many dozens of lectures. Speakers have come from within Stanford, but more often hail from other universities—some as far away as Europe or Asia. Attendance varies, Dr. Villeneuve says, but a wide range of faculty members, postdocs, and more senior graduate students usually attend in addition to students enrolled in the Frontiers course.

"A lot of our faculty think the habit of going to talks is really something to cultivate," says Dr. Villeneuve. "There are several senior members of our community who set a wonderful tone of lifelong learning."

At the height of the COVID-19 pandemic, the Frontiers seminars and corresponding course were moved online. While that allowed more flexibility for international speakers, Dr. Villeneuve says she's glad to see the series back in person for the fall of 2022.

"It's much more energizing to be there together in a room," she says.

CANCER BIOLOGY SEMINAR SERIES

Getting diverse researchers together in one room is a common theme for Beckman-supported seminars, and the Cancer Biology Seminar Series is no exception.

"Cancer biology is a pretty broad theme," says Julien Sage, Ph.D., the Elaine and John Chambers Professor of Pediatric Cancer and professor of genetics, and co-director of both the Cancer Biology Ph.D. Program and the Cancer Biology Seminar Series. "We have people in the Cancer Biology program from over twenty departments, ranging from very basic researchers to clinician scientists and medical residents. The seminar series is one way we strive to bring people together in this broad interdepartmental program."

First- and second-year Ph.D. students affiliated with the Cancer Biology program are required to attend a weekly Tuesday Cancer Talk Series. Some weeks, the Tuesday Talk is a casual presentation by one of the graduate students or a postdoctoral research fellow about their own research. But about a dozen times each academic year, the weekly talk features an invited speaker who shares recent advances in cancer research, as part of the Cancer Biology Seminar Series. These more formal talks by outside scientists are sponsored by the Beckman Center.

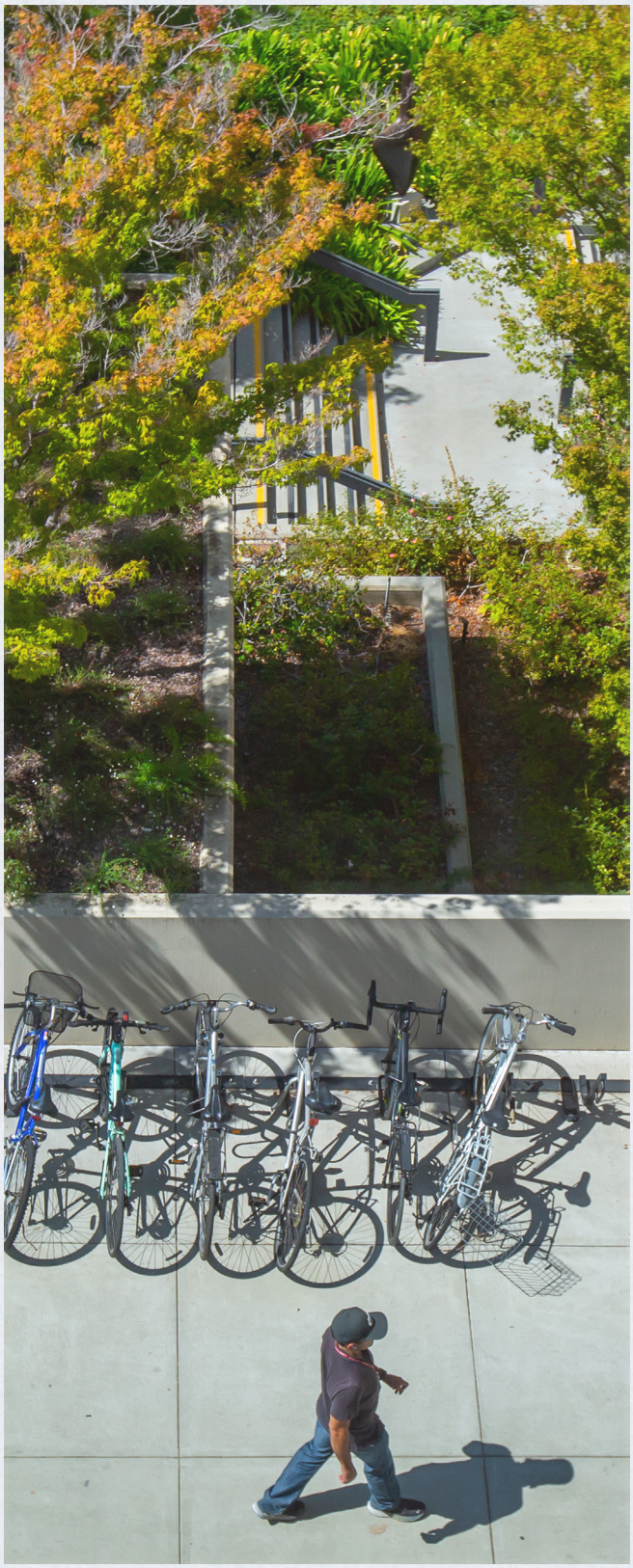
"To decide who to invite, we survey both students and faculty each year and compile an initial list of names," says Dr. Sage. "From there, we make sure we select a balanced group of speakers—East Coast and West Coast researchers, some junior faculty along with senior professors."

Like the breadth of the Cancer Biology training program, speakers range in their chosen topics—from basic science topics like single-cell epigenomics of cancer cells and the role of DNA repair proteins, to more clinical research topics on immunotherapy resistance and cancer vaccination.

Students are asked to help run the seminars—they introduce each speaker and then have lunch with them afterward, giving the students an opportunity to ask about not only science but their career trajectories.

"I think a critical part of it is that the students feel like they can ask a speaker about all these other things they might be wondering—How do you have a family and run a lab? How do you cope with moving far away for your career? How do you select a postdoc lab?" says Dr. Sage.

Faculty also get a chance to interact with invited speakers at breakfast and dinner on the day of the seminar, as well as schedule one-on-one meetings.



REGENERATIVE MEDICINE SEMINAR SERIES

While the focus of many seminars at Stanford is on outside speakers, the Regenerative Medicine Seminar Series (ReMS) puts just as much emphasis on the inclusion of speakers from within the university.

"I wanted to do something that reached beyond building boundaries, beyond school boundaries, beyond department boundaries, to bring together Stanford researchers doing related work who can learn from each other," says Dr. Fuller. "I think there is a tremendous opportunity for sparking new collaborations."

ReMS is held most Thursdays during the academic year, and often involves talks by two different speakers whose work—very broadly—falls under the general umbrella of regenerative medicine, which might mean anything from basic cell biology to tissue engineering.

"A lot of it is developmental biology, but it might also be transcriptional decisions; it might be cell surface interactions, it might be how do you wire a nervous system, it might be how do tissues interact with each other," says Dr. Fuller.

Dr. Fuller and her co-organizers try to select speakers who might not often be represented in a seminar series—such as new faculty members without a long list of publications—and pair up two researchers with complementary work. With such diversity in researchers, their backgrounds, and their scientific expertise, it is inevitable that attendees learn about new techniques and approaches by attending ReMS, says Dr. Fuller.

"I've invited people to talk in the series specifically because I knew they had a new technique that others might like to use," she says. "We had W.E. Moerner, Ph.D., the Harry S. Mosher Professor of Chemistry and a Nobel Laureate, talk about being able to visualize single proteins in cells because, wow, wouldn't people working on differentiating cells like to hear about that?"

During the COVID-19 pandemic, ReMS temporarily merged with the Cancer Biology Seminar Series, expanding the breadth of the science discussed even further. Researchers involved in each department realized the large overlap in topics between, in particular, stem cell biology and cancer stem cells.

"In cancer, you want to stop cells from growing, while in regenerative medicine you want to coax the cells to grow, and it's really two sides of the same coin," says Dr. Fuller.

As of the fall of 2022, the two series have once again returned to their normal—and separate—schedules, but Dr. Fuller suspects faculty and students affiliated with each topic will continue visiting each other's lectures.

> "I wanted to do something that reached beyond building boundaries, beyond school boundaries, beyond department boundaries, to bring together Stanford researchers doing related work who can learn from each other."
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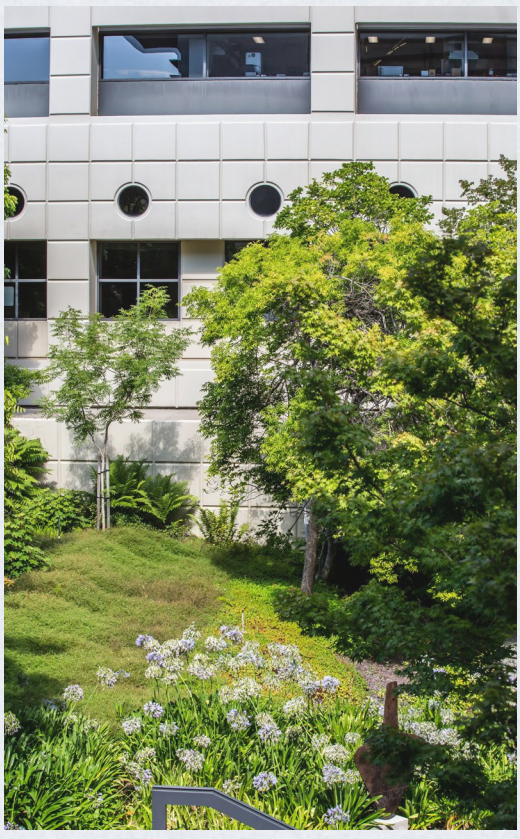


BECKMAN MEDICAL SCHOLARS FORGE CONNECTIONS

Not everyone with the potential to contribute to the goal of the Beckman Center—efficient, translational research—is housed in the center itself or belongs to one of the Beckman basic science departments. Physicians in the Stanford community are also vital to the bench-to-bedside pipeline, and training doctors how to work with more basic researchers can only enhance this kind of collaboration.

The Beckman Center Medical Scholars Program helps to fulfill this need. It provides funds to Stanford medical students who are carrying out translational biomedical research, allowing them to spend time during their medical training immersed in basic science labs.

"It works in two directions," says Dr. Shapiro. "The medical scholars learn to appreciate and use the results that are coming out of basic research, but at the same time, the basic scientists learn from our scholars—What are the most important questions and challenges in medicine right now? What are young physicians thinking about?"

Stanford is unique among U.S. medical schools in that all of its future doctors carry out research as part of a required Scholarly Concentration program. The scholarly work varies—some research revolves around social justice and health equity, global health, or quality improvement projects—but a significant portion of the medical students choose work in basic biomedical science.

"The idea behind this is really to provide an in-depth learning exposure for our students to develop critical thinking and get hands-on experience in a research area of their choice," says Daniel Bernstein, M.D., the Alfred Woodley Salter and Mabel G. Salter Endowed Professor of Pediatrics and associate dean for curriculum and scholarship at the School of Medicine.

Medical students can choose how to fit their research projects into the rest of their medical curriculum. Some still complete their degree in four years, but many add a year or two to their time in school so they can fully develop and carry out a more detailed longitudinal project. Funds awarded to two or three Beckman Center Medical Scholars each year allow those students to add this time without incurring more debt.

"A big question for students is how they manage the financial aspect of taking on these longer research projects," says Dr. Bernstein. "Medical school is expensive enough as it is."

Beckman funds not only provide financial help for the students selected each year, but also establish important ties between Beckman researchers and the next generation of clinician researchers, adds Laurence Baker, Ph.D., the Bing Professor of Human Biology and senior fellow at the Stanford Institute For Economic Policy Research.

"These students are future leaders in medicine; many of them will end up working in translational research from the clinical side for decades to come," says Dr. Baker. "We often see them carry their research forward over multiple years, even after they've left Stanford."

The training opportunity not only boosts their skills as researchers—it can also make them better doctors, more able to integrate the latest findings into how they treat patients.

"Having clinicians who have a good understanding of research and how to interpret medical developments is really valuable," says Dr. Baker.

> "These students are future leaders in medicine; many will end up working in translational research from the clinical side for decades to come."
>
> — Laurence Baker, Ph.D.

BECKMAN SYMPOSIA EXPLORE BIG IDEAS

If the goal of Beckman educational programs is to bring together a wide swath of the Stanford community to share ideas, brainstorm, collaborate, and learn about the future of science, then the crown jewel of this effort is perhaps the Beckman Symposium. Held on a fluid schedule—whenever ideas emerge from the community—these day-long symposia tackle large themes from many angles.

A recent Beckman Symposium, "The Revolution in Diagnostics," included presentations on how various approaches, from liquid biopsies to new imaging technologies, are changing medical diagnosis. Earlier symposia tackled diseases of the brain and the effect of emerging infectious diseases on global health, presaging a pandemic that has had a lasting impact on medical science.

"With our symposia, we've tried to incorporate all the latest and most exciting explorations in an area like diagnostics, genomics, or big data," says Dr. Shapiro. "We couple the future of where we're going in basic science with how it will help medicine and global health in the long run."

The fluctuating schedule of the symposia—some are held just a few months apart while others are spaced years apart—means that researchers don't force topics that aren't yet ready. Instead, symposia ideas emerge organically, as Beckman scientists start to notice an area that they want to discuss in more depth.

"It's a constant ongoing discussion among faculty in all the departments in the building," says Dr. Shapiro.

> "With our symposia, we've tried to incorporate all the latest and most exciting explorations in an area like diagnostics, genomics, or big data."
>
> – Lucy Shapiro, Ph.D.

WHERE SCIENCE MEETS ART

> I love to express my infinite wonder of the complexities and beauty of nature by combining fine art and scientific illustration.
>
> — Neil Murphy

The cover of this annual report is more than an eye-catching mash of colors and words; it's a look into the brain of artist Neil Murphy, who has painted and designed custom art for Beckman publications and symposia for more than 15 years. Look at the cover—or any art he's done for the Beckman Center—more closely, and details emerge that you might have missed at first.

At his home studio in San Carlos, acrylic paintings of neurons hang alongside sketches of tiny beasts. In many pieces, constellations and trap doors hide in the shadows. In some, he places detailed diagrams of scientific ideas on top of fluid, colorful fine art paintings.

The inclusion of Murphy's art in Beckman publications is not mere happenstance. Art that merges the beautiful with the scientific, and makes people look twice, is an aspect of scientific education, says Dr. Shapiro.

"The wonderful artwork that he does for us is a way of advertising and attracting attention," she says. "But it also stands for who we are; it conveys this excitement we have behind the science and puts our research into this broader context."

Murphy has been an artist since his Hawai'i childhood; he attended the San Francisco Art Institute in the 1960s, before shifting to a career in audio engineering, graphic design, and web development. But when his day job had him spending hours on Photoshop designing corporate websites, he started to miss art—and wondered what would happen if he combined traditional art with new digital tools. About 20 years ago, he started more seriously creating art again, using a technique that merged acrylic painting, ink, and Photoshop.

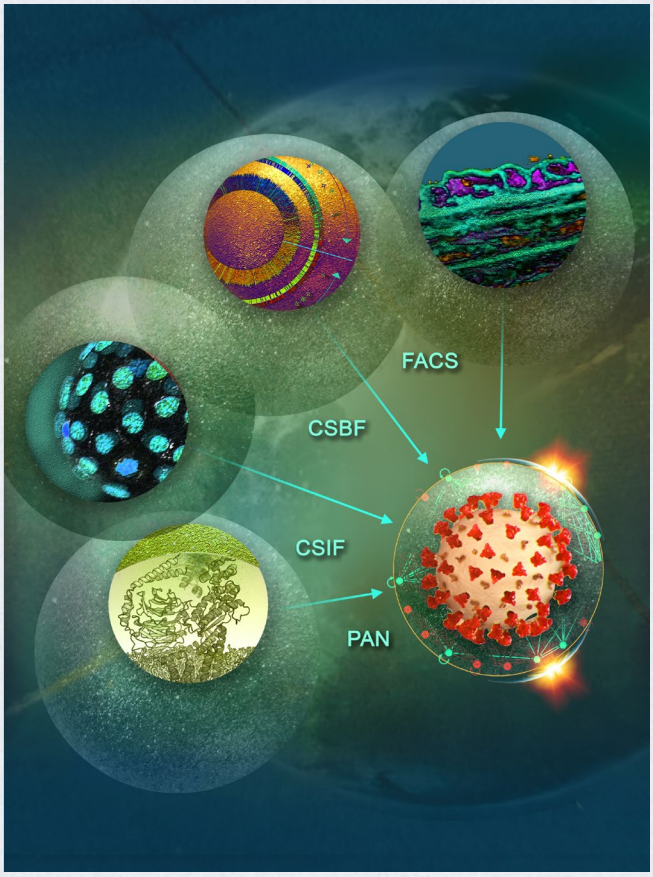
"With traditional media, if you want to change the color of an area, you have to paint over it," says Murphy. "But in Photoshop, you can highlight something and play with the colors. You can add layer after layer and then delete it if it doesn't work."

This image is adapted from the 2019 Beckman Center annual report cover designed by Neil Murphy.

At the same time, Murphy's absolute fascination with the natural and biological world drew him to scientific topics, and the blend of painting and digital manipulation let him easily capture both the fluid beauty of the world and the more concrete patterns of basic science.

"I love to express my infinite wonder of the complexities and beauty of nature by combining fine art and scientific illustration," he says.

Most of Murphy's projects—the cover of this report being no exception—begin with washes of acrylic paint flooding across a thin canvas. Slowly, wash after wash, an image builds up. Then, both on canvas and on screen, he adds shapes, lines, dots, diagrams, or words. But Murphy doesn't just have an eye for the attractive; his art is full of both metaphors for human biology and precise representations of science. A tiny creature outlined in ink might represent the stigma that people with neurological differences face, for instance.



2020 BECKMAN CENTER ANNUAL REPORT ARTWORK

"The 2020 annual report cover was inspired by the collaboration and coordination demonstrated by the four Beckman Centers as they worked together to find solutions to the Covid-19 pandemic." —Neil Murphy

2015 BECKMAN SYMPOSIUM ARTWORK: INNOVATION IN THE BIOSPHERE

"The 2015 Beckman Symposium brought together what may seem to be disparate biological fields of discovery, and showed how they overlap, influence one another, and unify shared knowledge to develop new treatments and understanding of biological processes." —Neil Murphy

NEIL MURPHY

Artist and designer

"When scientists look at my paintings, they're often just delighted by them," says Murphy. "They're able to see the real science—the ion channels, the molecular diagrams—but they also appreciate the chaos and the wonder and craziness that goes beyond the science."

