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Nanotools for Neuroscience and Brain Activity Mapping

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Abstract

Neuroscience is at a crossroads. Great effort is being invested into deciphering specific neural interactions and circuits. At the same time, there exist few general theories or principles that explain brain function. We attribute this disparity, in part, to limitations in current methodologies. Traditional neurophysiological approaches record the activities of one neuron or a few neurons at a time. Neurochemical approaches focus on single neurotransmitters. Yet, there is an increasing realization that neural circuits operate at emergent levels, where the interactions between hundreds or thousands of neurons, utilizing multiple chemical transmitters, generate functional states. Brains function at the nanoscale, so tools to study brains must ultimately operate at this scale, as well. Nanoscience and nanotechnology are poised to provide a rich toolkit of novel methods to explore brain function by enabling simultaneous measurement and manipulation of activity of thousands or even millions of neurons. We and others refer to this goal as the Brain Activity Mapping Project. In this Nano Focus, we discuss how recent developments in nanoscale analysis tools and in the design and synthesis of nanomaterials have generated optical, electrical, and chemical methods that can readily be adapted for use in neuroscience. These approaches represent exciting areas of technical development and research. Moreover, unique opportunities exist for nanoscientists, nanotechnologists, and other physical scientists and engineers to contribute to tackling the challenging problems involved in understanding the fundamentals of brain function.

The Brain Activity Mapping (BAM) Project¹⁻⁵ has three goals in terms of building tools for neuroscience capable of (1) measuring the activity of large sets of neurons in complex brain circuits, (2) computationally analyzing and modeling these brain circuits, and (3) testing these models by manipulating the activities of chosen sets of neurons in these brain circuits.

As described below, many different approaches can, and likely will, be taken to achieve these goals as neural circuits of increasing size and complexity are studied and probed.

The BAM project will focus both on dynamic voltage activity and on chemical neurotransmission. With an estimated 85 billion neurons, 100 trillion synapses, and 100 chemical neurotransmitters in the human brain,⁶ this is a daunting task. Thus, the BAM project will start with model organisms, neural circuits (*vide infra*), and small subsets of specific neural circuits in humans.

Among the approaches that show promise for the required dynamic, parallel measurements are optical and electro-optical methods that can be used to sense neural cell activity such as Ca^{2+} ,⁷ voltage,⁸⁻¹⁰ and (already some) neurotransmitters;¹¹ electrophysiological approaches that sense voltages and some electrochemically active neurotransmitters;¹²⁻¹⁷ next-generation photonics-based probes with multifunctional capabilities;¹⁸ synthetic biology approaches for recording histories of function;¹⁹⁻²¹ and nanoelectronic measurements of voltage and local brain chemistry.²²⁻³⁹ We anticipate that tools developed will also be applied to glia and more broadly to nanoscale and microscale monitoring of metabolic processes.

Entirely new tools will ultimately be required both to study neurons and neural circuits with minimal perturbation and to study the human brain. These tools might include “smart”, active nanoscale devices embedded within the brain that report on neural circuit activity wirelessly and/or entirely new modalities of remote sensing of neural circuit dynamics from outside the body. Remarkable advances in nanoscience and nanotechnology thus have key roles to play in transduction, reporting, power, and communications.

One of the ultimate goals of the BAM project is that the knowledge acquired and tools developed will prove useful in the intervention and treatment of a wide variety of diseases of the brain, including depression, epilepsy, Parkinson’s, schizophrenia, and others. We note that tens of thousands of patients have already been treated with invasive (*i.e.*, through the skull) treatments. While we hope to reduce the need for such measures, greatly improved and more robust interfaces to the brain would impact effectiveness and longevity where such treatments remain necessary.

Neuroscience at a Crossroads

Understanding how the brain works is one of the greatest challenges facing science and engineering. After more than a century of sustained progress in biological sciences and medicine, one could argue that mankind has made significant advances in our understanding of how biological systems operate and how different parts of the body function and, when damaged, generate disease. At the same time, a comprehensive understanding of the brain remains an elusive, distant frontier. To arrive at a general theory of brain function would be an historic event, comparable to inferring quantum theory from huge sets of complex spectra and inferring evolutionary theory from vast biological field work. Not only would a theory of brain function be a fundamental advance in biology, but it would enable understanding of the pathophysiology of neurological and neuropsychiatric diseases. Many of these devastating brain-based pathologies have neither cures nor effective treatments, in large part because it is difficult to provide a treatment for a dysfunctional organ when one does not

know how it works. Finally, the historic importance of generating a theory of brain function is highlighted by the fact that as humans, more than any other species, we are defined by the higher cognitive abilities generated by our brains. Thus, scientific understanding of our brains will enable deeper knowledge of ourselves and of our minds.

Neuroscientists have worked on this key problem for the last century, and yet a comprehensive theory of brain function remains elusive. Enormous progress has been made in understanding the molecular and cellular components of neural circuits in humans and experimental animals. One goal to this end is to drive the development and testing of theories of brain function that require better spatial and temporal sampling and intervention than is presently possible. Greater precision and parallelism in electrical and chemical sensing, as well as the ability to excite and to probe neural circuits actively, as proposed here, would bridge the nanoscale to the microscale to the macroscale and would complement ongoing connective mapping of brain circuits.

Need for High-Resolution, Network-Level Brain Activity Mapping Approaches

There has been remarkable progress in the ability to ascribe specific functional roles to specific neuroanatomical regions, axonal tracts, cells, synapses, and molecules. For example, large-scale maps of gene expression in the brain, such as the Allen Brain Atlas⁴⁰ or GENSAT Project⁴¹ provide enormous insight into the brain's architecture at the genetic level with precise anatomical resolution. However, no comparably high-resolution maps of brain-wide neuronal activity are available. On one hand, noninvasive mapping techniques such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and electroencephalography (EEG) reveal a wealth of information about functional brain organization and connectivity.^{42–44} These methods offer coarse-grain views that do not fully capture the underlying networks' properties. On the other hand, our ability to perceive and to ponder the cosmos, to remember information, to feel pleasure from daily experiences, to make decisions—and deficits in performing some of these tasks when faced with disease—involves a complex interplay of large, distributed neuronal populations signaling on millisecond time scales. Science has barely scratched the surface of this fast, network-level regime. The huge potential payoff for understanding the brain and diagnosing and treating neurological disorders means that techniques for measuring brain activity are scaling up at a rapid pace.⁴⁵

Thanks in part to these advances, the development of technology to enable a paradigm shift from experiments that routinely record tens of neurons at a time to experiments that can record millions of cells is important and timely. From a computational perspective, it is obvious that information processing in the brain relies on a cascade of events,⁴⁶ and sampling these events a few cells at a time, as has been the norm in neuroscience, cannot capture the emergent properties of such a deeply interconnected network as the brain. The search for spatiotemporal patterns and correlations in spike trains of recorded neurons can be enormously enhanced by raising the number (N) of simultaneously accessible units.^{22,47} For example, assuming a uniform connection probability, the likelihood of finding synaptically coupled cells increases quadratically with N . Testing the functional implications of small-world models of interacting neural networks⁴⁸ would likewise benefit from having access to greater N . Molecular-level analyses of cellular organization reveal the immense heterogeneity of neuronal subpopulations. Many of these subpopulations—such as cholinergic interneurons in the striatum⁴⁹—represent only a small fraction of cells in a given area, yet they are known to play important roles in regulating behavior.⁵⁰ This suggests that in order to sample several of these rare but important units reliably, so as to understand their function *in vivo*, large-scale measurements of neuronal activity are necessary. Further,

because the brain is composed of many specialized neuron types that perform specific functions within microcircuits, techniques must be developed that enable both the identities and activities of neurons to be measured.

