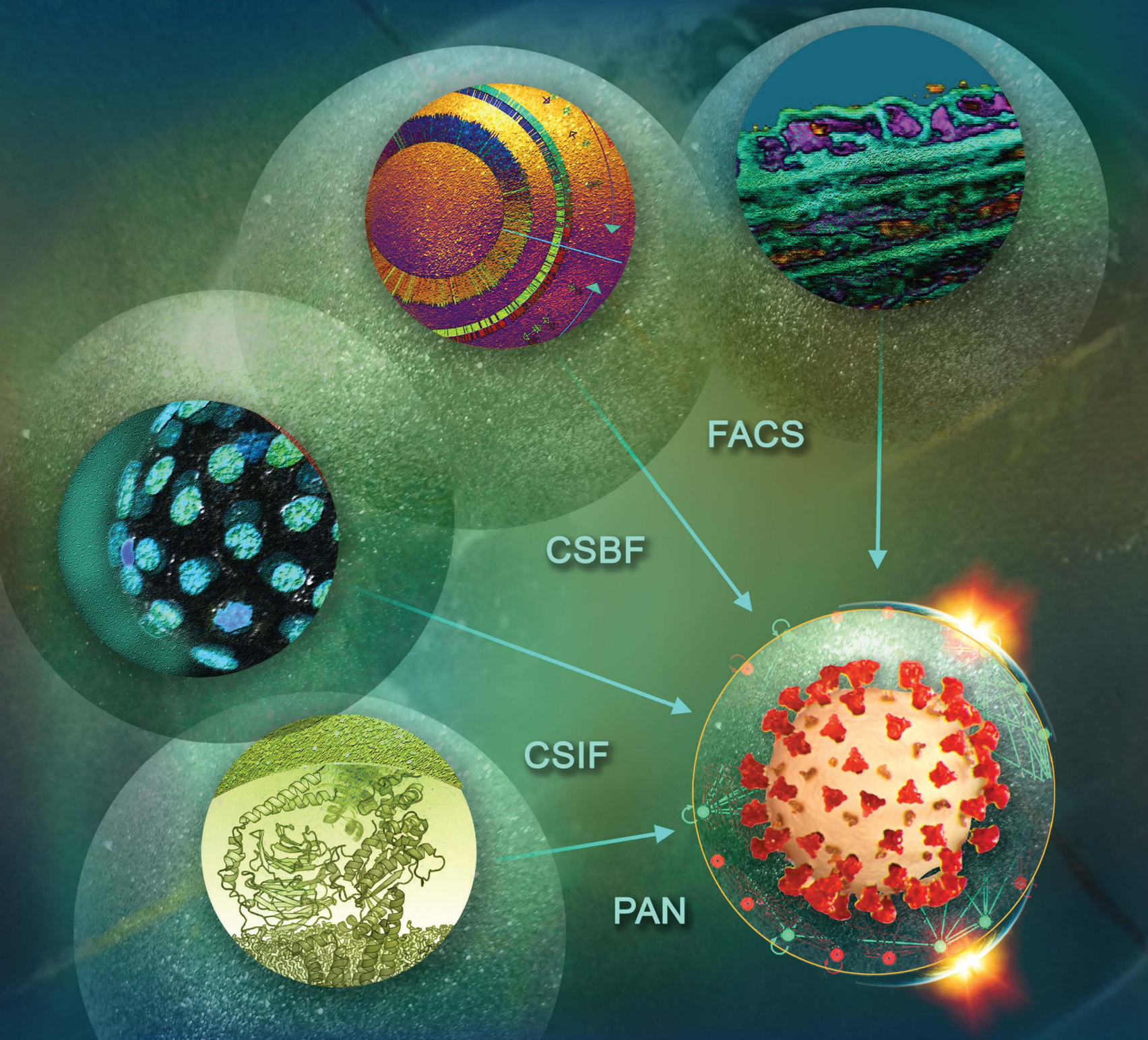
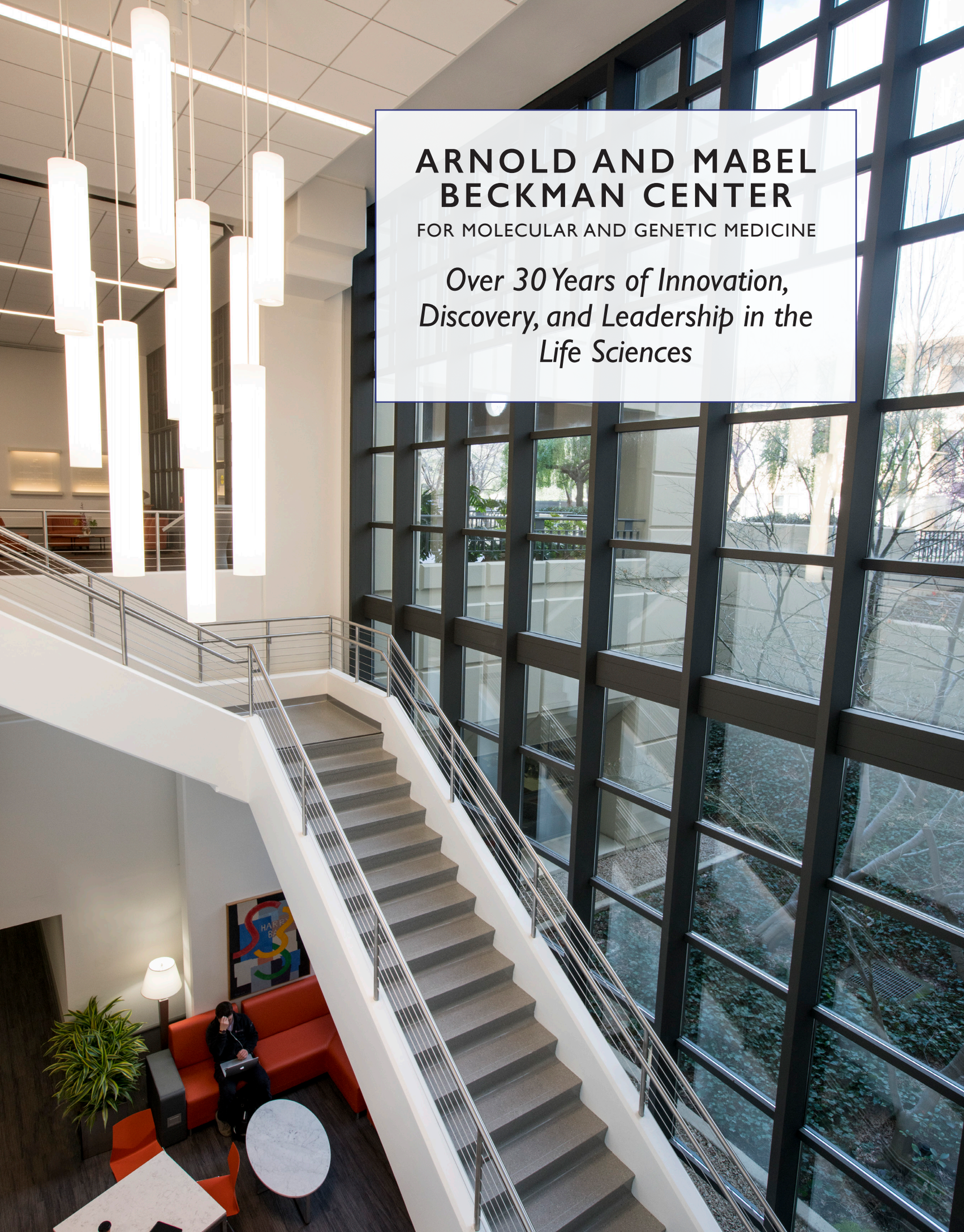
Stanford | Beckman Center
for Molecular and Genetic Medicine



Beckman Service Centers: Providing the Most Advanced Technologies to Stanford Researchers & Beyond

2020 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,

2019-2020 has been a highly productive year for the Arnold and Mabel Beckman Center for Molecular and Genetic Medicine at Stanford University School of Medicine.

Among the many achievements this year, the center recruited an outstanding new faculty member, provided Technology Development Grant funding for five high risk/high reward seed grant projects, and received National Institutes of Health (NIH) funding for the purchase of new cutting-edge technologies in the Beckman Service Centers, which advance basic research and translational medicine. In addition, the service centers greatly expanded the outreach and training opportunities they provide to researchers across the Stanford campus, launching two new bimonthly seminar series on the application and use of the centers' advanced technologies. Also this year, the training of young scientists in our Medical Scholars Program continued to

be a high priority in promoting translational medicine. Finally, in conjunction with the Chan Zuckerberg Biohub, the Beckman Center has invited renowned scientists to participate in our upcoming 30th anniversary Beckman Symposium, "Climate Change and World Health," to be held in the near future.

Our new faculty member, Silvana Konermann, Ph.D., was recruited in October 2019 to join the Beckman Center as an assistant professor in the Department of Biochemistry. Dr. Konermann's interests lie in dissecting key molecular pathways in human neurodegenerative diseases. Dr. Konermann did her graduate work with Feng Zhang, Ph.D., at the Broad Institute of MIT and Harvard, where she focused on the development of technologies to enable efficient, generalizable, and precise perturbation of mammalian gene expression. As a postdoc in the laboratory of Patrick Hsu, Ph.D., at the Salk Institute for Biological Studies, she discovered a new family of RNA-targeting CRISPR nucleases and harnessed it as an efficient tool for programmable RNA perturbation and the reduction of pathological tau isoforms in a model of frontotemporal dementia. As a new member of the Department of Biochemistry, Dr. Konermann will develop and apply technologies for high-throughput transcriptional perturbations, to understand the cellular and molecular pathways driving human genetic risk in neurodegenerative disease.

Funds from the Arnold and Mabel Beckman Foundation leverage our continued ability to obtain additional funding from federal sources. One of our service centers, the Cell Sciences Imaging Facility (CSIF), was awarded an NIH Shared Instrumentation Grant in the amount of $196,083; the funds will used to purchase a OneView scintillator-coupled sCMOS transmission electron microscope (TEM) camera and a OneView 16bit computer running Gatan Microscopy Suite V3 software. The sCMOS TEM camera represents a new class of state-of-the-art, fast, and sensitive sCMOS scintillator-coupled cameras that offer a large field of view and real-time drift correction. The availability of this camera will greatly advance Stanford's biomedical electron-microscopy-dependent research.

Another service center, the Fluorescence Activated Cell Sorting (FACS) Facility, also received an NIH Shared Instrumentation Grant. That award, in the amount of $510,431, is for the purchase of a FACSymphony 5-laser, 30-parameter analyzer. The facility will also purchase a Symphony S6 sorter. Both purchases reflect the growing trend for high-parameter, high-throughput, single-cell analysis. The number of distinct fluorescent dyes on the market has increased substantially, while at the

same time we've seen the utilization of faster electronics and high-end optics in modern cytometers. These advancements now allow researchers to extract in-depth information from complex biological samples at a throughput that is far greater than any other available technology (20,000+ cells per second).

Expanding our outreach efforts, the FACS Facility and CSIF have each initiated a new bimonthly seminar series to provide education and training on the application of the advanced technologies offered in those service centers. The "Get the FACS" seminar series features lectures from FACS staff and outside experts on a range of topics in flow cytometry. CSIF's "What's the SCOPE?" imaging seminar series features in-depth discussions by CSIF scientists and guest speakers on new and existing advanced imaging technologies. Both seminar series have been exceedingly well attended and have contributed to an increase in demand for services in the facilities.

Stay tuned for updates on the 30th anniversary Beckman Symposium, on the topic of "Climate Change and World Health." The symposium is being hosted jointly by the Beckman Center and the Chan Zuckerberg Biohub. Experts in the field will give presentations on the role climate change is playing in altering the geographical locations of the world's disease vectors and the spread of deadly pathogens, and the need for breakthrough technologies to respond to this threat. More information on this exciting event to come.

I want to thank the Beckman Foundation again this year for their valuable partnership with us and their support for our programs and facilities. Beckman Center programs and activities play a vital role in supporting research and state-of-the-art technology at Stanford. We look forward to continuing our partnership with the foundation in the coming years.

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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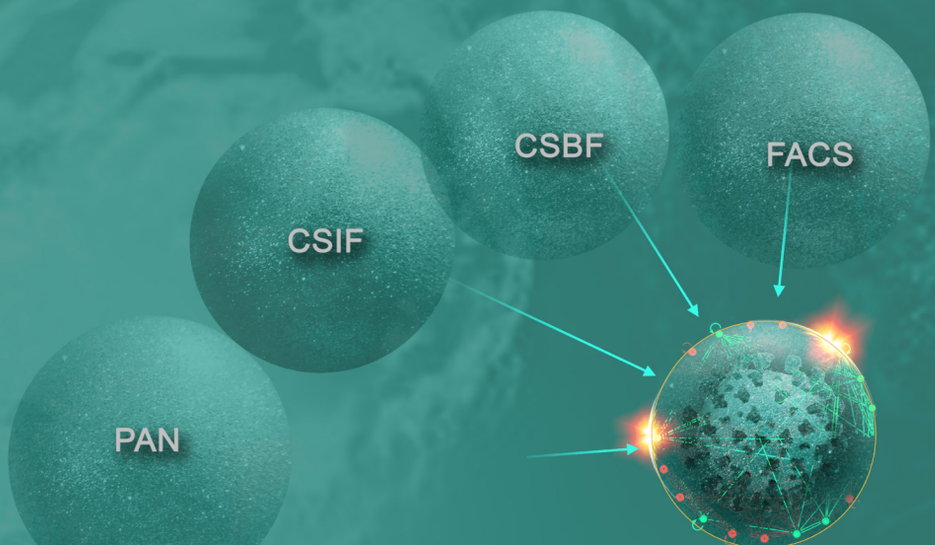


BECKMAN SERVICE CENTERS: PROVIDING THE MOST ADVANCED TECHNOLOGIES TO STANFORD RESEARCHERS & BEYOND

By Sarah C.P. Williams

Lucy O'Brien, Ph.D., a Stanford University molecular biologist, studies what happens to the stem cells inside fruit fly intestines as flies eat and digest their food. To learn how the gut is able to continuously generate new cells—a process that could teach researchers about organ regeneration and wound healing—Dr. O'Brien needs to create high-resolution videos of these stem cells and then use powerful software to analyze those images. But Dr. O'Brien's laboratory doesn't have the microscopy equipment that is needed for these experiments, and buying a license for the software—which costs upwards of $20,000 per user—would stretch her budget thin.

Luckily for Dr. O'Brien, she can take advantage of the Beckman Center's shared technology resources—four core service centers that serve departments and laboratories throughout Stanford University. Dr. O'Brien is a regular at one of the service centers, the Cell Sciences Imaging Facility (CSIF), where staff have helped develop new ways of imaging the insides of living fruit flies. She's also worked with another service center, the Computational Services and Bioinformatics Facility (CSBF), to get access to the 4-D visualization software she needs.

"I don't know what I would have done without these facilities," said Dr. O'Brien. "Having access to cutting-edge microscopy and the training to use it has really let my lab explore in directions we wouldn't have been able to otherwise."

The ability to explore—it's a sentiment that's echoed by every faculty member who uses the Beckman Service Centers. The facilities provide not only equipment and software, but training and support that's hard to put a price tag on.

Without the centers, many faculty say, their research would either have to take another direction or they'd have to find more funding.

"The service centers especially offer a lot of help for new and emerging researchers," said Kara Brower, a bioengineering graduate student whose research relies on equipment and expertise at another Beckman Service Center, the Fluorescence Activated Cell Sorting (FACS) Facility.

Beckman's original shared technology resources were established in 1989—before the advent of genome sequencing, before stem cells could be generated from human skin, and before some of the highest-resolution forms of microscopy had even been dreamed of. For three decades, the service centers have been keeping up with advances in technology and adapting to meet the needs of Stanford scientists.

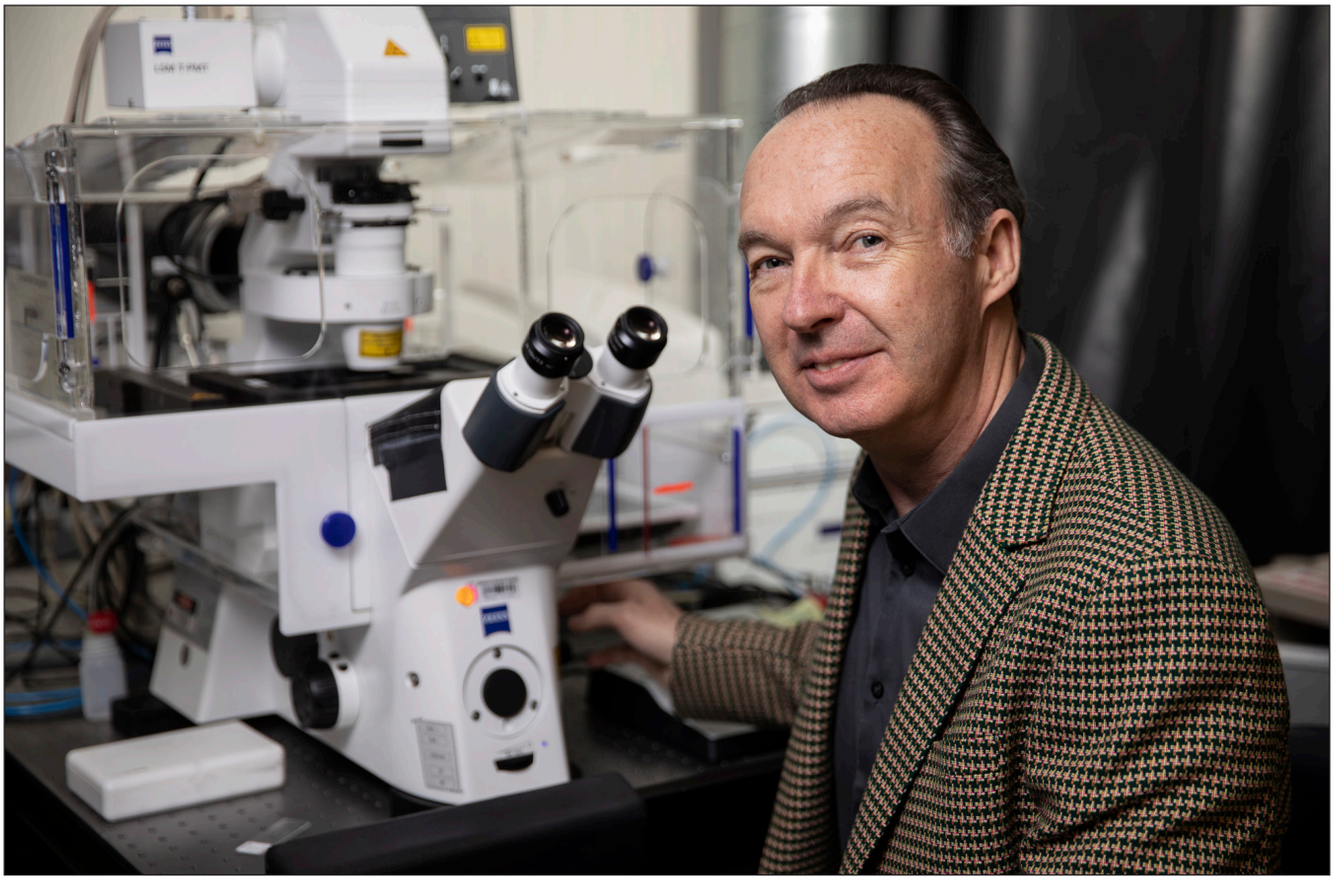
Today, the directors of the service centers are not only acquiring the newest technologies. They are also forging new collaborations that allow researchers to easily use more than one service center for a single line of investigation, such as when Dr. O'Brien snaps shots of cells at CSIF and then uses software from CSBF to parse her data. The service center directors are working together to streamline these collaborative workflows and offer new combination services to their customers.

Cell Sciences Imaging Facility: A High-Resolution View

Since the 17th century, when Anton van Leeuwenhoek carefully ground glass into microscope lenses and discovered a miniscule living world inside pond water, microscopy has been a mainstay of biology. But today's biological investigations make use of equipment far more sophisticated than handmade glass lenses. Microscopes now allow researchers to see the three-dimensional structure of subcellular organelles in living cells, simultaneously follow dozens of molecular markers, and locate cells deep within living tissues. Each scope, though, costs at least half a million dollars to buy, plus tens of thousands of dollars a year for the service contracts needed to keep the equipment running smoothly.

"From a taxpayer and institutional perspective, it just doesn't make sense for every individual lab to have this kind of instrumentation," said Jonathan Mulholland, director of the CSIF. "It's much more cost-effective to have a central facility."