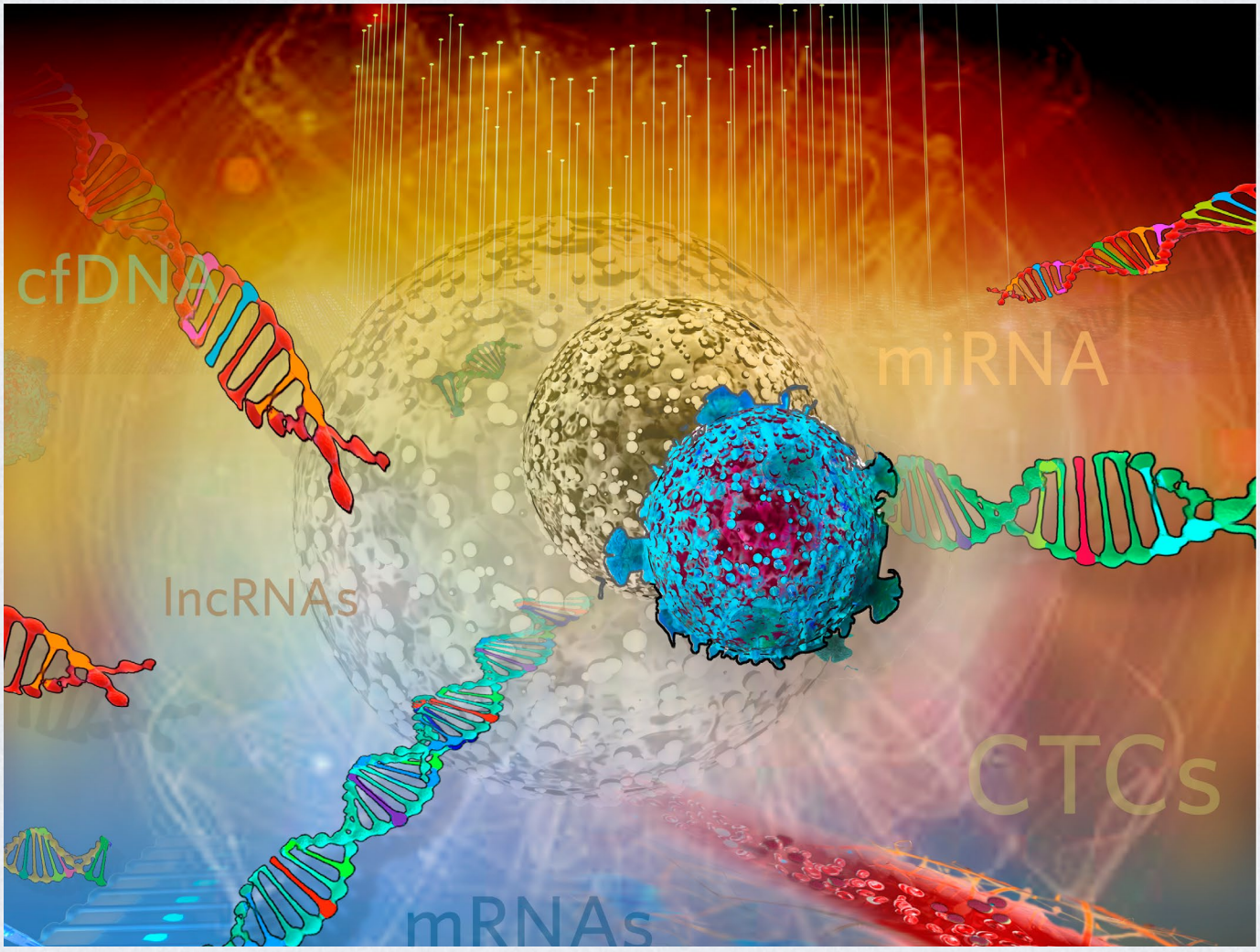
For each piece of Beckman symposia art, Murphy does extensive research; he not only talks to scientists who will present at the symposia, he also watches online lectures on related subjects.

"I try to just be a sponge and absorb a lot of these ideas about the research," says Murphy. "And then I get an idea and run with it."

There are similarities between the way Murphy works and the way Beckman scientists work—in both, ideas from different disciplines merge to captivate us and move us forward. Whether it is the combination of art and neuroscience, Photoshop and painting, new technology with traditional biological methods, or two fields of science, these innovative connections are what inspire and educate.

"Science is beautiful in and of itself and you could always find a striking microscopy image to use as art on its own," Murphy says. "By there's something about the juxtaposition of science and fine art that is fascinating and adds new dimension."

"The Beckman Center educational programs, with contributions from so many different people, disciplines, and venues, are enriching the scientific community at Stanford by helping current and future scientists to explore the universe of the living world," says Dr. Shapiro.



2018 BECKMAN SYMPOSIUM ARTWORK: REVOLUTION IN DIAGNOSTICS

"Diagnostics have become far more accurate, timely, and safe. In this symposium we learned, for example, that amniocentesis, which had required a needle biopsy with its safety concerns, could now be accomplished with a simple blood draw and genetic sequencing."
— Neil Murphy

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 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, as well as the Howard Hughes Medical Institute's local administrative offices. It has a world-class faculty of research scientists that includes three Nobel Laureates and 24 members of the National Academy of Sciences. The Howard Hughes Medical Institute oversees more than 20 investigators across campus.

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 advent of full genome sequencing of viruses, bacteria, protists, and mammals, powerful gene editing technologies, and breakthrough 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 that includes an ad hoc advisory committee chosen by the Beckman Center director and drawn from Stanford's School of Medicine, School of Engineering, and School of Humanities and Sciences, to evaluate Technology Development Seed Grant applications and assist with decision-making as needed. This year, the PMGM continued to support an exciting array of innovative programs, including:

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

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

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

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

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

2021–2022 HIGHLIGHTS

Despite the continuing limitations imposed by the COVID-19 pandemic, 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

New Technology Development Seed Grants Awarded

The Beckman Center awarded five new interdisciplinary Technology Development Seed Grants in the biomedical sciences. These grants support the development of new and improved instruments or devices, or the development of new methodologies to be used in biomedical research and diagnostics. Each seed grant provides funding of $100,000 per year for a two-year period.

Applicants to the Technology Development Seed Grant program are encouraged to submit proposals that have a disease focus, involve collaboration between basic and physician scientists, and have a translational medicine (bench-to-bedside) emphasis.

Twenty-three outstanding grant applications were submitted from 65 faculty members, representing 32 separate disciplines drawn from the schools of Medicine, Engineering, and Humanities and Sciences. The selection committee was composed of Beckman Center advisory committee members.

New Faculty Member Joins the Beckman Center

A new faculty member, Florentine Rutaganira, Ph.D., was recruited to join the Beckman Center. Dr. Rutaganira joined the departments of Biochemistry and Developmental Biology as an assistant professor in the fall of 2022.

Dr. Rutaganira obtained her Ph.D. in chemical biology from the University of California, San Francisco, where she trained with Kevan Shokat, Ph.D., developing chemical tools to probe signal transduction mechanisms in pathogens. She then did postdoctoral work with Nicole King, Ph.D., professor of genetics, genomics, and development at the University of California, Berkeley, examining how cell-cell communication drives complex organismal assemblies. Dr. Rutaganira's research uses choanoflagellates (the closest living single-celled relatives to animals) and will apply chemical, genetic, and cell biological tools to probe the mechanisms that these cells use to communicate. Her work has wide-ranging implications for cell communication and organismal evolution.

Beckman Faculty Member Discovers That Face, Brain Development are Tightly Linked

Joanna Wysocka, Ph.D., the Lorry Lokey Professor and professor of developmental biology and of chemical and systems biology, in collaboration with researchers at KU Leuven, a university in Belgium, have identified more than 70 genes that affect variation in both brain and facial structure. The genes don't influence cognitive ability, further debunking beliefs that intelligence can be assessed by facial features.

Although developmental biologists are used to thinking about the developing face as a receptacle for the embryonic brain—morphing and stretching as the growing brain pushes outward—it turns out that the face is an active participant in biological cross talk during development that affects the three-dimensional features of both structures.

"We were astonished to find 76 genetic regions that affect both face and brain shape in the human population," says Dr. Wysocka. "That's an amazing degree of overlap, and it shows how closely these two structures affect each other during development. However, nothing in our data suggests that it's possible to predict behavior, cognitive function, or neuropsychiatric disorders like schizophrenia or ADHD simply by looking at a person's face."

The reason is that these regions are not the same as those that determine brain structure in ways that affect cognitive function. It's an important distinction, if only to once again discredit the idea that a person's intelligence is reflected in their facial features—a belief that's been used to promote racial and ethnic discrimination.

Beckman Faculty Member Assists in Developing Algorithm That Predicts Biological Structures

Rhiju Das, Ph.D., associate professor of biochemistry, working in conjunction with postdoctoral fellow Andrew Watkins, Ph.D., and collaborators Ron Dror, Ph.D., associate professor of computer science, and postdoctoral fellows Stephan Eismann, Ph.D., and Raphael Townshend, Ph.D., used clever new machine

learning techniques to develop an algorithm that can determine the 3D shapes of biological molecules.

The algorithm designed by the researchers predicts accurate molecular structures and, in doing so, can allow scientists to explain how different molecules work, with applications ranging from fundamental biological research to informed drug design practices.

"Proteins are molecular machines that perform all sorts of functions," says Dr. Eismann. "To execute their functions, proteins often bind to other proteins. If you know that a pair of proteins is implicated in a disease and you know how they interact in 3D, you can try to target this interaction very specifically with a drug."

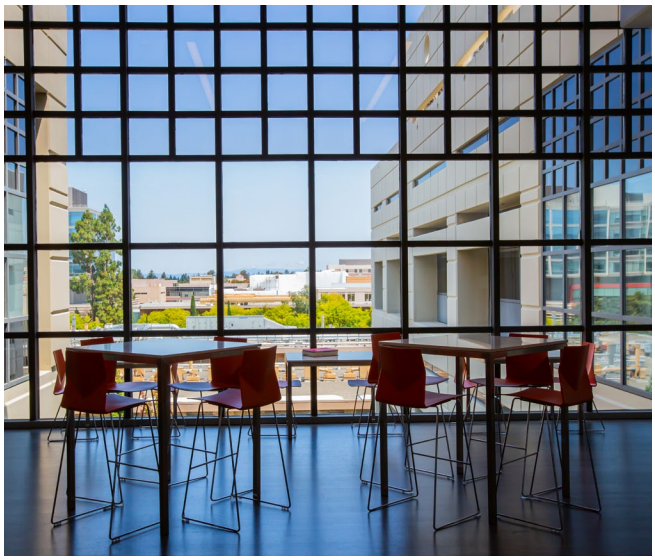
Having shown success with proteins, the researchers next applied their algorithm to RNA. They tested their algorithm in a series of "RNA Puzzles" from a long-standing competition in their field, and found that the tool outperformed all other puzzle participants and did so without being designed specifically for RNA structures.

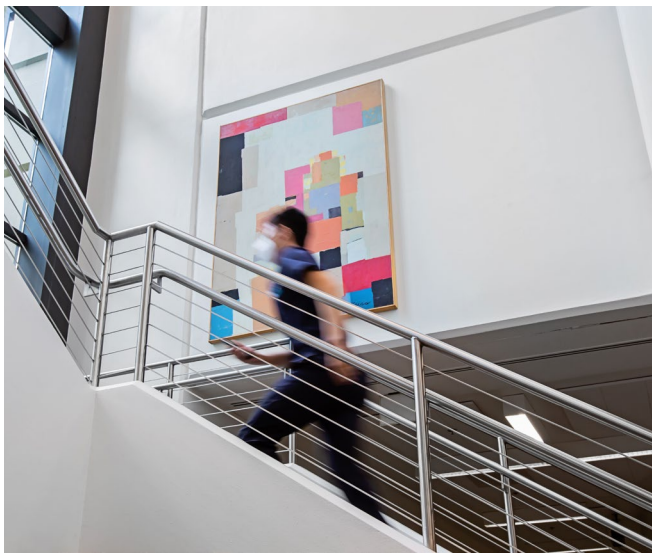
The researchers hope to see the new technology used to explore designing new molecules and medicines.

Beckman Faculty Member Develops Tool to Speed Up the Study of Enzymes

Daniel Herschlag, Ph.D., a professor of biochemistry who recently received the Stein & Moore Award from The Protein Society, working in collaboration with Polly Fordyce, Ph.D., assistant professor of bioengineering and of genetics, developed a new technique dubbed HT-MEK—short for High-Throughput Microfluidic Enzyme Kinetics. The new technique allows scientists to compress years of work into just a few weeks by enabling thousands of enzyme experiments to be performed simultaneously, and to deeply probe beyond the small "active site" of an enzyme where substrate binding occurs.

HT-MEK combines two existing technologies to rapidly speed up enzyme analysis. The first is microfluidics, which involves molding polymer chips to create microscopic channels for the precise manipulation





of fluids. The second is cell-free protein analysis, a technology that takes only those crucial pieces of biological machinery required for protein production and combines them into a fluid extract that can be used to create enzymes synthetically.

Using the new technology, it's possible to engineer thousands of variants of an enzyme in a single device and study them in parallel. It is now possible to systematically study how different modifications to an enzyme affects its folding, catalytic ability, and ability to bind small molecules and other proteins.

The adoption of HT-MEK could not only improve our basic understanding of enzyme function, but also catalyze advances in medicine and industry, the researchers say.

PMGM Faculty Member Determines Brain's Navigation Center Calls on Mental State as Well as Physical Environment

Research by Lisa Giocomo, Ph.D., assistant professor of neurobiology and a 2012 Beckman faculty recruit, and Isabel Low, a doctoral candidate in the Neurosciences Program working in Dr. Giocomo's lab, suggests that the earliest stages of memory formation may incorporate awareness of our surroundings as well as the internal context—such as emotions or thoughts—that mark those memories.

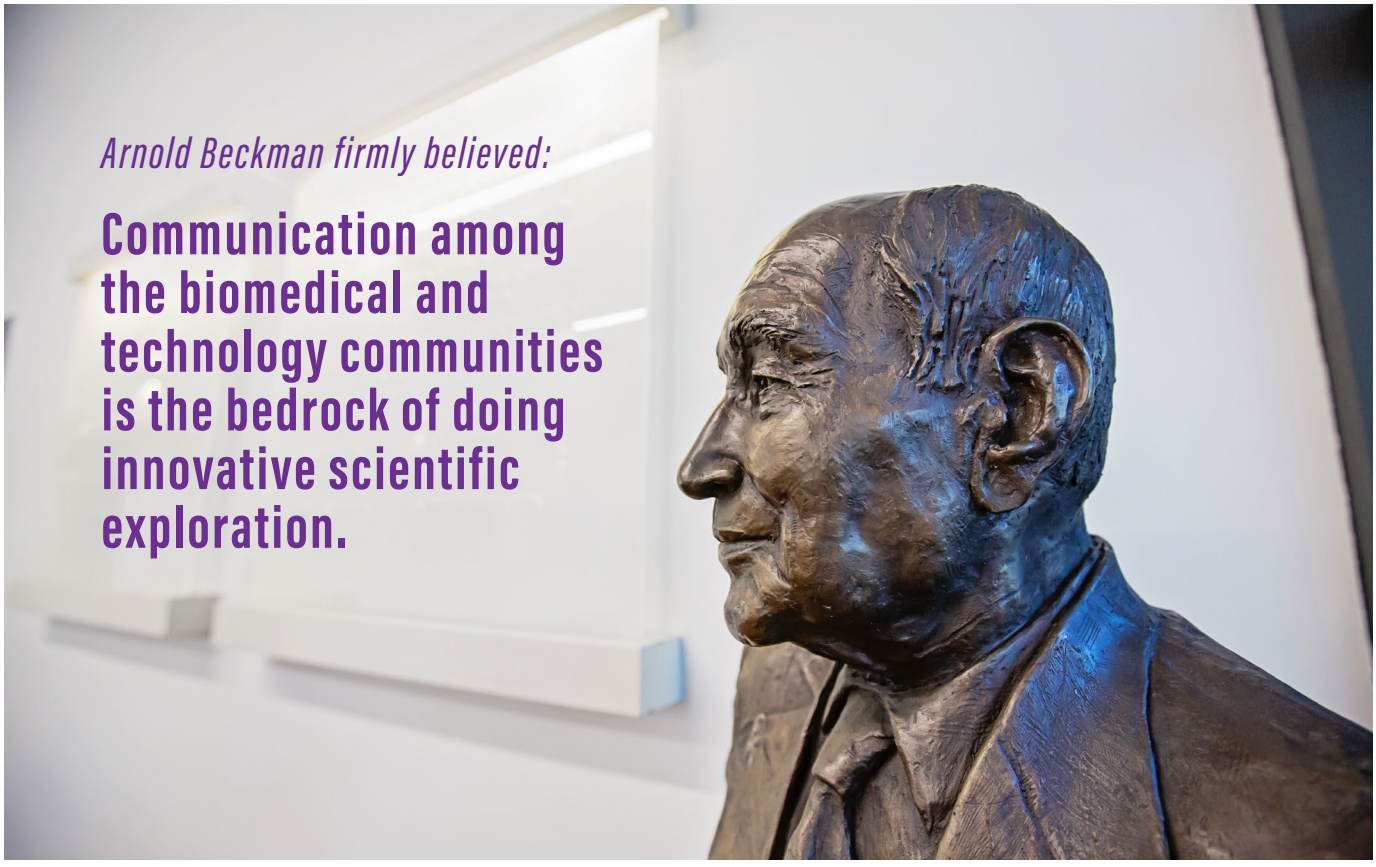
"The traditional view was that, like a GPS device in your car, the entorhinal cortex could get you to your favorite restaurant, but it didn't care if you were going because you're hungry, meeting a friend, or using it as a landmark on your way to somewhere else," says Dr. Giocomo. "That richer context was thought to be added later in the memory centers of the hippocampus." To see how the entorhinal cortex's spatial maps behaved in the absence of any environmental changes, the researchers studied mice, designing the animals' virtual space to be an infinite linear track with features that repeated about every four meters. To their surprise, the animals alternated between two distinct maps of the same place, and all in perfect coordination.

