

Boston University Neurophotonics Center Annual Report 2019



Neurophotonics
Center

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Message From the Director

I am extraordinarily pleased to describe here the activities of the Boston University Neurophotonics Center (NPC) during its second academic year of operation, 2018-2019. During this second year of operation, we continue to engage more than 30 active faculty members, we've grown an active community of 67 PhD and 15 post-doctoral trainees, 14 PhD students are jointly mentored by NPC faculty, many of the initial Center supported projects have submitted grant proposals, 6 new projects have been initiated, 17 joint papers engaging two or more member labs were published, and 19 Center and Collaborative grants were awarded totaling \$7.2 million. All-in-all, we are building momentum delivering on the mission of the Center to build and support an interdisciplinary community that can develop and broadly deploy impactful photonics technologies in the neurosciences to advance our understanding of how the brain works in health and in disease.

A major success of the NPC this past year has been utilizing its funds to initiate collaborative projects that bring together 2 or more member labs to develop new neurophotonics technology to address open challenges in studying the brain. Noteworthy accomplishments of the Center supported projects from the last year include:

1. **Development of a Wearable Functional Near Infrared Spectroscopy (fNIRS) System for**



Imaging Brain Activity in Freely Behaving Humans – We are developing the next generations (plural!) of fNIRS systems that are wearable and integrated with EEG and other biosensors to provide a more complete understanding of brain activity to revolutionize Neuroscience and Neuroimaging in the Everyday World with wearable fNIRS.

2. **Development of a Computational Miniature Mesoscope for Imaging Neural Activity in Freely Behaving Animals** – Recognizing the need for a wearable mesoscope that could image neural activity over an area up to 1 cm², Professors Tian and Davison initiated a collaboration to produce the solution and now have NIH funding.

3. **Increasing the Volumetric Imaging Rate of Confocal Microscopy to Image Voltage Indicators with Millisecond Temporal Resolution** – This project, led by Professor Mertz, is being developed in the Center to facilitate interaction with neuroscience faculty. Successful progress of this project has led to

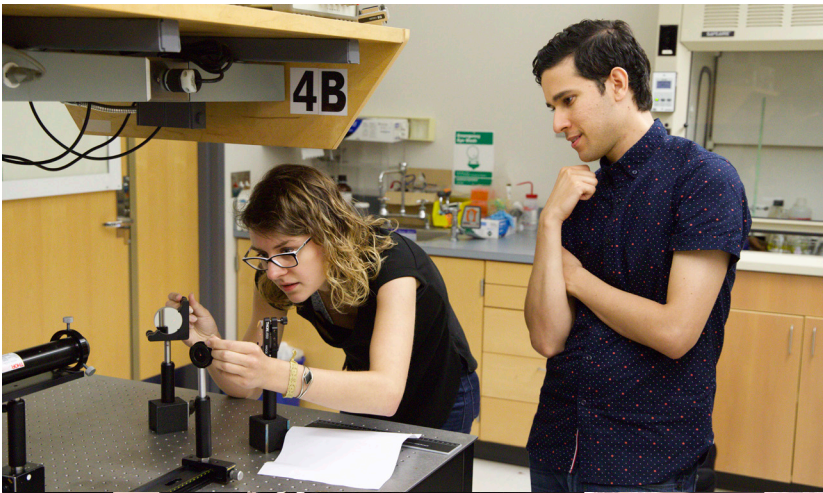
initiation of a new project – 2-Photon Reverberation Microscopy – that is a core part of an NIH P41 Biomedical Technology Resource Center that was submitted in September of 2019.

4. **Developing Functional Ultrasound for Imaging the Brain and Other Organs** – Professors Wong and Boas have been actively using and developing the technology. Professors Roblyer and Porter are beginning to use the systems this year. And it formed a core part of the NIH P41 proposal that was just submitted.

5. **Investigation of a Novel Laser for 3-Photon Microscopy For Deep Brain Imaging** – This pilot project was started with Professor Ramachandran and has now graduated to building a full-blown 3-photon microscope that is being driven by new junior faculty member Scott with Center support.

Finally, I am very grateful for the fantastic trainees working with the NPC and the collegial Center faculty who are eagerly working together to develop and apply the cutting edge technologies needed to advance our understanding of the brain. I can't imagine a better environment for open, collaborative, and multi-disciplinary science to take place!

David Boas
Director, Boston University Neurophotonics Center



At a Glance

39

Faculty Members

55

Students in NRT

17

Projects Supported

19

Publications from
NPC Faculty
Collabrations

14

External Speakers

Events



The Neurophotonics Center offers an exciting array of events throughout the year to engage the community and offer enriching opportunities to BU, Boston area universities and local companies. These events foster interdisciplinary discussion and encourage faculty and students to collaborate with a variety of professionals on fundamental research. Check out our website at <http://www.bu.edu/neurophotonics/events> for upcoming events.

Boot Camp

This summer, Boot Camp was held Monday, July 8 – Wednesday, July 10, 2019. Thirteen of our first year doctoral National Science Foundation (NSF) NRT UtB: Neurophotonics trainees attended the three day intensive sessions. The day started with Helen Fawcett, Program Coordinator of the NSF NRT UtB: Neurophotonics leading Science Communications training, where trainees were purposefully partnered with another

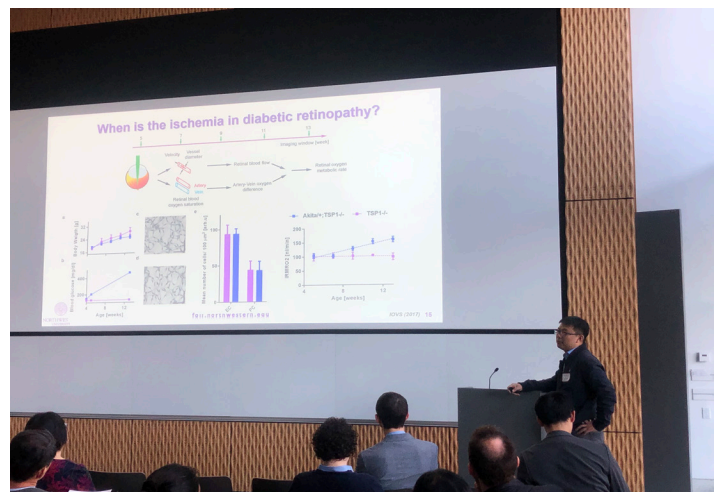
not in their same department, to begin discussions about their research to a person outside their field. The trainees learned about how to communicate their message without using jargon and each ended their session with a 15 second research pitch that can be found on the Neurophotonics Center website Graduate Students page. Yarden Cohen then led an Introduction to Photonics Seminar that included the NRT trainees as well as participants in the NSF Research Experiences for Teachers and Undergraduates Sites in Integrated Nanotechnology and the NSF REU participants from the Engineering Research Center CELL-MET. After the lecture, the NRT trainees participated in a hands on optics session in the Photonics Center Teaching Lab. Tushare Jinadasa led the overview, and two NRT Trainees, Jenny Sun and Havva Begum Kabagoz, assisted with questions from the new trainees. The afternoon was filled with lectures on Introduction to Neuroscience, led by Mark Howe and Introduction to Neurophotonics led by Mike Economo. On Tuesday

and Wednesday, the students spent ½ days in lectures and hands on sessions on Microscopy led by Jerome Mertz, Laser Surgery and C. elegans led by Chris Gabel, Fluorescent Indicators led by Tushare Jinadasa, and Optogenetics in Neurophotonics led by Steve Ramirez. A new section of Boot Camp was added this year based on the feedback from the previous year’s participants: hands on microscopy. We included this session, introducing the trainees to four different optical imaging techniques. Tushare Jinadasa prepared identical samples for viewing with Widefield and Confocal Microscopes available at the Life Science and Engineering Building. Todd Blute demonstrated and discussed with students the merits and limitations of the two different microscopes. The same sample was imaged with the 2-Photon microscope located at the Neurophotonics Center, as NRT Trainee, Jack Giblin, led the trainees through the operation of the microscope. NRT Trainee, Jiarui Yang, also at the Neurophotonics Center, introduced the NRT trainees to the OCT microscope as the final imaging system demonstrated to the first year trainees. To end three days of intensive learning, the trainees all received Certificates of Completion as their PI’s gathered to support their introduction to the NRT program and engagement with the Neurophotonics Center Community. Boot Camp continues to be a pivotal point of engagement and training for our first year graduate students as they embark on their research and join the neurophotonics community.

Neurophotonics Center 2nd Annual Symposium

The Neurophotonics Center organized the **2nd Annual Symposium** focused on the topic of Neurophotonics. The symposium, held on Jan, 15 2019, drew 200 attendees from BU, other academic institutions, and industry. The agenda for this year’s symposium featured presentations by researchers from leading academic institutions.

- Dr. Yarden Cohen, Boston University
- Dr. Diego Restrepo, UC Denver
- Dr. Ben Scott, Boston University
- Dr. Adam Cohen, Harvard University
- Dr. Jerome Mertz, Boston University
- Dr. Kaspar Podgorski, Janelia Farm Research Campus-
- Dr. Xavier Intes, RPI
- Dr. Maria Angela Franceschini, MGH
- Xinge Li, Boston University



At the conclusion of this year's conference, a reception and electronic poster board session was held where participants, students and speakers discussed their research in an informal setting.

Seminar Series

The Neurophotonics Center organizes a monthly seminar series, generally in coordination with affiliated Departments and Centers.

September 10, 2018

Lin Tian , University of California
“Watching the Brain in Action: Creating Tools for Functional Analysis of Neural Circuitry”

October 6, 2018

Vijay Iyer, Neuroscience Community Liaison at Math-Works
“MATLAB as a Platform for Neuroscience & Neurophotonics”

November 16, 2018

Darcy Peterka, Columbia University
“SLM-based Methods for 3D Control and Imaging in the Brain”

December 3, 2018

Nozomi Nishimura, Cornell University
“Exploring behaviors of cells “in the wild” with in vivo multiphoton microscopy

May 14, 2019

Dawen Cai, University of Michigan
“Mapping neuronal identities and connections in neural circuits by light microscope.”

fNIRS Workshop

The BU Neurophotonics Center organized a 3-day course on functional Near-Infrared Spectroscopy (fNIRS) on November 7-9, 2018. The course included lectures, hands-on sessions with fNIRS instruments, and a strong emphasis on data analysis exercises using the Homer2 software.

Neurophotonics Social

A monthly social is held in CILSE on the 8th floor lounge to bring together students and faculty to meet and catch up on the latest activities in their labs.



Faculty



Thomas Bifano
Professor (ME, MSE, BME)

Deformable mirrors; Microelectromechanical systems (MEMS); Adaptive optics; Biphotonic microscopy; Astronomical telescope instrumentation; Laser wavefront control



Alberto Cruz-Martin
Assistant Professor of Biology

Neural circuits; Sensory processing; Visual pathways



Irving Bigio
Professor (BME, ECE)

Medical application of optics, lasers and spectroscopy; Biophotonics; Nonlinear optics; Applied spectroscopy; Laser physics



Ian Davison
Assistant Professor of Biology

Pheromones and innate social behaviors; Cortical computations and plasticity



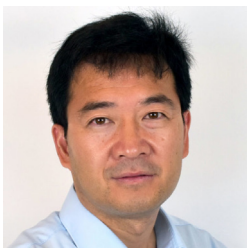
David Boas
Professor (BME, ECE)
Director of Neurophotonics Center

Neurophotonics; Biomedical Optics; Oxygen delivery and consumption; Neuro-vascular coupling; Physiological Modeling



Allison Dennis
Assistant Professor (BME, MSE)

Nanobiotechnology; Fluorescent biosensing; Fluorescence resonance energy transfer (FRET); Quantum dot chemistry; Fluorescence microscopy; Single molecule sensing/imaging



Ji-Xin Cheng
Professor (ECE, BME, MSE)

Molecular spectroscopic imaging technologies; Label-free microscopy; Medical photonics; Neurophotonics; Cancer metabolism; Photonics for infectious diseases



Anna Devor
Assistant Professor (BME)

Cellular and systems-level neuroscience, microscopy, physiological underpinning of noninvasive imaging



Jerry Chen
Assistant Professor of Biology

Large-scale neuronal networks; Sensorimotor integration; Decision making; Neurodevelopment; Non-linear microscopy



Michael Economo
Assistant Professor (BME)

Neural circuits, Cognitive function, Neurodevelopmental disorders



Alice Cronin-Golomb
Professor (Psychological & Brain Sciences)

Neural correlates of perception and cognition in aging and age-related neurodegenerative disease



Chris Gabel
Assistant Professor (Physiology & Biophysics)

Femtosecond laser surgery and optical neurophysiology for the study of the nervous system of the nematode worm *C. elegans*

Faculty



Lee Goldstein

Assoc. Professor (Neurology, BME, ECE)

The role of abnormal protein aggregation in chronic degenerative disorders of aging



Laura Lewis

Assistant Professor (BME)

Develop computational and signal processing approaches for neuroimaging data that enable new kinds of analyses of human brain physiology and function at subsecond timescales



Xue Han

Assoc. Professor (BME)

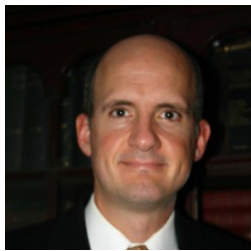
Neurotechnology; Optical neural modulation; Optogenetics; Neural prosthetics; Neural network dynamics; Brain rhythms; Neurological and psychiatric diseases; Cognition



Sam Ling

Assistant Professor (Psychological & Brain Scientists)

Imaging human behavior to explore how the brain mediates between the “buzzing confusion” of the visual world and our limited processing power



Michael Hasselmo

Director of Center for Systems Neuroscience

The role of oscillatory dynamics and neuromodulatory regulation in cortical mechanisms for memory-guided behavior



Jerome Mertz

Professor (BME, ECE)

Development and applications of novel optical microscopy techniques for biological imaging