The CSIF is that central facility. With two electron microscopes, eight light microscopes, an atomic force microscope, and other specialized equipment, the CSIF can support nearly every state-of-the-art microscopy approach that's in use today. As scopes push the limits



Jonathan Mulholland
Director of the Cell Sciences Imaging Facility

of what's possible to view—with super-resolution imaging now able to overcome the resolution limit set by the diffraction of light—Mulholland makes sure his facility keeps up.

"The tendency has been to move toward higher and higher resolution," he said. "It's a cliché, but everyone wants to do it. So we do quite a bit of super-resolution imaging these days."

The light microscopes are the most readily available and easy-to-use pieces of CSIF equipment. Light microscopes use glass lenses and narrow, focused beams of light to visualize the fine details of cells. Researchers often use light microscopes when they've marked molecules with fluorescent tags; the microscopes allow them to locate and follow the fluorescent dots that indicate those tagged molecules.

Each light microscope in the CSIF has slightly different specs; some have wider fields of view, some can take videos with many frames per second, and some are better when fluorescent tags are dim and hard to see. Most can image at super-resolution, allow imaging of live cells, and let researchers visualize several different color fluorescent tags at once.

To reserve a CSIF light microscope, a researcher new to the facility first has to get trained on how to use the equipment. Mulholland and his staff—which includes seven scientists, each with a different area of expertise—offer individual training as well as more formal courses. Each year, for instance, Mulholland and two colleagues run an eight-week course in light microscopy, stressing hands-on experience. The CSIF also holds a bimonthly seminar series, "What's the Scope?," which presents the latest technologies available at the facility.

When Mulholland's team trains users on the light microscopes, they try to ensure that the researchers not only know what buttons to press on the scope, they also know how to prepare a sample and choose the right microscope. Once a researcher finishes their training, they can book microscope time in the CSIF on their own.

"We're always here and peeking in on people as they're imaging, though," said Mulholland. "If they have problems, they know they can always ask us for help."

When researchers want to use electron microscopy (EM), the facility staff is not quite as hands-off. Electron microscopes use beams of electrons, rather than light, to illuminate samples. Before being placed under the microscope, cells or tissues must be prepared—frozen, in some cases, and cut into ultra-thin slices.

"I hate to call electron microscopy an art, but it really is a craft," said Mulholland. "People need years of experience to really get good at it."

If a researcher wants to be trained in every step of sample preparation for electron microscopy, CSIF staff will train them. But more often, Mulholland said, researchers drop off tissue samples and his staff prepare them for EM, using freezers and ultramicrotomes at the service center to slice the tissue. When the samples are ready to go under the scope, the researcher comes back for the imaging.

When it comes to the third, and newest, class of imaging available at CSIF—multiplexed imaging—Mulholland's team takes over more fully, running the microscopes themselves and providing final images for researchers. CSIF acquired a multiplexed CODEX platform in late 2018, with financial support from the Beckman Center, the Stanford School of Medicine Dean's Office, and the Stanford Cancer Institute. In early 2019, the CODEX service became available to researchers.

CODEX, which was developed at Stanford by microbiology and immunology professor Garry Nolan, Ph.D., allows the simultaneous visualization of many more proteins than was previously possible. If a researcher wants to locate, say, 50 different proteins in a single tissue, other light microscopy

"This technology is a great example of how cutting-edge technologies can be developed at Stanford and then we bring them back into a shared facility and provide them as a resource to researchers more broadly." –Jonathan Mulholland

methods fall short; the fluorescent tags' signals overlap and blend together, making it difficult to distinguish the molecules. CODEX, though, uses unique tags coupled with molecular barcodes that let researchers visualize a handful of proteins at once and repeat the experiment with different sets of proteins and tags.

"This technology is a great example of how cutting-edge technologies can be developed at Stanford and then we bring them back into a shared facility and provide them as a resource to researchers more broadly," said Mulholland.

The CODEX platform, he said, is particularly a boon to cancer researchers who are trying to visualize complex, heterogeneous tumors and understand the roles of dozens of genes and proteins in the tumors' microenvironments.

Also in 2019, CSIF installed a lattice light sheet microscope, thanks to a $1 million grant from the Howard Hughes Medical Institute awarded to faculty member Joanna Wysocka, Ph.D., the Lorry Lokey Professor in the School of Medicine, professor of developmental biology, and of chemical and systems biology, and a Howard Hughes Medical Institute investigator. Dr. Wysocka secured the funding to help support her own research on the DNA inside stem cells, but the microscope will be housed in the CSIF and will have a dedicated technician to help run it for other researchers.

"There's a lot of buzz about lattice light sheet microscopy," said Mulholland. "So this is a very exciting acquisition."

What's the difference? Most light microscopes aim a beam of light at a sample from the same direction as the microscope. But as researchers image this illuminated spot, the fluorescent tags are photobleached—they disappear over time. In a lattice light sheet microscope, in contrast, the light comes from the side, illuminating a large, thin plane of a sample. This setup helps minimize photobleaching and gives researchers more time to study a tissue. It generates brighter, clearer images than other microscopy methods and—in the process—massive amounts of data.

Many researchers, once they have obtained images using the CSIF equipment, move forward with analysis of their images on their own. Mulholland is now increasing

CSIF's role in that area with a new bioimage analysis service. The new service has, for example, helped researchers to count the number of nerves in a ganglion nerve bundle, follow cells infected by the parasite *Toxoplasma gondii*, and identify all the cells' nuclei within cultures of human embryonic stem cells, among other projects.

"Right now, a big focus is to expand that analysis and processing service to also offer research support on the front end of experiments," said Mulholland.

While CSIF's existing training includes generic suggestions on sample preparation, and the staff provides help when asked, researchers frequently come to the facility armed with samples that, unfortunately, won't help them to get the data they need. To solve that problem, Mulholland envisions a more formal and advertised service where staff could work one-on-one with researchers before they ever begin experiments, to help fine-tune the details.

"We would sit down, find out the objective of the experiments and help determine what kind of analysis and routines and statistics are needed," he said. "That would ensure that researchers end up with data that's adequate."

Many heavy users of CSIF, though, already see the facility as informally offering this kind of help. Dr. O'Brien, for instance, consulted frequently with Mulholland and his staff as she developed new ways to image fruit fly intestines.

"They are really great at working with researchers and talking to us about what we need," she said. Her group, for example, wanted to develop custom apparatuses that fit onto the CSIF light microscopes and held fruit flies in place while they were being imaged. She came up with a prototype and the CSIF staff helped her adjust it to fit.

For Mulholland—and other service center directors—this kind of back-and-forth with researchers to get things just right is one of the rewarding parts of the job. "The best part, at the end of the day, is working with students and researchers to get their results," he said.

Mulholland also loves staying on top of new technology, and helping guide decisions on what new instrumentation will be acquired next at CSIF. It's a job that never ends. "There is always new and cool instrumentation we want to get," he said. "The technology is developing so quickly."

"The best part, at the end of the day, is working with students and researchers to get their results."
—Jonathan Mulholland

Fluorescence Activated Cell Sorting Facility: Expanding the Rainbow

Each year, more than 800 different researchers from 200 labs around Stanford visit the FACS Facility at the Beckman Center. There, they use florescence activated cell sorting technologies (also known as FACS) to sort cells into distinct subsets based on their molecular properties. An immunologist, for example, might want to sort immune cells based on which ones respond to a vaccine and which don't. A cancer researcher may want to differentiate groups of diverse tumor cells from each other.

"This is a very valuable and widely used technology," said Lisa Nichols, Ph.D., director of the FACS Facility. "But it's not practical for every lab to have their own equipment."

FACS is a high-throughput technology that separates cells based on molecular characteristics or fluorescent tags; the machines at the Beckman service center can sort 10,000 to 20,000 cells per second. Five years ago, most researchers using the technology were sorting these large samples of cells using only five to six different fluorescent colors. Now, the FACS Facility at Beckman has more advanced machines that are capable of analyzing individual cells with up to 40 parameters and using that information to sort up to six distinct populations of cells. The facility is also in line for a new analyzer that can handle more than 30 colors; in collaboration with a faculty member, the facility recently secured funding from the National Institutes of Health for that equipment.

Even the average user of the Beckman facility now uses 10 to 12 fluorescent tags at a time, Dr. Nichols estimated. "We have everyone from people who come in with a very simple assay—a couple of colors—to very high-end users who are doing complex, large, multi-parameter experiments," she said.

With the wide range of backgrounds among the facility's users, training is critical to make sure everyone is able to access FACS equipment and generate quality data. The facility offers individual instruction on equipment use, as well as monthly "Get the FACS" seminars on more

"This is a very valuable and widely used technology, but it's not practical for every lab to have their own equipment."
—Lisa Nichols, Ph.D.

Lisa Nichols, Ph.D.
Director of the Fluorescence Activated Cell Sorting Facility

advanced topics, such as how to create controls in a FACS experiment and how to improve data analysis workflow and methods.

"We want to move past 'what buttons do you push?' to really cover how to get the best data," said Dr. Nichols.

In part, this push for more and better training is related to the age of FACS technology. The approach was developed—in part at Stanford by geneticist Leonard Herzenberg, Ph.D.—in the late 1960s and early 1970s. The first commercial cell sorting machines became available in the 1970s, and FACS was a mainstream laboratory tool by the 1980s.

Thirty years later, many of the people who have expertise in the original technology are retiring, said Dr. Nichols. "We have a lot of knowledge here at Stanford, but it's important right now to develop tools to retain and share that technical knowledge so it stays within the community."

"The more things you can ask about a cell using this kind of technology, the more you can study not only what a cell is, but what it does."
–Lisa Nichols, Ph.D.

Dr. Nichols also regularly teams up with researchers who want to use FACS in new and inventive ways. "The more things you can ask about a cell using this kind of technology, the more you can study not only what a cell is, but what it does," she said.

Graduate student Kara Brower and Polly Fordyce, Ph.D., an assistant professor of bioengineering and of genetics, for example, have developed a new technique that combines FACS and single-cell genomics. Rather than sending whole, intact cells through the FACS sorter, they envelop cells in tiny droplets of water surrounded by a thin shell of oil; each droplet is about half the width of a strand of hair. Inside each droplet, molecules react with the cells, opening them up, exposing their genetic material, and reacting with DNA.

"Instead of using FACS to analyze proteins or the cell's morphology, we can analyze what happens in this droplet volume," said Brower. "The big advance we made was putting droplets through the flow cytometer instead of cells."

Brower added that it took "a huge amount of back and forth" with the FACS Facility to get the technique working. Both Dr. Nichols and one of her staff members were co-authors on the paper describing the new technology, called single droplet double emulsion flow cytometry. "We spent hundreds of hours with them thinking about every parameter we could adjust on the FACS instrument so that anyone at any university will be able to do this."

For Dr. Nichols—a former immunologist who "got caught with her hands in the FACS instruments too many times"—this kind of innovation and research success keeps her job interesting. While FACS has existed for several decades, its use is still growing and expanding to new areas, she said.

Protein and Nucleic Acid Facility: Adapting to Changing Needs

Director Michael Eckart, Ph.D., describes the Protein and Nucleic Acid (PAN) Facility as an "all-encompassing molecular biology center." The facility offers gene sequencing, peptide and oligonucleotide synthesis, mass spectrometry, and single-cell genomics. If a researcher wants to make or study DNA, RNA, or proteins, there's probably a service at the PAN Facility that they can use.

"We are unlike many service centers in that we're not based around one technology platform," said Dr. Eckart. "We have a variety of different technology platforms in different areas, and we're always expanding the number of applications or methodologies that we can run on a particular instrument."

Many of the services offered at the PAN Facility run together into a natural workflow, where researchers use one service after another. For example, someone might want to synthesize primers for a particular gene, run polymerase

Michael Eckart, Ph.D.
Director of the Protein and Nucleic Acid Facility

"Our big advantage is our geographic location within the university. Researchers know who's making their peptides and who's running their experiments and can come sit down with us. I encourage the facility staff to work very closely with individual researchers to fully understand their needs."
—Michael Eckart, Ph.D.

chain reaction (PCR) using the primers, confirm the resulting sequence that's been amplified, express protein corresponding to the gene of interest, and characterize the protein. Surface plasmon resonance (SPR) technology, originally developed to characterize the interactions between antibodies and antigens, can be used to determine whether proteins bind with each other or with other biomolecules. In each step of that gene and protein analysis, the PAN Facility can help.

How is all of this helpful to researchers? Elucidating the biological differences between cells at the molecular level in tumor tissue, stem cells, or rare subpopulations of immune cells can provide significant insight into the development of specific diseases in oncology, neurology, and immunology. To study the differential gene expression in the different biological systems, PAN also provides next-generation genetic sequencing, microarray, and quantitative real-time PCR technologies.

Stanford researcher Charles Chan, M.D., for example, studies the role of stem cells in aging and cancer. He often wants to compare normal stem cells to those affected by diseases such as osteoarthritis, to understand their differences.

"The difficulty in trying to do these types of experiments is they require a lot of specialized equipment and a lot of technical know-how that takes years to accumulate," he said. "We rely on the PAN Facility to help us conduct a wide variety of molecular analyses."

Indeed, while researchers at the CSIF and the FACS Facility can use equipment on their own, the PAN Facility is more of a drop-off shop. In most cases, Dr. Eckart and his staff run the experiments requested by researchers and provide them with the resulting product or data. But that doesn't mean they rarely interact with those researchers. On the contrary, Dr. Eckart sees the facility's one-on-one assistance as an advantage over, for example, mail-off molecular biology services.

"Our big advantage is our geographic location within the university," said Dr. Eckart. "Researchers know who's making their peptides and who's running their

experiments and can come sit down with us. I encourage the facility staff to work very closely with individual researchers to fully understand their needs."

Brian Kobilka, M.D., a professor of molecular and cellular physiology, points to that personal interaction as one reason he uses the PAN Facility instead of other options for sequencing and synthesizing molecules.

"They're willing to listen and provide advice, even if it goes beyond their exact services offered," he said.

Dr. Chan agrees. He often talks to the PAN Facility staff before he even begins experiments, he said, to make sure he's heading in the right direction when it comes to the genetics and proteomics he has planned.

Dr. Eckart recently added to the PAN Facility the capability to synthesize entire genes, in addition to short oligonucleotides. This means that researchers, rather than relying on PCR to amplify gene sequences, can request DNA sequences that don't exist in nature or that come from pathogens they don't want to handle and, in synthetic biology design, test and learn about genes in a high-throughput manner.

The PAN Facility is also moving toward offering more single-cell technologies, which are becoming widely used by biologists. While most genomic and proteomic approaches in the past relied on average measurements across pools of cells, single-cell technologies allow researchers to study how cells within a population differ, or take a close look at rare outliers. The Fluidigm C1 machine at the PAN Facility offers the ability to sequence DNA or RNA in single cells arranged in a 96- or 800-cell chip.

When deciding what new technologies to offer, Dr. Eckart notes he must ride a fine line—providing cutting-edge approaches, but not investing in something that won't be used. "We're always in close communication with researchers and scientists on campus, and in discussions with them it becomes very apparent where the science is headed," he said. "But there needs to be a critical mass of researchers that would use a new technology."

"We're always in communication with researchers on campus, and in discussions it becomes very apparent where the science is headed. But there needs to be a critical mass of researchers that would use a new technology."
—Michael Eckart, Ph.D.

Computational Services and Bioinformatics Facility: More than a Help Desk

In 1989, when the Computational Services and Bioinformatics Facility came into existence, it consisted of a computer room with a terminal. Researchers could visit the terminal to use a handful of data analysis tools or search through national gene and protein databases, which were available on CDs. Now, of course, computing is drastically different. Today, more than 5,000 Stanford researchers rely on CSBF as a one-stop shop for sophisticated software that can be accessed on their own computers, even when offline.

"Most of the software runs on the cloud now, so people don't even need their own computing clusters," said Lee Kozar, director of the CSBF. "It's really made access very easy."

The CSBF runs as a membership-based center. Labs pay an annual fee for access to software ranging from the most basic, such as Microsoft Office, to advanced data and image analysis programs.

In recent years, Kozar has spent much of his time negotiating with software vendors. Many companies eliminated their shared licenses—a mainstay of central facilities like CSBF—and began requiring users to buy a license for each computer that would use a given program. For some costly programs, Kozar found a workaround that saved Stanford researchers significant amounts of grant money: he negotiates a bulk price for licenses, buying up to a thousand at once, and sells them to CSBF members at a steep discount.

"Instead of paying hundreds of dollars for a license, this means a lab might pay more like $35," said Kozar.

Lee Kozar
Director of the Computational Services and Bioinformatics Facility

"Instead of paying hundreds of dollars for a license, this means a lab might pay more like $35."
—Lee Kozar

He likens the business model to joining Costco—some labs, he says, don't see the benefit in paying for a membership while others recognize the savings right away.

Josh Elias, Ph.D., a former assistant professor of chemical and systems biology at Stanford, is in the latter group. Dr. Elias, who is now the mass spectrometry platform leader at the Chan Zuckerberg Biohub, uses mass spectrometry to study proteomes—the collections of proteins present in any given cell. At Stanford, he used software that analyzes mass spectrometry data, identifying molecules that were in a mixture in a test tube.

"The biggest advantage of the CSBF was access to software that would otherwise have been prohibitive to a small lab like ours," said Dr. Elias.

Without Kozar's help, Dr. Elias said, his lab likely would have had only one license for the key piece of software needed. That would have meant that just one lab member or collaborator at a time was able to work on analyzing their data. Instead, Kozar negotiated a deal for five licenses. "It was really good to have access to that," said Dr. Elias.

When it comes to technical support, Kozar and his two staff members often act as intermediaries between researchers and software companies, getting questions answered and working out kinks in the programs. But while other Beckman service centers are ramping up their training efforts, the CSBF has seen a diminishing demand for software training.

"It used to be we would do a lot of training, because the software was very difficult to use," Kozar said. "Nowadays, a lot of the software just walks you right through all the steps and has very graphical, user-friendly interfaces."

The CSBF has dozens of commercial software programs available to researchers, including many that do the same thing, but with different approaches. Kozar recognizes that when it comes to software, people have individual preferences for what they like to use.

"People come from other universities and have spent years being comfortable with a certain software package," he said. "We have multiple software packages so users don't have to relearn how to do something. They can access the software they are familiar with."

With science becoming more collaborative and interdisciplinary, the CSBF has also expanded its software options from those

that were strictly biomedical to a broader suite of programs. They've recently added simulation software that's used by engineers, for instance. And Kozar is always on the lookout for new packages that his customers want—as well as those that could attract new CSBF customers.

Collaboration: Bringing Technologies Together

As single-cell genomics technologies become standard practice, and as molecular biology and imaging approaches generate more and more data, there's an increasing intersection between technologies. That also means an increasing intersection between Beckman's four service centers.

In 2019, for example, the PAN Facility began offering researchers a new service: spatial transcriptomics. The technology combines imaging and protein expression patterns to describe the diversity of transcription that occurs throughout a tissue. A thin slice of frozen tissue is imaged and then protein expression is measured while the tissue is still fixated on a slide, using a series of chemical reactions that make cells release mRNA. The final data paints a picture of how expression patterns fit within the morphology of the tissue.

With the heavy load of imaging integrated into spatial transcriptomics technology, PAN Facility director Dr. Eckart couldn't offer the new service all on his own—so he teamed up with Mulholland at the CSIF.

"This is an example where microscopy and transcriptomics work really nicely together," said Dr. Eckart. "We're working closely with CSIF on that."

Similarly, CSIF relies on the PAN Facility to help researchers use the new CODEX system. Dr. Eckart and his colleagues at the PAN Facility can custom-make the oligonucleotides that are used to barcode proteins of interest. Researchers can prepare for CODEX by ordering the oligos from Dr. Eckart, and then bring their cells and antibodies to CSIF for imaging.

It's also natural for researchers to move from the FACS Facility to other service centers—once cells are sorted using an FACS machine, they can be analyzed in a variety of ways. That may involve imaging cells, sequencing their DNA or measuring data on their gene expression, or running statistical analyses on the cells' groupings.

"We're all working together as technologies start to overlap," said Dr. Eckart.

The services offered by the CSBF also work hand-in-hand with the other service centers. "The other facilities help researchers generate data, but they generally want the users to analyze their data elsewhere," said CSBF director Kozar. "They don't want them to spend time

analyzing data in front of these highly-in-demand instruments." That's where Kozar's ability to provide image analysis software at the CSBF comes in handy.