The researchers went on to demonstrate that the parts of the brain's memory circuits that care about a stable representation of space could ignore the distinctions between the alternating maps, while other parts of the circuit could extract the internally generated contextual information—such as goals or emotions—the maps represent.

"This is a new understanding of how an animal can maintain representations of the world that can be dynamically changing, but stable at the same time," says Low.

PMGM Faculty Member Details How Bacterial Cells Pack in Their Chromosomal DNA

A new study by Christine Jacobs-Wagner, Ph.D., a former postdoctoral fellow at the Beckman Center who is



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.

now the Dennis Cunningham Professor and professor of biology in the School of Humanities and Sciences, as well as a recently elected member of the National Academy of Sciences, reveals details about how bacterial cells manage to pack in their chromosomal DNA, which is a thousand times longer than the cells and—even more amazingly—to do so in a highly organized manner. The research offers new insights into the fundamental, yet poorly understood process whereby genomes fold up in a way that allows specially tailored access by certain biomolecules.

The study modeled how DNA as a polymer would behave when interacting with the cell's cytoplasm as a solvent.

Results showed that the nucleoid avoids becoming a jumble and instead attains organization, in part because of repulsive interactions between DNA and RNA. This apparent repulsive interaction causes DNA to spread out in areas where the genes that make RNA are active and RNA is abundant, and scrunch up in areas where RNA is scarce. As a result, in sections of DNA where the genes that make RNA are particularly active, the nucleoid has a bigger mesh size, conveniently making it easier for ribosomes to access and assemble on the RNAs emerging from these active DNA regions. In contrast, in DNA regions where genes are not transcribed frequently, the nucleoid is dense, making these active regions less accessible. Overall, this delicate interplay between biomolecules forms a super-squished, yet structured, nucleoid.

"This study just goes to show that there is still a lot about cells we don't know," says Dr. Jacobs-Wagner. "How a chromosome gets folded inside a cell in such a precise, organized manner is really fundamental to life, but is not something we fully understand."

PMGM Faculty Member Discovers Entirely New Kind of Biomolecule

Carolyn Bertozzi, Ph.D., the Anne T. and Robert M. Bass Professor in the School of Humanities and Sciences, the Baker Family Director of Stanford ChEM-H, and a Howard Hughes Medical Institute investigator, in collaboration with Ryan Flynn, M.D., Ph.D., a former postdoctoral fellow in her lab who is now an assistant professor at Boston Children's Hospital, discovered a new kind of biomolecule that could play a significant role in the biology of all living things.

The novel biomolecule, dubbed glycoRNA, is a small ribbon of ribonucleic acid (RNA) with sugar molecules, called glycans, dangling from it. Up until now, the only kinds of similarly sugar-decorated biomolecules known to science were fats (lipids) and proteins. Glycolipids and glycoproteins appear ubiquitously in and on animal,

plant, and microbial cells, contributing to a wide range of processes essential for life.

"This is a stunning discovery of an entirely new class of biomolecules," says Dr. Bertozzi. "It's really a bombshell, because the discovery suggests that there are biomolecular pathways in the cell that are completely unknown to us."

"What's more," Dr. Bertozzi adds, "some of the RNAs modified by glycans to form glycoRNA have a sordid history of association with autoimmune diseases."

The presence of glycoRNAs in different organisms suggests they perform fundamentally important functions. Furthermore, the RNAs are structurally similar in creatures that evolutionarily diverged hundreds of millions to billions of years ago. This suggests glycoRNAs could have ancient origins and may have had some role in the emergence of life on Earth, explains Dr. Bertozzi.

SERVICE CENTER HIGHLIGHTS AND TECHNOLOGY UPGRADES

Cell Sciences Imaging Facility

The Cell Sciences Imaging Facility (CSIF) received grant funding in the amount of $265,000 from Stanford University's new Community of Shared Advanced Research Platforms (c-ShARP) initiative (PI, Jonathan Mulholland, director of the CSIF). The award was for the purchase of a second CODEX microfluidics highly multiplexed imaging platform, with additional funding for an Opentrons robotic automation device and Visiopharm AI software. The availability of this second CODEX platform with automation and AI-enhanced software will double the throughput of the facility's spatial proteomics services. In addition, to further support the CSIF's educational mission, c-ShARP awarded the facility $30,000; those funds will support experiential learning on the CSIF's advanced microscopes. Lastly, the School of Medicine's small equipment funding program awarded the facility $50,000 for a Leica, Inc., Ultracut UC7 ultramicrotome. The new ultramicrotome will be used to cut ultrathin (50-100nm) sections for electron microscope imaging.

Protein and Nucleic Acid Facility

The Protein and Nucleic Acid Facility (PAN) has expanded its surface plasmon resonance capabilities with the acquisition of a $368,984 Biacore T200 instrument system. The new system offers researchers the opportunity to work confidently at the limits of kinetic, molecular weight, and concentration ranges, bringing improvements in data quality to a wide range of new applications.

The Protein and Nucleic Acid Facility has also implemented the BD Rhapsody Single-Cell Analysis System, which 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.

Fluorescence Activated Cell Sorting Facility

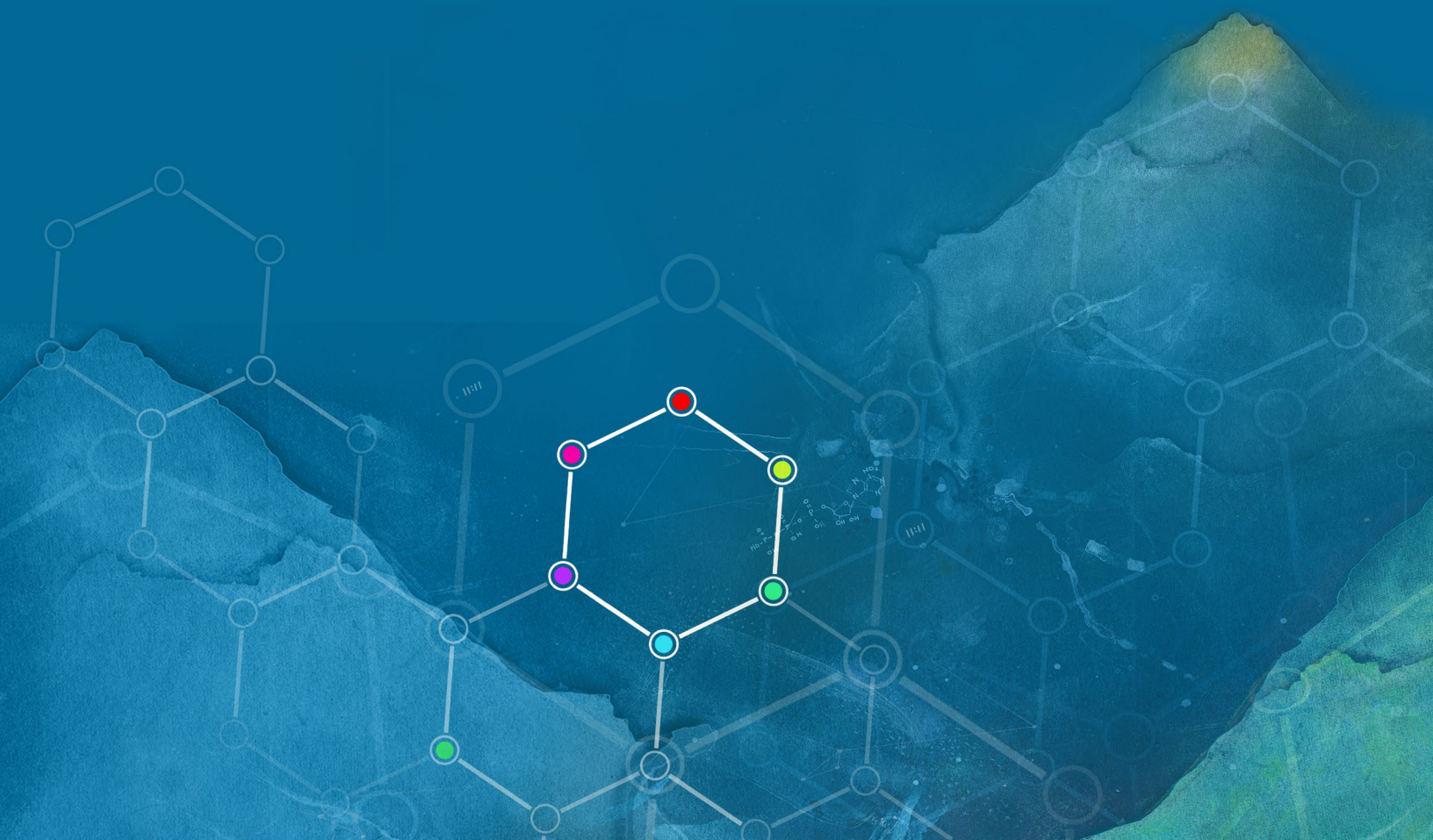
With high demand for analyzer access quickly filling instrument schedules as researchers return to labs, the Fluorescence Activated Cell Sorting (FACS) Facility accelerated its plans for the update and expansion of its flow cytometry analyzers.

Two additional 5-laser analyzers were purchased for the High-Parameter Analysis lab. The 5-laser Agilent Penteon with Auto-Sampler ($259,719) provides a second workhorse instrument for screen assay from plates/tubes. With quick setup and accessible training, as well as low operator charges, it has helped shift many routine assays to drop-off service for researchers—allowing them to return their focus to the lab sooner. The second instrument purchased is the 5-laser, 29-color FACSymphony ($367,882). This instrument is a match for the 2020 Symphony, purchased with a National Institutes of Health Shared Instrumentation Grant, which has already reached capacity. Finally, with more high-parameter analysis and for more efficient use of critical clinical samples, FACS has purchased a 50-parameter FACSymphony S6 spectral sorter ($814,340). This sorter is in a biosafety hood, providing support for clinical, COVID-19, infectious disease, and other Biosafety Level 2 sorts. With 6-way sort capability and both spectral and traditional analysis capabilities, it complements the analyzer capabilities in the High-Parameter Analysis Lab and allows researchers a mechanism to transfer panels to sorting for downstream applications.

Computational Services and Bioinformatics Facility

The Computational Services and Bioinformatics Facility (CSBF) received an anonymous grant of $17,000, to purchase a server to support the new multiplex imaging technology system installed in the 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 faculty, and the broader research community of the university.

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

In recognition of the unique role the center plays with respect to the basic sciences, the Stanford University School of Medicine Office of the Dean provides an annual operating budget to the Beckman Center to cover the cost of administering the programs funded by the center. In addition, the School of Medicine 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 $4.3 million in user fees this year (despite setbacks due to the COVID-19 pandemic), continuing a level of service that sets the standard at Stanford University. One-time emergency support, in the amount of $544,118, has been requested from the School of Medicine to cover COVID-19-related revenue losses resulting from the delta and omicron waves. 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 pairs of investigators (one a basic scientist and the other a clinician scientist) propose risky, but potentially high-pay-off 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

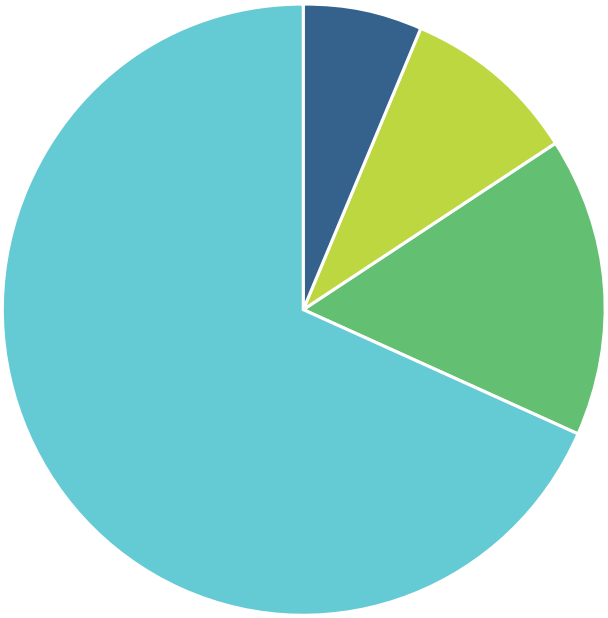
To foster the training of medical students in translational research, the center provides a stipend to selected students doing research in top-tier research labs with Program in Molecular and Genetic Medicine faculty. This is a competitive program, closely monitored by the Beckman Center.

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 along with the annual Beckman Symposium, which focuses on a critical area in scientific innovation. These seminars and symposia 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.



6.6%
Research Education & Training

9.5%
Faculty Recruitment

15.7%
Technology Development Seed Grants

68.2%
Technology Resources

EXTERNAL REVIEW

The Beckman Center director has drafted a formal external review process for all center programs, which will take place every five years. Given the exigencies of the current COVID-19 pandemic response, and the importance of bringing reviewers onsite to observe the facilities and speak with researchers firsthand, the initial review will take place as soon as an onsite review becomes feasible.

Reviewers from both within and outside the university will be chosen by the director and presented with an overview of center research, including the contributions made to that research by each of the four service centers operated by the Beckman Center. Also reviewed will be the impact of the center's Technology Development Seed Grant program, educational activities, medical scholars training program, and symposia held over the preceding years. Reviewers will be invited to provide the director with their expert feedback on center research operations, along with suggestions they may have for new programs and/or changes and improvements to existing programs. The results of this review, along with an appraisal by the center director, will be provided under separate cover to the Beckman Foundation.

The Beckman Center runs several technology resource programs—the Beckman Service Centers—that provide support for outstanding technological and scientific advances. These service centers require ongoing monitoring, review, and assessment. The centers provide services in 1) state-of-the-art imaging technologies (CSIF); 2) protein and nucleic acid molecular analyses (PAN Facility); 3) fluorescence activated cell-sorting technologies (FACS Facility); and 4) computer and biocomputational work (CSBF). All four service centers provide cutting-edge, high-tech resources to scientists on a fee-for-service basis. The demand for these services, as measured by the revenue generated as well as acknowledgments of the work done by these facilities in peer-reviewed journals, is an important measure of their overall success and value to the scientific community at Stanford.

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

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

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

The Cores Advisory Board meets at least once a year, and includes faculty members from the School of Medicine (often chairs of departments), appointed by the senior associate dean for research. The board's goals are to:

- Review and approve detailed business plans for proposed new service centers
- Invite existing service center directors, in rotation, to present their budgets, revenues/expenses, and lists of users
- Analyze overall subsidies required to operate each facility, including the cost-to-income ratio of each service being provided
- Evaluate the overall demand for services in a given facility over time
- Review the list of users for each facility and the dollar volume of activity per user, in order to determine the scope of demand for those services
- Assess the degree of duplication of services between service centers across the campus
- Evaluate which high-cost technologies should be subsidized
- Determine the need for new services or new service centers
- Evaluate whether certain services have outlived their usefulness, are readily available outside the university, or should be discontinued

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

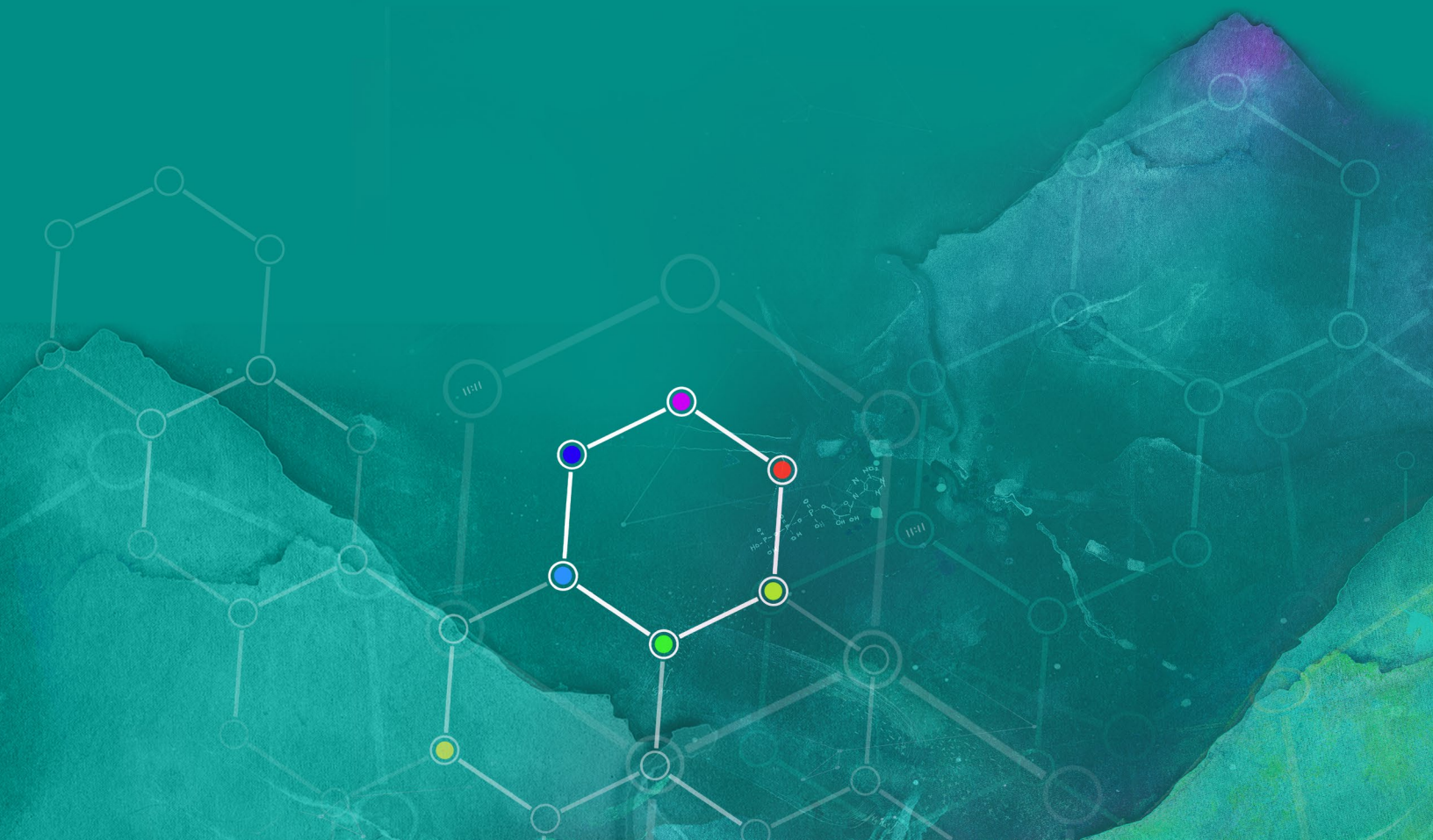
In addition to these review committees, Stanford University's Department of Audit, Compliance, and Privacy conducts internal financial audits of the facilities, and the Office of Research Administration oversees compliance of the facilities with the university's cognizant agency, the Office of Naval Research.