Mark Howe

Assistant Professor (Psychological & Brain Sciences)

Basal ganglia circuit mechanisms for learning and action



Tim Otchy

Assistant Professor of Biology

Experiments in probing, manipulating and modeling the neural circuits involved in song learning and production



Melissa Kibbe

Assistant Professor (Psychological & Brain Sciences)

Representation of information about objects (e.g., perceptual features, animacy, group statistics, numerosity, verbal labels)



Siddharth Ramachandran

Professor (ECE, MSE)

Optical physics of guided waves; Micro- and nano-structured optical fibers; High-power fiber lasers and fiber sensors; Biomedical imaging and microscopy with optical fibers



Swathi Kiran

Professor (Department of Speech, Language, and Hearing Sciences)

Bilingual aphasia; Aphasia rehabilitation; Functional neuroimaging; Language recovery; Impairments in naming, reading, writing



Steve Ramirez

Assistant Professor (Psychological & Brain Sciences)

Revealing the neural circuit mechanisms of memory storage and retrieval, and artificially modulating memories to combat maladaptive states

Faculty



Darren Roblyer
Assistant Professor (BME)

Optical functional imaging; Diffuse optics and spectroscopy; Monitoring of therapies in oncology; Non-invasive monitoring of tumor metabolism

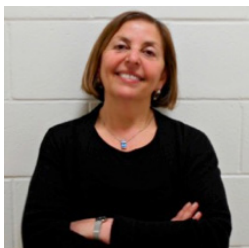


Chantal Stern
Professor (Psychological & Brain Sciences)
Using fMRI to study how the normal brain encodes, stores and subsequently recognizes visual, spatial and verbal information



Michelle Sander
Assistant Professor (ECE, MSE)

Femtosecond lasers; Ultrafast photonics and nonlinear processes; Fiber and integrated optics; Frequency combs; Infrared spectroscopy and biomedical applications



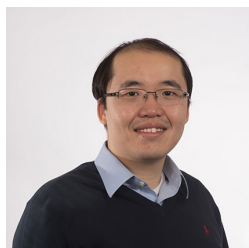
Helen B. Tager-Flusberg
Director of Center for Autism Research

The phenotypic characteristics of the language, communication and associated social-cognitive deficits in autism (ASD) and other neurodevelopmental disorders



Benjamin Scott
Associate Professor (Psychological & Brain Sciences)

Develop and apply new technologies to study the neural basis of cognition and complex learned behavior



Lei Tian
Assistant Professor (ECE)
Computational imaging and sensing; Gigapixel, 3D microscopy; Compressive imaging; Phase retrieval; Imaging through complex media; X-ray phase imaging



Kamal Sen
Associate Professor (BME)

Neural coding of natural sounds; Neural discrimination; Population coding of natural sounds



John A. White
Professor & Chairman (BME)

Mechanisms of episodic memory; Pathophysiology of epilepsy; Computational neuroscience; Design of real-time instrumentation; Imaging of activity in neurons and astrocytes



David Somers
Professor & Chair (Psychological & Brain Sciences)

Functional MRI, psychophysics, and computational modeling to investigate the mechanisms underlying visual perception and cognition



Ji Yi
Assistant Professor (Medicine)

Development of novel optical techniques for fundamental research and clinical applications with a focus on cancer detection, glaucoma, and aging



Robert Stern
Professor (Neurology)
Director of CTE

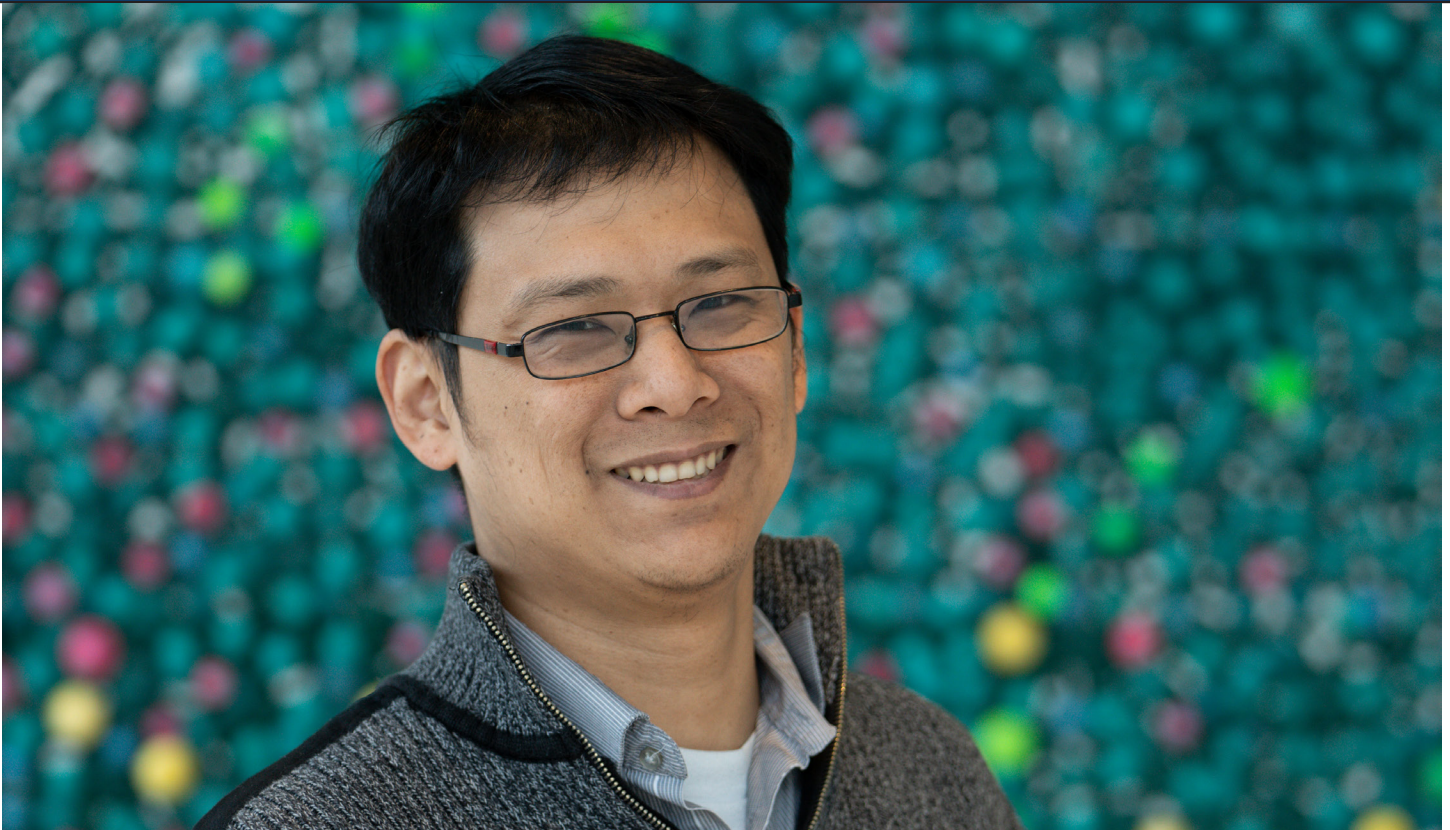
Long-term effects of repetitive brain trauma in athletes, including the neurodegenerative disease, CTE



Meryem Yücel
Assistant Professor (BME)

Functional neuroimaging (fNIRS, fMRI, EEG); fNIRS signal processing; cognitive neuroscience

Anderson Chen: The Man Behind the Microscopes



Among Chen's responsibilities at BU is teaching users of the Micro and Nano Imaging Core how best to utilize the microscopes there. Photo by Bernhard Zimmermann.

At the heart of the BU Neurophotonics Center and all of its endeavors lies an important mission: support a range of imaging methods from early stage development to broader adoption and on again to turn-key core facility usage. Meeting this goal involves working with the dozens of photonics faculty at BU who are advancing novel optical imaging technologies—solutions in search of problems, to use the old saying—as well as the many biology faculty who are pursuing research prob-

lems that do not yet have a solution. And not only working with the two disciplines, but bridging them and finding ways for the respective investigators to collaborate in as fruitful a way as possible. In short, furthering the mission is not as easy as it might seem. It takes a particular type of person to understand the gamut of both problems and solutions in play; to envision all the possible ways they might intersect; and finally to support both the developers and users of the optical imaging technologies, to guide the former in adopting

their solutions for diverse biological problems and the latter in understanding how to implement those solutions.

Meet Anderson Chen. Trained in experimental physics, Chen is the Senior Imaging Scientist with the Neurophotonics Center and manager of the Micro and Nano Imaging Core facility, which straddles the Neurophotonics Center and the Biomedical Engineering Department at BU. As the Senior Imaging Scientist, he spearheads

the building of new microscopes and interacts with biologists about their research. As the manager of the Micro and Nano Imaging Core facility, he interfaces with core users, assists in teaching them how to use the core microscopes, and when needed helps to design their experiments. “I kind of wear many hats,” he says. Indeed, it is this mix of roles and responsibilities that makes him so integral to the Center and its mission, facilitating the development of imaging methods from their earliest stages to their final integration into core facilities for simple, turn-key use by a broad swath of biology and other faculty.

A True Renaissance Man

Chen’s path to his current position in the Neurophotonics Center was a winding and altogether fascinating one, and in many ways prepared him for the unique set of responsibilities he now has.

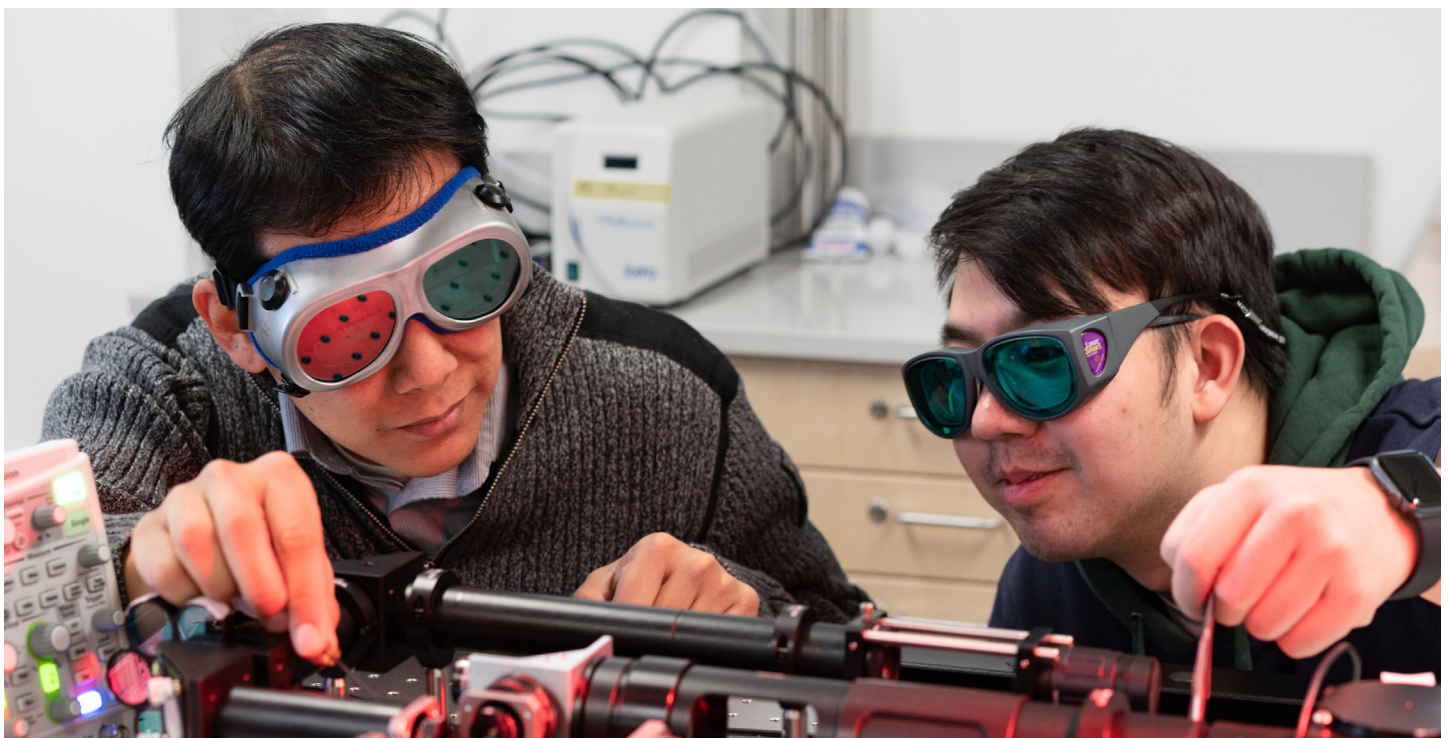
After receiving his PhD in experimental physics from Stevens Institute of Technology, in Hoboken, N.J., he began a postdoctoral fellowship at North Carolina State University as a National Research Council scholar. Here, he continued his research with deep sensors, improving the sensors for military applications: developing, for example, a low-cost “hyper-spectral” camera sensor that images the UV through the THz regions on a single, inexpensive imaging chip. (He is quick to point out that the camera bore some resemblance to a device featured in the sci-fi action movie *Predator* and to the VISOR from *Star Trek: The Next Generation*.)

The fellowship lasted a year, forcing him to move on, taking a job with the photonics company Newport as a senior electrical optical engineer. The move to industry gave him a new understanding of best practices in bringing developing technologies to market. “I

learned a lot about engineering documentation and engineering manufacturing,” he says, “and I gained a new appreciation for time efficiency and the art of design—how to design something that’s easy and cost-effective to build.”

Chen worked with Newport for a year and a half, at the Oriel Instruments Division in Stratford, Connecticut. He might have stayed with the company longer but it was planning to split the division between two existing facilities: one in Irvine, Calif., and the other in Bozeman, Mt. The higher-ups wanted to move him to California but Chen, a street car racer ever since his days in the asphalt jungle of New Jersey, chafed at the idea of moving to a state with such strict emissions standards.