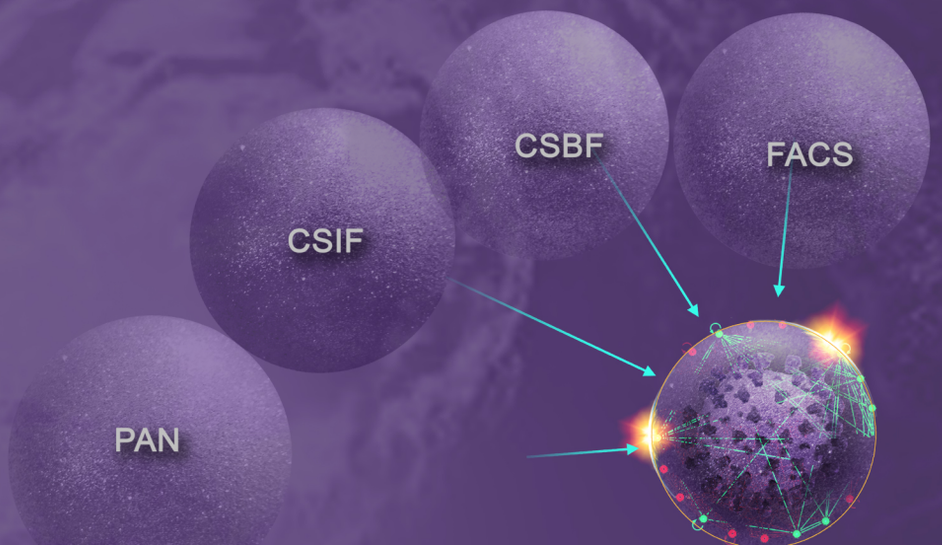
In addition, when new equipment arrives at the CSIF or the PAN Facility, those centers rely on Kozar and his colleagues to make sure users will have the right software to use the data generated. "I always try to make sure I have it on my end and buy additional licenses," said Kozar.

Collaboration between the centers can also come in the form of referrals. Mulholland said when he sits down with a researcher to discuss their goals at the CSIF, it sometimes becomes clear that they'd be better off counting cells at the FACS Facility instead of under a scope, so he'll send them to Dr. Nichols.

"There's a sense of comradery between the service centers," Mulholland said. Although they all charge user fees and strive to break even each year, the directors all have the ultimate goal of helping researchers, even if that means sending their potential customers to another center.

"I think the main thing is that we always feel we're part of the research that goes on at Stanford," said Dr. Eckart. "We make contributions to allow folks to establish their careers or get grant funding or make scientific breakthroughs, and that's really rewarding."

OVERVIEW & HIGHLIGHTS



OVERVIEW & HIGHLIGHTS

BECKMAN CENTER OVERVIEW

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

Recognizing the impact that this new body of knowledge would have on improving the diagnosis, prevention, and treatment of disease, Paul Berg, Ph.D., a Stanford University School of Medicine professor and Nobel Laureate, sought to establish a center at Stanford that would integrate the basic, clinical, and applied sciences. His vision was that the rapid advancements taking place in the fields of molecular biology and genetics might become more readily available to clinical scientists, and thus hasten the translation of scientific discovery into new medical technologies and clinical applications. In 1989, with the inauguration of the Arnold and Mabel Beckman Center for Molecular and Genetic Medicine, Dr. Berg's vision became reality and Stanford ushered in a new era of rapid advancement in the field of molecular and genetic medicine.
Under the leadership of its current director, Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research in the Department of Developmental Biology, the Beckman Center continues to be at the vanguard of basic science, translational medicine, and technological discovery. The Beckman Center today houses three academic departments and the Howard Hughes Medical Institute (HHMI) Unit in Molecular and Genetic Medicine, and has a world-class faculty of research scientists that includes three Nobel Laureates, 26 members of the National Academy of Sciences, and 21 HHMI investigators.

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

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

PROGRAMS AT A GLANCE

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

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

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

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

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

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

2019-2020 HIGHLIGHTS

The Beckman Center has enjoyed an exciting and productive year of scientific achievement. This year's highlights are as follows.

New Faculty Member Joins the Beckman Center

A new faculty member, Silvana Konermann, Ph.D., was recruited to join the Beckman Center. Dr. Konermann joined the Department of Biochemistry as an assistant professor in October 2019. Dr. Konermann obtained her Ph.D. in neuroscience at MIT, doing doctoral work with Feng Zhang, Ph.D., at the Broad Institute. She then did postdoctoral work with Patrick Hsu, Ph.D., at the Salk Institute for Biological Studies, where she discovered a new family of RNA-targeting CRISPR nucleases and harnessed it as an efficient tool for programmable RNA perturbation and the reduction of pathological tau isoforms in a model of frontotemporal dementia. As a graduate student, Dr. Konermann focused on the development of technologies to enable efficient, generalizable, and precise perturbation of mammalian gene expression. Dr. Konermann developed the first tool for optogenetic manipulation of endogenous mammalian transcriptional and epigenetic states in the mouse brain. She also uncovered the biochemical mechanism of the first single effector RNA-targeting CRISPR enzyme, C2c2. Currently,

she is applying multiple modes of targeted transcriptional perturbations to understand genetic interactors of APOE in late-onset Alzheimer's disease.

30th Anniversary Beckman Symposium

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

New Research by Beckman Faculty Member Could Lead to Lifetime Flu Vaccine

A new approach to the flu vaccine—one that teaches the body to recognize a portion of the virus that stays the same year to year—could shake up the yearly vaccination ritual and protect people against a flu pandemic like the one that killed 40 to 50 million people in 1918. Last year, the flu virus killed more than 50,000 people in the United States alone. The team working on this new approach is led by Beckman Center scientist Peter S. Kim, Ph.D., the Virginia and D.K. Ludwig Professor of Biochemistry and the lead investigator of the infectious disease initiative at the Chan Zuckerberg Biohub.

The new approach involves the use of a monoclonal antibody that can bind to the exact spot on the flu virus protein that researchers want the immune system to recognize. Using the monoclonal antibody as a stencil, the rest of the protein can be "painted" with molecules that act as a chemical cloak, rendering it invisible to the immune system. Removing the stencil then leaves only a tiny portion of the protein visible for the immune system to learn to recognize and eventually attack.

Lab animals that received the cleverly cloaked flu protein also showed an immune response to other strains of the flu—something that would happen only if their immune systems had learned to recognize the viral core. Animals that received a normal vaccine didn't respond well to other flu strains.

For more information about Dr. Kim's research, see the MEDIA section of this report.

Beckman Faculty Member Identifies Immune Cells That are Protective in Mouse Model of Multiple Sclerosis

A seldom-studied class of immune cells may reduce the friendly fire that drives autoimmune disease, according to a new study by researchers in the Beckman Center laboratory of Mark M. Davis, Ph.D., a professor of microbiology and immunology and Howard Hughes Medical

Institute investigator.

Researchers tracked immune cells in the blood of mice with a disease akin to multiple sclerosis. They discovered a rise in CD8 T cells, typically known for killing infected or cancerous cells. To their surprise, injecting mice with peptides recognized by these CD8 T cells reduced disease severity and killed disease-causing immune cells.

While the bulk of the study was done in mice, the researchers also showed that one of their central findings—an increase in CD8 T cells derived from single cells—held true in cells from people with multiple sclerosis.

"We absolutely think that something like this is happening in human autoimmune diseases," said Dr. Davis. "It represents a mechanism that nobody's really appreciated. If we could mobilize those cells to function more effectively in patients with autoimmunity, then we'd have a novel treatment for diseases like multiple sclerosis."

For more information about Dr. Davis's research, see the MEDIA section of this report.

Beckman Faculty Member Uncovers Genetic Similarities Among Species That Use Sound to Navigate

Evolutionary adaptations like echolocation that are shared by unrelated species arose in part due to identical, independently acquired genetic changes, according to a new study of whole genome sequences spearheaded by Beckman Center scientist Gil Bejerano, Ph.D., professor of developmental biology, of computer science, and of pediatrics (genetics).

Dr. Bejerano's group developed an unbiased way to sift through whole genome sequences and spotlight concerted genetic changes shared by animals with unusual abilities or traits. Remarkably, the researchers found that their unbiased analysis homed in on the cochlear ganglion as the single most affected tissue among echolocating mammals. "Pulling the cochlear ganglion—a real poster child for the development of echolocation—out of a hat containing more than 4,000 possible gene sets, based on genomic sequence alone, was pretty spectacular," said Dr. Bejerano.

"This study is a great example of what we can accomplish when we combine the data in whole genome sequences from multiple species with functional information about specific genes," he said.

For more information about Dr. Bejerano's research, see the MEDIA section of this report.

Beckman Faculty Member Discovers Antibody Treatment Allows Transplant of Mismatched Stem Cells, Tissues in Mice

Researchers in the lab of Irving Weissman, M.D., the Virginia and D.K. Ludwig Professor for Clinical Investigation in Cancer Research and professor of developmental biology, have determined that a combination of six antibodies can successfully prepare mice to accept blood and immune stem cells from an immunologically mismatched donor.

The researchers found that treating mice with a combination of those six specific antibodies safely and efficiently eliminated several types of immune cells in the animals' bone marrow, and allowed pure hematopoietic stem cells to engraft and begin producing blood and immune cells without the need for continued immunosuppression.

"Radiation and chemotherapy are the current standard for preparing patients for a bone marrow transplant," said Dr. Weissman. "For the past decade, we have been working to step-by-step replace these nonselective and dangerous treatments with targeted antibodies."

For more information about Dr. Weissman's research, see the MEDIA section of this report.

Beckman Faculty Member Discovers New "Don't Eat Me" Signal That May Provide Basis for Cancer Therapies

Researchers led by Irving Weissman, M.D., the Virginia and D.K. Ludwig Professor for Clinical Investigation in Cancer Research and professor of developmental biology, have also found that a protein called CD24 acts as a "don't eat me" signal, and is used by cancer cells to protect themselves.

The scientists have shown that blocking this signal in mice implanted with human cancers allows immune cells to attack the cancers. Blocking other "don't eat me" signals, such as CD47, has become the basis for other possible anti-cancer therapies.

Of particular interest was the discovery that ovarian and triple-negative breast cancer, both of which are very hard to treat, were highly affected by blocking the CD24 signaling, pointing to a potential vulnerability for those very dangerous cancers.

For more information about Dr. Weissman's research, see the MEDIA section of this report.

Beckman Faculty Member Discovers That Human Response to Flu Vaccine is Influenced by Gut Microbes

A recent study in healthy adults suggests that antibiotics may reduce the effectiveness of the flu vaccine.

The depletion of gut bacteria by antibiotics appears to leave the immune system less able to respond to new challenges, such as exposure to previously unencountered germs or vaccines, said Bali Pulendran, Ph.D., the Violetta L. Horton Professor and professor of microbiology and immunology.

"To our knowledge, this is the first demonstration of the effects of broad-spectrum antibiotics on the immune response in humans—in this case, our response to vaccination—directly induced through the disturbance of our gut bacteria," said Dr. Pulendran.

For more information about Dr. Pulendran's research, see the MEDIA section of this report.

PMGM Faculty Member Finds Cure for the Common Cold in Human Cells and Mice

Researchers led by Jan Carette, Ph.D., a 2010 Beckman faculty recruit and associate professor of microbiology and immunology, working in collaboration with colleagues at the University of California, San Francisco, found that disabling a single, apparently noncritical protein in cells may foil replication of the viruses that cause half of all common colds, polio, and other diseases.

For more information about Dr. Carette's research, see the MEDIA section of this report.

TECHNOLOGY RESOURCES HIGHLIGHTS

The Beckman Service Centers are continually undergoing key technological enhancements in order to better serve our research community. Beckman Foundation funds have been instrumental in this process, building the infrastructure and providing the financial leverage that has made the facilities competitive for both internal and external grant awards. These service centers operate on a fee-for-service basis.

CELL SCIENCES IMAGING FACILITY

The CSIF has secured funding (~$200K) from the National Institutes of Health (NIH) Shared Instrumentation Grant Program (PI, Jonathan Mulholland) for a new Gatan, Inc. OneView scintillator-coupled sCMOS transmission electron microscope (TEM) camera, a OneView 16bit computer running Gatan Microscopy Suite V3 software. This new sCMOS TEM camera and computer will replace and significantly upgrade the aging and obsolete CCD TEM camera now at the CSIF Electron Microscopy Core (EMC).

The funded sCMOS TEM camera represents a new class of state-of-the-art fast and sensitive sCMOS scintillator-coupled cameras that offer a large field

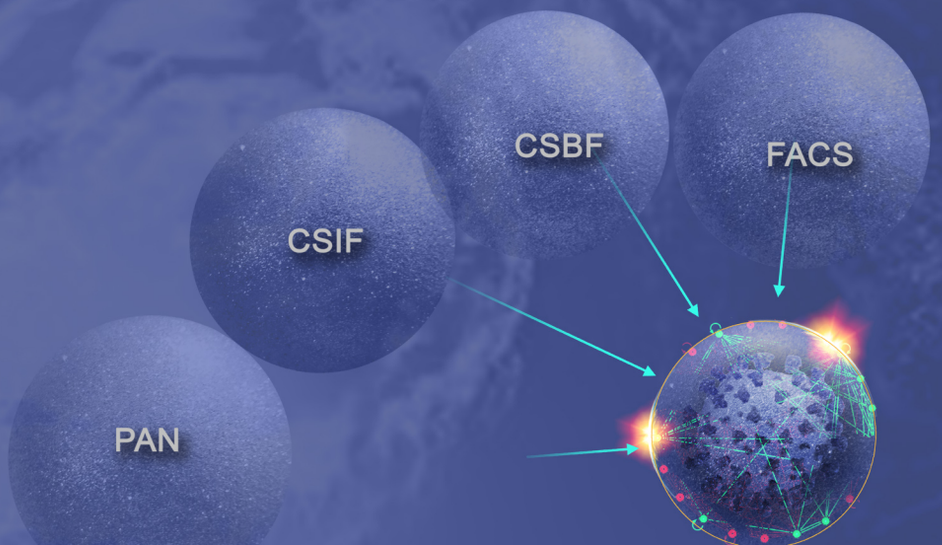
of view and real time drift correction.

The availability of this camera to Stanford's researchers and microscopists will greatly advance Stanford's biomedical electron microscopy dependent research. Importantly, having the new OneView TEM camera managed and supported by the Beckman Center's CSIF will greatly enhance and expand Stanford's openly accessible, biological and biomedical electron microscopy resources. In addition, it will provide an on-campus TEM and camera combination that will support low-level single particle sample screening. This is an important part of the training and screening process for trainees and their projects that hope to do higher resolution imaging and analysis with the Stanford-SLAC Cryo-EM facilities. As Wah Chiu, Ph.D., director of the Stanford-SLAC Cryo-EM Facility, made clear in his NIH letter of support, the CSIF EMC provides electron microscopy training and initial sample screening to novice and future Cryo-EM Facilities users. Thus, the new camera greatly supports the missions of both the CSIF Electron Microscopy Core and the Stanford-SLAC Cryo-EM facilities. Installation is expected summer 2020.

FLUORESCENCE ACTIVATED CELL SORTING FACILITY

The FACS Facility has grown at a rapid pace and has continued to add instruments to provide the latest technologies and easy access for researchers. Using funds provided by the Beckman Foundation, the facility's spectral analyzer has undergone its final upgrade and now has five excitation lasers. The additional two lasers will allow researchers to expand their queries to up to 40 different parameters (up from 20). In addition, in collaboration with faculty advisor Garry Nolan, Ph.D., National Institutes of Health funding for purchase of an FACSymphony 5-laser 30-parameter instrument has been awarded. This will be paired with the facility purchase of a state-of-the-art 5-laser sorter, the S6 Symphony.

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 Beckman Foundation of $12 million. Another $50 million in funding from private sources made it possible to complete the center on time and under budget.

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

The center plays an important role in Stanford's scientific community by providing funding that would not otherwise be available for interdisciplinary research, for technology development, and for securing cutting-edge resources and services for the research community. The center's programs and initiatives serve to complement and enhance the research efforts of the resident departments, the PMGM faculty, and the broader research community of the university. Without the Beckman Foundation support, many of our highly successful programs simply would not exist.

In recognition of the unique role the center plays with respect to the basic sciences, the Stanford University School of Medicine Office of the Dean provides an annual operating budget to the Beckman Center to cover the cost of administering the programs funded by the center. In addition, the School of Medicine recently funded a complete overhaul of the Beckman Center building.

The four Beckman Service Centers—the Cell Sciences Imaging Facility (CSIF), the Protein and Nucleic Acid (PAN) Facility, the Fluorescence Activated Cell Sorting (FACS) Facility, and the Computational Services and Bioinformatics Facility (CSBF), which are used by scientists throughout the campus and are managed by the Beckman Center—are expected to generate more than $5 million in user fees this year, continuing a level of service that sets the standard at Stanford University. The service centers operate at or close to break-even each year.

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

SERVICE CENTERS

Major advances in new imaging, bioinformatics, and genomics technologies are having a remarkable impact on our ability to translate basic research into medical applications. These new technologies are very expensive and many investigators find themselves unable to purchase state-of-the-art instrumentation. We have created service centers that provide these instruments and technologies on a fee-for-service basis, underwritten and administered by the Beckman Center.

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

TECHNOLOGY DEVELOPMENT GRANTS

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

MEDICAL SCHOLARS PROGRAM

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

RESEARCH COMMUNICATION AND EDUCATION

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

EXTERNAL REVIEW

The Beckman Center runs several programs—the Beckman Service Centers—that provide support for outstanding technological and scientific advances. The centers provide services in 1) state-of-the-art imaging technologies (CSIF); 2) protein and nucleic acid molecular analyses (PAN Facility); 3) fluorescence activated cell sorter technologies (FACS Facility); and 4) computer and biocomputational work (CSBF) technologies. 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 for 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:

- 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 provided
- Evaluate the overall demand for services in a given facility over time
- Review the list of users for each facility and the dollar volume of activity per user, in order to determine the scope of demand for those services
- Assess the degree of duplication of services between service centers

across the campus
- Evaluate which high-cost technologies should be subsidized
- Determine the need for new services or new service centers
- Evaluate whether or not certain services have outlived their usefulness, are readily available outside the university, or should be discontinued

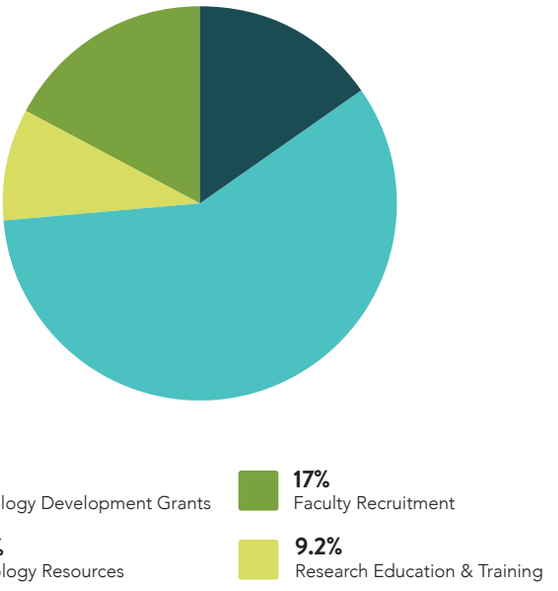
The board's recommendations are summarized and relayed to the Beckman Center director for consideration. This evaluation provides important feedback that allows the center director to consider changes (expansion or elimination) to the services provided by the four service centers.

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

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

EXPENDITURE OF DIRECTOR FUNDS

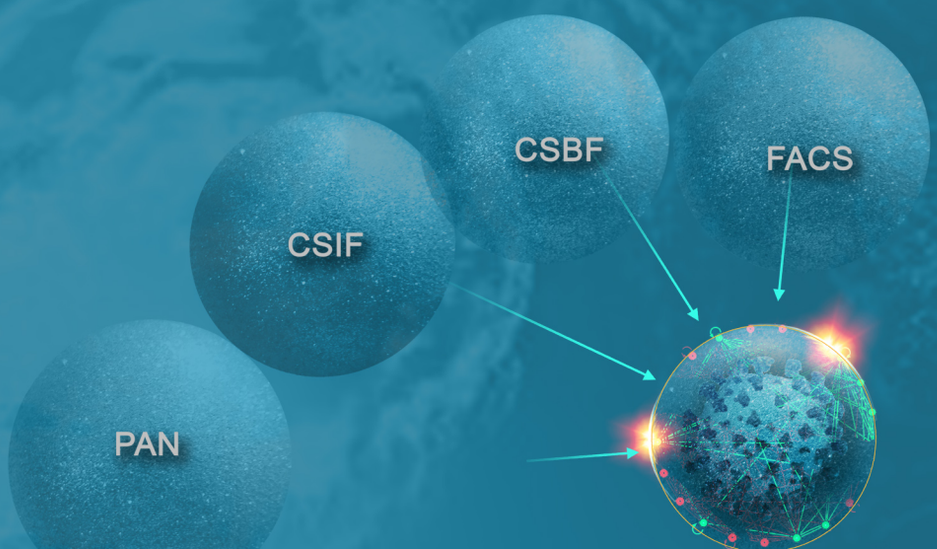
The Beckman Center receives an annual gift from the Beckman Foundation that is disbursed to its programs in technology development, technology resources, faculty recruitment, and research education and training. The chart below shows how the Beckman funds were disbursed this fiscal year.