In spite of this progress, why does neuroscience still lack a general theory? One contributor is the sheer complexity of brain circuits. Even in the simplest organisms, nervous systems are composed of circuits built with many different subtypes of neurons connected in patterns of prohibitive complexity. These “impenetrable jungles where many investigators have lost themselves”, as Ramon y Cajal, one of the earliest neuroanatomists termed them,⁵¹ have difficult experimental access, and the sheer diversity of neural circuits and their components makes it difficult to draw strong conclusions or generalizations on which to build general theories. In fact, neuroscientists have traditionally analyzed the structure and function of these circuits one neuron at a time, using electrical recordings from individual neurons, for example, while an experimental animal is performing a specific behavior. At the same time, any neural circuit is composed of thousands or hundreds of thousands of neurons, which are heavily interconnected. Because of these structures, it is likely that neural circuits operate at an emergent level, one generated by the functional interactions between large populations of neurons. Thus, measurements from individual neurons would not give insight into function, just as one cannot understand the function of a building by analyzing the molecular structures of its bricks. In fact, emergent properties have been encountered in many areas of science and engineering. The laws of thermodynamics, statistical mechanics, and the generation of magnetic properties are examples of fields of science that require understanding of emergent phenomena that result from interactions across many individual particles. The goal of the BAM project is to provide the data sets and the critical tests to enable the development and testing, respectively, of theories and models of neural circuits and brain function.

To elucidate emergent properties, neuroscientists will need novel techniques that enable simultaneous monitoring of the activities of many or all of the cells in neural circuits. While whole-brain imaging techniques, such as fMRI, enable bird’s eye views of the activity of brain areas, they lack the spatial and temporal resolution required to provide functional information on individual neurons and their interactions. New techniques are needed, and while neuroscientists are generating many novel approaches, we believe that nanoscience and nanotechnology are ideally poised to make fundamental contributions to this problem and to help generate the toolkits of methods that could be used to measure and to manipulate the activities of increasingly larger sets of neurons in complex and widespread neural circuits.

The Nanoscience and Nanotechnology Revolution

The nanoscience and nanotechnology revolution began with the ability to “see” at the atomic scale with the inventions of the scanning tunneling microscope, the atomic force microscope, and related tools.^{52–55} It then progressed with the ability to manipulate individual atoms and molecules, as well as to direct assemblies of molecules into precise structures.^{56–60}

In the years since, remarkable progress has been made in developing novel materials, tools, and methods that have opened up new possibilities across science, engineering, and medicine. Some progress has already been made toward addressing problems in neuroscience *via* nanotechnology.

In the past decade, substantial investments have been made through the National Nanotechnology Initiative in the United States and similar programs in countries around the world. Support continues in the hope that the dramatic advances we have seen in

nanoscience and nanotechnology will continue and will now be applied to other fields of science, engineering, and medicine, as well as to manufacturing and commercialization.⁶¹

Top-Down (Lithographic) Devices

Miniaturization through lithographic and other means has been a continuing trend fueled largely by the need to develop ever more functional systems. Over the past decades, these systems have catered to the needs of consumer electronics, with the opportunities of using microchips to control and to interpret information on massive scales. More recently, this trend has been driven by our need to be connected and to communicate, leading to more portable systems in which size, weight, and power are at a premium. The same trend that enables the miniaturization of electronic and radio frequency communications systems has also influenced optical and fluidic systems, with the emergence of printed silicon photonics and microfluidics that can decrease the sizes of lasers, modulators, and detectors, as well as pumps, valves, and mixers. As a general rule, the size of printed systems has been reduced by a factor of $100 \times$ in volume every 10 years (Bell's Law).⁶² These reductions in system size correspond to improvements in the fidelity of lithographic processes, enabling the geometric doubling in the number of individual devices on a chip every 18 months (Moore's Law).⁶³ It could be argued that Moore's Law is driven by the "real-estate" value on the chip, which in the case of silicon electronics has remained constant over the past 40 years, at a cost of approximately $\$5/\text{cm}^2$.

Miniaturization in electronic devices has led to the ability to create structures with 22 nm lateral width over 300 mm wafers, produced on commercial scales at approximately 20 wafers/h. Individual transistors are now 200 nm in size, and amplification circuits are on the order of micrometers. For the specific application of studying the brain, it is now possible to contemplate using these capabilities to increase the numbers of neurons interrogated by reducing the sizes of electrophysiological probes and to develop systems that can manage large amounts of data accumulated and/or transmitted during such measurements. This dramatic reduction in the size of electronic systems enables the construction of devices that can be implanted with less intrusiveness and enables the development of small electrophysiological tools to measure and to control individual neurons. Simultaneously, this miniaturization is associated with increases in the operating frequencies of electronics and reductions in the sizes of antennas and power required, resulting in smaller communications systems.

Indeed, systems being developed for future use in communications may be of great value for neuroscience, as well. Multiferroic antennas are one example in which devices can be $1000 \times$ smaller than conventional antennas and may be able to be powered remotely.⁶⁴ More broadly, ultrasmall nanoelectronic chips might be used to combine modalities of detection of signals from operating neural circuits and the wireless broadcasting of this information at extremely high data rates to decoders for real-time recording and deciphering of neural codes.

Bottom-Up Methods, Self-Assembly, and Chemical Patterning

Smaller-than-standard lithographic scales can be reached using self- and directed assembly.^{65–68} Tremendous progress has been made in functionalizing a wide variety of materials, including semiconductors, insulators, metals, glasses, nanoparticles, and porous materials. With a single molecular layer, the chemical, physical, and biological properties of materials can be controlled and tailored.

Given the need for more than electronic or optical function, it is critical to control the exposed chemistry on devices. Tremendous advances have been made in the last 30 years in

the chemical functionalization on a wide range of substrates.^{65–67} Such advances can be applied both to guide assembly and to control biological and other interactions.