Next, they offered him a position in Montana, but “Montana is just too freaking cold and the hail storms would ruin my cars.” So, in 2014, he moved on again, joining the lab of Na Ji at the Howard Hughes Medical Institute Janelia Research Campus in Ashburn, Va. Here he began



his journey as a microscopist, developing a simple and robust adaptive optics add-on module for use with two-photon microscopes. “My critical thinking skills sort of took off,” he says. “Dr. Ji really guided me in learning how to formulate processes: how do you establish research questions and how do you answer those questions step by step in microscopy.” His work in the lab was formative, and he might have continued with Dr. Ji when she launched a new lab a couple of years later. But the new lab was in California and, well, the emissions standards.

As the adaptive optics add-on module project at Howard Hughes was winding down, Chen, by happenstance, met David Boas, director of the Neurophotonics Center at BU, at an NIH BRAIN Initiative Conference. Boas was quick to recruit him to join the then-newly formed Center. Thus began the next stage of his journey. Today he looks back fondly on all of the experiences he has had, and all of the mentors and others who have helped to shape him and his career. “Without them,” he says, “I would probably still be hacking away at my little sensor from my PhD. The jobs I’ve had and the people I’ve met along the way have all really helped to expand my horizons.”

A Better Mousetrap

In addition to training and providing assistance to the many faculty, staff, and students who use the Micro and Nano Imaging Core, Chen is helping to acquire new shared resources for the facility. This past fall, he and other faculty received a “shared instrumentation grant” from the NIH to purchase a



Chen’s career path and robust skillset have been shaped by his many, varied interests and experiences. Just one example: His passion for street car racing, which he cultivated in the hidden back streets and industrial stretches of New Jersey, indirectly led him to his current position at BU.

Photo by Anderson Chen.

new laser scanning confocal microscope. The microscope will replace the one the faculty have been using for the past 12 years, which has now reached the end of its service lifetime, while adding several features previously unavailable to them. The new instrument will be much faster, capturing images at a video frame rate of almost 30 frames per second on average, as opposed to acquiring an image every two seconds or so with the current instrument. In addition, upgraded detectors in the microscope will also offer improved sensitivity.

That’s not all. Chen’s vision of the facility is to bring cutting-edge equipment and training to the BU campus by partnering with industry leaders. In cooperation with the purchasing department at BU, he negotiated with Olympus, a leading microscope company, to acquire two additional microscopes to complement the new confocal

microscope. One of these is a slide scanner that can autonomously and effortlessly image one hundred image histology slides. Previously, this work was generally left to students, who would manually perform the grueling task—which could take many hours to complete and was prone to human errors.

The combination of these new instruments will allow a number of BU faculty to continue their NIH-funded research programs that depend on confocal imaging-based experiments while opening the door to any number of new studies. All of which is good news to Chen, who always appreciates the opportunity to interact with people and learn more about their work. “What makes me especially happy is when I’m able to help them achieve and visualize and finish their projects. The fact that I had a hand in it really speaks to me.”

From Cupcakes to Custom-made Microscopes: The many talents of Kivılcım Kılıç



Team work (and fun) at Boston University. From left to right: John 'Jack' Giblin, Kivılcım Kılıç, Shen Ning, Nathan Perkins, Evren Erdener, Xiaojun Cheng, Sreekanth Kura, Chang Liu, Jiarui Yang

In just over a year, Kivılcım Kılıç has established herself as an indispensable member of the Neurophotonics Center at Boston University: filling such essential roles as providing expertise in both physiology and neuroscience, interfacing with establishing connections between other investigators throughout the Center and the university, and frequently pampering researchers with her homemade desserts. As if that weren't enough, her passion for art in science has led to her creating medical illustrations to be

published in prestigious academic journals. But how did she come to possess such wildly diverse talents, and how is she able to wield them so effectively? We checked in with Kılıç to learn more about her background and her integral role in the Center.

Developing a research mindset

Kılıç's background is anything but ordinary. One of the first-ever joint MD-PhD candidates in Turkey, she began her medical training at 18

and her PhD at 19. She graduated from Hacettepe University Medical School five years later and immediately began her two years of obligatory medical service, overseeing an emergency department in Merzifon, Turkey. This proved a dramatic change from her student days, when she and others in her cohort were "constantly watched over by people who are more experienced in the area." Now only 24 years old, she found herself making life-and-death decisions almost hourly while supervising everyone in the emer-

gency room – even though all of the other staff members had at least 10 years more on-the-job experience than she did.

At first, she says, she often had doubts about herself and her work. With the experience and support of the other staff, though, her doubts slowly disappeared. The sense of comradery among the staff – the ‘we’re all in the same boat together’ ethos – imparted lessons she carries with her to this day. “This was the moment that I started appreciating being a part of the team,” she says, “training others, learning from others and leaning on others regardless of age, seniority or degree.”

After her service ended, Kılıç returned to Hacettepe University Neurological Sciences and Psychiatry Institute to continue her doctoral training. Over the next several years she devoted herself to training in the neurobiological sciences but also to developing a mindset that would lead her to a

successful career in research. “It is not the techniques you have already learned how to use,” she says, “but the willingness to try something different on a daily basis. It is sometimes being stubborn and arguing your point of view when you have enough data to prove it, and other times accepting that you simply do not know the answer to the question. Most people see a PhD as a way to get respect or a better salary, but I was always opposed to this point of view. It is about being open-minded and accepting change as it comes. It is more of a lifestyle than a bunch of nonsensical letters following your name.”

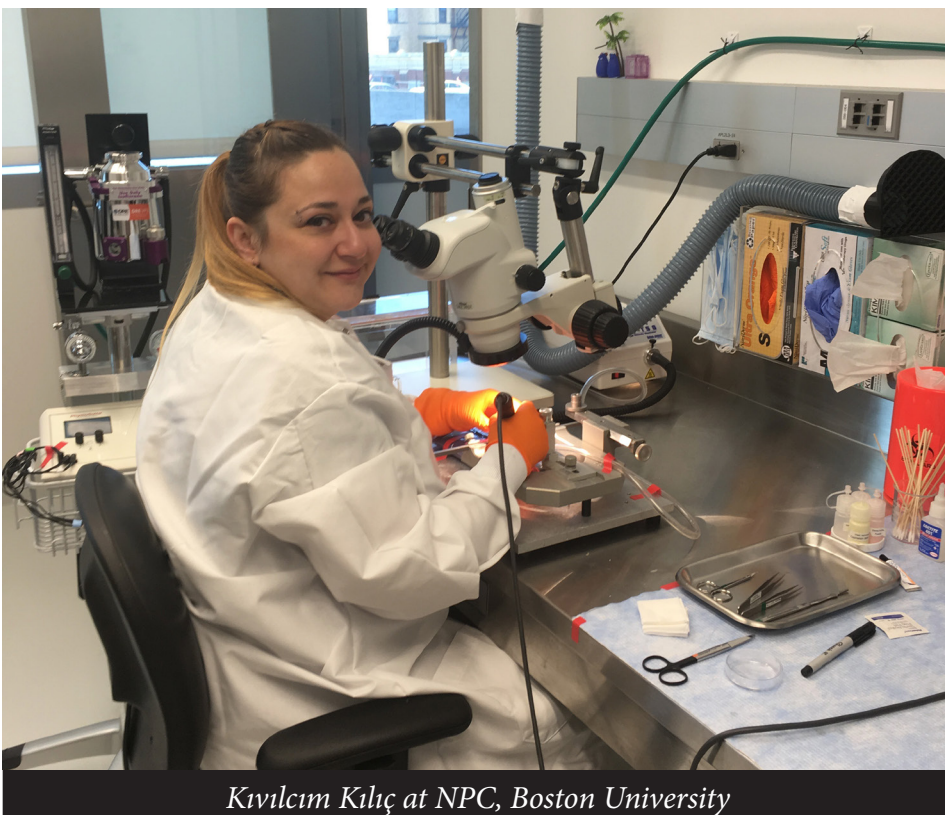
Her choices paid off. As she was wrapping up her doctoral training, she was awarded a postdoctoral fellowship from the International Headache Society to work at the University of California, San Diego, working with Anna Devor, principal investigator of the Neurovascular Imaging Lab there.

With Devor, whom Kılıç describes as “an excellent mentor as well as a brilliant scientist,” she focused mainly on dissecting the neural circuitry to see the specific effects of neurons on neurovascular coupling. She also spent time developing various awake imaging techniques in chronic settings (> 6 months) in mouse models. It was through the latter project that she met and started collaborating with David Boas, director of the Neurophotonics Center at BU. As her postdoctoral fellowship was winding down, Boas offered her a job.

The “principal emotional stabilizer” and baker of the Center

Kılıç’s position in the BU Neurophotonics Center encompasses two important roles. As a research scientist working in the Biomedical Engineering Department, she engages in a range of studies aiming to develop novel or improved imaging tools especially for neuroscience and apply these tools to better understand physiological or pathologic processes. She also serves as the manager for the Center. In this position, she interacts with faculty, students and staff about a range of internal projects and collaborations, teaching, writing grants and other, related work.

Currently, she is working on or collaborating with more than 30 projects. These run the gamut from developing novel imaging technologies – including custom-made two- and three-photon microscopes and a variety of wearable microscopes – to applying techniques such as optical coherence tomography and laser speckle contrast imaging to better understand the brain mechanisms of stroke, traumatic brain injury



Kivilcim Kılıç at NPC, Boston University



Some of Kivilcim Kılıç's sweet creations

and chronic traumatic encephalopathy, among other diseases.

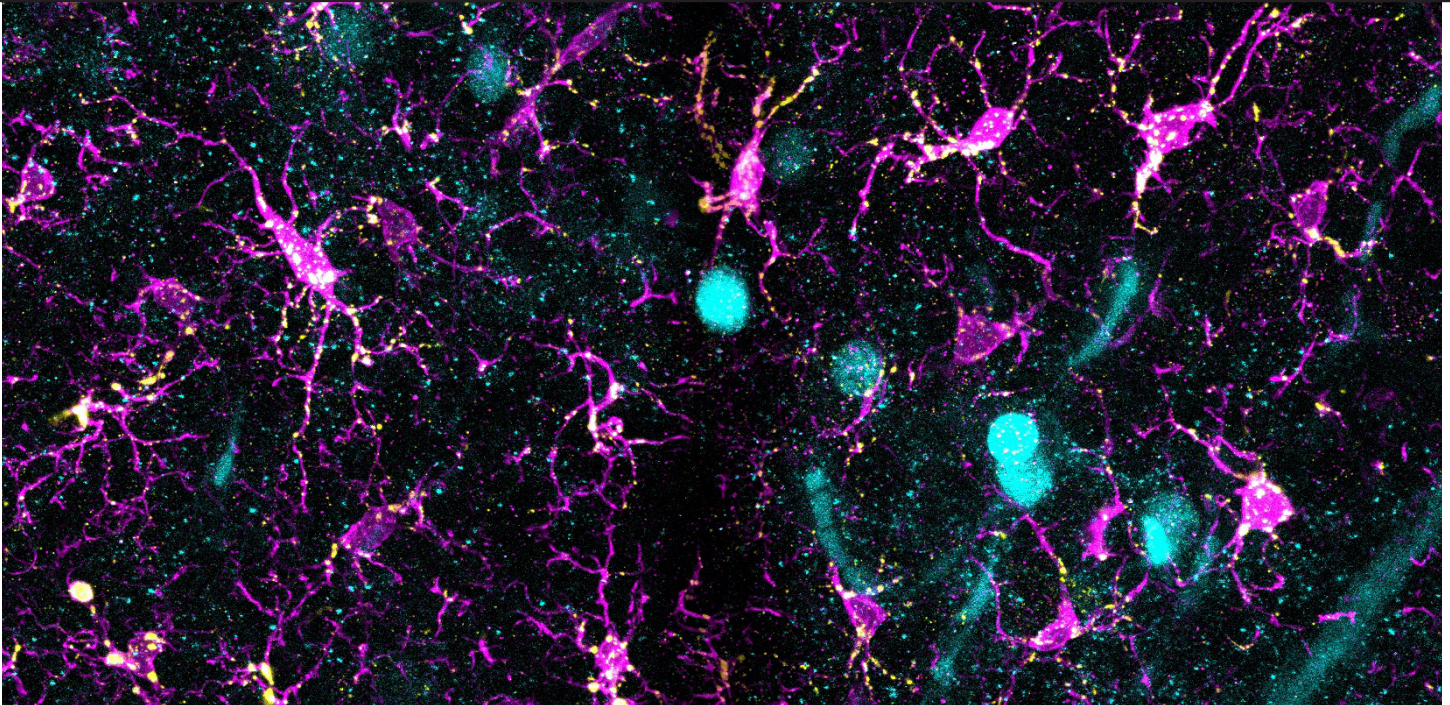
Kılıç takes a moment to walk us through a couple of the projects currently under way. One is a collaboration with graduate student Smrithi Sunil and research technician Blaire Lee in which they are seeking to understand the vascular and cellular changes in the acute and chronic phases following a stroke in a mouse model and to find new treatments that might ultimately yield better outcomes. She adds: "Another project that I am really excited about – with another of our graduate students, John 'Jack' Giblin – is measuring the oxygen pressure in vasculature using phosphorescent two-photon imaging. This tool will allow us to track the changes in the levels of oxygen pressure due to vascular pathologies and provide a mechanism for cellular death or compromise."

Giblin notes just how integral Kılıç has been, both to this project and to the Center generally. With respect to the latter, he describes her as a "major resource" in the Center for both physiology and neuroscience – in no small part because of her formal training as both an MD and a neuroscience PhD – and as an important conduit connecting people and ideas across labs. "There are very few people in the community she does not seem to know or have at least interacted with or advised," he says.

Indeed, Kılıç's contributions to the Center extend well beyond her responsibilities as a research scientist and NPC manager. Postdoctoral fellow Evren Erdener describes her as both "the principal emotional stabilizer and the baker of the team," adding that the two roles are surely related.