ENDOWMENT FUNDS

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

PROGRAMS



PROGRAMS

TECHNOLOGY DEVELOPMENT GRANTS

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

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

In October 2018, the Beckman Center awarded five new ITRP Technology Development Grants that were geared toward support for technology development in the biomedical sciences. Each Technology Development Grant provided funding of $100,000 per year for a two-year period.

Applicants were encouraged to submit proposals to support innovative applications for 1) the development of new and improved instruments or devices, or 2) the development of new methodologies to be used in biomedical research.

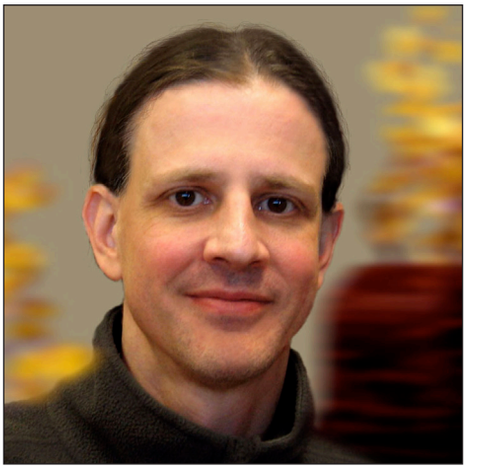
Preference was given to applications that had a disease focus, were truly innovative, and met the interdisciplinary and translational criteria for the ITRP grant program. Part of the selection process for the Technology Development Grants was based on an assessment of the likelihood that the pilot research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

The grant recipients chosen in 2018 have all made great progress on their research projects. First-year progress reports from each group follow.

A Next-Generation Imaging Technology for Human Tissue Atlases

Pehr A.B. Harbury, Ph.D.
(Biochemistry)

Tushar Desai, M.D., MPH
(Medicine-Pulmonary and Critical Care)



Pehr A.B. Harbury, Ph.D.
Department of Biochemistry

Tushar Desai, M.D., MPH
Department of Medicine (Pulmonary and Critical Care

Many human diseases cannot be modeled in animals and must therefore be studied in human subjects—requiring direct molecular analysis of cells and tissues from patients. The strategy of using humans as their own "model organism" has been greatly advanced in the last decade by high-throughput sequencing technology. An ongoing phase of this revolution is the introduction of single-cell molecular profiling methods, which enable high-dimensional, quantitative analysis of the body's fundamental unit—the isolated cell. In animals, single-cell profiles must be synthesized into a systems-level picture of multicellular structures. Importantly, current single-cell sequencing approaches operate on dissociated tissues, and lack information about the 3D organization and interactions of the cells. This missing information is essential for moving beyond cell classification to an understanding of the global programs that control animal biology. Mapping technologies that molecularly profile single cells in intact tissues are therefore needed. It is already clear that *in situ* mapping will uncover cell classes and functional specializations that are invisible to dissociative sequencing approaches. If *in situ* mapping could be implemented on a genome-wide scale and in fully intact biological structures, it could rival sequencing as an upfront discovery tool.

The present state of *in situ* mapping is similar to the early days of high-throughput sequencing. The transition from being a scientific curiosity to a

game-changing technology will require performance advances, specifically in data quality, information throughput, and the range of applications. This Beckman-funded project addresses two severe limitations of current 3D volumetric mapping methods: reliance on traditional fluorescence microscopy for imaging, and reliance on passive transport for delivery of imaging agents. The operational consequences are 1) poor detection of low-abundance molecular features in tissue due to autofluorescence background and non-specific retention of labeling reagents—a particularly vexing problem in human tissue; 2) low information throughput due to the small number of spectrally-resolvable fluorescence data channels; and 3) a minimal 3D mapping capability in large, anatomically-intact tissue structures due to slow reagent penetration.

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

Specific Aims

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

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

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

Targeting the Influenza Matrix Layer Through Hyper-Stabilization

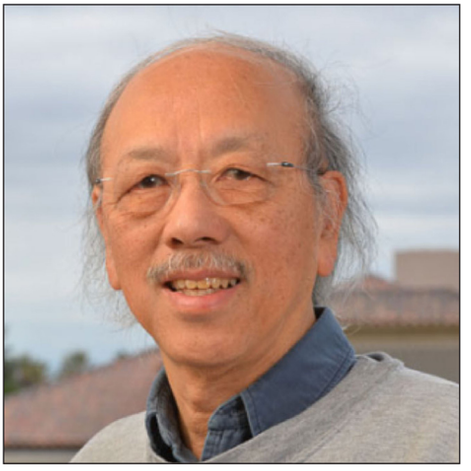
Karla Kirkegaard, Ph.D.
(Genetics, and Microbiology and Immunology)

Wah Chiu, Ph.D.
(Bioengineering, and Microbiology and Immunology)

The influenza A virus M1 protein is a crucial component of virion structure and function. The matrix layer assembles on the cytoplasmic surface of the membrane of the infected cell during virion formation, protecting the viral genome from extracellular stresses. Subsequently, upon entry into a new cell via an acidified compartment, the matrix layer undergoes structural rearrangement to release the viral RNA genome.

To obtain high-resolution structure of the matrix layer structural transitions during virus assembly and uncoating, oligomer formation by recombinant full-length matrix protein was investigated: two different filamentous structures, a single-layered and a multi-layered helical oligomer, were identified. Using crosslinking and mass spectrometry, the researchers have shown that the single-layered oligomer is structurally similar to the matrix layer observed in intact virions and that the multi-layered oligomer resembles matrix layers observed in low-pH treated and disrupted virus particles.

Karla Kirkegaard, Ph.D.
Department of Genetics, and of Microbiology and Immunology



Wah Chiu, Ph.D.
Departments of Photon Science, of Bioengineering, and of Microbiology and Immunology

Progress Report

Aim 1. Determine atomic resolution structures of M1 within assembled oligomers. Since submission of the

grant, the research team has achieved their goal of determining the high-resolution structure of the matrix protein within assembled structures. Using cryo-electron microscopy, they solved the high-resolution (3.4 angstrom) structure of highly ordered tube-like oligomers formed by a matrix protein with a single mutation. They identified this V97K variant and showed that it forms matrix layers in viruses using biochemical methods. Solving the structure of the mutant oligomer revealed a helical arrangement of M1 proteins and led to the discovery of the full-length structure of M1.

In addition, the structure revealed important interfaces for matrix protein assembly and a cluster of functional amino acid residues that is located at the base of three adjacent monomeric subunits. These residues have been shown to respond to changes in pH and thus could be highly important during uncoating of the matrix layer in the acidic environment of the endosome.

These findings have greatly contributed to the team's knowledge about the structure and contacts of M1 within the matrix layers of viruses and will aid future drug discovery targeting the matrix layer.

Aim 2. Implement a high-throughput screen for small molecules that target M1-M1 interfaces. Compounds were sought that hyper- or destabilized M1 oligomers. At the Stanford High-Throughput Bioscience Center, 3,000 compounds were evaluated. With support from this funding and an arrangement with the Stanford Chemistry, Engineering & Medicine for Human Health (ChEM-H) institute, the researchers also expanded the drug screen to a larger, publicly available compound library in collaboration with the Novartis Institutes for BioMedical Research in Boston.

A library of 50,000 compounds was screened for changes in melting temperature of M1 oligomers at neutral and low pH. The screen identified 954 hit compounds that increased the melt temperature of M1 by three standard deviations above the average. The hit compounds were secondarily screened in dose response, which identified 135 compounds for further analysis. The team is currently developing a phenotypic screen to test these compounds for their inhibition of influenza A virus in tissue culture.

Developing FFPE-Optimized CODEX to Reveal the Cutaneous T Cell Lymphoma Tumor Microenvironment in Response to Immunotherapy

Garry Nolan, Ph.D.
(Microbiology and Immunology)

Youn Kim, M.D.
(Dermatology)

Cutaneous T cell lymphoma (CTCL) is a rare, but potentially fatal malignancy of the skin for which there are no curative systemic therapies. New treatment strategies are therefore needed, and a recent phase II clinical trial of pembrolizumab immunotherapy (a PD-1-blocking antibody) suggests great promise (Khodadoust, MS, et al. *J Clin Oncol*, Sept 2019, PMID:31532724). While 38% of CTCL patients treated with pembrolizumab achieved a sustained clinical response, 40% of those who did not respond experienced rapid disease progression. Given the high economic costs and potentially devastating side effects of pembrolizumab, it is critical to identify predictive biomarkers that enable CTCL patients to be stratified into probable responders and non-responders prior to initiating therapy. This team has implemented new experimental and computational approaches to accomplish that feat.

Garry Nolan, Ph.D.
Department of Microbiology and Immunology

Youn Kim, M.D.
Department of Dermatology

During year one of this project, the researchers adapted and optimized a new imaging technology called CODEX (CO-Detection by indEXing) for use in formalin-fixed, paraffin-embedded (FFPE) tissue, which is the most common type of clinical specimen. They also developed a 55-marker antibody panel specific to CTCL and created a 70-core tissue microarray (TMA) with matched CTCL skin biopsies before and after pembrolizumab

treatment. Additionally, they implemented a computational pipeline to analyze the resultant multidimensional data.

This work identified a spatial signature that predicts pembrolizumab response, which underscores the importance of characterizing the spatial organization of the TME. This signature involves the proximity of reactive T cells, immunosuppressive regulatory T cells, and tumor cells. While a 55-marker antibody panel was required to discover this spatial signature, the researchers are currently working to validate this signature with a clinically accessible platform, Vectra Polaris, using a seven-marker antibody panel. Accomplishing this will allow widespread clinical use of this spatial signature to predict pembrolizumab response for CTCL patients.

In sum, year one of this project focused on developing the FFPE-CODEX technology, which provides a robust and scalable platform that is especially valuable for identifying biomarkers in the clinical trial setting.

Year two of this project will focus on correlating CODEX and RNAseq data to simultaneously visualize protein expression, quantify cellular interactions, and obtain matched gene expression profiles. To date, the researchers have performed laser capture microdissection and RNAseq for each TMA core, identifying a total of 22,845 genes. In-depth analysis of the RNAseq data is ongoing. Additionally, they are developing the computational tools to correlate specific genes with the predictive spatial signature identified with CODEX. They are especially interested in chemokines, since these functional molecules induce the migration of immune cell subsets within the TME so that they can target and destroy cancer cells. Thus, identifying chemokines associated with pembrolizumab response may provide a combinatorial treatment avenue for enhancing immunotherapy success.

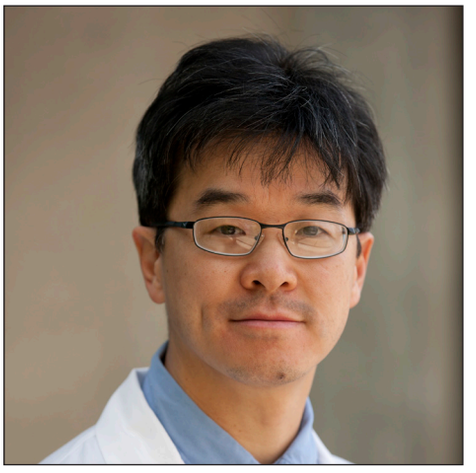
Ultimately, the CODEX-RNAseq platform developed in this project allows the spatial and molecular activity of the CTCL TME to be characterized in response to pembrolizumab, paving the way toward improved treatment for CTCL patients and the broad leveraging of cancer immunotherapies.

Scalable Long-Term DNA Storage with Error Correction and Random-Access Retrieval

Tsachy Weissman, Ph.D.
(Electrical Engineering)

Hanlee P. Ji, M.D.
(Medicine-Oncology)

Tsachy Weissman, Ph.D.
Department of Electrical Engineering



Hanlee P. Ji, M.D.
Department of Medicine (Oncology)

With the amount of data being stored increasing rapidly, there is significant interest in exploring alternative storage technologies. In this context, DNA-based storage systems can offer significantly higher storage densities (petabytes/gram) and durability (thousands of years) than current technologies. Specifically, DNA has been found to be stable over extended periods of time, which has been demonstrated in the analysis of organisms long since extinct.

Recent advances in DNA sequencing and synthesis pipelines have made DNA-based storage a promising candidate for the storage technology of the future. However, the synthesis and sequencing processes are error prone, leading to a need for error-correction coding. Furthermore, significant work needs to be done on the automation and scalability of the pipeline to achieve a practical system.

Part of this research group's work has focused on the development of novel error-correction schemes optimized for DNA storage. In their work on Illumina sequencing-based DNA storage, they studied the tradeoff between the writing and reading costs involved in DNA-based storage and proposed a practical scheme to achieve an improved tradeoff between these quantities. Unfortunately, state-of-the-art Illumina sequencing is too costly, slow, and bulky to deliver on the promises of DNA-based storage. Meanwhile, third-

generation nanopore sequencing offers affordable, high-throughput sequencing using devices as small as a USB thumb drive. But as of now, raw error rates remain prohibitively high, necessitating the development of novel error-correction techniques. The researchers' proposed approach uses additional information present in raw current signal for nanopore sequencing. While the nanopore sequencing process is quite complex and directly exploiting the raw signal is non-trivial, they circumvent this problem by using the strength of machine learning-based basecallers. This provides a significant reduction in sequencing cost over previous state-of-the-art systems. These works are available on bioRxiv and have been published/submitted in conferences.

Beyond optimized error-correction coding schemes, the researchers are exploring automation of the entire pipeline in collaboration with Miroculus, and alternative synthesis strategies, which can be significantly cheaper and scalable. Finally, they are also looking into other aspects of a practical storage system, such as random access to parts of the stored data and the ability to repeatedly read the data without depleting the material.

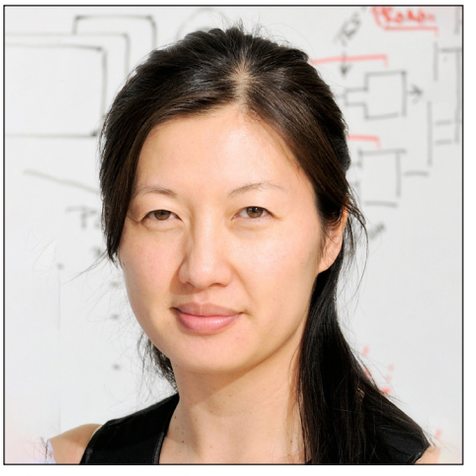
The potential of DNA storage and the team's work on this were prominently featured in the 2019 Stanford Compression Workshop, in an invited talk by Hanlee P. Ji, M.D. The error correction aspect was presented at ISMB/ECCB 2019, by graduate student Shubham Chandak. The researchers have also applied for a provisional patent with the Stanford Office of Technology Licensing on the nanopore sequencing error-correction code.

Optical Tools to Assess Neuronal Function in Human Stem Cell-Based Disease Models

Marius Wernig, M.D., Ph.D.
(Pathology)

Alice Ting, Ph.D.
(Genetics and Biology)

Marius Wernig, M.D., Ph.D.
Department of Pathology
(Stem Cell Institute)



Alice Ting, Ph.D.
Department of Genetics and
of Biology

The first goal of this research project was to evaluate the already existing FLARE tool (Ca2+-light coincidence detector) in human neurons. The researchers reported that they initially ran into a few unexpected problems. They tested the AAV constructs already available and verified to work in mouse cultures and in mouse brains *in vivo* and infected human neuron cultures derived from human iPS cells using the Ngn2 method. Unfortunately, they could not detect any Ca2+/light-dependent activity of the transcriptional reporter. They then investigated the expression levels of the three components needed for the system to work and essentially found no expression. After a few replicates and assurance that no reagents were mixed up, and continued lack of expression, they then tested other AAV constructs with GFP reporters and made the surprising discovery that human iPS-cell derived Ngn2-neurons cannot be infected with AAV viruses. This was completely unexpected, since undifferentiated human iPS cells are readily infectable, and AAV vectors targeting the CNS are even in clinical trials with verified expression.

The researchers then resorted to lentiviral delivery. They cloned the three main components into a standard lentiviral vector that they successfully use routinely in their cultures. They delivered the three flare components in these lentiviruses together with the Ngn2 virus needed to convert iPS cells into neurons. Of note, they needed to delay infection with the tetO-mCherry FLARE readout reporter because they needed the doxycycline-

inducible system to express Ngn2. The researchers therefore first needed to test various timings to make sure that there was no interference with the doxycycline needed to differentiate the cells and the FLARE readout. Indeed, following some optimization of viral delivery time points of the tetO-mCherry reporter, they did find a "beautiful" response to electrical stimulation that was both Ca2+ and light dependent.

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

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

In addition, during this reporting period, the group made significant progress with regard to further technological development—more specifically, the development of an optical integrative activity-history detector. Starting with their original FLARE tool, they have made several modifications and improvements to adapt it for the proposed application in stem cells. First, they used directed evolution to improve the catalytic efficiency of the TEV protease component; this improved the temporal resolution of the tool to just one minute. Second, they improved the LOV domain by combining mutations in eLOV with mutations in iLID to improve the dark-state blockage of the TEV cleavage site (TEVcs). Third, they developed a single-chain version of FLARE (scFLARE), which is easier to use and reduces expression level variability (FLARE is a two-component tool and its performance varies a great deal, depending on component expression levels and relative stoichiometry of the two components). The improved performance of scFLARE at high- and low-expression levels is now being prepared for submission to *Nature Chemical Biology.*

SEMINARS AND SYMPOSIA

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

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

BECKMAN SYMPOSIUM

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

GET THE FACS SEMINAR SERIES

"Get the FACS" is held throughout the calendar year and features lectures from staff from the Fluorescence Activated Cell Sorting Facility as well 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 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 SERIES

The Frontiers in Biological Research Series focuses on cutting-edge research involving interdisciplinary approaches to bioscience and biotechnology.

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

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

CANCER AND TUMOR BIOLOGY SEMINAR SERIES

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

REGENERATIVE MEDICINE SEMINAR SERIES

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

The seminars bring together students, postdocs, faculty, and trainees from diverse Stanford disciplines, including bioengineering, engineering, medicine, and the biological sciences to hear about and discuss work in progress. The seminars have been a tremendous help in making the Stanford research community aware of the broad range of research going on in regenerative medicine 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 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 helped recruit an outstanding new faculty member: Silvana Konermann, Ph.D.

Dr. Konermann joined the Department of Biochemistry as an assistant professor in October 2019. Dr. Konermann obtained her Ph.D. in neuroscience at MIT, doing doctoral work with Feng Zang, Ph.D., at the Broad Institute. She then did

postdoctoral work with Patrick Hsu, Ph.D., at the Salk Institute for Biological Studies, where she discovered a new family of RNA-targeting CRISPR nucleases and harnessed it as an efficient tool for programmable RNA perturbation and the reduction of pathological tau isoforms in a model of frontotemporal dementia. As a graduate student, Dr. Konermann focused on the development of technologies to enable efficient, generalizable, and precise perturbation of mammalian gene expression. Dr. Konermann developed the first tool for optogenetic manipulation of endogenous mammalian transcriptional and epigenetic states in the mouse brain. She also uncovered the biochemical mechanism of the first single effector RNA-targeting CRISPR enzyme, C2c2. Currently, she is applying multiple modes of targeted transcriptional perturbations to understand genetic interactors of APOE in late-onset Alzheimer's disease.

"The Beckman Center has been a vital source of support for faculty leaders seeking to promote broad-based scientific interaction and training."

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 (PMGM) 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 four Beckman Center Medical Scholars.

Khristian Erich Bauer-Rowe Ramos

Academic Year: 2019-2020

Year At Stanford Medical School: 2

Undergrad Education: Massachusetts Institute of Technology; Mathematics, BS

Hometown: Stamford, CT

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

Research Description

Inflammatory bowel disease (IBD) is a set of autoimmune diseases characterized by recurrent and destructive episodes of inflammation in the digestive tract; IBD affects three million people in the United States. Repeated cycles of inflammation cause intestinal fibrosis, characterized by the formation of thick scar tissue in the bowel, which leads to epithelial dysfunction and stricture formation. In the IBD subtype Crohn's disease, 30% of patients eventually require a bowel resection to manage strictures, and recurrence rates are estimated at 50-70%. Currently, there are no anti-fibrotic treatments, and anti-inflammatory therapies often fail to mitigate disease progression. Consequently, there is a significant clinical need to understand the pathophysiology of intestinal fibrosis to improve clinical management.

One of the hallmarks of intestinal fibrosis is the formation of creeping fat by the mesentery. This organ consists of a peritoneal fold that attaches the bowel to the posterior abdominal wall and supplies lymphatics, blood vessels, and nerves. During chronic inflammation, the mesentery wraps around the inflamed bowel, the bowel wall thickens, and

the lumen constricts. In addition, the mesentery is a rich source of adipocytes and fibroblasts, which are thought to mediate fibrotic responses. An intriguing hypothesis is that creeping fat formation is a wound-healing response whereby mesenteric adipocytes transdifferentiate into fibroblasts and infiltrate the bowel to cause fibrosis. Whether mesenteric adipocyte remodeling plays a direct role in the formation of intestinal fibrosis remains unknown.

