Technology Road Map for the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative

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Overview

In 2014, the National Photonics Initiative (NPI) founded an innovative multidisciplinary industry cohort, the Photonics Industry Neuroscience Group, to work collaboratively with the research community and federal government on next generation imaging technologies in support of the federal government's Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative. Optics and photonics, the science and application of light, are essential to achieving the goals of the BRAIN Initiative. Recognizing this fact, the NPI united industry leaders across optics and photonics fields to offer strategic guidance and recommendations for federal, private and joint funding opportunities in key optics and photonics areas where advances in technology and training would significantly accelerate progress toward achieving the BRAIN initiative goals of mapping neurons and circuits. This document serves as a technology road map and provides an overview of recommendations made by the NPI Photonics Industry Neuroscience Group for federal and private sector investment in critical optics and photonics technologies.

Role of Optics and Photonics Technologies in the BRAIN Initiative

On April 2, 2013, President Barack Obama launched one of his flagship programs, the BRAIN Initiative, to "accelerate the development and application of new technologies that will enable researchers to produce dynamic pictures of the brain that show how individual brain cells and complex neural circuits interact at the speed of thought." In response to President Obama's charge, the National Institutes of Health (NIH) convened a panel to recommend how best to proceed. The panel recommended a focus on accelerating the development of technology necessary to acquire fundamental insight into how the nervous system functions in health and disease. Photonics forms the basis of almost all medical diagnostic imaging devices: CT, MRI, X-ray imaging, microscopy, cell sorting and most devices used in ophthalmology. Further, photonics will play a pivotal role in achieving the goals of the BRAIN Initiative.

The enormous technical challenges presented by the BRAIN Initiative are difficult to comprehend. A network of 100 billion neurons exists in the human brain. Each neuron is connected to its neighbors by up to 1000 neural connections, axons and dendrites. Using a traffic analogy, making an atlas of this network is akin to drawing a three-dimensional map with 100 billion intersections (neurons) with 1000 streets (axons and dendrites) converging at each intersection. Extending this analogy, the signals propagating in this living network can be viewed as roughly 1000 billion cars simultaneously traveling on the dendritic "streets," moving at about 200 miles per hour. An ultimate goal of the BRAIN Initiative is to map accurately the locations of all of the intersections and streets, determine their functions and track the trajectories of the 1000 billion cars simultaneously moving along these pathways. A second goal of the BRAIN Initiative is to develop methods using light that will control the traffic flow at specific intersections in important regions of the brain, much as traffic lights do on city streets.

Current technology allows monitoring of only several dozen neurons simultaneously of the 100 billion neurons present in a human brain. Researchers have been able to use existing imaging instruments to locate portions of the brain that respond to simple stimuli (such as different aromas or a bright light) and in a few cases map connections between these regions and other important regions of the brain, showing their relationships. As we expand the volume of the brain and the number of monitored neurons to several thousand we can begin to understand how the neural networks respond to more complicated sensory stimulus patterns, learning how the brain deciphers language and intricate visual images, acquires complicated new skills, and forms complex memories, all at the fundamental neural network level.

Mapping and controlling neurons are central to the BRAIN Initiative and will require new optics and photonics technologies to enable development of new systems and tools such as:

- Quantitative microscope instruments;

- Miniature implantable probes;

- New high-speed imaging devices;

- Advances in molecular cell biology and protein engineering; and

- New software to handle massive amounts of image data necessary to construct these maps.

Translational Value

The development of new optics and photonics technologies is critical to realizing the translational value of the BRAIN Initiative. Once mapping and neuron control are achieved, the neuroscience community should be able to better understand the disruption of traffic caused by a stroke or traumatic brain injury and accelerate the redirection of neural signaling or traffic around these damaged regions to speed recovery from injury. We will be able to pinpoint the location and potentially prevent the explosions of local neural activity that cause epileptic seizures or the pulsation in neural signaling causing the tremors of Parkinson's disease. We can better understand the hyper neural activity that may be related to autism and the origin of many learning disabilities such as dyslexia. And, when the capability of controlling neural traffic is fully realized, we will be able to insert interface devices that can connect external sensors directly to the nervous system, restoring sight in certain types of blindness through artificial retinas or regaining muscle movement and providing prosthesis control to paralyzed patients.

