DELVING DEEPLY INTO THE BRAIN'S MYSTERIES

Biocompatible microelectrode arrays interface with the brain, promising greater understanding of brain function and new treatments for neurological disorders.

Livermore's Biomedical Foundry includes a clean room that is recognized nationally as a unique thin-film neural interface facility. Here researchers fabricate components for the Livermore Flexible Probes. (Photo by Randy Wong.)

UNDERSTANDING the brain, especially that of humans, is one of the most complex challenges in science today. With an estimated 100 billion neurons joined together by 100 trillion connections in a hugely interconnected network of circuits, the human brain is extraordinarily formidable to investigate. For instance, a brain circuit can encompass not just a group of neighboring neurons but also individual neurons located as far as centimeters away.

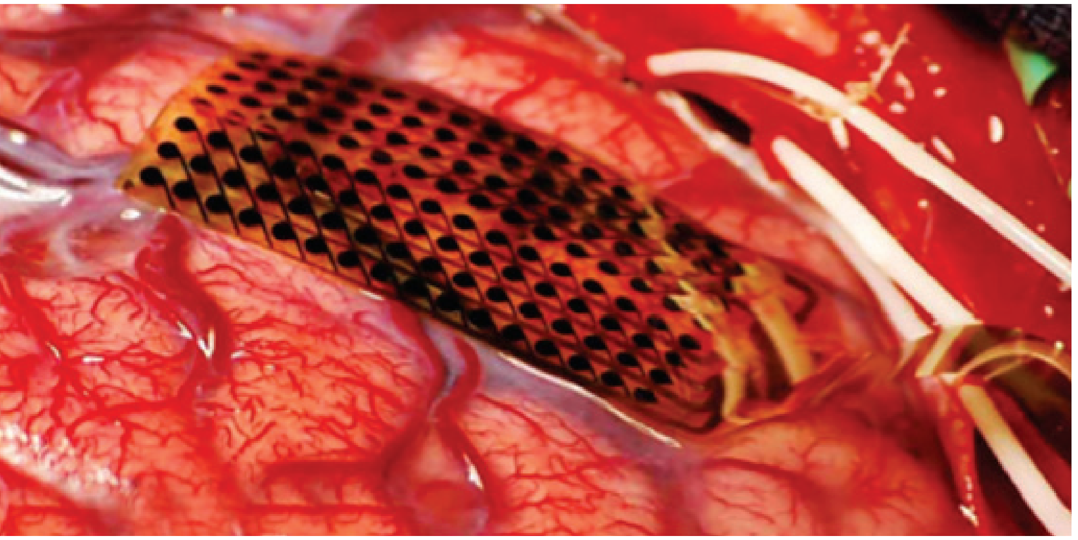
Researchers in Lawrence Livermore's Neurotechnology Program, part of the Laboratory's Center for Bioengineering, are responding to the need for innovative approaches to further understanding of brain function and neural communication dynamics. The group is focused on designing and building extremely small and biocompatible devices called Livermore Flexible Probes—microelectrode arrays that are implanted directly into the brain. Also known generically as neural interfaces, Livermore Flexible Probes monitor and optionally stimulate neural activity. Soft and flexible, the probes do not interfere with normal functions or behavior, allowing for long-term studies of brain circuitry. In animal studies, the probes are already proving to be exceptionally stable and useful.

In fact, research laboratories across the nation are using Livermore Flexible Probes to record neural activity in the brains of both animals and humans. Small-scale animal studies are done at Livermore, while more complex and long-term experiments are being conducted at University of California (UC) campuses and other institutions nationwide with strong neurophysiological programs. There, researchers test and characterize the mechanical and electrical properties of the implants, as well as their suitability for long-term use. At UC San Francisco (UCSF), the Livermore probes have been successfully tested for short periods on patients undergoing surgery to treat severe epilepsy.

Bolstering a National Effort

Shivshankar Sundaram, director of Livermore's Center for Bioengineering, says, "By providing high-quality,

A researcher holds an example of a Livermore Flexible Probe. The probes are designed to record data on the neural activity of animal and human brains in situ. (Photo by Randy Wong.)