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

ENDOWMENT FUNDS

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

PROGRAMS



PROGRAMS

TECHNOLOGY DEVELOPMENT 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. Descriptions of 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

The enteric nervous system (ENS) populates the gastrointestinal (GI) tract and controls GI functions such as motility, luminal secretion, and absorption. Recent studies have revealed differential distribution of neuronal subtypes throughout the GI tract, with some subtypes enriched in the small intestine and others more prevalent in the colon. Understanding how different neuron subtypes contribute to GI functions requires causally manipulating their respective activity and observing functional responses of the GI tract.

However, a major challenge for dissecting the ENS arises from limited tools for effectively manipulating different neuron subtypes in the GI tract. Specifically, despite the success in the central nervous system, optogenetics cannot be readily utilized in the ENS *in vivo* due to the lack of cranial exposure for fiber fixation, the need to illuminate a broad area over which enteric neurons are distributed, and the inability to reposition the illuminated target after implantation.

To this end, in this seed grant we aim to transform the conventional paradigm of *in vivo* light delivery with an internal systemic light source, thus enabling selective optogenetic activation in the ENS with region-specific illumination patterns and neuron subtype specificity in live mice. This systemic light source is implemented by intravenously delivered optically stimulated luminescence nanoparticles (OSLNPs), which emit bright 470-nm light under focused, transdermal infrared illumination. We will use a fecal pellet expulsion assay and immunostaining of c-fos in GI tissue to validate the feasibility and regional specificity of this technology.

We envision that this selective *in vivo* activation method could eventually lay the foundation for GI pacing via a noninvasive pacemaker, which will alleviate enteric disorders such as constipation or other GI dysfunctions.

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.

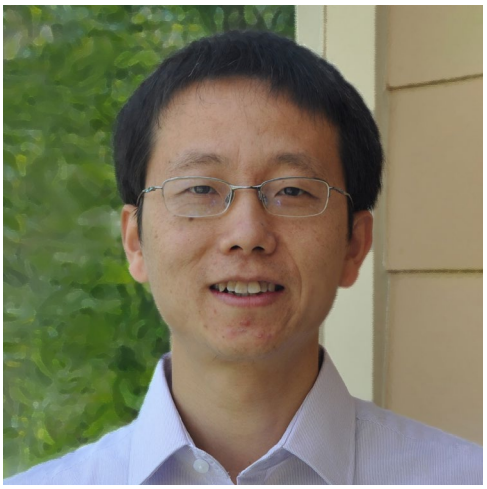
The first step to single-cell sequencing of solid tumors is the dissociation of tumor tissues into single-cell suspensions. Standard dissociation methods involving multiple manual steps are laborious, time consuming, and highly variable in cell recovery. The lengthy process can also introduce cellular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

The goal of this proposal 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. Single cells are collected as soon as they are dissociated to prevent further mechanical or biochemical stress. We will evaluate our system on kidney and prostate tumor tissues.

The team is interdisciplinary with complementary skill sets, with Dr. Tang in mechanical engineering with expertise in microfluidics and microscale cutting devices, and Dr. Brooks in urology with clinical expertise and extensive experience using genomic approaches to understand prostate and kidney cancers.

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

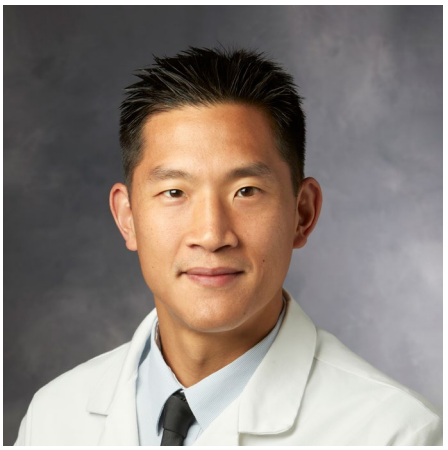
Membrane proteins, which account for approximately 23 percent of the human proteasome, play crucial roles in diverse biological and physiological processes. Many human diseases are associated with their mutations, and they are the targets for approximately 60 percent of drugs in use today. Biologics that can specifically target membrane proteins from the extracellular side represent an ever-growing list of best-selling drugs that treat sundry disorders. Currently, developing such biologics is technically demanding and time-consuming, thus presenting a major barrier to advancing therapeutic applications and to probing membrane proteins' physiological and pathophysiological functions.

Here, we will take an interdisciplinary approach to circumvent these challenges. By combining membrane protein biology and computational genomics, we will establish a new technology that enables efficient, scalable, and low-cost discovery for biologics that target membrane proteins, such as receptors, enzymes, ion channels, transporters, and scaffolds.

Overall, we envision our interdisciplinary project will transform the discovery of therapeutics across a variety of diseases and health conditions and will enable us to provide easy access to a comprehensive collection of valuable reagents for biomedical research across many fields.

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

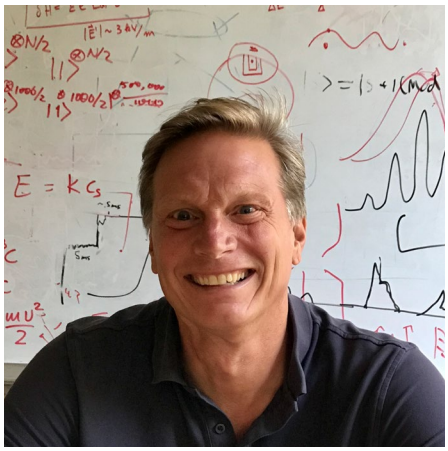
Pediatric patients with tetralogy of Fallot (ToF) with major aortopulmonary collateral arteries (ToF with MAPCAs) require extensive surgery to reconstruct the pulmonary vascular tree, typically using a combination of autologous vasculature and synthetic material. A biofabricated vascular tree graft could offer treatments with reduced risks of bleeding, thrombosis, and restenosis, and if made with living cells, could grow with the patient and obviate the need for repeated surgeries through life. While natural scaffold biomaterials, such as fibrinogen and collagen, demonstrate excellent biocompatibility, remodelability, and cell adhesion, these native materials cannot be rendered into complex shapes using light.

We have recently demonstrated the feasibility of a new enzymatic 3D bioprinter to manufacture complex and 3-dimensional vascular grafts using natural fibrinogen, enabling both micron-scale resolution and high biocompatibility. By employing a photothrombin system to render wholly natural fibrin scaffolds, our bioprinting system overcomes the need to use free-radical crosslinking chemistry and synthetic photopolymers, both of which are poorly biocompatible.

Here, we propose to first characterize and optimize the kinetics and concentrations of our photothrombin system to optimize 3D printing speed and biocompatibility. Using optimal settings, we will generate branched pulmonary artery tree grafts laden with smooth muscle cells and endothelial cells. We will install these grafts in our custom perfusion bioreactor to apply flow and cyclic mechanical stresses to stimulate the maturation of the tissue. We will characterize the matrix remodeling, cell alignment, burst pressure, and suture strength of these 3D-printed grafts to optimize maturation conditions and assess clinical readiness.

While our proposal focuses on the development of fibrin vascular grafts, since fibrin is a popular tissue engineering material for many organ systems, our enzymatic 3D bioprinter will find broad application across many disciplines. The success of this proposal depends on interdisciplinary expertise, including 3D printing and tissue engineering (Dr. Skylar-Scott), chemistry (Dr. Boxer), and vascular surgery (Dr. Ma).

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

The preliminary data and experiments described in this proposal are a collaborative product of the Kasevich, Wakatsuki, and Yang groups and their complementary expertise, that hereinafter shall be referred to collectively as "we."

We aim to develop a novel approach for the detection and characterization of mitochondria containing extracellular vesicles using high-throughput 3D fluorescence lifetime imaging microscopy (FLIM). 3D FLIM will provide an in-depth understanding of mitochondrial transfer as a novel therapeutic approach for treating heart failure (HF) in patients.

HF patients represent the leading diagnosis of hospital admission in the U.S., with high mortality and morbidity; therefore, there is a strong clinical need to develop new effective therapeutic interventions. The disruption of bioenergetic balance underlies the pathogenesis of HF. Mitochondria, known as the cellular power plants, exhibit structural abnormalities and diminished ATP production in HF, despite increased metabolic energy demands in the failing heart. We recently discovered the mitochondria-rich extracellular vesicles (M-EVs), which restore the cardiomyocyte bioenergetic balance and subsequently improve contractile function.

In this proposal, we will employ a new type of FLIM technique allowing for the first-of-its-kind multiparametric, 3D visualization, and characterization of M-EVs. FLIM will be utilized to characterize and track M-EV packaging, uptake into recipient cells, and intracellular integration of M-EV contents. It will also allow for in-line detection and separation of M-EV, using distinct fluorescence lifetime signals.

Studying these key biological processes will allow us to gain an in-depth understanding of how mitochondrial and non-mitochondrial contents of M-EVs restore myocardial function in HF as a basis for the future rational design of M-EV-based therapeutics.

SEMINARS AND SYMPOSIA

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

In 2020 and 2021, when many researchers were working from home due to the COVID-19 pandemic, Beckman Center program leaders redesigned several programs as online webinars and video tutorials, allowing vital scientific interactions and training to continue throughout the pandemic. Some of those programs are now being restarted and expanded, and the program leaders are looking forward to soon 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 COVID-19 pandemic. We are looking forward to possibly rescheduling in 2023 the program that was planned for 2020.

Top and Bottom: Get the FACS: Intro to Flow Cytometry seminar, September 2022.

GET THE FACS SEMINAR SERIES

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

WHAT'S THE SCOPE? SEMINAR SERIES

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

FRONTIERS IN BIOLOGICAL RESEARCH SEMINAR SERIES

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

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

CANCER 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.

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 a new assistant professor of biochemistry and developmental biology: Florentine Rutaganira, Ph.D. She began her work at Stanford in the fall of 2022.

Dr. Rutaganira did her Ph.D. in chemical biology working with Kevan Shokat, Ph.D., at the University of California, San Francisco, and her postdoctoral work with Nicole King, Ph.D., at the University of California, Berkeley. Dr. Rutaganira's laboratory is studying choanoflagellates (the closest living single-celled relatives to animals), which form complex colonial organizations through intercellular signaling, to understand how assemblies of cells signal to one another. Her work has wide-ranging implications for cell communication and organismal evolution. Dr. Rutaganira has also played an important role in advocacy for women and underrepresented minorities in STEM fields, and is recognized for her excellence in mentorship.

The Beckman Center is actively recruiting additional faculty—one each in the departments of Biochemistry and Developmental Biology, and two in the Department of Molecular and Cellular Physiology. Announcements concerning these new faculty recruits will be made in upcoming issues of *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, and selection is made through the Stanford Medical Scholars Program by the Medical Scholars Committee, which is composed of leading PMGM faculty members drawn from the basic and clinical sciences in the School of Medicine. Applications are reviewed on a quarterly basis. Student awardees are required to make an oral presentation of project results to an audience of their faculty advisor and others with expertise in the field, and must also prepare a written summary of their project results.

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

CARMEL G. MCCULLOUGH

Academic Year: 2022-2023

Year at Stanford Medical School: 2b

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 is 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% 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: 2022-2023

Year at Stanford Medical School: 3

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 pressures 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.

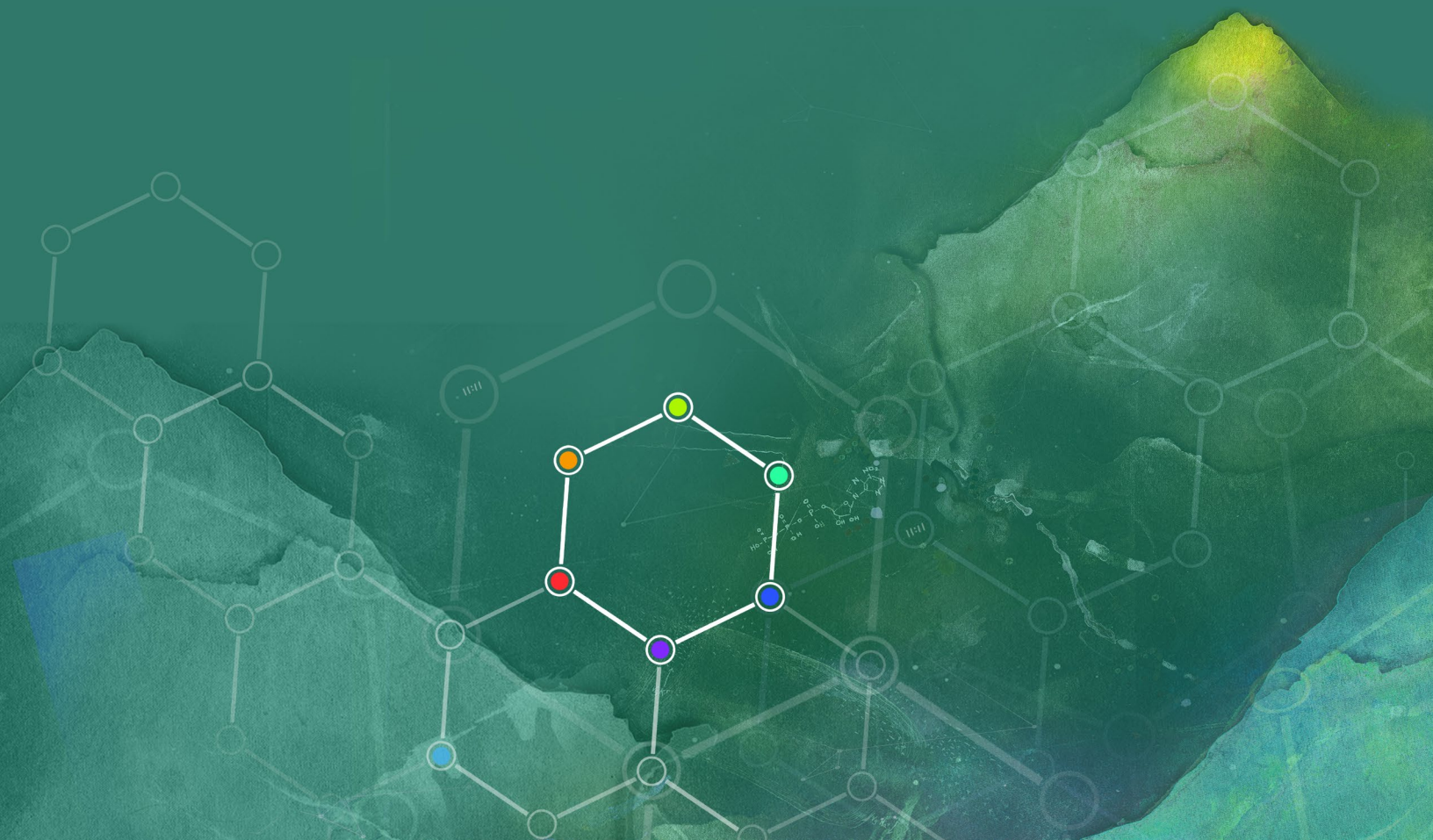
Clinically, PAH is characterized by elevated pulmonary arterial pressure and remodeling of pulmonary vessels that lead to the narrowing of pulmonary blood vessels. Some forms of PAH are caused by heritable, familial mutations, one of which is in KCNK3. This gene, which encodes an outward rectifying potassium 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 pressures 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 million in annual revenues from faculty, postdoctoral fellow, and graduate student users campus-wide, as well as from the broader scientific community. This allows the service centers to operate at or close to break-even. The service centers include:

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

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

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

CELL SCIENCES IMAGING FACILITY

OVERVIEW

The Cell Sciences Imaging Facility (microscopy.stanford.edu) provides high-resolution, state-of-the-art technologies for imaging and analyzing the molecular and structural organization of cells and tissues, as well as bioengineered materials. The facility offers sophisticated and demanding microscopy techniques to Stanford University and industry researchers, including super-resolution, confocal, FLIM, FRET, FRAP, 2-photon and live cell imaging, as well as spatial proteomics, atomic-force measurements, immuno-electron microscopy, and high-pressure freezing.