Likewise, nanoparticles and other nanostructures can be specifically functionalized so as to target specific locations and to be stabilized there such as lodging themselves in cell membranes.^{69,70} In this way, physical placement of nanostructures will not necessarily be required, but instead reporting or *post-mortem* analyses can be used to determine the absolute and relative locations of nanostructures used in BAM sensing. Potential uses of nanoparticles and nanostructures are discussed further below.

In addition, functional molecules can be used to sense and to transduce potentials, pressure, and specific aspects of chemical environments. We anticipate that artificial and hybrid neurotransmitter receptors will be critical in reporting the local environment in the brain. Tremendous opportunities exist if one can explore the 10 nm synapse scale to understand dynamic neuro-transmission while simultaneously recording the activities of thousands of neurons in a neural circuit. If nanostructures can be targeted to specific cell or synapse types by means of chemical signatures on the cell surfaces, it may be possible to monitor and to control the activities of neurons in cell-type-specific manners. We anticipate that, ultimately, such measurements could also be used in feedback circuits to control diseases in which local chemistries play critical roles, such as Parkinson's and schizophrenia. The implications of developing these and other BAM technologies are significant.

Development of Nanoscale Tools for Neuroscience

In light of the advances in resolution of these top-down and bottom-up miniaturization strategies, the endeavor toward matching the sizes of devices that measure and control neuronal activity with the sizes of individual neurons is compelling and appears inevitable. Below, we enumerate a few of the areas in which these contributions are anticipated.

Electrophysiology

The major obstacles that presently limit the use of nanoscale probes are the engineering challenges of building efficient power and communications systems to interface such neural probes with the outside world and at the same time avoid tissue damage and undesirable cell responses. Whether quantum dots or wafer-bonded microsystems are used, it is important to avoid heating and toxicity in the vicinity of the measurement probes. The most important physical barrier that limits the sizes of intracellular neural interfaces is the impedance of the electrodes—whether these are on nano-particles embedded within cell walls or on more conventional electro-physiological patch-clamp systems. For extracellular recording, the impedance is even more important, as it determines signal-to-noise characteristics and, thus, the sensitivities of the neural probes. The sensitivity toward neuronal signals depends on the impedance, which can then be transduced to measurement devices outside of the brain.

Brain Activity Mapping with Nanofabricated Electrode Arrays

One of the technologies that can greatly facilitate brain activity mapping is the extracellular microelectrode, which can resolve single-neuron firing *in vivo* without penetrating the cell. Once inserted in the brain, the detection range of this type of sensor is typically limited to neurons whose cell bodies are closer than $\sim 50 \mu\text{m}$ from the microelectrode surface.^{23,71,72} Thus, in order to construct systems-scale views of brain function from such measurements, the objective has been to increase the number and density of recording sites. Scaling up these device attributes has been spurred by parallel advances in electronic instrumentation for reading out signals *via* low-noise amplifiers, multiplexers, and wireless transmitters^{24–30} and performing basic signal processing functions on-chip to reduce the burden of data

collection.^{31,32} Innovations in microelectrode manufacturing techniques have made it possible to deploy simultaneously nearly 1000 measurement sites distributed across several cortical areas of the same animal, allowing the firing patterns of hundreds of neurons to be monitored in parallel.³³ Leveraging micro- and nanofabrication technology raises the prospect for creating vastly greater numbers of electrodes and smaller, less invasive implantable devices.^{34–37} A promising category of these micromachined devices is the planar electrode array, which is patterned on a crystalline,^{38,73} ceramic,³⁹ or polymer^{74,75} support structure (Figure 1). Positions of micro-electrodes on these thin penetrating shafts and spacing between two or more shafts can be tailored to simplify targeting of multiple anatomical areas or subregions in tandem.^{76–78} The measurement of neuronal activity with three-dimensional (3D) microelectrode arrays represents a major advance in brain activity mapping techniques, by providing a tool to probe how intra- and inter-regional neural circuits behave cooperatively to compute information. We envision scaling up this 3D architecture to sample arbitrarily complex networks.

Advantages and Challenges of Electrode-Array-Based Mapping Approaches

The use of implantable electrodes is complementary to optical-based brain activity mapping: Electrodes can access deep brain structures that are challenging to reach with optical methods; they do not require labeling cells with a dye; and they offer higher sampling speed than voltage or calcium indicators (although advances in optical recording techniques are circumventing many of these issues).^{7,79,80} Furthermore, the manufacturing processes used in voltage-sensing electrode development can translate to other modes of interrogating neuronal activity, such as chemical sensors.^{81,82}

Electrodes also present two major challenges for brain activity mapping. First, as they measure extracellular electric fields from all nearby active neurons, deriving single-unit information from these signals is not trivial,⁸³ and a fast, automated “spike sorting” algorithm for handling data from a large number of electrodes receiving correlated signals remains elusive. Even after spike sorting is successful, extracellular signals cannot directly differentiate the origins of action potentials at the level of genetically specific neuronal subpopulations. One approach that partially addresses this limitation is to rely on indirect identification methods such as extracellular action potential shape, spike time characteristics, and pharmacological response. This may be valuable for identifying cells along broadly defined categories, such as pyramidal neurons *versus* interneuron⁸⁴ or dopaminergic *versus* nondopaminergic.⁸⁵ However, this method is not without pitfalls.⁸⁶ Perhaps an early goal of the BAM electrode technology effort would be to catalog exhaustively extracellular electrophysiological markers of genetically identified neuronal sub-populations to lend greater validity to this indirect approach. Alternatively, a more direct way of identifying neurons is by probing their responses to a gene, region, or pathway-specific pharmacological or optogenetic modulator of activity.^{87–89} Microfabricated neural probes that can record activity and deliver drugs or light have been developed and can help address this issue.^{90–94}

New developments in nano–bio interfacing and 3D microfabrication techniques might provide the means to overcome some of the limitations of planar microelectrode-based extracellular electrophysiology. Nanoscale needle electrodes (Figure 2) can provide high-fidelity electrophysiological interfaces to cardiomyocytes^{95,96} and mammalian neurons,⁹⁷ with clear cell-to-electrode registry. These electrodes can even perform intracellular recording and stimulation of neurons in a highly scalable fashion *in vitro* and *ex vivo*.⁹⁷ One possibility is to couple these nanoscale electrodes together with the modular 3D brain interfacing technology that has recently been developed.⁹⁸ These 3D devices, which are

assembled from lock-and-key digitally designed elements, enable positioning of electrodes with micrometer-scale precision yet can extend a centimeter or more in linear dimension and deliver and collect information from thousands of points throughout the brain. While the technology was originally developed for brain-wide optogenetic mapping,⁹⁹ it can also be used for practically any sensing and stimulation modalities, including electrodes, chemical sensors, and camera pixels. When coupled together, the nanoscale electrodes and 3D interface system will enable high-precision observation of synapse- and subthreshold-resolution neural activity in neurons throughout complex intact brain circuits, thereby addressing many of the early technological goals of the BAM project.