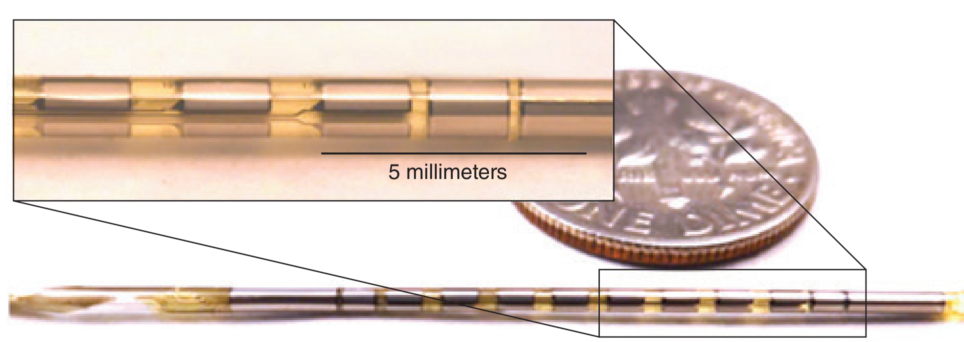
A 128-electrode Livermore Flexible Probe temporarily implanted in the hippocampus of a patient at the University of California at San Francisco. (Photo courtesy of Dr. Eddie Chang.)

long-term, and continuous recordings of brain activity with high resolution, the Livermore work enhances a larger national effort to revolutionize understanding of the brain and uncover ways to diagnose, treat, and prevent brain disorders." Researchers are hopeful that the vast amounts of data being collected from the arrays will point the way to innovative treatments for both neurological disorders—such as Parkinson's disease—and neuropsychiatric conditions, such as generalized anxiety, depression, and post-traumatic stress disorder (PTSD). The arrays may even one day restore lost neural functions such as sight, hearing, mobility, and memory.

Lawrence Livermore's long history of developing innovative bioengineering systems dates back to the 1970s, when researchers developed the first laser-based cell sorters, and continues through the development of miniaturized polymerase chain reaction systems that revolutionized molecular diagnostics. These pioneering achievements took advantage of a core Livermore strength—precisely recording, measuring, and analyzing data from complex systems, ranging from air turbulence to inertial confinement fusion reactions—and led to Livermore's involvement in both fabricating implanted biomedical devices and handling the large amounts of data the devices collect. The neural interface work also underscores the Laboratory's growing leadership in precision medicine initiatives such as the Accelerating Therapeutics for Opportunities in Medicine Consortium, the national Cancer Moonshot initiative, and human organ models that use three-dimensional bioprinting and microchips to re-create human physiology outside the body.

Much of the foundational research and development on flexible materials for microelectrodes was originally supported by the Department of Energy's Retinal Prosthesis Program, with additional support from the National Institutes of Health (NIH), UC's Office of the President, and Livermore's own Laboratory Directed Research and Development Program. Current research is funded by the Department of Defense's Defense Advanced Research Projects Agency (DARPA) and the federal Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative. Launched in 2013, the BRAIN Initiative aims to revolutionize scientific understanding of the human brain by discovering how individual cells and neural circuits interact in both time and space and uncovering ways to treat, prevent, and cure brain disorders and traumatic brain injuries. Participating in the initiative are federal agencies, national laboratories, foundations, universities, institutes, and private industry.

A strong relationship with bioengineers, neuroscientists, and surgeons nationwide is critical to Livermore's research effort. For example, Livermore's microfabrication know-how complements clinical science expertise at UCSF. A Livermore–UCSF team developed a method to implant the Livermore Flexible Probes into deep brain tissue with the aid of a removable device.



Although commercial devices commonly feature only 4 electrodes, Livermore designs offer 32, 64, and even 128 electrodes, enabling the capture of more data at higher rates.

At UCSF, the Livermore team works closely with neurosurgeon Eddie Chang and with Loren Frank, who is focused on neural circuits that underlie learning and decision making. Other collaborators include electronics researchers at Lawrence Berkeley National Laboratory and Professor Charles Della Santina at Johns Hopkins University.

Recording at High Density

Acquiring an in-depth understanding of how the brain functions and how signals flow within and between brain regions requires recording signals at high density, in multiple brain regions, and for long durations—up to several months or even years. Researchers need high-density neural interfaces implanted for long periods to understand brain networks. However, most commercial implants are bulky, handmade devices that provide limited resolution and are typically made of rigid and breakable silicon, which can damage brain tissue. Such devices are often quickly rejected by the immune system as foreign objects, making it nearly impossible to take long-term recordings of awake animals. "The body is a harsh environment for electronics," notes Sundaram. "We can't simply embed something and not expect the body to attack it as foreign." Moreover, components must be sealed off from bodily fluids that would cause electronics to short circuit.