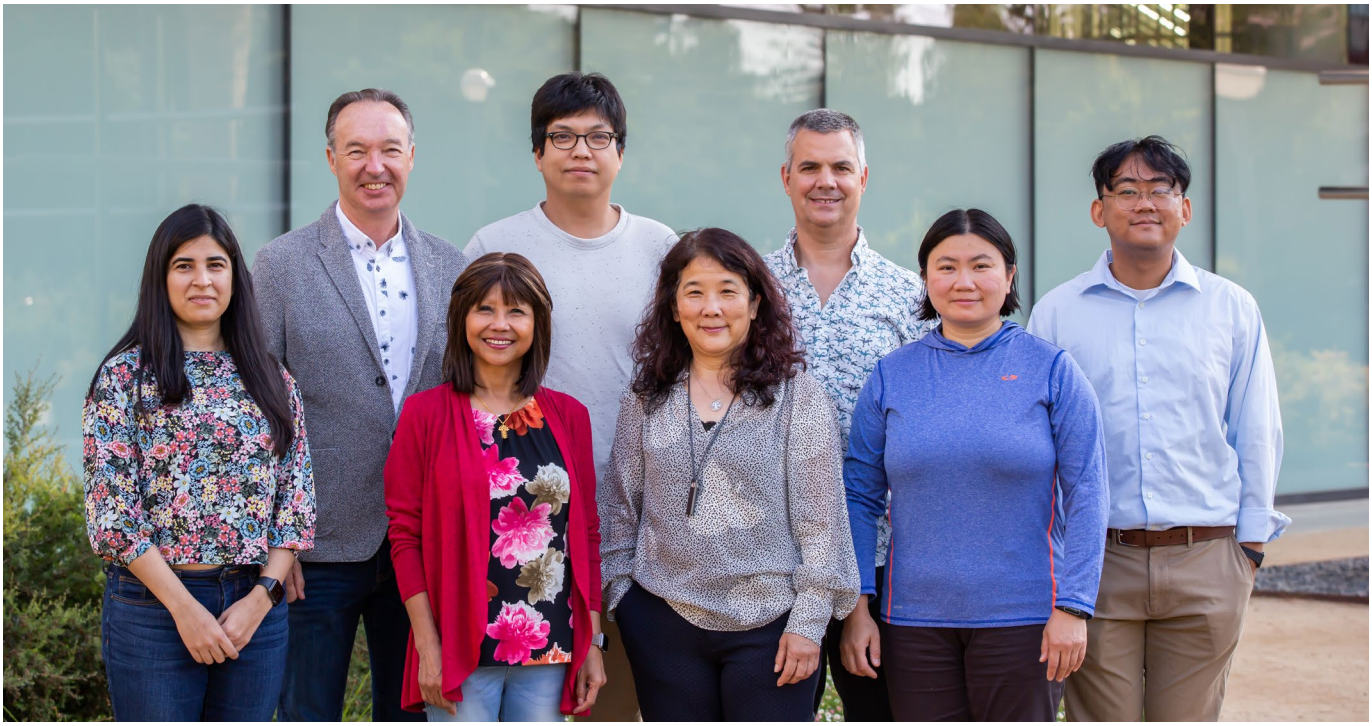
The CSIF is organized into three interdependent imaging labs: the Fluorescence Microscopy Core (FMC), which houses multi-photon, confocal, super-resolution, fluorescence lifetime and deconvolution microscopes, as well as image analysis software; the Electron Microscopy Core (EMC), which houses high-resolution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

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

JON MULHOLLAND
Director

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



CELL SCIENCES IMAGING FACILITY

Front Row, Left to Right: Anum Khan, Ibanri Phanwar-Wood, Ruth Yamawaki, Kitty Lee

Back Row, Left to Right: Jon Muholland, Youngbin Lim, John Perrino, Wes Alejandro

EXPERTISE

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

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

SERVICES

Fluorescence Microscopy Services

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

- Super-resolution (API OMX-SIM, STORM, Leica SP8-gSTED, Zeiss Airyscan)
- Laser scanning confocal (Zeiss LSM 880, LSM 780, Leica SP8, Leica SP5)
- Spinning disk confocal (Nikon-Yokogawa)
- Deconvolution (API OMX Delta Vision)
- 2-photon (Zeiss LSM 780, Leica SP5, each with Spectra Physics DeepSee laser)
- Lattice light sheet microscope (3i, Inc., LLSM V2)
- Fluorescence lifetime imaging (FLIM) light microscopy technologies
- Bio-atomic force microscopy (Bio-AFM, Bruker Resolve BioScope)

Super-resolution technologies allow researchers to exceed the diffraction-limited resolution limits of conventional light microscopy (<200nm). This allows researchers to image and resolve structures and cellular dynamics that were previously unresolvable with other optical technologies. Two-photon, confocal, and deconvolution technologies allow optical sectioning while eliminating out-of-focus fluorescence. This makes the precise 3D localization of fluorescently labeled proteins within the cell or tissue possible. Lattice light sheet microscopy is the standard for fast live-cell imaging with low phototoxicity. FLIM allows researchers to measure changes in a molecule or protein's fluorescence lifetime, in addition to its

Clockwise from Top Right: John Perrino with the Gatan OneView TEM microscope and OneView 16bit computer; Ibanri Phanwar-Wood with the Leica Ultracut UC7; Youngbin Lim with the Zeiss Lattice Lightsheet; Youngbin Lim with the LSM 900 Airyscan 2.

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 material. Using epifluorescence, brightfield, and phase contrast optical imaging, these measurements can be directly correlated with macromolecules, proteins, and subcellular structures, as cells sense and respond to mechanical cues and environmental changes. The CSIF also has capabilities for total internal reflection microscopy (TIRF) and fast, wide-field, live-cell imaging.

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

Electron Microscopy Services

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

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

Ancillary equipment includes four ultramicrotomes for cutting ultra-thin sample sections (less than 100nm), a cryo-ultramicrotome for sectioning ultra-thin frozen sections, all equipment necessary for sample preparation, and computers for image analysis. Additionally, the EMC houses a new state-of-the-art Leica 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.

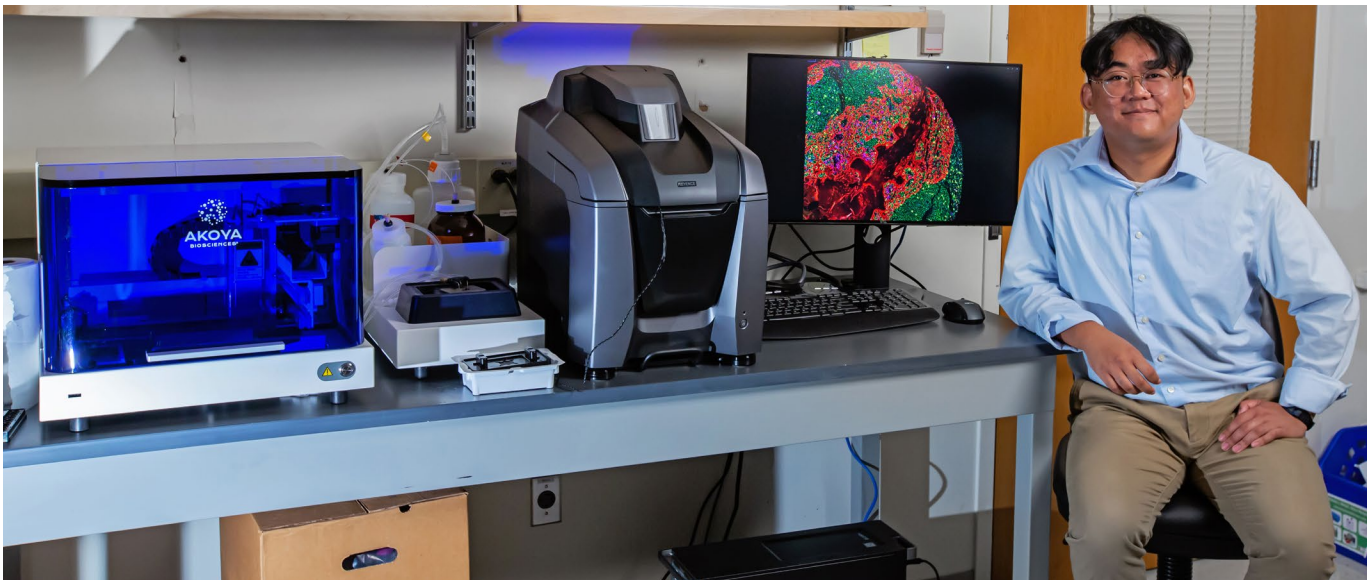
Spatial Proteomics Services

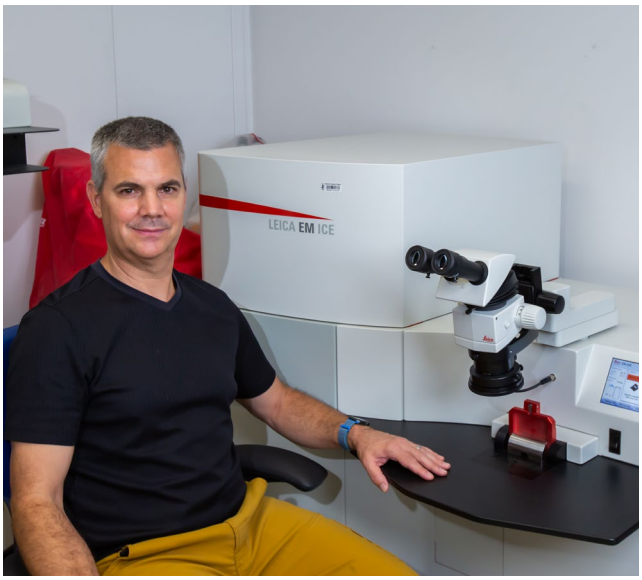
The facility's Spatial Multiplexing Core provides complete multiplexing epitope localization (CODEX) and array tomography (AT) services.

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 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. Array tomography permits easy acquisition

John Perrino and Ibanri Phanwar-Wood with the Gatan OneView TEM microscope and OneView 16bit computer.





Top: Wes Alejandro; ***Bottom Left***: Anum Khan with the Opentrons robotic device; ***Bottom Right:*** John Perrino with the Leica EM ICE.

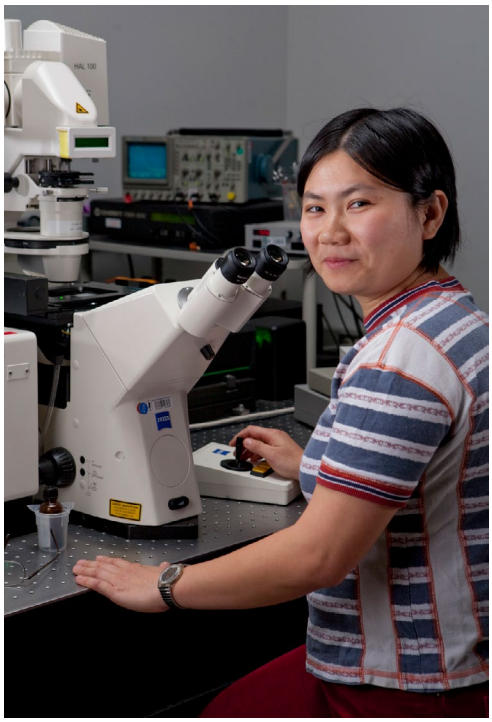
of electron microscopic images in register with immunofluorescence. Array tomography thus promises an opportunity to explore the 3D molecular architectures of tissue at an unprecedented level of detail.

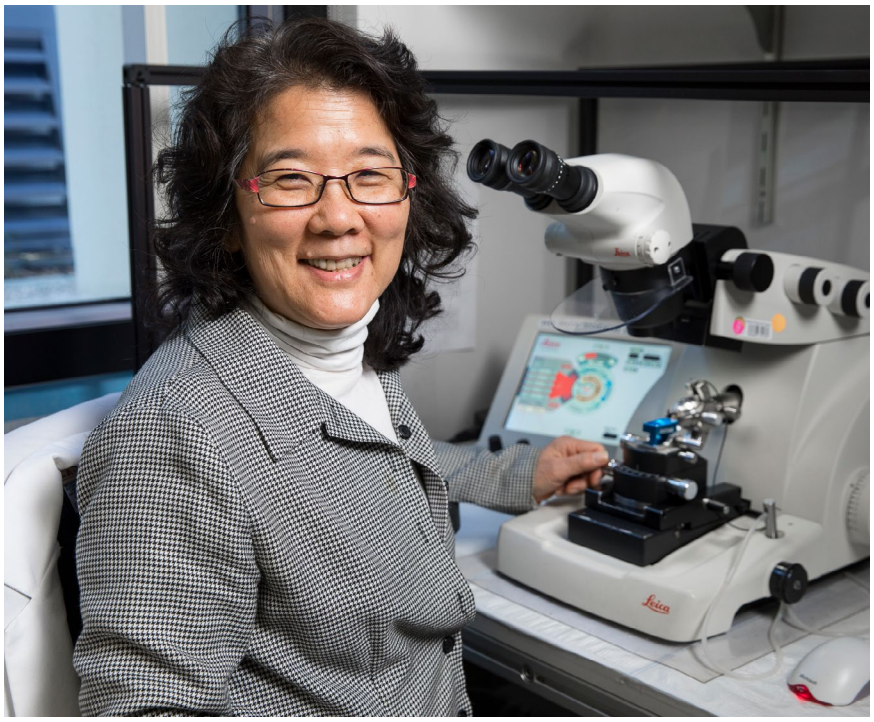
RECENT DEVELOPMENTS

Experiential Learning

Stanford University's c-ShARP (Community of Shared Advanced Research Platforms) initiative is providing funding to support experiential learning in Stanford facilities. CSIF has been awarded $30,000 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 marks the sixth year of this course.
- EE235A: Analytical Methods in Biotechnology I. The overall 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.
- EE235B: Analytical Methods in Biotechnology II. This course seeks to further equip students with





Left: Kitty Lee; ***Right:*** Ruth Yamawaki

biochemistry and molecular biology techniques by providing them with hands-on facility training in flow cytometry, fluorescence activated cell sorting, advanced microscopy, mass spectrometry, and affinity measurements.

Instrument Upgrades

The Stanford c-ShARP initiative also awarded CSIF funding for instrument upgrades, in the amount of $265,000. The award was for the purchase of a second CODEX microfluidics highly multiplexed imaging platform, with additional funding for an Opentrons robotic automation device and Visiopharm AI software. It was installed in August 2021. The availability of this second CODEX platform with automation and AI-enhanced software will double the throughput of the facility's spatial proteomics services.

In addition, the School of Medicine's small equipment funding program awarded the facility $50,000 for a Leica, Inc., Ultracut UC7 ultramicrotome. The new ultramicrotome replaces 15-year-old equipment and will be used to cut ultrathin (50-100nm) sections for electron microscope imaging.

FUTURE VISION

Several new programs and services are now in development.

- The CSIF is collaborating with the Department of Biochemistry and the Sarafan Chemistry, Engineering & Medicine for Human Health (ChEM-H) institute to further develop and expand the university's light sheet microscope (LSM) imaging program. A significant aspect of this development is seeking funding to fill a major gap in LSM instrumentation and provide support for expanded data science services.
- The CSIF will be working with the PAN Facility to establish standardized validation protocols for the antibody-probe conjugation chemistries being used for CODEX multiplexing.

PROTEIN AND NUCLEIC ACID FACILITY

OVERVIEW

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

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

EXPERTISE

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

The PAN Facility is staffed, in addition to its director, by five full-time, experienced research professionals who have been trained in all the services provided and who also offer expertise in specific service areas. The PAN Facility is organized into number of interdependent services, as listed below.

MICHAEL ECKART, PH.D.
Director

SERVICES

The PAN Facility offers a number of interdependent services:

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

PROTEIN AND NUCLEIC ACID FACILITY

Left to Right: Ian Anderson, Jessica Tran, Michael Eckart, Katia Alvarez, Jennifer Okamoto, Yen Tran

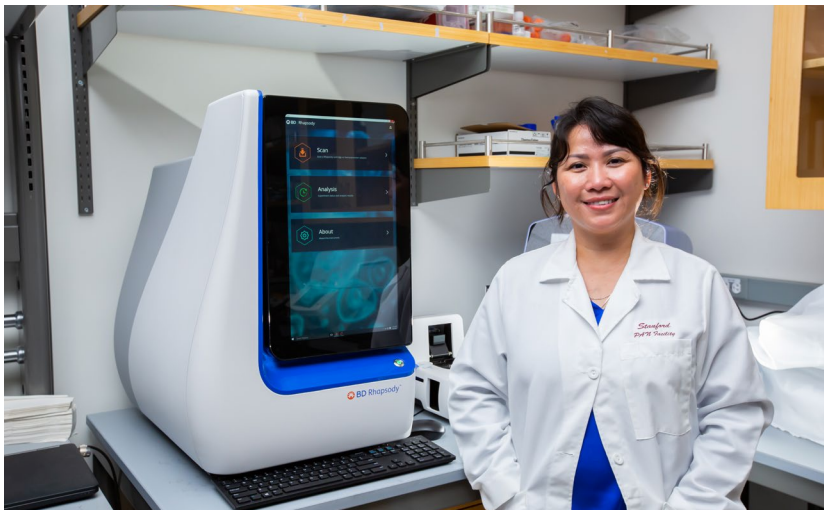
Shared Services

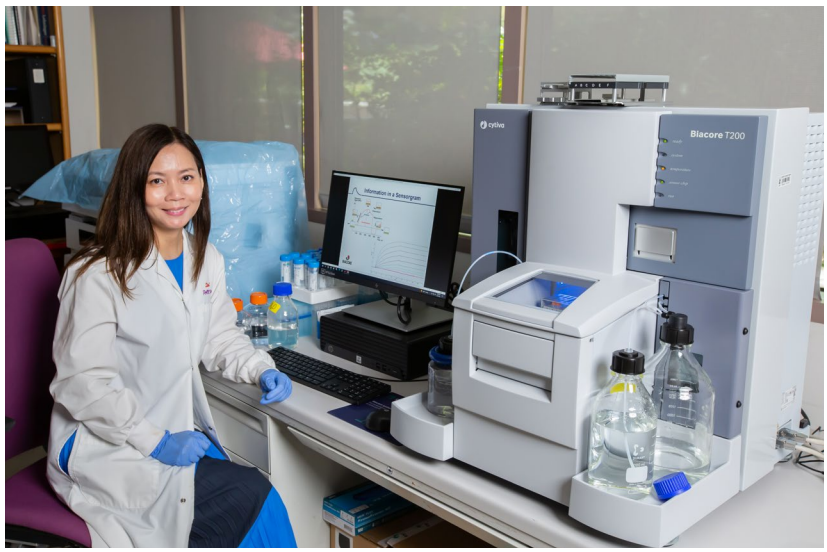
The core services offered by the PAN Facility enable and facilitate efficient and economical biomedical research by providing users with technology, without the necessity of major capital or staffing expense. With the organized and efficient infrastructure that shared resources such as the PAN Facility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

The core strength of the PAN Facility comes from its talented, highly experienced, and dedicated scientific staff. The dedication of PAN staff in advancing our mission of education and research was again very evident this year, as the COVID-19 pandemic continued to present challenges. The staff has viewed the continued changing circumstances as an opportunity to identify new ways to support the Stanford scientific community's research.