Flexible and active electronics offer another potential option for interfaces to neural circuits and the brain.^{100–106} Significant progress has been made in the areas of ultrathin, flexible, light, biocompatible circuits. Hundreds of contacts to the brain can be made on double-sided active semiconductor nano-membrane electronics transferred onto silk or other flexible substrates (Figure 3).¹⁰⁶ Early examples of these devices already integrate electrodes, sensors, amplifiers, and multiplexers at the hundreds- to thousands-of-devices scale and have been tested *in vivo*. Substrates and other parts can be made to be biodegradable/bioresorbable.¹⁰⁵ The possibilities of folding these flexible structures may enable less invasive means of introduction.

An additional challenge is electrode longevity, which is critical for ensuring the success of brain machine interfaces and long-term studies linking brain activity to behavior.^{105,107–109} The ability to record units from chronically implanted microelectrodes degrades on the time scale of days to years,^{110–112} and the mechanisms underlying this degradation have not been fully determined. On short time scales, disruption of the blood-brain barrier and cellular milieu from the stab wound may negatively affect recording performance^{113–115} but do not appear to prohibit high-yield measurements.¹¹⁶ On longer time scales, this injury triggers a cascade of molecular signaling events leading to a sustained inflammatory response around the implant.^{117,118} A variety of approaches are being explored to mitigate signal-to-noise reduction resulting from acute and chronic injury responses. Some of these include using flexible or wireless interconnects to decouple the electrode from shearing forces caused by brain micromotion in the skull,^{119,120} anti-inflammatory probe coatings,^{121,122} and techniques to coax neurons to form stable interfaces with the implanted devices.^{123,124} Nanotechnology has the potential to augment these efforts significantly by enabling extremely miniaturized sensors that have negligible adverse interactions with surrounding neural tissue or vasculature.¹²⁵ An important milestone in addressing electrode longevity challenges could be a minimally invasive wireless nanoscale probe that can attach itself to a single neuron and report its firing activity for greater than 1 year. Finally, many important observations of brain activity can take place on short behavioral time scales of minutes or hours. In this acute recording situation, the interface longevity issue is less critical than the need to sample as many neurons as possible.^{126,127} This creates an intriguing opportunity to pursue in parallel a different set of BAM tools for acute, ultra-large-scale electrophysiology and tools for chronic, ultrastable electrophysiology. Insights from these different technological development strategies could be combined in the final embodiment of the BAM electrode initiative.

Nanoparticle Labeling and Reporting

Over the past few decades, the development of more reproducible and accurate tools for monitoring and controlling chemical reactions has enabled the synthesis of an enormous variety of nanoparticles, nanomaterials, and nanostructures, with controlled composition, organization, shape, and functionalization. Examples include the evolution of fullerenes, carbon nanotubes, and graphene, materials systems in which desirable electrical and

mechanical attributes can be obtained by careful vapor deposition of carbon with accurate control over geometry and bonding. Similarly, carefully controlled chemical reactions have led to light-emitting materials defined in nanoparticles whose surfaces can be passivated to ensure high luminescence efficiencies. Today, quantum dots with emission wavelengths spanning the spectrum from ultraviolet to infrared can be found and are used for optical imaging, as efficient light sources, for photovoltaic energy harvesting systems, and for quantum physics experiments. As these particles are small enough to quantum-confine carriers in the semiconductor materials from which they are made, their geometries determine the blue shift in the band gap of the light emitter, and their emission wavelength can be tuned geometrically by changing their size or shape. Moreover, as the dimensions of these nanoparticles are small, it is possible to build up extremely large electrical or optical fields in quantum dots and wires, leading to switching and sensing opportunities far beyond simple photoluminescent light sources.¹²⁸ For imaging purposes, nanoparticle surfaces have been functionalized with specific binding chemistries to target and highlight local chemistries.^{129,130} Unlike more conventional organic dye molecules, quantum dots are less subject to many of the stability problems that can plague fluorophores, such as photobleaching and oxidation. We can also envision nanodevices with active optoelectronic properties, so that they become self-powered neural signal transmitters, perhaps tapping into the brain's metabolic pathways for operating power.

Chemical Measurements in the Brain

Direct chemical measurements can be made in the brains of live, behaving animals with probes that extract analytes (*e.g.*, microdialysis)^{131–134} or extracellular fluid¹³⁵ or that take measurements locally and directly (*e.g.*, electrochemical measurements for electroactive compounds such as dopamine).^{12–17} Currently, the spatial and temporal resolutions for chemical measurements based on extraction are $\sim 100\ \mu\text{m}$ and tens of seconds to minutes, respectively, whereas local electrochemical methods can measure at the few micrometer scale and hundreds of milliseconds, respectively.

Indirect *in vivo* measurements also can be made of nonelectrochemically active neurotransmitters (*e.g.*, glutamate, acetyl choline) and other molecules by coupling enzymatic reactions that produce electrochemically active products to sensors.^{136–139} These biosensor methods have somewhat lower spatial and temporal resolution than direct electrochemical sensors but could be coupled to the parallel platforms envisaged for the BAM project. Likewise, as platforms are developed for this project, artificial receptors will be developed^{140,141} and coupled for electronic measurements^{142,143} to sense the spatial and temporal profiles of neurotransmitters *in vivo*.

New Imaging Tools

Optical interrogation of populations of neurons in intact animals critically depends on two things: the ability to deliver light efficiently to the brain and the ability to get light out of the brain. These problems are difficult in the single-cell context and become more challenging when parallel simultaneous measurements are required. Today, both conventional fluorescence microscopy and nonlinear optical microscopy play important roles in imaging neural activity in behaving animals.¹⁴⁴ High-speed, miniaturized epifluorescence microscopes (<2.0 g mass) have been fabricated from mass-producible optical parts, such as light-emitting diodes (LEDs) and nanofabricated semiconductor sensors (Figure 4).¹⁴⁵ An adult mouse can readily bear such a microscope on the head during active behavior, which routinely allows imaging of calcium dynamics in >1000 neurons per mouse (Figure 5).¹⁴⁶ To reach deep brain areas, this approach relies on optical needles based on microlenses.