This project will explore how mesenteric tissue remodels itself after intestinal injury and the association of creeping fat with fibrosis. Specifically, it will leverage a murine surgical model that recapitulates key characteristics of IBD fibrosis in conjunction with lineage tracing to determine the cellular origins of bowel wall fibroblasts. By understanding how mesenteric remodeling leads intestinal fibrosis, this project may eventually provide novel anti-fibrotic therapeutic targets.

Anthony S. Buzzanco III

Academic Year: 2019-2020

Year at Stanford Medical School: 2

Undergrad Education: University of California, Los Angeles; Biochemistry, BS

Hometown: Long Beach, CA

Title of Medical Scholars Project: Synthesis of a Proximity Ligation-Based Reporter for the Characterization of Afucosylated IgG

Research Description

Each year, approximately 390 million people worldwide develop dengue fever. Spread by mosquitoes, the dengue virus is primarily endemic to developing countries in tropical climates. But as climate change continues to warm regions farther from the equator, cases in previously temperate regions have multiplied.

For many people, the virus develops into nothing more than a flu-like illness. However, for about 1 percent of patients, the infection progresses to dengue hemorrhagic fever (DHF), a severe complication that leads to systemic bleeding, shock, and often death; DHF claims 25,000 lives each year.

Currently, no treatments beyond supportive care are available to those who progress to DHF. However, numerous DHF patients who would otherwise die may survive when hospitalized early in the course of their illness. Clinicians estimate that prompt supportive care can reduce the case fatality rate from 20 percent to less than 1 percent. The trouble is that many regions where dengue is endemic suffer from limited medical resources and are unable to hospitalize every suspected case of dengue. Thus, it becomes critical to prioritize resources for the patients most

at risk for DHF, for whom early care is likely to make the difference between life and death. Unfortunately, current methods offer few clues for which patients are likely to develop DHF—until it is too late.

One emerging technique offers hope that better predictive methods are on the horizon. Previous work by Taia Wang, M.D., Ph.D., assistant professor of Medicine and of Microbiology and Immunology, and her colleagues, suggests that progression to DHF is more likely in patients who, having previously been infected with a different dengue serotype, went on to produce antibodies against the virus that were un-decorated by the sugar known as fucose. It is thought that these afucosylated, or fucose-lacking, antibodies actually aid viral entry into cells rather than blocking it. Thus, measuring the proportion of antibodies that are afucosylated in dengue patients is a promising avenue to predict which patients are most likely to develop DHF and will, therefore, benefit most from lifesaving care.

To this end, this research project is developing a rapidly deployable, easy-to-use platform to measure antibody fucosylation that does not require expensive equipment or specialized training. The platform uses proximity ligation-based reagents that can be added to a blood sample to give a clinician a quantitative measurement of the proportion of a patient's antibodies that are afucosylated. In addition to these clinical applications, this platform will also help researchers further study how fucosylation and other sugar modifications impact human immunity and disease.

Ekaterina Tkachenko

Academic Year: 2019-2020

Year at Stanford Medical School: 2

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

Hometown: Tyumen, Western Siberia region, Russia

Title of Medical Scholars Project: Immunorepertoire of Human Gut T and B Cells with Fiber Treatment in a Longitudinal Cohort Study

Research Description

Diet plays a crucial role in the modulation of the gut microbiota, significantly impacting microbial gene abundance and expression, and host metabolic traits. There is a growing amount of scientific evidence linking the gut microbiome to a variety of conditions, such as type 2 diabetes, obesity, Alzheimer's disease, hyperlipidemia, graft-versus-host disease, and inflammatory bowel disease. Despite this, few studies have addressed the role of specific commensal gut microbes in the production and regulation of metabolically active compounds derived from phytochemicals consumed in the diet. In particular, microbial-derived phenolic compounds can be synthesized into beneficial secondary metabolites by gut microbiota and have been associated with antioxidant and anti-inflammatory effects. However, this response appears to be dependent on the composition of microbes in the gut that can be modulated with prebiotics (fibers digestible by microbes but not humans).

Prebiotic fibers have been shown to improve key metabolic parameters

in mice, including glucose tolerance and oxidative stress, potentially through the attenuation of low-grade inflammation. It has previously been shown that phenolic compounds of microbial origin alter the intensity of the inflammatory response by modulating cellular inflammatory mediators including cytokines (TNF-alpha, IL-6, IL-8, and PGE2 are among the most studied), nuclear factor kappa-B (NF-kB), monophosphate-activated protein kinase (MAPK), and leukocytes. To date, there are no clear mechanisms to describe the way phenolic compounds interact systemically in the host to create such a response. The preliminary results of this research program indicate that as prebiotics stimulate differential microbial growth, they are in turn stimulating an immune response.

In this study, the researchers intend to correlate gut immunology, microbiota composition, and microbial metabolites using modern epigenomic tools and bioinformatics to identify microbial species and genes responsible for host physiological changes. They will characterize the subsets of T cells and B cells found in a human gut with prebiotic treatment and correlate metagenomics, metabolomics, and clinical data produced from stool samples of a select patient cohort. They hypothesize that the immunologic data set describing VDJ regions of T cells and B cells present in the gut will correlate with particular subsets of gut bacteria present in the patient, as well as certain clinical observations.

This integrative gut microbiota and host omics analysis will help reveal the genetic and environmental factors implicated in the modulation of the immune response induced by diet both on an individual and a population level.

Deborah Tsao

Academic year: 2019-2020

Year at Stanford Medical School: 2

Undergrad Education: McGill University; Honours Physiology

Hometown: Winnipeg, Manitoba, Canada

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

Research Description

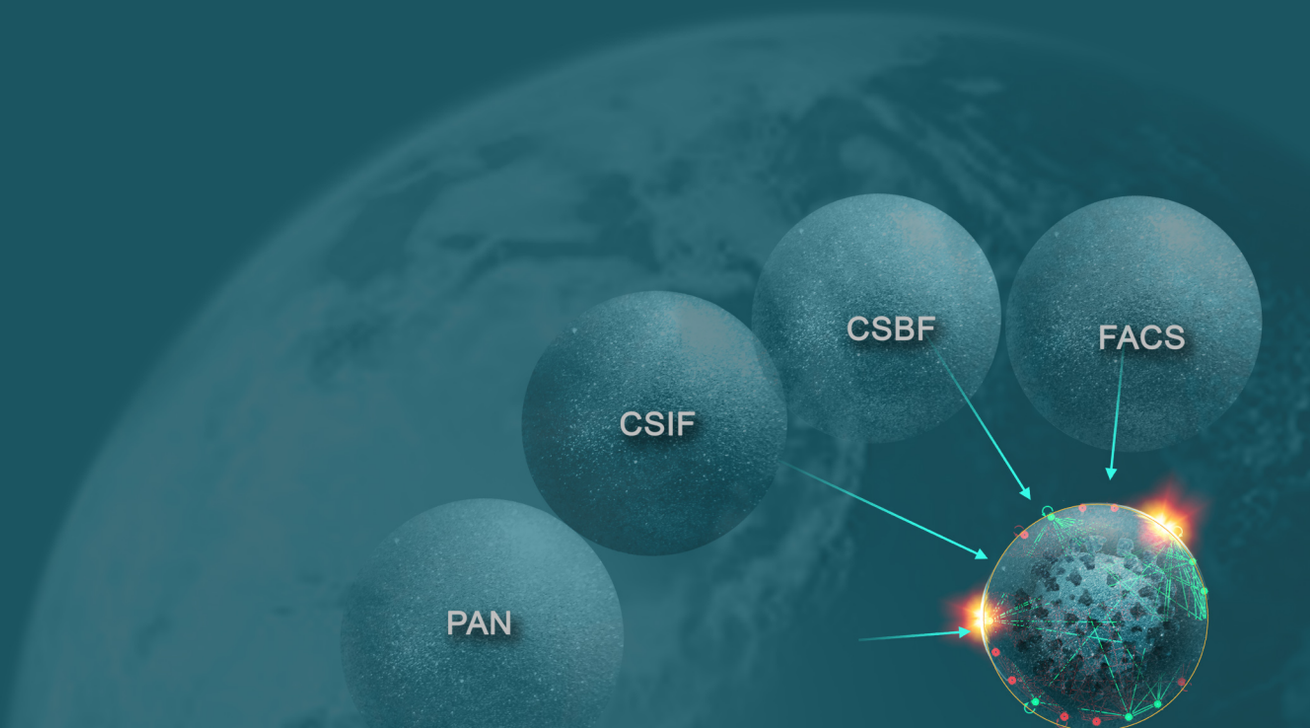
The incidence of diabetes mellitus has risen rapidly in the past half-century, reaching epidemic proportions in many areas of the world. This increasing incidence, and the recognition that diabetes mellitus is a genetic disease, has generated intensive effort in deciphering the genetic basis of diabetes.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

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

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

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

TECHNOLOGY RESOURCES



TECHNOLOGY RESOURCES

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

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

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

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

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

CELL SCIENCES IMAGING FACILITY

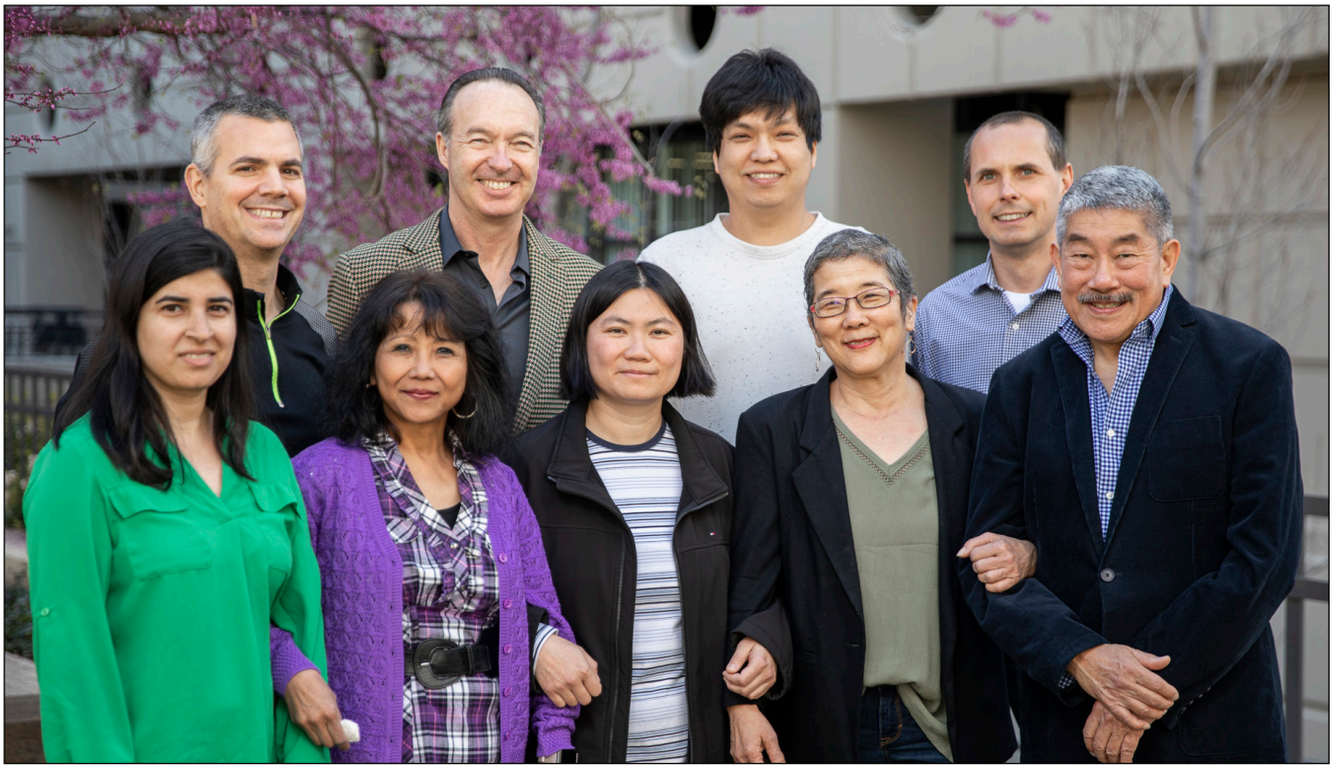
OVERVIEW

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

Jon Mulholland
Director

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, and image analysis software; the Electron Microscopy Core (EMC), which houses high-resolution scanning and transmission electron microscopes; and the Multiplexing and Array Tomography Core (M-ATC), which provides multiplexed marker imaging and array tomography services.

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



Cell Sciences Imaging Facility
Front Row, Left to Right: *Anum Khan, Ibanri Phanwar-Wood, Kitty Lee, Ruth Yamawaki, Philip Huie Jr*
Back Row, Left to Right: *John Perrino, Jon Mulholland, Youngbin Lim, Marcin Walkiewicz*

More recently (2014), in a collaborative effort with the Stanford School of Engineering (SOE), the CSIF opened a satellite light microscopy facility in the SOE's Shriram Center. This new facility brings much needed biological imaging instrumentation and expertise to the departments of Bioengineering and Chemical Engineering. In addition, the CSIF recently added a new service: bioimage analysis. The new service provides expertise in assembling individual methods into complete bioimage analysis/processing pipelines for specific projects using Python/SciPy. Lastly, in 2019 the CSIF added highly multiplexed antibody marker fluorescence imaging, thus creating the Multiplexing and Array Tomography Core.

Today, the CSIF's mission remains the same as when it was first established: to provide access to and training in high-resolution, state-of-the-art imaging technologies. While these technologies have evolved substantially over the last 25-plus years, they remain essential, basic tools for studying molecular, sub-cellular, and cellular biology and disease. A major element of the CSIF's commitment to its mission is the continuous and ongoing process of upgrading technologies, equipment, and expertise, to remain at the forefront of cell sciences imaging.

EXPERTISE

A ten-member advisory committee provides leadership and direction for the CSIF. The committee is chaired by the Beckman Center director, Lucy Shapiro, Ph.D., and includes Beverly Mitchell, M.D., director of the Stanford Cancer Institute, Tim Stearns, Ph.D., chair of Biological Sciences and an expert in microscopy, and seven other researchers from the Beckman Center, the Stanford Cancer Institute, and Stanford's School of Medicine, School of Engineering, and School of the Humanities and Sciences.

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

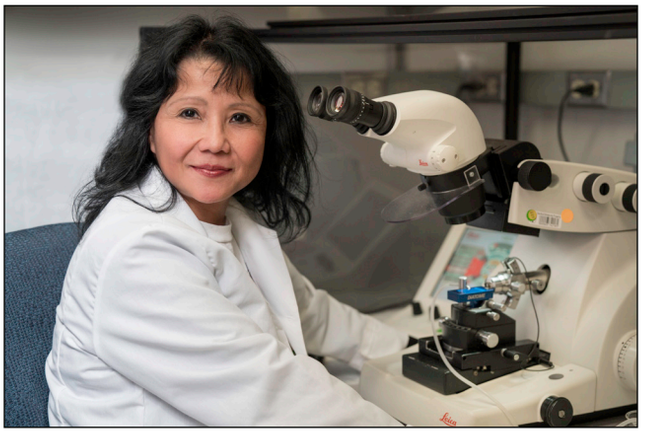
SERVICES

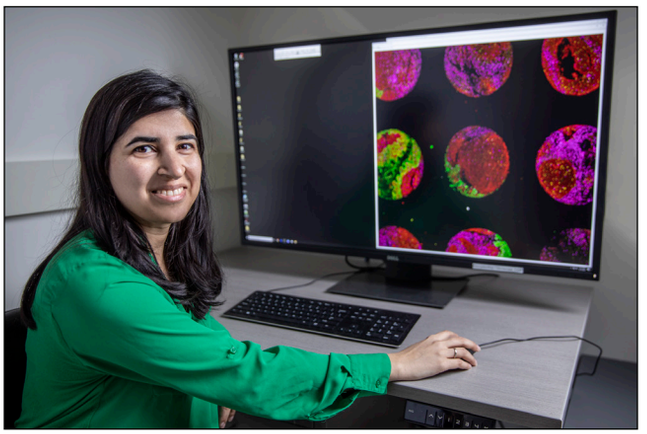
Fluorescence Microscopy Services

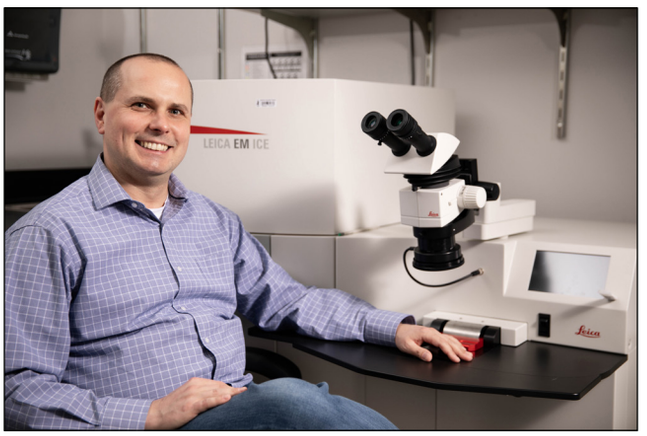
The CSIF's Fluorescence Microscopy Core provides training and consultation in the application of numerous microscopy technologies:

- Super-resolution (API OMX-SIM, STORM, Leica SP8-gSTED, Zeiss AiryScan)
- Laser scanning confocal (Zeiss LSM880, LSM780, Leica SP8, Leica SP5)
- Spinning disk confocal (Nikon-Yakogawa)
- Deconvolution (API OMX Delta Vision)
- 2-photon (Zeiss LSM780, Leica SP5, each with Spectra Physics DeepSee laser)
- 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. FLIM allows researchers to measure changes in a molecule or protein's fluorescence lifetime, in addition to its fluorescence intensity. Bio-AFM enables innovative live cell experiments that provide high-resolution force measurements and mapping over the surface of soft materials, cells, and other biological material. Using epifluorescence, brightfield, and phase contrast optical imaging, these measurements can be directly correlated with macromolecules, proteins, and subcellular structures, as cells sense and respond to mechanical cues and environmental changes.







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

The CSIF also has capabilities for total internal reflection microscopy (TIRF) and super-fast, wide-field, live cell imaging. Additionally, time-lapse software allows 3D localization of labeled proteins over time, thus providing 4D data sets. The CSIF also provides advanced software resources for 3D, 4D interactive, volume imaging (Improvision Volocity, Bitplane Imaris) of data sets, as well as advanced deconvolution software packages (SoftWoRx and SVI Huygens).

Electron Microscopy Services

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

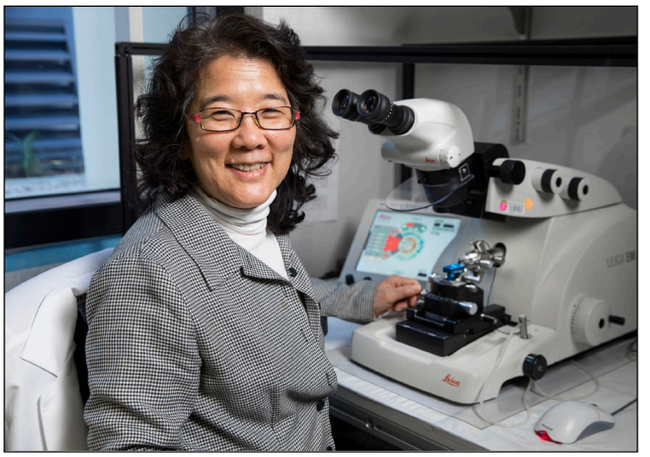
The EMC houses a transmission electron microscope (TEM) equipped with high-resolution, cooled CCD cameras for digital acquisition of images (JEOL 1400-TEM). The CSIF's TEM can produce a resolution of two angstroms, thus making it possible to image and study isolated macromolecules and subcellular structures. 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.