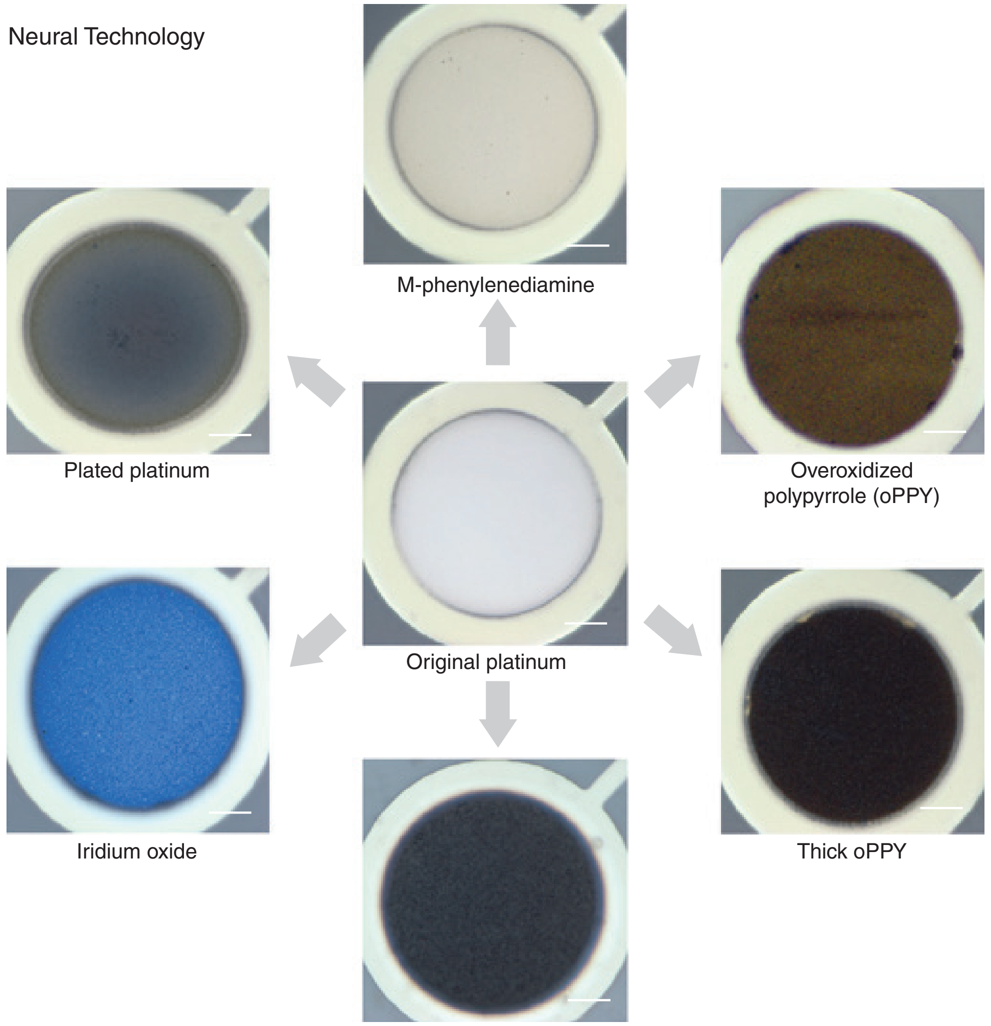
Once implanted, Livermore Flexible Probes can last for long periods without their performance degrading. Indeed, animal experiments conducted at several university laboratories are demonstrating that the probes uniformly achieve long-term, high-density recordings from multiple brain regions. One key to this longevity is the thin polymer film that insulates the device and allows it to move naturally with the micromotions of the brain. "Brain tissue is flexible like gelatin, so we need to build devices that match those mechanical properties," explains Razi Haque, head of Livermore's Neurotechnology Program and leader of several projects involving neural implantable probes. Haque says, "The devices' flexibility allows placement underneath imaging windows without danger of breakage, making possible combined electrophysiology and imaging studies." The Livermore devices also support optogenetic stimulation, a technique that uses light and light-sensitive proteins to manipulate neural activity.