Optics and Photonics Technology Roadmap

The NPI Photonics Industry Neuroscience Group was formally assembled on September 30, 2014, during a meeting at the White House Office for Science and Technology Policy

(OSTP). The group met a second time on November 15, 2014, in Washington, DC, and a third time on February 10, 2015, in San Francisco, California. At each of the three meetings, representatives of the neurosciences research community and federal government officials participated in dialogue with a multidisciplinary group of optics and photonics industry leaders from companies including Accumetra LLC, Agilent, Applied Scientific Instrumentation, Coherent, Hamamatsu, Inscopix, Inc., Spectra-Physics and THORLABS. Pursuant to these meetings, the NPI Photonics Industry Neuroscience Group identified and detailed technology priorities within each of the four BRAIN Initiative imaging goals required to map and understand the function of complex systems of neurons in the human brain and translate this understanding into medical advances.

The four BRAIN Initiative imaging goals are as follows:

- Developing a detailed morphological map showing the interconnections of thousands of neurons at the single-cell level in any given location of the brain;

- Measuring the dynamic interactions of large groups of neurons in alert behaving animals;

- Probing and controlling the dynamics of neural circuits by injecting and modifying neuron signaling and modifying neural circuit geometry; and

- Creating effective and practical neural/external-world interface technologies that allow the translation of the advances provided by the BRAIN Initiative into important medical devices.

Essential photonics technologies identified by the Photonics Industry Neuroscience Group necessary to achieve each of these goals are summarized below.

Goal I. Developing a Detailed Morphological Map

Developing a map of the brain at the single-neuron level has required new sample preparation methods to reduce scattering due to optical inhomogeneity in the brain tissue, while maintaining the delicate interconnect structures in the neural circuitry. Fat bodies, small peripheral cells (glial cells) and capillaries present a forest of objects that obscure the neurons and their interconnections. New methods have been developed to "clarify" the brain, removing many of these obscurations and allowing imaging of neurons in excised brain tissue samples up to 1mm thick. To image the neurons, new designs of scanning laser microscopes using specially shaped laser beams and state of the art ultra-short pulsed lasers illuminate extended subsurface regions of the brain while suppressing scattered light from intervening cells. To create an overall view of larger portions of the neural networks, these slices need to be "assembled" by registering and aligning separate three-dimensional data sets using three-dimensional image processing and registration software.

Neurons are essentially transparent in the visible region of the spectrum in their native state. Neurons can be genetically reprogrammed to produce fluorescent proteins that emit light of a specific wavelength when exposed to a laser or LED. These reprogrammed neurons function normally in vivo while the fluorescent proteins allow precise imaging of the neuron locations and interconnections. Researchers can selectively genetically engineer specific neurons to study their precise function or can map all neurons in a given section of the brain. Using these techniques, neurons can be precisely located and identified both in living animals and in excised tissue.

Researchers have begun to map portions of the complex brain neural network that encompass dozens of neurons using existing microscope and laser designs to generate quantitative three-dimensional images of neural circuits at depths of fractions of a millimeter. Extending this approach to tens of thousands of neurons/interconnections and larger brain volumes (~1 mm3 and greater) will require more powerful lasers, advances in beam shaping and steering technology, and feature extraction software to identify different neuron types, their location and their interconnections. This mapping of larger brain volumes will also require developing new software that will allow three-dimensional registration of multiple image datasets taken in separate experiments mapping contiguous regions of the brain.

Recommendations

In order to develop a detailed morphological map, the NPI Photonics Industry Neuroscience Group recommends the following:

- New methods for forming laser beams with intensity and phase properties optimized for locating neurons and their interconnections through Bessel beam optics and light sheet microscope sources;

- New higher efficiency, fast response, protein and small molecule fluorophores and associated highly specific binding agents to provide labeling of specific targeted regions in the brain;

- Improved methods to remove optical scattering material from the brain while preserving circuit morphology (i.e., second generation CLARITY);

- New software to register multiple two-dimensional data sets to create complex three-dimensional maps of the brain;

- New methods to register data from optical imaging with functional MRI, PET and CT datasets;

- Simultaneous, mixed modality imaging methods that combine optical imaging with acoustic and MR imaging; and

Goal II. Measuring the Dynamic Interactions of Large Groups of Neurons

Neuroscientists are now creating dynamic pictures of neuron signaling in animals reacting to carefully controlled stimuli. These experiments require genetic engineering methods to transform neurons so that they emit fluorescent light only when the neurons are activated. To this end, neuroscientists have developed methods to genetically reprogram neurons to assemble proteins that fluoresce when calcium (Ca) ions are present. The number of Ca ions increases during neuron activation. Other engineered proteins are sensitive to the voltage drop or action potential that develops across the neuron cell membrane when the neuron is activated. These proteins become fluorescent or change the color of their fluorescence when the neuron fires and alters its membrane potential.