And then, of course, there are her contributions as a teacher and mentor, laced with the lessons about community and helping one another she learned during her medical service in Turkey. Shen Ning, a GPN MD-PhD candidate and professional development co-chair of New England Graduate Women in Science and Engineering (NEGWISE), of which Kılıç is a member, sums up these contributions nicely: "Kivilcim is a dedicated mentor for students and postdocs in the lab," Shen says. "She sets an example for what research ought to be: fun but rigorous. As a teacher, her patience and attention to detail is unparalleled. As a mentor, she brings out the passion and excellence that make the Neurophotonics Center so productive. She inspires her students to not only contribute to science, but to change science to be a more inclusive and invigorating environment."

Alberto Cruz-Martín Lab: Fostering the Future of Neuroscience



Led by Alberto Cruz-Martín, Assistant Professor of Biology, the Cruz-Martin Lab is seeking a better understanding of the cellular and molecular mechanisms that guide the development of synaptic connections in the neocortex. The lab launched in 2015, when Alberto joined the faculty at BU.

Alberto traces his interest in neuroscience to his early college days in Puerto Rico. Born in San Juan, Puerto Rico, where he also enjoyed surfing, discovering hidden beaches and watching old movies, he was fortunate to have had an inspiring and influential science mentor. Guided by his mentor, he was able to do undergraduate research in one of a handful of laboratories at the University of Puerto Rico, Río

Piedras. His earliest training using electrophysiological recordings aimed at understanding how excitation and inhibition are arranged within small local microcircuits and how the specific molecular makeup of a synapse determines its synaptic release properties. He also investigated a calcium-binding protein, Neuronal Calcium Sensor-1 (NCS-1), whose role in the mammalian nervous system was unknown at that time.

Eager to expand his expertise in imaging techniques and to incorporate the study of brain disorders in mouse models, he joined Dr. Carlos Portera-Cailliau's laboratory while working on his PhD. at UCLA. There, he used in vivo two-photon microscopy imaging through cra-

nial windows in neonates to show that in the mouse model of fragile X syndromes (FXS) spines are abnormally unstable and there is an overabundance of immature protrusions. Alberto recounts, "I still remember seeing my first filopodia moving in vivo in the intact brain, it blew my mind that synapses in the brain were dynamic and I wanted to understand how the motility of these structures regulated synaptic formation."

After receiving his Ph.D., he joined Dr. Anirvan Ghosh's laboratory in September 2010 to further pursue his interests in linking the function of specific circuits to sensory processing and perception. Through his research experiences, he says, "with the right tools in my hand I

proceeded to do a very challenging experiment that was needed to finish my story, to record thalamocortical axons in vivo in the visual cortex.

I have failed miserably many times in my life but this time I got lucky and my experiments worked. I showed for the first time pretty axonal boutons activating in response to a visual stimulus! Not thinking too much about what I had accomplished at the time, it later sank in my mind that I also made an important contribution in our understanding of sensory perception.”

In launching his lab at BU, he is expanding upon his early successes. His start at BU coincided with the submission of the NSF Research Training (NRT) grant “Understanding the Brain: Neurophotonic,” which BU was fortunate to obtain in 2016. The grant has been instrumental in helping to establish the foundation of his lab at BU. As he says, “the NSF grant has really made a difference in creating a more connected community between all the systems neuroscience labs at BU.” It has also created a collegiate environment where postdocs and graduate students can talk about their science. Lab members Lisa Kretsge and Ashley Comer are NRT Trainees and have already presented their work at various science meetings including The Cold Spring Harbor Meeting: Molecular mechanisms of Neuronal Connectivity.

Lisa Kretsge is a third year Ph.D. student in the Graduate Program for Neuroscience. She earned her B.A. in Neuroscience and Behavior from Vassar College in 2014. In the

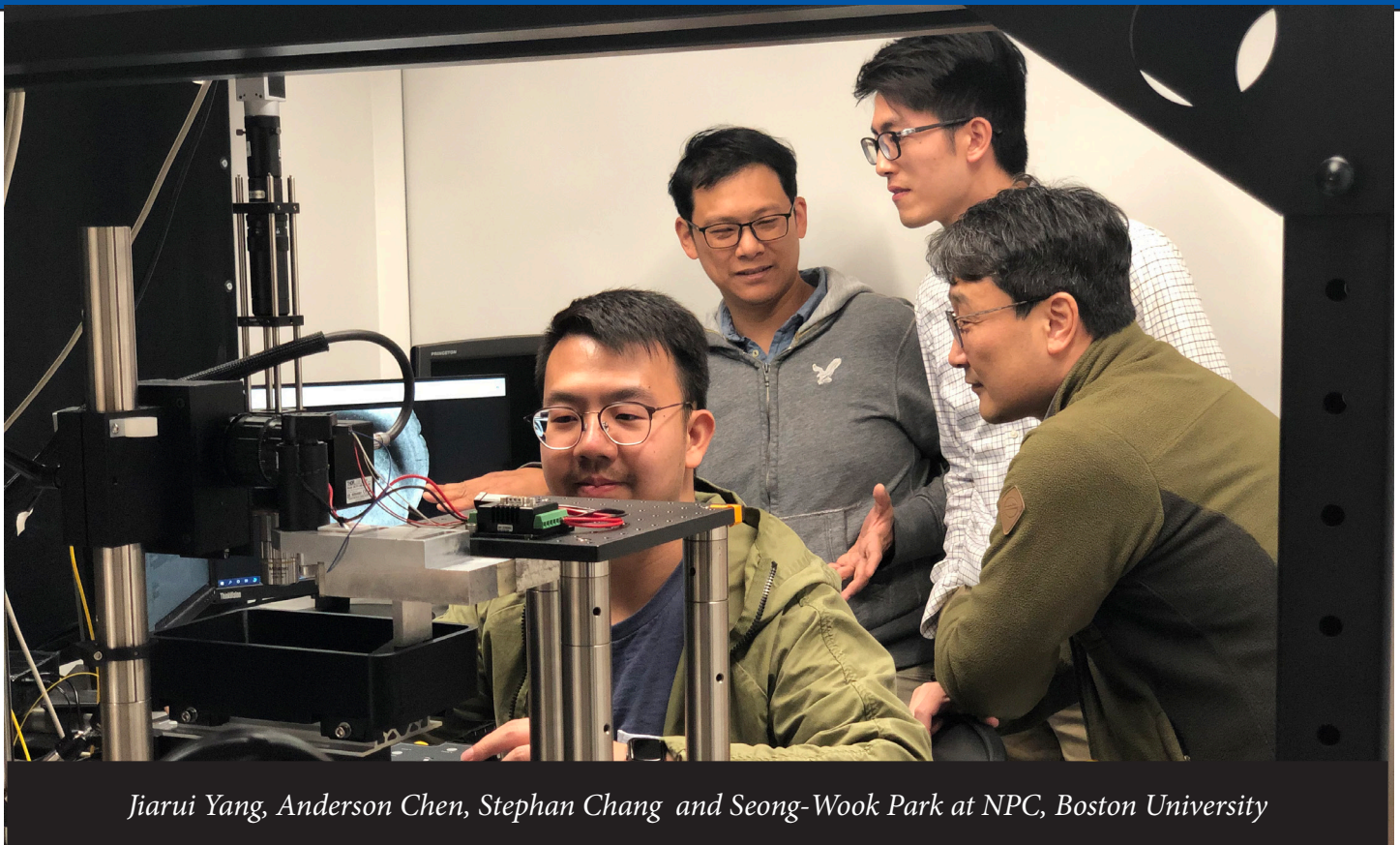
Cruz-Martín lab, she uses in vivo 2-photon calcium imaging to better understand whether exposure to early life stress alters network-level activity in the developing prefrontal cortex. The NSF NRT Neurophotonic program has fostered a collaborative research community, which facilitated her work on a project with a photonics-focused laboratory. Additionally, the traineeship includes coursework and Boot Camp, which have provided Lisa with more practice explaining her work to scientifically diverse audiences and have made her more comfortable discussing the optics involved in her research.

Ashley Comer, also a NRT Trainee and member of Alberto’s lab, is a fourth-year graduate student in the Graduate Program for Neuroscience. She graduated from the University of Houston in 2015 with a B.S. in Psychology and a minor in Biology. She was first introduced to neurophotonic as a first-year graduate student in Alberto Cruz-Martín’s lab where she learned how to use two-photon microscopy to image pyramidal neurons in vivo during development in mice. For her dissertation, she is studying the development and plasticity of neural circuits and how these processes are perturbed in diseased states. The NSF NRT Neurophotonic community has challenged Ashley to perceive her project from multiple perspectives, ultimately strengthening the project through an interdisciplinary approach to the research. Additionally, the NSF NRT has fostered a larger community of people which has allowed Ashley to communicate her research with those she would not have encountered otherwise.

Tushare Jinadasa, a postdoctoral research fellow in Alberto’s laboratory, received his PhD in Physiology from McGill University studying neuron firing in relation to pH regulation during metabolic shifts associated with ischemia. In the Cruz-Martín laboratory, he is taking a vertical approach to studying the physiology and pathophysiology of cortex. A portion of his research uses optical tools to elucidate social and cognitive circuitry of the cingulate cortex. The remainder of his work focuses on the connectivity, behavioral and functional changes of the prefrontal cortex in association with schizophrenia.

These projects use advanced in vivo imaging techniques but also leverage biochemical techniques to examine cellular and molecular mechanism. As a member of Boston University Neurophotonic Center, Tushare is involved with training and teaching; lecturing on the topic of fluorescent indicators as tools in neuroscience. The Cruz-Martín lab is highly engaged with the Neurophotonic Community. In addition to the support and professional development that he, his students, and postdoctoral researchers have received through the Biology Department and Graduate Program for Neuroscience, his lab has the support of many groups at Boston University. Alberto and all of his researchers are highly engaged in programs associated with all of these groups and are excited to highlight the lab and some of the members who are connected closely with the NRT program and the Neurophotonic Center.

Mesoscale OCT Could Provide Cellular-resolution Images of the Whole Human Brain



Jiarui Yang, Anderson Chen, Stephan Chang and Seong-Wook Park at NPC, Boston University

Despite significant advances in imaging that have allowed researchers to peer ever deeper into the body, fundamental questions remain about human brain anatomy. Not least: How many cytoarchitectural areas are there (that is, parcellations of the brain based on the properties of individual cells)? What cell types can be found in these areas? To what extent do the areas vary between regions of the brain or across subjects? Answering these questions requires an imaging technology that can visualize the morphological and molecular properties of

individual cells directly and without significant distortion. To date, though, no such technology exists

This could soon change. In a recently funded grant, a team of researchers in the BU Neurophotonics Center described a means to bridge microscopic volumetric histological imaging, which offer cellular resolution but with inherent distortion due to sectioning of the tissue prior to imaging, and macroscopic MRI. The new tool will enable registration of the histological cell typing to an MRI-based

atlas coordinate system – allowing reconstruction of undistorted 3D images of the human brain with high enough sensitivity and resolution to directly measure cells and their molecular properties in the brain.

“We’re thrilled to begin work on this grant,” says project lead David Boas. “The funding will enable development of cell type atlases of the human brain, showing expected variability across populations as well as changes induced by disease, all of which can be subsequently



used to improve interpretation of anatomical MRIs of the living human brain.”

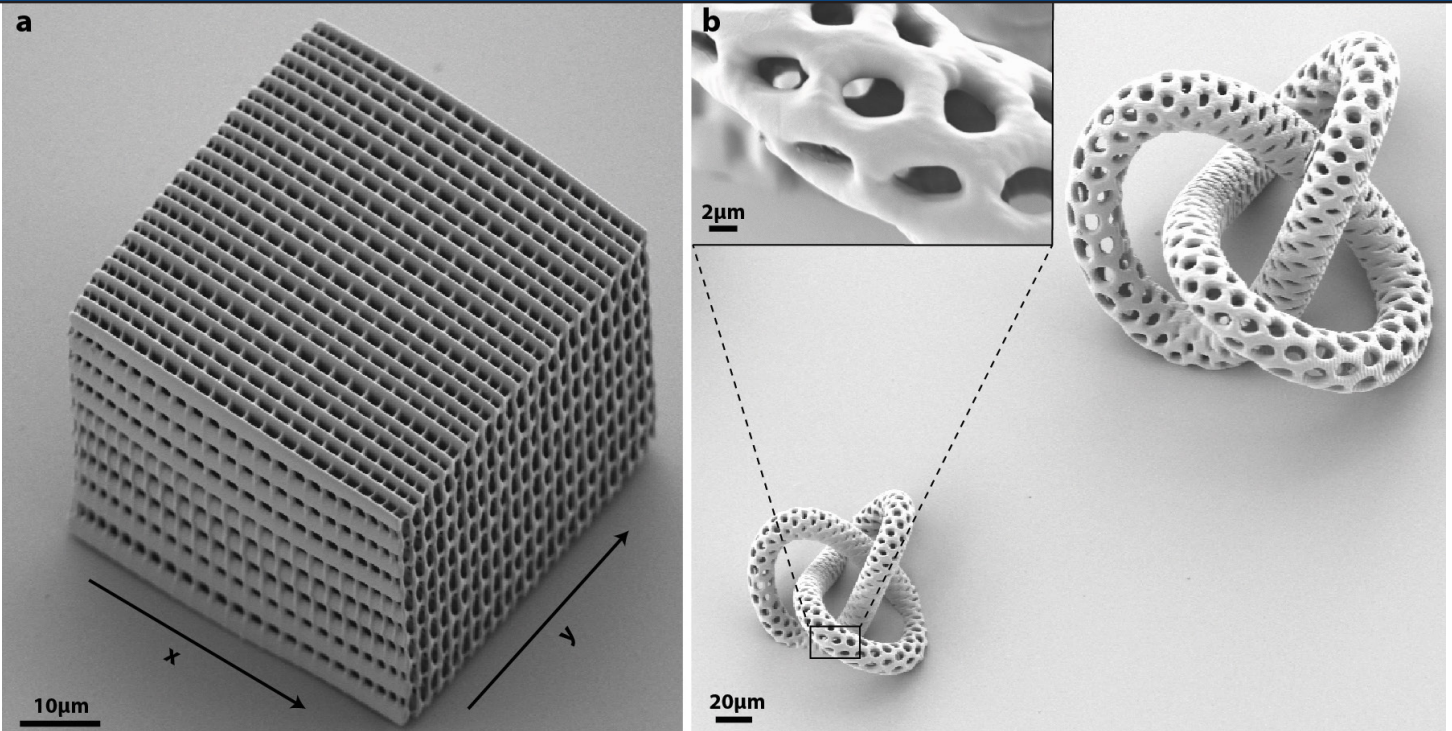
The key to the new approach is the use of mesoscopic optical coherence tomography (OCT). OCT is an optical technology enabling high-resolution cross-sectional imaging and 3D reconstruction in biological tissue up to several hundred micrometers in depth, non-invasively and contact-free. Integrated with a vibratome, an instrument used for sectioning slices of biological samples, OCT can be used to image the block-face prior to sectioning – thus preserving the spatial information across slices and removing the distortions and tears that are inevitable in histology sections of tissue. Histology is easily performed on the sectioned slices. At the same time, because OCT was performed prior to any cutting, avoiding introduction of deformations, OCT is easily registered to the MRI of the brain. Thus, the technology facilitates registration of subsequent histological cell typing to an MRI-based atlas coordinate system.