A researcher in the clean room holds wafers on which Livermore Flexible Probes electronics have been manufactured. The probes consist of metal layers separated and insulated by flexible, biocompatible polyimide films. Fabrication employs the same photolithographic process that the electronics industry uses to manufacture integrated circuits, building a device layer by layer. (Photo by Randy Wong.)

Manufacturing in Clean Rooms

Livermore Flexible Probes are produced with Laboratory microfabrication techniques—many of them patented—at the Biomedical Foundry, in the Laboratory's Center for Micro- and Nanotechnologies. The foundry includes a clean room with dedicated processing and characterization equipment and is recognized nationally as a unique thin-film neural interface facility. "We have a singular skill set in microfabrication coupled with tight control over manufacturing," declares Haque. In fact, quality control is key for the Livermore Flexible Probes that has been approved for use in humans. The clean room–produced probes consist of metal layers separated and insulated by flexible, biocompatible polyimide films. Fabrication employs the same photolithographic process that the electronics industry uses to manufacture integrated circuits, building a device layer by layer.

Once the patterned metal layers are embedded in the flexible polymer, a tiny portion of the metal traces are exposed so they can record neural activity or stimulate brain tissue. In all, manufacturing the implants takes



Special electrochemical methods have been developed for applying polymer and metal coatings to transform the electrodes into biosensors. Photos show probes coated with the original platinum surface (center) and other biosensor-enabling coatings. Each scale bar represents 20 micrometers.

four to six weeks. The final device used in many studies consists of a polymer probe nearly as narrow as a human hair and a connector attached to the array, providing a direct, passive connection to external devices. In cases of animal use, the connector is typically secured to a headstock mounted atop the skull with a biocompatible material. For long-term human use, the researchers envision data from the implant being routed by a wireless transmitter located elsewhere in the body, much like a heart pacemaker.

Leveraging the Artificial Retina

Haque notes that the Neurotechnology Program first gained national recognition by playing a critical role in developing the world's first artificial retina. Also known as the "bionic eye," this retinal prosthesis used the same biocompatible polyimides as the current brain probes and was developed for people blinded by retinitis pigmentosa or macular degeneration. Livermore engineers developed a flexible microelectrode array that conforms to the curved shape of the retina. Approved by the U.S. Food and Drug Administration (FDA) as the first high-density, microfabricated, and fully implantable neural prosthetic ever produced, the device partially restores sight to blind individuals. The artificial retina received an R&D 100 Award in 2009 and a *Popular Mechanics* Breakthrough Award in 2010. "We are leveraging much of that retinal prosthetic endeavor," says Haque. "The artificial retina is a great foundation from which to work."

One of the most important features of Livermore Flexible Probes are their large number of electrodes. Whereas commercial devices commonly feature 4 electrodes, Livermore designs offer 32, 64, or even 128 electrodes. With this arrangement, a single electrode is likely to detect the activity of more than one neuron, and the activity of a single neuron could be detected by multiple electrodes. Software algorithms are often used to differentiate signals from individual neurons when the number of electrodes would make the job overwhelming for humans. "The human brain has 100 billion neurons, and a 128-electrode Livermore device can pick up activity from several hundred of the cells," explains Haque.

Efforts are underway to increase the electrode count. Electrical engineer Angela Tooker, who is spearheading the creation of ultrahigh-density arrays, says, "Scientists would like more electrodes—1,000 per device—to record at very high densities throughout entire brain structures, such as the cortex, and across multiple brain areas." The group's long-term goal is to develop systems that record activity from 10,000 neurons or more to study memory, learning, addiction, and anxiety. The hippocampus, the region of the brain associated with memory, is composed of 100,000 neurons, so several high-electrode-count arrays could record approximately 1 in 10 of those cells.

Tracking Neurotransmitters

Neurons interact through both chemical and electrical signaling, but the majority of electrode arrays from Livermore and elsewhere in the neuroscience community have so far focused on monitoring only the electrical component. Livermore chemist

Anna Belle is integrating biosensors onto the standard Livermore Flexible Probe platform to understand the chemical dynamics of neuron signaling. The biosensors allow the Livermore probe to record extremely small changes in chemical concentrations in addition to electrical activity.