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

The training and professional development of PAN staff is a top priority, to ensure both personal and research project success. This is often achieved through an active, open exchange of ideas between PAN Facility staff and researchers that enables leveraging the full potential of the available technologies. Development and implementation of new applications and technologies at Stanford are often achieved when a research group and the PAN staff engage in a joint project, with all contributing their individual strengths. The results of these efforts are often highlighted in publications to which PAN scientists have made contributions. Indeed, the consultation provided by PAN staff is often as important as the data obtained, since biomedical





Top: Yen Tran with the BD Rhapsody Single-Cell Analysis System; ***Middle:*** Jessica Tran with the Biacore T200; ***Bottom***: Katia Alvarez.

researchers not trained in a specific technique or field can find it difficult to interpret specialized data without help from PAN scientists.

Single-Cell Genomics

The field of single-cell analysis continues to evolve rapidly.

There have been many technological and methodological developments in single-cell genomics, which is the application of genomic technologies to understanding biology at the level of an individual cell, rather than an entire population of cells. Single-cell genomics has revealed how much variation there is between individual cells at the molecular level in, for example, tumor tissue, stem cells, or rare subpopulations of immune cells. However, analyzing genomic DNA or RNA at the single-cell level may provide only genome, methylome, chromatin, or transcriptome information. Although these individual sets of information are valuable, by themselves they do not provide a full understanding of all the genomic, transcriptomic, epigenomic, and proteomic activities of individual cells. Together with costs, one of the main challenges is sample acquisition and preparation, which can be very laborious and time consuming. The development of new technologies and the modification of existing single-cell protocols have addressed some of the challenges, such that different types of both genetic and protein molecules can be analyzed simultaneously.

The PAN Single-Cell Genomics Laboratory, which was established by the Beckman Center together with a group of research programs in cancer, stem cells, and immunology, provides a full range of services aimed at advancing discoveries and the development of methods to analyze genomes and transcriptomes in single cells.

Single-cell sequencing is performed in three major steps: cell isolation, whole genome/transcriptome library construction, and high-throughput sequencing. The first step, the successful, rapid isolation of single cells for genomic analysis, is a critical step for obtaining meaningful results. It can be achieved by using, for example, fluorescence activated cell sorting, by simple micromanipulation, or by capture using microfluidic technology.

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, the C1 Single Cell Auto Prep instrument (Fluidigm), which processes 96 or 800 single cells, and the ddSEQ Single-Cell Isolator instrument (BioRad), which performs rapid single-cell isolation using droplet partitioning technology. Cell acquisition is confirmed via an EVOS Cell Imaging System.

More recently, the PAN Facility has implemented 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 (NGS).

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.

Spatial Transcriptomics

Spatial biology provides genomic and proteomic information from cells with regard to their specific location in biological tissue.

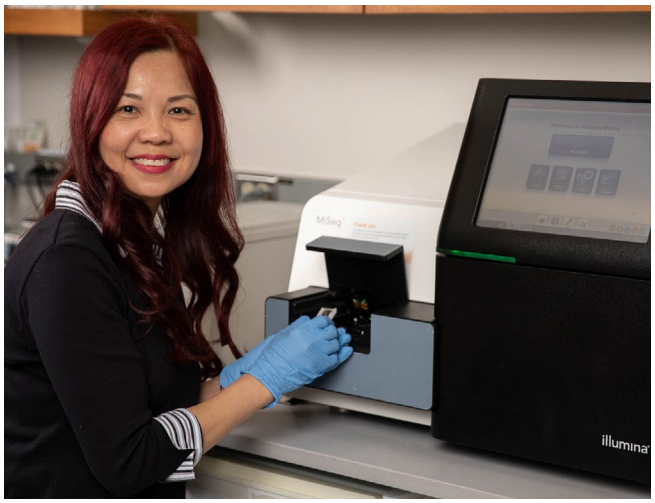
It Is known that interactions among cells, proteins, and expressed genes in biological microenvironments are very important in understanding disease states in oncology, neurology, and immunology, as well as organism development. However, spatial information is lost when single cells are collected from suspensions of dissociated tissue. In 2020, the journal *Nature Methods* named spatially resolved transcriptomics as its "Method of the Year."

Spatially resolved gene expression in tissue sections is traditionally analyzed using immunohistochemistry (IHC) or *in situ* hybridization (ISH); however, these technologies, aside from being laborious and challenging, are low-throughput and nonquantitative. To overcome these limitations, many different spatial biology platforms, using different approaches, are being developed within both academic and biopharmaceutical environments. Thus, the PAN Facility is evaluating and implementing different spatial transcriptomics technologies.

For example, the Visium platform provided by 10X Genomics combines traditional histology with high-throughput, single-cell RNA sequencing (scRNA-seq), whereby intact tissue sections are captured on an array containing spatially barcoded, complementary DNA primers for the capture of either full-transcriptome or transcript subsets. Subsequent RNA library generation for next-generation sequencing of a single intact tissue sample utilizes the existing instrumentation in PAN's Single-Cell Genomics Laboratory. PAN is also working with Resolve Biosciences to evaluate the Molecular Cartography platform; Resolve Biosciences is providing

access to the technology, via three competitive Molecular Cartography RNA profiling projects, to Stanford scientists who proposed projects that will advance their field of study.

Many spatial technology platforms are limited to a single modality, typically gene expression or protein expression. However, the technology is evolving whereby a few platforms enable the detection of genes and proteins from a single intact tissue sample. These workflows encompass microscopy techniques and RNA sequencing to generate protein biomarker and transcriptome data, respectively. The PAN Facility 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.



Top: Jennifer Okamoto; ***Bottom***: Ian Anderson

The collaboration of PAN with different research programs and technologies in other shared resources (FACS and CSIF), in accordance with our mission, adapts and takes advantage of single-cell tools, protocols, and technologies, including equipment acquisition, as they become available, so that scientists and clinicians within the Stanford scientific community remain on the cutting edge of scientific research. It is anticipated that advances made using PAN's scientific resources will enable researchers to obtain a deeper understanding of the underlying causes of diseases such as cancer and immune disorders, and the differentiation of stem cells, which have the promise of developing diagnostics and therapeutics in the different areas.

Other Technologies

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

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

PAN's portfolio of technologies also includes those required for the validation of genes and proteins identified in large-scale genomic and proteomic

studies. We believe that the need for such validation technologies will continue to grow, as they are key to demonstrating how genetic or proteomic differences have effects in a specific disease. Quantitative-PCR continues to be a popular 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 other unrelated cell lines. Misidentification of cell lines produces misleading results and has a significant negative impact on research costs. Journals and funding agencies now require proof that the cell lines being used are authentic and have remained so over the course of a study. We have seen an increase in the demand for fragment analysis in STR analysis workflow that uses the capillary electrophoresis technology in our DNA sequencer, since it is a simple, economical method, and the gold standard for establishing the identity of human samples.

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

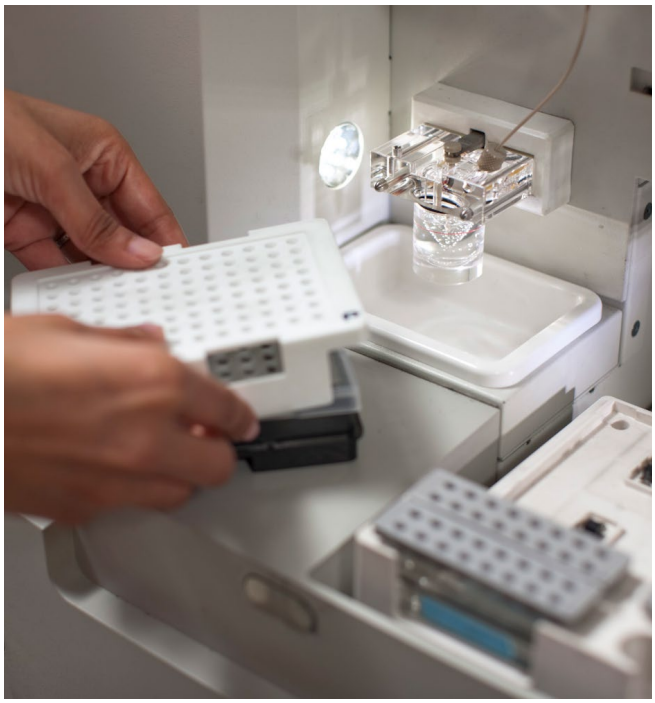
SPR is a key technology in support of our efforts to meet the post-genomic biological challenge of understanding the complex networks of interacting genes, proteins, and small molecules that give rise to biological form and function. Demand for biophysical characterization of protein and small molecule compound interactions is growing, and SPR technology has emerged as a powerful tool for hit identification, hit validation, and lead optimization.

Hit identification has been achieved primarily by using the existing Biacore T200 instrument to screen 384-well plates of the Stanford fragment library containing 5,000

low-molecular weight compounds (<300 Da) for several different fragment-based ligand and drug discovery (FBLD) programs at Stanford. Hit validation using SPR refers to the verification of the compound-target interaction, whether identified using FBLD SPR in the PAN Facility or in high-throughput screening campaigns and characterizing the interaction by its affinity and other properties. 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 small chemical entity, PAN's Biacore T200 instrument system is extensively used in biotherapeutic antibody discovery and development, from selection of first candidates to clinical lead for treatment of a variety of diseases. Due to researchers' increased demand for performing SPR studies, PAN's SPR capabilities have been expanded through the acquisition of an additional T200 instrument system.

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

The PAN Facility environment allows the Stanford research community to bridge the technical diversity gap and encourages collaborations that apply different technologies to biomedical research. PAN Facility scientists reach out for new technical opportunities to broaden horizons by working closely with scientists from different disciplines in implementing scientific breakthroughs and associated methodologies in genomics and proteomics. This enables researchers to make connections between basic and clinical research that will benefit the field of translational medicine. PAN Facility scientists have made significant contributions to many different scientific programs in the form of publications in peer-reviewed journals.



FUTURE VISION

The PAN Facility will continue, in an ever-changing scientific environment that encompasses a wide range of biological, chemical, engineering, and physical sciences, to focus on providing solutions to advance and further the science of Stanford researchers. With the existing strengths and expertise in the different areas of the PAN Facility, the collaborative efforts between the different Beckman Center shared technology resources will enable multidisciplinary innovation and strategies that will broaden the application of different technologies to the multi-omic sciences.

Overall, despite the continued challenges and changes presented by the COVID-19 pandemic, PAN will continue to play a role in supporting and ensuring that Stanford researchers have access to technological capabilities to perform their research.

FLUORESCENCE ACTIVATED CELL SORTING FACILITY

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 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 at once. In addition to the typical applications using mammalian cells, FACS is also valuable for work with yeast, bacteria, plankton, and other small particles.

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

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

LISA NICHOLS, PH.D.
Director

laboratory continued to be a major source of innovation in the field throughout the subsequent years. Dr. Herzenberg initiated the precursor to the current facility in the mid-1980s and joined the Beckman Center when it opened. The FACS Facility, which was then part of the Herzenberg group, also moved to the Beckman Center at that time and was reorganized into a service center.

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

FLUORESCENCE ACTIVATED CELL SORTING FACILITY

From Left: Tom Nozaki, Catherine Cheng, Dave Parks, Ricardo Zermeno, Bianca Gomez, Lisa Nichols, Rudy Wycallis, Cindy Jiang, Melody Wang

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 currently offers 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

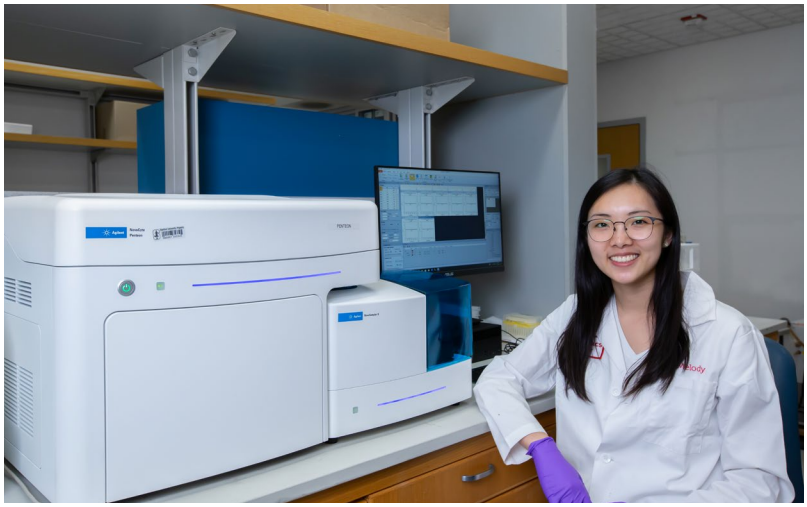
The facility manages a site license for FlowJo data analysis software. This license provides Stanford researchers with a discount of approximately 60 percent off the cost of an individual license. Additionally, the facility administers the FlowJo SeqGeq license to support high-parameter single-cell analysis.

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.



Top: Melody Wang with the 5-laser Agilent Penteon; ***Middle:*** Rudy Wycallis with the 5-laser, 29-color FACSymphony; ***Bottom:*** Catherine Cheng with the 5-laser, 29-color FACSymphony.

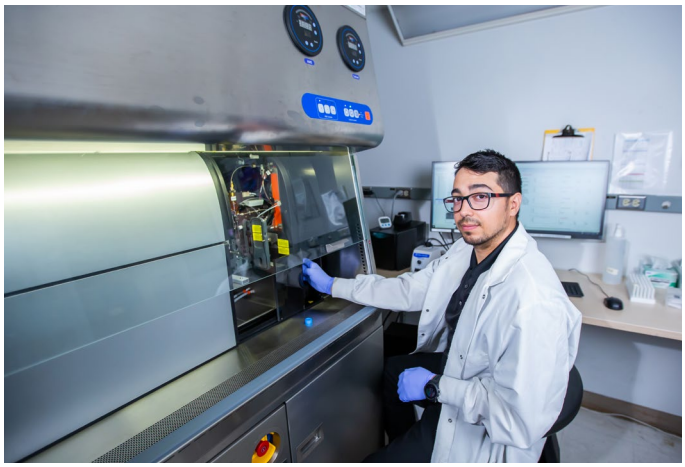
RECENT DEVELOPMENTS

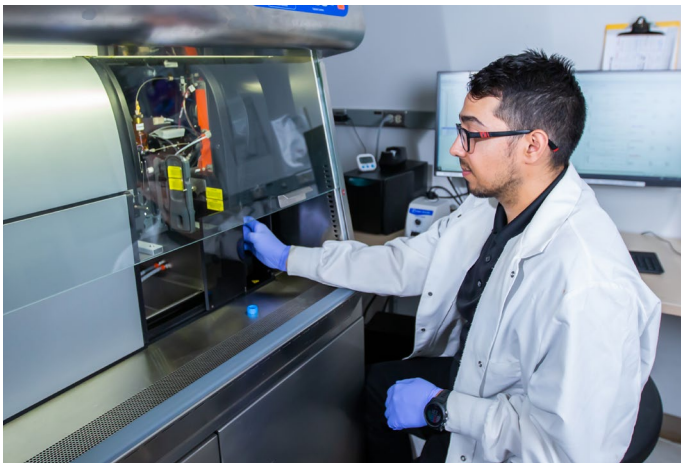
The past year has been one of refocus. As the turmoil of the pandemic years has settled down, the FACS Facility has reenvisioned which technologies and services are most critical.

As researchers returned to labs, all analyzers—especially the 30-parameter BD FACSymphony analyzer purchased with a National Institutes of Health award in 2020—quickly reached capacity. The High-Parameter Analysis Lab, which opened in 2020, is now heavily trafficked. This tight scheduling prompted a new priority: rapid expansion of the center's flow cytometry analyzer options. In response, the FACS Facility accelerated plans for update and expansion of its suite of analyzers. An additional 5-laser system ideal for screening assays was added in late 2021, and a second FACSymphony was brought online in early 2022.

The first instrument is an Agilent Penteon system with multi-tube and -plate autosampler. This system is best suited for one to 15 color assays; it is similar to the previously added Quanteon, but includes an ultraviolet excitation laser for maximum flexibility in fluorochrome/label selections. With robust sample handling, it has provided additional capacity for multi-plate assays and is primarily used by researchers preferring the sample drop-off service. Drop-off service takes advantage of staff assistance for setup, monitoring, and basic data analysis, allowing researchers the flexibility to return to the lab and await results. Remote live monitoring is also available.

The second new system, the FACSymphony2, is a match for the previously purchased FACSymphony, with five excitation lasers and up to 30-parameter analysis. This redundancy





Top Left and Right: Ricardo Zermeno with the FACSymphony 50-detector, 5-laser, 6-way sorter.

offers an invaluable option for clinical research which, due to its unpredictable scheduling, is dramatically impacted when critical flow cytometry tools are limited. With the additional lab space available in the High-Parameter Analysis Lab, we have seen ready instrument access facilitate research progress, and clinical research has been directly supported.

In lockstep with the addition of high-parameter analyses, the facility has also added a high-parameter sorter. These high-end instruments enable researchers to drill down and identify key populations in complex microenvironments, such as within tumors or in specialized tissues such as brain or gut. The FACSymphony S6 sorter was purchased and became available in late 2021. With 50 detectors and the ability to spectrally analyze more than 35 different cell identifiers, this sorter will allow the separation of up to six separate simultaneous populations. This functionality enhances researchers' efficiency in utilizing invaluable clinical samples and collecting cell subsets required for downstream assays.

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. In keeping with this demand, we will continue on a path of increased updating and expansion of capability. As new technologies allow, we

Attendees arrive at a Get the FACS seminar, September 2022.

are supporting a gradual shift for researchers away from tedious, hands-on data acquisition tasks, in support of more automated data analysis for routine assay, thus promoting more efficient research time. This allows both staff and researchers to shift focus to education, and to provide more direct support for high-parameter assays that can be technically challenging to develop.

With a strong focus on expanding our educational and technical support outreach, the upcoming year will include a relaunch of in-person seminars. Participation in lab-based instruction for engineering graduate students is already in progress, and this year we will add two hands-on lab sessions to provide students with additional learning opportunities. 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.