Scanning laser microscopes read out the dynamic fluorescent signals emitted by the neurons as a result of environmental stimuli. Miniaturized microscopes, smaller than a thumb drive, and insertable fiber optic and LED/detector probes have been developed and can be attached to live animals. These tiny light-weight devices excite the fluorescent proteins, providing real-time images of neurons firing in specific regions of the brain. Smaller, more efficient "wearable" microscopes that can image larger regions of the brain will be necessary to accomplish the goals of the BRAIN Initiative.

Following the dynamic signals of large ensembles of neurons requires the development of new solid-state cameras that combine high sensitivity (single photon), high speed (~10,000 frames/second) and high resolution (1 megapixel or higher). More efficient and stable wavelength-versatile Ca ion and voltage-sensitive dyes are also needed. Employing recent advances in high-throughput protein engineering methods should allow development and optimization of these improved protein-based compositions of matter.

Utilizing these imaging methods in animal models or in human subjects will require more detailed knowledge of the safe exposure limits for excitation of these fluorescent indicators and the development of standard protocols and methods to determine the safety and toxicity of new protein fluorophores.

Recommendations

To measure the dynamic interactions of large groups of neurons, the NPI Photonics Industry Neuroscience Group recommends the development of the following technology to reach this goal:

- High-power, wavelength-agile, ultra-short pulsed infrared laser sources for use in multi-photon microscopes (20 W, NIR, Tunable 650 nm to 1110 nm, 100 fsec);

- Fixed wavelength, high-power (50 W to 100 W) infrared lasers emitting pulses of about 100 fsec at variable repetition rates from 1 MHz to 1 GHz;

- High-resolution spatial light modulators (10 million to 100 million pixels) providing amplitude and phase modulation of high-power lasers to independently address and control thousands of neurons simultaneously;

- Synchronized ultra-short pulsed sources for simultaneous scanning of multiple animal model brains or multiple regions within a brain;

- Improved calcium indicator and membrane potential dyes such as voltage and calcium-sensitive protein fluorophores with higher quantum efficiency, greater photostability, higher voltage/ion sensitivity and that have excitability at various visible and IR wavelengths;

- Miniaturized wearable single- and multi-photon microscopes to monitor neural activity in alert animals such as self-powered or wireless coupled microscopes weighing a few grams, with better than 1 micron resolution and several hundred micron fields of view; and

- Software to extract transient signals from large groups of neurons and determine temporal and spatial correlations through pattern recognition indicative of information flow.

Goal III. Probing and Controlling the Dynamics of Neural Circuits

To control the dynamics of neural networks, neuroscientists genetically engineer neurons to produce light-sensitive regulating proteins. Certain proteins activate the neurons when they are exposed to light and other proteins suppress the neural activity upon light exposure. This technique is referred to as optogenetics. A single neuron or groups of neurons can be controlled by selectively genetically engineering just the neuron subgroups or by focusing the controlling light on only selected neurons.

The controlling laser beams need to be precisely pulsed in synchrony with the signaling patterns in the neural network and must be aimed precisely at the target neurons. Combining this technique with voltage sensing fluorescent indicators of neuronal activation, researchers will be able to determine the precise function of neural circuit elements and how they determine behavior in response to environmental stimulation. Extending optogenetics to control the function of networks consisting of thousands of neurons will require new methods for fast and precise laser beam steering, high-speed intensity modulation of hundreds of laser beams, methods for delivering these light sources to freely moving, behaving animals, and high-speed, high-resolution, low-noise cameras.