Alternatively, to maintain single-cell precision and localized excitation volumes within tissue that scatters light, one can use nonlinear optical microscopies in the near-infrared (NIR) region because of higher transmission relative to visible wavelengths.¹⁴⁷

Despite the reduced scattering of NIR photons compared with visible photons, the exponential loss of ballistic photons in scattering media greatly limits our ability to penetrate deep within the brain.¹⁴⁸ One strategy to increase image contrast and total depth is to increase the nonlinearity of the excitation process, which can dramatically reduce background signal. This can be done directly, as in the case of long-wavelength three-photon fluorescence microscopy,¹⁴⁹ or through cascaded nonlinearities enabled by new fluorophores.¹⁵⁰ Both techniques still require delivery of ballistic photons and suffer in scattering media. However, in the past decade, there have been notable improvements in the ability to deliver light deep within complex media.^{151,152} In these experiments, the wavefront of the incoming light beam is dynamically modified to compensate effectively for the aberrations and scattering bodies in the sample. The more complex the media, the more complex the wavefront modifications required for correction, and the greater the number of iterations of corrections that will be required to compensate. The most dramatic examples have been performed using single-photon illumination with high-speed deformable mirror devices (DMD), which can modulate the wavefront at rates of ~20 kHz, and have achieved near diffraction-limited performance imaging through a dynamically changing turbid environment. The same methods have been used successfully in directing light and imaging through multimode fibers,¹⁵³ offering the potential of minimally invasive imaging of deep internal structures. Unfortunately, the same successes have not yet been achieved with nonlinear microscopies, as the modulation devices are significantly slower. This difference occurs because, in order to maintain excitation efficiency, phase-only modulators are used, and the best devices for this purpose are phase-only liquid-crystal spatial light modulators where the liquid crystal properties limit speed to frame rates up to 500 Hz. Within the materials/nanoscience communities, there is a tremendous opportunity for applying solid-state or polymer technologies to address this problem. For optical switching, many materials can provide fast phase modulation, with switching times in the GHz. The development of a high resolution (>1 Mpixel) phase modulator array might enable deep-tissue optical imaging. These significantly higher speeds make digital/optical phase conjugation or compressive sampling more powerful when working with scattering media and enable deeper imaging in dynamically changing samples with temporal resolution below the decorrelation time.

High-resolution, high-speed arrays would also enable the creation of holograms for multisite excitation throughout the volume, which is a possible means of attaining high-speed, parallel measurements. Temporal coding of individual target sites might also enable high-speed, parallel measurements. However, in nonlinear optical imaging, holograms that illuminate large volumes of tissue have the notable disadvantage of reducing the instantaneous light intensity at any one location. In comparison, time-multiplexing schemes, such as those using acousto-optic deflectors, have the distinct advantage that they keep the instantaneous illumination high. Furthermore, as the focal point moves deeper into the sample, the emitted photons scatter during escape. When the focal depth increases beyond ~10 scattering lengths, the escaping photons lose memory of their origins and approach the photon diffusion limit. Here, the effective source size of the illuminated object scales proportionally with the depth of the target, making simple imaging insufficient to recover the individual signals. With fast modulation of the hologram, one could beat individual targets at different rates, enabling temporal encoding of the multiplexed illumination such that unique signatures exist for each overlapping target.

Along with improvements in the input of light to the system, we also need to improve the detectors measuring light coming out of the sample. Custom-designed microcavities and photonic crystals offer the possibility of extremely sensitive detection of the local environment and activity.¹⁸ Additionally, these microcavities can be used to enhance the brightness and spectral properties of coupled fluorophores, through the Purcell effect.¹⁵⁴ Large fields of view (FOVs) are required to collect emitted photons efficiently from deep within the samples. Here, there are opportunities to harness the nanoscale electronics community to develop large FOV multichannel avalanche photodiode (APD) arrays that would offer significant multiplexing advantages over traditional detectors. The high time resolution and channel independence provided by such arrays would be well-suited to demultiplex complex signals arising from deep *in vivo* multisite excitation.

Many cellular structures regulating neuronal activity (including synapses, tight junctions, actin, microfilaments, and receptor complexes) are composed of densely arrayed components with spacings that are complex and far below the diffraction limit (Figure 6). New optical imaging tools with nanoscale resolution, such as PALM¹⁵⁵ and STORM,¹⁵⁶ are helping scientists explore these nanoscale objects within cells. These techniques can resolve structures in microscopic images with ~20 nm or better spatial precision. They thus promise to help uncover the organizational principles of macromolecular complexes within specialized cells of the nervous system. As an example, recent work employing these techniques has revealed the dynamic behavior and organization of the actin cytoskeleton inside cells, which is relevant for understanding how neurons probe their involvement during neuronal outgrowth and in response to injury¹⁵⁷ and how they differentiate axonal processes.¹⁵⁸ These techniques also permit characterization of receptor clustering and stoichiometry at the plasma membrane under diverse conditions^{159,160} as well as protein organization inside synapses,¹⁶¹ which are critical for understanding how synapses respond to changes in neuronal activity.

Optogenetics

Brain activity mapping is tightly linked to optogenetics—the use of light to control well-defined events within targeted elements of intact biological systems (reviewed in ref 162)—in several important ways (Figure 7).¹⁶³ Control of brain activity in a precise, targeted, high-speed manner (*e.g.*, as enabled by optogenetics with microbial opsin genes,^{89,162,164–166} which encode light-activated channel and pump regulators of transmembrane ion conductance) in principle will allow assessment of the causal significance of brain activity patterns observed with recording or imaging. In this way, activity mapping becomes not simple, passive observation of activity correlating with behavior but observation coupled with insight into causal significance—a key distinction.

Further, optogenetics enables the identification of cellular genetic identity by “phototagging”. This method could help liberate high-speed readouts of neural activity from the need to be intrinsically genetically targeted. For example, multiunit electrical recording itself carries essentially no information on the genetic phenotype of the neurons generating the recorded spikes; however, when linked to phototagging (in which a genetically encoded control tool is introduced to drive spiking), corresponding electrical spikes with their own unique waveforms are observed after light pulse initiation at sufficiently brief intervals and can in some cases thereafter be inferred to belong to the genetically targeted class.¹⁶⁷ In this way, optogenetics can enhance and enable pure activity mapping itself.

Optogenetic tools are themselves nanoscale devices that can be engineered for new classes of brain activity mapping function, building on molecular structure–function relationships. Not only can many different kinds of ion flow, spectral responses, and kinetics be achieved

through directed engineering,^{162–164} but also fundamentally new classes of function can arise from this kind of work (*e.g.*, turning a microbial opsin into a voltage sensor for activity mapping⁹). Moreover, the success of optogenetics has inspired discussions of other possible classes of control (*e.g.*, magnetogenetics, acoustogenetics) in which other modalities of energy delivery would be captured by distinct classes of engineered, targetable-molecular-energy antenna-like elements expressed in specific classes of cells.^{163,168,169}

Optogenetics has already found widespread utility in mapping circuits causally involved in both normal function and in the elicitation and correction of disease-related phenomena including anxiety, depression, fear memory, parkinsonism, and social dysfunction.^{170–174} We have pointed out that additional technologies also need to be developed further for this approach to reach its full potential.²⁰ (1) methods for determination of global (brain-wide) wiring diagrams of cells that are observed and controlled *in vivo*; (2) volumetric, genetically targeted methods both to visualize and to control activity within intact tissue,^{98,175,176} and (3) non-optical methods that leave a recoverable trace of activity within cells to sidestep the light-scattering problem, which could involve a gene encoding a designer polymerase transduced into a genetically targeted subset of neurons,^{19–21} especially if the polymerase were engineered for increased error rate in elevated Ca²⁺,²⁰ which can track neural activity patterns at high speeds, even in the nucleus.¹⁷⁷ Together, these concepts highlight how optogenetics approaches could address key goals of brain activity mapping but require integration with other established and novel technologies.