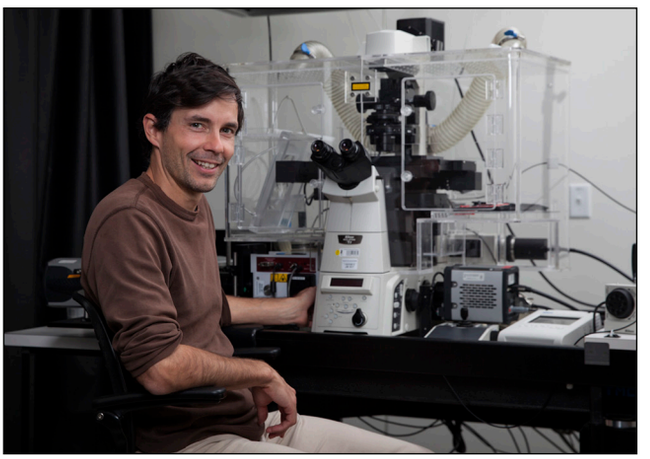
Ancillary equipment includes three ultramicrotomes for cutting ultra-thin sample sections (less than 100nm), a cryo-ultramicrotome for sectioning ultra-thin frozen sections, all equipment necessary for sample preparation, and computers

for image analysis. Additionally, the EMC houses a new state-of-the-art Leica ICE high-pressure freezing machine. High-pressure freezing is the gold standard for fixation of biological microscopy samples; in the numerous studies where it has been applied, high-pressure freezing has extended our understanding of the structural and molecular organization of cells and tissues.

Multiplexed and Array Tomography Services

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





Top: *Ruth Yamawaki,* ***Bottom:*** *Cedric Espenel*

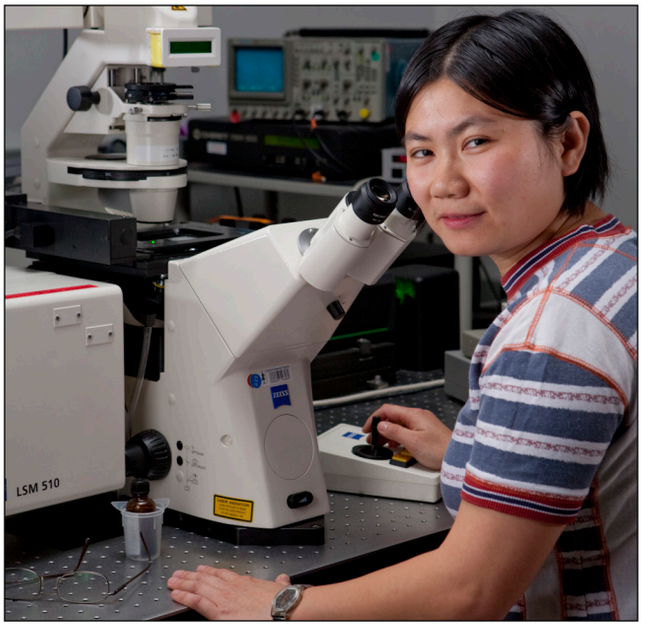
CODEX, a highly multiplexed imaging platform, allows automated, multiplexed, antibody localizations of a potentially unlimited number of proteins on tissue sections or tissue arrays, with cellular-level resolution. The CODEX instrument provides greatly increased throughput and analysis of multiple cancer,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

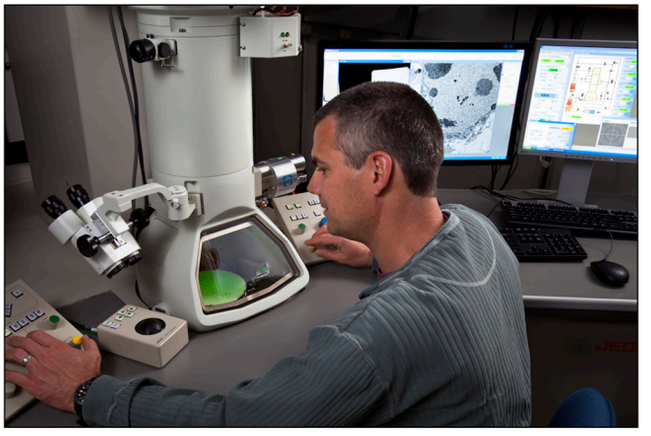
The AT imaging method was invented at the Beckman Center in the Department of Molecular and Cellular Physiology by neuroscientists Stephen J. Smith, Ph.D., and Kristina D. Micheva, Ph.D. Compared to previous microscopic methods for 3D imaging of fixed tissue, array tomography offers increased resolution (z resolution of 200-50nm), quantitative reliability, antibody multiplexing capacity and throughput and volume (automated image acquisition). Array tomography also complements live, whole animal, or tissue explant imaging studies, providing higher-resolution 3D data with many more molecular markers, which can extend the molecular interpretation of *in vivo* dynamics. Array tomography permits easy acquisition of electron microscopic images in register with immunofluorescence. Array tomography thus promises an opportunity to explore the 3D molecular architectures of tissue

at an unprecedented level of detail. This methodology is applied by many Stanford researchers and provides insights into structural organization and protein location in tissue of numerous organisms and disease models.

RECENT DEVELOPMENTS

The CSIF is collaborating with the Protein and Nucleic Acid (PAN) Facility to correlate, in fresh-frozen tissue slices, protein-localization specific cell phenotypes with RNA expression patterns, using data from multiplexed, epitope-based tissue imaging (CODEX, Akoya, Inc.) combined with spatial transcriptomics (Visium, 10X Genomics, Inc.) technologies. This correlative approach will be done using tissue slices taken from both cancer and normal patient samples, using the CSIF's validated marker panels and the PAN Facility's next-generation sequencing (NGS) methodologies. Correlating highly multiplexed tissue imaging to deep spatial transcriptomics is expected to identify new phenotypic classifications, based on the protein-RNA expression correlation maps, which will in turn lead to the identification of new molecular subtypes of cancer with identifiable treatment targets. It is expected that this combined proteomic and transcriptomic spatial analysis will provide more precise diagnoses of cancer types and stages and subsequently, earlier diagnosis and therapeutic interventions.





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

Instrument Upgrades

The CSIF has received funding from the National Institutes of Health's Shared Instrumentation Grant (SIG) Program to replace and upgrade the Electron Microscopy Core's aging and obsolete CCD TEM camera. The current TEM camera is a 9-year-old, cooled CCD camera that no longer meets the demanding imaging needs of Stanford's electron microscopy imaging community. The new equipment, a OneView sCMOS TEM camera, will be used on EMC's transmission electron

microscope to digitally record TEM images of biomedical samples. It will be used in both high- and low-electron-dose TEM imaging applications and will be used to correct, in real time, the inherent drift of TEM samples. The new sCMOS camera will significantly advance the research projects of 15 different facility users, including two Nobel Laureates. These projects cover a diverse range of biomedically important areas of inquiry, including, for example, the biomechanics of hearing, neural stem cell maintenance and homeostasis, ventricular hypertrophy and failure, and mechanisms of viral infection and olfaction in sensory neurons, as well as the development of biomedically useful nanoprobes and super-resolution cryogenic correlative light and electron microscopy.

FUTURE VISION

Several new programs and services are now in development.

- The CSIF is collaborating with several faculty members as well as the director of the imaging facility at Stanford's Wu Tsai Neurosciences Institute to further develop and expand the university's light sheet microscope (LSM) imaging program and bioimage data science services. 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.
- CSIF director Jonathan Mulholland, Richard Lewis, Ph.D., professor of molecular and cellular physiology, and Andrew Olson, Ph.D., director of the Neuroscience Microscopy Service at the Wu Tsai Neurosciences Institute, will continue to teach their annual eight-week course in biological light microscopy, which serves as a foundation for supporting the next generation of light microscopists. The CSIF is also now developing a short course on conventional biological electron microscopy, following up on the success of the biological light microscopy course.

PROTEIN AND NUCLEIC ACID FACILITY

OVERVIEW

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

Michael Eckart, Ph.D.
Director

The advancement and expansion of the PAN Facility's services since its inception in 1989 has been the result of a team effort by the Beckman Center administration and PAN Facility staff to expand services to support the increasing variety of Stanford research programs.

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

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

Protein and Nucleic Acid Facility

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

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

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 (NGS)
- DNA sequencing
- Synthetic nucleic acid synthesis
- Biomolecular interaction analysis (surface plasmon resonance)
- Peptide synthesis
- Mass spectrometry

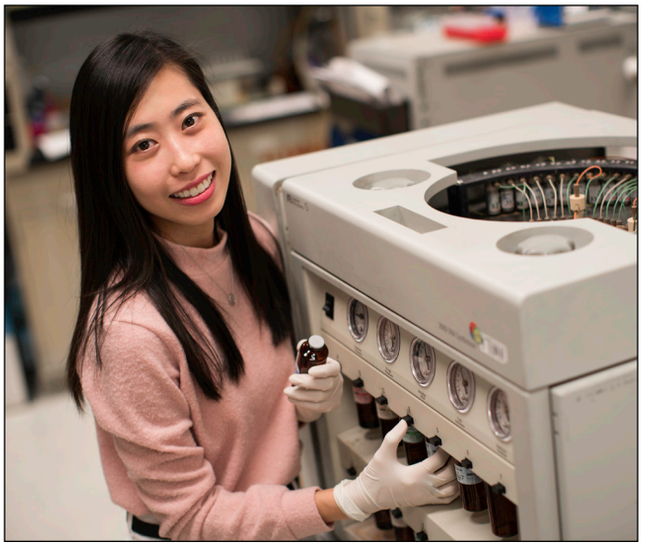
Shared Services

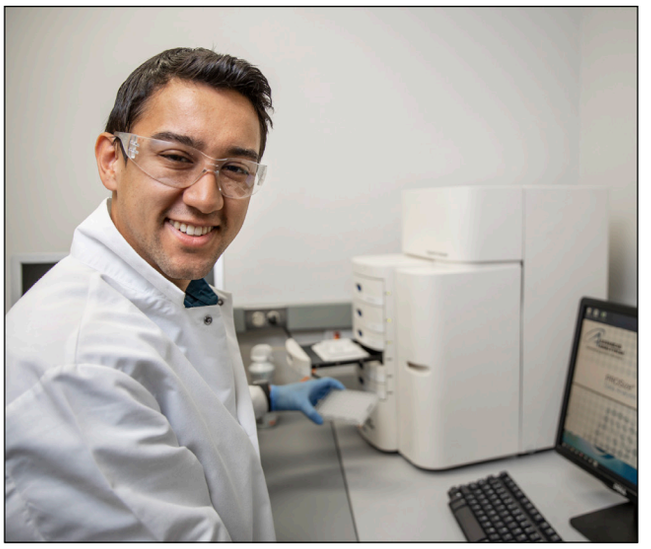
The core services offered by the PAN Facility enable and facilitate efficient and economical biomedical research by providing users with technology, without the necessity of major capital or staffing expense. With the organized and efficient infrastructure that shared resources such as the PAN Facility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

To leverage the full potential of the available technologies, each of PAN's services is staffed and maintained

by highly experienced and dedicated scientists. These 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 are always interested in developing new assays or adapting existing established assays to address a specific research question.

Researchers are encouraged to interact closely with PAN Facility staff to obtain maximum benefit of services. Development and implementation of new applications and technologies at Stanford are often achieved when a research group and the PAN staff engage in a joint project, with all contributing their individual strengths. The results of these efforts are often highlighted in publications to which PAN scientists have made contributions. Indeed, the consultation provided by PAN staff is often as important as the data obtained, since biomedical researchers not trained in a specific technique or field can find it difficult to interpret specialized data without help from PAN scientists (who frequently update their skills through appropriate training courses).





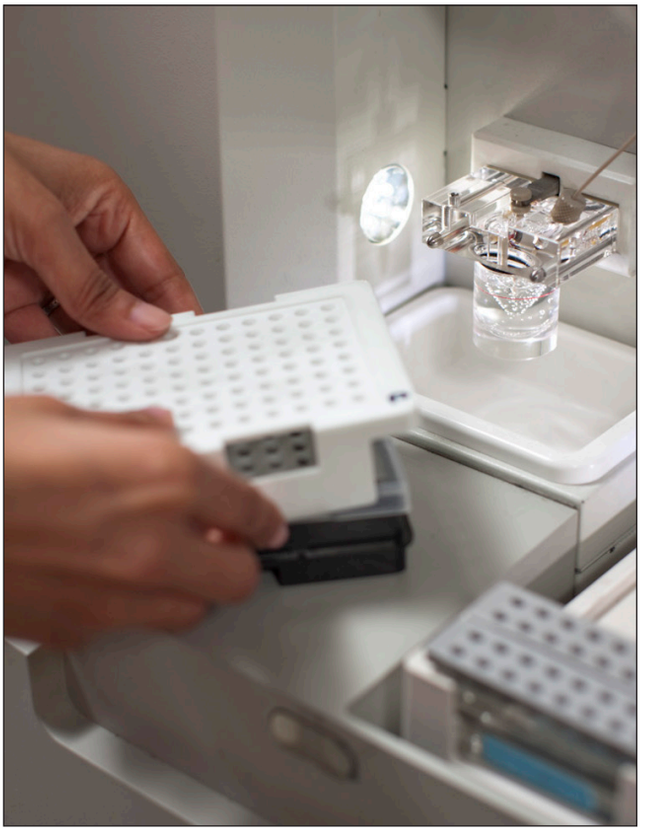
Top: *Nghi Cat Bao Le,* ***Bottom:*** *Kyle Fukui*

Single-Cell Genomics

In the past few years, science has become increasingly interdisciplinary, and as a shared resource that aims to be at the forefront of new developments and ideas, we see the PAN Facility as part of this progression. This is especially true in the field of single-cell genomics, which is the application of genomic technologies to understanding biology at the level of an individual cell, rather than an entire population of cells. There is currently strong enthusiasm to pursue activities

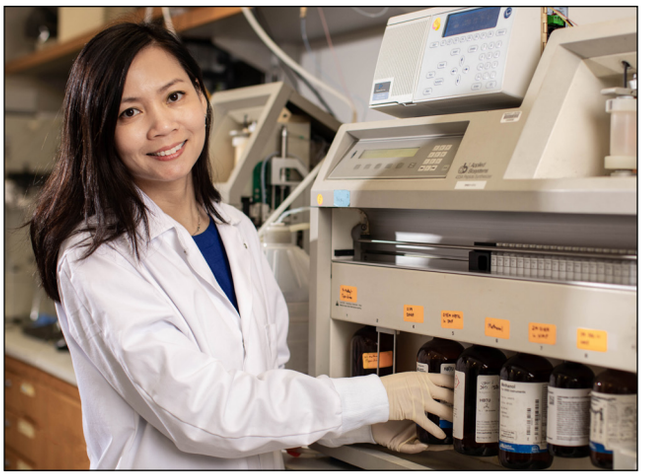
that will develop and implement a suite of integrative multimodal technologies that simultaneously measure multiple types of information from the same cell. This will enable researchers to achieve a better understanding of the cellular and molecular mechanisms governing cellular function in healthy, developmental, and disease states.

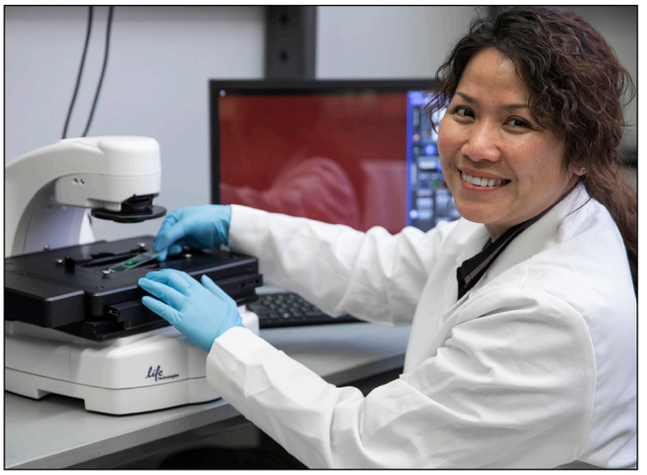
Single-cell genomics has revealed how much variation there is between individual cells at the molecular level in, for example, tumor tissue, stem cells, or rare subpopulations of immune cells. However, analyzing genomic DNA or RNA at the single-cell level may provide only genome, methylome, chromatin,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



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

Currently, the PAN Facility provides a full range of services aimed at advancing discoveries and the development of methods to analyze genomes and



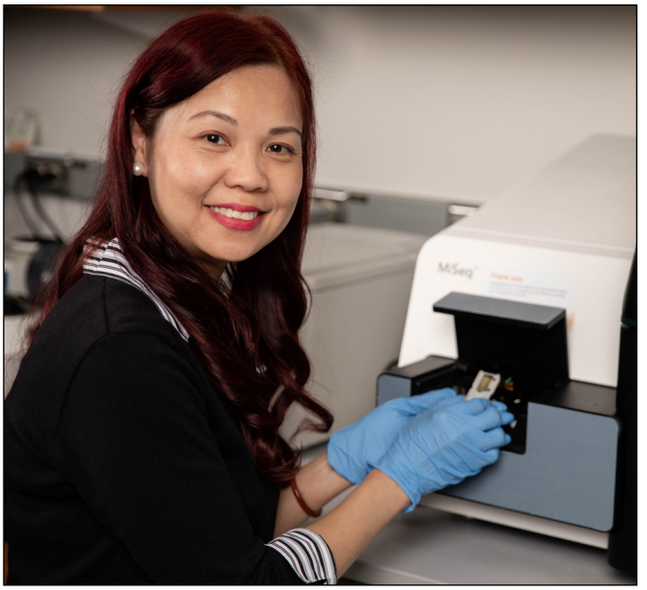


Top: *Ian Anderson,* ***Middle:*** *Jessica Tran,* ***Bottom:*** *Yen Tran*

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 (FACS), by simple micromanipulation, or by capture using microfluidic technology. The PAN single-cell genomics resource features single-cell capture microfluidic technology, the C1 Single Cell Auto Prep instrument (Fluidigm), which processes 96 or 800 single cells, and the ddSEQ Single-Cell Isolator instrument (BioRad), which performs rapid single-cell isolation using droplet partitioning technology.

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

Subsequently, PAN processes the templates generated from individual cells for analysis by next-generation sequencing. The conversion to next-generation sequencing libraries is accomplished using automated liquid handling instruments. The nanoliter Mosquito HTS liquid handler (TTP Labtech) allows us to significantly decrease library preparation costs and increase throughput. To ensure quality



Jennifer Okamoto

control at different steps in all the workflows, a fragment analyzer instrument is used to perform nucleic acid quality control.

Spatial Transcriptomics

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

To overcome these limitations, the PAN Facility is now implementing a recently developed technology known as spatial transcriptomics. This technology combines traditional histology with high-throughput, single-cell RNA sequencing (scRNA-seq), whereby intact tissue sections are captured on an array containing spatially barcoded, complementary DNA primers for the capture of either full-transcriptome or transcript subsets. Subsequent RNA library generation for next-generation sequencing of a single intact tissue sample utilizes the existing instrumentation in PAN's Single-Cell Genomics Laboratory. The spatial transcriptomics workflow bridges new microscopy techniques and RNA sequencing to generate protein biomarker and transcriptome data, respectively, from a single intact tissue sample. To accomplish this, the PAN Facility is working closely with the Cell Sciences Imaging Facility (CSIF) (https://microscopy.stanford.edu), which has implemented the CODEX (CO-Detection by indEXing) technology; that technology enables the analysis of at least 50 different protein biomarkers.

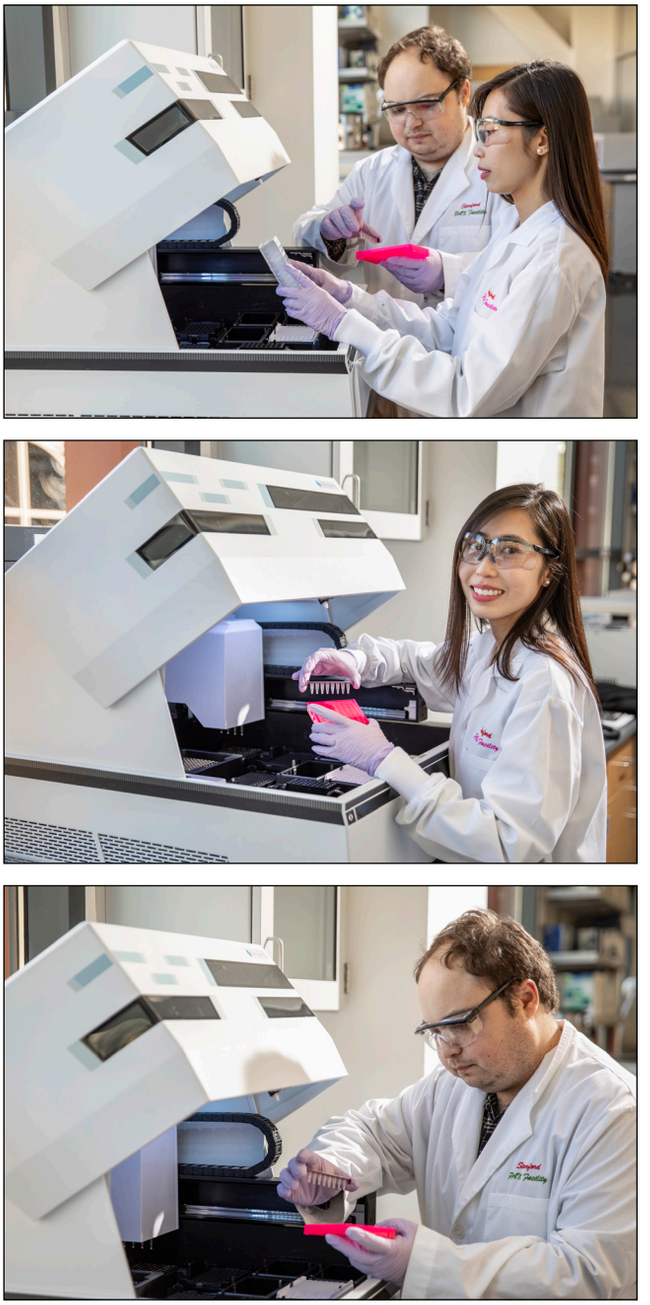
The collaboration of PAN with different research programs and technologies in other shared resources (FACS and CSIF), in accordance with our mission, adapts and takes advantage of single-cell tools, protocols, and technologies, including

equipment acquisition, as they become available so that scientists and clinicians within the Stanford scientific community remain on the cutting edge of scientific research. It is anticipated that advances made using PAN's scientific resources will enable researchers to obtain a deeper understanding of the underlying causes of diseases such as cancer and immune disorders, and the differentiation of stem cells, which have the promise of developing diagnostics and therapeutics in the different areas.