The proposed technology is complex and, not surprisingly, involves a number of challenges for the developers. One of the most significant of these is making the OCT data acquisition pipeline both robust and sustainable. This will be especially tricky because, in order to reduce the total acquisition time, the researchers are pushing the limits of acquisition speed with the OCT system they are using – namely, by using a larger field of view and less overlap between tiles. At the same time, they are pushing the sustainability of the acquisition pipeline – that is, the data management. Raw data from one human brain block measuring 4 cubic centimeters acquired with the technology could be more than 10 TB. Here, in order to reduce the data flow and disk load, they have implemented real-time pre-processing steps on the acquisition computer and then pushing the data to the Massachusetts Green High Performance Computing Center for further volumetric registration and reconstruction of the thousands of acquired image tiles.

The Neurophotonics Center at BU is well-suited to address these challenges: not least, says Jiarui Yang, a PhD student at the university who will be working with the technology, because it is an interdisciplinary center, well-suited to the multi-disciplinary scope of the project. “For example, the NPC has optical engineering specialists that can build an OCT system that meets our needs, wet lab technicians that can help with sample preparation, and engineering students and faculty who can work on experimental design and image processing.” And in cases where particular resources aren’t available within the Center, the developers can turn to the myriad other facilities within the University. As just one example, the IT department at BU plays an essential role in setting up the data management solution while the researchers are using the campus shared computing cloud (SCC) service, which provides data storage space and computation power.

When completed, the proposed technology could help advance a broad range of questions pertaining to cell types and cytoarchitectural areas. “A complete human brain cell census could not only provide detailed brain anatomy information for ex vivo studies but also possible neuropathology insights for in vivo studies,” says Yang. “For example, our solution will lay the groundwork for automated whole-brain laminar modeling and architectonic segmentation, the investigation of structure-function relationships, and the assessment of histologic variability across healthy and disease populations, as well as conditions such as development and aging.”

3D Printing: The Unsung Hero of Neuroimaging Studies



Complex geometric objects printed in acrylic with a two-photon resonant-scanning direct laser writing (rDLW) system developed by BU researchers. (a) Woodpile structure with dimensions $60 \times 60 \times 60 \mu\text{m}$. (b) A torus knot design printed at $100 \times 100 \times 150 \mu\text{m}$ (top right) and $50 \times 50 \times 75 \mu\text{m}$ (bottom left). The inset shows details within the circumscribed region of the bottom left structure. (Images from: Pearre, B.W., Michas, C., Tsang, J.-M., Gardner, T.J., and Otchy, T.M. (2018). Fast Micron-Scale 3D Printing with a Resonant-Scanning Two-Photon Microscope. *ArXiv:1803.07135*.)

3D printing has advanced rapidly in recent years. Whereas once the technology was suitable mostly only for prototyping and other applications that did not require high precision or repeatability, today it is widely used for the production of parts across a number of sectors. Not least of these is the biomedical imaging arena, where 3D printing is enabling quick, affordable fabrication of components for an array of imaging devices. In the following pages, researchers in the

Neurophotonics Center at Boston University describe several projects that are benefitting from use of the technology, progressing in ways that otherwise might not have been possible.

Studying birdsong with 3D-printed miniature microscopes

Neuroscientists are increasingly interested in imaging animals' brains as they go about their daily business— playing, exploring their

environment, interacting with other animals. Studying these types of behaviors can reveal information about the brain that likely would not be accessible if the animals were constrained for use with conventional microscopes. To this end, companies have introduced miniature microscopes that, because of their size and mobility, enable monitoring of freely behaving animals in a range of different contexts.

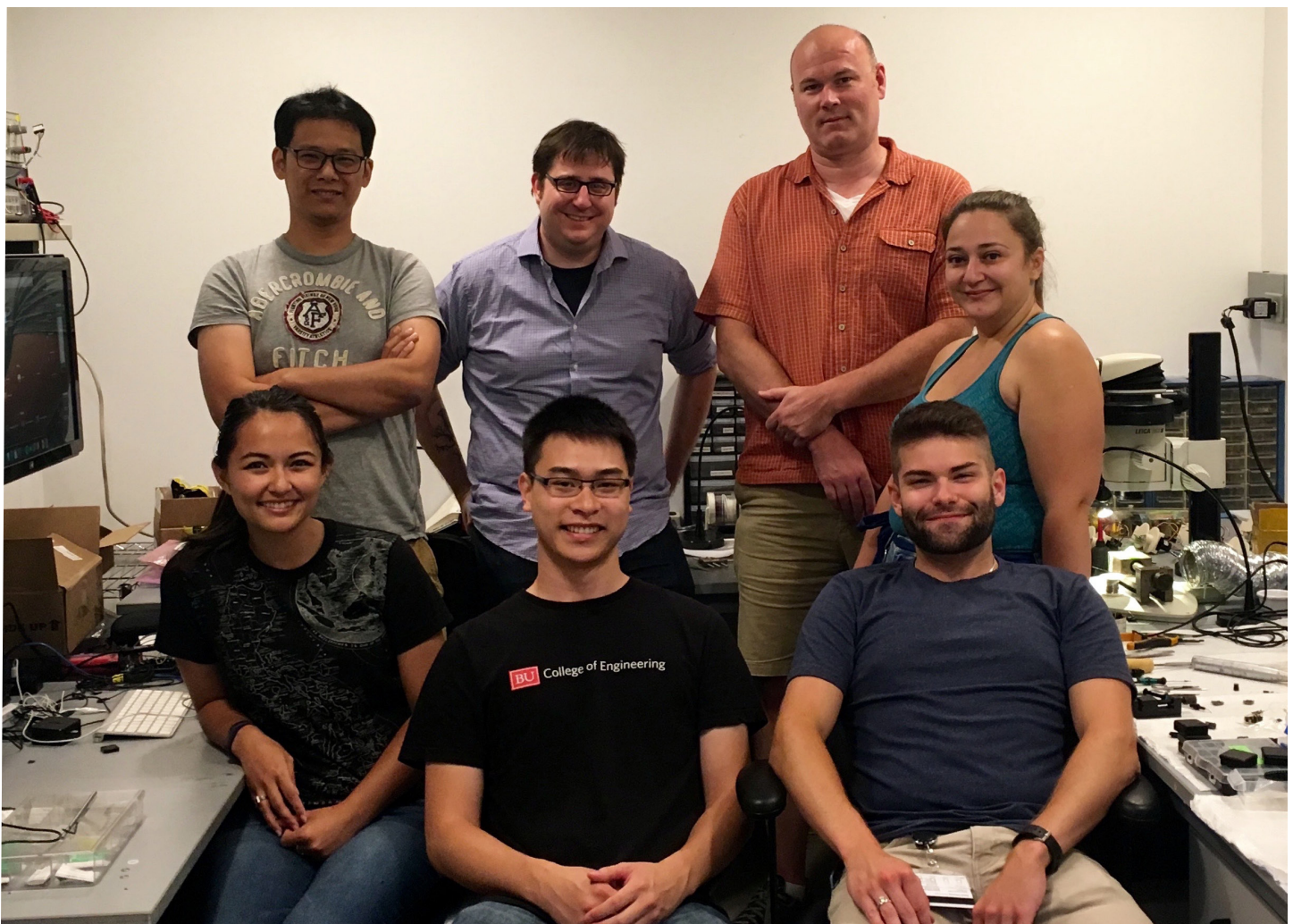
At BU, researcher Ian Davison and colleagues wanted to study bird-song in the small, boisterous zebra finch, so they could better understand learning-related changes in the relevant region of the brain. Because most zebra finches will not sing if they are constrained, the researchers turned to miniature microscopes, borrowing a couple of commercially available instruments from other groups in the Department of Biomedical Engineering. They found, though, that these did not offer all of the features they were looking for, and the instruments' closed designs meant they couldn't tweak them as needed.

Enter 3D printing

The investigators decided they could fabricate a microscope with the specs they wanted using a commercially available, consumer-grade desktop 3D printer. After surveying a number of instruments they selected the Formlabs Form 2 stereolithography 3D printer. With the Form 2, they would be able to print components with exceptionally small feature size (25 μm layers, 140 μm laser spot size), thus enabling printing of the high-resolution threads they would need to adjust the focal length of the microscope. For the 3D printing resins,

they tested a number of resin types for light-blocking capacity, minimum print resolution, and auto-fluorescence in response to imaging wavelengths and chose FGPBLK01 and FGPBLK02. The final design incorporated the 3D-printed components and inexpensive off-the-shelf electronics.

Other options were available for the fabrication of the miniature microscope—including milling and other, related processes—but 3D printing offered significant time and cost savings over these. Using the technology, “an optical engineer can design an optical pathway



The team advancing the miniature microscope includes: (top, L-R) Anderson Chen, Tim Otchy, Ian Davison, Kivilcim Kılıç; (bottom, L-R) Jasmine Clevenger, Will Yen, Dan Leman.

and have a finished product in a couple of days,” says Daniel Leman, a researcher in Davison’s group at BU and one of the developers of the miniature microscope designed for the birdsong study. “To be able to do all of that inhouse is really incredible.”

Ultimately, the advantages of 3D printing for the fabrication of the miniature microscope extend well beyond the study of birdsong. It could enable advances in wearable technologies, generally, advances that could open up a range of new applications—including, says Davison, the study of neurochemical processes underlying various social behaviors in rodents.

Advances could enable 3D printing on smaller scales and with complex materials

3D printing can be considerably less expensive and less time-consuming than other available processes. Just as importantly, though,

it can enable fabrication of shapes that in many cases would be impossible to produce otherwise. “You can mold shapes and crevices and tolerances in 3D that you could never actually make with milling,” says Leman. “Many of the designs we have produced for the microscope thus far would be frankly unfeasible using traditional techniques.”

And they have done so using commercial, off-the-shelf 3D printers. Timothy Otchy, a researcher in the Biology Department at BU, is developing an approach that will facilitate 3D printing on much smaller scales. The approach takes advantage of a technique called two-photon polymerization, where a pulsed laser is focused down to a tiny spot and swept through a liquid polymer in three dimensions, essentially writing the geometry of the desired structure in the polymer.

The approach could open the door to a number of new applications

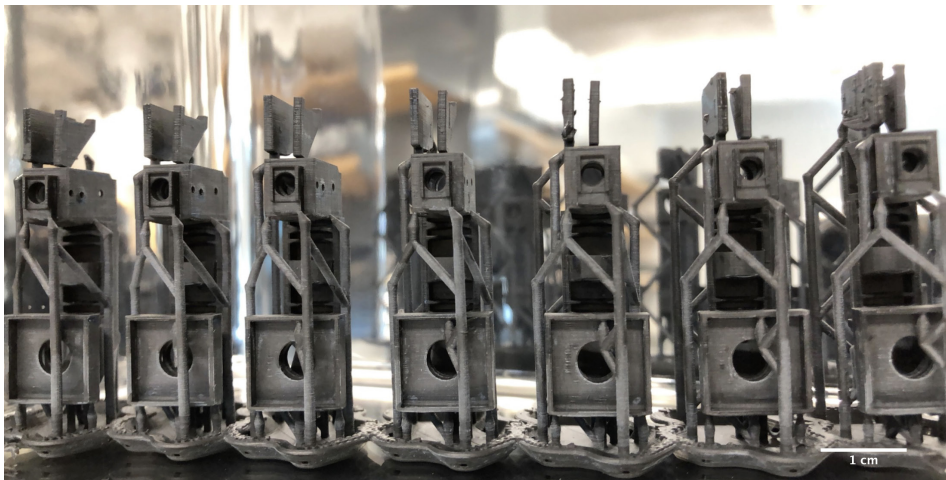
for 3D printing, Otchy says. “The off-the-shelf system we use to print the miniscopes is wonderful, and relatively inexpensive to get up and running, but the tolerances it can hold are probably, at best, submillimeter—maybe 250 microns, depending on the post-processing steps. A lot of the things we’re interested in printing—implantation devices, microlenses, other optical components requiring highly smooth surfaces—call for tolerances on the submicron scale.” Right now, he and colleagues are mostly using the new approach to print mechanical components like those found in the miniscope, but they plan to start exploring the other possibilities this year.

At the same time, they are exploring ways to incorporate complex materials into 3D printing. Currently, printing is only possible with a single material—acrylic, polymer, in some cases even metal. Being able to fabricate multi-material objects in a single step—patterning polymers and metals together, for example—could prove tremendously useful, allowing the researchers to interleave conductive materials into optodes or other optical elements.