COMPUTATIONAL SERVICES AND BIOINFORMATICS FACILITY

OVERVIEW

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

EXPERTISE

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

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

SERVICES

Available Software

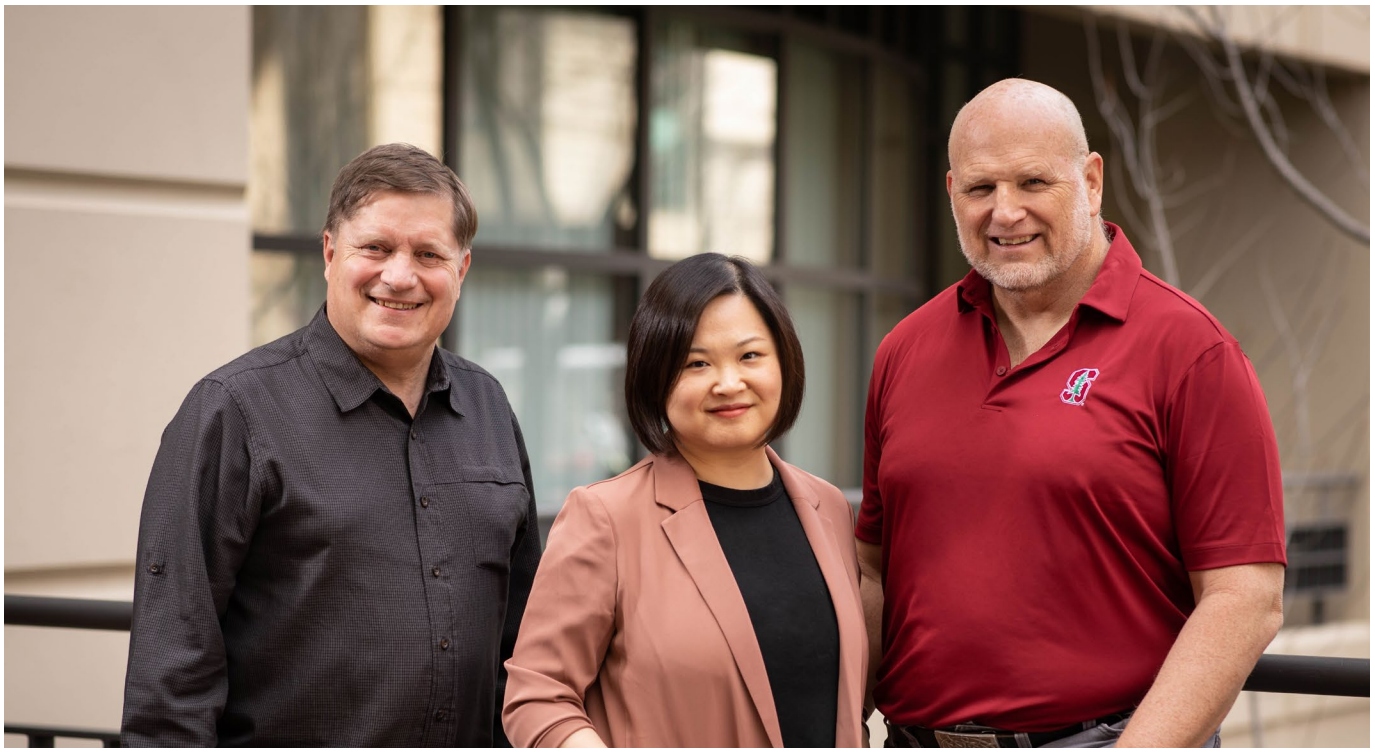
The CSBF provides a variety of Macintosh, Windows, and Linux software for scientific research and general administrative use.

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

LEE KOZAR
Director

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

While there are many public domain software packages available for doing scientific research, the CSBF has focused on providing access to commercial software because, in most cases, commercial scientific software



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

has significant advantages over its public domain counterparts. Commercial software offers technical support, is usually easier to install and run, is updated more frequently, and is less prone to errors.

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

- Sequence analysis (DNAstar, SnapGene, MacVector, Sequencher, Geneious, CLCBio)
- Microarray analysis (GeneSpring, Partek)
- Genomics analysis (Geneious, Golden Helix, Partek, JMP Genomics, iPathwayGuide)
- Mass spectrometry (Mascot, PEAKS, ProteinMetrics)
- Database (FileMaker, EndNote, Paperpile)
- Statistical and mathematical analysis (SPSS, Matlab, Mathematica, SigmaPlot, GraphPad)
- Graphics (Illustrator, Photoshop, BioRender)
- Microscope imaging (Volocity, Imaris, Metamorph)
- Gel electrophoresis imaging (Nonlinear Dynamics)
- Electronic lab notebooks (LabArchives, Benchling)

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

This has been especially useful in recent years, as many people had to work remotely at home due to the COVID-19 pandemic. A full list of the software offered by the CSBF can be seen at csbf.stanford.edu/software.

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

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

CSBF Membership

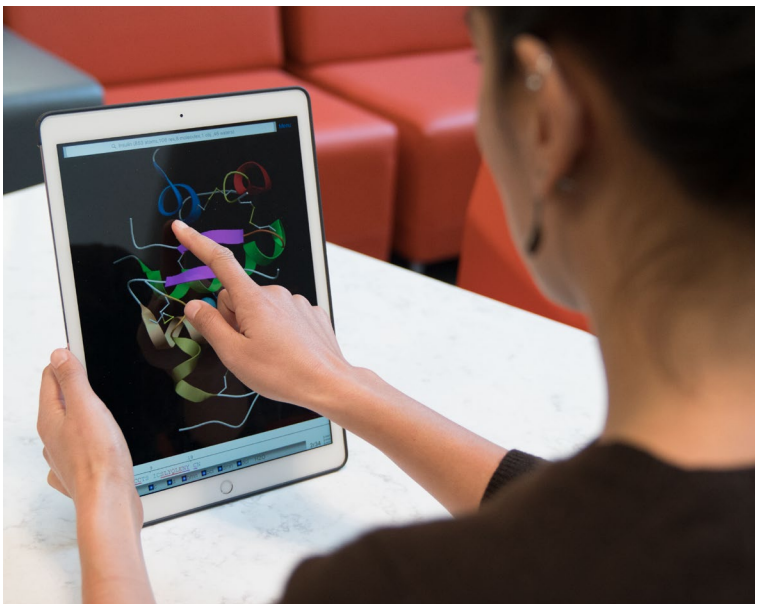
To access CSBF software, researchers must first obtain a CSBF membership. This can be done at csbf.stanford.edu/membership.

The CSBF has two levels of membership:

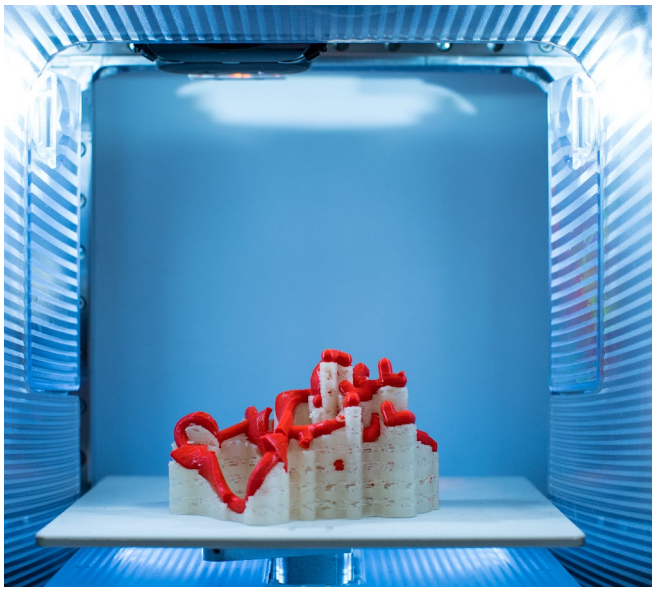
- A Level 1 membership gives everyone in a specific lab access to the bioinformatics computer facilities, including the large library of commonly used Mac, PC, and UNIX software packages.
- A Level 2 membership gives a specific lab access to all CSBF software, including the more expensive software packages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

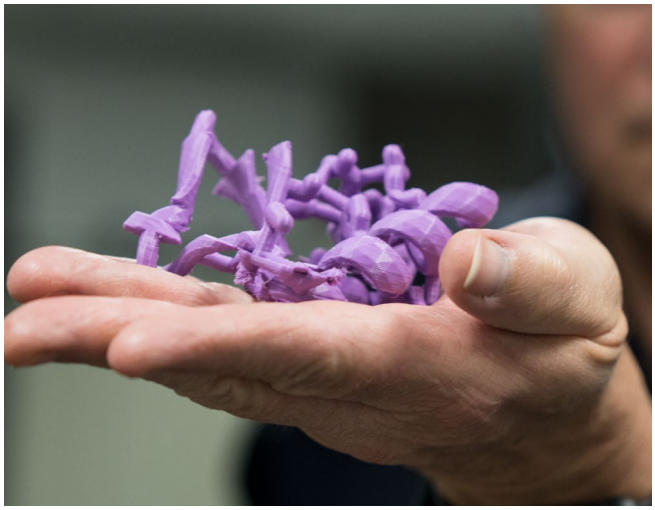
It is possible to join at Level 1 and upgrade to Level 2 at a later date with a prorated charge. More information about the different levels of software is available online at csbf.stanford.edu/membership/Level1.html and csbf.stanford.edu/membership/Level2.html.

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 also protected by a Halon™ fire suppression system. The server room also houses computer equipment from other labs and service centers in the Beckman Center, providing a secure location to store important computer hardware and research data. The server room is equipped with a variety of environmental monitors and CSBF staff members are alerted by email or text message if there is a problem in the room.

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

ACADEMIC DEPARTMENTS

ACADEMIC DEPARTMENTS

DEPARTMENT OF BIOCHEMISTRY

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

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

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. **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 is interested in how assemblies of cells signal to one another. Her research group studies choanoflagellates (the closest living single-celled relatives to animals), which form complex colonial organizations through intercellular signaling. **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.

2021-2022 FACULTY HONORS, AWARDS AND APPOINTMENTS

Rhiju Das—associate professor of biochemistry, was among 33 scientists from 21 institutions announced as new Howard Hughes Medical Institute investigators. Investigators are invited for a seven-year term to pursue highly innovative biomedical research. In addition to covering the researchers' full salary and benefits, and a research budget, the institute covers other expenses, including research space and the purchase of critical equipment.

Dr. Das was named for his work exploring the three-dimensional structures of biological molecules. His team began by adapting computational methods that previously had been used to predict protein shapes. Bringing these tools together with experimental data from collaborators helped reveal the shapes of entire viruses and key intracellular, RNA-based molecular machines.

Daniel Herschlag—professor of biochemistry, was awarded the 2022 Stein & Moore Award, which is sponsored by The Protein Society. The award recognizes eminent leaders in protein science who have made sustained high-impact research contributions to the field. The award was made to Dr. Herschlag for his work on the frontiers of protein science and biology, and for his distinctive style of scientific inquiry, applying fundamental chemical biophysical and enzymological principles to long-standing and emerging questions in protein science and biology.

Lingyin Li—assistant professor of biochemistry, received the 2022 Eli Lilly Award in Biological Chemistry for her contributions to discovering the molecular basis for innate immune activation through the STING pathway.

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.

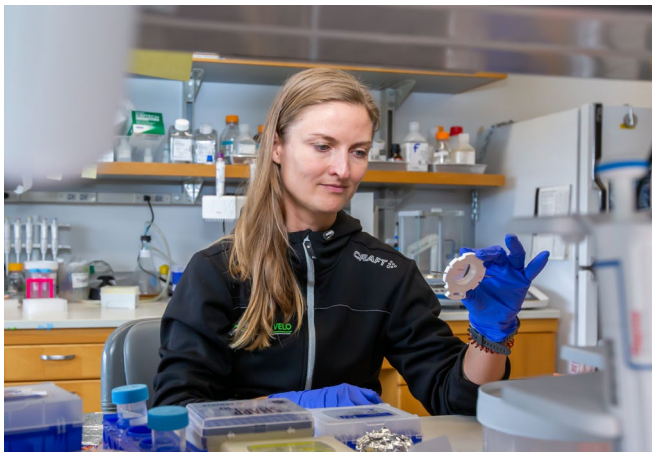
FACULTY RESEARCH

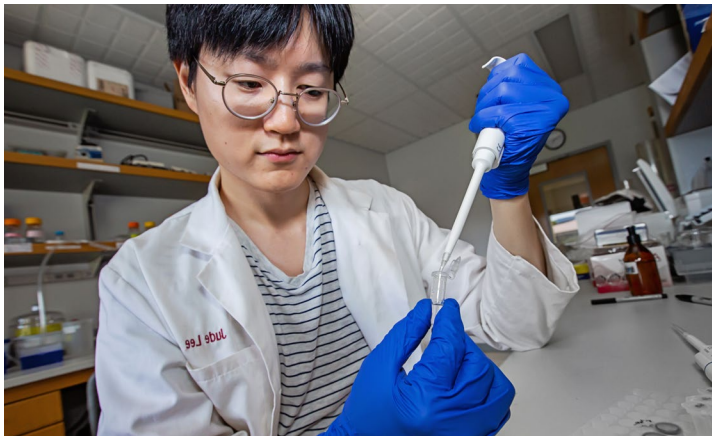
Philip Beachy's group studies the function of hedgehog proteins and other extracellular signals in injury repair and regeneration, primarily through effects on stem cell physiology. They also study abnormal signaling and perturbed stem cell physiology as it occurs in tissue disorder and in the formation and expansion of cancer stem cells. The members of **Gill Bejerano**'s lab focus on a fundamental question in human genomics: the relationship between geno(me) type and phenotype. The group studies genome function in human and related species by mapping genome sequence (variation) to phenotype (differences) and extracting specific genetic insights from deep sequencing measurements. **Alistair Boettiger**'s lab aims to understand how long-range interactions between nonconsecutive parts of the genome are regulated to control gene expression. **James Chen**'s group integrates synthetic chemistry and developmental biology to interrogate the molecular mechanisms that control embryonic patterning, tissue regeneration, and oncogenesis. The focus of research in the **Gerald Crabtree** laboratory is the role of chromatin regulation

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

in development and human cancer. **Margaret Fuller**'s research group seeks to understand the mechanisms that regulate stem cell behavior, and in particular, the mechanisms that regulate and mediate cellular differentiation during male gametogenesis, using spermatogenesis in *Drosophila* as a powerful genetic model system. **Daniel Jarosz**'s lab aims to gain insight into the interplay among genetic variation, phenotypic diversity, and environmental fluctuations in complex cellular systems. **Seung K. Kim**'s lab has created unprecedented opportunities for harnessing knowledge about the molecular and cellular basis of pancreatic development and growth to restore pancreas islet function and to diagnose pancreas cancers. They trust their discoveries will provide the tools and expertise needed to produce islet regeneration therapies for type 1 diabetes, improve treatments and tests to mitigate or prevent type 2 diabetes, and generate new therapeutic strategies for endocrine or exocrine pancreas cancers.

David Kingsley is using a combination of genetic and genomic approaches to identify the detailed molecular mechanisms that control evolutionary change in vertebrates. **Kyle M. Loh**'s lab aspires to understand how different human cell types form from stem cells, and how developing tissues incipiently take shape and form. **Nicole M. Martinez** and her research group are interested in RNA-based mechanisms of gene regulation. They are currently studying mechanisms and functions of RNA modifications in pre-mRNA processing and their roles in development and disease. **Roeland Nusse**'s

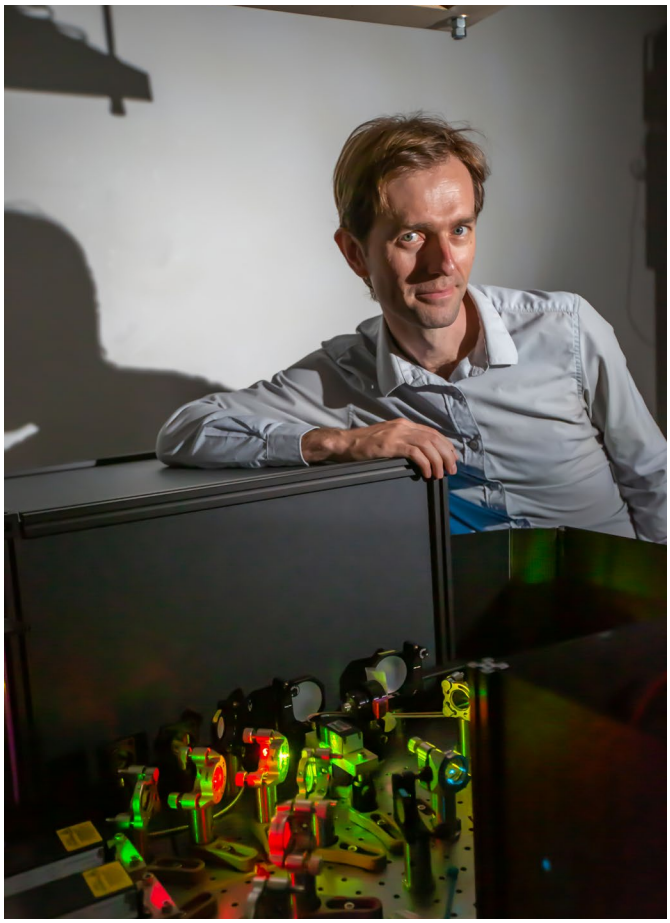


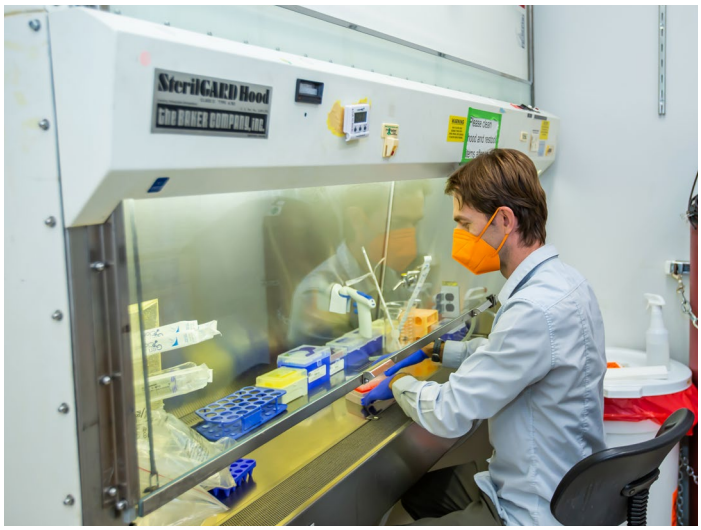


LAB OF ALISTAIR BOETTIGER, PH.D.