Other Technologies

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

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



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

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 an effect in a specific disease. Quantitative-PCR (Q-PCR) continues to be a popular technique to validate array study data. The use of pyrosequencing, using the Qiagen PyroMark Q24 instrument for real-time, sequence-based detection for quantification of sequence variants (SNPs/mutation detection) and epigenetic methylation, has also increased. The validation of methylation events identified by microarray and high-throughput, massively parallel sequencing technologies has been the main driver in pyrosequencing services.

Other research phases involve the use of technologies such as peptide synthesis, mass spectrometry, and surface plasmon resonance (SPR) to facilitate a more detailed and more comprehensive molecular study focusing on the complex of proteins expressed in biological systems, their structures, interactions, and post-translational modifications. SPR is a key technology in support of our efforts to meet the post-genomic biological challenge of understanding the complex networks of interacting genes, proteins, and small molecules that give rise to biological form and function. PAN's Biacore T200 instrument offers researchers the opportunity to work confidently at the limits of kinetic, molecular weight, and concentration ranges, bringing improvements in data quality to a wide range of new applications. Using the capabilities of the T200 instrument, PAN scientists have and will continue to work with investigators to perform fragment-based lead discovery (FBLD) to discover small-molecule drug candidates for a variety of drug targets in different disease indications.

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

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

FUTURE VISION

The PAN Facility will continue, in an ever-changing scientific environment that encompasses a wide range of biological, chemical, engineering, and physical sciences, to focus on providing solutions to the scientific technological needs that confront researchers. With the existing strengths and expertise in the different areas of the PAN Facility, the collaborative efforts between the different Beckman Center shared technology resources will enable multidisciplinary innovation and strategies that will broaden the application of different technologies to the multi-omic sciences. Overall, the PAN Facility will continue to support and seize opportunities that lay the foundation for the research and discovery efforts of the Stanford scientific community.

FLUORESCENCE ACTIVATED CELL SORTING FACILITY

OVERVIEW

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

In this technology, biological cells are labeled with one or more fluorescent reagents, often antibodies that detect specific molecules inside cells or on their surfaces. These labeled cells are then streamed at a high rate through a sequence of laser beams, and the resulting fluorescence is measured on a per-cell basis. Quantitative evaluation of multiple reagents on each cell enables resolution and analysis of complex mixtures of cell types, such as tumor and bone marrow cells. Cell sorters, an advanced subset of flow cytometers, can utilize the quantitative criteria provided by the fluorescent labels 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.

Lisa Nichols, Ph.D.
Director

The Fluorescence Activated Cell Sorting (FACS) Facility 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., of the Stanford department of Genetics, was one of the main developers of FACS instrumentation and techniques in the late 1960s and early 1970s, and the Herzenberg laboratory continued to be a major source of innovation in the field throughout the subsequent years. Dr. Herzenberg initiated

Fluorescence Activated Cell Sorting Facility

Front Row, Left to Right: *Meredith Weglarz, Tom Nozaki, Bianca Gomez, Cindy Jiang, Lisa Nichols, Dave Parks,* ***Back Row, Left to Right:*** *Edgar Henriquez, Tim Knaak, Kenneth Quayle, Ricardo Zermeno*

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 together have more than 100 years of experience in flow cytometry, and are available to assist facility users in designing experiments and in data analysis. Staff members also maintain the facility's instruments and support facility operations, as well as develop improved technology for advanced applications and instrumentation.

EXPERTISE

The FACS Facility is under the general oversight of a faculty advisory committee chaired by Garry Nolan, Ph.D., professor of microbiology and immunology, 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, Lisa Nichols, Ph.D., is a flow cytometry expert with more than 15 years of experience, as well as a scientist with expertise in T cell immunology and cancer immunotherapy. The rest of the staff members have similarly long histories in flow cytometry, and contribute a level of expertise available at no other site, enabling researchers to perform innovative and top-quality work using flow cytometry.

SERVICES

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

Cell Analysis

Cell analysis services include both BSL-1 and BSL-2 samples and measurements of up to 40 simultaneous fluorochromes, and support acquisition from either single sample tubes or automated from 96-well plates. Analyzers are also available to trained users 24/7. Four analyzers are currently available with an additional 25-color instrument coming online soon and a 30-color instrument funded for purchase in 2020.

Cell Sorting

Cell sorting services include BSL-1 and BSL-2 samples, aseptic sorting, single cell sorting into 96-well and 384-well plates (cloning), and measurement and sorting using up to 18 simultaneous fluorochromes. Sorting is either operator-supported during normal business hours or self-operated 24/7 upon completion of training. Nine sorters are available, each with different capabilities.

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.

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.

Data Management Services

Data collected in the facility is stored and archived in a secure, highly redundant system, and made available over the internet. This service is available to the

entire Stanford community. The Stanford Institute for Stem Cell Biology and Regenerative Medicine and the Canary Center at Stanford for Cancer Early Detection utilize this service for their flow cytometry data.

Federated Sites

Some research laboratories, because of the nature of their work, need to have flow cytometry equipment on-site. The facility offers individualized contracts to provide management and technical consulting for these groups. In 2018-2019, the facility provided services to 779 researchers from 290 different labs.

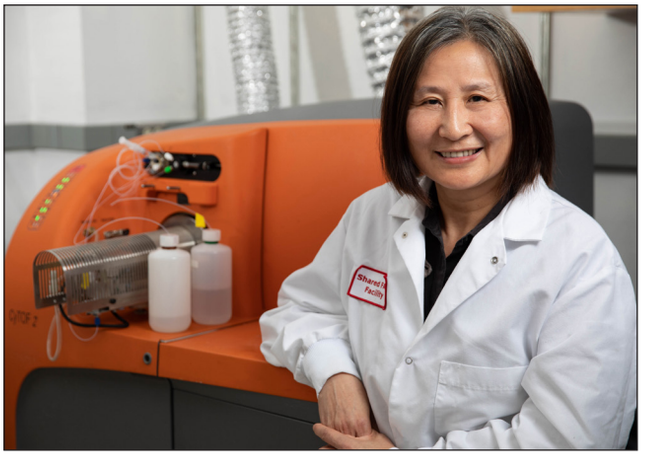
RECENT DEVELOPMENTS

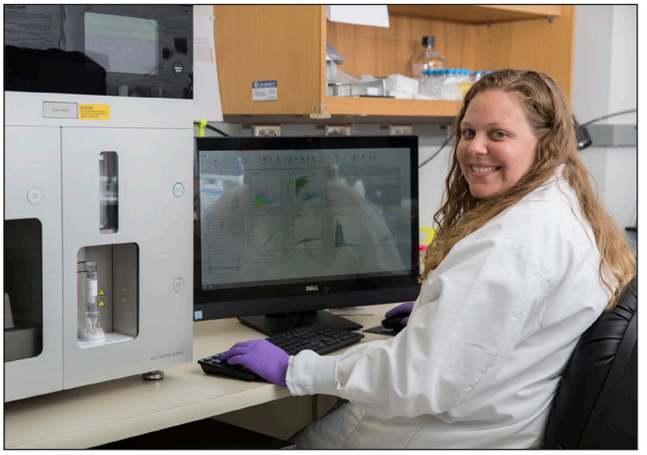
The FACS Facility this year continued to enhance its user access and training to support the growing researcher needs for flow cytometry services.

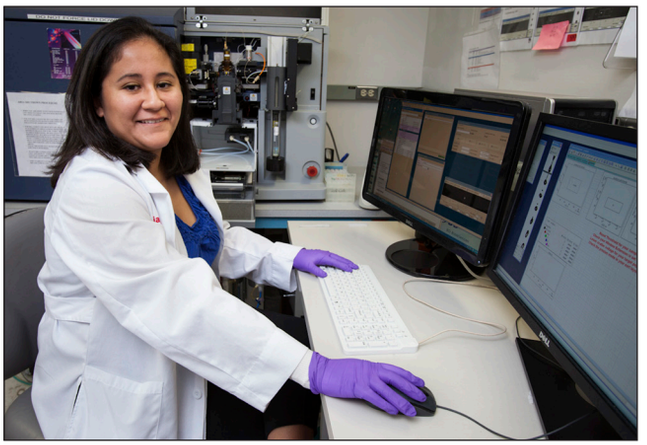
A number of infrastructure updates to facility scheduling, as well as an overhaul of our training program, were implemented. The FACS Facility now has a number of new faculty who are available to researchers who need assistance in collecting experimental data. A revamped training program now ensures quick access to small group learning sessions, which are combined with one-on-one sessions tailored to experimental needs. As a result, even new researchers who need instrument access are able to reserve the machines and begin collecting quality data quickly.

Another area of emphasis has been the ongoing process of updating and expanding the fleet of cytometers. Expansion began in 2017 with the purchase of two bench-top “walk-up” sorters, and the Parker Institute for Cancer Immunotherapy provided funds to purchase another Aria class sorter. These purchases were followed by the addition of a satellite facility, at 1651 Page Mill Road. This additional location continues to support the research community at the School of Medicine Technology & Innovation Park, with one analyzer and one Aria class sorter, in space shared with the Human Immune Monitoring Center.

New this year is the final upgrade of the Cytek Aurora spectral cytometer. With the ever-growing demand for FACS Facility services, analyzer schedules are often filled with more than 12 hours of continuous operation each day. To help meet user needs, in 2018, the Beckman Foundation generously provided funding for the purchase of an additional high-end analytical instrument. The Cytek Aurora spectral cytometer implements state-of-the-art technologies to provide high-quality cytometry data. It utilizes arrays of detectors with high-efficiency light collection for analysis of the emitted fluorescence photons, and is also a full-spectrum, or spectral, cytometer. While traditional cytometers use narrow filters to collect the peak of the fluorescent labels’ emission, the Cytek Aurora, as a spectral cytometer, has an array of detectors/filters







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

evenly spaced across the spectrum of light emission to collect not only the peak, but the majority of photons emitted from each label on the cells. This approach provides the instrument with excellent sensitivity, allowing researchers to resolve more than 20 fluorescent labels simultaneously, even for low-level signals. This year, the Cytek Aurora received its final update, to a five-laser system capable of analyzing up to 40 different labels. Panels of this size are revolutionary for flow cytometry, allowing more in-depth scientific queries and more efficient use of limited experimental samples.

Design of 30+ color cytometry panels is not a trivial task, and can be a barrier for researchers needing to complete in-depth queries. With larger panels becoming more routine, the need for panel design and experimental consultation has grown. Staff at the FACS Facility have therefore optimized a core panel for researchers to use as a jumping-off point for development of their unique high-parameter characterization of cell subsets. This level of experimental support is not available outside of the shared resource lab.

Finally, as education is a primary goal, the training program and consultation support available at the facility are now complemented by a seminar series. Bimonthly seminars, supported by the Beckman Foundation, allow our large user group to receive both basic and advanced instruction. Presentations by staff and guest speakers outline fundamentals of the instrumentation required for collection of quality data, as well as advanced techniques and workflows. Researcher feedback from these sessions has been

overwhelmingly positive. In addition, post-seminar social hours have provided additional opportunities to share ideas and experimental approaches.

FUTURE VISION

In the next several months, we will continue on a trajectory of rapid growth and updating of older equipment. We have already purchased and are now testing a new four-laser, 25-color analyzer cytometer, the Acea Quanteon. The strength of this platform is its user-friendly software, along with robust auto-sampling, which is available from up to 40 tubes, or 96- or 38-well plates. We anticipate this instrument will be heavily used as we phase out older instruments such as the DxP digital FACScan.

In additional good news, in collaboration with faculty advisor Dr. Nolan, National Institutes of Health funds have been awarded for the purchase of a Becton Dickinson 30-color, five-laser analyzer that represents the state-of-the-art for traditional analyzers. This instrument should be in place this year, and will be complemented by the purchase of an FACSymphony S6 sorter capable of sorting up to six populations selected, using up to 30 parameters.

With excellent staffing levels and a commitment to both education and updating to the latest technologies, the FACS Facility is positioned to provide users a wide range of support.

We look forward to another productive year supporting more than 200 labs both at Stanford and in the surrounding Bay Area community.

COMPUTATIONAL SERVICES AND BIOINFORMATICS FACILITY

OVERVIEW

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

EXPERTISE

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

The CSBF works closely with other service centers at the Beckman Center to ensure that the CSBF has the necessary hardware and software for analyzing the wide variety of data that is generated by the different

Lee Kozar
Director

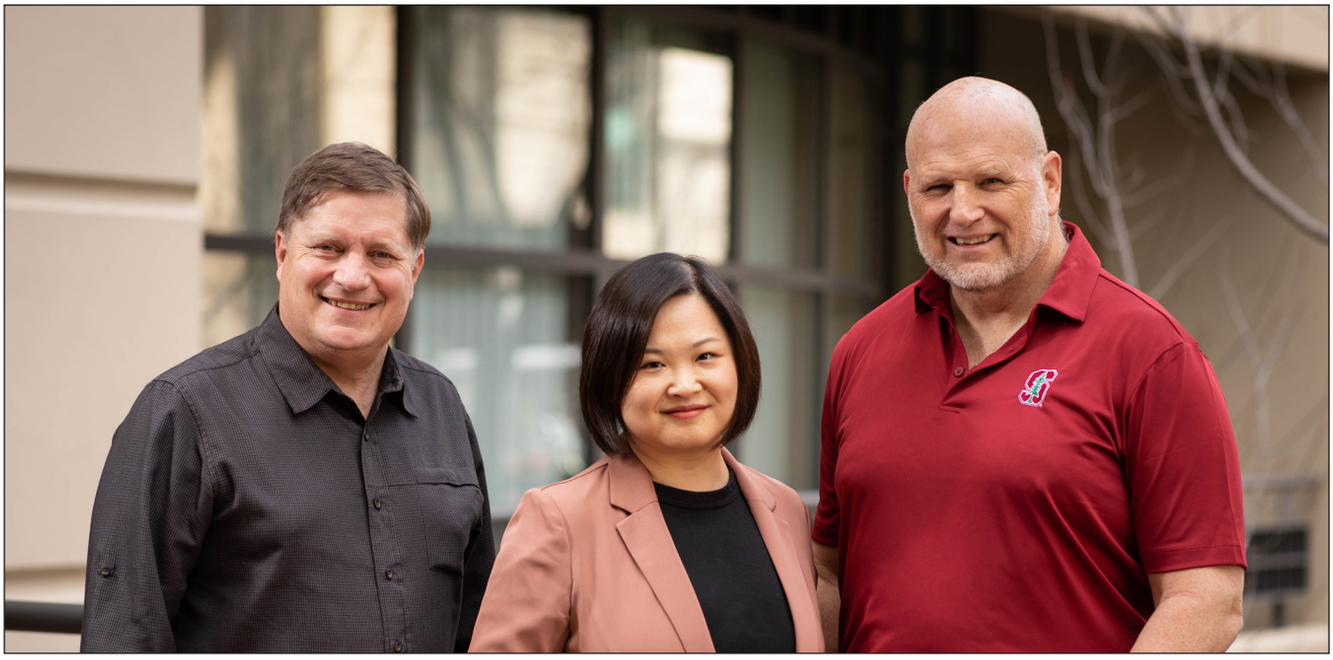
facilities. In essence, the other service centers provide the instrumentation for generating data, and the CSBF provides the computer hardware and software for analyzing the data flowing out of these facilities.

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



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

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

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

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

- Sequence analysis (DNAstar, SnapGene, MacVector, Sequencher, Geneious, CLCBio)
- Microarray analysis (GeneSpring, Partek)

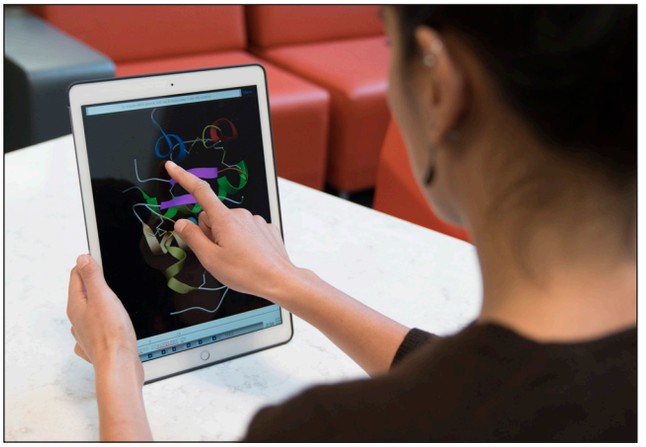
- Genomics analysis (Geneious Server, Golden Helix, Partek Flow, JMP Genomics, Nexus, iPathwayGuide)
- Mass spectrometry (Mascot, PEAKS, ProteinMetrics)
- Database (FileMaker, EndNote)
- Statistical and mathematical analysis (SPSS, Matlab, Mathematica, Systat, GraphPad)
- Graphics (Illustrator, Photoshop, BioRender)
- Microscope imaging (Volocity, Imaris, Metamorph)
- Gel electrophoresis imaging (Nonlinear Dynamics)
- Electronic lab notebooks (LabArchives, Benchling)

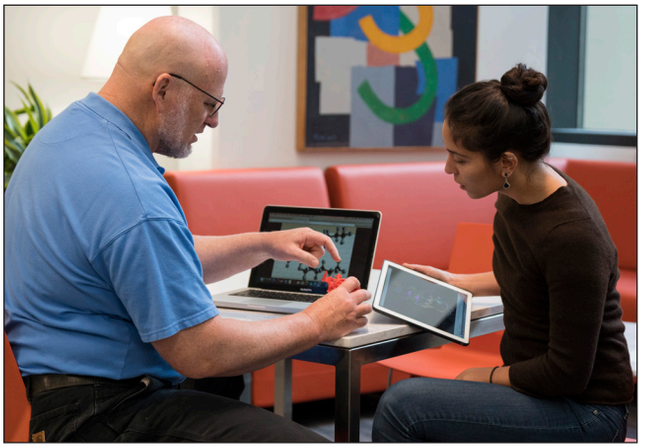
These software programs are repackaged by the CSBF so they can be easily downloaded from the facility's website and installed, already configured for use within the Stanford network. Many of these software programs can be used off-campus; special licensing arrangements can be made so the software will 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. A full list of the software offered by the CSBF can be seen at http://csbf.stanford.edu.

Researchers may also 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 the facility is able to purchase the license and make it available on the CSBF server.

The Beckman Center offers Technology Innovation Grants to faculty members to fund the development of new core facility services that can be used for the benefit of all core facility users. In the past year, we have funded two proposals to bring new software into the core facility. Along

Top: *Ling Xie,* ***Bottom:*** *Alan Hebert*





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

with benefitting the individual lab that requested the grant, this software can be shared with other users of the CSBF software library.

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

CSBF Membership

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

The CSBF has two levels of membership:

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

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

In the past year, more than 300 labs from 36 different departments have had memberships in the CSBF. On average, more than 5,000 computers per month utilize this 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.

Additional Services

In addition to the software library, the CSBF also provides a variety of other services for CSBF members.