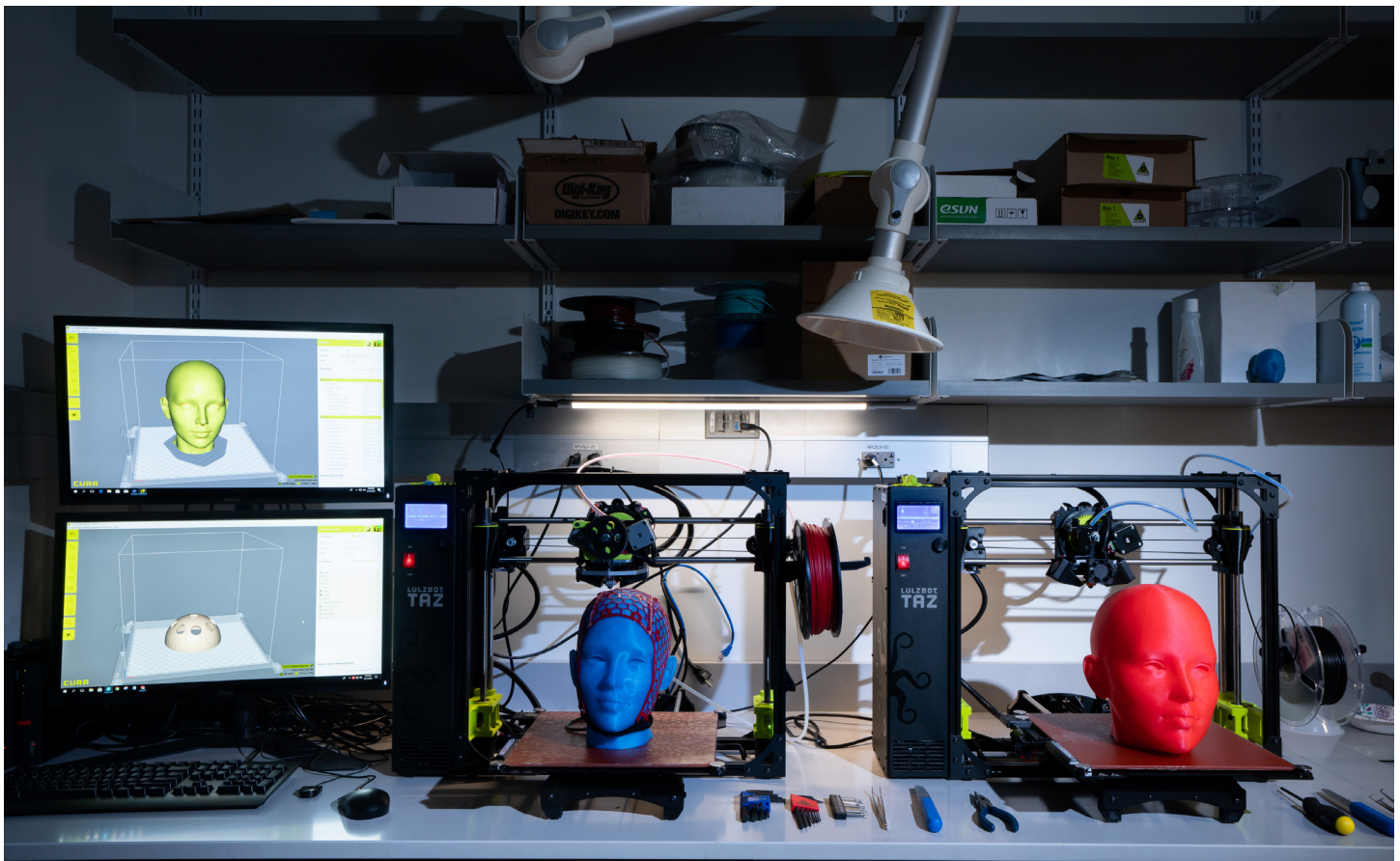
3D printing caps for functional imaging of the human brain

Elsewhere on the BU campus, researchers are working with 3D printing on a very different scale, using it to produce caps to hold imaging probes on the heads of human subjects.

The neuroimaging technique functional near-infrared spectroscopy (fNIRS) helps to shed light on the neural underpinnings



Printed and post-cured chassis for implantable fluorescent microscopes. Once pruned from support structures, fitted with optical components, and instrumented with imaging sensors, these microscopes are capable of making chronic recordings of calcium dynamics in hundreds of individual neurons over a 4-mm-wide field of view. Image courtest of Daniel Leman and Ian Davison



BU researchers are also using 3D printing to fabricate caps used to hold imaging probes on subjects' heads during functional near-infrared spectroscopy (fNIRS) measurements.

of brain function by monitoring cerebral oxygenation—a proxy of neural activity—by transmitting near-infrared light into the head and monitoring it as it emerges. For many years, investigators have relied on plastic caps developed for EEG applications to position the light sources and detectors on subjects' heads, punching new holes in the caps to accommodate the placement. But the most widely used caps—known as Easy Caps—are far from ideal for use with fNIRS.

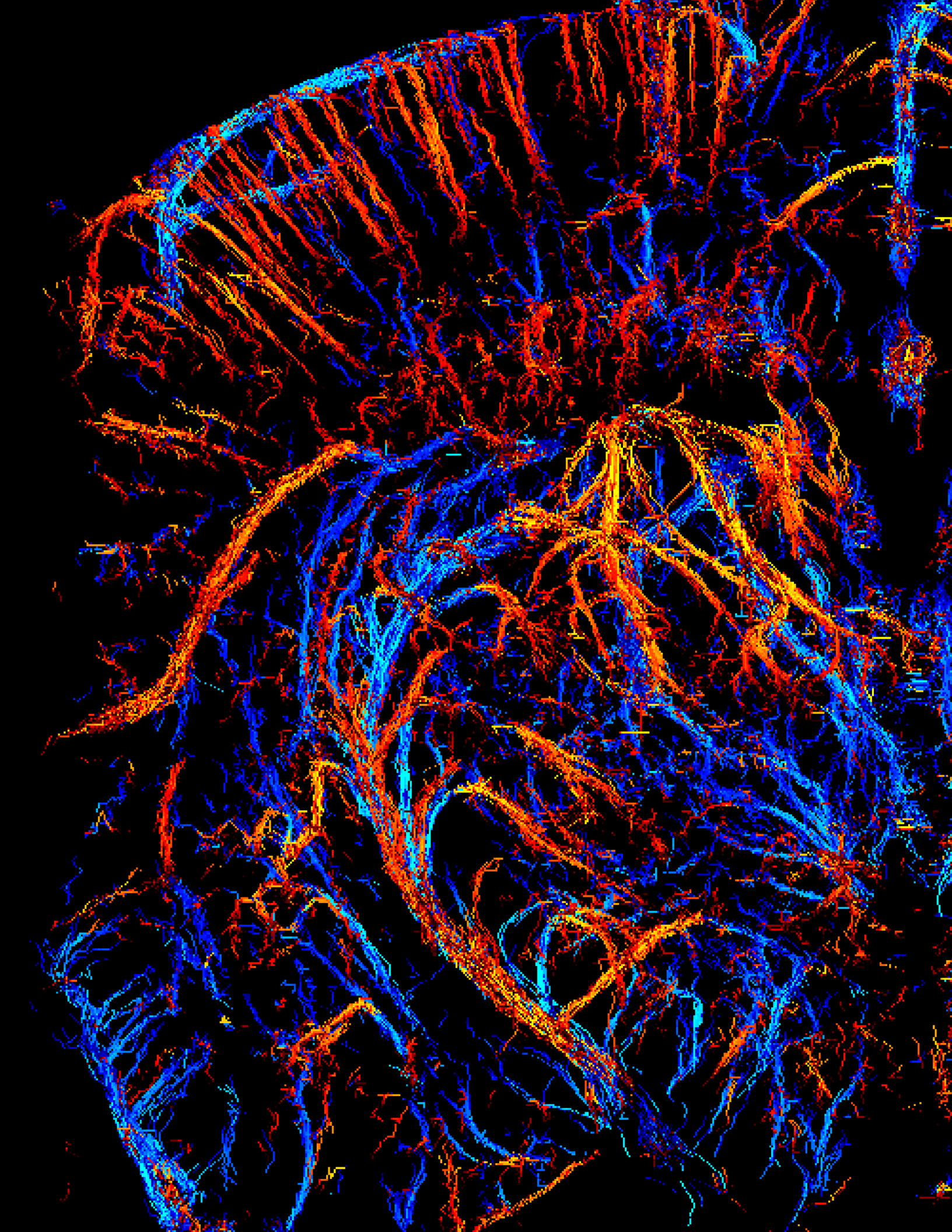
“The thing is,” says Antonio Ortega Martinez, a graduate student in the BU Department of Biomedical Engineering, “the probe location is different depending on the

experiment. It can be cumbersome making holes in caps all the time, especially if you want to have several caps for the same experiment. The holes might be inconsistent across the caps.”

Early last year, as part of a BME Senior Design project, undergraduate students Wesley Rivera, Erin Landry, Jessica Chou, Nikita Bhatia and Dale Tollman produced a design for an fNIRS cap that could be fabricated on demand using 3D printing. Here, the researcher needs only to tell the fNIRS-specific software package what area of the brain he or she wants to measure and the software calculates the position of the probes on the cap and sends the design to the printer. Not only does

this enable customization of the probe's location, it allows researchers to specify the size of the head for which they are making the cap. Being able to tailor caps to fit subjects' heads is especially important because so many fNIRS investigators work with infant populations as well as adults.

The initial design and tests of the fNIRS cap were done by Zack Starkweather at the MGH Martinos Center for Biomedical Imaging, a collaborating Institution with the BU Neurophotonics Center. Other contributors to the project at BU include Cameron Snow, Nathan Perkins and Alexander Lühmann, as well as Antonio Ortega Martinez.





Neurophotonics Center-Supported Research Projects

Supporting new research directions between faculty is an important component of the *Center's mission: to build and support an interdisciplinary community that can develop and broadly deploy impactful photonics technologies in the neurosciences to advance our understanding of how the brain works in health and in disease.* Neuroscientists are dependent on new technologies that enable them to measure previously inaccessible aspects of brain function and structure. Scientists advancing photonics methods are on the lookout for impactful applications to motivate and direct their technological developments. The Center aims to connect the technology developers with the users to accelerate the advancement and early adoption of novel photonic methods. The following pages describe seven such innovative efforts that the Center is supporting.

Image by Jianbo Tang

Summary of Supported Projects

During the last year, the Neuro-photonics Center has supported thirteen projects, which are indicated in Figure 5. We summarize activities for each of these projects here, following the start date order in Figure 5. This is then followed by short descriptions of four of the support projects.

Development of a Wearable Functional Near Infrared Spectroscopy (fNIRS) System for Imaging Brain Activity in Freely Behaving Humans – In parallel with supporting NPC faculty (Tager-Flusberg, Kiran, Cronin-Golomb, Somers, Awad, and Sen) in the adoption of fNIRS for their varied applications, we've be developing the next generations (plural!) of fNIRS systems that are wearable and integrated with EEG and other biosensors to provide a more complete understanding of brain activity in human subjects engaged in everyday world activities. In addition to resulting in the award of an NIH R24 (Boas) and an NIH F31 (Gilmore) grant, this team of faculty submitted on September 3, 2019, a major \$7.0 million NIH Brain Initiative U01 proposal to revolutionize Neuroscience and Neuroimaging in the Everyday World.

Development of a Multi-ROI Two Photon Microscopy for Imaging Functionally Connected Brain Regions – Jerry Chen received an NSF NeuroNex award in 2017 to develop this microscope. The NPC is providing technical expertise from NPC staff Anderson Chen and

Start	Faculty	Title	Funding Status
6/7/17	Boas / fNIRS Users (Awad etc)	Development of a Wearable fNIRS System for Imaging Brain Activity in Freely Behaving Humans	R24 (Boas). Submitted U01 (Boas / Kiran / Cronin-Golomb / Somers)
9/20/17	Chen	Development of a Multi-ROI Two Photon Microscopy for Imaging Functionally Connected Brain Regions	NSF Neuronex (Chen)
9/20/17	Boas / Mertz / Wong*	Developing Functional Ultrasound Imaging of the Brain and Other Organs	R01 (Wong), R21 (Mertz). Submitted P41 (Boas / Devor / Han / Mertz)
10/3/17	Scott / Ramachandran	Three Photon Microscopy for Deep Brain Imaging	Evolving to next generation. NSF MRI not awarded.
1/1/18	Gardner / Howe / Boas	Development of Splaying Fibers for Sensing and Modulating Neural Activity in Deep Brain Regions	Resubmission of R01 planned (Boas / Howe / Otchy)
5/20/18	Mertz / ERC	Increasing the Volumetric Imaging Rate of Confocal Microscopy to Image Voltage Indicators	NSF ERC
5/20/18	Tian / Davison	Developing a Computational Miniature Mesoscope for Imaging Neural Activity in Freely Behaving Animals	Dean's Catalyst, R21
8/16/18	Bigio / Rosene* / Boas	Birefringence Microscopy for Quantifying Myelin Content in Brain Slices	
9/1/18	Boas / Cheng	Serial Sectioning Volumetric Histology of Post-Mortem Human Brain	U01 (Boas)
1/1/19	Chen / Sanders	Ultra-Fast Two Photon Microscopy for Imaging of Voltage Sensors	UF1 (Chen / Sander)
1/1/19	Mertz	Reverberation Multi-Photon Microscopy for High Speed Volumetric Imaging	Submitted P41 (Boas / Devor / Han / Mertz)
1/1/19	Dennis	Nanoparticles for Neuroscientific Applications	R01 (Dennis)
2/1/19	Boas / Davison / Goldstein	Widefield Dynamic Laser Speckle Imaging of Traumatic Brain Injury	

* Indicates not an NPC Faculty member

Thirteen projects currently supported by the NPC. Funding from NIH unless otherwise stated.