Biological Hybrids and Synthetic Biology

Functional metagenomics can survey the biosphere for extraordinary new nanocomponents (typically proteins), which, *via* protein engineering and laboratory selections, can be fused and optimized to make complex systems very much at home in nanometer- to centimeter-scale biological networks. These bio-nano “parts” can include not only fluorescent ion sensors and light-responsive channels (*vide supra*) but also light-emitting sensors (luciferases) and fluorescent voltage (action potential) indicators like Archaeorhodopsin 3 (Arch) with an optical signal-to-noise ratio >10 and 41 ms time response.⁹

Synthetic biology can potentially provide hybrid system interfaces with inorganic fabricated components, including building or bridging 3D optical fiber arrays^{91,98} to provide effectively high optical surface area and multiplexing—for example, thin natural light wave guides in glass sponges.¹⁷⁸ Building optical fibers around the extensive brain vasculature might be less disruptive than inserting them as solid arrays from the surface (or could complement such arrays). Dynamic viral capsids, DNA nanorobots,¹⁷⁹ and/or engineered cells can navigate the blood-brain barrier, and cerebrospinal fluid or trans-synaptic clefts¹⁸⁰ can provide targeting specificity for brain activity input/output and/or neuronal connectivity data. Polymerases can provide analog-to-digital “ticker tape” recording sensors for light, ATP (*e.g.*, in active synapses), and ions.^{1,181} Fluorescent *in situ* sequencing (FISSEQ)¹⁸² or hybridization (FISH)¹⁸³ could enable alignment of electrode or optical fiber arrays with the anatomical connectome, RNA transcriptome, and ticker tapes all in one set of serial sections—reminiscent of the Allen Brain atlas,⁴⁰ but without the artifacts (and cost) of aligning “similar” regions from numerous different brains. Insertion of (up to billions of) synthetic DNA barcodes is helpful not only for neuronal lineage analysis¹⁸⁴ and synaptic connectivity¹⁸⁰ but also for integrating the multidisciplinary brain data (*vide supra*) with the controlled sensory inputs and behavioral outputs in BAM experiments on individual animals over diverse (normal and pathological) genetic and pharmacological backgrounds.

Connecting Neuroscience at the Molecular and Dynamical Systems Levels

Brain activity mapping electrode technology will enable scientists and clinicians to generate an inherently different type of data set than what genomic sequencing or brain-wide gene expression maps provide, which are static or slowly evolving snapshots of molecular information. In contrast, BAM technology will reveal an extremely complex choreography of neuronal activation that is constantly in flux. One of the major challenges for BAM technology development is to unify this electro-physiological view with the equally complex and important molecular landscape of the brain. After all, behavior, learning, and cognition require synergy between chemical neurotransmission, biochemical reactions, and electrical impulses. It is critical that, in addition to enabling recordings from unprecedentedly large numbers of neurons, BAM also enhances these incredibly powerful complementary levels of analysis. The development of multifunctional nanoscale probes that can simultaneously record and pharmacologically or optogenetically perturb molecularly defined neurons, with high spatial and temporal precision, may be a necessary step in providing critical tests of proposed mechanisms and theories of neural circuit and brain function.

Theory, Modeling, and Computation

One of the goals of brain activity mapping is to provide large data sets to be used in modeling and developing theories of the emergent properties of neural circuits and detailed connections between different levels of operations and hierarchical abstractions, including natural, synthetic, and practical spatiotemporal patterns. Likewise, technology to be developed can provide critical tests of these models and theories by active interaction with neural circuits. We anticipate that open access to these data sets will draw worldwide interest in and attention to the task of developing this understanding. The data sets will be large but comparable to those being produced by current astronomical observations, in genomics, and other areas. As in these other large data sets, compression and leveraging tools developed in the field of sparsity will likely be used heavily.¹⁸⁵

Equally important for achieving a deeper understanding of brain function is the comparable development of a conceptual framework and mathematical theory for brain activity in high-dimensional spaces. Current theories of brain function based on recordings from single neurons are limited in scope. Population codes have been deduced by combining recordings from many neurons recorded separately. Observing population codes as they unfold in real time should reveal deeper and dynamically shifting relationships between the neurons and the ongoing processing occurring in dense brain circuits. The population dynamics of the neural ensemble carries a far richer representation of sensory stimuli and actions.

Simulations of brain activity will be another important tool for refining our ideas about brain function, based on experimental recordings and additional constraints from the cellular and molecular levels of brain organization. The new methods for recording and manipulating neurons, including intracellular biochemical pathways, will enable us to design experiments that test competing hypotheses. For example, the responses of neurons in the visual cortex have latencies that range from 20 to 100 ms, but we have the impression of a single moment when a stimulus flashes. Is there a deeper neural correlate among a large population of neurons that is more closely associated with our subjective impression of time? How do we store and recall the temporal structures that occur in music? The BAM Project may bring us closer to answering ultimate questions about how we think and make decisions, which involve the coordinated activity in large numbers of neurons widely distributed throughout the human brain. Understanding the principles of neural computation will also lead to new devices based on these principles.

PROSPECTS

In these and other areas, there are tremendous opportunities for nanoscience and nanotechnology to contribute to neuroscience. We have collected answers to frequently asked questions on the BAM Project as this exciting proposal now stands. We hope that the BAM Project will bring the past decade's national and international investments in science, technology, and people in nanoscience and nanotechnology to bear on important and challenging problems in brain science.

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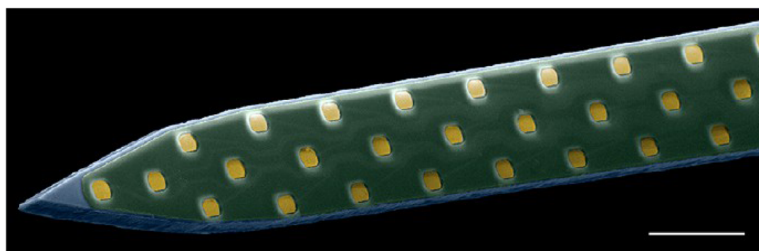


Figure 1. Nanofabricated planar electrode array for high-density neuronal voltage recording. False-color SEM image of a portion of a 64-channel array patterned on a silicon substrate. Scale bar = 50 μm . Modified from ref 37. Copyright 2011 Du *et al.*

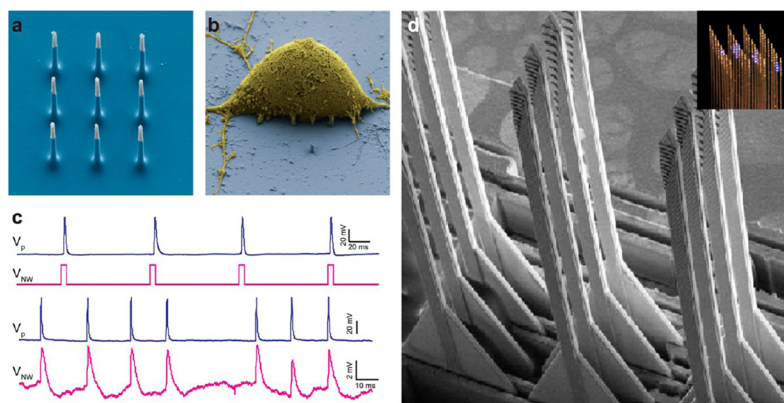


Figure 2.