Clockwise from Top: Tonia Hafner; William Roman; Aleena Patel; Jude Lee; Sedona Murphy

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 is interested in how assemblies of cells signal to one another. Her research group studies choanoflagellates (the closest living single-celled relatives to animals), which form complex colonial organizations through intercellular signaling. **Lucy Shapiro**'s laboratory studies the





Left and Bottom Right: Alistair Boettiger

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.

2021-2022 FACULTY HONORS, AWARDS AND APPOINTMENTS

Margaret T. Fuller—the Reed-Hodgson Professor of Human Biology and professor of developmental biology and of genetics, won the 2022 Genetics Society of America Medal for her pioneering work on cellular mechanisms that underlie *Drosophila* spermatogenesis.

Seung K. Kim—professor of developmental biology, and director of the Stanford Diabetes Research Center, was awarded the 2020-21 Stanford Biosciences Award for Excellence in Graduate Teaching at the School of Medicine commencement on June 12, 2021.

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.

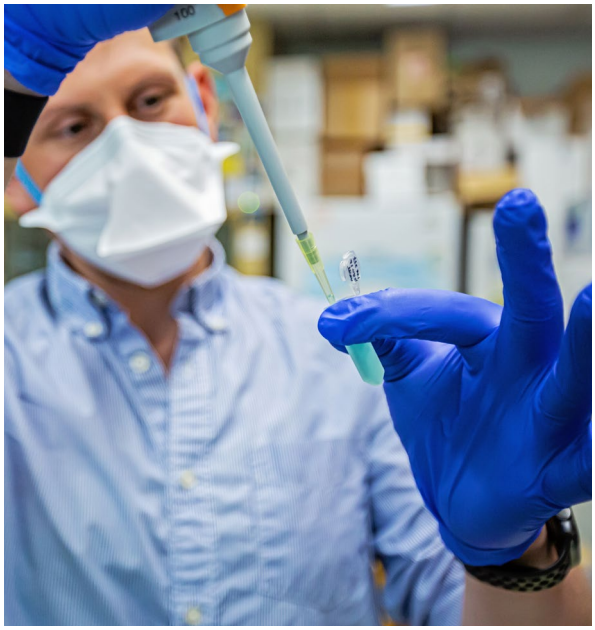
By uncovering molecular and cellular processes, MCP scientists have established new paradigms in the biology of signaling and communication, such as the relationship between the structure and function of G-protein-coupled receptors (GPCRs), and the presynaptic molecular mechanisms underlying neuronal 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.

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

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 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






LAB OF BRIAN KOBILKA, M.D.

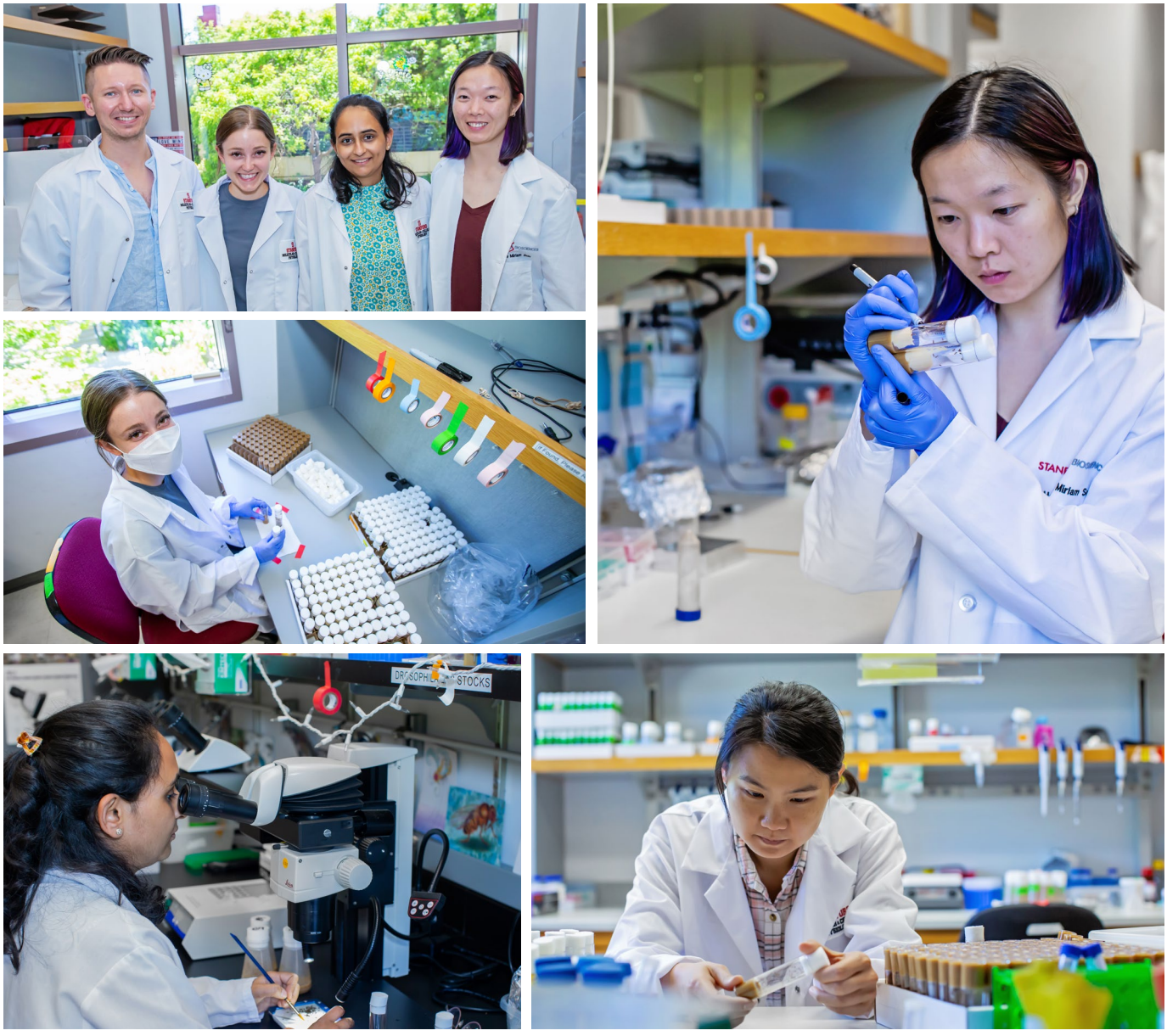
Clockwise from Top Right: Betsy White; Brian Kobilka; John Janetzo; Marina Casiraghi

microscopy. **Brian Kobilka**'s laboratory investigates the molecular mechanisms of G-protein-coupled receptor signaling. G-protein-coupled receptors are responsible for the majority of cellular responses to hormones and neurotransmitters, as well as the senses of sight, olfaction, and taste. The laboratory of **Richard Lewis** investigates calcium signaling mechanisms and their consequences for cell behavior, with a focus on store-operated calcium channels. **Daniel Madison**'s laboratory uses electrophysiological techniques to study the mechanisms of synaptic transmission and plasticity in the mammalian hippocampus. A major focus of the lab is the study of long-term potentiation and mechanisms underlying memory formation in the central nervous system. The goal of research in **Merritt Maduke**'s lab is to determine the molecular mechanisms of chloride selective ion channels and transporters. These membrane proteins are ubiquitously expressed in humans and are necessary for proper cardiovascular, muscular, neuronal, and epithelial function. **Lucy O'Brien**'s lab uses a stem-cell-based *Drosophila* epithelium, the intestinal lining of the adult midgut, as a system to explore the regulatory interface of stem cell and epithelial tissue biology. **Georgios Skiniotis** and his research group are using electron cryo-microscopy (cryoEM) to study the mechanisms of transmembrane signal instigation, with a particular focus on G-protein-coupled

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

2021-2022 FACULTY HONORS, AWARDS AND APPOINTMENTS

Chris Garcia—professor of structural biology and the Younger Family Professor, won the 2021 ICIS-BioLegend William E. Paul Award for Excellence in Cytokine Research. The honor is for leading biomedical researchers who have made important contributions to the field. Dr. Garcia was cited for his enormous contributions to understanding receptor-ligand interactions in the area of cytokine and interferon research.



LAB OF LUCY O'BRIEN, PH.D.

Clockwise from Top Right: 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

2021-2022 FACULTY HONORS, AWARDS AND APPOINTMENTS

Helen M. Blau—director of the Baxter Laboratory for Stem Cell Biology and professor of microbiology and immunology, was elected to the board of directors of the International Society for Stem Cell Research. She has been a member of the society since its inception in 2002 and has served on many of its committees.

John Boothroyd—the Burt and Marion Avery Professor of Immunology, won the Alice and C.C. Wang Award in Molecular Parasitology from the American Society for Biochemistry and Molecular Biology. Dr. Boothroyd studies the pathogenesis of parasitic infections, particularly *Toxoplasma gondii*.

Christina Curtis—associate professor of oncology and of genetics, received the Award for Outstanding Achievement in Basic Cancer Research from the American Association for Cancer Research. The honor is in recognition of her paradigm-shifting research on tumor evolution, including the "big bang" model, which explains how treatment-naive cancers grow in the absence of therapeutic influence.

Dr. Curtis was also named a Komen Scholar by the Susan G. Komen foundation. Her work in systems biology has defined new biomarkers of aggressive breast cancer and has led to new insights in tumor progression.

Mark M. Davis—the Burt and Marion Avery Family Professor, professor of microbiology and immunology, and director of the Stanford Institute for Immunity, Transplantation and Infection, has been elected as the 2022-2023 president of the American Association of Immunologists, which is the oldest immunology society in the U.S., founded in 1913.

Karl Deisseroth—the D.H. Chen Professor and a professor of bioengineering and of psychiatry and behavioral sciences, has been awarded an honorary doctorate from Erasmus University Rotterdam for his discovery of optogenetics, a technique that makes it possible to use light to activate specific brain cells.

Anna L. Gloyn—professor of pediatrics, is the recipient of the 2022 Outstanding Scientific Achievement Award from the American Diabetes Association; the award recognizes research in diabetes that demonstrates independence of thought and originality. Dr. Gloyn studies the genetic basis of diabetes and pancreatic islet cell dysfunction.

Mark Kasevich—professor of physics and of applied physics, and a 2021 Beckman Center Technology Development Seed Grant awardee, was elected to membership in the prestigious National Academy of Sciences at the academy's 159th annual meeting in April 2022. Scholars are elected in recognition of their outstanding contributions to research. The National Academy of Sciences is a private organization, created in 1863 to advise the nation on issues related to science and technology. Dr. Kasevich is an experimental physicist whose work informs development of high-accuracy navigation and sensing, and whose interests include, among many other things, advanced microscopy techniques.

Lloyd B. Minor—professor of otolaryngology – head and neck surgery, and dean of the Stanford University School of Medicine, received the 2022 Award of Merit from the American Otological Society. The award recognizes his advances in the understanding of the neurophysiology of the vestibular system and for the discovery of superior canal dehiscence syndrome.

Michelle Monje—associate professor of neurology, was among 33 scientists from 21 institutions announced as new Howard Hughes Medical Institute investigators. Investigators are invited for a seven-year term to pursue highly innovative biomedical research. In addition to covering the researchers' full salary and benefits, and

a research budget, the institute covers other expenses, including research space and the purchase of critical equipment.

Dr. Monje was named for her research targeting a group of aggressive and deadly brain tumors. Called gliomas, these tumors arise from the glial cells that surround and support neurons. Dr. Monje's work on understanding healthy and cancerous glia is providing new hope for better glioma therapies, as well as a better understanding of a common side effect of cancer chemotherapy, cognitive impairment known as "chemobrain."

Sergiu P. Pasca—associate professor of psychiatry and behavioral sciences and the Bonnie Uytengsu and Family Director of the Stanford Brain Organogenesis Program, received the Theodore Reich Young Investigator Award from the International Society of Psychiatric Genetics. Dr. Pasca pioneered a way to integrate 3D brain-region-specific organoids and study neural circuit formation in preparations known as assembloids.

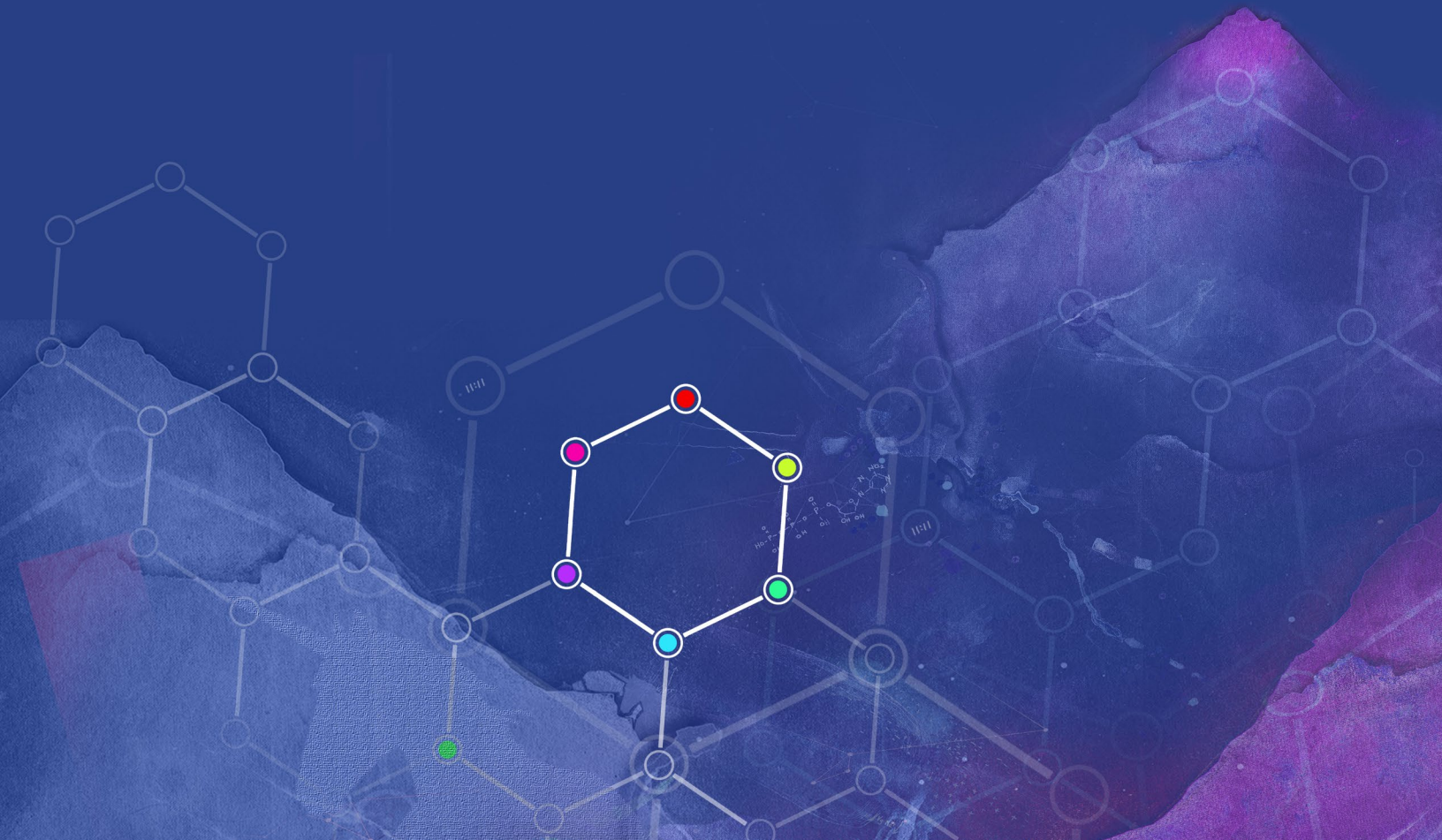
Dr. Pasca also received the 2022 IBRO-Dargut and Milena Kemali International Prize for Research in the field of Basic and Clinical Neurosciences from the International Brain Research Organization. The award is in recognition of his innovative research work using stem cell technology to create human brain organoids and assembloids, which allow researchers to study the cellular mechanisms of human brain development and disease.

Dr. Pasca also received the Joseph Altman Award in Developmental Neuroscience from the Japan Neuroscience Society. He was recognized for pioneering human stem-cell-based models of brain development and for insights into the molecular mechanisms leading to disease.

Kristy Red-Horse—associate professor of biology, was among 33 scientists from 21 institutions announced as new Howard Hughes Medical Institute investigators. Investigators are invited for a seven-year term to pursue highly innovative biomedical research. In addition to covering the researchers' full salary and benefits, and a research budget, the institute covers other expenses, including research space and the purchase of critical equipment.

Dr. Red-Horse was named for her research on blood vessels of the heart and a special subtype called collateral arteries, which can function as natural coronary artery bypasses. Dr. Red-Horse and her team have been studying how and when collateral arteries form, and whether inducing their growth might pave the way for a therapy for individuals with coronary heart disease.

MEDIA COVERAGE



MEDIA COVERAGE

Included in the appendix are the following articles referenced in the 2021-2022 Highlights section.

"Beckman Center Awards Seed Funding to Five Projects"
The Beckman Center News
December 13, 2021
https://med.stanford.edu/beckman/news/NewsletterFall2021-BeckmanCenterAwardsSeedFundingtoFiveProjects.html

"Flora Rutaganira, Ph.D., Joins the Beckman Center"
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"Face, brain development tightly linked, study finds"
Stanford Medicine News Center
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https://med.stanford.edu/news/all-news/2021/03/Face-brain-development-tightly-linked-study-finds.html

"Stanford machine learning algorithm predicts biological structures more accurately than ever before"
BIO-X News
August 26, 2021
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August 6, 2021
https://med.stanford.edu/news/all-news/2021/08/brain-navigation-center-calls-on-mental-state-as-well-as-physical-environmen-.html

"Secrets of how cells cram in oversized genomes revealed"
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August 5, 2021
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"Stanford researchers make 'bombshell' discovery of an entirely new kind of biomolecule"
Stanford News
May 17, 2021
https://news.stanford.edu/2021/05/17/stanford-study-reveals-new-biomolecule/

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