Seong-Wook Park to assist in the development of this microscope. This resource will be made available to interested BU faculty.

Developing Functional Ultrasound for Imaging the Brain and Other Organs – Identifying the broad community interest in and the potential to further advance, the Center invested in functional Ultrasound technology early in 2018. Professors Joyce Wong and David Boas have been actively using and developing the technology. Boas leveraged the system to obtain an ~\$160,000 NIH R01 supplement to further develop the technology for imaging neurodegenerative diseases. Professors Roblyer and Porter are beginning to use the systems this year. And it formed a core part

of the NIH P41 proposal that was just submitted by the NPC.

Investigation of a Novel Laser for 3-Photon Microscopy For Deep Brain Imaging – This pilot project was started with Professor Siddharth Ramachandran and has now graduated to building a full-blown 3-photon microscope that is being driven by new junior faculty member Ben Scott with Center support. Several other Center faculty are interested in adopting this technology once Ben demonstrates its utility. An important novel opportunity that investing in this infrastructure enabled is that it allowed the launch of a novel project by Professor Mertz to develop 3-Photon Reverberation Microscopy.

Development of Splaying Fibers for Sensing and Modulating Neural Activity in Deep Brain Regions – This was a project started by Tim Gardner with an R21. David Boas took over mentoring the graduate student on the project when Tim Gardner was on leave. Pilot data is being collected with Mark Howe and Tim Otchy. An R01 was submitted and reviewed well. A resubmission is being planned.

Increasing the Volumetric Imaging Rate of Confocal Microscopy to Image Voltage Indicators with Millisecond Temporal Resolution – This project, led by Professor Jerome Mertz, is supported by the ERC and is being developed in the Center to facilitate interaction with neuroscience faculty. Successful progress of this project has led to initiation of a new project – 2-Photon Reverberation Microscopy – that is a core part of a \$6.2 million NIH P41 Biomedical Technology Resource Center that was submitted September 25, 2019.

Development of a Computational Miniature Mesoscope for Imaging Neural Activity in Freely Behaving Animals – Recognizing the need for a wearable mesoscope that could image neural activity over an area up to 1 cm², Professors Lei Tian and Ian Davison initiated a collaboration to produce the solution. Supported by the Center, they received the Dean's Catalyst Award in 2018, followed by an NIH R21 award in 2019, that will then lead to an NIH R01 application in the near future. As an outgrowth of this project, Boas and Tian continue to discuss novel methods to advance neurophotonics and successfully obtained an ~\$100,000 NIH R01

supplement to develop machine learning approaches in 2018.

Birefringence Microscopy for Quantifying Myelin Content in Brain Slices – This is a new project initiated by Irving Bigio. It is important because methods do not exist for quantifying myelin content. Exciting results are being obtained with Doug Rosene at BUMC. There is potential for synergy with funded work for serial sectioning histological imaging of the human brain with David Boas.

Serial Sectioning Volumetric Histology of Post-Mortem Human Brain – This is funded by a major NIH Brain Initiative U01 with the ultimate goal to perform volumetric histological imaging of the entire human brain. This is a phase 2 effort which, if successful, can compete for a very large scale Phase 3 effort. To compete for Phase 3, David Boas is hoping to engage Irving Bigio, Ji-Xin Cheng, and any other faculty who can contribute.

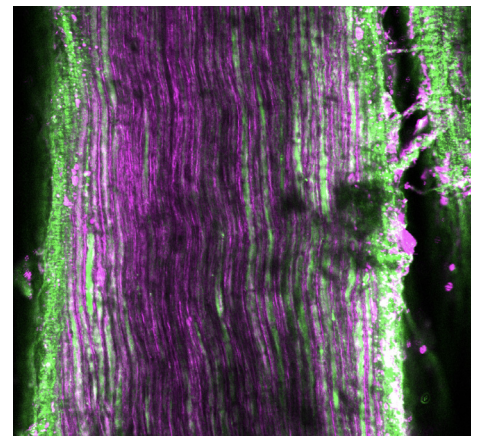
Ultra-Fast Two Photon Microscopy for Imaging of Voltage Sensors – Jerry Chen and Michelle Sanders met through the NPC and competed for and were awarded an NIH BRAIN Initiative UF1 to develop new lasers and microscopy methods for two photon microscopy of voltage sensors in the brain. The NPC is helping them with technical expertise and will help disseminate the developed technology to interested faculty.

Reverberation Multi-Photon Microscopy for High Speed Volumetric Imaging – This is being led by Jerome Mertz and has the potential to have broad impact in the neurosciences generally and for many

BU faculty specifically. It has grown from the confocal microscopy project mentioned above, that was supported by the NPC. This project is a core part of a \$6.2 million NIH P41 Biomedical Technology Resource Center that was submitted September 25, 2019.

Nanoparticles for Neuroscientific Applications – The NPC engaged Allison Dennis early on to help figure out applications for her novel nanoparticles. With NPC support, she was just awarded an NIH R01. The NPC will continue to support her activities and assist in translating her technologies to neuroscience applications.

Widefield Dynamic Laser Speckle and Optical Coherence Tomographic Imaging of Traumatic Brain Injury – This is technology from David Boas' lab that is of great utility for studying traumatic brain injury in rodents. The NPC has been supporting Ian Davison and Lee Goldstein in the utilization of this technology for such studies.



Longitudinal mouse nerve. FOV is 480x480 um, green is 3-photon signal from the YFP-labeled axons and magenta is THG signal from the myelin. Image courtesy of Lars Rishøj and Siddharth Ramachandran.

Birefringence Imaging Microscopy

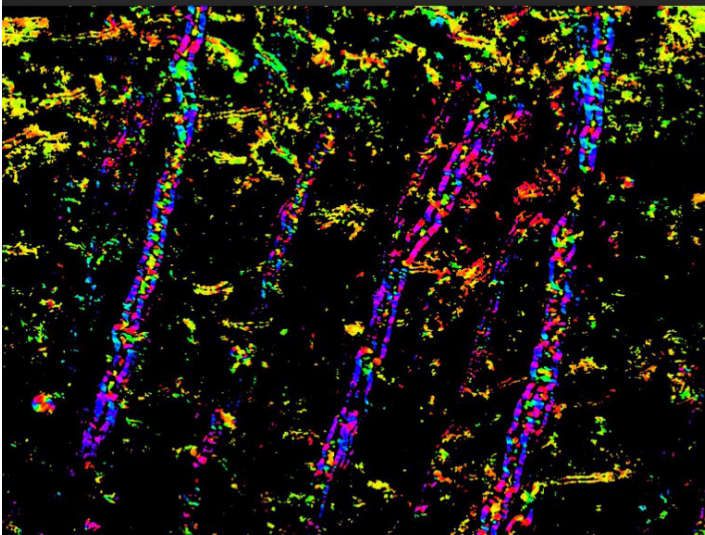
The study of neurodegenerative processes requires careful consideration of a variety of factors, but perhaps one of the most important is the status of myelin. Myelin is abundant in the brains of vertebrates, serving as an electrical insulator along the lengths of axons, thus facilitating efficient propagation of action potentials. As such, myelin is vital for maintaining complex neural function. However, due to its compact, layered structure, studying myelin with conventional molecular labeling techniques proves to be difficult. Fortunately, when using polarized-light techniques to image myelin birefringence, the same properties that hinder molecular labeling can be leveraged to image details of the structural integrity of the myelin sheath.

With support from the Neurophotonics Center, in the form of startup components and project advisory services, the Bigio lab has been developing a birefringence imaging microscope. This method of wide-field microscopy uses polarized light, in a transmission geometry, to obtain high contrast, label-free images of birefringent structures. Birefringence is the property of anisotropic media, by which light experiences different indices of refraction for different orientations of the optical polarization. It is a consequence of the inherent difference in polarizability of anisotropic media along different axes, corresponding to the structure's direction of anisotropy. The birefringence imaging setup that offers the most flexible method of sample vi-

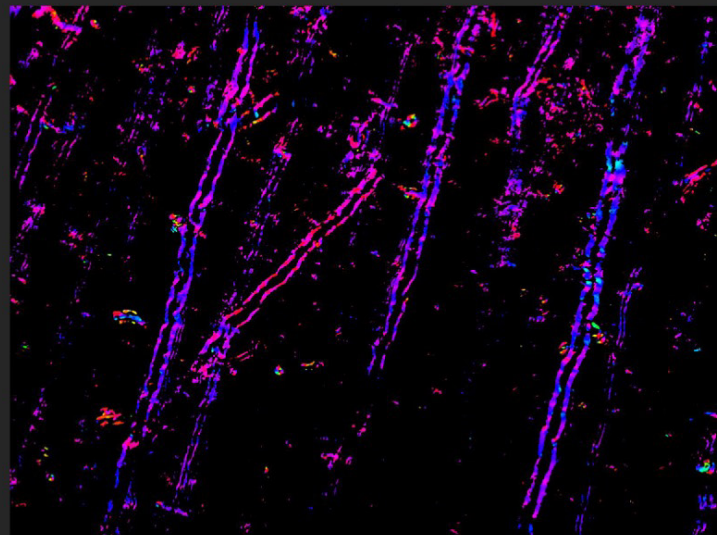
sualization employs crossed circular polarizers. This operates on the same principles as a conventional polarized-light microscope with crossed linear polarizers, but has the advantage of providing orientation-independent images. In short, birefringence microscopy is dark-field imaging, in which the sample is illuminated with polarized light. Birefringent (anisotropic) structures modify the polarization state of the illumination field, whereas non-birefringent (isotropic) media do not modify the polarization state. Thus, with a crossed polarizer in the detection arm, only birefringent structures will appear against the dark background.

During the myelination process in the central nervous system, oligodendrocytes form projections and

Birefringence microscope images of myelin organization in monkey brains: age-related dementia vs young brain



Degenerated (age = 28 years)



Healthy (age = 6 years)

wrap around axons up to 60 times. The tight compaction of concentric layers of lipid membrane render the myelin sheath highly anisotropic, and therefore birefringent. (In peripheral nerves, similar myelin wrapping is effected by Schwann cells.) When imaging with polarized light, all anisotropic structures generate birefringence contrast, except for when the structural optic axis is parallel to the direction of light propagation. Given that the orientation of the optic axis in the myelin sheath is radial (to the axis of the axon), when the length of an axon is in the plane of a sample section, the myelin sheath appears as a pair of parallel lines on either side

of the axon. When the axis of the axon is oriented normal to the sectioning plane, the myelin appears as a circular structure surrounding the axon. However, when the myelin sheath begins to degenerate, as a result of, for example, neuropathies or age-related dementia, it becomes fragmented and the direction of structural anisotropy randomizes.

Expected Outcomes

It is anticipated that birefringence-imaging microscopy will become a valuable tool for tracking and understanding the structural degeneration of myelin in both the central and peripheral nervous sys-

tems. In particular, given the nature of polarized-light interaction with birefringent media, this imaging framework will allow for extraction of quantitative information, which will facilitate structural comparisons between tissue types and states of neuropathy. While our initial focus has been on studying myelin, birefringence imaging microscopy will likely find utility in a wide array of biomedical applications.

Ultrafast Functional Ultrasound

Functional quantitative in vivo imaging of the entire brain with high spatial and temporal resolution remains an open quest in biomedical imaging. Ultrafast ultrasound is a fast developing novel technology promising to fulfill the unmet demands of imaging the cerebral hemodynamics of the entire rodent brain with 10-100 μm resolution, and even holds the promise of measuring neuronal activity directly with the advent of acoustic reporter genes. Since the introduction of Power Doppler-based functional ultrasound imaging (fUS), an increasing number of researchers are exploiting the truly impressive capabilities of fUS for functional brain imaging studies.

A conventional ultrasound image is usually acquired by sequentially scanning a focused beam across

the image plane and a 2D image is typically made of 64-512 such lines. Such serialized architecture limits the frame rate to ~ 60 frames/s. In contrast, ultrafast ultrasound imaging utilizes the parallelization paradigm that emits plane waves at different angles and a resolution enhanced 2D image is obtained with coherent plane wave compounding. The image frame rate is no longer limited by sequential scanning of the focused beam but by the travel time of the pulsed plane wave to propagate through the medium and get back to the transducer array, and the achievable frame rate is ~ 30 KHz for an imaging depth of 15 mm. Such a high frame rate opens a wide range of possible neuroscience applications.