Three-dimensional nanoelectrode array (3D-NEA) for *in vivo* interrogation of neuronal networks. (a) Scanning electron microscope (SEM) image of the nine silicon nanoneedles that constitute the active region of a 3D-NEA. Dimensions of the nanoneedle electrodes are designed to facilitate single-cell intracellular electrical coupling. False colors show metal-coated tips (gray) and insulating silicon oxide (blue). Reprinted with permission from ref 97. Copyright 2012 Nature Publishing Group. (b) Scanning electron micrograph of a rat cortical cell (3 days *in vitro*, false colored yellow) on top of an electrode pad (false colored blue). Reprinted with permission from ref 97. Copyright 2012 Nature Publishing Group. (c) Stimulation and recording of rat cortical neurons. Upper traces show that action potentials (blue: measured by a patch pipet) could be reliably stimulated by voltage pulses applied to the nanoelectrodes (magenta). Similarly, lower traces show that the nanoelectrodes can record action potentials (magenta) stimulated by a patch pipet (blue). Reprinted with permission from ref 97. Copyright 2012 Nature Publishing Group. (d) Scanning electron micrograph of a representative 3D brain-interfacing device consisting of 24 probes, each containing arrays of active sites distributed along their length. Inset: optical image of the 3D probe array. Reprinted with permission from ref 98. Copyright 2012 Optical Society of America.

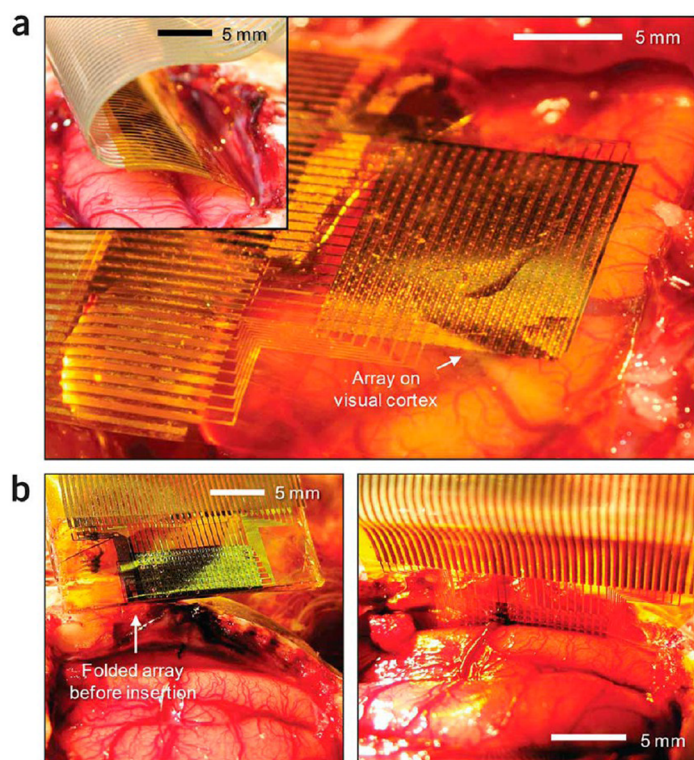


Figure 3. (a) Flexible, high-density active electrode arrays composed of semiconductor nanomembrane electronics transferred onto polymer substrates were placed on the visual cortex of a cat brain or (b) into the interhemispheric fissure (inset). Reprinted with permission from ref 105. Copyright 2011 Nature Publishing Group.

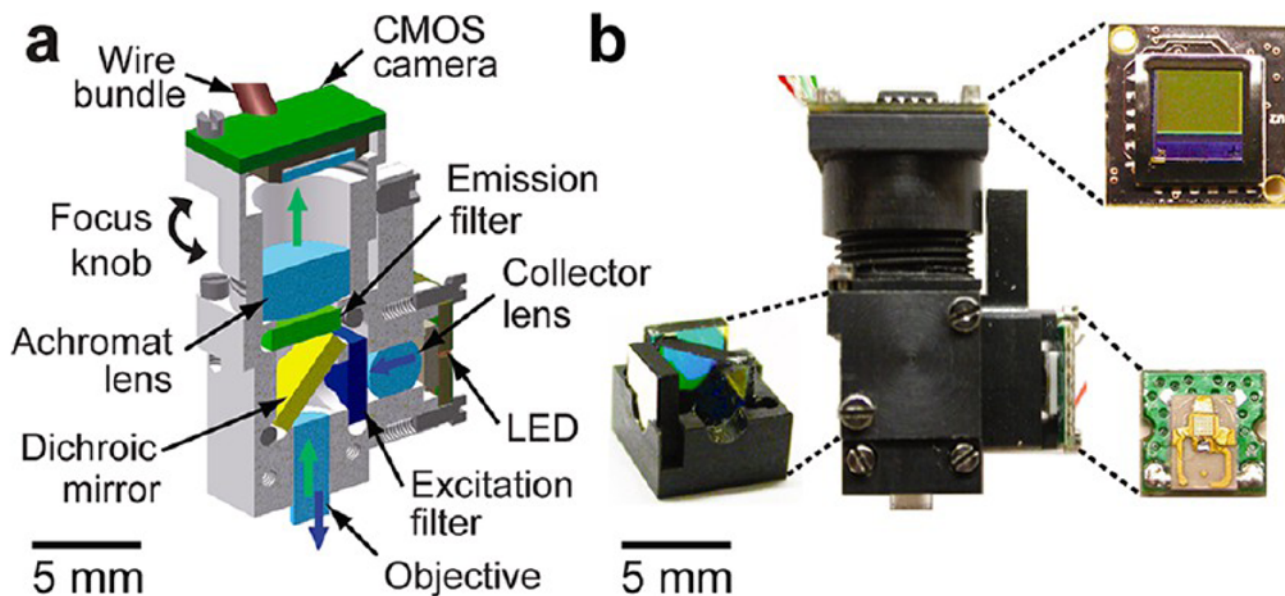


Figure 4. Miniature, mass-producible fluorescence microscope. (a) Cross-sectional schematic of the microscope design. Purple and green arrows show excitation and emission pathways, respectively. (b) Microscope (1.9 g) shown fully assembled with its LED light source, micro-optics, and camera. Insets show, clockwise from bottom-left, the fluorescence filter cube with excitation and emission filters and dichroic mirror; the mounted camera chip; and the LED light source. Scale bars for a, b, and insets are 5 mm. Reprinted with permission from ref 145. Copyright 2011 Nature Publishing Group.