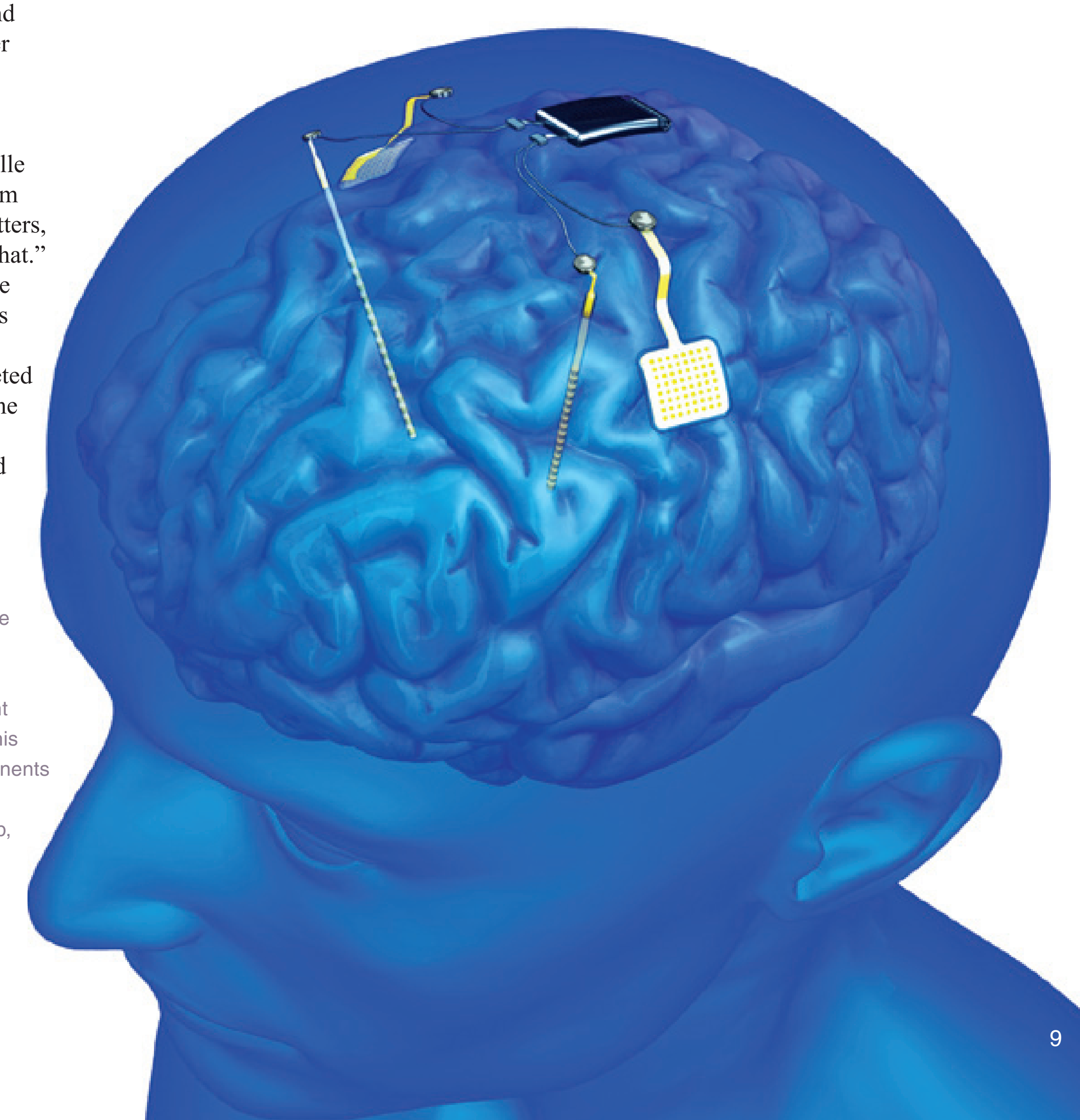
Current research indicates that many neurological conditions such as Parkinson's disease, depression, PTSD, and drug addiction are related to imbalances in the production of neurotransmitters such as dopamine and serotonin. Livermore Flexible Probes are among the few tools that allow scientists to continuously measure real-time changes in neurotransmitters for extended lengths of time in ambulatory animals. By integrating chemical sensors with the probes, scientists hope to determine—first in animals and then in humans—how neurotransmitter concentrations change during various activities and compare those changes in normal brains with those of people suffering from a particular disease. Belle says, "We don't know what an optimum level is for a lot of these neurotransmitters, but our biosensors can help establish that."