Recommendations

To successfully probe and control the dynamics of neural circuits, the NPI Photonics Industry Neuroscience Group recommends the following:

- Develop fixed wavelength, high-power (50 W to 100 W) infrared lasers emitting pulses of about 100 fsec at variable repetition rates from 1 MHz to 1 GHz;

- Improve beam shaping and aiming technologies providing high-speed intensity modulation, dynamic aiming and focusing of multiple laser beams with millisecond or shorter response times. For example: high-bandwidth (1 msec pixel response or less), high-power ($10\ W/cm2$ to $100\ W/cm2$ power handling capability), spatial light amplitude and phase modulators with 1080P, 4K and 8K spatial resolution (2 megapixels, 8 megapixels, 33 megapixels, respectively);

- Develop new more sensitive, wavelength-versatile, protein fluorophores that allow multiplexed in vivo control of neuron dynamics;

- Design apparatus for controlling animal stimulation and simultaneously and automatically optically monitoring behavioral and neural circuit response in real time;

- Develop new highly efficient, low-power, laser and LED sources for one and two photon excitation of suitable protein fluorophores to allow individual neural control

such as stimulation and blocking of neural activity in multiple locations at the single-cell level; and

- Develop miniaturized optical probes (LED arrays and detectors, fiber optic probes) that can be inserted minimally invasively into the brain in specific locations and can simultaneously modulate and monitor neural circuitry in live animals.

Goal IV. Creating Effective and Practical Neural/External-World Interface Technologies

Understanding neural circuit morphology and function is the first critical step in developing effective intervention strategies to help patients impacted by traumatic brain injury (TBI), paralysis or neural degenerative diseases such as Parkinson's and Alzheimer's, and certain types of blindness. Optical monitoring of neural function may provide an effective way to provide precise dosage and timing information for the delivery of drugs to help control epilepsy and depression.

Effective intervention will require the development of new, safe and effective methods to monitor and control multiple neurons in specific areas of the brain in real time. Examples of the promise of this technology have already been demonstrated in animal models of Parkinson's disease, retinal degeneration, optogenetic stimulation and control of muscle groups, modification of animal behavior through optogenetic control of anxiety centers, and artificial limb activation.

Practical utilization of these optogenetic control techniques in humans will require extensive testing and modeling of safe exposure limits for laser irradiation of the brain. Monitoring and stimulating neural networks to access motor control function will require inserting devices deep within the brain using fiber optics and micro-electromechanical (MEMS) optical devices with extremely high electrical efficiency.

Recommendations

In order to create effective and practical neural/external-world interface technologies for the brain, the NPI Photonics Industry Neuroscience Group recommends the following:

- Design and develop optoelectronic coupling to thousands of neurons in a neural network. For example: design and testing of artificial retinal implants;

- Develop methods to test the long term safety and efficacy of implantable optics and photonics technology;

- Develop microminiature, implantable optically activated drug delivery and detection systems;

- Create optomechanical methods to measure traumatic brain injury and relate these measurements to external pressure, shock and acceleration; and

- Develop methods to safely and effectively test optical methods for phototoxicity in vivo, including optogenetic modification of neurons in animal models and humans.

Potential Areas of Joint Private and Federal Investment

The NPI Photonics Industry Neuroscience Group believes federal, private and joint investment in the following areas will significantly accelerate the progress toward achieving the BRAIN Initiative goals outlined above:

- Sponsored intern programs for student training in technology translation to facilitate transfer of technology and know-how from academic to commercial environment;

- Sponsored visiting industrial scientist programs to support academic/industry collaborations;

- High-risk product development programs to develop instrumentation specific to accomplishing BRAIN Initiative goals;

- Joint National Institute of Standards and Technology (NIST)/industry programs to develop new consensus standards and measurement science infrastructure in neuroscience;

- Joint NIH/industry programs to support translation of BRAIN technology into clinical practice; and

- Joint Food and Drug Administration (FDA)/industry programs to develop safety standards for optogenetics and related methods.

Conclusion

Achieving the goals of the BRAIN Initiative will require significant advances in optics and photonics technology and collecting the necessary data will require employing the precision, non-damaging properties of imaging using visible light-based, advanced microscopic instruments. Joint development efforts involving academia and industry, encouraged and funded in part by the federal government, will greatly accelerate progress in the neuroscience field, establish a training ground for the next generation of neuroscience researchers and provide US industry with the appropriate guidance and perspective to be the leading supplier of next generation research and clinical neuroscience tools.

ABOUT THE NPI: The National Photonics Initiative (NPI) is a collaborative alliance among industry, academia and government to raise awareness of photonics and the impact of photonics on our everyday lives; increase cooperation and coordination to advance photonics-driven fields; and drive US funding and investment in areas of photonics critical to maintaining US economic competitiveness and national security. The initiative is being led by top scientific societies including the American Physical Society (APS), the IEEE Photonics Society (IEEE), the Laser Institute of America (LIA), The Optical Society (OSA) and SPIE, the International Society for Optics and Photonics (SPIE).

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