fUS is readily applied to brain imaging of awake mice. With this

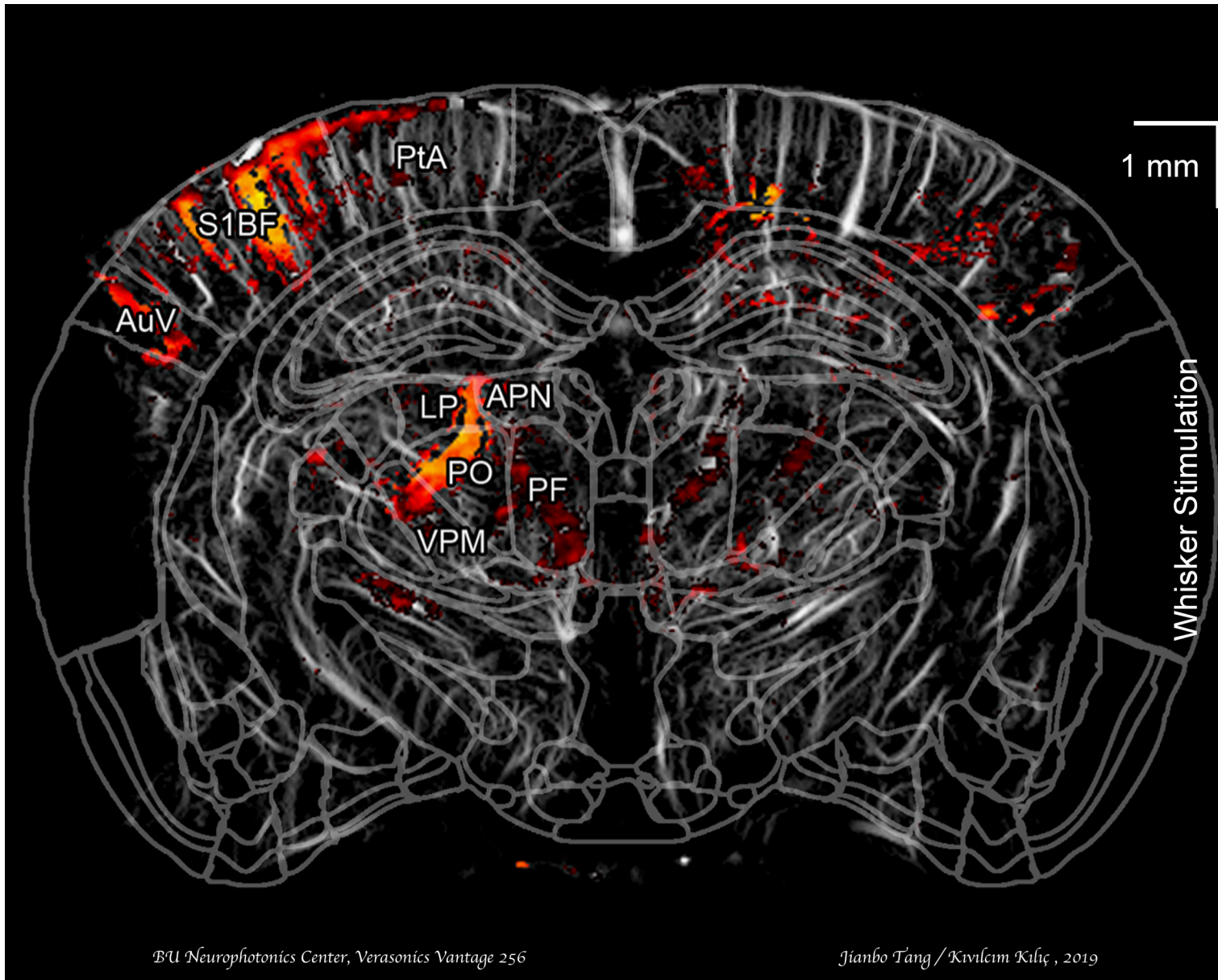
technology, the blood signal arises from Doppler shifts and is obtained either by high pass filtering or spatiotemporal clutter rejection, and the final image is formed by summing up the power of the blood signal for each pixel. The figure on the next page shows an fUS image of brain activation in a mouse acquired by Jianbo Tang using the instrument at the Neurophotonics Center.

Microbubble-based ultrasound localization microscopy (ULM) is an ultrasound analogue to optical super resolution technologies (e.g. photoactivated localization microscopy (PALM), fluorescence photoactivation localization microscopy (FPALM) and stochastic optical reconstruction microscopy (STORM)). It locates the centroid of flowing microbubbles and ac-

cumulates tens of thousands of such events to form a coronal plane blood vasculature map with ~10 micron in-plane resolution. The same technology can be used for quantification of cerebral blood flow velocity (vULM) by tracking the microbubbles. Ultrafast ultrasound has been commercialized, and as of today there are multiple commercial platforms supporting fUS and ULM applications.

Recently, Jianbo Tang from the Neurophotonics Center has advanced this technology by introducing a novel ultrasound speckle decorrelation-based velocimetry (vUS) method as a quantitative alternative to fUS and a faster velocimetry method than vULM. He developed a comprehensive theory for vUS image analysis and the data processing stream for cerebral blood flow velocity quantification. He validated vUS with numerical simulations, phantom experi-

ments, and in vivo measurements compared with the established ULM velocimetry. Finally, with the support of Kivlicim Kilic, he demonstrated the functional imaging ability of vUS by quantifying blood flow velocity changes during whisker stimulation in awake head restrained mice (see the figure below), something not possible with the slower vULM technology.



Wearable functional NIRS Systems

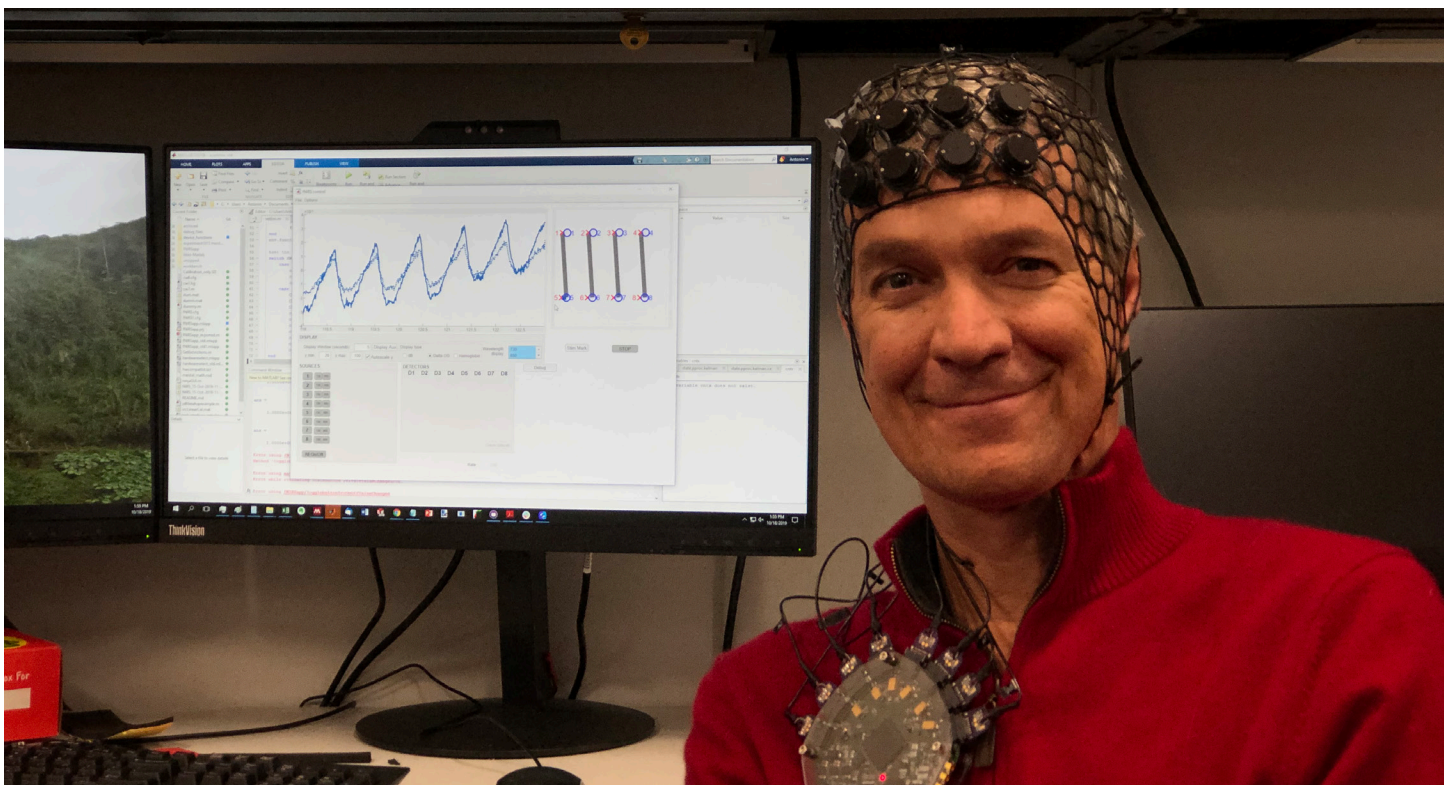
Advances in wearable fNIRS are transforming the technology, not only enabling studies of brain activity associated with natural behaviors in natural settings, but also dramatically reducing the cost of fNIRS systems. The past decade has seen a growing effort by several research groups and fNIRS companies to develop wearable systems. Because they typically replace the bulky optical fibers of conventional systems with light-weight electrical wires, by placing light emitters and receivers directly on the scalp, wearable devices are much lighter and more flexible than conventional fNIRS equipment. While most wearable systems have been restricted to fixed source-detector separations and to forehead measurements, novel approaches have appeared in recent years, allowing multi-channel high dynamic range acquisition with promising results.

Building on these recent advances, the Neurophotonics Center is developing a wearable, fully scalable and modular system based on a new compact optode. Each optode consists of one dual wavelength LED (730 nm and 850 nm) and one photodiode (7.5 mm² active area) 5 mm away, on a circular printed circuit board (PCB) of 14.9 mm diameter. Each optode PCB is encapsulated in a 3D printed case where light pipes guide the light from the LED to the scalp, and from the scalp to the photodiode. Multilevel LED output power and high dynamic range detection allow the photodiode to act both as short-separation detector for the source on the same optode and as longer separation detector for surrounding optodes. When completed, the system will be functional with as little as 2 optodes and can easily be expanded to as many as 128 opto-

des. This truly scalable approach will allow users to begin with a very simple low-cost device and from there, if needed, extend to high density whole-head coverage with complete flexibility to design probe arrays specifically relevant to given applications.

The latest version of this wearable system is shown in the figure below. Bernhard Zimmerman has been leading the development of the circuitry, Alexander von Luhmann has lead the development of the 3D printed cap, and Antonio Ortega is leading the software development and human testing.

Upon completion of this wearable technology, it will be possible to fabricate several fNIRS systems and provide them to BU research groups to perform measurements in their own labs.



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NPC Faculty Collaborative Grants

The table below summarizes the NPC Center, New Collaborative, and Ongoing Collaborative grants. Additional grants to NPC Director Boas are included as well as they contribute to the resources of the Center that are made available to NPC members. During the 2019 academic year, there were 19 such grants with a total of \$7.2 million awarded during the 2019 academic (and fiscal) year. The increase in funding between ongoing collaborative grants and the new collaborative grants from \$1.1 million to \$4.0 million, and number of grants from 3 to 11, is a strong indication that the Center is having a growth spurt. This is only the second year of operation for the Center and collaborations are just being formed and initial awards are being made. In steady state, when the Center is neither growing nor shrinking in terms of center and collaborative grant activities, we would expect that ongoing collaborative grant funding would be approximately 2 to 3 times larger than the new collaborative grant activity, if we assume that grants on average last for 3 to 4 years.

Grant Type	PI	AWARD TITLE (FULL)	SPONSOR	ADDITIONAL FUNDS THIS BUDGET PERIOD
Center	Boas	2018 WORKSHOP: INTEGRATING NEUROPHOTONICS, STATISTICAL PHYSICS, AND CONTROL THEORY FOR ADVANCING NEUROSCIENCE	NSF	88,350.00
Center	Mertz	ADVANCED LASER SCANNING CONFOCAL MICROSCOPE FOR MULTIPLE USERS	NIH	448,479.00
New Collaborative	Boas / Tian	SUPPLEMENT - MICROSCOPIC IMAGING OF TISSUE OXYGEN DELIVERY ALTERED BY MICROVASCULAR CHANGES	NIH	254,924.00
New Collaborative	Boas / Devor	THE IMPACT OF MICROVASCULAR (DYS)REGULATION ON CEREBRAL FLOW AND OXYGEN HETEROGENEITY	NIH	671,300.00
New Collaborative	Boas / Yucel	NONINVASIVE FAST OPTICAL CORRELATES OF NEURAL AND NEUROVASCULAR ACTIVITY	Sponsored	249,500.00
New Collaborative	Boas / Yucel	NONINVASIVE FAST OPTICAL CORRELATES OF NEURAL AND NEUROVASCULAR ACTIVITY	Sponsored	614,009.00
New Collaborative	Chen / Sander	POPULATION IMAGING OF ACTION POTENTIALS BY NOVEL TWO-PHOTON MICROSCOPES AND GENETICALLY ENCODED VOLTAGE INDICATORS	NIH	938,851.00
New Collaborative	Cheng / Han	UNVEILING THE MECHANISMS OF ULTRASOUND NEUROMODULATION VIA SPATIALLY CONFINED STIMULATION AND TEMPORALLY RESOLVED RECORDING	NIH	679,100.00
New Collaborative	Han / Sen	CORTICAL SPATIAL PROCESSING FOR SOLVING THE COCKTAIL PARTY PROBLEM	NIH	360,113.00
New Collaborative	Tian / Davison / Boas	A COMPUTATIONAL MINIATURE MESOSCOPE FOR LARGE-SCALE BRAIN MAPPING IN BEHAVING MICE	NIH	247,500.00
Collaborative	Boas / Devor	MICROSCOPIC FOUNDATION OF MULTIMODAL HUMAN IMAGING	NIH	159,350.00
Collaborative	Boas / Devor	THE IMPACT OF MICROVASCULAR (DYS)REGULATION ON CEREBRAL FLOW AND OXYGEN HETEROGENEITY	NIH	636,136.00
Collaborative	Davison / Gardner	A PLATFORM FOR INNOVATION IN MINIATURE MICROSCOPY	NIH	280,236.00
	Boas	MICROSCOPIC IMAGING OF TISSUE OXYGEN DELIVERY ALTERED BY MICROVASCULAR CHANGES	NIH	641,940.00
	Boas	GATED DIFFUSE CORRELATION SPECTROSCOPY FOR FUNCTIONAL IMAGING OF THE HUMAN BRAIN	NIH	82,500.00
	Boas	IMPROVING HUMAN FMRI THROUGH MODELING AND IMAGING MICROVASCULAR DYNAMICS	NIH	113,167.00
	Boas	IMAGING AND ANALYSIS TECHNIQUES TO CONSTRUCT A CELL CENSUS ATLAS OF THE HUMAN BRAIN	NIH	430,118.00
	Boas	ESTABLISHING AN FNIRS ECOSYSTEM FOR OPEN SOFTWARE-HARDWARE DISSEMINATION	NIH	247,607.00
	Yucel (Boas)	ADVANCING A NOVEL PORTABLE DETECTION METHOD FOR CANNABIS INTOXICATION	NIH	17,490.00