The chemical-sensing electrodes are plated with different coatings—such as enzymes and polymers—that act like selective amplifiers to enable the targeted detection of a single chemical out of the many in a brain region. At Livermore, glutamate sensors surgically implanted into rat brains can detect very small, localized changes in glutamate concentration as the animals move around, providing far more data than is possible with current diagnostic techniques. Such data has the potential to help researchers understand a wide range of conditions.

The specialized coating processes used at Livermore also allow a single probe to be equipped with multiple types of biosensors to monitor a diverse population of biomolecules simultaneously. For example, combinations of biosensors can simultaneously measure several biomarkers, including pH, oxygen, and glucose—indicators of blood flow and metabolism—and neurotransmitters such as dopamine and glutamate, which reflect normal learning and memory formation. Such biosensor combinations can also provide more information than single biosensors can on the neural dynamics of both healthy and diseased brains. Integrating multiple biosensors onto Livermore probes will help establish optimum neurotransmitter levels and accelerate development of therapies to maintain these levels in diseased brains.

Stimulating Selected Neurons

One therapeutic use of present Livermore Flexible Probe designs is deep brain stimulation (DBS). Currently used to treat some neurological disorders such as Parkinson's disease, essential tremor, and epilepsy, DBS is also being clinically investigated as a possible treatment for depression, neuropsychiatric disorders, PTSD, traumatic brain injury, and chronic pain, among other conditions. In DBS, surgically implanted electrical leads deliver



Livermore researchers are investigating the potential of implantable microelectrodes to alleviate severe depression by disrupting accompanying neural signals, an emergent treatment called deep brain stimulation. This artist's concept depicts a variety of components that such therapy might feature, including Livermore Flexible Probes, a telemetry hub, and associated electronics. (Illustration by Kwei-Yu Chu.)

Researchers such as Allison Yorita can use Livermore Flexible Probes to collect an extraordinary amount of neural signaling, as seen on the monitor. (Photo by Randy Wong.)

pulses to specific brain tissue to disrupt certain electrical signals associated with the disorder.

A Livermore effort to better understand DBS and enhance its effectiveness is part of DARPA's Systems-Based Neurotechnology for Emerging Therapies (SUBNETS) program, part of the BRAIN Initiative. DARPA recognizes that surgery, medications, and psychotherapy can help to alleviate the worst effects of neurological conditions but are still imprecise and not universally effective. Through SUBNETS, the agency hopes to reach an unprecedented ability to record, analyze, and stimulate multiple brain regions for therapeutic purposes and even cure some conditions. Haque says, "Although DBS can be used today as an FDA-approved therapy to treat Parkinson's, the current technique is imprecise, affecting broad regions of the brain, and can cause significant side effects during use." Microfabricated DBS probes could better target a brain region while avoiding unwanted effects. Haque adds, "One of the questions we are attempting to answer in support of SUBNETS is whether we can disrupt certain neural signals while someone is suffering from an episode of severe depression or PTSD. For example, we might want to search for specific electrical activity when a patient is in a particular mood state, such as severe depression, and then send a signal to a group of associated neurons to disrupt that activity." The device would be similar to a pacemaker, which is activated when the heart beats abnormally, and would not require patient intervention. Researchers in Livermore's Neurotechnology Program and its clinical collaborators are investigating the underlying mechanisms by which DBS works to treat major depressive disorders by stimulating cells to release dopamine, for example. Haque explains, "DBS would probably not replace pharmaceuticals and would be reserved for severe cases, although data recorded by the probe could aid the development of new pharmaceuticals."

