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.

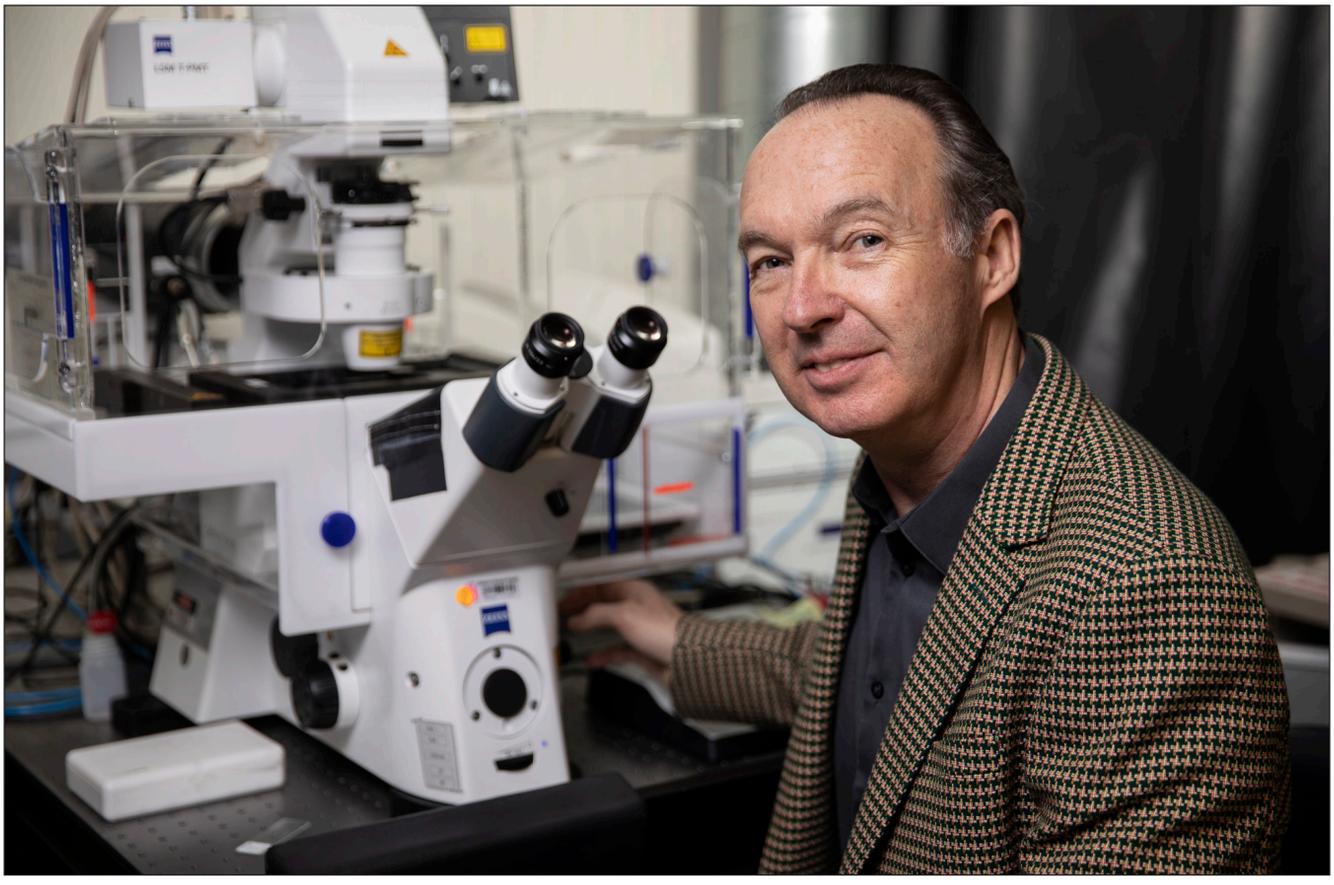
Today, the directors of the service centers are not only acquiring the newest technologies. They are also forging new collaborations that allow researchers to easily use more than one service center for a single line of investigation, such as when Dr. O'Brien snaps shots of cells at CSIF and then uses 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

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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."

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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

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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."

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–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

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Director of the Protein and Nucleic Acid Facility

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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.

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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."

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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

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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."