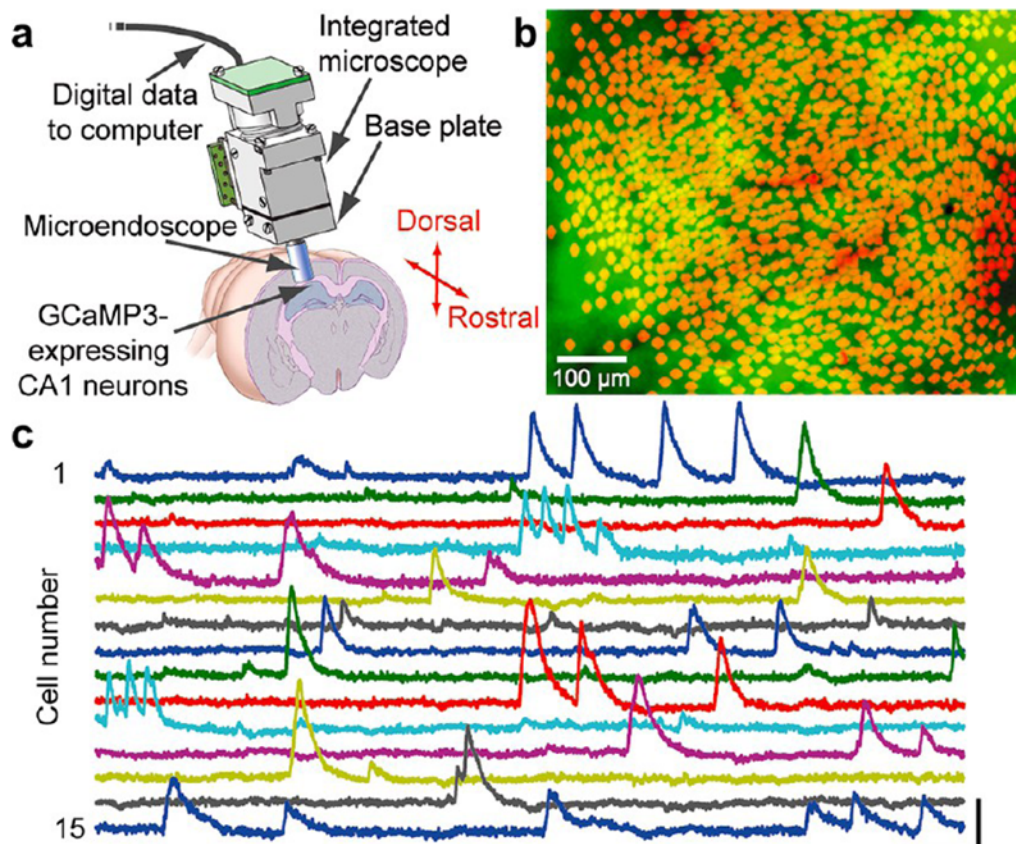


Figure 5.

Ca^{2+} imaging in >1200 CA1 pyramidal cells in freely moving mice. (a) Integrated microscope (Figure 4) is equipped with a microendoscope and images CA1 neurons expressing the Ca^{2+} indicator GCaMP3 via the *Camk2a* promoter. The base plate and microendoscope are fixed to the cranium, for repeated access to the same field of view. Reprinted with permission from ref 146. Copyright 2013 Nature Publishing Group. (b) 1202 CA1 pyramidal cells (red somata) identified by Ca^{2+} imaging in a freely moving mouse, atop a mean fluorescence image (green) of CA1. Vessels appear as dark shadows. Image courtesy of Yaniv Ziv and Lacey Kitch, Stanford University. (c) Example traces of Ca^{2+} dynamics from 15 cells. Scale bars: 5% $\Delta F/F$ (vertical) and 10 s (horizontal). Reprinted with permission from ref 146. Copyright 2013 Nature Publishing Group.

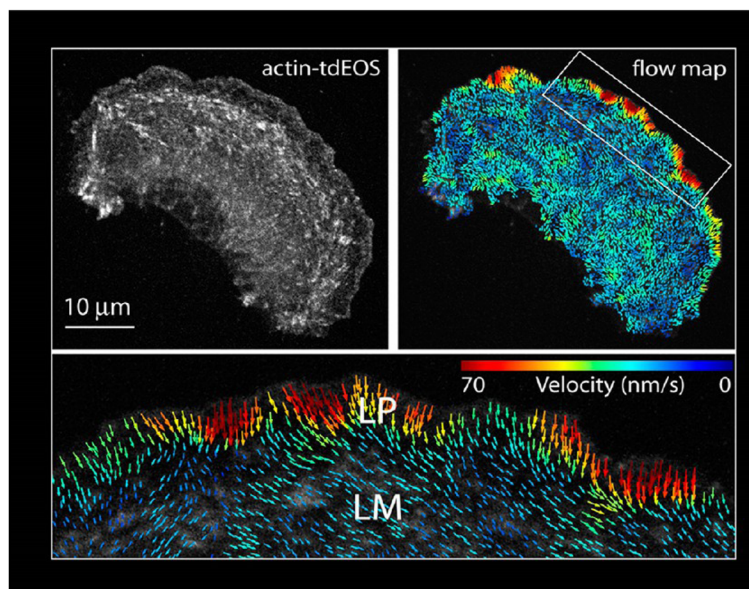


Figure 6.

Actin retrograde flow rates at the leading edge of a PtK1 cell. The photoactivatable protein tdEos tagged to actin was expressed in PtK1 cells. Individual molecules were visualized through photoactivation with ultraviolet light. They were then tracked over time to reveal movement of individual actin molecules within actin filaments at the edge of the cells. A flow map of rates of actin filament movement from the cell surface is shown. Vector colors reflect flow speed (color bar), and arrows reflect direction. The scale bar is 10 μm. Reproduced with permission from ref 157. Copyright 2011 Nature Publishing Group.



Figure 7. Brain activity mapping may be enabled, in part, by optogenetic preparations as shown here in a freely moving mouse; green light is delivered to deep or superficial brain areas *via* fiber optics. Optogenetic control of microbial protein-expressing targeted neurons enables (1) determination of causal significance of activity patterns; (2) in some cases, phototagging identification of cells from which electrical spikes are recorded; and (3) in some preparations, imaging of neural responses to control or stimulation. Advances in computational optics and nanoscale device engineering will further enable delivery of complex spatially modulated light patterns to the target tissue. Figure adapted from Inbal Goshen and Karl Deisseroth, Stanford University/HHMI.