As the number of electrodes increases, the amount of information captured grows exponentially. Collaborators using Livermore Flexible Probes have helped develop an automated system called MountainSort, which sorts brain activity by individual neurons, making it possible to record and analyze unprecedented amounts of neural signals. Part of a joint project among Livermore's Neurotechnology Program, UCSF, the Simons Foundation Flatiron Institute, and Intan Technologies, the work features an algorithm and open-source software that automatically sort neural activity detected by implanted electrodes. "Current analytical approaches are time-consuming because they involve manual intervention," explains Tooker. Using Livermore's 32- and 64-channel probes, Frank's UCSF team demonstrated MountainSort's ability to collect and sort information from about 1.1 million neural events per hour. The system separated neural signals from background noise and performed better than humans manually separating these same signals, a tedious and labor-intensive process. Tooker predicts that the ability to automatically sort neural activity data at unprecedented rates will in turn drive the development of microelectrodes with higher electrode counts.

Human Use on the Horizon

By all accounts, the Livermore Flexible Probes seem ready for human use. Sundaram points out that Livermore's 128-channel probes have done exceedingly well when implanted for short periods in UCSF patients who underwent surgery

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for epilepsy and who agreed to the temporary implants. During the operation, neurosurgeons recorded high-resolution electrical activity while patients responded to verbal questions. Haque explains, "The neurosurgeons said the data collected was the best they had ever seen and that recording from the hippocampus was possible for the first time." He is hopeful that the Livermore probes will be implanted in humans for longer term studies through the SUBNETS program.

"The question is how to transition from the animal to human world," says Sundaram. "Until a qualified commercial provider emerges, the Laboratory can fill the gap." He adds that Livermore's work remains unattractive to private companies because of technical challenges, high costs, a perceived small market, and the steep regulatory hurdles involved in commercially manufacturing a device for internal human use. In addition, many university groups that perform neurophysiology research on animals lack manufacturing expertise and the facilities required—such as a clean room with strict quality control standards—to extend their work from animals to humans.

Pushing the Frontiers

Sundaram states, "The Livermore team is pushing the frontiers of neurotechnology by furthering understanding of the brain and speeding development of new neurological therapies." To enable widespread adoption of Livermore Flexible Probes, the team plans to collaborate with additional laboratories in optimizing the devices and conducting studies on high-density, long-term recording. Feedback from such studies in different species and across different brain regions will provide a deeper understanding of normal and pathological brain function. Sundaram is also in discussions with NIH to designate Lawrence Livermore a national research resource center that widely distributes the Livermore Flexible Probes. At the same time, planning has begun on forming a neuroscience consortium that brings together the Laboratory and several other institutions in the San Francisco Bay Area.

In the meantime, Livermore's Neurotechnology Program continues to progress toward the long-term goal of devices that map pathways in the brain

"The Livermore team is pushing the frontiers of neurotechnology by furthering understanding of the brain and speeding development of new neurological therapies."

with thousands of electrodes. Researchers continue to take advantage of advances in microfabrication and new materials to develop new generations of devices with substantially increased electrode density. Tooker says, "We want to push the size limit and manufacture smaller devices with more electrodes so that each device can deliver more information." To that end, engineer and electronics packaging expert Susant Patra is leveraging Livermore's cutting-edge capabilities in lasers and manufacturing. He notes that applications inside the human body require a small, high-density package with performance that can survive the body's closed, device-unfriendly environment. One avenue of research is using Livermore's femtosecond laser to seal the components. The laser emits a pulse lasting only 50 to 1,000 femtoseconds (quadrillionths of a second)—too brief to transfer heat or shock to the materials being sealed.

Patra is investigating the use of another core Livermore manufacturing strength, additive manufacturing, in which components are built up layer by layer according to computerized direction. Here the goal is to eschew conventional planar printed circuit boards in favor of an additively manufactured architecture that takes advantage of the third dimension—depth—to achieve the smallest possible package. Patra explains, "We want to assemble all electronics in the smallest possible form factor, with less than 500 micrometers of depth, then cover the entire device with soft materials and seal them with femtosecond lasers."

Sundaram predicts that therapies such as DBS will someday be widely adopted for the treatment of serious neurological problems. When that milestone is reached, the next step will likely be devising performance augmentation—that is, learning—techniques based on new information gleaned from implant-collected data. Such data may make possible the next human–machine interface. "Today, we have to speak or use a keyboard to communicate with a computer," says Sundaram. "Perhaps someday we will communicate using our thoughts, with a skullcap that detects neural activity and translates it to words." Haque comments, "So much about the brain still waits to be discovered. We do not even know what we are going to learn."

—Arnie Heller

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