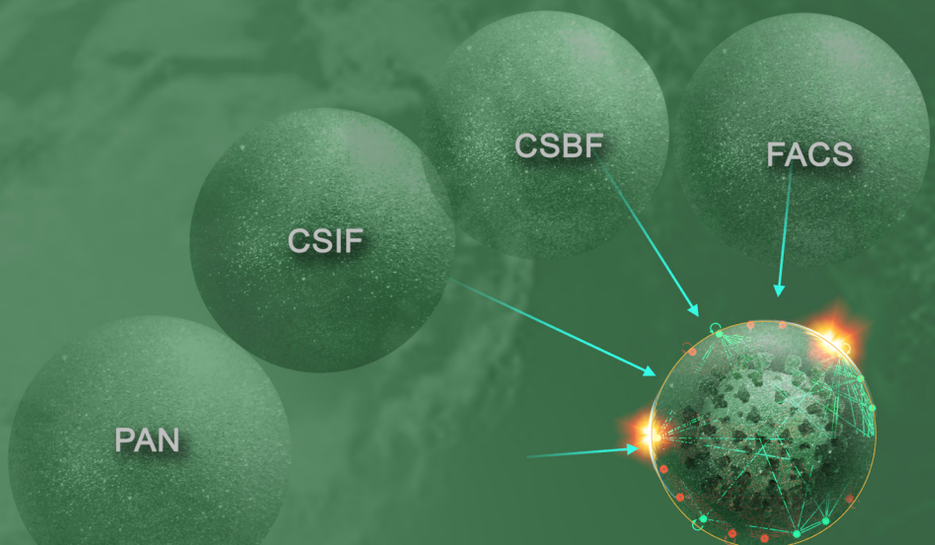
The CSBF moved most of its hardware and storage to the Stanford cloud as a cost-saving measure. The CSBF web server is the primary route for distributing software to users at Stanford; it also hosts many lab and departmental websites. The CSBF also has a large Linux system that hosts proteomics and genomics software.

The CSBF also 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 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 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 offerings. The private Wiki can be accessed only by members of the CSBF and contains important information regarding policies, procedures, license codes, troubleshooting techniques, and any 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.

Aaron Straight, Ph.D.
Professor and Chair of Biochemistry

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.

FACULTY RESEARCH

Steve Artandi's lab is interested in unraveling the molecular and cellular mechanisms with which telomeres and telomerase modulate stem cell function and carcinogenesis. **Onn Brandman**'s lab studies how cells ensure protein quality and how they signal stress. The lab uses an integrated set of techniques, including single cell analysis of proteotoxic stress pathways, structural studies, *in vitro* translation, and full genome screens. **Gil Chu**'s laboratory studies cellular responses to damaged DNA. The group focuses on pathways for the repair of UV-damaged DNA and the repair of DNA double-strand breaks induced by ionizing radiation and V(D) J recombination in order to understand the mechanisms that generate immunological diversity. **Rhiju Das**'s research group strives to predict how RNA sequence determines the folding properties of proteins, nucleic acids, and heteropolymers and establishes their ultimate structure. **Ron Davis** is using *Saccharomyces cerevisiae* and human DNA to conduct whole genome analysis projects. The **James Ferrell** lab has been studying the system of regulatory proteins that drives the cell cycle, through a combination of quantitative experimental approaches, computational modeling, and the theory of nonlinear dynamics. **Pehr Harbury** aims to measure and understand dynamic structural changes in proteins, and their role in the functional biology of macromolecular machines. **Dan Herschlag**'s laboratory is aimed at understanding the chemical and physical behavior underlying biological macromolecules and systems, behaviors that define the capabilities and limitations of biology. **Peter Kim** studies the process by which proteins cause viral membranes to fuse with cells, designs molecules that stop membrane fusion by HIV, and pioneers efforts to develop vaccines based on similar principles. **Silvana Konermann**'s lab is applying multiple modes of targeted transcriptional perturbations to understand genetic interactions of APOE in late onset Alzheimer's disease. The research in **Mark Krasnow**'s laboratory is focused on understanding lung development, stem cells, and diseases, including cancer, and the neural circuits that control lung function, including breathing and speaking. **Lingyin Li** uses chemical biology to uncover biochemical mechanisms in innate immunity and, in parallel, develop therapeutic hypotheses and lead compounds. **Suzanne Pfeffer**'s group is investigating the molecular mechanisms by which proteins are targeted to specific membrane compartments. They seek to

understand how transport vesicles select their contents, bud, translocate through cytoplasm, and then fuse with their targets, as well as other similar processes.
Rajat Rohatgi's lab is working to elucidate the biochemical and cell biological principles that govern signaling pathways that sit at the intersection between developmental biology and cancer.
Julia Salzman's research group develops statistical and experimental tools to construct a high dimensional picture of gene regulation, including cis and trans control of the full repertoire of RNAs expressed by cells. The broad research interest of the **James Spudich** lab is the molecular basis of cell motility. Research interests include the molecular basis of energy transduction that leads to ATP-driven myosin movement on actin, the biochemical basis of regulation of actin and myosin interaction and their assembly states, and the roles these proteins play *in vivo*, in cell movement and changes in cell shape. The **Aaron Straight** group studies the process of cell division in eukaryotes, focusing on the mechanisms of chromosome segregation.
Ellen Yeh's research goal is the elucidation of apicoplast biology, function, and role in pathogenesis, with the ultimate goal of realizing the potential of the apicoplast as a therapeutic target.

2019-2020 FACULTY HONORS, AWARDS AND APPOINTMENTS

Ellen Yeh—assistant professor of biochemistry, of pathology and of microbiology and immunology, has been named an Investigator in the Pathogenesis of Infectious Disease (PATH) by the Burroughs Wellcome Fund. PATH is a highly competitive award that supports assistant professors who study the interplay between infectious agents and their hosts, shedding light on how both are affected by their encounters.

DEPARTMENT OF DEVELOPMENTAL BIOLOGY

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

FACULTY RESEARCH

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

Roel Nusse, Ph.D.
Professor and Chair of Developmental Biology and Howard Hughes Medical Institue Investigator

The group studies genome function in human and related species by mapping genome sequence (variation) to phenotype (differences) and extracting specific genetic insights from deep sequencing measurements. **Alistair Boettiger**'s lab aims to understand how long-range interactions between nonconsecutive parts of the genome are regulated to control gene expression. **James Chen**'s group integrates synthetic chemistry and developmental biology to interrogate the molecular mechanisms that control embryonic patterning, tissue regeneration, and oncogenesis. The focus of research in the **Gerald Crabtree** laboratory is the role of chromatin regulation in development and human cancer. **Margaret Fuller**'s research group seeks to understand the mechanisms that regulate stem cell behavior and in particular the mechanisms that regulate and mediate cellular differentiation during male gametogenesis, using spermatogenesis in *Drosophila* as a powerful genetic model system.

Daniel Jarosz's lab aims to gain insight into the interplay among genetic variation, phenotypic diversity, and environmental fluctuations in complex cellular systems.

Seung K. Kim's lab has created unprecedented opportunities for harnessing knowledge about the molecular and cellular basis of pancreatic development and growth to restore pancreas islet function and to diagnose pancreas cancers. They trust their discoveries will provide the tools and expertise needed to produce islet regeneration therapies for type 1 diabetes, improve treatments and tests to mitigate or prevent type 2 diabetes, and generate new therapeutic strategies for endocrine or exocrine pancreas cancers.

David Kingsley is using a combination of genetic and genomic approaches to identify the detailed molecular mechanisms that control evolutionary change in vertebrates. **Kyle M. Loh**'s lab aspires to understand how different human cell types form from stem cells, and how developing tissues incipiently take shape and form. **Roeland Nusse**'s laboratory is interested in the growth, development, and integrity of animal tissues. The group studies multiple different organs, trying to identify common principles and extend these investigations to cancer and injury repair. The laboratory has a long-standing interest in the activity of Wnt proteins during embryogenesis and other processes. **Lucy Shapiro**'s laboratory studies the mechanisms used to generate the three-dimensional organization of a cell from a one-dimensional genetic code. Their goal is to define the complete genetic circuitry that regulates cell cycle progression in time and space.

Will Talbot's lab focuses on the development and function of glial cells in the vertebrate nervous system.

Anne Villeneuve's lab group is interested in elucidating the events required for the orderly segregation of homologous chromosomes during meiosis, the crucial process by which diploid germ cells generate haploid gametes.

Bo Wang's research group is working at the interface between statistical physics, developmental biology, and bioengineering. They seek to understand, quantitatively, the fundamental rules that control stem cell collective behavior to optimize tissue regeneration, 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.

2019-2020 FACULTY HONORS, AWARDS AND APPOINTMENTS

Philip Beachy—the Ernest and Amelia Gallo Professor and professor of developmental biology and of urology, received the bladder cancer research innovation award from the Bladder Cancer Advocacy Network. The two-year, $300,000 award supports novel and creative projects with the potential to produce breakthroughs in the management of bladder cancer. His research seeks to reduce bladder cancer recurrence by identifying and replacing diseased cells that persist in the bladder lining with healthy progenitor cells.

Alistair Boettiger—assistant professor of developmental biology, and Leslie Mateo, a graduate student in developmental biology, were awarded a Gilliam Fellowship for Advanced Study. They will receive $50,000 a year, for up to three years, to promote inclusivity in laboratories and increase diversity in the life sciences. The goal of the Gilliam Fellowships for Advanced Study is to increase the diversity among scientists who are prepared to assume leadership roles in science, particularly as college and university faculty. The program provides awards to pairs of students and their dissertation advisers who are selected for their scientific leadership and commitment to advancing diversity and inclusion in the sciences.

Seung Kim—professor of developmental biology, will serve as a member of the National Institutes of Health Center for Scientific Review's Cellular Aspects of Diabetes and Obesity Study Section for the term that began July 1 and ends June 30, 2023. Members are selected on the basis of their demonstrated competence and achievement in their scientific disciplines.

Kyle Loh—assistant professor of developmental biology, received a David & Lucile Packard Foundation Fellowship for Science and Engineering. The $875,000 grant, to be issued over five years, is for his work in building human cells from pluripotent stem cells.

In addition, Dr. Loh has been named a Pew Scholar in the Biomedical Sciences. The program provides funding to young investigators of outstanding promise. He will receive $300,000 over four years to support his research focused on embryonic stem cell differentiation and tissue transplants.

Also, Dr. Loh received a grant from the international Human Frontier Science Program. The three-year, $250,000-per-year grants are awarded to teams of researchers from different countries. Loh's team will study the role of vasculature in the development of brain tissue.

Roeland Nusse—the Reed-Hodgson Professor of Human Biology, the Virginia and Daniel K. Ludwig Professor in Cancer Research, and professor of developmental biology, was the recipient of the 2020 Canada Gairdner International Award for his work on understanding the role of the Wnt signaling pathway in normal development and in cancer. The award recognizes excellence in fundamental research that affects human health.

Irving Weissman—professor of developmental biology and pathology and director of the Ludwig Center for Cancer Stem Cell Research at Stanford, was awarded the 2019 Albany Medical Center Prize in Medicine and Biomedical Research for his pioneering work in stem cell and cancer biology, including the identification of blood-forming stem cells and their role in blood cancers, as well as the discovery of a "don't eat me" signal on the surface of many cancer cells that protects them from being eliminated by the immune system.

Joanna K. Wysocka—the Lorry Lokey Professor and professor of chemical and systems biology and of developmental biology, was elected as an associate member of the European Molecular Biology Organization, a group of researchers who promote excellence in the life sciences in Europe and beyond.

DEPARTMENT OF MOLECULAR AND CELLULAR PHYSIOLOGY

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

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

Miriam 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 Goodman**'s research investigates the biophysics and mechanics of touch sensation by combining *in vivo* electrophysiology with genetics and novel tools for mechanical stimulation, through quantitative behavioral studies, light and electron microscopy.

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

Georgios Skiniotis and his research group are using electron cryo-microscopy (cryoEM) to study the mechanisms of transmembrane signal instigation with a particular focus on G-protein-coupled receptors and cytokine receptors.

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

2019-2020 FACULTY HONORS, AWARDS AND APPOINTMENTS

Miriam Goodman—professor and chair of molecular and cellular physiology, received the midcareer Landis Award for Outstanding Mentorship from the National Institute of Neurological Disorders and Stroke. The award recognizes faculty members who have shown superior mentorship and training. It provides $100,000 to foster career development of additional trainees in her lab, where the research focuses on how skin and its embedded neurons give rise to touch sensation, and how sensory neurons bend without breaking.

Richard Lewis—professor of molecular and cellular physiology, was elected to membership in the prestigious National Academy of Sciences at the academy's 157th annual meeting in April 2020. 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.

PROGRAM IN MOLECULAR AND GENETIC MEDICINE

2019-2020 FACULTY HONORS, AWARDS AND APPOINTMENTS

Scott Boyd; Michelle Monje; and Carolyn Rodriguez—Boyd, associate professor of pathology; Monje, associate professor of neurology and neurological sciences; and Rodriguez, associate professor of psychiatry and behavioral sciences, are recipients of the Presidential Early Career Award for Scientists and Engineers. The award is the highest honor bestowed by the U.S. government to outstanding scientists and engineers who are beginning their independent research careers and who show exceptional promise for leadership in science and technology.

Howard Chang—the Virginia and D.K. Ludwig Professor of Cancer Genomics and Genetics and professor of dermatology and of genetics, was elected to membership in the prestigious National Academy of Sciences at the academy's 157th annual meeting in April 2020. 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.

Hongjie Dai—the J.G. Jackson and C.J. Wood Professor in Chemistry, was elected a member of the National Academy of Medicine. Dai's research spans chemistry, physics, and materials and biomedical sciences and has led to materials with properties useful in electronics, energy storage, and biomedicine.

Lisa Giocomo—assistant professor of neurobiology and a 2012 Beckman Center faculty recruit, received a scholars award from the Vallee Foundation. The $300,000 grant is for basic biomedical research. Giocomo researches the neural mechanisms of cognition, specifically navigation.

Aaron Gitler—professor of genetics, received the 2019 Sheila Essey Award from the American Academy of Neurology, the ALS Association, and the American Brain Foundation. The $50,000 prize recognizes significant contributions in the search for the causes of amyotrophic lateral sclerosis, also known as Lou Gehrig's disease, as well as ways to prevent and cure the disease. His research uses genetic screening to focus on mechanisms of neurodegenerative diseases, including Parkinson's, Alzheimer's, and ALS.

Chaitan Khosla—the Wells H. Rauser and Harold M. Petiprin Professor of chemistry, and the Baker Family Co-director of Stanford ChEM-H, was elected to membership in the prestigious National Academy of Sciences at the academy's 157th annual meeting in April 2020. 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.

Michael Longaker—the Deane P. and Louise Mitchell Professor in the School of Medicine and co-director of the Institute for Stem Cell Biology and Regenerative Medicine, was awarded the Lifetime Achievement award by the Society of University Surgeons. The award recognizes individuals who have had a sustained career in academic surgery with contributions to surgical science and have demonstrated a commitment to the society.

Stephen Montgomery—associate professor of pathology and of genetics, received a 2019 early-career award from the American Society of Human Genetics. The award, which includes a $10,000 prize, recognizes the contributions of genetics and genomics scientists in the first 10 years of their careers as independent investigators.

Julie Parsonnet—the George DeForest Barnett Professor in Medicine, was elected a member of the National Academy of Medicine. Parsonnet researches how microbial exposures in children affect long-term health, the role played by the skin microbiome, and other microbial studies.

Stanley Qi—assistant professor of bioengineering and of chemical and systems biology, was named to *Science News'* Scientists to Watch 2019 list. The honor is for scientists age 40 and younger who are making outstanding contributions in their fields and show promise of making future advances.

Thomas Quertermous—the William G. Irwin Professor in Cardiovascular Medicine, received a three-year Seed Networks project grant from the Chan Zuckerberg Initiative. As a member of one of 38 collaborative teams funded by this grant, his work will support the continued development of the Human Cell Atlas, an international effort to map all cells in the human body. His team will work to create an atlas of single cell transcriptomic and epigenomic features of the human vasculature to define the cellular composition and key regulatory features of these vessels.

Peter Sarnow—professor of microbiology and immunology, was elected to membership in the prestigious National Academy of Sciences at the academy's 157th annual meeting

in April 2020. 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.

Michael Snyder—the Stanford W. Ascherman, M.D., FACS, Professor in Genetics, chair of genetics and director of the Stanford Center for Genomics and Personalized Medicine, received the 2019 George W. Beadle Award from the Genetics Society of America. The award recognizes significant sustained service to the genetics community. He was recognized for developing and disseminating widely used technology for the simultaneous analysis of thousands of genes, RNA molecules, and proteins.

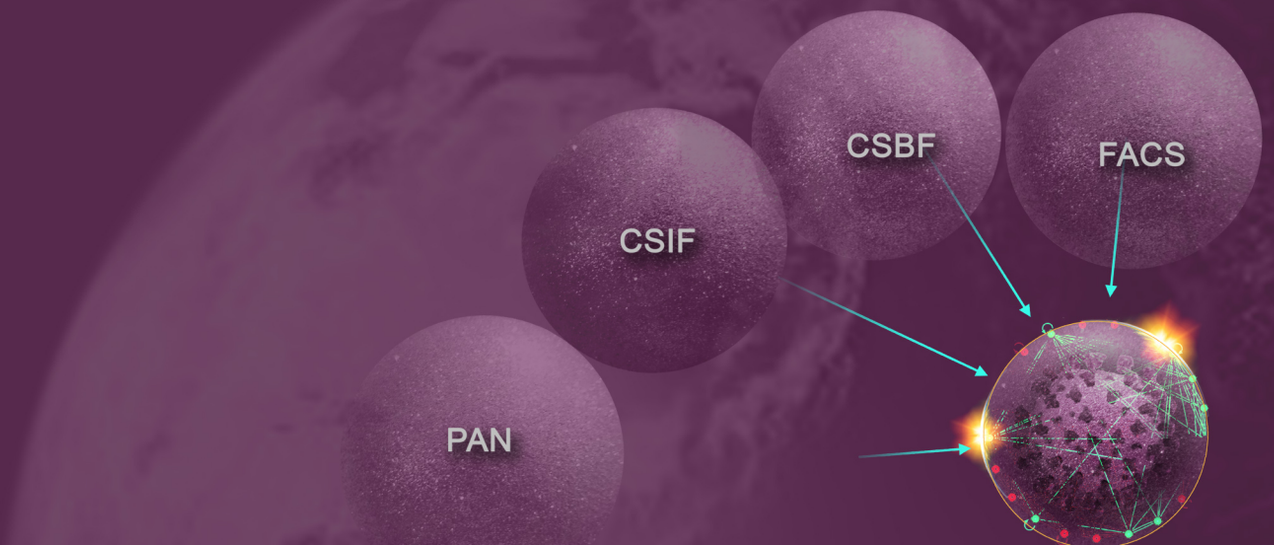
Marc Tessier-Lavigne—the Bing Presidential Professor and Stanford University President received the 2020 Gruber Neuroscience Prize. The award recognizes Tessier-Lavigne's groundbreaking work on axon guidance processes in mammals and their role in spinal cord development. His research has helped reveal molecular mechanisms that are critical to axon development throughout the animal kingdom. Tessier-Lavigne shared the prize with Friedrich Bonhoeffer, emeritus director of the Max Planck Institute for Developmental Biology, and Corey Goodman, a founding partner of venBio Partners in San Francisco.

Robert Tibshirani—professor of biomedical data science and of statistics, was elected a fellow of The Royal Society, the world's oldest independent scientific academy. He was recognized for his seminal contributions to the fields of bioinformatics and statistics, namely his invention of statistical tools for extracting information from data.

Bo Wang—assistant professor of bioengineering, received a grant from the international Human Frontier Science Program. The three-year, $250,000-per-year grants are awarded to teams of researchers from different countries. Wang's team will study how an immune response can shift from being beneficial to being harmful.

Joseph Wu—the Simon H. Stertzer, M.D., Professor, professor of medicine and of radiology, and director of the Stanford Cardiovascular Institute, was elected a member of the National Academy of Medicine. His research attempts to understand cardiovascular disease mechanisms.

MEDIA COVERAGE



MEDIA COVERAGE

THE FOLLOWING ARTICLES WERE REFERENCED IN THE HIGHLIGHTS SECTION

"New Research Could Lead to Lifetime Flu Vaccine"

STANFORD MEDICINE NEWS CENTER
MAY 1, 2019

"Forgotten Immune Cells Protective in Mouse Model of Multiple Sclerosis"

STANFORD MEDICINE NEWS CENTER
AUGUST 7, 2019

"Scientists Uncover Genetic Similarities Among Species that Use Sound to Navigate"

STANFORD MEDICINE NEWS CENTER
OCTOBER 3, 2019

"Antibody Treatment Allows Transplant of Mismatched Stem Cells, Tissues in Mice"

STANFORD MEDICINE NEWS CENTER
JUNE 13, 2019

"New 'Don't Eat Me' Signal May Provide Basis for Cancer Therapies"

STANFORD MEDICINE NEWS CENTER
JULY 31, 2019

"People's Response to Flu Vaccine Influenced by Gut Microbes"

STANFORD MEDICINE NEWS CENTER
SEPTEMBER 5, 2019

"In Human Cells and Mice, a Cure for the Common Cold, Stanford-UCSF Study Reports"

STANFORD MEDICINE NEWS CENTER
SEPTEMBER 16, 2019

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